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PHYSIOLOGICAL ECOLOGY - ORIGINAL RESEARCH

# Terrestrial birds in coastal environments: metabolic rate and oxidative status varies with the use of marine resources

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**Abstract**

Life in saline environments represents a major physiological challenge for birds, particularly for passerines that lack nasal salt glands and hence are forced to live in environments that do not contain salty resources. Increased energy costs associated with increased salt intake, which in turn increases the production of reactive oxygen species, is likely a major selection pressure for why passerines are largely absent from brackish and marine environments. Here we measured basal metabolic rates (BMR) and oxidative status of free-ranging individuals of three species of *Cinclodes*, a group of passerine birds that inhabit marine and freshwater habitats in Chile. We used a combination of carbon, nitrogen, and hydrogen isotope data from metabolically active (blood) and inert (feathers) tissues to estimate seasonal changes in marine resource use and infer altitudinal migration. Contrary to our expectations, the consumption of marine resources did not result in higher BMR values and higher oxidative stress. Specifically, the marine specialist *C. nigrofumosus* had lower BMR than the other two species (*C. fuscus* and *C. oustaleti*), which seasonally switch between terrestrial and marine resources. *C. fuscus* had significantly higher total antioxidant capacity than the other two species (*C. nigrofumosus* and *C. oustaleti*) that consumed a relatively high proportion of marine resources. Nearly all studies examining the effects of salt consumption have focused on intraspecific acclimation via controlled experiments in the laboratory. The mixed results obtained from field- and lab-based studies reflect our poor understanding of the mechanistic link among hydric-salt balance, BMR, and oxidative stress in birds.

**Keywords** Basal metabolic rate · Birds · *Cinclodes* · Oxidative stress · Osmoregulation · Passerines · Stable isotopes

## Introduction

Songbirds (Passeriformes) have diversified in all continents and occupy nearly all terrestrial ecosystems (Barker et al. 2004), but only a few species in this group inhabit marine and coastal environments (Wheelwright and Rising 1993; Martinez del Rio et al. 2009). A major physiological constraint limiting the geographic distribution of this diverse group of birds is the maintenance of osmotic homeostasis

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in environments where freshwater is scarce or unavailable (Johnston and Bildstein 1990; Casotti and Braun 2000; Sabat 2000; Bozinovic et al. 2011; Williams et al. 2012; Gutiérrez 2014). For passerines, marine and other saline environments are essentially dry environments as they cannot drink saline water, and most of the available prey (e.g., intertidal invertebrates, Larsen et al. 2014) are in osmotic equilibrium with environmental water. Indeed, feeding on salty marine foods and drinking seawater is especially challenging because these birds lack functional salt glands (Shoemaker 1972), and most of them have a limited ability to concentrate urine (Goldstein and Skadhauge 2000; Sabat and Martínez del Rio 2002; Sabat and Martinez del Rio 2006), which likely explains the paucity of obligate marine passerine species.

Some authors have suggested that the ingestion of salts and other osmotically active compounds represents an important energetic cost to seabirds and shorebirds, and may limit their distribution as well as colonization of new habitats (Nehls 1996; Gutiérrez et al. 2011). Although these osmoregulatory costs have been extensively studied in ectothermic animals (Tseng and Hwang 2008; Evans 2009), the topic has received little attention in endothermic vertebrates. For birds, a scarce amount of experimental evidence shows that consumption of saltwater for days or weeks increases basal (or resting) metabolic rate (BMR) in comparison to individuals that consume freshwater (Gutiérrez et al. 2011; Peña-Villalobos et al. 2013). This increase in BMR is assumed to be the cost of living in saline habitats, which is offset in seabirds and shorebirds by the osmotic work performed by the salt gland and kidneys.

Indirect evidence suggests that the costs of osmoregulation can be significant in birds and thus an important factor in prey selection and associated energy budgets in the field. For example, McNab (2009) concluded that seabirds have higher BMR than terrestrial species that inhabit forest or desert/grassland habitats. Furthermore, Gutierrez et al. (2012) found that BMR was significantly lower in shorebirds (*Calidris alpina*) inhabiting inland freshwater aquatic habitats in comparison to those that reside in coastal environments. Gutierrez et al. (2012) also found a similar pattern at the interspecific level among shorebirds after accounting for potential climatic effects. It is important to note that abiotic (e.g., temperature) and biotic (e.g., diet) factors known to influence BMR (Tieleman and Williams 2000; Tieleman et al. 2003; Cavieres and Sabat 2008; Swanson 2010; Maldonado et al. 2012; Naya et al. 2013) vary among habitats. Altogether, these studies suggest that saltwater consumption or consuming prey with high salt loads affects energy expenditure (BMR) in unpredictable ways, a topic that needs further research. In this context, obtaining an independent estimate of marine resource use is essential to identify the mechanisms that underlie physiological changes associated with salt intake in wild free-ranging populations.

