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 (δ^2H) 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 δ^2H for inference of bat movements is that the relationship between δ^2H 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 δ^2H of hair (δ^2H$_{hair}$) and long-term δ^2H of growing-season precipitation (δ^2H$_{GSprecip}$) 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 δ^2H$_{hair}$ and δ^2H$_{GSprecip}$ 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 δ^2H$_{hair}$ and δ^2H$_{GSprecip}$. These results establish the relationship between δ^2H$_{hair}$ and δ^2H$_{GSprecip}$ for red bats, which is necessary for the use of δ^2H$_{hair}$ 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.

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8   **Introduction**

9   Bats living in temperate zones display a range of strategies for escaping unfavorable

10   winter conditions. Species that survive through winter by hibernating in caves or buildings often

11   have relatively sedentary populations or undertake regional migrations, whereas other species

12   undertake seasonal migrations of hundreds or thousands of kilometers to find suitable winter

13   habitat (Fleming and Eby 2003; Cryan et al. 2004). However, despite the importance of

14   movement and migration patterns to bat ecology and conservation, such behaviors remain

15   difficult to quantify for these highly dispersive animals.

16   Understanding patterns of movement and migration is key to the conservation of bats

17   experiencing threats associated with global environmental change, including the rapid worldwide

18   expansion of wind-energy facilities, emerging infectious disease, and habitat loss (Kuvelsky et al.

19   2007; NRC 2007; Cryan and Barclay 2009; Boyles et al. 2011). For example, such information

20   may aid bat conservation by helping to reveal migration pathways, population connectivity,

21   regional habitat use, and the spatial extent of the impacts from these threats (Webster et al. 2002;

22   O'Shea et al. 2003). Unfortunately, existing tracking methods are of limited use for

23   understanding movement and migration of bats that migrate long distances. For example, mark-

24   recapture studies suffer from low recapture rates (Holland and Wikelski 2009). The use of radio

25   transmitters suffers from small ranges of detection and short life spans of batteries that limit their

26   ability to track bats capable of migrating long distances (Cryan and Diehl 2009; Taylor et al.

27   2011; McGuire et al. 2012). Furthermore, geolocation by light is of limited use for nocturnal or

28   crepuscular organisms (Lisovski et al. 2012), such as bats that roost in heavy foliage and are

29   active when there is little to no sunlight. Alternatively, intrinsic markers, such as stable hydrogen

30   isotope ratios (δ^2H), overcome these challenges and are a viable method to infer the summering

31   grounds of bats (Cryan et al. 2004; Fraser et al. 2012; Sullivan et al. 2012). The basis of this

32   approach is that continental-scale variation in δ^2H of precipitation (Dansgaard 1964) is

33   incorporated into hair keratin through drinking water and diet (Estep and Dabrowski 1980; Fogel

34   and Cifuentes 1993), and this incorporation occurs during summer when temperate bats undergo

35   their annual molt (Quay 1970; Cryan et al. 2004; Cryan et al. 2012; Fraser et al. 2013). The use of

36   stable isotopes has several advantages: it can be applied to live and dead bats, only small

37   quantities of hair are required, and geographic origins of summering grounds can be assigned to

38   bats captured outside the period of molt because hair is metabolically inert (Rubenstein and

39   Hobson 2004).

40   Application of δ^2H to infer the geographic origin of bats requires the prior estimation of

41   isotopic discrimination between δ^2H of their tissues and δ^2H of precipitation incorporated into

42   their drinking water and diet. To validate this relationship, bats are sampled at their known

43   summering grounds and values of δ^2H of hair (δ^2H_{hair}) are compared with values of δ^2H of

44   growing-season precipitation ($\delta^2H_{GSprecip}$) at the same locations. Prior studies have shown strong

45   positive relationships between δ^2H_{hair} and $\delta^2H_{GSprecip}$ for hoary bats (*Lasiurus cinereus*; Cryan et

46   al. 2004; Cryan et al. 2014), tri-colored bats (*Perimyotis subflavus*, Fraser et al. 2012), little

47   brown bats (*Myotis lucifugus*, Britzke et al. 2009; Sullivan et al. 2012), and others (Britzke et al.

48   2009; Popa-Lisseanu et al. 2012; Table 1). However, the relationship between δ^2H_{hair} and

49   $\delta^2H_{GSprecip}$ is often species specific as the result of differences in life history and/or physiology, so

50   the δ^2H relationship established for one species is often not transferable to another species (Table

51   1; Britzke et al. 2009; Hobson et al. 2012).

52   The eastern red bat (*Lasiurus borealis*) is among the bat species experiencing the highest

53   levels of mortality at wind-energy facilities in the eastern United States (Arnett et al. 2008). Red

54   bats are thought to undertake long-distance migrations from their winter grounds along coastal

55   regions of the southeastern United States and the Gulf of Mexico to widely distributed

56   summering grounds located throughout eastern North America (Cryan 2003). Their northern

57   range limit is southern Canada and their western range limit is the Rocky Mountains (Shump and

58   Shump 1982; Cryan 2003). In contrast to most prior studies, Britzke et al. (2009) found a

59   negative relationship between δ^2H_{hair} and $\delta^2H_{GSprecip}$ for male red bats, but a positive relationship

60   for females. These results suggest that male red bats may have unusual migration patterns and/or

61   isotopic discrimination relative to female red bats and other species, such as hoary bats (Cryan et

62   al. 2004), a sister taxa (Roehrs et al. 2010). Since this intraspecific difference is unusual and red

63   bats are a species of conservation concern because of wind-turbine mortality, additional studies

64   are required to assess the relationship between δ^2H_{hair} and $\delta^2H_{GSprecip}$ for red bats and the

65   applicability of this relationship to assigning geographic origins of migrants. We hypothesized

66   that if δ^2H_{hair} is useful for inferring locations at which red bats summer, then individuals from

67   regions with more negative $\delta^2H_{GSprecip}$ values should have more negative δ^2H_{hair} values than

68   individuals from regions with more positive $\delta^2H_{GSprecip}$ values. Further, if male and female red bats

69   exhibit similar migration patterns and patterns of isotopic discrimination, then we expect no

70   difference in their relationships between δ^2H_{hair} and $\delta^2H_{GSprecip}$.

