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# Osmoregulatory capacity and the ability to use marine food sources in two coastal songbirds (*Cinclodes*: Furnariidae) along a latitudinal gradient

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**Abstract** *Cinclodes nigrofumosus* and *C. oustaleti* are two closely related songbirds that inhabit the northern Chilean coast during the austral fall and winter.This stretch spans a dramatic north to south latitudinal gradient in rainfall and temperature. Whereas *C. nigrofumosus* lives exclusively on coastal environments, *C. oustaleti* shifts seasonally from coastal environments to inland freshwater ones. We used the $\delta^{13}C$ of these two species' tissues to investigate whether the reliance on marine versus terrestrial sources varied from the hyperarid north to the wet south. We also investigated latitudinal variation in the renal traits that mediate how these birds cope with dehydration and a salty marine diet. Both species increased the incorporation of terrestrial carbon, as measured by $\delta^{13}C$, as terrestrial productivity increased southwards. However, *C. nigrofumosus* had consistently more positive (i.e. more marine) and less variable $\delta^{13}C$ values than *C. oustaleti*. The osmoregulatory traits of both species varied with latitude as well. Urine osmolality decreased from extremely high values in the north to moderate values in the south, while *C. nigrofumosus* produced more concentrated urine than *C. oustaleti*. In both species, the proportion of kidney devoted to medullary tissue decreased from north to south, and kidney size increased significantly with latitude. *Cinclodes nigrofumosus* had larger kidneys with larger proportions of medullary tissue than *C. oustaleti*. *C. nigrofumosus* and *C. oustaleti* are terrestrial organisms subsidized by a rich marine environment where it is adjacent to an unproductive terrestrial. Variation in the reliance on marine food sources seems to be accompanied by adjustments in the osmoregulatory mechanisms used by these birds to cope with salt and dehydration.

**Keywords** *Cinclodes* · Latitudinal gradient · Marine-terrestrial nutrient transfer · Osmoregulation · Stable isotopes

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## Introduction

Marine environments can be very productive and often subsidize consumers in more unproductive contiguous terrestrial ecosystems (Polis and Hurd 1996). However, the terrestrial animals that take advantage of marine foods must get rid of high salt loads (Nystrom and Pehrsson 1988; Mahoney and Jehl 1985). Diets of marine origin can impose an exceptional osmoregulatory challenge to songbirds (Order Passeriformes), which lack functional salt glands (Shoemaker 1972) and have a limited ability to concentrate urine (Goldstein and Skadhauge 2000). Among passerines, the genus *Cinclodes* (Furnariidae) is well suited to investigate the interplay of osmoregulation, water availability, and diet in terrestrial organisms that feed on marine diets. This genus is unique because it includes coastal species that feed almost exclusively on marine invertebrates (Sabat et al. 2003), species that inhabit inland freshwater environments and feed on insects, and species that shift seasonally between coastal and inland environments and feed on both insects and marine invertebrates (Sabat and Martinez de Río 2002; Sabat and Martínez del Rio 2005).

P. Sabat (unpublished results) contends that *Cinclodes* represents an adaptive radiation in which ecological diversification has been mediated by evolutionary changes in osmoregulatory function. They compared the traits of five *Cinclodes* species and found that the renal traits associated with the capacity to concentrate urine were tightly correlated with reliance on a marine diet as estimated by the carbon isotope composition of the bird's tissues (P. Sabat, unpublished results). The objective of this contribution is to describe the variation in reliance on marine sources as estimated by stable isotopes in two *Cinclodes* species, *C. nigrofumosus* and *C. oustaleti*, along a dramatic latitudinal gradient in water availability and temperature (Fig. 1). We also portray changes along this gradient in two renal features that are associated with the capacity to cope with salty diets and dehydration: kidney size and the proportion of renal tissue allocated to medulla (Sabat and Martínez del Rio 2002; P. Sabat, unpublished results). We ask whether the patterns revealed by these interspecific comparisons also hold within a single species.

We chose *C. nigrofumosus* and *C. oustaleti* because they are syntopic during the austral winter and, because in addition to inhabiting the same habitat seasonally, they seem to differ in their specialization to a marine diet (P. Sabat, unpublished results). *C. nigrofumous* inhabits only wave-swept rocky shorelines, where it feeds on marine invertebrates including molluscs and crustaceans (Sabat et al. 2003). To our knowledge, *C. nigrofumosus* is the only documented passerine species to have a primarily marine diet (Sabat and Martínez del Rio 2002). *C. oustaleti* shifts habitats seasonally. During the winter, it shares *C. nigrofumosus'* habitat, but in contrast with this habitat specialist, *C. oustaleti* also occupies freshwater and brackish estuaries (P. Sabat and C. Martínez del Rio, unpublished observations). During the austral spring and summer, *C. oustaleti* moves inland to freshwater streams and bogs. *C. nigrofumosus* and *C. oustaleti* can be characterized as a habitat specialist and a generalist, respectively.

