5-1-2010

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

Matthew Baumann

Matthew J. Baumann
Candidate

Biology
Department

This thesis is approved, and it is acceptable in quality and form for publication:

Approved by the Thesis Committee:

Blair O. Wolf, Chairperson

Christopher C. Witt

Seth D. Newsome

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

by

MATTHEW J. BAUMANN

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

THESIS

Submitted in Partial Fulfillment of the
Requirements for the Degree of

Master of Science
Biology

The University of New Mexico
Albuquerque, New Mexico

May, 2014

ACKNOWLEDGMENTS

I would like to first thank my advisor, Dr. Blair Wolf, for the academic guidance he provided me during my tenure as a graduate student at the University of New Mexico. I would also like to thank my committee members, Drs. Christopher C. Witt and Seth D. Newsome. They provided valuable guidance and advice for my thesis project. Andy Johnson has been a mentor to me since I was an undergraduate at the University of New Mexico and has provided invaluable support during my undergraduate and graduate careers.

I thank Bill Talbot, Ian Murray, Cole Wolf, Chuck Hayes, and Shane DuBay for help in the field and valuable discussions and evaluations of my thesis work. I thank my parents, Mark and Tammy Baumann, for the support they have provided in any endeavor that I have undertaken. Lastly, I thank Traci Baumann and Madison Warren for helping me through my graduate career, especially the last few months.

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

by

Matthew J. Baumann

B.S., BIOLOGY, UNIVERSITY OF NEW MEXICO, 2009
M.S., BIOLOGY, UNIVERSITY OF NEW MEXICO, 2014

ABSTRACT

Animals incorporate local δ^2H 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 δ^2H as an indicator of altitudinal movements by examining multiple tissues (δ^2H_{bw} (body water), $\delta^2H_{feather}$, δ^2H_{fur}, and δ^2H_{claw}) in birds and mammals in the Sangre de Cristo Mountains. Additionally, we measured δ^2H_{sw} (stem water) and insect δ^2H_{bw} and δ^2H_{wb} (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. δ^2H_{sw} and δ^2H_{bw} of plants, birds, and mammals decreased with increasing elevation. δ^2H_{fur}, $\delta^2H_{feather}$, δ^2H_{claw}, and δ^2H_{bw} of insects did not show any discernable pattern with increasing elevation. Only one animal species, the least chipmunk (*Tamias minimus*), showed significant decreases in δ^2H values with increasing elevation. Additionally, $\delta^2H_{feather}$ 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 δ^2H for elevational movements will require careful selection of species, tissues, and extensive validation efforts to ensure accuracy of using this technique. Local variability in δ^2H among species and tissues needs to be understood in detail before elevational movements can be assessed.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

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 (δ^2H), and its distribution in keratinous tissue, e.g. feathers, fur or claws, have been used extensively to track migratory animals based on distinct δ^2H 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 δ^2H 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 δ^2H values of multiple tissues (body water, blood, feather, fur, claws) can provide temporal information on animal movements and locations. Body water and plasma δ^2H 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 δ^2H 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 δ^2H values become increasingly more depleted with increasing elevation (Bowen and Wilkinson 2002). These changes in δ^2H 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 δ^2H 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 δ^2H 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 δ^2H 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 δ^2H 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 δ^2H 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 δ^2H 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 δ^2H 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 δ^2H along elevational gradients, it is essential to understand variation of δ^2H 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 $\delta^2H_{feather}$ to local precipitation δ^2H 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 δ^2H values, and how different tissue δ^2H values vary accordingly.

In this study we examined how faithfully δ^2H values correlated with elevation along a 1200m elevation gradient in the mountains of north-central New Mexico. Plant δ^2H_{sw} (stem water), insect δ^2H_{bw} (body water) and δ^2H_{wb} (whole body), bird (δ^2H_{bw} and $\delta^2H_{feather}$), and mammal tissue (δ^2H_{bw}, $\delta^2H_{fur,}$ and δ^2H_{claw}) were sampled continuously along the gradient. Because δ^2H_{bw} turns over rapidly we expected to see a strong correlation between body water of the animals sampled and δ^2H_{sw} values with increasing elevation. In addition, we predicted a direct correlation between groundwater, plant tissue water, fur and feather, and claw δ^2H 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 δ^2H values and predicted that ground foraging birds will show higher δ^2H values compared to non-ground foragers. If precipitation patterns with elevation are faithfully recorded by animal tissues then the unique δ^2H 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 (35.77O N, 105.55O 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 ($^2H/^1H=R$) were expressed in standard δ notation. Units of per mil (‰) were compared to a standard that is defined as $[(R_{sample}/R_{standard})/-1]*1000$, 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^2H values to the known δ^2H 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 $\delta^2H_{feather}$ values in bird species. Expected δ^2H 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 δ^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 $\delta^2H_{feather}$ 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 δ^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 δ^2H with increasing elevation yet high variation (P=0.02, r^2=0.08, Fig. 3). Predicted precipitation δ^2H lapse rate was estimated at 4.8‰/500m whereas observed stem water δ^2H was found to be 4.0‰/500m. Slopes of the predicted precipitation δ^2H values did not differ from observed δ^2H_{sw} (P=0.8). Out of the plants sampled (*P. tremuloides*, *Q. gambelii*, *Rosaceae sp.*), only *Q. gambelii* (n=15) showed a significant decreasing δ^2H_{sw} with increasing elevation (P=0.005, r^2=0.4).

Insects

Insects δ^2H_{bw} and δ^2H_{wb} treated as a whole were found to not show any significant relationship with increasing elevation (P=0.3 and P=0.4, Table 1, respectively). Additionally, δ^2H_{bw} was noticeably higher compared to δ^2H_{wb} (Fig 4). Slopes between

δ^2H_{bw} and δ^2H_{wb} did not significantly differ (P=0.7). δ^2H_{wb} was found to decrease with higher hydrogen content in sample (P=0.0009, r^2=0.2, Fig. 10).

Birds

When all avian species and individuals were combined, δ^2H_{bw} decreased with increasing elevation (P=0.002, r^2=0.3, Fig. 1. Table 1) but $\delta^2H_{feather}$ showed no discernible pattern with elevation (P=0.3, r^2=0.02) and was lower compared to δ^2H_{bw} (Fig. 1). Slopes between δ^2H_{bw} and $\delta^2H_{feather}$ 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 δ^2H_{bw} values with increasing elevation (robin: P=0.06, r^2=0.1; junco: P=0.05, r^2=0.3; Fig. 7, Fig. 8) but non-significant relationships were found in $\delta^2H_{feather}$ 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 δ^2H_{claw} data for birds because it likely represents non-breeding locations during or prior to migration since we sampled in late spring.