On the other hand, an increase in metabolic activity may result in increased production of reactive oxygen species (ROS), a standard byproduct of the formation of ATP (Costantini and Verhulst 2009), whose accumulation ultimately leads to cell damage, disease, and even death (Dowling and Simmons 2009; Monaghan et al. 2009; van de Crommenacker et al. 2010; Selman et al. 2012). Increasing osmoregulatory function accelerates metabolic rates in several organisms including birds, but the precise effects of this acceleration on cellular oxidative status are unknown. Recently, Sabat et al. (2017) reported that when acclimated to drinking water of moderate salinity (200 mM NaCl), the rufous-collared sparrow (*Zonotrichia capensis)* exhibited changes in total oxidative status. Specifically, both antioxidant capacity and lipid peroxidation were higher in the liver of animals acclimated to saltwater than in those acclimated to freshwater. In addition, urine concentration and BMR increased in birds that drank salt water. Thus, it is important to note that there are intra- and interspecific differences in the resistance to environmentally mediated oxidative stress (Cohen et al. 2008; Beaulieu and Costantini 2014). Thus, an unresolved question is whether there are differences in the antioxidant capacity among phylogenetically related species if they differ in their seasonal use of marine resources, and more broadly what are the consequences of oxidative damage induced by salt consumption on physiological integrity.

Here we explore the presumed links between osmoregulation, BMR, and enzymatic mechanisms of oxidative stress in three species of *Cinclodes*, the only passerine genus that exhibits intra- and interspecific differences in marine resource use. By quantifying the physiological effects associated with the consumption of marine resources, we explored the proximate factors explaining the use of coastal habitats and dietary preferences in a widespread group of songbirds, which has implications for the question of why passerines as a group infrequently use saline resources. Furthermore, studying the effects of high salt loads experienced by *Cinclodes* will provide an understanding of how plastic animals can be in coping with diverse environmental stressors.

## Materials and methods

### Field collection

Birds were captured using mist nest and floor traps on the central coast of Chile in Algarrobo (33°22′09″S 71°40′05″W), El Quisco (33°24′S 71°42′W), and Los Molles (32°14′22″S 71°30′54″W) during austral winter (May–August). Blood samples were obtained with heparinized capillaries from the humeral vein of each bird immediately after capture. Blood samples were kept at 4 °C until centrifugation, which occurred within 12 h of capture. Blood was centrifuged at 10,000*g* for 10 min, and the plasma was separated and transported in liquid $N_2$ to the laboratory and then stored at − 80 °C until analysis. In addition, a subsample of whole blood was collected for isotope analysis, which corresponded to the dietary resources assimilated during ~ 1–2 months prior to capture (Martinez del Rio et al. 2009), which occurred in the austral winter. Primary feathers (P9) were collected from each individual, which corresponds to the assimilated resources during the molting period that occurs during the austral summer in *Cinclodes* (Bertolero and Zavalaga 2003).

## Basal metabolic rate

Basal metabolic rate (BMR) was measured via oxygen consumption using a continuous flow respirometry system (FoxBox, Sable Systems®, Nevada), as reported in Sabat et al. (2017). Briefly, birds were placed in metabolic chambers kept at a constant temperature (30 ± 0.5 °C) that received air free of water and $CO_2$ removed via Drierite and $CO_2$ absorbent at a flow of 750 mL/min; air flow was maintained at ± 1%. The $O_2$ concentration in the chamber was recorded for 8 h per individual during the period of inactivity between 20:00 and 06:00. Because water vapor and $CO_2$ were removed before breath samples entered the $O_2$ analyzer, and the flow rates are measured downstream from the metabolic chamber, oxygen consumption was calculated according to equation (11.1) in Lighton (2008): $VO_2 = \mathrm{FR} \times 60 \times (\mathrm{F_iO_2} - \mathrm{F_eO_2})/(1 - \mathrm{F_iO_2})$, where FR is the flow rate in mL $\mathrm{min}^{-1}$, and $\mathrm{F_iO_2}$ and $\mathrm{F_eO_2}$ are the fractional concentrations of inflow and outflow $O_2$ in the metabolic chamber, respectively.

