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RESEARCH ARTICLE

The influence of drinking water on the δD and $\delta^{18}O$ values of house sparrow plasma, blood and feathers

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SUMMARY

We investigated the relationships between the δdeuterium (δD) and the δ^{18}oxygen ($\delta^{18}O$) of drinking water and the δD and $\delta^{18}O$ of blood plasma, red blood cells and feathers in house sparrows (*Passer domesticus*) fed on diets with identical hydrogen and oxygen isotopic compositions and five isotopically distinct drinking water treatments. We expected and, with only one exception (^{18}O in blood plasma), found linear relationships between the δD and $\delta^{18}O$ values of drinking water and those of bird tissues. The slopes of these relationships, which estimate the percentage contributions of drinking water to the tissue isotopic signatures, were lower than those of previous studies. We found significant differences in the δD and $\delta^{18}O$ values of feathers, red blood cells and plasma solids. In feathers and red blood cells, δD and $\delta^{18}O$ values were linearly correlated. Our results have three implications for isotopic field studies: (1) if the isotopic composition of drinking water differs from that of food, its effect on tissue isotope values can confound the assignment of animals to a site of origin; (2) comparisons of the δD and $\delta^{18}O$ values of different tissues must account for inter-tissue discrimination factors; and (3) δD/$\delta^{18}O$ linear relationships are probably as prevalent in animal systems as they are in geohydrological systems. These relationships may prove to be useful tools in animal isotopic ecology.

Key words: δD, $\delta^{18}O$, feathers, precipitation, discrimination, migration ecology.

INTRODUCTION

It is often stated by isotope ecologists that animals are what they eat plus a discrimination factor. This observation recognizes that animals incorporate the isotopic composition of their resources, but that the match between the isotopic makeup of animal's tissues and its diet is rarely perfect (Bearhop et al., 2002; Pearson et al., 2003). Knowing the tissue-to-diet discrimination factors (denoted by $\Delta X=\delta X_{tissues}-\delta X_{diet}$, where X is an element) between an animal's tissues and its food sources is an essential aspect of most stable isotope applications in ecology (reviewed by Martinez del Rio et al., 2009b). For hydrogen and oxygen, discrimination factors are not measured or expressed in the traditional sense as the difference in delta values between consumer and diet (McCutchan et al., 2003). Instead they are reported as the difference in isotopic composition of an animal's tissue (typically bird feathers) and the delta value of local precipitation [Δdeuterium $(D)_{feather-precipitation}$ or $\Delta^{18}O_{feather-precipitation}$ (Hobson, 2005)]. This usage is unorthodox but reflects researchers' interests in using hydrogen and oxygen isotopes to measure animal movements. If the discrimination factors for hydrogen and/or oxygen are known, we can calibrate the relationship between the isotopic composition of a tissue and the isotopic composition of precipitation in an 'isoscape' (*sensu* West et al., 2006) to determine where the tissue was grown (Hobson, 2008; Hobson et al., 2009). Wunder describes in detail the state-of-the-art methods currently used to assign a likely site of origin for a sample given its isotopic composition (Wunder, 2010).

For deuterium, discrimination values are often estimated from the linear relationship between the delta values of feathers of wild-caught birds and the average value of growing season precipitation at the site of feather growth estimated from modeled values derived from long-term (40+ years) delta values of precipitation available in the International Atomic Energy Agency – Global Network of Isotopes in Precipitation (IAEA-GNIP) database (reviewed by Hobson, 2008). Wunder highlighted the importance of calibrating the isotopic value of feathers for correct geographical assignment and identified the complexities involved in these calibrations. He also emphasized how mechanistic understanding can clarify some of the issues involved in the estimation of tissue to precipitation discrimination factors (Wunder, 2010).

One of the important issues that clouds the estimation of tissue $\Delta D_{feather-precipitation}$ or $\Delta^{18}O_{feather-precipitation}$ values is the existence of multiple sources for hydrogen and oxygen in organic matter. Hobson et al. observed that the source of hydrogen in the organic molecules that make up animal tissues can be ultimately derived from two potential sources: the hydrogen in the organic compounds in food and pre-formed water (including free drinking water and water in food) (Hobson et al., 1999). Similarly, Hobson et al. noted that the possible sources for oxygen in the organic molecules that make up animal tissues are food, pre-formed water (including free drinking water and water in food), and air (Hobson et al., 2009). Hobson et al. in their previous paper also noted that sometimes there are large differences in the isotopic signatures between drinking water and food at a single locality and speculated that this disparity could lead to variation in the relationship between the isotopic content of an animal's tissues and that of the precipitation of the area in which this animal lives (Hobson et al., 1999).

Several experiments have attempted to measure the relative contribution of preformed water and diet to the hydrogen and oxygen

in animal tissues. Hobson et al. reported that only 18–32% of the hydrogen in bird tissues (blood, muscle, liver, lipids, feathers and claws) was derived from preformed water (Hobson et al., 1999). Wang et al., Solomon et al. and Nielson and Bowen reported similar ranges (20–39%) for aquatic invertebrate tissues (including chitin produced by chironomids, mosquitoes and brine shrimp) and muscle from salmonid fishes (12%) (Wang et al., 2009; Solomon et al., 2009; Nielson and Bowen, 2010). Wang et al. and Nielson and Bowen also found that 70% of the oxygen in chitin produced by larval chironomids and brine shrimp was derived from water (Wang et al., 2009; Nielson and Bowen, 2010). This value is approximately twice as high as the values found in two previous studies investigating human hair keratin [35% (Ehleringer et al., 2008) and 27% (O'Brien and Wooller, 2007)].

