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Multi-tissue δ^2H 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 (δ^2H) values to characterize animal movement and migration strategies. Most studies measure δ^2H 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 δ^2H 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 δ^2H 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 δ^2H 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 δ^2H 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 $\delta^2H_{muscle\text{-}feather}$ offsets. Lastly, a positive correlation between feather δ^2H and $\delta^{15}N$ values in *Cinclodes* that consume a mixed marine-freshwater diet confirms that δ^2H is a useful proxy for quantifying marine resource use. We anticipate that comparison of δ^2H 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 δ^2H patterns among tissues.

Key words: δ^2H; $\delta^{13}C$; $\delta^{15}N$; 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 (δ^2H) and to a lesser extent oxygen ($\delta^{18}O$), carbon ($\delta^{13}C$), nitrogen ($\delta^{15}N$), and strontium ($\delta^{87}Sr$) 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. 1999*b*, Rubenstein et al. 2002, Rubenstein and Hobson 2004, Cryan et al. 2004, Bowen et al. 2005*a*, Wunder et al. 2005, Sellick et al. 2009, Graham et al. 2010). The hydrogen isotope approach relies on correlating the δ^2H 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 $\delta^2H_{precipitation}$ 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 $\leq$3% of body weight for terrestrial species. Other advantages of δ^2H analysis include the need for only one capture event to collect tissues and that this approach can be combined with carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) 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 δ^2H to study animal movement exploit latitudinal δ^2H isoscapes; however, δ^2H also varies predictably with altitude (Poage and Chamberlain 2001) and has been used to examine altitudinal distributions in birds. Hobson et al. (2003) used δ^2H to examine altitude of origin in a community of hummingbirds collected across an altitudinal gradient from ~300m to ~3300m in the Ecuadorean Andes. Ecuadorean hummingbirds are an ideal group for using δ^2H 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 nectivorous and thus any potential δ^2H variation related to trophic level is minimized. This study found a significant negative correlation between $\delta^2H_{precipitation}$ and altitude ($r^2 = 0.68$, $n = 11$), but noted that the relationship between hummingbird $\delta^2H_{feather}$ values and altitude was not linear; most variation in $\delta^2H_{feather}$ occurred between 1300 and 3300 m. A more recent study in the Ecuadorean Andes examined feather and blood δ^2H 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 nectivorous foraging guilds, but sample sizes were low for most species (<3 individuals). Both feather and blood showed a significant negative trend in δ^2H with increasing altitude, but the percentage of δ^2H variation explained by altitude was low, ranging from 10% to 62% depending on foraging guild and tissue type. Lastly, Boyle et al. (2011) used δ^2H 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 δ^2H 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 δ^2H 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 δ^2H 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 (Wassenaar and Hobson 2000), analytical protocols that account for hydrogen exchange with ambient water vapor are interchangeable and provide reliable δ^2H 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. 2009*a*). 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 or habitat use over a variety of timescales. For example, Martinez del Rio et al. (2009*b*) 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 δ^2H 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 δ^2H 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 δ^2H values. Mean muscle, liver, and plasma δ^2H values reported by Wolf et al. (2012) were also significantly lower than mean feather δ^2H values, a pattern observed across all dietary treatments. The physiological mechanisms that control δ^2H 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 δ^2H 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 δ^2H variation, and a multiple-tissue approach requires a better understanding of tissue-specific δ^2H discrimination and how it varies in natural settings.

Here we examine δ^2H 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. (2006*a*), and Martinez del Rio et al. 2009*b*. *Cinclodes nigrofumosus* are residents that consume invertebrates in intertidal

marine ecosystems throughout the year. *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 δ^2H values that served as a proxy for altitude, we measured tissue carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) 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 $\delta^{13}C$ and $\delta^{15}N$ values of primary producers in each ecosystem (Kelly 2000). Consumers that rely on marine ecosystems typically have higher $\delta^{13}C$ and $\delta^{15}N$ 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 δ^2H related to diet, habitat use, and altitudinal movement. Specifically, our study addressed three questions. First, can δ^2H 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 δ^2H 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 $\delta^{13}C$ and $\delta^{15}N$ values. Second, for species that remain in the same environment throughout the year (*nigrofumosus*), are tissue-specific δ^2H discrimination patterns similar to those observed for captive birds grown on diets and drinking water that had a consistent δ^2H composition? Finally, what is the effect of a marine diet on tissue δ^2H values? We expected that tissue $\delta^{15}N$ values, a commonly used proxy for marine resource use, would positively covary with tissue δ^2H 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 δ^2H 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. 2006*a*, 2006*b*) and ecological (e.g., Martinez del Rio et al. 2009*b*) 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 δ^2H 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 ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) 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.

