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ESTIMATING BIRD/BAT FATALITY RATES AND USING STABLE-HYDROGEN ISOTOPES TO ASSESS HOARY BAT MIGRATORY PATTERNS ASSOCIATED WITH A WIND-ENERGY FACILITY IN WESTERN OKLAHOMA

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ESTIMATING BIRD/BAT FATALITY RATES AND USING STABLE-HYDROGEN ISOTOPES TO ASSESS HOARY BAT MIGRATORY PATTERNS ASSOCIATED WITH A WIND-ENERGY FACILITY IN WESTERN OKLAHOMA

A DISSERTATION APPROVED FOR THE
DEPARTMENT OF BIOLOGY

BY

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I dedicate this dissertation to my husband, Chris Burba, and our three children, Caleb, Adi, and Isaac, who are more than just my contribution to global *r*.

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PREFACE

This dissertation is presented in four chapters, each formatted for submission to different journals. The first chapter is formatted for *American Midland Naturalist*. The second chapter and its associated appendix are formatted for *Ecological Modelling*. The third chapter and its supplemental material are formatted for *Methods in Ecology and Evolution*. The fourth chapter is formatted for *Journal of Mammalogy*.

ABSTRACT

Offsetting a portion of the energy deficit of the United States with emission-free, renewable energy is driving a rapid increase in the number of wind-power installations across the country. Although wind energy has environmental benefits, the associated turbine-related fatalities have generated concern for the impact on bird and bat populations. Estimating the number of fatalities is key to determining the population impact, but fatality patterns routinely violate assumptions of the methods commonly used to estimate fatality rates, namely non-uniform death rates and/or searcher-induced perturbations on steady-state processes. Therefore, four new mathematical models were developed to reduce bias in fatality estimates, as were new methods using exponential-decay functions to estimate carcass-removal rates. Compared to the new models, earlier methods can result in estimated fatality-rate errors ranging from -100.0 to 65.7%. The degree of error is related to the carcass-removal rate, search interval, and carcass-detection probabilities. In general, error is likely to increase with carcass size. Estimates of bat-fatality rates typically will be less biased than those for large-sized birds. Examples illustrating the application of the models are given, as well as conditions in which each is appropriately applied.

Approximately one-third of all United States wind energy is generated in the south-central portion of the country, yet little effort has been devoted to determining fatality risk in this region. Bird and bat fatality rates were assessed for Blue Canyon II Wind Power Project (the study site), an array of 84 1.8-MW turbines in Caddo and Kiowa counties, Oklahoma. Weekly fatality searches were conducted over a period of 8 months (Mar.-Jun. and Aug.-Nov.) during 2006-2008 at 50 wind turbines. For the study site, we estimate 97.7 bird fatalities/year (1.16/turbine). Almost half of detected bird fatalities were of turkey vultures (*Cathartes aura*, 47.8%), with others involving 15-17 species. Bat fatalities involved 8 species, with hoary bats (*Lasiurus cinereus*, 60.8%) and eastern red bats (*L. borealis*, 21.3%) being the majority; fatalities of these species were male biased. Overall, bat fatalities rates were estimated to be 641.3 bats/year (7.63/turbine). Bird fatalities were evenly spread among survey months, with bat fatalities concentrated during late summer and fall migration (66% occurring within 3-week interval in late Aug. and early Sep.). Our derived mathematical models demonstrate that such migratory events exert little bias in fatality estimates. Bird fatalities at the study site were lower than found at most facilities nationwide and not likely to be biologically significant. Bat fatality rates were moderate and in the range found at other facilities, yet cumulative impacts are of concern.

While estimating the number of fatalities at individual wind-energy facilities is important, mitigation strategies are needed to reduce fatality risk. Hoary bats (*Lasiurus cinereus*) are among the most susceptible species and our limited knowledge of bat migration hinders our ability to (1) determine the reason for their susceptibility, (2) implement mitigation strategies, and (3) assess long-term population impacts. Bat carcasses collected from the study site were also used to assess migratory patterns of the hoary bat with stable-hydrogen isotopes, a technique that can connect hydrogen-isotope ratios of an animal's keratin-based tissues (i.e., fur and claws) to the geographic region in which it was grown. Thus, large-scale movements of individuals can be tracked to identify locations of summer and/or winter residency of migratory species. The reliability of stable-isotope methods is influenced by patterns and timing of tissue

growth, which is unknown for many species. To circumvent potential bias of fur samples, techniques were developed to appropriately sample claw tissue to assess migratory patterns.

Claws are composed of multiple layers and our analysis indicates only unguis tissue should be used in isotope analysis. Otherwise, assignments of geographic locations will be biased to latitudes further north than where tissues were actually grown. Recently grown claw tissue of fall migrants can be used to infer location of summer residency and fall-migratory origins. The claw tip may be useful for inferring locations of winter residency, but further research is needed to confirm this relationship. Furthermore, congruency of δD_{fur} (i.e., the hydrogen-isotope ratio of fur) and δD_{claw} (i.e., the hydrogen-isotope ratio of claws) in female hoary bats suggests molt occurs at location of summer residency. However, in male hoary bats, δD_{claw} was significantly more depleted than δD_{fur}, indicating males do not molt at location of summer residency; thus, claws may be more reliable for assigning migratory origins.

Hoary bats have a wide-spread distribution across the entire contiguous United States; thus, it is unknown from where bats that are killed at wind-energy installations may have migrated. Using claw keratin, hydrogen-isotope ratios revealed all female and 87.8% of male hoary bats recovered at the wind farm were not summer residents, but migrants from other geographic locations. The represented locations of summer residency were from a broad range of latitudes extending from Oklahoma into northern Canada (δD_{claw} from -43.6‰ to -153.0‰). The change in δD from the claw root to the claw tip within the same individual appears to be an indirect measure of migratory distance, as individuals from more northern localities had the greatest change in δD.

Individuals with summer residency at greater latitudes migrate greater distances than those from lower latitudes, suggesting the species exhibits a differential migration. These differences were not explained by sex as there was no difference in latitudinal distributions of males and females. There was also a significant relationship between the arrival date at the study site and the geographic region from which individuals likely migrated. Individuals with summer residency in greater latitudes arrive later than individuals from lower latitudes.

While the cumulative, nation-wide impact of wind energy is of concern, our studies highlight characteristics that are at least best-case scenarios for minimizing population impacts. Unlike some bird fatalities, bat fatalities are not likely to suffer from substantially underestimated fatality rates and the biased removal of males will have less impact on the population's reproductive potential. Furthermore, individuals killed are representatives of many geographic regions, which likely disperse the risk among many populations and may minimize localized population reductions. We highlight the use of wind farm fatalities and claw keratin to address migratory patterns that, in turn, may provide insights to the development of mitigation strategies.

Three-Year Assessment of Bird and Bat Fatalities for a Wind-Energy Installation in Western Oklahoma

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ABSTRACT.—Wind-energy production is growing rapidly in the south-central United States, yet few studies have assessed impacts on bird and bat populations in this region. A bird and bat fatality study was conducted at the Blue Canyon II Wind Power Project, an array of 84 1.8-MW turbines in Caddo and Kiowa counties, Oklahoma. Weekly fatality searches were conducted over a period of 8 months (Mar.-Jun. and Aug.-Nov.) during 2006-2008 at 50 wind turbines of which 23 had Federal Aviation Administration (FAA) lights. Searcher-efficiency and carcass-removal rates were estimated six times during the 3-y study. Exponential decay functions provided excellent fit to data on carcass disappearance. Over the 8-month survey period for the Blue Canyon II facility, we estimate 82.9 ± 19.00 (mean±SD) bird fatalities/y (0.99 ± 0.226/turbine, 0.55 ± 0.126/MW; 12-month projections 97.7/y, 1.16/turbine, 0.65/MW). Almost half of detected bird fatalities were of turkey vultures (*Cathartes aura*, 47.8%), with others involving 15-17 species. Bat fatalities involved 8 species, with hoary bats (*Lasiurus cinereus,* 60.8%) and eastern red bats (*L. borealis,* 21.3%) being the majority; fatalities were male biased for these species. Overall, 8-month bat fatalitity estimates were 623.5 ± 48.73/y (7.42 ± 0.580/turbine, 4.12 ± 0.322/MW; 12-month projections 641.3/y, 7.63/turbine, 4.24/MW). The evening bat (*Nycticeius humeralis*) and cave myotis (*Myotis velifer*) were documented as turbine-related fatalities for the first time. FAA obstruction lighting did not influence bird or bat fatality rates. The south-central section of the facility had more bat fatalities and the western section fewer than predicted by chance, whereas spatial distribution of bird fatalities did not differ from random. Bird fatalities were evenly spread among survey months, with bat fatalities concentrated during late summer and fall migration (66% occurring within 3-wk

interval in late Aug. and early Sep.). Bird fatalities at Blue Canyon II were lower than found at most facilities and not likely to be biologically significant. Bat fatality rates were moderate and in the range found at other facilities.

INTRODUCTION

Since the 1960s, energy consumption has exceeded production in the United States, with the disparity increasing in recent years (Energy Information Administration, 2011a). Offsetting a portion of the energy deficit in the United States with emission-free, renewable energy is driving a rapid increase in the number of wind-power installations across the country. Nationally, wind-energy production grew from 10 megawatts (MW) in 1981 to 40,181 MW in 2010, or about 34,000 turbines (American Wind Energy Association, 2011). Despite this very substantial increase, wind energy only accounts for about 1% of current energy production in the United States (Energy Information Administration, 2011b). However, the Department of Energy (2008) has introduced an initiative to have 20% of the nation's energy generated by wind by 2030.

Although wind energy has environmental benefits, impacts to birds and bats have been associated with wind-energy installations. Initially, wind energy was thought to pose the greatest fatality risk to birds, particularly after numerous raptor fatalities were reported at Altamont Pass, California, one of the largest and earliest wind-farm installations (California Energy Commission, 2003). Mortality of non-raptor bird species also was documented for the site (Smallwood and Thelander, 2008). Mortality investigations at other facilities have recorded widely varying numbers of bat fatalities, with migratory tree- and foliage-roosting bats being most vulnerable (Kunz *et al.*, 2007; National Research Council, 2007). Thus, studies to date indicate that relatively few birds are killed by wind turbines, except for high mortality of raptors at older facilities in California and relatively greater passerine mortality rates at some forested sites in the eastern United States (reviewed by Kerlinger *et al.*, 2010). However, bat fatalities are of increasing concern as fatality rates have been high among wind-energy facilities along the forested Appalachian mountain ridges in the eastern United States (Kunz *et al.,* 2007; National Research Council, 2007).

Relatively little effort has been devoted to determining fatality risks in the south-central portion (*i.e.*, Texas, Oklahoma, Kansas) of the nation (Tierney, 2009; Miller, 2008; Piorkowski and O'Connell, 2010; Graham and Hudak, 2011), even though one-third of wind energy in the United States is generated in this region (American Wind Energy Association, 2011).

Herein, we report on a 3-y post-construction study conducted at the Blue Canyon II from 2006-2008 to assess the species composition and numbers of bird and bat fatalities occurring at the project site. We also developed a new analytical method for assessing carcass removal by scavengers.

METHODS

STUDY SITE

The Blue Canyon II Wind Power Project, located in the Slick Hills of southwestern Oklahoma, began commercial operations in Dec. 2005 and consists of 84 V80 Vestas turbines (Fig. 1), each having a rated capacity of 1.8 MW; 38 have red-flashing strobe-like aviation obstruction beacons (FAA L-864) and 46 do not. Turbines are located along ridge tops of the Slick Hills (elevation 520-610 m) with slopes varying from level to relatively steep grades.

Vegetation is primarily mixed-grass prairie among large limestone outcroppings, but also includes several cactus and brushland species. Mesic woodland is found in the valleys where water runoff flows seasonally. Cattle grazing is permitted, including areas around the turbines, and hunting leases are maintained for wild turkey (*Meleagris gallopavo*), white-tailed deer (*Odocoileus virginianus*), and elk (*Cervus elaphus*). Landscape surrounding the Slick Hills is mostly agricultural with both plowed and cattle-grazed fields. The Wichita Mountains Wildlife Refuge, 6.4 km to the south, supports a diversity of wildlife.

TURBINE SEARCHES

Searches were conducted at 50 (59.5%) of 84 turbines (Fig. 1), with 23 (46.0%) of the 50 being lighted—a percentage similar to that for the entire project. Sampled turbines were selected using a stratified-random process. Based on number of turbines, the project was divided into four nearly equal sections (Fig. 1): (N) north-central; (S) south-central; (E) east; and (W) west. Within each section, we randomly selected among lighted and among unlighted turbines.

Other wind-turbine studies have suggested that most bird and bat fatalities fall within 63 m of the turbine base (Orloff and Flannery, 1992; Johnson *et al.*, 2002; Young *et al.*, 2003; Kerns and Kerlinger, 2004; Jain *et al.*, 2009). Therefore, we adopted a search procedure covering the area within such a radius. Search paths of four concentric circles with radii of 15, 30, 45, and 60 m centered on the turbine base were established. Each circle was marked with four 0.6-m brightly colored stakes, one in each cardinal direction. For the 60-m circle, additional stakes were placed midway between each adjacent pair of original stakes. Searchers followed the four circular paths, looking side-to-side for carcass remains. On average, searchers walked about 40 m/min, requiring about 25 person·min to search one turbine. When a carcass was found, basic specimen and location data were recorded, and it was collected and taken to the Sam Noble Oklahoma Museum of Natural History for archival purposes and further study.

All carcasses and biological material were collected but not all specimens found were judged to be wind-farm fatalities. Operationally, a bird was considered a documented fatality if we found at least 10 feathers or some skeletal material. Carcasses found incidentally at non-sample turbines were included in documented fatalities, but excluded from numbers used when estimating total wind-farm fatalities based on sampled turbines.

In total, 23 rounds of fatality searches were conducted during 8 months of each year, with about weekly intervals between searches. Dates were focused during peak periods of bird and bat migration in spring (mid-Mar. through Jun.) and fall (mid-Aug. through Nov.) Surveys were not conducted in Jul. or Dec.-Feb. The search effort resulted in 1,150 turbine searches/y (total 3,450 for 3-y study). Surveys were not conducted in Jul. or Dec.-Feb. Each survey round took 2-3 d to search all sample turbines. Surveys were conducted on 51 d in 2006, 48 in 2007, and 49 in 2008 (total 148 d). Occasionally, weather conditions, personal interruptions, and site restrictions resulted in an interval between searches being more than 1 wk. For 2006-2008, respectively, mean between-search intervals (± SD) were 9.1 ± 3.87, 8.2 ± 2.60, and 9.8 ± 3.68 for spring and 8.9 ± 2.91, 9.7 ± 4.37, and 9.9 ± 5.25 for fall. In addition to carcass surveys, passive acoustical monitoring for bats was conducted on 10 nights (with no precipitation and low wind speeds) from May 2007 through Oct. 2008 using an Anabat SD1 bat detector.

CARCASS-REMOVAL ASSESSMENT

A proportion of fatalities go undetected because some carcasses are removed by scavengers prior to a survey. To correct this bias, carcass-removal trials were conducted each spring and fall except for spring 2006; a second trial was completed in spring 2008. Previously frozen bird carcasses with a small attached leg tag were placed 50 m from the center of a turbine base in each cardinal direction, resulting in adjacent ones being about 100 m apart. Birds were grouped in three size classes—small (smaller than a brown-headed cowbird, *Molothrus ater*), medium (cowbird to pigeon size), and large (larger than a pigeon). Rates for small-sized birds were used as surrogates for bats; in at least two previous studies (Fiedler, 2004; Brown and Hamilton, 2006), no differences were found for scavenging time or in searcher efficiency using bat carcasses or small-bird carcasses. Specimens were monitored daily for scavenging whenever searchers were on site.

Typically eight small-, eight medium-, and up to five large-sized birds were used in each trial. For a trial, specimens were placed at a minimum of five turbines dispersed across the wind farm, with four carcasses placed per turbine (no more than two of a given size). Number of carcasses per trial ranged from 20-25 (mean 21.5). For a few trials, this resulted in an additional carcass being placed at one of the turbines between adjacent specimens. Maximum density of carcasses was 0.25/ha.

Carcasses were monitored daily when investigators were at the site and for at least 4 wk after placement or until specimens disappeared. During checks, carcass condition was recorded as found, disappeared, and/or decomposed. When a specimen was not found at the designated location, the surrounding area was searched; if a carcass had been moved by a scavenger, the carcass was returned to its original location. Often specimens were scavenged, but still remained detectable by some biological remains. To be consistent with our fatality definition, a specimen was considered undetectable only when all skeletal material was removed and no more than 10 feathers could be located.

SEARCHER-EFFICIENCY ASSESSMENT

A searcher will not detect every carcass. Searcher-efficiency trials were conducted to estimate probability a carcass would be detected during regular surveys. Detection probability may be influenced by size and/or color of the carcass, so birds of variable size and color were used in trials. Carcasses were divided among size classes consistent with those used in carcass-removal trials. Typically, for each searcher-efficiency trial, 46 bird carcasses (*i.e.,* 21 small-, 21 medium-, and 4 large-sized birds) were placed under five randomly selected turbines. Searchers were not aware as to which turbines were selected nor the number of carcasses placed at a given turbine. Large carcasses were not included in the first three searcher-efficiency trials but were in the last three. Over the 3-y study except for spring 2006, searcher efficiency was assessed once in spring and once in fall, with an additional trial in spring 2008. Detection probabilities for small-sized birds were used for bats as well.