The methods used by Hobson et al., Solomon et al. and Wang et al. rely on a basic mixing model, which does not account for several potential process that can affect the hydrogen and oxygen signatures of tissues (Hobson et al., 1999; Solomon et al., 2009; Wang et al., 2009). For example, a simple two-end mixing model does not account for the potential contribution of atmospheric oxygen to the isotopic composition of body water (Gretebeck et al., 1997) and, in terrestrial animals, for the potential effect of an isotopically fractionated loss of body water (McKenchnie et al., 2004). Ehleringer et al. and Bowen et al. developed a more complete, but also more complex, model to explain the relationship between δD and $\delta^{18}O$ in human hair and local tap water (Ehleringer et al., 2008; Bowen et al., 2009). Unfortunately, despite recent advances (Nielson and Bowen, 2010), some of the many parameters of this model are unknown for most species, and can be difficult to measure.

Rather than attempting to estimate the fractional contribution of drinking water, we adopted a phenomenological strategy and tried to determine the relationship between the isotopic composition of drinking water and the δD and $\delta^{18}O$ of bird tissues. We offered drinking water with contrasting δD and $\delta^{18}O$ values to house sparrows (*Passer domesticus*) fed on the same diet. Based on the results of the studies discussed above we expected a linear relationship between the δD and $\delta^{18}O$ of drinking water and bird tissue.

MATERIALS AND METHODS

Fifteen house sparrows, *Passer domesticus* L. [initial body mass 23.6±2.0 g, final body mass 24.5±2.4 g; means ± s.d.) were captured with mist nets in Albany County, WY, USA in October and November 2007. The birds were marked with unique combinations of colored leg bands and housed in groups of three per cage (0.6 m×0.6 m×0.6 m) at the University of Wyoming's Animal Care Facility at the Department of Zoology and Physiology. Throughout the experiment, the birds were housed at 26.0°C. This temperature is within the thermal neutral zone (25.0–28.0°C) reported for house sparrows (Nzama et al., 2010). For the first 180 days, all five groups received the same millet (*Pennisetum glaucum*) diet (δD=–65.3±6.1‰, $\delta^{18}O$=32.5±0.4‰) and drinking water (δD=–133.5‰, $\delta^{18}O$=–17.4‰) *ad libitum*. Following this 180-day equilibration period, the birds were randomly assigned to one of five treatment groups of three birds each. The five treatment groups were fed the same millet diet, but each group received a drinking water treatment with different δD and $\delta^{18}O$ values [δD($\delta^{18}O$) equaled –132.0(–17.4)‰, –96.7(–15.1)‰, –59.0(–12.3)‰, –23.3(–10.2)‰, 8.3 (–8.1)‰]. The coupled variation in δD and $\delta^{18}O$ values of these waters approximates the natural variation in environmental waters lying along the Global Meteoric Water Line (Craig, 1961). The δD and $\delta^{18}O$ of the drinking water in each treatment were achieved by adding 99 atom percent deuterated water (D_2O) and 98 atom percent ^{18}O water (H_2O^{18}) to distilled local tap water.

Following the water switch, we took weekly blood samples from each bird until the tissues reached constant asymptotic values (16 weeks). Blood samples (≈50 μl) were taken from the brachial vein using a 0.5-ml syringe with a 30-gauge needle, and centrifuged (2 min in a microhematocrit centrifuge) to separate red blood cells and plasma. Red blood cells and plasma were dried to a constant mass in an oven at 50°C, ground into a fine powder and loaded into silver capsules (<0.1–0.2 mg). Samples, packed in capsules, were equilibrated alongside protein reference materials (two horse hair keratins) with known non-exchangeable δD values (horse hair keratin 1: δD=142.0±2.0‰ and $\delta^{18}O$=4.5±0.3‰; horse hair keratin 2: δD=–76.0±2.0‰ and $\delta^{18}O$=13.1±0.3‰) for at least 1 week, then dried under vacuum for 1 week prior to analysis. The D/H and $^{18}O/^{16}O$ ratios of the samples were analyzed at the Purdue University Stable Isotope Facility (West Lafayette, IN, USA) using a Zero Blank Auto-sampler (Costech Analytical, Valencia, CA, USA) interfaced with a thermo-chemical elemental analyzer (Thermo Fisher Scientific, Waltham, MA, USA) and a Delta Plus XP isotope-ratio-monitoring mass spectrometer (IRMS; Thermo Fisher Scientific). Co-analyzed reference materials were used to calibrate the measured values to the Vienna standard mean ocean water–Vienna standard light Antarctic precipitation (VSMOW–VSLAP) reference scale and achieve an approximate correction for exchangeable hydrogen present in the sample matrix, following the guidelines proposed by Wassenaar and Hobson (Wassenaar and Hobson, 2003) and Bowen et al. (Bowen et al., 2005). We followed these guidelines strictly to avoid the differences between laboratory results reported by Smith et al. (Smith et al., 2009).

Once the sparrow blood samples had reached constant asymptotic values, we plucked several tail feathers from each bird and allowed them to re-grow. These new-grown feathers were sampled to determine the contribution of drinking water to the D and O^{18} signatures of feathers, the most widely used tissue in isotope-based avian movement studies. Feathers were cleaned with distilled water and dried [see Paritte and Kelly (Paritte and Kelly, 2009) for discussion of the effect of cleaning methods on feather δD values]. We used surgical scissors to remove the feather barbs from the rachis. The barbs were then minced and homogenized. The rachis was not included in the isotope analyses. Feathers were prepared and analyzed for D/H and $^{18}O/^{16}O$ ratios using the same methods as used for the blood samples. Replicate analyses of a commercial reference keratin (δD=–115.0‰ and $\delta^{18}O$=11.1‰) were run throughout the sample analyses and yielded an accuracy and precision of δD=–114.9±2.0‰, $\delta^{18}O$=11.1±0.3‰.