71   **Materials and Methods**

72   We searched the Smithsonian Institution National Museum of Natural History's Division

73   of Mammals Collections database (http://collections.nmnh.si.edu/search/mammals) for red bat

74   specimens that (1) had sufficiently detailed information to be able to geo-reference the location of

75   collection, and (2) were collected during June−August. This period includes the time of year

76   when red bat individuals are most likely to be resident on their summering grounds, as

77   approximated from the estimated period of molt in hoary bats (Cryan et al. 2004) and other bat

78   species (see Fraser et al. 2013 for a review of published molt dates). The pool of potential

79   specimens was selected to maximize geographic coverage throughout the known distribution of

80   red bats (Fig. 1) and to minimize overrepresentation of samples from similar locations. When

81   available from specimen labels, we recorded the sex of each individual.

82   We removed approximately 1 mg of hair from the axillary region of each specimen to

83   minimize visible damage to the specimens. We cleaned the samples of natural oil and residues

84   using 1:200 Triton X-100 detergent and 100% ethanol. Then, each sample was air dried at

85   ambient temperature, as recommended by Coplen and Qi (2012). To account for exchange of

86   keratin hydrogen with ambient vapor we used a comparative equilibration approach (Wassenaar

87   and Hobson 2003) in which samples were equilibrated and analyzed alongside international hair

88   standards (USGS42, Tibetan hair, and USGS43, Indian hair; Coplen and Qi 2012) and an internal

89   keratin standard (porcine hair and skin, Spectrum Chemical product # K3030). Approximately 0.3

90   mg of cleaned hair from each bat sample, as well as each standard, was weighed into silver

91   capsules and exposed to ambient air for >72 hrs to allow for equilibration of exchangeable

92   hydrogen. Samples were analyzed for δ^2H using a ThermoFisher high temperature

93   conversion/elemental analyzer pyrolysis unit interfaced with a ThermoFisher Delta V+ isotope

94   ratio mass spectrometer at the Central Appalachians Stable Isotope Facility

95   (http://casif.al.umces.edu). Values of δ^2H are expressed in parts per mil (‰) using the following

96   equation: δ^2H(‰) = [($R_{sample}/R_{standard} - 1$) x 1000], where R is the ratio of $^2H/^1H$. δ^2H sample data

97   were normalized to the Vienna Standard Mean Ocean Water-Standard Light Antarctic

98   Precipitation (VSMOW-SLAP) scale using a two-point normalization curve with USGS42 and

99   USGS43, whose δ^2H values of non-exchangeable hydrogen are -78.5 and -50.3‰, respectively.

100   Most of the δ^2H_{hair} values of the specimens were > -50.3‰, but prior studies suggest that linear

101   extrapolation of normalization relationships for δ^2H is appropriate for values within ~100‰ of

102   the range of the standards used for normalization (Kelly et al. 2009, Wiley et al. 2012). The

103   analytical precision of the internal keratin standard was ±1.9‰.

104   We used Google Earth to determine the approximate latitude, longitude and elevation of

105   the collection location of each specimen, based on information provided on the specimen labels.

106   Where information was restricted to broader geographic regions (e.g., counties, national parks)

107   we used values for a central point. Latitude, longitude and elevation values were entered in the

108   Online Isotopes in Precipitation Calculator (http://waterisotopes.org; Bowen and Revenaugh

109   2003; Bowen et al. 2005) to determine average δ^2H values of precipitation for June−August (i.e.,

110   δ^2H$_{GSprecip}$) for each collection site. The small uncertainties associated with our approach for

111   approximating the latitude, longitude and elevation of sample locations had little influence on the

112   δ^2H values of precipitation that were calculated for each site because δ^2H values of precipitation

113   exhibit greater variation across large than small environmental gradients (e.g., of latitude).

114   Specimen collection years spanned a period from 1900−2009. We subset samples by sex for

115   initial analyses to assess potential intersex differences; specimens of unknown sex were excluded

116   from these analyses.

117   The variance of the difference between δ^2H$_{hair}$ and δ^2H$_{GSprecip}$ values should decrease during

118   the period of molt. Therefore, to attempt to more precisely estimate the range of days during

119   which new pelage was presumably synthesized, we empirically evaluated the interval of time

120   during the June−August period for which the standard deviation (created by grouping the Julian

121   days of collection into 5-day intervals) of the difference between individual δ^2H$_{hair}$ and δ^2H$_{GSprecip}$

122   values was minimized. To do this, we calculated the standard deviation of the difference between

123   individual δ^2H$_{hair}$ and δ^2H$_{GSprecip}$ values. We determined the presumed period of molt by visually

124   identifying where the standard deviation was the lowest. We included samples collected during

125   the presumed period of molt in subsequent reduced major axis (RMA) regressions. We performed

