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Tracing Deuterium through the food web and into Birds and Mammals along an elevational gradient in the Southern Rocky Mountains

Matthew Baumann

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TRACING DEUTERIUM THROUGH THE FOODWEB AND INTO BIRDS AND MAMMALS ALONG AN ELEVATIONAL GRADIENT IN THE SOUTHERN ROCKY MOUNTAINS

by

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B.S., BIOLOGY, UNIVERSITY OF NEW MEXICO, 2009

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TRACING DEUTERIUM THROUGH THE FOODWEB AND INTO BIRDS AND MAMMALS ALONG AN ELEVATIONAL GRADIENT IN THE SOUTHERN ROCKY MOUNTAINS

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ABSTRACT

Animals incorporate local δ²H values from available water and dietary sources during tissue synthesis, which can provide evidence of elevational movements if multiple tissues with different turnover times are examined. Here we test the applicability of using δ²H as an indicator of altitudinal movements by examining multiple tissues (δ²Hbw (body water), δ²Hfur, δ²Hfeather, and δ²Hclaw) in birds and mammals in the Sangre de Cristo Mountains. Additionally, we measured δ²Hsw (stem water) and insect δ²Hbw and δ²Hwb (whole body) values to assess changes with elevation. We sampled 15 species of birds and two small mammal species along with potential food sources (three plant families and four insect groups) over an ~1200 m elevational gradient. δ²Hsw and δ²Hbw of plants, birds, and mammals decreased with increasing elevation. δ²Hfur, δ²Hfeather, δ²Hclaw, and δ²Hbw of insects did not show any discernable pattern with increasing elevation. Only one animal species, the least chipmunk (*Tamias minimus*), showed significant decreases in δ²H values with increasing elevation. Additionally, δ²Hfeather differed based on foraging guild. Other contributing factors such as diet, physiology, ecology, and within site variation may all contribute wide variation in values observed at a specific elevation. Given the amount of variability in each tissue type sampled in this study, we conclude that the use
of δ²H for elevational movements will require careful selection of species, tissues, and extensive validation efforts to ensure accuracy of using this technique. Local variability in δ²H among species and tissues needs to be understood in detail before elevational movements can be assessed.
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Introduction

Over the last decade, stable isotope approaches have been used as a proxy to understand continent-wide population connectivity and to track movements and origins of animals (Marra et. al. 1998, Hobson et. al. 1999, Hobson et. al. 2001, Kelly et. al. 2002). In particular, stable hydrogen isotopes, specifically deuterium (δ²H), and its distribution in keratinous tissue, e.g. feathers, fur or claws, have been used extensively to track migratory animals based on distinct δ²H values of resources found on breeding or wintering grounds (Meehan 2001, Kelly et. al. 2002, Hobson et. al. 2004a, Fraser et. al. 2008a). Hydrogen isotopes provide a useful tool for establishing links between animals and biological resources because there is a latitudinal gradient in the δ²H values of growing season precipitation, which are transferred from groundwater through the food web and into animal tissues (Hobson 1999, Meehan et al. 2004). Intrinsic stable isotope methods have become favored over mark and recapture studies because they provide a much more efficient way to establish connectivity. They involve no recapture, resighting, or body size limitations associated with extrinsic markers such as bands, global positioning system (GPS) locators, or geolocation devices (Hobson 1999). In birds and mammals, the application of stable hydrogen isotope techniques has revealed novel migratory and connectivity patterns (Hobson et al. 2001, Rubenstein et. al. 2002, Britzke et. al. 2009). Sampling the δ²H values of multiple tissues (body water, blood, feather, fur, claws) can provide temporal information on animal movements and locations. Body water and plasma δ²H are turnover rapidly (McKechnie et. al. 2004, Wolf et. al. 2012) while feathers, fur, and claws are inert after synthesis (Hobson 1999), and reflect the location of the animal during the period of synthesis.
In addition latitudinal changes, precipitation δ²H values also change with increasing elevation due to Rayleigh distillation, a process by which heavier isotopes “rain out” of clouds with decreasing temperature and elevation because the heavier isotope favors the condensed state (the altitude effect) (Poage and Chamberlain 2001). Based on the physical properties of deuterium, precipitation δ²H values become increasingly more depleted with increasing elevation (Bowen and Wilkinson 2002). These changes in δ²H with elevation should be reflected in animal tissues, however, very few data examining this phenomenon. The few studies that have examined this issue are confined to mesic environments and vary in their conclusions. Although this work confirms that δ²H values of avian tissues show a significant depletion with increasing elevation (Hobson et. al. 2003, Hardesty and Fraser 2010), in the latter study the observed variation in δ²H among individuals at a specific elevation was very large. The observed depletion, in birds, ranges from depletion of deuterium has been documented to be -3.8 to -5.3‰ per 500m increase in elevation based on feathers and blood from nectarivores and insectivores along an elevational gradient in Ecuador (Hardesty and Fraser 2010). In mammals, a δ²H lapse rate with elevation is unknown and elevational movements in small mammals are thought to be minimal (Bartels and Thompson 1993, Verts and Carraway 2001). Elevational movements in birds are thought to be fairly common and related to food resource availability or environmental conditions (Dixon and Gilbert 1964, Powell and Bjork 1994). Elevational movements may also be related to energetically-expensive life history events such as molting or breeding (Hobson 2003). Just as with latitudinal movements, vertical movements of animals are hard to detect due to the constraints of traditional mark and recapture methods. Thus, measurements of
tissue δ²H values are a potential source of information on altitudinal movements in animals.

