

ANALYSIS OF STABLE ISOTOPES OF HYDROGEN TO DETERMINE MIGRATIONAL SOURCE OF
SILVER-HAIRED BATS (*LASIONYCTERIS NOCTIVAGANS*) IN ALABAMA

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THESIS ABSTRACT

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Samuel James Hirt

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Signatures of deuterium obtained from claws of 21 silver-haired bats (*Lasionycteris noctivagans*) were used to estimate the nearest probable pre-migratory location of these bats. For 5 consecutive weekends beginning 28 September 2007, bats were captured in mist nets at Walls of Jericho and near Paint Rock, Jackson Co., Alabama. In addition to the 21 silver-haired bats, 2 hoary bats (*Lasiurus cinereus*) and 2 big brown bats (*Eptesicus fuscus*) were preserved as museum specimens. Claw, wing, feces, and hair samples were taken from 1 big brown bat and 2 silver-haired bats (1 male and 1 female) for comparison between species. Mean value of signatures of deuterium in claws of silver-haired bats was -87.07‰ (SE = 14.67). I used GIS maps of the distribution of stable isotopes of hydrogen to determine estimates of distance traveled during migration using signatures of hydrogen isotopes. Data from analysis of stable isotopes support previous observations that silver-haired bats are migratory and provide evidence that they migrate great distances, possibly >1,600 km. There was a significant

interaction of sex by night captured on signatures of deuterium ($F_{2,20} = 5.7$, $P = 0.022$). One-way ANOVAs revealed no difference between sexes ($F_{1,20} < 0.001$, $P = 0.998$) or night of capture ($F_{7,20} = 0.46$, $P = 0.846$) indicating no sex-specific differences in migration patterns or time-dependent variation in those patterns. Preliminary comparisons between 2 specimens of silver-haired bats and 1 big brown bat for claw, wing, hair, and fecal samples indicated that claws may be the best tissue to use for analysis of stable isotopes in studies of long-distance dispersal, and that male and female silver-haired bats may molt at different times; however a larger sample is needed to fully elucidate these relationships.

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Style of *Journal of Mammalogy* was followed.

SPSS statistical software 16.0 for Windows was used for statistical analyses.

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INTRODUCTION

Isotopes are elements with the same number of electrons and protons, but with differing numbers of neutrons. Those with more neutrons are heavier and, therefore, require slightly more energy to move during processes such as evaporation. Isotopes occur in different abundances in nature. For elements found in water, hydrogen and oxygen, the more abundant isotope is the lighter isotope. Oxygen-16 is the more abundant isotope and oxygen-18 occurs in trace amounts; hydrogen-1 is the more abundant isotope and hydrogen-2 (deuterium) is less abundant. Using stable isotopes in research is safe and convenient because there is no handling of radioactive substances and ratios are predictable in certain processes in nature.

Stable isotopes of hydrogen and oxygen in water occur in varying degrees depending on size, volume, surface area, elevation, latitude, longitude, and location of the water source. This gives a unique isotopic signature to bodies of water and creates general patterns according to geographical areas (Bowen et al. 2005). For example, in the United States, bodies of water that are more inland have less deuterium than bodies of water closer to the ocean, which is why they have a more negative value than coastal bodies of water (Bowen et al. 2005). This is due to weather patterns, evaporation and precipitation events, and the fact that water with hydrogen-1 evaporates slightly more

readily than water with deuterium (Gat 1996). Tissues of animals reflect isotopic signatures occurring in the geographic region of their source of water (Gannes et al. 1998). This has been confirmed in controlled laboratory experiments with woodrats (*Neotoma*) where signatures of hydrogen reflected signatures of their drinking water, food, and atmospheric water (Podlesak et al. 2008).

Use of stable isotopes for studying migration is progressing rapidly and is being used more frequently with different animals. Stable isotopes have been evaluated to estimate frequency of migration and provide rough estimates of natal origins after long-distance dispersal (Nathan et al. 2003). Research evaluating signatures of hydrogen and oxygen have used feathers of birds to track origins of migration (Hobson and Wassenaar 2001). Stable isotopes also have been used as evidence that monarch butterflies (*Danaeus plexippus*) migrate from the eastern United States to Cuba (Dockx et al. 2004) and from the midwestern United States to Mexico (Wassenaar and Hobson 1998). However, little research has been done with signatures of isotopes of hydrogen and oxygen in mammals, especially bats.

Many studies using other stable isotopes, such as signatures of carbon and nitrogen, have been conducted on bats, but relatively few of these studies have attempted to determine routes of migration. One study compared diets of bats and stable isotopes of carbon to determine importance of fruit and CAM (crassulacean acid metabolism) plants in migratory and non-migratory bats (Fleming et al. 1993). Analysis of hydrogen isotopes in hair (Cryan et al. 2004) provided evidence of geographic migratory patterns of hoary bats (*Lasiurus cinereus*). However, most studies have assessed stable isotopes to ascertain food consumption in C₃ and C₄ foodwebs (Ceballos et al. 1997, Herrera et al.

1993, Nassar et al. 2003, Sullivan et al. 2006, Voigt and Kelm 2006, Voigt et al. 2007).

As an ecological tool, stable isotopes are increasing in their importance and their applications to research a variety of questions.

Bats have varying migratory and hibernation strategies. The silver-haired bat (*Lasionycteris noctivagans*) enters a state of torpor when cold (Dunbar 2007), but museum records indicate that at least some migrate (Kunz 1971, Cryan 2003). There has not been a detailed study of seasonal movements of these bats, and there is no evidence that they migrate to and from North and South America, but there seems to be a difference in movement patterns between eastern and western populations (Cryan 2003).

Silver-haired bats apparently migrate through Nebraska, but also may have some populations that stay there all year (Geluso et al. 2004). Studies on their winter range indicate that silver-haired bats can be active or hibernating during November-February as far north as eastern or western Canada (Cryan 2003, Izor 1979). Wintering strategies of silver-haired bats seem to be variable and inconsistent within different geographic areas.