Our study was guided by three questions: (1) Does reliance on marine sources vary with the availability of freshwater along the gradient; (2) does the latitudinal pattern of variation of *C. nigrofumosus*, a habitat specialist differ from that of *C. oustaleti*, a habitat generalist; and (3) is water availability and/or reliance on a marine diet associated with the traits that correlate with the ability to cope with salty diets, such as kidney size and the proportion of kidney devoted to medullary tissue (P. Sabat, unpublished results)? Question 1 cannot be answered unambiguously a priori. On the one hand, the availability of freshwater can facilitate a marine diet by providing water to flush out dietary salts and by "diluting" the salt loads in osmoconforming invertebrates in areas close to estuaries (Sabat and Martinez del Rio 2005, and references therein). On the other, rainfall is accompanied by increased productivity in terrestrial habitats (Lieth 1978; Polis and Hurd 1996) and hence with increased availability of terrestrial prey. We hypothesized that, during the winter, when the two

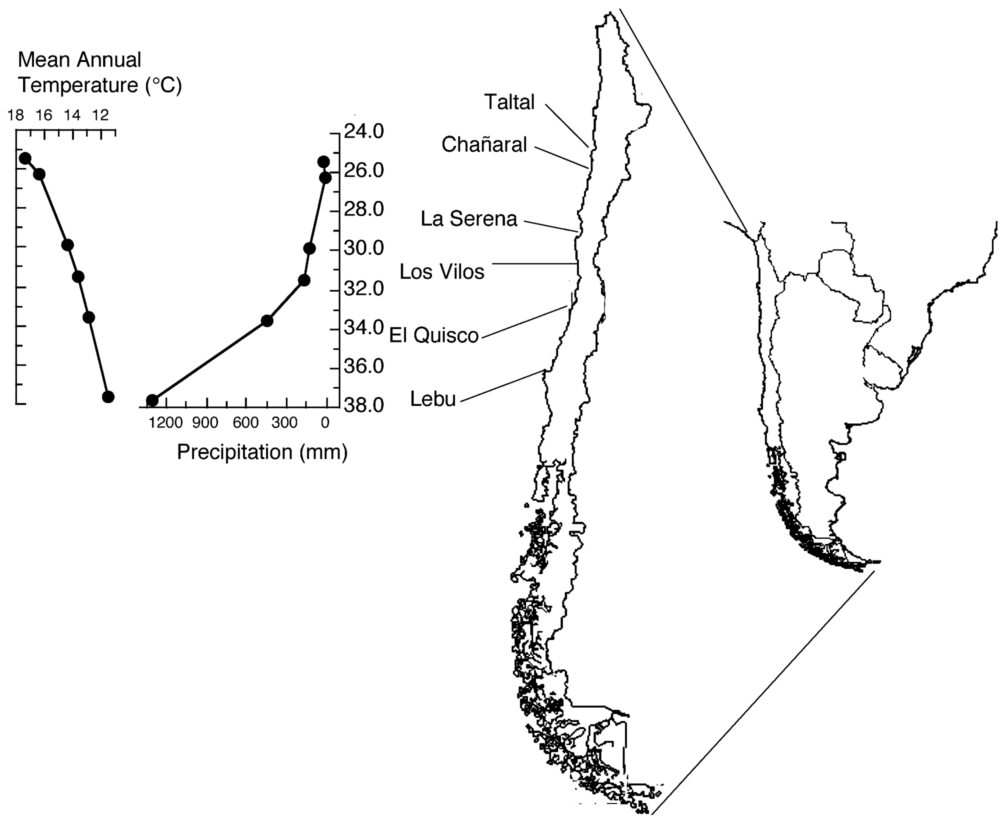


**Fig. 1** During the austral fall and winter, *Cinclodes nigrofumosus* and *C. oustaleti* inhabit the northern coast of Chile. We collected birds at six sites along an approximately 1,600 km, north to south, latitudial gradient. Average annual rainfall increased from our northernmost to the most southernmost site by more than three orders of magnitude. Annual average temperature decreased by approximately 6°C

species are syntopic, *C. nigrofumosus* which lives year round in inter-tidal environments, would be more dependent on marine sources than *C. oustaleti*, which migrates seasonally between freshwater and marine environments. We also hypothesized that *C. oustaleti* would have higher variance in the use of marine and terrestrial sources than *C. nigrofumosus*. In a previous comparative study, Sabat and Martínez del Rio (2002) found that within the genus *Cinclodes*, the ability to produce concentrated urine, and hence to cope with aridity and with diets with high salt loads, was correlated with both the relative size of the kidney, with the fraction of the kidney allocated to medullary tissue, and with the relative number of medullary cones (see also Goldstein and Skadhauge 2000). The positive association between relative kidney size and percentage of medullary tissue with concentrating ability is consistent with previous observations on both passerines (reviewed by Casotti and Braun 2000; Sabat 2000) and non-passerines (Hughes 1970). Consequently, we also predicted that kidney size and the proportion of kidney devoted to medullary tissue would decrease from north to south with increased precipitation (P. Sabat, unpublished results).

