

Osmoregulation and adaptive radiation in the ovenbird genus *Cinclodes* (Passeriformes: Furnariidae)

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Summary

1. The genus *Cinclodes* is unique among passerines because it includes two species that can be considered marine/coastal and also includes several species that inhabit freshwater streams or that shift habitats between terrestrial/fresh water and marine habitats. The *Cinclodes* clade satisfies two criteria of an adaptive radiation: it is monophyletic and it experienced recent speciation accompanied by rapid phenotypic diversification.
2. We focused on the osmoregulatory traits of five *Cinclodes* species to determine if the clade also satisfies the criterion of adaptive phenotype–environment correlation that characterizes adaptive radiations. We used the $\delta^{13}\text{C}$ of tissues to estimate reliance on a marine diet. We predicted that $\delta^{13}\text{C}$ would be positively correlated with the renal traits responsible for urine concentration (relative kidney size, fraction of the kidney comprising medulla, and number of medullary cones per unit of kidney mass).
3. Our analyses confirmed these hypotheses. We concluded that *Cinclodes* satisfies the adaptive phenotype–environment correlation criterion. *Cinclodes* seems to represent an example of an avian adaptive radiation in osmoregulatory function.

Key-words: Carbon isotopes, kidney, marine birds, renal function, salt

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Introduction

Although songbirds (Order Passeriformes) have diversified in all continents and now occupy nearly all terrestrial ecosystems (Barker *et al.* 2004), few songbird species inhabit marine environments (Sabat 2000). To our knowledge, and with the exception of one of the species described here, there are no reports of passerine species that rely exclusively on marine diets (Sabat, Fariña & Soto-Gamboa 2003). Several subspecies of Savannah Sparrows (*Passerculus sandwichensis*) can be considered ‘marine’ because they inhabit salt marshes with scarce fresh water (Wheelwright & Rising 1993). Salt-marsh Savannah Sparrows have remarkable abilities to tolerate dry and salty environments (Poulson & Bartholomew 1962; Goldstein, Williams & Braun 1990), but because they feed primarily on the seeds of terrestrial plants and on insects they cannot be considered strictly marine (Wheelwright & Rising 1993). Some of the reasons for the extreme paucity of marine species among the passerines may be ecological and include competition with non-passerine birds. There is, however, a potential physiological cause

for the relative scarcity of marine species among passerines. Feeding on salty marine foods may be challenging for passerines without functional salt glands and with limited ability to concentrate urine (Goldstein & Skadhauge 2000).

The species in the genus *Cinclodes* (Furnariidae) are well suited to investigate the role that osmoregulatory traits may play in the evolution of a marine habit. *Cinclodes* includes coastal species, including at least one species, *C. nigrofumosus*, whose diet is constituted exclusively or almost exclusively of marine molluscs and crustaceans (Sabat *et al.* 2003). The genus also includes species that inhabit freshwater streams and species that shift seasonally between marine and freshwater habitats (reviewed by Sabat 2000). All *Cinclodes* species feed on invertebrates. The study of osmoregulation and its evolution in *Cinclodes* is facilitated by Chesser’s (2004) recent phylogenetic study. Chesser’s study suggests that the marine habit evolved independently twice in the genus. It also suggests that a freshwater habit is the ancestral condition and that seasonal use of coastal environments evolved three times. Chesser (2004) and Villeumier (1986) hypothesized that *Cinclodes* constitutes an adaptive radiation. Futuyma (1989) defined the phenomenon of adaptive radiations as ‘...evolutionary divergence of members of a single

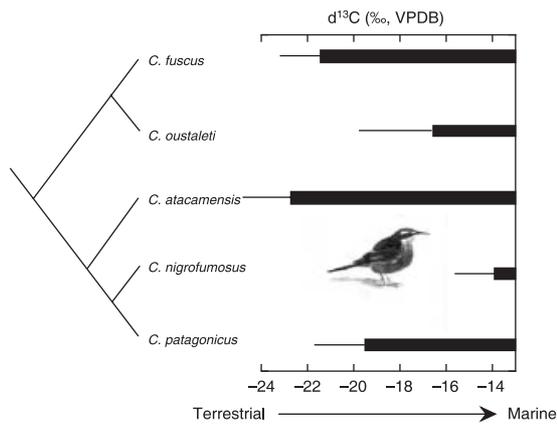


Fig. 1. The carbon isotope composition of pectoralis muscle reveals significant interspecific variation in the use of marine and terrestrial diets among five *Cinclodes* species. The phylogenetic relationships among the five species are from Chesser's (2004) hypothesis.