Statistical analysis

To assess whether the delta values of bird tissues had achieved a relatively constant value, we used repeated measures ANOVA on the δD and $\delta^{18}O$ measurements of red blood cell samples collected on weeks 13–16 after the drinking water shift. Before doing these analyses we tested whether the covariance matrix satisfied sphericity assumptions using Mauchly's criterion (Mauchly, 1940). We used standard least squares linear regression to establish the relationship between the isotopic composition of drinking water and the asymptotic value (week 16) of tissues. Unless otherwise stated, average values are accompanied by standard errors. All statistical analyses were performed using JMP 8 (SAS Institute Inc., Cary, NC, USA).

RESULTS

There were no significant differences in red blood cell δD or $\delta^{18}O$ for weeks 13–16 (δD: $F_{(3,48)}$=0.26, P=0.85; $\delta^{18}O$: $F_{(3,48)}$=0.52, P=0.67). Two birds died before week 13. Consequently, we assumed that the red blood cell δD and $\delta^{18}O$ values for these birds had not reached asymptotic values, and did not include any data from these birds in our analyses. With only one exception ($\delta^{18}O$ in plasma), the δD and $\delta^{18}O$ values of bird tissues were linearly related to the hydrogen and oxygen isotopic values of drinking water (Fig. 1). The slope of the relationship between the delta value of a tissue and that of drinking water has been interpreted as a first order approximation of the percentage contribution of drinking water to the tissue hydrogen and oxygen content (see Ehleringer et al., 2008). The estimated percentage contributions (±s.e.m.) of drinking water to the δD values of plasma, red blood cells and feathers were 17±4%, 14±2% and 18±3%, respectively (Fig. 1). For oxygen, only red blood cell and feather $\delta^{18}O$ were significantly linearly correlated with the $\delta^{18}O$ values of drinking water (Fig. 1). The estimated percentage contributions (±s.e.m.) of drinking water to the $\delta^{18}O$ values of blood cells and feathers were 15±1% and 27±1%, respectively (Fig. 1).

Feathers were significantly enriched in deuterium compared with red blood cells and plasma ($\Delta D_{feathers–rbc}$=21.0±1.5‰ and $\Delta D_{feathers–plasma}$=18.6±4.4‰; paired t_{12}=13.70 and paired t_{12}=4.20, P<0.05, respectively), but the δD value of plasma and red blood cells did not differ significantly (paired t_{12}=0.72, P=0.75; Fig. 1). By contrast, feathers were significantly depleted in ^{18}O relative to blood cells and plasma ($\Delta^{18}O_{feathers–rbc}$=–1.4±0.3‰ and $\Delta^{18}O_{feathers–plasma}$=–3.5±0.7‰; paired t_{12}=4.71 and paired t_{11}=5.29, P<0.01, respectively; Fig. 1). Additionally, plasma was significantly enriched in ^{18}O relative to blood cells ($\Delta^{18}O_{plasma–rbc}$=2.0±0.6‰; paired t_{11}=3.33, P<0.01). δD and $\delta^{18}O$ were significantly correlated in feathers and red blood cells (r^2=0.46 and 0.65, P<0.05, respectively; Fig. 2). However, there was no correlation between δD and $\delta^{18}O$ in plasma (r^2=0.00, P=0.95; Fig. 2).

DISCUSSION

The δD of house sparrow plasma proteins, red blood cells and feathers, and the $\delta^{18}O$ of house sparrow red blood cells and feathers varied linearly with drinking water δD and $\delta^{18}O$, despite the provision of identical diets. Following the model of Ehleringer et al. we used the slope of these relationships as a ‘first-order estimate’ of the fractional contribution of drinking water to the hydrogen and oxygen content of tissues (Ehleringer et al., 2008). In the first section of this discussion, we use Ehleringer et al.’s model to examine the factors that might influence the value of these estimates. Our estimates were lower than those for similar tissues from previous studies (Hobson et al., 1999; O’Brien and Wooller, 2007; Ehleringer et al., 2008; Solomon et al., 2009). In the second section, we consider potential explanations for this observation. Our experiment also revealed a positive correlation between δD and $\delta^{18}O$ in feathers and red blood cells, but not in plasma. In the third section, we examine the possibility of using the δD/$\delta^{18}O$ relationship in keratin-containing tissues, such as hair and feathers, as a potential source of ecological information and discuss the implications of our results for the interpretation of animal movements that rely on δD and $\delta^{18}O$ as markers for site of origin. Finally, in the fourth section, we consider possible reasons for the variations in isotopic compositions we found between tissue types.

