Variation in catchment areas of Indiana bat (*Myotis sodalis*) hibernacula inferred from stable hydrogen (δ^2H) isotope analysis

E.R. Britzke, S.C. Loeb, C.S. Romanek, K.A. Hobson, and M.J. Vonhof

Abstract: Understanding seasonal movements of bats is important for effective conservation efforts. Although female Indiana bats (*Myotis sodalis* Miller and Allen, 1928) have been documented to migrate >500 km, knowledge of their migratory patterns is still extremely limited. We used the relationship between latitude and stable hydrogen isotope ratio in bat hair (δ^2H_{hair}) to estimate the north–south extent of the summer range (catchment area) of bats hibernating in 14 Indiana bat hibernacula in eight states throughout its range. Range of δ^2H_{hair} values varied substantially among hibernacula, suggesting large differences among sites in the north–south distance travelled by bats between summer and winter habitats. In particular, hibernacula in the southern portion of the range had greater catchment areas than those in the central and northern portions of the range. Variability in movement distances among sites was not associated with the number of hair samples analyzed or colony size. Significant year-to-year variation (2007–2008 to 2008–2009) in the distribution of δ^2H_{hair} for two sites in Tennessee was observed. Currently, hibernacula considered important for species conservation are largely determined by population size, but our results suggest that migratory diversity should also be considered.

Key words: catchment area, deuterium, hibernacula, Indiana bat, *Myotis sodalis*, migration, stable isotope analysis.

Résumé : Il importe de bien comprendre les déplacements saisonniers des chauves-souris pour assurer l'efficacité des efforts de conservation visant ces animaux. Bien que la migration de chauves-souris de l'Indiana (*Myotis sodalis* Miller et Allen, 1928) femelles sur plus de 500 km soit documentée, les connaissances sur leurs patrons de migration demeurent extrêmement limitées. Nous avons utilisé la relation entre la latitude et les rapports d'isotopes stables d'hydrogène dans les poils de chauve-souris (δ^2H_{poils}) pour estimer l'étendue nord–sud de l'aire de répartition estivale (aire de recrutement) de chauves-souris hibernant dans 14 hibernaculums de chauves-souris de l'Indiana répartis dans huit États à l'échelle de leur aire de répartition. Des variations substantielles de la fourchette de valeurs du δ^2H_{poils} entre hibernaculums suggèrent de grandes différences entre les sites en ce qui concerne les distances de déplacement nord–sud parcourues par les chauves-souris entre leurs habitats estivaux et hivernaux. Plus particulièrement, les hibernaculums dans la partie méridionale de l'aire de répartition étaient caractérisés par des aires de recrutement plus grandes que ceux situés dans le centre et le nord de l'aire de répartition. La variabilité des distances de déplacement d'un site à l'autre n'était pas reliée au nombre d'échantillons de poil analysés, ni à la taille de la colonie. Des variations interannuelles significatives (de 2007–2008 à 2008–2009) dans la distribution du δ^2H_{poils} pour deux sites au Tennessee ont été observées. Si, actuellement, la détermination des hibernaculums considérés comme importants pour la conservation de l'espèce repose principalement sur la taille de la population, nos résultats suggèrent que la diversité migratoire devrait également être prise en considération.

Mots-clés : aire de recrutement, deutérium, hibernaculum, chauve-souris de l'Indiana, *Myotis sodalis*, migration, analyse des isotopes stables.

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Introduction

Migration is a fundamental aspect of the life cycles of many organisms. Because migratory animals utilize a variety of habitats at different stages of their annual cycle, understanding patterns of migratory movements in both space and time, as well as connections among specific summer and winter habitats (i.e., degree of migratory connectivity), is critical to effective conservation efforts (Marra et al. 2006; Hobson and Norris 2008). Strong connectivity occurs when most individuals breeding in one area also spend the winter together at a particular location, while connectivity may be dif-

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fuse if a winter population is composed of individuals from many different summer populations (or vice versa; Webster et al. 2002). Response of migratory animals to habitat change and other anthropogenic pressures is therefore complicated by the geographic scale of their annual cycle because they must contend with habitat alterations on both summer and winter habitats, as well as any areas used while migrating between the two. Variation in the scale of movements and levels of connectivity within or between species may determine the nature and trajectory of any response to natural or anthropogenic environmental change, and may also strongly influence various aspects of their ecology and evolution, including population dynamics, mating and dispersal behavior, population genetics, and the transmission of diseases and parasites.

