



# Intraspecific basal metabolic rate varies with trophic level in rufous-collared sparrows

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## ABSTRACT

One of the most controversial hypotheses that associate basal metabolic rate (BMR) with food habits and habitat productivity is the food habit hypothesis (FHH). Here we examined the relationship between BMR, diet, and climate among populations of the omnivorous passerine, *Zonotrichia capensis* (Emberizidae). We used nitrogen stable isotopes to estimate each individual's relative trophic level. To tease apart the effect of climatic variables and diet on BMR, we also used structural equation modeling. After the effect of body mass and climatic variables was taken into account, a significant effect of trophic level as estimated by  $\delta^{15}\text{N}$  on BMR was found. Our result seems to support the FHH at the intraspecific level, i.e., birds from the lower trophic levels – feeding on seeds and bud – had higher BMR than individuals from higher trophic levels.

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## 1. Introduction

Comparative physiologists have measured the basal metabolic rate (BMR) of endothermic vertebrates for decades (Kleiber, 1932). BMR is measured under standard conditions (i.e., in postabsorptive adults, within their thermoneutral zone and during inactivity) and therefore it is commonly used to compare energy demands among individuals, populations, and species (Speakman and Thomas, 2003). BMR is allometrically related to body mass, but the relationship between BMR and mass has large residual variation (McNab, 2002). Indeed, when the effects of body mass are removed statistically, a perceptible residual variation still remains; mass-corrected BMR can still vary by nearly tenfold (McNab, 2002). Using both the traditional comparative approaches as well as phylogenetic comparative methods, several studies have sought to correlate these differences in mass-corrected BMR with abiotic environmental (e.g., ambient temperature, rainfall), morphological (e.g. organ mass) and ecological factors (e.g., habitat productivity) and to determine whether observed patterns reflect genetic adaptation, phenotypic plasticity or phylogenetic constraints (McNab, 2002). Among the extrinsic biotic factors that affect the level of mass-independent BMR, the so called food habits hypothesis (FHH) postulate the existence of an evolutionary correlation between diet and mass-independent BMR. Although the ecological and evolutionary significance of this variation has received much attention

(Bozinovic and Rosenmann, 1988; White et al., 2007), as yet there is no consensus about the factors that generate it. McNab (1986) hypothesized that diet quality, food availability, and the predictability of food supplies should determine the value of BMR (Cruz-Neto and Bozinovic, 2004). Briefly, according to FHH, animals that feed on diets with low assimilable energy content and/or that live in habitats where food is scarce and/or unpredictable should have low mass-independent BMRs (Bozinovic et al., 2007a,b; McNab, 1986; Cruz-Neto et al., 2001). Alternatively, birds faced with low quality diets tend to have larger guts, which in turn could lead to an increase in BMR by the higher cost of maintenance of such organs. Because food availability and predictability are difficult to quantify, researchers have relied on other factors such as latitude, temperature, aridity and habitat productivity as proxy variables for food quality (Lovegrove, 2000; Tieleman and Williams, 2000; Mueller and Diamond, 2001; McNab, 2002; Tieleman et al., 2002a,b; Lovegrove, 2003; Wikelski et al., 2003; Cruz-Neto and Jones, 2005; Rezende et al., 2004; Williams et al., 2004).

In spite of decades of research, McNab's (1986) FHH remains controversial (see McNab, 2000, 2003, 2009; Cruz-Neto and Jones, 2005). The controversy stems from the difficulty of testing apart the multiple possible factors that impinge on BMR from comparative data sets that use species as data points (Cruz-Neto and Bozinovic, 2004). Cruz-Neto and Bozinovic (2004) have argued that interspecific comparative tests of FHH rely in monotypic diet categories that ignore the potential variation in diets and habitats of a species.

Several experimental studies have been performed to test how dietary quality and availability shape the energy budget in endotherms (mostly in mammals), and only few studies have evaluated the FHH at the intraspecific level in birds. Indeed, Geluso and Hayes (1999) found

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no effect of chronic dietary acclimation (insects versus fruits) on BMR in *Sturnus vulgaris* while Piersma et al. (2004) found a reduction in BMR in *Calidris canutus* when shifted from a soft texture diet (trout chow diet) to a hard-texture (mussels) diet (see also Piersma et al., 1996). Bech et al., 2004 reported no effect of food quality on BMR during early development in zebra finches (*Taeniopygia guttata*), while Moe et al. (2005) observed that after diet restriction the BMR level of ducklings (*Anas platyrhynchos*) exhibits a significant decrease.