Mammals

δ^2H_{bw} of both small mammal species combined decreased with increasing elevation (P=0.01, r^2=0.2; Fig. 2, Table 1) but showed no relationship in δ^2H_{fur} (P=0.3, Fig. 2) or δ^2H_{claw} (P=0.8, Fig. 2). Just as in insects and birds, δ^2H_{fur} and δ^2H_{claw} were noticeably lower compared to δ^2H_{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 (δ^2H_{bw}: P=0.001, r^2=0.6; δ^2H_{fur}: P=0.01, r^2=0.4; δ^2H_{claw} : P=0.007, r^2=0.5; Fig. 6). Golden-mantled ground squirrel (*Callospermophilus lateralis*) (n=19) showed non-significant relationships in each tissue (δ^2H_{bw}: P=0.1, r^2=0.1; δ^2H_{fur}: P=0.9, r^2=0.05; δ^2H_{claw}: P=0.6, r^2=0.02; Fig. 5).

Foraging Guilds

Since $\delta^2H_{feather}$ 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 $\delta^2H_{feather}$ values compared to non-ground foraging species (P=0.02, Fig. 9). δ^2H_{bw} 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 δ^2H values along an elevational gradient in an arid environment. As expected, we found a significant depletion of δD_{sw} with increasing elevation. We also found a similar negative relationship between elevation and the δ^2H_{bw} pools of small mammals and birds, but not in insects. In all cases, δ^2H_{bw} values were enriched compared to δ^2H_{sw}. In contrast to our original hypothesis and the current literature (Hobson et al. 2003, Hardesty and Fraser 2010), we found no significant relationship in $\delta^2H_{feather}$, δ^2H_{fur}, or δ^2H_{claw} with increasing elevation. Interestingly, we found significant differences

between δ^2H_{fur} and δ^2H_{bw} values among mammals. We also found differences between $\delta^2H_{feather}$ and δ^2H_{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 δ^2H to track altitudinal movements of wildlife in arid ecosystems.

Changes in δ^2H of water in plants, insects, and animals with elevation

Globally, as elevation increases, precipitation δ^2H 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 δ^2H lapse rate of 4.0‰/500m. The lapse rates were similar but elevation explained little of the variation seen in δ^2H_{sw}. We also found δ^2H_{sw} values to be the most depleted of the different pools we sampled and were generally more depleted than the predicted annual precipitation δ^2H values predicted for our site (Bowen 2003; Fig. 3). Our observed δ^2H_{sw} values may have been more depleted because observed values may reflect large inter-annual differences of δ^2H in precipitation at this site. Additionally, runoff from higher elevations may also play a factor in the observed discrepancy between predicted and observed δ^2H values (Hobson 2003). When all plant samples were pooled, we found the expected significant relationship of increasing depletion δ^2H_{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 δ^2H_{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 δ^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 δ^2H_{sw} values. Dawson (1996) reported that small sugar maples (<5m) were found to have enriched δ^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 δ^2H values with elevation.

The insects sampled (*Apidae*, *Diptera*, *Caelifera*, and *Lasiocampidae*) showed no discernible pattern of δ^2H_{bw} with increasing elevation, and had some of the most enriched δ^2H_{bw} values observed. Insects have small body water pools and potentially high evaporative rates that may result in greater δ^2H enrichment in comparison to stem water, and the tissues of birds, and mammals. Interestingly, grasshopper δ^2H_{bw} were found to be significantly more enriched in comparison to the other insects sampled. Variation in soil water δ^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 δ^2H values than deep ground water (Allison 1983, Dawson 1996).

Birds and mammal body water

Bird and small mammal δ^2H_{bw} pools were significantly enriched compared to δ^2H_{sw} and this may represent trophic discrimination in δ^2H values (Birchall et al. 2005). Stem water δ^2H are closely tied to the local precipitation δ^2H values and we expected to see similar relationships with the bird and mammal δ^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 δ^2H of feathers, fur, and δ^2H_{wb} of birds, mammals, and insects with elevation

Although feather δ^2H values also have been shown to track precipitation δD values with increasing elevation (Hobson et al. 2003, Fraser et al 2008b, Hardesty and Fraser 2010); elevational δ^2H patterns have not been examined in mammal tissues. In birds, δ^2H 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 δ^2H values with no predictable pattern of depletion in $\delta^2H_{feather}$ with increasing elevation. Our $\delta^2H_{feather}$ and δ^2H_{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 δ^2H also showed no relationship with elevation and showed large variation. Hardesty and Fraser (2010) reported similar results where $\delta^2H_{feather}$ values showed a weaker elevational trend compared to δ^2H of plasma in birds from the Ecuadorian Andes. A disconnect between body water and keratinous tissue δ^2H 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 from ~20-30%, (Hobson et al. 1999, Podlesak et a. 2008, Wolf et al. 2011, Wolf et al.

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 δ^2H values compared to body water, but had similar values compared to the insect δ^2H_{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 δ^2H_{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 δ^2H_{bw} and $\delta^2H_{feather/fur}$ and variation seen in keratinous tissues is likely a byproduct of the composition of food items ingested.

Within-site variation in the δ^2H of dietary resources may also play an important role in driving the observed variation in $\delta^2H_{feather}$ or δ^2H_{fur}. Betini et al. (2009) found ~ 20‰, variation in insect δ^2H values in differing microsites at a single location in Ontario, which they hypothesized contributed to the variation in nestling Tree Swallow (*Tachycineta bicolor*) δ^2H_{blood}. Our insect δ^2H_{bw} and δ^2H_{wb} samples showed large variation (~100‰) and undoubtedly has an effect on $\delta^2H_{feather}$, δ^2H_{fur} , $\delta^2H_{claw,}$ and the

δ^2H of other tissues. Given the large variation in δ^2H 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 $\delta D_{feather}$ 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 δ^2H in mammals

We found species- and taxon-specific differences in patterns of δ^2H values along the elevational gradient of the small mammals we sampled. In least chipmunks we observed significant depletion in δ^2H of all tissues sampled with increasing elevation. In contrast, the δ^2H 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 δ^2H_{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 δ^2H_{fur} between these two molts.

Body size may also have an effect on the tissue δ^2H 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 δ^2H 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 δ^2H 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 δ^2H values (Smith and Epstein 1970, Verts and Carraway 2001).

Species-specific differences feather δ^2H in birds- potential effects of foraging substrate

We found differences in $\delta^2H_{feather}$ values between foraging substrate of sampled birds. Ground foraging birds were found to have more enriched (±SD, Fig. 9) $\delta^2H_{feather}$ 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 δ^2H 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 δ^2H_{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). δ^2H 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 δ^2H values based on foraging substrate. Our ground dwelling insects (grasshoppers) were found to have significantly enriched δ^2H_{bw} values, which agree with the pattern described above.

Conclusions

This study has implications for the use of δ^2H 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 $\delta^2H_{feather}$, δ^2H_{fur}, and δ^2H_{claw} values did not follow the body water pools and instead showed no discernible pattern over this elevational gradient. Insect δ^2H_{bw} and δ^2H_{wb} also did not show a discernible pattern with increasing elevation. These findings suggest that other ecological factors are contributing to the variable δ^2H values of different tissues and that these mask the expected relationship of δ^2H and elevation. We found that foraging substrate had an effect on δ^2H feather value. Ground foraging versus canopy feeding birds had more enriched δ^2H 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 δ^2H_{wb} showed large variation (~100‰), which likely contributed to the variation in keratinous tissues. Also, hydrogen rich lipids cause lower δ^2H, values which likely added to the discrepancy between body water and keratin δ^2H values. Least chipmunks were found to have higher body water, fur, and claw δ^2H 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 δ^2H 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, δ^2H 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:

Allison, G.B., C.J. Barnes, and M.W. Hughes. 1983. The distribution of deuterium and 18O in dry soil. Exp. J. Hydrol. 64:377-397.