## Oxidative stress

One limitation of using blood serum or plasma biochemical assays to characterize oxidative status in wild birds is the uncertainty that such measurements reflect the oxidative profile of the whole body. Although different tissues may show different biomarker responses to stressors in birds (e.g., Sabat et al. 2017), it is believed that plasma adequately reflects stressful situations, and hence the blood oxidative profile could be appropriate for field studies in birds and other vertebrate species (Costantini 2008; Costantini et al. 2012; Margaritelis et al. 2015). Finally, considering the short turnover time of blood plasma (Martinez del Rio et al. 2009), the use of this substrate for biochemistry assays allowed us to compare the oxidative status of birds in the winter months when the birds were captured. We used two common biomarkers of oxidative stress to assess the oxidative status in plasma samples: (1) the total antioxidant capacity (TAC) that is an indicator of the presence of molecular and enzymatic antioxidants in tissues, and (2) lipid peroxidation that is an indicator of oxidative damage. Total antioxidant capacity was measured by a colorimetric reaction between copper (I), which results of the reduction of copper (II) by antioxidants present in the sample, and a chromogenic reagent, generating a complex with a maximum absorbance at 490 nm (Cell Biolabs OxiSelect™ STA-360, San Diego, CA, USA). Lipid peroxidation was measured via formation of the malondialdehyde:thiobarbituric acid adduct at 532 nm (Cell Biolabs OxiSelect™ STA-330).

## Stable isotope analysis

Whole blood was dried at 60 °C for 1 week and feathers were cleaned with a 2:1 chloroform:methanol solvent solution to remove surface contaminants. Approximately 0.5–0.6 mg of dried whole blood or feather tissue was weighed into tin capsules and carbon ($\delta^{13}$C) and nitrogen ($\delta^{15}$N) isotope values were measured on a Costech 4010 elemental analyzer coupled to a Thermo Scientific Delta V Plus isotope ratio mass spectrometer. Tissue hydrogen isotope ($\delta^{2}$H) values were measured with a Thermo Scientific thermal conversion elemental analyzer (TCEA) coupled to a Thermo Scientific Delta V Plus isotope ratio mass spectrometer. All isotope measurements were conducted at the University of New Mexico Center for Stable Isotopes (Albuquerque, NM). Stable isotope data are expressed as $\delta$ values using the equation $\delta X = (R_{\mathrm{Sample}}/R_{\mathrm{Standard}}) - 1$, where $X$ is any isotope system of interest (e.g., H, C, or N) and $R_{\mathrm{Sample}}$ and $R_{\mathrm{Standard}}$ are the ratios of the heavy to light isotope (e.g., $^{2}$H/$^{1}$H, $^{13}$C/$^{12}$C, $^{15}$N/$^{14}$N) for each sample and standard, respectively. The internationally accepted standards are Vienna Standard Mean Ocean Water (V-SMOW) for $\delta^{2}$H, Vienna Pee Dee Belemnite (V-PDB) for $\delta^{13}$C, and atmospheric nitrogen for $\delta^{15}$N; units are per mil (‰). Within-run analytical precision (SD) for $\delta^{2}$H measurements of organic materials was determined by analysis of internal reference materials described below and measured to be ≤ 3‰. Within-run precision (SD) for both $\delta^{13}$C and $\delta^{15}$N was estimated via analysis of internal reference materials and measured to be ≤ 0.2‰ for both isotope systems.

Approximately 8–15% of the organically bound hydrogen in proteins can potentially exchange with hydrogen in atmospheric water vapor at ambient temperature (Wassenaar and Hobson 2000; Bowen et al. 2005). To correct for exchangeable hydrogen in unknown samples, we used three keratins and two whole blood internal reference materials for which the $\delta^{2}$H composition of the non-exchangeable hydrogen fraction ($\delta^{2}\mathrm{H}_{\text{non-ex}}$) was determined via comparative equilibration experiments and statistical analyses identical to those described in Bowen et al. (2005). $\delta^{2}\mathrm{H}_{\text{non-ex}}$ values of these reference materials ranged from − 55 to − 175‰ for the feather keratins and from − 80 to − 160‰ for whole blood, which brackets the $\delta^{2}$H values of the *Cinclodes* tissue samples we analyzed. Analyses of these four organic reference materials, two oil reference materials, and pure stearic acid were used to determine the within-run precision (standard deviation), which was determined to be ≤ 3‰ relative to V-SMOW.

