Multi-tissue $\delta^2$H analysis reveals altitudinal migration and tissue-specific discrimination patterns in *Cinclodes*

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**Abstract.** One of the fastest growing uses of stable isotope analysis in ecology is using hydrogen isotope ($\delta^2$H) values to characterize animal movement and migration strategies. Most studies measure $\delta^2$H values in metabolically inert tissues such as feathers, which are typically grown during or just after the summer breeding season and provide a limited snapshot of an individual’s annual life history. In contrast, isotopic analysis of metabolically active tissues can provide ecological information integrated over weeks to months prior to sampling. Here we characterize $\delta^2$H patterns among multiple metabolically inert and active tissues in *Cinclodes*, a genus of South American songbirds noted for variation in altitudinal movement and foraging strategies. We also coupled $\delta^2$H with carbon ($\delta^{13}$C) and nitrogen ($\delta^{15}$N) isotope data to combine information on movement with marine versus terrestrial resource use at the individual level. We find that a combination of physiological and ecological factors control $\delta^2$H patterns among tissues, which mirrors results of feeding experiments on captive birds. For example, in the coastal resident *C. nigrofumosus*, metabolically active muscle collected during the winter has higher $\delta^2$H values than feathers grown the previous summer, a tissue-specific discrimination pattern previously observed in captive birds. This pattern is reversed to various degrees for altitudinal migrants such as *C. fuscus* and *C. oustaleti* that spend winters foraging in marine intertidal habitats but migrate to high elevation and forage in stream habitats during the summer. We also find that among altitudinal migrants, individuals that forage sympatrically in intertidal habitats during the winter appeared to summer at a wide range of elevations, as evidenced by large differences of $>50\%$ in $\delta^2$H\textsubscript{muscle-feather} offsets. Lastly, a positive correlation between feather $\delta^2$H and $\delta^{15}$N values in *Cinclodes* that consume a mixed marine-freshwater diet confirms that $\delta^2$H is a useful proxy for quantifying marine resource use. We anticipate that comparison of $\delta^2$H values in metabolically active and inert tissues may allow for the reconstruction of animal movement and foraging strategies within the annual life cycle; however, more work is required to better understand the physiological mechanisms responsible for the observed $\delta^2$H patterns among tissues.

**Key words:** $\delta^2$H; $\delta^{13}$C; $\delta^{15}$N; altitudinal migration; isotope discrimination.

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INTRODUCTION

Characterizing animal movement and connecting it to habitat and resource utilization is a research area that has implications for both conceptual and applied questions in ecology and wildlife management. Over the past two decades, ecologists interested in this area have been aided by the development of both extrinsic and intrinsic technologies that are now routinely used to track animal movement. While costly and potentially intrusive to deploy and retrieve, extrinsic radio and satellite tags offer high-resolution spatial and temporal data that can be correlated with both abiotic and biotic information, which allows ecologists to connect movement patterns with environmental conditions and habitat use across landscape scales. With the exception of tagging technologies that include cameras (Heithaus et al. 2001, Naito et al. 2013), directly connecting tag-derived movement information with resource use remains a challenge.

In step with the development of extrinsic tagging technologies, stable isotopes of hydrogen (δ²H) and to a lesser extent oxygen (δ¹⁸O), carbon (δ¹³C), nitrogen (δ¹⁵N), and strontium (δ⁸⁷Sr) have become a common tool for assessing animal movement and migration strategies (e.g., Koch et al. 1995, Chamberlain et al. 1997, Hobson 1999, Hobson and Wassenaar 1997, Hobson et al. 1999b, Rubenstein et al. 2002, Rubenstein and Hobson 2004, Cryan et al. 2004, Bowen et al. 2005a, Wunder et al. 2005, Sellick et al. 2009, Graham et al. 2010). The hydrogen isotope approach relies on correlating the δ²H of animal tissues with that of long-term amount-weighed local precipitation integrated over annual or seasonal timescales, which is primarily controlled by abiotic factors such as temperature, altitude, and the source(s) of precipitation that varies predictably across continental scales. Maps of spatial variation in δ²Hprecipitation values are called hydrogen isoscapes sensu West et al. (2006). An obvious advantage to this approach is that isotopes can be used to study animals for which satellite tags are not feasible because of weight limitations, since extrinsic tags must be ≤3% of body weight for terrestrial species. Other advantages of δ²H analysis include the need for only one capture event to collect tissues and that this approach can be combined with carbon (δ¹³C) and nitrogen (δ¹⁵N) analysis to directly connect movement, habitat and resource use at the individual level (Yohannes et al. 2007, Wunder et al. 2012).

The vast majority of studies that use δ²H to study animal movement exploit latitudinal δ²H isoscapes; however, δ²H also varies predictably with altitude (Poage and Chamberlain 2001) and has been used to examine altitudinal distributions in birds. Hobson et al. (2003) used δ²H to examine altitude of origin in a community of hummingbirds collected across an altitudinal gradient from ~300 m to ~3300 m in the Ecuadorean Andes. Ecuadorean hummingbirds are an ideal group for using δ²H to examine altitude of origin because (1) altitudinal gradients in the Andes are large (500–6000 m) in comparison to most mountainous regions of the world, and (2) hummingbirds are primarily nectarivorous and thus any potential δ²H variation related to trophic level is minimized. This study found a significant negative correlation between δ²Hprecipitation and altitude (r² = 0.68, n = 11), but noted that the relationship between hummingbird δ²Hfeather values and altitude was not linear; most variation in δ²Hfeather occurred between 1300 and 3300 m. A more recent study in the Ecuadorean Andes examined feather and blood δ²H values of a diverse group (18 species) of passerines collected along an altitudinal gradient from 1350 to 3500 m (Hardesty and Fraser 2010). Species were grouped into insectivorous and nectarivorous foraging guilds, but sample sizes were low for most species (<3 individuals). Both feather and blood showed a significant negative trend in δ²H with increasing altitude, but the percentage of δ²H variation explained by altitude was low, ranging from 10% to 62% depending on foraging guild and tissue type. Lastly, Boyle et al. (2011) used δ²H values to characterize altitudinal movement in white-ruffed manakins (Corapipo altera), but examined a narrow altitudinal range (<1000 m) that resulted in a small but significant mean difference of ~15% between claws of birds collected from high and low elevation sites.