126   RMA regressions to assess the relationship between δ^2H$_{hair}$ and δ^2H$_{GSprecip}$ because of symmetry

127   between the dependent and independent variables (i.e., it is arbitrary which variable is plotted on

128   the X and Y axes, because δ^2H$_{hair}$ is influenced by δ^2H$_{GSprecip}$, but δ^2H$_{GSprecip}$ is also calculated from

129 δ^2H_{hair}; Smith 2009) and because both variables contain measurement uncertainty (McArdle

130 1988). We examined model residuals across collection dates to check for non-uniform variance

131 (e.g., heteroscedasticity) across the period of molt. In light of the potential for delayed molt in

132 reproductive female bats (Fraser et al. 2012), we also determined the relationship between δ^2H_{hair}

133 and $\delta^2H_{GSprecip}$ for female red bats collected between July 1 and August 31 and between July 1 and

134 August 7. We performed all statistical analyses in R (R Core Team 2013).

135 **Results**

136   We obtained a total of 112 red bat specimens (41 male, 67 female, 4 sex unknown) for

137 evaluation of the relationship between δ^2H_{hair} and $\delta^2H_{GSprecip}$ (Table S1). For male red bats, the

138 standard deviation for δ^2H_{hair} - $\delta^2H_{GSprecip}$ values for days 160−164 was 33.0 (Fig. 2a). The standard

139 deviation dropped to 4.9 at day 165 and remained low (range: 0.5−7.5) between days 165−219

140 (June 14−August 7). Standard deviations were generally high between days 220−240 (August 8−

141 August 28), averaging 19.9 during this period. The lower standard deviations of δ^2H_{hair} - $\delta^2H_{GSprecip}$

142 values between days 165−219 (June 14−August 7) suggest that this is the approximate period

143 during which male red bats are typically resident on their summering grounds and synthesize new

144 annual pelage. Males collected before June 14 or after August 7 were more likely to have molted

145 at a location other than where they were collected. There was no clear trend in temporal variation

146 of the standard deviation of δ^2H_{hair} - $\delta^2H_{GSprecip}$ values for female red bats (Fig. 2b).

147   A total of 64 male and female specimens were collected between June 14 and August 7.

148 δ^2H_{hair} values for male red bats exhibited a strong positive relationship with $\delta^2H_{GSprecip}$ during this

149 period ($R^2 = 0.69$, $p < 0.001$, $n = 20$; Fig. 3a). Assuming an identical period of molt for female

150 red bats, δ^2H_{hair} from females also exhibited a positive relationship with $\delta^2H_{GSprecip}$, although the

151 variance explained was lower than in males ($R^2 = 0.29$, $p < 0.001$, $n = 44$; Fig. 3a). The mean

152 slope and intercept for males (1.48 and 13.95, respectively) fall within the 95% confidence

153   interval of the slope and intercept for females (1.29-2.21 and 5.09-30.95, respectively), and the

154   mean slope and intercept for females (1.75 and 18.02, respectively) fall within the 95%

155   confidence interval of the slope and intercept for males (1.07-1.89 and 1.89-26.0, respectively).

156   For female red bats, the relationships between δ^2H_{hair} and $\delta^2H_{GSprecip}$ for individuals collected

157   between July 1 and August 31 ($R^2 = 0.33$, $p < 0.001$, $n = 46$) and between July 1 and August 7

158   ($R^2 = 0.39$, $p < 0.001$, $n = 30$) were stronger than the relationship between δ^2H_{hair} and $\delta^2H_{GSprecip}$

159   between June 14 and August 7. When male and female bats (from June 14-August 7) were

160   combined, there was a strong positive relationship between δ^2H_{hair} and $\delta^2H_{GSprecip}$ ($R^2 = 0.37$, $p <$

161   0.001, $n = 64$; Fig. 3a), with no consistent trend in model variance across day of collection (Fig.

162   3b). Conversion of δ^2H_{hair} values obtained from the four red bats of unknown sex (which were

163   collected between June 14 and August 7; Table 1) to $\delta^2H_{GSprecip}$ using the combined relationship for

164   males and females (Fig. 3a) produced $\delta^2H_{GSprecip}$ values within 5‰ of those calculated for these

165   sites at http://waterisotopes.org.

166   To assess the species-specific nature of the relationship between δ^2H_{hair} and $\delta^2H_{GSprecip}$ we

167   compared likelihood-of-origin maps produced based on the separate regression equations

168   estimated for red bats and their sister taxa, hoary bats. For this exercise, we used as an example a

169   representative δ^2H_{hair} value of -40‰. For red bats we converted this δ^2H_{hair} value to $\delta^2H_{GSprecip}$ using

170   the relationship between δ^2H_{hair} and $\delta^2H_{GSprecip}$ for our combined male and female sample (Fig. 3a),

171   which yielded a $\delta^2H_{GSprecip}$ value of -34.1‰. For hoary bats, there currently exist two published

172   estimates of the relationship between δ^2H_{hair} and $\delta^2H_{GSprecip}$ during their presumed molting period

173   (20 June–23 August). The first, from Cryan et al. (2004) is based on δ^2H_{hair} data from museum

174   specimens and estimates of $\delta^2H_{GSprecip}$ from Meehan et al. (2004). The second, from Cryan et al.

175   (2014), contains the δ^2H_{hair} data from Cryan et al. 2004, along with additional samples (Table 1).

