

Physiological responses in rufous-collared sparrows to thermal acclimation and seasonal acclimatization

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Abstract A large number of physiological acclimation studies assume that flexibility in a certain trait is both adaptive and functionally important for organisms in their natural environment; however, it is not clear how an organism's capacity for temperature acclimation translates to the seasonal acclimatization that these organisms must accomplish. To elucidate this relationship, we measured BMR and TEWL rates in both field-acclimatized and laboratory-acclimated adult rufous-collared sparrows (*Zonotrichia capensis*). Measurements in field-acclimatized birds were taken during the winter and summer seasons; in the laboratory-acclimated birds, we took our measurements following 4 weeks at either 15 or 30°C. Although BMR and TEWL rates did not differ between winter and summer in the field-acclimatized birds, laboratory-acclimated birds exposed to 15°C exhibited both a higher BMR and TEWL rate when compared to the birds acclimated to 30°C and the field-acclimatized birds. Because organ masses seem to be similar between field and cold-acclimated birds whereas BMR is higher in cold-acclimated birds, the variability in BMR

cannot be explained completely by adjustments in organ masses. Our findings suggest that, although rufous-collared sparrows can exhibit thermal acclimation of physiological traits, sparrows do not use this capacity to cope with minor to moderate fluctuations in environmental conditions. Our data support the hypothesis that physiological flexibility in energetic traits is a common feature of avian metabolism.

Keywords Energetics · Water economy · Physiological flexibility · Passerines · Seasonality

Abbreviations

BMR	Basal metabolic rate
Cw	Wet thermal conductance
CWL	Cutaneous water loss
RWL	Respiratory water loss
Tb	Body temperature
TEWL	Total evaporative water loss

Introduction

Measuring an organism's response to a changing environment can yield insight into the adaptive value of phenotypic adjustments. Using this rationale, several studies have shown that the environmental temperature shapes the biogeographical ranges of birds via temperature's influence on physiological demands (Porter et al. 2000; Root 1988). For instance, evolutionary adjustments appear to have occurred in rates of energy expenditure (e.g., basal metabolic rate, BMR) at both intra- and inter-specific levels (Dawson et al. 1983b; Hinsley et al. 1993; Repasky 1991; Swanson 1993; Wiersma et al. 2007; Withers and Williams 1990). Total evaporative water loss (TEWL) also appears to be responsive to both environmental temperature and water availabil-

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ity (Haugen et al. 2003a, b; Muñoz-Garcia and Williams 2005; Tieleman and Williams 2002; Williams 1996, 1999). Studies on energy and water budgets have shown that birds from arid areas have lower TEWL and BMR compared to birds from mesic areas (Sabat et al. 2006; Tieleman and Williams 2000; Tieleman et al. 2003a). A proximal mechanism proposed to explain the lower TEWL in desert birds is to reduce the water permeability through the skin (Muñoz-Garcia and Williams 2005). Additionally, it has been proposed that the high environmental temperatures and water scarcity in arid environments is a selective pressure that favors individuals with lower BMR because endogenous heat production is lower and, therefore, the water requirements for evaporative cooling are reduced (Dawson 1984; Tieleman and Williams 2000).