Region, season, and species	Altitudinal migrant?	Diet (winter/summer)	Feather δ^2H	Muscle δ^2H	Liver δ^2H	Blood δ^2H
Central Chile						
Winter						
fuscus	yes	mixed/freshwater	−106 ± 18 (*9*)	−81 ± 8 (*9*)	. . .	. . .
nigrofumosus	no	marine/marine	−64 ± 9 (*24*)	−80 ± 6 (*24*)	−54 ± 9 (*16*)	. . .
oustaleti	yes	mixed/freshwater	−104 ± 21 (*32*)	−77 ± 11 (*32*)	−47 ± 13 (*27*)	−117 ± 10 (*12*)
Southern Chile						
Winter						
oustaleti	yes	mixed/freshwater	−78 ± 14 (*18*)	−81 ± 8 (*18*)	−49 ± 10 (*17*)	−113 ± 4 (*14*)
patagonicus	no	mixed/freshwater	−61 ± 10 (*27*)	−79 ± 6 (*27*)	−45 ± 7 (*18*)	−115 ± 8 (*14*)
Tierra del Fuego						
Summer						
oustaleti	no*	mixed/freshwater	−80 ± 7 (*18*)	−89 ± 8 (*18*)	−67 ± 9 (*16*)	−92 ± 10 (*11*)
patagonicus	no*	mixed/freshwater	−74 ± 11 (*16*)	−90 ± 8 (*16*)	−68 ± 14 (*15*)	−92 ± 10 (*11*)

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 δ^2H for at least three weeks prior to analysis to account for exchangeable hydrogen (Wassenaar and Hobson 2000, Bowen et al. 2005*b*). δ^2H values of the non-exchangeable portion of hydrogen were determined by comparative equilibration (Wassenaar and Hobson 2003) using three internal keratin δ^2H reference materials, Turkey (−54‰), Chicken (−94‰), and Moose (−165‰) for which non-exchangeable δ^2H values were measured by comparative bench-top equilibration experiments (e.g., Bowen et al. 2005*b*) 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 δ^2H composition (Wassenaar and Hobson 2000).

δ^2H 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 $\delta^{13}C$ and $\delta^{15}N$ analysis, dried tissue samples were sealed in 3 × 5 mm tin capsules. $\delta^{13}C$ and $\delta^{15}N$ 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,

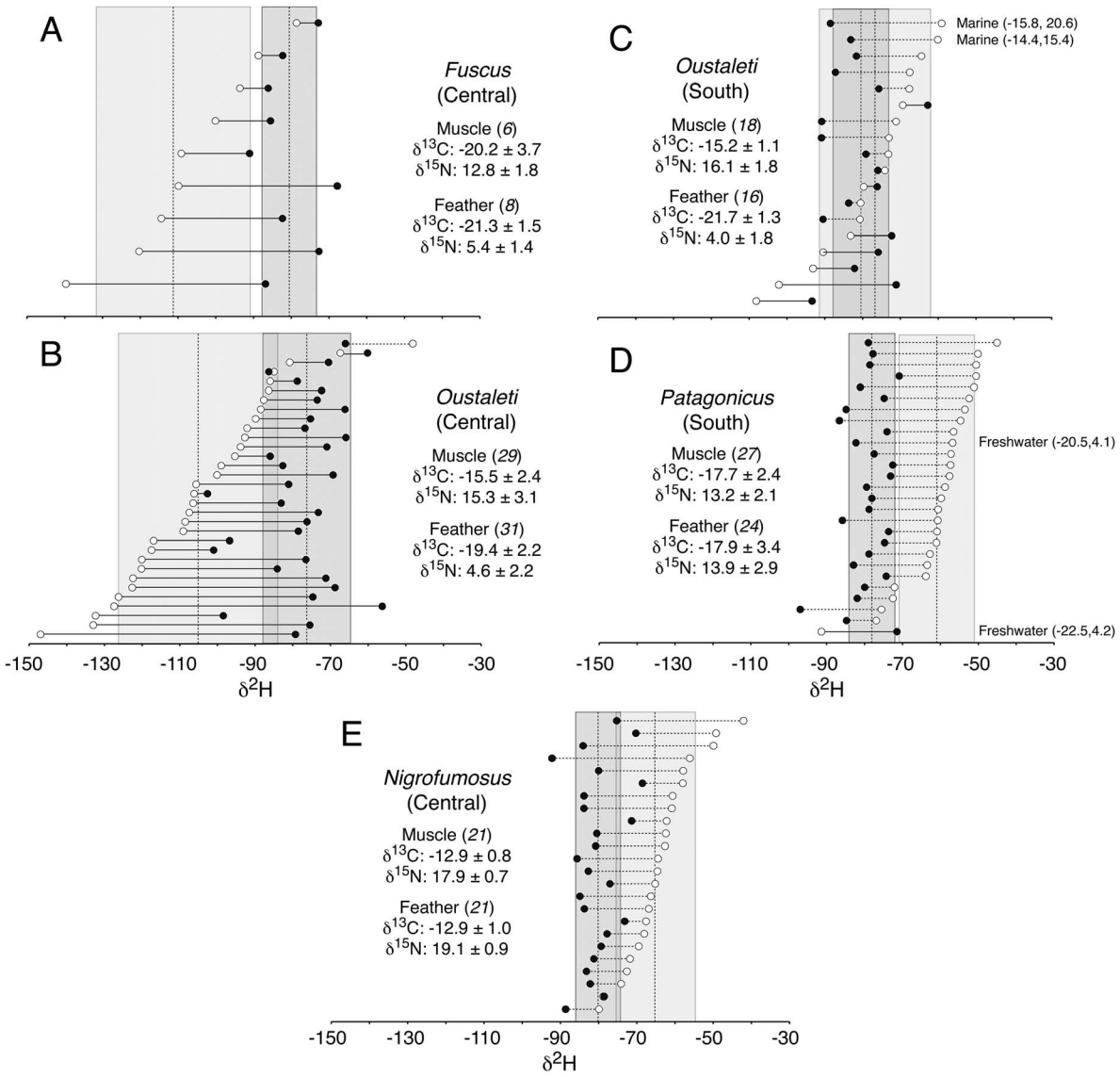

Fig. 1. Paired muscle (closed circles) and feather (open circles) δ^2H values of four species of *Cinclodes* collected in coastal environments during the winter months (July–August) in central (29–34° S) and southern (37–41° S) Chile. Metabolically active muscle tissue represents winter ecological information, while feather δ^2H values represent ecological information from the summer prior to collection. Paired samples in each panel are sorted by highest (top) to lowest (bottom) feather δ^2H values. Dashed vertical lines denote mean δ^2H values and shaded areas represent standard deviation. Horizontal lines denote when feather δ^2H values are lower (solid) or higher (dashed) than associated muscle. Mean (±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.