176   In Cryan et al. (2014), the δ^2H_{hair} data were recalibrated to different standards and estimates of

177 δ^2H$_{GSprecip}$ were derived from the same model (Bowen et al. 2005) that we used for deriving δ^2H-

178 $_{GSprecip}$ values for the locations from which our red bat samples were collected. Conversion of a

179 δ^2H$_{hair}$ value of -40‰ using Cryan et al. (2004) and Cryan et al. (2014) yields δ^2H$_{GSprecip}$ values of

180 -19.2 and 3.6‰, respectively. Based on these conversions of δ^2H$_{hair}$ to δ^2H$_{GSprecip}$ values, we

181 produced likelihood-of-origin maps for each bat using the Isoscapes Modeling, Analysis, and

182 Prediction tool, IsoMAP (http://www.isomap.org; Bowen et al. 2014). The likelihood-of-origin

183 maps based on a common δ^2H$_{hair}$ value of -40‰ were substantially different when using the

184 species-specific equations for red and hoary bats and applying a liberal estimate of uncertainty in

185 the δ^2H$_{GSprecip}$ values (10‰, based on the variation in our regression equations). The difference

186 between the maps for red and hoary bats remained regardless of whether the Cryan et al. (2004)

187 or Cryan et al. (2014) relationship was used for hoary bats (Fig. 4).

188 **Discussion**

189 Stable isotope analysis has emerged as an important tool for studies of movement,

190 migration, population connectivity, and habitat use of animals not amenable to traditional

191 tracking methods (Hobson 1999; Cryan et al. 2004; Rubenstein and Hobson 2004; Fraser et al.

192 2012). However, applying isotope data to make such inferences requires accurate knowledge of

193 the relationship between δ^2H$_{hair}$ and δ^2H of precipitation. This relationship is often species-

194 specific for different animals (Hobson et al. 2012), including bats (Britzke et al. 2009; Table 1),

195 which makes it important to establish this relationship for focal species of interest or conservation

196 concern. Given the recent impact of wind turbines on the migratory red bat, and the growing

197 interest among conservation biologists and natural resource managers in applying stable isotopes

198 to track the origins of *Lasiurus* spp. killed at wind-turbine facilities, it is essential to establish the

199 reliability of δ^2H for tracking the summering grounds of red bats. Our data showed positive

200   relationships between δ^2H_{hair} and $\delta^2H_{GSprecip}$ for both male and female red bats, which indicates that

201    $\delta^2H_{GSprecip}$ values deduced from δ^2H_{hair} may be used to infer the summering locations of bats

202   captured (or killed) at distant sites, such as at wind turbines or on their overwintering grounds.

203   We estimated a period of molt of June 14−August 7 for male red bats based on

204   comparison of δ^2H_{hair} and $\delta^2H_{GSprecip}$ values. Greater variation in δ^2H_{hair} - $\delta^2H_{GSprecip}$ values for male

205   red bats collected before June 14 and after August 7 suggests that individuals collected outside of

206   the approximate timeframe of June 14−August 7 were less likely to have molted at the site of

207   capture. This estimated period of molt is similar to the δ^2H-inferred period of molt (June 20−

208   August 23) reported for the hoary bat (Cryan et al. 2004), a close relative of the red bat (Roehrs et

209   al. 2010). In contrast to males, there was no distinct period of low variability in δ^2H_{hair} - $\delta^2H_{GSprecip}$

210   values for female red bats. This lack of a period of low variability may indicate that females molt

211   outside of June-August, such as during migration. Another explanation is that female red bats

212   undertake long-distance dispersal or even begin to migrate soon after molt, which would decrease

213   our ability to detect a distinct molt period with δ^2H, particularly if there exists geographic

214   variation in the seasonal timing of molt and/or migration. Indeed, studies suggest that some

215   female bats (including hoary bats, Cryan et al. 2004) delay molt until after parturition and

216   lactation (Quay 1970; Jones and Genoways 1976) when they then synthesize pelage rapidly at the

217   end of the growing season, within ~ 2 weeks of autumn migration (Cryan et al. 2004). Regardless

218   of its precise cause(s), the lack of a distinct period of low variability in δ^2H_{hair} - $\delta^2H_{GSprecip}$ values

219   for female red bats does preclude the use of δ^2H_{hair} for identifying their summering grounds.

220   Within the estimated period of molt, we found significant positive relationships between

221   δ^2H_{hair} and $\delta^2H_{GSprecip}$ for red bats that were similar for males and females. However, the

222   relationship between δ^2H_{hair} and $\delta^2H_{GSprecip}$ for female red bats explained less of the variance (e.g.,

223   lower R^2) compared to male red bats. The weaker relationship for females might be a function of

224   delayed molt in reproductive females, as discussed above. Indeed, $\delta^2H_{GSprecip}$ had a stronger

225 relationship with δ^2H_{hair} for female red bats collected only in July and August than for females

226 from June 14-August 7. Although the precise timing of molt of female red bats warrants further

227 study, the regression slopes and intercepts for males and females were not different (Fig. 3a) and

228 there was only a small (5‰) maximum difference in $\delta^2H_{GSprecip}$ between the respective equations

229 for males and females for δ^2H_{hair} values ranging between -10 and -60‰. Thus, our results suggest

230 that male red bats do not display aberrant migratory patterns or isotopic discrimination relative to

231 female red bats (as suggested by Britzke et al. 2009) or other bat species (Table 1). These results

232 also suggest that a single relationship may be used for conversion between δ^2H_{hair} and $\delta^2H_{GSprecip}$

233 for both sexes of red bats. A single relationship applicable to either sex implies that this approach

234 may be used for assessing the origin of red bats of unknown sex. For example, $\delta^2H_{GSprecip}$ values

235 derived from δ^2H_{hair} values for four red bats of unknown sex in our dataset (Table 1) were within

236 5‰ of the actual $\delta^2H_{GSprecip}$ values at these sites, which is less than the estimated uncertainty

237 (10‰) in the relationship between δ^2H_{hair} and $\delta^2H_{GSprecip}$ for red bats.

