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Inter- and intraspecific variation in the use of marine food resources by three *Cinclodes* (Furnariidae, Aves) species: carbon isotopes and osmoregulatory physiology

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Summary

The avian genus *Cinclodes* (Furnariidae) includes species that inhabit both inland and marine shores. We compared the carbon isotopic composition and osmoregulatory capacities of field caught individuals of three *Cinclodes* species in Chile. *Cinclodes nigrofumosus* is a resident of coastal shores, whereas *C. oustaleti* and *C. patagonicus* inhabit both coastal and inland environments. The tissues of *C. nigrofumosus* exhibited distinctively marine $\delta^{13}C$ values, whereas those of *C. oustaleti* and *C. patagonicus* were intermediate between marine and terrestrial values. The differences in carbon isotopic composition among these three species were paralleled by differences in osmoregulatory characteristics. The species that carbon isotopes revealed to be strictly marine, *C. nigrofumosus*, had relatively larger kidneys with a higher fraction of total renal tissue occupied by medullary cones than its congeners *C. oustaleti* and *C. patagonicus*. *Cinclodes nigrofumosus* individuals also produced more concentrated urine. In addition to interspecific differences in osmoregulation, we found intraspecific differences. *Cinclodes nigrofumosus* collected at an arid site with limited or no available fresh water exhibited larger kidneys and higher relative medullary thickness than individuals collected at a mesic site. *Cinclodes nigrofumosus*, like all passerines, lacks functional salt glands. This species appears to be unique among passerines in its ability to live in extreme arid coastal environments and to cope with a marine diet that imposes high osmotic loads.

Key words: Cinclodes, marine passerine, osmoregulation, renal function, stable isotopes

Introduction

Birds living in marine environments can take advantage of a productive habitat, but their feeding may be constrained by the need to get rid of the high salt loads that can accompany marine foods (Nystrom and Pehrsson, 1988; Mahoney and Jehl, 1985). Feeding on diets of marine origin can impose a significant osmoregulatory challenge (Janes, 1997). Feeding on salty marine foods may be especially challenging for passerines, which lack functional salt glands (Shoemaker, 1972) and that have a limited ability to concentrate urine (Goldstein and Skadhauge, 2000). Perhaps the paucity of truly marine passerine species can be attributed to the absence of a functional salt gland that prevents them from utilizing foods of marine origin in the absence of fresh water.

Several subspecies of Savannah sparrows (*Passerculus sandwichenis*) can be considered "marine" because they inhabit salt marshes with scarce fresh water (Wheelwright and Rising, 1993). The physiological traits that allow these birds to inhabit a dry and salty environment have been relatively well studied in one of these subspecies, *P. s. beldingi* (Poulson and Bartholomew, 1962; Goldstein et al., 1990). Although salt marsh Savannah sparrows have remarkable abilities to tolerate dry and salty environments, they cannot be

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considered strictly marine. They feed primarily on the seeds of terrestrial plants and on insects (Wheelwright and Rising, 1993). An exceptional marine passerine is *Cinclodes nigrofumosus* (Furnariidae), a species that inhabits the intertidal and sandy beaches of Chile where it feeds primarily on marine osmoconforming invertebrates (Goodall et al., 1946; Paynter, 1971). Over 90% of the diet of *C. nigrofumosus* is comprised of marine mollusks and crustaceans (we will describe the diet of *C. nigrofumosus* in detail in a subsequent report). *Cinclodes nigrofumosus* is also remarkable because populations of this species can inhabit hyperarid coastal habitats that lack fresh water (Sabat, 2000). In this study we used stable isotopes to document the reliance of *C. nigrofumosus* on a marine diet and conducted field physiological measurements to determine the traits that allow this species to feed on an osmotically challenging marine diet.

The genus *Cinclodes* may be well-suited to study osmoregulation in birds from a comparative perspective. This genus includes species that are strictly marine (*C. nigrofumosus*), species that are strictly riverine (*C. atacamensis*), and species that may shift seasonally between marine and riverine habitats (*C. oustaleti* and *C. patagonicus*, among others; Housse, 1945; Goodall et al., 1946; Stotz et al., 1996). Here, we take advantage of the variation in habitat use and salt loads experienced by birds in the genus *Cinclodes* to investigate how habitat and diet can shape osmoregulation. Our study included three *Cinclodes* species with significant ecological differences. *Cinclodes nigrofumosus* inhabits intertidal zones and feeds almost exclusively on marine invertebrates (Goodall, et al. 1946; Paynter, 1971; Hockey et al., 1987). *Cinclodes oustaleti* and *C. patagonicus* live in both coastal and riverine habitats and can include insects and other terrestrial arthropods in their diet in addition to marine invertebrates (Housse, 1945; Goodall et al., 1946). Some individuals of *C. patagonicus* and *C. oustaleti* probably move seasonally from the coast to inland streams during the dry austral summer (December to March; Sielfeld et al., 1996; Jorge et al., 1998).

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. Using stable isotopes to determine the origin of dietary sources is not a new idea (Fry et al., 1978; Bouton et al., 1980). The method has been used by Hobson (1987) to document historical diet shifts in gulls, and by Bilby et al. (1996) to determine the contribution of anodramous fishes to the carbon and nitrogen balance of terrestrial ecosystems (see also Ambrose and DeNiro, 1986; Hilderbrand et al., 1996). Using the carbon isotope ratio of a consumer's tissues to assess the relative contribution of marine and terrestrial sources to its diet relies on two observations: Firstly, tissues often reflect the isotopic composition of an animal's diet (Hobson and Clark, 1992), and secondly, although there can be significant inter-habitat variation, marine food sources are significantly enriched in ^{13}C relative to sources from contiguous terrestrial habitats (i.e., $\delta^{13}C$ is more positive; Griffiths, 1991; Marra et al., 1998). Chisholm and Nelson (1982), for example, reported that $\delta^{13}C$ of tissues from Canadian terrestrial mammals averaged –25.5‰ whereas that of marine mammals averaged –17.5‰. A larger isotopic difference was observed between fresh water (–28.8‰) and marine fish (–18.7‰; Chisholm and Nelson, 1982). In addition, marine seagrasses and macroalgae have typical positive values (–10 to –15‰), compared with those of plants living at the adjacent salt marsh (–27 to –13‰, Boutton; 1991). We predicted that the tissues of *C. nigrofumosus*, the strictly marine species, would be consistently enriched in ^{13}C relative to those of its congeners that use both marine and fresh water habitats. We also predicted that species that move seasonally between marine and fresh water habitats would exhibit intermediate $\delta^{13}C$ values. We expected that tissues with higher carbon turnover, such as liver, would reflect diet at the time of collection, whereas tissues with slower turnover, such as bone collagen, would reflect past diets.