Because of climatic seasonality, bird species that do not migrate must often cope with fluctuating environmental conditions (e.g., environmental temperature, water availability). These species are expected to exhibit reversible phenotypic variation (Dawson et al. 1983b; Piersma and Vezina 2007; Tieleman et al. 2002) as an adaptive response to their variable environments (Kingsolver and Huey 1998). This kind of response has been termed phenotypic flexibility (Piersma and Drent 2003), and is believed to have evolved via natural selection to enhance the biological performance of individuals in a population (de Jong 1995; Futuyma and Moreno 1988; Moran 1992; Via 1993). Short-term changes in BMR (seasonal acclimatization and acclimation) have been documented in several birds species (Cooper 2002; Cooper and Swanson 1994; Piersma et al. 1995; Swanson 1991; Tieleman et al. 2003b; Williams and Tieleman 2000). In general, short-term changes in BMR are thought to arise from adjustments in (1) the mass of metabolically active organs and (2) the specific metabolic activity of such organs (Daan et al. 1990; Dawson et al. 1983b; Kersten et al. 1998; Piersma et al. 1999; Piersma and Lindstrom 1997; Weber and Piersma 1996). In addition, the flexibility of both BMR and internal organs is seemingly a common adaptive syndrome to variable environments (Piersma and Lindstrom 1997; McKechnie 2008). However, lesser efforts have been made to investigate physiological flexibility in TEWL in the laboratory (Tieleman et al. 2003b; Williams and Tieleman 2000) and in the field (Tieleman et al. 2002). The physiological mechanism responsible for adjustments in TEWL appears to be attributable to changes in cutaneous water loss (CWL; Menon et al. 1989). For example, it has been documented that water deprived birds have a reduced CWL and can modify both the deposition and composition of lipids in the stratum corneum (Menon et al. 1989; Muñoz-Garcia and Williams 2005). We tested if birds in the field also exhibit phenotypic flexibility in TEWL to enhance water conservation in response to water shortage.

The ecological and evolutionary significance of physiological acclimation can only be judged properly comparing the laboratory responses with the response of animals in the field. In birds, studies integrating laboratory and field responses are few. To address this gap, we compared the changes in BMR and TEWL induced by both seasonal acclimatization and laboratory acclimation. We performed this study in rufous-collared sparrows (*Zonotrichia capensis*, Passeriformes: Emberiziidae) from central Chile. The climate of central Chile is characterized as Mediterranean with seasonal fluctuations in both ambient temperature and precipitation. Rufous-collared sparrows are omnivorous and distributed between southeast Mexico and Cabo de Hornos in the southern Chile (Goodall et al. 1951). In Chile, its distribution is almost ubiquitous and includes both deserts and rain forests (Araya 1996).

Methods

Field methods and study site

Rufous-collared sparrows were collected from Quebrada de la Plata (33°31'S, 70°50'W), a region in central Chile with a Mediterranean climate that is characterized by hot, dry summers and cold, rainy winters (di Castri and Hajek 1976). Some vertebrate species from this area have previously been shown to exhibit seasonal changes in morphology, physiology and behavior (Sabat and Bozinovic 1994; Sabat et al. 1998; Kenagy et al. 2002; Bozinovic et al. 2004). Annual mean precipitation in this area is 367 mm with the majority occurring during the austral winter (208 mm from June to August); during the austral summer (December–March), this area only receives 3% of the annual mean precipitation. Highest ambient temperatures occur during the austral summer with a mean of 18.9°C and maximum daily range from 26.9 to 29.7°C. The lowest ambient temperatures occur during the austral winter (June–August) with a mean of 8.3°C and maximum daily range from 14.9 to 16.7°C.

Acclimatization

A total of ten adult birds were captured with a mist net in July 2006 (Austral winter); ten were captured in January 2006 (Austral summer). Following capture, we transported them 30 km to a laboratory in Santiago, Chile (33°27'S, 70°42'W). Here, birds were held in individual plastic-mesh cages (35 × 35 × 35 cm) in a constant temperature room (22 ± 2°C) on a 12L:12D photoperiod. While in captivity, the sparrows consumed mealworms, bird seed and water, which were available ad libitum. After habituating birds to laboratory conditions for 1 day, we measured BMR

(mL O₂ h⁻¹) and TEWL (mg H₂O h⁻¹) at 30°C, which is within the thermoneutral zone for this species (Sabat et al. 2006). Temperature data from a meteorological station near the site of capture for 30 days prior to capture indicated that the mean maximum temperature of the winter (July) and summer (January) periods were $15.8 \pm 5.1^\circ\text{C}$ and $28.1 \pm 2.9^\circ\text{C}$, respectively (Pudahuel meteorological station, Dirección Meteorológica de Chile). Mean minimum temperatures were $5.5 \pm 3.0^\circ\text{C}$ and $11.3 \pm 2.0^\circ\text{C}$ for winter and summer, respectively (Pudahuel meteorological station, Dirección Meteorológica de Chile). The range of minimum temperatures on the days of capture were: $12.3\text{--}19.5^\circ\text{C}$ (mean: 15°C), $0.6\text{--}7.1^\circ\text{C}$ (mean 5.5°C) for summer and winter, respectively; maximum temperatures on the day of capture were $21.7\text{--}29.7^\circ\text{C}$ (mean 27.2°C) and $10.3\text{--}12.6^\circ\text{C}$ (mean 11.2°C) for summer and winter, respectively.