238   In contrast to our results, Britzke et al. (2009) found a negative relationship between

239 δ^2H_{hair} and $\delta^2H_{GSprecip}$ for male red bats. Although precise reason for this discrepancy this

240 uncertain, we offer two potential explanations. The Britzke et al. (2009) dataset included samples

241 from red bats collected between May 15 and August 1 during the years 2001-2005, whereas we

242 identified a molt period of June 14-August 7 using samples from the years 1900-1972. Thus, one

243 explanation for these differing results is that some of red bats analyzed in Britzke et al. (2009)

244 may have been sampled before they reached their summering grounds and molted new pelage,

245 which means that δ^2H_{hair} values from such bats would partly indicate their location the prior

246 summer rather than of the year in which they were collected. A second possible explanation is

247 that bats used in Britzke et al. (2009) were samples across a smaller number of years. Although

248 there is no long-term trend in $\delta^2H_{GSprecip}$ during the last ~100 years (Hobson et al. 2010; Hobson et

249 al. 2014), there can be inter-annual spatial variation in $\delta^2H_{GSprecip}$. Such variation may be

250 minimized when using samples from a large number of years (i.e., 1900-1972), whereas it may

251 have a larger impact when using samples from a relatively small number of years (i.e., 2001-

252 2005).

253 Our results provide confidence for using $\delta^2 H_{hair}$ to identify the location of the summering

254 grounds (i.e., the location where new pelage was synthesized) of red bats of unknown geographic

255 origin. In contrast to intraspecific similarities, our results underscore the species specificity of the

256 $\delta^2 H_{hair}$ and $\delta^2 H_{GSprecip}$ relationship, even among closely related bat species. For example, a $\delta^2 H_{hair}$

257 value of -40‰ yielded distinct $\delta^2 H_{GSprecip}$ values and likelihood-of-origin maps for red and hoary

258 bats based on using the regression presented here for red bats and those of Cryan et al. (2004) and

259 Cryan et al. (2014) for hoary bats (Fig. 4). Thus, our study provides critical calibration data for

260 the use of $\delta^2 H_{hair}$ to infer the movement and migration patterns of red bats, and will enable future

261 studies on red bat ecology and conservation, especially in the context of assessing the impacts of

262 threats associated with global environmental change.

263 **Acknowledgments**

264 We thank John Hoogland for providing feedback on an earlier version of the manuscript. Suzanne

265 Peurach at the Smithsonian National Museum of Natural History facilitated the sampling of

266 museum specimens and Robin Paulman assisted with stable isotope analyses.

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Figure 1

Likelihood-of-origin maps for a δ^2H_{hair} value of -40‰ that was transformed into $\delta^2H_{GSprecip}$ 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 $\delta^2H_{GSprecip}$ 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*).

