EXPLORING MIGRATORY CONNECTIVITY IN THE CALLIOPE HUMMINGBIRD THROUGH STABLE ISOTOPE ANALYSIS OF TAIL FEATHERS

by

Clare E. Brown

A Thesis
Submitted in partial fulfillment
of the requirements for the degree
Master of Environmental Studies
The Evergreen State College
August 2012

This Thesis for the Master of Environmental Studies Degree

by

Clare E. Brown

has been approved for

The Evergreen State College

by

_____

Alison R. Styring, Ph.D.
Member of the Faculty

_____

Robert H. Knapp, Jr., D.Phil.
Member of the Faculty

_____

Jonathan A. Moran, Ph.D.
Associate Professor, School of Environment & Sustainability,
Royal Roads University

_____

Date

ABSTRACT

Exploring migratory connectivity in the Calliope Hummingbird through stable isotope analysis of tail feathers

Clare E. Brown

Knowing where individuals and populations of a migratory species are throughout their annual cycle, and how those individuals and populations are connected to each other in both the breeding and non-breeding seasons, is important for understanding the ecology, evolution, and conservation needs of that species. Stable isotopes can be used as endogenous markers in animal movement studies, with deuterium being particularly useful in assigning migratory origins. In this study I use feather deuterium to explore migratory connectivity in Calliope Hummingbirds (Stellula calliope). First, I used feathers collected from juveniles in the breeding range to determine the species-specific relationship between feather deuterium and deuterium in precipitation. Then, I used feathers collected in the breeding range from adult Calliope Hummingbirds, which molt in the winter, to examine the strength of migratory connectivity in this species, and to establish wintering locations of sampled breeding populations. Juvenile feather deuterium values were not correlated with predicted deuterium values of capture site precipitation, possibly due to the rugged topography of the study area and the contribution of snowmelt to growing season water supply. Adult feather deuterium values revealed no differences among populations from different regions, or between sexes, suggesting that sampled populations of this species have weak or no migratory connectivity. My ability to detect connectivity, however, was limited by the low resolution of the isotope data. Approximately twenty percent of adult females had significantly more depleted feather deuterium values than the remainder of adults, perhaps due to retained juvenile flight feathers in second-year birds, elevational or latitudinal differences in molt location, or differences in molt timing. Mapping the range of potential molt locations predicted by feather deuterium values onto deuterium isoscapes of Mexico and North America suggests that the most likely explanation is that some Calliope Hummingbird individuals molt their flight feathers during migration.

Table of Contents

List of figures…….....v
List of tables……..vi
Acknowledgements……..vii

Introduction……1
Avian migration and migratory connectivity
Studying migratory connectivity in birds
Stable isotope methods
Calliope Hummingbirds

Methods……....10
Feather collection
Isotope analysis
Statistical and isoscape analysis
Figures and tables

Results……...17
Adults
Adult males
Adult females
Enriched cluster
Juveniles
Isoscape analysis
Alabama
Figures and tables

Discussion……37
Feather-deuterium values of juvenile hummingbirds
Migratory connectivity in Calliope Hummingbirds
Depleted cluster

Summary……..41

References……42

List of Figures

Figure 1. Map of hummingbird capture sites.

Figure 2. Distribution of δ^2H values of adult and juvenile Calliope Hummingbird tail feathers.

Figure 3. Distribution of δ^2H values of adult Calliope Hummingbird tail feathers.

Figure 4. Distribution of δ^2H values of adult Calliope Hummingbird tail feathers, divided into an enriched and a depleted cluster by k-means clustering.

Figure 5. Distribution of δ^2H_f values of juvenile Calliope Hummingbirds and depleted-cluster adults. δ^2H_f values of juvenile birds differ significantly from δ^2H_f values of individuals in the depleted cluster.

Figure 6. Distribution of δ^2H_f values of enriched cluster adult female and adult male Calliope Hummingbirds. The mean δ^2H_f values of enriched cluster males and females do not differ significantly.

Figure 7. Distribution of δ^2H_f values of adult female Calliope Hummingbirds.

Figure 8. Distribution of δ^2H_f values of enriched cluster Calliope Hummingbirds, divided by region.

Figure 9. Distribution of δ^2H values of juvenile Calliope Hummingbird tail feathers.

Figure 10. Mean annual δ^2H values of precipitation predicted by the OIPC for feather collection sites vs. δ^2H values of tail feathers of juvenile Calliope Hummingbirds captured at those sites.

Figure 11. Mean annual δ^2H values of precipitation predicted by the OIPC for feather collection sites vs. δ^2H values of tail feathers of Rufous Hummingbirds captured at those sites.

Figure 12. δ^2H_f values of enriched cluster females and males mapped onto a groundwater-deuterium isoscape of Mexico at one and two standard deviations about the mean.

Figure 13. δ^2H_f values of enriched cluster individuals from British Columbia, Northern Washington and Southern Washington mapped onto a groundwater-deuterium isoscape of Mexico at one and two standard deviations about the mean.

Figure 14. δ^2H_f values of individuals in the enriched and depleted clusters mapped onto a North American isoscape of mean-annual deuterium in precipitation at one and two standard deviations about the cluster means.

List of Tables

Table 1. Hummingbird capture sites.

Table 2. Summary of *Stellula calliope* captures by region, showing number of sites within each region, period of field work, and individuals captured.

Table 3. Summary statistics.

Acknowledgements

I am indebted to many people for making this project possible. I am grateful to my advisor, Alison Styring, for both her guidance and her patience. The input of my committee members Jonathan Moran and Rob Knapp was invaluable; Jonathan provided advice, training, and financial support, and Rob's insightful comments on this manuscript were extremely helpful. Dan Harville devoted an enormous amount of time and effort to training me to handle and band hummingbirds, and he and Jan Harville were instrumental in making my field work both successful and enjoyable. My mother, Rachel Lawson, became my unofficial co-investigator, and spent weeks traveling to far flung corners of Washington State with me in search of hummingbirds. I am also grateful to Mark and Colleen Myers, Scott Heinz, Don Norman, and Alison Brown for their assistance in the field.

My hummingbird capture sites were almost all at private residences, so my project relied on the willingness of homeowners to open their homes to me. The hospitality of many of these people, their enthusiasm for hummingbirds, and their interest in my study made my field work a truly wonderful experience. I am very grateful to Dianne and Rick Edmonds, Betty and Ralph Hagenbuch, Teri Pieper and Ken Bevis, Ruthella Skagen, Libby and Victor Glick, Scott Fitkin, Tom Lamb, Cricket Webb, Jill Streeter, Julia O'Connor, Gordon Kent, and Doris and Larry Robinson. I also appreciate the help of Nancy Wallwork in coordinating my visits to Brooks Memorial State Park.

I was able to expand the geographic scope of feather collection through the willingness of other hummingbird banders to collect feathers for me. In Canada, Alison Moran, Cam Finlay, and other members of the Hummingbird Project of British Columbia collected feathers in Alberta and British Columbia, and Ida Bacon collected feathers in Alberta. Rob Faucett at the Burke Museum helped me with the import process. In the United States, Fred Bassett collected feathers in Alabama, and Lisa Goldberg sent me feathers samples from California.

I also owe thanks to the people that assisted me with analysis of my feathers and of my data. Vasiliki Demas at Lifespan Biosciences and Paul Quay and Mark Haught at the stable isotope laboratory in the University of Washington School of Oceanography provided me with lab space and the equipment I needed to prepare my samples for analysis, and Gabriel Bowen at Purdue University made the isotopic analysis of my feathers possible. Once I had data in hand, Joseph Brown was an enormous help in managing those data, determining how best to proceed with analysis, and in putting the results of that analysis in context.

Finally, no project is possible without funding, and I am grateful for the financial support of the Mountaineers Foundation and The Evergreen State College Foundation.

INTRODUCTION

Avian migration and migratory connectivity

Avian migration is one of the world's great natural phenomena. Every year, billions of individual birds from thousands of species undertake a round-trip journey between their breeding and wintering ranges, flying from breeding to wintering site in the fall and returning to the breeding site the following spring (Berthold 1993). While the breeding and non-breeding ranges of a species as a whole may be well described, there is often little known about the year-round geographic distribution of individuals and populations within that species. One question in particular is how strong migratory connectivity is in a given species. Migratory connectivity is the extent to which individuals that breed in the same geographic area also migrate to spend the non-breeding period in the same wintering area, and can be ranked on a continuum from strong to weak (Webster et al. 2002).

The total range of a seasonally migratory species can be roughly broken into two usually geographically distinct areas: the breeding range and the wintering range. Within each of those areas, individuals are grouped geographically into populations which may be quite distinct from each other. In a species with strong migratory connectivity, most individuals from one breeding population migrate to the same area in the non-breeding range so that population structure in the non-breeding range reflects that in the breeding range. In a species with weak migratory connectivity, the geographic distribution of individuals in the breeding range bears little relationship to their distribution in the non-breeding period, and non-breeding populations will be a mixture of individuals from multiple breeding populations and vice versa. Beyond a strict geographic definition, migratory connectivity can also be a function of age or sex class, if males and females or birds of different ages are segregated geographically or occupy different habitats in the winter range.

Understanding the ecology and evolution of a migratory species is impossible in the absence of knowledge about where individuals and populations of that species are throughout their annual cycle, and how those individuals and populations are connected to each other. As outlined by Webster and Marra (2005), the strength of migratory connectivity in a species is important at both immediate and evolutionary scales. Cross-seasonal interactions act at the individual or population level, with events or conditions in one season affecting individual survival and reproduction or population dynamics in another. Studying these interactions in migratory species is complicated in that they occur across space as well as across time, meaning that measuring cause (e.g. wintering ground conditions) and effect (e.g.

reproductive success) requires monitoring the same individuals in both the breeding and non-breeding range. Seasonal interactions at the individual level have been most successfully demonstrated in the American Redstart (*Setophaga ruticilla*). Redstart males that winter in higher quality habitat, as determined by stable-carbon isotope measurement of claws, arrive at breeding sites earlier in the subsequent breeding season (Marra et al. 1998) and, in turn, experience lower rates of extra-pair paternity in their own nests and higher rates of polygyny (Reudink et al. 2009).

At the population level, survival or mortality in the non-breeding season may affect population dynamics in the breeding season, or vice versa, by increasing or decreasing the density of breeding populations. The importance of this effect depends on the strength of migratory connectivity. With strong connectivity, a localized event that causes high mortality in a specific wintering population will have a significant effect on the corresponding breeding population. When connectivity is weak, winter mortality will be diffused across the breeding range. At the evolutionary scale, migratory connectivity may determine a species' degree of local adaptation to its non-breeding environment (Webster and Marra 2005). In a species with weak connectivity, gene flow among wintering populations that mix and interbreed in the breeding range will prevent those wintering populations from adapting to their local environments. If connectivity is strong, discrete winter populations will also be separated from each other in the breeding season, preventing gene flow and allowing local adaption to non-breeding conditions to occur. In practical terms, studies on migratory connectivity could inform conservation planning. When connectivity is strong, the restricted gene flow that enables local adaptation of wintering populations may prevent populations from adapting to habitat alteration or loss through adopting alternate migration routes or wintering areas. Further, in a species with strong connectivity, populations that occupy stable habitats in the breeding range may show declines due to local disturbances in the non-breeding range or vice versa. If a species' patterns of connectivity are understood, conservation and management plans can target key breeding or wintering sites or migration routes in order to protect specific populations identified as in decline, or to maintain stable populations across the breeding range (Martin et al. 2007).

Studying migratory connectivity in birds

Studies of migratory connectivity present the formidable obstacle of linking individual birds captured at their breeding sites to their specific wintering sites, or vice versa. There are several potential approaches to this problem, including mark-recapture methods such as leg-banding, satellite tracking, geolocator

devices, and endogenous markers such as genetic markers and stable isotope analysis (Webster et al. 2002). Leg-banding programs, in which captured birds are fitted with printed metal bands that serve as unique identifiers for those individuals in subsequent encounters, began in Europe and North America at the end of the nineteenth century, and hundreds of millions of birds have been banded in countries across the globe (Berthold 1993). Only a very small proportion of banded birds, however, are ever encountered in locations other than the original capture site (Webster et al. 2002). This means that the great majority of band recoveries of migratory birds are from individuals that disappear on migration and then reappear the next year, providing no information about where they have been during the intervening period. Enormous, long term datasets of bird captures and recaptures from across the multiple countries often included in a migratory species' range are thus required to generate sufficient data points for robust analyses of migratory connectivity from banding data. For example, Ambrosini et al. (2009) used encounter records of banded Barn Swallows (*Hirundo rustica*) in Europe and Africa from 1911 to 1998 to evaluate migratory connectivity in that species. Such datasets are not available for most bird species, meaning that studies of migratory connectivity that are able to rely on banding data alone are unusual.

Satellite tracking was first applied to bird movements in the late 1980s (Jouventin and Weimerskirch 1990), and it revolutionized migration studies by allowing investigators to remotely track the movements of individual birds virtually anywhere on the planet in real time. Satellite tags are attached to birds and transmit location information via the ARGOS satellite system. The tags can have lifespans of up to several years, meaning that individuals can be followed over multiple migratory journeys (Webster et al. 2002). The broad application of satellite technology to migration studies has, however, been constrained by both high costs and the size of the transmitters themselves. The combined cost of transmitter and satellite data acquisition is typically in the thousands of dollars for each tracked individual, and the smallest transmitters available weigh at least five grams (Bridge et al. 2011). The high cost has kept sample sizes in satellite tracking studies small, and the size of transmitters limits their use to birds larger than 100 grams, which excludes many migratory passerine species. The ICARUS project (Wikelski et al. 2007), expected to launch in 2014, plans to install an antenna in the International Space Station that will detect radio signals from transmitters attached to small animals. First generation transmitters are anticipated to weigh less than five grams, with future transmitters scaled down even further. This reduction in transmitter weight relative to currently available satellite tags will greatly increase the number of taxa that can be tracked remotely.

Geolocator devices differ from satellite tags in that they record and store data rather than transmitting it. Solar geolocators are attached to birds and record the time of every sunrise and sunset,

which can then be used to determine approximate latitude and longitude (Stutchbury et al. 2009). The size of the batteries needed to satisfy the power requirements of data transmission is the major obstacle to miniaturizing satellite tags. Because geolocators do not transmit, they can be much smaller than satellite transmitters. Geolocator devices have been produced that weigh less than one gram, opening up the possibility of using them to study migratory movements of all but the smallest avian taxa (Bridge et al. 2011). Geolocators also have a much lower cost per individual than do satellite transmitters, and are independent units that do not rely on a satellite infrastructure. However, the lack of transmission means that a bird must be recaptured in order to download stored data from its geolocator. Research using geolocators to study bird migration thus relies on the fact that many migratory bird species exhibit high site fidelity, with individuals returning to the same breeding site year after year (e.g., Stutchbury et al. 2009, Bairlein et al. 2012, Beason et al. 2012). Unfortunately, not all migratory species are site-faithful, and some species such as hummingbirds are too tiny for even the smallest geolocators.