For the first three trials, the evening before a scheduled survey a person other than the searchers placed bird carcasses at randomly selected turbines. Birds of each size class were distributed equally among the four concentric rings and among distance classes from search path (*i.e.*, ≤3 m, 4-5m, and ≥6 m from walking line). A thin red string was tied to a leg of each trial specimen to ensure it was not counted as a wind-farm fatality. Basic survey data were recorded if a trial specimen was recovered. After completion of an entire survey round (*i.e.*, all 50 turbines), each trial turbine was revisited by the searchers who were then able to consult a map showing placement of each carcass used in the trial. Searchers retrieved carcasses not found during the routine search or noted that an initially undetected carcass still could not be found. Given trial specimens were placed a day or two before searchers surveyed a particular turbine, specimens potentially were subject to scavenging before a turbine was surveyed. To correct for this potential loss, we used equations developed in the carcass-removal assessment to estimate the number of carcasses likely present when searchers surveyed a particular turbine. Thus, searcher-efficiency rate was calculated as the proportion of carcasses detected of total number estimated to be present at the time surveyors reached a particular turbine used in the trial.

Because of the confounding nature of carcass removal during initial searcher-efficiency trials, procedures were altered for the last three trials. For these trials, a subset of half the turbines was selected (*i.e.*, those that could be surveyed in 1 d), and a person other than the searchers placed carcasses under randomly selected turbines early in the morning on which the survey commenced. Carcass placement followed the same procedures described above. At the end of the day, the individual who placed carcasses returned to retrieve those undetected.

Searchers endeavored to ensure that search effort was the same for regular and searcher-efficiency surveys. Mean search times per turbine for searcher-efficiency trials

(25.6 person·min) and regular surveys (23.4 person·min) were not significantly different (F_s = 1.45, P = 0.256). Given that more specimens were encountered at turbines used for searcher-efficiency trials than at other turbines, an increase in search time is actually expected because of the need to record specimen data.

STATISTICAL ANALYSES

We used the following model (Shoenfeld, 2004) to estimate fatality rates:

$$N_K = N_F\left(\frac{T_S}{pT_R}\right)\left(\frac{e^{T_S/T_R}-1+p}{e^{T_S/T_R}-1}\right), \tag{1}$$

where N_K is estimated number of fatalities for survey period, N_F is observed number of carcasses, T_S is mean search interval between surveys, T_R is mean scavenging time for carcasses, and p is probability of searcher detecting a carcass. Detection probability (p) was calculated from searcher-efficiency trials as proportion of carcasses detected by searchers. Mean scavenging time (T_R) was estimated from carcass-removal trials. N_K was divided by number of turbines surveyed (n = 50) to obtain an estimated number of fatalities per turbine per survey period. To estimate expected number of fatalities for the whole Blue Canyon II Wind Power Project, fatality rates per turbine were multiplied by total number of turbines (n = 84). Fatality rates also were evaluated per megawatt based on turbines having a rated capacity of 1.8 MW each.

Mean scavenging time (T_R) was determined by using an *XY* scatter plot with number of days since placement as the independent variable and probability a carcass remained unscavenged as the dependent variable. For each carcass-removal trial, an exponential decay function was established for each bird size class. The mean of an exponential function is 1/λ, where λ is rate of exponential decay; thus, mean removal times were calculated directly from time-dependent carcass-removal data. This technique does not require assignment of a removal time to specimens that disappear at an unknown time between regular searches, nor does it require right-censoring of data like the Barnard (2000) method used in most studies.

For bats, fatality rates were estimated with a modified approach because, unlike birds, bats often were found near the turbine base where searcher efficiency was higher. Numbers of retrieved bat carcasses were considered in two categories: carcasses found ≤37 m the turbine center (*i.e.,* inner two concentric search circles); and those at ≥38 m. Carcass-detection probabilities were calculated separately. Using equation 1, fatality rates were estimated for the two categories and resulting values summed.

While surveys were carried out during 8 months each year and fatalities outside survey periods were judged infrequent, annual fatalities are of interest. Estimates for non-survey months were extrapolated from numbers of fatalities that occurred a few weeks before survey periods ended (Jun. and Nov.) and the few weeks after surveys resumed (Aug. and Mar.). Adding these values to those for the 8-mon period, annual fatality rates were estimated using equation 1.

We also conducted 1,000 parametric bootstrap simulations with replacement (Manly, 2006). The only deviation from standard bootstrap methods was that any value within range of our estimates was allowed to be randomly selected. Typically, only known estimates are used. Because carcass-removal and searcher-efficiency rates fluctuate depending on several variables, theoretically, any value within range of our data could be the best estimate for any given year. The method employed assumes a uniform distribution of values (*i.e.*, any value is equally probable). From 1,000 simulations, 90% confidence intervals (CI) were calculated for estimated fatality rates by eliminating the highest 5% and lowest 5% of values obtained.

For analyses, values are reported as mean ± SD. Several standard statistical tests were employed, including goodness-of-fit *G*-tests (Sokal and Rohlf, 2012).

RESULTS

DOCUMENTED FATALITIES OF BIRDS AND BATS

During standard mortality surveys, 51 carcasses were found in 2006 (15 birds and 36 bats), 57 in 2007 (16 birds and 41 bats), and 58 in 2008 (15 birds and 43 bats; Table 1). The 46 bird carcasses included 16-18 species, with 47.8% being turkey vultures. Passerines accounted for 17.4% of bird specimens, upland game birds 6.5%, and waterfowl 13.0%. At least 6 of the 46 carcasses (13.0%) were night-migrating passerines. Three raptor carcasses (6.5%) were found.

The 120 bat carcasses included eight species (Table 1). Fifteen (10 hoary bats and 5 eastern red bats) were from non-sample turbines. Hoary bats were the most frequently encountered. Solitary, migratory tree- and foliage-roosting bats (hoary, eastern red, and silver-haired bats) accounted for 101 (84.2%) of bat carcasses. Mexican free-tailed bats, tri-colored bats, and cave myotis—cave-roosting bats—accounted for 13.3% of bat fatalities.

Passive acoustical monitoring resulted in 159 recorded bat calls. Calls characteristic of each of species with documented fatalities were documented acoustically, although eastern red and Seminole bat calls cannot be distinguished. Also, geographic ranges of canyon bats (*Parastrellus hesperus*) and tri-colored bats overlap at our study site, and they are difficult to distinguish acoustically. No canyon bat carcasses were found, but the species may have produced some of recorded calls. Some species of *Myotis* can be difficult to distinguish acoustically; given geographic location and previously collected specimens (Caire *et al.*, 1989), *Myotis* calls with a characteristic frequency of 40 Khz were judged to be cave myotis and those with 45 Khz to be small-footed bats (*M. leibii*). Calls of Townsend's big-eared bats (*Corynorhinus townsendii*) also were recorded.

All hoary bats were adults; 65.8% could be sexed, with 75.0% being males and 25.0% females. All eastern red bats were adults and, of the 46.2% that could be sexed, all were males. Most carcasses were partially decomposed. If external genitalia remained intact, males could easily be distinguished. However, for specimens lacking visible genitalia, it sometimes was difficult to determine whether they were females or males with decomposed reproductive organs. Thus, recorded sex ratios indicate more fatalities for males than females.

SEASONAL DISTRIBUTION OF FATALITIES

Bird fatalities were evenly distributed throughout the year. Of 46 carcasses, 21 (45.7%) were found in spring and 25 (54.3%) in fall. Relatively few night-migrating songbirds or songbird-like species were found dead; only nine carcasses (19.6%) were recovered during migration seasons. Four of six waterfowl carcasses were found during the migration period.

For bats, there was seasonal disparity in fatality rates. Of 120 carcasses recovered, only 15 (12.5%) were from spring, with 105 (87.5%) from fall (Fig. 2). In 2006, 69.4% of all fatalities occurred 22 Aug.-14 Sep. In 2007, 65.9% of fatalities occurred 25 Aug.-16 Sep. and, in 2008, 62.8% from 23 Aug.-14 Sep. Thus, 66.0 ±

3.30% of bat fatalities occurred within a 3-wk period from late Aug. to early Sep., corresponding to peak bat migration.

SPATIAL DISTRIBUTION OF FATALITIES

The null expectation was that geographically 24% of fatalities would occur in section N (12 turbines checked; Fig. 1), 26% in Section S (13), 26% in section E (13), and 24% in section W (12). For birds, the relatively small numbers of carcasses (Table 2) precluded meaningful yearly evaluation of fatalities by section. For the 3 y combined, numbers of birds found in different sections did not differ from chance expectation ($P = 0.227$; goodness-of-fit *G*-test), suggesting bird fatalities were randomly or uniformly distributed across the wind farm.

For bats, there were notably nonrandom distributions of fatalities among wind-farm sections in 2006 ($P < 0.001$; Table 2), with more fatalities than predicted in section S. We investigated whether a particular turbine or temporal event skewed fatality rates geographically; however, no unusual patterns were apparent. Bat fatalities in section S during 2006 were spread among multiple turbines over a two-month period. The within-section spatial and temporal patterns mirror those of other sections of the facility, only with greater frequencies.

Among-section differences also were found for 2007 ($P = 0.024$) and 2008 ($P = 0.015$), with lower than expected number of bat fatalities from section W. For the 3 y combined, there were more bat fatalities recorded for section S and many fewer in section W than predicted by chance ($P < 0.001$; Table 2).

Proportions of bird fatalities were nearly identical for the first three concentric circles (15, 30, and 45 m from the turbine base) and lower for the 60-m circle (Fig. 3a).

In contrast, 54.2 ± 10.09% of bat carcasses were found along the first concentric circle and 33.1 ± 9.68% along the second (Fig. 3a). Thus, 87.3% of bat carcasses were retrieved within 37.5 m of the turbine base. Bat fatalities seldom were recorded along the outer circles (45 m, 9.7 ± 3.91% of total; 60 m, 3.0 ± 3.39%). For bats, as distance from turbine base increased, proportion of documented bat fatalities decreased almost linearly (Fig. 3a).

FATALITIES RELATIVE TO FAA LIGHTS

Given the number of survey turbines with and without FAA lights, the null hypothesis was that 46% of fatalities would occur at lighted turbines. For birds, statistical tests for individual years and the 3 y combined were nonsignificant ($P > 0.05$). Numbers of bat carcasses found at lighted and unlighted turbines were similar to random expectations (for the 3 y, 50 from lighted and 55 from unlit turbines; $P = 0.740$). Comparisons for individual years also were nonsignificant (all $P > 0.05$).

SEARCHER-EFFICIENCY AND CARCASS-REMOVAL RATES

Detection probability for carcasses varied with distance of a carcass from the turbine base, being about equal for the inner two concentric circles, but lower for the outer two rings (Fig. 3b). For the six searcher-efficiency trials combined, detection probabilities (p) were 0.117 for small-sized birds (also used as surrogates for bats), 0.320 for medium-sized birds, and 0.833 for large-sized birds (Table 3). For bats, separate detection probabilities were derived for the inner two and outer two concentric rings for each survey period (Table 4); mean detection probability was 0.173 for the inner two circles and 0.075 for the outer rings.

Exponential decay functions were derived for each of six carcass-removal trials (Fig. 4). For the six trials combined, small-sized birds disappeared most quickly (mean 6.2 d), followed by medium- and large-sized birds (8.5 and 24.1 d, respectively; Table 3). For large-sized birds in fall 2007, no birds were scavenged, resulting in an infinite mean; this trial was excluded when calculating the overall mean for large-sized birds. As indicated by high R^2-values (Fig. 4), exponential decay functions provided excellent fit to data on carcass disappearance.

ESTIMATED FATALITIES

Mean detection probabilities (p) and scavenging times (T_R) for the six trials were used when calculating fatality estimates. Search intervals specific to season were applied to calculate seasonal fatality rates, with annual rates being the sum of estimated rates for spring and fall. In this way, estimated fatalities each season and year were obtained, as were fatality rates per turbine and per megawatt (Table 5). Results from bootstrap simulations are provided in Table 6.

For all birds, we estimate 82.9 ± 19.00 birds were killed each year during the 8-month survey period for the 84-turbine array (0.99 ± 0.226 birds/turbine, 0.55 ± 0.126 birds/MW). For 12 months, projections indicate these rates are underestimated by 17.9% (extrapolated 12-month rates 97.7 birds/y, 1.16 birds/turbine, 0.65 birds/MW). Bootstrapped estimates for the 84 turbines were 76, 8, and 23 birds/y for small-medium-, and large-sized birds, respectively (Table 6). When considering all birds, complexity introduced by different detection probabilities and carcass-removal rates for size classes precluded obtaining reliable confidence limits through bootstrap simulations.

For the 3 y combined, estimated mean bat fatality rates for the 84 turbines for the 8-month survey period were 623.5 bats/y, 7.42 bats/turbine, and 4.12 bats/MW (Table 5). We estimate 12-month rates to be 2.9% higher, resulting in 641.3 bats/y, 7.63 bats/turbine, and 4.24 bats/MW. Bootstrap simulations estimate 710 bats/y, which is 8.46 bats/turbine and 4.70 bats/MW (Table 6).

DISCUSSION

FACTORS INFLUENCING FATALITY ESTIMATES

Primary fatality estimates were for 8 months when surveys were conducted; these months include migration periods for birds and bats. Sampling periods included seasons when most fatalities have been found at wind farms by other researchers (Kerns and Kerlinger, 2004; Johnson *et al.*, 2002; Jain *et al.*, 2009; Kerlinger *et al.*, 2006; Fiedler *et al.*, 2007).

Projecting to annual 12-month bird fatalities, 8-month values were considered to be 17.9% low. However, since relatively few bird carcasses were found, 12-month values still are small. For bats, the adjustment of fatality values to 12 months also resulted in little change, in this case because projected increases were only 2.9%. Few, if any, bats are active in Dec.-Feb. in southwestern Oklahoma in that most are hibernating or have migrated. Documented fatalities for Jun. and early Aug. in all years were low and should be indicative of Jul. fatalities given that bat migrants likely were not present.

Reliability of fatality estimates depends on accuracy of detection probabilities and scavenging rates. Compared to other studies (Arnett *et al.*, 2008), detection probabilities were relatively low and variable, particularly for small-sized birds. Low-detection probabilities can lead to wider confidence intervals but this effect was offset by sampling a high percentage (59.5%) of turbines in Blue Canyon II.

Variability across trials in our detection probabilities may be explained by interannual variation in precipitation, vegetation height, and vegetation density. Western Oklahoma had one of the driest years on record in 2006 followed by a very wet 2007. Vegetation in 2006 was sparse due to lack of rainfall, grazing, and the fact that the area was in the process of revegetating following wind-farm construction. Abundant rainfall in 2007 led to dramatic increases in vegetative cover, which in some instances was 2 m high. Average rainfall in 2008 led to considerable dead vegetative cover because the lush vegetation of the previous year did not survive, reducing ground visibility and detection probabilities.

Scavenging rates for bats, based on small-sized birds, may have been somewhat biased. Kerns *et al.* (2005) concluded that fresh bat carcasses were scavenged almost twice as fast as those previously frozen, and previously frozen bats were removed more rapidly than previously frozen birds. Such a bias, if present, would result in underestimating bat fatalities.

Bootstrap simulations for small-sized birds and bats resulted in mean fatality rates higher than those obtained using traditional calculations (28.9% higher for small-sized birds and 10.7% higher for bats). This may be because we allowed any value to be selected within the range of our known parameters, which may not have been uniformly distributed. Therefore, fatality estimates using mean searcher-efficiency and carcass-removal data may be less biased than bootstrapped means. However, bootstrap simulations were useful in providing confidence limits and a range of possible fatalities that could be anticipated.

IMPACT OF FAA LIGHTING

No difference was found in fatalities at Blue Canyon II for turbines with L-864 red flashing, strobe-like obstruction beacons and turbines without such lights. This finding is consistent with those at other wind-turbine sites for birds (Kerlinger *et al.*, 2010) and bats (Kerns and Kerlinger, 2004; Arnett *et al.*, 2008; Horn *et al.*, 2008). Specifically, the study by Kerns and Kerlinger (2004) demonstrated bat fatalities were not statistically greater at turbines with the same type of lights as are mounted on Blue Canyon II turbines. These findings, together with those of Gehring *et al.* (2009) for guyed communication towers in the Midwest, strongly suggest that red flashing FAA obstruction lighting does not attract or disorient birds and does not lead to birds colliding with turbines, towers, and support structures.

SIGNIFICANCE OF BIRD FATALITIES AT BLUE CANYON II

Overall, bird mortality was relatively low. Aside from turkey vultures, few individuals of the other 15-17 species were found. While some song birds (*e.g.*, golden-crowned kinglet, least flycatcher, yellow warbler, spotted towhee) and waterfowl recovered probably were night migrants, others (*e.g.*, red-tailed hawk) are known to reside of Blue Canyon and are not night migrants. No rare, threatened, or endangered species were found. The published national average for fatalities at wind farms is about 2.0 birds/(turbine·y) (National Research Council, 2007), although Kerlinger *et al.* (2010) estimated upward to about 4 night migrants/(turbine·y). The estimate of 0.99 birds/(turbine·y) for Blue Canyon II is near the lower bound for previous studies. Thus, impacts of Blue Canyon II fatalities on night migrants, raptors, waterbirds, and gamebirds were minimal. To put bird-fatality rates at Blue Canyon II in perspective, it is helpful to examine hunting harvest statistics for Oklahoma. Whereas two blue-winged teal, one mallard, and one ring-necked duck carcasses were found on site during 3 y, annual harvest for these species in Oklahoma averages 11,000-23,000, 126,000-219,000, and 12,000-15,000, respectively (Richkus *et al.* 2008). Therefore, there is no suggestion of biologically significant impacts on birds resulting from Blue Canyon II.

