

USING HYDROGEN ISOTOPES TO ASSIGN ORIGINS OF BATS IN THE EASTERN UNITED STATES

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Stable hydrogen isotopes (δD s) in metabolically inert tissues such as feathers and hair provide a set of endogenous markers that may be useful for establishing migratory connectivity in animals. We tested the assumption of a clear relationship between δD values of growing-season-weighted average precipitation (δD_p) derived from 2 geographic information system (GIS) models or latitude (LAT) and δD values in bat hair (δD_h), and examined intra- and interspecific variation in δD_h of 4 bat species in the eastern United States. We analyzed 251 hair samples from 1 long-distance migrant (eastern red bat [*Lasiurus borealis*]) and 3 regional migrants (Indiana bat [*Myotis sodalis*], northern long-eared bat [*M. septentrionalis*], and little brown bat [*M. lucifugus*]) captured during the reproductive period (pregnancy and lactation) when bats are resident. LAT explained more of the variation in δD_h than δD_p derived from either of the GIS-based models, although 1 model of δD_p performed better for some species. We found significant intraspecific differences in the relationships between δD_h and LAT and between δD_h and estimates of δD_p derived from Bowen et al. (δD_{pB}) for *L. borealis*, and significant variation in the regression equations of δD_h and LAT and δD_h and δD_p among species for adult females and adult males. Stable hydrogen isotope analysis may be a valuable tool for studying migratory connectivity in bats, but significant intra- and interspecific variation in δD_h values suggests that pooling across sex and age categories and use of surrogate species should be avoided.

Key words: bats, interspecific variation, intraspecific variation, *Lasiurus borealis*, migration, *Myotis lucifugus*, *Myotis septentrionalis*, *Myotis sodalis*, stable hydrogen isotope analysis

Establishing levels of connectivity between breeding and nonbreeding populations of migratory wildlife is essential to our understanding of their ecology, life history, and behavior. Such information also is essential for their effective conservation and management. Species vary in the strength of connectivity along a continuum ranging from weak, where individuals from a single breeding population migrate to several different wintering locations, to strong, where most individuals from a breeding population migrate to the same wintering location (Rubenstein and Hobson 2004; Webster et al. 2002). Variation in the pattern and dynamics of migration has broad implications for the demography and evolutionary dynamics of populations of migratory animals, and for the

identification and protection of critical habitats used by these species during migration (Webster and Marra 2005).

Stable isotopes provide a set of endogenous markers that may be useful for establishing migratory connectivity in animals (Hobson and Wassenaar 2008; Rubenstein and Hobson 2004). The isotope approach has advantages over the use of exogenous markers because stable isotopes provide time-integrated information that can often be linked directly to geographical regions or habitats, and do not rely on recapture or resighting previously captured individuals. Isotopic analyses of migratory animals in North America have typically used stable hydrogen isotopes (δD s) because, unlike those of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$), geographic variation in weighted mean precipitation δD (δD_p) values is reasonably well characterized and shows a characteristic latitudinal and elevational pattern across North America (Bowen et al. 2005; Hobson 1999) and these patterns are transferred to tissues of plants and animals (Hobson and Wassenaar 2008). Use of δD values to assess migratory connectivity is based on the fact

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that δD values in metabolically inert keratinous tissues such as feathers or hair (δD_f or δD_h , respectively) reflect δD_p , thus maintaining an isotopic record reflecting the location where they were synthesized. To assign individuals captured during or after migration to a source locality, tissue δD values are compared with expected δD_p values for various geographic regions. Geographic assignments are facilitated by geographic information system (GIS) tools depicting δD_p corrected for elevation across North America or Europe (Bowen et al. 2005; Meehan et al. 2004). These models use spatial interpolation or kriging of long-term (45+ years), weighted averages of growing-season precipitation values from a limited number of monitoring sites, primarily those of the Global Network of Isotopes in Precipitation (http://www-naweb.iaea.org/napc/ih/GNIP/IHS_GNIP.html), and have been used extensively to examine patterns of migratory connectivity in birds as well as some other taxa (Hobson 2005a, 2005b, 2008). Recently, more probabilistic approaches have been developed to propagate assignment error inherent in isotopic variance among individuals (Wunder and Norris 2008).

