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Using Stable Isotope Analysis to Study Altitudinal and Latitudinal Bat Migration

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ABSTRACT

The general lack of knowledge on basic aspects of the biology of temperate and tropical bats, their low reproductive rates, and threats such as white-nose syndrome, wind farms, and habitat loss, make them very susceptible to population declines. My research uses an innovative technique, the analysis of stable isotopes, to study the ecology of bat migration with the main goals of contributing significantly to the understanding of bat biology and assessing the conservation status and susceptibility of bats.

In the first chapter, I measured the content of hydrogen isotopes in fur samples of migratory bat species killed at a wind farm in northern Indiana to determine their geographic origin. North American tree bats (*Lasiurus borealis, L. cinereus,* and *Lasionycteris noctivagans*) are considered long distance migrants. In North America, peaks in bat mortality at wind farms occur between mid-July and mid-September. This period is associated with fall migration of bats from their summer (breeding) grounds to their wintering grounds. Thus, wind turbines may have serious negative effects on a strategic event in the life of bats by interrupting migratory connectivity and thereby imperiling the long-term persistence of migratory bat species at large scales. The analysis accurately predicted the known origin of control samples and estimated that non-control bats killed at the wind farm originated from several populations in the United States as well as in Canada. My results highlighted the threat of wind farms to local bat populations as well as to bats originating far from those farms, and emphasized the need for conservation policies across borders. High variation in stable hydrogen isotopes in migrant individuals of all 3 species was observed, suggesting that individuals or populations from a variety of regions pass through the wind farm.

In the second chapter, I evaluated the triple-isotopic (hydrogen, carbon, and nitrogen) composition of the tissues of 7 bat species collected at 3 altitudes in the Central Andes of Peru, and the variation of these isotopes across an altitudinal gradient, the application of isotope analysis to migration studies, and trophic effect. Previous studies had demonstrated that

hydrogen isotopes were a reliable tool to track altitudinal movements of birds, and there was evidence from soil and plant studies that nitrogen and carbon isotopes could serve the same purpose. However, studies focused on bats were lacking. Hydrogen and nitrogen isotopes in the sanguinivorous control were found to be enriched relative to those of the syntopic frugivores. Carbon isotopes in the sanguinivorous bat were depleted when compared to frugivores. Differences in hydrogen found between trophic groups are the first reported for the species studied and support results found elsewhere in the Neotropics. My results demonstrated that, in spite of the wide array of physiological and environmental factors producing temporal and spatial variation, the analysis of hydrogen isotopes is a promising tool to study altitudinal movements of bats when used over long distances. Neither stable isotopes of nitrogen or carbon appear to be reliable to track movements along short gradients such as those along mountains. The contrast of these findings with the results of previous studies suggests that isotopic gradients may be specific to given taxon and localities. My results contributed to the understanding of bat movement patterns and therefore to assessing their sensitivity to potential threats such as habitat loss and connectivity.

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Finally, I would like to thank my family in Peru. My parents, Jesus and Maria, my siblings Christian, Fiorella, and Rolando were always there to support me through this process.

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Chapter I

Using hydrogen stable isotopes to assess geographic origins of bats killed by wind turbines in Northern Indiana

North American tree bats (*Lasiurus borealis, L. cinereus,* and *Lasionycteris noctivagans*) are considered long distance migrants. Their seasonal movements between breeding and non-breeding regions occur twice a year; they move northward each spring and return south in the fall. In North America, peaks in bat mortality at wind farms occur between mid-July and mid-September. This period is associated with fall migration of bats from their summer (breeding) grounds to their wintering grounds. Thus, wind turbines may also have serious negative effects on a strategic event in the life of bats by interrupting migratory connectivity and thereby imperiling the long-term persistence of migratory bat species at large scales. Therefore, the goal of this study is to identify the potential geographic origin (summer grounds) of North American bat populations of *L. cinereus, L. borealis,* and *La. noctivagans* killed at the Fowler Ridge wind farm by analyzing hydrogen stable isotopes in their tissues. High variation in stable hydrogen isotopes in migrant individuals of all 3 species was observed, suggesting that individuals or populations from a variety of regions pass through the wind farm. Regions of geographic origin included the United States and Canada highlighting the importance of cooperation among countries in the development of conservation policies, especially regarding the implementation of wind farms that have been demonstrated to cause the death of thousands of bats and birds.

Lack of knowledge regarding timing of migration and molting patterns may bias the results of stable isotope analysis oriented to locate geographic origins of bat populations.

Introduction

Bats in temperate ecosystems display two strategies to deal with low winter temperatures: hibernation and migration. Most species hibernate in underground shelters such as caves and mines, and a few species often hibernate in buildings. Alternatively, relatively few species travel over 1000 km between summer and winter roosts (Bisson et al. 2009). North American tree bats (*Lasiurus borealis, L. cinereus,* and *Lasionycteris noctivagans*) are considered long distance migrants. Their seasonal movements between breeding and non-breeding regions occur twice a year; they move northward each spring (Cryan 2003, Cryan et al. 2004) and return south in the fall (Fleming and Eby 2003).

The distributional range of *L. cinereus,* the hoary bat, is broad; it occurs from northern Canada south to Argentina and Chile (Cryan 2003, Shump and Shump 1982). The distribution of *L. noctivagans*, the silver-haired bat, includes southeastern Alaska and much of Canada, with the southeastern United States as the southernmost part of its range (Kunz 1982, Hall 1981). These two species present differential sex distribution at the continental scale (Cryan 2003). In North America, the distribution of *L. borealis*, the Eastern red bat, includes areas east of the Continental Divide from southern Canada south to northeastern Mexico (Cryan 2003). Males and females of *L. borealis* also exhibit differential distribution, but at smaller scales (Cryan 2003). Populations of *L. borealis* seem to be declining throughout much of its range (Winhold and Kurta 2006).

Filling the knowledge gap on migratory timing, routes, distance traveled, stopover ecology, and population-specific differences in migratory strategies is vital to the understanding of the ecology and life history of migratory bat species. Migration could strongly influence population structure and dynamics (Popa-Lisseanu et al. 2009), and therefore, information on migration is essential for the effective design of conservation policies that focus on protecting the habitats species use during their migration (Webster and Marra 2005). These conservation policies will ensure the long term persistence of the populations.

This study will not answer all the questions related to bat migration but it will contribute to building the knowledge of bat migration ecology.

Lack of information on migratory bats

Lack of knowledge

The lack of knowledge on basic aspects of the ecology of NorthAmerican tree bats reflects their cryptic lifestyle. Neither local nor total population sizes for these species are known, nor are very many aspects of their migratory behavior (Holland 2007). These bat species have received little attention when compared with social and more conspicuous bat species (e.g. *Eptesicus fuscus*, *Myotis sodalis*). When compared with birds, the incidence and extent of migration is lower among bats (fewer species and potentially shorter distances; Fleming and Eby 2003). In the particular case of tree bats, evidence of migration is scattered, circumstantial, and often based solely on observation of diurnal flocks (a rare event because bat migration occurs mostly at night), records of individuals that collide with buildings, temporal and spatial variation in local abundance, and the appearance of individuals on distant locations such as islands (Cryan

2003) or even boats at sea. In recent years, investigation on bat fatalities at wind farms has provided some additional information about how environmental variables affect bat migration or potential factors that could attract bats to turbines during their migration (Cryan and Brown 2007, Kunz et al. 2007). In addition to the general lack of knowledge on bat migration, their low reproductive rates make them very susceptible to population declines (Kunz et al. 2007).

Lack of efficiency of traditional methods

The capture rate of migratory tree bats is very low (with exception of *L. borealis* in some localities); probably because of their solitary roosting habits (Griffin 1970) their foraging habits (Cryan 2003, Kunz et al. 2007) and flight style (i.e. high altitudes, above the tree canopy; Norberg and Rayner 1987, but see Barbour and Davis 1969, Sealander and Heidt 1990). Banding studies in Europe have provided useful data on the movements of bats (Hutterer et al. 2005). In contrast, there has been a lack of coordinated banding efforts in North America (Holland and Wikelski 2009). Moreover, banding is no longer recommended as a safe alternative to track bats because it may cause injuries on their forearm (Ellison 2008) and may also interfere with hunting activities (Popa-Lisseanu et al. 2012). However, the main issue with banding techniques is that bats are rarely recaptured (Hobson and Norris 2008.). Therefore, banding is not an effective method to study long distance movements of migratory bats in North America (Cryan and Veilleux 2007). Satellite telemetry is a fairly recent technique designed to track wildlife over long distances, but it remains limited to large bird species because of the size of the transmitters (Wikelski et al. 2007). The size of transmitters used in radio-tracking is better suited to North American migratory bats; however, this technique is limited by the small range they cover, the

short battery life and the considerable cost, time, and effort required to obtain large amounts of data. Bat movements at high altitude can be observed with radar, but it is easy to confuse migratory bats with birds (Holland and Wikelski 2009), and it is also difficult or impossible to identify them to species (Hobson and Norris 2008).

Stable isotope analysis

Chemical elements such as C, H, O, and N are present in biological systems in different forms called isotopes. These isotopes have the same number of protons but differ in the number of neutrons, which makes them differ in mass. Isotopes can be stable or unstable (Wassenaar 2008). The stable isotopes of C, H, O, and N are the most useful as biological tracers because they are found in the tissues of all living organisms. Unstable or radioactive forms decay over time, which makes them unsuitable for most biological studies.

Isotopes are represented with a superscript number to the left of the element indicating its mass (number of protons plus neutrons); for instance, ^{1}H means that the mass number of this isotope is 1, which is its number of protons. Stable isotopes are either abundant and light in mass (i. e. ^{1}H and ^{16}O) or rare and heavy (i. e. ^{2}H and ^{18}O). The ratio between the light and the heavy isotope is the measurement of interest (Wassenaar 2008), because variation in this ratio can be linked to temporal and spatial variation of many ecological variables. These ratios are expressed as delta values (δ) in parts per thousand (‰). These ratios compare the isotope ratio of a sample to that of an international standard (that depends on the isotope used) according to the formula (illustrated here for hydrogen):

$$\delta D(‰) = \left[\frac{{}^{1}H/{}_{2}H\ sample}{{}^{1}H/{}_{2}H\ standard} - 1 \right] \times 1000\ per\ mil$$.....Equation 1

Hydrogen isotopes are expressed as H or D (for deuterium, the name of the hydrogen isotope with mass equal 2, ^{2}H). From here on I will use the D notation. Research evaluating signatures of hydrogen and oxygen has proven useful in estimating geographic origins of animal populations and migration distances, and it is relatively inexpensive when compared to other methods (Hobson and Norris 2008). This method also overcomes the issue of having to capture the individuals twice to get information on their migratory route; by capturing the animals only once, reliable information on their location during the previous molting season (see below) can be gathered.

Fundamentals of the analysis

Stable isotopes of hydrogen (δD) in water vary depending on factors such as elevation and location of the water source. Thus, bodies of water are characterized with a unique isotopic signature creating general patterns according to geographical areas (Bowen et al. 2005). Since animal tissues represent the isotopic signature of their water source (Gannes et al. 1998) a match can be made between the signatures of hydrogen in animal tissues and the signatures from the region where the isotopes were assimilated. Keratin from tissues such as fur and feathers is the target of stable isotope analysis because it incorporates the local δD and is metabolically inert once synthesized (Hobson and Wassenaar 1997). Since the original signature gets locked in, it will not change even if the animal moves to a new geographic location or if it changes its diet; it

is only replaced when molting occurs. For instance, δD in bat hair reflects values acquired at the time of molt, which occurs once a year during the summer season in temperate forest species (Quay 1970, Cryan et al. 2004).

Empirical bases for the use of stable isotope analysis

Early studies by Epstein et al. (1976) demonstrated the relationship between δD in precipitation and δD in plants. The same relationship can be found in beetles (Schimmelmann et al. 1993) and deer (Cormie et al. 1994), and now isotopes are being used in investigations of animal migration. Hobson et al.'s (1999) work on monarch butterflies demonstrated that rain determines the isotopic composition of milkweed and ultimately is incorporated into tissues of the monarch butterfly caterpillars that feed upon milkweed. Therefore, the known pattern of δD in precipitation across North America should provide information on the geographic origin of the butterflies. These results were validated by captive-rearing studies. Similarly, Hobson and Wassenar (1997) found a strong correlation between δD of feathers of wild insectivorous songbirds and δD of local precipitation and this relationship was not influenced by the species' trophic level. Several other studies have found a strong correlation ($r > 0.8$) between δD of feathers and δD of local precipitation (Wassenaar and Hobson 2000, Chamberlain et al. 1997, Meehan et al. 2001). The accumulation rate of hydrogen in keratin from claws has been estimated for Golden-winged and Cerulean Warblers (Fraser et al. 2008), and the geographic origin of bird populations such as American Kestrels (Hobson et al. 2009a) and European Woodpigeons (Hobson et al. 2009b) has been estimated.

In contrast, few studies have used stable isotopes to investigate migration characteristics of bats. Britzke et al. (2009) compared three models to explain the variation in δD in hair from

bats collected across eastern United States. They found that latitude explains δD variation better than two other models that take into account elevation and latitude. They also found evidence that females migrate to their summer breeding grounds earlier than males, and that reverse migration occurs (south to north during the fall). They concluded that isotopic analyses should be run independently for species by age and sex classes. Hirt (2008) estimated the migration distance of *La. noctivagans* to be more than 1600 km and that females and males had different arrival times to Alabama. This study was based on the analysis of keratin from claw tips; however this tissue is not the best target for isotope analysis because claws grow continuously and, therefore, keratin is deposited there continuously (Ethier et al. 2010). Based on stable isotope analysis, Cryan et al. (2004) first demonstrated with physical evidence (isotope analysis) long-distance migration by *L. cinereus*; they recorded that a female of this species that had traveled > 1,800 km during a one-way migration from Chihuahua, Mexico to Canada. In Europe, Popa-Lisseanu et al. (2012) tracked the breeding origins of bat species using a triple-isotope approach (δD, δC, and δN). By using hair samples from known-sedentary species (validation of the isotope technique), they developed an equation that was later used to predict the origin of non-sedentary species.

Wind farms

Wind turbines are a new and important source of renewable energy, and wind farms are rapidly spreading all over Europe and North America. However, the impact of wind farms on bats is of great concern. A large number of bat fatalities, especially among migratory bats, have been reported at wind farms (Arnett et al. 2008). It has been estimated that as many as 225,000

hoary bats, 90,000 eastern red bats and 90,000 silver-haired bats might be killed at wind farms each year in North America (Cryan 2011). The reasons why these bats are attracted to the turbines are still unknown, but knowledge of migratory behavior should help in the design of appropriate conservation strategies.

In North America, peaks in bat mortality at wind farms from mid-July and mid-September. This period is associated with fall migration of bats from their summer (breeding) grounds to their wintering grounds (Cryan 2011). Therefore, it has been assumed that most bats killed at wind farms are migratory individuals. This assumption has been tested only once in Europe (Voigt et al. 2012) with strong results supporting the hypothesis of the large scale effects of wind farms in killing not only local but also non-local bat populations. Thus, wind turbines may also have serious negative effects on a strategic event in the life of bats by interrupting migratory connectivity and thereby imperiling the long-term persistence of migratory bat species at large scales (Cryan 2011). Additional knowledge about such large-scale effects of wind farms would lead to a better understanding of bat mortality and to the implementation of better conservation strategies for migratory bat populations. Therefore, the goal of this study is to identify the potential geographic origin (summer grounds) of North American bat populations of *L. cinereus*, *L. borealis*, and *La. noctivagans* killed at the Fowler Ridge wind farm by analyzing hydrogen stable isotopes in their tissues. I hypothesize that the geographic scale of the effects of Indiana wind farms on bat populations is not only local but includes a much wider geographical area.

Methods

Study Area

The Fowler Ridge farm is located in Benton County, near the city of Fowler, Indiana, about 140 km northwest of Indianapolis, and currently consist of 355 turbines in an area of 54,880 acres (USFWS 2012). Most of the site is open farm land, as is the county in general (harvested cropland in 1997 was 241,562 acres, almost 93% of the total land area for the entire county; U.S. Census of Agriculture 1999).

Based on records from the nearby Indianapolis International Airport weather station, in the last five years (2008-2012), in average, annual temperatures in the area have ranged from a minimum of -18.33 °C to a maximum of 33.89 °C. Annual precipitation has ranged from a minimum of 0 inches (0 mm) to a maximum of 3.81 inches (96.77 mm) (Wunderground.com).

Methodology

The U.S. Fish and Wildlife Service monitors the effect of wind turbines on wildlife at the Fowler Ridge wind farm, Benton County, Indiana. Most bats collected at the site belong to the three North American migratory species, *L. borealis, L. cinereus,* and *La. noctivagans*. This monitoring creates an opportunity to investigate migratory patterns of the bat population(s) passing through the wind farm. Bats killed by the turbines were collected daily or weekly by the U. S. Fish and Wildlife Services and stored at -20 °C. Samples were analyzed at the Cornell University Stable Isotope Laboratory. In September and October 2009 and 2010, when bats are

supposed to be migratory, USFWS collected 13 *L. borealis* (6 females, 5 males, 1 unknown), 13 *L. cinereus* (4 females, 5 males, 4 unknown), and 30 *La. noctivagans* (11 females, 17 males, 2 unknown). Additional individuals collected during the summer of 2009 and 2010, when they are supposed to be residents, also had their tissues analyzed. Since only migratory species were collected at the wind farm, I took samples from *Eptesicus fuscus*, the big brown bat (n=13, 7 females and 6 males), a species with known sedentary habits netted in 2009 at the properties of the International Indianapolis Airport, Hendricks County to serve as control. δD of precipitation depends on latitude and elevation (Dansgaard 1964). The wind farm and the airport are only 160 km away; the difference between their latitudes is only 1 degree and between their elevations is only 60 m, thus the δD in precipitation at the airport and at the wind farm should be fairly similar (J. Sparks, Cornell University, pers. comm.). The control provided information about resident populations that should reflect local δD values in their fur. Samples collected from bats killed at the wind farms in September and October will reflect δD values at the time of hair synthesis, when isotopes are incorporated into the keratin. Molting in bats occurs in summer; therefore, these samples provided information of the summer breeding grounds of the individuals killed during their fall migration. Before performing parametric tests, a Shapiro-Wilks W test determined whether the distribution of δD_k values followed a normal distribution.

Lab analysis—Stable-isotope analyses of all bat hair samples were conducted at the Cornell University Isotope Laboratory (COIL). For carbon, nitrogen, and hydrogen analyses, hair samples were washed in a 2:1 chloroform: methanol solution for 24 h to remove lipids and particles and then dried in a fume hood. Samples then were weighed using a Sartorius MC5 microbalance, placed in small tin capsules, folded to form ca. 1 mm^3 sized packets, and then

sorted into port micro titer trays, in which they were allowed to equilibrate with ambient air over a day.

For δD analysis, 0.2 mg of hair sample was placed into the tin capsules and then flushed in an autosampler for at least 1 h with chemically pure helium. Samples were then pyrolyzed at high-temperatures (~1400 °C) in a ThermoScientific Finnigan TC/EA interfaced into the same IRMS used for carbon and nitrogen analyses. A comparative equilibration method (Wassenaar and Hobson 2000, 2003, Wassenaar 2008), was used to account for the potential exchange of hydrogen isotopes between keratin in the samples and ambient laboratory air moisture. Through this method, hair samples and in-house standards of known hydrogen isotope ratios were analyzed together. Therefore, the values reported are non-exchangeable hydrogen. Four animal and chemical in-house standards were weighed into silver capsules at 0.2 mg and allowed to equilibrate with local water vapor for 24 h. These in-house standards were: keratin, kudu hoof standard, benzoic acid, and caribou keratin standard, and had -117, -54, -114, and -196 ‰ δD values respectively. Results are expressed in the delta notation (δD), in units per mil (‰), and normalization was based on the Vienna Standard Mean Ocean Water standard scale. Measurement error is estimated at ± - 3‰ for δD values. Stable isotope values were calculated following Equation 1.

Precipitation models

The assignment of migrating individuals to their geographic origin can be done by following different approaches, each with advantages and disadvantages (see Wunder and Norris 2008). For the purposes of my study, I decided to use a likelihood-based assignment which

determines geographic origin based on probability density assignments, has an intermediate level of complexity, and incorporate some sources of error (Wunder and Norris 2008). For this assignment I used IsoMAP, which is a recently developed web-GIS portal (http://isomap.org) for isoscape modeling, analysis, and prediction, that assigns samples to a geographic origin. Models commonly used to predict hydrogen precipitation values in studies of wildlife migration are those developed by Bowen et al. (2005) and Meehan et al. (2004). Instead of using these general models, IsoMAP allowed me to created my own, and in this way, adjust temporal and spatial variables and parameters to the specific needs of my study.