The Indiana bat (*Myotis sodalis* Miller and Allen, 1928) is a migratory species that was listed as endangered by the US Fish and Wildlife Service in 1967 (US Fish and Wildlife Service 2007). Reasons for listing include destruction and degradation of winter hibernacula, disturbance in the winter hibernacula, and loss and fragmentation of summer maternity habitat, migratory habitat, and swarming sites (US Fish and Wildlife Service 2007). Indiana bats are found throughout the eastern US and migrate from winter hibernacula in the cave and karst regions of the midwestern and northeastern US to summer habitats where they roost in trees (Clawson 2002). Summer maternity colonies may be close to hibernacula (within 40 km; Britzke et al. 2006) or require relatively long migrations of >500 km (Kurta and Murray 2002; Winhold and Kurta 2006). Since 2006, Indiana bats are one of several hibernating bat species that have experienced severe population declines in the northeastern US due to White-nose Syndrome (WNS; Blehert et al. 2009; Turner et al. 2011). WNS has spread rapidly from its origin in upstate New York in 2006 to hibernacula in Tennessee, North Carolina, Alabama, and Missouri in winter of 2011–2012, suggesting that migrating or dispersing bats, as well as human transmission, may be involved in its rapid spread (Szymanski et al. 2009). Thus, gaining information on the migratory patterns of bats and levels of connectivity among populations is a critical step in understanding the transmission of WNS and its dynamics.

Unfortunately, we know very little about the migratory patterns of Indiana bats. Banding efforts have provided researchers with a few connections between winter and summer ranges (Hall 1962; LaVal and LaVal 1980; Gardner and Cook 2002; Kurta and Murray 2002; Whitaker and Brack 2002; Winhold and Kurta 2006). Although genetic analysis has been successful in determining migratory patterns of some bat species (Wilkinson and Fleming 1996; Popa-Lisseanu and Voigt 2009), preliminary analyses failed to find sufficient genetic structuring to allow Indiana bat hibernacula to be linked with summer maternity sites (M.J. Vonhof, Western Michigan University, unpublished data). Radiotelemetry has been successful in tracking Indiana bats from hibernacula to their summer range, but is very resource intensive (Britzke et al. 2006) and is not a practical method for understanding range-wide patterns. Stable hydrogen isotope analysis has been proposed as a means to document origins and migratory patterns of wildlife in general (Hobson and Wassenaar 2008) and bats in particular (Cryan et al. 2004), and it has recently been shown to be useful for Indiana bats and other species (Britzke et al. 2009; Popa-Lisseanu and Voigt 2009).

Stable isotopes are intrinsic markers that have been used to study long-distance migration in a number of animal taxa and have several advantages compared with other techniques (Hobson and Wassenaar 2008). Isotope analysis does not require recapture, is less labor intensive than radio-tracking techniques, and is not biased by the choice of the initially marked population. Stable hydrogen isotopes, in particular, are a useful intrinsic marker in regions where large-scale meteorological patterns create spatial gradients in isotopic signatures in precipitation (Bowen et al. 2005). The ratio of heavy to light hydrogen isotopes (^2H:^1H, expressed as δ^2H) in the environment varies with many factors including latitude, altitude, and distance from the ocean (Dansgaard 1964), and isotope ratios of the local environment are incorporated into various animal tissues through food and water intake. Systematic spatial variation is then used to predict an animal's location when a specific tissue was formed, and the time scale represented by the isotopic ratios depends on the turnover of the tissue sampled (Hobson 2008). Because temperate zone bats molt in summer (Constantine 1957, 1958; Cryan et al. 2004; Tiunov and Makarikova 2007), hair δ^2H values (δ^2H_{hair}) reflect the isotope values of the summer range and hair samples taken during the winter can provide insight into the summer origins, and thus, the migratory patterns of bats (Britzke et al. 2009).