Although use of tissue δD measurements for assigning migrants to source populations rests on the key assumption that these values reflect the δD_p values at the site of tissue formation, this relationship has been tested for only a small number of taxa. Among birds, regression equations describing the relationship between δD_p and δD_f at locations of known feather growth, although often explaining as much as 80% of the variance, are quite variable (reviewed in Hobson 2008). Such variation may result from ecophysiological differences among individuals or taxa associated with incorporation of hydrogen from the environment into tissues (isotopic discrimination), variation among food webs in the contribution of groundwater versus precipitation to the hydrogen pool, influence of interannual difference in δD_p , influence of individuals assumed to be local but that have dispersed into populations from elsewhere, analytical error associated with the measurement of tissue δD values, or shortcomings of the models used to develop location-specific δD_p values (Hobson 2005b, 2008; Lott and Smith 2006; Smith and Dufty 2005; Wunder and Norris 2008). Furthermore, intraspecific variation in tissue δD values may affect the accuracy of geographic assignments if different sex or age classes form their tissues at different times and places, or have different physiological processes influencing deuterium incorporation (Langin et al. 2007; McKechnie et al. 2004; Meehan et al. 2003; Smith and Dufty 2005). Consequently, calibration of models based on long-term averages of precipitation values (Bowen et al. 2005; Meehan et al. 2004) with tissue δD values for known locations may be required to account for these sources of variation and to more accurately assign individuals to their source populations using a map lookup (Hobson 2005b; Lott and Smith 2006) or probabilistic (Wunder and Norris 2008) approach.

Migration is an important part of the ecology and life history of many temperate-zone bat species (Fleming and Eby 2003). Because of the methodological challenges of studying

small nocturnal animals that fly over large distances, knowledge of the migratory patterns of most bats is extremely limited. Based on limited recapture data, migratory bats in North America can be classified as sedentary, moving <50 km between summer and winter roosts; regional migrants, migrating moderate distances (100–500 km); or long-distance migrants, moving >500 km between summer and winter habitats (Fleming and Eby 2003). Although few studies of molt patterns in bats have been conducted, evidence suggests that temperate bats molt during the summer (Constantine 1957, 1958; Cryan et al. 2004; Tiunov and Makarikova 2007). Thus, hair of migratory bats should reflect the isotope signatures of their summer range. In the only study to apply the stable isotope approach to migration in bats, Cryan et al. (2004) found that δD_h of hair from hoary bats (*Lasiurus cinereus*) collected during late summer was correlated with expected δD_p values in the area from which the bats were collected, and based on this relationship, they estimated that hoary bats travel up to 2,000 km during migration. Thus, the δD approach may be suitable for use with bats, but its general applicability requires further testing. For example, before this method can be used to assign summer ranges to animals captured during autumn and winter (e.g., Wunder et al. 2005), potential sources of isotopic variation, such as those based on age or sex, must be understood.

We examined variation in δD_h in 4 bat species in the eastern United States to further the development of using δD analysis to study bat migratory patterns in North America. We collected hair samples during the summer when females were pregnant or lactating and most likely to be resident. Our objectives were to test the assumption of a clear relationship between modeled δD_p or latitude (LAT) and δD_h ; whether there was significant intraspecific variation in the relationship between δD_h and LAT or δD_p based on age and sex; and whether there was significant interspecific variation in the relationship between δD_h and LAT or δD_p . The 4 species represent 1 long-distance migrant (eastern red bat [*Lasiurus borealis*]—Cryan 2003), and 3 regional migrants (Indiana bat [*Myotis sodalis*], northern long-eared bat [*M. septentrionalis*], and little brown bat [*M. lucifugus*]—Fenton and Barclay 1980; Kurta and Murray 2002; Thomson 1982; Whitaker and Hamilton 1998).

MATERIALS AND METHODS

We analyzed hair samples from bats captured during the reproductive period (pregnancy and lactation) when bats are resident. Hair samples of *M. septentrionalis*, *M. sodalis*, and *M. lucifugus* were collected between 15 May and 1 August 2004 and 2005 and hair samples of *L. borealis* were collected between 15 May and 1 August 2001–2005. A small area of fur (up to 1 × 1 cm) was removed from between the scapulae. The extended period of sampling resulted in samples representing either the current or previous year of molt. Samples were stored in numbered microcentrifuge tubes. Sample number, species, sex, age, date of collection, location, and latitude and longitude were recorded for each sample. Capture and

handling of bats followed guidelines approved by the American Society of Mammalogists (Gannon et al. 2007). Estimates of growing-season δD_p were obtained using the GIS models of Meehan et al. (2004) and Bowen et al. (2005). We created a point-data layer of our sampling points and overlaid this on the raster data sets for each δD_p model in ArcGIS (version 9.0; ESRI, Redlands, California) and obtained δD_p estimates for each of the grids in which our points occurred.