## Statistical analysis

We tested the effect of species on logarithmic transformed basal metabolic rate (log BMR) using ANCOVA with body mass (mb) as a covariate. A post hoc Fisher test was then performed to compare the least squares means (mass-corrected basal metabolic rate, $BMR_c$) obtained from the ANCOVA analysis. We also tested the effect of species on total BMR and mass-specific metabolic rate ($BMR_m$) by means of a one-way ANOVA. Significant differences in tissue isotope values and biomarkers of oxidative stress biomarkers in plasma were assessed with a Kruskal–Wallis test and a post hoc Wilcoxon test. Finally, we used linear regression to examine the relationship between plasma TAC (dependent variable) and whole blood $\delta^{13}C$ values (independent variable).

# Results

## Stable isotope analysis

Both feather and whole blood $\delta^{13}C$, $\delta^{15}N$, and $\delta^{2}H$ values were significantly different among species (Table 1, Fig. 1); see supporting Table S1 for statistical output of the Kruskal–Wallis tests. *C. nigrofumosus* had higher $\delta^{13}C$, $\delta^{15}N$, and $\delta^{2}H$ values than *C. fuscus* in both whole blood and feathers indicative of winter and summer foraging, respectively. *C. oustaleti* had intermediate whole blood $\delta^{13}C$ and $\delta^{15}N$ values relative to *C. nigrofumosus* and *C. fuscus*; *C. oustaleti* and *C. fuscus* had similar whole blood $\delta^{2}H$ values. Feather $\delta^{13}C$ and $\delta^{15}N$ values were similar for *C. fuscus* and *C. oustaleti*, and were much lower than for *C. nigrofumosus*. *C. oustaleti* had lower feather $\delta^{2}H$ values than *C. fuscus* and *C. nigrofumosus*.

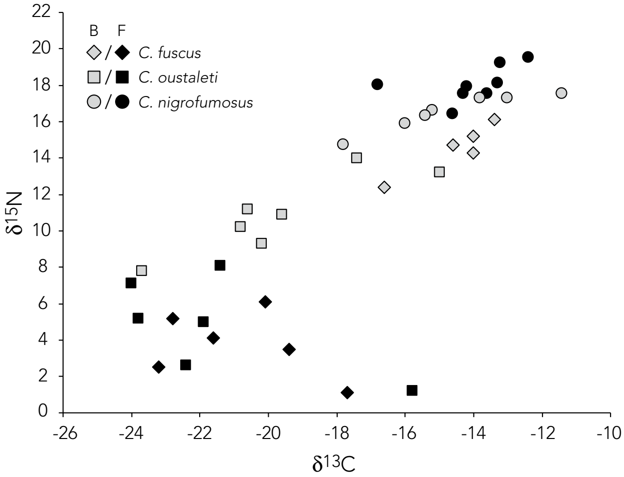

**Fig. 1** $\delta^{13}C$ and $\delta^{15}N$ values of whole blood (B; gray symbols) representing winter diet and feathers (F; black symbols) representing summer diet for each *Cinclodes* species: *C. fuscus* (diamonds) and *C. oustaleti* (squares), and *C. nigrofumosus* (circles)

**Table 1** Mean (± SD) $\delta^{13}C$, $\delta^{15}N$ and $\delta^{2}H$ values for two tissues in the three *Cinclodes* species

| | Whole blood | Feathers |
|---|---|---|
| *C. nigrofumosus* ($n = 8$) | | |
| $\delta^{13}C$ | $-14.4 \pm 2.1^{a,1}$ | $-14.1 \pm 1.3^{a,1}$ |
| $\delta^{15}N$ | $16.5 \pm 1.0^{a,1}$ | $18.0 \pm 1.0^{a,2}$ |
| $\delta^{2}H$ | $-70 \pm 16^{a,1}$ | $-54 \pm 16^{a,1}$ |
| *C. fuscus* ($n = 8$) | | |
| $\delta^{13}C$ | $-19.6 \pm 2.8^{b,1}$ | $-22.3 \pm 3.4^{b,2}$ |
| $\delta^{15}N$ | $10.9 \pm 2.2^{b,1}$ | $4.6 \pm 2.5^{b,2}$ |
| $\delta^{2}H$ | $-95 \pm 5^{b,1}$ | $-100 \pm 20^{b,1}$ |
| *C. oustaleti* ($n = 9$) | | |
| $\delta^{13}C$ | $-14.5 \pm 1.2^{a,1}$ | $-20.8 \pm 2.1^{b,2}$ |
| $\delta^{15}N$ | $14.5 \pm 1.4^{c,1}$ | $3.8 \pm 1.8^{b,2}$ |
| $\delta^{2}H$ | $-79 \pm 12^{a,b,1}$ | $-118 \pm 11^{c,2}$ |