Laboratory acclimation

A total of 19 birds were captured in September 2006. In the laboratory, birds were housed individually in plastic-mesh cages ($35 \times 35 \times 35$ cm) under a 12L:12D photoperiod at $22 \pm 1^\circ\text{C}$. Following capture, the animals were habituated to the laboratory conditions for 1 day. During this period, the birds consumed water, seed mixture, and mealworms which were provided ad libitum. After habituation, each bird was assigned to one of two treatments: one group was housed in a constant temperature room at $15 \pm 1^\circ\text{C}$ (cold-acclimated group, $n = 10$) and the other at $30 \pm 1^\circ\text{C}$ (warm-acclimated group, $n = 9$). These two temperatures are comparable to the mean of daily maximum temperatures experienced by birds in the field in winter and summer, respectively.

We were confident that these two temperatures present birds with contrasting thermoregulatory demands because the rise in oxygen consumption over the BMR at 15°C is near 64% in *Z. capensis* (Sabat et al. 2006). The body mass at the beginning of experiments was similar in both groups ($F_{1,8} = 0.193$, $P = 0.67$). After an acclimation period of 30 days, we measured BMR and TEWL in both the cold- and warm-acclimated birds at 30°C , a temperature which is within the thermoneutral zone for this species (Sabat et al. 2006).

Oxygen consumption and TEWL measurements

Measurements of BMR were made in post-absorptive, resting birds during the inactive phase using standard flow-through respirometry and hygrometry methods. Briefly, birds were weighed, placed in a dark metabolic chamber (1 L), and then placed in a controlled temperature cabinet at a constant temperature ($T_a = 30 \pm 0.5^\circ\text{C}$). Oxygen consumption was measured using a computerized, open-flow

respirometry system (Sable Systems, Henderson, Nevada) calibrated with a known mix of oxygen (20%) and nitrogen (80%) that was certified by chromatography (INDURA, Chile). The measurement and calibration protocols we followed were after Williams and Tieleman (2000). Because CO₂ was scrubbed before entering the O₂ analyzer, oxygen consumption was calculated as (Withers 1977, p. 122): $VO_2 = [FR \times 60(F_iO_2 - F_eO_2)] / (1 - F_iO_2)$, where FR is the flow rate in mL/min after STP correction, and F_i and F_e are the fractional concentrations of O₂ entering and leaving the metabolic chamber, respectively. We calculated absolute humidity (kg/m³) of air entering and leaving the chamber as $\rho = P / (T \times R_w)$, where P is water vapor pressure of the air in Pascal, T is the dewpoint temperature in Kelvin and R_w is the gas constant for water vapor (461.5 J/kg K, Lide 2001). The P_{in} was determined using the average value of the vapor pressure of the air entering the empty chamber (i.e., the baseline period of 15 min) before and after each experiment with a dew-point hygrometer (RH 200, Sable System, Henderson, Nevada). TEWL was calculated as $TEWL = (V_e \rho_{out} - V_i \rho_{in})$, where TEWL is in mg/mL, ρ_{in} and ρ_{out} are the absolute humidity in kg/m³ of the inlet air and the outlet air, respectively, V_i is the flow rate of the air entering the chamber as given by the mass flow controller (500 mL min^{-1}), and V_e is the flow of exiting air. V_e was calculated following Williams and Tieleman (2000) as: $V_e = V_i - [VO_2 (1 - RQ)] + VH_2O$; V_{in} and VO_2 (mL min^{-1}) are known.