The method that IsoMAP follows to assign wildlife to geographic origins involves the use of weighted growing-season precipitation data collected at monitoring stations through out the world (IAEA/WMO 2011) as well as other variables to create precipitation models "isoscapes" that predict expected stable hydrogen isotopes in precipitation (δD_p) values for different geographic regions (Bowen et al. 2005, Meehan et al. 2004). IsoMAP allows the visualization of the isoscapes through maps of predicted isotopic values. Subsequently, individuals are assigned to these isoscapes based on the isotopic values measured in their tissues.

To create the isoscapes, I first developed a series of models that described the spatial variation in hydrogen isotope ratios (dependent variable) on a world map as a function of several independent variables to find the one that best predicted the origin of the control species of known geographic origin. IsoMAP provides a series of variables (e.g latitude, longitude, elevation, temperature, etc.) gathered from different sources (e. g. CRU [University of East Anglia, Climatic Research Unit], ETOPO [U. S. National Geophysical Data Center 1998], monitoring stations) that could potentially be included in the model. I tried three main models,

each with a different set of independent variables. My first model included altitude, minimum temperature, and amount of precipitation. These variables were used by Hobson et al. (2012) to test the relationship between tissue δD and global hydrologic δD patterns. My second model included the variables used by Bowen et al. (2005): altitude, latitude (absolute value), and latitude (squared root). These authors did not consider amount of precipitation when developing their global model because this variable presents regional patterns. In my study, regional patterns are important; therefore, my third model included the variables used by Bowen et al. (2005) along with amount of precipitation. I ran these three models for each of three temporal scales (this temporal scale refers to months of the year included in the analyses), determined mostly by the transfer functions that were going to be used in the "Assignment" stage of the IsoMAP process. Bowen et al. (2014) cautions against the use of models that include a temporal range that does not coincide with the temporal range used to develop the transference function (discrimination factor, see below). The first of these temporal scales included the months of May, June and July and was developed to be used with the transfer function developed by Britzke et al. (2009) for *L. borealis*. The second model included all 12 months based on the transfer function developed by Popa et al. (2012) for European species of the genus *Eptesicus*. The third model included growing-season precipitation months (when the average daily temperature is above 0°C, considered here to be from April through September), based on the transfer function developed by Cryan et al. (*in review*) for *L. cinereus*.

The spatial scale selected for the model encompassed North America and was decided *a priori* because this is the region in which the bat species studied occur. Precipitation in other regions of the world could have very different patterns and therefore be isotopically different.

The temporal scale of the model went from 1990 to 2003 (this temporal scale refers to the years included in the analyses). This range was selected because precipitation data from the last 20 years probably represents better the year when the bats were captured (2009-2010) and few records were available after 2003. The drawback of restricting the spatial and temporal scales was that fewer stations, therefore fewer data, were included in the model. Next in the process of developing the isoscape, I created a map of precipitation isotope ratios for the region and time interval of interest using the models previously developed. In this way I was able to visualize the geographic regions and their corresponding isotopic values.

The next step was to assign bats of known origin to the predicted isoscapes by developing a likelihood-of-origin map for each bat sample based on its measured isotopic composition. For this assignment I first had to transform the raw isotope values measured in bat tissues (hair in this case; δD_h) into precipitation units (as isoscapes are given in these units). This transformation was made via equations that take into account the biochemical differences between animal tissues and the water that they incorporate (Bowen et al. 2005). Early studies assumed a net isotopic discrimination between δD in precipitation and that in animal tissues that reflected the difference or depletion caused by biochemical processes (fractionation). This discrimination factor was estimated to be 25‰ for birds (Wassenaar and Hobson 2001, Hobson et al. 2003), and 24.7‰ for bats (Cryan 2004). However, more recent studies assume a more complex relationship between δD_p and δD in tissues than a simple "net difference" or fixed value. I chose the following regression equations published in the literature to represent these relationships: δD_h=-42.6+0.73[δD_p] for *L. cinereus* (Cryan et al. *in Review*), δD_h= -26.10 + 0.48[δD_p] for *L. borealis* (equation for both males and females, Britzke et al. 2009), and δD_h=-16.8391+1.0699[δD_p] for

La. noctivagans (Popa-Lisseanu et al. 2012), where δD_h is hydrogen isotope in bat hair and δD_p is hydrogen isotope in precipitation. I tried these three different functions in the control species *E. fuscus* because there is not one developed specifically for it. Since all these species are insectivorous, there was not any concern about trophic effects which are known to have a strong effect on δD_h values (Birchall et al. 2005).The likelihood-estimate model incorporates sources of error that sometimes are hard to calculate. In the analyses, I tried four different values of standard deviations for each of the runs I made. The values were 0 (to test what happens when no error is assumed), 3 (the analytical error associated with analysis of stable hydrogen isotopes), the mean standard error from the regression analysis, and the mean standard error from the geostatistical analysis. These two last sources of errors are obtained when developing the isoscapes.

After these transformations, δD_h samples were assigned to geographic regions following two approaches: the multiple linear regression model and the geostatistical model. Both models are based on the assumption that the precipitation hydrogen isotope ratios (dependent variable) and a vector of geographic location and elevation data (independent variable) are observed at a given number of sites. The multiple linear regression method assumes the data arise from independent observations, but the geostatistical method assumes they do not (spatial autocorrelation). The geostatistical model describes the data based on the independent variable values plus the spatial correlation structure of the dataset (kriging). I tried both models for each individual analyzed and chose the one that best predicted the known geographic origin of the control. The statistical analyses run by IsoMAP are described in Bowen et al. (2012).

IsoMAP runs the regression and geostatistical analysis either for single individuals or for groups of individuals. The geographic location of the sample mean is not the same as the mean of the geographic locations structure (Wunder 2010). Wunder and Norris (2008) suggested one should first assign geographic origins to individual bats (process known as fitting a surface) and then average those surfaces, rather than find a location that is associated with the (single) mean isotope value for the sample. Therefore, I ran the analysis for each individual in IsoMAP first, and then compiled the resulting maps in ArcGIS. This resulted in one likelihood-of-origin maps for each species.

After finding the model that better estimated the known origin of the control, I applied its variables to the target species. The assignation of *L. borealis* was done using Britzke's equation for all individuals (Britzke et al. 2009) and the temporal range included summer months only (as Britzke's equations was developed based on that range). I decided not to use the equations developed for males and females separately but the one given for all individuals because preliminary analysis showed that the areas of origin based on those equations were biologically implausible (in the ocean). The assignation of *L. cinereus* was done using Cryan et al. (in review) and the temporal range included growing-season precipitation months (April to September). The assignation of *La. noctivagans* was based on Popa et al. (2012) equation and the temporal range included all 12 months. Each assignment map was laid over with known bat distribution maps (IUCN 2008, Bat Conservation International/PASDA 2003) and known bird flyways. Flyways are used by birds during their migration from their breeding grounds in the northern hemisphere to the wintering regions in the tropics. They have been delimited based on different techniques such as genetic analyses, band recovery, survey data, stable-isotope analysis, and geolocation

tracking. The flyway that passes through Indiana is the Mississippi Flyway which follows the McKenzie River in Canada and the Mississippi River in the United States.

To create the isoscapes and develop the maps for visualization, IsoMAP uses data from different sources and with different resolutions (ETOPO [five minute gridded world elevation], CRU [5°×5° and 2.5°×3.75° grids], GNIP/IAEA stations [extrapolation data collected from over 1,000 meteorological stations randomly distributed in more than 125 countries and territories since 1962]). Therefore, to create the final isoscape, IsoMAP chooses the data with the coarsest resolution and resamples all the data to that resolution. In this study, the final resolution of maps for each species was 40 (longitude) X 60 (latitude) km grid cells.

Since the precipitation models have a very fine-scale resolution and cover a large geographic area (North America in this case), the scale of the probability density surfaces, i.e. the probability of each grid cell of being the geographic origin of the bats (likelihood-of-origin maps) obtained can be very small (the cumulative probability of the 40 X 60 km grid cells is 1).

Additionally, I also tested the relationship between ordinal dates and δD ratios to see if there was any migratory pattern, such as individuals from northern regions start their migration earlier than those in the south.

Results

Validation

For the control, *E. fuscus*, δD values for males were slightly more depleted than females, but the differences were not significant ($p=0.33$). Therefore, in all subsequent analyses, data

from all individuals were pooled together. Varying the temporal scales and the independent variables, several precipitation models were developed to assess the provenance of the control species with known origin (*E. fuscus* from Indiana). The model that best described the origin of the control included altitude, minimum temperature, and amount of precipitation (referred as the Hobson model from here on, because these are the variables used by Hobson et al. [2012]) and this was the model used to assign the migratory species to their geographic origin. For developing the model, the source of all 3 variables was CRU, and for preparing the map elevation data came from ETOPO and the source of the other two variables was still CRU. The analysis covered the North American region only, and included data from 1990-2003, and annual precipitation data (all months in contrast to summer precipitation data or growing-season precipitation data). The Hobson model was tested with three different set of equations, the ones described by Britzke et al. 2009, Cryan et al. (*in Review*), and Popa et al. (2012). The equation that along with Hobson model, best predicted the origin of the control was Popa et al. (2012). The error value that best fit the equation was the regression error, 15.15‰, although all the candidate error values tried resulted in very similar results. From the two analyses that IsoMAP ran, the one that best fit the known origin of the bats was the geospatial analysis (kriging). The comparison among models was made visually by analyzing the resulting assignments in ArcGIS. It is true that the assignment was not specific to predict only Indiana as potential origin; it consisted of a band that included locations such as Texas, Oklahoma, Missouri, Illinois, North Carolina and Virginia (Figure 1). However, Indiana was within the areas with the highest probability to be the origin. The other models included several states but did not include Indiana or assigned it a low probability compared to other areas.

Therefore, for the assignation of the target species I used Hobson model and kriging analysis. Transformation equations and regression errors were specific for each migratory species.

Average of the hydrogen isotope values in the 13 samples included in the analysis was -30.24‰ and the standard deviation was 5.02‰ (after transformation to correct for fractionation).

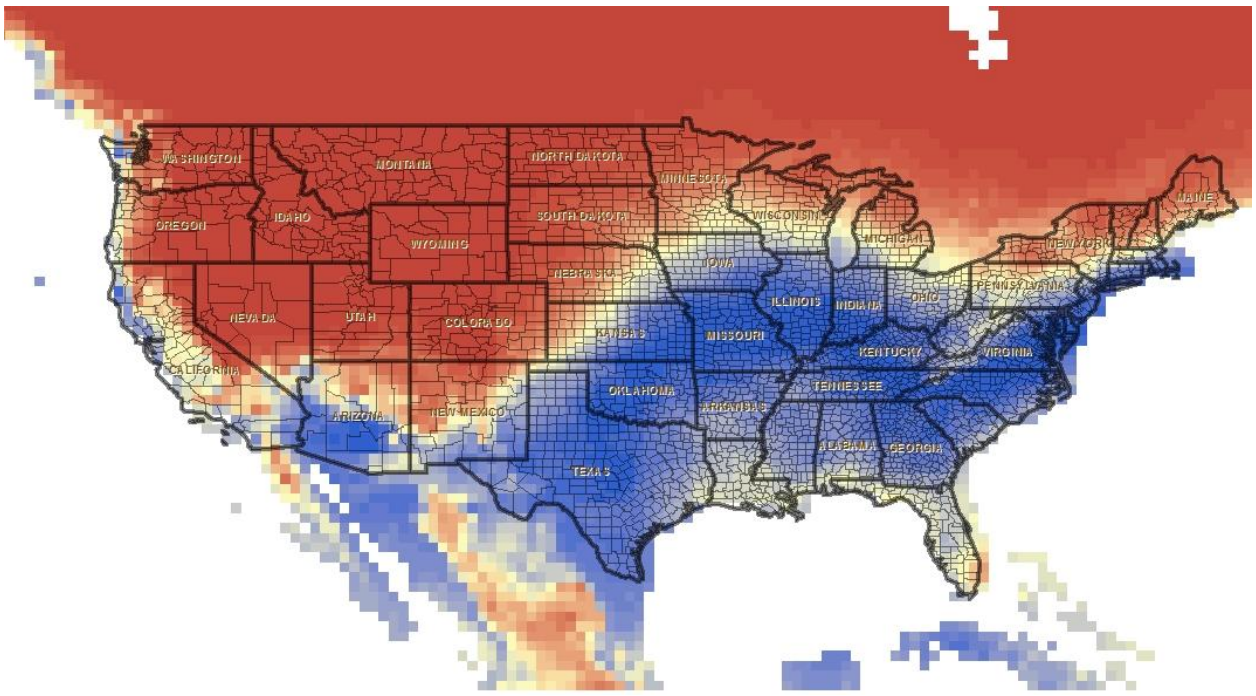

Figure 1. Map of geographic origin for the control species *Eptesicus fuscus*. Blue represents high probability of being geographic origin of the species, red represents low probability.

Variation

Tissues of all three migratory species (residents and migrants) were clearly depleted in δD (*L. borealis* residents = -81.25, migrants = -73.45; *L. cinereus* residents = -87.02, migrants = -75.86; *La. noctivagans* residents = -69.27, migrants = -96.58) in relation to the control (-30.25). The control did not exhibit much variation (range from -19.28 to -38.59; standard deviation = 5.02) but all the migratory species did (*L. borealis* residents = 26.65, ranged from -8.96 to -145.17, migrants = 23.53; *L. cinereus* residents = 41.57, ranged from -35.83 to -98.13, migrants = 24.37; *La. noctivagans* residents = 18.89, ranged from -54.41 to -90.53, migrants = 36.50). Values of the control were characteristics of the region where Indiana is located. Values of all individuals in the three migratory species were always more negative than the control, indicating the assimilation of isotopes in northern latitudes relative to Indiana.

Lasiurus borealis

Females were slightly depleted in δD compared to males but the difference was not significant (t-test p = 0.70), therefore, data from each sex were pooled together for all analyses.

The geographic origin of *L. borealis* predicted by the model included regions in the United States and Canada (Figure 2). The regression error was 13.80. Due to the large variation in hydrogen values, individuals were divided among 4 δD_h bins: -50± 10‰, -70±10‰, -90±10‰, and -110±10‰. The analysis predicted the individuals were from a broad range of latitudes; however, based on the known distribution of the species, this range got narrowed down. I also made the assumption that the direction of migration was only east-south, west-south, or north-south; therefore eliminating localities that suggest south-north migration because

this kind of movement during the fall when bats should move to warmer areas is biologically unlikely. Consequently, regions of summer residence for bats in the first bin are Nebraska, North Dakota, South Dakota, Minnesota, Michigan, New York and Wisconsin in the U. S. and southern Ontario in Canada (red circles in Figure 2).

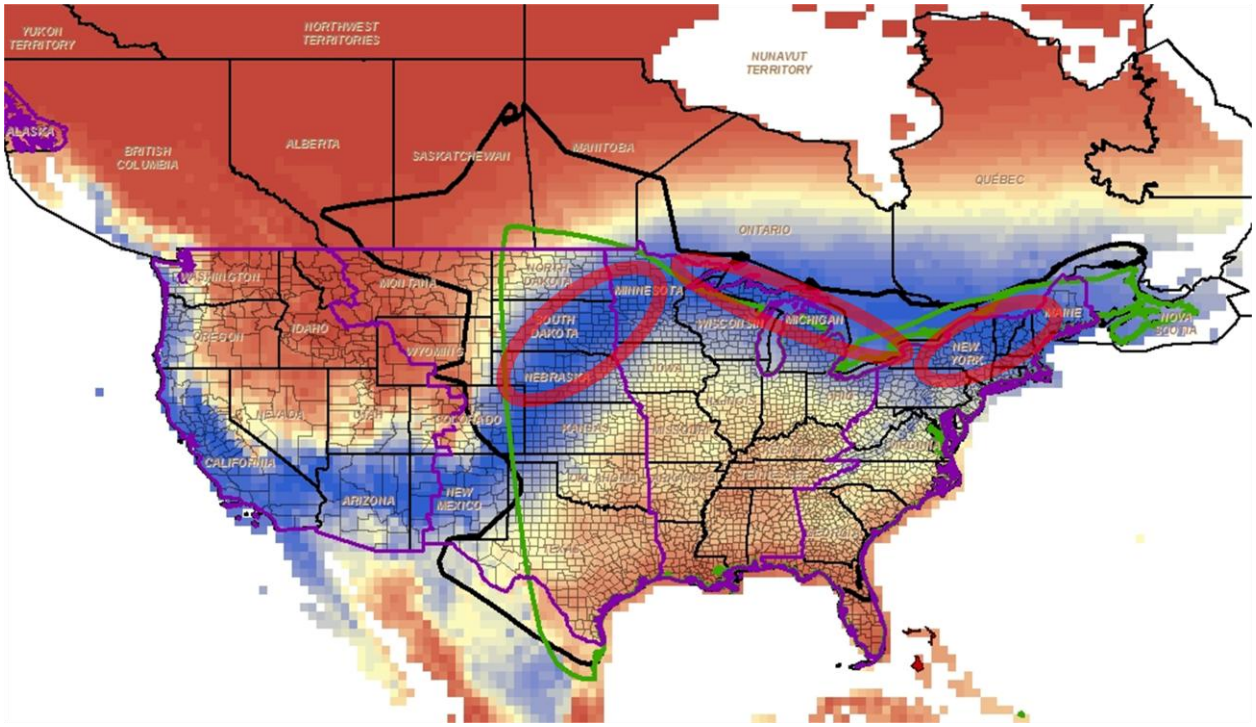

Figure 2. Origin of first bin of *L. borealis* whose values ranged from -50±10. The green line represents the bat distribution according to IUCN 2008a, the black line represents the distribution according to Bat Conservation International/PASDA 2003), the purple lines represent the flyways used by migratory birds. The red circles represent the area of potential origin based on the isotope analysis, known distribution, and assumed southward migration. Blue represents high probability of being geographic origin of *L. borealis*, red represents low probability.

Based on the known distribution and assumed direction of migration, the resulting regions of summer residence for bats in the second bin are North and South Dakota in the U. S., south eastern Manitoba and south western Ontario (red circle in Figure 3).

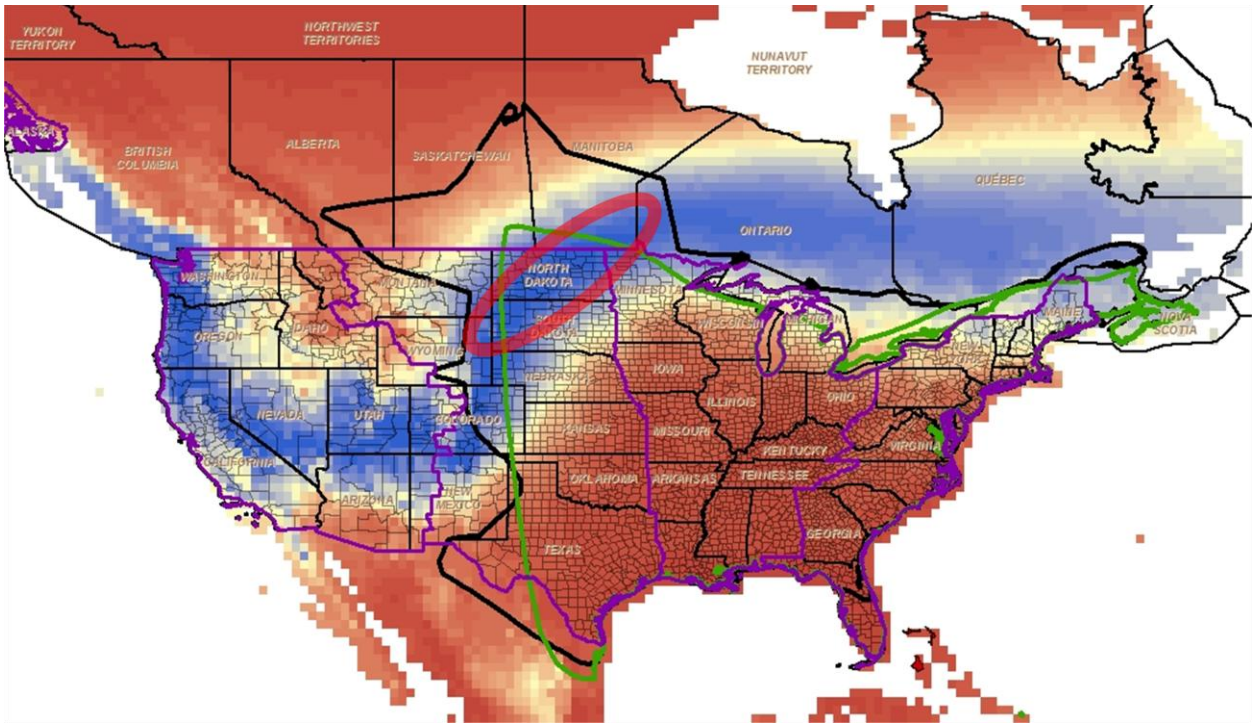

Figure 3. Origin of second bin of *L. borealis* whose values ranged from -70±10. The green line represents the bat distribution according to IUCN 2008a, the black line represents the distribution according to Bat Conservation International/PASDA 2003), the purple lines represent the flyways used by migratory birds. The red circles represent the area of potential origin based on the isotope analysis, known distribution, and assumed southward migration. Blue represents high probability of being geographic origin of *L. borealis*, red represents low probability.