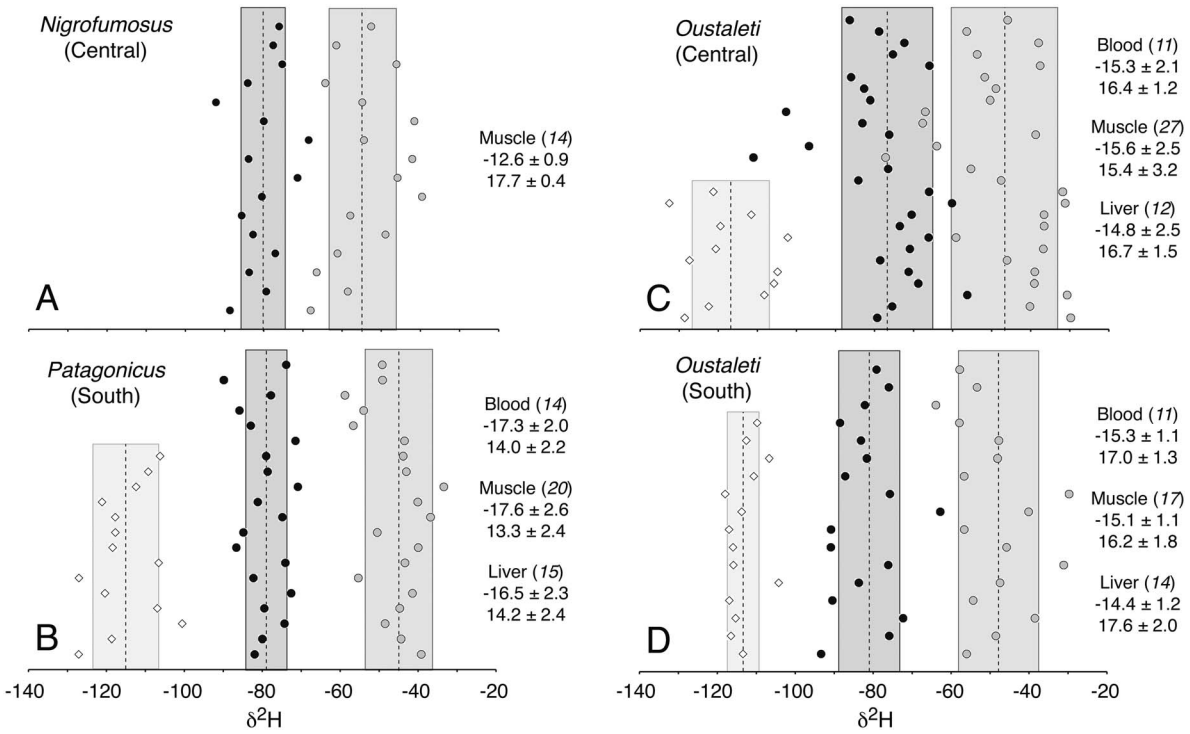

Fig. 2. Paired liver (gray circles), muscle (black circles) and blood (white diamonds) δ^2H values of three *Cinclodes* species collected in winter (July–August) from coastal environments in central (29–34° S) and southern (37–41° S) Chile. Dashed vertical lines denote mean δ^2H values and shaded areas represent standard deviation. Mean blood, muscle, and liver $\delta^{13}C$ (negative numbers) and $\delta^{15}N$ (positive numbers) values (±SD) are provided to the right; italic numbers in parentheses represent sample sizes.

δ^2H or $\delta^{13}C$ or $\delta^{15}N = 1000 \times [(R_{sample} - R_{standard}/R_{standard})]$, where R_{sample} and $R_{standard}$ are the $^2H/^1H$ or $^{13}C/^{12}C$ 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 δ^2H was determined by analysis of the three exchangeable (keratin) reference materials described above; within-run δ^2H variation (SD) of these reference materials on the mass spectrometer system described above was ≤4‰. All samples for δ^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 ≤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).

Statistical analysis

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

Results

Feather δ^2H

Mean $\delta^2H_{feather}$ values varied by 45‰ among species, ranging from −61‰ to −106‰ (Table 1),