Compared to other stable isotopes, particularly Carbon and Nitrogen, deuterium has only recently been used in ecological studies and comparatively much less is known about its distribution and behavior. As a consequence, the movement and incorporation of deuterium into animal tissues and the variation within individuals and even populations is still poorly understood (Wolf et al. 2012). The hydrogen incorporated into animal tissues is derived from several sources, which include drinking water, and preformed water and organic hydrogen from diet (Hobson 1999). Recent studies have shown that the contribution of hydrogen atoms to animal tissues from preformed or drinking water is relatively small (Hobson et al. 1999, Wang et al. 2009, Wolf et al. 2011), but this may vary by species drinking requirements (Wolf et al. 2013). Relatively little hydrogen allocated towards tissue synthesis from drinking water potentially provides variability in δ²H values of animal tissues due to other contributing factors such as diet, environmental conditions, or physiology which may confound predictable spatial deuterium patterns. Additionally, recent studies have shown that foraging substrate and small scale microhabitat also have an effect on δ²H values. Differences between ground versus non-ground foraging birds may account for some uncertainty in origin estimates as well because ground foraging birds have shown higher δ²H tissue values (Fraser et al. 2011, Hobson et al. 2012). Testing multiple tissues may uncover just how much variation exists at a single site. Since few studies have assessed δ²H along elevational gradients, it is essential to understand variation of δ²H from different water and food sources within an ecosystem and how the role of increasing elevation affects both sources as they are
transferred into animal tissues. Previous studies have simply compared $\delta^{2}H_{\text{feather}}$ to local precipitation $\delta^{2}H$ values, which does not account for many other biological factors that occur between feather synthesis and precipitation. Sampling potential dietary items, local ground water, and multiple tissues from animals at a single site allows a look at the dynamics affecting local $\delta^{2}H$ values, and how different tissue $\delta^{2}H$ values vary accordingly.

In this study we examined how faithfully $\delta^{2}H$ values correlated with elevation along a 1200m elevation gradient in the mountains of north-central New Mexico. Plant $\delta^{2}H_{\text{sw}}$ (stem water), insect $\delta^{2}H_{\text{bw}}$ (body water) and $\delta^{2}H_{\text{wb}}$ (whole body), bird ($\delta^{2}H_{\text{bw}}$ and $\delta^{2}H_{\text{feather}}$), and mammal tissue ($\delta^{2}H_{\text{bw}}$, $\delta^{2}H_{\text{fur}}$, and $\delta^{2}H_{\text{claw}}$) were sampled continuously along the gradient. Because $\delta^{2}H_{\text{bw}}$ turns over rapidly we expected to see a strong correlation between body water of the animals sampled and $\delta^{2}H_{\text{sw}}$ values with increasing elevation. In addition, we predicted a direct correlation between groundwater, plant tissue water, fur and feather, and claw $\delta^{2}H$ values from mammals and birds with increasing elevation, in particular small mammals because of their presumed limited elevational movements. We also examined how foraging substrate may lead to differing $\delta^{2}H$ values and predicted that ground foraging birds will show higher $\delta^{2}H$ values compared to non-ground foragers. If precipitation patterns with elevation are faithfully recorded by animal tissues then the unique $\delta^{2}H$ tissue values could be used to discern elevational movements and assign origin to animals along our elevational gradient and also expanded to other similar elevational gradients.

**Methods:**
Our study was conducted along a ~1200 meter elevational transect at Elk Mountain in the Sangre de Cristo Mountains, San Miguel County, New Mexico (35.77°N, 105.55°W). Animal, insect, and plant samples were collected (Bird: IACUC-11-100742-MCC, Mammal: 12-100764-MCC) opportunistically throughout the transect from late May to early June in 2011 and 2012, or until fire restrictions closed the Santa Fe National Forest, to ensure we were sampling breeding individuals. Individual specimens were marked using a GPS to record latitude, longitude, and elevation. Muscle and liver were extracted from birds (15 spp.) and mammals (2 spp.), respectively, in the field and were immediately placed in sealed vials and frozen for water extraction. Plant (3 families) and insect (4 families.) specimens were also placed in sealed vials and immediately frozen for water extraction. Feather (rectrix 1) or fur from the distal end of the tail and claw material were sampled from each bird and mammal specimen and placed in individually labeled envelopes for isotopic analysis.