We used a respiratory quotient (RQ) of 0.71, assuming that fasting sparrows rely mainly on stored lipids (King and Farner 1961; Walsberg and Wolf 1995). Body mass was measured before the metabolic measurements using an electronic balance (± 0.1 g) and cloacal body temperature (T_b) was recorded at the end of each measurement with a Cole-Palmer copper-constantan thermocouple attached to a Digisense thermometer (Model 92800–15). Output from the H₂O (kPa) and oxygen analyzers (%) was digitized using a Universal Interface II (Sable Systems) and recorded on a personal computer using EXPEDATA data acquisition software (Sable Systems). Our sampling interval was 5 s. Birds remained in the chamber until a visual inspection of the recorded data allowed us to determine when steady-state conditions had been achieved (Tieleman et al. 2003b; Tieleman 2007). This typically occurs after 3 h in *Z. capensis* (Fig. 1). We averaged water vapor pressure and O₂ concentration of the excurrent air stream over a 20 min period after steady-state was reached (following Tieleman et al. 2002). Using BMR and body temperature data we calculated the wet thermal conductance (C_w , see Schleucher and Withers 2001) as $BMR / (T_b - T_a)$. After these trials, both field and laboratory animals were weighed, euthanized by over-exposure to CO₂, and dissected. Organs were then removed and massed (± 0.005 g).

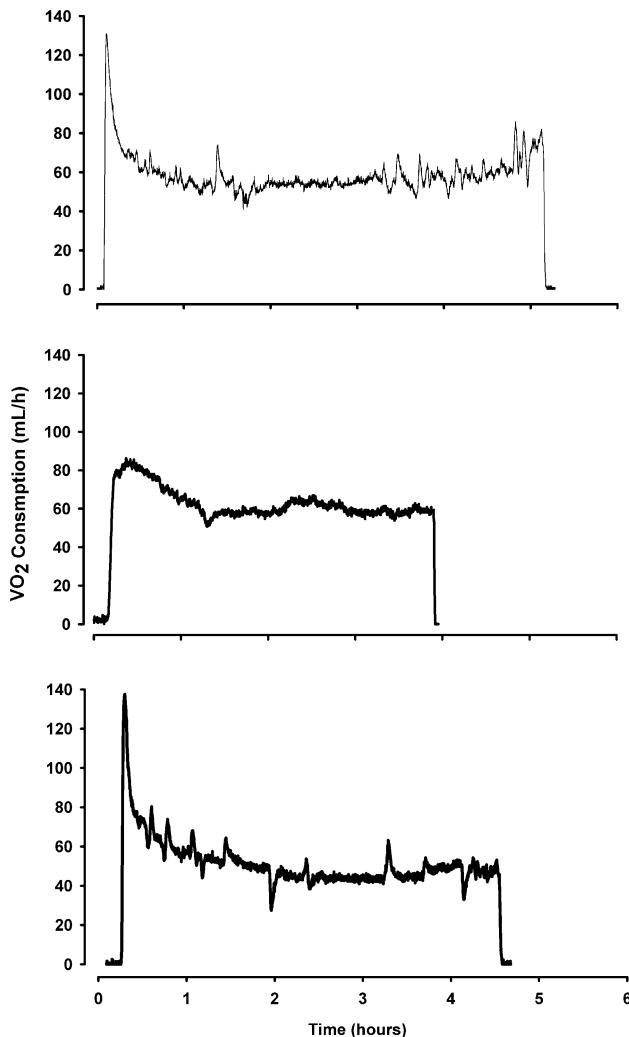


Fig. 1 Three representative VO_2 records in individuals of *Zonotrichia capensis* during the resting phase. Note that the lower consumption rates are achieved between 2 and 4 h of measurements

Data analysis

To test if BMR and TEWL differed between field and laboratory birds, and also between the temperature-acclimated and seasonal-acclimatized birds, we performed an analysis of covariance (ANCOVA), using the a posteriori Tukey test for multiple comparisons among groups. This design used the treatment (winter, summer, cold and warm) as the independent variable, and logTEWL and logBMR as dependent variables. Because dependent variables (TEWL, BMR) were correlated with body mass, the effect of body mass was removed using log of body mass as the covariate. Because changes in BMR may be associated with the mass of metabolically active organs (Karasov 1996; Mueller and Diamond 2001), we performed an ANCOVA to see if changes in liver, kidney, and heart masses were related to BMR variation. For the analysis of organ masses, we used

body mass minus organ mass as the covariate to avoid problems associated with part-whole correlations (Christians 1999). A preliminary model was run to test for homogeneity of slopes of the dependent variable versus the covariate among treatments. To explore the association between variables, we performed linear regressions and residuals analyses on double-log-transformed data. Statistical analyses were performed using STATISTICA 6.0. Data are presented as mean $\pm$ SD.

