Stable hydrogen isotopes record the summering grounds of eastern red bats (*Lasiurus borealis*)

Bats face numerous threats associated with global environmental change, including the rapid expansion of wind-energy facilities, emerging infectious disease, and habitat loss. An understanding of the movement and migration patterns of these highly dispersive animals would help reveal how spatially localized the impacts from these threats are likely to be on bat populations, thus aiding in their conservation. Stable hydrogen isotope ratios ($\delta^2$H) can be used to infer regions where bats have been foraging during the summer molt season, thus allowing an assessment of summering location and distance of movement of bats sampled during other times of year. However, a major impediment to the application of $\delta^2$H for inference of bat movements is that the relationship between $\delta^2$H of bat hair and precipitation tends to be species specific and is still unknown for some key species of conservation concern. We addressed this issue by using geo-referenced museum specimens to calibrate the relationship between $\delta^2$H of hair ($\delta^2$H$_{\text{hair}}$) and long-term $\delta^2$H of growing-season precipitation ($\delta^2$H$_{\text{GSprecip}}$) at the site of collection for eastern red bats (*Lasiurus borealis*), one of the main species of bats experiencing large numbers of fatalities at wind-energy facilities in North America. Based on comparison of $\delta^2$H$_{\text{hair}}$ and $\delta^2$H$_{\text{GSprecip}}$ values for males we estimated a period of molt of June 14–August 7. Within this period, male and female red bats exhibited a significant positive relationship between $\delta^2$H$_{\text{hair}}$ and $\delta^2$H$_{\text{GSprecip}}$. These results establish the relationship between $\delta^2$H$_{\text{hair}}$ and $\delta^2$H$_{\text{GSprecip}}$ for red bats, which is necessary for the use of $\delta^2$H$_{\text{hair}}$ to infer the movement and migration patterns of this important species. These results provide a critical resource to conservation biologists working to assess the impacts of environmental change on bat populations.
Cortney L. Pylant, David M. Nelson*, Stephen R. Keller

Frostburg State University, Department of Biology, 101 Braddock Road, Frostburg, MD, 21532 USA (CLP)

University of Maryland Center for Environmental Science, Appalachian Laboratory, 301 Braddock Road, Frostburg, MD, 21532 USA (CLP, DMN, SRK)

* = Corresponding Author (dnelson@umces.edu, 301-689-7171)
Introduction

Bats living in temperate zones display a range of strategies for escaping unfavorable winter conditions. Species that survive through winter by hibernating in caves or buildings often have relatively sedentary populations or undertake regional migrations, whereas other species undertake seasonal migrations of hundreds or thousands of kilometers to find suitable winter habitat (Fleming and Eby 2003; Cryan et al. 2004). However, despite the importance of movement and migration patterns to bat ecology and conservation, such behaviors remain difficult to quantify for these highly dispersive animals.

Understanding patterns of movement and migration is key to the conservation of bats experiencing threats associated with global environmental change, including the rapid worldwide expansion of wind-energy facilities, emerging infectious disease, and habitat loss (Kuvelsky et al. 2007; NRC 2007; Cryan and Barclay 2009; Boyles et al. 2011). For example, such information may aid bat conservation by helping to reveal migration pathways, population connectivity, regional habitat use, and the spatial extent of the impacts from these threats (Webster et al. 2002; O’Shea et al. 2003). Unfortunately, existing tracking methods are of limited use for understanding movement and migration of bats that migrate long distances. For example, mark-recapture studies suffer from low recapture rates (Holland and Wikelski 2009). The use of radio transmitters suffers from small ranges of detection and short life spans of batteries that limit their ability to track bats capable of migrating long distances (Cryan and Diehl 2009; Taylor et al. 2011; McGuire et al. 2012). Furthermore, geolocation by light is of limited use for nocturnal or crepuscular organisms (Lisovski et al. 2012), such as bats that roost in heavy foliage and are active when there is little to no sunlight. Alternatively, intrinsic markers, such as stable hydrogen isotope ratios (δ²H), overcome these challenges and are a viable method to infer the summering grounds of bats (Cryan et al. 2004; Fraser et al. 2012; Sullivan et al. 2012). The basis of this...
approach is that continental-scale variation in δ²H of precipitation (Dansgaard 1964) is incorporated into hair keratin through drinking water and diet (Estep and Dabrowski 1980; Fogel and Cifuentes 1993), and this incorporation occurs during summer when temperate bats undergo their annual molt (Quay 1970; Cryan et al. 2004; Cryan et al. 2012; Fraser et al. 2013). The use of stable isotopes has several advantages: it can be applied to live and dead bats, only small quantities of hair are required, and geographic origins of summering grounds can be assigned to bats captured outside the period of molt because hair is metabolically inert (Rubenstein and Hobson 2004).

Application of δ²H to infer the geographic origin of bats requires the prior estimation of isotopic discrimination between δ²H of their tissues and δ²H of precipitation incorporated into their drinking water and diet. To validate this relationship, bats are sampled at their known summering grounds and values of δ²H of hair (δ²H_{hair}) are compared with values of δ²H of growing-season precipitation (δ²H_{GSpresip}) at the same locations. Prior studies have shown strong positive relationships between δ²H_{hair} and δ²H_{GSpresip} for hoary bats (Lasiurus cinereus; Cryan et al. 2004; Cryan et al. 2014), tri-colored bats (Perimyotis subflavus, Fraser et al. 2012), little brown bats (Myotis lucifugus, Britzke et al. 2009; Sullivan et al. 2012), and others (Britzke et al. 2009; Popa-Lisseanu et al. 2012; Table 1). However, the relationship between δ²H_{hair} and δ²H_{GSpresip} is often species specific as the result of differences in life history and/or physiology, so the δ²H relationship established for one species is often not transferable to another species (Table 1; Britzke et al. 2009; Hobson et al. 2012).