Our study relied on carbon stable isotope ratios as indicators of diet (marine vs terrestrial) and as indirect indices of the salt loads experienced by birds (Sabat and Martínez del Rio 2002). Using the carbon isotope ratio of a consumer's tissues to assess the relative contribution of marine and terrestrial sources relies on two observations: (1) tissues reflect the isotopic composition of an animal's diet (Hobson and Clark 1992), and (2) marine food sources are significantly enriched in $^{13}$C relative to sources from contiguous terrestrial habitats (i.e., $\delta^{13}$C is more positive; Blundell et al. 2002,and references therein). Our study depends on the large difference in isotopic composition between marine seagrasses and macroalgae at the base of intertidal food webs ($\delta^{13}$cranges from $-10$ to $-15‰$) and the C3 vegetation of the coast of Chile ($-24$ to $-27‰$).

## Materials and methods

Animals were collected between 2000 and 2003 in the austral winter and fall (between June and September) at five sites along the coast of Chile (Fig. 1): Taltal (25°25′S, 70°34′W), Chañaral(26°20′S, 70°37′W), La Serena (29°54′S, 71°15′W), Los Vilos (31°53′S, 71°30′W), El Quisco (33°34′S, 71°37′W), and Lebu (37°37′S, 73°40′W). We collected *C. nigrofumosus* at all sites, but we were unable to collect *C. oustaleti* at Taltal. Our study sites vary not only in rainfall and temperature, but also in the length of the season during which each site can be considered arid. For example, Taltal and Chañaral are dry year round, whereas Lebu's dry season extends only from December to March (di Castri and Hayek 1976). De-Martonne's aridity index (DMAI; di Castri and Hayek 1976) characterizes Tal Tal and Chañaral as very dry (DMAI equals 0.92 and 0.06, respectively). It characterizes La Serena and los Vilos as dry (DMAI = 5.13 and 8.33, respectively), and El Quisco and Lebu as humid (DMAI = 33.43 and 56.62, respectively). The vegetation along the gradient ranges from very scanty in the hyperarid north, to Mediterranean scrub with increased productivity from La Serena to Lebu. Immediately after capture, a sample of ureteral urine was obtained by inserting a small closed-ended cannula into the birds' cloaca (Goldstein and Braun 1989; Sabat and Martinez del Río 2002). Urine drained into the cannula via a window placed dorsally over the ureteral orifices (Goldstein and Braun 1989). Urine samples were centrifuged and the osmolality of the supernatant was measured (Wescor 5130B). Kidneys were weighed (±0.001 g) and preserved in paraformaldehyde-glutaraldehyde. We estimated the area of medulla by point counting using the Cavalieri Principle on the right kidney, which was processed for routine light microscopy (Gundersen et al. 1988; Wauri 1989). The percentage of renal medulla was determined as the area of medullary tissue divided by the total area of the kidney section. A sample of pectoralis muscle was defatted by petroleum ether extraction, freeze-dried, ground into a fine powder, and loaded (≈0.15 mg) into pre-cleaned tin capsules for isotopic analysis. We determined the diet of a sub-sample of collected individuals. Food was extruded from the crop, gizzard and proventriculus and stored frozen in liquid nitrogen. Gut contents were thawed and prey items were separated, weighed (±0.0005 g) and identified to the lowest taxonomic level possible. In addition to documenting diet composition qualitatively (Table 1), we also estimated the percent by weight of items of marine or terrestrial origin. Carbon isotope ratios were measured on a continuous flow isotope ratio mass spectrometer (VG Isotech, Optima) with samples combusted in a Carlo Erba NA 1500 elemental analyzer at the Columbia University Biosphere 2 stable isotope facility. The precision of these analyses was ±0.3 (‰) (SD). Laboratory standards, vacuum oil ($\delta^{13}$C = $-27.5$ ‰) and sucrose ($\delta^{13}$C = $-10.5‰$, National Institute of Standards and Technology 8542) were included with each run. Stable isotope ratios were expressed using standard delta notation ($\delta$) in parts per thousand (‰) as:

$$\delta^{13}\mathrm{C} = \left(\frac{R_{\mathrm{sample}}}{R_{\mathrm{standard}}} - 1\right) \times 1{,}000$$

where $R_{\mathrm{sample}}$ and $R_{\mathrm{standard}}$ are the molar ratios of $^{13}$C/$^{12}$C of the sample and reference, respectively. Samples were referenced against the international standard, the Vienna Pee DeeBelemnite (VPDB).