Interpretation of the linear relationship between tissue and drinking water isotopic values

Can the slopes of tissues *versus* drinking water regression lines be used to estimate the contribution of drinking water to the hydrogen and oxygen content of tissues? To answer this question, we consider the models proposed by Ehleringer et al. (Ehleringer et al., 2008). For a detailed description of these models, we refer readers to Ehleringer et al. (Ehleringer et al., 2008) and to Bowen et al. (Bowen et al., 2009). Briefly, the models account for all possible sources of hydrogen and oxygen in hair keratin, and identify possible steps in

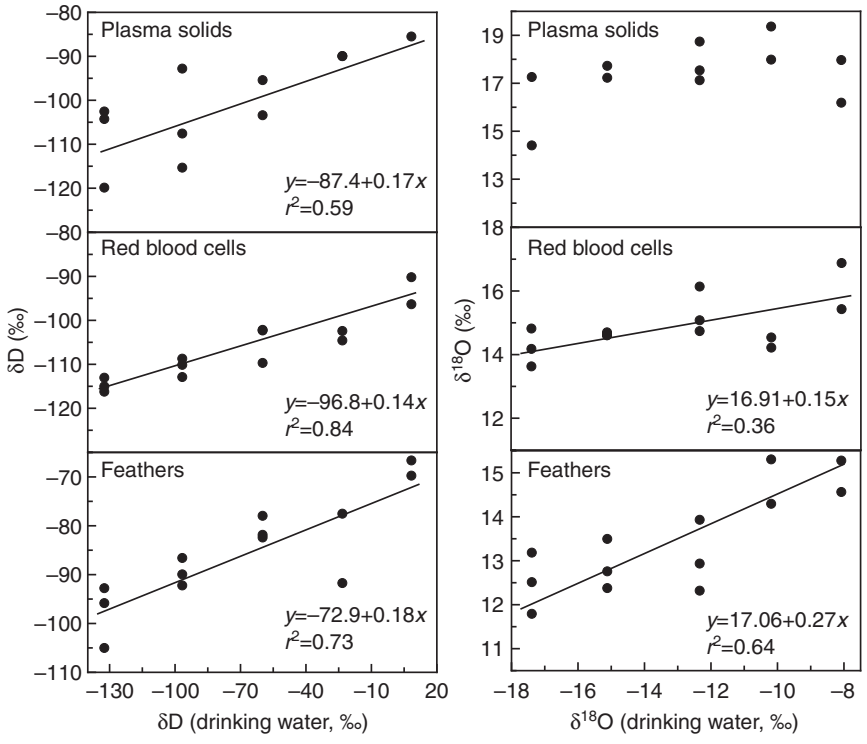

Fig. 1. Relationships between the δD (left) and $\delta^{18}O$ (right) of drinking water and that of three tissue types of house sparrows: plasma solids, red blood cells and feathers. The isotopic values of tissues were significantly correlated with those of drinking water in all cases except the $\delta^{18}O$ of plasma solids.

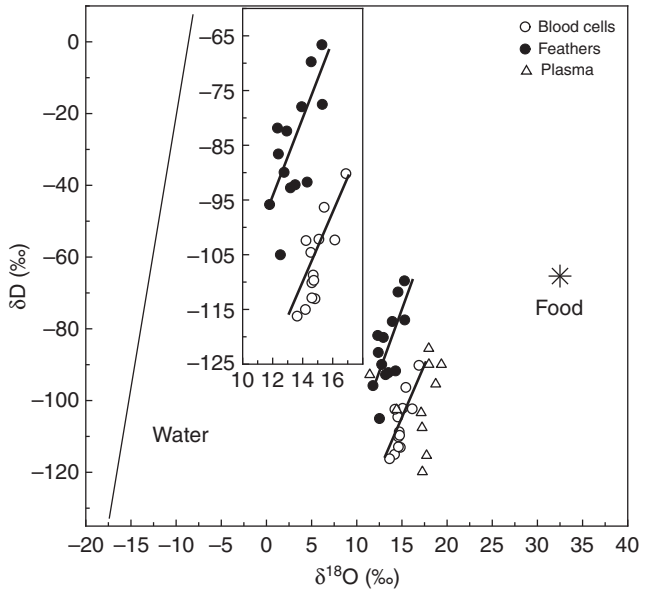

Fig. 2. The δD and δ^{18}O values of feathers, blood cells and plasma. The feather and blood cell values were positively and linearly correlated (y=–170.9+6.4x, r^2=0.46, y=–214.0+7.2x, r^2=0.65, P<0.01). The δD and δ^{18}O values of plasma solids were not statistically correlated. For reference, we included the meteoric water line and the isotopic composition of food (*). The inset shows an enlarged view of the scatter plots for feathers and blood cells.

the incorporation of these elements that can lead to fractionation of an isotope. The models predict a linear relationship between the δD and δ^{18}O values of drinking water and those of protein. Our results verified these predictions (Fig. 1). Furthermore, these models allow for the derivation of explicit expressions to describe the factors that influence the slope of the relationship between the D and ^{18}O values of drinking water and tissues.

For D, the value of this slope equals:

$$\text{Slope}_{\text{tissue–drinking water}} = \left\{\frac{\alpha_{\text{hw}} ed}{m\alpha_{\text{fwlh}} + n}\right\}(1 - f_{\text{d}}), \quad (1)$$

where f_{d} is the fraction of the H atoms in a tissue derived directly from food, and hence 225 (1–f_{d}) equals the fraction of H atoms in the tissue derived from other sources including drinking water [the meanings of the remaining parameters are defined in the List of symbols and abbreviations, following the nomenclature used by Ehleringer et al. (Ehleringer et al., 2008)]. Because the values of the fractionation factors α_{hw} and α_{fwlh} are very close to 1 (Gretebeck et al., 1997), and because (m+n)=1 (Gretebeck et al., 1997), we can approximate Eqn 1 as:

$$\text{Slope}_{\text{tissue–drinking water}} = ed\,(1 - f_{\text{d}}), \quad (2)$$

where d and e are as defined above, but in the case of feathers, e is the fraction of body water in follicular cells. The values of these parameters are unknown in house sparrows. However, it is reasonable to assume the d is directly related (if not identical) to the proportion of body water derived from drinking water. Consequently, in birds, d depends on water intake and can vary widely from 0.22 in water-deprived granivores (MacMillen, 1990) to <1 in nectar feeding birds (McWhorter et al., 2003). The value of e depends on the relative rates of metabolic water production and water exchange across the cell plasma membrane in each tissue (Kreuser-Martin et al., 2003; Kreuser-Martin et al., 2005).