In this study we examined the relationship between BMR, diet, and climate among populations of the omnivorous passerine, *Zonotrichia capensis* (Emberizidae). To our knowledge, this is the first study that tested the FHH within a single bird species under field conditions. We measured BMR on freshly caught birds and took advantage of stable isotopes to estimate each individual's relative trophic level (Schondupe et al., 2001). Using the nitrogen isotope ratio of a consumer's tissues to assess the relative position in the food web relies on two observations: 1) tissues often reflect the isotopic composition of an animal's diet (Hobson and Clark, 1992), and 2) food sources are significantly depleted in  $^{15}\text{N}$  relative to consumers (Gannes et al., 1997; Robbins et al., 2005). Because a consumer's nitrogen is enriched in  $^{15}\text{N}$ ,  $\delta^{15}\text{N}$  values in animals' tissues at the top of the trophic web tend to be more positive than that of animals at the bottom of the food web. To tease apart the effect of climatic variables and diet on BMR, we used structural equation modeling. Rather than assuming a single model of the causal relationships among diet, climate, and BMR, we examined two models and used an information theoretic criterion to determine the weight of evidence in favor of each model given the data (Stephens et al., 2006).

## 2. Materials and methods

### 2.1. Animals and capture

Rufous-collared sparrow, *Z. capensis* (Passeriformes: Emberizidae) has a generalist diet (from seeds to insects) and shows population differences in the use of resources, (Lopez-Calleja, 1995; Novoa et al., 1996). Consequently, *Z. capensis* is a well-suited model to study population differences in physiological and ecological variables (see Sabat et al., 2009). Birds were caught during 2005 and 2006 from four localities in Chile along a geographic gradient: 1) Copiapo ( $27^{\circ} 21' \text{ S}$ ,  $70^{\circ} 24' \text{ W}$ ,  $n=10$ ), 2) La Serena ( $29^{\circ} 54' \text{ S}$ ,  $71^{\circ} 15' \text{ W}$ ,  $n=6$ ), 3) Quebrada de la Plata ( $33^{\circ} 31' \text{ S}$ ,  $70^{\circ} 50' \text{ W}$ ,  $n=9$ ) and 4) Valdivia ( $39^{\circ} 48' \text{ S}$ ,  $73^{\circ} 14' \text{ W}$ ,  $n=8$ ). Because seasonal variations may be associated with changes in reproductive hormones which in turn may influence BMR (Chastel et al., 2003), we study birds only during the non reproductive seasons. Capture sites varied in mean annual rainfall from Copiapo (400 m.a.s.l., arid habitat with a rainfall of 29 mm/year), La Serena (sea level, semi-arid, and rainfall of 100 mm/year), Quebrada de la Plata (450 m.a.s.l., mesic with a rainfall of 367 mm/year) and Valdivia (44 m.a.s.l., temperate rainforest with 2300 mm/year). We also estimated the aridity index of Martone (di Castri and Hajek, 1976; Cavieres and Sabat, 2008) during month of capture ( $\text{DMI} = \text{PP}/(T_A + 10)$ ), where PP is the monthly precipitation in mm, and  $T_A$  is mean monthly temperature in  $^{\circ}\text{C}$ ). Birds were transported to the laboratory in Santiago, Chile ( $33^{\circ} 27' \text{ S}$ ,  $70^{\circ} 42' \text{ W}$ ) within the two days after capture and housed in individual plastic-mesh cages ( $35 \times 35 \times 35 \text{ cm}$ ). Under laboratory conditions, temperature and photoperiod were held at  $22 \pm 2^{\circ}\text{C}$  and 12L: 12D, respectively. Birds had *ad lib* access to mealworms, bird seeds and water.