Silver-haired bats in the southeastern United States are rare throughout most of the year. The southeastern United States is the southernmost part of its range (Kunz 1982). Silver-haired bats have been observed in northeastern Alabama beginning in late autumn and may be there all winter or in spring (personal observation). Because of their unknown wintering strategy and broad geographic range, it is not known where these bats are coming from as they arrive in Alabama. Analyses of stable isotopes, may allow us to approximate the migrational distance traveled by these bats.

Traditional methods for studying migratory patterns are expensive, lengthy in time, and tedious. In contrast studies of stable isotopes are relatively inexpensive, can

be done in less time, and require less field work. Results of my study may prove important for ecologists studying potential migratory routes of bats. In areas with species of unknown migratory status, or areas that border migratory and non-migratory populations, it would be especially easy to identify migratory status using analyses of stable isotopes rather than using conventional capture-mark-release or similar methods (Cockrum 1969).

Using stable isotopes in claws of bats to show evidence of migration, although suggested (Gannes et al. 1998), has not been used previously. The only prior study of migration in bats using the stable isotopes of hydrogen examined hair samples (Cryan et al. 2004). Hair, however, may not work well for species for which molt patterns are unknown, for which molts are irregular in timing, or for which the times of molt differ among species or sexes. Conversely, examining stable isotopes in claws should show consistency because of continuous growth in all species; however, hair and claw growth assumptions need further testing. Claws may provide a more reliable tissue standard for use of stable isotopes in studying migration. This method could be used for other species and possibly other orders or classes of mammals.

In addition to using claws to determine distance of migration, I compared variation in stable isotopes among bat species and types of tissues. Exactly how these isotopes transfer in bats from drinking water or food to tissues is unknown and may vary depending on the tissue being tested. I examined a non-migratory species, the big brown bat (*Eptesicus fuscus*), which, because it is non-migratory, is expected to have an isotopic signature that correlates with the geographic region where it lives throughout the year. I evaluated isotopic signatures of multiple tissues comparing silver-haired bats and a big

brown bat. I also took claw samples from 2 migrating hoary bats (*Lasiurus cinereus*) to compare with the silver-haired bats.

The primary objective of this study was to use claws to determine approximate migratory distance traveled of silver-haired bats by analyzing signatures of deuterium and GIS precipitation maps provided for studies of long distance dispersal (Meehan et al. 2004). Furthermore, I wanted to test for differences between signatures of deuterium in males and females and between nights of capture of silver-haired bats in Alabama. In addition to analysis of silver-haired bats, I did a preliminary comparison of the variation in signatures of deuterium among samples of 3 different species of bat. I compared variation in stable isotopes of claws between silver-haired bats and a migratory species (*L. cinereus*) and of hair, claw, wing, and feces of silver-haired bats and a non-migratory species (*E. fuscus*).

MATERIALS AND METHODS

Beginning 27 September 2007, 21 silver-haired bats (12 males, 9 females), 2 hoary bats (2 males), and 2 big brown bats (*Eptesicus fuscus*; 1 male, 1 female) were captured during 5 consecutive weekends at Walls of Jericho (34°59'01.0"N, 86°05'40.3"W) and near Paint Rock (34°46'53.8"N, 86°18'20"W), Jackson Co., Alabama. The 2 sites were about 40 km apart. Upon capture, each bat was placed into a cotton bag and transported to the lab. In the laboratory, fecal pellets were collected from the bags, and blood samples were taken and separated into blood and plasma using a centrifuge. Fecal and blood samples were stored in a standard deep-freeze at 4° C. All specimens, according to Auburn University IACUC protocol PRN 2006-1101, were prepared as museum specimens and deposited in the Auburn University Collection of

Mammals. After specimens were prepared as vouchers and dried, the entire claw was cut from the first three phalanges of the right foot of each individual, however only one claw was used in the analysis. Hair was collected from the rump, and wing membrane was collected between the first and second phalanges of the right wing, where no hair was present using a hole punch. Feces also were taken from 2 silver-haired bats and 1 big brown bat for comparison of stable isotopes among types of tissues. Samples were rinsed with a 2:1 chloroform:methanol solution to remove lipids. Samples were then allowed to dry for 48 h in a 25°C drying oven. After drying, all samples were cut so that they weighed about 0.5 mg, wrapped in silver capsules, and sent to the Cornell Isotope Laboratory, Ithaca, New York. Whole claws of silver-haired bats weighed ca. 0.5 mg; thus, whole claws were used in analyses. Some claws of big brown bats and hoary bats were subsampled because mass of their claws exceeded 0.5 mg; all samples were taken from the proximal part of each claw. Extra claws, feces, and wing punches were then stored in the freezer for future analysis.

Other tissues were taken from 2 silver-haired bats and 1 big brown bat. After specimens were prepared as voucher specimens, I took a 3-mm-diameter sample of wing membrane and a sample of hair from each of these 3 bats. Hair was taken from the rump, just above the uropatagium, of all three bats, and cut as close to the skin as possible. Fecal samples also were taken from these individuals. The 2 silver-haired bats were captured on 4 (specimen Ln 2) and 16 (specimen Ln 16) October. The big brown bat was captured on 6 October (specimen Ef2).

At the Cornell Isotope Laboratory, 2 in-house standards were used as part of the quality-assurance-and-control protocol; benzoic acid and RPG (feather) and three

international reference materials; IAEA CH7 (δ 2H), IAEA CO-1 (δ 18O), and IAEA CO-8 (δ 18O). All results were expressed as deuterium in parts per thousand (ppt, or ‰) relative to Vienna Standard Mean Ocean Water and will hereafter be referred to as the signature of deuterium. Samples were pyrolyzed in a thermal-combustion elemental analyzer (Finnigan MAT, Thermo Finnigan, San Jose, California) prior to analysis by continuous-flow, isotope-ratio, mass spectrometry (DeltaPlus XL, Thermo Finnigan, San Jose, California).