Results

Morphology

After the period of habituation to laboratory conditions, field birds lost in average 4% of their body mass ($t_{18} = 4.03$, $P = 0.001$ for all field birds). However, after 1 month of acclimation either to warm or cold conditions, final body masses did not differ from the initial weight ($t_9 = 1.10$, $P = 0.29$; $t_8 = 0.53$, $P = 0.60$ for cold and warm-acclimated birds, respectively). As a result, body masses of *Z. capensis* were significantly higher in the laboratory birds acclimated to cold compared to the field birds at the time the experiments were performed ($F_{1,35} = 4.055$; $P = 0.01$; Table 1). In addition, the ANCOVA revealed that organ masses were affected differentially by the experimental treatment: heart mass was affected by the experimental treatment ($F_{3,34(\text{treatment})} = 3.94$; $P = 0.02$, $F_{3,31(\text{treatment} \times \text{mb})} = 0.03$; $P = 0.99$), and the a posteriori analysis revealed that cold-acclimated birds had the heaviest hearts (Table 1). Liver mass was also affected by the experimental treatment ($F_{3,34} = 4.24$; $P = 0.01$, $F_{3,31(\text{treatment} \times \text{mb})} = 1.31$; $P = 0.29$). Here, an a posteriori analysis revealed that warm-acclimated birds had smaller livers (Table 1). Kidney mass was affected by the experimental treatment ($F_{3,34} = 3.63$; $P = 0.01$, $F_{3,31(\text{treatment} \times \text{mb})} = 2.12$; $P = 0.11$), and the a posteriori analysis showed that in the laboratory, cold-acclimated birds had heavier kidneys than warm-acclimated birds (Table 1).

TEWL and BMR

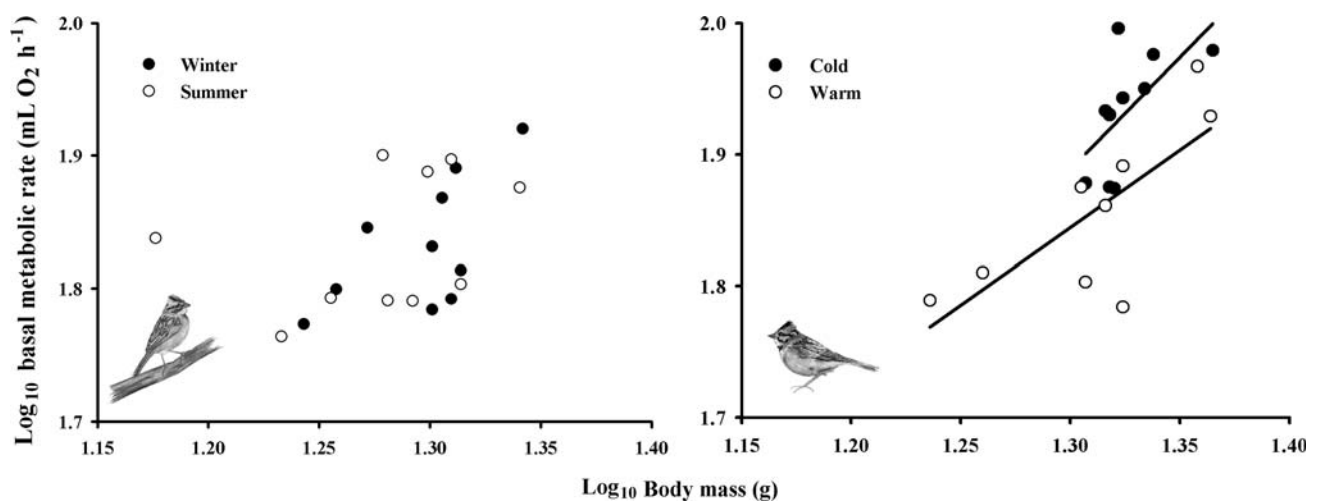
Comparisons of TEWL measured at 30°C revealed that means were statistically different between groups ($F_{3,34(\text{treatment})} = 5.07$; $P = 0.003$, $F_{3,31(\text{treatment} \times \text{mb})} = 0.70$; $P = 0.56$); TEWL was higher in summer birds and cold-acclimated birds than in warm-acclimated birds (Table 1). The difference in TEWL between summer and winter birds was not significant (Tukey test $P = 0.09$).