### Statistical analyses

We were interested in finding out the potential effect of a latitudinal gradient in rainfall and temperature on the

**Table 1** The organisms found in the gastrointestinal contents of *Cinclodes nigrofumosus* and *C. oustaleti* at all study sites included both marine invertebrates and insects

| | *C. oustaleti* ($n=36$) | | *C. nigrofumosus* ($n=20$) | |
|---|---|---|---|---|
| Prey | FO | % FO | FO | % FO |
| Algae | | | | |
| *Ulva* sp. | 6 | 16.7 | 11.0 | 55.0 |
| *Chondrus caniculatus* | 1 | 2.8 | 1.0 | 5.0 |
| Crustaceans | | | | |
| *Allopetrolisthes spinifrons* | | – | 7.0 | 35.0 |
| *A. angulosus* | 1 | 2.8 | 3.0 | 15.0 |
| *A. punctatus* | | – | 1.0 | 5.0 |
| *Grapsus grapsus* | | – | 2.0 | 10.0 |
| *Leptograpsus variegatus* | | - | 5.0 | 25.0 |
| *Cyclograpsus cinereus* | 1 | 2.8 | 2.0 | 10.0 |
| *Rinchocinettes typus* | | – | 1.0 | 5.0 |
| *Paraxanthus barbiger* | 1 | 2.8 | | – |
| *Acanthocyclus gay* | 2 | 5.6 | | – |
| Barnacles | | 0.0 | 1.0 | 5.0 |
| Mollusks | | | | |
| *Nodilittorina araucana* | 4 | 11.1 | 10.0 | 50.0 |
| *N. peruviana* | | - | 2.0 | 10.0 |
| *Prisogaster niger* | 1 | 2.8 | 1.0 | 5.0 |
| *P. gigas* | | - | 1.0 | 5.0 |
| *Scurria variabilis* | | - | 2.0 | 10.0 |
| *S. ceciliana* | 4 | 11.1 | 1.0 | 5.0 |
| *S. zebrina* | | - | 1.0 | 5.0 |
| *Semimytilus algosus* | | - | 1.0 | 5.0 |
| Insects | | | | |
| Diptera (adults) | 15 | 41.7 | 4.0 | 20.0 |
| Diptera (larvae) | 8 | 22.2 | 9.0 | 45.0 |
| Coleoptera | 3 | 8.3 | 3.0 | 15.0 |
| Isopoda | | – | 6.0 | 30.0 |

FO is the absolute frequency of occurrence in gut contents and % FO is the percent of occurrence. Although the marine algae, *Ulva* sp. and *Chondrus caniculatus*, appear frequently in gut contents, their contribution by mass was small and we suspect that they were ingested accidentally (1.6 and 0.2% of total mass in *C. oustaleti* and *C. nigrofumosus*, respectively)

characteristics of *C. nigrofumosus* and *C. oustaleti*. However, both of these variables covary tightly along our gradient, and both covary tightly with De Martonne's aridity index (di Castri and Hayek 1976; Fig. 1). Thus, we used latitude as a surrogate variable for aridity. Latitude has the added virtue that it is measured without error, whereas rainfall, temperature, and DMAI are measured as averages with associated variances. Using latitude allowed us to use least squares regression and its associated linear models (Sokal and Rohlf 1995). Analyses conducted using average annual rainfall and DMAI as independent variables are consistent with the results described here. Our study compares the responses of two closely related congeners to a gradient in aridity. In most comparisons we used a linear model to assess the effect of latitude on a trait and to compare among species. Whenever the interaction term between species and latitude was non-significant, we dropped it from the model (Zar 1996). Two species comparisons have been belittled as uninformative (Garland and Adolph 1994). Although we recognize their limitations, we find two species comparisons informative. Our comparisons reveal whether *C. nigrofumosus* and *C. oustaleti* differ—or not—along a latitudinal gradient. They do not permit inferring whether habitat generalists differ from habitat specialists in their response to a latitudinal gradient in aridity.