Similarly, regions of summer residence for bats in the third bin are north eastern Montana, southern Saskatchewan and Manitoba (red circle in Figure 4).

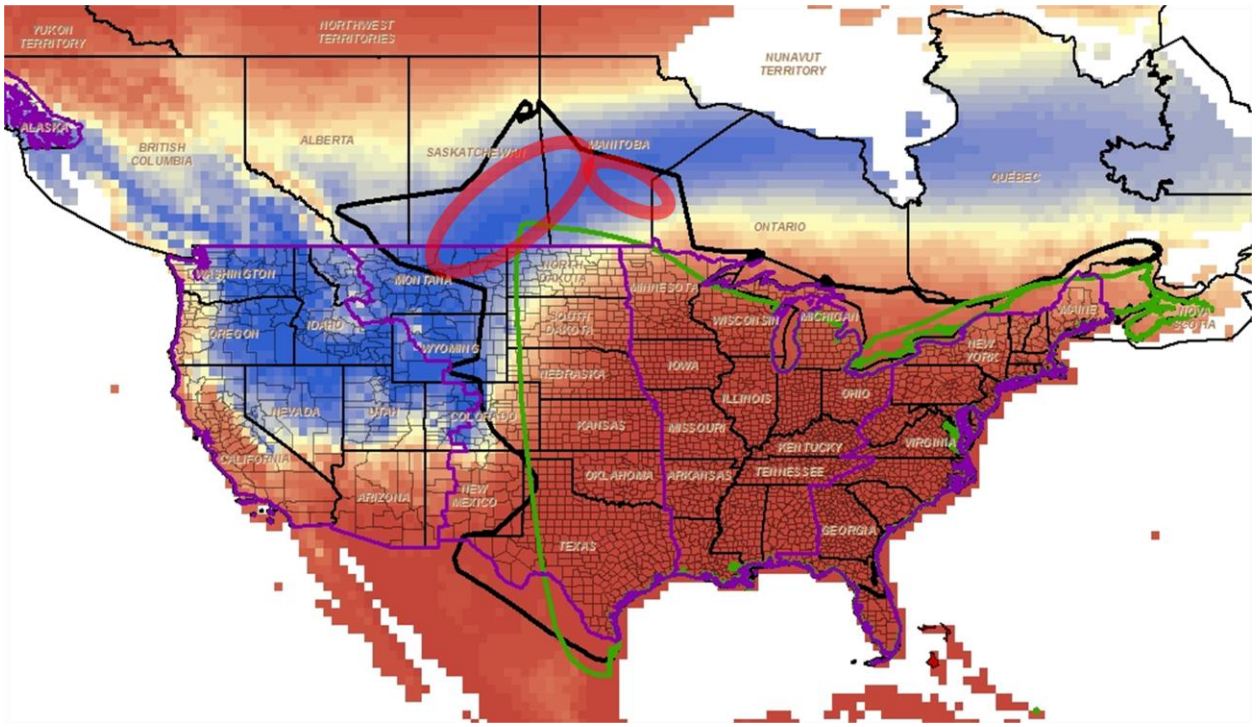

Figure 4. Origin of third bin of *L. borealis* whose values ranged from -90±10. The green line represents the bat distribution according to IUCN 2008a, the black line represents the distribution according to Bat Conservation International/PASDA 2003), the purple lines represent the flyways used by migratory birds. The red circles represent the area of potential origin based on the isotope analysis, known distribution, and assumed southward migration. Blue represents high probability of being geographic origin of *L. borealis*, red represents low probability.

I did not find any significant correlation between ordinal days and δD ratios (p=0.14, Figure 5). Therefore, the hypothesis that bats from more distant location start their migration earlier than other bats was not supported by the data.

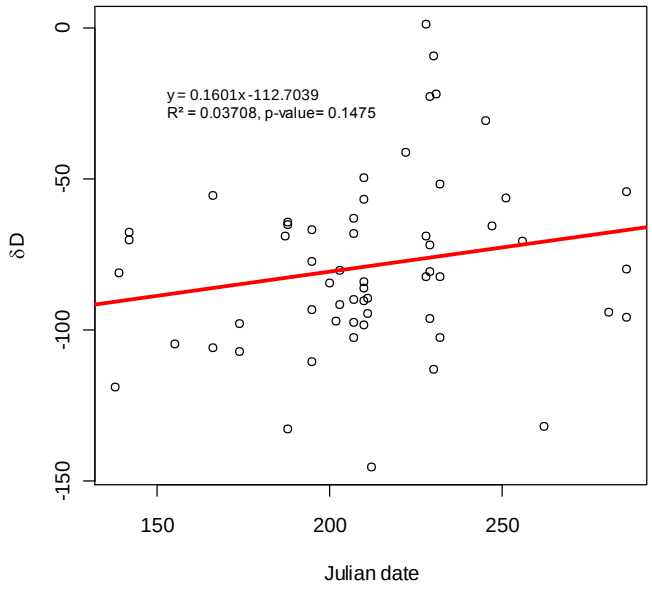

Figure 5. Relationship between Julian dates and δD values in *L. borealis.*

Lasiurus cinereus

In the present study, females were slightly depleted in their δD values compared to males but the difference was not significant (t-test, p=0.55). Therefore, subsequent analyses were run for all the individuals together.

The geographic origin of *L. cinereus* predicted by the model included regions in the United States and Canada. The regression error was 12.29‰. The distribution of the species in this case did not help to narrow down the potential origin. Due to the large variation in hydrogen values, individuals were divided among 3 δD_h bins: -50± 10‰, -70±10‰, -90±10‰, and -110±10‰. Based on the same assumption, that migration does not follow a south-north direction; the regions of summer residence for bats in the first bin are northeastern Colorado, South Dakota, Minnesota, northern Wisconsin, Nebraska, Michigan, New York, in the United States, and southern Ontario and Quebec in Canada (red circles in Figure 6).

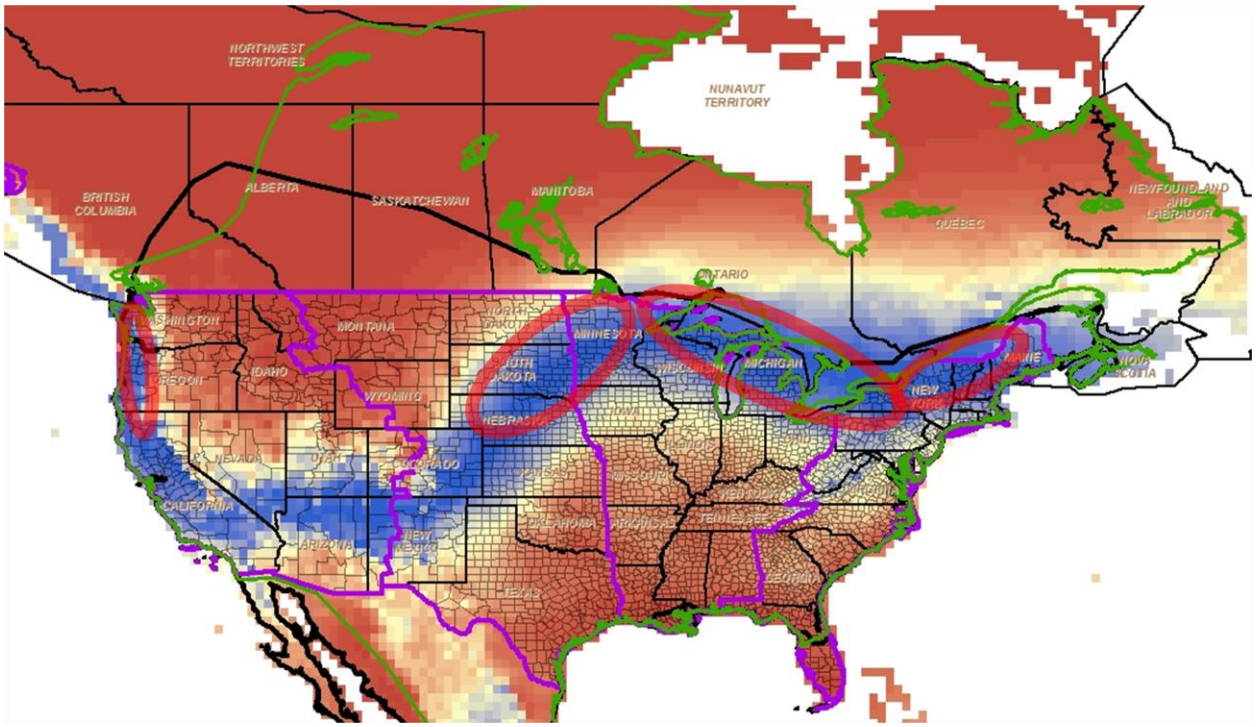

Figure 6. Origin of first bin of *L. cinereus* whose values ranged from -50±10. The green line represents the bat distribution according to IUCN 2008b, the black line represents the distribution according to Bat Conservation International/PASDA 2003), the purple lines represent the flyways used by migratory birds. The red circles represent the area of potential origin based on the isotope analysis, known distribution, and assumed southward migration. Blue represents high probability of being geographic origin of *L.cinereus*, red represents low probability.

Regions of summer residence for bats in the second bin are North and South Dakota, Nebraska, Minnesota, and southern Ontario and Quebec (red circles in Figure 7).

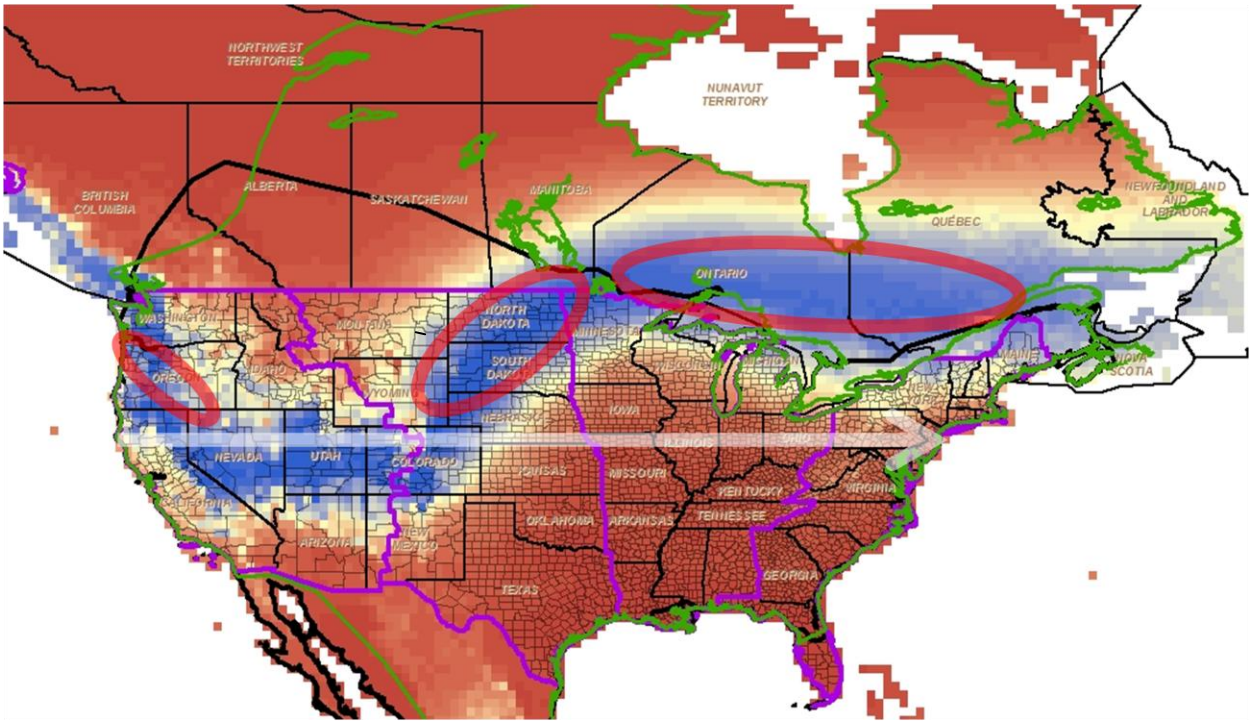

Figure 7. Origin of second bin of *L. cinereus* whose values ranged from -70±10. The green line represents the bat distribution according to IUCN 2008b, the black line represents the distribution according to Bat Conservation International/PASDA 2003), the purple lines represent the flyways used by migratory birds. The red circles represent the area of potential origin based on the isotope analysis, known distribution, and assumed southward migration. Blue represents high probability of being geographic origin of *L. cinereus*, red represents low probability.

Regions of summer residence for bats in the third bin are Montana, southern British Columbia, southern Alberta, southern and central Saskatchewan, and northern and central Manitoba (red circle in Figure 8).

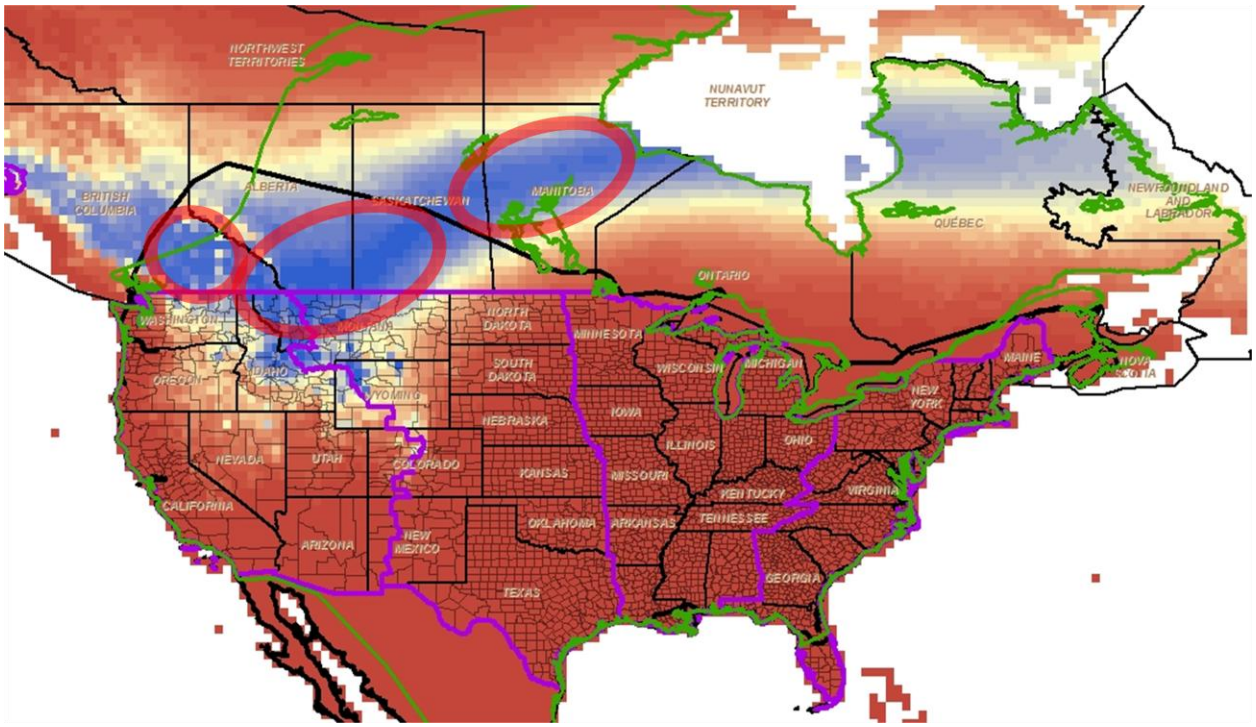

Figure 8. Origin of third bin of *L. cinereus* whose values ranged from -90±10. The green line represents the bat distribution according to IUCN 2008b, the black line represents the distribution according to Bat Conservation International/PASDA 2003), the purple lines represent the flyways used by migratory birds. The red circles represent the area of potential origin based on the isotope analysis, known distribution, and assumed southward migration. Blue represents high probability of being geographic origin of *L. cinereus*, red represents low probability.

The relationship between δD and ordinal dates was not significant ($p = 0.6$; Figure 9). Therefore, the hypothesis that bats from more distant locations start their migration earlier than other bats was not supported by the data.

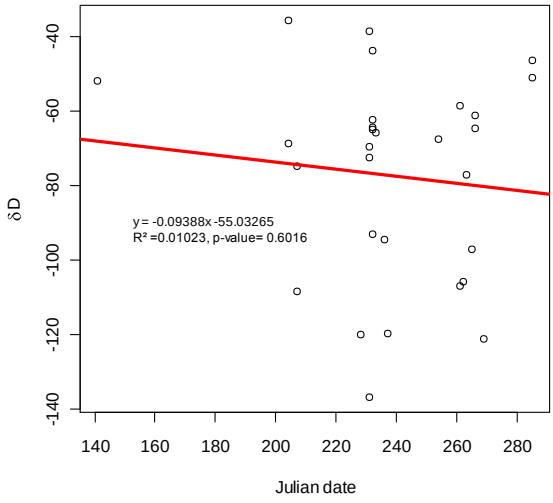

Figure 9. Relationship between ordinal dates and δD values in *L. cinereus.*

Lasyonicteris noctivagans

Females were more depleted in δD than males but the difference was not significant (t-test, p = 0.26). Due to the large variation in hydrogen values, individuals were divided among 3 δD_h bins: -50± 10‰, -70±10‰, -90±10‰, and -110±10‰. The geographic origin of *La. noctivagans* predicted by the model included regions in the United States and Canada. The regression error was 15.15‰. Regions of summer residence for bats in the first bin are Washington, Oregon, Nebraska, South Dakota, Michigan, Wisconsin, Minnesota, and New York (red circle in Figure 10).

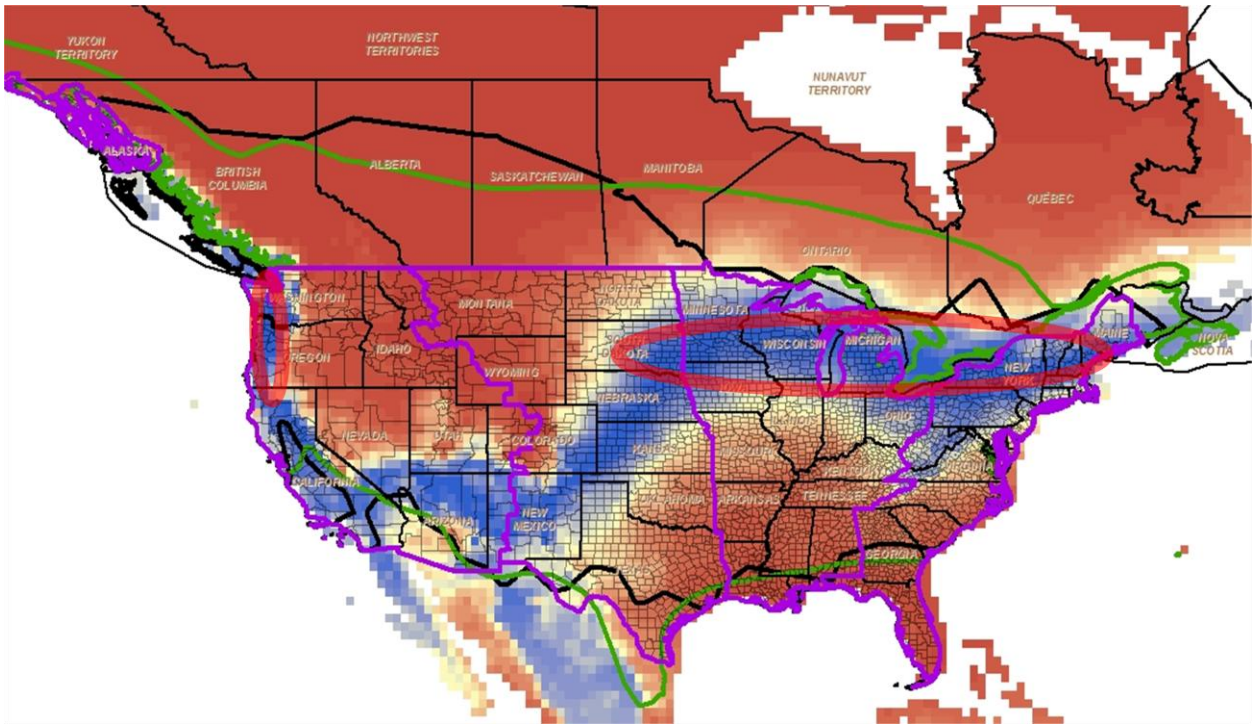

Figure 10. Origin of first bin of *La. noctivagans* whose values ranged from -50±10. The green line represents the bat distribution according to IUCN 2008c, the black line represents the distribution according to Bat Conservation International/PASDA 2003), the purple lines represent the flyways used by migratory birds. The red circles represent the area of potential origin based on the isotope analysis, known distribution, and assumed southward migration. Blue represents high probability of being geographic origin of *La. noctivagans*, red represents low probability.