Most studies that use δ²H to examine latitude or altitude of origin in avian species have analyzed feathers, a tissue that is grown by most passerines in the late summer after breeding. Because feathers are metabolically inert and do not exchange with the body pool of hydrogen (or
carbon and nitrogen) after formation, they can be collected during migration or on wintering grounds to estimate the latitude or altitude of breeding (Kelly et al. 2002, Rubenstein et al. 2002, Wunder et al. 2005, Yohannes et al. 2007). Ecologists have devised clever strategies for examining temporal variation in the type of information gained from $\delta^2$H analysis. For example, analysis of different feathers (e.g., primary sequence) or portions of the same feather collected from a single individual can provide a within-breeding season time series of ecological information (e.g., Knoff et al. 2002). Furthermore, analysis of different types of feathers (primaries and rectrices) in species that undergo two molts per year can provide information from two discrete time periods during the year (Mazerolle and Hobson 2005). Because these approaches are based on feathers, they largely record ecological information within the breeding season. In contrast, comparison of $\delta^2$H values of metabolically active (e.g., blood, liver, muscle) versus inert (feathers) tissues collected during a single capture event may allow ecologists to track shifts in movement and foraging throughout the annual life cycle. And since metabolically active tissues have similar amounts of exchangeable hydrogen (~15–20%) as feather keratins (Wasse-naar and Hobson 2000), analytical protocols that account for hydrogen exchange with ambient water vapor are interchangeable and provide reliable $\delta^2$H measurements of a wide range of tissue types.

Previous work quantifying isotopic incorporation rates for metabolically active tissues that continuously turn over has shown that tissues have vastly different incorporation rates, varying from days to years (Martinez del Rio et al. 2009a). Liver and plasma proteins have high isotopic incorporation rates and their isotopic composition reflects recent ecological information integrated over several weeks prior to collection, while other metabolically active tissues like muscle integrate over longer timescales (months). In contrast, metabolically inert tissues like feathers only record information during the short and discrete period of growth, which may only last a few days. Thus, isotopic analysis of different tissues can provide information on changes in resource and habitat use over a variety of timescales. For example, Martinez del Rio et al. (2009b) analyzed $\delta^{13}$C and $\delta^{15}$N values of both metabolically inert and active tissues in the genera of South American passerines (Cinclodes) we studied in this paper to document temporal shifts in resource use between freshwater and marine habitats in Chile. Though this is a potentially powerful approach, one must control for isotopic differences among tissues that occur irrespective of ecology, a physiological phenomenon often referred to as tissue-specific discrimination.

Evidence of $\delta^2$H tissue-specific discrimination has been previously found in both field and laboratory studies. Hardesty and Fraser (2010) found that whole blood collected from Andean birds had significantly lower $\delta^2$H values at a given altitude than feathers, a pattern similar to that found by Wolf et al. (2012) for captive Japanese quail (Cortunix japonica) fed diets and water with consistent $\delta^2$H values. Mean muscle, liver, and plasma $\delta^2$H values reported by Wolf et al. (2012) were also significantly lower than mean feather $\delta^2$H values, a pattern observed across all dietary treatments. The physiological mechanisms that control $\delta^2$H tissue-specific discrimination have not been rigorously examined. As is the case for $\delta^{13}$C (Hare et al. 1991, Newsome et al. 2014) and $\delta^{15}$N (Popp et al. 2007), the $\delta^2$H of individual amino acids, which form the building blocks of proteinaceous tissues, varies depending on how they are synthesized or routed by an organism (Fogel et al. 2010). Thus, the isotopic composition of bulk tissues vary as a function of their amino acid concentration irrespective of ecologically related isotopic variation in food or water sources. Regardless, both ecological and/or physiological factors may cause inter-tissue $\delta^2$H variation, and a multiple-tissue approach requires a better understanding of tissue-specific $\delta^2$H discrimination and how it varies in natural settings.

Here we examine $\delta^2$H patterns among metabolically inert (feathers) and active (muscle, liver, blood) tissues collected from species in the genus Cinclodes, a group of South American passerines (Furnariidae) that include species with a diverse set of ecological characteristics. The following discussion of Cinclodes natural history is based on Jaramillo (2005), Sabat et al. (2006a), and Martínez del Rio et al. 2009b. Cinclodes nigrofumosus are residents that consume invertebrates in intertidal
C. patagonicus consume invertebrates in both marine and freshwater ecosystems year-round, but segments of the population likely undertake latitudinal migrations to forage in these ecosystems at high latitudes (>45° S) during the summer months. C. fuscus undertake altitudinal migrations, foraging in coastal marine and freshwater habitats during the winter and then migrate upslope during the summer to forage in streams at high elevation. C. oustaleti likely have populations that undertake both latitudinal and altitudinal movements and either forage in freshwater streams at high altitudes in central Chile (29–34° S) or in coastal marine and/or freshwater ecosystems at high latitudes (>45° S) during the austral summer, but then return to winter at low altitudes in central Chile.

In addition to δ²H values that served as a proxy for altitude, we measured tissue carbon (δ¹³C) and nitrogen (δ¹⁵N) values to discriminate between the relative use of freshwater versus marine resources within and among Cinclodes at different times of the year. Carbon and nitrogen isotope values are particularly useful for differentiating between marine from freshwater resource use because of differences in the structure (number of trophic levels) and δ¹³C and δ¹⁵N values of primary producers in each ecosystem (Kelly 2000). Consumers that rely on marine ecosystems typically have higher δ¹³C and δ¹⁵N values than freshwater aquatic ecosystems, a pattern that has been previously examined in Chile (Ehleringer et al. 1998, Sabat and Martinez del Rio 2002).

Such variation in movement and foraging ecology among species makes Cinclodes an ideal group to examine inter-specific differences in δ²H related to diet, habitat use, and altitudinal movement. Specifically, our study addressed three questions. First, can δ²H patterns among metabolically active (blood, muscle, liver) and inert (feathers) tissues be used to examine inter-individual variation in altitudinal migration? We predicted that for species that undertake altitudinal migrations (fuscus and oustaleti), the offset in δ²H values between feathers grown in the summer and that of metabolically active tissues (muscle) collected in the winter months would be greater than for resident species that remain at or near sea level throughout the year and consume a mixed marine-terrestrial (patagonicus) or fully marine (nigrofumosus) diet as indicated by their tissue δ¹³C and δ¹⁵N values. Second, for species that remain in the same environment throughout the year (nigrofumosus), are tissue-specific δ²H discrimination patterns similar to those observed for captive birds grown on diets and drinking water that had a consistent δ²H composition? Finally, what is the effect of a marine diet on tissue δ²H values? We expected that tissue δ¹⁵N values, a commonly used proxy for marine resource use, would positively covary with tissue δ²H in Cinclodes that consume a mixed marine-terrestrial diet. We anticipate that as the use of hydrogen isotopes to examine movement and foraging ecology expands to include the analysis of metabolically active tissues, the patterns observed here are useful for differentiating between ecological and physiological sources of δ²H variation in wild animal populations.