Stable isotope analysis

Feather, fur, and claw material were cleaned in a 2:1 Chloroform: Methanol solution and allowed to air dry in a fume hood for 48 hours. The samples (0.12-0.2 mg) were then cut with sharp scissors and were loaded into silver capsules for isotopic analysis. Insects (0.12-0.2 mg) were dried for 24 hours at 60°C and ground into a fine powder with a mortar and pestle and loaded into silver capsules. Feather and fur samples were run at the Colorado Plateau Stable Isotope Laboratory (CPSIL) with a Thermo-Finnigan Delta Plus XL isotope ratio mass spectrometer, while claw and insect samples were run at the Carnegie Institution of Washington (Washington D.C). Samples were allowed to equilibrate with the local water vapor for at least 72 hours before analysis.
Normalization keratin standards (-121.6‰) along with Caribou hoof (CHS, -197‰) were used as reference standards at CPSIL to calibrate isotopic values. Stable-hydrogen isotope ratios ($^{2}$H/$^{1}$H=R) were expressed in standard δ notation. Units of per mil (‰) were compared to a standard that is defined as $[(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 1000$, where the sample is the measured ratio of D:H and the standard is of known value compared to a standard scale of the Vienna Standard Mean Ocean Water- Standard Light Antarctic Precipitation (VSMOW-SLAP). Precision of normalized keratin standards were empirically determined to fall within a standard deviation of 1.5‰, while standard deviation of CHS samples was 0.1‰. Water from each tissue (muscle and liver) sample, insect, and plant stem was extracted using a cryogenic vacuum extraction line and analyzed by a Liquid Water Isotope Analyzer, Los Gatos Research, LGR DLT-100 (Lis et al. 2008). Two working standards (-154.1±1.0 ‰ and -9.8 ±1.0‰) were used to normalize unknown sample δ$^{2}$H values to the known δ$^{2}$H values of the standards.

Assessment of stable isotope values

All animals, pants and insects were analyzed as a function of elevation and species. Birds, mammals, plants, and insects were broken down by species, and when sample samples sizes were sufficient, were run as a function of elevation. For birds, foraging substrate (ground vs. non-ground) was assigned to each species based species accounts in Birds of North America (Poole 2005). These life history accounts were used for analysis of foraging substrate to assess this ecological function of δ$^{2}$H$_{\text{feather}}$ values in bird species. Expected δ$^{2}$H precipitation values were generated along the elevation gradient using estimates from waterisotopes.org following Bowen (2003) and were compared to observed values of samples collected. Statistical tests were performed in R.
2.13.1 (R Development Core Team 2011) for all samples collected. Linear regressions of \( \delta^2H \) values and elevation were performed on birds, mammals, insects, and plants independently and when appropriate by species. Unpaired \( t \)-tests were used to compare foraging guild \( \delta^2H_{\text{feather}} \) values of birds. Additionally, slopes of linear regressions were compared with ANCOVA analyses in GraphPad Prism version 6 for Windows, GraphPad Software, San Diego California USA.

**Results:**

*Stem water*

Predicted precipitation \( \delta^2H \) values were higher than observed stem values, but when all plant sample stem water values were combined there was a significant decreasing trend of \( \delta^2H \) with increasing elevation yet high variation \( (P=0.02, r^2=0.08, \text{Fig. 3}) \). Predicted precipitation \( \delta^2H \) lapse rate was estimated at 4.8‰/500m whereas observed stem water \( \delta^2H \) was found to be 4.0‰/500m. Slopes of the predicted precipitation \( \delta^2H \) values did not differ from observed \( \delta^2H_{\text{sw}} \) \( (P=0.8) \). Out of the plants sampled (\( P. \) tremuloides, \( Q. \) gambelii, Rosaceae sp.), only \( Q. \) gambelii \( (n=15) \) showed a significant decreasing \( \delta^2H_{\text{sw}} \) with increasing elevation \( (P=0.005, r^2=0.4) \).

*Insects*

Insects \( \delta^2H_{\text{bw}} \) and \( \delta^2H_{\text{wb}} \) treated as a whole were found to not show any significant relationship with increasing elevation \( (P=0.3 \text{ and } P=0.4, \text{Table 1, respectively}) \). Additionally, \( \delta^2H_{\text{bw}} \) was noticeably higher compared to \( \delta^2H_{\text{wb}} \) (Fig 4). Slopes between
δ²H}_{bw} and δ²H}_{wb} did not significantly differ (P=0.7). δ²H}_{wb} was found to decrease with higher hydrogen content in sample (P=0.0009, r²=0.2, Fig. 10).

Birds

When all avian species and individuals were combined, δ²H}_{bw} decreased with increasing elevation (P=0.002, r²=0.3, Fig. 1, Table 1) but δ²H}_{feather} showed no discernible pattern with elevation (P=0.3, r²=0.02) and was lower compared to δ²H}_{bw} (Fig. 1). Slopes between δ²H}_{bw} and δ²H}_{feather} were significantly different (P=0.02). The two bird species with the largest sample size, American Robin (Turdus migratorius, n=20) and Dark-eyed Junco (Junco hyemalis, n=12), were each found to have marginally significant decreasing δ²H}_{bw} values with increasing elevation (robin: P=0.06, r²=0.1; junco: P=0.05, r²=0.3; Fig. 7, Fig. 8) but non-significant relationships were found in δ²H}_{feather} values for each species (P=0.1 and 0.9; Table 3, respectively). This is the same trend seen with birds grouped as a whole. Because claw material represents a time period roughly 2-5 months prior to synthesis (Bearhop et al. 2003) we chose not to include δ²H}_{claw} data for birds because it likely represents non-breeding locations during or prior to migration since we sampled in late spring.