All samples were soaked in a 2:1 chloroform:methanol solution for 24 h to remove surface oils and internal lipids. Each sample was then rinsed and air-dried. Approximately 1 mg of material was placed in a precleaned silver capsule (3.5×5 mm), equilibrated with room air for several weeks along with 2 in-house standards of ground feather keratin (chicken: CCHIX = -53.9% Vienna standard mean ocean water [V-SMOW]; and turkey: TURK-1 = -93.4% V-SMOW; University of Georgia, Savannah River Ecology Laboratory) that were isotopically calibrated to the keratin laboratory standard BWBII in the laboratory of Len Wassenaar from Environment Canada. The δD value of BWBII (whale baleen: -108% V-SMOW) used in the calibration represents the nonexchangeable fraction of H in the standard (Wassenaar and Hobson 2003). Measured hydrogen isotope values of the CCHIX and TURK-1 standards were compared to their nonexchangeable δD values to develop a functional relationship that was used to correct for the effect of exchangeable H and to normalize the scale for each analysis. Calibration by this technique ensured that reported δD values of the samples represent the nonexchangeable fraction of H in the samples assuming the kinetics of H isotope exchange are similar between the sample and standard materials. Samples were analyzed at the University of Georgia, Savannah River Ecology Laboratory, and the laboratory of Len Wassenaar from Environment Canada.

All samples and standards were analyzed by pyrolysis gas chromatography using a Finnigan TC/EA (Thermo Fisher Scientific Inc., Waltham, Massachusetts) coupled to an isotope ratio mass spectrometer (Delta^{PLUS}XL; Thermo Fisher Scientific Inc., Waltham, Massachusetts). Multiple in-house standards were at the beginning of a sequence and every 5th sample with a precision of better than $\pm 2\%$ (1 SD) to monitor for possible drift in isotope composition, but none was observed. Approximately 10% of the samples were run in duplicate and all were within 4% of each other. All δD values are reported versus the V-SMOW standard by calibration through the BWBII standard. All of our hair samples were calibrated to a 3-point calibration curve as per the comparative equilibration technique described by Wassenaar and Hobson (2003).

Statistical analysis.—We developed species-specific regression equations between δD_h and LAT, estimates of δD_p derived from Meehan et al. (2004; δD_{pM}), and estimates of δD_p derived from Bowen et al. (2005; δD_{pB}) for all samples. Because of potential physiological and behavioral differences among age and sex classes, we divided samples for each species into 3 sex and age categories (adult males, adult females, and juveniles) and tested whether there were significant differences among the

regression lines for each group. Specifically, we were interested in whether the slopes or intercepts or both differed among groups. Thus, we used PROC GLM (SAS Institute Inc. 2002) to develop regression equations between δD_h and δD_{pM} , δD_{pB} , and LAT for each age and sex group. We then used the LSMEANS statement to test for differences among intercepts and used linear contrasts to test for differences among slopes. We also used this approach to test whether the slopes and intercepts of regression lines differed among species. Because δD_{pM} accounted for little of the variation observed in δD_h (see below), we did not use these estimates in the intraspecific or interspecific comparisons. We used $\alpha = 0.05$ to determine significance of all statistical tests.

RESULTS

We analyzed hair samples from 251 individuals from 73 locations across the eastern United States (Fig. 1). LATs ranged from 29.71°N to 45.07°N for *L. borealis*, 34.98°N to 47.82°N for *M. lucifugus*, 34.92°N to 43.95°N for *M. septentrionalis*, and 35.64°N to 43.96°N for *M. sodalis*. Number of hair samples for each species ranged from 33 for *M. septentrionalis* to 81 for *L. borealis* (Table 1).

The regression equations between δD_h and LAT and between δD_h and δD_{pB} were statistically significant for every species (Table 1). However, equations based on δD_{pM} were not statistically significant for *L. borealis* or *M. lucifugus*. Further, although equations using LAT and δD_{pB} were statistically significant for *L. borealis* and *M. lucifugus*, they explained very little of the variation in δD_h . In general, LAT explained far more of the variation in δD_h than did δD_{pM} or δD_{pB} , and δD_{pB} explained more of the variation in δD_h than did δD_{pM} (Table 1). Because δD_{pM} performed poorly, we excluded it from further analyses.