**Materials and Methods**

**Sample collection**

We analyzed tissues from Cinclodes specimens collected over the last ten years for related ecophysiological (Sabat et al. 2006a, 2006b) and ecological (e.g., Martinez del Rio et al. 2009b) studies. Hereafter, Cinclodes are identified by species (not genus). All birds were collected in Chile between 29° S and 41° S or in Tierra del Fuego, Argentina (54° S). Specimens from Chile were subdivided into two latitudinal regions: central Chile (29–34° S) and southern Chile (37–41° S). We also subdivided the dataset based on the season of capture/collection: austral winter corresponds to July–August, and austral summer corresponds to January–February. Tissues of C. fuscus and C. oustaleti, and C. nigrofumosus from central Chile as well as C. oustaleti and C. patagonicus from southern Chile were collected during the austral winter from birds at low (<300 m) elevation near the coast. We also collected tissues during the summer months from C. oustaleti from central Chile and Tierra del Fuego and C. patagonicus from southern Chile and Tierra del Fuego. See Table 1 and Figs. 1–4 for sample sizes of tissues analyzed from each species.
Table 1. Mean (± SD) tissues δ²H values for Cinclodes species by region and season of collection; italic numbers in parentheses represent sample sizes. Also included is information prevalence of altitudinal migration based on previous literature and the seasonal use of marine versus freshwater resources consumed by each species or population based on carbon (δ¹³C) and nitrogen (δ¹⁵N) isotope data presented in Figs. 1-4; mixed resource use represents a combination of marine and freshwater resource use as defined in Materials and Methods. See Introduction for more detailed information with associated references on the natural history of each species. Asterisks denote that oustaleti and patagonicus that summer in Tierra del Fuego are likely latitudinal (not altitudinal) migrants.

<table>
<thead>
<tr>
<th>Region, season, and species</th>
<th>Altitudinal migrant?</th>
<th>Diet (winter/summer)</th>
<th>Feather δ²H</th>
<th>Muscle δ²H</th>
<th>Liver δ²H</th>
<th>Blood δ²H</th>
</tr>
</thead>
<tbody>
<tr>
<td>Central Chile Winter fuscus</td>
<td>yes</td>
<td>mixed/freshwater</td>
<td>−106 ± 18 (9)</td>
<td>−81 ± 8 (9)</td>
<td>…</td>
<td>…</td>
</tr>
<tr>
<td>nigrofusus</td>
<td>no</td>
<td>marine/marine</td>
<td>−64 ± 9 (24)</td>
<td>−80 ± 6 (24)</td>
<td>−54 ± 9 (16)</td>
<td>…</td>
</tr>
<tr>
<td>oustaleti</td>
<td>yes</td>
<td>mixed/freshwater</td>
<td>−104 ± 21 (32)</td>
<td>−77 ± 11 (32)</td>
<td>−47 ± 13 (27)</td>
<td>−117 ± 10 (12)</td>
</tr>
<tr>
<td>Southern Chile Winter oustaleti</td>
<td>yes</td>
<td>mixed/freshwater</td>
<td>−78 ± 14 (18)</td>
<td>−81 ± 8 (18)</td>
<td>−49 ± 10 (17)</td>
<td>−113 ± 4 (14)</td>
</tr>
<tr>
<td>patagonicus</td>
<td>no</td>
<td>mixed/freshwater</td>
<td>−61 ± 10 (27)</td>
<td>−79 ± 6 (27)</td>
<td>−45 ± 7 (18)</td>
<td>−115 ± 8 (14)</td>
</tr>
<tr>
<td>Tierra del Fuego Summer oustaleti</td>
<td>no</td>
<td>mixed/freshwater</td>
<td>−80 ± 7 (18)</td>
<td>−89 ± 8 (18)</td>
<td>−67 ± 9 (16)</td>
<td>−92 ± 10 (11)</td>
</tr>
<tr>
<td>patagonicus</td>
<td>no</td>
<td>mixed/freshwater</td>
<td>−74 ± 11 (16)</td>
<td>−90 ± 8 (16)</td>
<td>−68 ± 14 (15)</td>
<td>−92 ± 10 (11)</td>
</tr>
</tbody>
</table>

Stable isotope analysis

For isotopic analysis of feathers, whole flank feathers were treated with a 2:1 chloroform:methanol solvent mixture to remove surface contaminants, barbs were cut into small (1 × 1 mm) pieces with scissors and then air-dried. Whole blood samples were air-dried in the field on glass microscope slides, scraped into microcentrifuge tubes, and homogenized by mixing. We did not lipid-extract whole blood samples because their mean weight-percent [C]/[N] ratios were within the range expected of unaltered protein containing negligible amounts of lipids (3.4–3.6). Homogenized sub-samples of pectoralis muscle and liver were lipid-extracted by three separate ~24 hour soaks in a 2:1 chloroform:methanol solvent mixture (Bligh and Dyer 1959); samples were subjected to ~15 minutes of sonication between each soak. Samples were then rinsed five times in deionized water and freeze-dried.

Approximately 0.1–0.2 mg of dried tissue was sealed in 3 × 5 mm silver capsules and subjected along with reference materials to bench-top equilibration to local water vapor δ²H for at least three weeks prior to analysis to account for exchangeable hydrogen (Wassenaar and Hobson 2000, Bowen et al. 2005b). δ²H values of the non-exchangeable portion of hydrogen were determined by comparative equilibration (Wassenaar and Hobson 2003) using three internal keratin δ²H reference materials, Turkey (~54%), Chicken (~94%), and Moose (~165%) for which non-exchangeable δ²H values were measured by comparative bench-top equilibration experiments (e.g., Bowen et al. 2005b) followed by external verification with other laboratories: University of Wyoming Stable Isotope Facility (Laramie, Wyoming, USA) and Savannah River Ecology Laboratory (Aiken, South Carolina, USA). Keratin reference materials have a similar proportion of exchangeable hydrogen (15–20%) as the metabolically active (muscle, liver, blood) tissues and thus are reliable proxies for correction of exchangeable hydrogen in samples of unknown δ²H composition (Wassenaar and Hobson 2000).

δ²H values were determined using a Thermo-Finnigan high-temperature conversion elemental analyzer (TCEA) coupled to a Thermo-Finnigan Delta Plus XL isotope ratio mass spectrometer at the Carnegie Institution of Washington (Washington, D.C., USA). For δ¹³C and δ¹⁵N analysis, dried tissue samples were sealed in 3 × 5 mm tin capsules. δ¹³C and δ¹⁵N values were determined using a Costech elemental analyzer (EA) coupled to a Thermo-Finnigan Delta Plus XL isotope ratio mass spectrometer at the University of Wyoming Stable Isotope Facility (Laramie, Wyoming, USA). Isotopic results are expressed as δ values,
Fig. 1. Paired muscle (closed circles) and feather (open circles) $\delta^{2}H$ values of four species of *Cinclodes* collected in coastal environments during the winter months (July–August) in central (29°–34° S) and southern (37°–41° S) Chile. Metabolically active muscle tissue represents winter ecological information, while feather $\delta^{2}H$ values represent ecological information from the summer prior to collection. Paired samples in each panel are sorted by highest (top) to lowest (bottom) feather $\delta^{2}H$ values. Dashed vertical lines denote mean $\delta^{2}H$ values and shaded areas represent standard deviation. Horizontal lines denote when feather $\delta^{2}H$ values are lower (solid) or higher (dashed) than associated muscle. Mean ($\pm$SD) muscle and feather $\delta^{13}C$ and $\delta^{15}N$ values are also provided to show seasonal marine versus freshwater resource use for each population; italic numbers in parentheses represent sample sizes. Also presented are feather (summer) $\delta^{13}C$ and $\delta^{15}N$ values for two *oustaleti* (panel C) and two *patagonicus* (panel D) individuals that had very different values than the mean feather $\delta^{13}C$ and $\delta^{15}N$ values of their respective populations.
\[ \delta^2H \text{ or } \delta^{13}C \text{ or } \delta^{15}N = 1000 \times \left( \frac{R_{\text{sample}} - R_{\text{standard}}}{R_{\text{standard}}} \right), \]