The eastern red bat (Lasiurus borealis) is among the bat species experiencing the highest levels of mortality at wind-energy facilities in the eastern United States (Arnett et al. 2008). Red bats are thought to undertake long-distance migrations from their winter grounds along coastal regions of the southeastern United States and the Gulf of Mexico to widely distributed summering grounds located throughout eastern North America (Cryan 2003). Their northern
range limit is southern Canada and their western range limit is the Rocky Mountains (Shump and
Shump 1982; Cryan 2003). In contrast to most prior studies, Britzke et al. (2009) found a
negative relationship between $\delta^{2}H_{\text{hair}}$ and $\delta^{2}H_{\text{GSprecip}}$ for male red bats, but a positive relationship
for females. These results suggest that male red bats may have unusual migration patterns and/or
isotopic discrimination relative to female red bats and other species, such as hoary bats (Cryan et
al. 2004), a sister taxa (Roehrs et al. 2010). Since this intraspecific difference is unusual and red
bats are a species of conservation concern because of wind-turbine mortality, additional studies
are required to assess the relationship between $\delta^{2}H_{\text{hair}}$ and $\delta^{2}H_{\text{GSprecip}}$ for red bats and the
applicability of this relationship to assigning geographic origins of migrants. We hypothesized
that if $\delta^{2}H_{\text{hair}}$ is useful for inferring locations at which red bats summer, then individuals from
regions with more negative $\delta^{2}H_{\text{GSprecip}}$ values should have more negative $\delta^{2}H_{\text{hair}}$ values than
individuals from regions with more positive $\delta^{2}H_{\text{GSprecip}}$ values. Further, if male and female red bats
exhibit similar migration patterns and patterns of isotopic discrimination, then we expect no
difference in their relationships between $\delta^{2}H_{\text{hair}}$ and $\delta^{2}H_{\text{GSprecip}}$. Materials and Methods

We searched the Smithsonian Institution National Museum of Natural History’s Division
of Mammals Collections database (http://collections.nmnh.si.edu/search/mammals) for red bat
specimens that (1) had sufficiently detailed information to be able to geo-reference the location of
collection, and (2) were collected during June–August. This period includes the time of year
when red bat individuals are most likely to be resident on their summering grounds, as
approximated from the estimated period of molt in hoary bats (Cryan et al. 2004) and other bat
species (see Fraser et al. 2013 for a review of published molt dates). The pool of potential
specimens was selected to maximize geographic coverage throughout the known distribution of
red bats (Fig. 1) and to minimize overrepresentation of samples from similar locations. When available from specimen labels, we recorded the sex of each individual.

We removed approximately 1 mg of hair from the axillary region of each specimen to minimize visible damage to the specimens. We cleaned the samples of natural oil and residues using 1:200 Triton X-100 detergent and 100% ethanol. Then, each sample was air dried at ambient temperature, as recommended by Coplen and Qi (2012). To account for exchange of keratin hydrogen with ambient vapor we used a comparative equilibration approach (Wassenaar and Hobson 2003) in which samples were equilibrated and analyzed alongside international hair standards (USGS42, Tibetan hair, and USGS43, Indian hair; Coplen and Qi 2012) and an internal keratin standard (porcine hair and skin, Spectrum Chemical product # K3030). Approximately 0.3 mg of cleaned hair from each bat sample, as well as each standard, was weighed into silver capsules and exposed to ambient air for >72 hrs to allow for equilibration of exchangeable hydrogen. Samples were analyzed for δ²H using a ThermoFisher high temperature conversion/elemental analyzer pyrolysis unit interfaced with a ThermoFisher Delta V+ isotope ratio mass spectrometer at the Central Appalachians Stable Isotope Facility (http://casif.al.umces.edu). Values of δ²H are expressed in parts per mil (‰) using the following equation: δ²H(‰) = [(R_{sample}/R_{standard} – 1) x 1000], where R is the ratio of ²H/¹H. δ²H sample data were normalized to the Vienna Standard Mean Ocean Water-Standard Light Antarctic Precipitation (VSMOW-SLAP) scale using a two-point normalization curve with USGS42 and USGS43, whose δ²H values of non-exchangeable hydrogen are -78.5 and -50.3‰, respectively. Most of the δ²H_{hair} values of the specimens were > -50.3‰, but prior studies suggest that linear extrapolation of normalization relationships for δ²H is appropriate for values within ~100‰ of the range of the standards used for normalization (Kelly et al. 2009, Wiley et al. 2012). The analytical precision of the internal keratin standard was ±1.9‰.
We used Google Earth to determine the approximate latitude, longitude and elevation of the collection location of each specimen, based on information provided on the specimen labels. Where information was restricted to broader geographic regions (e.g., counties, national parks) we used values for a central point. Latitude, longitude and elevation values were entered in the Online Isotopes in Precipitation Calculator (http://waterisotopes.org; Bowen and Revenaugh 2003; Bowen et al. 2005) to determine average $\delta^2\text{H}$ values of precipitation for June–August (i.e., $\delta^2\text{H}_{\text{GSprecip}}$) for each collection site. The small uncertainties associated with our approach for approximating the latitude, longitude and elevation of sample locations had little influence on the $\delta^2\text{H}$ values of precipitation that were calculated for each site because $\delta^2\text{H}$ values of precipitation exhibit greater variation across large than small environmental gradients (e.g., of latitude).