Because the equations for δD_h and LAT and δD_h and δD_{pB} were not significant for male and juvenile *M. lucifugus*, no interspecific tests were run for this species. There was no significant intraspecific variation in the relationships between δD_h and either LAT or δD_{pB} for *M. septentrionalis* (Table 2). There were significant intraspecific differences in the relationships between δD_h and LAT and between δD_h and δD_{pB} for *L. borealis* (Table 2). Contrary to the expected relationships, δD_h of adult male *L. borealis* increased with LAT (Fig. 2) and decreased with δD_{pB} . Although there were no significant differences among *M. sodalis* age and sex classes in the relationship between δD_h and LAT, the intercepts and slopes of the lines for δD_{pB} differed significantly between adult females and juveniles (Table 2).

Because of intraspecific variation in the relationships between δD_h and LAT and δD_h and δD_{pB} for some species, we examined interspecific variation by age and sex class. There was significant interspecific variation in the regression equations of δD_h and LAT and δD_h and δD_{pB} for adult females and adult males (Table 2). There was no interspecific variation among juveniles. Using LAT as a predictor, the intercept for adult female *L. borealis* differed significantly from that for *M. lucifugus* but not from the other species; the intercept for adult



FIG. 1.—Sampling locations for each of the 4 species examined. Circles represent sites where adult females were sampled, triangles represent sites where adult males were sampled, and squares represent sites where juveniles were sampled.

female *M. lucifugus* also differed significantly from that of adult female *M. septentrionalis*. The slopes for adult female *M. lucifugus* also differed significantly from the slope for *L. borealis*. Similar differences occurred when using δD_{pB} as the predictor. The slopes and intercepts for adult male *L. borealis* differed significantly from those for all other species for both predictors (Table 2).

DISCUSSION

The broad application of the stable isotope approach to establishing migratory connectivity generally rests on several important assumptions, including a clear relationship between δD_p and δD values for the tissue of interest, and relatively low variance in tissue δD values among individuals growing

TABLE 1.—Regression equations for stable hydrogen isotopes in hair (δD_h) and latitude (LAT), estimates of weighted mean precipitation δD (δD_p) from Meehan et al. (2004; δD_{pM}), and estimates of δD_p based on Bowen et al. (2005; δD_{pB}) for *Lasiurus borealis*, *Myotis lucifugus*, *M. septentrionalis*, and *M. sodalis* captured during summer 2001–2005.

Species	<i>n</i>	Latitude	Meehan	Bowen
<i>L. borealis</i>	81	$\delta D_h = 8.09 - 1.25LAT$ $P = 0.0224, R^2 = 0.06$	$\delta D_h = -28.07 + 0.24\delta D_{pM}$ $P = 0.1530, R^2 = 0.02$	$\delta D_h = -26.10 + 0.48\delta D_{pB}$ $P = 0.0201, R^2 = 0.07$
<i>M. lucifugus</i>	78	$\delta D_h = 35.53 - 2.10LAT$ $P < 0.0001, R^2 = 0.32$	$\delta D_h = -37.51 + 0.24\delta D_{pM}$ $P = 0.3608, R^2 = 0.01$	$\delta D_h = -30.82 + 0.52\delta D_{pB}$ $P = 0.0002, R^2 = 0.17$
<i>M. septentrionalis</i>	33	$\delta D_h = 82.55 - 2.76LAT$ $P < 0.0001, R^2 = 0.63$	$\delta D_h = 40.48 + 1.32\delta D_{pM}$ $P < 0.0001, R^2 = 0.53$	$\delta D_h = 5.48 + 0.98\delta D_{pB}$ $P < 0.0001, R^2 = 0.54$
<i>M. sodalis</i>	59	$\delta D_h = 110.78 - 3.47LAT$ $P < 0.0001, R^2 = 0.57$	$\delta D_h = 29.54 + 1.23\delta D_{pM}$ $P < 0.0001, R^2 = 0.28$	$\delta D_h = -2.97 + 0.83\delta D_{pB}$ $P = 0.0001, R^2 = 0.49$

TABLE 2.—Intercepts (β_0) and slopes (β_1) of linear regression equations between stable hydrogen isotopes in hair (δD_h) and latitude (LAT) and estimates of weighted mean precipitation based on Bowen et al. (2005; δD_{pB}) for adult female, adult male, and juvenile *Lasiurus borealis*, *Myotis lucifugus*, *M. septentrionalis*, and *M. sodalis*. *P*-values ≤ 0.05 indicate that the coefficients differ significantly from 0. Coefficients within a species followed by the same lowercase letter do not differ significantly across age and sex groups ($P > 0.05$). Coefficients followed by the same uppercase letter within an age and sex group do not differ significantly across species ($P > 0.05$).