### 2.2. Basal metabolic rate

After habituating birds to laboratory conditions for 1 d, we measured rates of oxygen consumption ( $V_{\text{O}_2}$ ) in post absorptive (four hours fasted), resting birds in the inactive phase (from 20:00 h

to 06:00 h), using standard flow-through respirometry. Inside dark metabolic chambers of 1000 mL, birds perched on a wire-mesh. Oxygen consumption was measured using a computerized, open-flow respirometry system (Sable Systems, Henderson, NV, USA) calibrated with a known mix of oxygen (20%) and nitrogen (80%) that were certified by chromatography (INDURA, Chile). Measurements were made at ambient temperatures ( $T_A$ ) of  $30.0 \pm 0.5^{\circ}\text{C}$  within the thermoneutral zone of this species (Sabat et al., 2006a). The metabolic chamber received dried air at  $500 \text{ mL min}^{-1}$  from a mass flow controller and through Bev-A-Line tubing (Thermoplastic Processes Inc.). This flow ensured adequate mixing in the chamber. The mass flowmeter was calibrated monthly with a volumetric (bubble) flowmeter. The excurrent air passed through columns of  $\text{CO}_2$ -absorbent granules of Baralyme, and Drierite before passing through an  $\text{O}_2$ -analyzer, model FC-10A (Sable System). Output from the oxygen analyzer (%) 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 for at least 3 h and visual inspection of the recorded data allowed us to determine when steady-state conditions had been achieved. We considered that the steady-state was reached when birds do not modify by more than 5% for 10 min its metabolic rate. We averaged  $\text{O}_2$  concentration of the excurrent airstream over a 20 min period after steady-state was reached (following Tieleman et al., 2002a; Maldonado et al., 2009). Because  $\text{CO}_2$  was scrubbed before entering the  $\text{O}_2$  analyzer, oxygen consumption was calculated as [Withers (1977: p 122)]:  $V_{\text{O}_2} = [\text{FR} \cdot 60 \cdot (\text{Fi O}_2 - \text{Fe O}_2)] / (1 - \text{Fi O}_2)$ , where FR is the flow rate in  $\text{mL min}^{-1}$ , Fi and Fe are the fractional concentrations of  $\text{O}_2$  entering and leaving the metabolic chamber, respectively. 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 ( $\pm 0.1 \text{ g}$ ) 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). We found that all animals were euthermic after the metabolic trials ( $T_b > 40^{\circ}\text{C}$ ).

After metabolism measurements, animals were weighed and euthanized by  $\text{CO}_2$  exposure. Organs (kidney, intestine, heart, liver and gizzard) were then removed and massed ( $\pm 0.005 \text{ g}$ ). Pectoralis muscles were dissected and de-fatted by ether extraction prior to isotopic analyses. Nitrogen isotope ratios were measured on a continuous flow isotope ratio mass spectrometer (VG Isotech, Optima) with samples combusted in a Carlo Erba NA 1500 elemental analyzer at the Columbia University Biosphere 2 stable isotope facility. Stable isotope ratios were expressed using standard delta notation ( $\delta$ ) in parts per thousand (‰) as:  $\delta^{15}\text{N} = (R_{\text{sample}}/R_{\text{standard}} - 1) \times 1000$ , where  $R_{\text{sample}}$  and  $R_{\text{standard}}$  are the molar ratios of  $^{15}\text{N}/^{14}\text{N}$  of the sample and reference, respectively. Samples were referenced against international standard, the atmospheric nitrogen. Because  $\delta^{15}\text{N}$  of primary producers could be affected by the rainfall regime of each habitat, and thus the isotopic signature of the consumers (Robinson, 2001), we performed a linear regression analysis between nitrogen signatures from primary producers and annual PP of each locality. This analysis revealed that  $\delta^{15}\text{N}$  was negatively correlated with annual PP ( $r = -0.63$ ,  $F_{(1,43)} = 28.8$   $p < 0.001$ ). Thus we decided to calculate the relative trophic level (TL) in each specimen of *Zonotrichia* specimens following Post (2002) as:  $\text{TL} = (1 + [\delta^{15}\text{N}_{\text{animal}} - \delta^{15}\text{N}_{\text{producers}}] / \Delta^{15}\text{N})$ , where the  $[\delta^{15}\text{N}_{\text{animal}}]$  represent the isotopic signature from pectoral muscle samples,  $\delta^{15}\text{N}_{\text{producers}}$  is the isotopic of the producers and  $\Delta^{15}\text{N}$  is the enrichment factor by trophic level. Recently, it has been reported that discrimination of  $^{15}\text{N}$  varies with dietary protein content (see Martínez del Río et al., 2009), so consumers at higher trophic levels have lower  $\Delta^{15}\text{N}$  than consumers at lower trophic level. For our calculations we used a mean of 3.4‰ which could at some extent underestimate the relative position of birds at higher level in the food web. We estimated