Regions of summer residence for bats in the second bin are Washington, Oregon, Nebraska, South Dakota, North Dakota, Minnesota, Wisconsin, Michigan, Maine, and New York (red circle in Figure 11).

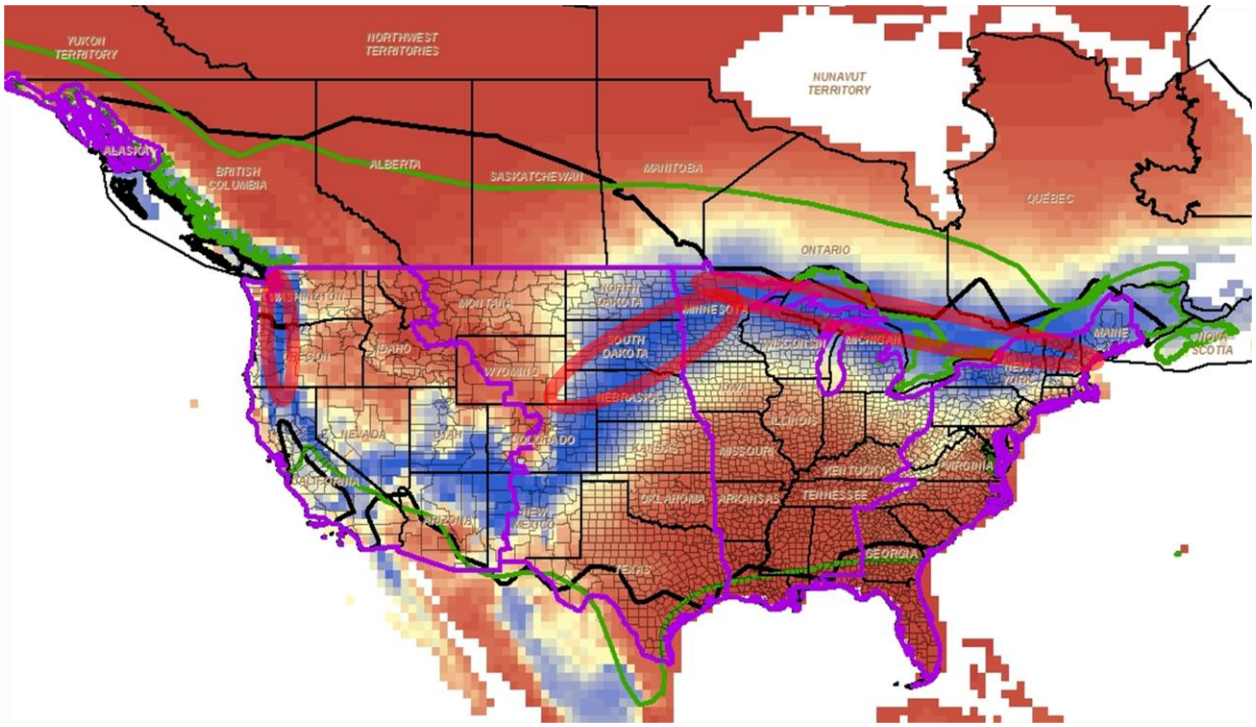

Figure 11. Origin of second bin of *La. noctivagans* whose values ranged from -70±10. The green line represents the bat distribution according to IUCN 2008c, the black line represents the distribution according to Bat Conservation International/PASDA 2003), the purple lines represent the flyways used by migratory birds. The red circles represent the area of potential origin based on the isotope analysis, known distribution, and assumed southward migration. Blue represents high probability of being geographic origin of *La. noctivagans*, red represents low probability.

Regions of summer residence for bats in the third bin are Washington, Oregon, Montana, Wyoming, North and South Dakota, southern Manitoba, Ontario, and Quebec (red circle in Figure 12).

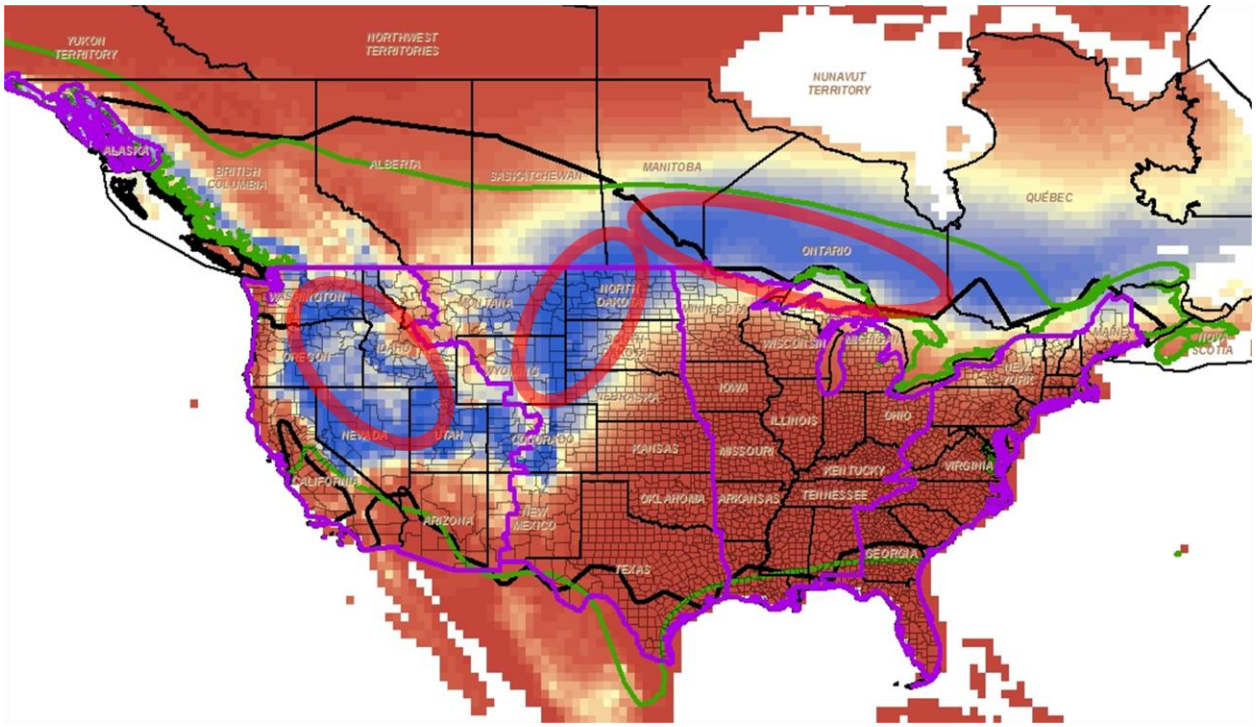

Figure 12. Origin of third bin of *La. noctivagans* whose values ranged from -90±10. The green line represents the bat distribution according to IUCN 2008c, the black line represents the distribution according to Bat Conservation International/PASDA 2003), the purple lines represent the flyways used by migratory birds. The red circles represent the area of potential origin based on the isotope analysis, known distribution, and assumed southward migration. Blue represents high probability of being geographic origin of *La. noctivagans*, red represents low probability.

Regions of summer residence for bats in the fourth bin are southwestern Alberta (red circle in Figure 13). This finding does not agree with the known distribution based on IUCN and BCI records, which do not include the northern half of Canada, but does it agrees with the distribution determined by Cryan (2003).

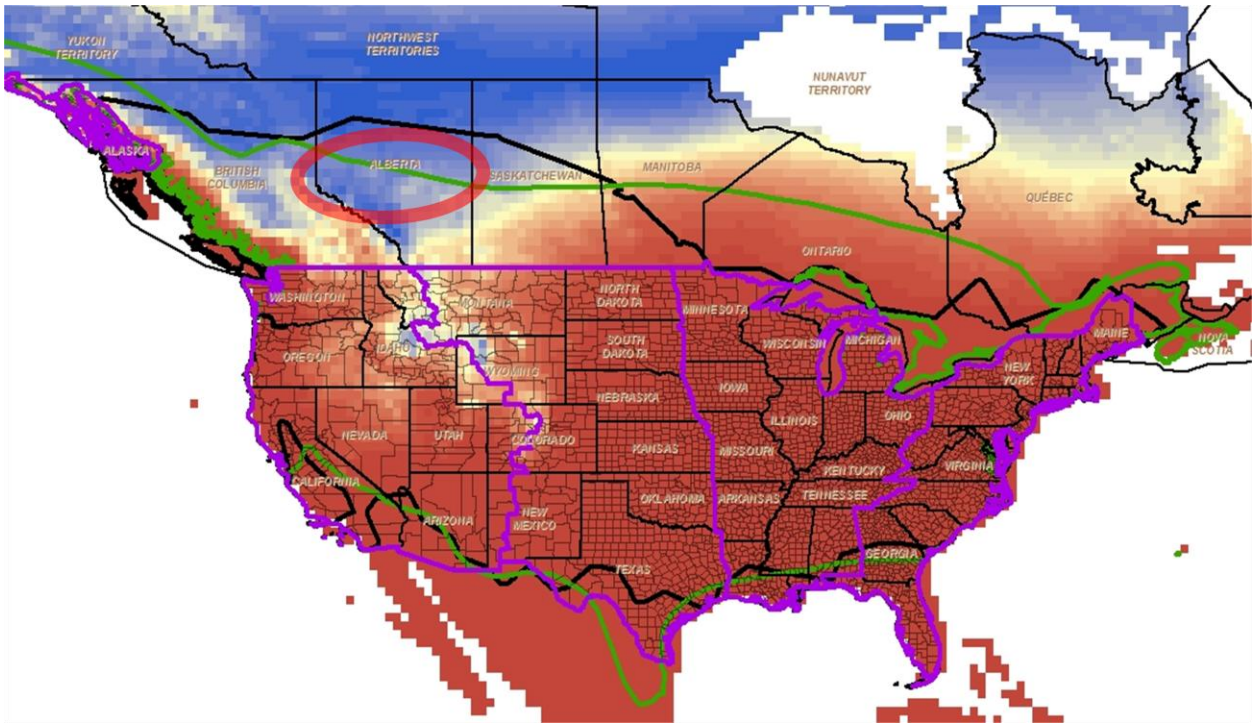

Figure 13. Origin of fourth bin of *La. noctivagans* whose values ranged from -110±10. The green line represents the bat distribution according to IUCN 2008c, the black line represents the distribution according to Bat Conservation International/PASDA 2003), the purple lines represent the flyways used by migratory birds. The red circles represent the area of potential origin based on the isotope analysis, known distribution, and assumed southward migration. Blue represents high probability of being geographic origin of *La. noctivagans*, red represents low probability.

The relationship between δD and ordinal dates were not significant (p=0.47, Figure 14). Therefore, the hypothesis that bats from more distant location start their migration earlier than other bats was not supported by the data.

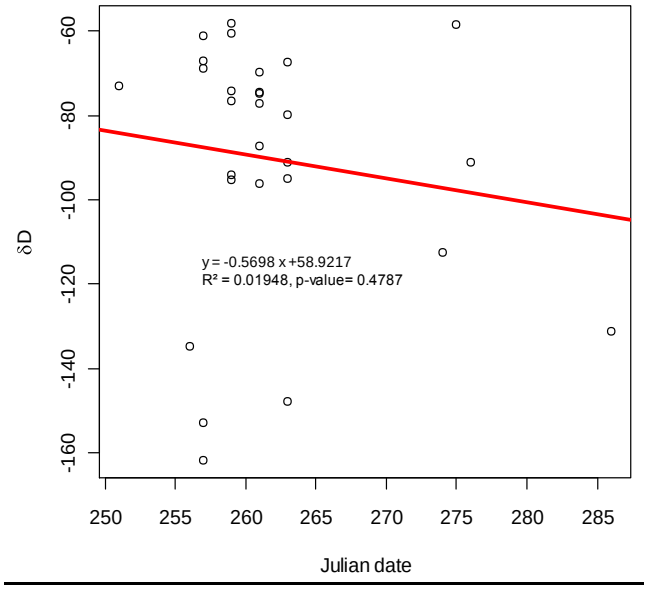

Figure 14. Relationship between ordinal dates and δD values in *La. noctivagans.*

Discussion

Here, I used a stable isotope approach and a geostatistical model to assess the geographic provenance of bats killed by wind turbines in Indiana. Results showed a large degree of variation within species suggesting the presence of several waves of migrating individuals passing through the wind farm. Hydrogen isotope values also suggest that origin of migrating populations of *L. borealis*, *L. cinereus*, and *La. noctivagans*, included regions in the United States and Canada. Records from these three bat species at the wind farm in Indiana were different from those observed in an extensive study (1894-2002; Cryan 2003) in terms of distribution, abundance, and fall and winter activity, highlighting the importance of updating previously known records.

Wind farms

Wind farms are being built all over the U.S. and are responsible for considerable bat mortality. Results of monitoring studies at wind farms indicate a clear pattern in which the highest mortality is observed in three migratory species *L. borealis*, *L. cinereus*, and *La. noctivagans* during the period these species are assumed to have their fall migration (Cryan and Barclay 2009).The latest estimate is 888,000 bat fatalities/year in the United States in 2012 (Smallwood 2013) with migratory species representing 75% of the reported deaths (Arnett et al. 2009), and with *L. cinereus* being the species killed in greater numbers, except for *L. borealis* in the Appalachians (Johnson 2005). However, many details on the migratory behavior of bats are lacking. Even very recently, *Perymyotis subflavus*, a bat species thought to be sedentary, was found to travel distances longer than those of local or short-distant migratory bats (Fraser 2011). We currently do not have precise information on movements of bat populations during the year. Most data on bat distribution comes from museum records (Cryan et al. 2003) that could be biased due to researcher's temporal or spatial ability to sample bats. The results of my study support the hypothesis that the geographic scale of the effects of Indiana wind farms on bat populations is not only local but includes a much wider geographical area.

Bat species

For the control species, *E. fuscus*, the Hobson model was tested with three different sets of equations, the ones described by Britzke et al. 2009, Cryan et al. (*in Review*), and Popa et al. (2012). The equation that along with Hobson model, best predicted the origin of the control was Popa et al. (2012) which is what I expected because this multi-species equation was developed

for European species of the genus *Eptesicus*. Hydrogen isotope values for the control species were those typical for Indiana's precipitation, and did not show much variation which was expected for a species that does not migrate.

According to Cryan (2003) *L. borealis* occurs east of the continental divide and in southern Canada, very close to the U. S. border. According to BCI (2008), this distribution also includes the lower half of Saskatchewan and Manitoba. The results of the isotope analysis showed that for this species the potential summer grounds could extend to western regions where the species is not present; however the predicted northern range matched the known distribution for all the bins in which these individuals occurred. This is because isoscapes determined by a single isotope such as hydrogen display low longitudinal resolution (Bowen et al. 2005). *L. borealis* is known to occur throughout the southeastern United States and northeastern Mexico during winter, extends into the Great Lakes and the Great Plains regions (Baker 1978, Whitaker and Hamilton 1998) in spring and early summer, and is followed by further expansion to the north and west in late summer. The analysis of all three bins suggested that the migration route could potentially have an east-west, west-east and north south direction, with the former being the more likely in most cases. Cryan (2003) determined that movements of *L. borealis* during fall are oriented towards the east and south which agrees with results found in this analysis.

Scattered records of *L. cinereus* have been found in the eastern half of the continent and only a couple in the Midwest during winter in a study that included records from 1894-2002 (Cryan 2003). In this study, a much larger number of individuals was collected in that area. This finding highlights the importance of collecting individuals at wind farms not only to document

migration but also absence/presence or activity of bats during fall or winter. My data did not provide information on bat activity during winter because FWS collected carcasses from late spring to late fall only. One individual was collected in May 2009 suggesting that bats are not only using this route during their fall migration but also in the spring.

Museum records of *L. cinereus* points towards movement from California to eastern regions during the spring and summer (Cryan 2003, Cryan et al. 2014 *in review*). Similarly, the results of my analysis point to, among others, areas to the west, specifically Washington and Oregon, as the origin of populations of this species. These east-west migration movements suggest that bats do not follow traditional north-south migratory flyways used by birds that usually consist of northern-southern movements. Cryan (2003) found no records of *L. cinereus* in Indiana from the months of July and August. The bat population analyzed in this study was collected in Indiana during the fall suggesting different summer grounds and migratory routes or the lack of efficiency of traditional methods such as mist-netting to record individuals of this species in the past.

Cryan (2003) reports that the distribution of *L. cinereus* during the summer includes the southern half of Canada and that the species is uncommon east of the Mississippi river and south of the Ohio River during this period. Samples collected during this study show that *L. cinereus* may be more common in the Midwest than previously known. Five individuals were collected in July and 13 in August and, thus bats collected at wind farms provide valuable information about the presence of bats in an area and their abundance.

The distribution of *La. noctivagans* recorded by Cryan (2003) includes southeastern Alaska, most of Canada, and in the United States south to central California and east through Georgia. The distribution based on IUCN records differs from that of BCI in that it includes the entire U. S. except for southern California and southern Canada and does not occur in Alaska. Cryan (2003) recorded only a few individuals from the Midwest region. In this study 30 individuals were recorded. This author states that as fall progresses, individuals generally move south. Some areas predicted by my analysis agree with a north-south direction of migration.

Interestingly, all analysis predicted similar regions of origin, regardless of the the temporal range applied (year around, summer months, growing-season precipitation months) and equation (Britzke et al. 2009, Cryan et al. *in review*, Popa et al. 2012), and the inclusion of bats from 2009 and 2010 which may suggest all these populations are using a similar flyway that passes through Indiana.

Sex differences

Differences at the continental scale between males and females of *L. borealis* and *L. cinereus*, and differences at the regional level in *L. borealis* have been observed (Cryan 2003). In contrast, I did not find significant sex-based differences in any of the 3 species analyzed, but this result may be due to small sample sizes. Britzke et al. (2009) found gender differences only in *L. borealis*. They pointed out that male *L. borealis* display reverse migration or migration to the north after summer molt. I did not find such evidence; all values from males suggested migration north to south.

Variation

Rocque et al. (2006) observed high variation in δD values in summer residents of the plover *Pluvialis dominica*, in feathers grown in a small region. Values ranged from -175 to -62%. Similar ranges of variation were found in this study, in all three migratory species during the summer, when they are supposed to be resident, feeding and drinking on sources with little isotopic variation. Fur molted during this period should reflect this restricted range of water sources. Factors such as unknown molting periods or unknown migration timing, and therefore the potential use of water or food from different isoscapes, may have increased variation. Here, I considered individuals to be residents during the months of June, July, and August. Results would be biased if bats started to migrate in August, for instance, because isotope values in their tissues would have been assumed to represent their summer grounds when in reality these values represent other region or regions. The control bat did not show much variation because it does not migrate.

Some researchers suggest that high variation in δD indicates a generalized diet and habitat use, and low variation indicates a specialized diet. Among the three species of bats studied here, the most generalist is *L. borealis*. It feeds on moths, beetles, various homopterans and hemipterans, ants, some dipterans, and many other items. The most specialized is *L. cinereus*, which is mostly a moth feeder, although it will eat other items (J. Whitaker pers. comm.). However, in this study, all three migratory species displayed high variation in their δD values suggesting that along with their diets, other factors influenced the observed variation. Several other physiological processes such as metabolic rate, fractionation, and assimilation rates

(Pearson et al. 2003, McKechnie et al. 2004,) could have added to the large variation found. Since these sources of variation are not well understood, they are not included in the assignation of individuals to regions of origin.

The large degree of variation in hydrogen values in the individuals captured in the fall suggests that bats passing through the wind farm in Indiana come from different populations or even migrate as single individuals. This should not be surprising because all three species, unlike other colonial bat species, are solitary and, therefore, they may migrate individually as well.