A novel contribution of this paper is the use of the carbon isotopic composition of a consumer's tissues as a potential covariate in a comparative physiological analysis. A diet of osmoconforming invertebrates is wet and salty. It is accompanied by a high ionic load and by a large amount of water (Gilles, 1987). Thus, we assumed that birds with marine isotopic signatures were subject to higher electrolyte and water loads and predicted that these animals would have osmoregulatory traits appropriate to deal with them. Savannah sparrows inhabiting salt marshes have larger kidneys with relatively large medullas than individuals of subspecies inhabiting nearby uplands (Goldstein et al., 1990), and than other species of sparrows living in mesic environments (Casotti and Braun, 2000). These traits seem to allow them to handle higher salt loads and to balance their water budget by drinking sea water (Poulson and Bartholomew, 1962). Thus, we predicted that in *Cinclodes*, kidney size and relative medulla size would increase with the $\delta^{13}C$ of the birds' tissues. In addition, we expected that differences in kidney morphology among species would be accompanied by differences in the degree to which birds concentrate urine in the field. In summary, we expected the marine species, *C. nigrofumosus*, to show ^{13}C enriched tissues, to have larger kidneys with a large fraction of renal tissue allocated to the medulla, and to produce more concentrated urine than its congeners, *C. oustaleti* and *C. patagonicus*, which inhabit marine habitats only seasonally.

Material and methods

Animals were collected with mist nets or shot between February and December 1999 at three sites: El Quisco (33° 34′ S, 71° 37′ W, *C. nigrofumosus* and *C. oustaleti*), Taltal (25° 25′ S, 70° 34′ W, *C. nigrofumosus*), and El Manzano (33° 39′ S, 70° 22′ W, C. *oustaleti and C. patagonicus*), Chile. The former two sites are coastal, whereas the latter is an inland fresh-water stream. El Quisco is relatively mesic (mean annual precipitation 441.3 mm; di Castri and Hajek, 1976), whereas Taltal is arid (mean annual precipitation 25.1 mm; di Castri and Hajek, 1976). 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). After urine collection was completed, a blood sample (50 to 100 µl) was collected into heparinized tubes. Birds were decapitated and their stomach (proventriculus and gizzard) contents were immediately removed and frozen in liquid nitrogen. Kidneys were removed from the synsacrum, weighed and preserved in paraformaldehyde-glutaraldehyde. The volume densities of kidney components (cortex and medulla) were estimated by point counting using the Cavalieri Principle on the right kidney which was processed for routine light microscopy (Gundersen et al., 1988). Volume density values were converted to absolute values by taking into account the volume of kidney estimated by kidney mass and assuming a tissue density of 1.

Gut contents were homogenized with a Potter-Elvehjem tissue grinder and centrifuged (12000 G, 5 min). The osmolality of the supernatant fluid was determined by vapor pressure osmometry (Wescor 5130B). The solid residue was used for determination of isotope ratios. Animal tissues (liver, pectoralis muscle and extracted bone collagen, ca. 0.15 mg) and solid gut contents were freeze-dried, ground into a fine powder, and loaded into pre-cleaned tin capsules for isotopic analysis. Bones were demineralized to obtain collagen (Tuross et al., 1988) and all tissues were de-fatted by ether extraction prior to isotopic analyses. 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. Lab standards, vacuum oil ($\delta^{13}C$ = –27.5‰ VPDB) and ANU sucrose ($\delta^{13}C$ = –10.5‰ VPDB, NAST 8542) were included with each run to correct raw values obtained from the mass spectrometer. Stable isotope ratios were expressed using standard delta notation (d) in parts per thousand (‰) as:

$$\delta^{13}C = (R_{sample}/R_{standard} - 1) \times 1000$$

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

Results

Diet isotopic composition: interspecific and habitat differences

At the moment of capture, the three species exhibited significant differences in the isotopic composition of their diets ($F_{2,28}$ = 10.5, $P < 0.001$). The diet of *Cinclodes nigrofumosus* had a significantly more positive isotopic composition ($\delta^{13}C \pm SE = –12.7 \pm 0.93$, $n = 15$, Tukey-Kramer HS test, $P < 0.05$) than that of *C. oustaleti* and *C. patagonicus* ($\delta^{13}C \pm SE = –16.1 \pm 1.2$, $n = 9$, and $\delta^{13}C \pm SE = –21.05 \pm 1.6$, $n = 15$, respectively). These latter two species did not differ significantly in diet isotopic composition (Tukey-Kramer HS test, $P > 0.05$). Within species, birds in different habitats also exhibited differences in diet $\delta^{13}C$. The diet of *C. nigrofumosus* individuals collected at an arid site (Taltal) was significantly enriched in ^{13}C ($\delta^{13}C \pm SE = –10.9 \pm 0.9$, $n = 8$, t = 3.05, $P < 0.01$) relative to the diet of individuals collected at a mesic site (El Quisco, $\delta^{13}C \pm SE = –14.8 \pm 1.2$, $n = 3$). The diet of *C. oustaleti* individuals collected at El Quisco was significantly enriched in ^{13}C ($\delta^{13}C \pm SE = –20.3 \pm 1.8$, $n = 8$, t = 5.1, $P < 0.01$) relative to the diet of individuals collected at the inland fresh-water stream (El Manzano)