Specimen collection years spanned a period from 1900–2009. We subset samples by sex for initial analyses to assess potential intersex differences; specimens of unknown sex were excluded from these analyses.

The variance of the difference between $\delta^2\text{H}_{\text{hair}}$ and $\delta^2\text{H}_{\text{GSprecip}}$ values should decrease during the period of molt. Therefore, to attempt to more precisely estimate the range of days during which new pelage was presumably synthesized, we empirically evaluated the interval of time during the June–August period for which the standard deviation (created by grouping the Julian days of collection into 5-day intervals) of the difference between individual $\delta^2\text{H}_{\text{hair}}$ and $\delta^2\text{H}_{\text{GSprecip}}$ values was minimized. To do this, we calculated the standard deviation of the difference between individual $\delta^2\text{H}_{\text{hair}}$ and $\delta^2\text{H}_{\text{GSprecip}}$ values. We determined the presumed period of molt by visually identifying where the standard deviation was the lowest. We included samples collected during the presumed period of molt in subsequent reduced major axis (RMA) regressions. We performed RMA regressions to assess the relationship between $\delta^2\text{H}_{\text{hair}}$ and $\delta^2\text{H}_{\text{GSprecip}}$ because of symmetry between the dependent and independent variables (i.e., it is arbitrary which variable is plotted on the X and Y axes, because $\delta^2\text{H}_{\text{hair}}$ is influenced by $\delta^2\text{H}_{\text{GSprecip}}$, but $\delta^2\text{H}_{\text{GSprecip}}$ is also calculated from
δ²H_{hair}; Smith 2009) and because both variables contain measurement uncertainty (McArdle 1988). We examined model residuals across collection dates to check for non-uniform variance (e.g., heteroscedasticity) across the period of molt. In light of the potential for delayed molt in reproductive female bats (Fraser et al. 2012), we also determined the relationship between δ²H_{hair} and δ²H_{GSprecip} for female red bats collected between July 1 and August 31 and between July 1 and August 7. We performed all statistical analyses in R (R Core Team 2013).

Results

We obtained a total of 112 red bat specimens (41 male, 67 female, 4 sex unknown) for evaluation of the relationship between δ²H_{hair} and δ²H_{GSprecip} (Table S1). For male red bats, the standard deviation for δ²H_{hair} - δ²H_{GSprecip} values for days 160–164 was 33.0 (Fig. 2a). The standard deviation dropped to 4.9 at day 165 and remained low (range: 0.5–7.5) between days 165–219 (June 14–August 7). Standard deviations were generally high between days 220–240 (August 8–August 28), averaging 19.9 during this period. The lower standard deviations of δ²H_{hair} - δ²H_{GSprecip} values between days 165–219 (June 14–August 7) suggest that this is the approximate period during which male red bats are typically resident on their summering grounds and synthesize new annual pelage. Males collected before June 14 or after August 7 were more likely to have molted at a location other than where they were collected. There was no clear trend in temporal variation of the standard deviation of δ²H_{hair} - δ²H_{GSprecip} values for female red bats (Fig. 2b).

A total of 64 male and female specimens were collected between June 14 and August 7. δ²H_{hair} values for male red bats exhibited a strong positive relationship with δ²H_{GSprecip} during this period (R² = 0.69, p < 0.001, n = 20; Fig. 3a). Assuming an identical period of molt for female red bats, δ²H_{hair} from females also exhibited a positive relationship with δ²H_{GSprecip}, although the variance explained was lower than in males (R² = 0.29, p < 0.001, n = 44; Fig. 3a). The mean slope and intercept for males (1.48 and 13.95, respectively) fall within the 95% confidence
interval of the slope and intercept for females (1.29-2.21 and 5.09-30.95, respectively), and the
mean slope and intercept for females (1.75 and 18.02, respectively) fall within the 95%
confidence interval of the slope and intercept for males (1.07-1.89 and 1.89-26.0, respectively).
For female red bats, the relationships between $\delta^2$H_{hair} and $\delta^2$H_{GSprecip} for individuals collected
between July 1 and August 31 ($R^2 = 0.33$, $p < 0.001$, $n = 46$) and between July 1 and August 7
($R^2 = 0.39$, $p < 0.001$, $n = 30$) were stronger than the relationship between $\delta^2$H_{hair} and $\delta^2$H_{GSprecip}
between June 14 and August 7. When male and female bats (from June 14-August 7) were
combined, there was a strong positive relationship between $\delta^2$H_{hair} and $\delta^2$H_{GSprecip} ($R^2 = 0.37$, $p <
0.001$, $n = 64$; Fig. 3a), with no consistent trend in model variance across day of collection (Fig.
3b). Conversion of $\delta^2$H_{hair} values obtained from the four red bats of unknown sex (which were
collected between June 14 and August 7; Table 1) to $\delta^2$H_{GSprecip} using the combined relationship for
males and females (Fig. 3a) produced $\delta^2$H_{GSprecip} values within 5‰ of those calculated for these
sites at http://waterisotopes.org.