phylogenetic line into a variety of different adaptive forms' and we follow this definition here. Schluter (2000) identified three criteria that characterize adaptive radiations: (1) common ancestry, (2) adaptive phenotype–environment correlation and (3) rapid speciation accompanied by phenotypic diversification. The genus *Cinclodes* satisfies criteria 1 and 3 (Chesser 2004 and Discussion). Our main purpose is to describe evidence for criterion 2. Specifically, the aim of our research was to determine whether changes in reliance from terrestrial to marine food diets were accompanied by evolutionary changes in renal structure and function. We investigated the renal morphology and function of five species of *Cinclodes* that range in ecological habits from strictly riverine to strictly marine (Fig. 1).

Because the feeding habits and natural history of Chilean *Cinclodes* are poorly known, it is risky to characterize their dependence on marine and freshwater/terrestrial sources from published observations. To avoid this problem, we measured the carbon isotope composition of the bird's tissues. $\delta^{13}\text{C}$ is more positive in marine-based food webs than in terrestrial and freshwater ones (Chisholm, Nelson & Schwarcz 1982; Anderson & Polis 1998). Our study depends on the large difference in isotopic composition between marine seagrasses and macroalgae at the base of intertidal food webs and the C3 vegetation of the coast of Chile and of the stream food webs that *Cinclodes* species may depend on. In contrast with the relatively ^{13}C depleted isotopic composition of terrestrial C3 plants ($\delta^{13}\text{C}$ ranges from -24 to -27‰ , Ehleringer *et al.* 1998), the plants that form the base of intertidal communities tend to have more positive $\delta^{13}\text{C}$ values (reviewed by Wang & Yeh 2003). For example, when *Cinclodes* spp. feed on the seashore, they feed primarily on the wave-swept upper-intertidal levels where the main primary producers are *Ulva rigida* and other foliose algae (Santelices 1990). Most marine algae have $\delta^{13}\text{C}$ values between -13 and -17‰ (reviewed by Wang & Yeh 2003), and the carbon isotopic composition of *Ulva* spp. ranges

from -10.5 to -16.3‰ (Wang & Yeh 2003). Samples of the macroalgae *Ulva lactuca* and *Enteromorpha compressa* measured at a Chilean coastal site within the range of *Cinclodes* spp. had $\delta^{13}\text{C}$ values ranging between -14 and -18‰ (Fariña 2000). *Cinclodes* species feed on stream invertebrates. Thus, there is the possibility of a mixture of autochthonous carbon (which has a C3 isotopic signature) with allochthonous carbon (with C4 or CAM isotopic signatures) at the bases of the food chains of the streams in which these birds feed. The influence of C4 and CAM signatures might spuriously have led us to overestimate the importance of marine items in their diets. This possibility, however, is unlikely in the system we studied for two reasons:

1. The climate of the geographical range species included in our study can be best characterized as Mediterranean with winter rains. Winter precipitation and relatively cool springs favour terrestrial primary productivity dominated by C3 photosynthesis (Hattersley 1983; Ehleringer *et al.* 1998).
2. The secondary productivity of streams in dry regions is almost entirely dependent on autochthonous inputs (Webster & Meyer 1997). Consequently, although CAM plants may be common in some of the most arid regions within the range of the *Cinclodes* species included in this study, their contribution to the carbon of stream food webs is likely very small.

We predicted (1) that the ability to concentrate urine would be positively correlated with $\delta^{13}\text{C}$ and (2) that the magnitude of renal traits associated with the ability to concentrate urine, such as relative kidney size (Hughes 1970), fraction of the kidney comprising medullary tissue (Casotti & Braun 2000) and number of medullary cones per unit kidney mass (Poulson 1965; Johnson & Ohmart 1973), would be positively correlated with $\delta^{13}\text{C}$. Although it is well known that urine can be modified in the lower intestinal tract by several bird species (Braun 1999), this possibility was not explored in this study. We focused on the renal traits of *Cinclodes*.