	<i>n</i>	LAT					δD_{pB}				
		β_0	<i>P</i>	β_1	<i>P</i>	<i>R</i> ²	β_0	<i>P</i>	β_1	<i>P</i>	<i>R</i> ²
<i>L. borealis</i>											
Adult females	36	96.48aA	0.0019	-3.64aA	0.0001	0.41	-6.30aA	0.4709	1.35aA	0.0003	0.31
Adult males	17	-127.94bC	0.0032	2.41bC	0.0281	0.40	-58.80bC	0.0001	-0.82bC	0.0482	0.33
Juveniles	28	17.78aF	0.5268	-1.61aE	0.0390	0.10	-23.97aF	0.0016	0.67aE	0.0143	0.16
<i>M. lucifugus</i> ^a											
Adult females	54	24.98B	0.2212	-1.86B	0.0002	0.21	-40.41aB	0.0001	0.33B	0.0492	0.06
Adult males	12	9.32	0.8175	-1.41	0.1557	0.16	-30.90	0.0162	0.49	0.1527	0.19
Juveniles	12	75.80	0.2023	-3.13	0.0552	0.56	-9.31	0.6356	1.09	0.1291	0.40
<i>M. septentrionalis</i>											
Adult females	16	110.74aA	0.0001	-3.40aAB	0.0001	0.74	18.48aA	0.0243	1.25aA	0.0001	0.71
Adult males	10	61.29aDE	0.0733	-2.27aD	0.0080	0.47	-4.73aDE	0.6462	0.79aD	0.0088	0.53
Juveniles	7	110.05aF	0.0162	-3.65aE	0.0046	0.70	17.64aF	0.3138	1.65aE	0.0258	0.47
<i>M. sodalis</i>											
Adult females	39	103.96aAB	0.0002	-3.29aAB	0.0001	0.40	-8.17aA	0.1513	0.71aAB	0.0001	0.35
Adult males	12	141.84aE	0.0032	-4.29aD	0.0008	0.74	-0.59abE	0.9418	0.90abD	0.0115	0.46
Juveniles	8	185.86aF	0.0089	-5.47aE	0.0036	0.59	30.33bF	0.1027	2.18bE	0.0046	0.63

^a Intraspecific comparisons were not attempted because of nonsignificant regression equations for adult males and juveniles. Interspecific comparisons were only made for adult females.

tissues at a given site (Hobson 2005b; Langin et al. 2007). In our analyses of δD_h collected from bats during the putative period of molt, we found that δD_p and LAT explained very little of the variance in δD_h in 2 of the 4 species examined (*L. borealis* and *M. lucifugus*). In contrast, the relationships between both δD_p and LAT and δD_h values were relatively strong for *M. septentrionalis* and *M. sodalis*. Although GIS-based models incorporate variation in δD_p due to altitude as well as LAT, in our analyses, LAT consistently explained more of the variation in δD_h than either δD_{pB} or δD_{pM} . In addition, we found significant intraspecific variation in the relationships between δD_h and LAT or δD_{pB} for some species (particularly *L. borealis*) and significant variation in these relationships among species. Here we discuss possible reasons for the observed variation in the relationship between δD_p and δD_h values, and the implications of our results for the use of stable isotopes to address migratory connectivity in bats.

Hydrogen isotopes in hair may be obtained directly from drinking water or through diet (Birchall et al. 2005; Hobson et al. 1999). A strong relationship between δD_p and tissue δD is expected only for food webs that are driven by precipitation providing hydrogen to shallow-rooted plants. This relationship may break down when precipitation is not the primary source of hydrogen to the food web used by bats, such as occurs in lake environments. Lakes can have higher δD values compared to δD_p as a result of seasonal loss of water due to evaporation. Additionally, animals may derive nutrition from aquatic food webs, thereby modifying the δD that they are exposed to in the environment (Hobson 2005b; but see Clark et al. 2006). Although bat foraging behavior may be relatively plastic (e.g., Ratcliffe and Dawson 2003), species may differ

in the relative proportion of nightly foraging bouts spent in aquatic versus terrestrial habitats. Thus, even though species are utilizing the same general area at the same time they may be exposed to foods derived from water with different δD values.