where \( R_{\text{sample}} \) and \( R_{\text{standard}} \) are the \(^2H/\text{H} \text{ or } ^{13}C/^{12}C \text{ or } ^{15}N/^{14}N \) of the sample and standard, respectively. The internationally accepted standard for hydrogen, carbon, and nitrogen isotope values are Vienna Standard Mean Ocean Water (V-SMOW), Vienna Pee Dee Belemnite (V-PDB), and atmospheric nitrogen respectively and the units are expressed as parts per thousand, or per mil (‰). Precision for \( \delta^2H \) was determined by analysis of the three exchangeable (keratin) reference materials described above; within-run \( \delta^2H \) variation (SD) of these reference materials on the mass spectrometer system described above was \( \leq 4\% \). All samples for \( \delta^2H \) analysis were run in duplicate; the absolute mean difference between duplicates was equal to or less than analytical precision. Precision for \( \delta^{13}C \) and \( \delta^{15}N \) was determined by analysis of acetanilide and alanine standards; within and among run variation (SD) was \( \leq 0.2\% \) for both \( \delta^{13}C \) and \( \delta^{15}N \). We also analyzed the weight-percent [C]/[N] ratios of each sample which for every tissue (including whole blood) were within the range of unaltered protein containing negligible amounts of lipids (3.0–3.6).

\textbf{Statistical analysis}

We used repeated measures multivariate analysis of variance or RM-MANOVA to test for significant differences in tissue \( \delta^2H \) within individuals of the same species (Martinez del Rio et al. 2009b). We also used a non-parametric Wilcoxon/Kruskal-Wallis one-way analysis of variance to identify significant differences in \( \delta^2H \) values of a particular tissue among species.

\textbf{RESULTS}

\textit{Feather} \( \delta^2H \)

Mean \( \delta^2H_{\text{feather}} \) values varied by 45\% among species, ranging from \(-61\% \) to \(-106\% \) (Table 1),
and there were significant differences among species ($\chi^2: 90.4$, DF = 6, $P < 0.0001$). Oustaleti feathers showed the widest range in $\delta^2$H values, but had significantly lower ($P < 0.01$) mean (±SD) $\delta^2$H values in central ($-104 \pm 21\%$) versus southern Chile ($-78 \pm 14\%$; z score: $4.19$, $P < 0.0001$) or Tierra del Fuego ($-79 \pm 6\%$; z score: $4.67$, $P < 0.0001$). Oustaleti feathers from southern Chile and Tierra del Fuego were statistically indistinguishable (z score: $-1.09$, $P = 0.28$). Oustaleti $\delta^2$H values were also more variable in central (SD = 21%) and southern (SD = 6%) Chile than in Tierra del Fuego (SD = 14%). Feathers of other species had $\delta^2$H standard deviations of $\leq 10\%$ (Levene F = 6.05, DF = 6, $P < 0.001$). The only exception to this pattern was fuscus collected from central Chile ($-106 \pm 18\%$), which had similar mean $\delta^2$H and associated standard deviation as Oustaleti collected from central Chile ($-104 \pm 21\%$; z score: $0.30$, $P = 0.76$). Patagonicus collected from southern Chile ($-61 \pm 10\%$) had significantly higher $\delta^2$H values than conspecifics from Tierra del Fuego ($-73 \pm 12\%$; z score: $-3.58$, $P < 0.001$) or Oustaleti collected from southern Chile ($-78 \pm 14\%$; z score: $4.14$, $P < 0.0001$). Mean $\delta^2$H values of nigrofumosus collected from central Chile ($-64 \pm 9\%$) were significantly higher than Oustaleti ($-104 \pm 21\%$; z score: $-5.84$, $P < 0.0001$) or fuscus ($-106 \pm 18\%$; z score: $4.26$, $P < 0.0001$) from this region, but were similar to $\delta^2$H values of patagonicus from southern Chile ($-61 \pm 10\%$; z score: $1.87$, $P = 0.06$). Lastly, $\delta^2$H values collected from Oustaleti ($-80 \pm 7\%$) and patagonicus ($-74 \pm 11\%$) in Tierra del Fuego were not significantly different (z score: $1.40$, $P = 0.16$).

**Muscle $\delta^2$H**

Mean $\delta^2$H values were surprisingly con-
sistent and varied by only 11% among species (Table 1). In addition, there were fewer significant differences among species ($\chi^2$: 34.5, DF = 6, $P < 0.0001$) in comparison to patterns among feather $\delta^2$H. Variance (SD) was also low and ranged from 6% to 11%. Muscle collected during the winter from oustaleti in central (−77 ± 11%) or southern (−81 ± 6%) Chile, patagonicus from southern Chile (−79 ± 6%), and fuscus (−81 ± 8%) or nigrofumosus (−80 ± 6%) from central Chile all had statistically indistinguishable $\delta^2$Hmuscle values; z scores: −1.64 to 1.85, $P > 0.10$. $\delta^2$Hmuscle values of oustaleti (−89 ± 8%) and patagonicus (−90 ± 8%) collected from Tierra del Fuego in the summer were identical (z-score: −0.07, $P = 0.95$), but significantly lower than mean $\delta^2$Hmuscle values collected during the winter from other species ($P < 0.05$).

**Blood $\delta^2$H**

Mean $\delta^2$Hblood values collected from central and southern Chile during the winter varied by 31% among species, ranging from −102% to −133% (Table 1), but we found no significant differences among species ($\chi^2$: 1.87, DF = 2, $P < 0.39$). There were no significant differences among oustaleti from central Chile (−117 ± 10%), oustaleti from southern Chile (−113 ± 4%), or patagonicus from southern Chile (−115 ± 8%; $P > 0.20$). Mean $\delta^2$Hblood values of oustaleti (−92 ± 10%) and patagonicus (−92 ± 10%) collected from Tierra del Fuego during the summer were identical (z score: 0.13, $P = 0.90$) but significantly higher than mean $\delta^2$Hblood values collected during the winter from oustaleti in central Chile ($P < 0.001$) and either oustaleti or patagonicus in southern Chile ($P < 0.001$).