Assumption of the analysis

All methods of isotopic assignation of individuals to geographic origin, including the likelihood approach used in this analysis, are based on the assumption of a strong relationship between δD in precipitation and δD in animal tissue. Caution should be taken when foodwebs are based on water from sources others than precipitation (Wassenar and Hobson 2000). Britzke et al. (2009) found interspecific differences in the relationship between both δD in precipitation and latitude and δD in bat fur and explained these findings based on the reliance of the species on terrestrial vs. more aquatic food webs. Britzke et al. (2009) found a strong relationship between δD in precipitation and δD in fur in *M. septentrionalis*, a species that depends mostly on terrestrial insects and that rarely forages over open bodies of water. This was, in contrast with the weak relationship they found in other species that largely rely on aquatic ecosystems. In my study, I expected the precipitation-tissue relationship to hold true because all the species analyzed depend mostly on terrestrial foodwebs rather than on wetlands or other standing water

body (J. Whitaker pers. comm.) except for *L. borealis*, which seems to prefer foraging areas that contain sources of water (Hutchinson and Lacki 1999, Sealander and Heidt 1990).

Molting patterns

A major assumption of wildlife forensic studies is that bats molt during the time they are residents, right before migration. If bats molt during migration, the isotopes in their fur would be a mixture of values of all the places visited during migration, between the region of residence and the wintering grounds.

Much of the literature suggests that species from temperate forest molt sometime between July and August (Constantine 1957). Cryan et al (2004) set this period to be sometime between June 20 and August 23 in *L. cinereus,* which is the period when the difference between observed and expected δD values was the lowest. However, Fraser (2011) failed to find a similar relationship in *L. borealis* from Canada based on the same temporal range of molting. To avoid errors due to the variation of timing and duration of molt encompassed by such wide ranges, and to ensure the animals from the study had already undergone molting and were actually migrating when they were killed. I only included in the analysis carcasses found at the wind farms between September and May.

Additionally, it has been observed that females of *L. cinereus* move east from California during the summer (Cryan 2003). Such large movements during the potential period of molt could have a strong influence in the correspondence between the presumed molting region and

the correspondent expected precipitation if the animals move through landscapes with different δD in precipitation. Britzke et al. (2009) mention that juveniles can display these movements, and, therefore, in my analysis I only included adults, and did not include decomposed animals of unknown gender or age, or unknown day of death.

Collecting fur from carcasses

Wassenaar and Hobson (2000a) found no relationship between storage time and isotopic ratios in bird tissues. Similarly, Cryan (2003) observed that year or day of capture did not influence the average relationship between δD in bat hair δD in precipitation. Therefore, not only museum specimens but also carcasses found near wind turbines are an important resource for isotopic studies and the fact that they have been stored or exposed to the environment should not affect the δD ratio once this has been assimilated into bat hair.

GIS models

Most studies based on stable isotopes to track wildlife migration use the GIS models developed by Meehan (2004) or Bowen et al. (2005). Britzke et al. (2009) did not find the GIS models to be appropriate to predict the relationship between δD in bat hair and δD in precipitation, even though these models include latitude as one of the factors, and latitude is assumed to explain a large amount of the variation in δD in precipitation. The model used in the present study does not include latitude as a factor, but instead uses minimum temperature as an indirect measure of latitude.

Flyways

I overlapped the Mississippi flyway into the maps resulting from the stable isotope analysis to determine if bats were following the flyways used by birds during their migration. Many of the regions of origin predicted by the isotope analysis fell outside the established route of the Mississippi flyway. Even if bats use this flyway, it is unlikely they do it in the same way or for the same reasons that birds do. Birds, especially waterfowl, fly through this region which is on the pathway of the Mississippi River, mostly because of the availability of water and food, and the fact that is an open area, with no mountains or ridges of hills that block their visibility during migration. Bats, on the other hand, migrate at night, and roost during the day. Therefore, these tree bats should favor woodlands which provide roosting places, over grasslands or wetlands, and areas with mountains or rivers that could serve as visual cues. Baerwald and Barclay (2009) observed that activity of *L. cinereus* and *L. noctivagans* in Alberta, Canada during migration was higher near the Rocky Mountains than in a nearby prairie. They suggested that bats may use geographic features such as rivers or mountains as visual aids for orientation and may need stopovers and roosting sites during migration.

Fractionation

Early studies assumed a net isotopic discrimination between δD in precipitation and that in animal tissues that reflected the difference or depletion caused by biochemical processes (fractionation). This discrimination factor was estimated to be 25‰ for birds (Hobson et al. 2003, Wassenaar and Hobson 2001), and 24.7‰ for bats (Cryan 2004). However, more recent studies assume a more complex relationship between δD in precipitation and δD in tissues than a

simple "net difference" or fixed value. Fractionation values established and widely used for birds do not necessarily apply to mammals (Hobson and Clark 1992). Fractionation processes can differ between species and regions (Bowen et al. 2005) and the use of a generic value has been discouraged. In the present study I used equations that took into account the fractionation between precipitation and bat tissues developed specifically for each of the migratory bat species (Britzke et al. 2009, Cryan et al. *in review*, Popa et al. 2012).

Wide regions of potential origin

The assignation of individuals to regions of origin based on a single isotope such as δD resulted in wide areas of potential origin. Voigt et al. (2012) used the same approach to track region of residence of bat species in Europe and observed wide bands of potential origin that included several countries. Wunder et al. (2005) concluded that assigning individuals to regions of origin based on δD alone was inadequate. Roque et al. (2006) concluded that the use of stable isotopes may not be an ideal approach for species that migrate at small spatial scales, but would be valuable in tracking intercontinental migrants. The inclusion of another isotope could narrow down the regions of predicted origin. Carbon isotopes discriminate between areas where C_3 and C_4 vegetation are dominant. In contrast, nitrogen isotopes may not provide additional information because they mostly distinguish between trophic levels and in this study all species belonged to the same trophic group.

Ordinal dates

I was expecting to find a relationship between δD values in bat hair and ordinal dates that demonstrated some migratory pattern, but none of the relationships were significant. Specifically, I was expecting that the earlier in the fall season individuals were killed at the wind farm, the greater the distance (from northern latitudes) from which they had migrated. This lack of relationship between long-distance migration and early arrival has been found previously in several bird species (MacMynowski and Root 2007, Hubalek and Capek 2008).

Cryan et al. (2004) observed that the difference between δD in hair of *L. cinereus* and precipitation was lower when the ordinal dates corresponded to the summer months.

Conservation policies across borders

The region of potential geographic origin of all 3 bat species killed at the wind farm in Indiana included provinces in Canada. In some cases, the region of origin includes areas in both countries, in other cases there was a 100% probability that the bats originated in Canada, such as the case of the fourth bin of *La. noctivagans*. Therefore, it is likely that wind farms in Indiana are disrupting populations from other geographic regions. This suggests the need for conservation policies that are not restricted by political boundaries and for international cooperation so when wind farms are installed in one country migratory populations in other countries are considered.

Conclusions

All three species showed more than a single region of potential origin. Many of these regions implied a west-east direction of migration. In contrast to other studies, no significant differences between males and females were found. The analysis of stable isotopes did not coincide with the known geographic distribution of bats in their western ranges but accurately predicted the most northern distributional limits for all 3 species. High variation in δD values in all 3 species was observed, suggesting that individuals or populations from a variety of regions pass through the wind farm. Regions of geographic origin included the United States and Canada, highlighting the importance of cooperation among countries in the development of conservation policies, especially regarding the implementation of wind farms that have been demonstrated to cause the death of thousands of bats and birds. Lack of knowledge regarding timing of migration and molting patterns may bias the results of stable isotope analysis oriented to locate geographic origins of bat populations. Future studies could include data on abundance to restrict even more the predicted origin of the bats. Regions where the abundance of the species is high would have a higher probability to be the place where bats spend the summer. Abundance data could be obtained from band recovery studies.

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Chapter II

Assessment of altitudinal movements and trophic effects in a bat community in central Peru using a triple-isotope approach

Altitudinal migration by birds is known to occur in temperate and tropical forests. This migration is likely associated with tracking food resources, especially fruit and nectar, available at different elevations at different times of the year. Bats that depend on similar resources could exhibit a similar migration pattern. Here, I evaluated the triple-isotopic (hydrogen, carbon, and nitrogen) composition of the tissues of 7 bat species collected at 3 altitudes in the Central Andes of Peru, and the variation of these isotopes at a smaller scale, across an altitudinal gradient, and its applications to migration studies. Hydrogen (δD) and nitrogen ($\delta^{15}N$) isotopes in the sanguinivore control were found to be enriched relative to those of the syntopic frugivores. Carbon isotopes in the sanguinivorous bat were depleted when compared to those of frugivores. Differences in δD found between trophic groups are the first reported for the species studied and support results found elsewhere in the Neotropics. Based on δD results, I present the first physical evidence of bat altitudinal movement in South America. Neither stable isotopes of nitrogen or carbon appear to be reliable to track movements along short gradients such as those along mountains. The contrast of these findings with the results of previous studies suggests that isotopic gradients may be specific to given taxon and localities.

Introduction

Due to physical and chemical processes (Dansgaard 1964), hydrogen isotopes of water vary systematically across latitudinal and altitudinal gradients, and this variation is passed to plants and then into animal tissues (Cormie et al. 1994). Latitudinal variation in hydrogen has been successfully applied to track migration of a variety of wildlife (see Chapter I). Variation of hydrogen in precipitation along altitudinal gradients has also been demonstrated (Bowen and Wilkinson 2002); however, the translation of this trend from precipitation to wildlife has not been well supported (Graves et al. 2002). This chapter focuses on the triple-isotopic (hydrogen, carbon, and nitrogen) composition of the tissues of seven bat species collected at three altitudes in the Central Andes of Peru, and the variation of these isotopes at a smaller scale, across an altitudinal gradient, and its applications to migration studies.

Altitudinal migration by birds is known to occur in temperate and tropical forests. This migration is likely associated with tracking food resources, especially fruit and nectar, available at different elevations at different times of the year (Loiselle and Blake 1991). Bats that depend on fruits and nectar, could exhibit a similar migration pattern (Fleming and Eby 2003). However, there is limited evidence of altitudinal movement in bats, especially in the Neotropics. Bat migration along altitudinal gradients is known to occur based on seasonal variation in species' abundances (Nadkarni and Wheelwright 2000) or occasional recapture of marked individuals at different altitudes (Esberard et al. 2011). However, there is a lack of knowledge regarding the

geographic origin or destination of the bats, when exactly the migration occurs, or if there are observable patterns at the intra or interspecific levels. Moreover, many Neotropical bat species have not yet been classified as migratory or sedentary (Fraser et al. 2010), especially in South America.

Research evaluating the signature of hydrogen has proven useful in estimating geographic origins of animal populations and migration distances, and it is relatively inexpensive when compared to other methods. This method also overcomes the issue of having to capture the individuals twice to get information on their migratory route; by capturing the animals only once and taking tissue samples at the time of capture, reliable information on the animal's location during the previous molting season can be gathered (Hobson 1999).

Stable isotopes and elevation

Due to the Rayleigh distillation effect (Gleixner and Mugler 2007), stable hydrogen (δD) and oxygen ($\delta^{18}O$) isotopes show strong depletion with altitude. If this pattern previously established for plants (Korner et al. 1991) is passed to consumers, then we would expect to see a similar trend in the tissues of animals. Animals incorporate local δD in precipitation (δD_p) through drinking water and diet, and $\delta^{13}C$ through diet. Keratin from tissues, such as fur and feathers, is the typical target of stable isotope analysis because these tissues are metabolically inert once synthesized (Hobson and Wassenaar 1997). Inert tissue means that the original isotopic signature gets locked in and does not change until the next molt, even if the animal moves to a new geographic location or changes its diet, and is only changed when molting occurs. Therefore, keratinous animal tissues synthesized at a given elevation should reflect the

isotopic signature of the diet and water at that elevation. Departure from the expected values at a given altitude would be evidence for the altitudinal migration of the animal from a feeding site to a capture site (Hobson et al. 2003, Fraser et al. 2008).

The analysis of stable isotopes has been used to successfully track latitudinal movements of a variety of wildlife (Cerling et al. 2006, Cyran et al. 2004, Hobson et al. 2009, 2010, Wassenaar and Hobson 1998). In contrast, this analysis has been applied only to a few studies focused on migration along altitudinal gradients. Graves et al. (2002) observed a positive relationship between $\delta^{13}C$ values of adult male black-throated blue warblers and altitude (790-1545 m) in the Appalachian Mountains; however, the large degree of variation observed raised some concern about the use of $\delta^{13}C$ in tracking the geographic origins of migratory birds. Hobson et al. (2003) documented a significant relationship between elevation and $\delta^{13}C$ and δD values in hummingbird feathers along an altitudinal gradient (300-3290 m) in Ecuador and pointed out the potential of these isotopes in studies of altitudinal migration. Hardesty and Fraser (2010) found the expected decrease of δD in rainwater and bird tissues with increasing altitude on a gradient (1350-3500 m) in Ecuador but concluded that the variability in feather δD obscured this relationship when some other factors such as diet, molting, and differences between global and local patterns of δD in precipitation are considered.

To date, only two studies have used stable hydrogen isotope analysis to track altitudinal migration of bats, and both studies were conducted in the Neotropics. Fraser et al. (2010) could not find evidence of a relationship between altitude and δD in bat claws and hair in Nicaragua; however, these interpretations were based on a very small sample size ($n \leq 5$) and a single

sampling location. Erzberger et al. (2011), working in Honduras, suggested that δD may vary across trophic levels in both sedentary and migratory species. They concluded that short altitudinal movement (less than 400 m) could not be detected by analysis of δD, and suggested the use of other isotopes in addition to δD. This question has been complicated by the absence of molting information for Neotropical bats. It has largely been assumed that molting in bats occurs after reproduction (Cryan et al. 2004), but studies on this topic have been focused on species that have a single reproductive period (North American bats, for instance, reproduce during the summer only). On the other hand, bats in the Neotropics can be monestrous, polyestrous, or reproduce all year around (Fleming et al. 1972). Therefore, molting in these species probably follows a different pattern than that established for species in temperate zones.

Another study focused on δD analysis in Neotropical bats did not address migration per se, but investigated several factors influencing the variation of δD at a single locality in Costa Rica (Voigt et al. 2013a). This represents the most extensive isotope study in the Neotropics (36 bat species and more than 400 individuals) and among its findings are the large intra (20‰) and interspecific (70‰) variation in δD in hair keratin. Voigt et al. (2013a) suggested that this level of variation should be considered by studies of migration using stable isotopes. Similar conclusions were reached by Graves et al. (2012).

These results suggest the need to test the relationship between hydrogen and altitude in other species, in ecosystems over larger altitudinal gradients, and that test whether trophic effects affect this relationship. Also, other isotopes could be used, such as, for instance, $\delta^{13}C$ or $\delta^{15}N$. Therefore, the general objectives of this study were to describe the triple-isotopic composition

(variation, gender and trophic effects on δD, $\delta^{13}C$ and $\delta^{15}N$, and body size effect on δD) of seven species (six frugivores and one sanguinivore) collected at three altitudes (1500, 1800, and 2300 m) and to investigate the potential use of stable isotope analysis to track movements of Neotropical bat species along an altitudinal gradient. The specific objectives of this study were:

1) To evaluate differences in δD, $\delta^{13}C$ and $\delta^{15}N$ between trophic groups. I expected that values of the sanguinivorous bat *D. rotundus* would be enriched in both δD and $\delta^{15}N$ in relation to frugivorous species, as has been shown in previous studies (Fraser et al. 2010, Erzberger et al. 2011, Voigt et al. 2013a).

2) To investigate migration in each of the bat species captured at each altitudinal point. Species of the genus *Carollia* and *Platyrrhinus* have found to be local (non-migratory) in forests of Central America (see Voigt et al. 2013a); however, there is no information on the migratory status of *Sturnira* species

3) To investigate the relationship between values of δD, $\delta^{13}C$ and $\delta^{15}N$ isotopes in bat hair and the altitude at which bats were captured. Evidence for a relationship between these isotopes and altitude has been found in soil (Mariotti et al. 1980), plants (Korner et al. 1991), and wildlife (Graves et al. 2002, Hobson et al. 2003). Based on the results of these studies, I expected an inverse relationship between δD and $\delta^{15}N$ with altitude, and a direct relationship between $\delta^{13}C$ and altitude.

Moreover, there is evidence of seasonal segregation of the sexes at different scales (Cryan 2003) and differences in patterns of latitudinal migration (Britzke et al. 2009) in several species of bats from temperate forests. In birds, differences in altitudinal migration based on

sex are common (Stiles 1988, Johnson and Maclean 1994). Therefore, analyses of stable isotopes should be run independently for species and sexes (Britzke et al. 2009). Additionally, contradictory results have been found in studies testing the assumption that δD_h is independent of body size. Betini et al. (2009) found that δD kinetic fractionation was linked to size of songbird nestlings; however, Voigt et al. (2013) did not find evidence for such a relationship in bats from Costa Rica when testing blood and keratin. These results suggest the need for more studies that test the differences in isotopic composition between the sexes and the correlation between body size and δD.

This study differs from previous research in that it (i) only targeted hair as the source of isotopes, (ii) tested samples collected over a large altitudinal gradient (850 m), and (iii) used a triple isotopic approach (δD, $\delta^{13}C$ and $\delta^{15}N$). The results of this investigation should contribute to our understanding of animal migration and elucidate unknown patterns in the life history and ecology of tropical bat species. Information on seasonal movements that result in changes in population sizes of species is crucial for the assessment of their susceptibility to changes in environmental conditions due to local human disturbance or global climate change and for the design and application of conservation strategies aimed to achieve the long-term persistence of populations. This research is the first to use stable isotopes to describe the isotopic composition and address altitudinal migration of bat populations in South America.

Methods

The study area is located in the Calabaza district of Pampa Hermosa, Province of Satipo, Department of Junín, Central Peru. Bats were mist-netted along an altitudinal gradient in the western slope of the Andes during the dry season of 2011. Bats were collected at three altitudinal sites (Table 1). Hair samples from each individual were collected from between the scapulae and placed in plastic bags to be sent to the Cornell University Stable Isotope Laboratory for analysis. Samples collected from the non-migratory vampire bat *D. rotundus* (Fleming and Eby 2003) were collected to serve as a control.

Table 1. Information about the location of the sampling sites, capture dates, and effort displayed

Site	Altitude (m)	Latitude	Longitude	Sampling dates (2011)	Capture effort (mist-nets X hours X night)
1	1533-1569	-11.4505	-74.7873	15-20 October	65
2	1821-1864	-11.4876	-74.7989	11-14 October	58
3	2333-2373	-11.5101	-74.836	September 29-October 4	70

Lab analysis—Stable-isotope analyses of all bat hair samples were performed at the Cornell University Isotope Laboratory (COIL). For carbon, nitrogen, and hydrogen analyses, hair samples were washed in a 2:1 chloroform: methanol solution for 24 h to remove lipids and particles and then dried in a fume hood. Samples then were weighed using a Sartorius MC5 microbalance, placed in small tin capsules, folded to form ca. 1 mm^3 sized packets, and then

sorted into port micro titer trays, in which they were allowed to equilibrate with ambient air over a day.

For the $\delta^{13}C$ and $\delta^{15}N$ analyses, approximately 0.5 mg of hair material was placed into the tin capsules. Samples were then combusted in a CarloErba NC2500 Temperature Conversion Elemental Analyzer (TC/EA) interfaced with a ThermoScientific Delta V isotope ratio mass spectrometer (IRMS). Here samples were run along with four in-house standards: Cayuga Brown Trout, corn, mink and methionine. For every 12 bat hair samples, one of each in-house standard was inserted in sequence for isotope data correction and to estimate instrument precision and linearity across runs.