Mammals

δ²H}_{bw} of both small mammal species combined decreased with increasing elevation (P=0.01, r²=0.2; Fig. 2, Table 1) but showed no relationship in δ²H}_{fur} (P=0.3, Fig. 2) or δ²H}_{claw} (P=0.8, Fig. 2). Just as in insects and birds, δ²H}_{fur} and δ²H}_{claw} were noticeably lower compared to δ²H}_{bw}. The two mammal species sampled showed different trends compared to when they both were combined (Table 2). Least chipmunk (Tamias
minimus) (n=13) showed significant relationships with elevation in all tissues sampled ($\delta^2$Hbw: $P=0.001$, $r^2=0.6$; $\delta^2$Hfur: $P=0.01$, $r^2=0.4$; $\delta^2$Hclaw: $P=0.007$, $r^2=0.5$; Fig. 6).

Golden-mantled ground squirrel (CallospERMophilus lateralis) (n=19) showed non-significant relationships in each tissue ($\delta^2$Hbw: $P=0.1$, $r^2=0.1$; $\delta^2$Hfur: $P=0.9$, $r^2=0.05$; $\delta^2$Hclaw: $P=0.6$, $r^2=0.02$; Fig. 5).

**Foraging Guilds**

Since $\delta^2$Hfeather showed no relationship with elevation, we combined all values together and compared across the two different foraging guilds in question (Table 4).

Ground foraging bird species were found to have higher overall $\delta^2$Hfeather values compared to non-ground foraging species ($P=0.02$, Fig. 9). $\delta^2$Hbw of grasshoppers, ground foraging, was significantly higher compared to all the other insects sampled ($P=0.01$).

**Discussion:**

This is the first study to incorporate an ecosystem-wide approach to examining variation in $\delta^2$H values along an elevational gradient in an arid environment. As expected, we found a significant depletion of $\delta$Dsw with increasing elevation. We also found a similar negative relationship between elevation and the $\delta^2$Hbw pools of small mammals and birds, but not in insects. In all cases, $\delta^2$Hbw values were enriched compared to $\delta^2$Hsw. In contrast to our original hypothesis and the current literature (Hobson et al. 2003, Hardesty and Fraser 2010), we found no significant relationship in $\delta^2$Hfeather, $\delta^2$Hfur, or $\delta^2$Hclaw with increasing elevation. Interestingly, we found significant differences
between $\delta^2$H$_{fur}$ and $\delta^2$H$_{bw}$ values among mammals. We also found differences between $\delta^2$H$_{feather}$ and $\delta^2$H$_{bw}$ among birds and insects that forage on different substrates. In the following paragraphs we discuss these findings in detail and assess the implications for using $\delta^2$H to track altitudinal movements of wildlife in arid ecosystems.

Changes in $\delta^2$H of water in plants, insects, and animals with elevation

Globally, as elevation increases, precipitation $\delta^2$H values become increasingly depleted (Poage and Chamberlain 2001, Bowen and Wilkinson 2002). The approximate lapse rate per 500m was estimated at 4.8‰ at our site in the Sangre de Cristo Mountains based on the model provided by Bowen (2003). We observed a stem water $\delta^2$H lapse rate of 4.0‰/500m. The lapse rates were similar but elevation explained little of the variation seen in $\delta^2$H$_{sw}$. We also found $\delta^2$H$_{sw}$ values to be the most depleted of the different pools we sampled and were generally more depleted than the predicted annual precipitation $\delta^2$H values predicted for our site (Bowen 2003; Fig. 3). Our observed $\delta^2$H$_{sw}$ values may have been more depleted because observed values may reflect large inter-annual differences of $\delta^2$H in precipitation at this site. Additionally, runoff from higher elevations may also play a factor in the observed discrepancy between predicted and observed $\delta^2$H values (Hobson 2003). When all plant samples were pooled, we found the expected significant relationship of increasing depletion $\delta^2$H$_{sw}$ with increasing elevation. When separated by plant species, only Gambel oak (Quercus gambelii) showed a significant negative trend with increasing elevation, and this species also had the most enriched $\delta^2$H$_{sw}$ values compared to the other samples. Gambel oak has an upper elevational limit of approximately 3000m (Brown 1958), which is lower than any other plant species we sampled. Because our highest sample was taken from 2648 meters, comparatively more
enriched $\delta^2H_{sw}$ values are not unexpected. Tree height was not measured from each plant sample, but previous work has shown that plant height can have an effect on $\delta^2H_{sw}$ values. Dawson (1996) reported that small sugar maples (<5m) were found to have enriched $\delta^2H$ values of xylem sap due to differential water source usage compared to large trees. The non-uniform height of the trees and shrubs sampled may have contributed to the observed variation such that it swamped or masked any trends of species-specific $\delta^2H$ values with elevation.

The insects sampled (Apidae, Diptera, Caelifera, and Lasiocampidae) showed no discernible pattern of $\delta^2H_{bw}$ with increasing elevation, and had some of the most enriched $\delta^2H_{bw}$ values observed. Insects have small body water pools and potentially high evaporative rates that may result in greater $\delta^2H$ enrichment in comparison to stem water, and the tissues of birds, and mammals. Interestingly, grasshopper $\delta^2H_{bw}$ were found to be significantly more enriched in comparison to the other insects sampled. Variation in soil water $\delta^2H$ with depth may play a role in explaining this trend. Grasshoppers generally feed on grasses and shrubs that draw water from shallow soil layers that are subject to high rates of evaporation and evaporative enrichment and more enriched $\delta^2H$ values than deep ground water (Allison 1983, Dawson 1996).