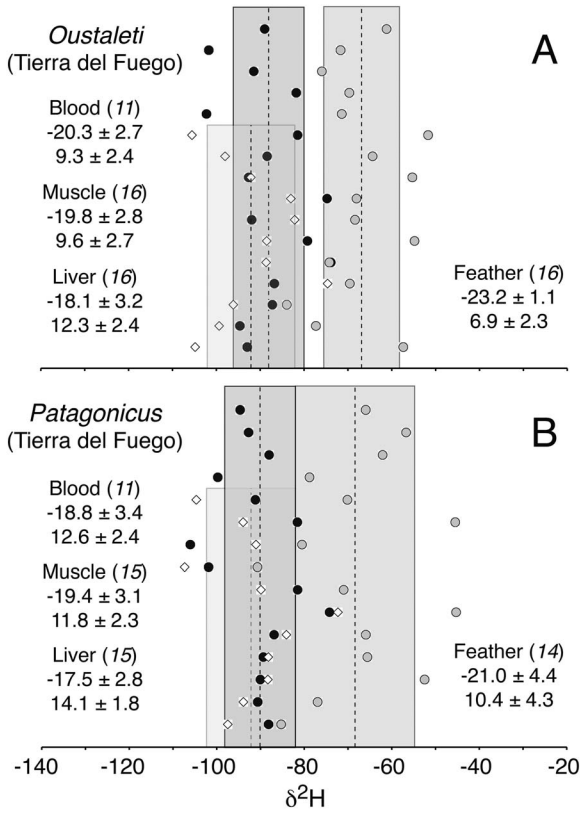

Fig. 3. Paired liver (gray circles), muscle (black circles) and blood (white diamonds) δ^2H values of *oustaleti* and *patagonicus* collected in coastal environments during the summer (February) at the southern margin of their range in Tierra del Fuego (54° S). Dashed vertical lines denote mean δ^2H values and shaded areas represent standard deviation. Mean liver, muscle, and blood $\delta^{13}C$ (negative numbers) and $\delta^{15}N$ (positive numbers) values (±SD) are provided to the left, mean feather $\delta^{13}C$ and $\delta^{15}N$ (±SD) are shown to the right; italic numbers in parentheses represent sample sizes.

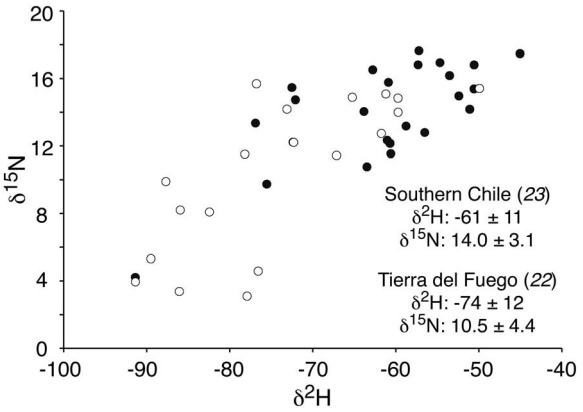

Fig. 4. Feather δ^2H versus $\delta^{15}N$ values of *patagonicus* collected during the summer from coastal environments in southern Chile (closed circles) and Tierra del Fuego (open circles). Mean (±SD) feather δ^2H (negative values) and $\delta^{15}N$ (positive values) values are also shown; numbers in parentheses note sample sizes for each group.

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

Muscle δ^2H

Mean δ^2H_{muscle} values were surprisingly con-

sistent and varied by only 11‰ among species (Table 1). In addition, there were fewer significant differences among species (χ^2: 34.5, DF = 6, $P < 0.0001$) in comparison to patterns among feather δ^2H. 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 ± 8‰) Chile, *patagonicus* from southern Chile (−79 ± 6‰), and *fuscus* (−81 ± 8‰) or *nigrofumosus* (−80 ± 6‰) from central Chile all had statistically indistinguishable δ^2H_{muscle} values; *z* scores: −1.64 to 1.85, $P > 0.10$. δ^2H_{muscle} 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 δ^2H_{muscle} values collected during the winter from other species ($P < 0.05$).

Blood δ^2H

Mean δ^2H_{blood} 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 (χ^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 δ^2H_{blood} 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 δ^2H_{blood} 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 δ^2H

Mean δ^2H_{liver} values collected from central and southern Chile during the winter (Fig. 2) ranged from −30‰ to −59‰ (Table 1). There were significant differences (χ^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 (χ^2: 36.1, DF = 4, $P < 0.001$). Mean δ^2H_{liver} 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 δ^2H patterns

Differences in mean δ^2H 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 $\delta^2H_{feather}$ 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, $P < 0.0001$) $\delta^2H_{feather}$ values were significantly higher than paired δ^2H_{muscle}. In contrast, paired *oustaleti* feather and muscle δ^2H 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 δ^2H_{muscle} values than paired $\delta^2H_{feather}$ values.

δ^2H_{liver} values were significantly higher than paired δ^2H_{muscle} across all species (Fig. 2). *Nigrofumosus* had mean δ^2H_{liver} values that were higher by 26‰ than paired δ^2H_{muscle} (*F* value: 8.29, DF = 15, $P < 0.0001$). *Patagonicus* from southern Chile had mean δ^2H_{liver} values that were higher by 34‰ than paired mean δ^2H_{muscle} values (*F* value: 26.00, DF = 17, $P < 0.0001$). *Oustaleti* from central Chile had mean δ^2H_{liver} values that were higher by 30‰ than paired mean δ^2H_{muscle} values (*F* value: 14.02, DF = 26, $P < 0.0001$). *Oustaleti* from southern Chile had mean δ^2H_{liver} values that were significantly higher by 33‰ than paired δ^2H_{muscle} (*F* value: 16.28, DF = 16, $P < 0.0001$).