Bartels, Molly A., and Doug P. Thompson. 1993. Spermophilus lateralis. Mammalian Species 440: 1-8.

Bearhop, S., R.W. Furness, G.M.Hilton, S.C. Votier, and S. Waldron. 2003. A forensic approach to understanding diet and habitat use from stable isotope analysis of (avian) claw material. Functional Ecology, 17(2), 270-275.

Betini, G. S., K. A. Hobson, L. I. Wassenaar, and D. R. Norris. 2009. Stable hydrogen isotope (δD) values in songbird nestlings: effects of diet, temperature, and body size. Canadian Journal of Zoology 87:767–772.

Birchall, J., T.C. O'Connell, T.H. Heaton, and R.E. Hedges. 2005. Hydrogen isotope ratios in animal body protein reflect trophic level. Journal of animal ecology, 74(5), 877-881.

Bowen G.J., and B. Wilkinson. 2002. Spatial distribution of 618O in precipitation. Geology 30(4):3 15-318

Bowen G. J., and J. Revenaugh. 2003. Interpolating the isotopic composition of modern meteoric precipitation. Water Resources Research 39(10), 1299, doi:10.129/2003WR002086. (for annual average values)

Britzke, E. R., S. C. Loeb, K. A. Hobson, C. S. Romnnek, and M. J. Vonhof. 2009. Using hydrogen isotopes to assign origins of bats in the eastern United States. Journal of Mammalogy 90:743–751.

Brown, H. E. 1958. Gambel oak in west-central Colorado. Ecology, 39(2), 317-327.

Dawson, T. E. 1996. Determining water use by trees and forests from isotopic, energy balance and transpiration analyses: the roles of tree size and hydraulic lift. Tree Physiology, 16(1-2), 263-272.

Dixon, K. L., and J.D. Gilbert.1964. Altitudinal migration in the mountain chickadee. The Condor, 66(1), 61-64.

Fraser, K.C., T.K. Kyser, R.J. Robertson, and L.M. Ratcliffe. 2008a. Seasonal patterns of hydrogen isotopes from claws from breeding wood warblers (Parulidae): Utility for estimating migratory origins. Avian Conservation and Ecology, 3, 2.

Fraser, K. C., T. K. Kyser and L. M. Ratcliffe. 2008b. Detecting altitudinal migration events in Neotropical birds using stable isotopes. Biotropica 40: 269-272.

Fraser, K. C., E. A. McKinnon, A. W. Diamond, and L. Chavarria. 2011. The influence of microhabitat, moisture and diet on stable-hydrogen isotope variation in a Neotropical avian food web. Journal of Tropical Ecology 27:563–572.

Graves, G. R., C.S. Romanek, and A.R. Navarro. 2002. Stable isotope signature of philopatry and dispersal in a migratory songbird. Proceedings of the National Academy of Sciences, 99(12), 8096-8100.

Greenwood, P. J., and P.H. Harvey. 1982. The natal and breeding dispersal of birds. Annual review of ecology and systematics, 13, 1-21.

Hardesty, J.L., and C. Fraser. 2010. Using deuterium to examine altitudinal migration by Andean birds. Journal of Field Ornithology. 81(1): 83-91.

Hobson, K. A., L. Atwell, and L.I. Wassenaar. 1999. Influence of drinking water and diet on the stable-hydrogen isotope ratios of animal tissues. Proceedings of the National Academy of Sciences, 96(14), 8003-8006.

Hobson, K.A., K.P. McFarland, L.I. Wassenaar, C.C Rimmer, and J.E. Goetz. 2001. Linking breeding and wintering grounds of Bicknell's thrushes using stable isotope analyses of feathers. Auk 118: 16–23.

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

Hobson K. A., L.I. Wassenaar, and O.R. Taylor. 1999. Stable isotopes (δD and δ13C) are geographic indicators of natal origins of monarch butterflies in eastern North America. Oecologia. 120, 397–404.

Hobson, K.A. 2003. Making migratory connections with stable isotopes. In: Berthold P, Gwinner P (eds) Avian migration. Springer, Berlin Heidelberg New York, pp 379-391

Hobson, K. A., L.I Wassenaar, B. Milá, I. Lovette, C. Dingle, and T.B. Smith. 2003. Stable isotopes as indicators of altitudinal distributions and movements in an Ecuadorean hummingbird community. Oecologia, 136(2), 302-308.

Hobson, K.A., G.J. Bowen, L.I. Wassenaar, Y. Ferrand, H. Lormee. 2004a. Using stable hydrogen and oxygen isotope measurements of feathers to infer geographical origins of migrating European birds. Oecologia 141: 477–488.

Hobson, K.A., L.I. Wassenaar, and E. Bayne. 2004b. Using isotopic variance to detect long-distance dispersal and philopatry in birds: An example with Ovenbirds and American Redstarts. Condor 106: 732–743.

Hobson, K. A., S. L. Van Wilgenburg, L. I. Wassenaar, and K. Larson. 2012. Linking hydrogen (δ2H) isotopes in feathers and precipitation: sources of variance and consequences for assignment to global isoscapes. PLoS ONE 7(4):e35137. http://dx.doi.org/10.1371/journal.pone.0035137

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

Lis, G., L.I. Wassenaar, and M.J. Hendry. 2008. High-precision laser spectroscopy D/H and 18O/16O measurements of microliter natural water samples. Analytical Chemistry, 80(1), 287-293.

Marra, P. P., K.A. Hobson, and R.T. Holmes. 1998 Linking winter and summer events in a migratory bird by using stable-carbon isotopes. Science 282, 1884–1886.

McKechnie, A. E., B.O. Wolf, and C.M. del Rio. 2004. Deuterium stable isotope ratios as tracers of water resource use: an experimental test with rock doves. Oecologia, 140(2), 191-200.

Meehan T.D., C.A. Lott, Z.D. Sharp, R.B. Smith, R.N. Rosenfield, A.C. Stewart, and R.K. Murphy. 2001. Using hydrogen isotope geochemistry to estimate the natal latitudes of immature Cooper's hawks migrating through the Florida Keys. Condor 103:11-20

Meehan, T. D., J. T. Giermakowski, and P. M. Cryan. 2004. A GIS-based model of stable hydrogen isotope ratios in North American growing-season precipitation for use in animal movement studies. Isotopes in Environmental and Health Studies. 40:291-300.

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

Podlesak, D.W., A.M Torregrossa, J.R Ehleringer, M.D. Dearing, B.H. Passey, and T.E. Cerling. 2008. Turnover of oxygen and hydrogen isotopes in the body water, CO2, hair, and enamel of a small mammal. Geochimica et Cosmochimica Acta, 72, 19–35.