Birds and mammal body water

Bird and small mammal $\delta^2H_{bw}$ pools were significantly enriched compared to $\delta^2H_{sw}$ and this may represent trophic discrimination in $\delta^2H$ values (Birchall et al. 2005). Stem water $\delta^2H$ are closely tied to the local precipitation $\delta^2H$ values and we expected to see similar relationships with the bird and mammal $\delta^2H_{bw}$, but environmental and/or
physiological factors potentially cofound this relationship. A potential source of the
difference between the plant water and body water pools of birds and mammals is likely
related to the aridity at this site. Evaporative water loss results in enrichment in body
water pools of animals and likely enriches body water pools further in arid conditions or
at high elevations where metabolic rates are increased (McKechnie et al. 2004).

Changes in \( \delta^2 H \) of feathers, fur, and \( \delta^2 H_{wb} \) of birds, mammals, and insects with elevation

Although feather \( \delta^2 H \) values also have been shown to track precipitation \( \delta D \)
values with increasing elevation (Hobson et al. 2003, Fraser et al 2008b, Hardesty and
Fraser 2010); elevational \( \delta^2 H \) patterns have not been examined in mammal tissues. In
birds, \( \delta^2 H \) fractionation between precipitation and non-exchangeable Hydrogen in
feathers has been reported at roughly 25‰ (Wassenaar and Hobson 2001). Our results
differ from previous studies; we found high variability in tissue \( \delta^2 H \) values with no
predictable pattern of depletion in \( \delta^2 H_{\text{feather}} \) with increasing elevation. Our \( \delta^2 H_{\text{feather}} \) and
\( \delta^2 H_{\text{fur}} \) values did fall, however, within the range of previously reported values for
fractionation between precipitation and feathers (Fig. 11). Fur and insect whole body \( \delta^2 H \)
also showed no relationship with elevation and showed large variation. Hardesty and
Fraser (2010) reported similar results where \( \delta^2 H_{\text{feather}} \) values showed a weaker elevational
trend compared to \( \delta^2 H \) of plasma in birds from the Ecuadorian Andes. A disconnect
between body water and keratinous tissue \( \delta^2 H \) values may be driven by the amount of
hydrogen atoms allocated to feather, fur, and claw growth from dietary versus drinking or
preformed water inputs. Contributions of Hydrogen atoms from drinking or preformed
water to the assembly of feather and fur keratin is relatively small and typically ranges
2013) and may be reduced even further due to increased metabolic rates in animals inhabiting colder environments at higher elevations (Storm-Suke et al. 2012). The contributions of dietary organic hydrogen to avian tissues ranges from 30-86% depending upon tissue type (Hobson et al. 1999, Wolf et al. 2011, Wolf et al. 2013). Our results showed that keratinous tissues from birds and mammals had much more depleted δ²H values compared to body water, but had similar values compared to the insect δ²H wb, which suggests that dietary hydrogen inputs are the primary contributor to keratinous tissue synthesis. In addition, lipids are known to have substantially more depleted deuterium values due to fractionation during biosynthesis (Smith and Epstein 1970), which was reflected in our insect whole body samples. Insect δ²H wb samples were increasingly depleted as hydrogen content in the samples increased (Fig. 10). Lipids are composed of long chains of hydrogen atoms and have lower C:H ratios than proteins and polysaccharides. Dietary items high in lipid content are sought out by birds and presumably small mammals, especially prior to hibernation. Given the large quantities of hydrogen from dietary items, the large discrepancy seen between δ²H bw and δ²H feather/fur and variation seen in keratinous tissues is likely a byproduct of the composition of food items ingested.

Within-site variation in the δ²H of dietary resources may also play an important role in driving the observed variation in δ²H feather or δ²H fur. Betini et al. (2009) found ~20‰, variation in insect δ²H values in differing microsites at a single location in Ontario, which they hypothesized contributed to the variation in nestling Tree Swallow (Tachycineta bicolor) δ²H blood. Our insect δ²H bw and δ²H wb samples showed large variation (~100‰) and undoubtedly has an effect on δ²H feather, δ²H fur, δ²H claw, and the
δ²H of other tissues. Given the large variation in δ²H of insects at a local scale, animal foraging and movements over a relatively small area might produce significant variation. We also cannot rule out the possibility that the lack of elevational trend in δD _feather_ values is caused by dispersal of immature individuals returning to a different area to breed. In birds, it is assumed that the feathers we sampled were grown on the previous year’s breeding area (Pyle 1997), but first year returning individuals may be driven away from their natal territory by competition or resource availability (Greenwood and Harvey 1982), which could add additional variation to our results. Previous studies have suggested that variation in stable isotope values of feathers associated with breeding populations of birds was likely an artifact of natal dispersal (Hobson et al. 2001, Graves et al. 2002).