## Results

Table 1 lists the identity and frequencies of prey items found in the gastrointestinal of both species at all sites. We were able to analyze the percentage of marine and terrestrial items in the gastrointestinal contents of 67 *C. nigrofumosus* individuals collected at all sites, except Chañaral. Unfortunately, the sample of *C. oustaleti* gut contents from which we were able to estimate the quantitative contribution of each prey type to the total mass of food in the gastrointestinal tract was restricted to 20 individuals from a single site (El Quisco). Because we did not have adequate representation of both species at all sites, we used inferential statistics to compare between the gut contents of *C. nigrofumosus* and *C. oustaleti* only at El Quisco. At El Quisco, the gut contents of *C. nigrofumosus* ($n=19$) had higher percentages of crustaceans (mean% $\pm$ SD $=57.2\pm34.2\%$, $t=3.6$, $P<0.002$) and of total marine foods ($62.8\pm35.9\%$, $t=3.8$, $P<0.001$) than those of *C. oustaleti* ($12.1\pm29.0\%$ and $18.7\pm31.8\%$ for crustaceans and marine items, respectively). In contrast, the guts of *C. oustaleti* contained a higher percentage of insects ($72.5\pm34.6$) than those of *C.* nigrofumosus ($19.3\pm22.4$). The percentage of molluscs in the gut contents of *C. oustaleti* ($10.6\pm23.4$) did not differ significantly from that in the gut of *C. nigrofumosus* ($23.3\pm27.3$, $t=1.3$, $P=0.21$).

Variation in reliance on marine against terrestrial sources along the latitudinal gradient was apparent in both *C. nigrofumosus* and *C. oustaleti*. The $\delta^{13}$C of *pectoralis* muscle decreased significantly with latitude in both species ($F_{1,84}$(latitude) $=10.24$, $P=0.002$; Fig. 2). The tissues of *C. nigrofumosus* were enriched in $^{13}$C over those of *C. oustaleti* by approximately 2.5‰ throughout the gradient ($F_{1,84}$(species) $=94.36$, $P<0.001$; Fig. 2). The variance in the residuals of the $\delta^{13}$C against latitude regression line estimates variance in the use of marine and terrestrial food sources. *Cinclodes oustaleti* was more variable in its incorporation of marine carbon than *C. nigrofumosus* ($F_{56,30}=36.63$, $P<0.001$; Fig. 2).

The changes in reliance on marine against terrestrial sources along the gradient appeared to also be accompanied by changes in the ingestion of salts and/or by changes in evaporative water losses and hence in the need to produce more concentrated urine. The osmolality of ureteral urine decreased significantly with latitude in both species ($F_{1,84}$(latitude) $=47.24$, $P<0.001$; Fig. 2). However, *C. nigrofumosus* produced urine that was $\approx$100 mOsm/kg more concentrated than that produced by *C. oustaleti* ($F_{1,84}$(species) $=8.15$, $P=0.006$; Fig. 2).

After visual analysis of residuals (Fig. 3), we deemed that the relationship between body mass and latitude was not adequately described by a linear function. Thus, we used Spearman rank correlations ($r_s$) to assess the effect of latitude on body mass. Body mass did not differ among study sites in *C. oustaleti* ($F_{4,24} = 2.4$, $P = 0.07$) and there was no relationship between body mass and latitude in this species ($r_s = 0.26$, $P = 0.11$). However, there were significant differences in mass among sites in *C. nigrofumosus* ($F_{5,\ 66} = 10.44$, $P = 0.006$; Fig. 2) and mass and latitude were positively correlated ($r_s = 0.62$, $P < 0.001$). This correlation reveals heterogeneity in size among sites, but it hides a complex relationship. Birds were of roughly the same size at the most northern and southern sites, but increased in mass from Chañaral to El Quisco (Fig. 3).

Because kidney mass increases isometrically with body mass within the genus *Cinclodes* (P. Sabat, unpublished results), we used the percentage of total body mass represented by the kidney as an estimate of relative kidney mass. The percentage of total body mass represented by the kidney increased significantly with latitude in both species ($F_{1,84}$(latitude) = 2.34, $P = 0.021$; Fig. 4) and *C. nigrofumosus* had kidneys that were 50% larger than those of *C. oustaleti* ($F_{1,84}$(species) = 8.48, $P < 0.001$; Fig. 4). The percentage of kidney tissue represented by medulla decreased significantly with latitude in both species ($F_{1,84}$(latitude) = 10.91, $P = 0.0013$, Fig. 4). *C. nigrofumosus* not only had larger kidneys throughout the gradient than *C. oustaleti*, but a higher percentage of its kidneys consisted of medulla ($F_{1,84}$(species) = 76.70, $P < 0.001$; Fig. 4).