Ehleringer et al.'s model assumes that essentially all oxygen atoms in amino acids are in the terminal (carboxylic) carbon (Ehleringer et al., 2008). This is not strictly the case. Many amino acids contain oxygen atoms in other positions (e.g. all acidic amino acids and their amides). Thus, the model misses a potentially important contribution of the δ^{18}O value of food to that of tissues. In this discussion, we will follow Ehleringer et al. and assume that the direct contribution of diet to the oxygen content of protein is negligible. Ehleringer et al.'s model also assumes that the oxygen in the carbonyl atom should reflect the isotopic composition of intestinal water (Ehleringer et al., 2008). This assumption stems from the observation that during the digestive hydrolysis of peptide bonds, the oxygen from water is incorporated into the C-terminus of carboxylic acids (Stewart et al., 2001). This assumption implies that the value of the slope equals:

$$\text{Slope}_{\text{tissue–drinking water}} = \alpha_{\text{ow}}\left(\frac{ag_1}{h\alpha_{\text{fwlo}} + j\alpha_{\text{CO}_2} + k} + g_2\right). \quad (3)$$

Because $h\alpha_{\text{fwlo}}$+$j\alpha_{\text{CO}_2}$ +k≈1, a_{ow}≈1, and g_2≈0, we can approximate Eqn 3 as:

$$\text{Slope}_{\text{tissue–drinking water}} \approx ag_1, \quad (4)$$

where a is the proportion of body water oxygen derived from drinking water and g_1 is as defined above.

Protein is digested into its constituent amino acids in the small intestine, and most of the water in the lumen of the small intestine is derived from body fluids (Soergel and Hofmann, 1972). Consequently, the value of g_1 should be close to 1, and the value of the slope should be ≈a (namely, the proportion of body water oxygen derived from drinking water). Ehleringer et al.'s model assumes that the contribution of drinking water and food to the oxygen content of tissues is exclusively through their effect on the isotopic composition of body water (Ehleringer et al., 2008). In the following paragraphs, we use these interpretations to examine why the D and ^{18}O isotopic composition of drinking water has a seemingly lower effect on the isotopic composition of sparrow tissues than that reported before for other species.

Low contribution of drinking water to the D and ^{18}O content of house sparrow tissues

The percentage contribution of drinking water to the hydrogen content of house sparrow tissues was lower than previous estimates for similar tissues in other bird species [20–32% (Hobson et al., 1999)] and humans [36% (O'Brien and Wooler, 2007) and 27% (Ehleringer et al., 2008)], but higher than those estimated by Solomon et al. for salmonid fishes (Solomon et al., 2009). Granivorous passerines, such as house sparrows, have relatively low drinking water requirements (MacMillen, 1990). Therefore parameters d in Eqn 2 and a in Eqn 4 are likely to have a low values in our study system. Thus, we should expect lower contributions of drinking water in these animals than in obligate drinkers or aquatic organisms. The low value estimated by Solomon et al. for salmonids is perplexing (Solomon et al., 2009). We also expect the contribution of drinking water to the oxygen content of tissues to be higher in aquatic animals than in terrestrial animals with low drinking water requirements. We emphasize that tests of this prediction must account for potential differences in tissue types because the sources of oxygen and hydrogen in the different biomolecules that constitute different tissues might differ (Nielson and Bowen, 2010).

The meaning of keratin δD and δ^{18}O lines

Ehleringer et al. documented a tight positive relationship between δD and δ^{18}O in the hair of humans in the United States (Ehleringer et

al., 2008). In a similar fashion, we found positive linear relationships between the δD and $\delta^{18}O$ of feathers and blood cells. As pointed out by Ehleringer et al. and Bowen et al., these linear relationships are expected if the hydrogen and oxygen isotopic composition of food is either relatively constant across a study group or co-varies with the isotopic composition of drinking water (Ehleringer et al., 2008; Bowen et al., 2009). Ehleringer et al. assume that humans in the United States ingest an isotopically homogeneous diet [the "supermarket diet" (Nardoto et al., 2006)]. This condition was satisfied in our experiment because birds in all treatments shared the same millet diet. If the isotopic composition of food is constant, then the slope of the relationship between the δD and $\delta^{18}O$ of tissues can be estimated by:

$$\text{Slope}_{\delta D/\delta^{18}O} \text{ of tissues} = \frac{ed(1-f_d)}{a}\,(\text{Slope}_{\delta D/\delta^{18}O} \text{ of drinking water source}) \quad (5)$$

(all parameters are defined in the List of symbols and abbreviations).

Eqn 5 establishes that the slope of the relationship between the δD and $\delta^{18}O$ of tissues equals the product of the slope of the relationship for drinking water and the ratio of the fractional contributions of drinking water to the hydrogen [$ed(1–fd)$] and oxygen (a) content of tissues. For example, the slope of the δD and $\delta^{18}O$ relationship for tap water in the United States is 7.9 and the ratio of the fractional contributions of drinking water to the hydrogen and oxygen content of hair equals 0.27/0.35=0.77 (Ehleringer et al., 2008). Thus, the expected slope of the δD and $\delta^{18}O$ relationship for hair is approximately 6.1, which is close to the 5.7 value reported by Ehleringer et al. (Ehleringer et al., 2008). In our experiment, the $\delta D/\delta^{18}O$ relationship for drinking waters had a slope of 15.0. For feathers and blood cells, the observed slopes of the δD and $\delta^{18}O$ relationships were 6.4±2.2 and 7.2±1.6 (±s.e.m.), respectively. Although these values differed somewhat from the expected slope values (9.9 and 14.0) for feathers and blood cells, respectively, they were within the 95% confidence intervals of these expected values (5.6–19.8 for feathers and 7.1–80.9 for blood cells).