PATTERNS OF BAT FATALITIES

Number of bat carcasses detected at sample turbines increased slightly from 2006 to 2007 to 2008 (32, 35, and 38 bats, respectively). Bat fatalities in 2007 likely were underestimated to some extent due to road grading that may have removed some carcasses on the day before one survey during peak bat migration (*i.e.*, 19 of 35 bats for 2007 were found in the previous survey). With bats, there were differences in fatalities among sections of the project, with greater risk in section S and lower in section W. Differences among years in precipitation may provide a partial, indirect explanation for why bat fatalities were much more frequent in section S in 2006 than in other years. A ravine, containing a small stream and riparian woodland, is centered in section S and provided a potential water source and roosting site for bat migrants. In times of drought, as in 2006, this may have been the principal water source in the project area. Insect abundance may also be concentrated in this region, and the ravine may have a channeling effect on flying bats. Piorkowski (2006) noted fatalities of Mexican free-tailed bats were associated with wooded ravines in May-Jun. 2004, but did not find a similar pattern in 2005.

The reasons for lower mortality in the western part of the wind farm are not obvious, and attempts at explanation are speculative. However, an important factor may be topography and the general configuration of the terrain. Just north and west of section W, land is relatively flat for an extended distance and 100 m lower than the uplifted topography on which the turbines of section W are based. Bats flying relatively low and approaching section W from the north during fall migration could readily skirt the uplift with the turbines by adjusting their flight path to a trajectory slightly to the west. In addition, about 2 km north, a narrow uplift extends to the south-southwest, which may serve to channel some bats to the east and away from section W. Other sections of the Blue Canyon II are not fronted to the north with such a topographic configuration, and low-flying bats would not be as well positioned to avoid flying over uplifted areas facing them to the south. Other factors, such as meteorological conditions, probably also influence the above-ground altitude at which migrating bats fly (Reynolds, 2006) and could explain some of the disparities in fatalities between fall and spring migration. Focused investigation would be of interest to determine the extent to which climatic and landscape characteristics contribute to bat-fatality risk.

Most bat fatalities on wind farms in the United States have involved tree- or foliage-roosting migratory bats, with highest numbers of fatalities often being hoary bats (Johnson *et al.*, 2003; Arnett *et al.*, 2008; Jain *et al.*, 2009). Fatalities also have been relatively high for eastern red bats, particularly at eastern sites (Kerns and Kerlinger, 2004; Kerns *et al.*, 2005; Arnett *et al.*, 2008). These two species accounted for most of fatalities at Blue Canyon II.

From acoustical monitoring, several cave species were documented as residing in the area, yet few carcasses of them were found. Some species, like Townsend's big-eared bats and small-footed bats do not appear susceptible to turbine collisions or are killed in such low numbers that they go undetected. The cave myotis and tricolored bat were the most common acoustically recorded species, yet few of their carcasses were recovered. There appear to be behavioral or biological differences that make migratory tree- and foliage-roosting bats more susceptible to wind-farm mortality. Specifically, most fatalities occurred during fall migration.

Male bats appeared more likely to be killed than females. Of carcasses identified to sex, males were 75% of hoary bats and 100% of eastern red bats. For both species, sex ratios could be equal only if nearly all unidentifiable carcasses were female, which is unlikely. Our results are similar to those for sites in the eastern United States that have reported a large bias towards males (Arnett *et al.*, 2008).

Based on bat-fatality patterns indicating carcasses are concentrated near the turbine and biased toward adult males during fall migration, Cryan (2008) proposed a roost-attraction hypothesis, under which bats are killed by turbine blades while attempting to use turbine towers as roosting sites. Copulation in hoary bats likely occurs during autumn migration (Cryan and Brown, 2007), and males may attempt to use turbines, like tall trees, for display during reproductive periods (Cryan, 2008). Our data are not at variance with this hypothesis, but it would not explain fatalities of Mexican free-tailed bats and other cave-roosting species that have been documented at Blue Canyon II and other facilities in the region (Piorkowski, 2006; Miller, 2008).

A northwestern Oklahoma wind-turbine facility is in close proximity to several nursery colonies of Mexican free-tailed bats; 85% of carcasses for a summer survey there were of this cave species (Piorkowski and O'Connell, 2010). For a facility in western Texas, 94% of fatalities were Mexican-free tailed bats (Miller, 2008). Although small caves are known in the Blue Canyon II area, the closest known nursery colony of Mexican free-tailed bats is 80 km northwest (Oklahoma Wind Power Initiative, 2007). At Blue Canyon II, fatalities of Mexican free-tailed bats were infrequent (9 of 120 bats) and all but one were in a single year (2006) and during the migration period. They roost almost exclusively in caves or appropriately configured manmade structures (Wilkins, 1989). Radar images from mid-Sep. 2006 (National Weather Center) showed Mexican free-tailed bats from known nursery colonies in Greer County making nightly flights extending into the Blue Canyon II area. We cannot be sure whether fatalities of this species involved migrants or local individuals.

SIGNIFICANCE BAT FATALITIES AT BLUE CANYON II

Species composition and adult-male bias at Blue Canyon II mirror findings for facilities in the eastern United States. Cave species have been relatively infrequent fatalities in the east, paralleling our finding (Kunz et al., 2007; National Research Council, 2007). We documented the first occurrences of evening bats and cave myotis as fatalities at a wind farm. The Seminole bat previously has been recorded as a wind-farm fatality only at Buffalo Mountain in Tennessee (Fiedler, 2004). There are only two other records of the species in Oklahoma (Caire *et al.*, 1989).

The average annual 12-month fatality rate of 7.63 bats/turbine is at the low end of those documented in the eastern United States, but higher than found in the west

(Kunz et al., 2007; National Research Council, 2007). Another Oklahoma wind-farm study estimated bat fatalities at 1.34/turbine (Piorkowski, 2006). Yearly fatality estimates from that study likely would have been considerably greater and with modified species composition had surveys extended into the fall migration period. Given the timing of surveys, findings are not fully comparable with our results. The fatality estimate of 641.3 bats/y for Blue Canyon II is moderate and in line with fatality values for other wind farms

Most bat fatalities at wind farms have involved relatively common species, yet impacts on bats are considered potentially significant, especially in the east where upwards of 63.9 fatalities/(turbine·y) have been reported (Fiedler *et al.*, 2007). Bats are relatively long-lived (Wilkinson and South, 2002) with relatively low reproductive rates (1.11-1.38 young/y for most U.S. species; Barclay and Harder, 2003), suggesting vulnerability to modest annual mortality rates.

Whereas off-setting a portion of energy production in the United States with renewable, emission-free energy is an imperative for the environment, there also is urgency in determining causes of bat fatalities associated at wind farms, assessing the extent of biological impact of such fatalities, and finding ways to mitigate such risk. Given wind-energy facilities in south-central United States are rapidly expanding both in size and number, cumulative effects of fatalities have potential to be significant, and adjustments at individual facilities to minimize bat mortality (Arnett *et al.*, 2008) should be given careful consideration. Bat mortality occurs at night and at most facilities, including Blue Canyon II, is concentrated during fall migration. At Blue Canyon II it appears fatalities occurred disproportionately across the wind farm. Alteration in operating procedures for a short time period in only a portion of Blue Canyon II likely would result in substantial reduction in bat mortality. Creative solutions can be pursued to reduce bat mortality at wind farms in general. For example, Arnett *et al.* (2011) have demonstrated that increasing the lowest speed at which turbines generate power can reduce bat mortality 44-99% with only 1% loss of total annual energy output. Given other stresses on bat populations and rapid growth of wind-power generation, it is prudent to look for imaginative approaches to reduce bat mortality.

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LITERATURE CITED

American Wind Energy Association. 2011. 2010 U.S. wind industry market update. 2 p. Available: http://www.awea.org/learnabout/publications/factsheets/upload/Market-Update-Factsheet-Final_April-2011.pdf. Accessed 13 Jan. 2012.

Arnett, E. B., W. K. Brown, W. P. Erickson, J. K. Fiedler, B. L. Hamilton, T. H. Henry, A. Jain, G. D. Johnson, J. Kerns, R. R. Koford, C. P. Nicholson, T. J. O’Connell, M. D. Piorkowski, and R. D. Tankersley, Jr. 2008. Patterns of bat fatalities at wind energy facilities in North America. *J. Wildl. Manage.*, **72:**61-78.

——, M. M. P. Huso, M. R. Schirmacher, and J. P. Hayes. 2011. Altering turbine speed reduced bat mortality at wind-energy facilities. *Front. Ecol. Environ.,* **9:**209-214.

Barclay, R. M. R. and L. M. Harder. 2003. Life histories of bats: life in the slow lane, p. 209-253. *In:* T. H. Kuntz and M. B. Fenton (eds.). Bat ecology. University of Chicago Press, Chicago, Illinois.

Barnard, D. 2000. Statistical properties of an avian fatality estimator. M.S. Thesis. University of Wyoming, Laramie, Wyoming. 59 p.

Brown, W. K., and B. L. Hamilton. 2006. Monitoring of bird and bat collisions with wind turbines at the Summerview Wind Power Project, Alberta 2005-2006. Prepared for Vision Quest Windelectric, Calgary, Alberta, Canada. Terrestrial & Aquatic Environmental Managers ltd., Calgary, Alberta, Canada. 42 p. Available: http://www.batsandwind.org/pdf/Brown2006.pdf. Accessed 30 Jan. 2012.

Caire, W., J. D. Tyler, B. P. Glass, and M. A. Mares. 1989. Mammals of Oklahoma. University of Oklahoma Press, Norman, Oklahoma. 567 p.

California Energy Commission. 2003. Bird strike indicator/bird activity monitor and field assessment of avian fatalities. 108 p. Available: http://www.energy.ca.gov/reports/2004-03-05_500-03-107F.pdf. Accessed 13 Jan. 2012.

Cryan, P. M. 2008. Mating behavior as a possible cause of bat fatalities at wind turbines. *J. Wildl. Manage.,* **72:**845-849.

—— and A. C. Brown. 2007. Migration of bats past a remote island offers clues toward the problem of bat fatalities at wind turbines. *Biol. Cons.*, **139:**1-11.

Department of Energy. 2008. 20% wind energy by 2030: increasing wind energy's contribution to U.S. electricity supply. U.S. Department of Energy, Washington, D.C. 248 p. Available: http://www.nrel.gov/docs/fy08osti/41869.pdf. Accessed 17 Jan. 2012.

Energy Information Administration. 2011a. Primary energy overview, selected years, 1949-2010. 1 p. Available: http://www.eia.gov/totalenergy/data/annual/pdf/sec1_5.pdf. Accessed 13 Jan. 2012.

——. 2011b. Primary energy production by source, selected years, 1949-2010. 1 p. Available: http://www.eia.gov/totalenergy/data/annual/pdf/sec1_7.pdf. Accessed 13 Jan. 2012.

Fiedler, J. K. 2004. Assessment of bat mortality and activity at Buffalo Mountain Windfarm, eastern Tennessee. M.S. Thesis. University of Tennessee, Knoxville, Tennessee. 166 p.

——, T. H. Henry, R. D. Tankersley, and C. P. Nicholson. 2007. Results of bat and bird mortality monitoring at the expanded Buffalo Mountain Windfarm, 2005. Tennessee Valley Authority, Knoxville, Tennessee. 42 p. Available: http://www.tva.gov/environment/bmw_report/results.pdf. Accessed 13 Jan. 2012.

Gehring, J., P. Kerlinger, and A. M. Manville II. 2009. Communication towers, lights, and birds: successful methods of reducing the frequency of avian collisions. *Ecol. Appl.*, **19:**505-514.

Graham, T. L. and P. F. Hudak. 2011. Potential hazards of wind energy for rare, threatened, and endangered birds and bats in Texas. *Int. J. Enviro. Res.*, **5:**917-922.

Horn, J. W., E. B. Arnett, and T. H. Kunz. 2008. Behavioral responses of bats to operating wind turbines. *J. Wildl. Manage.*, **72:**123-132.

Jain, A. A., P. Kerlinger, R. Curry, L. Slobodnik, and M. Lehman. 2009. Annual report for the Maple Ridge Wind Power Project: post-construction bird and bat fatality study–2008. Prepared for Idberdrola Renewables, Inc, and Horizon Energy, and Technical Advisory Committee for the Maple Ridge Project Study. Curry & Kerlinger, L.L.C., McLean, Virginia. 80 p. Available: http://www.batsandwind.org/pdf/Jain_2009b.pdf. Accessed 16 Jan. 2012.

——, R. R. Koford, A.W. Hancock, G. G. Zenner. 2011. Bat mortality and activity at a northern Iowa wind resource area. *Am. Midl. Nat.*, **165:**185-200.

Johnson, G. D., W. P. Erickson, M. D. Strickland, M. F. Shepherd, D. A. Shepherd, and S. A. Sarappo. 2002. Collision mortality of local and migrant birds at a large-scale wind power development on Buffalo Ridge, Minnesota. *Wildl. Soc. Bull.*, **30:**879-887.

——, **——**, **——**, **——**, **——**, and **——**. 2003. Mortality of bats at a large-scale wind power development at Buffalo Ridge, Minnesota. *Am. Midl. Nat.*, **150:**332-342.

Kerlinger, P., R. Curry, L. Culp, A. Jain, C. Wilkerson, B. Fischer, and A. Hasch. 2006. Post-construction avian and bat fatality monitoring study for the High Winds Wind Power Project, Solano County, California: two year report. Prepared for High Winds, L.L.C., and FPL Energy, Livermore, California. Curry & Kerlinger, L.L.C., McLean, Virginia. 136 p. Available: http://www.batsandwind.org/pdf/Kerlinger_etal_2006.pdf. Accessed 16 Jan. 2012.

——, J. Gehring, W.P. Erickson, R. Curry, A. Jain, and J. Guarnaccia. 2010. Night migrant fatalities and obstruction lighting at wind turbines in North America. *Wilson J. Ornithol.*, **122:**744-754.

Kerns, J., W. P. Erickson, and E. B. Arnett. 2005. Bat and bird fatality at wind energy facilities in Pennsylvania and West Virginia, p. 24-95. *In*: E. B. Arnett (Ed.). Relationships between bats and wind turbines in Pennsylvania and West Virginia: an assessment of fatality search protocols, patterns of fatality, and behavioral interactions with wind turbines. Prepared for the Bats and Wind Energy Cooperative. Bat Conservation International, Austin, Texas. Available: http://www.batsandwind.org/pdf/postconpatbatfatal.pdf. Accessed 14 Jan. 2012.

—— and P. Kerlinger. 2004. A study of bird and bat collision fatalities at the Mountaineer Wind Energy Center, Tucker County, West Virginia: annual report for 2003. Prepared for FPL Energy and Mountaineer Wind Energy Center Technical Review Committee. Curry & Kerlinger, LLC, McLean, Virginia. 39 p. Available: http://www.wvhighlands.org/Birds/MountaineerFinalAvianRpt-%203-15-04PKJK.pdf. Accessed 16 Jan. 2012.

Kunz, T. H., E. B. Arnett, W. P. Erickson, A. R. Hoar, G. D. Johnson, R. P. Larkin, M. D. Strickland, R. W. Thresher, and M. D. Tuttle. 2007. Ecological impacts of wind energy development on bats: questions, research needs, and hypotheses. *Fron. Ecol. Environ.,* **5:**315-324.

Manly, B. F. 2006. Randomization, bootstrap and Monte Carlo methods in biology. 3rd edition. Chapman and Hall/CRC, New York, New York. 455 p.

Miller, A. 2008. Patterns of avian and bat mortality at a utility-scaled wind farm on the Southern High Plains. M.S. Thesis. Texas Tech University, Lubbock, Texas. 113 p.

National Research Council. 2007. Environmental impacts of wind-energy projects. National Academies Press, Washington, D.C. 394 p.

Oklahoma Wind Power Initiative. 2007. Interactive maps: Oklahoma wind and wildlife. Available: http://www2.ocgi.okstate.edu/website/owpi2/viewer.htm. Accessed 13 Jan. 2012.

Orloff, S. and A. Flannery. 1992. Wind turbine effects on avian activity, habitat use, and mortality in Altamont Pass and Salano County Wind Resource Areas, 1989-1991. Final Report. Prepared for Alameda, Costra Costa and Solano counties and the California Energy Commission. Biosystems Analysis, Inc., Tiburon, California. 199 p. Available: http://www.altamontsrc.org/alt_doc/orloff_and_flannery_1992.pdf. Accessed 16 Jan. 2012.

Piorkowski, M. D. 2006. Breeding bird habitat use and turbine collisions of birds and bats located at a wind farm in Oklahoma mixed-grass prairie. M.S. Thesis. Oklahoma State University, Stillwater, Oklahoma. 100 p.

—— and T. J. O'Connell. 2010. Spatial pattern of summer bat morality and collisions with wind turbines in mixed-grass prairie. *Am. Midl. Nat.*, **164:**260-269.

Reynolds, D. S. 2006. Monitoring the potential impact of a wind development site on bats in the Northeast. *Am. Midl. Nat.*, **70:**1219-1227.

Richkus, K. D., K. A. Wilkins, R. V. Raftovich, S. S. Williams, and H. L. Spriggs. 2008. Migratory bird hunting activity and harvest during the 2006 and 2007 hunting seasons: preliminary estimates. U.S. Fish and Wildlife Service, Laurel, Maryland. 65 p.

Shoenfeld, P. 2004. Suggestions regarding avian mortality extrapolation. Prepared for Mountaineer Wind Energy Center Technical Review Committee. 6 p. Available: http:// www.wvhighlands.org/Birds/SuggestionsRegardingAvianMortalityExtrapolation.pdf. Accessed 16 Jan. 2012.

Smallwood, K. S. and C. Thelander. 2008. Bird mortality in the Altamont Pass Wind Resource Area, California. *J. Wildl. Manage.*, **72:**215-223.