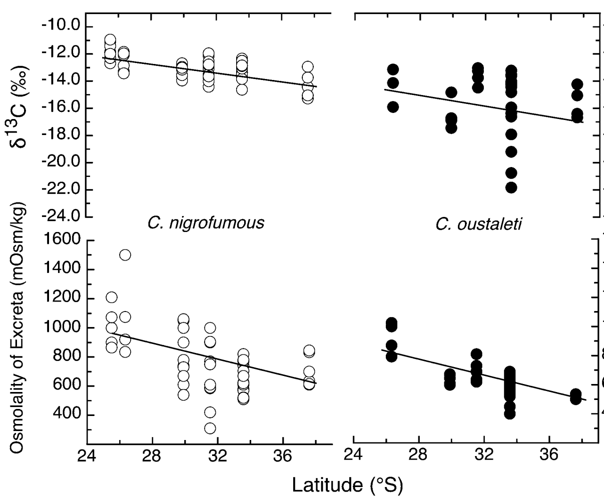


**Fig. 2** *Upper panels*. The tissues of both *C. nigrofumosus* (*open points*, $y = -10.76 - 0.15x$) and *C. oustaleti* (*closed points*, $y = -8.51 - 0.15x$) became increasingly depleted in $^{13}C$ from north to south along a latitudinal gradient. Because a linear model revealed that the effect of the interaction between species and latitude on $^{13}C$ was not significant, these relationships were fitted using a common slope. *Lower panels*. The osmolality of urine decreased significantly from north to south in *C. nigrofumosus* ($y = 1{,}877.1 - 35.4x$) and *C. oustaleti* ($y = 1{,}778.3 - 35.4x$). These relationships were also fitted using a common slope

## Discussion

*Cinclodes nigrofumosus* and *C. oustaleti* varied in reliance on marine food sources and in the renal traits that seem to be associated with a salty marine diet along a latitudinal gradient. Although the latitudinal patterns of both species were similar qualitatively, we also found significant interspecific differences. Here, we consider the answers that our study gave to the questions that guided us. We first discuss the potential causes of the north to south gradient in reliance on marine sources. Then, we explore the ecological consequences of the differences between *C. nigrofumosus* and *C. oustaleti* along this gradient. Finally, we examine the variation in renal form and function along the gradient.

### Does reliance on marine sources vary along a north–south gradient?

The tissues of both species became more depleted in $^{13}C$ at more southern sites. We interpret this gradient in $\delta^{13}C$ as evidence of latitudinal variation in the reliance of terrestrial against marine sources in the diets of *C. nigrofumosus* and *C. oustaleti*. It can be argued that the southward decrease in $\delta^{13}C$ is the result of latitudinal changes in the carbon composition of marine prey rather than of higher reliance on terrestrial prey. To examine this possibility, we correlated the percentage of marine items in the gut contents of *C. nigrofumosus* with latitude and found a significantly negative correlation ($r = 0.4$, $P = 0.02$, $n = 37$). This correlation is direct evidence of a latitudinal decline in reliance on marine sources in this species. Schreiber (1968) has emphasized the dramatic contrast between the vast productivity of coastal marine environments and the barrenness of terrestrial ones along the coast of the Atacama desert. Our observations emphasize this contrast. In the hyper-arid northern side

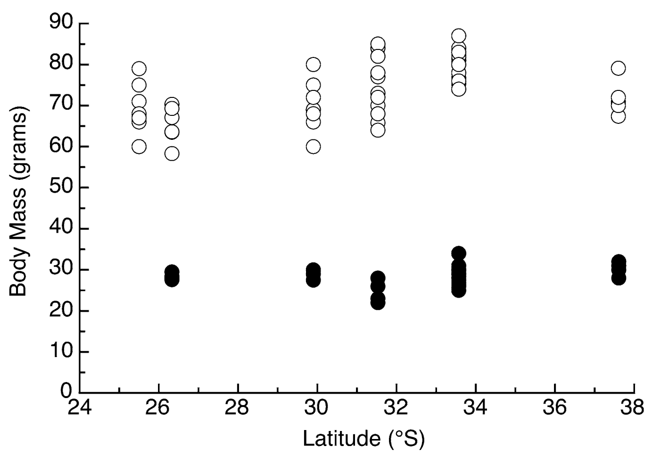


**Fig. 3** Body mass increased significantly ($r_s = 0.62$, $P < 0.001$), albeit irregularly, from north to south in *C. nigrofumosus* (*open points*). There was no significant effect of latitude on the body mass of *C. oustaleti* (*closed points* )