Poole, A, ed. (2005) The Birds of North America Online: http://bna.birds. cornell.edu/BNA/. Cornell Laboratory of Ornithology, Ithaca, NY.

Pyle, P. 1997. Identification Guide to North American Birds, part I. Slate Creek Press, Bolinas, CA.

Powell, G. V., and R.D. Bjork. 1994. Implications of altitudinal migration for conservation strategies to protect tropical biodiversity: a case study of the resplendent quetzal Pharomachrus mocinno at Monteverde, Costa Rica. Bird Conservation International, 4(2-3), 161-174.

R Development Core Team (2011). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org/.

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(5557), 1062-1065.

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 4(3): e4735. doi:10.1371/journal.pone.0004735

Smith, B. N., and S. Epstein.1970. Biogeochemistry of the stable isotopes of hydrogen and carbon in salt marsh biota. Plant Physiology, 46(5), 738-742.

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. Funct Ecol., 26: 1111–1119.

Thorington, R.W., J.L. Koprowski, M.A. Steele, and J.F. Whatton. 2012. Squirrels of the world. Johns Hopkins University Press, Baltimore.

Verts, B. J., and L. N. Carraway. 2001. Tamias minimus. Mammalian Species 653:1-10.

Wang, Y. V., D.M. O'Brien, J. Jenson, D. Francis, and M.J. Wooller. 2009. The influence of diet and water on the stable oxygen and hydrogen isotope composition of Chironomidae (Diptera) with paleoecological implications. Oecologia, 160(2), 225-233.

Wassenaar, L. I., and K.A. Hobson. 2001. A stable-isotope approach to delineate geographical catchment areas of avian migration monitoring stations in North America. Environmental science & technology, 35(9), 1845-1850.

Wolf, N., G.J. Bowen, and C. Martínez del Rio. 2011. The influence of drinking water on the δD and δ18O values of house sparrow plasma, blood, and feathers. J. Exp. Biol. 214, 98-103.

Wolf, N., S.D Newsome, M.L. Fogel, and C.M. Del Rio. 2013. The relationship between drinking water and the hydrogen and oxygen stable isotope values of tissues in Japanese Quail (Cortunix japonica). The Auk, 130(2), 323-330.

Table 1. Average δ^2H_{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.

	2400-2800m(‰) (mean±SD(N))	2800-3200m(‰) (mean±SD(N))	3200-3600m(‰) (mean±SD(N))
Stem Water	-108.5±9(33)	-114.4±6.1(21)	-113.7±13.9(13)
Birds	-5.2±14(19)	-14.8±13.8(17)	-23.5±17.6(27)
Mammals	-27.6±16.1(9)	-30.6±15.5(10)	-45.8±15.3(13)
Insects	17.4±19.5(6)	26.8±15.8(19)	12.5±31.4(19)

Table 2. Average δ^2H_{bw}, δ^2H_{fur}, and δ^2H_{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.

	2400-2800m(‰) (mean±SD(N))	2800-3200m(‰) (mean±SD(N))	3200-3600m(‰) (mean±SD(N))
S. lateralis δ^2H_{bw}	-32.8±13.4(7)	-43.3±8.6(5)	-47.6±18.3(7)
S. lateralis δ^2H_{fur}	-95.1±11.5(7)	-88.6±9.9(5)	-93.7±7(7)
S. lateralis δ^2H_{claw}	-94.4±4.5(7)	-77.8±16.7(5)	-86.8±15.5(7)
T. minimus δ^2H_{bw}	-9.4±12.4(2)	-17.9±8.1(5)	-43.6±12.4(6)
T. minimus δ^2H_{fur}	-72.3±7.5(2)	-80.1±7.8(5)	-82.6±4.4(6)
T. minimus δ^2H_{claw}	-71.8(1)*	-69.2±11.3(5)	-85.9±6.0(6)

*One *T.minimus* claw sample was too large for δ^2H analysis

Table 3. Average δ^2H_{bw} and $\delta^2H_{feather}$(‰) 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

	2400-2800m(‰) (mean±SD(N))	2400-2800m(‰) (mean±SD(N))	2400-2800m(‰) (mean±SD(N))
J. hyemalis δ^2H_{bw}	-3.7±13.3(2)	0.4±13.3(3)	-20.4±16.0(7)
J. hyemalis $\delta^2H_{feather}$	-82.1±3.0(2)	-89.7±4.7(3)	-85.2±12.6(7)
T. migratorius δ^2H_{bw}	-7.9±18.4(7)	-21.9±11.4(10)	-29.0±9.5(3)
T. migratorius $\delta^2H_{feather}$	-74.8±13.6(7)	-81.7±11.8(10)	-82.7±14.6(3)

Table 4. All fifteen species of bird species analyzed with corresponding foraging guild used in foraging substrate analysis.

Species	Foraging Guild
Dendragapus obscurus	Ground
Vireo gilvus	Non-Ground
Perisoreus canadensis	Non-Ground
Cyanocitta stelleri	Non-Ground
Poecile gambeli	Non-Ground
Myadestes townsendi	Non-Ground
Catharus guttatus	Ground
Turdus migratorius	Ground
Setophaga coronata	Non-Ground
Spizella passerina	Ground
Pooecetes gramineus	Ground
Junco hyemalis	Ground
Piranga ludoviciana	Non-Ground
Pheucticus melanocephalus	Non-Ground
Coccothraustes vespertinus	Non-Ground

Fig 1. All bird δ^2H_{bw} (blue open circles) values plotted as a function of elevation showing decreasing δ^2H 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. $\delta^2H_{feather}$ (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).