**Liver $\delta^2$H**

Mean $\delta^2$Hliver values collected from central and southern Chile during the winter (Fig. 2) ranged from −30% to −59% (Table 1). There were significant differences ($\chi^2$: 8.0, DF = 2, $P = 0.02$) among oustaleti from central Chile (−38 ± 8%), oustaleti from southern Chile (−47 ± 9%), or patagonicus from southern Chile (−43 ± 6%). We also found significant differences between blood samples collected from Tierra del Fuego in the summer with those collected in the winter from central and southern Chile ($\chi^2$: 36.1, DF = 4, $P < 0.001$). Mean $\delta^2$Hliver values collected from oustaleti (−66 ± 10%) and patagonicus (−68 ± 15%) in the Tierra del Fuego during the summer (Fig. 3) were lower than liver collected in the winter from oustaleti in central Chile ($P < 0.001$) and either species in southern Chile ($P < 0.005$).

**Inter-tissue $\delta^2$H patterns**

Differences in mean $\delta^2$H values among muscle, blood, and liver tissues within one species collected in a single region varied by as much as −70% (Fig. 2), a range that was larger than differences in mean $\delta^2$Hfeather values among species (Fig. 1). Nigrofumosus from central Chile ($F$ value: 3.34, DF = 23, $P < 0.0001$) and patagonicus from southern Chile ($F$ value: 3.16, DF = 26, $P < 0.0001$) $\delta^2$Hfeather values were significantly higher than paired $\delta^2$Hmuscle. In contrast, paired oustaleti feather and muscle $\delta^2$H values from southern Chile were not significantly different ($F$ value: 0.05, DF = 17, $P = 0.37$). Oustaleti ($F$ value: 1.76, DF = 31, $P < 0.0001$) and fuscus ($F$ value: 2.04, DF = 8, $P = 0.004$) from central Chile had significantly higher $\delta^2$Hmuscle values than paired $\delta^2$Hfeather values.

$\delta^2$Hliver values were significantly higher than paired $\delta^2$Hmuscle across all species (Fig. 2). Nigrofumosus had mean $\delta^2$Hliver values that were higher by 26% than paired $\delta^2$Hmuscle ($F$ value: 8.29, DF = 15, $P < 0.0001$). Patagonicus from southern Chile had mean $\delta^2$Hliver values that were higher by 34% than paired mean $\delta^2$Hmuscle values ($F$ value: 26.00, DF = 17, $P < 0.0001$). Oustaleti from central Chile had mean $\delta^2$Hliver values that were higher by 30% than paired mean $\delta^2$Hmuscle values ($F$ value: 14.02, DF = 26, $P < 0.0001$). Oustaleti from southern Chile had mean $\delta^2$Hliver values that were significantly higher by 33% than paired $\delta^2$Hmuscle ($F$ value: 16.28, DF = 16, $P < 0.0001$).

$\delta^2$Hblood values were also significantly higher than paired $\delta^2$Hmuscle across all species (Fig. 2). Patagonicus from southern Chile had mean $\delta^2$Hmuscle values that were significantly higher by 36% than paired $\delta^2$Hblood ($F$ value: 25.91, DF = 13, $P < 0.0001$). Oustaleti from central Chile had mean $\delta^2$Hmuscle values that were significantly higher by 47% than paired $\delta^2$Hblood ($F$ value: 22.68, DF = 11, $P < 0.0001$). Lastly, oustaleti from southern Chile had mean $\delta^2$Hmuscle values that were significantly higher by 31% than paired $\delta^2$Hblood ($F$ value: 10.07, DF = 13, $P < 0.0001$).
For *oustaleti* and *patagonicus* collected in Tierra del Fuego during the summer (Fig. 3), $\delta^2H_{\text{liver}}$ values were higher than $\delta^2H_{\text{muscle}}$ or $\delta^2H_{\text{blood}}$. *Patagonicus* had mean $\delta^2H_{\text{liver}}$ values that were significantly higher by 23% than paired $\delta^2H_{\text{muscle}}$ ($F$ value: 4.16, $DF = 10, P < 0.0001$). *Oustaleti* had mean $\delta^2H_{\text{liver}}$ values that were significantly higher by 21% than paired $\delta^2H_{\text{muscle}}$ ($F$ value: 2.77, $DF = 10, P < 0.001$). Paired muscle and blood $\delta^2H$ values were similar in *patagonicus* ($F$ value: 0.12, $DF = 10, P = 0.31$) and *oustaleti* ($F$ value: 0.39, $DF = 10, P = 0.08$) collected from Tierra del Fuego in the summer. This similarity is in direct contrast to the observed differences between paired muscle and blood $\delta^2H$ values seen in *Cinclodes* tissues collected in the winter from central and southern Chile.

**Tissue $\delta^{13}C$ and $\delta^{15}N$**

Mean $\delta^{13}C$ and $\delta^{15}N$ values for *Cinclodes* tissues are reported in Table 1 and Figs. 1–3. $\delta^{13}C$ and $\delta^{15}N$ values have been previously utilized to characterize the relative use of marine versus terrestrial resources in *Cinclodes* (Sabat et al. 2006a, 2006b, Martinez del Río et al. 2009b). In agreement with previous work, we found that *nigrofumosus*, a coastal resident that forages on marine invertebrates in intertidal habitats, have high mean tissue isotope values that range from approximately −12‰ to −13‰ for $\delta^{13}C$ and from 18‰ to 19‰ for $\delta^{15}N$ (Fig. 1). In contrast, feather $\delta^{13}C$ and $\delta^{15}N$ values of migratory species such as *fuscus, oustaleti*, and *patagonicus* are much lower and range from −19‰ to −22‰ for $\delta^{13}C$ and from 4‰ to 10‰ for $\delta^{15}N$ (Fig. 1). $\delta^{13}C$ and $\delta^{15}N$ values of metabolically active tissues like muscle, liver, and blood of seasonal migrants such as *fuscus, oustaleti*, and *patagonicus* that were collected during the winter when these species may forage in intertidal marine habitats have intermediate mean isotope values that range from −14‰ to −18‰ for $\delta^{13}C$ and from 13‰ to 16‰ for $\delta^{15}N$ (Fig. 2). Note that variance in mean $\delta^{13}C$ and $\delta^{15}N$ values as measured by standard deviation is high in migratory species and ranges from 1.5‰ to 3.7‰, while variance is <1‰ for both isotopes in resident *nigrofumosus*. Lastly, metabolically active tissues for *oustaleti* and *patagonicus* collected from Tierra del Fuego in the summer also have intermediate mean isotope values that range from −17‰ to −20‰ for $\delta^{13}C$ and from 9‰ to 14‰ for $\delta^{15}N$ (Fig. 3).