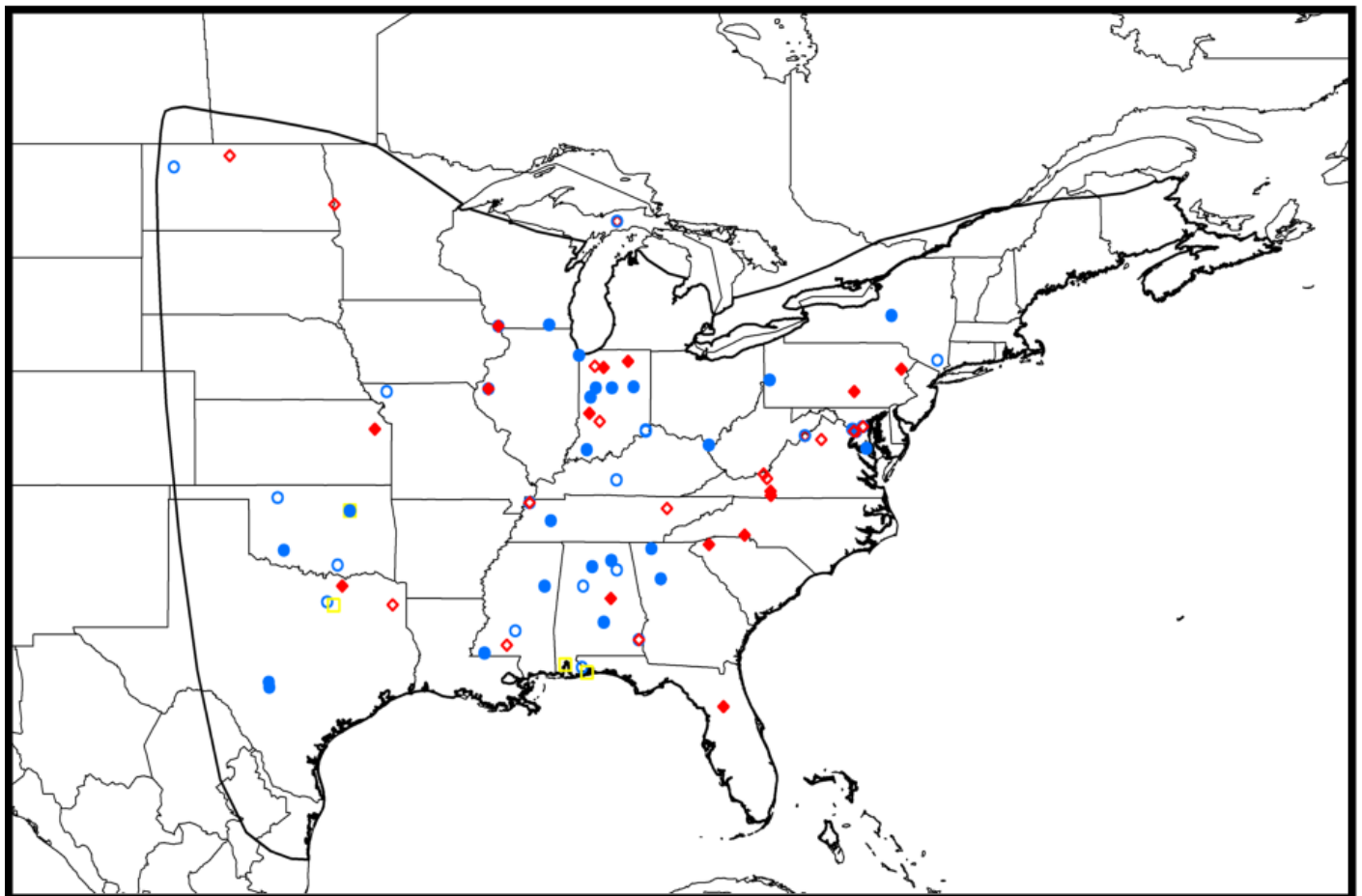



Figure 2

Differences between δ^2H_{hair} and $\delta^2H_{GSprecip}$.