Results are expressed in delta notation (δD), in units per mil (‰), and normalized relative to the PDB (Pee Dee Belemnite) standard for $\delta^{13}C$ and to atmospheric air for $\delta^{15}N$ standard. Measurement error is estimated at ±- 0.1% and 0.3‰ for $\delta^{13}C$ (Panarello 2002) and $\delta^{15}N$ respectively (Tesdal et al. 2013) values, respectively. δC and δN values are calculated from:

$$\delta^{13}C(‰) = \left[\frac{{}^{13}C/{}_{12}C\ sample}{{}^{13}C/{}_{12}C\ standard} - 1 \right] \times 1000\ per\ mil$$

$$\delta^{15}N(‰) = \left[\frac{{}^{15}N/{}_{14}N\ sample}{{}^{15}N/{}_{14}N\ standard} - 1 \right] \times 1000\ per\ mil$$

For δD analysis, 0.2 mg of hair sample was placed into tin capsules and then flushed in an autosampler for at least 1 h with chemically pure helium. Samples were then pyrolyzed at high-temperatures (~1400 °C) in a ThermoScientific Finnigan TC/EA interfaced into the same IRMS used for carbon and nitrogen analyses. A comparative equilibration method (Wassenaar and Hobson 2000, 2003, Wassenaar 2008), was used to account for the potential exchange of hydrogen isotopes between keratin in the samples and ambient laboratory air moisture. Through this method, hair samples and in-house standards of known hydrogen isotope ratios were analyzed together. Therefore, the values reported are non-exchangeable hydrogen. Four animal and chemical in-house standards were weighed into silver capsules at 0.2 mg and allowed to equilibrate with local water vapor for 24 h. These in-house standards were: keratin, kudu hoof standard, benzoic acid, and caribou keratin standard, and had -117, -54, -114, and -196 ‰ δD values respectively. Results are expressed in the delta notation (δD), in units of per mil (‰), and normalization was based on the Vienna Standard Mean Ocean Water standard scale. Measurement error is estimated at +/- 3‰ for δD values. δD values are calculated as:

$$\delta D(‰) = \left[\frac{{}^{1}H/{}_{2}H\ sample}{{}^{1}H/{}_{2}H\ standard} - 1 \right] \times 1000\ per\ mil$$

To address potential confounding effects of sex and body size, I ran preliminary analyses for all species. First, I tested for differences in δD, $\delta^{13}C$ and $\delta^{15}N$ values between males and females from each species using *t*-tests whenever the sample size was large enough (at least 10 individuals per species). I also tested the correlation between body size, expressed as mass, and δD in hair keratin. I ran a linear regression between δD and body mass across all species disregarding the altitude at which they were captured, their trophic level or sex.

To address Goal 1, the evaluation of the differences in isotopes between trophic groups, ANOVA's were used to determine whether frugivorous and sanguinivorous bats captured at the same elevation (i.e. syntopic species) differed in δD, $\delta^{13}C$ and $\delta^{15}N$. To estimate the actual difference in terms of parts per mil (‰) I calculated the mean for each isotope in the sanguinivore and frugivores (Table 2) and then subtracted those values.

To address Goal 2, to look for evidence of altitudinal migration, I first established a relationship between δD_p and elevation to obtain expected values across the gradient. To calculate the relationship between δD_p and elevation, I plotted a graph of mean weighted annual δD_p versus elevation. Data on mean weighted annual δD_p were collected from 67 stations

distributed along an altitudinal range from 98 to 4477 m (IAEA 2001) in Peru. Mean annual δD_p values were available for 18 stations; for the other 49 sites, only values for a single year were available. These data were used to derive the tendency of depletion with elevation. As a result, the equation

$$\delta D_p = B_0 + B_1(\text{Elevation}) \quad \text{..... Equation 1}$$

predicted the expected relationship between elevation (E) and δD_p, which is known to be linear . Next, I compared these expected δD_p to observed δD_h for each individual species. In order to compare δD_p obtained from the above equation to observed δD_h, δD_h was transformed into precipitation units by adding a discrimination factor or through an equation. Early studies assumed a net isotopic discrimination between δD in precipitation and that in animal tissues that reflected the difference or depletion caused by biochemical processes (fractionation). This discrimination factor was estimated to be 25‰ for birds (Hobson et al. 2003, Wassenaar and Hobson 2001), and 24.7‰ for bats (Cryan 2004). However, more recent studies assume a more complex relationship between δD_p and δD in tissues than a simple "net difference" or fixed value. Therefore, the equation used here was:

$$\delta D_h = 1.0699\ (\delta Dp) - 16.8391$$

where δD_p comes from equation 1. This is a multiple-species model developed by Popa-Lisseanu et al. (2012) which was based on δD_h values of European sedentary insectivorous bats. In addition to this equation, the only other fractionation constant for bats has been proposed for *Lasiurus cinereus* (Cryan et al. 2004), a North American insectivorous species (-24.7%); there is

not a fixed value or equation to account for the hydrogen fractionation in frugivore species or Neotropical bats. I chose not to use fractionation values that are established and widely used for birds, because work shows that these values do not necessarily apply to mammals (Hobson and Clark 1992). Fractionation processes can differ between species and regions (Bowen et al. 2005) and the use of a generic value has been discouraged; however, the equation I used was able to accurately predict the average hydrogen values of the *D. rotundus*, a species that is usually considered sedentary due to its dependence on sedentary cattle (Soriano 2007). The resulting δD_h from the above equation was then corrected for trophic differences so it can be applied to frugivorous species. Fraser et al. (2010) suggested that trophic level may have an effect on δD of animal tissues. Therefore, to obtain the final expected δD_h values of a species at a given altitude, factors of -40 (Fraser et al. 2010) and -45‰ (Erzberger et al. 2011, Voigt et al. 2013) were applied to the result of the above equation, as reported in the literature to be the difference in δD between the tissues of insectivores and frugivores. Finally, expected δD_h values were compared to the actual observed δD_h values resulting from the isotope analysis using t-tests. If species were sedentary, their δD values in hair should correspond to those predicted by this equation. If species were migratory, their δD values in hair should be depleted (more negative, suggesting incorporation of isotopes into keratin at higher elevations and then migration down slope) or enriched (more positive, suggesting incorporation of isotopes into keratin at lower elevations and then migration upslope) relative to those values expected at the site they were captured. If any species appeared to be migratory, then it was potentially excluded from further analyses that tested the relationship between δD, $\delta^{13}C$ and $\delta^{15}N$ and altitude because these relationships assume the species are sedentary and their tissues reflect local values. I did

not correct the control species, the sedentary bat *D. rotundus* for trophic differences. The trophic level of the control *D. rotundus* (sanguinivorous) and that of the insectivorous bats used to create the equation are very similar, in fact more similar to each other than each of them is to frugivores (Fraser et al. 2010).

To address Goal 3, I used linear regression models to evaluate the relationship between values of δD, $\delta^{13}C$ and $\delta^{15}N$ isotopes in bat hair and the altitude at which they were captured. To achieve sufficient sample sizes, I pooled species from the same trophic group.

In birds, it is known that there is variation within single feathers (Smith et al. 2008). I do not expect this intra-individual source of variation to affect the individual bat hairs, but to minimize any effect of this type of samples were collected from the same spot on the back in all the individuals.

Quantile-quantile (Q-Q) plots determine, in a graphic way, whether the residuals of the data follow a normal distribution by comparing the observed quantiles with the theoretical quantiles. If the data follows a normal distribution, the observed points should fall approximately on the straight line formed by the expected quantiles. Q-Q plots were used for post-hoc testing; if residuals deviated from normality, variables were log-transformed. All tests were analyzed via Q-Q plots but these are only mentioned in the text when they deviated from normality. Statistical analyses were conducted using R (Version 2.14.1, Vienna, Austria). The chosen alpha level was 0.05.

Results

Ninety-four individuals of 9 species and 3 trophic groups were captured (Table 2). Frugivorous species included *Sturnira erythromos* (n = 36), *S. oporaphilum* (n = 10), *Carollia brevicauda* (n = 11), *C. perspicillata* (n = 9), *Platyrrhinus masu* (n = 1), and *P. albericoi* (n = 2); nectarivorous species included *Anoura geofroyii* (n = 4) and *A. aequatoris,* and the only sanguinivorous species collected was *Desmodus rotundus,* (n = 20). See minimum and maximum δD, δC, and δN values varied at different altitudes for each of the species in the Appendix (Tables S1, S2, and S3)

Table 2. List of all bat species captured at low, medium, and high elevations with corresponding stable carbon, nitrogen, and hydrogen isotope ratios in hair keratin ($\bar{x} \pm SD$) in ‰. The total number of individuals captured from each species at the corresponding site is also reported (n).

	Low (1500 m)				Medium (1800 m)				High (2300 m)			
	($\bar{x} \pm$ SD)				($\bar{x} \pm$ SD)				($\bar{x} \pm$ SD)			
Species	δD	δC	δN	n	δD	δC	δN	n	δD	δC	δN	n
Carollia perspicillata	-108.41 ± 18.58	-18.91 ± 4.61	2 ± 1.4	9								
Carollia brevicauda	-117.82 ± 12.2	-19.67 ± 6.25	-1.76 ± 0.91	10					-108.12	-25.32	1.69	1
Sturnira erythromos	-112.3	-21.55	2.15	1	-102.28 ± 9.20	-21.98 ± 5.71	2.7 ± 1.11	4	-133.64 ± 15.71	-25.24 ± 0.89	2.61 ± 1.2	31
Sturnira oporaphilum	-126.75 ± 30.12	-18.75 ± 6.43	-3.8 ± 0.24	5	-144.18 ± 22.86	-14.47 ± 0.96	3.43 ± 1.02	2	-120.58 ± 21.96	-25.23 ± 1.2	4.01 ± 1.8	3
Platyrrhinus albericoi					-154.95 ± 21.86	-14.01 ± 0.51	3.7 ± 0.23	2				
Platyrrhinus masu	-157.32	-15.84	2.78	1								
Anoura aequatoris	-94.31	-15.55	5.99	1								
Anoura geoffroyii					-96.38 ± 21.11	-25.31 ± 0.37	6.6 ± 1.17	2	-123.64 ± 23.09	-24.70 ± 0.92	5.54 ± 1.68	2
Desmodus rotundus	-72.53 ± 23.39	-24.81 ± 0.84	8.02 ± 1.05	20								
			Total	47			Total	10			Total	37

Species showed different degrees of intraspecific variation in δD, δC, and δN at each of the 3 altitudes studied (Figure 1).

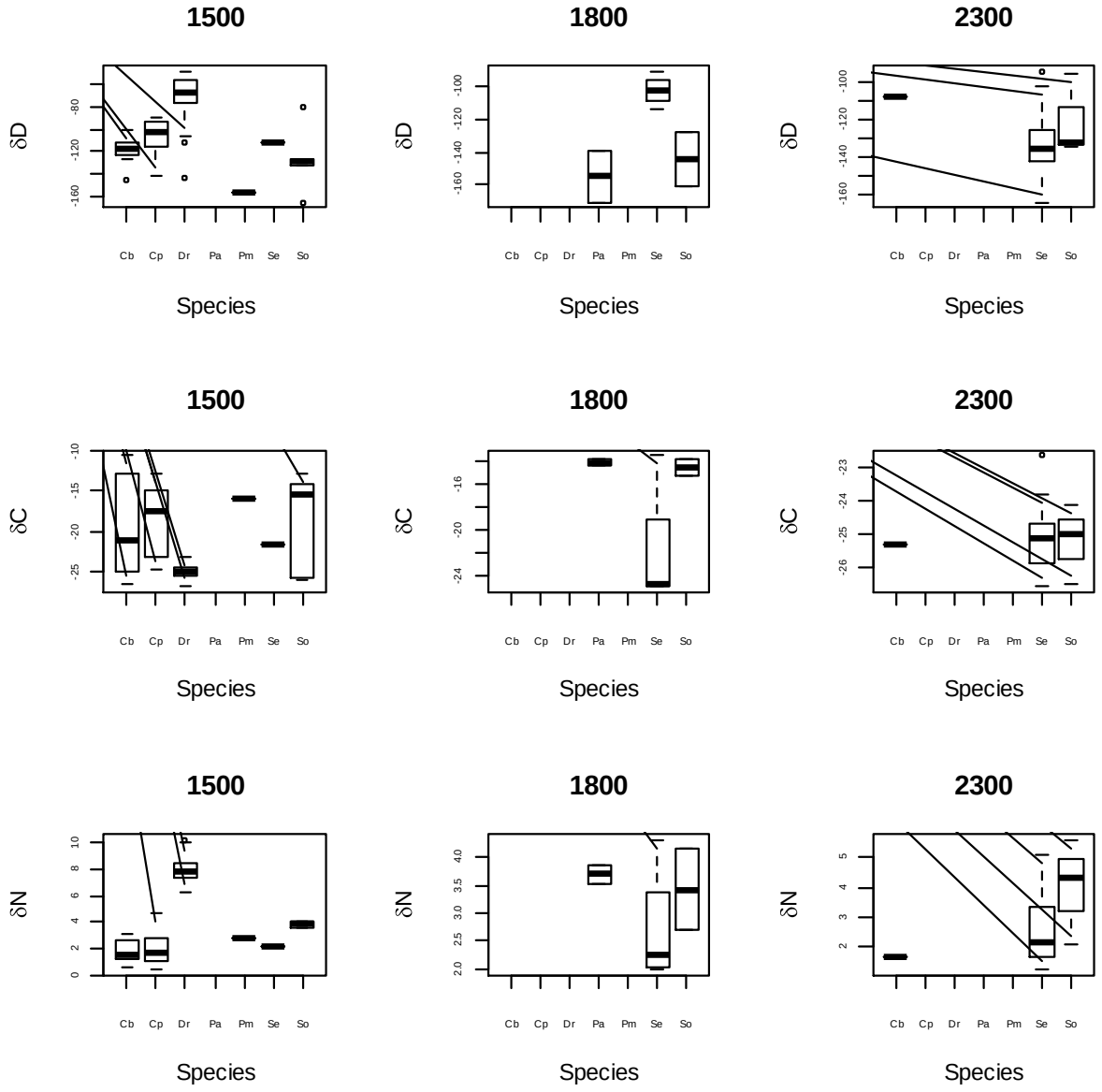

Figure 15. δD, δC, and δN ratios in frugivore species captured at 1500, 1800, and 2300 m. Boxes show 25th and 75th percentile whiskers, 5th and 95th percentiles, and solid lines in the boxes represent the median. Outliers are represented as points outside the range encompassed by the percentiles.

Differences between males and females were analyzed for species which had enough individuals for statistical analysis (Table 3). The only significant difference found was in $\delta^{13}C$ between males and females of *C. brevicauda.*

Table 3. Differences in isotope values between males and females of each species. A hyphen denotes that the test could not be run due to low samples sizes.

	δD			$\delta^{13}C$			$\delta^{15}N$		
Species	Females	Males	p-values	Females	Males	p-values	Female	Male	p-values
C. brevicauda	-117.0054	-118.3698	0.8503	-24.9225	-16.16833	0.0128*	2.2825	1.4033	0.1657
S. erythromos	-136.022	-132.1326	0.5536	-25.49083	-25.08579	0 .2075	3.165	2.2589	0.06
D. rotundus			-			-			-
A. geoffroyii			-			-			-
C. perspicillata			-			-			-
S. oporaphilum			-			-			-

Results of the relationship between body size and δD were significant (F-value = 5.09, p = 0.0287; Figure 2), but body mass explained relatively little of the variation observed in δD (R^2 = 0.0785). All species were included in this analysis, regardless of their trophic level or migratory status. After removing two individuals of *P. albericoi* with large body weights from the regression, the relationship turned to be not significant (F-value = 0.9324, p = 0.3399, R^2 = 0.0222; graph not shown).

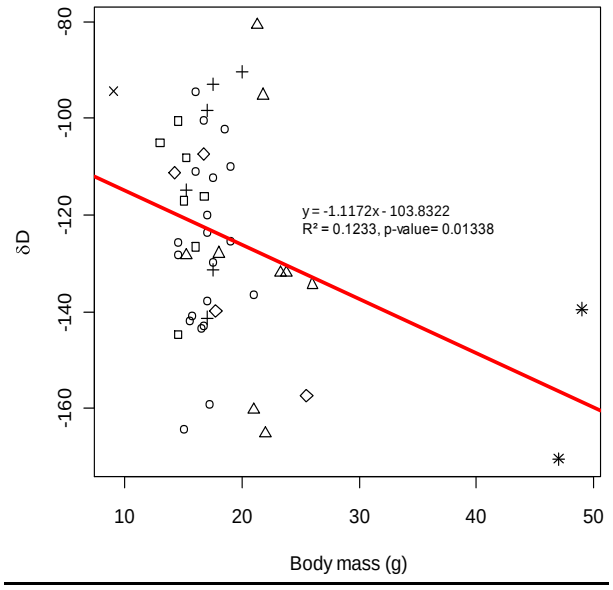

Figure 16. The relationship between δD values in bat hair and body mass (g). Different symbols represent different species (*S. erythromos*, ○, *S. oporaphilum*, Δ, *C. brevicauda*, □, *C. perspicillata*, +, *P. albericoi*, *, *P. masu*, ◊.

Goal 1: Trophic differences between frugivores and sanguinivores

Significant differences in isotope values (δD, $\delta^{13}C$, and $\delta^{15}N$) were found between the sanguinivorous *D. rotundus* and the 3 frugivores (*C. brevicauda, C. perspicillata*, and *S. oporaphilum*), except for $\delta^{13}C$ betwen *D. rotundus* and *S. oporaphilum* (Table 4). ANOVAs could not be run between *D. rotundus* and *S. erythromos* and *A. geoffroyii* (1500 m) because of low number of individuals.

δD enrichment (more positive) in keratin of sanguinivores relative to those of frugivores that co-existed at an altitude of 1500 m. ranged from 35.88 to 54.21‰. In terms of δC, there was a large difference between *D. rotundus* and that of the syntopic fruit-eating species (6.06‰ for *S.*

oporaphilum, 5.14‰ for *C. brevicauda*, and 5.9‰ for *C. perspicillata*). Differences in $\delta^{15}N$ between the sanguinivore and the 3 syntopic frugivores were 4.22‰ for *S. oporaphilum*, 6.02‰ for *C. perspicillata*, and 6.26 ‰ for *C. brevicauda*.

Table 4. Significance values for each ANOVA comparing *D. rotundus* to frugivorous bat species. A hyphen denotes that the test could not be run due to low sample sizes. * and ** represent a significant and highly-significant p-values respectively.

Species compared		p values		
		δD	$\delta^{13}C$	$\delta^{15}N$
D. rotundus	*C. perspicillata*	0.0008**	0.0071*	0.0002**
D. rotundus	*C. brevicauda*	<0.0001**	0.0287*	0.0001**
D. rotundus	*S. oporaphilum*	<0.0001*	0.0967	<0.0001**
D. rotundus	*S. erythromos*	-	-	-
D. rotundus	*A. geoffroyii*	-	-	-
C. brevicauda	*C. perspicillata*	0.7712	0.7645	0.657
C. brevicauda	*S. oporaphilum*	0.8693	0.7997	<0.0001**
C. perspicillata	*S. oporaphilum*	0.4210	0.9639	0.0046*

Goal 2: Relationship between altitude and δD in precipitation

Based on monitoring stations located along an altitudinal gradient in Peru (Figure 3), I derived an equation that predicts the expected relationship between elevation (E) and δD_p:

$$\delta Dp = -0.0255(Elevation) - 17.53$$

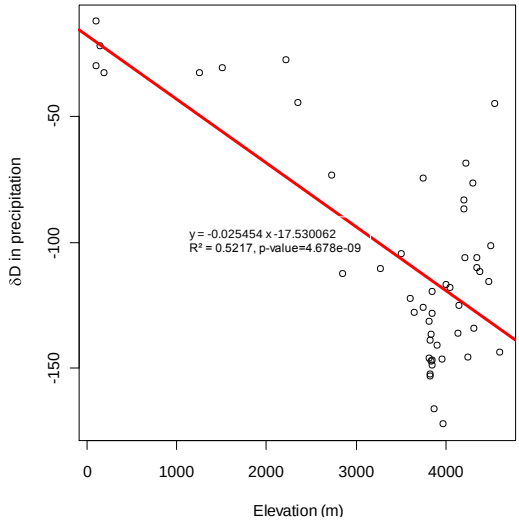

Figure 17. δD in precipitation based on data from Peruvian stations across an altitudinal gradient.

Significant differences were found when expected δD_h values (from above equation) for species at a given altitude were compared to the actual observed values using a t-test. δD_h values for *S. erythromos* captured at 1800 m (t = 5. 174, p = 0.0138 when using a -40‰ trophic factor, and t= 6.2593, p = 0.0081 when using a -45‰ trophic factor) and at 2300 m (t = 2.0523, p = 0.0491 when using a -40‰ trophic factor, and t = 3.8242, p = 0.0006 when using a -45‰ trophic factor) were significantly different from expectations; these values were more negative than expected (see Appendix, Tables S4 and S5). None of the other species compared resulted in significant differences. These values suggest that hair of *S. erythromos*, in both cases, was synthesized at higher elevations, potentially between 70 and 274 m higher than the 1800 m point (i.e. at altitudes of 1870-2074 m) and between 478 to 684 m higher than the 2300 m point (i.e. at altitudes of 2778- 2984 m) depending on the trophic factor used.

However, the short distance plus the lack of information on the isotopic composition of this species' diet or drinking water prevent a conclusive designation of *S. erythromos* as a migratory species. Therefore, analyses that tested the relationship between δD, $\delta^{13}C$ and $\delta^{15}N$ and altitude (goal 3) were run for frugivores under two scenarios, one that includes *S. erythromos*, and one that excludes it.