Differences in isotopic composition of tissues

Feathers are inert after they finish growing, and, as a result, their isotopic composition reflects that of the drinking water and food ingested before the molt. By contrast, blood cells and plasma are constantly renewed (Wolf et al., 2009). In house sparrows, the average residence time of carbon in plasma solids is approximately 10 days, whereas that of blood cells is approximately 29 days (Carleton et al., 2008). The turnover of D and ^{18}O in these tissues is unknown. By measuring the isotopic composition of different tissues, we can examine the resources (and potentially the habitats) used by animals at different times (see Martínez del Rio et al., 2009a). This application of stable isotopes demands that we know whether tissues differ in isotopic composition in a single animal.

In our experiment, feathers were significantly enriched in D relative to red blood cells and plasma. Feathers were also significantly depleted in ^{18}O relative to blood cells and plasma. A D enrichment of approximately 20.0‰ or a depletion of approximately –1.4‰ in ^{18}O in feathers relative to blood cells does not indicate a change in habitat or diet (see Results). We emphasize that the magnitude of the inter-tissue discrimination factors for D and ^{18}O reported here must be considered preliminary as they are based on a single study on one species. However, these values suggest that there are significant differences in D and ^{18}O composition in different tissues, and that these differences are large enough to complicate comparisons between the δD and $\delta^{18}O$ values of different tissues. The δD of different amino acids in avian tissues can vary widely (M. L. Fogel and S. D. Newsome, unpublished data). Hence the differences in the δD of different tissues probably results from differences in the amino acid composition of the tissues (Martínez del Rio et al., 2009b).

Conclusions and implications for the interpretation of field data

Our results point to three areas that field researchers must consider when interpreting field data. These areas also represent potentially fruitful arenas of experimental research. First, drinking water has a significant effect on both the δD and $\delta^{18}O$ values in animal tissues. This effect seems to depend on the contribution of drinking water to the animal's body water and hence probably differs between aquatic and terrestrial animals. In terrestrial animals it probably depends on water intake. Available sources of drinking water sometimes differ in δD and $\delta^{18}O$ values from food; for example, when animals feed on crops irrigated with ground water or snow melt or feed on marine sources but drink freshwater (Lott et al., 2003). In these cases, it is unsafe to assume that there is a direct relationship between the isotopic composition of precipitation and that of animal tissues. Differences in the δD and $\delta^{18}O$ values of food and water can confound the assignment of animals to a site of origin. In addition, the δD and $\delta^{18}O$ values of different tissues might differ in animals fed on identical diets and drinking the same water. Newsome et al. have argued that comparing tissues with different time courses of isotope incorporation can yield insights on both the degree and timing of resource specialization of individual animals (Newsome et al., 2007). Comparisons of tissues must be informed by differences in the time course of isotopic incorporation (see Wolf et al., 2009) and account for differences in discrimination factors in the different tissues. We know little about the time course of incorporation of hydrogen and oxygen into animal tissues (see Podlesak et al., 2008) and our results suggest that different tissues will have different D and ^{18}O discrimination factors. Interpreting differences in δD and $\delta^{18}O$ in the tissues of a single animal requires more experimental data on the magnitude of inter-tissue discrimination factors. We speculate that these differences are the result of variation in δD and $\delta^{18}O$ values in different amino acids and in the amino acid compositions of tissues (Martínez del Rio et al., 2009b). Future explorations of inter-tissue discrimination factors may benefit from the insights provided by compound specific analysis (Howland et al., 2003). Finally, there seems to be a clear relationship between the δD and $\delta^{18}O$ values of different body tissues (Ehleringer et al., 2008; Bowen et al., 2009). The $\delta D/\delta^{18}O$ meteoric water line has proved invaluable as a reference in hydrology and geochemistry (Clark and Fritz, 1997). We hypothesize that similar lines will be found for animals and will prove to be important tools in animal isotopic ecology.

LIST OF SYMBOLS AND ABBREVIATIONS

a	proportion of body water oxygen derived from drinking water (*)
ANOVA	analysis of variance
d	proportion of body water hydrogen derived from drinking water (*)
D	hydrogen isotope (1 proton, 1 neutron; moles)
D_2O	99 atom percent deuterated water
e	proportion of follicle water hydrogen derived from body water (*)
f_d	fraction of hydrogen atoms derived from diet (*)
g_1	proportion of gut water derived from body water (*)
g_2	proportion of gut water derived from drinking water (*)
h	proportion of oxygen in body water that is lost as fractionated water associated with breathing and/or evaporation (*)
H	hydrogen isotope (1 proton, 0 neutrons; moles)