Relationship between $\delta^{13}C_{diet}$ and $\delta^{13}C_{tissues}$

We used a linear model to compare the relationship between the isotopic composition of diet with that of tissues among species. This model included the tissue's isotopic composition as a dependent variable, and the diet's isotopic composition ($\delta^{13}C_{diet}$), species and the interaction between the diet's isotopic composition and species as independent variables. None of our analyses exhibited a significant interaction and hence this term was dropped from the model. We found remarkably different results for each tissue (Fig. 1). Liver $\delta^{13}C$ increased significantly with $\delta^{13}C_{diet}$ ($F_{1,32}$ = 36.1, $P < 0.001$) but was not affected by species ($F_{2,32}$ = 2.4, $p > 0.1$). The same regression line related $\delta^{13}C_{diet}$ and tissue $\delta^{13}C$ for all species (Fig. 1). In contrast, the analysis for both muscle and collagen demonstrated significant effects of both $\delta^{13}C_{diet}$ ($F_{1,32}$ = 10.4, $p < 0.003$ and $F_{1,32}$ = 12.7, $P < 0.05$, respectively) and species ($F_{2,32}$ = 15.2, $P < 0.001$, and $F_{2,32}$ = 20.6, $P < 0.001$, respectively, Fig. 1). The slope of the relationship between $\delta^{13}C_{diet}$ and tissue $\delta^{13}C$ differed among tissues. The steepness of the relationship between $\delta^{13}C_{diet}$ and

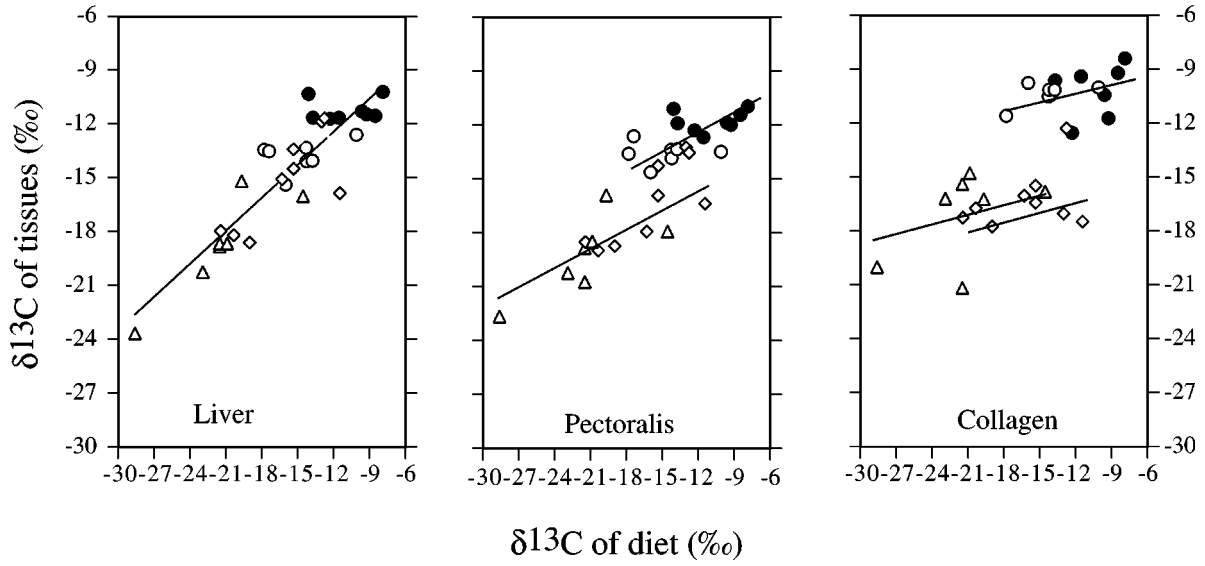

Fig. 1. In birds of the genus *Cinclodes*, tissue and diet $\delta^{13}C$ covaried, but the relationship between these two variables differered among liver, pectoralis muscle, and bone collagen. Circles represent data for *C. nigrofumosus*, triangles represent data for *C. patagonicus*, and diamonds data for *C. oustaleti*. Open circles represent *C. nigrofumosus* individuals collected at a mesic site (El Quisco), whereas closed circles represent individuals collected at an arid site (Taltal). Because we found no significant interaction effects, we used a common slope in all regressions for each tissue. Note that the analysis for liver showed no significant species effect (the regression line for all species shared a common intercept) whereas both pectoralis and collagen showed a significant species effect. For pectoralis and collagen, no significant differences were found between *C. oustaleti* and *C. patagonicus* and hence a common regression line was fitted. Note that the relationship between $\delta^{13}C_{diet}$ and $\delta^{13}C_{liver}$ is the steepest (slope ± SE = 0.48 ± 0.07) whereas that between $\delta^{13}C_{diet}$ and $\delta^{13}C_{collagen}$ is the shallowest (slope ± SE = 0.19 ± 0.07). The value of the $\delta^{13}C_{diet}$ Vs $\delta^{13}C_{tissue}$ slope for pectoralis had an intermediate value (slope ± SE = 0.35 ± 0.07).