δ^2H_{blood} values were also significantly higher than paired δ^2H_{muscle} across all species (Fig. 2). *Patagonicus* from southern Chile had mean δ^2H_{muscle} values that were significantly higher by 36‰ than paired δ^2H_{blood} (*F* value: 25.91, DF = 13, $P < 0.0001$). *Oustaleti* from central Chile had mean δ^2H_{muscle} values that were significantly higher by 47‰ than paired δ^2H_{blood} (*F* value: 22.68, DF = 11, $P < 0.0001$). Lastly, *oustaleti* from southern Chile had mean δ^2H_{muscle} values that were significantly higher by 31‰ than paired δ^2H_{blood} (*F* value: 10.07, DF = 13, $P <$

0.0001).

For *oustaleti* and *patagonicus* collected in Tierra del Fuego during the summer (Fig. 3), δ^2H_{liver} values were higher than δ^2H_{muscle} or δ^2H_{blood}. *Patagonicus* had mean δ^2H_{liver} values that were significantly higher by 23‰ than paired δ^2H_{muscle} (F value: 4.16, DF = 10, $P < 0.0001$). *Oustaleti* had mean δ^2H_{liver} values that were significantly higher by 21‰ than paired δ^2H_{muscle} (F value: 2.77, DF = 10, $P < 0.001$). Paired muscle and blood δ^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 δ^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. 2006*a*, 2006*b*, Martinez del Rio et al. 2009*b*). 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 migrant species that forage in freshwater aquatic habitats during the summer when feathers are molted and regrown, 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 migrant 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 δ^2H values. There are two general patterns apparent in our dataset that highlight what ecologists can learn from δ^2H analysis of multiple tissues. First, our study shows that comparing δ^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 δ^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 δ^2H variation among tissues within resident species (*nigrofumosus*), suggesting that tissue-specific discrimination mediated by physiological processes is an important factor to consider a when comparing δ^2H values among tissues. In the following sections, we focus on patterns in δ^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 δ^2H values.

Examining individual variation in altitudinal migration with δ^2H: a multi-tissue approach

Southern South America is an ideal region to study altitudinal (or latitudinal) movement patterns with δ^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 $\delta^2H_{precipitation}$ isoscape with nearly 100‰ of variation (Bowen et al. 2005*a*). 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 $\delta^2H_{precipitation}$ isoscape of southern South America is driven by altitude rather than latitude (Bowen et al. 2005*a*).

δ^2H 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 δ^2H 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 $\delta^{13}C$ and $\delta^{15}N$ data presented here (Fig. 1E) and in previous studies (Sabat et al. 2006*a*, Martinez del Rio et al. 2009*b*) show that this species resides in coastal habitats and consumes marine intertidal invertebrates year-round. We consider the mean $\delta^2H_{feather\text{-}muscle}$ 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 $\delta^2H_{feather\text{-}muscle}$ 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 $\delta^2H_{feather\text{-}muscle}$ offset observed in *C. nigrofumosus* and previously reported for captive quail is probably primarily driven by tissue-specific δ^2H discrimination related to physiology (i.e., tissue biosynthesis) rather than ecology. Variation in the magnitude of the δ^2H 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 δ^2H 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 $\delta^2H_{feather\text{-}muscle}$ offset (+15‰) as *nigrofumosus* from central Chile; mean feather and muscle δ^2H values are identical between the two species. *Patagonicus* feather (Fig. 1D) and muscle (Fig. 2B) $\delta^{13}C$ and $\delta^{15}N$ 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 $\delta^2H_{feather\text{-}muscle}$ offsets, a result that suggests physiology rather than ecology is primarily responsible for the observed pattern in δ^2H values between tissues. Two *patagonicus* individuals, however, had relatively low feather $\delta^{13}C$ and $\delta^{15}N$ 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 δ^2H values indicating that it specializes on freshwater habitats at low altitudes throughout the year.

In contrast to the patterns for *nigrofumosus* and *patagonicus*, feather δ^2H 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 δ^2H values decrease by ~8–10‰ for every 500-m increase in altitude (Hardesty and Fraser 2010, IAEA/WMO 2011). Since *fuscus* and *oustaleti* feather $\delta^{13}C$ and $\delta^{15}N$ values indicate terrestrial resource use, the apparent switch in the direction of the $\delta^2H_{feather\text{-}muscle}$ 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 $\delta^2H_{feather\text{-}muscle}$ 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 δ^2H values that were +15‰ higher than paired muscle tissue (Fig. 1B), a $\delta^2H_{feather\text{-}muscle}$ offset similar to that observed in *nigrofumosus* and *patagonicus* (Fig.

1D, E). Feather $\delta^{13}C$ (–21.2‰) and $\delta^{15}N$ (5.4‰) values from this *oustaleti* individual also indicate that it consumes freshwater resources during the summer, and likely remains at low altitudes throughout the year.

Feather δ^2H 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 δ^2H 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 δ^2H-based breeding altitude estimates, however, should be viewed with caution for several reasons. First, previous studies have noted nonlinearity in altitudinal $\delta^2H_{precipitation}$ lapse rates in the Andes (Hobson et al. 2003), and altitudinal δ^2H 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 δ^2H values requires further consideration since δ^2H 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* δ^2H *values*.

Oustaleti from southern Chile have the most variable pattern (Fig. 1C), with a near equal split in the direction of the $\delta^2H_{feather\text{-}muscle}$ offset among individuals that ranges from +30‰ to −30‰. This suggests that *oustaleti* in this region use a wider range of altitudinal movement strategies than their counterparts from central Chile (Fig. 1B). Six of the eighteen (33%) *oustaleti* from southern Chile, however, have $\delta^2H_{feather\text{-}muscle}$ offsets (+15‰) that are indicative of coastal residents and mirror offsets seen in *patagonicus* from southern Chile (Fig. 1D) and *nigrofumosus* from central Chile (Fig. 1E). Only two of these six individuals have feather $\delta^{13}C$ and $\delta^{15}N$ values indicative of marine resource use, all other *oustaleti* analyzed have feather $\delta^{13}C$ and $\delta^{15}N$ values indicative of terrestrial (freshwater) resource use during the summer. Thus, the other four individuals with $\delta^2H_{feather\text{-}muscle}$ offsets of +15‰ likely forage exclusively in terrestrial freshwater habitats at low altitudes.