Goal 3: Relationship between altitude and δD, δC and δN in bat tissues

Analyses including *S. erythromos*

The relationship between δD values in bat hair along an altitudinal gradient was negative and significant, but elevation did not explain much of the variation in δD ($R^2 = 0.1001$, $p = 0.008$; Figure. 4).

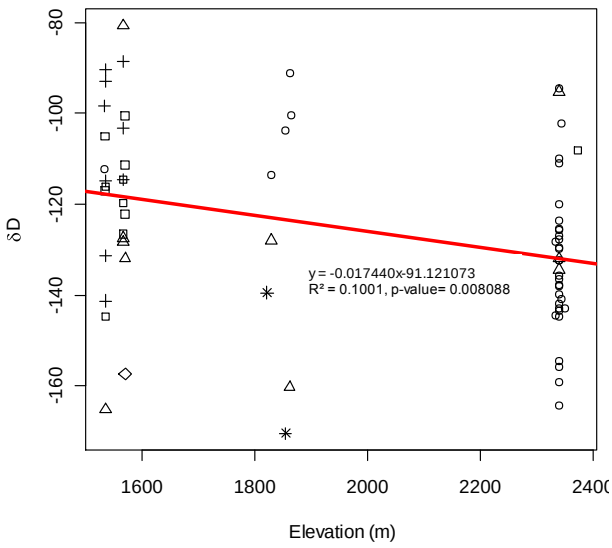

Figure. 18. The relationship between δD values in bat hair of all frugivore species along an altitudinal gradient. Different symbols represent different species (*S. erythromos*, ○, *S. oporaphilum*, Δ, *C. brevicauda*, □, *C. perspicillata*, +, *P. albericoi*, *, *P. masu*, ◊.

The relationship between $\delta^{13}C$ isotope values in bat hair along an altitudinal gradient was negative and significant, and elevation explained much of the variation in $\delta^{13}C$ (before log-transformation: $R^2 = 0.385$, $p < 0.0001$; after log-transformation: $R^2 < 0.0001$, $p = 8.64$ e-09; Figure. 5).

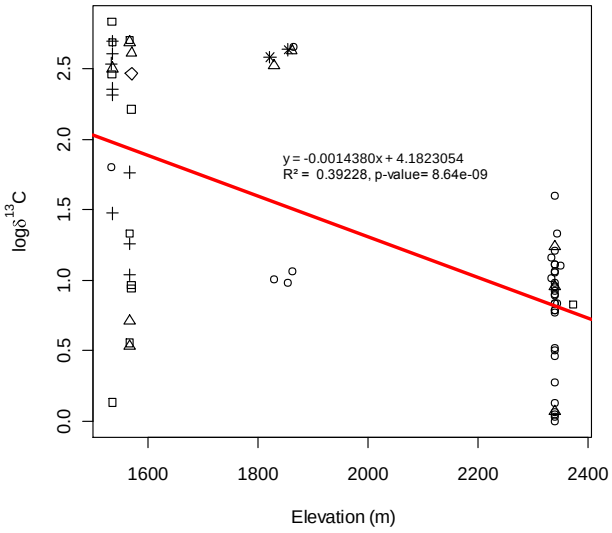

Figure. 19. The relationship between log $\delta^{13}C$ values in bat hair of all frugivore species along an altitudinal gradient. Different symbols represent different species (*S. erythromos*, ○, *S. oporaphilum*, Δ, *C. brevicauda*, □, *C. perspicillata*, +, *P. albericoi*, *, *P. masu*, ◊.

The relationship between $\delta^{15}N$ values in bat hair along an altitudinal gradient was positive and not significant (before log-transformation $R^2 = 0.0177$, p=0.2751; after log-transformation: $R^2 = 0.03413$, p = 0.1286; Figure. 6).

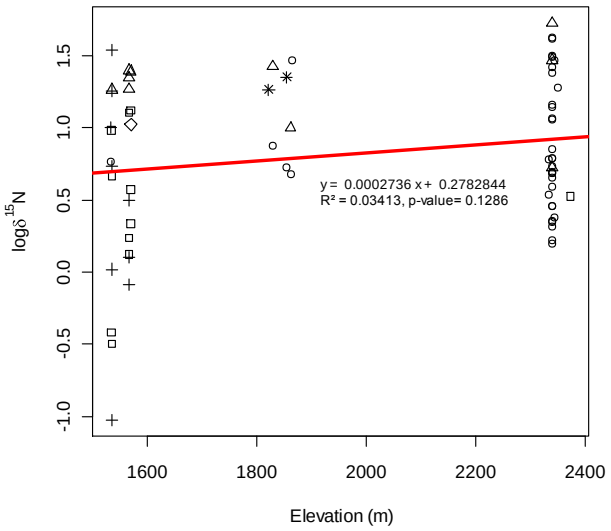

Figure 20. The relationship between $\delta^{15}N$ values in bat hair along an altitudinal gradient. Different symbols represent different species (*S. erythromos*, ○, *S. oporaphilum*, Δ, *C. brevicauda*, □, *C. perspicillata*, +, *P. albericoi*, *, *P. masu*, ◊.

Analyses run excluding S. *erythromos*

In addition to the analysis above, I decided to take a conservative approach and run analyses that tested the relationship between δD, $\delta^{13}C$ and $\delta^{15}N$ and altitude (goal 3) for frugivores excluding *S. erythromos*. It was excluded because the short distance plus the lack of information on the isotopic composition of this species' diet or drinking water prevented its conclusive designation as a migratory species.

The relationship between δD values in bat hair along an altitudinal gradient was not significant ($R^2 = 0.0098$, $p = 0.5824$; Figure 7).

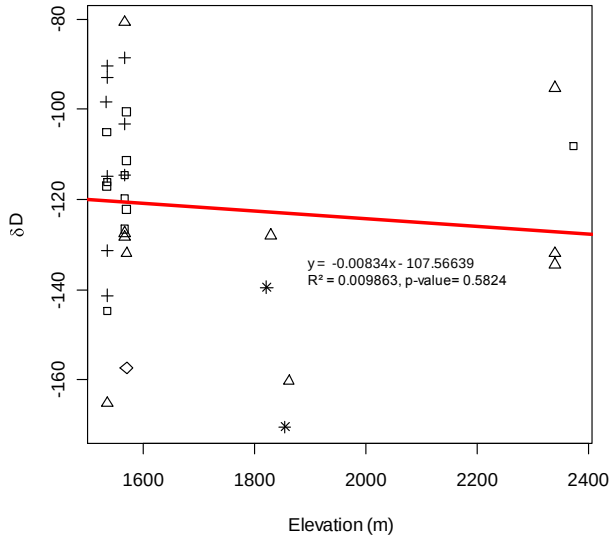

Figure 21 The relationship between δD values in bat hair along an altitudinal gradient. Different symbols represent different species (*S. erythromos*, ○, *S. oporaphilum*, Δ, *C. brevicauda*, □, *C. perspicillata*, +, *P. albericoi*, *, *P. masu*, ◊.

The relationship between $\delta^{13}C$ values in bat hair along an altitudinal gradient was negative and not significant (before log-transformation: $R^2 = 0.0938$, $p = 0.0828$; after log-transformation $R^2 = 0.1108$, $p = 0.05842$; Figure 8).

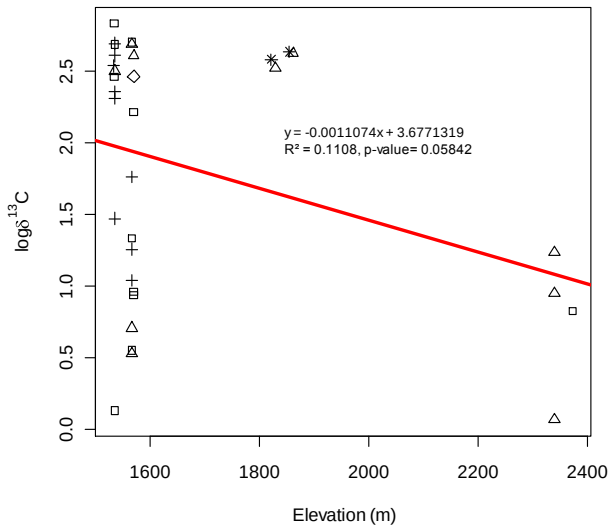

Figure 22. The relationship between log $\delta^{13}C$ values in bat hair along an altitudinal gradient. Different symbols represent different species (*S. erythromos*, ○, *S. oporaphilum*, Δ, *C. brevicauda*, □, *C. perspicillata*, +, *P. albericoi*, *, *P. masu*, ◊.

The relationship between $\delta^{15}N$ values in bat hair along an altitudinal gradient was not significant (before log-transformation: $R^2 = 0.1054$, p= 0.06529; after log-transformation $R^2 = 0.08466$, p = 0.1009; Figure 9).

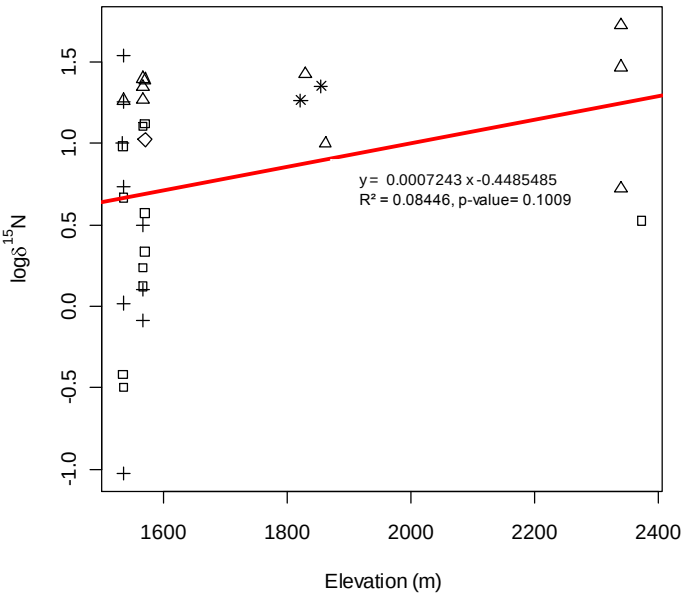

Figure 23. The relationship between log δ^{15}N values in bat hair along an altitudinal gradient. Different symbols represent different species (*S. erythromos*, ○, *S. oporaphilum*, Δ, *C. brevicauda*, □, *C. perspicillata*, +, *P. albericoi*, *, *P. masu*, ◊.

Discussion

I found that hydrogen and nitrogen isotopes in the sanguinivore control were enriched relative to those of the syntopic frugivores. Carbon isotopes in the sanguinivorous bat were depleted when compared to those of frugivores. In general, no significant differences in any of the three isotopes were found between sexes. Body mass and hydrogen isotopes were found to be correlated with each other. There is isotopic evidence that *S. erythromos* displays short altitudinal movements. The expected tendency for depletion of hydrogen isotopes along altitudinal gradients holds true for precipitation and this tendency was passed to bats. I conclude that in spite of the wide array of physiological and environmental factors producing temporal and spatial variation, the analysis of hydrogen isotopes is a promising tool to study altitudinal

migration when used over large distances and including large sample sizes. Neither stable isotopes of nitrogen or carbon appear to be reliable to track movements along short gradients such as those along mountains. The contrast of these findings with the results of previous studies suggests that isotopic gradients may be specific to given taxon and localities.

In this study, the characterization of the isotopic composition of the population was emphasized with the overall goal of gaining a deeper understanding of the intra and interspecific variation that exists in bat population at different altitudes. Recent studies have raised concerns regarding the assumptions underlying the use of stable isotopes to study migration (Betini et al. 2009, Voigt et al. 2013a). As such, I investigated whether a strong relationship between isotopes and altitude existed that could be used in studies of altitudinal migration. Differences in δD found between trophic groups are the first reported for the species studied and support results found elsewhere in the Neotropics (Fraser et al. 2010; Erzberger et al. 2011). In addition, I presented the first physical evidence of bat altitudinal movement in South America.

Considering only frugivores, greater intraspecific variation in all 3 isotopes (δD, 85‰, $\delta^{15}N$, 4.29‰, $\delta^{13}C$, 15.85‰) was found at low altitudes (1500 m), which likely corresponds with greater resource variety as compared to higher altitudes which may contain fewer plants and/or fruits. This observation is particularly true for $\delta^{13}C$ values which are incorporated into tissues from the animal's diet. Therefore, bat altitudinal movements suggested in this study could be displayed to take advantage of food resources as has been widely observed in birds.

No significant differences were found between sexes except for $\delta^{13}C$ in *C. brevicauda*. All females of this species (n = 4) showed more depleted tissues corresponding to a C_3

photosynthetic pathway. Among males (n = 6), they show a range that seems to represent a variety of C_3 and C_4 plants (Kelly 1999). These results point towards differences in diet, but the small sample size prevents firm conclusions. Differences in migratory patterns and seasonal distribution between males and females have been observed along latitudinal (Britzke et al. 2009, Cryan 2003) and altitudinal (Barclay 1991, Cryan 2000) gradients in temperate ecosystems. In the Neotropics, gender segregation has been recorded in *Pygoderma bilobatum*, where females are more abundant at intermediate and high elevations, and males at low elevations. Differences in food resources found at these elevations could explain the observed statistical difference in $\delta^{13}C$.

A significant correlation between body mass and δD values ($p = 0.02$) was found; however, body mass did not explain much of the variation in δD values ($R^2 = 0.07$). When the two largest individuals were removed from the relationship, the resulting correlation was not significant. Betini et al. (2009) suggested that body size could influence δD through evaporative water loss. Their hypothesis was that larger birds are more impacted by temperature and display higher rates of water loss due to evaporation than small birds, meaning that light isotopes evaporate at a higher rate than heavy isotopes (kinetic fractionation), resulting in δD-enriched tissues. Similarly, Topalov et al (2013) found a strong relationship between body size and bone collagen in marine and terrestrial vertebrates. If the largest individuals are considered, my results agree with these two aforementioned studies, but contradict those found in bats by Voigt et al. (2013a) who could not find a relationship between either body water (blood) or keratin (fur) and log-transformed body mass.

Goal 1: Trophic differences between syntoptic bats

Trophic differences in δD

Results show that trophic differences in δD between frugivores and sanguinivores ranged between 35.88‰ and 54.21‰. These are the first values reported for these particular species, the first reported for bats in South America, and agree with recent observations in other Neotropical bats.

δD enrichment (more positive) in sanguinivorous relative to those of frugivores ranged from 35.88 to 54.21‰. Fraser et al. (2010) did not directly address trophic differences, but they provided δD values for the hair keratin of the sanguinivorous *D. rotundus* (-37.4‰) and *Artibeus toltecus* (82.4‰), a species that has been also considered sedentary (Erzberger et al. 2011). The values reported represent a difference of ~ 45‰ between trophic groups. Ezberger et al. (2011) found a very similar enrichment rate (37.8‰) when comparing the sedentary and syntopic species *A. toltecus*, a fruit-eating bat, with the insectivorous *Mycronycteris microtis* in Honduras. Similarly, Voigt et al. (2013a) reports the enrichment in δD of secondary consumer tissues of 44‰ relative to those of primary consumers.

In central Peru, *D. rotundus* keratin was enriched by up to 55‰ relative to that of frugivorous *S. oporaphilum*, *C. brevicauda*, and *C. perspicillata* that co-existed at an altitude of 1500 m. These values are slightly higher than those previously reported (Ezberger et al. 2011, Fraser et al. 2010, Voigt et al. 2013a). In addition to trophic effects, other factors could have

increased the expected level of enrichment pattern in tissues of secondary consumers. Fraser et al. (2011) observed differences in δD values between birds foraging on the ground in the forest interior vs. those foraging above ground in open areas. These δD patterns resulted from the differences between enriched above-ground soil moisture which is influenced by fog drips (fog droplets that accumulate in foliage and drip to the ground), and depleted on the ground soil moisture influenced by precipitation and ground water (Liu et al. 2007) and differences in temperature between forest interior and open areas. This mechanism would result in tissues of frugivorous and nectarivorous bats being depleted relative to those of the sanguinivorous bat. Frugivores feed on plants with deeper roots that connect to depleted ground water while *D. rotundus* feeds on cattle that feeds on grasses with shallow roots that are mostly supplied with enriched forest fog mist (i.e., continual evapotranspiration increases δD over time; K. Fraser pers. comm.). The impact that these microgeographic differences could have on migration studies have resulted in the improvement of the North American latitudinal model of δD patterns by considering ground vs. above ground foraging species separately (Hobson et al. 2012).

It has been suggested that evaporative water loss produces enriched δD values in tissues such as blood (McKechnie et al. 2004) and potentially fur, which is particularly true in places with low levels of humidity (Hardesty and Fraser 2010). The dehydration that hummingbirds undergo overnight results in higher δD values (Hardesty and Fraser 2010). Similar dehydration might occur in *D. rotundus*, which is known to starve to death if it does not feed in periods of time as short as two days (Wilkinson 1984). Lack of information on the humidity level of the study area precludes further conclusions about the physiological effects of evaporation on the enrichment of δD values in *D. rotundus*.

This enrichment pattern due to trophic effects had previously been suggested by Birchall et al. (2005) for carnivores and herbivores but to a larger degree than the one found in this study. Moreover, in a review of a variety of marine and terrestrial species, Topalov et al. (2013) reported similar enrichment in bone collagen in higher trophic levels.

Trophic differences in $\delta^{13}C$

No significant differences in $\delta^{13}C$ were expected between frugivores and sanguinivores because trophic levels have shown to have little or no effect (0-1‰) on the assimilation of $\delta^{13}C$ in tissues (McCutchan et al. 2003). The large difference between *D. rotundus* and that of the three syntopic fruit-eating species (5.14‰ for *C. brevicauda*, 5.9‰ for *C. perspicillata,* and 6.06‰ for *S. oporaphilum*) found in this study likely results from the consumption of foods derived from both C_3 and C_4 plants by frugivorous bats, which produced a wide range of $\delta^{13}C$ values. $\delta^{13}C$ values reported for terrestrial C_3 plants (mean $\delta^{13}C$ = –27‰, range from –35 to –21‰) can be easily distinguished from those of C_4 plants (mean $\delta^{13}C$ = –13‰, range from –14 to –10‰; Kelly 1999). It seems that the frugivores in this study consumed fruits and insects associated with a variety of C_3 and C_4 plants ($\delta^{13}C$ ranges for *C. prespicillata* = -24.77 to -12.8‰, *C. brevicauda* = -26.46 to -10.61‰, and *S. oporaphilum* = -25.9 to -12.91‰) and that surprisingly, the sanguinivorous bat ($\delta^{13}C$ range -23.23 to -26.73‰) fed on cows that foraged mainly on C_3 plants. Plants from the following families have been recorded near the sampling areas: Piperacea, Rubiacea, Clusiacea, Melastomatacea, Malvacea, Sapindacea, Fabacea, Aracea, Lauracea, Cecropiacea, Euforbiacea, some pteridophytes and Poaceae (S. Refulio pers.comm.). Most C_4 plants are members of the Poaceae family which includes several species of grasses.

Most species of the other families are C_3 plants (shrubs, herbs, trees). An exhaustive classification and quantification of plant types in the study area was not conducted, so the contribution of each of them to the local foodweb remains unknown.

Trophic differences in $\delta^{15}N$

Differences in $\delta^{15}N$ between the sanguinivore and the 3 syntopic frugivores (4.22, 6.02, and 6.26 ‰) were larger than average differences reported for bats in Costa Rica (~3.7‰; Voigt et al. 2013a). Studies focused on other taxa have reported trophic differences in the range of 3-4 ‰ (see Vanderklift and Ponsard 2003), but these studies usually compared consumers and their diets, rather than two different consumers with different diets as in the present study. Moreover, high variability in this trophic effect on $\delta^{15}N$ has been reported (1 - 6‰; Peterson and Fry 1987, Post 2002).

The $\delta^{15}N$ values for individual species were within the ranges expected for primary (*C. brevicauda*, 0.61-3.07‰, *C. perspicillata,* 0.36-4.65‰, *S. oporaphilum* 3.54-4.04‰) and secondary consumers (*D. rotundus,* 6.88-10.27‰). However, these values were lower than those of species captured in a national park in Costa Rica (7-8 ‰ for frugivores; Voigt et al. 2013a). Depleted values of $\delta^{15}N$ are likely caused by the fact that bats in this study were captured near areas potentially impacted by agriculture, where fertilizers, which are known to have a depletion effect have been applied ($\delta^{15}N$ values of synthetic fertilizers fall within a narrow range between -2 and 2 ‰; Bateman and Kelly 2007).

Another factor that could have created this large difference between trophic groups is the fact that starved animals (Scrimgeour et al. 1995) or animals under nutritional stress (Hobson and Clark 1992, Voigt and Matt 2004) show enriched values of $\delta^{15}N$. The dependence of *D. rotundus* on a single and very specific food item could produce short periods of starvation (Freitas et al. 2005) that could enrich the $\delta^{15}N$ in its tissues and therefore, increase the difference with values of frugivores.