After receiving the analyses of tissues from the Cornell Isotope Laboratory, statistical comparisons were made using SPSS statistical software 16.0 (Green and Salkind 2008). I calculated a 95% confidence interval for the mean signature of deuterium of the 21 claws of silver-haired bats. A GIS map provided by Meehan et al. (2004) contained average estimates of signatures of deuterium from precipitation measurements calculated from stations across North America. To determine the likely source of the bats I matched up the confidence interval with the band of the map that contained the same values. Using the Haversine formula, I calculated the nearest linear distance from Hytop, Alabama, approximate location of capture sights, for the nearest western (Pueblo, Colorado) and eastern origin (Kenora, Ontario, Canada).

I conducted a two-way ANOVA on data from the silver-haired bats with gender and night captured as independent variables and signatures of deuterium as the dependent variable. Night 1 was the first night a silver-haired bat was captured and all other nights were expressed relative to that night. Night caught had 8 levels (one for each of the nights that silver-haired bats were collected) and sex had 2 levels. Because of the significant interaction between sex and night caught (see Results), I conducted one-way

ANOVAs to test if there was a significant difference between signatures of deuterium of male and female silver-haired bats and to test if there was a difference among the different nights of capture (Green and Salkind 2008).

RESULTS

The overall mean for signatures of deuterium from claws of silver-haired bats was -87.069‰ ($SD = 11.584$; range, -108.13 to -66.934‰). A less negative signature correlates with signatures that are more southern in the southeastern United States. Big brown bats had less negative signatures of deuterium of claws than any silver-haired bat (-43.237 and -18.519‰). The two hoary bats had signatures of deuterium (-91.166 and -80.467‰) which were within the same range of signatures of deuterium for silver-haired bats (Table 1). The 95% confidence interval of signatures of deuterium for claws of silver-haired bats was -81.796‰ to -92.342‰ (Fig. 1) and distribution of the sample was normal (Fig. 2). Using the map provided by Meehan et al. (2004), the closest linear distance from Hytop, Alabama, to a point within the band that contains the calculated confidence interval (Pueblo, Colorado) was 1,688 km. However, the closest linear distance from an eastern origin (Kenora, Ontario, Canada) was 1,788 km.

Results of a two-way ANOVA to test the interaction of sex by night captured on signatures of deuterium indicated a significant interaction between sex and night captured ($F_{2,20} = 5.696, P = 0.022$:Fig. 3). Mean signature of deuterium was -87.063‰ for males and -87.076‰ for females (Table 2). One-way ANOVAs revealed no difference between sexes $F_{1,20} < 0.001, P = 0.998$ or night of capture $F_{7,20} = 0.461, P = 0.846$. Therefore, I combined males and females to estimate the nearest geographic origin.

Comparisons of tissues from the 2 silver-haired bats and 1 big brown bat showed interesting trends (Fig. 4). Combining data for all tissues of each bat, 1 silver-haired bat (Ln 2) had a mean signature of deuterium of -108.30‰, the lowest of the 3 that were sampled for multiple tissues, and had the largest standard deviation of 49.610. The mean of the silver-haired bat Ln 16 was -74.220‰, which was between the other 2 bats, and had the smallest standard deviation of 16.704. The big brown bat (Ef2) had the least negative mean of -71.669‰ and the median standard deviation of 36.437 (Table 3).

DISCUSSION

Using the growing-season estimation of signatures of deuterium provided by Meehan et al. (2004) and the 95% confidence interval of signatures of deuterium, the nearest probable pre-migratory location of silver-haired bats I examined was west of Alabama. However, migration in temperate zones is usually north to south and not east to west for birds (Wiltschko and Wiltschko 1999), and also, bats such as *T. brasiliensis* (Cockrum 1969). Additionally, capture sites of museum specimens compiled by Cryan (2003) indicate that silver-haired bats in my sample are probably an eastern population coming from the north, which would indicate their nearest probable eastern origin was northwestern Ontario or eastern Manitoba (Fig. 1). This is the northernmost part of the geographic range of silver-haired bats in eastern North America, and Alabama is a southernmost extreme of their range (Kunz 1982). If silver-haired bats are coming from the west, they would be coming from as close as southern Colorado. However, my results, do not allow me to determine precise origins within that band.

There is variation from year to year in signatures of deuterium based on amounts of precipitation, and the maps derived from patterns of precipitation are only estimates

(Bowen et al. 2005, Meehan et al. 2004). Thus, to determine a more specific pre-migratory geographic origin of silver-haired bats that appear in Alabama in autumn, more studies should be conducted. Evidence presented herein, however, demonstrates that silver-haired bats are migrating great distances to Alabama and that they are not resident bats.

Biologically, there are many questions about migration by bats. The ability to use claws to study migration could provide an effective way to track populations of bats within a range of similar habitats. Potentially, the migrational movements of a single bat could be determined by examining different pieces of one claw. Information from stable isotopes might tell us where bats are coming from. Additionally, stable isotopes might help us understand if there is a difference in movement of current populations of bats compared to historic populations represented in biological research collections.

Males and females, in my study, seem to have different arrival times based on their distance of origin. However, the interaction between night captured by gender on signatures of deuterium needs to have a larger sample to fully elucidate this relationship. The last 2 bats captured on 26 October, which were 1 male and 1 female, had the greatest effect on the interaction. Because there was about 2 weeks between when these 2 bats were captured and when others were captured previously, this may be a false interaction or some unknown factor may be contributing to separation of these 2 bats from the others (Fig. 3). If bats were caught within those 2 weeks and the trend was still consistent, I could more confidently conclude that male bats were migrating from greater distances and that female bats were arriving from nearer origins earlier in the migratory season. I could also confidently conclude that males and females switch in this trend as the season

progresses. Additional, but weak evidence supporting the idea of differential timing of migration between sexes is that I also did not catch a female and caught 4 males in the first 2 weekends of netting. This could have been because males tend to migrate sooner than others, or by random chance females were not captured. My sample was too small to confidently conclude what trends were present. More research is needed to determine if these patterns are consistent.