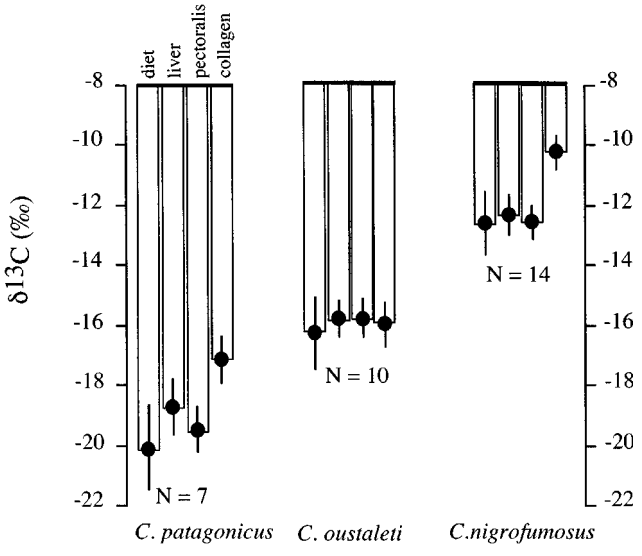

Fig. 2. Carbon isotopic composition of diet, liver, pectoralis muscle, and bone collagen differed among three *Cinclodes* species. In all species the carbon isotopic composition of collagen was more positive. Points are means and bars are standard errors. Repeated measures using ANCOVA revealed significant inter-specific differences in $\delta^{13}C$ as well as differences among tissue types.

tissue $\delta^{13}C$ seemed to vary with tissue turnover (Fig. 1). It was highest in liver, the tissue with the fastest turnover, and lowest in bone collagen, which is a tissue with slow turnover (Fig. 1).

The value of $\delta^{13}C$ differed significantly among species (repeated measures ANOVA $F_{2,28} = 31.5$, $P < 0.001$, Fig. 2) and tissues (repeated measures ANOVA $F_{3,84} = 6.5$, $P < 0.001$, Tukey-Kramer HS test, $P < 0.05$, Fig. 2). Because our analyses revealed no significant interaction ($F_{6,84} = 1.7$, $P > 0.1$), we compared mean differences among species and tissues. The tissues of *Cinclodes nigrofumosus* were significantly enriched in ^{13}C ($\delta^{13}C$ ± SD = –11.9 ± 2.0) relative to those of *C. oustaleti* and *C. patagonicus* (Tukey-Kramer HS test, $P < 0.05$). The latter two species did not exhibit significant differences ($\delta^{13}C$ ± SD = –15.8 ± 2.6 and –18.9 ± 3.4, respectively, Tukey-Kramer HS test, $P > 0.05$). The difference in isotopic composition among tissues was the result of the significantly more positive $\delta^{13}C$ of collagen ($\delta^{13}C$ ± SD = –13.6 ± 3.6, Tukey-Kramer HS test, $P > 0.05$). Diet, liver and muscle did not differ significantly in isotopic composition ($\delta^{13}C$ ± SD = –15.0 ± 4.7, –14.6 ± 3.3, –15.3 ± 3.3, re-

spectively). Data for *Cinclodes nigrofumosus* allowed comparison between habitats. We found that the tissues of *C. nigrofumosus* were significantly enriched in ^{13}C at the arid site ($\delta^{13}C \pm SD = -11.0 \pm 1.5$, N = 7) relative to the mesic site ($\delta^{13}C \pm SD = -13.1 \pm 2.1$, repeated measures ANOVA $F_{1,2} = 31.5$, $P < 0.001$). Again, collagen was significantly enriched in ^{13}C ($\delta^{13}C \pm SD = -10.3 \pm 1.1$, $n = 14$) relative to diet, liver and pectoralis (repeated measures ANOVA $F_{3,36} = 11.7$, $P < 0.001$).

Does $\delta^{13}C_{diet}$ predict the ability to cope with a high osmotic load?

The tissues of the marine crustaceans and mollusks ingested by *Cinclodes* are in osmotic equilibrium with seawater (Gilles, 1987). Thus, the osmolality of gut contents should increase with the fraction of marine organisms in diet as estimated by $\delta^{13}C$. Our limited data set of three species does not allow a proper test of this conjecture. However, it provides preliminary evidence that supports it: when data are separated into species and habitats, an apparent positive correlation emerges (Fig. 3). We call this correlation apparent because our data does not permit the use of inferential statistics without violating independence assumptions. Thus, this correlation must be considered preliminary and more a hypothesis than a well established pattern. The data in Figure 3 revealed an unexpected result. Although *C. nigrofumosus* individuals ingested primarily osmoconforming mollusks and crustaceans, the osmolality of their stomach contents (mean Osm/kg ± SD = 771 ± 176, $n = 14$) was significantly lower than that of seawater (≈1100 mOsm/kg, $t = 7.0$, $P < 0.001$).

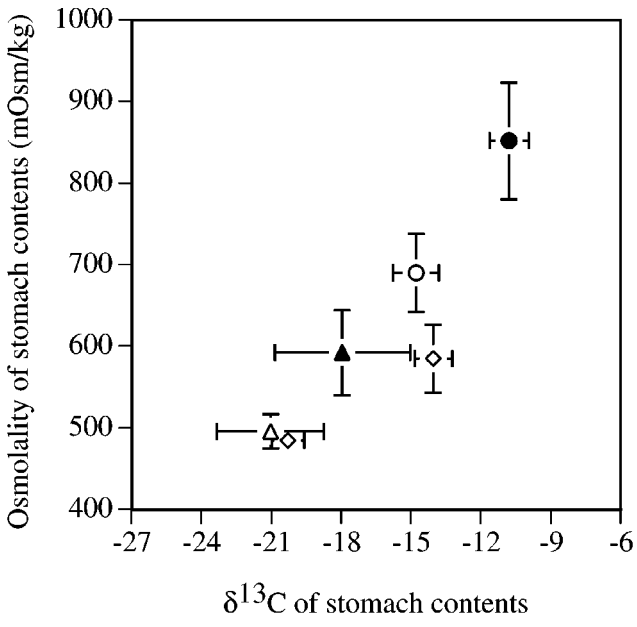

Fig. 3. The osmolality of stomach contents increased as the carbon isotopic composition ($\delta^{13}C$) became more positive in three species of *Cinclodes*. Points are means and bars are standard errors. Circles represent data for *C. nigrofumosus* (closed circles are data for birds collected at Taltal, whereas open circles are data for birds collected at El Quisco). Triangles represent data for *C. patagonicus* at El Manzano (open symbols) and El Quisco (closed symbols). Diamonds represent data for *C. oustaleti* at El Manzano (open) and El Quisco (closed).