Previous works have shown that $\delta^{15}N$ values in soil are enriched relative to leaves of high trees (Hyodo et al. 2010). Assuming that fruits in the canopy are similarly depleted relative to soil, then frugivores that forage at different levels of the forest would have depleted $\delta^{15}N$ values relative to those of *D. rotundus* that feeds on cattle that feed at ground level.

Goal 2: Potential evidence of migration

Data regarding the migratory status of Neotropical bats is either missing or contradictory. In general, bats have usually been classified as sedentary, medium, or long-distance migrants (Fleming and Eby 2003). Sedentary bats are said to move within ranges of less than 50 km. In the Andes, where the maximum altitudinal distance to move across is 6 km (the height of the highest mountain in the Andes), smaller scales should be applied to classify the movement of bat species. Lack of information on several species, especially in the Andes and Amazon forest, makes the classification between sedentary and migratory species a difficult task with sometimes confusing results. For instance, Voigt et al. (2013a) classified species in Costa Rica as local

(those that forage within a 4 km radius) or regional (those that go beyond 4 km). Species of *Carollia* and *Platyrrinus* were classified as local as opposed to a regional or migratory status for *D. rotundus*. *Carollia* and *Sturnira* were classified as "sedentary" as opposed to nomadic by Soriano (2007) in a study of Neotropical bat assemblages where boundaries in flying distances that distinguish these two classes were not provided. Erzberger et al. (2011) classified several species of *Sturnira* as "potential migrants" because no data about their movements was available. Based on the large scale classification (50 km), *D. rotundus* is a sedentary species; however, it is known to have a flight capacity that allows it to move among roosts within a 2-3 km radius (Trajano 1996) or to cover foraging distances over 5 km (Soriano 2007). On the other hand, it is assumed that *D. rotundus* is very dependent on their cattle prey, which suggests the species has a sedentary life-style (Bejarano-Bonilla et. al. 2007).

Similar to what happens with their migratory status, not much is known about the home ranges of Neotropical bat species, especially in South America. It usually is assumed that smaller species (e.g., *Glossophaga* and *Mesophylla*) have ranges up to a radius of 1 km (Klingbeil and Willig 2010). Insectivorous birds are less likely to display altitudinal movements than their frugivorous or nectarivorous counterparts (Levey and Stiles 1992), and this observation seems to apply to bats as well, with gleaning insectivorous, such as *Lophostoma silvicolum*, foraging within only 500 meters from its roosts (Kalko et al. 1999). In this study all species, except *D. rotundus*, were frugivores or nectarivores, which makes them potential migrants of distances probably longer than 1 km.

The same approach used by Hobson et al. (2003) and Fraser et al. (2010) was followed here to evaluate potential migration. δD values in precipitation were plotted against the elevation of the monitoring stations that collected the precipitation and obtained a highly significant regression equation. The resulting equation, which agrees with the known global tendency for depletion in δD with altitude, was very similar to the one found by Hobson et al. (2003) in the eastern slope of the Andes of Ecuador. I found that the keratin of *S. erythromos* captured both at 1800 and 2300 m were significantly more enriched (positive) than expected, suggesting synthesis at higher elevations than the site of capture, potentially between 70 to 680 m higher up. The maximum altitudinal distance potentially traveled from the 2300 capture site to the top of the mountain at 2800 m was 500 m, which is within the range predicted by the analysis. Moreover, the fact that δD values of populations of the same species at two different altitudinal points deviated from the expected values would support their migratory condition and the hypothesis that their tissues were synthesized at a different place from where they were captured. The assimilation of hydrogen isotopes in hair keratin is a process that takes several days or weeks depending on the length of the molting period. Assuming that during this period bats do not display migratory movements, just as it happens with bats from temperate forest, then, differences found between observed and expected hydrogen values should represent seasonal rather than daily movements. A study by Fleming et al. (1972), reported that average horizontal distance traveled by bats was as follows: 167 m. for *C. perspicillata*, 270 m. for *S. lilium*, and 249 m. for *D. rotundus* which potentially means very small home ranges. Therefore, the reported movement distance (70-680 m) by *S. erythromos*, a medium-sized bat, would not be considered too short. However, the intraspecific variation in δD in this study was found to be too large (SD

= 15.71) to conclusively say that the difference between expected and observed δD values was due to altitudinal movements. Even the control species showed high variation values (SD = 23.38). In Honduras, Erzberger et al. (2011) found that the large variation in δD of *A. toltecus* was too large to distinguish two populations (SD=23.9 and 17.4) captured at altitudinal points that differed by 400 m.

The causes of animal migration along altitudinal gradients are unknown (Boyle et al. 2010). In birds, migration involves the movement of individuals between higher elevation breeding ranges and lower elevation nonbreeding ranges and there is evidence that uphill and downhill movements are driven by different reasons (Boyle 2010). There is a series of hypotheses that have been postulated to explain altitudinal migration in birds. Food abundance has been pointed as the main factor driving uphill and downhill migration, mainly because altitudinal migrants are usually frugivores and nectarivorous that depend on resources whose abundance varies seasonally (Boyle 2010). The dominance hypothesis (Lundberg 1985) explains the migration of younger individuals driven by their competition with older, more dominant ones. The arrival time hypothesis (Ketterson and Nolan 1976) refers to male competition for mating sites. Predation risk has been another factor used to explain altitudinal migration (Boyle 2008a). In spite of the low numbers of bats killed by predators, this mortality has a strong effect on the populations because these are long-lived animals. The limited foraging opportunities hypothesis (Boyle 2008b, Boyle et al. 2010) states that individuals are forced to migrate because the weather (e. g. storms) reduces their foraging opportunities, and it's the hypothesis that has been better supported by evidence. Among these hypotheses, the ones that could potentially explain migratory movement in bats are the predation risk, resource use and the limited foraging

opportunities hypotheses, because bats do not compete for mating sites. The lack of information on age of the individuals precludes conclusions that could support the dominance hypothesis.

In the Atlantic forest, the bat species *P. bilobatum* seems to prefer warmer temperatures through the year and migrates from higher elevations during the colder months (Esberard et al. 2011). Similar observations have been made on *S. lilium* in southeastern Brazil, even though food resources did not decrease at higher elevations during the colder months (Mello et al. 2008). Temperature plays an important role in the physiology of bats (Speakman and Thomas 2003); especially at higher elevations where low temperatures have been shown to shorten the reproductive season (Mello et al. 2009). Lower temperatures, which relates to the limited foraging opportunities, could have driven the observed migration of bats in this study from higher elevations to lower regions. Even short distances with differences in temperatures as low as 2°C have been observed to drive bat altitudinal movements (Esberard et al. 2011).

Goal 3: Relationship between altitude and δD, $\delta^{13}C$ and $\delta^{15}N$ in bat tissues

Hydrogen

A negative relationship between δD and altitude (22% per 1000 m or 2.2% per 100 m) was predicted, according to what has been found in sedentary hummingbirds in Ecuador (Hobson et al. 2003). Interestingly, a strongly significant and negative correlation was found when the potentially migratory species was included in the analysis, even though altitude did not explain much of the variation in δD. When this species was removed, the results followed the same trend

but were not significant. It is concluded this is because of an "averaging effect", large sample sizes are required to be able to observe trends that are otherwise masked by factors such as trophic level, intraspecific variation, variability in precipitation, and more importantly the daily movements of single or few individuals or the population as a whole within their regular home range for foraging or roosting purposes. Similar conclusions were reached by Topalov et al. (2013) in a study covering a large group of aquatic and terrestrial animals. Fraser et al. (2010) and Erzberger et al. (2011) were not able to find a relationship between δD and altitude which was possibly caused by the narrow altitudinal ranges addressed (a single point or a range <800 m). More recently Voigt et al. (2013b) did not find a correlation between δD in bat fur and keratin and altitude along the Kilimanjaro Mountain.

In this study, I hypothesized that the observed tendency for δD depletion with altitude is passed through the food web from precipitation to plants and then into animals. The global depletion tendency was supported for Peru from data collected from monitoring stations. Additionally, this study focused on the Eastern slope of the Andes, a region where values of δD in precipitation are known to display a strong altitudinal gradient (Hardesty and Fraser 2010); therefore there was a high probability of finding the expected trend of δD depletion with altitude. In spite of potential local or regional variation (Hardesty and Fraser 2010) and other limitations of the analysis of stable isotopes (Farmer et al. 2008), a strong relationship between δD and altitude was found along a slope in the Peruvian section of the eastern Andes even when species from different genera where pooled together.

However, intraspecific variation in all species was observed to be higher than the 2.2% per 100 m expected increase of δD. I could not run any comparison between populations of the same species at two different altitudes due to low number of individuals captured but the high variation would have affected any analysis of this type.

Below, the factors that could have caused the observed levels of intra and interspecific variation in δD values are explored in detail.

Environmental factors

One hypothesis to account for the high variation observed in this study is that resources at a single altitudinal point could display a high degree of variation in δD and that this variation could be passed to consumers (Erzberger et al. 2011). Indeed, species of the genera *Carollia* and *Sturnira* are known to consume a large variety of fruits (Bejarano-Bonilla et al. 2007) and also insects (Arias 2008, Giannini and Kalko 2004). This variation could not only be produced by different plant species having variable δD values but also by different degrees of insect consumption, which even in small quantities, could add variation to the overall δD values in bat tissues. An isotopic analysis of potential local foods, fruits and insects, would increase the level of understanding that their variation in δD has on bat tissues.

Another factor that could influence the observed variation in δD in bat tissues is the large degree of short-term, intra and inter-annual variation in δD values in precipitation observed even within a single monitoring station (IAEA 2013). It is known that at some degree this variation is linked to the amount of precipitation, a mechanism known as the "amount effect", which implies higher levels of heavy isotopes during months with low rainfall (Dansgaard 1964). This

mechanism probably does not affect latitudinal or migrational estimates of larger scale but might have a large impact on short-distance estimates.

δD in the keratin of bat hair originates from drinking water (1/3) and diet (2/3) (Hobson et al. 1999). If bats forage in an area and drink water from another, and these two sources differ in their isotopic composition, then this would result in the assignment of animals to wrong sites of origin.

Physiological factors

The most important factor that could hinder the interpretation of the results is the lack of information on molting and reproduction. Molting is a key factor underlying the assumptions of the use of stable isotopes and it is related to reproduction and migration in birds and temperate forest bats. It is known that these bats and birds molt before migration (Voigt et al. 2013a) and that molting does not overlap with reproduction; species may interrupt the molting cycle to reproduce (Dwyer 1963). Molting has a very high energetic cost (Lindstrom et al. 1993), which may force individuals to move to places where resources are more abundant (Hobson et al. 2003). This need for additional resources would be magnified by the increase in energy requirements due to reproduction which also happens before migration. There is a complete absence of records on molting in tropical species and it has even been suggested that molting does not occur (V. Pacheco and S. Solari [Museum of Natural History, Peru], pers. comm.).

Physiological differences could have also been the source of the observed δD variation. Individuals in this study were separated based on species and gender; parameters such as age or reproductive status were not recorded. Lactating female bats require more water than non-

reproductive females (Adams and Hayes. 2008). This could have a large effect on the non-exchangeable δD of bat tissues as these values depend on diet and drinking water. Furthermore, first-time bird breeders have depleted δD in feathers relative to adults (Studs et al. 2012) and in marine mammals bone collagen of adults are enriched in δD relative to those of pups (Topalov et al. 2013). Bats were mist-netted during the dry season (October), when young bats (born and lactated during the previous rainy season) could have potentially been present. In Costa Rica, for instance, *C. perspicillata* and *S. lilium* have their young and lactate between January and July (Fleming et al. 1972). Juveniles may have different diets than their parents and their first keratin is probably influenced by the lactation period when the food source of the mother is passed to the young with the resulting additional fractionation process. Therefore, first-year bats may have different, depleted, δD values than adults.

In other studies (Hardesty and Fraser 2010), the lack of correspondence between the standards used during the running of the stable isotope analysis in the lab and the samples was identified as a factor contributing to the variation in δD. In this study, the δD values of the standards (-54, -114, -117, and -196) showed great correspondence with the range values of the samples (from -48 to -170).

Carbon

The expected tendency for $\delta^{13}C$ was an enrichment of 1.1-1.5% per 1000 m increase in elevation (Graves et al. 2002, Hobson et al. 2003). The altitudinal range in this study covered 800 m, and thus, the expected variation in carbon was probably too small to be observed. Moreover, my results show a significant depletion tendency rather than enrichment. The large

variation in values of $\delta^{13}C$ in tissues of frugivores (from -10 to -25%) suggests the utilization of fruits from both C_3 and C_4 plants. Graves et al. (2002) conducted their study in a forest known to be dominated by C_3 plants where agricultural crops, which are mostly C_4, had not been cultivated in decades. Hobson et al. (2003) did not have information on the proportion of C_3 or C_4 plants along their altitudinal transect but their results demonstrated the dominance of C_3 plants. In the study area, plants from both photosynthetic pathways have been observed (see Goal 1) and their contribution to bat diets is unknown. My results contrast with those found by Voigt et al. (2013b), who observed a correlation between $\delta^{13}C$ and altitude and tissues of frugivorous bats, but not for insectivorous, in the Kilimanjaro Mountain. Similarly, Chang et al. (2011) reported an increase in $\delta^{13}C$ with increasing elevation for herbivorous and omnivorous but not for insectivorous birds in the mountains of Taiwan.

Nitrogen

No significant correlations were found between $\delta^{15}N$ and altitude. Previous studies showed weak evidence of depletion of $\delta^{15}N$ with altitude in soil (Mariotti et al. 1980), birds in the Andes (Graves et al. 2002) and grazers and grasslands in the European Alps (Männel et al. 2007). Trophic effects are the main mechanism influencing $\delta^{15}N$ (Kelly 2000) which probably masks any other type of relationship. Voigt et al. (2013b) reported a correlation between $\delta^{15}N$ and frugivorous and insectivorous bats along the Kilimanjaro Mountain. Similarly, Chang et al. (2011) found that in Taiwan, $\delta^{15}N$ decreases with elevation in tissues of insectivorous birds but not in herbivores or omnivores. In the Eastern slope of the Andes, Hobson et al. (2003) did not find an elevational gradient in $\delta^{15}N$ in tissues of hummingbirds.

Recommendations

Future studies should focus on species that display fewer movements, such as insectivorous bats and *D. rotundus*, to establish a better relationship between isotopes and altitude. Isotopic values in the tissues of sedentary species should not vary due to migratory movements and are expected to reflect values characteristic of the altitude where they were captured. Bats with different diets should also be examined to clarify the influence of trophic effects. Active tissues, such as blood, correspond better with the site of capture due to a shorter half-life (a few days) and could add additional information to that obtained from inert tissues such as hair. These two tissues should be collected together at different points during the species' annual cycle, especially if no information on molting is available. Sources of variation such as gender, age, and reproductive status should be investigated, preferably within each species and each trophic group. Local precipitation should be sampled for a more accurate representation of local values and to avoid spatial and temporal variation due to the use of records from monitoring stations (see Farmer et al. 2008 and Fraser et al. 2010). Local sources of food (insects and fruits) along gradients should be analyzed to see if they correspond with the expected local isotope values. I also recommended evaluating the relationship between isotopes and altitude over larger distances and including larger sample sizes.

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Appendix 1: Minimum and maximum δD, δC, and δN values at different altitudes for each of the species at 1500 m.

Table S1. δD, δC, and δN range values in frugivorous species captured at 1500 m. The difference between the minimum and maximum isotope values is "difference" and this value expressed as percentage is "difference (%)".

	Low (1500 m)											
	δD				δC				δN			
Species	Min	Max	Difference	Difference (%)	Min	Max	Difference	Difference (%)	Min	Max	Difference	Difference (%)
Carollia perspicillata	-141.3	-88.67	-52.63	62.75	-24.77	-12.8	-11.97	51.68	0.36	4.65	-4.29	1290.92
Carollia brevicauda	-144.71	-100.57	-44.14	69.50	-26.46	-10.61	-15.85	40.10	0.61	3.07	-2.46	503.28
Sturnira erythromos												
Sturnira oporaphilum	-165.17	-80.67	-84.5	48.84	-25.9	-12.9	-13	49.81	3.54	4.04	-0.5	114.12
Platyrrhinus albericoi												
Platyrrhinus masu												
Anoura aequatoris												
Anoura geoffroyii												
Desmodus rotundus	-48.45	-143.95	95.5	297.11	-23.23	-26.73	3.5	115.07	6.24	10.3	-4.03	164.58

Appendix 2: Minimum and maximum δD, δC, and δN values at different altitudes for each of the species at 1800 m.

Table S2. δD, δC, and δN range values in frugivorous species captured at 1800 m.

	Medium (1800 m)											
	δD				δC				δN			
Species	Min	Max	Difference	Difference (%)	Min	Max	Difference	Difference (%)	Min	Max	Difference	Difference (%)
Carollia perspicillata												
Carollia brevicauda												
Sturnira erythromos	-113.52	-91.23	-22.29	80.37	-29.94	-13.42	-16.52	44.82	1.97	4.33	-2.36	219.80
Sturnira oporaphilum	-160.34	-128.01	-32.33	79.84	-15.15	-13.79	-1.36	91.02	2.71	4.15	-1.44	153.14
Platyrrhinus albericoi	-170.43	-139.4	-31.03	81.80	-14.37	-13.65	-0.72	94.99	3.54	3.86	-0.32	109.04
Platyrrhinus masu												
Anoura aequatoris												
Anoura geoffroyii												
Desmodus rotundus												

Appendix 3: Minimum and maximum δD, δC, and δN values at different altitudes for each of the species at 2300 m.

Table S3. δD, δC, and δN range values in frugivorous species captured at 2300 m.

	High (2300 m)											
	δD				δC				δN			
Species	Min	Max	Difference	Difference (%)	Min	Max	Difference	Difference (%)	Min	Max	Difference	Difference (%)
Carollia perspicillata												
Carollia brevicauda												
Sturnira erythromos	-164.42	-94.56	-69.86	57.51	-26.6	-22.65	-3.95	85.15	1.22	5.08	-3.86	416.39
Sturnira oporaphilum	-134.45	-95.26	-39.19	70.85	-26.53	-24.16	-2.37	91.07	2.06	5.62	-3.56	272.82
Platyrrhinus albericoi												
Platyrrhinus masu												
Anoura aequatoris												
Anoura geoffroyii												
Desmodus rotundus												

Appendix 4: Observed and expected values of δD found in *S. erythromos* captured at 1800 m.

Table S4. Observed and expected values of δD found in *S. erythromos* captured at 1800 m.

Individual ID	Observed δD	Expected δD	
		Correction factor of -40	Correction factor of -45
1	-100.4979	-126.4489	-131.4489
2	-113.5185	-125.4668	-130.4668
3	-103.8643	-126.1761	-131.1761
4	-91.2293	-126.3671	-131.3671

Appendix 5: Observed and expected values of δD found in *S. erythromos* captured at 2300 m.

Table S5. Observed and expected values of δD found in *S. erythromos* captured at 2300 m.

Individual ID	Observed δD	Expected δD	
		Correction factor of -40	Correction factor of -45
1	-140.8224	-139.5172	-144.5172
2	-136.4630	-139.4354	-144.4354
3	-109.9229	-139.4354	-144.4354
4	-123.5555	-139.4354	-144.4354
5	-110.8951	-139.4354	-144.4354
6	-159.1001	-139.4081	-144.4081
7	-102.2065	-139.5172	-144.5172
8	-125.5858	-139.4081	-144.4081
9	-143.5844	-139.4354	-144.4354
10	-142.0416	-139.4354	-144.4354
11	-125.4962	-139.4354	-144.4354
12	-120.0260	-139.4354	-144.4354
13	-137.7926	-139.4354	-144.4354
14	-94.5571	-139.4354	-144.4354
15	-128.1525	-139.2444	-144.2444
16	-164.4213	-139.4354	-144.4354
17	-129.9433	-139.4081	-144.4081
18	-142.9611	-139.4081	-144.4081
19	-132.4997	-139.4354	-144.4354
20	-139.9115	-139.4354	-144.4354
21	-144.4604	-139.2444	-144.2444
22	-142.8262	-139.7082	-144.7082
23	-138.1556	-139.4081	-144.4081
24	-132.2837	-139.4081	-144.4081
25	-129.4491	-139.4081	-144.4081
26	-127.0596	-139.4081	-144.4081
27	-135.8180	-139.4081	-144.4081
28	-155.8968	-139.4081	-144.4081
29	-127.6537	-139.4354	-144.4354
30	-154.4522	-139.4081	-144.4081
31	-144.7904	-139.4081	-144.4081