Sokal, R. R. and F. J. Rohlf. 2012. Biometry: the principles and practice of statistics in biological research. 4th edition. W. H. Freeman and Company, New York, New York. 937 p.

Tierney, R. 2009. Buffalo Gap 2 Wind Farm avian mortality study July 2007–December 2008 final study report. Prepared for Buffalo Gap Wind Farm 2, LLC. TRC, Albuquerque, New Mexico. 49 p. Available: http://www.batsandwind.org/pdf/Buffalo%20Gap%202%20Final%20Report.pdf. Accessed 16 Jan 2012.

Wilkins, K. T. 1989. *Tadarida brasiliensis*. *Mamm. Species*, **331:**1-10.

Wilkinson, G. S. and J. M. South. 2002. Life history, ecology and longevity of bats. *Aging Cells,* **1:**124-131.

Young, D. P., Jr., W. P. Erickson, R. E. Good, M. D. Strickland, and G. D. Johnson. 2003. Avian and bat mortality associated with the initial phase of the Foote Creek Rim Windpower Project, Carbon County, Wyoming. November 1998-June 2002. Prepared for Pacificorp, Inc., Portland, Oregon, Sea West Windpower Inc, San Diego, California, and Bureau of Land Management, Rawlins, Wyoming. WEST, Inc, Cheyenne, Wyoming. 50 p. Available: http://www.west-inc.com/reports/fcr_final_mortality.pdf. Accessed 16 Jan. 2012.

TABLE 1. Number (percent of total in parentheses) of bird and bat carcasses found at Blue Canyon II Windpower Project each year of study

Species	2006	2007	2008	Total
	Birds			
Turkey vulture (*Cathartes aura*)	11 (73.3%)	8 (50.0%)	3 (20.0%)	22 (47.8%)
Red-tailed hawk (*Buteo jamaicensis*)	2 (13.3%)	0	1 (6.7%)	3 (6.5%)
Spotted towhee (*Pipilo maculatus*)	1 (6.7%)	0	1 (6.7%)	2 (4.3%)
Mourning dove (*Zenaida macroura*)	0	1 (6.3%)	1 (6.7%)	2 (4.3%)
Yellow-billed cuckoo (*Coccyzus americanus*)	0	1 (6.3%)	1 (6.7%)	2 (4.3%)
Blue-winged teal (*Anas discors*)	0	0	2 (13.3%)	2 (4.3%)
Yellow warbler (*Setophaga petechia*)	0	0	2 (13.3%)	2 (4.3%)
Golden-crowned kinglet (*Regulus satrapa*)	1 (6.7%)	0	0	1 (2.2%)
Canada goose (*Branta canadensis*)	0	1 (6.3%)	0	1 (2.2%)
Cattle egret (*Bubulcus ibis*)	0	1 (6.3%)	0	1 (2.2%)
House wren (*Troglodytes aedon*)	0	1 (6.3%)	0	1 (2.2%)
Sparrow sp.	0	1 (6.3%)	0	1 (2.2%)
Wild turkey (*Meleagris gallopavo*)	0	1 (6.3%)	0	1 (2.2%)
Duck sp.	0	1 (6.3%)	0	1 (2.2%)
American coot (*Fulica americana*)	0	0	1 (6.7%)	1 (2.2%)
Mallard (*Anas platyrhynchos*)	0	0	1 (6.7%)	1 (2.2%)
Least flycatcher (*Empidonax minimus*)	0	0	1 (6.7%)	1 (2.2%)
Ring-necked duck (*Aythya collaris*)	0	0	1 (6.7%)	1 (2.2%)
Total	15	16	15	46
	Bats			
Hoary bat (*Lasiurus cinereus*)	20 (55.6%)	31 (75.6%)	22 (51.2%)	73 (60.8%)
Eastern red bat (*Lasiurus borealis*)	6 (16.7%)	6 (14.6%)	14 (32.6%)	26 (21.7%)
Mexican free-tailed bat (*Tadarida brasiliensis*)	8 (22.2%)	1 (2.4%)	0	9 (7.5%)
Tricolored bat (*Perimyotis subflavus*)	1 (2.8%)	0	5 (11.6%)	6 (5.0%)
Silver-haired bat (*Lasionycteris noctivagans*)	1 (2.8%)	1 (2.4%)	0	2 (1.7%)
Evening bat (*Nycticeius humeralis*)	0	1 (2.4%)	1 (2.3%)	2 (1.7%)
Seminole bat (*Lasiurus seminolus*)	0	1 (2.4%)	0	1 (0.8%)
Cave myotis (*Myotis velifer*)	0	0	1 (2.3%)	1 (0.8%)
Total	36	41	43	120

TABLE 2. Spatial distribution of bird and bat fatalities at Blue Canyon II Wind Power Project

Section[a]	No. turbines surveyed	No. turbines with fatalities		No. carcasses recovered (deviation from expected)	
		Birds	Bats	Birds	Bats
		2006 (bats, P < 0.001)[b]			
N	12	3	5	4	7 (-0.7)
S	13	4	10	6	18 (9.7)
E	13	2	1	3	2 (-6.3)
W	12	2	5	2	5 (-2.7)
Total	50	11	21	15	32
		2007 (bats, P = 0.024)			
N	12	4	6	7	9 (+0.6)
S	13	1	8	1	10 (+0.9)
E	13	3	8	4	14 (+4.9)
W	12	2	2	2	2 (-6.4)
Total	50	10	24	14	35
		2008 (bats, P = 0.015)			
N	12	4	6	5	13 (+3.9)
S	13	3	7	4	10 (+0.1)
E	13	2	6	2	13 (+3.1)
W	12	2	2	3	2 (-7.1)
Total	50	11	21	14	38
		3 y combined (birds, P = 0.227; bats, P < 0.001)			
N	12	8	9	16 (+5.7)	29 (+1.4)
S	13	7	13	11 (-0.2)	38 (+10.7)
E	13	5	10	9 (-2.2)	29 (+1.7)
W	12	5	7	7 (-3.3)	9 (-16.2)
Total	50	25	39	43	105

[a] Turbines in sections N, S, E, and W were in north-central, south-central, eastern, and western parts of project, respectively (Fig. 1)

[b] Probability that distribution of fatalities across sections was random (goodness-of-fit *G*-test), with numbers of turbines surveyed in sections being basis for null expectations of carcass numbers

TABLE 3. Parameters for equations used to estimate bird mortality at the Blue Canyon II Wind Power Project

Period	Parameter[a]			
	N_F	p	T_R	T_S
		Small-sized birds		
Spring 2006	0	–	–	9.1 ± 3.87
Fall 2006	2	0.147	13	8.9 ± 2.91
Spring 2007	2	0.166	8.4	8.2 ± 2.60
Fall 2007	0	0.167	2.6	9.7 ± 4.37
Spring 2008	0	0.130, 0.044	4.8, 6.8	9.8 ± 3.68
Fall 2008	3	0.045	1.5	9.9 ± 5.25
Mean		0.117 ± 0.0573	6.2 ± 4.21	9.3 ± 3.74
		Medium-sized birds		
Spring 2006	0	–	–	9.1 ± 3.87
Fall 2006	0	0.163	17.9	8.9 ± 2.91
Spring 2007	2	0.267	6.2	8.2 ± 2.60
Fall 2007	0	0.44	4.6	9.7 ± 4.37
Spring 2008	0	0.286, 0.619	7.0, 10.1	9.8 ± 3.68
Fall 2008	2	0.143	5.2	9.9 ± 5.25
Mean		0.320 ± 0.1810	8.5 ± 4.99	9.3 ± 3.74
		Large-sized birds		
Spring 2006	6	–	–	9.1 ± 3.87
Fall 2006	7	–	17	8.9 ± 2.91
Spring 2007	5	–	25.7	8.2 ± 2.60
Fall 2007	5	–	>40.0	9.7 ± 4.37
Spring 2008	4	0.750, 0.750	38.0, 13.5	9.8 ± 3.68
Fall 2008	5	1	26.1	9.9 ± 5.25
Mean		0.833 ± 0.1443	24.1 ± 8.99	9.3 ± 3.74

[a] N_F is observed number of carcasses, p is probability of searcher detecting carcass, T_R is mean scavenging time for carcasses in days, and T_S (± SD) is mean search interval between surveys in days

TABLE 4. Parameters for equations used to estimate bat fatalities at Blue Canyon II Wind Power Project

Period	Parameter[a]					
	N_F for concentric rings		p for concentric rings		T_R	T_S
	1 and 2	3 and 4	1 and 2	3 and 4		
Spring 2006	0	0	–	–	–	9.1 ± 3.87
Fall 2006	26	6	0.14	0.154	13	8.9 ± 2.91
Spring 2007	11	0	0.244	0.102	8.4	8.2 ± 2.60
Fall 2007	20	4	0.267	0.121	2.6	9.7 ± 4.37
Spring 2008	3	1	0.222, 0.083	0.071, 0.000	4.8, 6.8	9.8 ± 3.68
Fall 2008	32	2	0.083	0	1.5	9.9 ± 5.25
Mean			0.173 ± 0.0819	0.075 ± 0.0638	6.2 ±4.21	9.3 ± 3.74

[a] N_F is observed number of carcasses, p is probability of searcher detecting carcass, T_R is mean scavenging time for carcasses in days (based on trials with small-sized birds), and T_S (± SD) is mean search interval between surveys in days

TABLE 5. Estimated number of fatalities for birds and bats by season and year for Blue Canyon II Wind Power Project (BCII)

	Small-sized birds		Medium-sized birds		Large-sized birds		Bats	
Parameter	Spring	Fall	Spring	Fall	Spring	Fall	Spring	Fall
Year 2006								
N_K for sampled turbines[a]	0	25.6	0	0	7.7	8.9	0	346.1
N_K/turbine	0	0.51	0	0	0.15	0.18	0	6.92
Estimated fatalities by season for all turbines[b]	0	43	0	0	12.9	15	0	581.4
Fatalities/turbine for 8 months[c]	0.51		0		0.33		6.92	
Fatalities/MW for 8 months[c]	0.28		0		0.18		3.85	
Total fatalities for BC II for 8 months[c]	43		0		27.9		581.4	
Year 2007								
N_K for sampled turbines[a]	23.6	0	7.2	0	6.2	6.5	89.4	274.9
N_K/turbine	0.47	0	0.14	0	0.12	0.13	1.79	5.5
Estimated fatalities by season for all turbines[b]	39.6	0	12.1	0	10.4	10.9	150.2	461.8
Fatalities/turbine for 8 months[c]	0.51		0.14		0.25		7.29	
Fatalities/MW for 8 months[c]	0.26		0.08		0.14		4.05	
Total fatalities for BC II for 8 months[c]	39.6		12.1		21.3		612	
Year 2008								
N_K for sampled turbines[a]	0	42.4	0	8.3	5.2	6.5	50.3	352.6
N_K/turbine	0	0.85	0	0.17	0.1	0.13	1.01	7.05
Estimated fatalities by season for all turbines[b]	0	71.2	0	13.9	8.7	10.9	84.5	592.4
Fatalities/turbine for 8 months[c]	0.85		0.17		0.23		8.06	
Fatalities/MW for 8 months[c]	0.47		0.09		0.13		4.48	
Total fatalities for BC II for 8 months[c]	71.2		13.9		19.7		676.9	

Table 5 Continued.

	Small-sized birds		Medium-sized birds		Large-sized birds		Bats	
Parameter	Spring	Fall	Spring	Fall	Spring	Fall	Spring	Fall
Years combined								
Mean fatalities/turbine for 8 months[c]	0.61 ± 0.207		0.10 ± 0.090		0.27 ± 0.052		7.42 ± 0.580	
Mean fatalities/MW for 8 months[c]	0.34 ± 0.115		0.06 ± 0.050		0.15 ± 0.029		4.12 ± 0.322	
Mean fatalities for BCII for 8 months[c]	51.3 ± 17.35		8.7 ± 7.57		23.0 ± 4.35		623.5 ± 48.73	
Mean fatalities/turbine for 12 months[d]	0.69		0.12		0.32		7.63	
Mean fatalities/MW for 12 months[d]	0.39		0.07		0.18		4.24	
Mean fatalities for BCII projected to 12 months[d]	58.6		10.5		27.3		641.3	

[a] N_K is estimated number of fatalities

[b] Fatality estimates were extrapolated from sample turbines to include all 84 turbines at Blue Canyon II Wind Power Project. Spring estimates were a survey period from Mar.-Jun. and fall estimates were a survey period from Aug.-Nov.

[c] Seasons and/or years were combined to estimate annual fatalities over the 8-month survey period covering Mar.-Jun. and Aug.-Nov.

[d] Compared to 8-month survey period, 12-month annual fatality rates were estimated to be 14.2% higher for small-sized birds, 20.4% higher for medium-sized birds, 19.0% higher for large-sized birds, and 2.85% higher for bats.

TABLE 6. Bootstrapped values (mean with 90% confidence interval in parentheses) of bird and bat fatalities per year associated with Blue Canyon II Wind Power Project

Category	2006	2007	2008	Overall
Bootstrap estimate of annual fatalities for all turbines in project				
Small-sized birds	63 (21-158)	63 (20-155)	101 (34-252)	76 (25-188)
Medium-sized birds	0 (0-0)	11 (6-21)	12 (6-25)	8 (4-15)
Large-sized birds	28 (25-31)	21 (19-24)	19 (18-23)	23 (21-26)
Bats	648 (285-1,420)	692 (286-1,599)	791 (332-1,839)	710 (301-1,619)
Bootstrap estimate of annual fatalities per turbine[a]				
Small-sized birds	0.75 (0.25-1.88)	0.75 (0.24-1.85)	1.20 (0.40-3.00)	0.90 (0.30-2.24)
Medium-sized birds	0.00 (0.00-0.00)	0.13 (0.07-0.25)	0.14 (0.07-0.30)	0.09 (0.05-0.18)
Large-sized birds	0.33 (0.30-0.37)	0.25 (0.23-0.29)	0.23 (0.21-0.27)	0.27 (0.25-0.31)
Bats	7.71 (3.39-16.90)	8.24 (3.40-19.04)	9.42 (3.95-21.98)	8.46 (3.58-19.28)
Bootstrap estimate of annual fatalities per megawatt[b]				
Small-sized birds	0.42 (0.14-1.04)	0.42 (0.13-1.03)	0.67 (0.22-1.67)	0.50 (0.17-1.25)
Medium-sized birds	0.00 (0.00-0.00)	0.07 (0.04-0.14)	0.08 (0.04-0.17)	0.05 (0.03-0.10)
Large-sized birds	0.19 (0.17-0.21)	0.14 (0.13-0.16)	0.13 (0.12-0.15)	0.15 (0.14-0.17)
Bats	4.29 (1.88-9.39)	4.58 (1.89-10.58)	5.23 (2.20-12.16)	4.70 (1.99-10.71)

[a] Values calculated by taking bootstrap estimate of annual fatalities and dividing by number of turbines (84)

[b] Values calculated by taking bootstrap estimate of total annual fatalities and dividing by product of number of turbines (84) and rated capacity of each turbine (1.8 MW)

FIGURE CAPTIONS

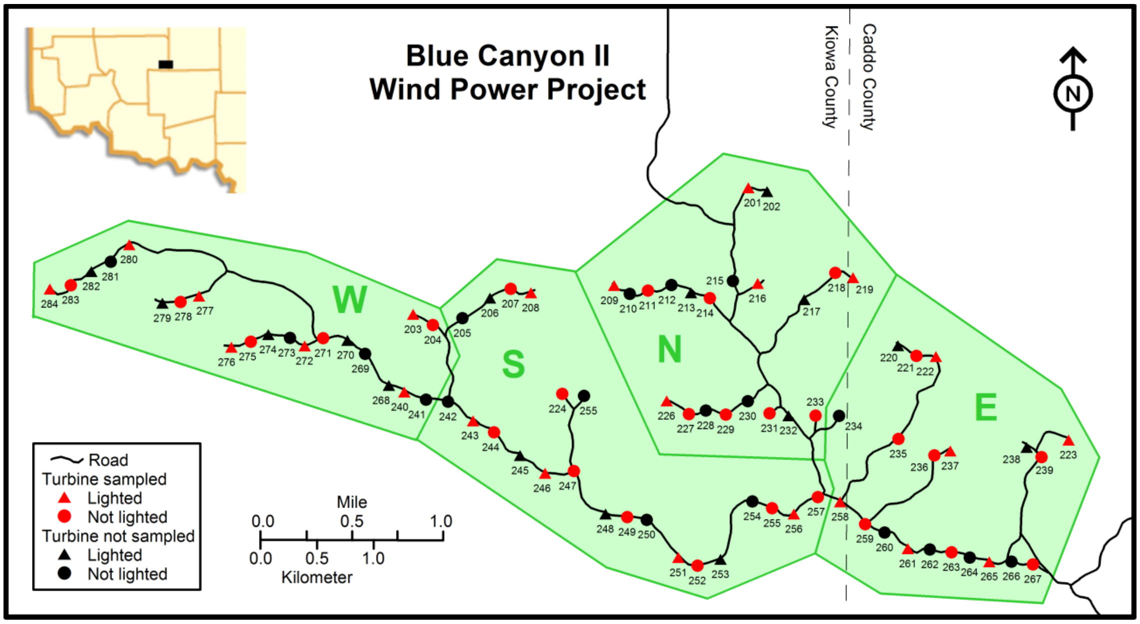
FIG. 1.—Map showing locations of 84 wind turbines in Blue Canyon II Wind Power Project, including 50 that were surveyed for bird and bat fatalities. Turbines with red strobe lights are indicated. Turbines were partitioned into four sections (N, S, E, W; *i.e.*, north-central, south-central, east, west) for some analyses. Lower edge of diagram corresponds to where Comanche County abuts Kiowa and Caddo counties. Inset map shows location of site in southwestern Oklahoma

FIG. 2.—Number of documented bat fatalities (all species) at Blue Canyon II Wind Power Project as function of time of year

FIG. 3.—(a) Mean proportion of documented fatalities as function of concentric circle on which specimen was found and (b) mean proportion of bird carcasses detected in searcher-efficiency trials on concentric circles along which specimens were placed. Whiskers indicate SD.