Populations within a species show varying degrees of genetic structure across space (Avise 2000). If populations can be distinguished from each other on the basis of population-specific genotypes at certain loci, those loci have the potential to be used as genetic markers in migration studies. For example, genetic variation at specific loci has been characterized across the range of some species of Pacific salmon, and the origin of individuals caught away from their spawning sites can be identified with genetic stock identification (e.g. Beacham et al. 2005). In migratory birds, if the geographic distribution of genotypes in the breeding range is known, birds captured during migration or in the non-breeding range can be assigned to a specific breeding population. Migratory bird species often have weak genetic structure, and studies to date suggest that genetic markers may be best suited to drawing broad conclusions about migratory connectivity at the continental scale rather than linking precise geographical areas (Bensch et al. 1999, Wennerberg 2001, Kimura et al. 2002, Rolshausen et al. 2009, Irwin et al. 2011). The creation of a distribution-wide phylogeography is a key first step in applying this method, and the sampling and sequencing effort required may be prohibitive.

Stable isotope methods

The use of stable isotopes emerged as a powerful tool for the study of animal movements in the mid-1990s with the application of hydrogen stable isotopes to the assignment of geographic origins of migratory birds (Chamberlain et al. 1996, Hobson and Wassenaar 1997) and butterflies (Wassenaar and Hobson 1998, Hobson et al. 1999). Isotopes are atoms with the same number of protons and electrons, but different numbers of neutrons, and thus different atomic masses. The most common isotope of hydrogen,

denoted ^{1}H, has one proton and no neutrons, and an atomic mass of one. Deuterium, denoted as ^{2}H or D, has one proton and also one neutron, and an atomic mass of two. Stable isotopes are distinguished from radioactive isotopes by their energetic stability, in that they do not decay. Isotopes of lighter elements such as hydrogen, carbon, nitrogen and oxygen are the most commonly used in ecological studies, both because these elements predominate in biological compounds and because the addition of a neutron to a lighter element causes a proportionately greater and easier to detect change in mass than the addition of a neutron to a heavier element.

Research using stable isotopes relies on biologically meaningful variation in the relative abundance of isotopes of a given element under different conditions. That variation is generally quantified as the deviation of the isotopic ratio (e.g. $^{2}H:^{1}H$) of the sample of interest from the isotopic ratio of an internationally accepted standard. In the case of hydrogen, the vast majority of hydrogen atoms in the natural world are ^{1}H, with deuterium atoms making up only about 0.0155% (Sulzman 2008). In precipitation, which is the ultimate source of hydrogen in most terrestrial plant and animal tissue, the abundance of deuterium relative to ^{1}H varies strongly and predictably with season, climate, elevation and latitude. Deuterium forms stronger hydrogen bonds in liquid water than does ^{1}H, meaning that it moves less easily to the vapor phase. This difference in bond strength and the resulting depletion of deuterium in water vapor relative to liquid water increases at lower temperatures (Marshall et al. 2008). In clouds, water that condenses and falls as precipitation is enriched in deuterium relative to the water vapor left behind, and over time this drives a "rainout" effect in which clouds become more and more depleted in deuterium (Marshall et al. 2008). Temperature and rainout effects lead to a depletion of deuterium in precipitation as elevation and latitude increase, in winter relative to summer, towards the middle of continents, and with overall increases in precipitation (Marshall et al. 2008). Measured geographic and climatic trends in the relative abundance of hydrogen isotopes in precipitation can be used to generate robust models that predict isotope ratios from environmental parameters, allowing the construction of detailed maps (isoscapes) describing that relative abundance across continents (Meehan et al. 2004, Bowen et al. 2005b).

Geographic patterns of deuterium abundance in precipitation are relevant to migration studies in that the isotope ratio of hydrogen incorporated into an animal's tissues as it eats and drinks is a function of the local isotope ratio of hydrogen in precipitation (Chamberlain et al. 1996). The hydrogen isotope ratio of a given tissue is not simply equivalent to the local hydrogen isotope ratio of precipitation, but the two ratios are often tightly correlated, such that isotope ratios in animal tissues depend on and can be predicted from isotope ratios in precipitation (Hobson 2008). As a result, an animal's tissues will carry an

isotopic signature characteristic of that animal's location. If the animal moves to a new location at which precipitation has a different hydrogen isotope ratio, persistence of the original location's isotopic signature will vary among tissue types. Hydrogen in tissues with rapid turnover rates, such as blood, will transition to a new isotopic ratio more quickly than will tissues with lower turnover rates, such as claws or hair (Hobson and Clark 1992). Feathers, which are metabolically inert after their growth is completed, retain the isotopic signature of the site where they were grown (i.e. the molt location) for the entire lifetime of the feather (Chamberlain et al. 1996). Thus, if both molt strategy and the relationship between feather hydrogen isotope ratios (δ^2H_f) and precipitation hydrogen isotope ratios (δ^2H_p) of feather-growth location are known for a species, an approximate geographic location of molt can be estimated for an individual captured far from the molt site. For example, if a bird species molts post-breeding, before beginning fall migration, feathers from individuals of that species captured on the wintering grounds will have hydrogen-isotopic ratios that reflect the breeding site. The hydrogen isotope ratio of those feathers can be measured, and then used to answer questions about migratory strategies and patterns. A pattern in which isotope ratios in feathers of individuals captured in the same area tend to be more similar to each other than to isotope ratios of feathers of individuals captured in other regions of wintering range suggests strong migratory connectivity, while the absence of such pattern suggests weak migratory connectivity. If the relationship between δ^2H_f and δ^2H_p is known, allowing feather values to be mapped onto a hydrogen isoscape, the distribution of individuals and populations across the breeding range can be compared to their distribution across the wintering range. The migratory pattern of a species can then be classified as, for example, chain migration, leapfrog migration, or telescopic migration.

The clear benefit of stable isotopic methods is that stable isotopes are endogenous markers that can be measured in every bird captured. Stable hydrogen isotopes can be used to infer the geographic location of parts of a bird's life cycle that take place far away from a capture site, without the need to mark and recapture an individual or to track individuals after capture. A substantial body of research has applied stable isotope analysis to the study of avian migration, beginning with Hobson and Wassenaar's (1997) demonstration that stable hydrogen isotope ratios of songbird feathers collected in the winter in Guatemala were consistent with stable hydrogen isotope ratios of water in the known breeding ranges of the sampled species. Analysis of deuterium abundance in feathers has been used to explore migratory connectivity and migratory patterns in numerous taxa, including Swainson's Hawks (*Buteo swainsoni*) (Sarasola et al. 2008), European Woodpigeons (*Colmuba palumbus*) (Hobson et al. 2009a), Loggerhead Shrikes (*Lanius ludovicianus*) (Perez and Hobson 2009), Bicknell's Thrushes (*Catharus bicknelli*) (Hobson et al. 2001, Hobson et al. 2004a), Veerys (Catharus *fuscescens*) (González-Prieto et al. 2011), Common Blackbirds (*Turdus merula*) (Evans et al. 2012), American Redstarts (*Setophaga ruticilla*)

(Norris et al. 2004b, Studds et al. 2008), and other wood warbler species (Parulidae) (Kelly 2006, Paxton et al. 2007, Jones et al. 2008, Langin et al. 2009).

The relative abundances of isotopes of carbon ($^{13}C/^{12}C$) and nitrogen ($^{15}N/^{14}N$) also vary in plant and animal tissues across landscapes in response to habitat parameters, diet, and trophic level (Marra et al. 1998, Hobson 1999, Rubenstein and Hobson 2004). Marra et al. (1998) and Norris et al. (2004a) used stable carbon isotopes to link quality of winter habitat with migration timing and measures of reproductive success in American Redstarts. Carbon and nitrogen isotopes in combination with each other and with hydrogen isotopes have been used to make broad-scale inferences about migratory connectivity in species such as Willow Warblers (*Phylloscopus trochilus*) (Chamberlain et al. 2000), Black-throated Blue Warblers (*Setophaga caerulescens*) (Rubenstein et al. 2002), Aquatic Warblers (*Acrocephalus paludicola*) (Pain et al. 2004), and Reed Warblers (*Acrocephalus scirpaceus*) (Prochazka et al. 2008).

Despite the potential utility of stable isotopes in studying animal migration, some caveats apply to the interpretation of feather-isotope values. First, hydrogen-isotope ratios measured in feathers give a range of possibilities for geographic assignment of molt site rather than a precise location, and must be interpreted in light of what is known about the occurrence and life history of the species of interest. Second, within-population individual variability in feather-deuterium values and within-site temporal variability in precipitation-deuterium values may render the assignment of anything beyond a broad geographic range for the molt site impossible (Langin et al. 2007, Farmer et al. 2008). Third, drawing conclusions about migratory differences among individuals based on measured differences in feather-isotope values assumes that sampled individuals do not vary in molt strategy, and this assumption may not be met. For example, Norris et al. (2004b) measured feather hydrogen-isotope ratios of male American Redstarts from a breeding site in Ontario that were known to have bred at the same location the previous year. This species molts post-breeding, and about forty percent of sampled individuals had feathers significantly enriched in deuterium relative to the rest, suggesting that they molted further south. Thus, while most birds molted near their breeding sites before beginning migration, a significant proportion of individuals employed a different strategy and molted at staging areas during migration. Fourth, a fundamental assumption of hydrogen-isotope studies is that the ultimate source of the isotope ratio of an animal's tissues, i.e. the water that that animal consumes either directly by drinking or indirectly through its food, is equivalent to precipitation sampled at the animal's capture site. This assumption is supported by the general correlation in stable isotope studies between feather-growth site δ^2H_p and δ^2H values of feathers, but has not been directly tested.

Calliope Hummingbirds

The Calliope Hummingbird (*Stellula calliope*) is the world's smallest long distance avian migrant. It breeds in western North America, ranging from central California east into Utah and north into Alberta and British Columbia, and migrates to southwestern Mexico to winter (Calder and Calder 1994). In the 1980s some individuals began wintering in the southeastern United States, where there is now a small but reliable winter population (Dittman and Demcheck 2006). This species occurs in primarily montane habitats in both the breeding and non-breeding ranges (DesGranges 1978, Calder and Calder 1994). In the Pacific Northwest, breeding Calliope Hummingbirds are most common in the Ponderosa pine habitats of the eastern slope of the Cascade mountain range and in the dry interior forests of the Okanagan Highlands, with relatively few records from the flat, low elevation Columbia Basin and the rainy Pacific slope (data were gathered using eBird (http://www.ebird.org)). In Mexico, the species occupies a range of habitats in the non-breeding season (Calder and Calder 1994). On Volcán de Colima, Jalisco, DesGranges (1978) recorded it as being most abundant in arid pine-oak forest habitat (about 1500 to 3000 meters above sea level). The non-breeding range is split in two by the Río Balsas depression.

Very little is known about Calliope Hummingbirds outside of the breeding season, and nothing is known about the nature of migratory connectivity in this species. Hundreds of Calliopes have been leg-banded in the western United States and Canada every year since the mid-1980s, with thousands of individuals banded annually since 2005 (North American Bird Banding Association/Western Bird Banding Association annual reports, 1980-2010). Despite this banding effort, no encounter of a banded Calliope Hummingbird has been ever been reported from Mexico (USGS Bird Banding Laboratory: Summaries of banding and encounter data, retrieved online 5/19/2012). This is no doubt due to a lack of capture effort in Mexico, and means that banding data cannot be used at this point to draw conclusions about migratory connectivity in this species. Calliope Hummingbirds weigh, on average, less than three grams (Calder and Calder 1994), rendering them too small to be tracked with any available transmitter or geolocation device. Investigations into their migratory movements are thus limited to endogenous markers. To date, there has been no phylogeographic or stable-isotopic study of this species.

This study applies stable isotope methods to an analysis of migratory connectivity in Calliope Hummingbirds. I ask if Calliope Hummingbirds that breed in the Pacific Northwest exhibit segregation on the wintering ground by sex or by breeding site, and use hydrogen-isotope ratios of feathers collected from individuals breeding in Washington State, British Columbia, Alberta, and California to evaluate if any such segregation exists. If this species has high migratory connectivity, individuals that breed in the

same area are expected to cluster together in the wintering range. If there is little or no migratory connectivity, individuals breeding near each will be spread across the wintering range, distributed panmictically with individuals from other breeding populations. These winter distribution patterns are, in theory, represented by the hydrogen-isotope ratios of the hummingbirds' feathers, which are molted in the non-breeding period. If individuals from one breeding site can be distinguished from individuals from another breeding site on the basis of the hydrogen-isotope values of their feathers, those individuals are presumably occupying different areas on the wintering grounds, suggesting high migratory connectivity.

In addition to comparing feather isotope values among groups, I seek to determine the geographic distribution of the sampled Calliope Hummingbirds' wintering sites by mapping their feather isotope values onto hydrogen isoscapes of Mexico and North America. First, I use measured deuterium values of feathers collected from juvenile Calliope Hummingbirds at their natal sites and deuterium values of precipitation predicted by isotope models for those sites to find the relationship between the hydrogen-isotope ratio of water at a site and the hydrogen-isotope ratio of feathers grown at that site. Once this relationship is known, measured hydrogen-isotope values of adult feathers can be converted to predicted hydrogen-isotope values of water at the feather growth site and mapped onto a hydrogen isoscape to produce a prediction of wintering site location.

METHODS

Feather collection

Feathers were collected from a total of 557 Calliope Hummingbird individuals at 23 sites in Washington State, British Columbia, Alberta, and California (Figure 1, Table 1) from May through August of 2009. Sites in Washington State were grouped geographically into "Northern" and "Southern" Washington (Fig. 1). Sampling at the Southern Washington sites did not begin until July, when most males had already disappeared post-breeding, and only one male was captured at the four sites in that region. Feathers were collected from an additional two individuals in Alabama during the subsequent winter.

All sites had established artificial hummingbird feeders. At each site, my field team and I captured hummingbirds with either a Hall trap or a cage-wire drop-door trap (see Russel and Russel 2001). All birds were sexed and classified as either hatch-year (juvenile) or after hatch-year (adult). As adult Calliope Hummingbirds are highly sexually dimorphic, adult males are easily identified. Adult females and juveniles are superficially similar in plumage, but juvenile hummingbirds can be reliably distinguished from adults by the presence of corrugations on the bill that persist for at least several months after fledging (Pyle 1997). To sex juvenile birds, I looked for rufous color along the edges of the central rectrices (R1). Rufous is present in juvenile males, and absent in juvenile females (Baltosser 1994). In addition to sex and age data, I measured mass, wing chord, tail length, and length of exposed culmen, and evaluated molt, degree of tail-feather wear, and furcular fat. I also noted if adult females were gravid – in hummingbirds, the developing egg can easily be seen through the skin of the abdomen. All individuals were banded with bands provided by the USGS Bird Banding Laboratory, and one fourth rectrix was pulled for isotopic analysis and placed in a paper envelope for storage. All birds were released following processing.

Field work in Washington State was carried out by John D. Harville and me. Members of the Hummingbird Project of British Columbia collected feathers in Canada. Fred Bassett collected feathers from hummingbirds wintering in Alabama. The feathers from hummingbirds captured in California were collected as part of a project at the University of California, Davis, and were breast feathers rather than tail feathers. Hummingbirds were handled in accordance with the guidelines laid out in the North American Bird Banding Manual (Gustafson et al. 1997) and the North American Banders' Manual for Banding Hummingbirds (Russel and Russel 2001).