*Species-specific differences in fur δ²H in mammals*

We found species- and taxon-specific differences in patterns of δ²H values along the elevational gradient of the small mammals we sampled. In least chipmunks we observed significant depletion in δ²H of all tissues sampled with increasing elevation. In contrast, the δ²H of golden-mantled ground squirrel tissues showed no relationship with increasing elevation. We speculate that differences in molt patterns and diet may produce the observed differences between the two species. Least chipmunks have two annual molts, one during the during summer and one in winter, while golden-mantled ground squirrels have a single molt that occurs after breeding in the late summer and early fall (Bartels and Thompson 1993, Verts and Carraway 2001). Our observations suggest that the least chipmunks we sampled had already molted into their summer pelage when collected, while the golden-mantled ground squirrels we sampled retained fur from the
previous year. This may have produced significant differences and greater variation because of dietary changes and the different precipitation periods that are integrated into the fur. The least chipmunk is a granivore with a relatively small home range compared to that of the omnivorous golden-mantled ground squirrel (Thorington et al. 2012). Greater variability in golden-mantled ground squirrel $\delta^2$H$_{fur}$ values may reflect a broader foraging niche. Least chipmunks, in contrast may have a more constrained diet especially in the early summer before plants green up. We only sampled the summer pelage of the least chipmunk, but it would be intriguing to sample both the summer and winter pelages to compare the differences in $\delta^2$H$_{fur}$ between these two molts.

Body size may also have an effect on the tissue $\delta^2$H values observed because least chipmunks are nearly three-fold smaller (~50 g compared to ~250g, Bartels and Thompson 1993, Verts and Carraway 2001) and have higher surface-area-to-volume ratios. We found least chipmunks to have more enriched $\delta^2$H values in all tissue values sampled compared to golden-mantled ground squirrels. Higher surface-area-to-volume ratios increase total amount of area exposed to the environment and would likely increase evaporative water loss rates potentially resulting in higher $\delta^2$H tissue values. Additionally, least chipmunk accumulate little fat prior to hibernation suggesting that this species would have reduced lipid content compared to the ground squirrel thus supporting the observed results based on the known effect of lipids on $\delta^2$H values (Smith and Epstein 1970, Verts and Carraway 2001).

*Species-specific differences feather $\delta^2$H in birds- potential effects of foraging substrate*
We found differences in $\delta^{2}\text{H_{feather}}$ values between foraging substrate of sampled birds. Ground foraging birds were found to have more enriched (±SD, Fig. 9) $\delta^{2}\text{H_{feather}}$ values compared to non-ground foraging birds, -82±2.2‰ and -91±3‰ respectively. This finding has previously been reported by other researchers (Hobson et al. 2004b, Fraser et al. 2011, Hobson et al. 2012) and likely represents differences in diet. Hobson et al. (2012) suggested that the insect prey utilized by ground foraging birds represented a higher trophic level.

Additionally, ground dwelling versus canopy or shrub feeding insects could potentially have different $\delta^{2}\text{H}$ tissue values due to the type of plant material assimilated (i.e., leaves of shrubs or trees vs. grasses). In our study, we found that the $\delta^{2}\text{H_{sw}}$ samples were some of the most depleted samples collected possibly due to the isotopically depleted deep ground water utilized by the root system of large trees (Allison 1983, Dawson 1996). $\delta^{2}\text{H}$ values of leaves from trees would then represent the deeper, more depleted ground water and be transferred into the insect consumers. Ground level plants utilize shallower soil water that is subject to evaporation within an arid environment, thus separating insect $\delta^{2}\text{H}$ values based on foraging substrate. Our ground dwelling insects (grasshoppers) were found to have significantly enriched $\delta^{2}\text{H_{bw}}$ values, which agree with the pattern described above.

**Conclusions**

This study has implications for the use of $\delta^{2}\text{H}$ to track or assess animal movements along elevational gradients. We found contradictory results from sampling multiple tissues. Body water pools of birds and mammals along with plant stem water followed the predicted spatial pattern of depletion with increasing elevation but showed
high amounts of variation. Contrary to these findings, we found that $\delta^2$H$_{\text{feather}}$, $\delta^2$H$_{\text{fur}}$, and $\delta^2$H$_{\text{claw}}$ values did not follow the body water pools and instead showed no discernible pattern over this elevational gradient. Insect $\delta^2$H$_{\text{bw}}$ and $\delta^2$H$_{\text{wb}}$ also did not show a discernible pattern with increasing elevation. These findings suggest that other ecological factors are contributing to the variable $\delta^2$H values of different tissues and that these mask the expected relationship of $\delta^2$H and elevation. We found that foraging substrate had an effect on $\delta^2$H feather value. Ground foraging versus canopy feeding birds had more enriched $\delta^2$H values compared to non-ground foraging birds. Ground-dwelling insects showed the same patterns as ground foraging birds in that they were more enriched than non-ground foraging insects. Insect $\delta^2$H$_{\text{wb}}$ showed large variation (~100‰), which likely contributed to the variation in keratinous tissues. Also, hydrogen rich lipids cause lower $\delta^2$H, values which likely added to the discrepancy between body water and keratin $\delta^2$H values. Least chipmunks were found to have higher body water, fur, and claw $\delta^2$H values compared to golden-mantled ground squirrel and also had significant trends with elevation whereas the ground squirrel did not. Differences in natural history and physiology between these two small mammals likely contributed to our observed patterns and must be taken into account for future studies. We urge caution when using $\delta^2$H as a proxy for assessing elevational movements of animals given the amount of variation we observed at this single site. We sampled a ~1200m elevational gradient in a temperate environment. A larger elevational gradient may be needed to truly assess altitudinal movements; for example, $\delta^2$H in avian tissues showed expected patterns along a 2,100 m elevational gradient in the Andes Mountains (Hardesty and Fraser 2010). The use of more than a single isotope may have benefited this particular study and additionally may
improve assignment based on multiple stable isotope values (Sellick et al. 2009). Our results also confirm the need for future laboratory and field studies to incorporate multiple tissues and an ecosystem wide sampling scheme to better understand the ecological processes driving stable isotope patterns.