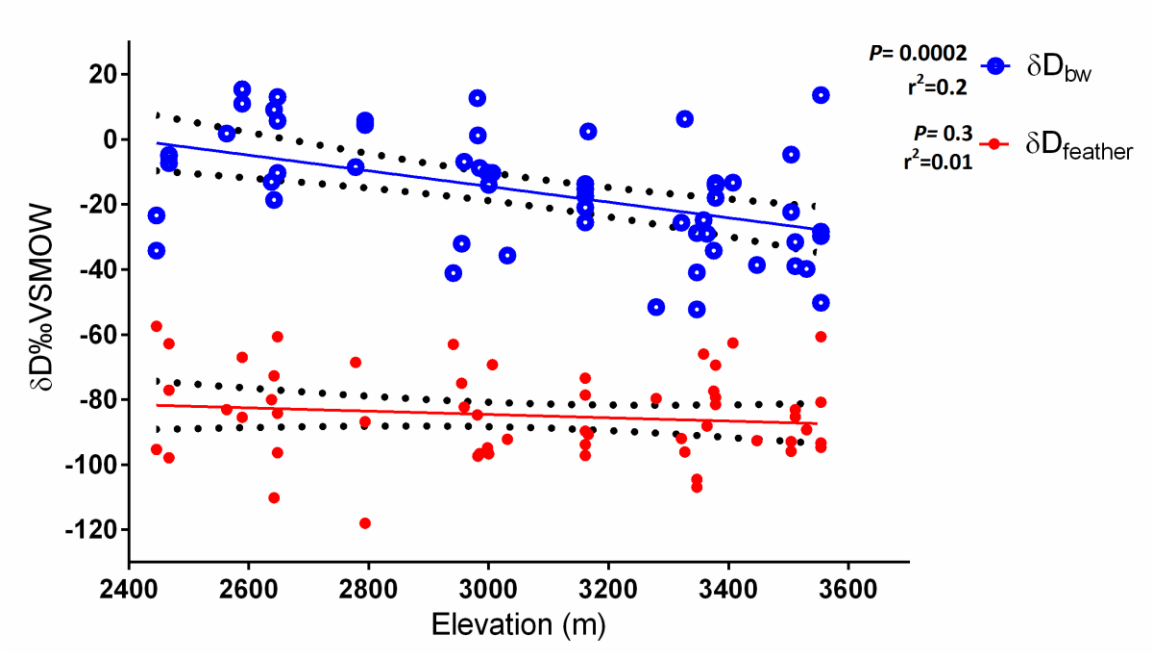

Fig 2. All mammal δ^2H_{bw} (blue open circles) values plotted as a function of elevation showing decreasing δ^2H values with increasing elevation (P=0.01, r^2=0.2, y=-0.02x+32.3) from animals collected at Elk Mountain in the Sangre De Cristo Mountains. δ^2H_{fur} (red circles, *P*=0.3 r^2=0.002, y=-0.001x-84.0) and δ^2H_{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.

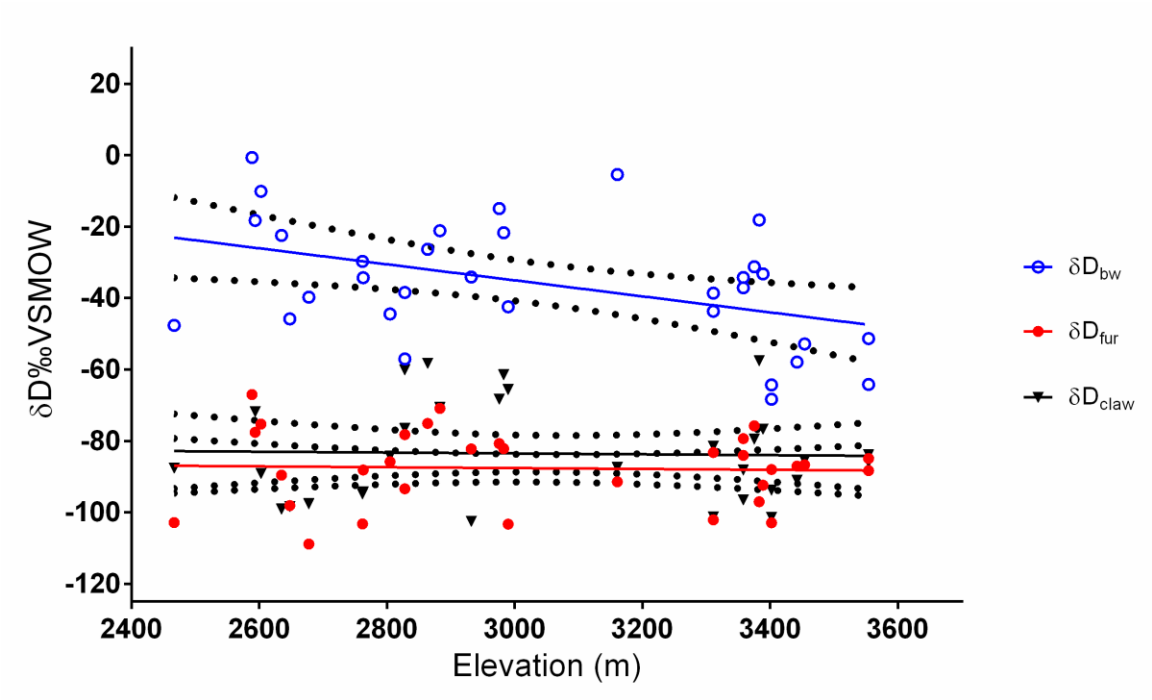

Fig 3. δ^2H_{sw} (blue circles) values plotted as a function of elevation showing decreasing δ^2H values with increasing elevation (P=0.02, r^2=0.1, y=-0.008x-88.2) from samples collected at Elk Mountain in the Sangre De Cristo Mountains. Expected precipitation values (red triangles) from Bowen (2003) (y=-0.014x-57.3).

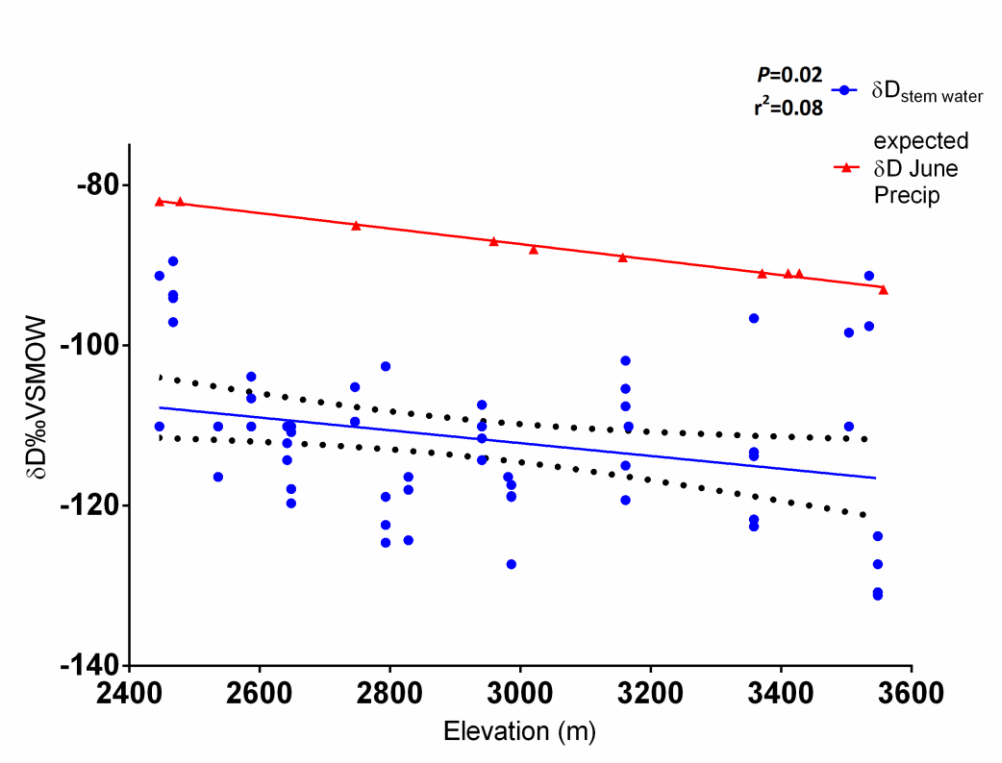

Fig 4. All insect δ^2H_{bw} (blue triangles, P=0.3, r^2=0.02, y=-0.01x+52.8) and δ^2H_{wb} (red circles, *P*=0.4, r^2=0.01, y=-0.006x - 81.5) plotted as a function of elevation.