Different letters represent significant differences between species, and numbers denote differences between tissues for each species

## Basal metabolic rate

There is a significant effect of species on log BMR after controlling for body mass (ANCOVA $F_{2,32} = 6.11$; $p = 0.006$). Log BMR was significantly related to Log mb ($F_{1,32} = 31.24$; $p < 0.0001$) and there was no interaction between Log mb and species ($F_{2,32} = 2.45$; $p = 0.11$). A posteriori analysis revealed that the mass-corrected basal metabolic rate ($BMR_c$) was higher in *C. oustaleti* than in *C. nigrofumosus* ($p = 0.002$) or in *C. fuscus* ($p = 0.015$). The log BMRc values of *C. nigrofumosus* and *C. fuscus* showed differences that are at the limit of significance ($p = 0.045$).

## Oxidative stress

Plasma TAC differed among species ($F_{2,15} = 6.67$, $p = 0.008$; Fig. 2). *C. fuscus* had higher TAC concentrations than *C. nigrofumosus* and *C. oustaleti*. However, there were no significant differences in the levels of lipid peroxidation among species ($F_{2,11} = 0.33$, $p = 0.73$). We found a negative relationship ($r = -0.64$, $F_{1,12} = 8.25$, $p = 0.014$) between plasma TAC and $\delta^{13}C$ value (Fig. 3), indicating

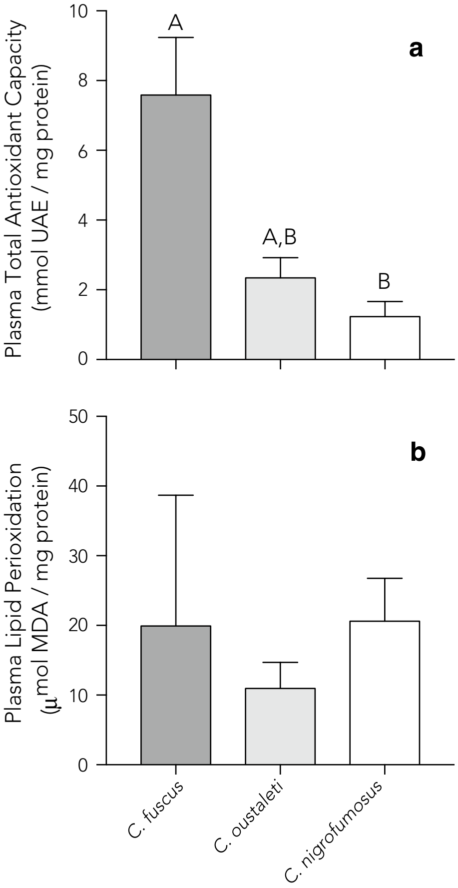

**Fig. 2** **a** Total antioxidant capacity (TAC) and **b** lipid peroxidation in three *Cinclodes* species; errors bars represent standard error. Different letters represent significant differences between species ($p < 0.05$)

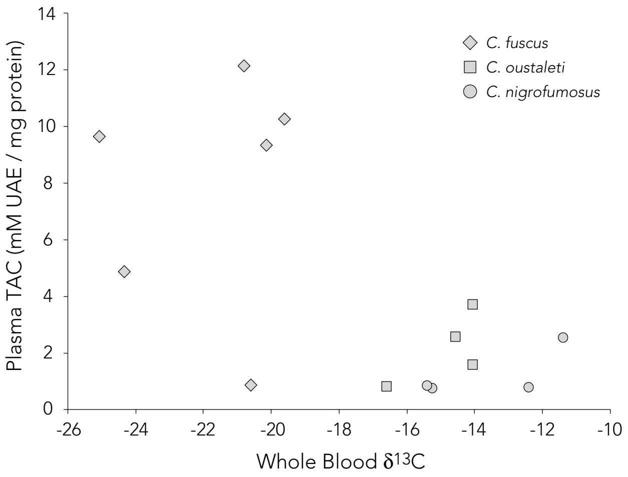

**Fig. 3** Relationship between plasma total antioxidant capacity (TAC) and whole blood $\delta^{13}C$ for each *Cinclodes* species: *C. fuscus* (diamonds) and *C. oustaleti* (squares), and *C. nigrofumosus* (circles)

that individuals with more terrestrial-based diets have higher levels of antioxidants.