The preliminary comparisons of multiple tissues showed interesting trends. The fecal samples showed the most similarity among all 3 specimens. This would be expected because all were caught in the same area of Alabama at about the same time, and thus, would have been eating insects with the same isotopic signature. For other tissues, I expected the big brown bat to have a more positive value compared to the silver-haired bats, indicating a more southern geographic range of the big brown bat during the growing season. As expected, samples of wing and claw revealed that the big brown bat had the most positive value and the silver-haired bats had the most negative. However, for samples of hair, the value for the big brown bat was between those of the 2 silver-haired bats. This may be a reflection of different molting times between male and female silver-haired bats, between silver-haired bats and big brown bats in general, or both. The female silver-haired bat had a more positive signature of deuterium for her hair sample, which indicates molt after or during migration, because the signature matches up with a more northern origin. The male silver-haired bat had a more negative signature of deuterium in his hair, indicating molt was before migration because the signature matches a more northern origin. In Kansas, big brown bats molt in late June (Phillips 1966) but there is no information on molting times of silver-haired bats.

The analysis of 2 species of bats in addition to silver-haired bats added validity to my conclusions. Hoary bats migrate long distances (Cryan et al. 2004). The 2 hoary bats I examined had signatures of deuterium within the same range as those of the silver-haired bats that I captured. This is evidence that hoary and silver-haired bats could have been migrating from the same place, but more testing should be done to verify this interpretation. Big brown bats are non-migratory and rarely move >80 km between summer and winter roosts (Mills et al. 1975). Signatures of deuterium for the big brown bat were within the range associated with precipitation signatures in northern Alabama (Meehan et al. 2004). This is evidence that claws may have little or no change in isotope ratios from water to tissues (no fractionation effect) as is suggested by other studies (Estep and Dabrowski 1980), but a larger sample and laboratory experiments should be conducted to verify these results.

This study has several implications for conservation of bats. As a migratory species, the silver-haired bat is one of the at-risk groups for mortality caused by wind turbines; recent research has elucidated correlations of weather and time of year with mortalities of migratory species of bats at wind turbines (Arnett et al. 2008, Cryan and Brown 2007, Horn et al. 2008). There is no way to assess how many wind turbines a bat may have to avoid to successfully migrate, but a longer distance would indicate a greater risk. Migratory bats may also be a vector diseases such as the white-nose syndrome that is killing bats in northeastern United States (T. Kelley in litt.) or rabies, a well known mammalian viral infection (Blanton et al. 2006). It is important to assess if these migratory bats could be carriers of such diseases, and how far they would be able to spread it.

More could be done with stable isotopes if more was known about growth and rates of turnover of tissues in bats. Rates of growth of claws need to be assessed to determine amount of time represented by parts of an individual claw. Knowing this also might allow us to assess different portions of the claw and associate growth of various parts of claws with different times before or during migration. Knowing rates of turnover of tissues also would help researchers understand the significance of values obtained from other tissues. If we knew the turnover of the hydrogen isotopes in different tissues we could determine where a bat was at different points in time by matching those signatures with GIS database information (Bowen et al. 2005, Meehan et al. 2004). Analysis of stable isotopes is not as simple as taking one sample and determining where hydrogen in that tissue originated, but it does support evidence that silver-haired bats are traveling long distances, perhaps >1,600 km.

Studies on growth of claws in bats would enhance my conclusions. However, this goal may be difficult to accomplish. One way to determine rate of claw growth is to conduct mark-recapture studies or to measure growth of claws in captive individuals. One difficulty in mark-recapture studies is the low probability of recapturing a bat after it is released (e.g., Geluso 2007, Glass 1982, Cockrum 1969). Moreover, it may be difficult to approximate an accurate rate of growth from studies of captive bats. Either of these methods would at least allow an estimate of the time required for a claw to grow, and thus, potentially provide a chronology of where a bat has been within its geographic range in North America.

An expansion of this project could include multiple tissues over multiple years with multiple species. Each tissue possibly has a different turnover rate, which could

provide a different time period for analysis; maps provided by Bowen et al. (2005) would be useful in determining location by month. Also, comparison to a non-migratory species, such as the big brown bat, would be beneficial. The analysis of claws using signatures of deuterium could be used for other migratory bats, such as the eastern red bat (*Lasiurus borealis*), western populations of the Brazilian free-tailed bat (*Tadarida brasiliensis mexicana*), or the hoary bat (*L. cinereus*).

My research has contributed to our understanding of migration in silver-haired bats. It is important to know the life history and ecology of a species to understand how it interacts with other species within ecosystems. When and where a species occurs can help us understand the impact it can have on associated species. This study has provided evidence that these bats are migrating and that they migrate long distances. In addition, this research also has raised many interesting questions that could inspire fruitful studies in the future. Use of stable-isotope technology to study migration in bats can lead to a better understanding of where these species are, where they have been.

LITERATURE CITED

- ARNETT, E. B., W. K. BROWN, W. P. ERICKSON, J. K. FIEDLER, B. L. HAMILTON, T. H. HENRY, A. JAIN, G. D. JOHNSON, J. KERNIS, R. R. KOFORD, C. P. NICHOLSON, T. J. O'CONNELL, M. D. PIORKOWSKI, AND R. D. TANKERSLEY. 2008. Patterns of bat fatalities at wind energy facilities in North America. *Journal of Wildlife Management* 72:61-78.
- BLANTON, J. D., J. W. KREBS, C. A. HANLON, AND C. E. RUPPRECHT. 2006. Rabies surveillance in the United States during 2005. *Journal of the American Veterinary Medical Association* 229:1897-1911.