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).

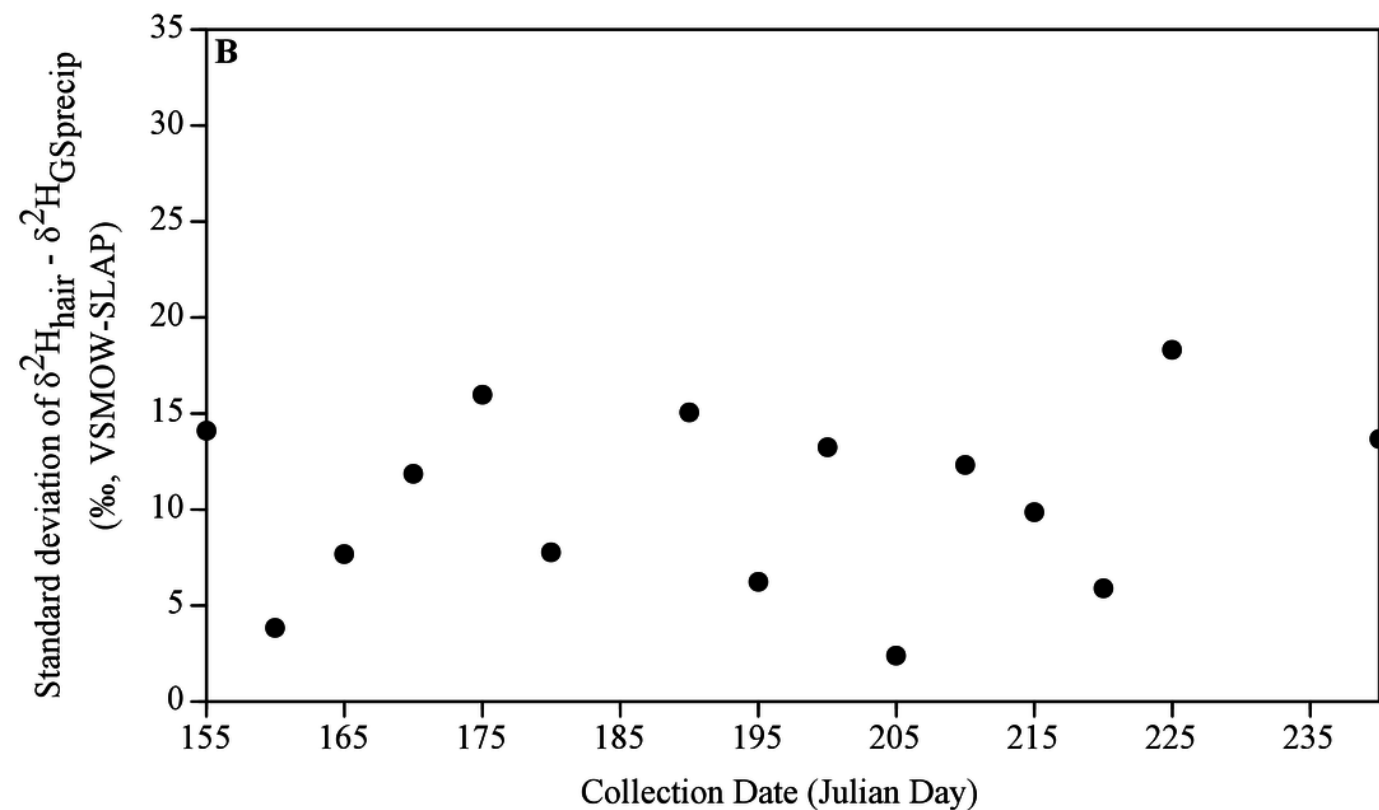
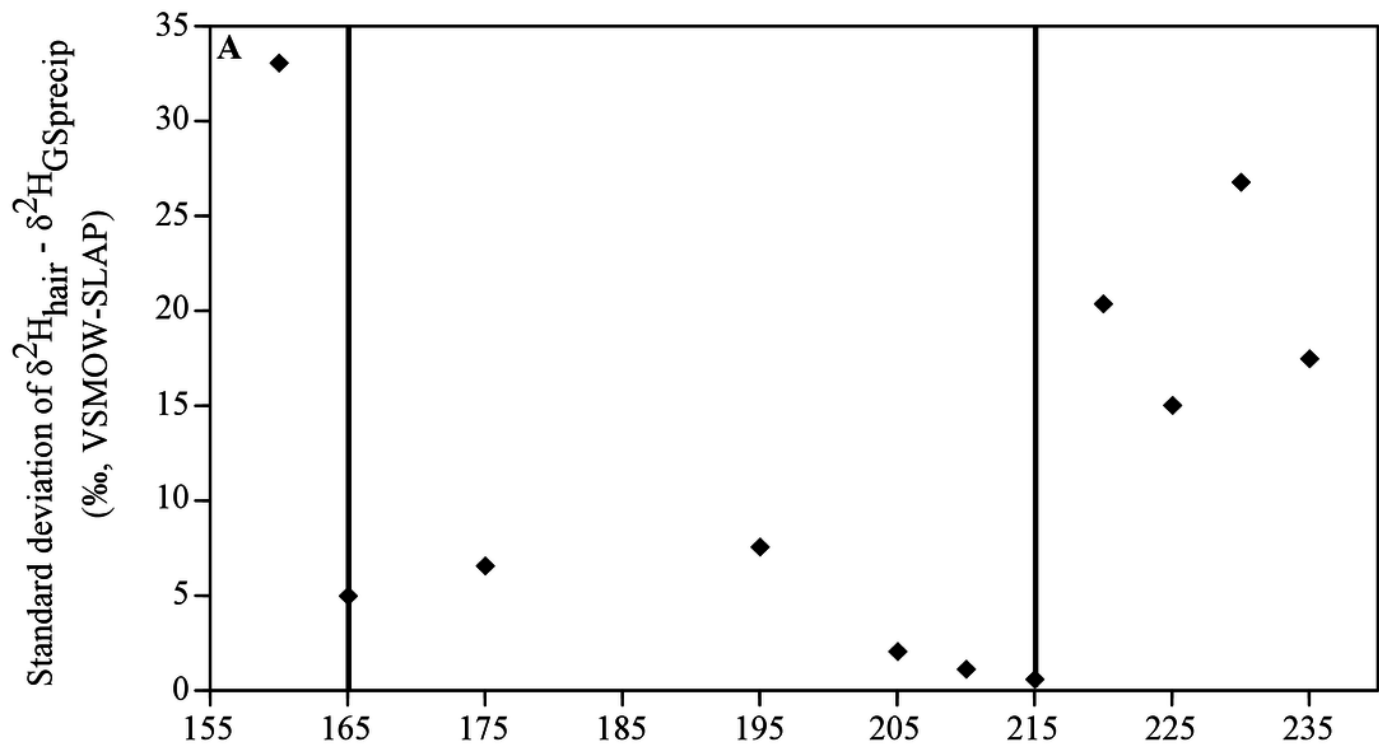