the  $\delta^{15}\text{N}_{\text{producers}}$  of the producers by two methods. First we obtained samples of plants from the localities where birds were collected and the nitrogen isotopic signature was also determined. Nevertheless, because some  $\delta^{15}\text{N}$  of plants were higher than the isotopic signatures of pectoralis, resulting in meaningless (i.e., negative) values of trophic level, we performed a linear regression analysis using the annual precipitation of each locality as independent variable and  $\delta^{15}\text{N}$  of plants as the dependent variable. Then we computed the predicted value for each locality using the equation resulting from the linear model ( $\text{TL} = -0.0025 \text{ PP} + 4.67$ ). This procedure allowed us to diminish the error of sampling. In order to confirm that such values were confident, we also estimated the predicted value of plants using a similar model for a rainfall gradient described by Robinson (2001). These two values results to be similar, so we are confident that our estimates of  $\delta^{15}\text{N}_{\text{producers}}$  are precise (see Table 3).

### 2.3. Data analysis

A preliminary analysis showed that body mass, the trophic level, and climatic data, but not the elevation of the capture sites, were significantly correlated with BMR. Hence, we removed from the models the altitude of the capture sites as an independent variable. To assess the potential causal relationships among variables (TL, PP, T, DMi,  $M_b$ ) and BMR, we constructed two path diagram (Fig. 2). We evaluated the adequacy of the hypothesized model using structural equation modeling (SEM) analysis under EQS (Bentler, 1995). This modeling procedure is a form of path analysis that allows one to analyze more complex sets of causal relationships including factors from multivariate principal components (Bozinovic et al., 2007a). This analysis besides showing the nature and direction of causal relationships also includes estimates of the strength of those relationships, the path coefficients (Scheiner et al., 2000). In short, we used TL, PP, T, DMi and  $M_b$  as predictors of BMR, and incorporated the possible relationships among them in a collection of plausible models. We computed standardized path coefficients among measured variables, which indicate the magnitude and direction of associations. Overall fit was assessed using each model's  $\chi^2$  (also called the discrepancy), and Bentler–Bonett normed fit index (NFI, Bentler and Bonnet, 1980). Finally, we used AICc (small sample Akaike's Information Criterion, Burnham and Anderson, 2002) to assess which model was better supported by data (customarily values in this index of less than 0.9 are considered unacceptable, Bentler and Stein, 1992). Because changes in BMR may be associated with the mass of metabolically active organs (Mueller and Diamond, 2001), we performed a regression analysis to test if changes in the organ masses were related to BMR level.

### 3. Results

Basal metabolism and  $M_b$  were allometrically correlated ( $\text{BMR (mL O}_2\text{/h)} = 2.51 M_b^{1.08 \pm 0.26} \text{ (g)}$ ,  $r^2 = 0.37$ ,  $F_{(1,33)} = 19.03$ ,  $p < 0.0001$ ,  $N = 37$ , Fig. 1).  $M_b$  accounted for just 37% of the variability in BMR. We proposed two models to causal relationship between predictor variables and BMR. In model 1, we incorporated the integrative effect of temperature and precipitations on BMR, through the de Martonne aridity index; in model 2, we proposed separately both temperature and annual precipitations as predictor variables of BMR (see Fig. 2).