Interspecific differences in osmoregulation

We used a linear model to compare the relationship between the osmolality of diet with that of urine. This model included urine osmolality as a dependent variable, and diet osmolality, species, and the interaction between the diet's osmolality and species as independent variables. None of our analyses exhibited a significant interaction term and hence this term was dropped from the model (Fig. 4). Urine osmolality increased in a 1 to 1 fashion with diet osmolality ($F_{1,15} = 12.5$, $P < 0.005$, slope ± SE = 1.0 ± 0.3) but was not affected by species ($F_{2,15} = 2.4$, $P > 0.1$). The same regression line related urine and diet osmolality for all species (Fig. 4). Because *C. nigrofumosus* ingested food with higher osmolality than *C. oustaleti* and *C. patagonicus*, the osmolality of its urine (mean mOsm/kg ± SD = 836 ± 238, $n = 9$) was also higher (mean mOsm/kg ± SD = 363 ± 23.1, $n = 3$, and 525 ± 105, $n = 8$, for *C. patagonicus* and *C. oustaleti*, respectively, $F_{2,19} = 10.9$, $P < 0.001$, Tukey HSD test $P < 0.05$). The sample sizes shown in Figure 4 are different from those described in this analysis because we could not obtain both urine and diet osmolality samples for all individuals captured.

Plasma osmolality varied significantly among species ($F_{2.31} = 8.5$, $p < 0.005$). *Cinclodes nigrofumosus* exhib-

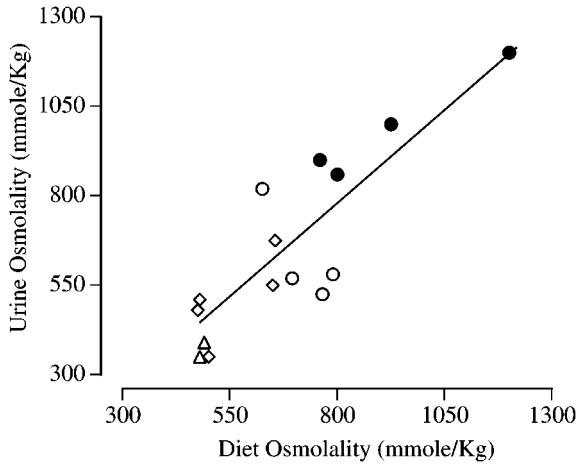

Fig. 4. ANCOVA revealed a significant effect of diet osmolality but not in *Cinclodes* species on urine osmolality. The regression line is $y = 4.4 + 0.97x$, $r^2 = 0.53$. Symbols for each species are as in Figures 1 and 3.

ited the highest plasma osmolality (mOsm/kg ± SD = 390 ± 27, N =14), followed by *C. patagonicus* (mOsm/kg ± SD = 382 ± 35, N =7), and *C. oustaleti* (mOsm/kg ± SD = 343 ± 23, N =10). In the field, *C. patagonicus* concentrated urine to an osmolality 1.4 to 3.2 times higher than that of plasma (mean urine to plasma ratio (U/P) ± SD = 2.14 ± 0.6). The average difference in osmolality between urine and plasma in *C. nigrofumosus* was 446 mOsm/kg (± 229.77, paired t = 5.83, p < 0.001). Although all *C. oustaleti* individuals concentrated urine to an osmolality higher than that of plasma (mean difference between urine and plasma ± SD = 164.7 ± 70.2 mOsm/kg, t = 6.2, p < 0.001, N = 7), the osmolality of urine was only 1.2 to 1.7 times higher than that of plasma (mean U/P ratio ± SD = 1.5 ± 0.2, N = 7). Our very limited data for *C. patagonicus* revealed a similar pattern to that exhibited by *C. oustaleti*. In the three *C. patagonicus* individuals for which we have data, urine and plasma osmolalities differed from only –15 to 20 mOsm/kg (mean U/P ratio ± SD = 0.99 ± 0.6, N = 3).

Kidney mass and morphology were consistent with differences in reliance on marine dietary sources. Kidney mass increased significantly with body mass (ANCOVA on log-transformed data $F_{1,30} = 8.8$, $P < 0.01$) and there were significant inter-specific differences in kidney mass when body mass was accounted for (ANCOVA on log-transformed data $F_{2,30} = 6.6$, $P < 0.01$, Fig. 5). *Cinclodes nigrofumosus* had relatively larger kidneys than *C. oustaleti* and *C. patagonicus* (Fig. 5). Because the exponent of the allometric relationship between body and kidney mass was not significantly different from 1 (exponent ± SE = 0.86 ± 0.29, $t = 0.5$, $P > 0.1$), it is appropriate to compare the percentage of body mass represented by the kidney among species. This percentage was significantly higher in *C. nigrofumosus* ($F_{2,31}$ =11.3, $P < 0.001$, mean percentage ± SD = 1.6 ± 0.2%, N = 14) than in *C. oustaleti* (mean percentage ± SD = 1.4 ± 0.2%, $n = 10$) and *C. patagonicus* (mean percent-