FIG. 4.—Probability small-, medium-, and large-sized bird carcasses remained (*Y*) at Blue Canyon II Wind Power Project plotted relative to number of days since carcass placement (*X*) for: (a-c) Fall 2006; (d-f) Spring 2007; (g-i) Fall 2007; (j-l) Spring 2008 in Mar.; (m-o) Spring 2008 in Jun.; and (p-r) Fall 2008. Exponential decay functions provided.

FIG. 1



Blue Canyon II
Wind Power Project
Kiowa County
Caddo County
N
W
S
N
E
Road
Turbine sampled
Lighted
Not lighted
Turbine not sampled
Lighted
Not lighted
Mile
0.0
0.5
1.0
0.0
0.5
1.0
Kilometer
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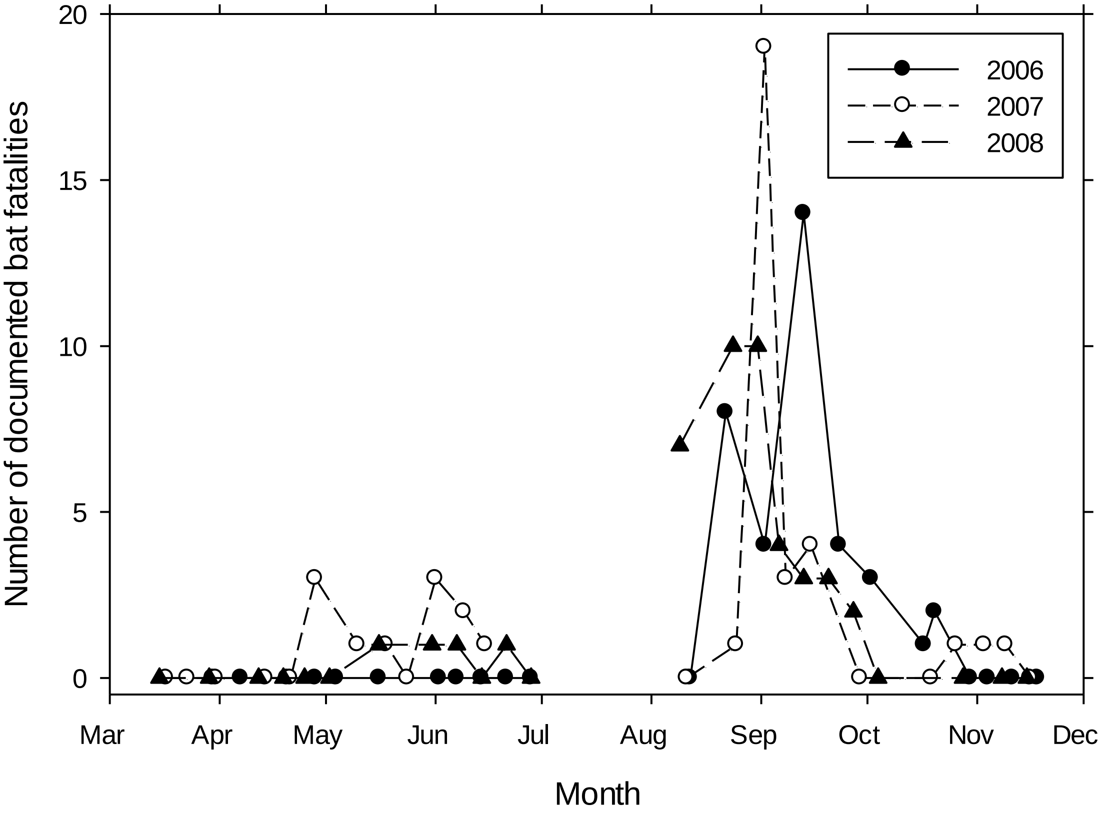
FIG. 2



Number of documented bat fatalities
0
5
10
15
20
Mar
Apr
May
Jun
Jul
Aug
Sep
Oct
Nov
Dec
Month
2006
2007
2008

FIG. 3

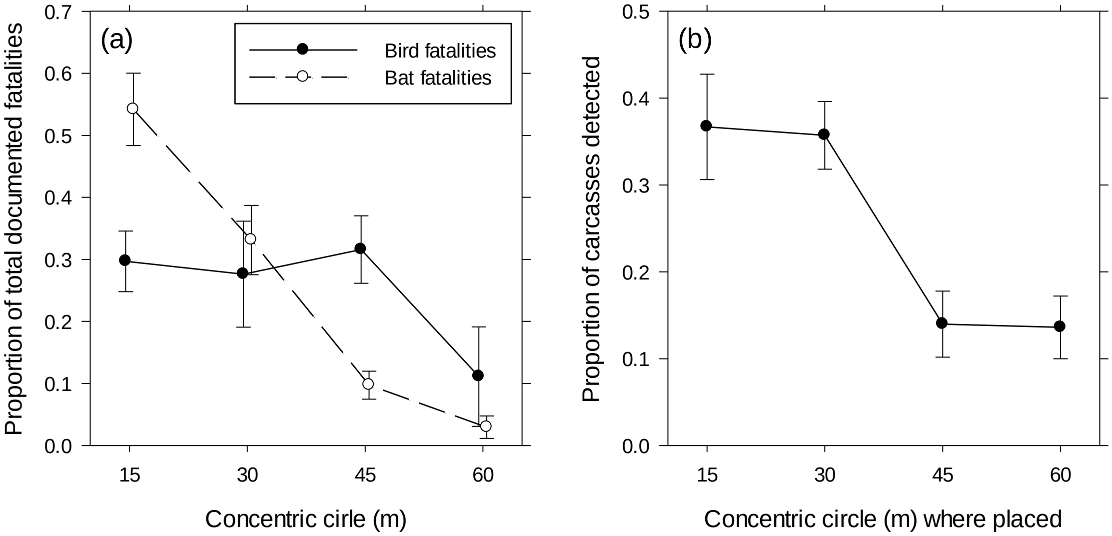
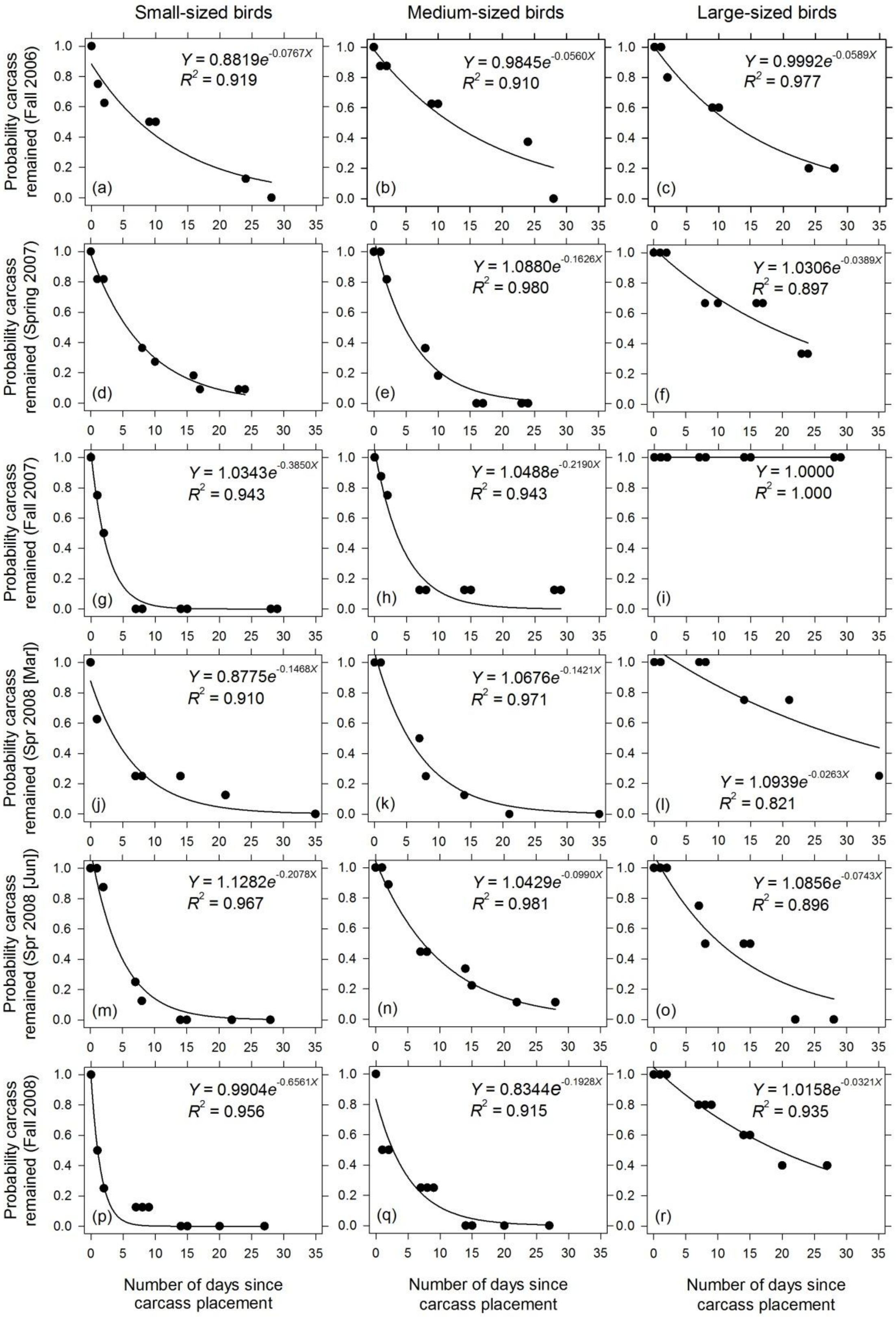

(a)
Bird fatalities
Bat fatalities
Proportion of total documented fatalities
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
15
30
45
60
Concentric cirle (m)
(b)
Proportion of carcasses detected
0.0
0.1
0.2
0.3
0.4
0.5
15
30
45
60
Concentric circle (m) where placed

FIG. 4


Small-sized birds
Medium-sized birds
Large-sized birds
Probability carcass remained (Fall 2006)
Probability carcass remained (Spring 2007)
Probability carcass remained (Fall 2007)
Probability carcass remained (Spr 2008 [Mar])
Probability carcass remained (Spr 2008 [Jun])
Probability carcass remained (Fall 2008)
(a) Y = 0.8819e^-0.0767X R^2 = 0.919
(b) Y = 0.9845e^-0.0560X R^2 = 0.910
(c) Y = 0.9992e^-0.0589X R^2 = 0.977
(d)
(e) Y = 1.0880e^-0.1626X R^2 = 0.980
(f) Y = 1.0306e^-0.0389X R^2 = 0.897
(g) Y = 1.0343e^-0.3850X R^2 = 0.943
(h) Y = 1.0488e^-0.2190X R^2 = 0.943
(i) Y = 1.0000 R^2 = 1.000
(j) Y = 0.8775e^-0.1468X R^2 = 0.910
(k) Y = 1.0676e^-0.1421X R^2 = 0.971
(l) Y = 1.0939e^-0.0263X R^2 = 0.821
(m) Y = 1.1282e^-0.2078X R^2 = 0.967
(n) Y = 1.0429e^-0.0990X R^2 = 0.981
(o) Y = 1.0856e^-0.0743X R^2 = 0.896
(p) Y = 0.9904e^-0.6561X R^2 = 0.956
(q) Y = 0.8344e^-0.1928X R^2 = 0.915
(r) Y = 1.0158e^-0.0321X R^2 = 0.935
0.0 0.2 0.4 0.6 0.8 1.0
0 5 10 15 20 25 30 35
Number of days since carcass placement

Mathematical models to estimate bird and bat fatality rates associated with wind turbines with an explanation of bias in earlier methods

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ABSTRACT

The rapid growth in wind-energy installations and associated turbine-related fatalities has generated concern for the impact on bird and bat populations. Estimating the number of fatalities is integral to determining the cumulative impact on affected species. The most commonly applied fatality estimators have fundamental assumptions that are routinely violated in wind-turbine data, namely uniform-population sizes that maintain a steady-state process. We assess the robustness of models to violation of these assumptions by deriving new mathematical models that remove at least one assumption of earlier estimators. In comparison to a null model (i.e., the simple-uniform model), we evaluate (1) conditions that perturb the steady-state process and (2) populations with non-uniform fatalities (i.e., migratory species). Using the simple-uniform model to estimate fatalities can result in errors ranging from –100.0 to 65.7%.

In general, migratory events exert minimal error except in atypical situations of short migrations relative to the search interval. However, perturbations that disrupt the steady-state process affect both uniform and migration models and can have a major influence on estimated fatality rates. Additionally, we provide means of correcting bias inherent in the null model and suggest that migration models have other applications, such as revealing migratory patterns from carcass counts. Examples illustrating the application of the models are given, as well as conditions in which each is appropriately applied. The diversity of our developed models and the ability for parameters to vary between searches provide broad applicability to a range of wind-farm conditions and study designs.

Keywords: Wind energy, Fatality estimators, Migration, Bat mortality, Bird mortality

1. Introduction

Energy production in the United States relies largely on fossil fuels, which raises concerns as to long-term sustainability and adverse environmental impacts. Growing energy consumption, in light of the concerns associated with non-renewable resources, has resulted in increased research efforts into alternative, renewable forms of energy, with wind energy being a major focus. Wind-energy capacity in the United States rose from 2,579 megawatts (MW) in 2000 to 46,919 MW by 2011 (American Wind Energy Association, 2009, 2012). Despite this rapid increase, commercial operations still only account for 1% of current energy production in the United States (Energy Information Administration, 2011). However, the Department of Energy (2008) has introduced an initiative to have 20% of the nation's energy generated by wind by year 2030.

Although wind energy has environmental benefits, fatalities of birds and bats have been documented for many wind-energy installations (National Research Council, 2007). Estimates of bird fatalities range from 0.49-7.70 birds/(turbine·yr) (Nicholson, 2003; Osborn et al., 2000) while those for bats range from 0.26-63.9 bats/(turbine·yr) (Fiedler et al., 2007; Johnson et al., 2004) The general pattern is that relatively few birds are killed by wind turbines, except for high mortality of raptors at older facilities in California and relatively greater passerine mortality rates at some forested sites in the eastern United States (Jain et al., 2009; Kerns and Kerlinger, 2004; Nicholson et al., 2005). Bat fatalities are also of concern as fatality rates have been relatively high at wind-energy facilities in the eastern United States, with migratory, tree-roosting bats being the most vulnerable (Kunz et al., 2007; National Research Council, 2007).

Based on current fatality rates and projected growth in the wind-energy industry, attempts have been made to project the number of annual bat fatalities anticipated in the mid-Atlantic highlands region of the United States, the area with highest documented fatality rates. Kunz et al. (2007) predicted that by year 2020, 33,000-110,000 bats would be killed per year in this region, with 63% being two species, the hoary bat (*Lasiurus cinereus*) and the eastern red bat (*L. borealis*). The projection for birds was 5,800-45,000 fatalities/year (National Research Council, 2007). The nation-wide cumulative impact on populations, especially for migratory, tree-roosting bats, is of concern.

Accurately estimating the number of bird and bat fatalities associated with a wind-power facility is important when characterizing potential biological impact on species. There is concern regarding potential bias and accuracy of commonly used methods to estimate fatalities. Wind-turbine mortality studies vary considerably in design. Intervals between carcass searches range from 1-28 days, and searches have been conducted over time periods from one season to 3 years (Arnett et al., 2008). Average persistence time of a carcass in the environment has ranged from 2-52 days (Fiedler et al., 2007; Tierney, 2007), with carcass-detection rates varying from 12-88% (Burba, 2013; Erickson et al., 2000). Many have recommended standardization in study designs (Anderson et al., 1999; Arnett et al., 2008; Kunz et al., 2007; Morrison, 2002; National Research Council, 2007), and some studies have been conducted to estimate biases that search procedures may create (i.e., timing of searches and types of carcasses used; Kerns et al., 2005). Attention also has been given to the models used to estimate fatality rates (Barnard, 2000; Huso, 2011; Korner-Nievergelt et al., 2011; Shoenfeld, 2004).

Mathematical models are an important foundation for understanding population dynamics and estimating population size. In the context of wind energy, there is interest in estimating the population size of dead animals, which is typically estimated via carcass counts. Carcass counts also are used to estimate fatalities associated with disease (Ward et al., 2006), environmental toxins (Balcomb, 1986), and man-made structures (Antworth et al., 2005; Lehman et al., 2007). However, carcass counts alone usually are an inadequate representation of the actual number of fatalities because carcass availability depends on decomposition rates and removal by scavengers; in addition, it is influenced by lag time between fatality occurrence and subsequent search as well as the probability of a carcass being detected by searchers (Wobeser and Wobeser, 1992).