Isotope analysis

Feather samples from 236 of the 559 hummingbirds captured were prepared and sent to the Purdue Stable Isotope Facility (PSIF) for isotopic analysis. I included all Calliope Hummingbird individuals from Canada, California and Alabama and a subset of individuals from Washington State in the isotopic analysis. For each site in Washington State, I analyzed a maximum of ten individuals each of juveniles, adult males, and adult females. Where possible for a site, I analyzed individuals that were captured on the same day. If more than ten individuals in one of those three categories had been captured at a site on the same day, I randomly selected ten for analysis. To reduce bias during analysis, I randomized the order in which feather samples were prepared and analyzed. Due to delays in the import process, however, all feathers from Canada were prepared and analyzed together.

Feathers were cleaned with a 2:1 chloroform: methanol wash in order to remove dirt and surface oils before isotopic analysis. This cleaning method is widely used in hydrogen-isotope studies (e.g., Hobson et al. 2009b, Langin et al. 2009, Hardesty and Fraser 2010, González-Prieto et al. 2011). I followed the protocols provided by the PSIF for cleaning and weighing feather samples, modified due to the small size of hummingbird feather. I placed each feather in a microcentrifuge tube, filled the tube with enough 2:1 chloroform:methanol solution to cover the feather, and left the feather in the solution for at least five minutes, agitating the tube at least once during the soak. I then decanted the solution and repeated the soaking and agitation step two more times. Finally, I rinsed both sides of the feathers with the chloroform:methanol solution and dried them on a sheet of aluminum foil under a fume hood. Feathers were stored in clean paper envelopes between cleaning and weighing. To prepare the samples for isotopic analysis, I weighed 0.15 ± 0.01 mg of material cut from the distal part of the cleaned feathers into individual 3.5 x 5 mm silver capsules and crushed the capsules.

Measurement of hydrogen-isotope ratios in organic materials such as feather-keratin is complicated by the presence of exchangeable hydrogen in the sample. While most hydrogen in keratin is bound to carbon and is non-exchangeable, a significant proportion of hydrogen atoms in feather-keratin are free to exchange with hydrogen atoms in ambient water vapor (Wassenaar and Hobson 2000). If the hydrogen-isotope ratio of ambient vapor differs from that of the sample, the replacement of exchangeable hydrogen atoms in the sample with atoms from the ambient environment will alter the sample's total hydrogen-isotope ratio. Feathers are metabolically inert, meaning that once feather growth is complete, the isotope ratio of non-exchangeable hydrogen in the feather does not change (see Hobson and Clark 1992). The isotope ratio of exchangeable hydrogen, however, and thus of the total hydrogen content in

feathers of migratory birds, will change as the feathers move to different locations, as when the bird leaves the site of feather growth and when feathers are collected and moved into a laboratory for analysis. Only isotope ratios of total hydrogen content can be measured directly, meaning that uncorrected sample isotope ratios measured at different facilities, or at different times within the same facility, are not comparable. The isotope ratio of non-exchangeable hydrogen alone can be determined using the comparative equilibrium method proposed by Wassenaar and Hobson (2000, 2003, also see Bowen et al. 2005a). In comparative equilibrium, keratin reference standards with known hydrogen isotope ratios and the samples of interest are simultaneously allowed to equilibrate with ambient laboratory conditions. The samples and the standards are then analyzed together, and a formula to correct for exchangeable hydrogen is determined using linear regression.

After cleaning and preparation, hummingbird feather samples were sent to the PSIF for analysis on an isotope ratio mass spectrometer. Samples were subjected to comparative equilibrium, so that reported δ^2H values can be interpreted as the isotope ratio of non-exchangeable hydrogen in the feathers. Analytical precision was determined by repeated analysis of two reference keratins. Hydrogen isotope ratios (^{2}H/^{1}H) are reported in terms of their deviation, in parts per thousand (‰), from the isotope ratio in Vienna standard mean ocean water-Vienna standard light Antarctic precipitation (VSMOW-VSLAP), a widely used international standard:

$$\delta^2\mathrm{H}_{\mathrm{sample}} = \left(\frac{R_{\mathrm{sample}}}{R_{\mathrm{standard}}} - 1\right) \mathrm{x}\, 1000$$

where R_{sample} is the measured ratio of deuterium:hydrogen in the sample, and R_{standard} is the known ratio of deuterium:hydrogen in VSMOW-VSLAP.

Statistical and isoscape analysis

All statistical analyses other than reduced major axis regressions were conducted in R version 2.11.1 (R Development Core Team 2011). δ^2H_f values of adult Calliope Hummingbirds samples are bimodally distributed. I segregated them into two groups based on their feather δ^2H values with k-means clustering, using the k-means clustering algorithm developed by Hartigan and Wong (1979), the default option in R. This algorithm groups data points into an *a priori* specified number of clusters such that the within-cluster sum of squares is minimized.

Calliope Hummingbirds molt in the winter after completing southward migration to their wintering sites (Calder and Calder 1994, Dittman and Demcheck 2006). The hydrogen isotope ratio measured in feathers (δ^2H_f) collected from an adult Calliope in the breeding range will thus reflect the hydrogen isotope ratio of precipitation (δ^2H_p) of that individual's wintering site. As hydrogen is incorporated into plant and animal tissues, however, isotope fractionation alters the hydrogen isotope ratio so that δ^2H_f will not simply equal δ^2H_p (Chamberlain et al. 1996). A key step, then, in discovering migratory origins is to translate measured δ^2H_f values into δ^2H_p values that can be mapped onto a hydrogen isoscape. This translation is straightforward - deuterium values of feathers and of feather growth site precipitation tend to have a strong linear correlation (e.g. Bowen et al. 2005b) that can be determined by analyzing feathers of known origin, i.e., feathers collected from adult birds at the site of feather growth, or from juvenile birds near the nesting site. Regressing δ^2H_p of the collection sites against δ^2H of these feathers will produce a linear equation that can then be used to convert measured δ^2H values of feathers of unknown origin into predicted δ^2H_p values for the sites where those feathers were grown. As there is error in both the model-based prediction of δ^2H_p (the dependent variable) and in the measurement of δ^2H_f (the independent variable), reduced major axis regression (RMA) is more appropriate in this case than simple least squares regression (Hobson et al. 2004b).

To determine the relationship between δ^2H_f and δ^2H_p of feather-growth location for Calliope Hummingbirds, I analyzed feathers collected from juvenile birds in Washington State and British Columbia. Mapping breeding Calliope Hummingbirds onto their putative wintering sites requires converting measured δ^2H_f into a predicted δ^2H_p value, so I performed RMA regressions with juvenile δ^2H_f as the independent variable and δ^2H_p of feather collection site as the dependent variable, using RMA for Java v1.21 (Bohonak and van der Linde 2004). I regressed both mean annual and June δ^2H_p values predicted by the Online Isotopes in Precipitation Calculator (OIPC) (Bowen et al. 2005b, Bowen 2011b) against juvenile δ^2H_f values. Juvenile δ^2H_f correlated poorly, however, with δ^2H_p of collection site in my data set (see Discussion), so I was unable to use juvenile Calliope feathers to predict the relationship between δ^2H_p and δ^2H_f in adults. Instead, I used data provided by Dr. Jonathan Moran for Rufous Hummingbird (*Selasphorus rufus*) feathers collected in Canada, the south-eastern United States, and Mexico. Feathers were collected from the individuals in this data set at the sites where the feathers were presumed to have been grown (i.e., juveniles in the breeding range, and adults or first-year birds in the wintering range following molt). Calliope and Rufous Hummingbirds are closely related (*Stellula* actually falls within the *Selasphorus* clade (McGuire et al. 2009)), and occur in the same habitats in both the breeding and non-breeding season. I assume that, due to their phylogenetic relatedness and ecological similarity, the effect of isotope fractionation on the isotope ratios of hydrogen incorporated into tissue will

be similar between the two species. It follows from this assumption that the relationship observed between Rufous Hummingbird δ^2H_f and collection site δ^2H_p should be a reasonable proxy for that relationship in Calliope Hummingbirds, and I use the equation obtained with the Rufous Hummingbird dataset to convert Calliope Hummingbird δ^2H_f to δ^2H_p.

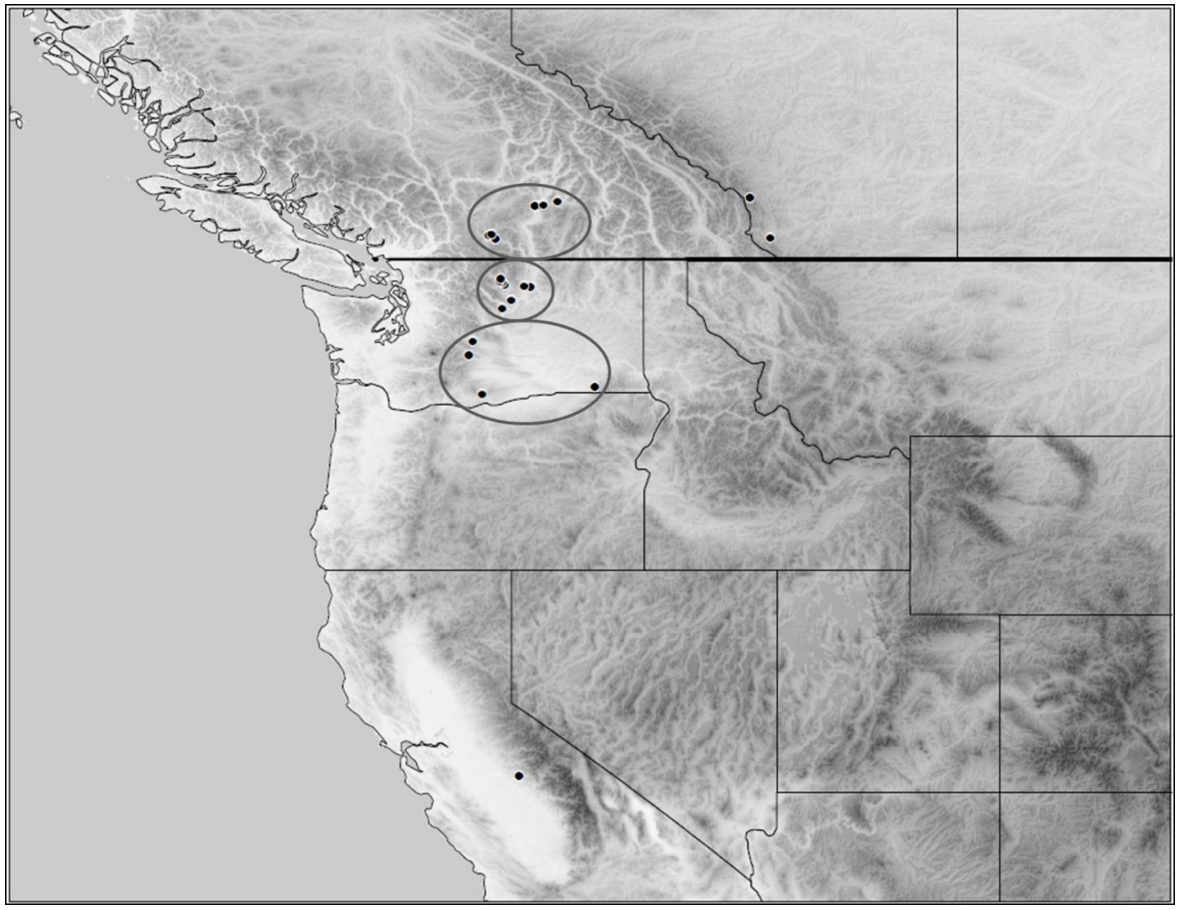
I performed an RMA regression on the Rufous Hummingbird data set with δ^2H_f as the independent variable and mean annual δ^2H_p estimated for feather collection sites with the OIPC as the dependent variable. Mean annual deuterium values are more appropriate for this regression than are monthly values, as the relationship obtained from the RMA regression is used to map adult Calliopes onto their wintering range with a hydrogen-isoscape map of Mexico constructed by Wassenaar et al. (2009) using deuterium in groundwater. Using groundwater effectively averages precipitation throughout the year, smoothing out the intra-and inter-annual variation of δ^2H_p values (Wassenaar et al. 2009). This means that groundwater-deuterium values are more comparable to annual than to monthly precipitation-deuterium values. I used the linear relationship produced with the RMA regression to convert measured δ^2H_f values of adult Calliope Hummingbirds to predicted δ^2H_p values of their feather-growth sites.

These δ^2H_p values fall into two distinct clusters, one with more enriched hydrogen-isotope ratios and the other with more depleted isotope ratios. I divided the enriched cluster by sex and by region (Fig. 1), and mapped both one standard deviation and two standard deviations about the mean δ^2H_p values of each group onto the hydrogen-isoscape of groundwater in Mexico (Wassenaar et al. 2009). The δ^2H_p values of the depleted cluster fall outside the range ofδ^2H_p values that occur in Mexico, so I used a global hydrogen-isoscape of precipitation (Bowen and Revenaugh 2003, Bowen 2011a) to map one standard deviation and two standard deviations about the mean δ^2H_p values of the enriched and depleted clusters. Both the global and Mexican isoscapes are available as ArcGIS rasters, and I mapped Calliope Hummingbird δ^2H_p values onto the rasters with ArcGIS 10.

TABLE 1 – Hummingbird capture sites

	Latitude	Longitude	Elevation (m asl)	Region
1	49.47	-114.19	1400	Alberta
2	50.38	-114.65	1510	
3	49.45	-120.36	600	British Columbia
4	49.54	-120.51	720	
5	49.54	-120.45	920	
6	50.19	-119.42	400	
7	50.20	-119.48	375	
8	50.20	-119.28	450	
9	50.29	-118.97	683	
10	48.08	-120.01	332	Northern Washington
11	48.37	-119.58	347	
12	48.37	-119.72	259	
13	48.38	-119.72	927	
14	48.42	-120.16	588	
15	48.47	-120.22	597	
16	48.5	-120.26	558	
17	49.45	-120.36	948	
18	45.95	-120.67	817	Southern Washington
19	46.12	-118.14	524	
20	46.84	-120.96	621	
21	47.15	-120.87	802	
22	47.89	-120.21	335	
23	37.37	-119.83	590	California
24	30.36	-87.49	8	Alabama
25	30.38	-87.46	13	

FIGURE 1 – Map of hummingbird capture sites. Site groupings used in the analysis are circled and are, from south to north, Southern Washington, Northern Washington, and British Columbia.



RESULTS

The overall precision in the isotopic analysis was 2‰ for one of two independent reference keratins and 3‰ for the other. The PSIF reported imprecision in the bracketing reference keratins for 12 samples, and I excluded those samples from further analysis. I also excluded three hatch year birds with feathers extremely enriched in deuterium (δ^2H_f = -71.5, -77.6, -80.3‰) relative to other juveniles, as my notes on either the bird or on the feather itself indicated that in all three cases the individuals were misidentified adult females. The two individuals from Alabama were not included in the general analysis. With the 15 questionable samples excluded, the following analysis includes a total of 219 Calliope Hummingbird individuals from the western United States and Canada, comprising 172 adults (128 females, 44 males) and 47 juveniles (14 females, 33 males) (Table 2).