**DISCUSSION**

Our multiple tissue and species approach provides unique insight into the interaction between ecological and physiological factors responsible for observed patterns in $\delta^2H$ values. There are two general patterns apparent in our dataset that highlight what ecologists can learn from $\delta^2H$ analysis of multiple tissues. First, our study shows that comparing $\delta^2H$ values in metabolically inert (e.g., feathers) to metabolically active (blood, muscle, or liver) tissues collected during the winter months is an informative approach to study variation in altitudinal movement patterns at the individual level. Second, observed $\delta^2H$ variation within feathers of migratory species (e.g., *fuscus or oustaleti*) that is likely attributable to altitudinal movement is of similar magnitude to the $\delta^2H$ variation among tissues within resident species (*nigrofumosus*), suggesting that tissue-specific discrimination mediated by physiological processes is an important factor to consider when comparing $\delta^2H$ values among tissues. In the following sections, we focus on patterns in $\delta^2H$ among tissues and species, but use associated $\delta^{13}C$ and $\delta^{15}N$ data to characterize temporal shifts in marine versus terrestrial resource use, which enables us to (1) determine whether tissues have equilibrated with local diet and water hydrogen sources at the time of collection, and (2) examine the influence of a marine versus terrestrial diet on tissue $\delta^2H$ values.

**Examining individual variation in altitudinal migration with $\delta^2H$: a multi-tissue approach**

Southern South America is an ideal region to study altitudinal (or latitudinal) movement patterns with $\delta^2H$. Specifically, Chile is exceptional because of its relatively narrow longitudinal (67°–73° W) range in comparison to large latitudinal (17–55° S) and altitudinal (0–4000 m) ranges. These geographical features combine to produce a $\delta^2H_{\text{precipitation}}$ isoscape with nearly 100% of variation (Bowen et al. 2005a). This variation is...
comparable to that observed across the continental United States, which is ~10 times larger than Chile in land area (IAEA/WMO 2011). Moreover, much of the variation in the δ²Hprecipitation isoscape of southern South America is driven by altitude rather than latitude (Bowen et al. 2005a).

δ²H data for tissues collected during the winter largely conformed to our expectations based on what is known about the annual life history of the species we examined. Specifically, our results show that the genus Cinclodes is composed of species that lie along the entire spectrum of movement strategies, from residents to altitudinal migrants. In addition, comparison of δ²H values from paired feather and muscle tissue collected during the winter allowed us to identify variation in movement strategies at the individual level within species. First, nigrofumosus feather and muscle δ¹³C and δ¹⁵N values presented here (Fig. 1E) and in previous studies (Sabat et al. 2006a, Martinez del Rio et al. 2009b) show that this species resides in coastal habitats and consumes marine intertidal invertebrates year-round. We consider the mean δ²Hfeather-muscle offset of +15‰ (Fig. 1E) observed in nigrofumosus to represent the non-migratory resident (marine) end-member offset for comparison to other Cinclodes species that are altitudinal migrants and switch between consuming marine resources in winter and foraging in freshwater streams at high elevation during the summer. A δ²Hfeather-muscle offset of similar direction but larger magnitude (~+30‰) was observed in captive quail (Wolf et al. 2012) that were fed isotopically homogenous food and drinking water. The δ²Hfeather-muscle offset observed in C. nigrofumosus and previously reported for captive quail is probably primarily driven by tissue-specific δ³H discrimination related to physiology (i.e., tissue biosynthesis) rather than ecology. Variation in the magnitude of the δ³H offset between tissues, however, may relate to differences in the diet (e.g., marine vs. terrestrial) and water (e.g., preformed vs. metabolic) consumed by different birds, but more δ³H data from a variety of tissues and species is needed to examine general trends related to physiology and/or ecology.

Patagonicus collected from southern Chile (Fig. 1D) also have a similar δ²Hfeather-muscle offset (+15‰) as nigrofumosus from central Chile; mean feather and muscle δ²H values are identical between the two species. Patagonicus feather (Fig. 1D) and muscle (Fig. 2B) δ¹³C and δ¹⁵N values indicate that most individuals analyzed (25/27 or 93%) consume a mixed diet of marine and freshwater resources in the summer and winter and thus are not altitudinal migrants. Even though these species consume different proportions of marine versus terrestrial resources they have similar δ²Hfeather-muscle offsets, a result that suggests physiology rather than ecology is primarily responsible for the observed pattern in δ²H values between tissues. Two patagonicus individuals, however, had relatively low feather δ¹³C and δ¹⁵N values, indicating consumption of terrestrial resources during the summer; individuals labeled Freshwater in Fig. 1D). One of these individuals had feather values that were ~20‰ lower than associated muscle, a pattern similar to that observed in species (fuscus and oustaleti) that undertake altitudinal migrations in the summer (see below). The other individual had feather δ²H values indicating that it specializes on freshwater habitats at low altitudes throughout the year.

In contrast to the patterns for nigrofumosus and patagonicus, feather δ²H values of fuscus and oustaleti collected from central Chile, which are commonly observed foraging in alpine streams at high altitudes (>2500 m) during the summer months, are typically lower than paired muscle tissue (Table and Fig. 1A, B). Andean datasets show that precipitation δ²H values decrease by ~8–10‰ for every 500-m increase in altitude (Hardesty and Fraser 2010, IAEA/WMO 2011). Since fuscus and oustaleti feather δ¹³C and δ¹⁵N values indicate terrestrial resource use, the apparent switch in the direction of the δ²Hfeather-muscle offset is likely driven by altitudinal migration in populations of these species that winter in central Chile. Furthermore, the degree of variation observed in the δ²Hfeather-muscle offset among fuscus and oustaleti individuals suggests that these species summer at a wide range of altitudes in central Chile. One oustaleti individual collected from central Chile appeared to be a low altitude resident and has feather δ²H values that were +15‰ higher than paired muscle tissue (Fig. 1B), a δ²Hfeather-muscle offset similar to that observed in nigrofumosus and patagonicus (Fig.
Feather δ²H values for all other *fuscus* and *oustaleti* individuals were lower than associated muscle by ~10–70‰. After application of an altitudinal lapse rate of 8–10‰/500 m for δ²H values of precipitation, estimated breeding altitudes for *oustaleti* and *fuscus* are between ~1400 and ~4800 m, a range that agrees with observed breeding ranges for these species in central Chile (Fjeldså and Krabbe 1990, Jaramillo 2005). Our δ²H-based breeding altitude estimates, however, should be viewed with caution for several reasons. First, previous studies have noted non-linearity in altitudinal δ²H lapse rates in the Andes (Hobson et al. 2003), and altitudinal δ²H lapse rates for Ecuadorean insectivorous passerines were found to be approximately half (4–5‰/500 m) that of precipitation (Hardesty and Fraser 2010). Reduced altitudinal lapse rates for bird tissues could be caused by the extremely large altitudinal gradients in large mountain ranges like the Andes, especially if birds are drinking directly from rivers/streams that are flowing at high velocity downhill and thus integrate precipitation (rain/snow) over large altitudinal ranges. Second, the influence of a marine versus freshwater diet on tissue δ²H values requires further consideration since δ²H values of marine invertebrates are likely higher than freshwater aquatic invertebrates; this issue is discussed in detail below, see section entitled *Influence of marine versus freshwater diet on δ²H values*. Another five *oustaleti* individuals from southern Chile have feather δ²H values that are ~10–30‰ lower than associated muscle and thus undertake altitudinal migrations during the summer. By applying a +15‰ correction to account for tissue-specific δ²H discrimination between feather and muscle and an altitudinal δ²H precipitation lapse rate of 8–10‰/500 m, we cautiously estimate breeding altitudes of between ~1400 and ~2500 m for these five *oustaleti* individuals from southern Chile that have lower feather δ²H values than associated muscle. This altitudinal range conforms to expectations based on personal observation and elevations of potential breeding habitat, which is lower in southern than central Chile (Fjeldså and Krabbe 1990). Overall, our data suggest that most *oustaleti* from southern Chile breed at low altitudes in terrestrial (freshwater) environments. More interesting is the apparent range in movement strategies used by the southern Chile *oustaleti* population and how variation in individual movement strategies differs from their counterparts in central Chile (Fig. 1B). Overall, differences among individual δ²H feather-muscle offsets highlight the potential to evaluate migration strategies at the individual level, which could be an effective approach to examine flexibility in individual physiological traits associated with altitudinal or osmoregulatory adaptation within (or among) species (Jessen et al. 1991, Sabat et al. 2006a, 2006b, Cheviron et al. 2008). In addition to highlighting flexibility in movement strategies within and among species of *Cinclodes*, our approach that compared metabolically active (muscle, liver, whole blood) to metabolically inert (feathers) tissues also represents a novel methodological approach, as there have been only two other studies to our knowledge that investigated inter-tissue δ²H variation in wild animal populations (Mazerolle and Hobson 2005, Hardesty and Fraser 2010).
Tissue specific δ²H discrimination: patterns among liver, muscle, and blood