In model 1, the hypothesized set of causal relationships among variables did not differ from the covariance structure of data (Bentler–Bonett normed fit index: 0.99, Fig. 2), which indicates that the hypothesized model was statistically supported ( $\text{AICc} = 0.5$ ,  $\chi^2 = 0.17$ ,  $\text{df} = 1$ ,  $p = 0.676$ ). In this model the results from SEM analysis revealed positive and significant direct effect between  $M_b$ , DMi and BMR (path coefficient 0.59 and 0.37, respectively). DMi had a negative indirect effect on BMR, and additionally showed a positive but not significant direct effect in  $M_b$ . The trophic level showed a negative and significant causal relationship on the response variable BMR (path

coefficient  $-0.475$ ). The second hypothesized set of causal relationships showed a minor statistic support (Bentler–Bonett normed fit index: 0.96,  $\text{AICc} = 0.77$ ,  $\chi^2 = 1.79$ ,  $\text{df} = 2$ ,  $p = 0.4$ ). In this model the results from SEM analysis revealed a positive and significant direct effect of  $M_b$ , T, PP and BMR and similarly with model 1 the trophic level showed a significant but negative effect on the response variable BMR. The temperature showed a positive but not significant effect on  $M_b$  (see Fig. 2). In this model both T and PP had a negative indirect effect on BMR (see Table 1). Based on the goodness of fit values and the lowest  $\chi^2$  (Burnham and Anderson, 2002) we chose to present the inferences derived from model 1 (Fig. 2).

Because the trophic level and DMi showed a positive and significant association ( $r = 0.75$ ,  $F_{(1,43)} = 56.77$ ,  $p < 0.001$ ) we performed a linear regression analysis between the residuals of TL against DMi and the residuals of BMR against body mass to assess the effect of diet on BMR after both body mass and climatic conditions were accounted for. The residuals from the regression between BMR and  $M_b$  were negatively correlated with the residuals of trophic level ( $r = -0.40$ ,  $F_{(1,35)} = 6.47$ ,  $p = 0.015$  (Fig. 3).

Since the morphological measurements exhibited high co-linearity we performed a principal component analysis (PCA) and then a correlation analysis between the factor scores generated by PCA and the residuals of BMR against  $M_b$ . PCA analysis revealed that the five variables in the model were reduced to two PCA axes, which accounted for 77.24% of the variance (Table 2). The first component axis (PCA 1) was strongly and positively correlated with the kidney, liver, gizzard and intestine masses whereas the second axis (PCA 2) was strongly positively correlated with the residuals of heart mass. Furthermore, the PCA1 was strongly and positively correlated with the residuals of BMR ( $r = 0.64$ ,  $F_{(1,27)} = 18.63$ ,  $p < 0.001$ ).

### 4. Discussion

Evolutionary physiological ecologists have hypothesized that, on evolutionary time, diet is a selective agent shaping rates of energy expenditure in endothermic vertebrates (McNab, 2002; Bozinovic and Martínez del Río, 1996). However, these comparative studies in general have focused on the ultimate (evolutionary) rather than proximate (mechanistic) factors responsible for differences in the rate at which energy is acquired, processed and expended. Indeed, the FHH can, and perhaps must, be tested through both approaches. The former explanations can be more useful for experimental designs that involve interspecific comparison but of limited utility when intraspecific comparison are involved. In this scenario, experimental approaches and intraspecific comparisons as we did here should be useful to advance the understanding of the proximal mechanism, and

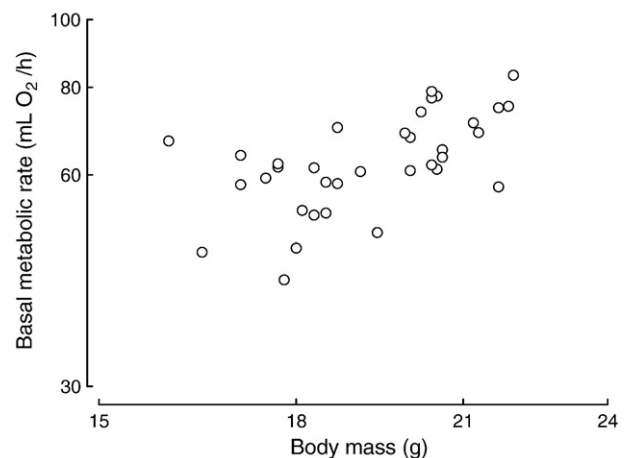


Fig. 1. Basal metabolic rate of *Zonotrichia capensis* as a function of body mass.