Another five *oustaleti* individuals from southern Chile have feather δ^2H 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 δ^2H discrimination between feather and muscle and an altitudinal $\delta^2H_{precipitation}$ 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 δ^2H 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 $\delta^2H_{feather\text{-}muscle}$ 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. 2006*a*, 2006*b*, 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 δ^2H variation in wild animal populations (Mazerolle and Hobson 2005, Hardesty and Fraser 2010).

Tissue specific δ^2H discrimination: patterns among liver, muscle, and blood

The consistent δ^2H 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, $\delta^{13}C$ and $\delta^{15}N$ data show that *nigrofumosus* are residents that spend the entire year foraging in coastal marine habitats (Fig. 1E). δ^2H_{liver} values for *nigrofumosus* were ~25‰ higher than mean δ^2H_{muscle} (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 δ^2H_{liver} values that were ~30‰ higher than mean δ^2H_{muscle} values (Fig. 2B). In addition, δ^2H_{blood} values in *patagonicus*, a tissue we did not collect from *nigrofumosus*, were ~30‰ lower than δ^2H_{muscle}.

The general δ^2H 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 $\delta^{13}C$ and $\delta^{15}N$ 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 $\delta^{13}C$ and $\delta^{15}N$ values indicative of a mixed marine and freshwater diet (Fig. 2). Similarity in δ^2H 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 δ^2H 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 δ^2H 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 δ^2H compositions. Quail liver δ^2H 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 δ^2H 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. 1999*a*, 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 δ^2H values, but these two tissues have δ^2H 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. δ^2H isotopic incorporation rates have not been quantified for liver, muscle, or whole blood, but McKinnon et al. (2012) found that δ^2H 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 δ^2H 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 δ^2H and $\delta^{13}C$

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 ~4–5 half-lives, we estimate that isotopic incorporation occurs in ~12–15 days for liver, but ~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 ~2‰ and ~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 ~1.5‰ and ~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. 2006*a*, Martinez del Rio et al. 2009*b*). 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 ~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) $\delta^{2}H_{feather}$ 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 $\delta^2H_{feather}$ 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 δ^2H values than invertebrates from freshwater aquatic habitats because the δ^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, δ^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 $\delta^2H_{feather\text{-}muscle}$ 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, *oustaleti* 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 *oustaleti* 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 δ^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 δ^2H data from multiple tissues. The general pattern observed among δ^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 δ^2H tissue-specific discrimination. As shown for $\delta^{13}C$ (Hare et al. 1991), comparing δ^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 δ^2H patterns with $\delta^{13}C$ 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 δ^2H values in *Cinclodes*. Using δ^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

Birchall, J., T. C. O'Connell, T. H. Heaton, and R. E. M. Hedges. 2005. Hydrogen isotope ratios in animal body protein reflect trophic level. Journal of Animal Ecology 74:877–881.

Bligh, E. G., and W. J. Dyer. 1959. A rapid method of total lipid extraction and purification. Canadian Journal of Biochemistry and Physiology 37:911–917.

Bowen, G. J., L. Chesson, K. Nielson, T. E. Cerling, and J. R. Ehleringer. 2005*b*. Treatment methods for the determination of δ^2H and $\delta^{18}O$ of hair keratin by continuous-flow isotope-ratio mass spectrometry. Rapid Communications in Mass Spectrometry

2005:2371–2378.

Bowen, G. J., L. I. Wassenaar, and K. A. Hobson. 2005*a*. Global application of stable hydrogen and oxygen isotopes to wildlife forensics. Oecologia 143:337–348.

Boyle, W. A., C. G. Guglielmo, K. A. Hobson, and D. R. Norris. 2011. Lekking birds in a tropical forest forego sex for migration. Biology Letters 7:661–663.

Carleton, S. A., and C. Martinez del Rio. 2005. The effect of cold-induced increased metabolic rate on the rate of ^{13}C and ^{15}N incorporation in house sparrows (*Passer domesticus*). Oecologia 144:226–232.

Chamberlain, C. P., J. D. Blum, R. T. Holmes, X. Feng, T. W. Sherry, and G. R. Graves. 1997. The use of isotope tracers for identifying populations of migratory birds. Oecologia 109:132–141.

Cheviron, Z. A., A. Whitehead, and R. T. Brumfield. 2008. Transcriptomic variation and plasticity in rufous-collared sparrows (*Zonotrichia capensis*) along an elevational gradient. Molecular Ecology 17:4556–4569.

Cryan, P. M., M. A. Bogan, R. O. Rye, G. P. Landis, and C. L. Kester. 2004. Stable hydrogen isotope analysis of bat hair as evidence for seasonal molt and long-distance migration. Journal of Mammalogy 85:995–1001.

Doucett, R. R., J. C. Marks, D. W. Blinn, M. Caron, and M. A. Hungate. 2007. Measuring terrestrial subsidies to aquatic food webs using stable isotopes of hydrogen. Ecology 88(6):1587–1592.