The δ^2H_f values for all individuals range from -36.6 to -169.4‰ with a clearly bimodal distribution (Fig. 2). As expected, juveniles, which grow their feathers in the nest in the breeding range, occupy the more depleted (more negative) peak of the distribution, while adults, which grow their feathers much further south in the wintering range, occupy the more enriched (less negative) peak.

Adults

The δ^2H values of adult feathers (N = 172) range from -148.1 to -36.6‰, and are bimodally distributed (Table 3, Fig. 3). A k-means cluster analysis with an initial assignment of two clusters groups the data in apparent agreement with the two peaks of the distribution (Table 3, Fig. 4). The cluster of individuals with feathers more depleted in deuterium ranges from -148.1 to -100.7‰ and comprises 1 male and 29 females. The remaining 43 males and 99 females make up the cluster of individuals with feathers more enriched in deuterium, with δ^2H values that range from -96.7 to -36.6‰. The means of the two clusters differ significantly from each other (t = 21.29, df = 42.38, $P < 0.001$), while the variances do not (F = 1.02, df = 29,141, P = 0.99).

One simple explanation for the presence of the depleted cluster is that these individuals are juvenile birds mistakenly identified as adult females. However, the δ^2H_f values of the birds in the depleted cluster differ significantly from the δ^2H_f values of juvenile birds (t = 7.83, df = 54.68, $P < 0.001$), and about half of the juveniles have more negative $\delta 2H_f$ values than the most depleted individual in the depleted cluster (Fig. 5). Additionally, if adult females with very negative δ^2H_f values are actually juveniles, cluster membership should depend on capture date, with birds in the depleted cluster tending to

be captured after juveniles began to visit feeders in early July. This is not the case, as cluster membership and capture date (defined as before July 1 or after July 1) of adult females are independent ($X^2 = 0.30$, df = 1, $P = 0.58$). These results indicate that the individuals in the depleted cluster are in fact adults, and not misidentified juvenile birds.

Adult males

All but one of the 44 adult males (δ^2H_f = -116.5‰, from British Columbia) were grouped into the more enriched k-means cluster. The δ^2H_f values of these enriched adult males appear normally distributed, and range from -36.6‰ to -96.7‰ (Table 3, Fig. 6). With the depleted-cluster male excluded, there is no significant difference in feather δ^2H between males from Northern Washington and males from British Columbia ($t = 0.94$, df = 18.58, $P = 0.36$), the only regions for which sample size allowed comparison. While the only male in the depleted cluster was captured in British Columbia, within the enriched cluster males from Northern Washington are slightly and non-significantly more variable than males from British Columbia ($F = 0.52$, df = 8,28, $P = 0.33$) (Table 3). Male feather δ2H is uncorrelated with the elevation of collection sites, both across all regions and within regions.

Adult females

The feather δ^2H values of adult females are bimodally distributed (Fig. 7), and drive the overall distribution of sampled adults – all but one of the 30 individuals in the depleted cluster are female. A k-means cluster analysis of adult females alone produced results similar to the cluster analysis of all adults, with all females assigned to the same cluster as before. As with adult males, adult female feather δ^2H is uncorrelated with collection site elevation, both across all regions and clusters and within regions and clusters.

As the sample sizes for Alberta and California are small, I focused on adult females from British Columbia, Northern Washington, and Southern Washington. For females, cluster membership is not independent of sampling locality (Fisher's Exact Test, $P < 0.001$), with more females from British Columbia and fewer females from Northern Washington in the depleted cluster than would be expected if cluster membership and sampling region were independent.

While Northern Washington has the lowest proportion of individuals in the depleted cluster, and thus the lowest overall variability, females from this region have the greatest variability within the

enriched cluster. The variance of females from Northern Washington is significantly greater than that of females from Southern Washington (F = 2.13, df = 28,53, P = 0.03), but is not significantly greater than that of females from British Columbia (F = 2.31, df = 7,53, P = 0.25). The variances of females from Southern Washington and British Columbia are virtually identical (Table 3). As the assumption of homoscedasticity for Fisher's analysis of variance is not met (Fligner-Killeen test: X^2 = 6.49, df = 2, P = 0.04) and the disparity in sample size among groups is substantial, I compared the means of the three regions using a one-way Welch's analysis of variance (Welch 1951). The mean δ^2H_f values of adult females do not vary significantly among the three regions (F = 2.48, df = 2.00,21.65, P = 0.11).

Only one male falls within the depleted cluster, and other than one individual from California (δ^2H_f = -107.1‰), all individuals in the depleted cluster are from British Columbia, Northern Washington, or Southern Washington. The variances of the δ^2H_f values for the depleted-cluster females from these three regions are homogeneous (Fligner-Killeen test: X^2 = 2.90, df = 2, P = 0.23), while the means are marginally significantly different (one-way analysis of variance, F = 3.18, df = 2,25, P = 0.06). This difference is driven by the more enriched mean δ^2H_f value of depleted-cluster females from Northern Washington (see Table 3).

Enriched cluster

Within the cluster of hummingbirds with feathers more enriched in deuterium, the mean (t = 0.78, df = 72.73, P = 0.44) and variance (F = 1.24, df = 42,98, P = 0.38) of δ^2H_f values do not differ significantly between males and females (Table 3, Fig. 6). Looking within regions, mean (t = 0.38, df = 54.41, P = 0.70) and variance (F = 1.13, df = 28,53, P = 0.68) of δ^2H_f do not differ between males and females from Northern Washington. I only captured one male from Southern Washington, so a comparison between sexes in that region is not possible. In British Columbia, the variance of δ^2H_f does not differ between sexes (F = 1.35, df = 7,8, P = 0.71), but females have a significantly more depleted mean δ^2H_f than do males (t = 2.49, df = 14.99, P = 0.02, see Table 3). There is one male from British Columbia with very enriched feather δ^2H (-41.3‰) relative to the other males from the region, but the difference between males and females is significant even with that male removed (t = 2.22, df = 13.47, P = 0.04).

As above, the following focuses on British Columbia, Northern Washington, and Southern Washington. For males and females together, as with females alone, the variance of δ^2H_f appears greatest in Northern Washington (Fig. 8). The difference in variance is significant between Northern and Southern Washington (F = 2.25, df = 29,82, P = 0.02), and is not significant between Northern Washington and

British Columbia (F = 1.53, df = 16,82, P = 0.34). The greater variance could be a factor of Northern Washington's large sample size relative to the other two regions. As for females alone, due to the unequal variances (Fligner-Killeen test: X^2 = 8.04, df = 2, P = 0.02) and the differences in sample size among groups, I compared the mean δ^2H_f of enriched-cluster adults among the three regions with a one-way Welch's anova. The means do not vary significantly (F = 0.20, df = 2.00,43.00, P = 0.82).

Juveniles

δ^2H values of juvenile feathers (N = 47) range from -169.4 to -121.8‰, and appear to be normally distributed, although with a slight positive skew (Table 3, Fig. 9). It is possible that juveniles with the most enriched δ^2H values are misidentified adult females. Feather deuterium values of juvenile males and of juvenile females do not significantly differ in either mean (t = -1.52, df = 29.17, P = 0.14) or variance (F = 1.43, df = 13,32, P = 0.50). Contrary to expectation, there is no correlation between juvenile δ^2H_f and either the mean annual or June precipitation deuterium (δ^2H_p) values predicted by the Online Isotopes in Precipitation calculator (Bowen et al. 2005b, Bowen 2011b) for the sites where the juvenile birds were captured (RMA regressions, N = 47: mean annual, R^2 = 0.04, P = 0.18; June, R^2 = 0.02, P = 0.31). Additionally, the difference between predicted δ^2H_p and measured juvenile δ^2H_f is greater than expected. The juvenile feather values tend to be depleted by about 50‰ or more relative to predicted precipitation values (Fig. 10), while the "standard" fractionation value expected from the literature is -25‰ (Wassenaar and Hobson 2001, Bowen et al. 2005b).

Isoscape analysis

In the *Selasphorus rufus* data set, which comprises 43 individuals, measured δ^2H feather values are tightly correlated with capture site mean annual δ^2H precipitation values predicted by the Online Isotopes in Precipitation Calculator (Fig. 11; $\delta^2H_p = 29.006 + 1.151\ \delta^2H_f$, R^2 = 0.92, $P < 0.001$). I used this fractionation equation to convert adult Calliope Hummingbird δ^2H feather values to the δ^2H_p values of the locations where adult birds grew those feathers, i.e., their putative wintering sites. In order to compare the geographic distributions of these putative wintering sites, I mapped one and two standard deviations about the mean delta2Hp values of the enriched and depleted clusters and of sexes and regions within the enriched cluster onto global annual precipitation and Mexican groundwater hydrogen-isoscapes, respectively.

Within the enriched cluster, there is no differentiation between the predicted wintering site of

Calliope Hummingbird males and females (Fig. 12) or among the wintering site of individuals breeding in different regions (Fig. 13). There is high variability in hummingbird feather-isotope values relative to the range of groundwater-isotope values modeled for Mexico by Wassenaar et al. (2009). The 95 percent confidence intervals (two standard deviations about the mean) of δ^2H_p of the groups in the enriched cluster each cover close to the entire range of modeled groundwater δ^2H in Mexico.

K-means clustering split adult Calliope Hummingbirds into a more enriched and a more depleted cluster, with hydrogen-isotope ratios greater than and less than -100‰, respectively (Fig. 4, Table 3). When mapped onto a global hydrogen isoscape, the two clusters show different and non-overlapping geographic distributions (Fig. 14). The known Calliope Hummingbird wintering range falls completely within the predicted geographic distribution of the enriched cluster at one standard deviation about the cluster mean, whereas one standard deviation about the mean of the depleted cluster almost entirely contains the known breeding range.

Alabama

Both birds captured in Alabama hatched in 2009, and were in juvenile plumage at the time of capture. The first, a female, was captured on 12/16/2009, while the second, a male, was captured a month later, on 1/21/2010. Their feather δ^2H values are -142.5 and 130.9‰, respectively. The first value falls squarely into the range of juveniles captured in Washington State and British Columbia, while the second falls towards the more enriched end of the range (Table 3).

TABLE 2 – Summary of *Stellula calliope* captures by region, showing number of sites within each region, period of field work, and individuals captured. The numbers in parentheses are the numbers of sites or individuals which were used in the final analysis. With the exception of sites in Alberta and Alabama, locations were visited multiple times during the indicated date range.

Region	Number of sites	Date range of captures	Adults		Juveniles	
			Males	Females	Males	Females
Alberta	2 (2)	5/28/2009-5/31/2009, 8/11/2009	2 (2)	5 (4)	0	0
British Columbia	7 (5)	6/31/2009-7/11/2009	13 (10)	27 (19)	4 (3)	8 (5)
Northern Washington	8 (8)	6/20/2009-7/25/2009	47 (29)	307 (60)	46 (28)	14 (8)
Southern Washington	4 (4)	7/8/2009-8/12/2009	1 (1)	70 (40)	3 (2)	1 (1)
California	1 (1)	5/3/2009	2 (2)	5 (5)	0	0
Alabama	2 (2)	12/16/2009, 1/21/2010	1 (1)	0	0	1 (1)

TABLE 3 – Summary statistics

	mean (‰)	standard deviation (‰)	N
JUVENILES (range -169.4 to -121.8‰)	-149.9	11.4	47
Males	-151.4	11.8	33
Females	-146.3	9.9	14
ADULTS (range -148.1 to -36.6‰)	NA	NA	172
Enriched cluster (range -96.7 to -36.6‰)	-69.6	13.5	142
Males	-68.2	13.0	43
Females	-70.2	14.5	99
British Columbia	-69.6	12.0	17
Males	-63.7	11.1	9
Females	-76.2	9.6	8
Northern Washington	-68.9	14.8	83
Males	-68.1	15.5	29
Females	-69.4	14.6	54
Southern Washington	-67.7	9.9	30
Males	-73.8	NA	1
Females	-67.5	10.0	29
Depleted cluster (range -148.1 to -100.7‰)	-126.8	13.3	30
Males	-116.5	NA	1
Females	-127.1	13.4	29
British Columbia (females)	-132.6	7.7	11
Northern Washington	-117.2	17.5	6
Southern Washington	-128.9	12.5	11

FIGURE 2 – Distribution of δ^2H values of adult and juvenile Calliope Hummingbird tail feathers. Juveniles occupy the more depleted peak to the left, adults the more enriched peak to the right.

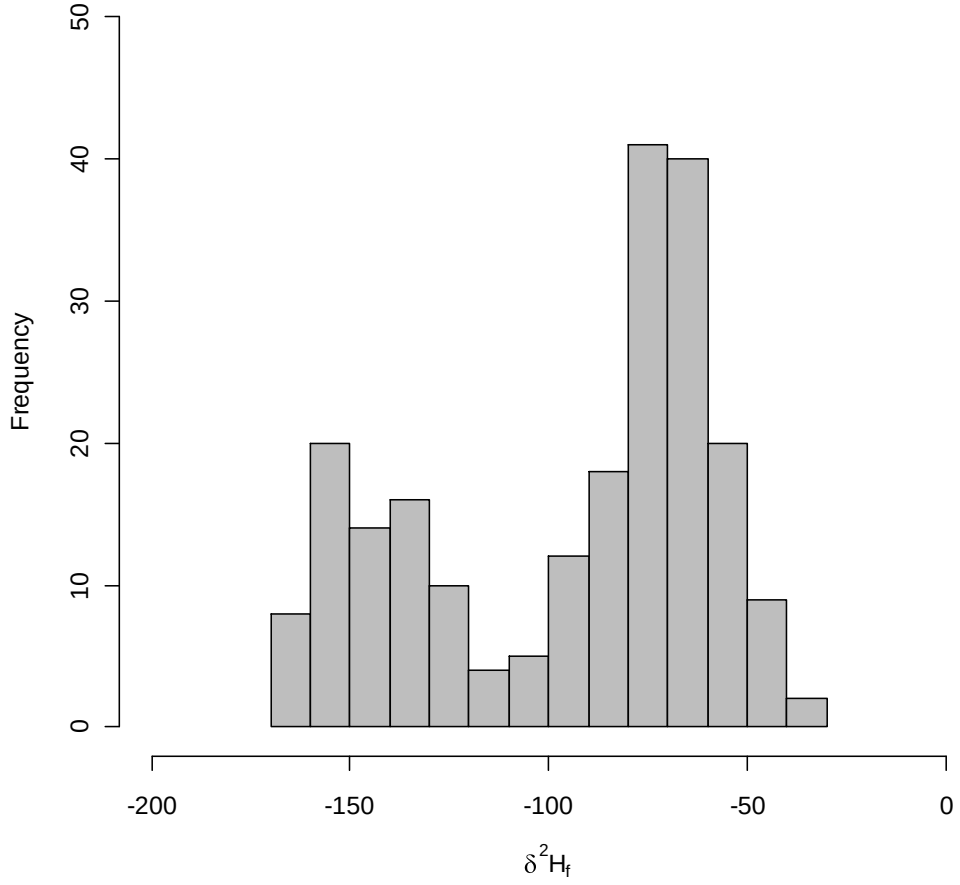


FIGURE 3 – Distribution of δ^2H values of adult Calliope Hummingbird tail feathers. The smaller peak to the left comprises the 30 individuals in the depleted cluster, while the larger peak to the right is the enriched cluster.