## Discussion

### Seasonal ecological patterns via $\delta^{13}C$ and $\delta^{15}N$ analysis

Stable isotope analysis is ideal for characterizing marine versus terrestrial resource use (Schoeninger and DeNiro 1984; Hobson 1987) and latitudinal and/or altitudinal migration (Hobson 2008). This approach has been successfully applied to study the ecology (Martinez del Rio et al. 2009; Newsome et al. 2015), environmental physiology (Sabat and Martinez del Rio 2002, 2006; Sabat et al. 2006), and evolutionary biology (Rader et al. 2017) of *Cinclodes*. Since we collected birds in winter, metabolically active whole blood represents ecological information integrated over 1–2 months prior to capture (Martinez del Rio et al. 2009), while metabolically inert feathers represent ecological information from the previous summer when feathers are molted and regrown in this genus (Bertolero and Zavalaga 2003).

In agreement with previous work (Sabat and Martinez del Rio 2006; Martinez del Rio et al. 2009; Newsome et al. 2015), we found high blood and feather $\delta^{13}C$ and $\delta^{15}N$ values in *C. nigrofumosus* (Table 1 and Fig. 1), suggesting that this species is a resident marine specialist. In contrast, *C. fuscus* had relatively low blood and feather $\delta^{13}C$ and $\delta^{15}N$ values indicating that terrestrial resources dominate its diet throughout the year. Note that marine resource use by *C. fuscus* was higher in winter than summer (Fig. 1), but relatively low in comparison to the proportion of marine resources used by the other two species during the winter months. *C. oustaleti* had blood (winter) $\delta^{13}C$ and $\delta^{15}N$ values that were intermediate in comparison to the other two species, but feather (summer) isotope values were similar to those of *C. fuscus*. This pattern suggests that *C. oustaleti* is a mixed marine and terrestrial forager that based on associated $\delta^{2}H$ data (see below), makes seasonal altitudinal movements to high elevations during summer.

Tissue $\delta^{2}H$ patterns agreed with expectations based on seasonal shifts in diet gleaned from associated $\delta^{13}C$ and $\delta^{15}N$ data. *C. nigrofumosus* had the highest tissue $\delta^{2}H$ values, which is likely the product of the ingestion of seawater that has a hydrogen isotope value of around 0‰. In contrast, *C. fuscus* had tissue $\delta^{2}H$ values that are relatively low suggestive of the use of terrestrial resources fuelled by coastal precipitation, which in central Chile varies between −20 and −40‰ throughout the year (Bowen 2018). The greatest difference in whole blood versus feather $\delta^{2}H$ was observed in *C. oustaleti*. Whole blood $\delta^{2}H$ was similar to that of the marine forager *C. nigrofumosus*, but *C. oustaleti* feather $\delta^{2}H$

values were significantly lower than the other species, indicating this species is an altitudinal migrant and spends the summer months foraging in freshwater aquatic environments at high altitude; a similar pattern was observed for this species by Newsome et al. (2015).

## Metabolic adjustments related to marine resource use

Previous studies on free-ranging wild birds suggest that consumption of salty (food and water) resources imposes energetic costs, which are consistent with the elevated BMR observed in salt-acclimated animals in experimental studies (Peña-Villalobos et al. 2014; Sabat et al. 2017). Here, we examined individuals of three closely related species in the same season (winter) collected from the same localities, and used a robust indirect dietary proxy (stable isotope analysis) to characterize how marine resource use, and thus osmotic load (Sabat and Martínez del Rio 2002, 2006), varies significantly among species.

