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The interplay between ambient temperature and salt intake affects oxidative status and immune responses in a ubiquitous Neotropical passerine, the rufous-collared sparrow

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ABSTRACT

Physiological traits associated with maintenance, growth, and reproduction demand a large amount of energy and thus directly influence an animal's energy budget, which is also regulated by environmental conditions. In this study, we evaluated the interplay between ambient temperature and salinity of drinking water on energy budgets and physiological responses in adult Rufous-collared sparrow (*Zonotrichia capensis*), an omnivorous passerine that is ubiquitous in Chile and inhabits a wide range of environments. We acclimated birds to 30 days at two ambient temperatures (27 °C and 17 °C) and drinking water salinity (200 mM NaCl and fresh water) conditions. We evaluated: 1) the aerobic scope and the activities of mitochondrial metabolic enzymes, 2) osmoregulatory parameters, 3) the skin-swelling immune response to an antigen, 4) oxidative status, and 5) the length of telomeres of red blood cells. Our results confirm that *Z. capensis* tolerates the chronic consumption of moderate levels of salt, maintaining body mass but increasing their basal metabolic rates consistent with expected osmoregulatory costs. Additionally, the factorial aerobic scope was higher in birds acclimated to fresh (tap) water at both 17° and 27 °C. Drinking water salinity and low ambient temperatures negatively impacted inflammatory response, and we observed an increase in lipid peroxidation and high levels of circulating antioxidants at low temperatures. Finally, telomere length was not affected by osmo- and thermoregulatory stress. Our results did not support the existence of an interplay between environmental temperature and drinking water salinity on most physiological and biochemical traits in *Z. capensis*, but the negative effect of these two factors on the inflammatory immune response suggests the existence of an energetic trade-off between biological functions that act in parallel to control immune function.

1. Introduction

How organisms allocate energy among biological functions is fundamental for understanding how they interact with their environment, which ultimately influences species life history traits and the persistence of populations (Karasov and Martínez del Río, 2007). Physiological processes associated with maintenance, growth, and reproduction require a large amount of energy and are considered the primary activities that influence an animal's energy budget (McNab, 2002). Energy acquisition is limited by morphological, physiological, and behavioral constraints and also by the availability of resources (Weiner, 1992). Thus, it is expected that free-living animals have to

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allocate this limited amount of energy among competing functions, where the allocation of energy to one component of the budget reduces its availability for another (see Buckley et al., 2015). In this context, animals challenged by changes in environmental conditions probably use their energy budget to maintain homeostasis to the detriment of alternative biological functions such as reproduction and immune function (Wieser, 1994; Cornelius et al., 2017).

Passerine birds confront a diverse array of physiological demands because they occur in nearly all types of terrestrial habitats and occupy a variety of ecological niches (Swanson and Olmstead, 1999; Swanson, 2010; Petit et al., 2016). Thermal environment has a strong influence on bird metabolic rate because thermoregulation at low ambient temperatures (T_a) is one of the most energetically costly functions to maintain (Martinez Del Rio et al., 2018). The dynamic nature of energy expenditure allows birds to survive in a broad range of contrasting environments from cold to desert climates (Dawson et al., 1983; Repasky, 1991; Hinsley et al., 1993; Swanson, 1993; Wiersma et al., 2007). Moreover, because their diurnal habits and high body temperatures, birds have proportionately high mass-specific rates of evaporative and excretory water loss (Williams and Tieleman, 2002; Sabat et al., 2006).

Birds also have a limited ability to concentrate urine (Goldstein and Skadhauge, 2000), and for passerines that lack functional salt glands (Shoemaker, 1972), maintaining osmotic balance is a challenge when feeding on salty food or living in arid habitats that contain scarce freshwater (Sabat and Martinez del Rio, 2002). Studies have suggested that osmoregulation in birds is related to high energy demands because the machinery required for metabolizing, processing, and excreting excess salts is usually associated with an increase in baseline energy requirements (Gutiérrez et al., 2011; Peña-Villalobos et al., 2013; Sabat et al., 2017).

As mentioned above, an increase in energy costs associated with maintaining homeostasis likely involves a reallocation of energy to meet those costs, limiting the animals' capacity to expend energy on other activities. The extent that energy production can be increased above baseline energy requirements represents an individual's potential to use energy to fuel activities such as locomotion, immune response, growth, and reproduction (Koteja, 2000; Maldonado et al., 2016). Among these activities, immune responses that enable animals to combat infectious agents and thus have direct impacts on fitness involve high investments in terms of energy (Mendes et al., 2006; Costantini and Møller, 2009; Hasselquist and Nilsson, 2012) and nutrients (Moller et al., 1990; Fitze et al., 2004; Barnard and Behnke, 2006; Ramirez-Otarola et al., 2018). Furthermore, some studies have shown that stressful conditions such as thermoregulation and osmoregulation, which typically consume high amounts of energy, reduce the immune response (Sheldon and Verhulst, 1996; Ilmonen et al., 2003). Nevertheless, knowledge on the complex linkage between the expression of immune responses and their impact on energy allocation patterns in birds is still limited. In addition to the energetic costs associated with immune responses, oxidative metabolism is also an energy demanding biological process. Oxidative metabolism generates reactive oxygen species (ROS), which can interact with macromolecules such as lipids, proteins, and nucleic acids when in excess (oxidative stress), ultimately disrupting multiple cellular processes (Dowling and Simmons, 2009; Monaghan et al., 2009; van de Crommenacker et al., 2010; Selman et al., 2012). For example, an excessive production of ROS may shorten the length of telomeres – repetitive non-coding sequences of DNA located at the ends of chromosomes – which are implicated in cellular aging (Blackburn, 1991; von Zglinicki, 2002; Kotrschal et al., 2007; Quirici et al., 2016). Consequently, cellular oxidative stress may affect the physiology of cells with implications for organism performance and life history (Beckman and Ames, 1998; Hulbert et al., 2007; Monaghan et al., 2009).

Our study seeks to increase the understanding of how environmental variables modulate the energy budget of a widely-distributed passerine, the Rufous-collared sparrow (*Zonotrichia capensis*), and how such changes in energy utilization impact selected physiological traits. To characterize an individual bird's energy budget, we estimated the aerobic scope, or the difference between the basal (BMR) and summit metabolic rates (M_{sum}), to identify which factors are associated with maintenance costs versus the upper limits of energy expenditure, respectively. The main objective of our study was to assess the impact of a 30-d acclimation to two contrasting ambient temperatures (17 °C and 27 °C) and exposure to salt on molecular/biochemical (mitochondrial metabolic enzymes, immune responses, oxidative stress), genetic (length of telomeres), and physiological responses (aerobic scope). We predict that birds exposed to high thermoregulatory- and osmoregulatory-associated energy demands should exhibit a reduced aerobic scope, enhanced activity of metabolic enzymes, higher plasma and urine osmolality, depressed immune response, oxidative damage and shortened telomeres. To the best of our knowledge, the integrated effect of temperature and salinity on physiological, genetic and biochemical features in passerine birds has, as of yet, not been studied. We antcipitate that our results will increase our understanding of how this cosmopolitan bird species and others like it that inhabit a wide variety of environments.

2. Materials and methods

2.1. Field collection and lab acclimation experiments

Our model species is the omnivorous passerine *Z. capensis*, which is ubiquitous in Chile and occurs in a diverse array of habitats from deserts to temperate rain forests (Araya and Millie, 2005). Furthermore, this species is found along a substantial elevation gradient, from coastal zones to areas > 4000 m.a.s.l. (Goodall et al., 1946). Our previous experiments showed that *Z. capensis* can tolerate the ingestion of solutions containing high concentrations of salt (200 mM NaCl) with no significant changes in body weight, however, increased salt intake did change this species basal metabolic rate (BMR) and oxidative status (Peña-Villalobos et al., 2013; Sabat et al., 2017).

In this study, we used 31 individuals that were captured using mist nets in the Quebrada de Macul (33° 29′S, 70° 29′W, 800 m.a.s.l, Central Chile); a locality with a Mediterranean climate. Following capture, we transported birds to the laboratory for a 21-d acclimatization period. The birds were maintained in individual cages (50 × 50 × 50 cm) and were fed *ad libitum* with mealworms (*Tenebrio molitor*), birdseed, and water. Seeds and water were offered in inverted 100 mL graduated plastic tubes that allowed birds to eat and drink in a small (~1 cm^2) container at the bottom of the tube. After this acclimatization time, birds were randomly separated into two experimental groups. One treatment group (n = 17) received tap water (TW-acclimated group) and the other (n = 14) received saltwater (SW-acclimated group) that contained 200 mM NaCl. We continued to feed both treatment groups with mealworms and seeds. Each of the experimental groups were divided into two subgroups maintained at 27 ± 2 °C or 17 ± 2 °C with a 12:12 light:dark photoperiod. These two temperatures are comparable to the mean of daily maximum temperatures experienced by wild birds in winter and summer, respectively. We were confident that these two temperatures represent contrasting thermoregulatory demands because we previously observed a 60% increase in oxygen consumption relative to BMR at 17 °C in *Z. capensis* from Central Chile (Sabat et al., 2006). Thus, our experiment included four total treatments: SW-27 (n = 8), SW-17 (n = 9), TW-17 (n = 7) and TW-27 (n = 7). Birds were kept under these experimental conditions for 30 days. Fluid intake rates were determined using the inverted graduated plastic tubes described above and corrected for evaporation by using control tubes located outside the experimental cages. After the incubation period, samples of blood (50–100 μL) were collected in the morning (09:00–11:00 h) from the humeral vein using hematocrit tubes with anticoagulant (heparine or EDTA), and samples were centrifuged at 9000*g* for 5 min to separate

plasma from red blood cells. Plasma and the cellular fraction were frozen at −80 °C until analysis. Ureteral urine was obtained by inserting a small closed-ended cannula into the bird cloaca, which was centrifuged and the supernatant was collected for osmometry analysis (Wescor 5130B).