BMR was affected by treatments ($F_{3,34(\text{treatment})} = 4.541$; $P = 0.01$, $F_{3,31(\text{treatment} \times \text{mb})} = 0.76$; $P = 0.52$; Table 1; Fig. 2). BMR values of cold-acclimated sparrows were on

Table 1 Morphological and physiological measures of *Z. capensis* from field (winter and summer) and laboratory experiments (acclimated at either 15 or 30°C)

	Field		Laboratory	
	Winter (10)	Summer (10)	Cold (10)	Warm (9)
Body mass (g)	19.79 ± 1.33b	19.06 ± 1.96b	21.22 ± 0.82a	20.52 ± 1.90a,b
TEWL (mg h ⁻¹ g ^{-0.67})	68.19 ± 10.49a,b	78.38 ± 12.10b	78.98 ± 11.06b	65.73 ± 8.76a
Kidney mass (g)	0.23 ± 0.04b	0.27 ± 0.02a,b	0.28 ± 0.04a	0.23 ± 0.061b
Liver mass (g)	0.73 ± 0.22a	0.86 ± 0.12a	0.76 ± 0.21a	0.53 ± 0.16b
Heart mass (g)	0.26 ± 0.03a,b	0.23 ± 0.02b	0.28 ± 0.03a	0.23 ± 0.04b
Tb (°C)	40.36 ± 0.54a,b	40.15 ± 0.87b	41.04 ± 0.27a	40.53 ± 0.45a,b
C _w (mL h ⁻¹ °C ⁻¹)	6.62 ± 0.91	6.85 ± 1.33	6.88 ± 0.93	7.81 ± 0.77

Values of TEWL were corrected by body mass elevated to the allometric exponent from the equation: $TEWL = 15.48 mb^{0.67}$ resulting from the regression for all birds. Different letters denotes significant differences between treatments after ANCOVA, at $P < 0.05$. Values are mean ± SD

**Fig. 2** Basal metabolic rate as a function of body mass for *Z. capensis* acclimated to winter and summer conditions (left plot), and acclimated to 15 and 30°C in the laboratory (right plot). The allometric

equation representing the linear curve for all birds is $BMR = 3.09 mb^{1.05}$; $BMR = 3.98 mb^{0.94}$ for field animals; $BMR = 2.06 mb^{1.1}$ and $BMR = 2.09 mb^{1.7}$ for warm and cold groups, respectively

average 19% higher than warm-acclimated birds (Tukey test $P = 0.004$, Table 1, Fig. 2). This cold-acclimated group also had higher values of BMR than the seasonal determinations (all Tukey test $P < 0.001$). Basal metabolic rate in the summer group did not differ significantly from that in the winter group, Tukey test $P = 0.91$). An analysis of the field pooled data indicated that the log of TEWL and the log of BMR were positive correlated with the log of body mass (TEWL, $r^2 = 0.23$, $P = 0.04$; BMR, $r^2 = 0.27$, $P = 0.02$). Hence, to analyze the association between BMR and TEWL, we calculated the correlation coefficient between both log-transformed variables and also between the residuals obtained of each transformed variable against body mass. These analyses revealed a poor and nonsignificant association between these variables in the field ($r^2 = 0.14$, $P = 0.11$ and $r^2 = 0.10$, $P = 0.18$ for log-transformed variables and residuals, respectively). When all