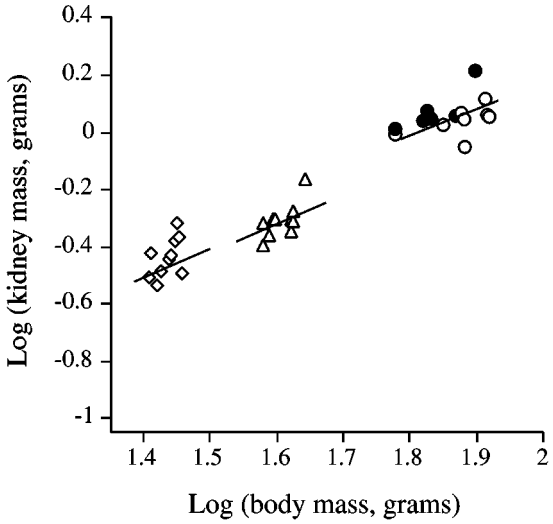

Fig. 5. Relative kidney mass was higher in *C. nigrofumosus* (Log(kidney mass) = –1.55 + 0.86Log(body mass)) than in *C. oustaleti* and *C. patagonicus* (Log(kidney mass) = –1.69 + 0.86Log(body mass)). A common regression was fitted through the latter two species because they exhibited no significant differences. Circles represent data for *C. nigrofumosus*, triangles represent data for *C. patagonicus*, and diamonds data for *C. oustaleti*. Open circles represent *C. nigrofumosus* individuals collected at a mesic site (El Quisco), whereas closed circles represent individuals collected at an arid site (Taltal).

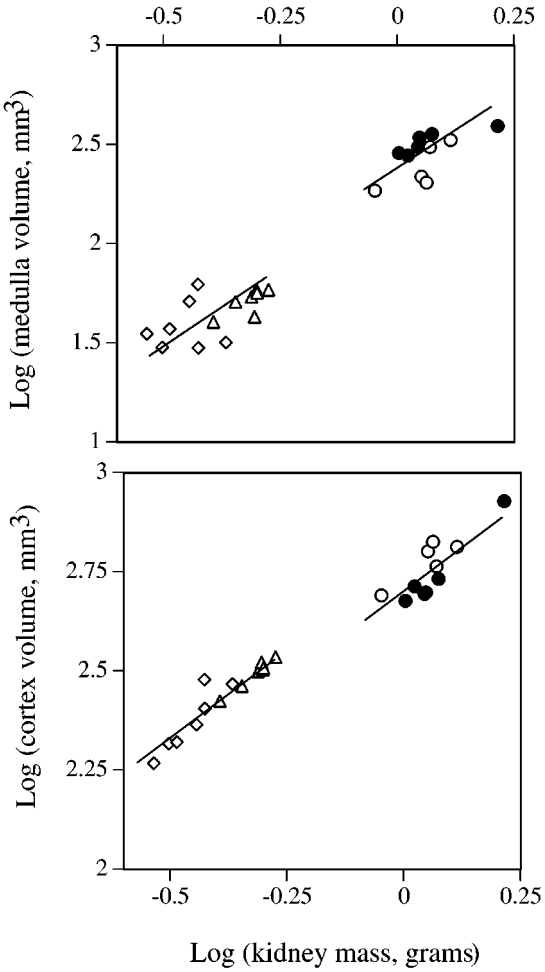

Fig. 6. Relative to kidney mass, medulla volume was higher in *C. nigrofumosus* (Log(medulla volume) = –0.7 + 0.87Log(kidney mass)) than in *C. oustaleti* and *C. patagonicus* (Log(medulla volume) = –1.02 + 0.87Log(kidney mass)). Relative to kidney mass, cortex volume was smaller in *C. nigrofumosus* (Log(cortex volume) = –0.31 + 1.01Log(kidney mass)) than in *C. oustaleti* and *C.* (Log(cortex volume) = –1.50 + 1.01Log(kidney mass)). A common regression was fitted through the latter two species because they exhibited no significant differences. Symbols are as in Figure 5.

age ± SD = 1.2 ± 0.1%, $n = 10$, Tukey-Kramer HSD test, $p < 0.05$). *Cinclodes oustaleti* and *C. patagonicus* did not show significant differences in relative kidney mass (Tukey-Kramer HSD test, $P > 0.05$). All analyses of percentages were done on arcsin$\sqrt{}$-transformed data.

Cinclodes nigrofumosus not only had relatively larger kidneys, this species also exhibited kidneys with a larger fraction of total renal tissue occupied by medullary cones. The volume occupied by medullary cones increased significantly with kidney mass (ANCOVA on log-transformed data $F_{1,21} = 6.6$, $P < 0.01$) and there were significant inter-specific differences in this volume when kidney mass was accounted for (ANCOVA on log-transformed data $F_{2,21} = 6.26$, $P < 0.02$, Fig. 6). *Cinclodes nigrofumosus* had relatively larger medullary volumes than *C. oustaleti* and *C. patagonicus* (Fig. 6). Curiously, the volume of renal cortex was relatively smaller in *C. nigrofumosus* than in *C. patagonicus* and *C. oustaleti*. The volume of renal cortex increased significantly with body mass (ANCOVA on log-transformed data $F_{1,21} = 4.31$, $P < 0.03$) and there were significant inter-specific differences in cortex volume when body mass was accounted for (ANCOVA on log-transformed data $F_{2,21} = 76.5$, $P < 0.001$, Fig. 6).

In *C. nigrofumosus* relative kidney size and the fraction of renal tissue allocated to the medulla and cortex differed significantly between sites. The kidneys of birds collected at the arid site were larger (mean % of total body mass ± SD = 1.7 ± 0.2%, $n = 6$, $t = 2.8$, $P < 0.05$) than those of birds collected at the more mesic site (mean % of total body mass ± SD = 1.5 ± 0.1%). When kidney mass was accounted for, birds at the arid site had a higher allocation to medullary tissue (ANCOVA on log-transformed data $F_{1,11} = 8.6$, $P < 0.02$), and lower allocation to cortex than birds at the mesic site (ANCOVA on log-transformed data $F_{1,11} = 10.04$, $P < 0.02$).

Discussion

Can stable isotopes be used to assess reliance on marine resources?