2.2. Immune response

The immune response of *Z. capensis* was evaluated by using the skin-swelling test that administers the plant lectin phytohaemagglutinin (PHA) with specific immunostimulatory properties (Vinkler et al., 2010). After the acclimation period, birds were injected in the left wing with 0.1 mL of 1 mg/mL PHA (Sigma L-8754, St. Louis, MO, USA) in phosphate-buffered saline (PBS) solution following the method of Smits et al. (1999). Prior to PHA injections, we measured the patagium thickness (to the nearest 0.01 mm in triplicate) with a digital micrometer. 24 h after injection of PHA, we again measured the patagium thickness in triplicate. The immune response was calculated as the difference between the mean values of pre- and post-injection thickness. We performed a repeatability analysis using the intraclass correlation coefficient as the ratio between the variance components due to individuals over total variance. When this coefficient was above 50–60% and significant, we considered the trait to be consistent and a good representation of an individual's capacity (Dohm, 2002). The zero hour measurement was 0.663 (i.e., 66% of the variance is explained by inter-individual variation; $F_{29,60} = 6.89$, $P < 0.00001$), whereas the 24 h measurement was 0.95 (i.e., 95% of the variance is explained by inter-individual variation; $F_{29,60} = 63.32$, $P < 0.00001$). These calculations indicate that these traits are highly repeatable and are a good representation of an animal's performance.

2.3. Metabolic rates

Metabolic rates were estimated via oxygen consumption rate ($\dot{V}O_2$) using a FoxBox respirometer (Sable Systems, Las Vegas, NV) as described in Sabat et al. (2017). BMR was measured in 4-h fasted animals using a dark metabolic chamber (2 L) located in a controlled temperature cabinet (Sable Systems, Henderson, Nevada) and kept at a constant ambient temperature within the thermal neutral zone for this species (Ta = 30 ± 0.5 °C). During measurement of BMR, we passed both incurrent and excurrent gas streams (flow rates = 500 mL min^{-1}) through columns of Drierite and Baralyme to remove H_2O and CO_2.

Output from the oxygen analyzer (%) was digitalized using a Universal Interface II (Sable Systems) and recorded on a computer using EXPEDATA data acquisition software (Sable Systems). Our sampling interval was 5 s. All measurements were made during the resting phase (18:00 and 07:00 h). Oxygen consumption was calculated according to Withers (1977) as:

$$\dot{V}O_2 = \frac{FR \times 60 \times (F_iO_2 - F_eO_2)}{1 - F_iO_2}$$

where FR is the flow rate in mL min^{-1}, and F_iO_2 and F_eO_2 are the fractional concentrations of inflow and outflow [O_2] in the metabolic chamber, respectively. Summit metabolism (M_{sum}), or the maximal metabolic rate during exposure to cold temperatures (King and Swanson, 2013) was measured in a He−O_2 (80–20%, INDURA, Chile) atmosphere according to Rosenmann and Morrison (1974) at −5 ± 2 °C following a similar protocol used for BMR measurement. The body temperature (T_b) of birds was checked with an intra cloacal thermocouple (± 0.1 °C) after each measurement to ensure that they were hypothermic; only measurements with hypothermic birds (i.e., $T_b < 35$ °C) were considered. Oxygen consumption during each trial was calculated as instantaneous $\dot{V}O_2$ from readings taking every 5 s, and we considered M_{sum} as the highest 5-min average oxygen consumption over the recording period (Swanson and Bozinovic, 2011).

2.4. Metabolic enzymes

Birds were sacrificed by CO_2 exposure, weighed, and dissected to remove the organs. Samples of pectoralis, liver, kidney, and heart tissue were homogenized on ice (1,10 *w/v*) in phosphate buffer 0.1 M supplemented with 2 mM EDTA (pH 7.3) using an Ultra Turrax (20,000 rpm). The sample was then sonicated at 130 watts using an Ultrasonic Processor VCX 130 on ice 14 times in 20-s cycles and 10-s interval between cycles. Homogenates were then centrifuged at 15,000 rpm for 15 min and 4 °C to obtain a post mitochondrial fraction. The supernatant was transferred into a new tube, to avoid transferring the upper lipid layer present in the homogenates. Protein concentration was determined using the method by Bradford (1976) with bovine serum albumin as the standard. We measured the activity of two mitochondrial enzymes: citochrome c Oxidase (COX; E.C. 1.9.3.1) and citrate synthase (CS; E.C. 4.1.3.7). An increase in the activity of these enzymes likely reflects changes in both the functional properties and the density of mitochondria (Guderley, 1998). The COX activity was quantified using a microplate-scale spectrophotometric method slightly modified from that reported by Moyes et al. (1997). In brief, enzyme activity was determined in a reaction mixture containing 10 mM Tris-HCl (pH 7), 120 mM KCl, 250 mM sucrose, and cytochrome *c* reduced with dithiothreitol in a final volume of 0.2 mL. The decrease in extinction at 550 nm was monitored in a Thermo Scientific Multiskan GO UV/VIS spectrophotometer at 25 °C. Enzyme activity was calculated using an extinction coefficient of 21.84 mM^{-1} cm^{-1} at 550 nm for cytochrome-c. The CS activity was measured according to Sidell et al. (1987) with slight modifications. The enzyme assay medium contained 10 mM Tris-HCl (pH 8.0), 10 mM 5,5′dithiobis- (2-nitrobenzoic acid), 30 mM acetyl Coenzyme A (acetyl CoA) and 10 mM oxaloacetic acid (OAA) in a final volume of 0.2 mL; these reagents were omitted in controls. Citrate synthase catalyzes the reaction between acetyl CoA and OAA to form citric acid. The increase in extinction at 412 nm was measured in a Thermo Scientific Multiskan GO at 25 °C. Enzyme activity was calculated using an extinction coefficient of 13.6 mM^{-1}cm^{-1}at 412 nm. All enzyme activities are reported as specific activity per gram of protein (μmol min $^{-1}$ mg protein^{-1}).

2.5. Biomarkers of oxidative stress

Oxidative status was assessed by measurement of three biomarkers in both tissue homogenates and plasma: (1) concentration of the free radical nitric oxide (NO), (2) total antioxidant capacity (TAC) that measures the presence of molecular antioxidants, and (3) lipid peroxidation as a measurement of oxidative damage. TAC was measured by a colorimetric reaction using the assay kit produced by Sigma Aldrich, (San Diego, CA; # CS0790). The assay is based on the formation of a ferryl myoglobin radical from metmyoglonin and hydrogen peroxide which oxidizes the ABTS (2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid)) to form the radical $ABTS^{\bullet+}$ which, in turn, produces a chromogen that can be detected spectrophotometrically at 405 nm. Lipid peroxidation was measured via formation of the malondialdehyde (MDA)-thiobarbituric acid adduct, which is detected at 532 nm (Cell Biolabs OxiSelect™ STA-330). Both tissue homogenates and plasma were spiked with 5% butylated hydroxytoluene (BHT) to prevent spontaneous oxidation and stored at −80 °C until MDA measurements. Nitric oxide (NO) concentration was measured according to Patton and Kryskalla (2011) using a commercially available kit (Biovision, Milpitas, CA; #K262) that measures the amount of azochromophore formed at 540 nm.

2.6. Telomere length

DNA was isolated from the red blood cells using the salting-out protocol described in Aljanabi and Martinez (1997). The quality of DNA was checked with integrity gel and the concentration of samples was

assessed using a Nanodrop-8000 spectrophotometer; all samples were within the acceptable purity values (absorbance ratio A260/ 280 > 1.7 and A260/230 > 1.8). Samples were run immediately after DNA extraction. The length of telomeres was measured using the real-time quantitative PCR technique described in Criscuolo et al. (2009) with some modifications by Angelier et al. (2013). The efficiency of the PCR reaction was checked using a standard curve generated by serial dilution from a DNA pool of birds (5–80 ng mL^{-1}, accepted efficiency range = 100 ± 15%). Validation of inter-plate measurements was performed using one sample (4-pooled individuals) as a reference and run in triplicate for every plate. We calculated the T/S ratio as described in Pfaffl (2001):

Eff_{Tel}^ΔCq_{Tel}/Eff_{GAPDH}^ΔCq_{GAPDH}.