- BOWEN, G. J., L. I. WASSENAAR, AND K. A. HOBSON. 2005. Global application of stable hydrogen and oxygen isotopes to wildlife forensics. *Oecologia* (Berlin) 143:337-348.
- CEBALLOS, G., T. H. FLEMING, C. CHAVEZ, AND J. NASSAR. 1997. Population dynamics of *Leptonycteris curasoae* (Chiroptera: Phyllostomidae) in Jalisco, Mexico. *Journal of Mammalogy* 78:1220-1230.
- COCKRUM, E. L. 1969. Migration in the guano bat, *Tadarida brasiliensis*. Miscellaneous Publications, University of Kansas Museum of Natural History 51:303-336.
- CRYAN, P. M. 2003. Seasonal distribution of migratory tree bats (*Lasiurus* and *Lasionycteris*) in North America. *Journal of Mammalogy* 84:579-593.
- CRYAN, P. M., AND A. C. BROWN. 2007. Migration of bats past a remote island offers clues toward the problem of bat fatalities at wind turbines. *Biological Conservation* 139:1-11.
- CRYAN, P. M., M. A. BOGAN, R. O. RYE, G. P. LANDIS, AND C. L. KESTER. 2004. Stable hydrogen isotope analysis of bat hair as evidence for seasonal molt and long-distance migration. *Journal of Mammalogy* 85:995-1001.
- DOCKX, C., L. P. BROWER, L. I. WASSENAAR, AND K. A. HOBSON. 2004. Do North American monarch butterflies travel to Cuba?--stable isotope and chemical tracer techniques. *Ecological Applications* 14:1106-1114
- DUNBAR, M. B. 2007. Thermal energetics of torpid silver-haired bats *Lasionycteris noctivagans*. *Acta Theriologica* 52:65-68.

- ESTEP, M. F., AND H. DABROWSKI. 1980. Tracing food webs with stable hydrogen isotopes. *Science* 209:1537–1538.
- FLEMING, T. H., R. A. NUNEZ, AND L. S. STERNBERG. 1993. Seasonal changes in the diets of migrant and non-migrant nectarivorous bats as revealed by carbon stable isotope analysis. *Oecologia (Berlin)* 94:72-75.
- GANNES, L. Z., C. M. DEL RIO, AND P. KOCH. 1998. Natural abundance variations in stable isotopes and their potential uses in animal physiological ecology. *Comparative Biochemistry and Physiology A, Molecular and Integrative Physiology* 119:725-737.
- GAT, J. R. 1996. Oxygen and hydrogen isotopes in the hydrologic cycle. *Annual Review of Earth and Planetary Sciences* 24:225-262.
- GELUSO, K. 2007. Winter activity of bats over water and along flyways in New Mexico. *Southwestern Naturalist* 52:482–492.
- GELUSO, K., J. J. HUEBSCHMAN, J. A. WHITE, AND M. A. BOGAN. 2004. Reproduction and seasonal activity of silver-haired bats (*Lasionycteris noctivagans*) in western Nebraska. *Western North American Naturalist* 64:353-358.
- GLASS, B. P. 1982. Seasonal movements of Mexican freetail bats *Tadarida brasiliensis mexicana* banded in the Great Plains. *The Southwestern Naturalist* 27:127-133.
- GREEN, S. B., AND N. J. SALKIND. 2008. Using SPSS for Windows and Macintosh : analyzing and understanding data. 5th ed. Pearson Education, Inc., Upper Saddle River, New Jersey.

- HERRERA, L. G., T. H. FLEMING, AND J. S. FINDLEY. 1993. Geographic variation in carbon composition of the pallid bat, *Antrozous pallidus*, and its dietary implications. *Journal of Mammalogy* 74:601-606.
- HOBSON, K. A., AND L. I. WASSENAAR. 2001. Isotopic delineation of North American migratory wildlife populations: loggerhead shrikes. *Ecological Applications*, 11:1545-1553.
- HORN, J.W., E. B. ARNETT, AND T. H. KUNZ. 2008. Behavioral responses of bats to operating wind turbines. *Journal of Wildlife Management* 72:123-132.
- IZOR, R. J. 1979. Winter range of the silver-haired bat. *Journal of Mammalogy* 60:641-643.
- KUNZ, T. H. 1971. Reproduction of some vespertilionid bats in central Iowa. *American Midland Naturalist* 86:477-486.
- KUNZ, T. H. 1982. *Lasionycteris noctivagans*. *Mammalian Species* 172:1:5.
- MEEHAN T. D., J. T. GIERMAKOWSKI, AND P. M. CRYAN. 2004. GIS-based model of stable hydrogen isotope ratios in North American growing-season precipitation for use in animal movement studies. *Isotopes Environmental Health Studies* 40:291-300.
- MILLS, R. S., G. W. BARRETT, AND M. P. FARRELL. 1975. Population dynamics of the big brown bat (*Eptesicus fuscus*) in southwestern Ohio. *Journal of Mammalogy*, 56:591-604.
- NASSAR, J. M., H. BECK, L. S. L. STERNBERG, AND T. H. FLEMING. 2003. Dependence on cacti and agaves in nectar-feeding bats from Venezuelan arid zones. *Journal of Mammalogy* 84:106-116.

- NATHAN, R., G. PERRY, J. T. CRONIN, A. E. STRAND, AND M. L. CAIN. 2003. Methods for estimating long-distance dispersal. *Oikos* 103:261–273.
- PHILLIPS, G. L. 1966. Ecology of the big brown bat (Chiroptera: Vespertilionidae) in northeastern Kansas. *American Midland Naturalist* 75:168-198.
- PODLESAK, D. W., A. TORREGROSSA, J. R. EHLERINGER, M. D. DEARING, B. H. PASSEY, AND T. E. CERLING. 2008. Turnover of oxygen and hydrogen isotopes in the body water, CO₂, hair, and enamel of a small mammal. *Geochimica et Cosmochimica Acta* 72:19–35.
- SULLIVAN, J. C., K. J. BUSCETTA, R. H. MICHENER, J. O. WHITAKER, JR., J. R. FINNERTY, AND T. H. KUNZ. 2006. Models developed from delta C-13 and N-15 of skin tissue indicate non-specific habitat use by the big brown bat (*Eptesicus fuscus*). *Ecoscience* 13:11-22.
- VOIGT, C. C., AND D. H. KELM. 2006. Host preference of the common vampire bat (*Desmodus rotundus*) assessed by stable isotopes. *Journal of Mammalogy* 87:1-6.
- VOIGT, C. C., P. GRASSE, K. REX, S. K. HETZ, AND J. R. SPEAKMAN. 2007. Bat breath reveals metabolic substrate use in free-ranging vampires. *Journal of Comparative Physiology B, Biochemical Systemic and Environmental Physiology* 178:9-16.
- WASSENAAR, L. I., AND K. A. HOBSON. 1998. Natal origins of migratory monarch butterflies at wintering colonies in Mexico: new isotopic evidence. *Proceedings of the National Academy of Sciences of the United States of America* 95:15436-15439.
- WILTSCHKO, R., AND W. WILTSCHKO. 1999. The orientation system of birds - III. Migratory orientation. *Journal fur Ornithologie* 140:273-308.