Marine and terrestrial organisms often exhibit distinctive, non-overlapping carbon isotopic compositions (Szepanski et al., 1999 and references there). Measurements of $\delta^{13}C$ (and $\delta^{15}N$; Ben-David et al., 1997) allow partitioning the proportion of marine and terrestrial sources in an animal's diet (Ambrose et al., 1997) and even the subsidy of marine ecosystems to insular terrestrial communities (Anderson and Polis, 1997). In our study, $\delta^{13}C$ differentiated between *C. nigrofumosus*, a species that relies primarily on marine food, and *C. patagonicus* and *C. oustaleti*, two species that use both terrestrial and marine sources. Our data also revealed significant intraspecific differences in isotopic composition: the diet of *C. nigrofumosus* individuals collected at an arid site was enriched in ^{13}C relative to the diet of individuals collected at a mesic site.

Differences in isotopic composition between these sites can be explained by two alternative hypothesis: 1) the proportion of terrestrial and marine dietary sources may have differed between sites, and 2) the isotopic composition of the sources may have differed between sites without a change in the combination of marine and terrestrial sources. The latter hypothesis probably explains the ca. 4‰ difference in carbon isotopic composition between the arid and the mesic site in *C. nigrofumosus*. Although the relative contribution of marine sources in diet (by dry mass) did not differ significantly between sites (> 90% at both sites), the carbon isotopic composition of marine items was significantly more positive at the arid than at the mesic site (Fig. 1). The carbon isotopic composition of the tissues of coastal producers, and hence of the tissues eaten by marine consumers, can show variation in space and time (Stephenson et al., 1984). The inter-site differences in $\delta^{13}C$ found in *C. nigrofumosus* highlights the importance of taking this variation into account when interpreting differences in isotopic composition.

Why does the relationship between diet and tissue $\delta^{13}C$ differ among tissue types?

The relationship between the carbon isotopic composition of diet and that of tissues, varied among tissue types. The slope of the relationship between diet and tissue $\delta^{13}C$ appeared to depend on carbon turnover rate (Fig. 1). Liver $\delta^{13}C$ increased steeply with diet $\delta^{13}C$, whereas the relationship between the carbon isotopic composition of diet and that of bone collagen was relatively flat (Fig. 1). The isotopic composition of a tissue is the result of the integration of isotopic inputs over time. The time window of isotopic incorporation integrated by the composition of a tissue depends on the turnover rate of its constituent carbon (Tieszen et al., 1983). The isotopic composition of tissues with high turnover, such as liver (Waterlow et al., 1978), reflects integration of recent inputs. The isotopic composition of tissues with low turnover, such as collagen (Stenhouse and Baxter, 1979), reflects integration of inputs over a longer time period (Hobson and Clark, 1992). Because diet $\delta^{13}C$ provides a point estimate for the carbon isotopic composition of recent diets, it should not be surprising that the slope relating diet and tissue $\delta^{13}C$ was steep for liver and shallow for collagen. The isotopic composition of tissues with high carbon turnover should be responsive to temporal changes in diet isotopic composition whereas the composition of tissues with low turnover should be relatively insensitive to temporally local diet changes.

Does $\delta^{13}C_{diet}$ predict osmotic load?

Our limited data set provided preliminary evidence that supports the notion that $\delta^{13}C$ can be used to predict ingested osmotic load (Fig. 3). Our conjecture is based on the observation that when *Cinclodes spp.* feed on marine habitats, they ingest osmoconforming invertebrates (primarily molusks and crustaceans, Sabat, 2000) whose tissues contain high ionic concentrations (Gilles, 1987). Because the tissues of marine teleosts tend to have low ionic concentrations (Evans, 1993), our conjecture applies to a much lesser degree to piscivorous birds and mammals. Only a larger comparative data set will support or falsify our conjecture of a positive correlation between osmotic load and the ingestion of marine invertebrates.

The data presented in Figure 3 revealed an unexpected result. Although *C. nigrofumosus* ingested primarily osmoconforming invertebrates, the osmolality of its stomach contents was lower than that of sea water. This observation can be explained by two non-exclusive hypotheses. Firstly, because the food in the stomach is mixed with gastrointestinal secretions (Denbow, 2000), its osmolality is decreased (Chang and Rao, 1994). Secondly, the ionic concentration of stomach contents can be diluted by drinking freshwater and by the ingestion of prey items with low osmolality. Available data does not allow assessing the relative importance of these two explanations. We suspect that the osmolality of the stomach contents provides a good relative estimate of ingested osmotic loads (note the interspecific differences in Fig. 4). However, dilution by gastrointestinal secretion and potential dilution by ingested freshwater probably render it a poor estimate of absolute osmotic load. The strength of the correlation between $\delta^{13}C$ and osmotic load may depend on the relative availability of fresh water and food with low ionic concentration.

Inter- and intraspecific variation in osmoregulatory capacity

The three species of *Cinclodes* included in this study seem to exhibit significant differences in kidney morphology (Figs. 5 and 6). The strictly marine *C. nigrofumosus* had relatively larger kidneys with a higher fraction of total renal tissue occupied by medullary cones than its congeners *C. oustaleti* and *C. patagonicus*. Previous studies have demonstrated that salt marsh savanah sparrows (*Passerculus sandwichensis*) have a higher volume of renal medulla than house sparrows (*Passer domesticus*) and song sparrows (*Melospiza melodia*). This pattern is expected from the limited availability of fresh water and the potentially high salt content of prey in marsh Savannah sparrow's habitats (Cassoti and Braun, 2000). Like marsh-dwelling savannah sparrows, *C. nigrofumosus* has a large kidney with a large medulla. The unique traits of *C. nigrofumosus* are highlighted by an allometric comparison (see data and equation in Cassoti et al. 1998). The mass of the cortex in *Cinclodes nigrofumosus* is similar of that expected, but the medulla is 370% and 670% higher than that expected from body mass (for individuals from El Quisco and Taltal respectively). In contrast, *Cinclodes patagonicus* and *C. oustaleti* have kidney, cortex, and medulla masses that are very close to those expected from Casotti et al. (1998) allometric equation.