The mean amplification efficiency (Eff) and cycle quantification (Cq) values were calculated using the software LinRegPCR (version 12.13), whereas the ΔCq was calculated as the Cq values of the reference sample subtracted by the Cq value of the target sample. Mean Eff_{Tel} was 1.89 ± 0.03 and mean Eff_{GAPDH} was 2.10 ± 0.09; both close to the expected efficiency value of 2.

2.7. Statistical analysis

Because, body mass remained unchanged among experimental treatments, the effect of environmental temperature and drinking water salinity on physiological (i.e. BMR, $M_{sum,}$ osmolality) and morphological traits were assessed with a MANOVA. The energy budget of individuals was analyzed through two indices of the aerobic scope: net aerobic scope (NAS: M_{sum} BMR) and factorial aerobic scope (FAS: M_{sum}/BMR) as both are indices of aerobic capacity.

Metabolic enzymes activity was evaluated using a two-way analysis of variance (ANOVA) with environmental temperature and drinking water salinity (TW vs SW) as the independent factors. Similarly, the effect of the interplay between temperature and salinity of drinking water on oxidative stress parameters of each tissues was analyzed using a two-way ANOVA, with the values of each parameters as the response variable. We then used a post hoc Tukey test to test for specific differences among means in physiological, morphological, and biochemical traits. We performed a principal component analysis (PCA) due the high degree of co-linearity between physiological and biochemical variables. Next, we performed a two-way ANOVA on the factor scores generated by PCA with environmental temperature and drinking water salinity as the independent factors.

All data were examined for assumptions of normality and homogeneity of variance using Kolmogorov–Smirnov and Levene tests prior to each statistical analysis. For variables that were not normally distributed, we logarithmically transformed data for statistical analysis. Statistical analyses were performed using the STATISTICA® statistical package for Windows.

3. Results

3.1. Morphology and osmometry

We found no effect of the drinking water treatment ($F_{(1,26)}$ = 0.36, p = 0.55), environmental temperature ($F_{(1,26)}$ = 1.03, p = 0.32) and the interaction between both independent variables ($F_{(1,26)}$ = 1.42, p = 0.24) on body mass of individuals. MANOVA analysis of morphological variables and osmometry revealed significant effects of treatment (Wilks λ = 0.013, $F_{(17,5)}$ = 23.16, p = 0.001), temperature (Wilks λ = 0.015, $F_{(17,5)}$ = 18.27, p = 0.002), and the interaction between both variables (Wilks λ = 0.034, $F_{(17,5)}$ = 8.2, p = 0.01). The results of the univariate test showed that birds acclimated to salt water conditions increased their small intestine size (p < 0.005, Table 1 and Table S1). Likewise, there was a significant effect of environmental temperature on the size of pectoralis muscle and food intake (p < 0.005, Table S1). Specifically, birds acclimated to 27 °C increased pectoralis muscle mass, but decreased food intake (Table 1). Finally, the osmolality of the excreta was affected by the interaction between treatment and temperature (p = 0.03; Table S1); specifically, urine osmolality was less concentrated in birds acclimated to 27 °C and exposed to tapwater (Table 1).

3.2. Energetics and metabolic enzyme activity

Analysis of energetic variables revealed two patterns. First, BMR was affected by the treatment of drinking water (p = 0.003; Table S2); SW-acclimated birds had the highest BMR values (Fig. 1A). In addition, environmental temperature significantly affected M_{sum} (p = 0.049; Table S2); birds acclimated to 17 °C showed an increase in their M_{sum} compared to birds acclimated to 27 °C (Fig. 1B). The factorial aerobic scope (FAS) was significantly lower in SW-acclimated birds in comparison to those that consumed tap water (Fig. 1A; Table S2). Second, the net aerobic scope (NAS) was not affected by treatment (p = 0.29), environmental temperature (p = 0.07), or the interaction between both variables (p = 0.37; Table S2). Finally, water treatment, environmental temperature, and the interaction between these factors did not affect cytochrome *c* oxidase activity in heart (p > 0.05), kidney (p > 0.05), pectoral muscle (p > 0.05), or liver (p > 0.05; Table 2 and Table S2). Similarly, the activity of citrate synthase (CS, Table 2) was not affected by these independent variables in all tissues (p > 0.05; Table S2).

3.3. Oxidative parameters and immune function

MANOVA analysis revealed that neither treatment nor environmental temperature and the interaction between both variables affected lipid peroxidation of plasma (p > 0.05; Fig. 2) and brain (p > 0.05; Fig. 2). However, a significant effect of environmental temperature was found on lipid peroxidation of liver (p = 0.049; Table S2). Specifically, birds acclimated to 27 °C showed decreased lipid peroxidation compared to that measured in birds acclimatized to 17 °C (Fig. 2). Liver or brain TAC did not change by treatment, environmental temperature or their interaction (p > 0.05, Fig. 2). However, plasma TAC was significantly affected by environmental temperature (p = 0.046; Table S2); lower TAC values were observed in birds acclimated to 27 °C in comparison to those acclimated to 17 °C (Fig. 2). Lastly, nitric oxide concentrations in plasma were not affected by drinking water treatment or environmental temperature (p > 0.05; Table S2).

Before injection of PHA, the thickness of the wing web was not affected by drinking water treatment ($F_{(1,26)}$ = 1.712, p = 0.2), environmental temperature ($F_{(1,26)}$ = 1.12, p = 0.3), or their interaction ($F_{(1,26)}$ = 0.16, p = 0.7). However, we found a significant effect of the interaction between drinking water treatment and environmental temperature after the injection of PHA (p = 0.032; Table S2), as the thickness of the wing web was higher in birds acclimated to fresh (tap) water at 27 °C (Fig. 3).

We also performed a correlation analysis between the oxidative parameters, energetic variables, metabolic enzymes, and immune response. The correlation matrix revealed a positive correlation between telomere length and M_{sum} (r = 0.38; p = 0.04), a negative relationship between lipid peroxidation of plasma and kidney (r = −0.38; p = 0.04) and liver (r = −0.44; p = 0.15) COX activity. Additionally, plasma TAC positively correlated with kidney (r = 0.55; p = 0.002) and liver (r = 0.54; p = 0.02) COX activity.

The principal component analysis (PCA) reduced the 21 variables into two axes, which accounted for the 30.9% of the variance (Table S3). The first principal component (PC_1), explained 16,3% of variance and was positively correlated with metabolic components M_{sum}, NAS, and FAS. PC_1 can be interpreted as the capacity for energy expenditure. The second principal component (PC_2) explained a smaller amount of variance (14,6%) and was negatively correlated with biochemical capacity, plasma TAC, and kidney and liver COX activities. Thus, PC_2 can

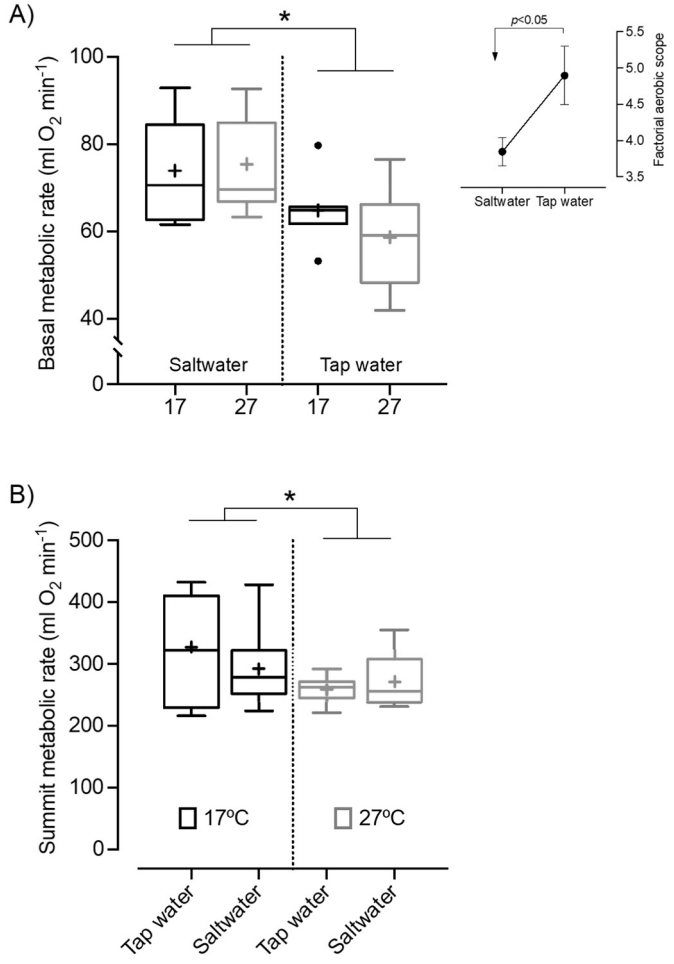

Fig. 1. Rates of energy expenditure in *Zonotrichia capensis* acclimated to environmental temperatures of 17 °C and 27 °C and drinking fresh (tap) water versus saltwater for 30 days. Box plots show the median, 25th, and 75th percentiles (box edges), the range (whiskers), and the mean values (crosses); black circles are outliers. The asterisk denotes significant differences after a posteriori Tukey test between tap water and saltwater acclimation (upper panel) and between environmental temperature (lower panel).

be interpreted as an index of oxidative status. The results of the ANOVA test revealed that the first axis was marginally affected by drinking water treatment ($F_{(1,27)} = 3.97$, $p = 0.06$), but not by environmental temperature ($F_{(1,27)} = 1.16$, $p = 0.29$) or the interaction between both variables ($F_{(1,27)} = 0.1$, $p = 0.75$). However, the second axis was affected by drinking water treatment ($F_{(1,27)} = 4.89$, $p = 0.04$), environmental temperature ($F_{(1,27} = 5.05$, $p = 0.03$), and the interaction between both independent variables ($F_{(1,27)} = 4.37$, $p = 0.05$). The post hoc test revealed that PC_2 increased in birds acclimated to fresh (tap) water at 27 °C (Fig. 4).