To assess the species-specific nature of the relationship between $\delta^2$H_{hair} and $\delta^2$H_{GSprecip} we
compared likelihood-of-origin maps produced based on the separate regression equations
estimated for red bats and their sister taxa, hoary bats. For this exercise, we used as an example a
representative $\delta^2$H_{hair} value of -40‰. For red bats we converted this $\delta^2$H_{hair} value to $\delta^2$H_{GSprecip} using
the relationship between $\delta^2$H_{hair} and $\delta^2$H_{GSprecip} for our combined male and female sample (Fig. 3a),
which yielded a $\delta^2$H_{GSprecip} value of -34.1‰. For hoary bats, there currently exist two published
estimates of the relationship between $\delta^2$H_{hair} and $\delta^2$H_{GSprecip} during their presumed molting period
(20 June–23 August). The first, from Cryan et al. (2004) is based on $\delta^2$H_{hair} data from museum
specimens and estimates of $\delta^2$H_{GSprecip} from Meehan et al. (2004). The second, from Cryan et al.
(2014), contains the $\delta^2$H_{hair} data from Cryan et al. 2004, along with additional samples (Table 1).
In Cryan et al. (2014), the $\delta^2$H_{hair} data were recalibrated to different standards and estimates of
Discussion

Stable isotope analysis has emerged as an important tool for studies of movement, migration, population connectivity, and habitat use of animals not amenable to traditional tracking methods (Hobson 1999; Cryan et al. 2004; Rubenstein and Hobson 2004; Fraser et al. 2012). However, applying isotope data to make such inferences requires accurate knowledge of the relationship between δ²H_{hair} and δ²H of precipitation. This relationship is often species-specific for different animals (Hobson et al. 2012), including bats (Britzke et al. 2009; Table 1), which makes it important to establish this relationship for focal species of interest or conservation concern. Given the recent impact of wind turbines on the migratory red bat, and the growing interest among conservation biologists and natural resource managers in applying stable isotopes to track the origins of *Lasiurus* spp. killed at wind-turbine facilities, it is essential to establish the reliability of δ²H for tracking the summering grounds of red bats. Our data showed positive
relationships between $\delta^2$H$_{hair}$ and $\delta^2$H$_{GSprecp}$ for both male and female red bats, which indicates that $\delta^2$H$_{GSprecp}$ values deduced from $\delta^2$H$_{hair}$ may be used to infer the summering locations of bats captured (or killed) at distant sites, such as at wind turbines or on their overwintering grounds.

We estimated a period of molt of June 14—August 7 for male red bats based on comparison of $\delta^2$H$_{hair}$ and $\delta^2$H$_{GSprecp}$ values. Greater variation in $\delta^2$H$_{hair}$ - $\delta^2$H$_{GSprecp}$ values for male red bats collected before June 14 and after August 7 suggests that individuals collected outside of the approximate timeframe of June 14—August 7 were less likely to have molted at the site of capture. This estimated period of molt is similar to the $\delta^2$H-inferred period of molt (June 20—August 23) reported for the hoary bat (Cryan et al. 2004), a close relative of the red bat (Roehrs et al. 2010). In contrast to males, there was no distinct period of low variability in $\delta^2$H$_{hair}$ - $\delta^2$H$_{GSprecp}$ values for female red bats. This lack of a period of low variability may indicate that females molt outside of June-August, such as during migration. Another explanation is that female red bats undertake long-distance dispersal or even begin to migrate soon after molt, which would decrease our ability to detect a distinct molt period with $\delta^2$H, particularly if there exists geographic variation in the seasonal timing of molt and/or migration. Indeed, studies suggest that some female bats (including hoary bats, Cryan et al. 2004) delay molt until after parturition and lactation (Quay 1970; Jones and Genoways 1976) when they then synthesize pelage rapidly at the end of the growing season, within ~ 2 weeks of autumn migration (Cryan et al. 2004). Regardless of its precise cause(s), the lack of a distinct period of low variability in $\delta^2$H$_{hair}$ - $\delta^2$H$_{GSprecp}$ values for female red bats does preclude the use of $\delta^2$H$_{hair}$ for identifying their summering grounds.