Passerines and non passerines inhabiting mesic and aquatic habitats have larger renal cortices that those inhabiting arid regions (Warui, 1989; Cassoti et al., 1998). The larger medulla and reduced cortices exhibited by *C. nigrofumosus* may indicate that these birds are better at conserving water than at than excreting high urine volumes. Our field results suggest that *C. nigrofumosus* individuals also produced more concentrated urine (Fig. 4). However, because diet and urine osmolality were correlated, it is unclear if the interspecific differences in urine concentration were the result of differences in ingested osmotic load or of differences in concentrating capacities (Braun and Dantzler, 1984). We predict that in the genus *Cinclodes* concentrating capacity measured in the laboratory will be higher in *C. nigrofumosus* than in its congeners *C. oustaleti* and *C. patagonicus*.

Although it is believed that vertebrates regulate plasma osmolality tightly, birds show both intraspecific and interspecific variation in this trait. For example Goldstein and Zahedi (1990) reported that dehydration in house sparrows led to an increase in plasma osmolality (from 347 $mOsm \cdot Kg^{-1}$ in well hydrated birds to nearly 400 $mOsm \cdot Kg^{-1}$ in water-deprived ones). Similarly, Goldstein et al. (1990) found significant variation in plasma osmolality between two subspecies *P. sandwichensis* which appeared to be related to water availability between Goldstein et al.'s study sites. Plasma osmolality can be influenced by diet, water availability and interspecific differences in how animals respond to these factors. Thus, the intespecific differences that we have documented for *Cinclodes* are difficult to interpret without more experimental data on the response of plasma osmolality to diet and hydration status.

In addition to demonstrating interspecific variation in osmoregulatory characteristics, our results demonstrated intra-specific variation. *Cinclodes nigrofumosus* individuals captured at an arid site had larger kidneys with larger medullas and smaller cortices than birds captured at a mesic site. It is unknown if these intraspecific differences are the result of population differentiation or if they are the result of phenotypic plasticity, i.e., of the acclimatization of individuals to variations in fresh water availability and higher evaporative water

losses. *Cinclodes nigrofumosus* inhabits a broad latitudinal coastal band that spans sites from the relatively wet and southern extreme of its distribution to the hyperarid sites at its northern limit (Goodall et al., 1946). At the northern limit of its range, *C. nigrofumosus* individuals are faced with relatively high temperatures, which impose high evaporative water losses, and no available fresh water (di Castri and Hajek, 1976). At the southern end of its range, precipitation is higher and fresh water is available. This species presents a unique opportunity to investigate the effect of the interaction between fresh water availability, an osmotically challenging salty diet and evaporative water losses on avian osmoregulatory function within a single species. We predict a south to north increasing gradient in the ability to concentrate urine and process large amounts of seawater in *C. nigrofumosus*.

Cinclodes nigrofumosus is remarkable for a passerine species because it feeds on marine foods containing high osmotic loads and because it can inhabit hyperarid environments (Sabat, 2000). Is *C. nigrofumosus* capable of surviving without drinking freshwater and/or ingesting osmoregulating terrestrial invertebrates? Because only one point in Figure 4 shows urine production more concentrated than sea water, our data appears to indicate that although *C. nigrofumosus* is relatively efficient among passerines in its ability to concentrate urine, it still has limited concentrating ability. Other studies have reported passerines with the ability to concentrate urine slightly above the concentration in sea water (Goldstein and Braun, 1989; Skadhauge and Bradshaw, 1974). However, the osmotic concentration of urine may underestimate a bird's ability to get rid of salts. Various studies have suggested that from 5% to 75% of urinary Na^+ and K^+ are associated with precipitated urate (reviewed by Goldstein and Skadhauge, 2000). In species that feed on invertebrates, urate excretion is substantial (Skadhauge, 1981). Hence urate-trapped ions that do not contribute to urine osmolality may present an important avenue for ion excretion. We hypothesize that a substantial fraction of the ionic output of *C. nigrofumosus* is excreted in association with urates. Given the presence of *C. nigrofumosus* at hyperarid sites, we hypothesize that these birds are capable of subsisting on a hyperosmotic diet and without access to fresh water.

It is noteworthy that the urine osmolality of *C. nigrofumosus* was significantly higher ($t = 3.6$, $p < 0.001$, d.f. = 39) than that produced by field caught Savannah sparrows (*Passerculus sandwichensis beldingi)* inhabiting salt marshes (mean mOsm/kg ± SD = 577.0 ± 163.6, N = 27, Goldstein et al., 1990). Salt-marsh Savannah sparrows have unusual physiological capacities for coping with high salt intake, can survive for extended periods drinking salt water exclusively, and have large kidneys with relatively large medullary tissue (reviewed by Goldstein et al., 1990). Like Savannah sparrows, *C. nigrofumosus* seems to be a passerine species with the exceptional capacity to cope with high salt loads and scarce fresh water without the aid of functional salt glands. Unlike marsh-dwelling Savannah sparrows that rely mainly on terrestrial food sources, *C. nigrofumosus* seems to be a passerine that relies almost exclusively on marine food sources.

There is considerable interspecific (and probably intraspecific) variation in the use of coastal environments by the genus *Cinclodes* (Sabat, 2000 and references there). Sabat (2000) has noted that in Chile, *C. oustaleti* and *C. patagonicus*, the two species that exhibit seasonal movements are present in coastal regions only during the austral winter when rain water is available and when mild temperatures reduce evaporative water losses. In the hot, dry summer they move inland to streams and lake shores (Sabat, 2000). We speculate that in the absence of fresh water *C. oustaleti* and C. *patagonicus* cannot take advantage of abundant, albeit salt-loaded, invertebrates. In contrast, *C. nigrofumosus*, which are resident of coastal areas, may be able to do so. Interspecific differences in osmoregulatory capacity may explain variation in temporal patterns of habitat use. The genus *Cinclodes* seems to offer unparalleled opportunities to investigate the evolution of osmoregulation in birds and to examine the ecological consequences of variation in osmoregulatory capacity.

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