4. Discussion

4.1. Aerobic scope and mitochondrial metabolic enzymes

The main objective of our study was to evaluate the integrated effect of environmental temperature and drinking water salinity on selected physiological and biochemical traits of *Z. capensis*. Our results confirmed that this passerine tolerated chronic exposure to moderate levels of saltwater, which was evidenced by the unchanged plasma osmolality and body mass at the two contrasting environmental temperatures. Nevertheless, these environmental variables (i.e., temperature and osmotic stress) did result in biochemical and physiological changes.

The ingestion of saltwater affected BMR, whereas M_{sum} was only impacted by environmental temperature. Although data from the literature generally show a positive relationship between BMR and M_{sum} among species in both field (e.g., seasonal acclimatization) and experimental conditions, some studies suggest that M_{sum} and BMR might be functionally independent at the intra-specific level (Dutenhoffer and Swanson, 1996; Petit et al., 2013; Barceló et al., 2017). In agreement with these studies, our results confirm the hypothesis that BMR and M_{sum} reflect adjustments in different physiological systems responding jointly to different constraints (Barceló et al., 2017).

The enhanced BMR of birds acclimated to saltwater was linked with higher values of urine osmolality and increased kidney mass relative to those that consumed fresh (tap) water, which corroborated previous results obtained for this species (Peña-Villalobos et al., 2014; Sabat et al., 2017). Therefore, it can be concluded that ingestion of hypersaline drinking water in *Z. capensis* triggers osmoregulatory mechanisms that have high energetic costs (Gutiérrez et al., 2012; Peña-Villalobos et al., 2014; Sabat et al., 2017). Surprisingly, the BMR of birds in our study was not influenced by T_a, which contrasts with previous findings by our research group that showed *Z. capensis* acclimated to 15 °C displayed higher BMR values than birds acclimated to 30 °C (Maldonado et al., 2009). However, these differences in the BMR response could be explained, at least in part, by intraspecific variation in BMR in acclimatized individuals sourced from different *Z. capensis* populations (Cavieres and Sabat, 2008). Birds in our study were captured from a site at the foot of the Andes (~ 1300 m altitude), while birds studied by Maldonado et al. (2009) were sourced from a lower altitude (~ 700 m) site at Quebrada de la Plata. We also cannot discard the possibility that the observed differences in how BMR responds to temperature acclimation could be due to differences in the experimental protocols used here and in Maldonado et al. (2009). In the current study, we habituated birds to captive conditions for 21 days before initiating the experiments, whereas in Maldonado et al. (2009) birds were only habituated to captivity for one day. This could be important because Barceló et al. (2009) documented that thermal history, which in this case was the time animals spent in the field before capture and the beginning of the acclimation period, affects metabolic adjustments to environmental temperature. Besides, M_{sum} was higher in sparrows acclimated to 17 °C in both saltwater and tap water treatments, which matches with the higher food intake observed in those experimental groups (Table 1, Fig. 1). Indeed, evidence suggests that maximum capabilities for energy expenditure in endotherms is primarily influenced by maximum food intake, which in turn likely enables animals to acquire more energy when resources are abundant (Maldonado et al., 2016).

4.2. Immune response and oxidative stress

Several authors have proposed the existence of functional trade-offs between thermoregulation and immune response (King and Swanson, 2013; Kokolus et al., 2013). For example, the magnitude of the immune response significantly decreases in endotherms acclimated to low environmental temperatures or during colder (winter) seasons (Svensson et al., 1998; Cichón et al. 2002). As expected, FAS was higher in birds acclimated to tap water at both 17° and 27 °C in our experiment, which was expected give the higher maintenance cost found in birds acclimated to saltwater. High values of aerobic scope would allow animals to use more of their available energy to fuel other activities such as thermoregulation or to trigger immune responses (Maldonado et al., 2016). Despite the finding that the birds acclimated to tap water in the 17 °C and 27 °C groups had the same FAS, they did not exhibit the same immune response. The group acclimated to tap water at 27 °C showed the strongest immune response compared to all other treatment groups. We thus suggest that birds acclimated to tap water at 17 °C are faced with higher thermoregulatory costs, thus reducing the amount of energy available to trigger an immune response (see Hawley et al., 2012).

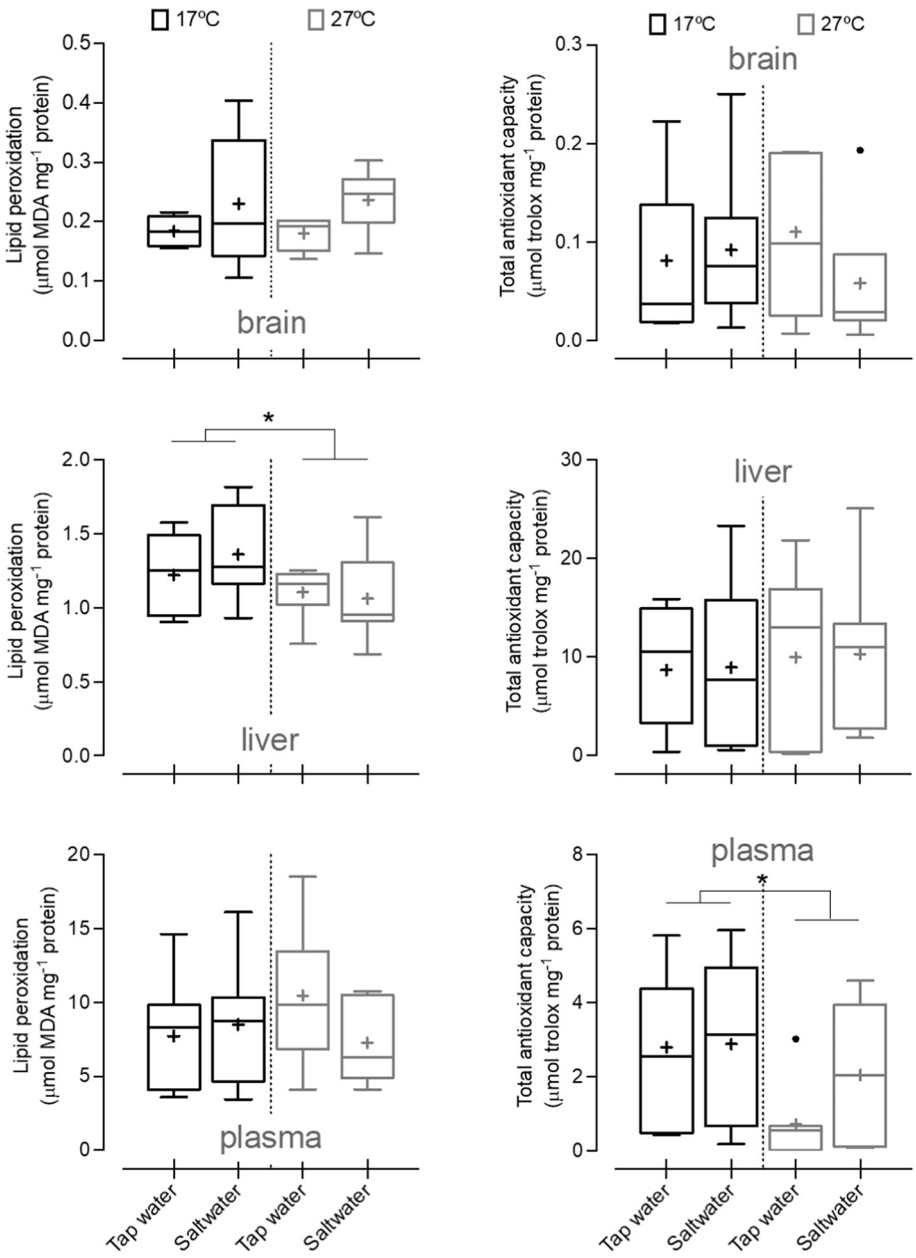

Fig. 2. Oxidative stress biomarkers in selected tissues of *Zonotrichia capensis* acclimated to environmental temperatures of 17 °C and 27 °C and drinking fresh (tap) water versus saltwater for 30 days. Box plots show the median, 25th, and 75th percentiles (box edges), the range (whiskers), and the mean values (crosses); black circles are outliers. Asterisks denote significant differences after a posteriori Tukey test between experimental groups for each tissue.