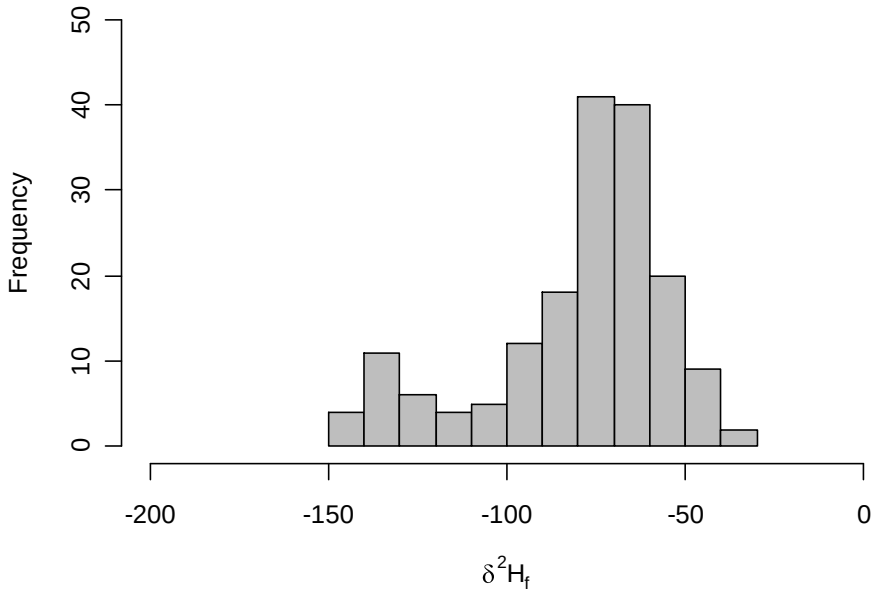


FIGURE 4 – Distribution of δ^2H values of adult Calliope Hummingbird tail feathers, divided into an enriched (upper panel) and a depleted cluster (lower panel) by k-means clustering with an initial assignment of two clusters.

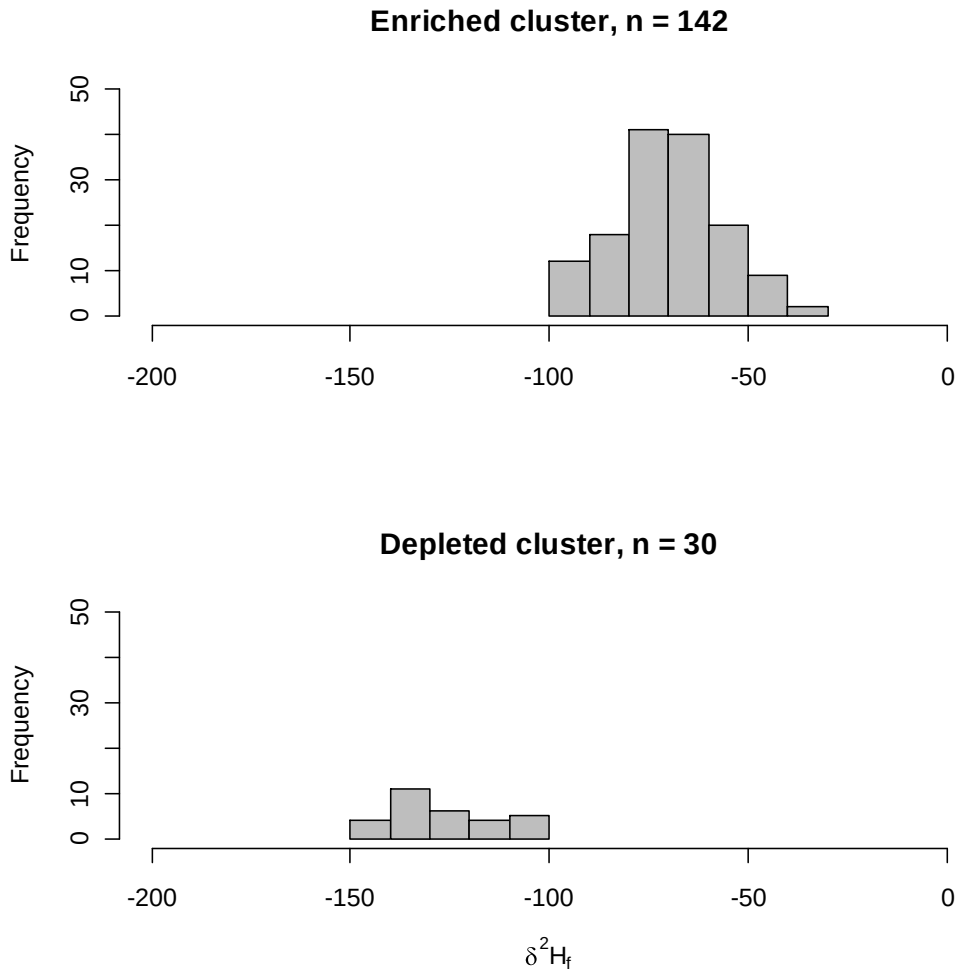


FIGURE 5 – Distribution of δ^2H_f values of juvenile Calliope Hummingbirds (upper panel) and depleted-cluster adults (lower panel). δ^2H_f values of juvenile birds differ significantly (t = 7.83, df = 54.68, $P < 0.001$) from δ^2H_f values of individuals in the depleted cluster.

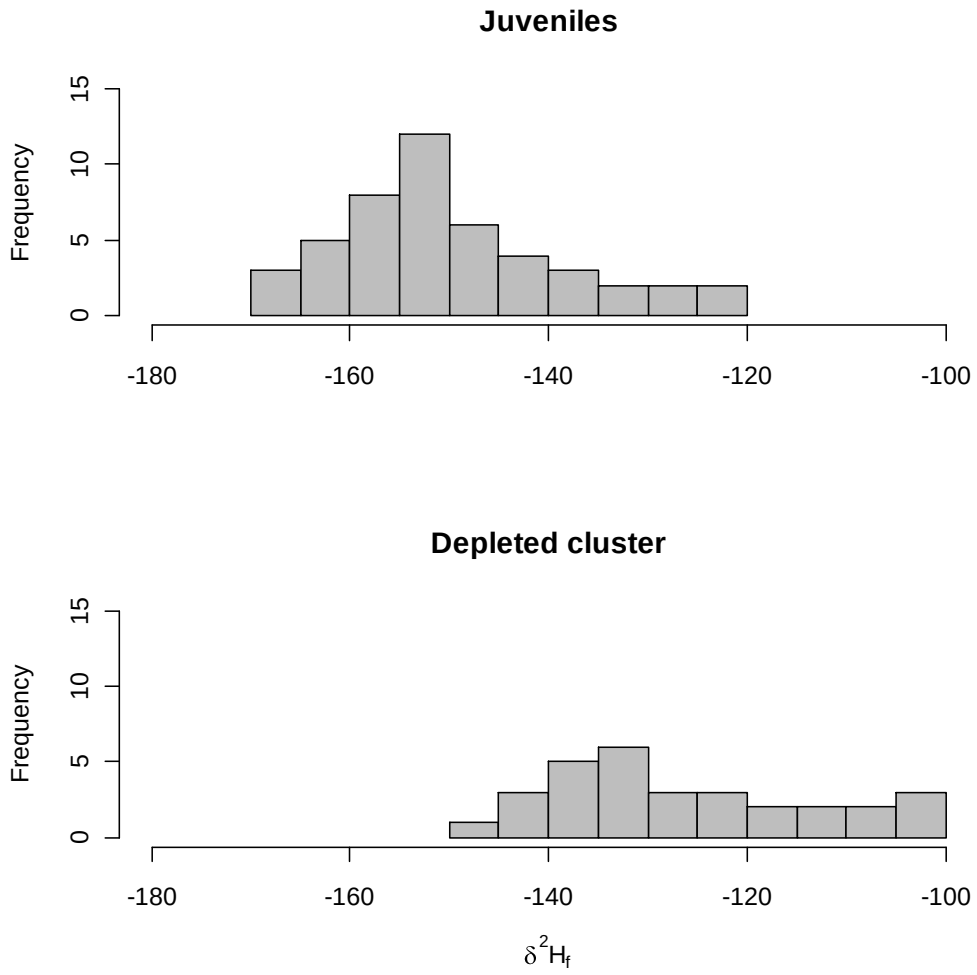


FIGURE 6 – Distribution of δ^2H_f values of enriched cluster adult female (upper panel) and adult male (lower panel) Calliope Hummingbirds. The mean δ^2H_f values of enriched cluster males and females do not differ significantly (t = 0.78, df = 72.73, P = 0.44).

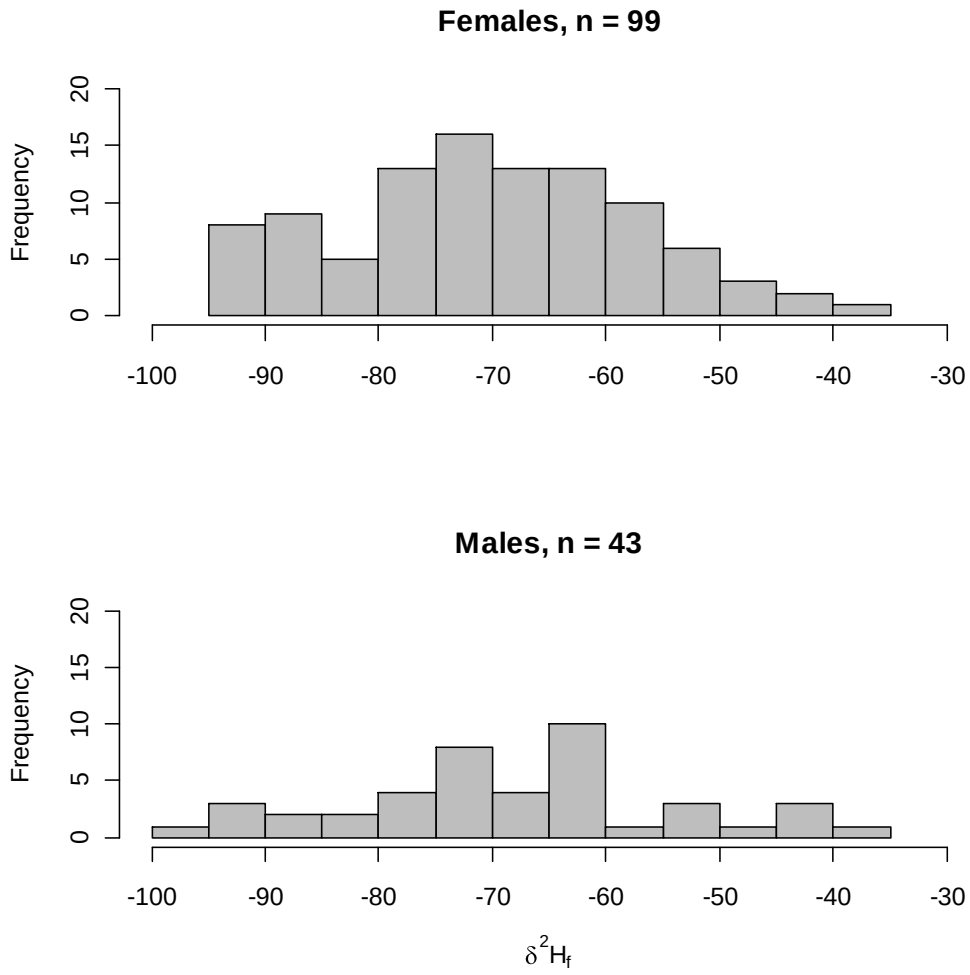


FIGURE 7 – Distribution of δ^2H_f values of adult female Calliope Hummingbirds. The peak on the left falls within the depleted cluster, while the peak on the right falls within the enriched cluster.

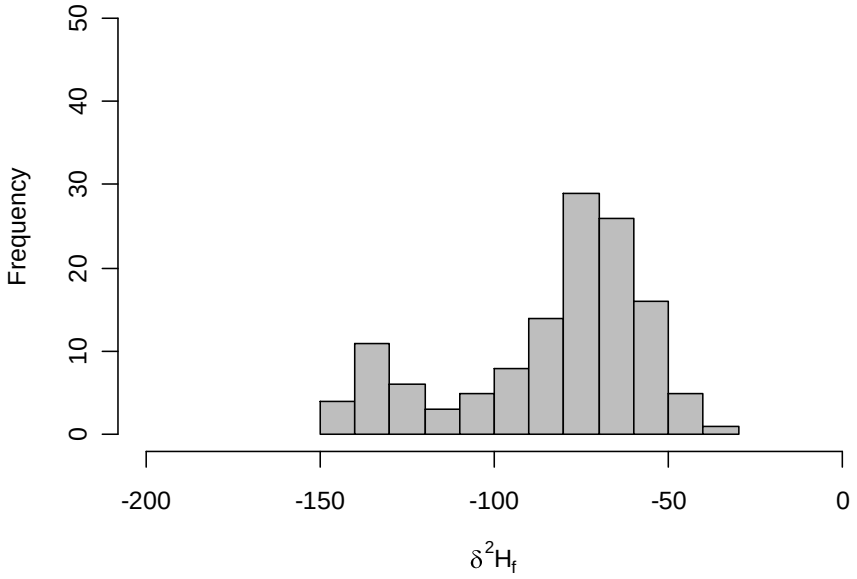


FIGURE 8 – Distribution of δ^2H_f values of enriched cluster Calliope Hummingbirds, divided by region.

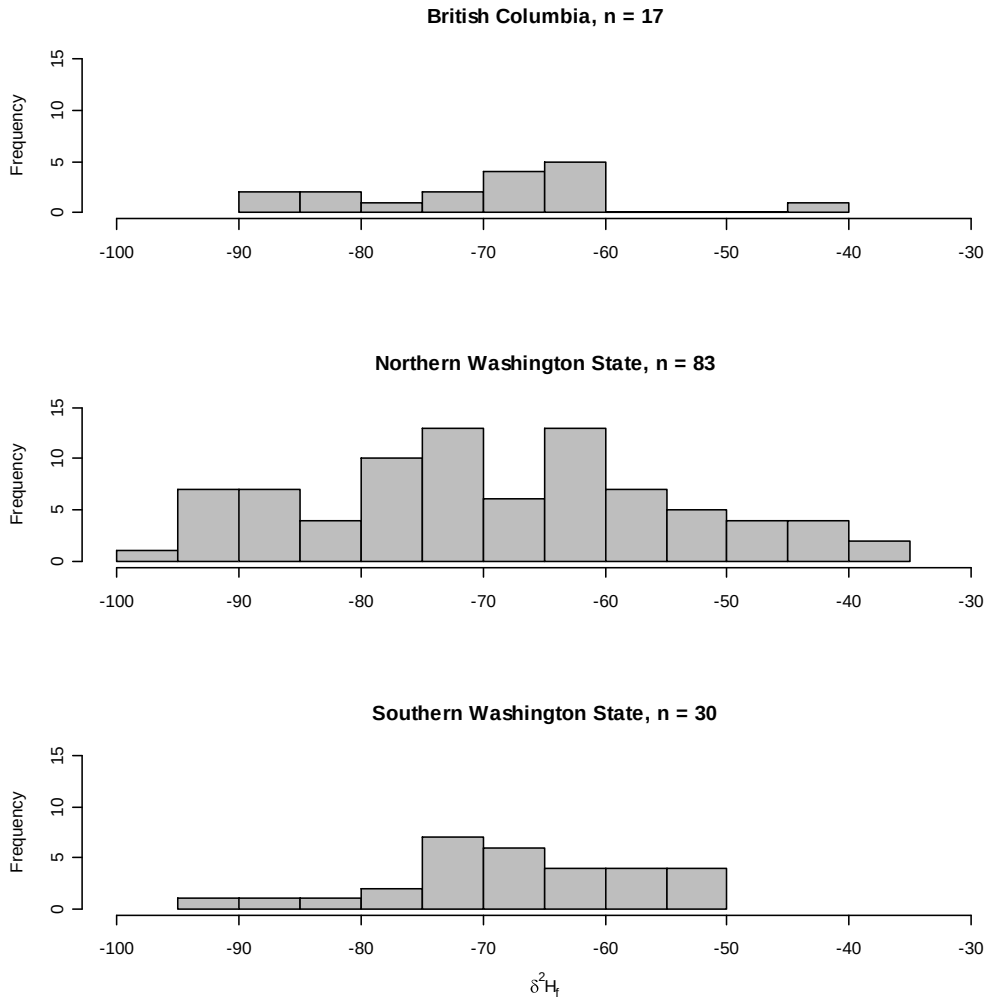


FIGURE 9 – Distribution of δ^2H values of juvenile Calliope Hummingbird tail feathers.