The relatively low BMR observed in the specialist marine forager *C. nigrofumosus* could result from phenotypic physiological adjustments that offset the osmoregulatory energy demands observed in birds subjected to acute osmotic stress (Gutiérrez et al. 2012; Sabat et al. 2017). For most birds, marine and other saline-rich environments (estuaries) are essentially dry habitats where freshwater is limiting because salt water is the only available source of drinking water and most marine invertebrate prey are in osmotic balance with environmental water. This seems to be the case for *C. nigrofumosus* (Sabat and Martínez del Rio 2005) that has a constant osmotic load in both the dry summer (735 mOsm/kg) and the more rainy winter (688 mOsm/kg) seasons. Thus, the water budget and specifically total evaporative water loss may also be a critical physiological variable constraining the consumption of salt-rich prey in *Cinclodes*. We hypothesize that the low BMR observed in *C. nigrofumosus*, which reduces endogenous heat production and water requirements (Dawson 1984; Bozinovic and Gallardo 2006; Maloney 2008), is a consequence of the consumption of marine resources with high osmotic loads (Sabat et al. 2004a). An interesting question for future research is to quantify the proportion of the total body water pool of *Cinclodes* species that use marine resources to various degrees (Rader et al. 2017) is composed of metabolic water versus pre-formed fresh or saline water sources.

Surprisingly, the highest BMR was observed in *C. oustaleti*, which may be associated with the high energetic and osmoregulatory costs of seasonally moving between coastal and high elevation Andean habitats (Table 2). *C. oustaleti* experiences seasonal changes in the osmotic load of its prey, which results in plastic modifications of its renal phenotype and function (Sabat et al. 2004b). Costs associated with such phenotypic plasticity (Pigliucci 2001) can be significant, and include changes organ mass, enzyme activities, and serum thyroid concentrations; such phenotypic changes have been observed in other bird species (Krebs 1950; Daan et al. 1990; Williams and Tieleman 2000; Vezina and Williams 2005; Swanson and Thomas 2007; Zheng et al. 2008; Maldonado et al. 2009; Sabat et al. 2017). Finally, tissue $\delta^2$H analysis revealed that *C. oustaleti* is an altitudinal migrant. Previous work has suggested that migratory species have higher BMR (Bushuev et al. 2018), however, the use of cold high-latitude breeding areas by migratory birds (Jetz et al. 2008) may also contribute to this pattern. In addition, some studies have reported that avian species from high elevation habitats have higher BMR compared with those inhabiting in low elevation areas (McNab 2009, 2013), but other studies did not find such a pattern (Londoño et al. 2015). We hypothesized that the relatively high basal metabolic rates found in *C. oustaleti* in comparison to low elevation resident species (e.g., *C. nigrofumosus*) could be explained by its elevational migratory behavior.

## Oxidative status in free-ranging birds

One of the primary objectives of our study was to assess whether there is a functional association between oxidative status and the differential use of marine and freshwater resources among three species of closely related passerines collected from the same localities. We observed a negative association between plasma antioxidant biomarkers and marine resource use (Fig. 3), so species that primarily consumed marine resources (*C. nigrofumosus and C. oustaleti*) had a lower mean TAC compared with *C. fuscus* that mostly consumed terrestrial resources (Fig. 2). Thus, the absence

**Table 2** Mean basal metabolic rate (BMR), mass-specific metabolic rate (BMR$_{m}$), corrected log basal metabolic rate (BMR$_{c}$) and mass of the three *Cinclodes* species; error bars represent standard deviation for BMR, BMR$_{m}$, and mass, but standard error for log BMR$_{c}$

| | BMR (ml O$_2$ h$^{-1}$) | BMR$_{m}$ (ml O$_2$ h$^{-1}$ g$^{-1}$) | Mass (g) | Log BMRc |
|---|---|---|---|---|
| *C. nigrofumosus* ($n=8$) | 147.1 ± 9.2$^{a}$ | 1.9 ± 0.2$^{a}$ | 74.9 ± 1.8 | 1.55 ± 0.17$^{a}$ |
| *C. fuscus* ($n=16$) | 83.9 ± 6.3$^{b}$ | 2.7 ± 0.2$^{b}$ | 32.2 ± 1.3 | 1.98 ± 0.02$^{b}$ |
| *C. oustaleti* ($n=11$) | 69.9 ± 7.8$^{b}$ | 2.8 ± 0.2$^{b}$ | 24.9 ± 1.6 | 2.36 ± 0.13$^{c}$ |

Different letters represent significant differences between species of differences in plasma lipid peroxidation concentrations among species in conjunction with the observed pattern in TAC, suggests that the three species are subjected to different levels of ROS, which are compensated for by production of antioxidants as indicated by TAC.