Our results show that both drinking water salinity and low environmental temperature had a negative effect on the inflammatory response of rufous-collared sparrows as birds acclimated to the least stressful conditions displayed the strongest immune response. This result is consistent with a previous study that showed a decreased in the inflammatory response in seawater-acclimated dunlins (Gutiérrez et al., 2013). The exact mechanism responsible for this pattern is not currently know, but it may be that the decrease in immune response is modulated by osmoregulatory hormones (e.g. prolactine, and corticosterone)that have an immunosuppressive effect (Gutiérrez et al., 2013; Gutiérrez, 2014; Masero et al., 2017).

We found signs of oxidative stress in *Z. capensis* exposed to low environmental temperature, probably because of increased energy requirements needed to maintain T_b. The increase in lipid peroxidation was coupled with high levels of circulating molecular antioxidants (i.e., plasma TAC). In fact, concentrations of NO did not vary among experimental groups, perhaps because there were similar levels of NO production and/or potential increases in NO were counteracted by the action of circulating antioxidants. Accordingly, animals acclimated to 17 °C exhibited high levels of plasma TAC (Fig. 2), which is concomitant with enhanced M_{sum} values. Likewise, plasma TAC concentrations were positively and significantly associated with liver and kidney COX activities. Lastly, our PCA showed that the oxidative status and metabolic intensity of tissues was impacted by the interaction of environmental temperature and drinking water salinity. In particular, birds acclimated to tap water at 27 °C had a remarkably high value for the second axis component of PCA, which was most closely related to oxidative stress capacity.

Contrary to our expectations, we did not find a clear relationship between (1) the levels of oxidative status and BMR (see Sabat et al., 2017) and (2) the levels of oxidative status, metabolic enzymes, and telomere length. First, some studies have found support for a functional

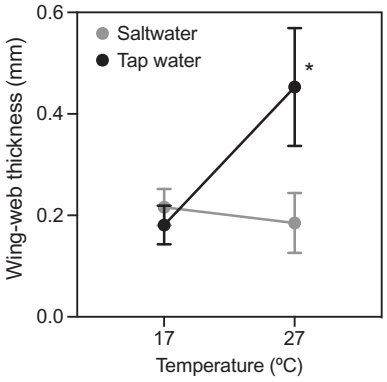

Fig. 3. Increase in wing-web thickness after the inoculation of phytohemagglutinin (PHA) in *Zonotrichia capensis* after acclimatization to tap water (black circles) and saltwater (grey circles) at 17 °C or 27 °C for 30 days. Symbols are the mean ± SE; asterisk denotes significant at $p < 0.05$.

relationship between mass-specific metabolic rates and oxidative status (Williams et al., 2010; Sabat et al., 2017), although this pattern has been the subject of debate (Selman et al., 2012) and is not supported by some empirical datasets (Brzęk et al., 2014). Interestingly, the primary assumption behind this proposition is that higher basal rates of metabolism are an unequivocal indicator of total rates of energy expenditure, which in turn leads to higher production of ROS. Some studies show, however, that daily avian total metabolism may be uncoupled from basal metabolic rates in certain experimental (and likely field) conditions. For example, a large (~100%) increase in energy use was observed in Zebra finches (*Taeniopygia guttata*) offered a restricted amount of food and water for 24 h in comparison to control birds, but BMR of birds in the restricted treatment decreased by ~15% (Rutkowska et al., 2016). Several factors acting alone or in concert could drive this pattern. First, the time required for acclimation in BMR may be longer than the experimental (treatment) period. Second, the thermal environment experienced by birds in the wild before acclimation affects the acclimation response to experimental environmental temperature (Barceló et al., 2009). Finally, this unexpected relationship between BMR and energy use under resource restricted conditions could result from a different expression of phenotypic flexibility between avian species and/or populations (Cavieres and Sabat, 2008).

4.3. Impact on genetic responses

Our study showed that energetic demands associated to osmo- and thermoregulatory stress did not impact telomere length. Low environmental temperatures (17 °C) were associated with higher M_{sum} and an increase in lipid peroxidation in the liver, an indicator of membrane damage. The lack of differences in telomere length among experimental groups could be explained by a high expression of antioxidants (e.g., Humans: Furumoto et al., 1998; Birds: Beaulieu et al., 2010; Sudyka et al., 2014; Badás et al., 2015; Kim and Velando, 2015). Alternatively, it is possible that the acclimation time used in the present study (30 days) was not long enough to cause a detectable shortening of telomere length (Sebastiano et al., 2017); note that previous work found evidence of telomere shortening over both short (e.g., weeks; Meillère et al., 2015; Salmón et al., 2016) and long time (e.g., months; Kotrschal et al., 2007; Boonekamp et al., 2014) time periods. In addition, telomere loss occurs at a higher rate early in life at the height of growth and development (Zeichner et al., 1999; Hall et al., 2004; Price et al., 2013), so the lack of change we observed among treatments may be related to the fact that all of the individuals in our experiment were adults. Despite the lack of significant differences, to our knowledge our study represents the first acclimatization experiment that evaluated the relationship between basal metabolic rate and teleomere length. We designed our experiment to examine this relationship because it has been proposed that increases in metabolic rate results in increased production of free radicals (Costantini et al., 2011), which theoretically should shorten telomeres (von Zglinicki, 2002). We did observe an unpredicted positive relationship between M_{sum} and telomere length, which could be due to age-associated variation in both telomere length and metabolic rate. It is well known that telomeres are shortened by cell division and therefore their length decreases with age (Haussmann and Vleck, 2002). As such, younger individuals with high antioxidant capacity would have longer telomeres (Monaghan and Haussmann, 2006). The effect of age on metabolic rate has not been as well studied, but it has been observed in zebra finches, metabolic rate declined with age (Moe et al., 2009). If metabolic rate also decreases with age in rufous-collared sparrows, then the positive association we observed between metabolic rate and telomere length may be an artifact of the age of individuals in our study.

Table 1
Body mass, osmolality of fluids, and organ masses and length (means ± SD) of *Zonotrichia capensis* acclimated to tap (fresh) water and saltwater and two contrasting environmental temperatures (17 °C and 27 °C) for 30 days. Asterisks denote significant differences between environmental temperature treatments. Ɨ Denotes significant differences between tap and salt water acclimation. Different letters denote significant differences between saltwater and tap water conditions and acclimation to different environmental temperatures.

	Salt Water		Tap Water		Salinity (pooled)		Temperature (pooled)	
	17	27	17	27	Salt	Tap	17 °C	27 °C
Body mass (g)	20.43 ± 1.37	20.51 ± 1.3	20.7 ± 1.01	19.7 ± 1.18	20.47 ± 0.31	20.2 ± 0.31	20.55 ± 0.31	20.11 ± 0.33
Pectoralis (g)	1.63 ± 0.5	2.06 ± 0.28Ɨ	1.67 ± 0.21	2.03 ± 0.15Ɨ	1.82 ± 0.09	1.85 ± 0.09	1.65 ± 0.07 *	2.04 ± 0.08
Liver mass (g)	0.59 ± 0.24	0.64 ± 0.17	0.59 ± 0.13	0.59 ± 0.13	0.62 ± 0.04	0.59 ± 0.05	0.60 ± 0.04	0.62 ± 0.05
Gizzard (g)	0.83 ± 0.05	0.82 ± 0.14	0.78 ± 0.12	0.76 ± 0.14	0.83 ± 0.03	0.77 ± 0.03	0.81 ± 0.03	0.79 ± 0.03
Heart (g)	0.27 ± 0.03	0.26 ± 0.04	0.28 ± 0.03	0.26 ± 0.02	0.26 ± 0.007	0.27 ± 0.008	0.27 ± 0.008	0.26 ± 0.008
Kidney (g)	0.22 ± 0.03	0.21 ± 0.01	0.2 ± 0.04	0.19 ± 0.01	0.22 ± 0.06	0.20 ± 0.07	0.21 ± 0.006	0.20 ± 0.007
Small Intestine (g)	0.77 ± 0.14	0.68 ± 0.07	0.61 ± 0.09	0.58 ± 0.12	0.73 ± 0.03 Ɨ	0.60 ± 0.03	0.70 ± 0.03	0.63 ± 0.03
Small intestine (cm)	15.7 ± 1.9	14.0 ± 1.0	13.12 ± 1.01	12.7 ± 1.7	14.86 ± 0.4 Ɨ	12.93 ± 0.42	14.50 ± 0.45	13.35 ± 0.48
Large intestine (g)	0.05 ± 0.02	0.06 ± 0.01	0.05 ± 0.01	0.06 ± 0.02	0.05 ± 0.004	0.06 ± 0.004	0.05 ± 0.004	0.06 ± 0.004
Large intestine (cm)	1.5 ± 0.33	1.5 ± 0.22	1.4 ± 0.3	1.6 ± 0.4	1.49 ± 0.08	1.50 ± 0.08	1.46 ± 0.08	1.53 ± 0.08
Urine Osm (mOsm/Kg)	385 ± 76.84[a]	401.75 ± 71.31[a]	383 ± 71.76[a]	299.57 ± 13.71[b]	393.94 ± 16.87 Ɨ	341.29 ± 18.59	384.5 ± 18.21	354.07 ± 18.81
Plasma Osm (mOsm/Kg)	320.53 ± 22.85	316.89 ± 15.88	324.57 ± 22.1	321.43 ± 14.89	318.43 ± 4.97	323 ± 4.97	322.57 ± 4.99	318.86 ± 4.99
Food intake (g/day)	3.72 ± 0.45	3.37 ± 0.68	3.77 ± 0.5	3.11 ± 0.27	3.57 ± 0.14	3.44 ± 0.16	3.40 ± 0.12 *	3.24 ± 0.13
Water intake (mL/day)	5.86 ± 1.87	7.79 ± 2.35	5.6 ± 2.05	5.86 ± 2.32	7.26 ± 0.51	5.73 ± 6.88	6.28 ± 0.56	6.89 ± 0.58
Plasma NO (nmol mg^{-1} protein)	0.33 ± 0.12	0.28 ± 0.21	0.40 ± 0.24	0.27 ± 0.14	0.30 ± 0.05	0.33 ± 0.06	0.35 ± 0.05	0.27 ± 0.05