Many early wind-turbine fatality studies used a simple formula, often referred to as the naïve or simple estimator, to estimate the total number of fatalities, N_K, as

$$N_K = \frac{YT_SN_F}{yT_Rp}\,, \tag{1}$$

where Y is total number of wind turbines, T_S is search interval, N_F is total number of carcasses detected, y is number of wind turbines surveyed, T_R is average persistence of a carcass in the environment, and p is detection probability of a carcass. The parameters T_S, T_R, and p are generally considered to be constants, though in practice one can adjust them throughout a survey period if sufficient data is available. This estimator, used in many company reports, was first formally published by Johnson et al. (2003). The naïve estimator, while intuitive in nature, has been criticized for lacking a theoretical basis (Barnard, 2000; Huso, 2011; Shoenfeld, 2004). Barnard (2000) was the first to address the bias of this estimator, demonstrating underestimation of fatalities by as much as 57%. Subsequently, Shoenfeld (2004) derived the first theoretical model for wind-turbine fatalities to address some issues inherent to the naïve model:

$$N_K = \frac{YT_S N_F}{yT_R p}\left(\frac{\mathrm{e}^{T_S/T_R} - 1 + p}{\mathrm{e}^{T_S/T_R} - 1}\right). \tag{2}$$

It is a modification of the naïve estimator that adjusts carcass-removal and detection probabilities, assuming carcass removal follows an exponential decay.

A fundamental assumption of the Shoenfeld and naïve estimators is that the animal death rate is constant. The combination of constant death rates and carcass removal following an exponential distribution results in a constant number of animal carcasses on the ground available for collection during carcass searching. Since the number of animals on the ground is assumed to be constant during the fatality survey, we define models that satisfy these two criteria as steady-state models. These two criteria are routinely violated under natural conditions (e.g., animal migration through the wind farm site). Huso (2011) and Korner-Nievergelt (2011) evaluated bias of the naïve and Shoenfeld estimators under various conditions, yet the robustness of these models to violation of the steady-state assumption has not been investigated.

While the naïve estimator (e.g., Miller, 2008; Tierney, 2009) and the modified estimator of Shoenfeld (2004) (e.g., Kerns and Kerlinger, 2004) are still used to estimate animal fatalities at wind farms, other fatality estimators have been proposed. Huso (2011) published the first peer-reviewed fatality estimator, followed shortly by an estimator developed by Korner-Nievergelt et al. (2011). In brief, Huso (2011) constructed a mathematical formula that estimates fatalities by dividing the survey carcass counts by the detection probability (*p*) and the probability that carcasses persist in the environment since the previous search. In addition, Huso (2011) argued that the effective search interval used to calculate fatalities may be smaller than the actual search interval if the animal carcasses have high removal rates. Korner-Nievergelt et al. (2011) modeled fatalities assuming that carcass-detection probability is dependent on the carcass-removal rate, such that carcasses can persist in the environment through subsequent searches.

The main goal of our study is to develop models that investigate how deviations from a steady-state distribution of animal carcasses on the ground bias fatality estimates. There are two main scenarios where steady-state conditions may be violated. First, if carcass-detection rates are high and search intervals short, there is not sufficient time for the number of carcasses on the ground to return to the initial value. Rather, one would expect fewer carcasses on the ground with each subsequent search; we refer to such conditions as perturbations on the steady-state number of carcasses. Second, migration events deviate from a steady-state assumption in that the number of animals killed is at or below the steady-state value prior to migration, quickly accelerates above the steady-state value during migration, and falls back to or below the original steady-state value after migration. Thus, the death rate is not constant during the migration event. Such a scenario is common for bat fatalities associated with wind-turbine collisions, with high concentrations during fall migration (Arnett et al., 2007). For example, 66% of all yearly bat fatalities occurred within a 3-week interval from late

August to early September at one wind-turbine facility; few, if any, bats were detected during spring survey months (Burba, 2013). We develop new models that incorporate deviations from steady-state distributions and compare the robustness of currently used models to violations of the steady-state assumptions. The development of these models not only expands our understanding of why bias occurs, but also provides new methods of estimating fatality rates that may be useful for wind-energy studies or in other ecological investigations where environmental disturbances result in altered populations.

2. Methods

Four separate mathematical models are developed to assess the bias associated with different population dynamics. Populations whose density is relatively constant over time in the vicinity of a wind farm are treated as having a uniform-population size over time. In contrast, migratory populations exhibit time-dependent variation in local population size in the vicinity of a wind farm, which we model as a Gaussian function. We provide derivations in the Appendix that are fully generalized so they may be applied to situations where search interval, carcasses-detection probabilities, and carcass-removal rates can change during the course of a study. However, for comparative purposes, the resulting equations are reduced to conditions of constant p, constant T_R, and a periodic search interval (T_S).

Much of the criticism surrounding the naïve estimator is its lack of theoretical backing. Therefore, we derive a model that incorporates necessary conditions to arrive at the naïve estimator. Such conditions require a constant death rate, an exponential distribution for carcass removal, and that the number of carcasses on the ground available for collecting is identical for each search. That is, the removal of carcasses by searchers does not influence the number of carcasses that may be detected on future searches. A complete description of assumptions and derivations is outlined in the Appendix (A.1.1). With a theoretical model developed, the naïve estimator is no longer appropriately named; thus, we refer to this estimator as the *simple-uniform model*:

$$N_K = \frac{N_F T_S}{p T_R}, \tag{3}$$

where variables and associated definitions used in Eq. (1) are also adopted here and below.

In many cases, removal of carcasses by searchers will influence the balance between new fatalities and their natural removal from the environment. When the steady-state process is perturbed, the number of carcasses on the ground at a given time is dependent on the conditions of the previous search. Thus, the conditions of each search (i.e., the searcher efficiency and the ratio of the search interval to the carcass-removal rates) will modify the expected number of carcasses collected on subsequent searches. All derivations are provided in Appendix A.1.2, and we refer to this model as the *perturbed-uniform* model:

$$N_K = \frac{N_F T_S}{p T_R}\left(\frac{1}{\frac{1}{1-p}+\frac{1}{n(1-p)}\sum_{i=1}^{n} C_i \exp\left[-\frac{(i-1)T_S}{T_r}\right]}\right). \tag{4}$$

In this equation, n represents total number of searches, and C_i is an integration constant that depends on initial conditions of number of carcasses on the ground after the i^{th}

search is conducted. Values of C_i are related through the recursion relationship:

$C_i = (1-p)C_{i-1} - p\exp\left(\frac{(i-1)T_S}{T_R}\right)$ where $C_0 = 0$.

Unlike in local populations that typically exhibit relatively uniform distributions, the abundance of migratory individuals varies temporally. To account for seasonal changes in population abundance, migratory models were developed using Gaussian distributions for the local population size as a function of time. The simple-migration model is analogous to the simple-uniform model in that it assumes number of the carcasses on the ground is not significantly affected by removal during fatality searches. The derivation of the *simple-migration model* is provided in Appendix (A.2.1):

$$N_K = \left(\frac{2N_F}{p}\right)\frac{1}{\sum_{i=1}^{n}\left\{W[0]\exp\left(-\frac{(i-1)T_S}{T_R}\right)-W[(i-1)T_S]\right\}}, \qquad (5)$$

where $W(t) = \exp\left[\frac{a^2}{2T_R^2} + \frac{(t_o-t)}{T_R}\right]\operatorname{erf}\left[\frac{a}{\sqrt{2}T_R} + \frac{(t_o-t)}{\sqrt{2}a}\right]$. The full-width-at-half-peak maximum (FWHM) is related to the parameter *a* through the equation

$FWHM = 2a\sqrt{2\ln 2}$. The lag time, t_o, represents the interval of time from the beginning of migration to its maximum peak.

A perturbed-migration model (Appendix A.2.2) is developed to explore searcher-induced bias associated with fatality estimates for migratory species. Fatality surveys may introduce significant discontinuities in the number of carcasses located beneath the wind turbines, which may impact the overall estimate beyond any bias associated with the non-uniform population structure. The expression for the total number of animals killed for the *perturbed-migration model* is given as:

$$N_K = \left(\frac{2N_F(1-p)}{p}\right)\frac{1}{\sum_{i=1}^{n}\left\{C_i \exp\left(-\frac{(i-1)T_S}{T_R}\right) - W[(i-1)T_S]\right\}}\,. \tag{6}$$

Variables used in this equation are defined above. The recursion relationship for the perturbed-migration model is $C_i = (1-p)C_{i-1} + pW[(i-1)T_S]\exp\left(\frac{(i-1)T_S}{T_R}\right)$.

For both migration models, the number of carcasses produced by wind-turbine-related deaths is described through a skewed Gaussian function with the degree of skew determined by carcass-detection probabilities, search interval, and persistence of carcasses in the environment. Depending on the combination of these parameters, birds or bats found on the ground may have been killed weeks earlier. Carcasses will continue to be detected by searchers even after a migration has ended, but the number of carcasses and duration of their detection will depend on p, T_S, and T_R. Therefore, the FWHM associated with the model is not synonymous with the width of the migration period as discerned from carcass survey counts (i.e., from the date the first carcass of a migratory animal is collected to the last). The observed width of the migration event is approximately twice the FWHM; a study with FWHM = $3T_S$ has an actual migration width of about $6T_S$. Thus, the predicted number of carcasses on the ground is not necessarily a representation of the true migration event. This is an important distinction when interpreting the ecological context of migration, especially given the selected search interval for the study of interest.

For simplicity, the derived fatality models assume all turbines and land area are surveyed; thus, estimates must be further corrected by multiplying by the proportion of wind turbines and/or to the fraction of land area surveyed as shown in Eqs. (1) and (2).

Confidence limits of estimated fatality rates are best established via bootstrap or Monte Carlo simulations (Manly, 1997).

To compare each model's performance under various conditions, percent-difference formulas were established. In calculations of percent difference, the simple-uniform model is used as a null model against which the other derived models are referenced. Specifically, we can quantify the error associated with applying a simple-uniform model to a population that violates one of the core assumptions of a steady-state process (i.e., animals enter the morbid population at a constant rate or carcass removal through searches is negligible). Using the percent difference also provides a convenient means for characterizing the impact of various fatality parameters (p, T_S, T_R, and n) within each model. In all cases, the percent difference is calculated with the following general formula:

$$X_{q_1,q_2} = 100\left(\frac{N_{K_{q_1}}}{N_{K_{q_2}}} - 1\right), \tag{7}$$

where q_1 and q_2 are the two models being compared, denoted as *su* (simple-uniform model), *pu* (perturbed-uniform model), *sm* (simple-migration model), or *pm* (perturbed-migration model). The percent difference is symbolized by X.

The percent difference between the simple-uniform model Eq. (3) and the perturbed-uniform model Eq. (4) is as follows:

$$X_{su,pu} = 100\left(\frac{p}{1-p} + \frac{1}{n(1-p)}\sum_{i=1}^{n} C_i \exp(-\frac{(i-1)T_S}{T_R}\right). \tag{8}$$

The parameters n, p, T_S, and T_R, will contribute to the percent differences between these models. The percent difference between the simple-uniform model Eq. (3) and the simple-migration model Eq. (5) is as follows:

$$X_{su,sm} = 100\left(\frac{T_S}{2T_R}\sum_{i=1}^{n}\left\{W[0]\exp\left(-\frac{(i-1)T_S}{T_R}\right) - W[(i-1)T_S]\right\} - 1\right). \qquad (9)$$

While the parameters n, T_S and T_R still contribute to the percent difference, p does not. Additionally, the resulting values are influenced by the FWHM of the migration through the function $W(t)$. The percent difference between the simple-uniform model Eq. (3) and the perturbed-migration model Eq. (6) is

$$X_{su,pm} = 100\left(\frac{T_S}{2T_R(1-p)}\sum_{i=1}^{n}\left\{C_i\exp\left(-\frac{(i-1)T_S}{T_R}\right) - W[(i-1)T_S]\right\} - 1\right). \qquad (10)$$

Because the perturbed-migration model combines the effects of perturbations and non-uniform population distributions, p, n, T_S, T_R, and FWHM of the migration all contribute to the resulting percent-difference values. To determine whether perturbations or the temporal skew in fatality rates has the greater influence in biasing fatality estimates, we also calculated the percent difference between the simple-migration model Eq. (5) and the perturbed-migration model Eq. (6) with the resulting equation

$$X_{sm,pm} = 100\left(\frac{\sum_{i=1}^{n}\left\{C_i\exp\left(-\frac{(i-1)T_S}{T_R}\right) - W[(i-1)T_S]\right\}}{(1-p)\sum_{i=1}^{n}\left\{W[0]\exp\left(-\frac{(i-1)T_S}{T_R}\right) - W[(i-1)T_S]\right\}}\right), \qquad (11)$$

in which only the parameters p, n, T_S, T_R, and FWHM may influence the percent difference. Furthermore, time units of T_S and T_R appear as ratios so that $T_S/T_R > 1$ indicates search intervals are longer than carcass persistence and $T_S/T_R < 1$ indicates carcass persistence exceeds the time of the search interval.

3. Results

3.1 Bias associated with uniform models

Low carcass-detection probabilities produce small changes in number of carcasses on ground (Fig. 1). If searches occur infrequently, the number of carcasses on the ground will return to the pre-search value before the subsequent search occurs (Fig. 1A). In contrast, high carcass-detection probability combined with frequent searches causes the number of carcasses under the wind turbine to deviate from the original steady-state conditions between searching events. Number of carcasses on the ground steadily declines to a new quasi-steady-state value as searcher perturbations become more significant (Fig. 1B). As expected, number of carcasses on the ground will recover its original steady-state value once fatality surveys cease.

If one misapplies a simple-uniform model to a population that exhibits a perturbed-uniform pattern, fatality rates will always be underestimated. When comparing the simple-uniform model to the perturbed form, percent difference in estimated fatality rates can reach as much as –96.7% (Fig. 2). The only conditions in which a population will return to a steady-state value are when search intervals (T_S) are longer than mean-removal times (T_R) with low detection probabilities (p). These conditions correspond to the lower right corner of Fig. 2. Thus, the simple-uniform model is appropriate for estimating fatality rates in situations where there is a high T_S/T_R ratio and a low p. In contrast, there is a large disparity between fatality estimates derived from the perturbed-uniform and simple-uniform models in the opposite scenario (i.e., when $T_S < T_R$, especially when combined with high p values). This disparity is reflected in an increase in bias for the simple-uniform model as p increases and T_S/T_R decreases (Fig. 2).

Effects of the number of surveys (n) on estimated fatality rates are not included in the percent-difference calculations depicted in Fig. 2. As shown in Eq. (8), n contributes to the percent difference; however, under most conditions the effects are negligible. The influence of n becomes important only when $n < 10$ (data not shown). In a perturbed-uniform distribution (Fig. 1B), periodic searches decrease number of animals on the ground from its original steady-state value, but these variations gradually approach a new quasi-steady-state value. It is over the transient time period when the number of dead animals does not regain the original steady-state value nor has it yet reached its new quasi-steady-state value that the number of fatality searches shows the greatest variation in percent differences. If $n = 1$, the perturbed-uniform model is reduced to the simple-uniform model, and there is no difference in estimated fatality rates between the two. However, there is a greater disparity between the simple-uniform and perturbed-uniform models (i.e., percent difference becomes more negative) with each additional search for any given set of T_S, T_R, and p values. Once the number of searches reaches $5 \leq n \leq 10$ (the exact number is dependent on the values of other parameters), changes in the percent difference become negligible with each additional search. For example, when $T_S/T_R = 1.0$ and $p = 0.50$, the percent error only increases an additional 1.83% between $n = 10$ and $n = 30$ searches. Thus, we ignore the effects of n on the percent-difference calculations in Fig. 2, as most studies involve more than 10 searches. Nevertheless, the percent-difference values recorded in Fig. 2 may not be accurate for studies with very low search effort.

The estimator of Shoenfeld (2004) is not directly compared to our models; however, note that the period of time needed to establish the quasi-steady-state value is a potential source of bias for his estimator. According to Shoenfeld (2004), his model assumes the process of animal death, scavenging, and searching has gone on indefinitely and uniformly. Thus, any searches conducted at the beginning of a fatality survey are assumed to have a minor influence on the total fatality estimates. This is a reasonable assumption for situations where T_S/T_R is high and p is low. However, small values of T_S/T_R produce long transient-time periods at the beginning of the fatality survey during which the carcass population on the ground adjusts to a new steady-state value. During these transient periods, assumptions undergirding the Shoenfeld estimator are violated. The problem is particularly exacerbated if the number of searches is small because the number of surveys conducted during the transient period constitutes a large proportion of the total number of surveys. For example, the percent difference for applying the Shoenfeld estimator to the simple-perturbed model yields an overestimate of 12.75% for $T_S/T_R = 0.20$, $p = 0.5$, and $n = 30$, whereas the overestimate reaches 38.23% for the same conditions with $n = 10$.

3.2 Bias associated with simple-migration model

The simple-migration model is illustrated in Fig. 1C. In the absence of significant perturbations, numbers of carcasses on the ground mimics the basic skewed Gaussian function that is predicted to occur in the absence of any searches. Percent difference between the simple-uniform model and the simple-migration model ranges from –100.0 to 65.7% (Fig. 3). If the simple-uniform model is applied to a population that exhibits a migratory distribution, fatality rates can be either over- or underestimated. The simple-uniform model overestimates fatality rates only if FWHM is $\leq \frac{1}{2} T_S$ and the search interval is longer than the mean carcass-removal rate ($T_S/T_R > 1$); the difference does not exceed 20% until FWHM $\leq 0.25\ T_S$ and if the $T_S/T_R \geq 2.0$ (Fig. 2). The later scenario corresponds to a very short migration event. For a study using weekly searches (T_S = 1 week) with a migration FWHM = 0.25 T_S and $T_S/T_R = 2.0$, the estimated fatality rate would be overestimated by more than 20% only if the entire migratory event occurred within 3 days and the $T_R \approx 3$ days. Such a scenario is not typical in nature, but could apply in special cases, such as a single large flock migrating through a wind farm over a very short period of time. Although spikes in fatalities from a rapid migratory event are possible, the associated bias only becomes significant if mean removal times are also very short.