A variety of methods have been used to depict the spatial extent of origins of populations of migratory animals using tissue δ^2H measurements (Hobson 2008). Initially, a constant isotopic discrimination factor between amount-weighted mean growing season precipitation δ^2H values ($\delta^2H_{precip.}$) and the tissue of interest was assumed, thereby allowing the $\delta^2H_{precip.}$ base map to be easily converted to the isotope values of interest. More recently, use of probabilistic approaches and GIS techniques that propagate all known sources of error, including those associated with the calibration algorithm, have been adopted within a largely Bayesian or likelihood-based framework (Hobson 2008; Hobson and Norris 2008; Van Wilgenburg and Hobson 2011).

An alternative approach to assignment of animals using a particular location or habitat feature following migration is to assess the relative size of the catchment area of that site. Catchment areas based on band recoveries have been used in other studies to define the area occupied by bats in summer that utilize the same hibernacula (Rivers et al. 2006). The concept has been applied using stable isotope values by examination of the variance structure within populations to identify sites that "collect" individuals from a relatively wide versus a relatively narrow latitudinal range (Sullivan et al. 2012; Voigt et al. 2012). This approach relies on the assumption that the δ^2H of the tissue in question varies consistently with latitude over the range of interest; an assumption that has been demonstrated across eastern North America for both bird feathers (Hobson and Wassenaar 1997; Meehan et al. 2001; Lott and Smith 2006) and bat hair (Cryan et al. 2004; Britzke et al. 2009). The approach also assumes that interindividual variance in tissue δ^2H values represent primarily differences in origins and not differences due to other factors such as nutritional state and physiology. The relationship

between δ^2H_{hair} and latitude allows the north–south extent of breeding populations that supply individuals to a particular winter population or hibernation site to be determined (cf. Parsons and Jones 2003; Rivers et al. 2005), and comparison of the variation in δ^2H_{hair} of different sites can be used to assess relative levels of connectivity and migratory diversity.

Our objective was to use δ^2H_{hair} measurements to compare the relative size of the catchment areas of Indiana bat hibernacula by measuring levels of δ^2H_{hair} variation of female Indiana bats collected at hibernacula across a latitudinal gradient. We utilized this approach because the probability of accurate geographic assignments was low due to low R^2 value between δ^2H_{hair} and $\delta^2H_{precip.}$ ($R^2 < 0.40$ for females; Britzke et al. 2009), and because the relatively small latitudinal range of the species negates the possibility of using more specific analyses utilizing probabilistic assignments. We predicted that because most observed seasonal movements of Indiana bats from hibernacula have been northward (Gardner and Cook 2002), variation in δ^2H_{hair} of bats using hibernacula in the southern portion of the range would be greater than in the northern portion of the range because the possible extent of movement from winter to summer habitats is greater for individuals using more southerly hibernacula. We also predicted that hibernacula with larger populations of Indiana bats would have greater variance in δ^2H_{hair} corresponding to larger catchment areas. Assuming no interannual variation in $\delta^2H_{precip.}$, changes in the north–south extent of catchment area over time would indicate changes in the number of bats migrating from different parts of the range. We investigated this by sampling two sites with inferred large catchment areas over successive years to examine interannual variation in δ^2H_{hair} and its possible relationship to the impact of WNS. Information on the north–south extent of catchment areas of Indiana bats will broaden our understanding of migratory connectivity in this species and may allow researchers and managers to distinguish hibernacula based on their importance for the spread of WNS.

Materials and methods

We collected hair from female Indiana bats from 14 hibernacula from across the range of Indiana bats (Table 1; Fig. 1). Five of the hibernacula were Priority 1 sites (>10 000 Indiana bats), four were Priority 2 sites (1 000 – 10 000 Indiana bats), and five were Priority 3 sites (<1 000 Indiana bats). Sampling was performed in conjunction with biannual population surveys during winter 2007–2008 or by harp trapping entrances during spring emergence of 2008. We resampled two hibernacula in Tennessee during the winter of 2008–2009. Hair was clipped from between the scapulae, placed in plastic 1.5 mL centrifuge tubes, and labeled with the species, sex, date, and location. We obtained the geographic coordinates and an estimate of the most recent Indiana bat population size for each hibernaculum from the US Fish and Wildlife Service (R.A. King, US Fish and Wildlife Service, personal communication). We used the total Indiana bat population of the hibernacula, as we assumed an equal sex ratio at each site (Hall 1962).