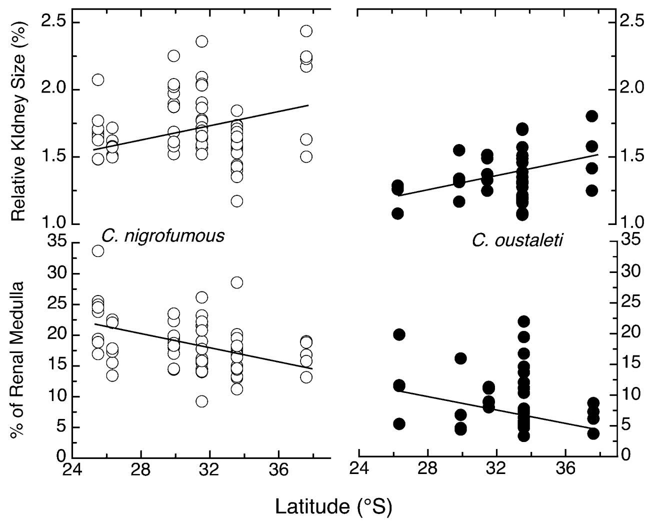


**Fig. 4** *Upper panels*. Relative kidney size, measured as percentage of body mass, increased significantly with latitude in *C. nigrofumosus* ($y = 1.23 + 0.16x$) and *C. oustaleti* ($y = 0.82 + 0.16x$). *Lower panels*. The percentage of kidney tissue represented by renal medulla decreased significantly in *C. nigrofumosus* ($y = 32.1 - 0.45x$) and *C. oustaleti* ($y = 23.8–0.45x$). Common slopes between species in latitudinal regressions imply that interaction terms were not statistically significant and were dropped from the linear model

of the gradient, terrestrial productivity is extraordinarily low and birds seemed to depend more on marine than on terrestrial foods. As terrestrial productivity increases to the south, both species increase their use of terrestrial sources. Our results also support Polis and Hurd's (1996) observation on the generality of transport phenomena between productive marine environments and unproductive terrestrial ones. Both *C. nigrofumosus* and *C. oustaleti* are terrestrial organisms that take advantage of, and are subsidized by, a marine environment where it is juxtaposed to a terrestrial one. The degree of marine subsidy seems to decrease as the disparity in productivity between these two environments decreases from north to south.

### Does the latitudinal pattern of variation of *C. nigrofumosus* differ from that of *C. oustaleti?*

Although the importance of terrestrial relative to marine food sources seemed to increase from north to south in both *C. nigrofumosus* and *C. oustaleti*, we found significant differences between these two species. Throughout the gradient, $\delta^{13}$C was more positive and less variable in *C. nigrofumosus* than in *C. oustaleti*. We used a linear mixing model to estimate the relative proportion of incorporation of terrestrial and marine and terrestrial organic carbon into the tissues of *C. nigrofumosus* and *C. oustaleti* (Phillips 2001). This model is based on preliminary measurements of the carbon isotopic composition of terrestrial and marine animals in the diet of *Cinclodes* species at our study sites (P. Sabat, unpublished data). We assumed that marine and terrestrial sources have $\delta^{13}$C values equal to–11‰ and –24‰, respectively.Using these values and a tissue to diet discrimination of 1.5‰ (Carleton and Martínez del Rio 2005), the percentage of carbon derived from marine sources in *C. nigrofumosus* ranged from 94% (± SD = 5%) at Taltal to 74%. (± SD = 7%) at Lebu. In *C. oustalet*, the percentage of carbon derived from marine sources ranged from 74% (± SD = 11%) at Chañaral to 65% (± SD = 9%) at Lebu. Although we find value in the use of a mixing model, we also warn about its reliance on a variety of assumptions, some of which may not be accurate. For example, even within a site there is variation in the $\delta^{13}$C of the prey consumed by *Cinclodes* (Sabat and Martínez del Rio 2002; P. Sabat, unpublished data). However, using different values for the end points of our mixing model would have changed its quantitative estimates, but would not have altered its qualitative conclusions. The fraction of terrestrial carbon incorporated into the tissues of *C. nigrofumosus* and *C. oustaleti* increased along a north to south gradient while the tissues of *C. nigrofumosus* had a more marine isotopic signature than those of *C. oustaleti*.

The tissues of *C. nigrofumosus* reflected not only a higher contribution of marine carbon sources but also a more consistent one. Throughout the gradient, the coefficient of variation in $\delta^{13}$C in *C. nigrofumosus* was only 6.8% whereas that of *C. oustaleti* was 14.0%. Our mixing model estimated that the incorporation of marine carbon ranged from 23 to 85% in *C. oustaleti*. In contrast, it estimated that it ranged from 65 to 100% in *C. nigrofumosus*. Because we measured $\delta^{13}$C in birds collected in coastal environments in the fall and winter, and because the residence time of carbon in muscle is approximately 18 days (Hobson and Clark 1992), this analysis probably underestimates the variation in reliance in terrestrial relative to marine environments in *C. oustaleti*. We predict that an analysis of tissues of birds collected in the spring and summer, when this species inhabits freshwater environments, will increase the variance in $\delta^{13}$C reported here. In contrast, the $\delta^{13}$C of *C. nigrofumosus* changes only slightly between seasons (Sabat and Martínez del Rio 2005).