Table 2
Metabolic enzymes (μmol min^{-1} mg^{-1} protein) in selected tissues of *Zonotrichia capensis* acclimated to environmental temperatures of 17 °C and 27 °C and drinking fresh (tap) water versus saltwater for 30 days.

	Salt Water		Tap Water		Salinity (pooled)		Temperature (pooled)	
	17	27	17	27	Salt	Tap	Salt	Tap
COX activity								
Heart	0.25 ± 0.11	0.20 ± 0.10	0.29 ± 0.13	0.32 ± 0.11	0.22 ± 0.03	0.3 ± 0.03	0.27 ± 0.03	0.27 ± 0.03
Kidney	0.05 ± 0.03	0.05 ± 0.03	0.05 ± 0.03	0.04 ± 0.01	0.05 ± 0.006	0.04 ± 0.007	0.05 ± 0.007	0.04 ± 0.007
Pectoral muscle	0.24 ± 0.12	0.28 ± 0.03	0.23 ± 0.16	0.21 ± 0.07	0.26 ± 0.03	0.22 ± 0.03	0.23 ± 0.03	0.25 ± 0.03
Liver	0.02 ± 0.02	0.03 ± 0.02	0.01 ± 0.01	0.006 ± 0.004	0.03 ± 0.005	0.01 ± 0.005	0.03 ± 0.005	0.02 ± 0.005
CS activity								
Heart	0.71 ± 0.19	0.68 ± 0.24	0.74 ± 0.21	0.81 ± 0.17	0.70 ± 0.05	0.77 ± 0.05	0.72 ± 0.05	0.75 ± 0.05
Kidney	0.28 ± 0.15	0.24 ± 0.06	0.23 ± 0.05	0.28 ± 0.08	0.26 ± 0.02	0.25 ± 0.03	0.26 ± 0.02	0.26 ± 0.03
Pectoral muscle	0.94 ± 0.34	0.94 ± 025	0.86 ± 0.16	0.87 ± 0.22	0.94 ± 0.06	0.87 ± 0.07	0.91 ± 0.06	0.91 ± 0.07
Liver	0.43 ± 0.14	0.37 ± 0.1	0.42 ± 0.11	0.36 ± 0.13	0.40 ± 0.03	0.39 ± 0.03	0.42 ± 0.03	0.37 ± 0.03

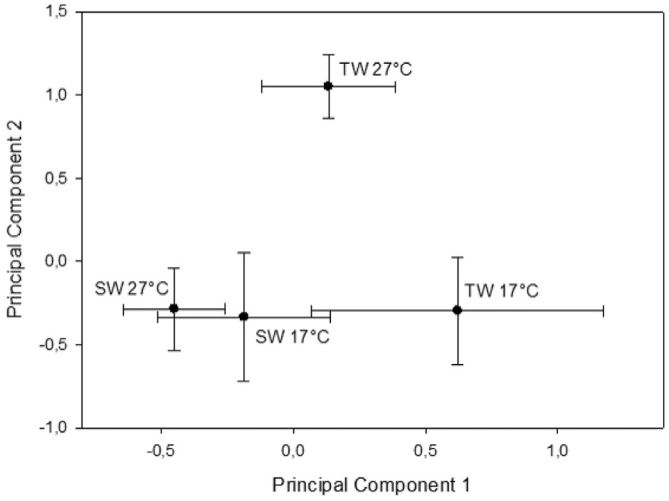

Fig. 4. Results of principal components analysis (PCA) on morphological and physiological variables studied in *Zonotrichia capensis* acclimated to environmental temperatures of 17 °C and 27 °C and drinking fresh (tap) water versus saltwater for 30 days. The first axis (PC_1) axis was positively correlated with M_{sum}, NAS, and FAS. The second axis (PC_2) was negatively correlated with plasma TAC concentrations, as well as COX activity in liver and kidney (Table S2).

4.4. Conclusions

Our study shows that environmental temperature and osmotic stress do not have a synergistic or antagonistic effect in *Z. capensis*, except when birds are presented with an immune challenge. Our results revealed that the ability to mount a strong immune response depended on the interaction of environmental temperature and salinity, with responses being greater in the treatment with the least stressful conditions in regards to thermo- and osmoregulation. These results suggest the existence of an energetic trade-off between biological functions that act in parallel to control immune function. It is increasingly imperative to understand the mechanistic basis of animal physiological responses to rapid environmental changes such as increased temperature and conditions that challenge osmoregulation constitute an acute form of environmental disturbance. Understanding how widespread and abundant bird species like *Z. capensis* respond to such disturbances is important for both fundamental and applied reasons, and will allow us to quantify how environmental change will influence physiological responses that in turn may ultimately impact fitness.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cbpa.2019.04.016.

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References

Aljanabi, S.M., Martinez, I., 1997. Universal and rapid salt-extraction of high-quality genomic DNA for PCR-based techniques. Nucleic Acids Res. 25, 4692–4693.
Angelier, F., Vleck, C.M., Holberton, R.L., Marra, P.P., 2013. Telomere length, non-breeding habitat and return rate in male American redstarts. Funct. Ecol. 27, 342–450.
Araya, B., Millie, G., 2005. Guía de campo de las aves de Chile. Editorial Universitaria, Santiago de Chile, pp. 406.
Badás, E.P., Martínez, J., Rivero de Aguilar Cachafeiro, J., Miranda, F., Figuerola, J., Merino, S., 2015. Ageing and reproduction: antioxidant supplementation alleviates telomere loss in wild birds. J. Evol. Biol. 28, 896–905.
Barceló, G., Salinas, J., Cavieres, G., Canals, M., Sabat, P., 2009. Thermal history can affect the short-term thermal acclimation of basal metabolic rate in the passerine *Zonotrichia capensis*. J. Ther. Biol. 34, 415–419.
Barceló, G., Love, O.P., Vézina, F., 2017. Uncoupling basal and summit metabolic rates in white-throated sparrows: digestive demand drives maintenance costs, but changes in muscle mass are not needed to improve Thermogenic capacity. Physiol. Biochem. Zool. 90, 153–165.
Barnard, C., Behnke, J., 2006. Behaviour, life history strategies and parasite infection in rodents. In: Morand, S., Krasnov, B.R., Poulin, R. (Eds.), Micromammals and Macroparasites. Springer-Verlag, Tokyo, Japan, pp. 475–511.
Beaulieu, M., Ropert-Coudert, Y., Le Maho, Y., Ancel, A., Criscuolo, F., 2010. Foraging in an oxidative environment: relationship between δ13C and oxidative status in Adelie penguins. Proc. R. Soc. Lond. B Biol. Sci. 277, 1087–1092.
Beckman, K.B., Ames, B.N., 1998. The free radical theory of aging matures. Physiol. Rev. 78, 547–581.
Blackburn, E.H., 1991. Structure and function of telomeres. Nature. 350, 569–573.
Boonekamp, J.J., Mulder, G.A., Salomons, H.M., Dijkstra, C., Verhulst, S., 2014. Nestling telomere shortening, but not telomere length, reflects developmental stress and predicts survival in wild birds. Proc. R. Soc. B 281, 20133287.
Bradford, M., 1976. A rapid and sensitive assay of protein utilizing the principle of bye dying. Anal. Biochem. 772, 28–264.
Brzęk, P., Książek, A., Ołdakowski, Ł., Konarzewski, M., 2014. High basal metabolic rate does not elevate oxidative stress during reproduction in laboratory mice. J. Exp. Biol. 217, 1504–1509.
Buckley, L.B., Ehrenberger, J.C., Angilletta, M.J., 2015. Thermoregulatory behaviour limits local adaptation of thermal niches and confers sensitivity to climate change. Funct. Ecol. 29, 1038–1047.
Cavieres, G., Sabat, P., 2008. Geographic variation in the response to thermal acclimation in rufous-collared sparrows: are physiological flexibility and environmental heterogeneity correlated? Funct. Ecol. 22, 509–515.
Cichoń, M., Chadzińska, M., Książek, A., Konarzewski, M., 2002. Delayed effects of cold stress on immune response in laboratory mice. Proc. R. Soc. Lond. B Biol. Sci. 269, 1493–1497.
Cornelius, E.A., Vezina, F., Regimbald, L., Hallot, F., Petit, M., Love, O.P., Karasov, W.H., 2017. Chikadees faced with unpredictable food increase fat reserves but certain components of their immune respose function decline. Phsiol. Biochem. Zool. 90,