All samples were soaked in a 2:1 chloroform:methanol solution for 24 h to remove surface oils, internal lipids, and absorbed water. Each sample was then rinsed in methanol and air dried for 24 h under a fume hood. Approximately 1 mg of finely chopped sample material was placed in a precleaned silver capsule (3.5 mm × 5 mm) along with two in-house ground feather keratin standards (TURK-1 = –53.9 ‰ V-SMOW and CCHIX = –93.4 ‰ VSMOW; University of Georgia's Savannah River Ecology Laboratory, Savannah River).

Although several keratin standards of relatively low δ^2H value are presently available (e.g., CCHIX), the TURK-1 standard was only one of a few ^2H-eriched standards that existed for hydrogen isotope analysis of keratin at the time of our analyses. These characteristics of the positive TURK-1 standard together with the other standards that we used permit the best possible scale correction and calibration for δ^2H values available for relatively ^2H-enriched samples such as bat hair. The hydrogen isotope composition of the non-exchangeable fraction of H for the TURK-1 and CCHIX standards was determined by calibration to the keratin laboratory standards CFS (–147.4‰ VSMOW), CHS (–187‰ VSMOW), and BWB (–108‰ VSMOW) in the laboratory of L.I. Wassenaar at Environment Canada (Wassenaar and Hobson 2003).

Once loaded, the capsules (samples and standards) were "virtually equilibrated" with laboratory air prior to isotope analysis according to Wassenaar and Hobson (2003). For this equilibration to be meaningful, the H bonding environments (e.g., OH, NH, CH_3 groups) and percentage of exchangeable H sites must be equivalent between samples and standards. Previous work has documented that full replacement of the exchangeable fraction of H in hair can be as short as 3–4 days (Bowen et al. 2005). Slight differences in exchange rates may also occur depending on particle size and shape (e.g., Bowen et al. 2005). In the present study, samples were finely chopped but standards were ground to a fine powder, which is the approach adopted by most laboratories using the comparative equilibrium technique for δ^2H measurements of keratins and this may introduce a small bias in the measurements. However, the hydrogen isotope composition of meteoric waters in the area where the laboratory analyses were performed is relatively stable, as no seasonal component to the hydrogen isotope composition of meteoric waters was observed over a 120 month period (i.e., –13‰ ± –15‰ (mean ± 1 SD); $n > 900$ separate observations). Based on previous H-exchange rates measurements for keratin reported by Carroll et al. (2006), all samples and standards were allowed to equilibrate for at least 3 weeks before the capsules were crimped and analyzed by pyrolysis GC using a Finnigan TC/EA coupled to an isotope ratio mass spectrometer (Delta^{PLUS}XL).

Multiple in-house standards were analyzed at the beginning of a sequence to condition the TC/EA and every fifth sample thereafter to monitor for possible drift in isotope composition, but none was observed. The precision of the standards was better than ±2‰ (1 SD). Approximately 10% of the samples were run in duplicate and all were within 4‰ of each other.

Statistical analysis

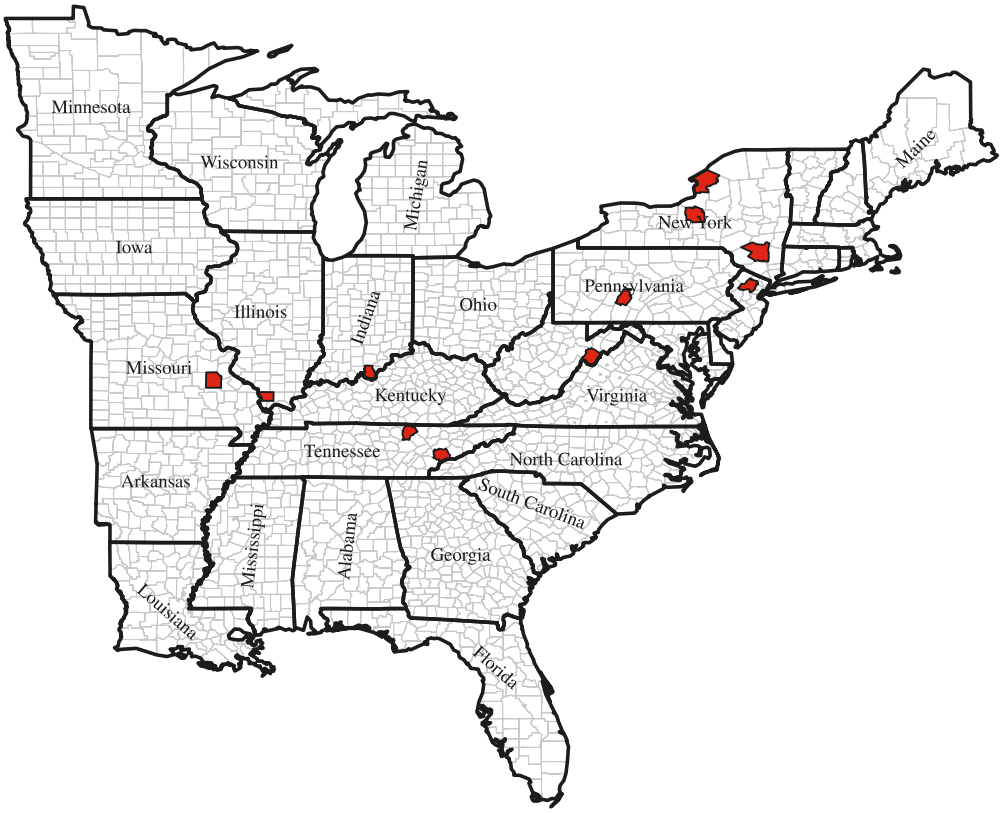
We used the coefficient of variation (CV) in δ^2H_{hair} for each hibernacula as a quantitative measure of the relative size of the catchment area and used Levene's homogeneity