Figure 3

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

The relationship of δ^2H_{hair} and $\delta^2H_{GSprecip}$ 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.

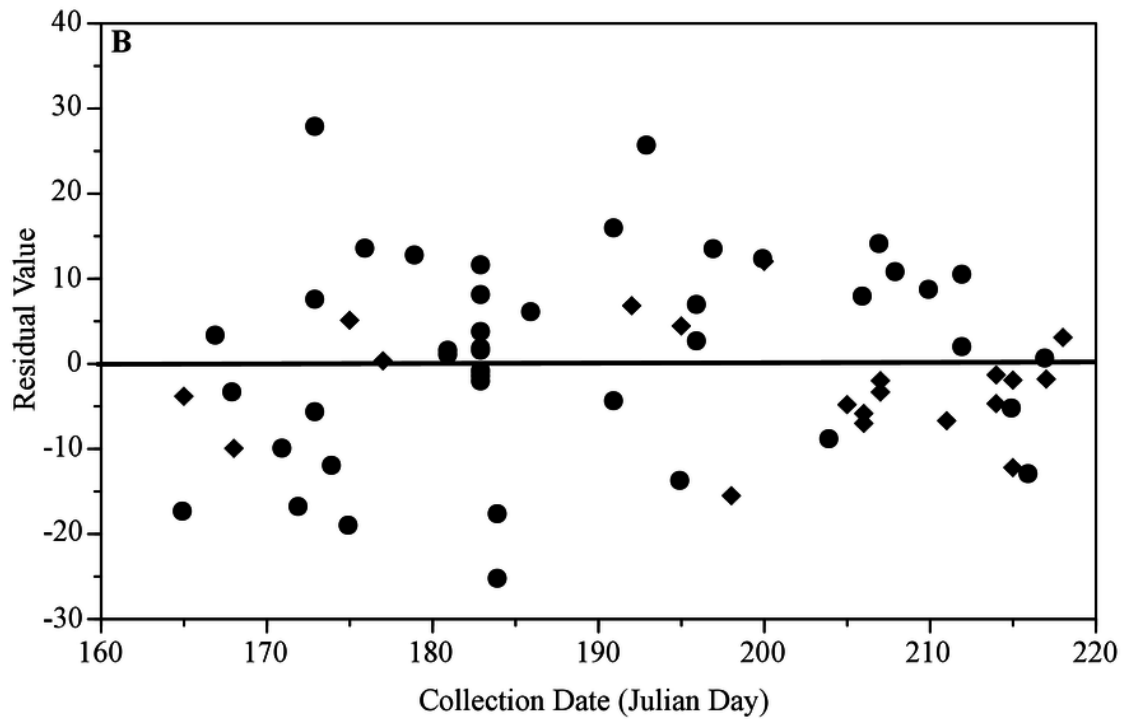
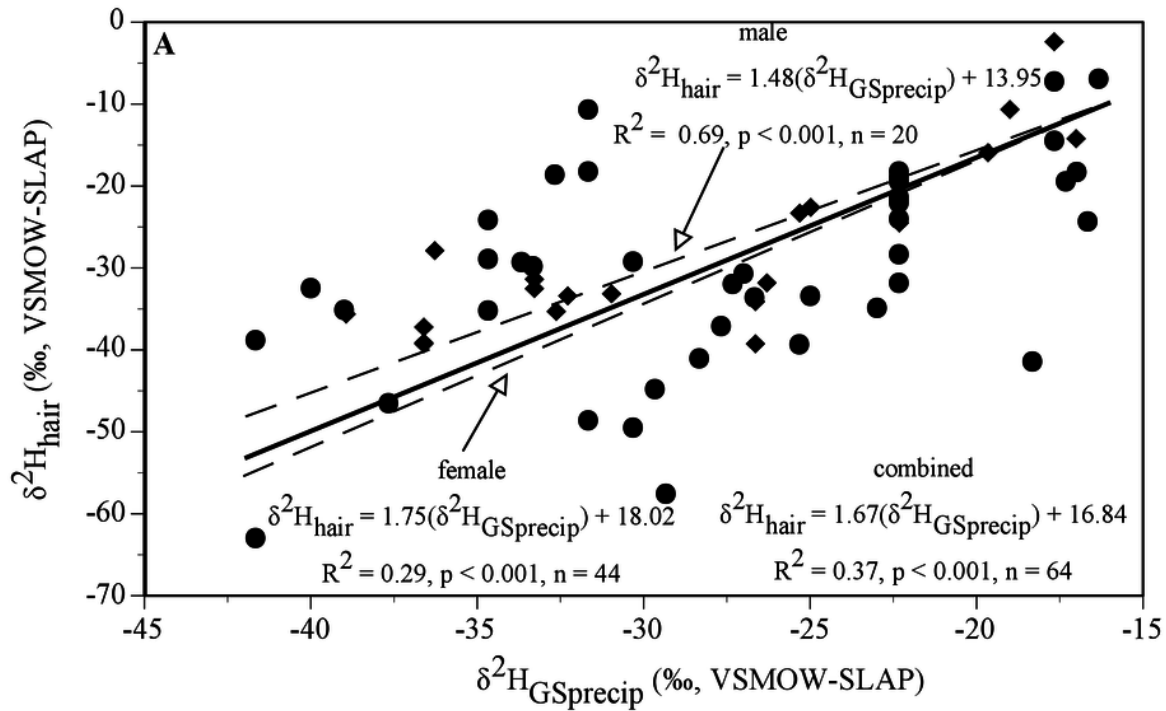
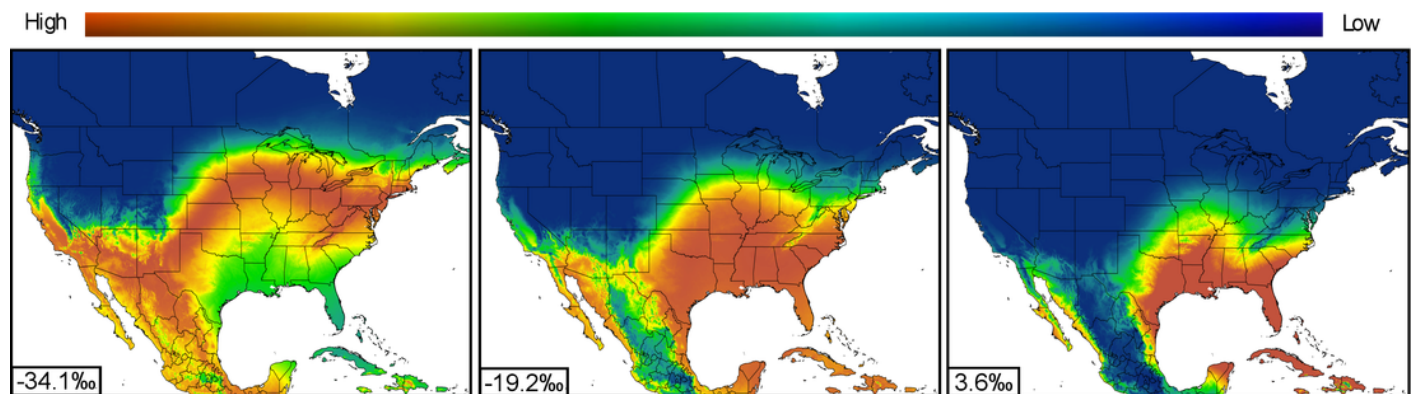



Figure 4

Likelihood-of-origin maps for a δ^2H_{hair} value of -40‰ that was transformed into $\delta^2H_{GSprecip}$ 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 $\delta^2H_{GSprecip}$ 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*).

Review of published relationships between δ^2H_{hair} and $\delta^2H_{GSprecip}$ for North American bats.

Note that the combined regressions from Britzke et al. 2009 include juvenile bats of unknown sex.

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