Within the estimated period of molt, we found significant positive relationships between $\delta^2$H$_{hair}$ and $\delta^2$H$_{GSprecp}$ for red bats that were similar for males and females. However, the relationship between $\delta^2$H$_{hair}$ and $\delta^2$H$_{GSprecp}$ for female red bats explained less of the variance (e.g., lower $R^2$) compared to male red bats. The weaker relationship for females might be a function of delayed molt in reproductive females, as discussed above. Indeed, $\delta^2$H$_{GSprecp}$ had a stronger
relationship with δ²Hₘₐᵣᵢₜ for female red bats collected only in July and August than for females from June 14-August 7. Although the precise timing of molt of female red bats warrants further study, the regression slopes and intercepts for males and females were not different (Fig. 3a) and there was only a small (5‰) maximum difference in δ²H₂Gₛₚₑᵣₑᵢₙ between the respective equations for males and females for δ²Hₘₐᵣᵢₜ values ranging between -10 and -60‰. Thus, our results suggest that male red bats do not display aberrant migratory patterns or isotopic discrimination relative to female red bats (as suggested by Britzke et al. 2009) or other bat species (Table 1). These results also suggest that a single relationship may be used for conversion between δ²Hₘₐᵣᵢₜ and δ²H₂Gₛₚₑᵣₑᵢₙ for both sexes of red bats. A single relationship applicable to either sex implies that this approach may be used for assessing the origin of red bats of unknown sex. For example, δ²H₂Gₛₚₑᵣₑᵢₙ values derived from δ²Hₘₐᵣᵢₜ values for four red bats of unknown sex in our dataset (Table 1) were within 5‰ of the actual δ²H₂Gₛₚₑᵣₑᵢₙ values at these sites, which is less than the estimated uncertainty (10‰) in the relationship between δ²Hₘₐᵣᵢₜ and δ²H₂Gₛₚₑᵣₑᵢₙ for red bats. In contrast to our results, Britzke et al. (2009) found a negative relationship between δ²Hₘₐᵣᵢₜ and δ²H₂Gₛₚₑᵣₑᵢₙ for male red bats. Although precise reason for this discrepancy this uncertain, we offer two potential explanations. The Britzke et al. (2009) dataset included samples from red bats collected between May 15 and August 1 during the years 2001-2005, whereas we identified a molt period of June 14-August 7 using samples from the years 1900-1972. Thus, one explanation for these differing results is that some of red bats analyzed in Britzke et al. (2009) may have been sampled before they reached their summering grounds and molted new pelage, which means that δ²Hₘₐᵣᵢₜ values from such bats would partly indicate their location the prior summer rather than of the year in which they were collected. A second possible explanation is that bats used in Britzke et al. (2009) were samples across a smaller number of years. Although there is no long-term trend in δ²H₂Gₛₚₑᵣₑᵢₙ during the last ~100 years (Hobson et al. 2010; Hobson et al. 2014), there can be inter-annual spatial variation in δ²H₂Gₛₚₑᵣₑᵢₙ. Such variation may be
minimized when using samples from a large number of years (i.e., 1900-1972), whereas it may have a larger impact when using samples from a relatively small number of years (i.e., 2001-2005).

Our results provide confidence for using $\delta^2$H$_{\text{hair}}$ to identify the location of the summering grounds (i.e., the location where new pelage was synthesized) of red bats of unknown geographic origin. In contrast to intraspecific similarities, our results underscore the species specificity of the $\delta^2$H$_{\text{hair}}$ and $\delta^2$H$_{\text{GSprecip}}$ relationship, even among closely related bat species. For example, a $\delta^2$H$_{\text{hair}}$ value of -40‰ yielded distinct $\delta^2$H$_{\text{GSprecip}}$ values and likelihood-of-origin maps for red and hoary bats based on using the regression presented here for red bats and those of Cryan et al. (2004) and Cryan et al. (2014) for hoary bats (Fig. 4). Thus, our study provides critical calibration data for the use of $\delta^2$H$_{\text{hair}}$ to infer the movement and migration patterns of red bats, and will enable future studies on red bat ecology and conservation, especially in the context of assessing the impacts of threats associated with global environmental change.

Acknowledgments

We thank John Hoogland for providing feedback on an earlier version of the manuscript. Suzanne Peurach at the Smithsonian National Museum of Natural History facilitated the sampling of museum specimens and Robin Paulman assisted with stable isotope analyses.

References
Arnett EB, Brown WK, Erickson WP, Fiedler JK, Hamilton BL, Henry TH, Jain A, Johnson GD, Kerns J, Koford RR, Nicholson CP, O’Connell TJ, Piorkowski MD, Tankersley RD. 2008. Patterns of bat fatalities at wind energy facilities in North America. *Journal of Wildlife Management* 72:61–78.

Bowen GJ, Liu Z, Vander Zanden HB, Zhao L, Takahashi G. 2014. Geographic assignment with stable isotopes in IsoMAP. *Methods in Ecology and Evolution* 5:201–206.

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

Bowen GJ, Wassenaar LI, Hobson KA. 2005. Global application of stable hydrogen and oxygen isotopes to wildlife forensics. *Oecologia* 143:337–348.

Boyles JG, Cryan PM, McCracken GF, Kunz TH. 2011. Economic importance of bats in agriculture. *Science* 332:41–42.

Britzke ER, Loeb SC, Hobson KA, Romanek CS, Vonhof MJ. 2009. Using hydrogen isotopes to assign origins of bats in the eastern United States. *Journal of Mammalogy* 90:743–751.

Coplen TB, Qi H. 2012. USGS42 and USGS43: Human–hair stable hydrogen and oxygen isotopic reference materials and analytical methods for forensic science and implications for published measurement results. *Forensic Science International* 214:135–141.
Cryan PM. 2003. Seasonal distribution of migratory tree bats (*Lasiurus* and *Lasionycteris*) in North America. *Journal of Mammalogy* 84:579–593.

Cryan PM, Barclay RMR. 2009. Causes of bat fatalities at wind turbines: hypotheses and predictions. *Journal of Mammalogy* 90:1330–1340.

Cryan PM, Bogan MA, Rye RO, Landis GP, Kester CL. 2004. Stable hydrogen isotope analysis of bat hair as evidence for seasonal molt and long-distance migration. *Journal of Mammalogy* 85:995–1001.