Table 1. Caves, locations, and the number of hair samples from Indiana bats (*Myotis sodalis*) analyzed for each cave.

Cave code	Cave Name	Estimated population size	State	Sample size
NYGP	Glen Park	2 000	New York	30
NYWI	Williams Complex	37 000	New York	39
NYJ	Jamesville	3 500	New York	19
NJH	Hibernia	122	New Jersey	59
PACC	Canoe Creek	774	Pennsylvania	21
WVH	Hellhole	12 858	West Virginia	32
INTD	Twin Domes	25 460	Indiana	12
ILBG	Barney Grace	400	Illinois	17
MOGS	Great Scott	5 100	Missouri	15
TNC	Cornstarch	450	Tennessee	60, 59
TNWR	Wolf River	1 500	Tennessee	154, 142
TNEF	East Fork Saltpeter	300	Tennessee	31
TNZ	Zarathustra's	80	Tennessee	10
TNWO	White Oak Blowhole	4 500	Tennessee	21

For Cornstarch and Wolf Rivers caves, numbers represent the number of samples taken in 2007–2008 and the number of samples taken in 2008–2009. Population sizes were obtained from the US Fish and Wildlife Service, Bloomington, Indiana.

Fig. 1. Map showing the counties that contain the 14 hibernacula of Indiana bats (*Myotis sodalis*) sampled during this study.