190–200.
Costantini, D., Møller, A.P., 2009. Does immune response cause oxidative stress in birds? A meta-analysis. Comp. Biochem. Physiol. A Mol. Integr. Physiol. 153, 339–344. https://doi.org/10.1016/j.cbpa.2009.03.010.
Costantini, D., Marasco, V., Møller, A.P., 2011. A meta-analysis of glucocorticoids as modulators of oxidative stress in vertebrates. J. Comp. Physiol. B. 181, 447–456.
Criscuolo, F., Bize, P., Nasir, L., Metcalfe, N.B., Foote, C.G., Griffiths, K., Gault, E.A., Monaghan, P., 2009. Real-time quantitative PCR assay for measurement of avian telomeres. J. Avian Biol. 40, 342–347.
Dawson, W.R., Marsh, R.L., Buttemer, W.A., Carey, C., 1983. Metabolic adjustments of small passerine birds for migration and cold. Am. J. Phys. 245, R755–R767.
Dohm, M.R., 2002. Repeatability estimates do not always set an upper limit to heritability. Funct. Ecol. 16, 273–280.
Dowling, D.K., Simmons, L.W., 2009. Reactive oxygen species as universal constraints in life-history evolution. Proc. R. Soc. B Biol. Sci. 276, 1737–1745.
Dutenhoffer, M.S., Swanson, D.L., 1996. Relationship of basal to summit metabolic rate in passerine birds and the aerobic capacity model for the evolution of endothermy. Physiol. Zool. 69, 1232–1254.
Fitze, P.S., Tschirren, B., Richner, H., 2004. Life history and fitness consequences of ectoparasites. J. Anim. Ecol. 73, 216–226.
Furumoto, K., Inoue, E., Nagao, N., Hiyama, E., Miwa, N., 1998. Age-dependent telomere shortening is slowed down by enrichment of intracellular vitamin C via suppression of oxidative stress. Life Sci. 3, 935–948.
Goldstein, D., Skadhauge, E., 2000. Renal and Extrarenal Regulation of Body Fluid Compartments Sturkie's Avian Physiology. Academic Press.
Goodall, L.J., Johnson, A.W., Philippi, R.A., 1946. Las Aves de Chile. Vol 1 Platt Establecimientos Gráficos, Buenos Aires.
Guderley, H., 1998. Temperature and growth rates as modulators of the metabolic capacities of fish muscle. In: Pörtner, H.O., Playle, R. (Eds.), Cold Oean Physiology. Cambridge University Press, Cambridge, pp. 58–87.
Gutiérrez, J.S., 2014. Living in environments with contrasting salinities: a review of physiological and behavioural responses in waterbirds. Ardeola. 61, 233–256.
Gutiérrez, J.S., Masero, J.A., Abad-Gómez, J.M., Villegas, A., Sánchez-Guzmán, J.M., 2011. Understanding the energetic costs of living in saline environments: effects of salinity on basal metabolic rate, body mass and daily energy consumption of a long-distance migratory shorebird. J. Exp. Biol. 214, 829–835.
Gutiérrez, J.S., Dietz, M.W., Masero, J.A., Gill, R.E.Jr., Dekinga, A., Battley, P.F., Sánchez-Guzmán, J.M., Piersma, T., 2012. Functional ecology of saltglands in shorebirds: flexible responses to variable environmental conditions. Funct. Ecol. 26, 236–244.
Gutiérrez, J.S., Abad-Gómez, J.M., Villegas, A., Sánchez-Guzmán, J.M., Masero, J.A., 2013. Effects of salinity on the immune response of an “osmotic generalist” bird. Oecologia. 171, 61–69.
Hall, M.E., Nasir, L., Daunt, F., Gault, E.A., Croxall, J.P., Wanless, S., Monaghan, P., 2004. Telomere loss in relation to age and early environment in long-lived birds. Proc. R. Soc. Lond. 271, 1571–1576.
Hasselquist, D., Nilsson, J.-Å., 2012. Physiological mechanisms mediating costs of immune responses: what can we learn from studies of birds? Anim. Behav. 83, 1303–1312.
Haussmann, M.F., Vleck, C.M., 2002. Telomere length provides a new technique for aging animals. Oecologia. 130, 325–328.
Hawley, D.M., DuRant, S.E., Wilson, A.F., Adelman, J.S., Hopkins, W.A., 2012. Additive metabolic costs of thermoregulation and pathogen infection. Funct. Ecol. 26, 701–710. https://doi.org/10.1111/j.1365-2435.2012.01978.x.
Hinsley, S.A., Ferns, P.N., Thomas, D.H., Pinshow, B., 1993. Black-bellied Sandgrouse (*Pterocles orientalis*) and pin-tailed Sandgrouse (*Pterocles alchata*): closely related species with differing bioenergetic adaptations to arid zones. Physiol. Zool. 66, 20–42.
Hulbert, A.J., Pamplona, R., Buffenstein, R., Buttemer, W.A., 2007. Life and death: metabolic rate, membrane composition, and life span of animals. Physiol. Rev. 87, 1175–1213.
Ilmonen, P., Hasselquist, D., Langefors, S.A., Wiehn, J., 2003. Stress, immunocompetence and leukocyte profiles of pied flycatchers in relation to brood size manipulation. Oecologia 136, 148–154.
Karasov, W.H., Martínez del Río, C., 2007. Physiological Ecology: How Animals Process Energy, Nutrients, and Toxins. Princeton University Press, Princeton, NJ.
Kim, S.Y., Velando, A., 2015. Antioxidants safeguard telomeres in bold chicks. Biol. Lett. 11, 20150211.
King, M.O., Swanson, D.L., 2013. Activation of the immune system incurs energetic costs but has no effect on the thermogenic performance of house sparrows during acute cold challenge. J. Exp. Biol. 216, 2097–2102. https://doi.org/10.1242/jeb.079574.
Kokolus, K.M., Capitano, M.L., Lee, C.T., Eng, J.W.L., Waight, J.D., Hylander, B.L., Sexton, S., Hong, C.C., Gordon, C.J., Abrams, S.I., Repasky, E.A., 2013. Baseline tumor growth and immune control in laboratory mice are significantly influenced by subthermoneutral housing temperature. Proc. Natl. Acad. Sci. 110, 20176–20181. https://doi.org/10.1073/pnas.1304291110.
Koteja, P., 2000. Energy assimilation, parental care and the evolution of endothermy. Proc. R. Soc. Lond. B Biol. Sci. 267.
Kotrschal, A., Ilmonen, P., Penn, D.J., 2007. Stress impacts telomere dynamics. Biol. Lett. 3, 128–130.
Maldonado, K., Cavieres, G., Veloso, C., Canals, M., Sabat, P., 2009. Physiological responses in rufous-collared sparrows to thermal acclimation and seasonal acclimatization. J. Comp. Physiol. B. 179, 335–343.
Maldonado, K., Sabat, P., Píriz, G., Bogdanovich, J.M., Nespolo, R.F., Bozinovic, F., 2016. Is maximum food intake in endotherms constrained by net or factorial aerobic scope? Lessons from the leaf-eared mouse. Front. Physiol. 7, 649.
Martinez Del Rio, C., Sabat, P., Cheviron, Z., 2018. Physiology. In: Morrisson, M.L., Rodewald, A.D., Voelker, G., Colón, M.R., Prather, J.F. (Eds.), Ornithology. Foundation, Analysis, and Application. John Hopkins University Press, pp. 173–199.
Masero, J.A., Abad-Gómes, J.M., Gutierrez, J.S., Santiago- Quesada, F., Senner, N.R., Sánchez- Guzmán, J.M., Piersma, T., Schroeder, J., Amat, J.A., Villegas, A., 2017. Wetland salinity induces sex-dependent carry-over effects on the individual performance of a long-distance migrant. Sci. Rep. 7, 6867.
McNab, B.I., 2002. The Physiological Ecology of Vertebrates: A View from Energetics. Comstock Publishing Associates, NY, USA.
Meillère, A., Brischoux, F., Ribout, C., Angelier, F., 2015. Traffic noise exposure affects telomere length in nestling house sparrows. Biol. Lett. 11, 20150559.
Mendes, L., Piersma, T., Hasselquist, D., Matson, K.D., Ricklefs, R.E., 2006. Variation in the innate and acquired arms of the immune system among five shorebird species. J. Exp. Biol. 209, 284–291.
Moe, B., Rønning, B., Verhulst, S., Bech, C., 2009. Metabolic ageing in individual zebra finches. Biol. Lett. 5, 86–89.
Moller, A.P., Allander, K., Dufva, R., 1990. Fitness effects of parasites on passerine birds: a review. In: Blondel, J., Gosler, A., Lebreton, J.D., McCleery, R.H. (Eds.), Population Biology of Passerine Birds: an Integrated Approach. Springer-Verlag, Berlin, pp. 269–280.
Monaghan, P., Haussmann, M.F., 2006. Do telomere dynamics link lifestyle and lifespan? Trends Ecol. Evol. 21, 47–53.
Monaghan, P., Metcalfe, N.B., Torres, R., 2009. Oxidative stress as a mediator of life history trade-offs: mechanisms, measurement and interpretation. Ecol. Lett. 12, 75–92.
Moyes, C.D., Mathieu-Costello, O.A., Tsuchiya, N., Filburn, C., Hans-ford, R.G., 1997. Mitochondrial biogenesis during cellular differentiation. Am. J. Phys. 272, C1345–C1351.
Patton, C.J., Kryskalla, J.R., 2011. Colorimetric determination of nitrate plus nitrite in water by enzymatic reduction, automated discrete analyzer methods: U.S. In: Geological Survey Techniques and Methods, Book 5, Chap, pp. B8.
Peña-Villalobos, I., Valdés-Ferranty, F., Sabat, P., 2013. Osmoregulatory and metabolic costs of salt excretion in the Rufous-collared sparrow *Zonotrichia capensis*. Com. Biochem. Physiol. A. 164, 314–318.
Peña-Villalobos, I., Nuñez-Villegas, M., Bozinovic, F., Sabat, P., 2014. Metabolic enzymes in seasonally acclimatized and cold acclimated rufous-collared sparrow inhabiting a Chilean Mediterranean environment. Curr. Zool. 60, 338–350.
Petit, M., Lewden, A., Vézina, F., 2013. Intra-seasonal flexibility in avian metabolic performance highlights the uncoupling of basal metabolic rate and Thermogenic capacity. PLoS ONE 8, e68292.
Petit, M., Clavijo-Baquet, S., Vézina, F., 2016. Increasing winter maximal metabolic rate improves Intrawinter survival in small birds. Physiol. Biochem. Zool. 90, 166–177.
Pfaffl, M.W., 2001. A new mathematical model for relative quantification in real-time RT-PCR. Nucleic Acids Res. 29, e45.
Price, L.H., Kao, H.T., Burgers, D.E., Carpenter, L.L., Tyrka, A.R., 2013. Telomeres and early- life stress: an overview. Biol. Psychiatry 73, 15–23.
Quirici, V., Guerrero, C.J., Krause, J.S., Wingfield, J.C., Vásquez, R.A., 2016. The relationship of telomere length to baseline corticosterone levels in nestlings of an altricial passerine bird in natural populations. Front. Zool. 13 (1).
Ramirez-Otarola, N., Espinoza, J., Kalergis, A.M., Sabat, P., 2018. Is there an effect of environmental temperature on the response to an antigen and the metabolic rate in pups of the rodent *Octodon degus*? J. Therm. Biol. 71, 17–23.
Repasky, R.R., 1991. Temperature and the northern distributions of wintering birds. Ecology. 72, 2274–2285.
Rosenmann, M., Morrison, P., 1974. Maximum oxygen consumption and heat loss facilitation in small homeotherms by HeO2. Am. J. Phys. 226, 490–495.
Rutkowska, J., Sadowska, E.T., Cichon, M., Bauchinger, U., 2016. Increased fat catabolism sustains water balance during fasting in zebra finches. J. Exp. Biol. 219, 2623–2628.
Sabat, P., Martinez del Rio, C., 2002. Inter-and intraspecific variation in the use or marine food resources by three Cinclodes (Furnariidae: Aves) species: carbon isotopes and osmoregulatory physiology. Zoology 105, 247–256.
Sabat, P., Cavieres, G., Veloso, C., Canals, M., 2006. Water and energy economy of an omnivorous bird: population differences in the Rufous-collared sparrow (*Zonotrichia capensis*). Com. Biochem. Physiol. A: Mol. Int. Physiol. 144, 485–490.
Sabat, P., Narváez, C., Peña-Villalobos, I., Contreras, C., Maldonado, K., Sanchez-Hernandez, J.C., Newsome, S.D., Nespolo, R., Bozinovic, F., 2017. Coping with salt water habitats: metabolic and oxidative responses to salt intake in the Rufous-collared sparrow. Front. Physiol. 8, 654.
Salmón, P., Nilsson, J.F., Nord, A., Bensch, S., Isaksson, C., 2016. Urban environment shortens telomere length in nestling great tits, *Parus major*. Biol. Lett. 12, 20160155.
Sebastiano, M., Eens, M., Angelier, F., Pineau, K., Chastel, O., Costantini, D., 2017. Corticosterone, inflammation, immune status and telomere length in frigatebird nestlings facing a severe herpes virus infection. Conserv. Physiol. 5, cow073.
Selman, C., Blount, J.D., Nussey, D.H., Speakman, J.R., 2012. Oxidative damage, ageing, and life-history evolution: where now? Trends Ecol. Evol. 27, 570–577.
Sheldon, B.C., Verhulst, S., 1996. Ecological immunology: costly parasite defences and trade-offs in evolutionary ecology. Trends Ecol. Evol. 11, 317–321.
Shoemaker, V., 1972. Osmoregulation and excretion in birds. In: Farner, D.S., King, J., Parkes, K. (Eds.), Avian Biology: 572–612. Academic Press, London.
Sidell, B.D., Driedzic, W.R., Stowe, D.B., Johnston, I.A., 1987. Biochemical correlations of power development and metabolic fuel preferenda in fish hearts. Physiol. Zool. 60, 221–232.
Smits, J.E., Bortolotti, G.R., Tella, J.L., 1999. Simplifying the phytohaemagglutinin skin-testing technique in studies of avian immunocompetence. Funct. Ecol. 13, 567–572.
Sudyka, J., Arct, A., Drobniak, S., Dubiei, A., Gustafsson, L., Chichoń, M., 2014. Experimentally increased reproductive effort alters telomere length in the blue tit