The consistent δ²H offsets observed among metabolically active tissues in both resident and migratory Cinclodes collected during the winter months (Fig. 2) strongly suggests that these patterns are driven by a physiological rather than ecological mechanism. As discussed above, δ¹³C and δ¹⁵N data show that nigrofumosus are residents that spend the entire year foraging in coastal marine habitats (Fig. 1E). δ²Hliver values for nigrofumosus were ~25% higher than mean δ²Hmuscle (Fig. 2A). A similar pattern was also observed in patagonicus from southern Chile, another species that is found year-round in coastal and freshwater habitats at low altitudes, which had mean δ²Hliver values that were ~30% higher than mean δ²Hmuscle values (Fig. 2B). In addition, δ²Hblood values in patagonicus, a tissue we did not collect from nigrofumosus, were ~30% lower than δ²Hmuscle.

The general δ²H pattern among tissues in nigrofumosus and patagonicus is also mirrored in oustaleti tissues collected from central and southern Chile (Fig. 2C, D), which migrate upslope during the summer to breed and forage in alpine stream habitats at >2000 m. During the winter in central Chile, oustaleti forage sympatrically with nigrofumosus and consume a mixed marine and freshwater diet, as indicated by the high mean δ¹³C and δ¹⁵N values and associated error (SD = 1.2–3.2‰) of liver, muscle, and blood tissues (Fig. 2C). Likewise, oustaleti and patagonicus co-occur in coastal habitats during the winter in southern Chile and also have high mean δ¹³C and δ¹⁵N values indicative of a mixed marine and freshwater diet (Fig. 2). Similarity in δ²H patterns among liver, muscle, and blood tissues in both migratory and resident species that forage sympatrically in central and southern Chile in the winter shows that tissue-specific discrimination is a major source of δ²H variation in our dataset; a conclusion that assumes that the migratory species have been on their wintering grounds long enough for their metabolically active tissues to equilibrate with local sources of hydrogen. We suggest that such physiologically mediated isotopic variation must be accounted for when using a multiple-tissue approach to examine movement patterns and temporal shifts in diet and/or habitat.

The δ²H offsets observed among tissues in Cinclodes (Fig. 2) are similar in direction but of higher magnitude than those found by Wolf et al. (2012) for captive quail fed diets and drinking water with consistent δ²H compositions. Quail liver δ²H values were on average 3–13% higher than muscle, which were 2–11‰ higher than red blood cells. At present it is difficult to identify why free-ranging Cinclodes have larger δ²H offsets among metabolically active tissues in comparison to captive quail; this contrast highlights the need for more experiments designed to understand how hydrogen isotopes are assimilated and sorted during tissue biosynthesis. For example, the relative proportion of hydrogen derived from water versus food appears to vary among tissues and bird species (Hobson et al. 1999a, Wolf et al. 2011, 2012, Storm-Suke et al. 2012). These patterns are likely caused by a combination of factors acting at both the molecular and organismal level, such as differences in amino acid composition among tissues as well as drinking water requirements and general dietary preferences among species.

Importance of isotopic incorporation: oustaleti and patagonicus from Tierra del Fuego

In contrast to Cinclodes tissues collected in the winter months from central and southern Chile, analysis of oustaleti and patagonicus tissues collected in the summer (early February) from Tierra del Fuego show that muscle and blood have similar mean δ²H values, but these two tissues have δ²H values that are ~20–25‰ lower than liver (Fig. 3). The most parsimonious explanation for this pattern and why it differs from that observed for winter-collected tissues discussed above is that muscle and blood have not equilibrated with local hydrogen sources when these birds were collected in Tierra del Fuego in early February. δ²H isotopic incorporation rates have not been quantified for liver, muscle, or whole blood, but McKinnon et al. (2012) found that δ²H half-life estimates for red blood cells in two species of migratory thrushes varied from 14 to 21 days. Wolf et al. (2012) and Storm-Suke et al. (2012) reported similar incorporation rates for δ²H of red blood cells in captive Japanese quail; half life estimates for plasma in quail were ~3.5 days. In addition, Wolf et al. (2012) found that δ²H and δ¹³C
incorporation rates were similar for both plasma and red blood cells. Hobson and Clark (1992) reported half-lives for $\delta^{13}$C in liver, muscle, and whole blood of 2.6, 12.4, 11.4 days, respectively, for captive Japanese quail, which are similar to $\delta^{13}$C incorporation rates for liver and muscle reported by Carleton and Martinez del Rio (2005) in house sparrows (Passer domesticus). Assuming that complete turnover occurs in $\sim$4–5 half-lives, we estimate that isotopic incorporation occurs in $\sim$12–15 days for liver, but $\sim$55–65 days for whole blood and muscle. Thus, liver tissue has likely equilibrated with local dietary and drinking water sources by early February, but muscle and whole blood do not entirely reflect local sources at this time.