Cryan PM, Diehl R. 2009. Analyzing bat migration. In: Kunz TH, Parsons S, editors. Ecological and behavioral methods for the study of bats. Johns Hopkins University Press, Baltimore, Maryland, US. pp. 476–488.

Cryan PM, Jameson JW, Baerwald EF, Willis CKR, Barclay RMR, Snider EA, Crichton EG. 2012. Evidence of late-summer mating readiness and early sexual maturation in migratory tree-roosting bats found dead at wind turbines. *PLoS ONE* 7:e47586.

Cryan PM, Stricker CA, Wunder MB. 2014. Continental-scale, seasonal movements of a heterothermic migratory tree bat. *Ecological Applications* 24:602-616.

Dansgaard W. 1964. Stable isotopes in precipitation. *Tellus* 16:436–468.

Estep MF. Dabrowski H. 1980. Tracing food webs with stable hydrogen isotopes. *Science* 209:1537–1538.
Fleming TH, Eby P. 2003. Ecology of bat migration. In: TH Kunz and MB Fenton, eds. Bat ecology. University of Chicago Press, Chicago, Illinois, USA. pp. 156–208.

Fogel ML, Cifuentes LA. 1993. Isotope fractionation during primary production. In: Engel MH, Macko SA, eds. Organic geochemistry. Plenum Press, New York, US. pp. 73–98.

Fraser EE, Longstaffe FJ, Fenton MB. 2013. Moulting matters: the importance of understanding moulting cycles in bats when using fur for endogenous marker analysis. *Canadian Journal of Zoology* 91:533–544.

Fraser EE, McGuire LP, Eger JL, Longstaffe FJ and Fenton MB. 2012. Evidence of latitudinal migration in tri-colored bats, *Perimyotis subflavus*. *PLoS ONE* 7:e31419.

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

Hobson KA, Van Wilgenburg SL, Wassenaar LI, Larson K. 2012. Linking hydrogen (δ²H) isotopes in feathers and precipitation: sources of variance and consequences for assignment to isoscapes. *PLoS ONE* 7:e35137.

Hobson KA, Greenberg R, Van Wilgenburg SL, Mettke-Hofmann C. 2010. Migratory connectivity in the rusty blackbird: Isotopic evidence from feathers of historical and contemporary Specimens. *Condor* 112:778-788.
Hobson KA, Van Wilgenburg SL, Faaborg J, Toms JD, Rengifo C, Llanes Sosa A, Aubry Y, and Brito Aguilar R. 2014. Connecting breeding and wintering grounds of Neotropical migrant songbirds using stable hydrogen isotopes: a call for an isotopic atlas of migratory connectivity. *Journal of Field Ornithology* 85:237-257.

Holland RA, Wikelski M. 2009. Studying the migratory behavior of individual bats: current techniques and future directions. *Journal of Mammalogy* 90:1324–1329.

International Union for Conservation of Nature (IUCN). 2008. *Lasiurus borealis*. In: IUCN 2013. IUCN Red List of Threatened Species. Version 2013.2. http://www.iucnredlist.org/details/11347/0.

Jones Jr. JK, Genoways HH. 1967. Annotated checklist of bats from South Dakota. *Transaction of the Kansas Academy of Science* 70:184–196.

Kelly JF, Bridge ES, Fudickar AM, Wassenaar LI. 2009. A test of comparative equilibration for determining non-exchangeable stable hydrogen isotope values in complex organic materials. *Rapid Communications in Mass Spectrometry* 23:2316-2320.

Kennedy CD, Bowen GJ, Ehleringer JR. 2011. Temporal variation of oxygen isotope ratios (δ18O) in drinking water: implications for specifying location of origin with human scalp hair. *Forensic Science International* 208:156–166.
Kuvlesky Jr. WP, Brennan LA, Morrison ML, Boydston KK, Ballard BM, Bryant FC. 2007. Wind energy development and wildlife conservation: challenges and opportunities. *Journal of Wildlife Management* 71:2487–2498.

Lisovski S, Hewson CM, Klaassen RHG, Korner-Nievergelt F, Kristensen MW, Hahn S. 2012. Geolocation by light: accuracy and precision affected by environmental factors. *Methods in Ecology and Evolution* 3:603–612.

McArdle BH. 1988. The structural relationship: regression in biology. *Canadian Journal of Zoology* 66:2329–2339.

McGuire LP, Guglielmo CG, Mackenzie SA, Taylor PD. 2012. Migratory stopover in the long-distance migrant silver-haired bat, *Lasionycteris noctivagans*. *Journal of Animal Ecology* 81:377–385.

Meehan TD, Giermakowski JT, Cryan PM. 2004. GIS-based model of stable hydrogen isotope ratios in North American growing-season precipitation for use in animal movement studies. *Isotopes in Environmental and Health Studies* 40:291-300.

National Research Council (NRC). 2007. Environmental impacts of wind-energy projects. Committee on Environmental Impacts of Wind Energy Projects. The National Academies Press, Washington DC.
O’Shae TJ, Bogan MA, Ellison LE. 2003. Monitoring trends in bat populations of the United States and territories: status of the science and recommendations for the future. *Wildlife Society Bulletin* 31:16–29.

Popa-Lisseanu AG, Sörgel K, Luckner A, Wassenaar LI, Ibáñez C, Kramer-Schadt S, Ciechanowski M, Görföl T, Niermann I, Beuneux G, Mysłajek RW, Juste J, Fonderflick J, Kelm DH, Voigt CC. 2012. A triple-isotope approach to predict the breeding origins of European bats. *PLoS ONE* 7:e30388.