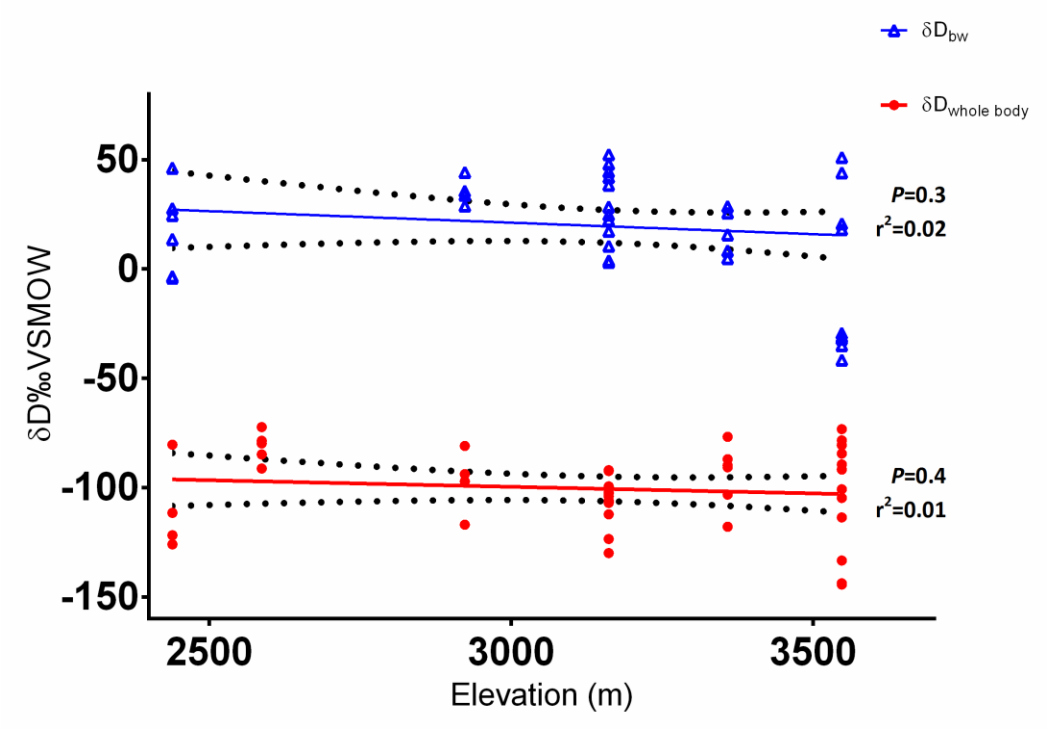

Fig 5. Golden-mantled ground squirrel δ^2H_{bw} (blue circles, P=0.1, r^2=0.1, y=-0.017x+10.3), δ^2H_{fur} (red circles, P=0.9, r^2=-0.05, y=0.00005x-92.9), and δ^2H_{claw} (P=0.6, r^2=0.02, y= 0.005445x - 103.5) plotted against elevation.

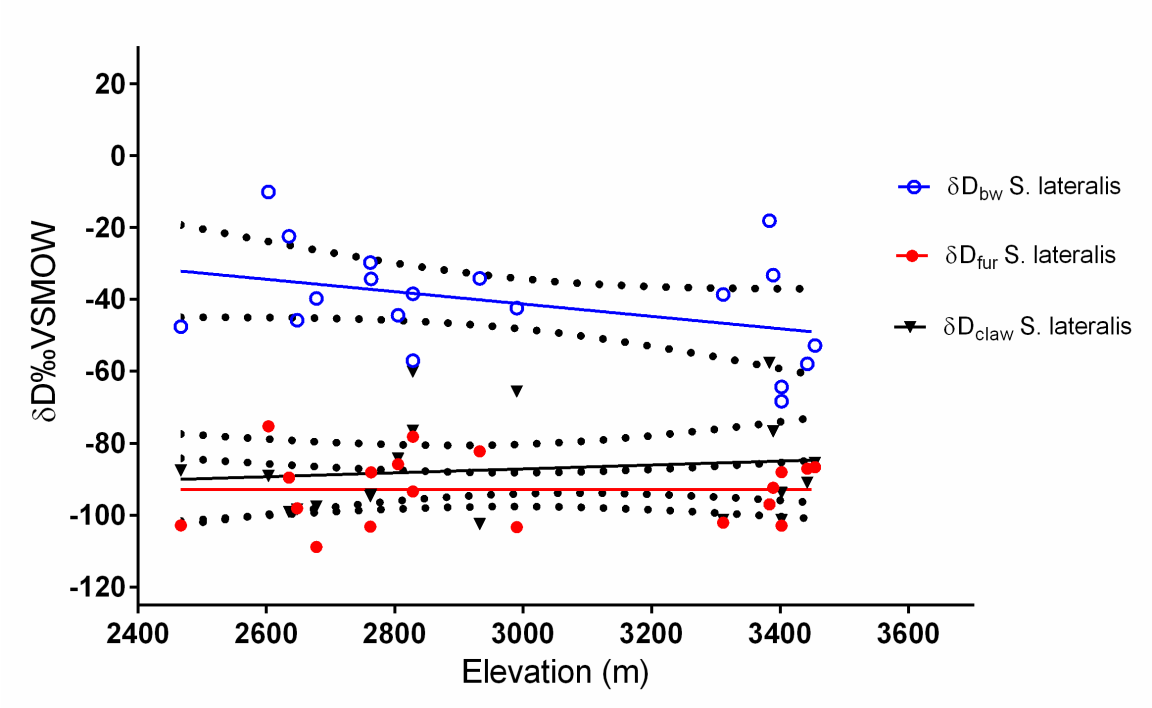

Fig 6. Least chipmunk δ^2H_{bw} (blue circles, P=0.001, r^2=0.6, y=-0.043x+106.7), δ^2H_{fur} (P=0.01, r2=0.4, y=-0.014x-37.9), and δ^2H_{claw} (P=0.008, r^2=0.4, y=-0.01349x - 37.93) plotted as a function of elevation.