acclimation data were pooled, both TEWL and BMR showed a positive and significant correlation with body mass (TEWL, $r^2 = 0.47$, $P = 0.001$; BMR, $r^2 = 0.49$, $P = 0.001$). The correlation analysis between logBMR and logTEWL, and between the residuals of both log-transformed variables against log body mass showed a positive and significant association ($r^2 = 0.60$, $P < 0.001$ and $r^2 = 0.32$, $P = 0.001$, respectively). We found that all animals were euthermic after the metabolic trials ($Tb > 40^\circ\text{C}$) and that Tb was affected by the experimental treatment ($F_{3,35} = 4.30$; $P = 0.01$). An a posteriori test revealed a significant difference ($P < 0.05$) of 0.84°C between the cold-acclimated and summer-acclimated groups (Table 1). Although wet thermal conductance seems to be higher in warm-acclimated birds, the ANCOVA analysis revealed that means did not vary among experimental groups ($F_{3,34} = 1.27$; $P = 0.30$).

Discussion

Evaporative water loss and metabolism

The rate of evaporative water loss is variable among birds species (Muñoz-García and Williams 2005; Tieleman and Williams 2000, 2002; Tieleman et al. 2002). However, the evidence for physiological flexibility in this trait is scant (Tieleman and Williams 2002; Tieleman et al. 2003b; Williams and Tieleman 2000). For example, Tieleman and Williams (2002) reported that among four larks species from desert and mesic areas, only one, the Hoopoe larks (*Alaemon alaudipes*), increased their mass-specific cutaneous water loss (CWL) after thermal acclimation. Additionally, Tieleman et al. (2002) found that both BMR and TEWL in Houbara bustards (*Chlamydotis macqueenii*) from Saudi Arabia were lower in summer than in winter. Our acclimation experiment revealed that, after correcting by body mass, cold-acclimated rufous-collared sparrows had higher TEWL values than those from the warm-acclimated group. Nevertheless, because 60% of the variation in TEWL is explained by variation in BMR (and 32% after removing the effect of body mass), the acclimation effect on TEWL could be the result of an indirect effect of temperature on oxygen consumption. In this sense, Williams and Tieleman (2000) suggested that an increase in oxygen consumption would increase respiratory frequency and therefore respiratory water loss (RWL), thus enhancing TEWL. This possibility is supported by the higher BMR found in cold-acclimated compared to warm-acclimated birds. On the other hand, a decrease in TEWL in response to higher ambient temperature and water deprivation has been associated with changes in the proportions of ceramides in the stratum corneum, which reduces CWL (Haugen et al. 2003a, b; Menon et al. 1989). Whether changes in skin permeability contribute to differences in TEWL in rufous-collared sparrows will require further study.

Several studies have shown that the size of internal organs may exhibit reversible changes in response to variations in energetic demands (Piersma and Lindström 1997). For example, increases in energy demands have been associated with enlargements in organs that contribute significantly to BMR, i.e., heart, kidneys, brain, and intestine (Daan et al. 1990; Kersten et al. 1998; Piersma et al. 1996; Williams and Tieleman 2000). Consistent with this idea, we found that both BMR and the masses of the kidney, liver and heart were higher in cold- than in warm-acclimated birds. Thus, adjustments of organ masses in acclimation experiments suggest that organ masses are modified to meet increased energetic demands. Nevertheless, compared with field birds, liver weight declined in the warm-acclimated group despite exhibiting similar BMRs. Additionally, cold-acclimated birds had higher BMRs compared to field ani-

mals, but their organ masses were similar among them. In summary, organ mass seems to be similar between field and cold-acclimated animals whereas BMR is higher in cold-acclimated birds. Thus, the variability in BMR cannot be explained completely by adjustments in organ masses, and additional variables might be considered, such as the specific metabolic activity of the internal organs.

Why do the energetic traits vary in the laboratory but not in the field?