Quay WB. 1970. Integument and derivatives. In: Wimsatt, W, ed. Biology of bats, volume II. Academic Press, New York, US. pp. 1–57.

R Core Team (2013) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org.

Roehrs ZP, Lack JB, Van Den Bussche RA. 2010. Tribal phylogenetic relationships within Vespertilioninae (Chiroptera: Vespertilionidae) based on mitochondrial and nuclear sequence data. *Journal of Mammalogy* 91:1073–1092.

Rubenstein DR, Hobson KA. 2004. From birds to butterflies: animal movement patterns and stable isotopes. *Trends in Ecology and Evolution* 19:256–263.

Shump KA, Shump AU. 1982. Mammalian species *Lasiurus borealis*. *Journal of Mammalogy* 183:1–6.
Smith RJ. 2009. Use and misuse of the reduced major axis for line-fitting. American Journal of Physical Anthropology 140:476–486.

Sullivan AR, Bump JK, Kruger LA and Peterson RO. 2012. Bat-cave catchment areas: using stable isotopes (δD) to determine the probable origins of hibernating bats. Ecological Applications 22:1428–1434.

Taylor PD, Mackenzie SA, Thurber BG, Calvert AM, Mills AM, McGuire LP, Guglielmo CG. 2011. Landscape movements of migratory birds and bats reveal an expanded scale of stopover. PLoS ONE 6:e27054.

Wassenaar LI and Hobson KA. 2003. Comparative equilibration and online technique for determination of non-exchangeable hydrogen of keratins for use in animal migration studies. Isotopes in Environmental and Health Studies 39:211–217.

Webster MS, Marra PP, Haig SM, Bensch S and Holmes RT. 2002. Links between worlds: unraveling migratory connectivity. Trends in Ecology and Evolution 17:76–83.

Wiley AE, Welch AJ, Ostrom PH, James HF, Stricker CA, Fleisher RC, Gandhi H, Adams J, Ainley DG, Duvall F, Holmes N, Hu DC, Judge S, Penniman J, Swindle KA. 2012. Foraging segregation and genetic divergence between geographically proximate colonies of a highly mobile seabird. Oecologia 168:119-130.

Wunder MB. 2012. Determining geographic patterns of migration and dispersal using stable isotopes in keratins. Journal of Mammalogy 93:360–367.
Figure 1

Likelihood-of-origin maps for a $\delta^{2}$H$_{\text{hair}}$ value of -40‰ that was transformed into $\delta^{2}$H$_{\text{GSprecip}}$ values for *L. borealis* and *L. cinereus*.

The likelihood-of-origin maps (on left for *L. borealis* and on middle and right for *L. cinereus*) were created using the geostatistical tool IsoMAP. Inset values represent the $\delta^{2}$H$_{\text{GSprecip}}$ values after transformation (using the combined relationship in Fig. 3a of this study for *L. borealis* and Cryan et al. 2004, middle, and Cryan et al. 2014, right, for *L. cinereus*).
Figure 2

Differences between $\delta^2\text{H}_{\text{hair}}$ and $\delta^2\text{H}_{\text{Gprecip}}$.

Standard deviations for A) male and B) female specimens of *L. borealis* as a function of Julian date. Dates were grouped in 5 day intervals. Solid vertical lines delineate the lowest period of variability (i.e., the estimated period of molt) for males (i.e., Julian days 165–219 or June 14–August 7).
Figure 3

Relationships of $\delta^{2}H_{\text{hair}}$ and $\delta^{2}H_{\text{GSpresp}}$ during the estimated period of molt for males and females of *L. borealis*.

The relationship of $\delta^{2}H_{\text{hair}}$ and $\delta^{2}H_{\text{GSpresp}}$ during the estimated period of molt for male (diamonds) and female (circles) red bats (A) and the resulting model residuals relative to sample collection date (B). The solid line in (A) represents the regression line for both sexes combined.
A

\[ \Delta^2 H_{\text{hair}} = 1.48(\Delta^2 H_{\text{Gprecip}}) + 13.95 \]

\[ R^2 = 0.69, p < 0.001, n = 20 \]

\[ \Delta^2 H_{\text{hair}} = 1.75(\Delta^2 H_{\text{Gprecip}}) + 18.02 \]

\[ R^2 = 0.29, p < 0.001, n = 44 \]

\[ \Delta^2 H_{\text{hair}} = 1.67(\Delta^2 H_{\text{Gprecip}}) + 16.84 \]

\[ R^2 = 0.37, p < 0.001, n = 64 \]

B

Residual Value

Collection Date (Julian Day)
Figure 4

Likelihood-of-origin maps for a $\delta^2H_{\text{hair}}$ value of -40‰ that was transformed into $\delta^2H_{\text{GSprecip}}$ for *L. borealis* and *L. cinereus*.

The likelihood-of-origin maps (on left for *L. borealis* and on middle and right for *L. cinereus*) were created using the geostatistical tool IsoMAP. Inset values represent the $\delta^2H_{\text{GSprecip}}$ values after transformation (using the combined relationship in Fig. 3a of this study for *L. borealis* and Cryan et al. 2004, middle, and Cryan et al. 2014, right, for *L. cinereus*).
Table 1 (on next page)

Review of published relationships between δ²H_{hair} and δ²H_{6Sprecip} for North American bats.