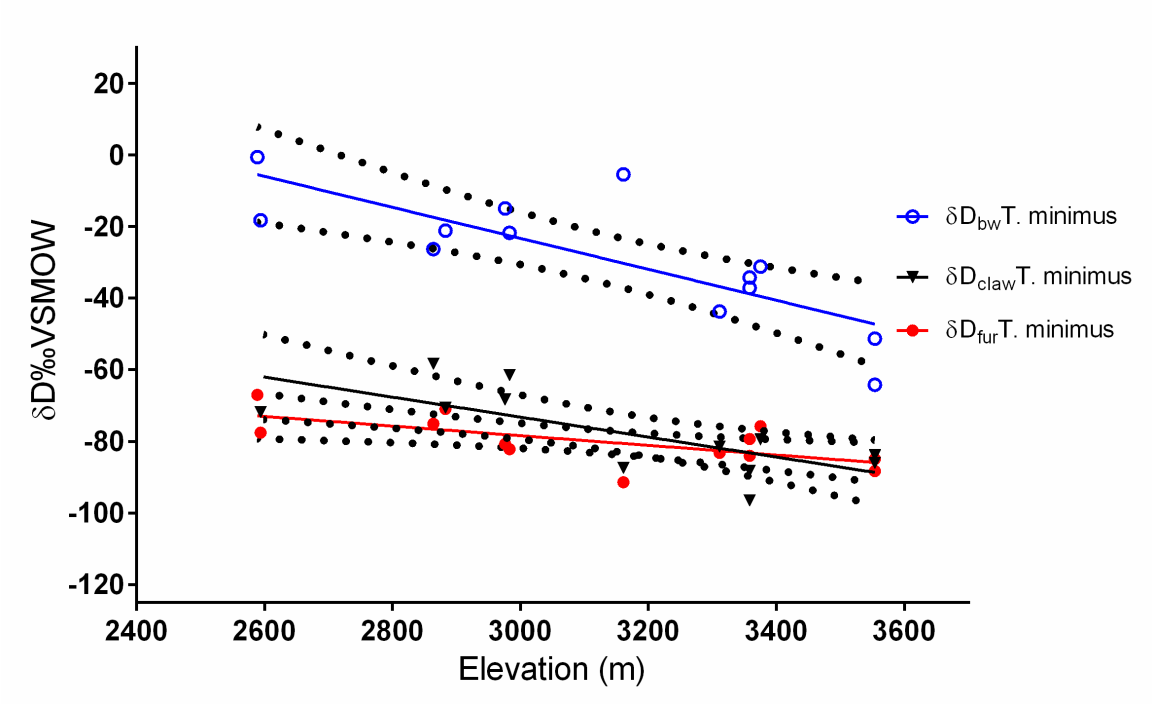

Fig 7. American Robin δ^2H_{bw} (blue triangles, P=0.06, r^2=0.1, y=-0.02x+39.8) and $\delta^2H_{feather}$ (red triangles, P=0.1, r^2=0.1, y=-0.012x-43.2) plotted against elevation.

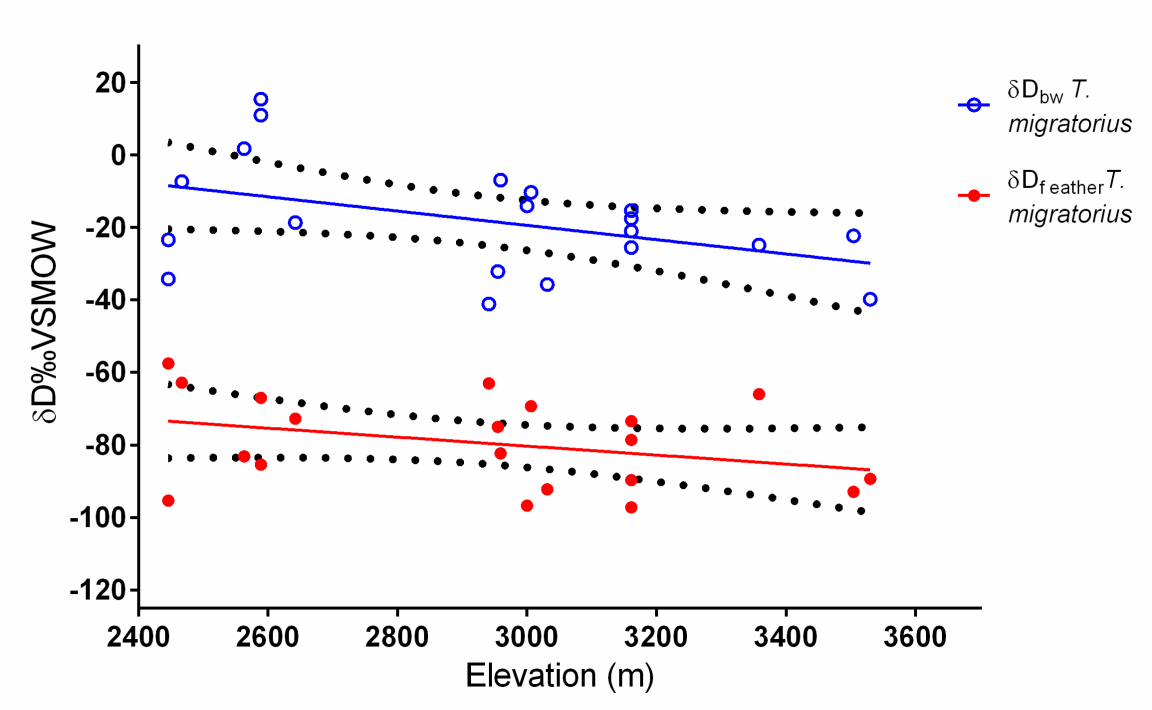

Fig 8. Dark-eyed Junco δ^2H_{bw} (blue circles, P=0.053, r^2=0.3, y=-0.03x+83) and $\delta^2H_{feather}$ (P=0.9, r^2=0.0001, y=-0.0004x-84.7) plotted as a function of elevation.