These results differ from our expectations because our recent work showed a significant increase of enzymatic antioxidant defences in rufous-collared sparrows experimentally acclimatized to saline resources (Sabat et al. 2017). Thus, we expected to find higher values of TAC in plasma of *C. nigrofumosus* than in that of its congeners. This expectation was based on the assumption that the response to acute acclimatization (in the laboratory) should be similar, both in magnitude and in the direction of change, to acclimatization or evolutionary adaptation to natural environmental conditions associated with increased salt intake (i.e., marine resource use). *C. nigrofumosus* can specialize on marine resources because it possesses a unique physiology that includes large kidneys with a high proportion of medullary tissue, which enables it to concentrate urine (Sabat and Martínez del Rio 2002, 2006), an adaptation that is absent in all other species in the order Passeriformes. *C. nigrofumosus* also has lower relative water losses as measured by the ratio between total evaporative water (TEWL) and BMR (Sabat et al. 2004a), which enables it to simultaneously cope with high saline loads and avoid dehydration. In contrast, *C. oustaleti* has a high degree of phenotypic plasticity in renal function, paired with structural changes in the kidneys (Sabat et al. 2004b) that facilitate seasonal movement between (freshwater) terrestrial and marine resources (Martinez del Rio et al. 2009) without conferring significant costs to maintaining water balance. The greater capacity of *C. nigrofumosus* to avoid dehydration and the phenotypic plasticity observed in *C. oustaleti* may explain why these species have lower TAC in the face of increased salt loads in comparison to *C. fuscus*. For *C. fuscus*, a reduced capacity to deal with high osmotic loads suggests that consumption of a relatively low proportion of marine prey in the winter (Table 1) would be enough to produce significant salt stress, resulting in increased glucocorticoids concentrations that ultimately enhances ROS formation (Lin et al. 2004). Future experiments should include acute acclimation experiments at different osmotic load levels in different bird species to compare with results observed in free-ranging individuals.

In addition to salt intake, there are other potential explanations for the high levels of total antioxidants observed in *C. fuscus* relative to the other species. First, variation in tissue metabolic activity and its relationship with body mass could influence TAC. Mass-specific metabolic rates are negatively related to body mass, i.e., smaller animals have higher cellular metabolic activity than larger ones. The two smallest species in our study, *C. fuscus* and *C. oustaleti*, had similar mass-specific metabolic rates (Table 2) but significantly different TAC values (Fig. 2). Thus, the prediction that higher metabolic rates produce more ROS, which leads to an increase in antioxidant expression or to greater oxidative damage is not supported by our results. Even though some studies have found support for a functional relationship between metabolic rate and oxidative state (Williams et al. 2010), this pattern has been the subject of debate (Selman et al. 2012) and is not supported by some empirical datasets (Brzęk et al. 2014). Finally, we cannot discard other potential reasons for the observed differences in TAC among *Cinclodes*, such as variation in the concentrations of dietary vitamin E and carotenoids, which are ubiquitous (Bhosale and Bernstein 2007) antioxidants that reduce oxidative damage (Catoni et al. 2008).

## Summary

Our study shows that some of the ecological traits unique to *Cinclodes*, such as persistent or seasonal marine resource use coupled with altitudinal migration, are correlated with organismal (e.g., BMR) and biochemical (e.g., TAC) physiological traits. We encourage future studies to include additional proxies of energy expenditure known to impact energy budgets in birds (e.g., metabolic scope or field metabolic rate), and additional indicators of oxidative status in a wider range of tissues or biological materials (urine, feces). The discrepancies between the patterns observed here and previous experimental data (Peña-Villalobos et al. 2014; Sabat et al. 2017) highlight substantial differences between intraspecific analyses that study the effects of acute acclimation in the laboratory versus interspecific comparative analyses of free-ranging species more indicative of evolutionary adaptation (Cruz-Neto and Bozinovic 2004). The disparity between these two levels of analysis may reflect our poor understanding of how the need to eliminate salts and conserve water influences oxidative status in birds.

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**Author contribution statement** PS, SDN, JCS-H, FB and RN conceived and designed the experiments. RT and PS conducted fieldwork. RT-M performed the experiments. RT-M, PS and SDN analyzed the data. RT-M, PS, SDN and JCS-H wrote the manuscript; other authors provided editorial advice.

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