Overestimating fatality rates becomes more likely with long search intervals (e.g., studies that use monthly search intervals; T_S = 4 weeks). In this case, an overestimation exceeding 20% will occur if $T_R \leq 2$ weeks, but the entire migratory event would still have to be short, within a 2-week period (FWHM $\leq 0.25\ T_S$). Such a scenario is more likely than that of a study using weekly searches because $T_R \leq 2$ weeks is within normal biological limits, although a migration event limited to within a 2-week period is probably not typical of most biological systems. Researchers should be aware of the possibility of overestimating fatality rates in atypical cases.

If a simple-uniform estimator is used during migratory events, it is possible that fatality rates will be underestimated. The FWHM value has very little impact on underestimated rates, yet the model becomes extremely sensitive in situations where the search interval is much shorter than the mean removal rate, $T_S/T_R < 0.20$ with $n = 30$

(Fig. 3). This situation is likely in studies that have used daily search intervals if $T_R \geq 5$ days or studies that use weekly search intervals with a very long mean removal rate ($T_R > 30$ days). Few studies use daily search intervals (e.g., Howe et al., 2002; Kerns et al., 2005) but long removal times can occur with large-sized birds (see Tierney, 2007). However, the extreme sensitivity in the far-left region of Fig. 3 most likely is due to n not being large enough to fully encompass the entire migration event (i.e., number of animals on the ground has not yet returned to zero before survey period ended). If T_S/T_R is small, $n = 30$ may not be sufficient to survey the entire migration period, which violates one of the assumptions of the migration model (see Appendices A.3 and A.4). Researchers finding themselves with these conditions are advised to increase the number of surveys.

Except for extreme cases, non-uniform fatality patterns caused by migratory events have little impact on estimated fatality rates compared to simple-uniform models. However, this does not evaluate impacts that perturbations may have on estimated fatality rates if the population is migratory. Searcher-induced perturbations produced notable bias in uniform distributions. While, the simple-uniform model appears relatively robust to temporal variation in fatality patterns, this is only in the absence of perturbations.

3.3 Bias associated with perturbed-migration model

The act of searching produces discontinuities in the skewed Gaussian distribution that is used to model the number of dead animals on the ground. There are significant distortions imposed on curves as searcher-induced perturbations become more pronounced (Fig. 1D; an example of perturbed-migration model). To evaluate effects of p, T_S, and T_R on disrupting the skewed Gaussian distribution, we compared percent difference between the simple-migration and perturbed-migration models, as was done between the two uniform models in Section 3.1. Conditions that perturb the migration model are nearly identical to those that perturb the uniform model; data are not shown because it is indistinguishable from Fig. 2. High p values and frequent searches relative to carcass persistence time ($T_S < T_R$) result in the largest percent differences between the two migration models. After carcasses have been removed by searchers, the only conditions in which a migratory population will not deviate from the skewed Gaussian distribution are at low p values and high T_S/T_R ratios (see Fig. 2 for comparable conditions). A perturbed-migration model will always produce estimated fatality rates less than or equal to a non-perturbed-migration model. Under most conditions, migratory populations are expected to follow a perturbed-migration model rather than a simple-migration model.

We evaluate perturbation effects within a migration model with percent difference in estimated fatality rates between a simple-uniform distribution and a perturbed-migratory distribution (Fig. 4). Similar to the simple-migration model, percent difference of the perturbed-migration model can be positive or negative, ranging from –100.0 to 48.6%. Unlike the simple-migration model, percent-difference calculations also depend on detection probability. Because p, T_S/T_R, and FWHM all influence percent difference between simple-uniform and perturbed-migration models, resulting values cannot be displayed on a single two-dimensional graph. Separate contour plots were created for four different FWHM widths across the spectrum of very broad to very narrow migration distributions ($4.0T_S$, $1.0T_S$, $0.5T_S$, and $0.125T_S$; Figs. 4A-4D, respectively). For broad migration widths (FWHM = $4.0T_S$), fatality rates will always be underestimated; percent difference increases with increasing p and a decreasing T_S/T_R ratio (Fig. 4A). There is little change in percent-difference calculation if migration widths narrow to FWHM = $1.0T_S$; however, limits in which there is no difference in estimated fatality rates between the simple-uniform and perturbed-migration models become apparent for low p values in conjunction with the $T_S > T_R$ (conditions represented in the lower right corner of Fig. 4B).

A visible shift occurs in percent error as migration widths narrow. When migration width at FWHM become $0.5T_S$, overestimated fatality rates of up to 15%, becomes possible but only when $T_S/T_R > 1$ (Fig. 4C). Otherwise, fatality rates are still underestimated when the carcass-removal rate is longer than the search interval ($T_S/T_R < 1$). With extremely narrow migration widths (FWHM = $0.125T_S$), fatality rates can be overestimated as much as 45% (Fig. 4D). As discussed in Section 3.2, such narrow migrations would only apply to a migratory flock that passed through in one day. In cases of rapid migration events, overestimated fatality rates are a potential concern. Under typical migratory conditions, fatality rates will be underestimated when a simple-uniform model is applied to a perturbed-migratory population.

The number of surveys (n) contributes to percent error in the perturbed-migration model (see Eq. (10)). As discussed in Section 3.1, n typically results in negligible changes in percent-difference values. For simplicity, variations of n were excluded from percent-difference calculations in Fig. 4. Like perturbed-uniform models, number of surveys is generally only of concern when $n < 10$. In uniform models, timing of a survey is of little importance because number of individuals dying is relatively constant over time. However, in migration models timing of the survey is very important given that the number of carcasses in one survey is not necessarily representative of any other time. Migration models assume that surveys cover the entire migration event. If the number of surveys is small, only a portion of the migration was likely surveyed, which will bias fatality estimates. The degree of bias will be influenced by the specific portion of the migration surveyed. Overall, failure to survey the entire migration deems the migration models unreliable. As long as the entire migration is surveyed, n will have negligible influence on resulting percent-difference values.

3.4 Differences between perturbation models

As demonstrated in Section 3.2, the simple-uniform model is relatively robust to temporal variation in fatality patterns in the absence of perturbation. On the contrary, perturbed systems can result in substantial deviations from the simple-uniform model. Except in cases of low p and high T_S/T_R ratios, populations will follow a perturbed pattern, whether uniform or migratory (see Sections 3.1 and 3.3). Thus, we evaluate effects of non-uniform fatality patterns in perturbed systems by comparing perturbed-uniform and perturbed-migration models. Because both models were already compared to the simple-uniform model in Sections 3.1 and 3.3, respectively, we present the resulting percent differences in Table 1.

Even if the population has a migratory distribution, in many situations a perturbed-uniform model may be applied with very little bias being introduced into the fatality estimates. The discrepancy between the perturbed-uniform and perturbed-migration models only exceeds 5% when $T_S/T_R = 0.2$, or $T_S/T_R = 2.0$; the latter case only applies if also coupled with a narrow migration width (FWHM $\leq 0.5T_S$; Table 1). In general, there is little variation in percent-difference values between perturbed-uniform and perturbed-migration models, regardless of the migration width. Only when parameters reach extreme limits, does migration have a notable effect.

As a general rule, the perturbed-migration model only needs to be applied if a migratory population has a $T_S/T_R < 0.5$, or if $T_S/T_R > 1.5$ and FWHM $\leq 0.5T_S$ (Fig.7). Using a perturbed-uniform model for a migratory population will underestimate fatalities when $T_S/T_R < 0.5$ and will overestimate fatalities when $T_S/T_R > 1.5$ Otherwise, the perturbed-uniform model will result in very similar fatality estimates even when the assumption of uniform fatality rates is violated. Perturbed systems, like uniform systems, are relatively robust to temporal variation in fatality patterns and analysis with a perturbed-migration model is only necessary in the atypical conditions described above.

3.5 Examples of correcting bias in estimated fatality rates

One of the benefits to our analysis is the ability to easily correct bias for studies that have used the simple-uniform model. While any study could be reanalyzed with our new models to evaluate the difference in estimated fatality rates, this will require a complete reanalysis of data. If only approximate corrections are of interest, our percent-difference calculations can be estimated from Figs. 2-4 and the resulting percent difference applied to reported values. For example, Tierney (2007) provided an estimated fatality rate of 16.7 birds/year for turkey vultures (*Cathartes aura*) based on a T_S = 21 days, T_R = 52 days, and $p = 0.77$. From Fig. 2, we can interpolate that T_S/T_R = 0.40 at $p = 0.77$ will yield approximately a –56% error. Using Eq. (7), an error of –56% corresponds to an estimated fatality rate of 38.0 turkey vultures/year, more than twice the reported value. In comparison, a complete analysis using our perturbed-uniform model (Eq. (4)) predicts a fatality rate of 37.4 turkey vultures/year. This is based on the additional parameters: $N_F = 10$, $n = 13$, and 21 of 67 turbines sampled. Number of searches (n) was never specifically stated in Tierney (2007) but was determined based on the search interval and number of months surveyed. While the latter example is the more precise way of determining estimated fatality rates, the former demonstrates that close approximation is attainable using the percent differences depicted in Fig. 2.

Corrections with a migration model are also feasible, but require determining the FWHM of each data set. An example for this model uses data published for Buffalo Ridge, Minnesota by Johnson et al. (2003) in which their table 3 records the temporal distribution of fatalities over a 2-year period. Because their table combines the number of carcasses over two years (number of turbines surveyed varied each year) and includes incidentally-collected carcasses that were excluded from their analysis of fatality estimates, our calculations will differ from their reported values because ours are not adjusted for proportion of turbines surveyed and use all carcasses found (N_F = 184). Using the simple-uniform model and based on reported parameters T_S = 14 days, T_R = 10.4 days, $p = 0.294$, we estimate 842.5 total bats fatalities.

The perturbed-migration model was also used to estimate the number bat fatalities from Johnson et al. (2003). The 184 bat carcasses were plotted as a function of time and a non-linear curve fit using Eq. (A.19) was constructed from the data to determine the FWHM ($2.68T_S$ = 37.5 days) and lag time ($4.59T_S$ = 64.3 days) of the Gaussian curve that would give rise to the skewed distribution of carcasses on the ground (Fig. 5). These values may then be used to reconstruct the population profile of the actual migration event, which is also plotted in Fig. 5. Using the same parameters above with $n = 13$ (the number of surveys that covered the entire migration), Eq. (6) yields an estimated fatality rate of 929.6 bats killed, a percent error of –9.4% from the simple-uniform model. For a quick approximation, the simple-uniform model for a T_S/T_R=1.35 and $p = 0.294$ will produce a percent error of about –10%; the estimated value obtained from either Fig. 5A or 5B because changes in percent differences between a FWHM = $1.0T_S$ and FWHM = $4.0T_S$ are negligible.

As noted in Section 3.5, the perturbed-uniform model often is suitable for estimating fatality rates even when the temporal distribution of fatalities clearly is non-uniform, as is the case for data from Johnson et al. (2003). When $T_S/T_R < 1.5$, especially with FWHM = $2.68T_S$ (i.e., not exceptionally narrow), conditions are such that the perturbed-uniform model is relatively unbiased to violation of an assumed uniform population size. The perturbed-uniform model (Eq. (4)) estimates a total of 924.6 bats killed, a –8.9% error. Likewise, percent error could be determined from Fig. 2 without calculation to be approximately –9%. These results represent a minor discrepancy to the perturbed-migration model, and the additional work required to implement a migration profile is likely not justifiable given this small discrepancy.

The data used to illustrate the migration model nicely follows a Gaussian distribution. However, it is suggested that some bat species migrate in waves with stopovers rather than continuous movements (McGuire et al., 2012). The shorter the search interval, the more apparent migration waves may become as daily activity of bats can be influenced by wind speed and precipitation. While we do not evaluate a published data set, multiple Gaussian distributions may be necessary for the best non-linear curve fit. If oscillations occur in the temporal distribution of fatalities, data may need to be analyzed for multiple migration waves. An example of simulated data is provided in Fig. 6. Similar to the migration model above, a non-linear curve fit was constructed for two migration events (Fig. 6A). The resulting FWHM and lag time can be used to construct the actual width and timing of each migration event (Fig. 6B). In the case of migration waves, migration widths will likely be narrower than a single curve and narrow migration widths are conditions in which fatality rates can be overestimated when using uniform models.

Using migration models to estimate fatalities is often unnecessary because uniform models are generally robust to temporal variation in fatalities. However, the two migration examples are an important demonstration of applying the model and illustrate the ability to determine the true migration profile of the population from carcass distributions on the ground. Studies that investigate migration patterns may find this a useful tool in evaluating the timing and characteristics of migration for a particular species, even if the number of fatalities is not of interest to their particular study.

4. Discussion

4.1 Implications of using the simple-uniform model

While other studies have demonstrated that the simple-uniform-model often produces underestimated fatality rates (Barnard, 2000; Huso, 2011; Korner-Nievergelt et al., 2011), the lack of a theoretical development for the simple-uniform model prevented a clear understanding for why these biases occur. Through our development of mathematical models, we demonstrate that it is necessary to assume (1) populations have temporally-uniform fatality rates and (2) the impact of carcass searching negligibly alters the number of carcasses on the ground in order to use the simple-uniform model. Thus, bias for this estimator occurs in situations that prevent the number of carcasses on the ground from returning to the steady-state value with each subsequent search. Such deviations are caused largely by sampling rapidly compared to carcass-persistence times, but are also negatively influenced by increasing detection probabilities. For a steady-state value to be maintained, search intervals need to be about twice as long as the carcass-persistence time ($T_S/T_R > 2.0$) and detection probabilities around 10%. If conditions for a given study do not meet these criteria, the simple-uniform model should not be used to evaluate fatality rates.

High carcass-detection values and long carcass-persistence times cause the greatest bias in the simple-uniform model. Such conditions are typical for large-sized birds; hence, studies that apply a simple-uniform model likely report fatality rates that are considerably underestimated. Because these reported values typically are low for large-size birds, the impact of wind farms on raptor and raptor-like populations are often ignored, with the exception being early studies at Altamont Pass, California that recorded large numbers of raptor fatalities (California Energy Commission, 2003). Studies in the south-central United States have reported notable numbers of turkey vulture fatalities (Burba, 2013; Tierney, 2007). The Tierney (2007) study is provided as an example in Section 3.6. The corrected value of 37.4 turkey vulture fatalities/year is substantially higher than their reported rate of 16.7 turkey vultures/year. Removing more than 37 turkey vultures/year from a local population could have significant impacts on population sustainability over time, especially given they are relatively long-lived animals with low reproductive output. If the reported values for large-sized birds are routinely underestimated, the biological significance of these fatalities may be of greater importance than currently perceived.

In contrast to large-size birds, bats typically have low carcass-detection rates and faster removal times, conditions which minimize bias. The number of reported bat fatalities on a national scale is already of concern as bat fatalities have been high in the eastern United States with as many as 63.9 bat fatalities/(turbine·yr) (Fiedler et al., 2007). Underestimated fatality rates in bats could have serious consequence. However, low p values in conjunction with the $T_S > T_R$, a condition that could be encountered for bat carcasses, produces small bias for a wide range of migration widths. The degree of underestimation would generally fall within –20% or less. Because p, T_S, and T_R vary greatly between sites, greater disparities are also expected. Moreover, for sites with high bat-fatality rates, even a small percent difference can result in a large estimated increase in the number of individuals killed.

4.2 Results compared to other fatality estimators

We did not evaluate percent relative bias directly (i.e., compare how well a model predicts a known number of fatalities); however, other studies have already done so for previously proposed estimators (Barnard, 2000; Huso, 2011; Korner-Nievergelt et al., 2011). Huso (2011) found the simple-uniform model underestimates fatalities as much as 98.5%, under conditions of long carcass-persistence times. The only time bias was close to zero was with long-search intervals and short average-persistence times (Huso, 2011). These mirror the conditions and degree of difference we find between the simple-uniform model and our perturbed-uniform model. Given the parallel findings between the percent relative bias described by Huso (2011) and our percent differences with the simple-uniform model, this suggests that our model has very little bias in estimating fatality rates. It would be beneficial in future studies to evaluate percent relative bias of our models if known fatality rates (or properly simulated data sets) are available. In addition, our estimated fatality rates should be compared to the results of other proposed estimators.

Inherent bias of a model can be evaluated by estimating fatalities when $p = 1$. Under this condition, the simple-uniform model reduces to $N_K = (T_S/T_R)N_F$; if the search interval is shorter than mean carcass persistence ($T_S/T_R < 1$), the estimated number killed will be less than the number of carcasses actually detected. For example, if 100 carcasses were found with $T_S/T_R = 0.1$, the estimated number of animals killed is 10, an estimate 90% lower than the actual number of carcasses collected during the survey period. Huso (2011) discussed this problem and indicated that her model is the only one for which the number of carcasses found yields the same estimated number of animals killed when $p = 1$. In contrast, Kromer-Nievergelt et al. (2011) illustrated that Huso's (2011) estimator tends to overestimate fatalities under the same conditions of short search intervals and long carcass-persistence times. We suggest that yielding an estimated number of fatalities equal to the carcass count, in fact, may overestimate fatalities under some conditions (see below).

For our perturbed-uniform and perturbed-migration models, the equations become undefined when $p = 1$. To circumvent this problem, we evaluated our models performance at $p = 0.9999$. This provides a reasonable approximation to the $p = 1$ scenario. Carcass counts for the perturbed-migration model yield the same number of estimated fatalities as T_S/T_R approaches zero, and there are no conditions in which the perturbed- migration model estimated number of fatalities to be less than the carcass count. In contrast, estimated number of fatalities was less than the number collected in some cases ($T_S/T_R \lesssim 0.25$) for the perturbed-uniform model; however, the degree of disparity is much less than observed for the simple-uniform model under the same conditions. For instance, if 100 carcasses were found during the entire fatality survey with $T_S/T_R = 0.1$ and $p = 0.9999$, estimated number killed using the perturbed-migration model is 80 individuals.