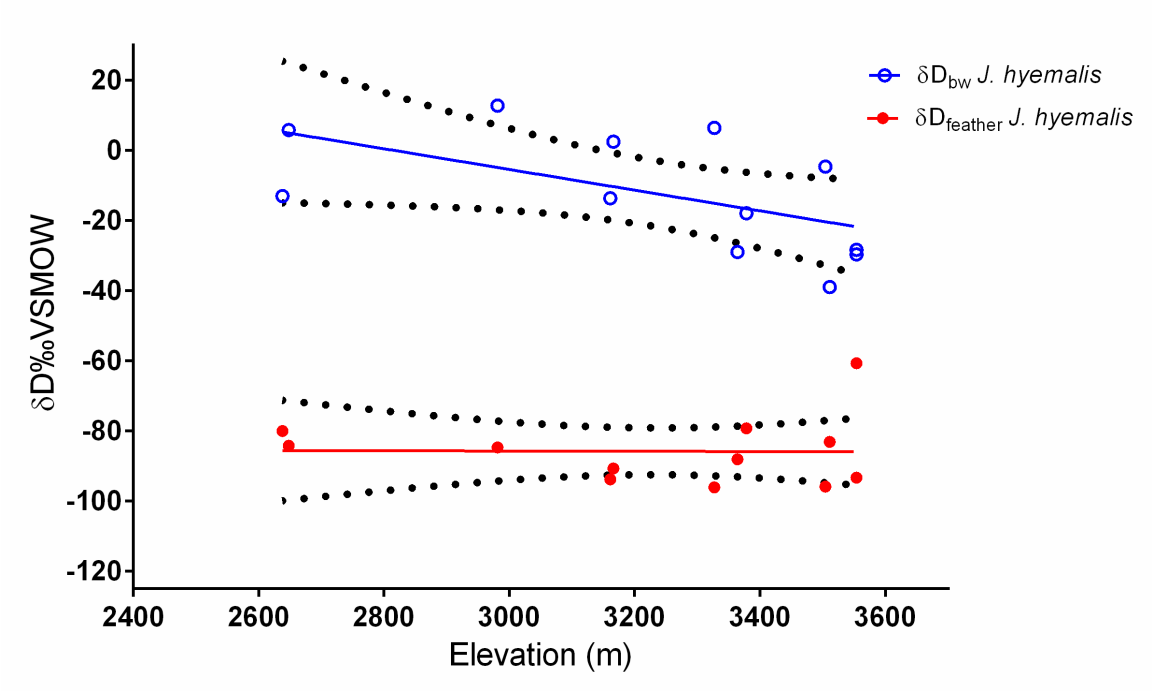

Fig 9. $\delta^2H_{feather}$ values of bird species separated into foraging guilds. Ground foraging birds have significantly higher (-82.3±13.6) $\delta^2H_{feather}$ values compared to Non-ground Foraging birds (-91.0±12.2), P=0.02.

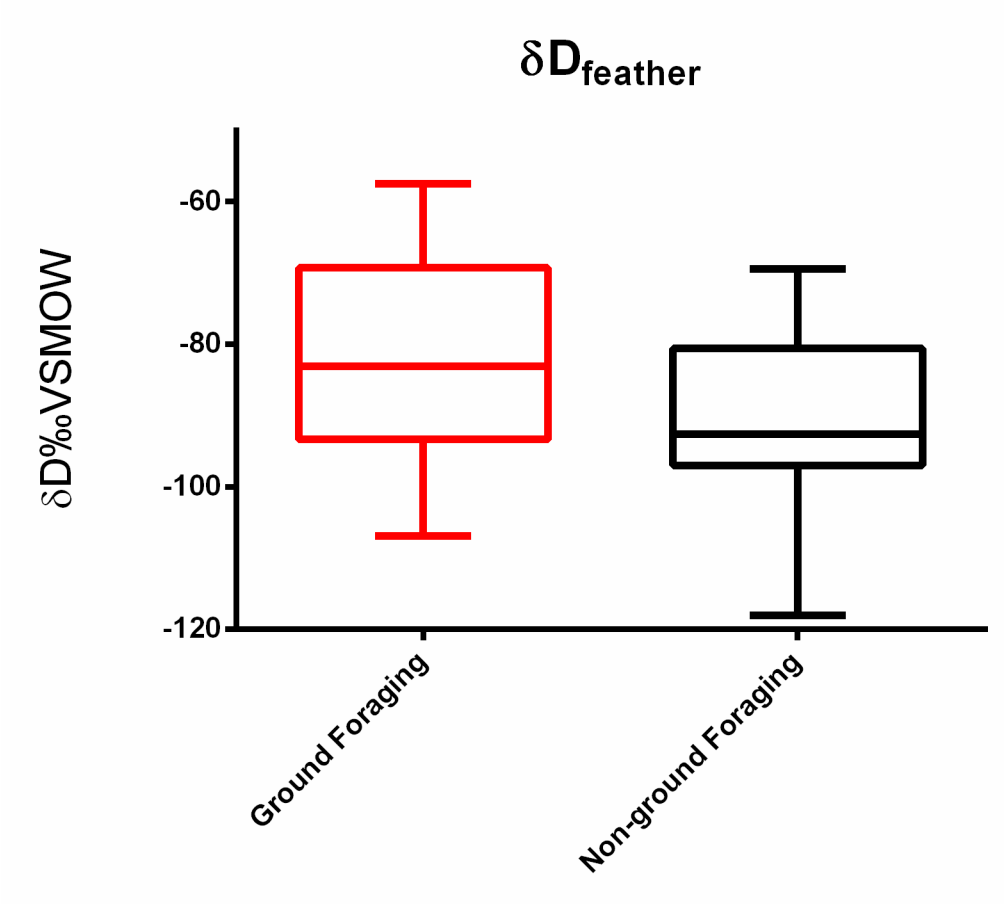

Fig 10. Insect δ^2H_{wb} plotted as a function of total hydrogen content of sample. All insect samples combined showing significant decreasing relationship with total hydrogen content (P=0.0009, r^2=0.2, y=-4.143x-71.19).

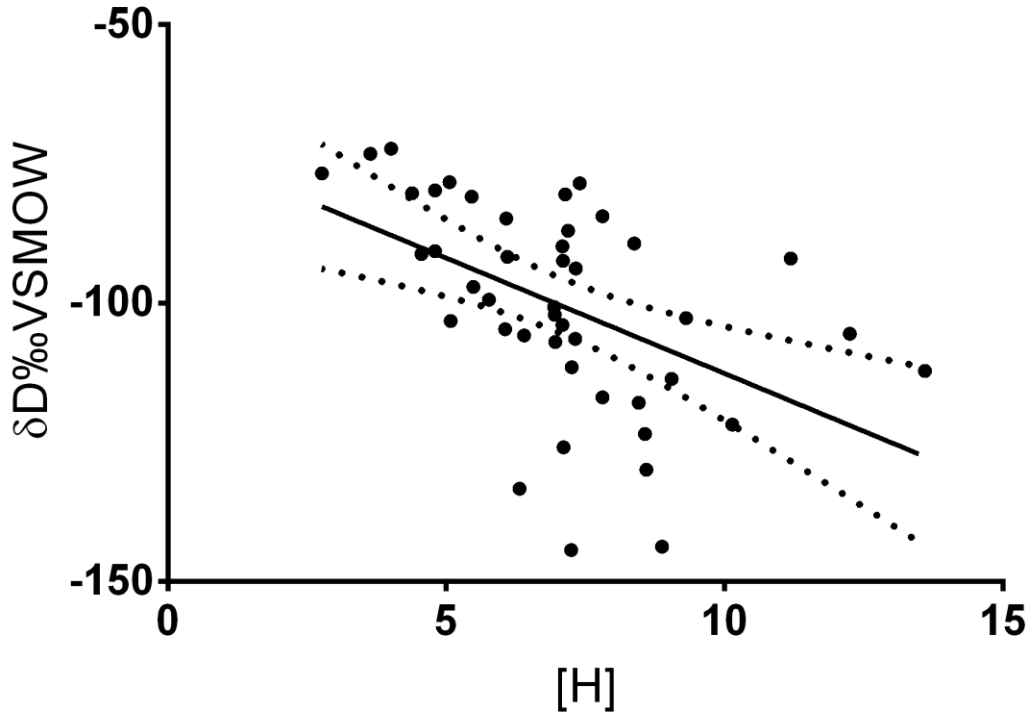

Fig 11. Bird (A) and mammal (B) δ^2H tissue values compared to δ^2H_{sw} and expected precipitaion δ^2H values along the elevational gradient. Keratin δ^2H values fall witin expected fractionation values from preciptiation to feathers previously reported at roughly 25‰ (Wassenaar and Hobson 2001).