Endotherms inhabiting seasonal cold environments often display adjustments in their levels of energy expenditure (Cooper 2002; Cooper and Swanson 1994; Dawson et al. 1983a). However, our results showed that BMR and TEWL in rufous-collared sparrows are relatively stable among seasons, although both variables were higher in cold than in warm conditions for laboratory-acclimated birds. In this vein, it is well known that laboratory studies involving short-term acclimation cannot completely simulate the conditions that birds experience in the field. Examples of uncontrolled factors in the laboratory influencing BMR are the body condition (Daan et al. 1990), hormone levels (Chastel et al. 2003) and diet (McNab 2003). Nevertheless, here we focused on one of the main factors influencing BMR in birds, i.e., environmental temperature (McKechnie 2008), in order to explain the seasonal and laboratory response of BMR in *Z. capensis*.

Usually any effect of thermal acclimation on physiology has been interpreted uncritically as the adaptive result of evolution by natural selection in response to variable environmental conditions (Bennett and Lenski 1999; Feder et al. 2000; Kingsolver and Huey 1998; Leroi et al. 1994). Nevertheless, the absence of acclimatization in BMR does not necessarily reflect environmental stability and/or physiological rigidity. Kingsolver and Huey (1998) argued that physical environments are not constant and consequently organisms can undergo fluctuations that may differ in time scales and severity. In this case, large scale oscillations could maintain the flexibility in thermogenic traits through generations. In addition, there is a precedent that environmental temperatures have a proximate role on regulation of avian metabolic acclimatization. Swanson and Olmstead (1999) found that winter BMR of three avian species varied among years and was negatively associated with mean winter temperature. They concluded that short-term temperatures (0–7 day preceding testing) to medium-term temperatures (14–30 day before testing) are better predictors of metabolic rates than long-term temperatures (100-year means average). Therefore, the magnitude of a flexible response would depend on the intensity of the environmental cues. A methodological consequence of this idea is that experimental analysis requires knowing the temperatures in

the natural habitats over a time window commensurate with the time it takes for the BMR to be acclimated. In our case this time window is 3–4 weeks (Gelineo 1964). Thus, seasonal variation in BMR may not be observed if the seasonal variations in temperature are insufficient to trigger adaptive responses. Considering daily maximum temperature of the 30 days prior to the capture, we could rule out this possibility because they are as different as the temperatures used in our experimental settings. Nevertheless, in spite of the seasonal difference in mean maximum temperature, the seasonal difference in daily minimum temperatures (ca. 5.8°C) is considerably smaller than the 15°C differential between cold- and warm-acclimated treatments. It is possible that daily minimum temperatures would have a greater influence on BMR than daily maximum temperatures since minimum temperatures would require higher rates of thermogenesis and therefore higher rates of energy expenditure. Because the seasonal difference in mean minimum temperature was only 5.8°C, this might not provide enough temperature difference to induce detectable seasonal changes in BMR.

Several studies have investigated seasonal changes in metabolic rates (including BMR) in response to environment temperature, but the conclusions from these studies are not in agreement (Hudson and Kimzey 1966; O'Connor 1995). For example, Dawson et al. (1985) studying free-living house finches (*Carpodacus mexicanus*) from Colorado and California reported no differences in BMR between seasons (winter and spring). As in our study, the temperature difference among seasons measured by Dawson et al. (1985) was moderate (15 and 10°C for maximum; 7 and 11°C for minimum) and much smaller than in those cases where seasonal acclimation in BMR reportedly occurs (20–25°C of difference between seasons, Cooper and Swanson 1994; Cooper 2000, 2002, Arens and Cooper 2005). In our study, an average temperature difference of 15°C for maximum temperatures in the field did not trigger a change in BMR, but the same difference elicited changes in the laboratory, that are comparable in magnitude with those reported for seasonal acclimatization (Cooper and Swanson 1994; Cooper 2002). As we have already stated, it is probable that the low difference in daily minimum temperatures could explain the lack of a seasonal difference in BMR. However, we found that laboratory animals exposed to cold, showed higher BMR than field animals. In conclusion, although *Z. capensis* can exhibit thermal acclimation of physiological traits, sparrows do not exhibit this capacity to cope with minor to moderate fluctuations in environmental conditions. Our data support the hypothesis proposed by Klaassen et al. (2004) that the physiological flexibility in energetic traits is a common feature of avian metabolism.

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