(*Cyanistes caeruleus*). J. Evol. Biol. 27, 2258–2264.
Svensson, E., Råberg, L., Koch, C., Hasselquist, D., 1998. Energetic stress, immunosuppression and the costs of an antibody response. Funct. Ecol. 12, 912–919.
Swanson, D.L., 1993. Cold tolerance and thermogenic capacity in dark-eyed juncos in winter: geographic variation and comparison with American tree sparrows. J. Therm. Biol. 18, 275–281.
Swanson, D.L., 2010. Seasonal metabolic variation in birds: Functional and mechanistic correlates. In: Thompson, C. (Ed.), Current Ornithology Volume 17. Current Ornithology. vol. 17 Springer, New York, NY.
Swanson, D.L., Bozinovic, F., 2011. Metabolic capacity and the evolution of biogeographic patterns in oscine and suboscine passerine birds. Physiol. Biochem. Zool. 84, 185–194.
Swanson, D.L., Olmstead, K.L., 1999. Evidence for a proximate influence of winter temperature on metabolism in passerine birds. Physiol. Biochem. Zool. 72, 566–575.
van de Crommenacker, J.N., Horrocks, P.C., Versteegh, M.A., Komdeur, J., Tieleman, B.I., Matson, K.D., 2010. Effects of immune supplementation and immune challenge on oxidative status and physiology in a model bird: implications for ecologists. J. Exp. Biol. 213, 3527–3535.
Vinkler, M., Schnitzer, J., Munclinger, P., Albrecht, T., 2010. Phytohaemagglutinin skin-swelling test in scarlet rosefinch males: low-quality birds respond more strongly. Anim. Behav. 83, 17–23.
von Zglinicki, T., 2002. Oxidative stress shortens telomeres. Trends Biochem. Sci. 27, 339–344.
Weiner, J., 1992. Physiological limits to sustainable energy budgets in birds and mammals: ecological implications. Trends Ecol. Evol. 7, 384–388.
Wiersma, P., Muñoz-Garcia, A., Walker, A., Williams, J.B., 2007. Tropical birds have a slow pace of life. Proc. Natl. Acad. Sci. U. S. A. 104, 9340–9345.
Wieser, W., 1994. Cost of growth in cells and organisms: general rules and comparative aspects. Biol. Rev. 69, 1–33.
Williams, J.B., Tieleman, B.I., 2002. Ecological and evolutionary physiology of desert birds: a Progress report. Integr. Comp. Biol. 42, 68–75.
Williams, J.B., Miller, R.A., Harper, J.M., Wiersma, P., 2010. Functional linkages for the pace of life, life-history, and environment in birds. Integr. Comp. Biol. 50, 855–868.
Withers, P.C., 1977. Measurement of VO_2, VCO_2, and evaporative water loss with a flow-through mask. J. Appl. Physiol. 42, 120–123.
Zeichner, S.L., Palumbo, P., Feng, Y., Xiao, X., Gee, D., Sleasman, J., Goodenow, M., Biggar, R., Dimitrov, D., 1999. Rapid telomere shortening in children. Blood 93, 2824–2830.