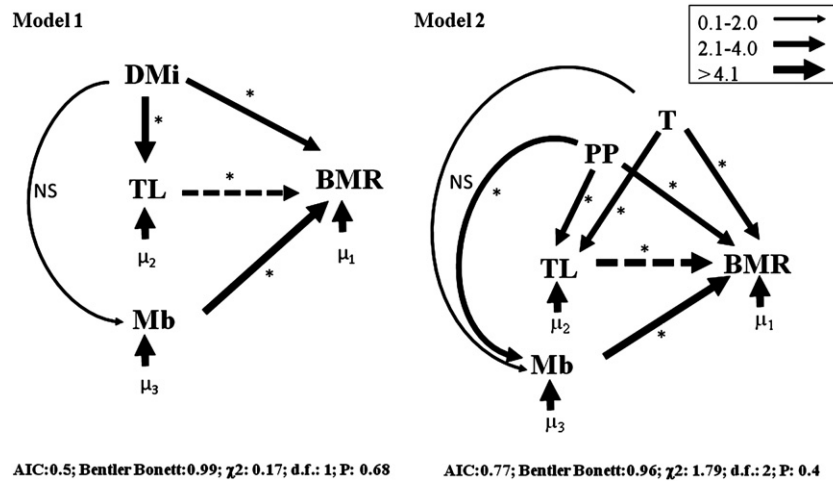


**Fig. 2.** Path diagrams of causal relationships among variables (TP, T, PP, Mb, DMi) and basal metabolic rate in adult sparrows. The arrows indicate the relative magnitude of path coefficient. Dashed lines indicate negative path coefficients. Asterisks indicate statistically significant coefficient (\* $p < 0.05$ ; NS, non significant);  $\mu$  = residual error.

their ecological significance, that support the quality component of the food-habit hypothesis.

Interestingly, our results revealed a significant negative effect of trophic level on BMR and both a positive direct effect and a negative indirect effect (through TL) of DMi on BMR. When we removed the effect of DMi on trophic level we obtained the negative effect on BMR (see Fig. 3). Nevertheless, it is possible that differences in climatic conditions (e.g., aridity, air temperature, rainfall) may also exert a significant effect on BMR, as it has been demonstrated previously in this species (Sabat et al., 2006a; Cavieres and Sabat, 2008). In fact, several studies reported correlations between  $T_A$  and PP and mass-independent BMR (e.g., Rezende et al., 2004). Essential to these analyses is the assumption that climatic variability directly reflects food availability and predictability. Here, the effect of climate (i.e., the aridity index in this study) on BMR may be both direct (see Cavieres and Sabat 2008) and indirect, through the effect on trophic level (Fig. 2).

On the other hand, few studies have assessed the effect of diet on physiology of animals in the field (e.g., Schondube et al., 2001; Sabat et al., 2006c), and more scarce are those that focus on the same species (Sabat et al., 2006b, Sabat et al., 2009). Recently, Bozinovic et al. (2007a) studying a natural population of a rodent species inhabiting seasonal Mediterranean habitats, reported that mass-independent BMR of freshly captured individuals were positively correlated with the seed proportion of fecal content, but that this relationship was an indirect effect of the environmental aridity. Here TL (as a proxy of the position in the food web) and aridity index were correlated with BMR (see Fig. 2). This last finding agrees with previous reports of metabolic adjustments along the aridity gradient for this species (Sabat et al.,

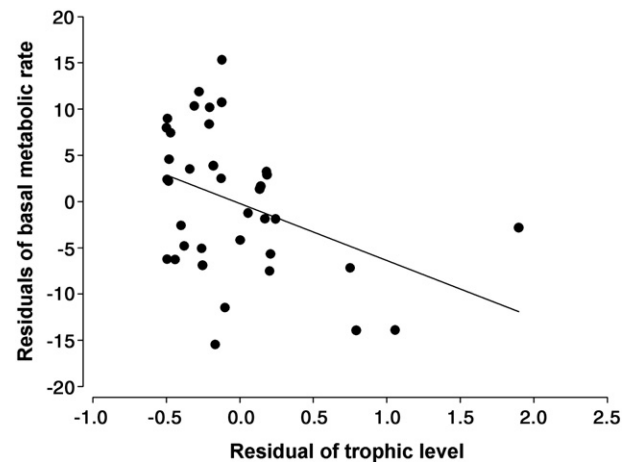


**Fig. 3.** Mass-independent basal metabolic rate as a function of residuals of trophic level against the de Martonne aridity index in *Zonotrichia capensis*.