Methods

Birds were captured from 2000 to 2003 using mist nets at several localities in Chile. *Cinclodes nigrofumosus* ($N = 15$), and *C. oustaleti* ($N = 13$) individuals were captured in two coastal localities from Central Chile (El Quisco and Los Vilos, $33^{\circ}34'S$, $71^{\circ}37'W$ and $31^{\circ}54'S$, $71^{\circ}31'W$, respectively). *Cinclodes patagonicus* ($N = 10$) individuals were captured at a freshwater stream in Central Chile (El Manzano, $33^{\circ}39'S$, $70^{\circ}22'W$). *Cinclodes atacamensis* ($N = 4$) was captured at freshwater site in northern Chile (Arroyo Chuzmiza, $19^{\circ}40'S$, $69^{\circ}10'W$) and *C. fuscus* ($N = 8$) at freshwater site in North/Central Chile (Rio Claro, $30^{\circ}22'55''S$, $70^{\circ}23'59''W$). A 50–100 μl blood sample was collected from the brachial vein into heparinized microhaematocrite tubes immediately after capture. Blood samples were centrifuged within 5 min

(9000 g, 5 min) of capture and the osmolality of plasma was measured. Then, animals were fed 0.02 ml g⁻¹ of a 600 mmol l⁻¹ NaCl solution. Ureteral urine samples were collected every 15 min after salt load administration for at least 2 h by inserting a small closed-ended cannula into the birds' cloaca. Urine drained into the cannula via an opening facing ureteral orifices (Goldstein & Braun 1989). Urine samples were centrifuged (9000 g, 5 min) and the osmolality of the supernatant was measured (Wescor 5130B, Logan, UT) to estimate maximal urine concentration (U_{\max}). After 2 h, birds were euthanized and dissected. Kidneys were removed, weighed and preserved in paraformaldehyde–glutaraldehyde. The area of medulla was estimated by point counting using the Cavalieri Principle on the right kidney, which was processed for routine light microscopy (Gundersen *et al.* 1988; Wauri 1989). Medullary cones were dissected from the left kidney using a dissecting microscope and the entire collection was counted. The characteristics of the kidney were estimated according to protocols described in detail in Sabat & Martínez del Río (2002). 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 & Martínez del Río 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 & Clark 1992) and (2) marine food sources are significantly enriched in ¹³C relative to sources from contiguous terrestrial habitats (i.e. ¹³C is more positive, Blundell, Ben-David & Bowyer 2002 and references there). Our study depends on the large difference in isotopic composition between marine seagrasses and macroalgae at the base of intertidal food webs (¹³C ranges from -10 to -15‰) and the C3 vegetation of Chile (-24 to -27‰). We chose pectoralis muscle for isotopic analyses because it has a relatively low turnover and thus reflects integration of inputs over a relatively long time period (Hobson & Clark 1992; Sabat & Martínez del Río 2002). Pectoralis muscle samples were processed as described by Sabat & Martínez del Río (2002). Carbon isotope ratios of food were measured on a continuous flow isotope ratio mass spectrometer (Finnigan Delta + XP Thermo Electron, Waltham, MA, University of Wyoming's light stable isotope facility) with samples combusted in a Costech elemental analyser (Costech, Valencia, CA). The precision

of these analyses was ±0.2‰. Our standards were vacuum oil ($\delta^{13}\text{C} = -27.5\text{‰}$, Vienna Pee Dee Belemnite (VPDB)) and ANU sucrose ($\delta^{13}\text{C} = -10.5\text{‰}$, VPDB, National Institute of Standards N° 8542). We included standards in every run to correct raw values obtained from the mass spectrometer. Isotope ratios in this paper are reported as δ -values on a per mil (‰) basis relative to the International Atomic Energy Agency carbon isotope standard, VPDB. Stable isotope ratios were expressed using standard delta notation (δ) in parts per mil (‰) as:

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

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

To examine correlations among variables we used both standard correlation and least squares regression (LSR) methods and correlation and regression on phylogenetically independent contrasts (PICs, Felsenstein 1985). PICs were calculated using Chesser's (2004) hypothesis for the phylogenetic relationships within the genus *Cinclodes* (Fig. 1) and COMPARE 4.6 (Martins 2004). Untransformed branch lengths used in our analyses were obtained from substitutions per site for combined sequences of cytochrome oxidase II and NADH dehydrogenase subunit 3 (Chesser 2004). We checked the diagnostic for phylogenetically independent contrasts as suggested by Díaz-Uriarte & Garland (1996) and found that these branch lengths standardized the contrasts adequately.

Because our study was conducted to test directional hypotheses on the relationship between diet (as estimated by ¹³C) and osmoregulatory traits, we used one-tailed tests. Data are presented as mean ± SD.

Results

We found significant interspecific variation in $\delta^{13}\text{C}$ ($F_{4,49} = 16.36$, $P < 0.001$, Fig. 1). In agreement with the marine diet of *Cinclodes nigrofumosus*, the tissue of this species had the most ¹³C-enriched isotopic composition. The tissue of the mostly riparian *Cinclodes atacamensis* had the most ¹³C-depleted isotopic composition (Fig. 1), and those of the three species that shift between freshwater and coastal habitats had intermediate $\delta^{13}\text{C}$ values. We also found significant variation in U_{\max} , P_{osm} and U/P ratios (Table 1). As predicted, the ability to concentrate

Table 1. Interspecific variation in the ability to concentrate urine (U_{\max} in mOsm kg⁻¹), in plasma osmolality (P_{osm} , mOsm kg⁻¹), in U/P ratios and carbon isotopic composition ($\delta^{13}\text{C}$) within the genus *Cinclodes*. The last column in this Table lists F and P -values for one way ANOVA

	<i>C. nigrofumosus</i>	<i>C. fuscus</i>	<i>C. patagonicus</i>	<i>C. oustaleti</i>	<i>C. atacamensis</i>	$F_{4,49}$
U_{\max}	1017.0 ± 101.1	878.7 ± 79.1	832.7 ± 99.3	878.6 ± 110.8	765.0 ± 31.1	9.4 ($P < 0.001$)
P_{osm}	390.9 ± 36.0	383.7 ± 25.9	366.5 ± 28.3	379.0 ± 20.6	327.5 ± 17.7	5.46 ($P = 0.01$)
U/P ratio	2.65 ± 0.4	2.12 ± 0.21	2.24 ± 0.23	2.31 ± 0.24	2.31 ± 0.2	7.39 ($P < 0.001$)
$\delta^{13}\text{C}$	-14.06 ± 1.42	-21.29 ± 1.23	-19.33 ± 2.06	-16.49 ± 3.26	-22.55 ± 2.16	16.4 ($P < 0.001$)
N	15	8	10	13	4	

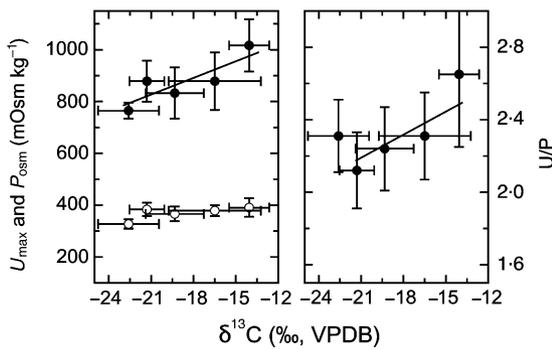


Fig. 2. In five species of the genus *Cinclodes* the ability to concentrate urine (filled circles) was positively correlated with $\delta^{13}\text{C}$ (left panel, $U_{\max} = 1303.4 + 22.9\delta^{13}\text{C}$, $r = 0.86$). The osmolality of plasma (open circles) is shown for reference. The ratio of U_{\max} and P_{osem} (U/P) was also positively correlated with $\delta^{13}\text{C}$ (right panel, $\text{U/P} = 3.1 + 0.04\delta^{13}\text{C}$, $r = 0.77$).