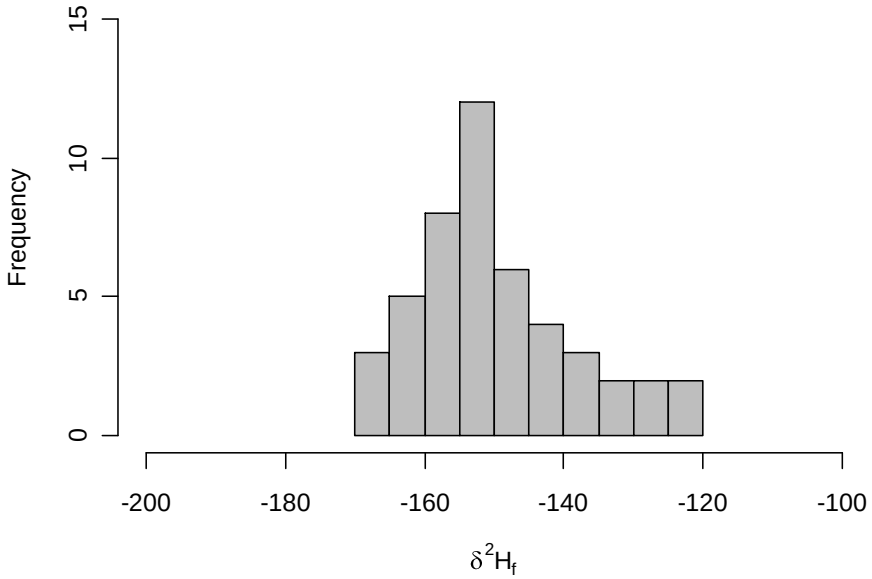


FIGURE 10 – Mean annual δ^2H values of precipitation predicted by the OIPC for feather collection sites vs. δ^2H values of tail feathers of juvenile Calliope Hummingbirds captured at those sites.

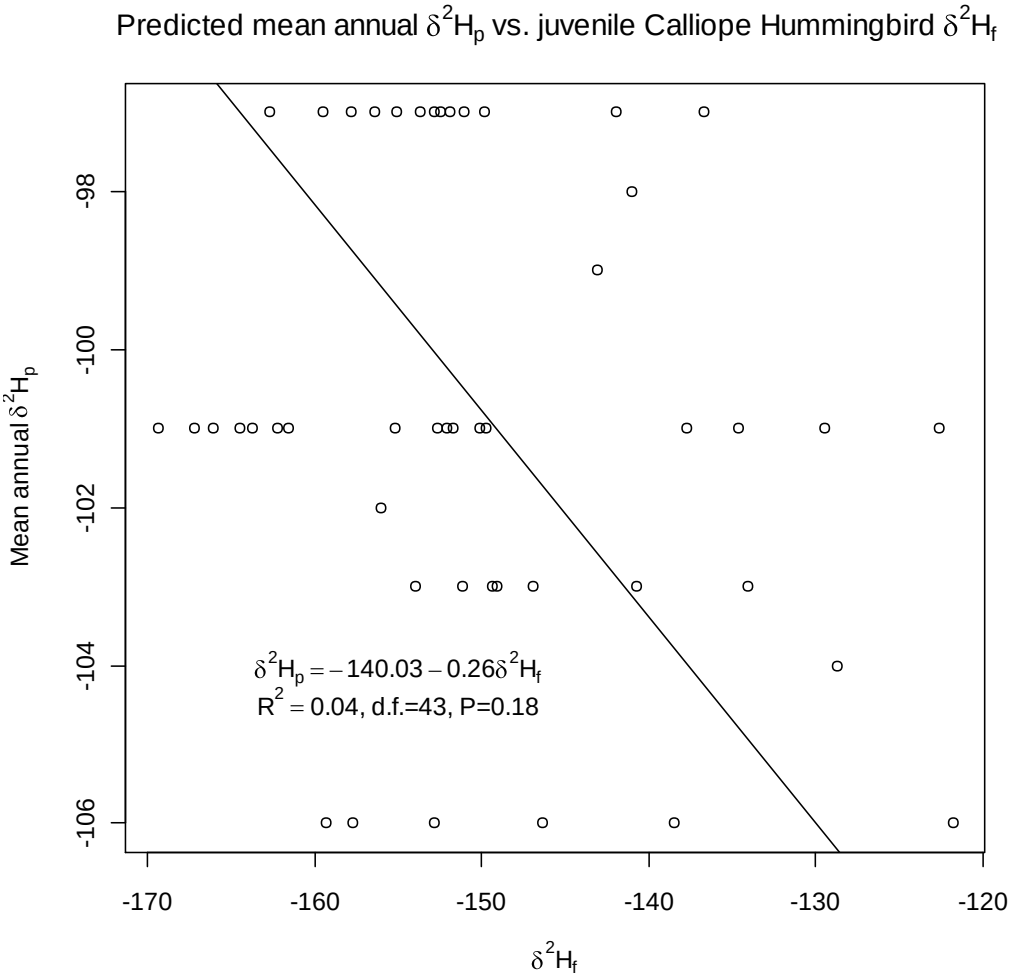


FIGURE 11 – Mean annual δ^2H values of precipitation predicted by the OIPC for feather collection sites vs. δ^2H values of tail feathers of Rufous Hummingbirds captured at those sites.

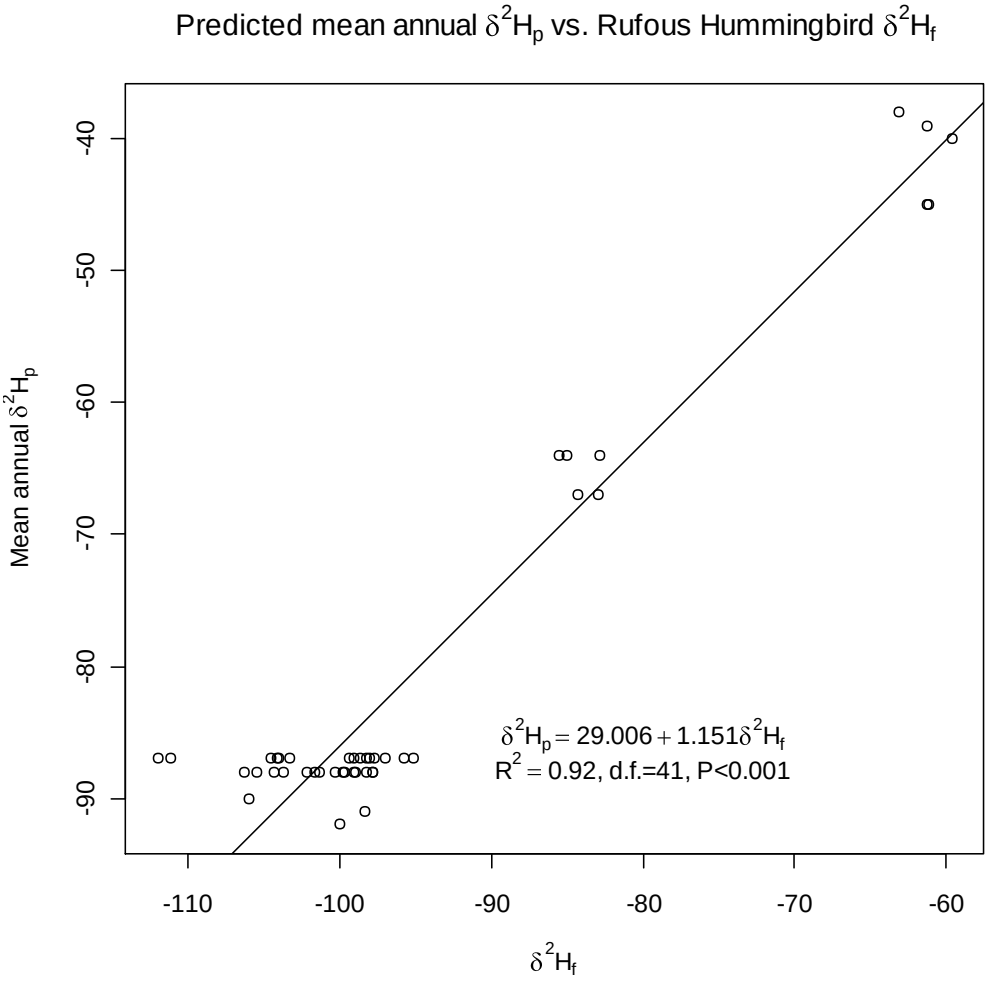


FIGURE 12 – δ^2H_f values of enriched cluster females (A, C) and males (B, D) mapped onto a groundwater-deuterium isoscape of Mexico. A, B – one standard deviation about the mean; C, D - two standard deviations about the mean.

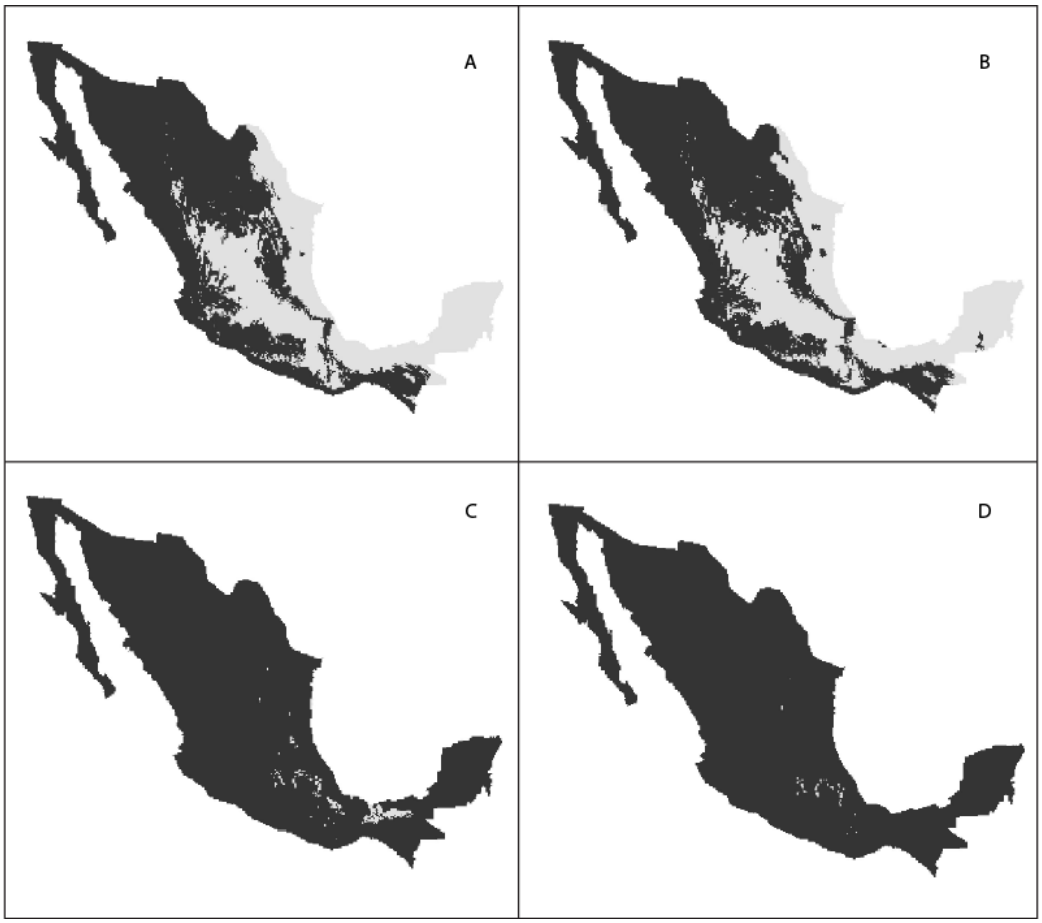


FIGURE 13 - δ^2H_f values of enriched cluster individuals from British Columbia (A, D), Northern Washington (B, E) and Southern Washington (C, F) mapped onto a groundwater-deuterium isoscape of Mexico. A-C – one standard deviation about the mean; D-F - two standard deviations about the mean.