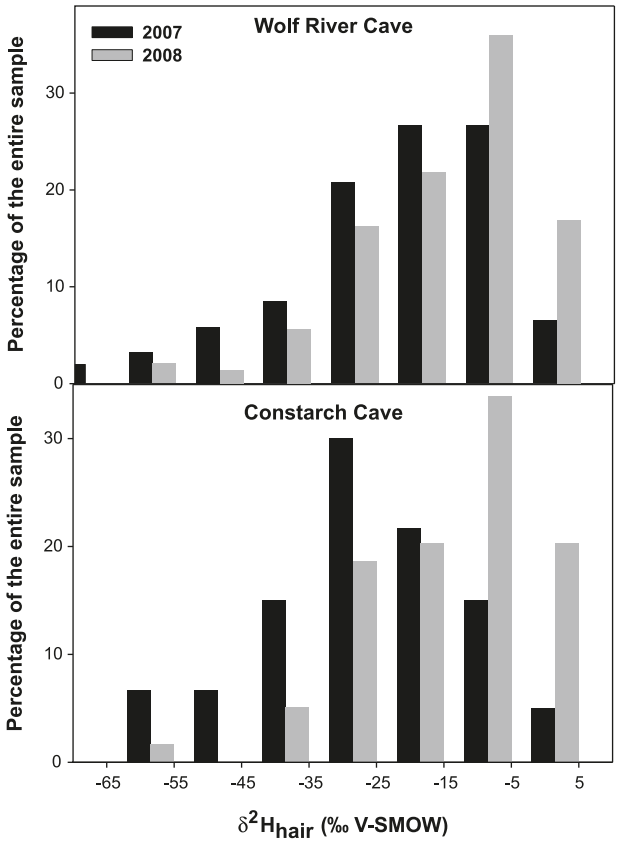


of variance test to compare variation in δ^2H_{hair} values among hibernacula. We used Pearson's correlation to test whether CV was related to the number of samples run and population size of the hibernacula. Distributions of δ^2H_{hair} values in samples taken from successive years for two hibernacula in Tennessee were compared using a Kolmogorov–Smirnoff test. Direction of change between the distributions was assessed using a departure index (Menning et al. 2007).

Results

A total of 520 female Indiana bat hair samples were analyzed with the number of hair samples analyzed from each hibernaculum ranging from 10 to 296 (Table 1). There were significant differences in the distributions of δ^2H_{hair} between years for both Cornstarch ($D = 0.249$, $p < 0.05$) and Wolf River ($D = 0.158$, $p < 0.05$) sites in Tennessee. This differ-

Fig. 2. Distribution of δ^2H_{hair} from Indiana bats (*Myotis sodalis*) taken in successive years from two sites in Tennessee. Tick values represent a range of 5 on either side of the presented value.



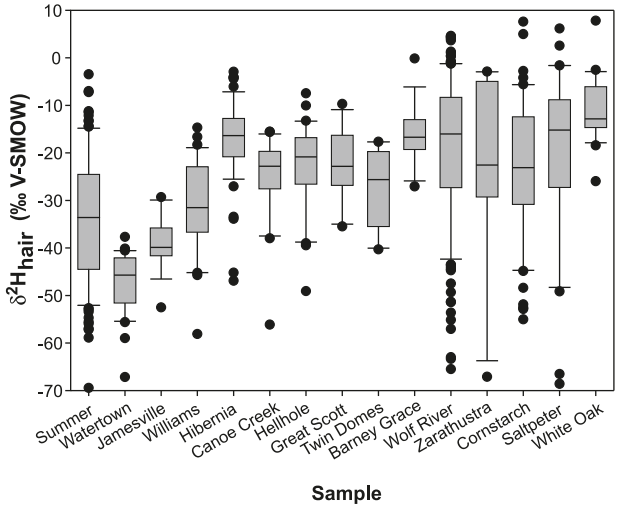
ence resulted from a loss of the more negative values for Cornstarch ($M = 0.35$; range –1.20 to 0.80) and Wolf River ($M = 0.19$; range –1.32 to 0.68) caves (Fig. 2). Because there was significant variation in δ^2H_{hair} between years in these caves, only samples taken during winter 2007–2008 were used in comparison with other sites.

As expected, bats sampled in the more northerly hibernacula (i.e., New York) had more negative δ^2H_{hair} values than individuals in hibernacula in the rest of the range (Fig. 3). Sampled populations in the large, Priority 1 hibernacula in West Virginia, Indiana, and Missouri had relatively narrow ranges of δ^2H_{hair} values, while four smaller hibernacula at the southern edge of the Indiana bat's range in Tennessee (Cornstarch, Wolf River, East Fork Saltpeter, and Zarathustra's) had relatively wide ranges. In some cases, bats from these small southern hibernacula had wider ranges in δ^2H_{hair} than the maximal spread of δ^2H_{hair} values from bats sampled during summer across the geographic range of this species (Fig. 3). Variance of δ^2H_{hair} was significantly different among hibernacula ($F_{[13,506]} = 5.398$, $p < 0.0001$; Fig. 3). The CV of δ^2H_{hair} was not significantly related to the number of samples analyzed ($r = 0.35$, $p = 0.22$) or the hibernating population size ($r = –0.36$, $p = 0.21$).