The differences in $\delta^{13}$C between *C. nigrofumosus* and *C. oustaleti* during the winter have two possible complementary and non-exclusive explanations. First, it is possible that *C. nigrofumosus*, which is abundant and territorial, excludes its smaller congener from marine environments. Our observations suggest that it is dominant over *C. oustaleti* in agonistic interactions (P. Sabat and C. Martínez del Rio, unpublished data). Second, *C. nigrofumosus* has traits that seem to facilitate the use of salty diets. It has relatively large kidneys with unusually well developed medullary tissue (Sabat et al. 2004a; P. Sabat, unpublished results), and it has lower rates of evaporative water losses than *C. oustaleti* (Sabat et al. 2004b). These differences in osmoregulatory capacity may also account for the segregation between these two species in the use of marine diets. Because evidence in favor of each of these explanations is scanty we offer them more as testable hypothesis than as established facts. *C. nigrofumosus* and *C. oustaleti* offer a good opportunity to investigate the role that agonistic behavior and physiological differences in the ability to use a resource play in determining niche breadth in two syntopic congeners (Taniguchi and Nakano 2000).

### Are aridity and/or reliance on a marine diet associated with relative kidney size and the proportion of kidney devoted to medullary tissue?

Both relative kidney size and the proportion of kidney devoted to medullary tissue varied from north to south. As expected, relative medullary size decreased from the more arid north where birds relied more on salty marine diets to the more mesic south where birds included more terrestrial prey in their diets. The significant latitudinal variation in urine osmolality along the gradient supports the notion that the dry north represents an environment where birds are challenged by both the absence of fresh water and the ingestion of salty prey (Cassoti and Richardson 1992). Indeed, at the two dry sites, Taltal and Chañaral, birds produced urine with osmolalities that are at the high end of, or even exceed, those reported for all bird species (Goldstein and Skadhauge 2000).

Against our expectations, birds had relatively larger kidneys in the mesic south than in the arid north. This result is concordant with the observation that animals that face antidiuresis chronically in dry environments tend to have smaller kidneys than those that live in wetter environments (reviewed by McNab 2002), but appears to contradict the interspecific patterns found by P. Sabat (unpublished results). Sabat also found a positive interspecific correlation between the $\delta^{13}$C of tissues and relative kidney size. The discrepancy may reveal a difference between interspecific and intraspecific levels of analysis. At the interspecific level, the positive association between $\delta^{13}$C and kidney size probably reflect the increased kidney size required by the amount of medullary tissue needed to process salty marine diets. Within *C. nigrofumosus* and *C. oustaleti*, the increase in kidney size from north to south probably reflects higher rates of water loads and hence higher water processing rates in the more mesic south (McNab 2002). The disparity in our interspecific and intraspecific analyses reflects our inadequate understanding of how the needs to dispose of and conserve water, and to get rid of salt, influence and are influenced by kidney morphology and function in birds (Goldstein and Skadhauge 2000).

### Are the differences in kidney morphology along the gradient the result of phenotypic plasticity or population differentiation?

Population differences in physiological traits may be due to genetic and/or environmental effects or their interaction (Garland and Adolph 1991). What is the contribution of each of these effects to the latitudinal variation observed in *C. nigrofumosus* and *C. oustaleti*? Sabat et al. (2004a) provided adult *C. nigrofumosus* and *C. oustaleti* individuals with either tap water or a salt solution (NaCl, 800 mOsm/kg) for 2 weeks. The *C. oustaleti* individuals exposed to salt water had larger renal medullae and were able to produce more concentrated urine than those exposed to tap water (Sabat et al. 2004a). However, the effect of exposure to fresh or salt water on medullary size was relatively small (≈22%) and smaller than the differences found along the extremes of the latitudinal gradient (≈120%). The renal characteristics of *C. nigrofumosus* individuals exposed to fresh or salt water did not differ (Sabat et al. 2004a). These experiments suggest that phenotypic flexibility (sensu Piersma and Drent 2003) in adult birds is unlikely to explain all the variation in renal traits observed along the latitudinal gradient. However, we cannot yet discount the possibility that exposure to different environments during early development (or developmental plasticity, sensu Piersma and Drent 2003) contributes to latitudinal variation in renal traits. At the moment, we cannot partition the effect of phenotypic plasticity and population differentiation on the magnitude of latitudinal gradient in kidney morphology, and hence in the capacity to cope with dehydration and salty diets, found in *C. nigrofumosus* and *C. oustaleti*.

Our results imply that variation in reliance on marine food, both along an aridity gradient and between species, is accompanied by adjustments in the osmoregulatory mechanisms that animals use to cope with salt and dehydration. Most studies on the subsidies by productive marine environments to unproductive terrestrial ones have emphasized nutrient transfer (reviewed by Polis et al. 2004). Our study reveals that sometimes this transfer of energy and nutrients also involves the movement of other less desirable materials, such as excessive salt. Our results highlight that marine-to-terrestrial nutrient transfer studies sometimes need to consider the physiological mechanisms that mediate how terrestrial organisms cope with marine diets.

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