urine as estimated by U_{\max} and U/P was positively correlated with $\delta^{13}\text{C}$ ($r = 0.86$ and $r = 0.77$, $P < 0.05$, respectively, Fig. 2). The results of phylogenetically independent contrasts were very similar to those of standard correlation ($r(\text{PIC}) = 0.87$ and $r(\text{PIC}) = 0.88$, $P < 0.05$, respectively). *Cinclodes nigrofumosus*, the coastal species, had U_{\max} values that were from 15 to 32% higher than those of all other species. Although the highest and lowest plasma osmolalities were recorded in the most marine and in the most freshwater species, respectively, the correlation between $\delta^{13}\text{C}$ and P_{osem} was not statistically significant ($r = 0.63$, $r(\text{PIC}) = 0.61$, $P > 0.1$).

The allometric exponent of the relationship between kidney mass and body mass did not differ from 1 when estimated by either standard regression analysis (exponent \pm SE = 1.10 ± 0.31 , $t = 0.6$, $P > 0.2$) or by PICs (exponent \pm SE = 1.12 ± 0.24 , $t = 0.88$, $P > 0.2$). Because kidney mass was isometrically related to body mass, the percentage of total body mass represented by the kidney is an adequate estimate of relative kidney mass. Using both standard regression and PICs we found that the percentage of body mass accounted by the kidneys was tightly correlated with $\delta^{13}\text{C}$ ($r = 0.99$ and $r(\text{PIC}) = 0.99$, $P < 0.001$, Fig. 3). As predicted, both the percentage of

kidney tissue comprising medulla ($r = 0.87$, $r(\text{PIC}) = 0.88$, $P < 0.05$) and the number of medullary cones per gram of kidney were positively correlated with $\delta^{13}\text{C}$ ($r = 0.90$, $r(\text{PIC}) = 0.93$, $P < 0.05$; Fig. 3). The maximal osmolality of urine (U_{\max}) was highly correlated with relative kidney size ($r = 0.86$, $r(\text{PIC}) = 0.87$, $P < 0.05$), with the percentage of the kidney comprising medulla ($r = 0.99$, $r(\text{PIC}) = 0.99$, $P < 0.05$), and with the number of medullary cones per gram of kidney ($r = 0.97$, $r(\text{PIC}) = 0.99$, $P < 0.05$).

Discussion

The range of interspecific variation in concentrating ability and renal morphology found in five species of the genus *Cinclodes* was almost as large as that observed in all other birds (Tables 1 and 2). Among birds, U_{\max} ranges from 600 to 1000 mOsm kg^{-1} (Goldstein & Skadhauge 2000), compared with 765–1017 mOsm kg^{-1} in *Cinclodes*. With the exception of only the Savannah Sparrows, *Passerculus sandwichensis* (Poulson & Bartholomew 1962), which has been reported to concentrate excreta to a singularly high degree (≈ 2000 mOsm kg^{-1}), no avian species seems to be able to concentrate urine above approximately 1000 mOsm kg^{-1} . It is noteworthy, however, that the urine osmolality produced by field-caught Savannah Sparrows inhabiting salt marshes is significantly lower (mean mOsm $\text{kg}^{-1} \pm$ SD = 577.0 ± 163.6 , $N = 27$, Goldstein *et al.* 1990) than that measured in the laboratory, and lower than the measurements reported for *Cinclodes* species. The percentage of the kidney comprising medullary tissue ranges from 5 to 22% in birds (Johnson & Mugaas 1970a, b), and the number of medullary cones per gram of kidney ranges from 63 to 600 (Goldstein & Braun 1989). Among the five species of *Cinclodes* that we studied, the percentage of medullary tissue and the number of medullary cones per gram of kidney ranged from 11 to 34% and from 141 to 840 cones g^{-1} of kidney, respectively. Interspecific phenotypic diversification is one of the criteria often associated with adaptive radiations (Losos & Miles 2002). The diversity in osmoregulatory traits in *Cinclodes* seems to fully satisfy this criterion.