$\delta^{13}$C and $\delta^{15}$N patterns among oustaleti and patagonicus liver, muscle, and blood tissues collected from Tierra del Fuego in the summer also support the conclusion that blood and muscle have not equilibrated with local dietary and drinking water sources (Fig. 3). For oustaleti, mean liver $\delta^{13}$C and $\delta^{15}$N values are higher than those associated values of muscle or blood by $\sim$2% and $\sim$3% respectively, while muscle and blood $\delta^{13}$C and $\delta^{15}$N values are identical. For patagonicus, mean liver $\delta^{13}$C and $\delta^{15}$N values are also higher than associated muscle or blood by $\sim$1.5% and $\sim$2.0% respectively, while mean muscle and blood $\delta^{13}$C and $\delta^{15}$N values are identical. The similarity in $\delta^{13}$C and $\delta^{15}$N patterns among tissues suggest that while both species consume a mixed terrestrial and marine diet, their summer diet in Tierra del Fuego has a higher proportion of marine resources than at other times of the year and that muscle and whole blood have not equilibrated to local diet (and water) sources by the time these birds were captured in early February.

Lastly, oustaleti and patagonicus do not occur in Tierra del Fuego in the winter months and are likely latitudinal migrants that winter at lower latitudes. Mean (±SD) $\delta^{13}$C (−23.2 ± 1.1%) and $\delta^{15}$N (6.9 ± 2.3%) values of old contour feathers collected from Tierra del Fuego in the summer of 2011, which represent dietary preferences during the summer prior to capture (2010), are indicative of terrestrial resource use. This pattern contrasts with mean $\delta^{13}$C (−18.1 ± 3.2%) and $\delta^{15}$N (12.3 ± 2.4%) values of liver from these same individuals that reflect summer dietary information in the weeks prior to capture and are indicative of a mixed marine and terrestrial diet. Furthermore, mean $\delta^{2}$H values of feathers (−79 ± 7%) collected from oustaleti in Tierra del Fuego were identical to those collected from their counterparts in southern Chile (−78 ± 14%). The discrepancy between oustaleti feather and liver isotope patterns from Tierra del Fuego suggest that there may be inter-annual flexibility in individual migratory and/or foraging strategies of this species in the southern margin of their range.

**Influence of a marine versus freshwater diet on $\delta^{2}$H values**

As mentioned above, Cinclodes is an ideal group to examine the influence of marine versus terrestrial resource use on tissue $\delta^{2}$H values. To simplify these patterns, we focus on patagonicus, which have mean $\delta^{13}$C and $\delta^{15}$N values indicative of a mixed marine and freshwater diet. In addition, variation in $\delta^{13}$C and $\delta^{15}$N among patagonicus individuals is larger than the other Cinclodes species analyzed here: standard deviations vary from 1.8% to 3.4% depending on tissue type, suggesting that different individuals specialize on marine or freshwater aquatic habitats; this large degree of variation has been observed in previous studies (Sabat and Martinez del Rio 2002, Sabat et al. 2006a, Martinez del Rio et al. 2009b). For patagonicus collected from southern Chile and Tierra del Fuego, feather $\delta^{2}$H and $\delta^{15}$N values are positively correlated (Fig. 4), and individuals with a terrestrial (freshwater) diet ($\delta^{15}$N < 10%) have $\delta^{2}$H values that are $\sim$25% lower than those patagonicus that consume a dominantly marine diet ($\delta^{15}$N > 14%).

The potential effects of a marine diet on tissue $\delta^{2}$H have implications for interpreting tissue $\delta^{2}$H values, as noted for other bird species (e.g., Lott et al. 2003). For example, mean (±SD) $\delta^{2}$Hfeather values of patagonicus collected from southern Chile (−61 ± 11%) are significantly higher than patagonicus collected in Tierra del Fuego (−74 ± 12%). This difference could be driven by (1) differences in marine versus freshwater foraging preferences between populations and/or (2) latitudinal variation in precipitation $\delta^{2}$H isoscapes. The significant positive trend between feather $\delta^{2}$H and $\delta^{15}$N values suggests that diet
plays an important role in explaining the difference in $\delta^2H_{\text{feather}}$ values between these two *patagonicus* populations (Fig. 4). Despite a high degree of variation in $\delta^{15}N$ values (SD = 3–4%) in both populations, mean $\delta^{15}N$ values show that *patagonicus* from southern Chile consume a higher proportion of marine resources, which likely have higher $\delta^2H$ values than invertebrates from freshwater aquatic habitats because the $\delta^2H$ value of seawater (0‰) is higher than river or stream waters (Kendall and Coplen 2001).

**Conclusions**

Our novel comparison of isotopic patterns in metabolically active and inert tissues within and among closely related species with divergent ecological characteristics allowed us to identify patterns attributable to physiology and ecology. First, $\delta^2H$ patterns between metabolically active muscle and inert feathers collected during the winter showed that variation in altitudinal migration occurs at the species, population, and individual level in this genus. The positive $\delta^2H_{\text{feather-muscle}}$ offsets found in resident species were opposite those observed in species that undertake altitudinal migrations during the summer. Moreover, we show that this approach can identify differences in strategies used by different individuals and/or populations within a species. For example, *oustalieri* individuals collected from central Chile were mostly altitudinal migrants, while most of their counterparts from southern Chile appeared to breed at low altitudes. Lastly, we were also able to characterize apparent variation in breeding altitude among individuals in a single population (e.g., *fuscus* and *oustalieri* from central Chile). In general, altitudinal movement is a commonly reported but poorly understood behavior in birds and the diverse avifauna of the Andes is no exception. The capability to characterize altitudinal movement with $\delta^2H$ is a powerful approach for examining variation in altitudinal movement within bird communities and possibly link it with physiological adaptations associated with life at high altitudes (Jessen et al. 1991, Weber 2007, Cheviron et al. 2008).

Second, our study demonstrates that tissue-specific discrimination is an important consideration in the interpretation of $\delta^2H$ data from multiple tissues. The general pattern observed among $\delta^2H$ values in *Cinclodes* was similar to but of higher magnitude than those shown in controlled feeding experiments. We suggest that further work is required to examine the mechanisms responsible for the observed patterns in $\delta^2H$ tissue-specific discrimination. As shown for $\delta^{13}C$ (Hare et al. 1991), comparing $\delta^2H$ values in individual amino acids with patterns in amino acid composition among tissues could provide some insight on the general bulk tissue patterns observed here and in previous studies.

Lastly, comparison of $\delta^2H$ patterns with $\delta^{15}N$ and $\delta^{15}N$ values from the same tissues shows that movement and diet can be studied simultaneously at the individual level. This was especially important in (1) assessing whether the tissues examined had equilibrated with local dietary and water sources at the time of collection, and (2) examining the relationship between a marine diet and tissue $\delta^2H$ values in *Cinclodes*. Using $\delta^2H$ to trace resource use rather than movement could expand the use of stable isotope analysis in animal ecology, but thus far this concept has only been examined in a limited number of contexts (Birchall et al. 2005, Doucett et al. 2007, Voigt et al. 2013).

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**Literature Cited**


Knoff, A. J., S. A. Macko, R. M. Erwin, and K. M.