Of the 4 species we examined, 2 (*M. lucifugus* and *M. sodalis*) regularly forage over open bodies of water, such as ponds, lakes, and slow-moving streams or rivers (Anthony and Kunz 1977; Broders et al. 2006; Humphrey et al. 1977; LaVal et al. 1977), and may derive significant portions of their diet from aquatic emergent insects. Differences between little brown bats and the other species may be a result of little brown bats being more tied to the aquatic system, whereas the other species eat more terrestrial insects. There is likely some fractionation that occurs in the terrestrial food webs, thereby explaining some of the observed differences in δD_h . *L. borealis* eats proportionately more moths than either of these 2 species (Whitaker 2004), but is regularly captured in both aquatic and terrestrial habitats while foraging. By contrast, although *M. septentrionalis* feeds on many of the same orders of insects as *M. lucifugus* and *M. sodalis* (Whitaker 2004), *M. septentrionalis* forages more commonly in forested ecosystems, and rarely forages over water (Broders et al. 2006). Dependence on terrestrial insects that are more closely tied into precipitation-based food webs may have resulted in a stronger relationship between δD_p and δD_h in *M. septentrionalis* relative to the other bat species that forage more regularly over aquatic habitats. Indeed, observed *R*² values for the relationship between δD_p and δD_f are often higher for songbirds feeding on insects found on vegetation in forested ecosystems (Chamberlain et al. 1997; Hobson and Wassenaar