**Literature Cited:**


Poage, M., and C.P. Chamberlain. 2001. Empirical Relationships between Elevation and the Stable Isotope Composition of Precipitation and Surface Waters: Considerations for Studies of


Table 1. Average $\delta^2$H$_{bw}$ (‰) values separated by 400 meter units for each group of animals and plants sampled grouped as a whole. The mean is reported with standard deviation (SD) and total number (N) of individuals for each elevational grouping.

<table>
<thead>
<tr>
<th></th>
<th>2400-2800m(‰) (mean±SD(N))</th>
<th>2800-3200m(‰) (mean±SD(N))</th>
<th>3200-3600m(‰) (mean±SD(N))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stem Water</td>
<td>-108.5±9(33)</td>
<td>-114.4±6.1(21)</td>
<td>-113.7±13.9(13)</td>
</tr>
<tr>
<td>Birds</td>
<td>-5.2±14(19)</td>
<td>-14.8±13.8(17)</td>
<td>-23.5±17.6(27)</td>
</tr>
<tr>
<td>Mammals</td>
<td>-27.6±16.1(9)</td>
<td>-30.6±15.5(10)</td>
<td>-45.8±15.3(13)</td>
</tr>
<tr>
<td>Insects</td>
<td>17.4±19.5(6)</td>
<td>26.8±15.8(19)</td>
<td>12.5±31.4(19)</td>
</tr>
</tbody>
</table>
Table 2. Average $\delta^2$H$_{bw}$, $\delta^2$H$_{fur}$, and $\delta^2$H$_{claw}$ (‰) values separated by 400 meter units for golden mantled ground-squirrel (*Spermophilus lateralis*) and least chipmunk (*Tamias minimus*). The mean is reported with standard deviation (SD) and total number (N) of individuals for each elevational grouping.

<table>
<thead>
<tr>
<th>Species</th>
<th>$\delta^2$H$_{bw}$ (mean±SD(N))</th>
<th>$\delta^2$H$_{fur}$ (mean±SD(N))</th>
<th>$\delta^2$H$_{claw}$ (mean±SD(N))</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. lateralis</em></td>
<td>-32.8±13.4(7)</td>
<td>-95.1±11.5(7)</td>
<td>-94.4±4.5(7)</td>
</tr>
<tr>
<td><em>T. minimus</em></td>
<td>-9.4±12.4(2)</td>
<td>-17.9±8.1(5)</td>
<td>-9.4±12.4(2)</td>
</tr>
</tbody>
</table>

One *T.minimus* claw sample was too large for $\delta^2$H analysis
Table 3. Average $\delta^2$H$_{bw}$ and $\delta^2$H$_{feather}$ (‰) values separated by 400 meter units for two of the largest sampled bird species, American Robin (*Turdus migratorius*) and Dark-eyed Junco (*Junco hyemalis*). The mean is reported with standard deviation (SD) and total number (N) of individuals for each elevational grouping.

<table>
<thead>
<tr>
<th></th>
<th>2400-2800m(‰) (mean±SD(N))</th>
<th>2400-2800m(‰) (mean±SD(N))</th>
<th>2400-2800m(‰) (mean±SD(N))</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>J. hyemalis</em> $\delta^2$H$_{bw}$</td>
<td>-3.7±13.3(2)</td>
<td>0.4±13.3(3)</td>
<td>-20.4±16.0(7)</td>
</tr>
<tr>
<td><em>J. hyemalis</em> $\delta^2$H$_{feather}$</td>
<td>-82.1±3.0(2)</td>
<td>-89.7±4.7(3)</td>
<td>-85.2±12.6(7)</td>
</tr>
<tr>
<td><em>T. migratorius</em> $\delta^2$H$_{bw}$</td>
<td>-7.9±18.4(7)</td>
<td>-21.9±11.4(10)</td>
<td>-29.0±9.5(3)</td>
</tr>
<tr>
<td><em>T. migratorius</em> $\delta^2$H$_{feather}$</td>
<td>-74.8±13.6(7)</td>
<td>-81.7±11.8(10)</td>
<td>-82.7±14.6(3)</td>
</tr>
</tbody>
</table>
Table 4. All fifteen species of bird species analyzed with corresponding foraging guild used in foraging substrate analysis.