Note that the combined regressions from Britzke et al. 2009 include juvenile bats of unknown sex.
| Species                        | Equation                                                                 | R²   | p-value | Source                  |
|-------------------------------|--------------------------------------------------------------------------|------|---------|-------------------------|
| *Perimyotis subflavus* (tri-colored bat) | male (n = 29) \( \delta^2 H_{\text{hair}} = (-0.036 \times \delta D_{\text{GSprecip}}^2) - (1.79 \times \delta^2 H_{\text{GSprecip}}) - 45.61 \) | 0.86 | < 0.01 | Fraser et al. 2012      |
|                               | female (n = 27) \( \delta^2 H_{\text{hair}} = (-0.034 \times \delta D_{\text{GSprecip}}^2) - (1.61 \times \delta^2 H_{\text{GSprecip}}) - 40.38 \) | 0.75 | < 0.01 | Fraser et al. 2012      |
| *Myotis lucifugus* (little brown bat) | male (n = 12) \( \delta^2 H_{\text{hair}} = (0.49 \times \delta^2 H_{\text{GSprecip}}) - 30.90 \) | 0.19 | 0.1527 | Britzke et al. 2009     |
|                               | female (n = 54) \( \delta^2 H_{\text{hair}} = (0.33 \times \delta^2 H_{\text{GSprecip}}) - 40.41 \) | 0.06 | 0.0492 | Britzke et al. 2009     |
|                               | combined (n = 78) \( \delta^2 H_{\text{hair}} = (0.52 \times \delta^2 H_{\text{GSprecip}}) - 30.82 \) | 0.17 | 0.0002 | Britzke et al. 2009     |
|                               | combined (n = ?) \( \delta^2 H_{\text{hair}} = (2.69 \times \delta^2 H_{\text{GSprecip}}) + 96.93 \) | 0.63 | < 0.001| Sullivan et al. 2012    |
| *Myotis septentrionalis* (northern long-eared bat) | male (n = 10) \( \delta^2 H_{\text{hair}} = (0.79 \times \delta^2 H_{\text{GSprecip}}) - 4.73 \) | 0.53 | 0.0088 | Britzke et al. 2009     |
|                               | female (n = 16) \( \delta^2 H_{\text{hair}} = (1.25 \times \delta^2 H_{\text{GSprecip}}) + 18.48 \) | 0.71 | 0.0001 | Britzke et al. 2009     |
|                               | combined (n = 33) \( \delta^2 H_{\text{hair}} = (0.98 \times \delta^2 H_{\text{GSprecip}}) + 5.48 \) | 0.54 | < 0.001| Britzke et al. 2009     |
| *Myotis sodalis* (Indiana bat)  | male (n = 12) \( \delta^2 H_{\text{hair}} = (0.90 \times \delta^2 H_{\text{GSprecip}}) - 0.59 \) | 0.46 | 0.0115 | Britzke et al. 2009     |
|                               | female (n = 39) \( \delta^2 H_{\text{hair}} = (0.71 \times \delta^2 H_{\text{GSprecip}}) - 8.17 \) | 0.35 | 0.0001 | Britzke et al. 2009     |
|                               | combined (n = 59) \( \delta^2 H_{\text{hair}} = (0.83 \times \delta^2 H_{\text{GSprecip}}) - 2.97 \) | 0.49 | < 0.0001| Britzke et al. 2009     |
| *Lasiurus cinereus* (hoary bat) | combined (n = 104) \( \delta^2 H_{\text{hair}} = (0.7884 \times \delta^2 H_{\text{GSprecip}}) - 24.81 \) | 0.60 | < 0.001| Cryan et al. 2004       |
|                               | combined (n = 117) \( \delta^2 H_{\text{hair}} = (0.73 \times \delta^2 H_{\text{GSprecip}}) - 42.61 \) | 0.55 | < 0.001| Cryan et al. 2014       |
| *Lasiurus borealis* (eastern red bat) | male (n = 17) \( \delta^2 H_{\text{hair}} = (-0.82 \times \delta^2 H_{\text{GSprecip}}) - 58.80 \) | 0.33 | 0.0482 | Britzke et al. 2009     |
|                               | female (n = 36) \( \delta^2 H_{\text{hair}} = (1.35 \times \delta^2 H_{\text{GSprecip}}) - 3.60 \) | 0.31 | 0.0003 | Britzke et al. 2009     |
|                               | combined (n = 81) \( \delta^2 H_{\text{hair}} = (0.48 \times \delta^2 H_{\text{GSprecip}}) - 26.10 \) | 0.07 | 0.0201 | Britzke et al. 2009     |
|                               | male (n = 20) \( \delta^2 H_{\text{hair}} = (1.48 \times \delta^2 H_{\text{GSprecip}}) + 13.95 \) | 0.69 | < 0.001| This study              |
|                               | female (n = 44) \( \delta^2 H_{\text{hair}} = (1.75 \times \delta^2 H_{\text{GSprecip}}) + 18.02 \) | 0.29 | < 0.001| This study              |
|                               | combined (n = 64) \( \delta^2 H_{\text{hair}} = (1.67 \times \delta^2 H_{\text{GSprecip}}) + 16.84 \) | 0.37 | < 0.001| This study              |