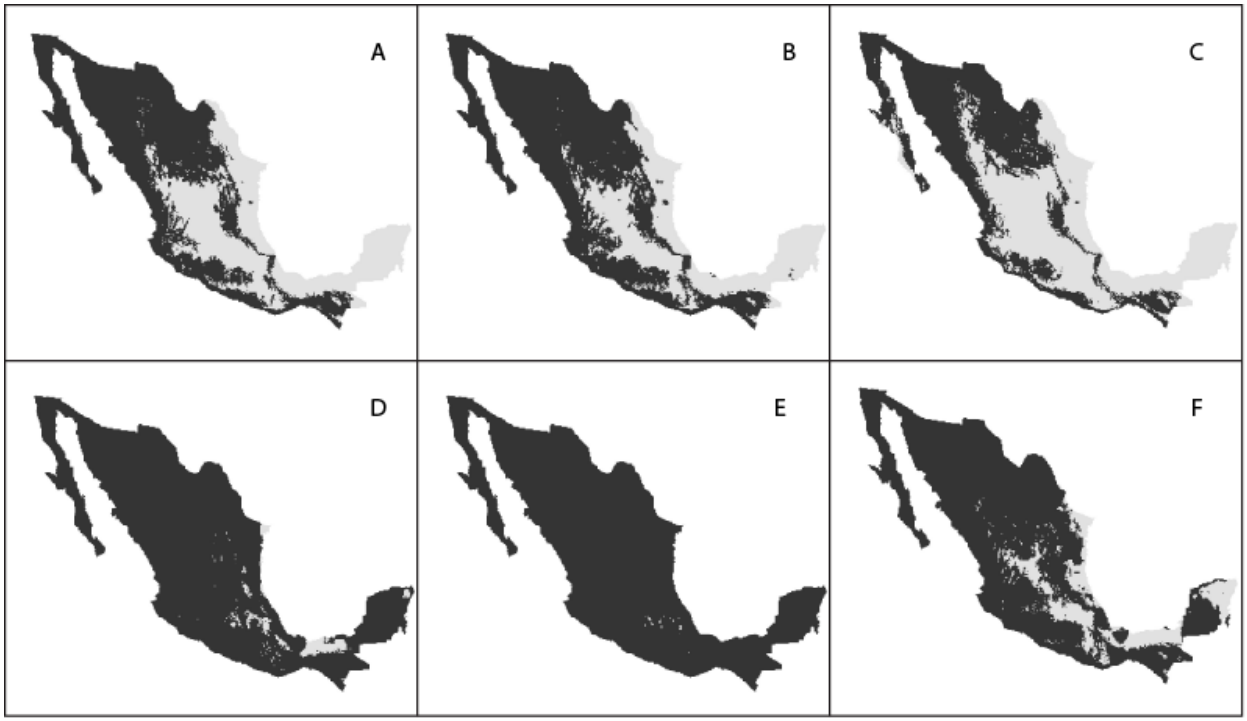
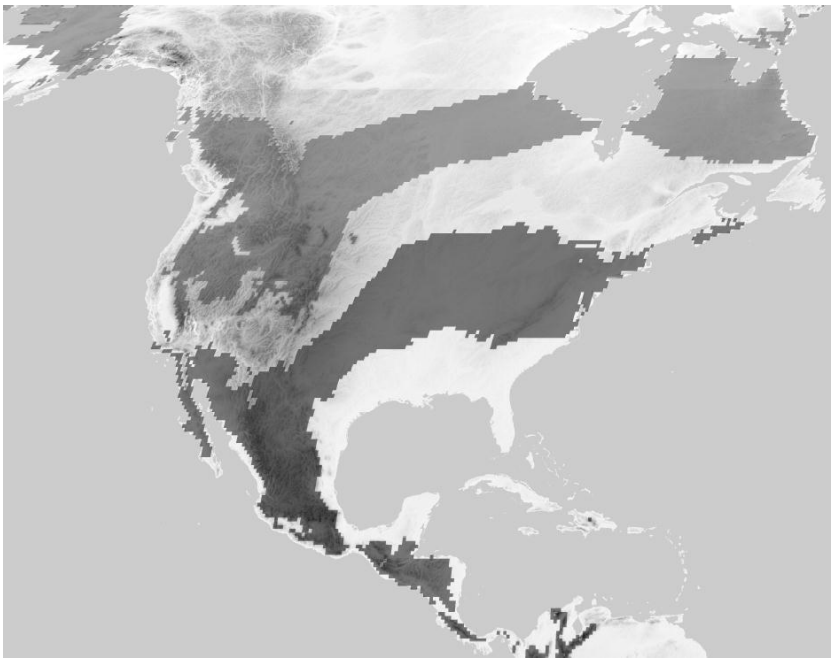
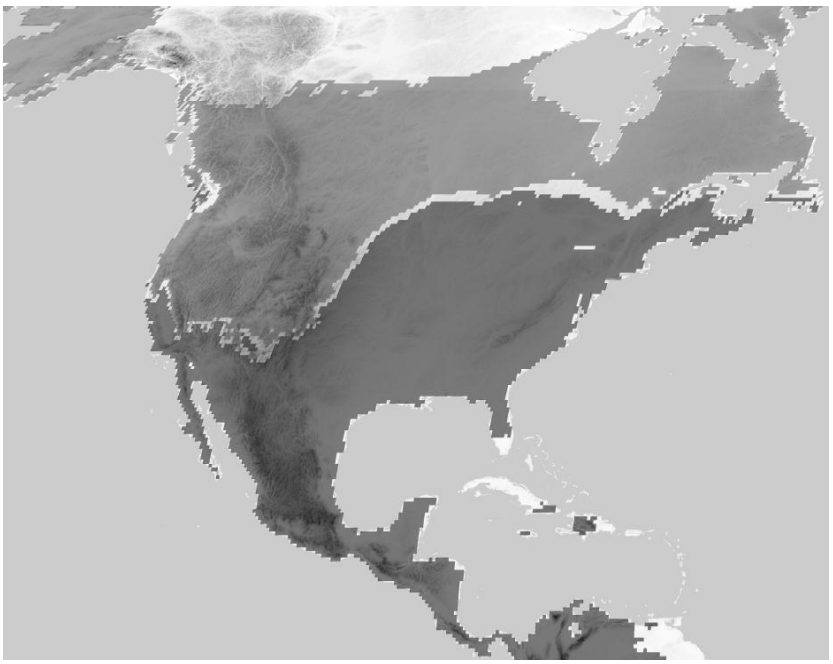


FIGURE 14 – δ^2H_f values of individuals in the enriched (darker grey) and depleted (lighter grey) clusters mapped onto a North American isoscape of mean-annual deuterium in precipitation. A - one standard deviation about the cluster means; B – two standard deviations about the cluster means.

A



B



DISCUSSION

Feather-deuterium values of juvenile hummingbirds

Hydrogen-isotope ratios of juvenile Calliope Hummingbird feathers are unexpected first in their lack of correlation with predicted hydrogen-isotope ratios of local precipitation and second in their distance from predicted precipitation values. The absence of a correlation is likely a product of both the limited geographic extent of feather sampling and the complex topography of the study area. Virtually all juveniles were captured at sites in the mountainous regions of northeastern Washington State and eastern British Columbia, spanning only a few degrees of latitude. There is little predicted variation in δ^2H_p across these sites; the range of OIPC-predicted mean annual δ^2H_p values for all sites at which juveniles were captured (-106‰ to -97‰) is much smaller than the range of δ^2H_f values measured in juvenile feathers (-169.4‰ to -121.8‰). This region is characterized by rugged topography, and elevation changes of thousands of feet can occur within a few miles. Hydrogen-isotope ratios in precipitation vary with elevation, so the region's high degree of variation in elevation across space is matched by a correspondingly variable hydrogen isoscape. For example, an increase in elevation of approximately 500 meters between two points about 5 km apart in the Methow Valley is matched by a depletion of approximately 8‰ in OIPC-predicted mean annual δ^2H_p. This change in δ^2H_p is as great as the total range of δ^2H_p values predicted for juvenile capture sites. The situation is further complicated by the rain shadow effect of the coastal ranges. Hummingbirds are very mobile, and females in the region may forage across a broad elevational range while raising nestlings. The resulting isotopic variation in nestling diet would create noise in the hydrogen-isotopic signal of juvenile feathers, preventing juveniles from sites without significant latitudinal separation from being distinguished on the basis of their δ^2H_f values and weakening the correlation between δ^2H_f and nesting site δ^2H_p. The mountainous nature of the study area thus enables variation in the elevational source of nestling diet, and this combined with the limited geographic extent of feather sampling likely contributes to the poor correlation between predicted δ^2H_p and measured δ^2H_f of juvenile Calliope Hummingbirds.

Beyond the lack of correlation, juvenile Calliope Hummingbird δ^2H_f values are more depleted relative to predicted capture site δ^2H_p values than expected. Measured δ^2H_f of the majority of juveniles in this study is at least 50‰ more depleted than capture site δ^2H_p, whereas correlations reported in the literature typically predict that δ^2H_f will be depleted by only about 25‰ relative to δ^2H_p (Wassenaar and Hobson 2001, Bowen et al. 2005b). The *Selasphorus rufus* correlation reported here is close to the expected, with a predicted depletion of about 30‰. One explanation for the greater depletion found in

juvenile feathers is that the source of water taken up by plants during the growing season may be melting winter snowpack from higher elevations rather than rainfall. The region where most juvenile Calliope Hummingbirds were captured is arid, with little rain in the summer months but with significant snow accumulation at higher elevations during the winter. OIPC predicted δ^2H_p of winter precipitation higher in the mountains near capture sites is negative enough to reach the expected value of 25‰ more enriched in deuterium than measured juvenile δ^2H_f. If winter snowpack is, in fact, a major source of water available to plants when juvenile hummingbirds are in the nest and growing their feathers, the gap between when and where precipitation falls and capture sites could be another factor contributing to the lack of correlation between capture site δ^2H_p and juvenile Calliope Hummingbird δ^2H_f.

Migratory connectivity in Calliope Hummingbirds

Hydrogen-isotope ratios of tail feathers collected from adult Calliope Hummingbirds in Washington State and British Columbia suggest that individuals from this part of the breeding range have weak or no migratory connectivity. Variation in feather-deuterium values of adults in the enriched cluster is high relative to the predicted variation in precipitation-deuterium in the species' winter range. Ninety-five percent confidence intervals of enriched cluster δ^2H_f values for all three regions and both sexes cover the virtually the entire known Calliope Hummingbird non-breeding range when converted to δ^2H_p values and mapped onto a hydrogen isoscape of Mexico (Figs. 12,13). There is no evidence that individuals are segregating by sex or by breeding site in the non-breeding season. However, while there is no observed difference in δ^2H_f either among regions or between sexes, high within-group variation may be masking among-group differences, and the presence of strong migratory connectivity cannot be ruled out.

Variation in groundwater δ^2H across Mexico is tied to elevation, with water becoming increasingly depleted in deuterium as elevation increases away from the coasts (Wassenaar et al. 2009). As with juvenile Calliope Hummingbird δ^2H_f values, complex topography combined with individual mobility may have resulted in the high variation found in hydrogen-isotope ratios of adult feathers. Other deuterium studies have had similar results. House Sparrows (*Passer domesticus*) sampled across Mexico had high within-site variability in δ^2H_f, leading to a weak relationship between δ^2H_f and interpolated δ^2H of groundwater (Hobson et al. 2009b). Bird species sampled along an elevation gradient in Ecuador showed an overall trend of decrease in δ^2H_f with increased elevation, but high within-site variability resulted in weak and non-significant correlations between δ^2H_f and elevation for all but one species (Hardesty and Fraser 2010). These studies and the present research suggest that for species that occur in mountainous areas where deuterium abundance in water is mainly a function of elevation, work with

feather hydrogen-isotope ratios may limited to general trends across broad geographic extents. The non-breeding range of Calliope Hummingbirds is quite small, and it is unlikely that further work with hydrogen isotopes will shed additional light on the strength of migratory connectivity in the species.

Depleted cluster

About twenty percent of adult female Calliope Hummingbirds have tail feathers significantly depleted in deuterium relative to the rest of the adults (Fig. 4). The two most plausible explanations for the presence of the depleted cluster are (1) that these individuals are second-year birds with retained tail feathers from the previous year, or (2) that these individuals molt at a more northern latitude than most other Calliope Hummingbirds.

Retained tail feathers

The δ^2H_f values of individuals in the depleted cluster map to the Calliope Hummingbird breeding range (Fig. 14). As such, the sampled feathers with depleted δ^2H_f values could be rectrices grown in the nest by juvenile birds that did not molt completely during their first winter. The individuals in the depleted cluster tend to have feathers more enriched in deuterium than juveniles sampled in the Pacific Northwest (Fig. 5). If the depleted cluster is made up of second-year birds with retained rectrices, these individuals have dispersed northward from natal sites in more southern parts of the breeding range. However, Pyle et al.'s (1997) study of molt in North American hummingbirds recorded only one percent of sampled individuals as having retained flight feathers. This renders it improbable that the depleted deuterium values of such a substantial proportion of individuals as in this study could be due to retained feathers. Additionally, only one male falls into the depleted cluster, with 29 females, and there is no reason to believe that a strong sex bias would be present in the retention of juvenile feathers.

Molt latitude

Precipitation becomes increasingly depleted in deuterium with increasing latitude, so the significantly more negative δ^2H_f values of depleted cluster individuals can be interpreted as meaning that these birds are molting at higher latitudes than the majority of sampled individuals. Calliope Hummingbirds winter in Mexico and, in recent decades, in the southeastern United States. The δ^2H_f values of the depleted cluster places the location of tail-feather growth for these birds far outside of the species' known wintering range (Fig. 14). Thus, these individuals are molting during migration, pausing for at least a partial molt either

during the southward migration after leaving their breeding sites or on their way north the following year. There is only one male in the depleted cluster, so females are more likely to adopt this alternate molt strategy. Cluster membership and sampling region are not independent, suggesting that a female's molting strategy is related to where she breeds. Females from British Columbia have a tendency to molt during migration, females from Northern Washington tend to molt at their wintering site, and females from Southern Washington do not appear to be biased in either direction.

Calliope Hummingbirds have been recorded as subordinate to virtually all other hummingbird species in the non-breeding season, but they do exhibit intraspecific territoriality (DesGranges 1978). Male hummingbirds often defend higher quality territories, leaving females to occupy less desirable resource patches (Lyon 1976, Kodric-Brown and Brown 1978, Kuban and Neill 1980). Molt is energetically costly, and females that are subordinate to both males and other females may be unable to obtain sufficient resources to complete molt before leaving their wintering sites to migrate northwards. Instead, they may devote energy to preparing for migration, and then pause en route to complete their molt. These females would make up the depleted cluster.

SUMMARY

This project used stable hydrogen isotopes to examine migratory connectivity in Calliope Hummingbirds. Study design was based on two predictions. First, I predicted that a correlation would exist between measured hydrogen-isotope ratios of juvenile Calliope feathers (δ^2H_f) and predicted hydrogen-isotope ratios of capture-site precipitation (δ^2H_p), such that measured δ^2H_f of adult birds could be transformed into δ^2H_p values of putative wintering sites. This prediction was not borne out. Such a correlation does not exist in this data set, and juvenile δ^2H_f values proved to be both more variable and more depleted relative to δ^2H_p than expected. The lack of correlation is likely due to the complex topography of the study area, the contribution of snowpack to water available to plants during the growing season, and the mobility of female hummingbirds foraging for nestlings. Research into animal movement that relies on isotopic analyses should take these factors into consideration. Second, I predicted that hydrogen-isotope analysis of tail feathers from adult birds would allow an evaluation of migratory connectivity in Calliope Hummingbirds. Variation in δ^2H_f values of adult birds, however, is too great to allow potentially distinct wintering populations to be distinguished; even if strong connectivity were present, it would be obscured by variability in feather-isotope values. This high variation is likely due to the topographic complexity and small size of the non-breeding range of this species. This result suggests that stable isotopic methods are better suited to broad-scale conclusions, and may be most appropriate for species that occupy large breeding and wintering ranges with ample water-isotopic variation.

Adult Calliope Hummingbird δ^2H_f values fell into two groups, an enriched and a depleted cluster. These two clusters likely reflect different molting strategies. Birds in the depleted cluster (about 20 percent of adult females) appear to grow their tail feathers well outside of the known wintering range for this species, probably molting during migration. Isotope studies typically rely on knowledge of the molt schedule of the species of interest, and this result suggests that homogeneity of molt strategy within a species cannot be assumed, and that potential differences in molt timing and location must be taken into account when interpreting feather isotope values.

REFERENCES

Ambrosini, R., A. P. Moller, and N. Saino. 2009. A quantitative measure of migratory connectivity. Journal of Theoretical Biology **257**:203-211.

Avise, J. C. 2000. Phylogeography. The history and formation of species. Harvard University Press, Cambridge, MA.