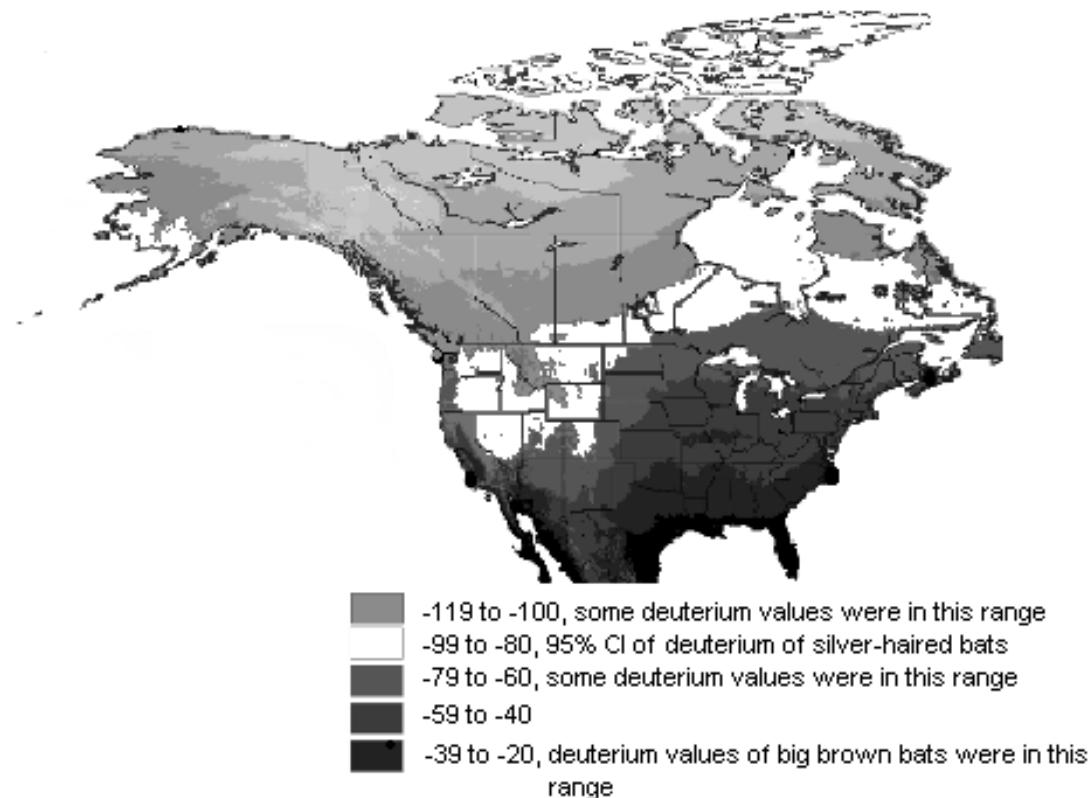


FIG. 1. Based on signatures of deuterium of claws, probable origins of silver-haired bats (*Lasionycteris noctivagans*) that migrate to Alabama are shown in white, (bodies of water are also in white). The 95% confidence interval of signatures of deuterium in claws of silver-haired bats is $-81.796\text{\textperthousand}$ to $-92.342\text{\textperthousand}$. Bands above and below the white band also had values that were present in claws of silver-haired bats. Values for the big brown bat (*Eptesicus fuscus*) were within the range of values associated with northern Alabama. Hoary bats (*Lasiurus cinereus*) had values within the range of silver-haired bats. This map was revised from Meehan et al. (2004) and values are based on approximate signatures of deuterium from precipitation stations located throughout North America.