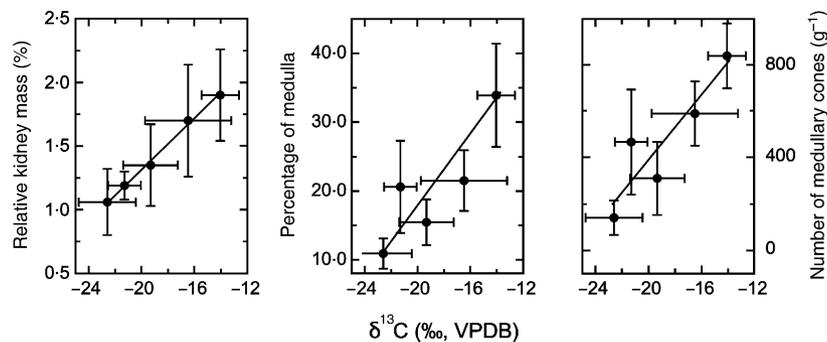


Fig. 3. In five species of the genus *Cinclodes* the relative mass of the kidney ($y = 3.35 + 0.101x$, $r = 0.99$), the percentage of the kidney comprising medullary tissue ($y = 60.83 + 2.2x$, $r = 0.89$) and the number of medullary cones per gram of kidney ($y = 1770.5 + 69.4x$, $r = 0.90$) were positively correlated with $\delta^{13}\text{C}$.

Table 2. Differences in body mass and renal morphology among five *Cinclodes* species. The last column in this Table lists *F* and *P*-values for one-way ANOVA. Sample sizes per species are listed in Methods. The number of medullary cones is for both kidneys

	<i>C. nigrofumosus</i>	<i>C. fuscus</i>	<i>C. patagonicus</i>	<i>C. oustaleti</i>	<i>C. atacamensis</i>	<i>F</i> _{4,49}
Body mass	69.5 ± 6.9	28.8 ± 2.5	38.8 ± 2.5	25.3 ± 2.4	53.2 ± 4.8	220.0 (<i>P</i> < 0.001)
Kidney mass (% of body mass)	1.91 ± 0.36	1.19 ± 0.11	1.35 ± 0.32	1.70 ± 0.44	1.06 ± 0.26	9.7 (<i>P</i> < 0.001)
Medulla (% of kidney mass)	33.9 ± 7.5	20.6 ± 6.7	15.45 ± 3.3	21.48 ± 4.4	10.91 ± 2.2	27.1 (<i>P</i> < 0.001)
Medullary cones	941.1 ± 157.5	128.9 ± 47.7	144.2 ± 57.6	225.3 ± 70.4	80.7 ± 21.9	–
Medullary cones g ⁻¹ kidney	840.2 ± 140.0	468.1 ± 227.1	311.1 ± 158.2	591.0 ± 139.1	141.3 ± 74.6	31.2 (<i>P</i> < 0.001)

The structure and function of the *Cinclodes* kidney varied predictably depending on each species' ecological characteristics. *Cinclodes nigrofumosus*, the most coastal species (Sabat *et al.* 2003), had the largest kidneys with the highest number of medullary cones and produced the most concentrated urine. In contrast, *C. atacamensis*, the most specialized inhabitant of freshwater streams, had the smallest kidneys with the lowest number of medullary cones and produced the most dilute urine. Within the genus *Cinclodes*, the ability to produce concentrated urine, and hence to cope 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 (Goldstein & 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 & Braun 2000 and Sabat 2000) and non-passerines (Hughes 1970).

Both renal structure and concentrating ability of *Cinclodes* were correlated with the carbon isotope composition of the birds' pectoralis. The significant correlations between $\delta^{13}\text{C}$ and the renal morphology and urine-concentrating ability among *Cinclodes* species are significant because (1) they support the notion that the stable isotope composition of animal tissues can be used as a surrogate variable for ecological characteristics (Schondube, Martínez del Río & Herrera 2001) and (2) they strengthen the evidence for a phenotype–environment association in *Cinclodes*. Rather than using a categorical variable that classified each species as coastal, freshwater or as shifting between these two environments, the well-documented strong difference in carbon isotope composition between terrestrial and marine food webs provided us with a continuous variable, $\delta^{13}\text{C}$, that 'diagnosed' the osmotic dietary load experienced by each species (see also Sabat & Martínez del Río 2002, 2006). Stable isotopes appear to be a powerful tool for comparative studies, albeit one that remains underutilized (see Schondube *et al.* 2001).