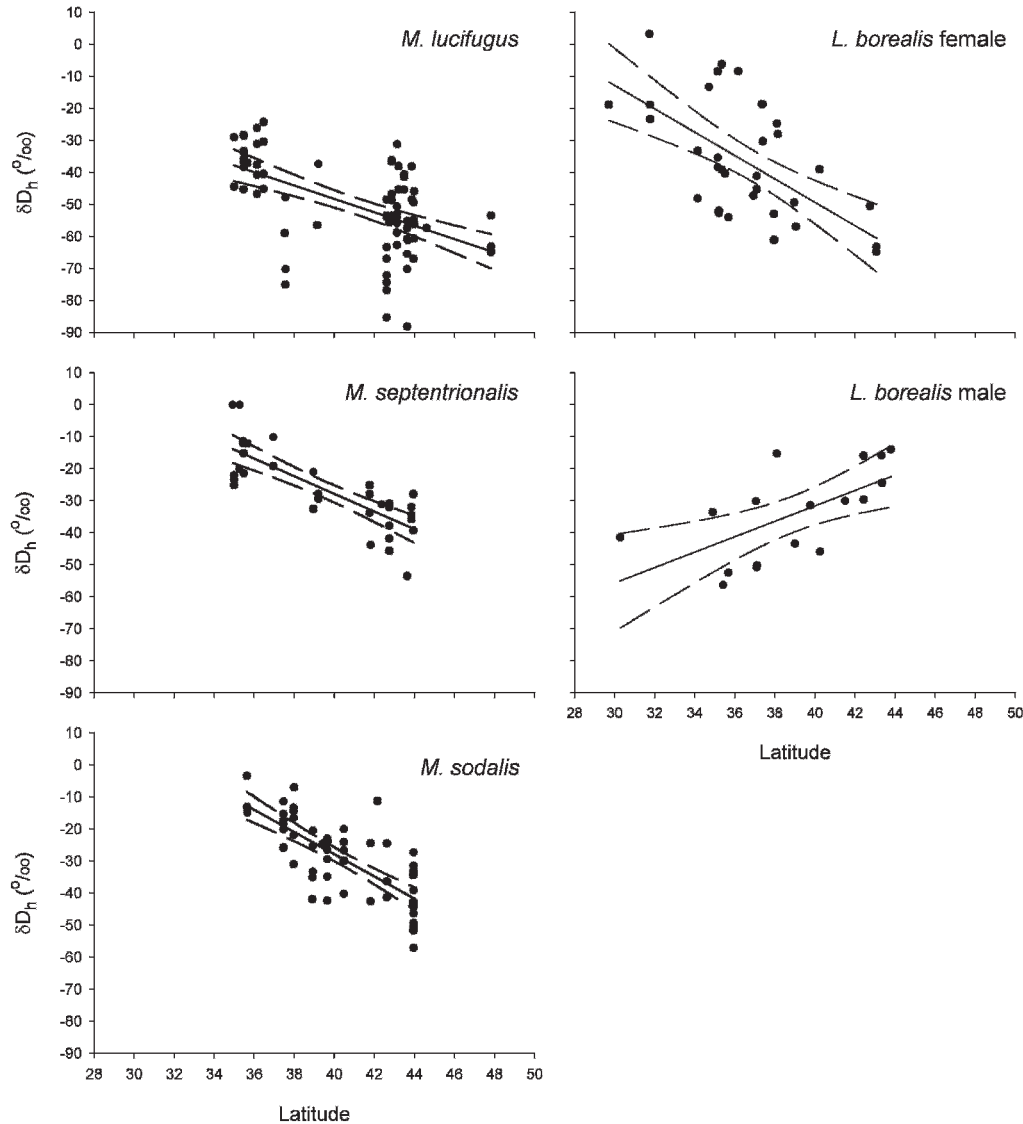


FIG. 2.—Regression lines with 95% confidence interval for the relationships between stable hydrogen isotopes in hair (δD_h) and latitude (LAT) for the 4 species examined. A single regression line combining all sex and age categories is provided for *Myotis sodalis*, *M. lucifugus*, and *M. septentrionalis*, whereas separate regression lines are provided for male and female *Lasiurus borealis* because of the significant intraspecific variation observed for this species.

1997; but see Clark et al. 2006; Hobson et al. 2001) than for raptors that are not as directly linked to precipitation-based food webs (Lott et al. 2003; Lott and Smith 2006).

The relationship between δD_p and δD_h also may be strongly affected by the dynamics of molt. Molting patterns in bats are poorly characterized, but evidence from direct examination of pelage for early growth stages of new hair or differences in color (faded versus nonfaded) in several species of bats suggests that molt takes place in early July to mid-August (Constantine 1957, 1958; Jones and Genoways 1967; Tiunov and Makarikova 2007), although in other species it may take place in late June (Phillips 1966). Cryan et al. (2004) inferred a single annual molt between 20 June and 23 August in *L. cinereus* by assuming that molt most likely occurred when the difference between δD_h and δD_p matched the expected offset for keratin-based tissues (-25% —Wassenaar and Hobson 2001). However, such wide

ranges of molting periods suggest that there may be considerable variation in the timing and duration of molt that may obscure the relationship between δD_h and δD_p , and may have led to the lack of fit we observed for *M. lucifugus* and *L. borealis*. In particular, if individuals molt at different times, and some individuals are sampled before the current year's molt, isotope values would represent the location of these bats during the previous summer. Although evidence suggests that at least some bats are philopatric to summer habitats between years (Gumbert et al. 2002; Kurta and Murray 2002; Willis and Brigham 2004), the degree of interannual philopatry for most species is unknown. If some bats do not exhibit site fidelity between years, then this could lead to significant variation in δD_h among individuals.

Intra-annual variation in timing of molt is an important consideration when using stable isotope methods to infer

origin. Variation in the duration of molt may lead to noise in the data if individuals make movements that would affect their exposure to different food-web δD values during the molting period. Depending on latitude, the presumed period of molt (late June to mid-August) encompasses the postreproductive period, when offspring become independent and females and offspring may make movements to new habitats and/or begin migrating to winter habitats (Barclay 1984; Cryan 2003; Cryan and Brown 2007; Walters et al. 2006). Large latitudinal movements during molt would result in an averaging of isotope values from different areas, and reduce our power to detect a relationship between δD_h and δD_p . Furthermore, such movements make assignments of migrating individuals to putative breeding habitats during the summer problematic, because an individual's δD_h may reflect the average between breeding and overwintering habitats or a specific location between them. Clearly, studies that systematically assess the timing, duration, and synchronicity of molt within bat species through longitudinal studies of known individuals that have had their pelage dyed or otherwise marked are required to refine our understanding of how and when bats may incorporate local hydrogen isotope ratios into their pelage. In terms of addressing migratory connectivity in bats, consistent differences among species in the timing and duration of molt will require species-specific assessment of models comparing δD_h and δD_p before this technique can be successfully applied. Researchers also are reminded that where possible, sampling of hair known to be grown locally will help constrain the variation associated with interannual departures of actual δD_p from that modeled from the long-term International Atomic Energy Association Global Network of Isotopes in Precipitation data set.