<table>
<thead>
<tr>
<th>Species</th>
<th>Foraging Guild</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Dendragapus obscurus</em></td>
<td>Ground</td>
</tr>
<tr>
<td><em>Vireo gilvus</em></td>
<td>Non-Ground</td>
</tr>
<tr>
<td><em>Perisoreus canadensis</em></td>
<td>Non-Ground</td>
</tr>
<tr>
<td><em>Cyanocitta stelleri</em></td>
<td>Non-Ground</td>
</tr>
<tr>
<td><em>Poecile gambeli</em></td>
<td>Non-Ground</td>
</tr>
<tr>
<td><em>Myadestes townsendi</em></td>
<td>Non-Ground</td>
</tr>
<tr>
<td><em>Catharus guttatus</em></td>
<td>Ground</td>
</tr>
<tr>
<td><em>Turdus migratorius</em></td>
<td>Ground</td>
</tr>
<tr>
<td><em>Setophaga coronata</em></td>
<td>Non-Ground</td>
</tr>
<tr>
<td><em>Spizella passerina</em></td>
<td>Ground</td>
</tr>
<tr>
<td><em>Pooecetes gramineus</em></td>
<td>Ground</td>
</tr>
<tr>
<td><em>Junco hyemalis</em></td>
<td>Ground</td>
</tr>
<tr>
<td><em>Piranga ludoviciana</em></td>
<td>Non-Ground</td>
</tr>
<tr>
<td><em>Pheucticus melanocephalus</em></td>
<td>Non-Ground</td>
</tr>
<tr>
<td><em>Coccothraustes vespertinus</em></td>
<td>Non-Ground</td>
</tr>
</tbody>
</table>
Fig 1. All bird $\delta^2$H$_{bw}$ (blue open circles) values plotted as a function of elevation showing decreasing $\delta^2$H values with increasing elevation ($P=0.002$, $r^2=0.3$, $y=-0.02x+39.2$) from animals collected at Elk Mountain in the Sangre De Cristo Mountains. $\delta^2$H$_{feather}$ (red circles) values plotted as a function of elevation showing no trend as a function of elevation ($P=0.3$, $r^2=0.02$, $y=-0.005x-69.3$).
Fig 2. All mammal $\delta^2$H$_{bw}$ (blue open circles) values plotted as a function of elevation showing decreasing $\delta^2$H values with increasing elevation ($P=0.01$, $r^2=0.2$, $y=-0.02x+32.3$) from animals collected at Elk Mountain in the Sangre De Cristo Mountains. $\delta^2$H$_{fur}$ (red circles, $P=0.3$ $r^2=0.002$, $y=-0.001x-84.0$) and $\delta^2$H$_{claw}$ (black circles, $P=0.9$, $r^2=0.0008$, $y=-0.001217x-79.85$) values plotted against elevation showing no significant trend as a function of elevation.
Fig 3. $\delta^2$Hsw (blue circles) values plotted as a function of elevation showing decreasing $\delta^2$H values with increasing elevation ($P=0.02$, $r^2=0.1$, $y=-0.008x-88.2$) from samples collected at Elk Mountain in the Sangre De Cristo Mountains. Expected precipitation values (red triangles) from Bowen (2003) ($y=-0.014x-57.3$).
Fig 4. All insect $\delta^2$H$_{bw}$ (blue triangles, $P=0.3$, $r^2=0.02$, $y=-0.01x+52.8$) and $\delta^2$H$_{wb}$ (red circles, $P=0.4$, $r^2=0.01$, $y=-0.006x - 81.5$) plotted as a function of elevation.
Fig 5. Golden-mantled ground squirrel $\delta^2$H_{bw} (blue circles, $P=0.1$, $r^2=0.1$, $y=-0.017x+10.3$), $\delta^2$H_{fur} (red circles, $P=0.9$, $r^2=-0.05$, $y=0.00005x-92.9$), and $\delta^2$H_{claw} ($P=0.6$, $r^2=0.02$, $y=0.005445x - 103.5$) plotted against elevation.
Fig 6. Least chipmunk $\delta^2 H_{bw}$ (blue circles, $P=0.001$, $r^2=0.6$, $y=-0.043x+106.7$), $\delta^2 H_{fur}$ ($P=0.01$, $r^2=0.4$, $y=-0.014x-37.9$), and $\delta^2 H_{claw}$ ($P=0.008$, $r^2=0.4$, $y=-0.01349x - 37.93$) plotted as a function of elevation.
Fig 7. American Robin $\delta^2$H$_{bw}$ (blue triangles, $P=0.06$, $r^2=0.1$, $y=-0.02x+39.8$) and $\delta^2$H$_{feather}$ (red triangles, $P=0.1$, $r^2=0.1$, $y=-0.012x-43.2$) plotted against elevation.
Fig 8. Dark-eyed Junco δ²Hbw (blue circles, $P=0.053$, $r^2=0.3$, $y=-0.03x+83$) and δ²H feather
($P=0.9$, $r^2=0.0001$, $y=-0.0004x-84.7$) plotted as a function of elevation.
Fig 9. $\delta^2 H_{\text{feather}}$ values of bird species separated into foraging guilds. Ground foraging birds have significantly higher ($-82.3\pm13.6$) $\delta^2 H_{\text{feather}}$ values compared to Non-ground Foraging birds ($-91.0\pm12.2$), $P=0.02$. 
Fig 10. Insect $\delta^2\text{H}_{wb}$ plotted as a function of total hydrogen content of sample. All insect samples combined showing significant decreasing relationship with total hydrogen content ($P=0.0009$, $r^2=0.2$, $y=-4.143x-71.19$).
Fig 11. Bird (A) and mammal (B) $\delta^2$H tissue values compared to $\delta^2$H$_{sw}$ and expected precipitation $\delta^2$H values along the elevational gradient. Keratin $\delta^2$H values fall within expected fractionation values from precipitation to feathers previously reported at roughly 25‰ (Wassenaar and Hobson 2001).