A fit between the diverse phenotypes of the members of a monophyletic lineage and their divergent environments fulfils the adaptive criteria of an adaptive radiation (Schluter 2000). Such a fit must fulfil two conditions. First, testing for correlations between phenotype and environment must control for the potential phylogenetic non-independence of species traits (Harvey & Pagel 1991).

In our study, the use of phylogenetically independent contrasts satisfied this condition. Second, phenotypic difference between species must be genetically based and not only the consequence of phenotypic plasticity. Sabat *et al.* (2004) investigated the plasticity in osmoregulatory traits in three of the species included in our investigation: *C. nigrofumosus*, *C. patagonicus* and *C. oustaleti*. They provided birds with either tap water or a salt solution (NaCl, 800 mOsm kg⁻¹) for 2 weeks. *Cinclodes nigrofumosus* and *C. patagonicus* individuals exposed to salt water or tap water did not show differences in renal morphology or in the ability to concentrate urine. Remarkably, *C. oustaleti* individuals exposed to salt water had significantly larger renal medullae and were able to produce more concentrated urine than those exposed to tap water (Sabat *et al.* 2004). The effect of exposure to fresh or salt water, however, was relatively small and smaller than the interspecific differences reported in Tables 1 and 2. Although it is possible that phenotypic plasticity contributes to explain some of the interspecific differences reported here, we hypothesize that this contribution is small. However, we cannot yet discount the possibility that exposure to different environments during early development (or developmental plasticity *sensu* Piersma & Drent 2003 and Schlichting & Pigliucci 1998) contributes to variation in renal traits. At the moment we cannot partition the effect of phenotypic plasticity on the magnitude of the interspecific differences, and hence in the capacity to cope with dehydration and salty diets, observed among *Cinclodes* species.

Schluter (2000) identified rapid speciation as one of the ingredients of an adaptive radiation. Did *Cinclodes* show rapid speciation relative to sister clades? Chesser (2004) suggests that they did. He observed that the amount of intrageneric genetic divergence in *Cinclodes* is small relative to that of other bird genera of similar species richness, and suggested a recent origin for the species within the clade. He also pointed to the remarkable differences in morphology and plumage between sister species. Two pairs of sister species are noteworthy: *C. nigrofumosus* and *C. patagonicus*, and *C. antarcticus* and *C. fuscus*. The species in these two pairs are genetically differentiated by less than 2% in sequence divergence, yet they differ greatly in size, plumage and ecological habits. Our results on *C. nigrofumosus* and *C. patagonicus* brought to light large differences in renal form and function. To our knowledge, there is no information on the osmoregulatory traits of *C. antarcticus*. We hypothesize

that this species, like *C. nigrofumosus*, will have relatively large kidneys with a high proportion of medulla, and the capacity to produce highly concentrated urine. The coastal habit of *C. antarcticus* and *C. nigrofumosus* evolved independently, presumably from an ancestor that shifted between freshwater and marine environments. Scrutiny of their osmoregulatory biology provides an unparalleled opportunity to test the possibility of convergent evolution in osmoregulatory function.

In conclusion, what is known of birds in the genus *Cinclodes* supports the notion that their monophyletic clade represents an adaptive radiation. Available phylogenetic data support the notion of rapid speciation accompanied by phenotypic diversification. The correlation between renal structure and function and reliance on terrestrial and marine resources shores up the criterion of adaptive phenotype–environment correlation. Perhaps the most characteristic feature of the clade is the diversity in the reliance of its member species on marine/coastal and freshwater environments. Because dependence on marine diets must be accompanied by adjustments in the capacity to excrete salt, the genus *Cinclodes* seems to represent an example of an adaptive radiation in osmoregulatory function. Our study focused on only 5 of 12 species and on only a handful of physiological traits. Further research on more *Cinclodes* species and on a wider array of traits may illuminate the interplay between physiological traits and ecological processes in avian adaptive radiations. It may hold the key to understanding why there are so few marine passerines that inhabit coastal habitats and that feed on marine sources.

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