Discussion

We observed significant variation in δ^2H_{hair} values among 14 Indiana bat hibernacula. Until recently, it was assumed that most movements of female Indiana bats involved north-

Fig. 3. Boxplots showing the range of values determined for each hibernacula of Indiana bats (*Myotis sodalis*). The first box represents samples that were collected while females were on the maternity range, thereby indicating the maximum range in values that would be expected throughout the north–south range of the species. Middle lines represent the median and the boxes represent the 25% and 75% quartiles. Circles represent outliers. Summer data were obtained from Britzke et al. (2009). Sites are arranged in a roughly northern–southern listing.



ward migration from the hibernacula (Gardner and Cook 2002). However, recently discovered maternity colonies in the southeastern US (Britzke et al. 2003) have suggested that patterns of movement between breeding and overwintering sites may be more complex than previously thought. If individuals primarily move northward from their hibernaculum to breed, and return to that same hibernaculum the following winter, we would expect a consistent relationship between the latitude of the hibernacula and variance in δ^2H_{hair} values, as bats hibernating in southern sites have the opportunity to move farther north than those already in the northern portion of the species' range. Instead, we observed that a small number of hibernacula, with relatively small population sizes, on the southern edge of the geographic range of Indiana bats contained individuals that used summer habitats across the presumed entire latitudinal range of this species, whereas sites in the northern portion of the species range had catchment areas encompassing a much narrower range of possible latitudes. We also found no relationship between CV of δ^2H_{hair} values within a site and population size. These results highlight the importance of relatively small hibernacula as centers of migratory diversity, and potential sites of disease transmission, gene flow, and recolonization of WNS-affected regions.

As a collection, bats using the southerly, high δ^2H_{hair} diversity sites are exposed to the full range of conditions present across the summer range before coming together each winter in the hibernaculum. While on the summer range, these bats may interact with other bats that used a variety of different habitats or hibernacula during the previous winter. Hibernacula with large north–south catchment areas are likely to be important sites for gene flow and the transmission of diseases and parasites, as they bring together individuals from a larger north–south area than sites with smaller catchments. Migra-

tion of hosts between populations is a key factor influencing the prevalence of parasites (Lopez et al. 2005; Garamszegi and Moller 2007), and contact between infected and uninfected hosts during periods of overlap on overwintering grounds or stop-over sites may lead to increased rates of parasite transmission (e.g., Altizer et al. 2000; Pérez-Tris and Bensch 2005). Depending on the biology and life cycle of the parasite or disease, infected individuals returning to a hibernaculum may transmit parasites or diseases to other individuals, and subsequent transmission over a much wider area may occur when newly infected individuals migrate to summer habitats the following season. Although it has been demonstrated that the fungus causing WNS, *Geomyces destructans*, has an upper growth limit of 20 °C (Blehert et al. 2009), it has not been demonstrated conclusively that survival of the fungal spores (conidia) is similarly temperature-limited. In fact, work at Fort Drum, New York, has shown the presence of *G. destructans* conidia on bats during the summer (Dobony et al. 2011). If spores can be transmitted from individual to individual during the summer months, then the hibernacula with high migratory diversity at the southern edge of the range may play a disproportionate role in the transmission of WNS to new areas.

The relatively wide catchment areas of the southern hibernacula indicated that at least some individuals using these sites migrated long distances between summer and winter habitats. These long movements are of special interest due to the risk posed by wind energy development for migratory bats. Annual mean mortality caused by wind turbines has been estimated to be approximately 10 bats/turbine (although it varies substantially among geographic regions) and is of major conservation concern with respect to population-level impacts for migratory bat species (Kunz et al. 2007; Arnett et al. 2008). Although long-distance migratory bat species such as eastern red (*Lasiurus borealis* (Müller, 1776)) and hoary (*Lasiurus cinereus* (Beauvois, 1796)) bats account for the greatest number of fatalities, two Indiana bat fatalities have recently been documented at wind power sites. Depending on the specific flight paths they utilize, bats that migrate longer distances may have an increased probability of encountering wind power developments and hence may be exposed to a greater risk of mortality. A greater understanding of the migratory behavior of Indiana bats can help in assessing the overall risk posed to this species.