H_2O^{18}	98 atom percent oxygen ^{18}O water
IAEA-GNIP	International Atomic Energy Agency – Global Network of Isotopes in Precipitation
j	proportion of oxygen in body water lost as CO_2 (*)
k	proportion of oxygen in body water that is lost as unfractionated water (*)
m	proportion of hydrogen in body water that is lost as fractionated water associated with breathing and/or evaporation (*)
n	proportion of hydrogen in body water that is lost as unfractionated water (*)
SMOW	standard mean ocean water
α_{CO2}	fractionation of oxygen between body water and CO_2 (*)
α_{fwlh}	fractionation of hydrogen in water lost through breathing and/or evaporation (*)
α_{fwlo}	fractionation of oxygen in body water lost through breathing and/or evaporation (*)
α_{hw}	hydrogen isotope fractionated during protein synthesis (*)
α_{ow}	fractionation of oxygen between carbonyl oxygen and water (*)
δD	$[(^2H/^1H_{sample}–^2H/^1H_{standard})\ ^2H/^1H_{standard}{}^{-1}]\times10^3$ (‰, SMOW)
$\delta^{18}O$	$[(^{18}O/^{16}O_{sample}–^{18}O/^{16}O_{standard})\ ^{18}O/^{16}O_{standard}{}^{-1}]\times10^3$ (‰, SMOW)
$\Delta^{18}O_{feather–precipitation}$	$\delta^{18}O_{feather}–\delta^{18}O_{precipitation}$ (‰, SMOW)
$\Delta D_{feather–precipitation}$	$\delta D_{feather}–\delta D_{precipitation}$ (‰, SMOW)
^{16}O	oxygen isotope (8 protons, 8 neutrons; moles)
^{18}O	oxygen isotope (8 protons, 10 neutrons; moles)

*Dimensionless.

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REFERENCES

Bearhop, S., Waldon, S., Votier, S. C. and Furness, R. W. (2002). Factors that influence assimilation rates and fractionation of nitrogen and carbon stable isotopes in avian blood and feathers. *Physiol. Biochem. Zool.* **75**, 451-458.

Bowen, G. J., Chesson, L., Nielson, K., Cerling, T. E. and Ehleringer, J. R. (2005). Treatment methods for the determination of δ^2H and $\delta^{18}O$ of hair keratin by continous-flow isotope-ratio mass spectrometry. *Rapid Commun. Mass Spectrom.* **19**, 2371-2378.

Bowen, G. J., Ehleringer, J. R., Chesson, L. A., Thompson, A. H., Podlesak, D. W. and Cerling, T. E. (2009). Dietary and physiological controls on the hydrogen and oxygen isotope ratios of hair from mid-20th century indigenous populations. *Am. J. Phys. Anthropol.* **139**, 494-504.

Carleton, S., Kelly, L., Anderson-Sprecher, R. and Martínez del Rio, C. (2008). Should we use one-, or multicompartment models to describe ^{13}C incorporation into animal tissues. *Rapid. Commun. Mass Spectrom.* **22**, 3008-3014.

Clark, I. and Fritz, P. (1997). *Environmental Isotopes in Hydrogeology*. Lewis Publishers, Boca Raton.

Craig, H. (1961). Isotopic variations in meteoric waters. *Science* **133**, 1702-1703.

Ehleringer, J. R., Bowen, G. J., Chesson, L. A., West, A. G., Podlesak, D. W. and Cerling, T. E. (2008). Hydrogen and oxygen isotope ratios in human hair are related to geography. *Proc. Natl. Acad. Sci. USA* **105**, 2788-2793.

Gretebeck, R. J., Schoeller, D. A., Socki, R. A., Davis-Street, J., Gibson, E. K., Schulz, L. O. and Lane, H. W. (1997). Adaptation of the doubly labeled water method for subjects consuming isotopically enriched water. *J. Appl. Physiol.* **82**, 563-570.

Hobson, K. A. (2005). Using stable isotopes to trace long-distance dispersal in birds and other taxa. *Divers. Distrib.* **11**, 157-164.

Hobson, K. A. (2008). Applying isotopic methods to tracking animal movements. In *Tracking Animal Migration with Stable Isotopes* (ed. K. A. Hobson and L. I. Wassenaar), pp. 45-78. London: Academic Press.

Hobson, K. A., Atwell, L. and Wassenaar, L. I. (1999). Influence of drinking water and diet on stable hydrogen isotope ratios of animal tissues. *Proc. Natl. Acad. Sci. USA* **96**, 8003-8006.

Hobson, K. A., DeMent, S. H., Van Wilgenburg, S. L. and Wassenaar, L. I. (2009). Origins of american kestrels wintering at two southern US sites: an investigation using stable-isotope (δD, $\delta^{18}O$) methods. *J. Raptor Res.* **43**, 325-337.

Howland, M. R., Corr, L. T., Young, S. M. M., Jones, V., Jim, S., Van Der Merwe, N. J., Mitchell, A. D. and Evershed, R. P. (2003). Expression of the dietary isotope signal in the compound-specific $\delta^{13}C$ values of pig bone lipids and amino acids. *Int. J. Osteoarcheol.* **13**, 54-65.

Kreuser-Martin, H. W., Lott, M. J., Dorigan, J. and Ehleringer, J. R. (2003). Microbe forensics: oxygen and hydrogen stable isotope ratios in *Bacilus subtilis* cells and spores. *Proc. Natl. Acad. Sci. USA* **100**, 815-819.

Kreuser-Martin, H. W., Ehleringer, J. R. and Hegg, E. L. (2005). Oxygen isotopes indicate most intracellular water in log-phase *Escherichia coli* is derived from metabolism. *Proc. Natl. Acad. Sci. USA* **102**, 17337-17341.

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

MacMillen, R. E. (1990). Water economy of granivorous birds: a predictive model. *Condor* **92**, 379-392.