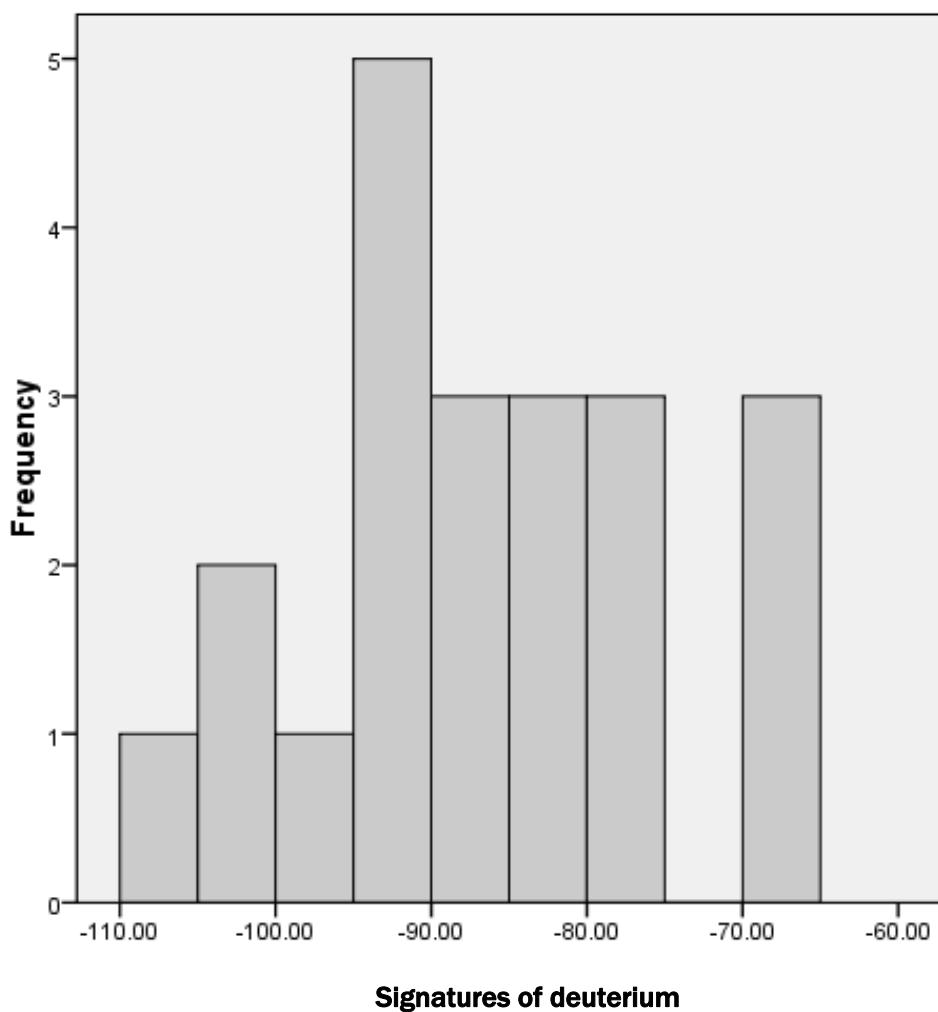


FIG. 2. Histogram of signatures of deuterium in samples of claws from silver-haired bats (*Lasionycteris noctivagans*) captured in Jackson Co., Alabama during autumn 2007.

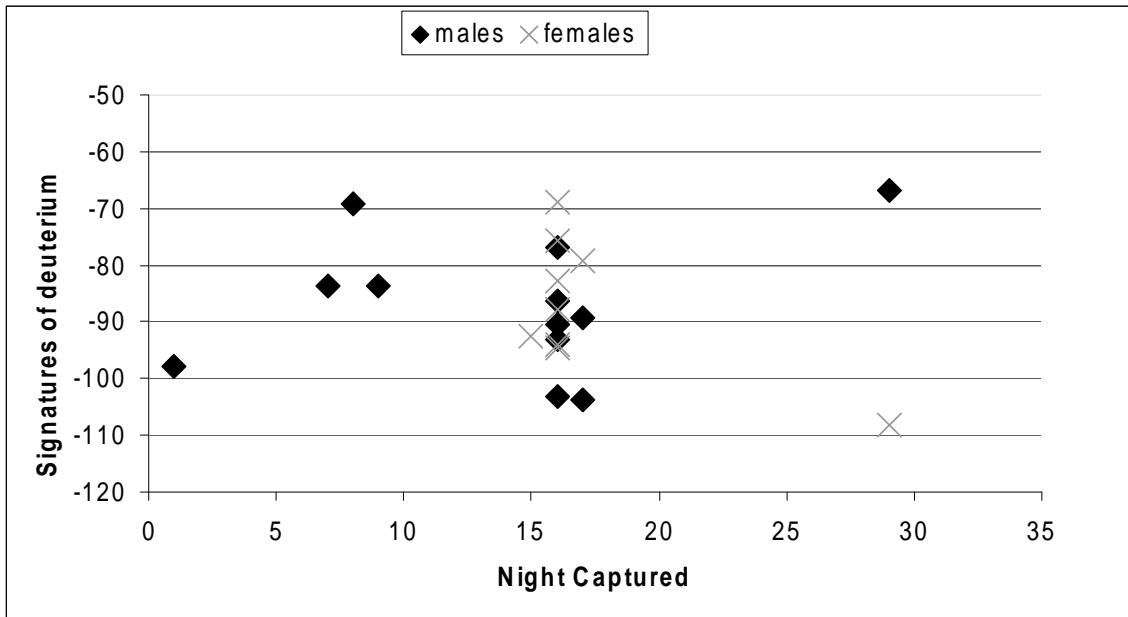


FIG. 3. Signatures of deuterium in male and female silver-haired bats (*Lasionycteris noctivagans*) by night captured. The significant interaction between sex by night captured on signatures of deuterium seems to be contributed primarily by the 2 bats on the right side of the figure.