Bairlein, F., D. R. Norris, R. Nagel, M. Bulte, C. C. Voigt, J. W. Fox, D. J. T. Hussell, and H. Schmaljohann. 2012. Cross-hemisphere migration of a 25 g songbird. Biology Letters.

Baltosser, W. H. 1994. Age and sex determination in the Calliope Hummingbird. Western Birds **25**:104-109.

Beacham, T. D., J. R. Candy, B. McIntosh, C. MacConnachie, A. Tabata, K. Kaukinen, L. Deng, K. M. Miller, R. E. Withler, and N. Varnavskaya. 2005. Estimation of Stock Composition and Individual Identification of Sockeye Salmon on a Pacific Rim Basis Using Microsatellite and Major Histocompatibility Complex Variation. Transactions of the American Fisheries Society **134**:1124-1146.

Beason, J. P., C. Gunn, K. M. Potter, R. A. Sparks, and J. W. Fox. 2012. The Northern Black Swift: Migration Path And Wintering Area Revealed. The Wilson Journal of Ornithology **124**:1-8.

Bensch, S., T. Andersson, and S. Akesson. 1999. Morphological and Molecular Variation across a Migratory Divide in Willow Warblers, Phylloscopus trochilus. Evolution **53**:1925-1935.

Berthold, P. 1993. Bird Migration. A general survey. Oxford University Press, Oxford.

Bohonak, A. J. and K. van der Linde. 2004. RMA: Software for reduced major axis regression, Java version. Version 1.21 http://www.kimvdlinde.com/professional/rma.html.

Bowen, G. J. 2011a. Gridded maps of the isotopic composition of meteoric waters. http://www.waterisotopes.org.

Bowen, G. J. 2011b. The Online Isotopes in Precipitation Calculator. Version 2.2 http://www.waterisotopes.org.

Bowen, G. J., L. Chesson, K. Nielson, T. E. Cerling, and J. R. Ehleringer. 2005a. Treatment methods for the determination of delta H-2 and delta O-18 of hair keratin by continuous-flow isotope-ratio mass spectrometry. Rapid Communications in Mass Spectrometry **19**:2371-2378.

Bowen, G. J. and J. Revenaugh. 2003. Interpolating the isotopic composition of modern meteoric precipitation. Water Resources Research **39**:1299.

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

Bridge, E. S., K. Thorup, M. S. Bowlin, P. B. Chilson, R. H. Diehl, R. W. Fléron, P. Hartl, K. Roland, J. F. Kelly, W. D. Robinson, and M. Wikelski. 2011. Technology on the Move: Recent and Forthcoming Innovations for Tracking Migratory Birds. Bioscience **61**:689-698.

Calder, W. A. and L. L. Calder. 1994. Calliope Hummingbird (Stellula calliope).*in* A. Poole, editor. The Birds of North America Online. Cornell Lab of Ornithology, Ithaca.

Chamberlain, C. P., S. Bensch, X. Feng, S. Åkesson, and T. Andersson. 2000. Stable isotopes examined across a migratory divide in Scandinavian willow warblers (Phylloscopus trochilus trochilus and Phylloscopus trochilus acredula) reflect their African winter

quarters. Proceedings of the Royal Society of London. Series B: Biological Sciences **267**:43-48.
Chamberlain, C. P., J. D. Blum, R. T. Holmes, X. Feng, T. W. Sherry, and G. R. Graves. 1996. The use of isotope tracers for identifying populations of migratory birds. Oecologia **109**:132-141.
DesGranges, J.-L. 1978. Organization of a tropical nectar feeding bird guild in a variable environment. Living Bird **17**:199-236.
Dittman, D. L. and D. K. Demcheck. 2006. Contributions to our knowledge of molt in the Calliope Hummingbird. Birding **38**.
Evans, K. L., J. Newton, K. J. Gaston, S. P. Sharp, A. McGowan, and B. J. Hatchwell. 2012. Colonisation of urban environments is associated with reduced migratory behaviour, facilitating divergence from ancestral populations. Oikos **121**:634-640.
Farmer, A., B. S. Cade, and J. Torres-Dowdall. 2008. Fundamental limits to the accuracy of deuterium isotopes for identifying the spatial origin of migratory animals. Oecologia **158**:183-192.
González-Prieto, A. M., K. A. Hobson, N. J. Bayly, and C. Gómez. 2011. Geographic Origins and Timing of Fall Migration of the Veery in Northern Colombia. The Condor **113**:860-868.
Gustafson, M. E., J. Hildenbrand, and L. Metras. 1997. The North American Bird Banding Manual (Electronic Version). Version 1.0.
Hardesty, J. L. and K. C. Fraser. 2010. Using deuterium to examine altitudinal migration by Andean birds. Journal of Field Ornithology **81**:83-91.
Hartigan, J. A. and M. A. Wong. 1979. A K-means clustering algorithm. Journal of the Royal Statistical Society: Series C (Applied Statistics) **28**:100-108.
Hobson, K. A. 1999. Stable-carbon and nitrogen isotope ratios of songbird feathers grown in two terrestrial biomes: implications for evaluating trophic relationships and breeding origins. The Condor **101**:799-805.
Hobson, K. A. 2008. Applying Isotopic Methods to Tracking Animal Movements. Pages 45-78 *in* K. A. Hobson and L. I. Wassenaar, editors. Tracking Animal Migration with Stable Isotopes, Terrestrial Ecology Vol. 2. Academic Press, London.
Hobson, K. A., Y. Aubry, and L. I. Wassenaar. 2004a. Migratory connectivity in Bicknell's Thrush: Locating missing populations with hydrogen isotopes. Condor **106**:905-909.
Hobson, K. A., G. J. Bowen, L. I. Wassenaar, Y. Ferrand, and H. Lormee. 2004b. Using stable hydrogen and oxygen isotope measurements of feathers to infer geographical origins of migrating European birds. Oecologia **141**:477-488.
Hobson, K. A. and R. G. Clark. 1992. Assessing avian diets using stable isotopes. I. Turnover of C-13 in tissues. Condor **94**:181-188.
Hobson, K. A., H. Lormee, S. L. Van Wilgenburg, L. I. Wassenaar, and J. M. Boutin. 2009a. Stable isotopes (delta D) delineate the origins and migratory connectivity of harvested animals: the case of European woodpigeons. Journal of Applied Ecology **46**:572-581.
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 analysis of feathers. The Auk **118**:16-23.
Hobson, K. A., S. L. Van Wilgenburg, K. Larson, and L. I. Wassenaar. 2009b. A feather hydrogen isoscape for Mexico. Journal of Geochemical Exploration **102**:167-174.

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

Hobson, K. A., L. I. Wassenaar, and O. R. Taylor. 1999. Stable isotopes (δD and δ 13C) are geographic indicators of natal origins of monarch butterflies in eastern North America. Oecologia **120**:397-404.

Irwin, D. E., J. H. Irwin, and T. B. Smith. 2011. Genetic variation and seasonal migratory connectivity in Wilson's warblers (Wilsonia pusilla): species-level differences in nuclear DNA between western and eastern populations. Molecular Ecology **20**:3102-3115.

Jones, J., D. R. Norris, M. K. Girvan, J. J. Barg, T. K. Kyser, and R. J. Robertson. 2008. Migratory connectivity and rate of population decline in a vulnerable songbird. Condor **110**:538-544.

Jouventin, P. and H. Weimerskirch. 1990. Satellite tracking of Wandering albatrosses. Nature **343**:746-748.

Kelly, J. F. 2006. Stable isotope evidence links breeding geography and migration timing in wood warblers (Parulidae). Auk **123**:431-437.

Kimura, M., S. M. Clegg, I. J. Lovette, K. R. Holder, D. J. Girman, B. Milá, P. Wade, and T. B. Smith. 2002. Phylogeographical approaches to assessing demographic connectivity between breeding and overwintering regions in a Nearctic−Neotropical warbler (Wilsonia pusilla). Molecular Ecology **11**:1605-1616.

Kodric-Brown, A. and J. H. Brown. 1978. Influence of economics, interspecific competition, and sexual dimorphism on territoriality of migrant Rufous Hummingbirds. Ecology **59**:285-296.

Kuban, J. F. and R. L. Neill. 1980. Feeding ecology of hummingbirds in the highlands of the Chisos Mountains, Texas. Condor **82**:180-185.

Langin, K. M., P. P. Marra, Z. Nemeth, F. R. Moore, T. K. Kyser, and L. M. Ratcliffe. 2009. Breeding latitude and timing of spring migration in songbirds crossing the Gulf of Mexico. Journal of Avian Biology **40**:309-316.

Langin, K. M., M. W. Reudink, P. P. Marra, D. R. Norris, T. K. Kyser, and L. M. Ratcliffe. 2007. Hydrogen isotopic variation in migratory bird tissues of known origin: implications for geographic assignment. Oecologia **152**:449-457.

Lyon, D. L. 1976. A montane hummingbird territorial system in Oaxaca, Mexico. The Wilson Bulletin **88**:280-299.

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.

Marshall, J. D., J. R. Brooks, and K. Lajtha. 2008. Sources of Variation in the Stable Isotopic Composition of Plants. Pages 22-60 Stable Isotopes in Ecology and Environmental Science. Blackwell Publishing Ltd.

Martin, T. G., I. Chadès, P. Arcese, P. P. Marra, H. P. Possingham, and D. R. Norris. 2007. Optimal conservation of migratory species. Plos One **2**:e751.

McGuire, J. A., C. C. Witt, J. V. Remsen, R. Dudley, and D. L. Altshuler. 2009. A higher-level taxonomy for hummingbirds. Journal of Ornithology **150**:155-165.

Meehan, T. D., J. T. Giermakowski, and P. M. Cryan. 2004. 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.

Norris, D. R., P. P. Marra, T. K. Kyser, T. W. Sherry, and L. M. Ratcliffe. 2004a. Tropical winter habitat limits reproductive success on the temperate breeding grounds in a migratory bird. Proceedings of the Royal Society of London Series B-Biological Sciences **271**:59-64.

Norris, D. R., P. P. Marra, R. Montgomerie, T. K. Kyser, and L. M. Ratcliffe. 2004b. Reproductive effort molting latitude, and feather color in a migratory songbird. Science **306**:2249-2250.

Pain, D. J., R. E. Green, B. Giessing, A. Kozulin, A. Poluda, U. Ottosson, M. Flade, and G. M. Hilton. 2004. Using stable isotopes to investigate migratory connectivity of the globally threatened aquatic warbler Acrocephalus paludicola. Oecologia **138**:168-174.

Paxton, K. L., C. Van Riper, T. C. Theimer, and E. H. Paxton. 2007. Spatial and temporal migration patterns of Wilson's Warbler (Wilsonia pusilla) in the southwest as revealed by stable isotopes. Auk **124**:162-175.

Perez, G. E. and K. A. Hobson. 2009. Winter habitat use by Loggerhead Shrikes (Lanius Ludovicianus) in Mexico: separating migrants from residents using stable isotopes. Journal of Ornithology **150**:459-467.

Prochazka, P., K. A. Hobson, Z. Karcza, and J. Kralj. 2008. Birds of a feather winter together: migratory connectivity in the Reed Warbler Acrocephalus scirpaceus. Journal of Ornithology **149**:141-150.

Pyle, P. 1997. Identification guide to North American birds, part I: Columbidae to Ploceidae. Slate Creek Press, Bolinas, CA.

Pyle, P., S. N. G. Howell, and G. M. Yanega. 1997. Molt, retained flight feathers, and age in North American hummingbirds. Pages 155-166 *in* R. W. Dickerman, editor. The Era of Allan R. Phillips: A Fetschrift. Horizon Communications, Albuquerque.

R Development Core Team. 2011. R: A Language and Environment for Statistical Computing. Version 2.11.1 http://www.R-project.org. R Foundation for Statistical Computing, Vienna, Austria.

Reudink, M. W., C. E. Studds, P. P. Marra, T. Kurt Kyser, and L. M. Ratcliffe. 2009. Plumage brightness predicts non-breeding season territory quality in a long-distance migratory songbird, the American redstart Setophaga ruticilla. Journal of Avian Biology **40**:34-41.

Rolshausen, G., G. Segelbacher, K. A. Hobson, and H. M. Schaefer. 2009. Contemporary Evolution of Reproductive Isolation and Phenotypic Divergence in Sympatry along a Migratory Divide. Current biology : CB **19**:2097-2101.

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

Rubenstein, D. R. and K. A. Hobson. 2004. From birds to butterflies: animal movement patterns and stable isotopes. Trends in Ecology & Evolution **19**:256-263.

Russel, S. M. and R. O. Russel. 2001. The North American banders' manual for banding hummingbirds. The North American Banding Council, Point Reyes Station, CA.

Sarasola, J. H., J. J. Negro, K. A. Hobson, G. R. Bortolotti, and K. L. Bildstein. 2008. Can a 'wintering area effect' explain population status of Swainson's hawks? A stable isotope approach. Diversity and Distributions **14**:686-691.

Studds, C. E., T. K. Kyser, and P. P. Marra. 2008. Natal dispersal driven by environmental conditions interacting across the annual cycle of a migratory songbird. Proceedings of the National Academy of Sciences of the United States of America **105**:2929-2933.

Stutchbury, B. J. M., S. A. Tarof, T. Done, E. Gow, P. M. Kramer, J. Tautin, J. W. Fox, and V. Afanasyev. 2009. Tracking Long-Distance Songbird Migration by Using Geolocators. Science **323**:896.

Sulzman, E. W. 2008. Stable Isotope Chemistry and Measurement: A Primer. Pages 1-21 Stable Isotopes in Ecology and Environmental Science. Blackwell Publishing Ltd.

Wassenaar, L. I. and K. A. Hobson. 1998. Natal origins of migratory monarch butterflies at wintering colonies in Mexico: New isotopic evidence. Proceedings of the National Academy of Sciences **95**:15436-15439.

Wassenaar, L. I. and K. A. Hobson. 2000. Improved Method for Determining the Stable-Hydrogen Isotopic Composition (δD) of Complex Organic Materials of Environmental Interest. Environmental Science & Technology **34**:2354-2360.

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**:1845-1850.

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

Wassenaar, L. I., S. L. Van Wilgenburg, K. Larson, and K. A. Hobson. 2009. A groundwater isoscape ([delta]D, [delta]18O) for Mexico. Journal of Geochemical Exploration **102**:123-136.

Webster, M. S. and P. P. Marra. 2005. The importance of understanding migratory connectivity and seasonal interactions. Pages 199-209 *in* R. Greenberg and P. P. Marra, editors. Birds of two worlds. The ecology and evolution of migration. Johns Hopkins University Press.

Webster, M. S., P. P. Marra, S. M. Haig, S. Bensch, and R. T. Holmes. 2002. Links between worlds: unraveling migratory connectivity. Trends in Ecology & Evolution **17**:76-83.

Welch, B. L. 1951. On the comparison of several mean values: an alternative approach. Biometrika **38**:330-336.

Wennerberg, L. 2001. Breeding origin and migration pattern of dunlin (Calidris alpina) revealed by mitochondrial DNA analysis. Molecular Ecology **10**:1111-1120.

Wikelski, M., R. W. Kays, N. J. Kasdin, K. Thorup, J. A. Smith, and G. W. Swenson. 2007. Going wild: what a global small-animal tracking system could do for experimental biologists. Journal of Experimental Biology **210**:181-186.