Ehleringer, J. R., P. W. Rundel, B. Palma, and H. A. Mooney. 1998. Carbon isotope ratios of Atacama Desert plants reflect hyperaridity of región in northern Chile. Revista Chilena de Historia Natural 71:79–86.

Fjeldså, J., and N. Krabbe. 1990. Birds of the high Andes. Zoology Museum, University of Copenhagen and Svendborg, Apollo Books, Copenhagen, Denmark.

Fogel, M. L., P. Griffin, and S. D. Newsome. 2010. Compound specific hydrogen isotope analysis of amino acids from microbes: new biosignatures. Astrobiology Science Conference. LPI Contribution 1538. http://www.lpi.usra.edu/meetings/abscicon2010/pdf/5466.pdf

Graham, B. S., P. L. Koch, S. D. Newsome, K. W. McMahon, and D. Aurioles. 2010. Using isoscapes to trace the movements and foraging behavior of top predators in oceanic ecosystems. *In* J. B. West, et al., editors. Isoscapes: understanding movement, pattern, and process on Earth through isotope mapping. Springer-Verlag, Berlin, Germany.

Hardesty, J. L., and K. C. Fraser. 2010. Using deuterium to examine altitudinal migration by Andean birds. Journal of Field Ornithology 831:83–91.

Hare, P. E., M. L. Fogel, T. W. Stafford, A. D. Mitchell, and T. C. Hoering. 1991. The isotopic composition of carbon and nitrogen in individual amino acids isolated from modern and fossil proteins. Journal of Archaeological Science 18:277–292.

Heithaus, M. R., G. J. Marshall, B. M. Buhleier, and L. M. Dill. 2001. Employing critter cam to study habitat use and behavior of large sharks. Marine Ecology Progress Series 209:307–310.

Hobson, K. A. 1999. Tracing origins and migration of wildlife using stable isotopes: a review. Oecologia 120:314–326.

Hobson, K. A., L. Atwell, and L. I. Wassenaar. 1999*a*. Influence of drinking water and diet on stable-hydrogen isotope ratios of animal tissues. Proceedings of the National Academy of Sciences USA 96:8003–8006.

Hobson, K. A., and R. G. Clark. 1992. Assessing avian diets using stable isotopes: turnover of ^{13}C in tissues. Condor 94:181–188.

Hobson, K. A., and L. I. Wassenaar. 1997. Linking breeding and wintering grounds of neotropical migrant songbirds using stable hydrogen isotopic analysis of feathers. Oecologia 109:142–148.

Hobson, K. A., L. I. Wassenaar, B. Mila, I. Lovette, C. Dingle, and T. B. Smith. 2003. Stable isotopes are indicators of altitudinal distributions and movements in an Ecuadorean hummingbird community. Oecologia 136:302–308.

Hobson, K. A., L. I. Wassenaar, and O. R. Taylor. 1999*b*. Stable isotopes (δD and $\delta^{13}C$) are geographic indicators of natal origins of monarch butterflies in eastern North America. Oecologia 120:397–404.

International Atomic Energy Agency [IAEA]/World Meteorological Organization [WMO]. 2011. Global Network of Isotopes in Precipitation (GNIP) database. http://www-naweb.iaea.org/napc/ih/IHS_resources_gnip.html

Jaramillo, A. 2005. Aves de Chile. Lynx Edicions, Barcelona, Spain.

Jessen, T. H., R. E. Weber, G. Fermi, J. Tame, and G. Braunitzer. 1991. Adaptation of bird hemoglobins to high altitudes: demonstration of molecular mechanism by protein engineering. Proceedings of the National Academy of Sciences USA 88:6519–6522.

Kelly, J. F. 2000. Stable isotopes of carbon and nitrogen in the study of avian and mammalian trophic ecology. Canadian Journal of Zoology 78:1–27.

Kelly, J. F., V. Atudorei, Z. D. Sharp, and D. M. Finch. 2002. Insights into Wilson's warbler migration from analysis of hydrogen stable isotope ratios. Oecologia 130:216–221.

Kendall, C., and T. B. Coplen. 2001. Distribution of oxygen-18 and deuterium in river waters across the United States. Hydrological Processes 15:1363–1393.

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

Brown. 2002. Stable isotope analysis of temporal variation in the diets of pre-fledged laughing gulls. Waterbirds 25:142–148.

Koch, P. L., J. Heisinger, C. Moss, R. W. Carlson, M. L. Fogel, and A. K. Behrensmeyer. 1995. Isotopic tracking of change in diet and habitat use in African elephants. Science 267:1340–1343.

Lott, C. A., T. D. Meehan, and J. A. Heath. 2003. Estimating the latitudinal origins of migratory birds using hydrogen and sulfur stable isotopes in feathers: influence of a marine prey base. Oecologia 134:505–510.

Martinez del Rio, C., P. Sabat, R. Anderson-Sprecher, and S. P. Gonzalez. 2009*b*. Dietary and isotopic specialization: the isotopic niche of three Cinclodes ovenbirds. Oecologia 161:149–159.

Martinez del Rio, C., N. Wolf, S. Carleton, and L. Gannes. 2009*a*. Isotopic ecology ten years after a call for more experiments. Biological Reviews 84:91–111.

Mazerolle, D. F. and K. A. Hobson. 2005. Estimating origins of short-distance migrant songbirds in North America: contrasting inferences from hydrogen isotope measurements of feathers, claws, and blood. Condor 107:280–288.