Hibernation sites with large catchment areas may also play an important role in promoting long-distance gene flow. Because mating takes place at swarming and hibernation sites, and many different breeding groups come together at these sites, mating may occur between females and males that did not originate from the same breeding group (Rivers et al. 2006). Therefore, swarming and hibernation sites have been suggested to represent "hot spots" of gene flow (Kerth and Reckardt 2003; Veith et al. 2004; Furmankiewicz 2008). Studies comparing genetic variation between breeding and overwintering groups of bats have demonstrated low levels of genetic differentiation than absence of genetic isolation by distance among summer colonies utilizing the same sites for mating (those falling within the same catchment area surrounding swarming or hibernation sites; Rivers et al. 2005; Furmankiewicz and Altringham 2007). Although δ^2H_{hair} values provide no information on the east–west extent of catchment areas and we cannot be sure of the overall degree of overlap among the catchment areas of different hibernation sites, the latitudinal extent of catchment areas of different sites clearly overlapped, and the latitudinal catchment area of the southerly sites overlapped those of all other sites. Presence of hibernation sites with large latitudinal catchment areas suggest that we might not observe genetic differentiation among summer populations across wide portions of the range. The only exception may be some northeastern populations and populations on the southeastern edge of the range (such as White Oak Blowhole Cave) which have narrow δ^2H_{hair} value ranges that minimally overlap those of hibernacula in the Midwest, and which, in the case of northeastern populations, have been shown to have small catchment areas based on radiotelemetry (Britzke et al. 2006).

Because of limited roost and food resources in the immediate vicinity of hibernacula, it has been assumed that the larger the hibernating population, the greater the seasonal movements of the colony. The largest hibernacula did not have the largest range of values of δ^2H_{hair} and the sites with the widest ranges of δ^2H_{hair} values had relatively small population sizes. Although sites with large population sizes may have had larger longitudinal catchment areas, and thus the catchment areas may be misrepresented in our study, the vast majority of known movements of this species are oriented north–south (US Fish and Wildlife Service 2007). Because our data failed to show a positive correlation with population size and extent of north–south origins, the catchment area of a hibernaculum cannot be inferred from the population size of the colony, but rather needs to be directly assessed for each hibernaculum.

We observed significant between-year variation in the distributions of δ^2H_{hair} in samples obtained during the winters of 2007–2008 and 2008–2009 at two sites in Tennessee despite similarity in samples sizes between years. Annual variation in the values at a site may occur in response to annual differences in $\delta^2H_{precip.}$ values. However, variation in $\delta^2H_{precip.}$ at a site would likely result in a shift in the mean of the distribution, not the shape, and does not explain the directional change in the shape of the distribution (loss of the more negative δ^2H_{hair} values presumably corresponding to a loss of more northern individuals) that we observed. One possibility is that bats from the sites in Tennessee that moved north to form maternity colonies in spring 2008 encountered WNS-infected bats at the maternity sites, contracted WNS, and died. Another possibility is that with the mass mortality due to WNS, there were suitable, unoccupied hibernacula in the northeastern region and bats may have simply chosen to hibernate there rather than return to the hibernacula in Tennessee. Decline of bats from the northeastern part of the range of Indiana bats between years mirrors similar observed declines of WNS-affected bat species in the summer range (Dzal et al. 2011; Frick et al. 2010; Ford et al. 2011), and is obviously of concern with respect to the population-level impacts of WNS.

Conservation actions for migratory species are inherently more complicated than those of sedentary species because migratory species use two or more spatially discrete habitats and have different resource requirements throughout their life cycle (Hobson and Norris 2008). Indiana bat conservation efforts have primarily focused on conservation and restoration

of hibernacula (US Fish and Wildlife Service 2007) and conservation actions have primarily been directed at larger hibernacula. For example, down-listing the Indiana bat from endangered to threatened requires that ≥80% of the Priority 1 hibernacula be protected and delisting requires that ≥50% of the Priority 2 hibernacula be protected (US Fish and Wildlife Service 2007). Because of the lack of a relationship between population size and size of the latitudinal catchment area, we propose that measures of migratory diversity should be integrated into the discussion of conservation priority of hibernacula.

Early attempts to determine the potential for bat transmission of WNS were limited because of the lack of understanding of bat migratory movements. One important factor considered in the assessments was population size because it was assumed that larger hibernating populations represent animals from a larger area, including those from infected sites. Although conservation efforts focused on larger hibernating populations may protect a greater number of individuals, in the long term and in the context of mass mortality due to WNS, conservation and containment efforts focused on small caves with large catchment areas may be equally important because they represent sources for recolonization throughout the north–south extent of the species range. Furthermore, if Indiana bats are a vector for WNS, focusing on hibernacula with small populations but large catchment areas may provide insight into the transmission dynamics of the disease. Thus, inclusion of information on inferred catchment areas and migratory diversity into management strategies may improve the effectiveness of conservation efforts for migratory bat species facing serious threats.

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