Martínez del Rio, C., Sabat, P., Anderson-Sprecher, R. and Gonzales, S. P. (2009a). Dietary and isotopic specialization: the isotopic niche of three Cinclodes ovenbirds. *Oecologia* **161**, 149-159.

Martínez del Rio, C., Wolf, N., Carleton, C. and Gannes, Z. (2009b). Isotopic ecology ten years after a call for more laboratory experiments. *Biol. Rev.* **84**, 91-111.

Mauchly, J. W. (1940). Significance test for sphericity of a normal n-variate distribution. *Ann. Math. Statist.* **11**, 204-209.

McCutchan, J. H., Jr, Lewis, W. M., Jr, Kendall, C. and McGrath, C. C. (2003). Variation in trophic shift for stable isotope ratios of carbon, nitrogen, and sulfur. *Oikos* **102**, 378-390.

McKenchnie, A. E., Wolf, B. O. and Martinez del Rio, C. (2004). Deuterium stable isotope ratios as tracers of water resource use: an experimental test with rock doves. *Oecologia* **140**, 191-200.

McWhorter, T. J., Martinez del Rio, C. and Pinshow, B. (2003). Modulation of ingested water absorption by Palestine sunbirds: evidence for adaptive regulation. *J. Exp. Biol.* **206**, 659-666.

Nardoto, G. B., Silva, S., Kendall, C., Ehleringer, J. R., Chesson, L. A., Ferraz, E. S., Moreira, M. Z., Ometto, J. P. and Martinelli, L. A. (2006). Geographical patterns of human diet derived from stable isotopes of fingernails. *Am. J. Phys. Anthropol.* **131**, 137-146.

Newsome, S. D., Martinez del Rio, C., Bearhop, S. and Phillips, D. L. (2007). A niche for isotopic ecology. *Front. Ecol. Environ.* **5**, 429-436.

Nielson, K. E. and Bowen, G. J. (2010). Hydrogen and oxygen in brine shrimop chitin reflect environmental water and dietary isotopic composition. *Geochim. Cosmochim. Acta* **74**, 1812-1822.

Nzama, S. N., Downs, C. T. and Brown, M. (2010). Seasonal variation in the metabolism-temperature relation of house sparrows (*Passer domesticus*) in KwaZulu-Natal, South Africa. *J. Therm. Biol.* **35**, 100-104.

O'Brien, D. M. and Wooler, M. J. (2007). Tracking human travel using stable oxygen and hydrogen isotope analyses of hair and urine. *Rapid Commun. Mass Spectrom.* **21**, 2422-2430.

Paritte, J. M. and Kelly, J. F. (2009). Effect of cleaning regime on stable-isotope ratios of feathers in japanese quail (*Coturnix japonica*). *Auk* **126**, 165-174.

Pearson, S. F., Levey, D. J., Greenberg, C. H. and Martinez del Rio, C. (2003). Effects of elemental composition on the incorporation of dietary nitrogen and carbon and isotopic signatures in an omnivorous songbird. *Oecologia* **135**, 516-523.

Podlesak, D. W., Torregrossa, A. M., Ehleringer, J. R., Dearing, D. M., Passey, B. H. and Cerling, T. E. (2008). Turnover of oxygen and hydrogen isotopes in the body water, CO_2, hair and enamel of a small mammal. *Geochem. Cosmochem. Acta* **72**, 19-35.

Smith, A. D., Lott, C. A., Smith, J. P., Donohue, K. C., Wittenberg, S., Smith, K. G. and Goodrich, L. (2009). Deuterium measurements of raptor feathers: Does a lack of reproducibility compromise geographic assignment? *Auk* **126**, 41-46.

Soergel, K. H. and Hofmann, A. F. (1972). Absorption. In *Pathophysiology. Altered Regulatory Mechanisms in Disease* (ed. E. D. Frohlich), pp. 423-453. Philadelphia: J. B. Lippincott.

Solomon, C. T., Cole, J. J., Doucett, R. R., Pace, M. L., Preston, N. D., Smith, L. E. and Weidel, B. C. (2009). The influence of environmental water on the hydrogen stable isotope ratio in aquatic consumers. *Oecologia* **161**, 313-324.

Stewart, I. I., Thomson, T. and Figeys, D. (2001). ^{18}O Labeling: a tool for proteomics. *Rapid Commun. Mass Spectrom.* **15**, 2456-2465.

Wang, Y. V., O'Brien, D. M., Jenson, J., Francis, D. and Wooller, M. J. (2009). The influence of diet and water on stable oxygen and hydrogen isotope composition of Chironomidae (Diptera) with paleoecological implications. *Oecologia* **160**, 225-233.

Wassenaar, L. I. and Hobson, K. A. (2003). Comparative equilibration and online exchange for determination of non-exchangable hydrogen of keratins for use in animal migration studies. *Isotopes Environ. Health Stud.* **39**, 211-217.

West, J. B., Bowen, G. J., Cerling, T. E. and Ehleringer, J. R. (2006). Stable isotopes as one of nature's ecological recorders. *Trends Ecol. Evol.* **21**, 408-414.

Wolf, N., Carleton, S. A. and Martinez del Rio, C. (2009). Ten years of experimental animal isotopic ecology. *Funct. Ecol.* **23**, 17-26.

Wunder, M. B. (2010). Using isoscapes to model probability surfaces for determining geographic origins. In *Isoscapes: Understanding Movement, Pattern, and Process on Earth Through Isotope Mapping* (ed. J. B. West, G. J. Bowen, T. E. Dawson and K. P. Tu), pp. 251-272. Heidelberg, London, New York: Springer Dordrecht.