2006a; Cavieres and Sabat 2008). The relationship between dietary habit and BMR is supported also by the significant and negative correlation between the residuals from the regression between BMR and  $M_b$  and the residuals of TL and DMi (Fig. 3).

Why is trophic level related with BMR in *Z. capensis* and the rodent investigated by Bozinovic et al. (2007a) in opposite ways? Bozinovic et al. (2007a) analyzed the dietary composition through feces analysis. Studies that include data from fecal and stomach content may be informative about the dietary habits of animals at a temporary scale different from those producing modifications in physiological (e.g., BMR) traits. Indeed the temporary resolution of fecal content can be a few hours or days, whereas the time required for BMR modifications

**Table 1**

Direct (DE), indirect (IE), and total effects (TE) of predictor variables on basal metabolic rate.

|                | DE     | IE     | TE     |
|----------------|--------|--------|--------|
| <i>Model 1</i> |        |        |        |
| DMi            | 0.379  | −0.262 | 0.117  |
| $M_b$          | 0.594  | –      | 0.594  |
| TL             | −0.475 | –      | −0.475 |
| <i>Model 2</i> |        |        |        |
| T              | 0.385  | −0.147 | 0.532  |
| PP             | 0.344  | −0.075 | 0.269  |
| $M_b$          | 0.516  | –      | 0.516  |
| TL             | −0.507 | –      | −0.507 |

The indirect effects were calculated from the product of sequential path coefficients.

**Table 2**

PCA axis derived from analyses from organ mass in *Zonotrichia capensis*.

| Variables                         | PCA axis 1 | PCA axis 2 |
|-----------------------------------|------------|------------|
| Factor loadings                   |            |            |
| Kidney mass                       | 0.812      | −0.265     |
| Liver mass                        | 0.866      | 0.225      |
| Gizzard mass                      | 0.848      | 0.194      |
| Heart mass                        | −0.087     | 0.977      |
| Intestine mass                    | 0.775      | −0.076     |
| Eigenvalues                       | 2.74       | 1.12       |
| Explained variance (%)            | 55.83      | 22.40      |
| Explained cumulative variance (%) | 55.83      | 74.24      |

is longer (e.g., Piersma et al., 2004; McKechnie, 2008). In fact, the time needed to reach a new steady-state condition after thermal acclimation of BMR is between 3 and 4 weeks (Barceló et al., in press). In this sense, the isotopic composition of pectoral muscle reflects integration of dietary inputs over longer time periods (Tieszen et al., 1983; Hobson and Clark, 1992). In fact, the average residence time of isotopes in the pectoralis muscle for a similar-size passerine (*Passer domesticus*) is about 3 weeks in *Z. capensis* (Carleton et al., 2008). Thus, it is expected that, if the features of dietary items influence the rates at which animals expend energy, it is more likely that such a relationship became apparent when the analyses comprise dietary information from a comparable (although not necessary exactly the same) temporary scales, such as the isotopic signature of pectoral muscle.

Nevertheless, which mechanism could explain the observed effect of dietary pattern on BMR? At the evolutionary scale, the FHH predicts that animals consuming foods with low digestibility and energy content might evolve lower BMR. The same rationale may be argued as an explanation at ecological timescale, (Daan et al., 1990). Consequently, when analyzing the isotope data, it seems that the relative position in the food web of *Z. capensis* at our study sites varies from almost strictly plant-eaters (e.g., seeds, fruits and buds) to animal-eaters (invertebrates). If we consider the range of trophic level computed for all individuals (see Table 3 for averages), our data suggests that the range in the trophic position of *Z. capensis* in the food web goes from near the second level, i.e., primary consumer ( $\lambda = 1.93$ ) to the fourth level, i.e., tertiary consumer ( $\lambda = 4.2$ ). When the stable isotope analysis is combined with previous studies of dietary ecology of *Z. capensis* (Lopez-Calleja, 1995; Sabat et al., 1998) it seems that individuals were probably consuming mainly seeds, a mixed diet of seeds and animals, and mainly insect prey. Our results suggest that animals from the lower trophic levels (consuming mainly seeds and buds) had higher BMR than individuals from higher trophic level, which presumably were preying on animal (mostly insects) resources.