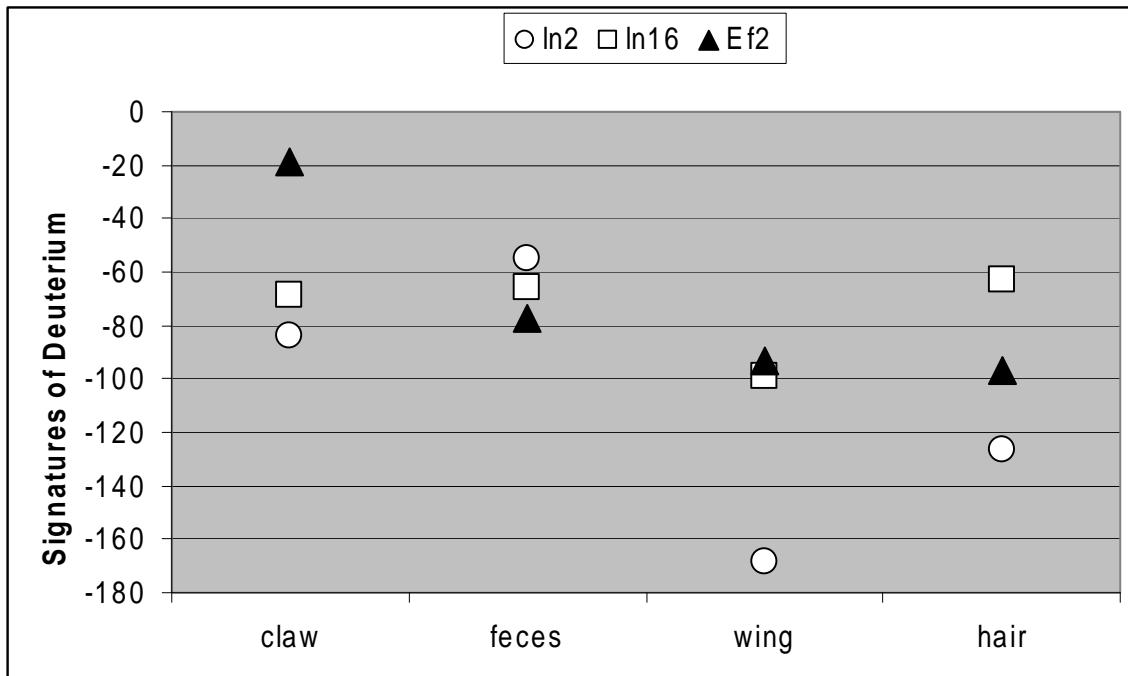


FIG. 4. Signatures of deuterium in tissues of 2 silver-haired bats (Ln2 and Ln16, *Lasionycteris noctivagans*) and a big brown bat (Ef2, *Eptesicus fuscus*). Fecal samples are similar for all 3 specimens and are indicative of a common foraging area (northern Alabama). The more positive values for deuterium in wing and claw tissues of silver-haired bats indicate a more northern geographic position in the growing season (summer) than the more negative values for the big brown bat. Amount of deuterium in hair of the big brown bat was between values for the 2 silver-haired bats, which may be a result of different molting periods between species, between sexes, or both.

TABLE 1. Species, type of sample, specimen number, date captured in 2007, sex, weight of sample, and signatures of deuterium in tissue of 21 silver-haired bats (*Lasionycteris noctivagans*), 2 big brown bats (*Eptesicus fuscus*), and 2 hoary bats (*Lasiurus cinereus*).

| Species | Type of tissue | Sample | Night captured | Sex | Weight (mg) | Signatures of deuterium |
|-----------------------|----------------|--------|----------------|-----|-------------|-------------------------|
| <i>L. noctivagans</i> | Claw | ln1 | 28 September | M | 0.56 | -97.801 |
| | | ln2 | 4 October | M | 0.47 | -83.685 |
| | | ln3 | 5 October | M | 0.42 | -69.094 |
| | | ln4 | 6 October | M | 0.39 | -83.768 |
| | | ln5 | 12 October | F | 0.40 | -92.632 |
| | | ln6 | 13 October | F | 0.55 | -75.694 |
| | | ln7 | 13 October | M | 0.36 | -103.225 |
| | | ln8 | 13 October | F | 0.34 | -82.660 |
| | | ln9 | 13 October | M | 0.48 | -90.572 |
| | | ln10 | 13 October | F | 0.30 | -87.860 |
| | | ln11 | 13 October | M | 0.58 | -86.465 |
| | | ln12 | 13 October | F | 0.36 | -94.633 |
| | | ln13 | 13 October | M | 0.59 | -76.919 |
| | | ln14 | 13 October | F | 0.55 | -93.911 |
| | | ln15 | 13 October | M | 0.39 | -93.245 |
| | | ln16 | 13 October | F | 0.44 | -69.032 |
| | | ln17 | 14 October | M | 0.56 | -89.186 |
| | | ln18 | 14 October | F | 0.59 | -79.136 |
| | | ln19 | 14 October | M | 0.31 | -103.872 |
| | | ln20 | 26 October | M | 0.62 | -66.934 |
| | | ln21 | 26 October | F | 0.43 | -108.127 |
| | feces | ln2 | 4 October | M | 0.57 | -54.759 |
| | | ln16 | 13 October | F | 0.41 | -65.666 |
| | wing | ln2 | 4 October | M | 0.61 | -168.080 |
| | | ln16 | 13 October | F | 0.49 | -99.015 |
| | Hair | ln2 | 4 October | M | 0.33 | -126.668 |
| | | ln16 | 13 October | F | 0.42 | -63.168 |
| <i>L. cinereus</i> | Claw | lc1 | 28 September | M | 0.54 | -91.166 |
| | | lc2 | 13 October | M | 0.66 | -80.467 |
| <i>E. fuscus</i> | Claw | ef1 | 28 September | M | 0.65 | -43.237 |
| | | ef2 | 6 October | F | 0.65 | -18.519 |
| | feces | ef2 | 6 October | F | 0.52 | -77.549 |
| | wing | ef2 | 6 October | F | 0.63 | -93.554 |
| | Hair | ef2 | 6 October | F | 0.69 | -97.056 |

TABLE 2. Signatures of deuterium in claws from male and female silver-haired bats (*Lasionycteris noctivagans*) captured in Jackson Co., Alabama during autumn 2007. Average deuterium for males was -87.063‰ ($SE = 11.938$) and for females was -87.076‰ ($SE = 11.812$).

| Sex | Date captured | Signatures of deuterium |
|---------|---------------|-------------------------|
| Males | 28 September | -97.801 |
| | 4 October | -83.685 |
| | 5 October | -69.094 |
| | 6 October | -83.768 |
| | 13 October | -103.225 |
| | 13 October | -90.572 |
| | 13 October | -86.465 |
| | 13 October | -76.919 |
| | 13 October | -93.245 |
| | 14 October | -89.186 |
| | 14 October | -103.872 |
| | 26 October | -66.934 |
| Mean | | -87.063 |
| Females | 12 October | -92.632 |
| | 13 October | -75.694 |
| | 13 October | -82.66 |
| | 13 October | -87.86 |
| | 13 October | -94.633 |
| | 13 October | -93.911 |
| | 13 October | -69.032 |
| | 14 October | -79.136 |
| | 26 October | -108.127 |
| Mean | | -87.076 |

TABLE 3. Signatures of deuterium (in parts per thousand) for various tissues from 2 silver-haired bats (*Lasionycteris noctivagans*, Ln2, Ln16) and 1 big brown bat (*Eptesicus fuscus*, Ef2) with overall average and standard deviation for each bat.

| Tissue sampled | Ln2 | Ln16 | Ef2 |
|--------------------|----------|---------|---------|
| Claw | -83.685 | -69.032 | -18.519 |
| Feces | -54.759 | -65.666 | -77.549 |
| Wing | -168.080 | -99.015 | -93.554 |
| Hair | -126.668 | -63.168 | -97.056 |
| Average | -108.298 | -74.220 | -71.669 |
| Standard deviation | 49.610 | 16.704 | 36.437 |