McKinnon, E. A., K. C. Fraser, A. W. Diamond, C. C. Rimmer, and J. M. Townsend. 2012. Stable-hydrogen isotope turnover in red blood cells of two migratory thrushes: application to studies of connectivity and carry-over effects. Journal of Field Ornithology 83:306–314.

Naito, Y., D. P. Costa, T. Adachi, P. W. Robinson, M. Fowler, and A. Takahashi. 2013. Unraveling the mysteries of a mesopelagic diet: a large apex predator specializes on small prey. Functional Ecology 27:710–717.

Newsome, S. D., N. Wolf, J. Peters and M. L. Fogel. 2014. Amino acid $\delta^{13}C$ analysis shows flexibility in the routing of dietary protein and lipids to the tissue of an omnivore. Integrative and Comparative Biology 54:890–892.

Poage, M. A., and C. P. Chamberlain. 2001. Empirical relationships between elevation and the stable isotope composition of precipitation and surface waters: considerations for studies of paleoelevational change. American Journal of Science 301:1–15.

Rubenstein, D. R., C. P. Chamberlain, R. T. Holmes, M. P. Ayres, J. R. Waldbauer, G. R. Graves, and N. C. Tuross. 2002. Linking breeding and wintering ranges of a migratory songbird using stable isotopes. Science 295:1062–1065.

Popp, B. N., B. S. Graham, R. J. Olson, C. C. S. Hannides, M. J. Lott, G. A. Lopez-Ibarra, F. Galvan-Magana, and B. Fry. 2007. Insight into the trophic ecology of yellowfin tuna, *Thunnus albacares*, from compound-specific nitrogen isotope analysis of proteinaceous amino acids. Terrestrial Ecology 1: 173–190.

Rubenstein, D. R., and K. A. Hobson. 2004. From birds to butterflies: animal movement patterns and stable isotopes. Trends in Ecology and Evolution 19(5):256–263.

Sabat, P., K. Maldonado, M. Canals, and C. Martinez del Rio. 2006*b*. Osmoregulation and adaptive radiation in the ovenbird genus *Cinclodes* (Passeriformes: Furnariidae). Functional Ecology 20:799–805.

Sabat, P., K. Maldonado, J. M. Fariña, and C. Martinez del Rio. 2006*a*. Osmoregulatory capacity and the ability to use marine food sources in two coastal songbirds (*Cinclodes*: Furnariidae) along a latitudinal gradient. Oecologia 148:250–257.

Sabat, P. and C. Martinez del Rio. 2002. Inter- and intra-specific variation in the use of marine food resources by three *Cinclodes* (Furnariidae, Aves) species: carbon isotopes and osmoregulatory physiology. Zoology 105:247–256.

Sellick, M. J., T. K. Kyser, M. B. Wunder, D. Chipley, and D. R. Norris. 2009. Geographic variation of strontium and hydrogen isotopes in avian tissue: implications for tracking migration and dispersal. PLoS ONE. http://dx.doi.org10.1371/journal.pone.0004735

Storm-Suke, A., L. I. Wassenaar, E. Nol, and D. R. Norris. 2012. The influence of metabolic rate on the contribution of stable-hydrogen and oxygen isotopes in drinking water to quail blood plasma and feathers. Functional Ecology 26:1111–1119.

Voigt, C. C., K. Schneeberger, and A. Luckner. 2013. Ecological and dietary correlates of stable hydrogen isotope ratios in fur and body water of syntopic tropical bats. Ecology 94:346–355.

Wassenaar, L. I., and K. A. Hobson. 2000. Improved method for determining the stable-hydrogen isotopic composition (δD) of complex organic materials of environmental interest. Environmental Science and Technology 34:2354–2360.

Wassenaar, L. I., and K. A. Hobson. 2003. Comparative equilibration and online technique for determination of non-exchangable hydrogen of keratins for use in animal migration studies. Isotopes in Environmental and Health Studies 39(3):211–217.

Weber, R. E. 2007. High-altitude adaptations in vertebrate hemoglobins. Respiration Physiology and Neurobiology 158:132–142.

West, J. B., G. J. Bowen, T. E. Cerling, and J. R. Ehleringer. 2006. Stable isotopes as one of nature's ecological recorders. Trends in Ecology and Evolution 21:408–414.

Wolf, N., G. J. Bowen, and C. Martinez del Rio. 2011. The influence of drinking water on the δD and $\delta^{18}O$ values of house sparrow plasma, blood, and feathers. Journal of Experimental Biology 214:98–

103.

Wolf, N., S. D. Newsome, M. L. Fogel, and C. Martinez del Rio. 2012. An experimental exploration of the incorporation of hydrogen isotopes from dietary sources into avian tissues. Journal of Experimental Biology 215:1915–1922.

Wunder, M. B., J. R. Jehl, and C. A. Stricker. 2012. The early bird gets the shrimp: confronting assumptions of isotopic equilibrium and homogeneity in a wild bird population. Journal of Animal Ecology 81:1223–1232.

Wunder, M. B., C. Kester, F. L. Knopf, and R. O. Rye. 2005. A test of geographic assignment using isotope tracers in feathers of known origin. Oecologia 144:607–617.

Yohannes, E., K. A. Hobson, and D. J. Pearson. 2007. Feather stable-isotope profiles reveal stopover habitat selection and site fidelity in nine migratory species moving through sub-Saharan Africa. Journal of Avian Biology 38:347–355.