Physiological differences among species or individuals within species also may be a source of variation in δD_h values, because they may result in differential body water enrichment, even under exposure to the same environmental isotope values. Isotopic enrichment in birds can occur due to evaporative loss of (lighter) body water during energetically demanding periods such as incubation and chick-rearing (McKechnie et al. 2004; see also Meehan et al. 2003; Smith and Dufty 2005). Presumably this also may occur during pregnancy and lactation in mammals. However, we only observed significant differences in the relationship between δD_h and LAT or δD_{pB} between the sexes for *L. borealis*, suggesting that intersexual physiological differences may not be generally important in bats. However, differences in seasonal energetic demands among species, and their interaction with the timing and duration of molt, may have contributed to the interspecific variation we observed.

The positive relationship between δD_h and LAT for male red bats was very surprising. Hair samples from male red bats collected in June from Kentucky had very negative values for that location. This might indicate that male red bats migrate to their summer range much later than females, and thus, males caught early in the season may still be far from more northern latitudes where they spent the previous summer. However, this

only explains part of the deviation from the expected because bats from northern sites had less negative values than expected for those summer sites. Male red bats would have to migrate north after going through a summer molt in a southern area for this to occur. Although there is no evidence of a "reverse migration" in red bats, there is some evidence that males congregate along migratory routes to mate with females in late summer and early fall (Cryan 2008). Thus, male red bats may not be following the normal migratory routes during the late summer resulting in the positive relationship between δD_h and LAT. However, other factors also may have contributed to this relationship and future research should examine potential causes of the different δD_h we found for male and female *L. borealis*.

We expected the GIS-based models using δD_p to be better predictors of δD_h because they describe the presumed isoscape experienced by the bats by incorporating variation in δD_p due to altitude as well as LAT (Bowen et al. 2005; Meehan et al. 2004). However, of the 3 predictors of δD_h we tested, LAT was the one factor that consistently explained the most variation in δD_h , whereas δD_{pM} explained the least. Such a pattern suggests that the bats incorporated the overall north-south gradient in δD_h , but that predicted δD_p values were not reflective of the ratios incorporated into the hair. Current GIS-based models are based on interpolated values of a limited number of sites, and coverage is particularly sparse in the eastern United States. The model grid size of Bowen et al. (2005) is larger than the model of Meehan et al. (2003), which allows for more appropriate interpolation of δD_{pB} , or alternatively the reduced grid size simply led to more error in the modeling process. Additionally, Bowen et al. (2005) used a larger number of sampling sites for their predictions. As the sampling density of precipitation sites increases, the ability of GIS-based models to estimate δD_p will likely improve, thereby maximizing the utility of these GIS-based models for the study of animal migration (Hobson 2008; Kelly et al. 2008). Another factor that may help to explain the weaker relationship between the 2 GIS-based model values and δD_h is intrinsic discrimination that occurs between precipitation and food resources. Thus, although the GIS-based models are appropriate estimators of δD_p (particularly for Bowen et al. 2005), we lack the discrimination rate for conversion of δD_p to δD_h . Wide confidence limits around our regression lines (Fig. 2) suggest that any geographic assignments of migrating individuals based on our models will be subject to moderate to high levels of uncertainty depending on the bat species.

Although δD_h analysis has not been used extensively in studies of bat migration (Cryan et al. 2004), there is increasing interest in the use of this technique in light of concern over widespread wind power development and the potential for high levels of bat mortality during migration (Arnett et al. 2008; Kunz et al. 2007). Our results suggest that δD_h measurements may be a valuable tool in studying migratory connectivity in bats. However, until controlled experiments can be conducted, several precautions are warranted. Because significant variation in δD_h exists among age and sex classes

in some species, pooling of data should be avoided in study design and analyses. Further, because of interspecific differences, relationships between δD_h and LAT or δD_p must be determined independently for each species. Finally, existing models of δD_p may not be adequate for studies of bats in the eastern United States, particularly for studies of bats in the mid-Atlantic and southeastern United States.

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