In general insects yield more energy than plant materials (Klasing, 1998; Karasov, 1990). On the other hand, digestibility of seeds by passerines is about 75%, and in *Z. capensis* the value is nearly 80% (Novoa et al., 1996), whereas, insects' digestibility in birds range from 50 to 80% (Karasov, 1990; Weiser et al., 1997). In addition, the exoskeleton of insects has variable amounts of chitin, whose digestibility in passerines is ca. 10% (Weiser et al., 1997). This fact might determine large differences in prey digestibility depending on the kind of insect consumed. Hence, it is possible that subtle differences in digestibility between seeds and insects, but not the net energy of prey, may explain some of the variation in BMR of *Z. capensis*.

Furthermore, because plant tissues have high levels of secondary chemical compounds, animals consuming allelochemicals may increase BMR as a consequence of an increase in detoxification cost (Cork and Foley, 1991; Foley and McArthur, 1994). In the case of *Z. capensis*, this hypothesis seems to be supported because the plant species commonly consumed by birds generally have high contents of secondary compounds (Lopez-Calleja, 1995; Norsworthy et al., 2007; Chang et al., 2008). Also, consumption of allelochemicals may increase liver mass (Sorensen et al., 2004), and the increase in liver mass may in turn increase BMR (Scott and Evans, 1992; Daan et al., 1990). Our results seem to support in part this hypothesis because we observed that liver and other organ masses were positively correlated with BMR.

In an evolutionary timescale the food-habit hypothesis predicts that the evolution of diets with low digestibility and/or energy content is thought to be correlated with the evolution of low rates of basal metabolism. Nevertheless, as far as the effects of reduced dietary digestibility and/or energy content are concerned, the results from within species studies showed mixed support for the food-habit hypothesis. At the proximate level, the direction of the response to a reduced diet quality seems to depend on whether or not animals can trigger the integrated processing responses and, if so, to the costs of such plasticity (Batzli et al., 1994). Nonetheless, results from intraspecific studies suggested that, whatever the factors responsible for the association between diet and BMR at an ecological timescale, they might not be the same as those that promoted the evolution of this correlation (Cruz-Neto and Bozinovic 2004). Analyses as the one we did here may help to enlighten how much of a role the proximate and ultimate processes have played in the evolution of BMR.

Finally, to our knowledge this is the first attempt to test the FHH in birds under field conditions. Also this is the first study that incorporates a continuous variable, as proxy estimation of the position in the food web ( $\delta^{15}\text{N}$ , trophic level). This procedure also resolves a common methodological problem when dietary categories are used instead of the some continuous variable. This is important because the loss of information about dietary variability in the field (see Klaasing, 1998) may mask a significant association between physiological traits and food habits. Finally the significant association between trophic position and BMR in *Z. capensis* could be explained both by the digestibility and/or by the presence of dietary allelochemicals.

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**Table 3**  
 $\delta^{15}\text{N}$  of primary producers (plants), bird tissues and the baseline predicted from the regression analysis of nitrogen isotopic signatures of plants against annual rainfall (\*) and using the Robinson (2001) model (\*\*).

| Locality  | $\delta^{15}\text{N}$ primary producers | $\delta^{15}\text{N}$ of pectoralis | $\delta^{15}\text{N}$ baseline predicted * | $\delta^{15}\text{N}$ baseline predicted ** | Trophic level |
|-----------|-----------------------------------------|-------------------------------------|--------------------------------------------|---------------------------------------------|---------------|
| Copiapó   | 4.58 ± 6.27 (4)                         | 9.07 ± 0.24 (10)                    | 4.64                                       | 4.46                                        | 2.41 ± 0.21   |
| La Serena | 12.54 ± 7.92 (4)                        | 11.78 ± 4.27 (6)                    | 4.37                                       | 4.14                                        | 3.34 ± 0.83   |
| Santiago  | 1.36 ± 3.83 (7)                         | 8.06 ± 1.09 (17)                    | 3.82                                       | 3.48                                        | 2.36 ± 0.32   |
| Valdivia  | − 1.19 ± 0.68 (4)                       | 7.55 ± 0.28 (9)                     | − 1.53                                     | − 2.83                                      | 3.1 ± 0.22    |

The trophic level of birds was calculated using the former model (see text for details).

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