For small values of T_S/T_R, estimating the number of fatalities less than the total carcass count is an expected consequence of the fundamental assumptions used to generate models for uniform populations. In these models, the number of animals on the ground is assumed to reach a steady-state value that is proportional to T_R. Searches are conducted over a discrete period of time, and estimated number of fatalities corresponds to those that have occurred within the specified survey period. Our uniform model accounts for a background population of carcasses existing on the ground prior to carcass searches. In the limit of very long carcass-persistence times, some carcasses in the environment that are collected during the first search may be fatalities from weeks or even months beforehand. Thus, it is possible for the number of carcasses collected in a survey to exceed the number of animals that were killed during the time covered by the survey. Korner-Nievergelt et al. (2011) recognized this problem and assumed that there were no carcasses on the ground prior to searches commencing; therefore, they suggested conducting a clean-out search during which all carcasses are discarded prior to routine sampling. Some studies have adapted this procedure (Kerlinger et al., 2006, 2007). However, performing clean-out searches before the survey period begins would violate one of the assumptions for our uniform model (i.e., a steady-state population exists at $t = 0$). Thus, it is not recommended to perform clean-out searches if our models are employed.

Similar problems of $N_K < N_F$ do not exist in the migration model because the survey period is assumed to span a sufficient period of time so that number of carcasses on the ground is zero for both the first and last searches. Therefore, there are no carcasses persisting on the ground at the first search. At $p = 1$, we expect $N_K = N_F$ in migration models and this expectation was verified to occur. Yet, a model’s ability to yield an estimated fatality rate equal to the number of carcasses found when $p = 1$ and as $T_S/T_R \rightarrow 0$, may not be indicative of an unbiased estimate. We suggest the modest difference of $N_K < N_F$ at the lower limits of T_S/T_R is expected and further supports the supposition that the perturbed-uniform model is relatively unbiased.

4.3 Potential biases in newly derived models

Our study did not evaluate methods used to obtain the parameters p and T_R, which can bias fatality estimates if these parameters themselves are biased. Others have investigated such issues (Barnard, 2000; Huso, 2011; Kerns et al., 2005). Barnard (2000) evaluated how various methods of calculating mean carcass-removal time affected fatality estimates. He concluded that right censoring carcass-removal data (i.e., eliminating any specimens that remained unscavenged at the end of a trial period) produced less bias in the simple-uniform model than using the true arithmetic mean. The recommendation of right censoring is a technique often used in these types of studies (e.g., Erickson et al., 2004). Right censoring data will reduce carcass-removal rates compared to the arithmetic mean because specimens with long removal times are often removed from the estimate. Mathematically, a shorter carcass-removal rate will result in higher fatality estimates. Because the simple-uniform model routinely underestimates fatalities, using an underestimated carcass-removal rate increases the resulting estimated number of fatalities, thereby making the simple-uniform model appear less biased under censored conditions. We suspect that, if an unbiased fatality estimator is used, right censoring carcass-removal data would result in over-estimated fatality rates for an otherwise unbiased estimator. While monitoring a carcass until it is completely decayed or scavenged can result in additional labor and costs, other methods used by Burba (2013) fit data to exponential decay functions and do not require right-censoring or indefinite carcass checks. Such methods may be a better solution to estimating carcass-removal time for unbiased estimators.

Shoenfeld's (2004) model estimates fatality rates by assuming exponential decay of carcasses, as do the new models presented herein. Huso (2011) evaluated other functional forms of carcass persistence (i.e., exponential, gamma, and half-normal distributions) as they relate to bias in estimated fatality rates. The simple-uniform model, Schoenfeld's (2004) estimator, and Huso's (2011) estimator all showed some bias if carcass persistence was modeled with exponential decay when, in fact, it had a gamma or half-normal distribution. Using an exponential-decay function will underestimate fatalities if carcass persistence has a gamma distribution and overestimate if it has a half-normal distribution; however, the degree of bias was often negligible, reaching a maximum difference of 20%. While Huso's (2011) model was the least biased overall, it was the one most sensitive to changes in the carcass-removal distribution and showed the greatest differences in percent relative bias depending on which decay function was used. For Shoenfeld's estimator, there was always less than a 9% difference in estimated fatality rates when using a carcass-removal distribution that was not exponential. Because our estimators follow the same type of exponential decay models as the Shoenfeld (2004) estimator, we suspect any bias in our model due to violations of the exponential decay assumption would be minor and in the range of differences found for his estimator. However, using the methods of Burba (2013) for estimating carcass persistence, one could evaluate whether the data are best fit by an exponential function. If data appear to exhibit more of a gamma or half-normal distribution, one would know the direction of bias in the estimation. A method for predicting the degree of bias has not been established, but the bias in estimated fatality rates increases with an increase in search interval (Huso, 2011).

4.4 Recommendations for study design and model selection

While the simple-uniform model performs best with very low searcher efficiency and infrequent searches, we do not advocate intentionally using such methods to reduce bias. Rather, a better solution would be to use models that are more robust and less biased to more thorough sampling effort. A flow chart is provided in Fig. 7 to guide researchers in selecting the appropriate model to use for their data. In many early wind-farm studies and some contemporary ones, the simple-uniform model was often used to estimate fatality rates. Because most studies have included site-specific searcher-efficiency and carcass-removal rates, as well as the search interval, our analysis provides for easy correction of former studies. While a complete reanalysis for data could be done, close approximations are attainable by determining the expected percent difference from the simple-uniform model for either perturbed-uniform or perturbed-migration populations (Figs. 2 and 4, respectively; see Section 3.6 for examples). This may be of interest to companies in which reports of biased fatality rates have already been submitted. Likewise, it allows the possibility of future studies to use the simple-uniform model, which requires little time and only basic math skills, to estimate fatalities and adjust the bias accordingly.

Whether fatality estimates are calculated with our more complex models or by adjusting the bias in the simple-uniform model, sufficient sampling is a high priority. From our analysis of error associated with the number of surveys, we suggest $n = 10$ as the minimum search effort for a given study. This is a general rule and, in cases where migratory models are employed, searches should begin before migration occurs and n

should be sufficiently large to cover the entire migration period (i.e., no carcasses should be detected on searches prior to or after migration).

The site-specific conditions that affect searcher-detection probabilities and carcass-removal rates resulting from scavenging and/or decay can vary substantially between sites. Because p, T_S, and T_R also influence error estimates, future studies should consider collecting preliminary data to assess the expected values of p and T_R for the site of interest before surveys begin. This would allow for better study designs to be developed for site-specific characteristics. The average persistence time of a carcass in the environment ranges from 2-52 days (Fiedler et al., 2007; Tierney, 2007). At the extremes, a weekly search interval could produce a T_S/T_R from 0.13 to 3.50. Thus, for instance, a search interval of 7 days may be appropriate for one site but less ideal for another.

Even within a single study, different types of animals and/or species exhibit different fatality patterns, and analysis with more than one model may be necessary. For example, individual bird and bat species may be migratory, but the timing of the migration may vary among species. Compiling all individuals into one category may ignore species-specific patterns. Analyzing each species individually with its own Gaussian curve and summing the estimated fatality rates together may provide more-reliable estimates in addition to revealing differences in migratory patterns among species. Likewise, multiple Gaussian curves may be necessary for species that migrate in waves rather than exhibiting a continuous flow. Situations may also exist in which a wind farm has a local uniform population in addition to a migratory population for the same species. For example, Piorkowski and O’Connell (2010) surveyed a wind farm in close proximity to several nursery colonies of Mexican free-tailed bats (*Tadarida brasiliensis*). Fatality patterns were uniform through the summer for local populations. While no surveys were conducted in the fall, it is likely migratory species would have been added to the fatalities and both uniform and migratory populations would occur at this site. Indeed, migrating bats were observed at a wind farm located to the south of the site (Burba, 2013) studied by Piorkowski and O'Connell (2010). For this scenario, the perturbed-uniform and perturbed–migration models may need to be considered in tandem.

In addition, all of our analyses assume that the parameters of p, T_S, and T_R are constant throughout a given study; however, the models themselves are derived so that these parameters could vary for each individual search period. Fiedler et al. (2007) conducted a study in which p, T_S and T_R all varied throughout the course of the study and the simple-uniform model was used in discrete time intervals to account for changes in these parameters. Not only would the perturbed models reduce bias for this study, but also allows for a continuous analysis of data by changing these parameters for each search (see the Appendix for the generalized forms).

As explained in Section 3.4, using migration models to estimate fatalities may not be necessary to arrive at unbiased estimates. However, methods used to develop the migration models also construct migration profiles from the skewed-Gaussian distributions of carcasses on the ground (see Section 3.5 for example). Relatively little is known about bat migratory behavior, and this lack of knowledge hinders abilities to mitigate wind-farm fatality risk. Wind-farm fatalities can provide data sets in which migration patterns can be investigated to decipher possible migration waves (McGuire et al., 2012), or to test predictions of bat-migration theory (Hendenström, 2009). Our model may be a useful tool to researchers interested in bat migratory behavior, even if the number of fatalities is not pertinent to the research question.

5. Conclusions

We are not suggesting that our proposed perturbation models are superior to other theoretical estimators (Huso, 2011; Korner-Nievergelt et al., 2011; Shoenfeld, 2004,), for this issue has yet to be evaluated. Our analysis differs from others in that we provide a theoretical framework for explaining why bias occurs and guidelines for determining the most appropriate model to use given the characteristics of a specific study. Bias in an estimator is not necessarily problematic, if the bias is known and can be corrected. Our analysis provides a means by which bias can be corrected for studies that apply the simple-uniform model. Given parallel findings of the percent-relative bias of the simple-uniform model (Huso, 2011) and our percent difference with the same suggests that our models are relatively unbiased in fatality estimates. The diversity of developed models, our explanations of conditions in which each is appropriately used, and the ability for parameters to vary between searches and/or season, provide broad applicability to a range of wind-farm conditions and study designs. While models were developed for use in wind-turbine related fatalities, the models can be applied to other disturbed systems in which the affected-population size is of interest.

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References

American Wind Energy Association, 2009. Annual Wind Industry Report, Year Ending 2008. URL http://www.awea.org/learnabout/publications/upload/AWEA-Annual-Wind-Report-2009.pdf

American Wind Energy Association, 2012. U.S. Wind Industry Fourth Quarter 2011 Market Report. URL http://www.awea.org/learnabout/publications/reports/upload/4Q-2011-AWEA-Public-Market-Report_1-31.pdf

Anderson R., Morrison M., Sinclair K., Strickland D., 1999. Studying wind energy/bird interactions: a guidance document, metrics and methods for determining or monitoring potential impacts on birds at existing and proposed wind energy sites. Technical Report, C National Wind Coordinating Committee, Washington D.C. URL http://www.nationalwind.org/assets/archive/Studying_Wind_Energy-Bird_Interactions_-_A_Guidance_Document__1999_.pdf.

Antworth R.L., Pike D.A., Stevens E.E., 2005. Hit and run: effects of scavenging on estimates of roadkilled vertebrates. Southeastern Naturalist 4,647–656.

Arnett, E.B., Brown, W.K., Erickson, W.P., Fiedler, J.K., Hamilton B.L., Henry, T.H., et al., 2008. Patterns of bat fatalities at wind energy facilities in North America. Journal of Wildlife Management 72, 61-78.

Balcomb R., 1986. Songbird carcasses disappear rapidly from agricultural fields. Auk 103, 817–820.

Barnard, D., 2000. Statistical properties of an avian fatality estimator. M.S. Thesis. University of Wyoming, Laramie, Wyoming.

Burba, E.A., 2013. Estimating bird/bat fatality rates and using stable-hydrogen isotopes to asses hoary bat migratory patterns associated with a wind-energy facility in western Oklahoma. Ph.D. Dissertation, University of Oklahoma, Norman, Oklahoma.

California Energy Commission, 2003. Bird strike indicator/bird activity monitor and field assessment of avian fatalities. URL http://www.energy.ca.gov/reports/2004-03-05_500-03-107F.PDF.

Department of Energy, 2008. 20% wind energy by 2030: increasing wind energy's contribution to U.S. electricity supply. U.S. Department of Energy, Washington, D.C. URL http://www.nrel.gov/docs/fy08osti/41869.pdf.

Energy Information Administration., 2011. Primary energy production by source, selected years, 1949-2010. URL http://www.eia.gov/totalenergy/data/annual/pdf/sec1_7.pdf.

Erickson W.P., Johnson G.D., Strickland M.D., Kronner K., 2000. Avian and bat mortality associated with the Vansycle Wind Project, Umatilla County, Oregon

1999 Study Year. Technical Report, WEST, Inc., Cheyenne; Wyoming. URL http://www.batsandwind.org/pdf/Ericksonetal2000.pdf.

Erickson, W.P., Jeffrey, J., Kronner, K., Bay, K., 2004. Stateline Wind Project wildlife monitoring final report, July 2001–December 2003. Technical report submitted to FPL Energy, the Oregon Office of Energy, and the Stateline Technical Advisory Committee. Western Ecosystems Technology, Inc., Cheyenne, Wyoming. URL http://www.batsandwind.org/pdf/Erickson_etal_2004.pdf.

Fiedler, J.K., Henry, T.H., Tankersley, R.D., Nicholson, C.P., 2007. Results of bat and bird mortality monitoring at the expanded Buffalo MountainWindfarm, 2005. Technical Report, Tennessee Valley Authority. URL http://www.batsandwind.org/pdf/Fiedler_etal_2007.pdf.

Hedenström, A., 2009. Optimal migration strategies in bats. Journal of Mammalogy 90, 1298–1309.

Howe, R.W., Evans, W., Wolf, A.T., 2002. Effects of wind turbines on birds and bats in northeastern Wisconsin. Wisconsin Public Service Corporation, Green Bay, Wisconsin, USA. URL http://www.batsandwind.org/pdf/Howe_2002.pdf.

Huso, M.M.P., 2011. An estimator of wildlife fatality from observed carcasses. Environmetrics 22, 318–329.

Jain, A.A., Kerlinger, P., Curry, R., Slobodnik, L., Lehman, M., 2009. Annual report for the Maple Ridge Wind Power Project: post-construction bird and bat fatality study–2008. Prepared for Idberdrola Renewables, Inc, and Horizon Energy, and Technical Advisory Committee for the Maple Ridge Project Study. Curry & Kerlinger, L.L.C., McLean, Virginia. URL http://www.batsandwind.org/pdf/Jain_2009b.pdf

Johnson G.D., Erickson W.P., Strickland M.D., Shepherd M.F., Shepherd D.A., Sarappo S.A., 2003. Mortality of bats at a large-scale wind power development at Buffalo Ridge, Minnesota. American Midland Naturalist 150, 332–342.

Johnson, G.D., Perlik, M.K., Erickson, W.P., Strickland, M.D., 2004. Bat activity, composition and collision mortality at a large wind plant in Minnesota. Wildlife. Society Bulletin 32, 1278-1288.

Kerlinger, P., Curry, R., Culp, L., Jain, A., Fischer, B., Hasch, A., 2006. Post-construction avian and bat fatality monitoring study for the High Winds Wind Power Project, Solano County, California: two year report. Prepared for High Winds, L.L.C., and FPL Energy, Livermore, California. Curry & Kerlinger, L.L.C., McLean, Virginia. URL http://www.batsandwind.org/pdf/Kerlinger_etal_2006.pdf.

Kerlinger, P., Curry, R., Culp, L., Fischer, B., Wilkerson, C. Hasch., A., 2007. Post-construction avian monitoring study for the Shiloh I Wind Power Project Solano County, California. Year one final report. Prepared for PPM Energy. Curry & Kerlinger, L.L.C., McLean, Virginia. URL http://nrm.dfg.ca.gov/FileHandler.ashx?DocumentVersionID=41927.

Kerns, J., Kerlinger, P., 2004. A study of bird and bat collision fatalities at the Mountaineer Wind Energy Center, Tucker County, West Virginia: annual report for 2003. Prepared for FPL Energy and Mountaineer Wind Energy Center Technical Review Committee. Curry & Kerlinger, LLC, McLean, Virginia. URL http://www.wvhighlands.org/Birds/MountaineerFinalAvianRpt-%203-15-04PKJK.pdf

Kerns, J., Erickson, W.P., Arnett, E.B., 2005. Bat and bird fatality at wind energy facilities in Pennsylvania and West Virginia, p 24-95. *In*: E. B. Arnett (Ed.). Relationships between bats and wind turbines in Pennsylvania and West Virginia: an assessment of fatality search protocols, patterns of fatality, and behavioral interactions with wind turbines. Prepared for the Bats and Wind Energy Cooperative. Bat Conservation International, Austin, Texas. URL http://www.batsandwind.org/pdf/postconpatbatfatal.pdf.

Korner-Nievergelt, F., Korner-Nievergelt, P., Behr O., Niermann, I., Brinkmann, R., Hellriegel, B., 2011. A new method to determine bird and bat fatality at wind energy turbines from carcass searches. Wildlife Biology 17, 350-363.

Kunz, T. H., Arnett, E.B., Erickson, W.P., Hoar, A.R., Johnson, G.D., Larkin, R.P., et al., 2007. Ecological impacts of wind energy development on bats: questions, research needs, and hypotheses. Frontiers in Ecology and the Environment 5, 315-324.

Lehman, R.N., Kennedy, P., Savidge, J., 2007. The state of the art in raptor electrocution research: a global review. Biological Conservation 136, 159–174.

Manly, B.F., 1997. Randomization, bootstrap and Monte Carlo methods in biology. 3rd edition. Chapman and Hall/CRC, New York, New York. 480 p.

McGuire, L.P., Guglielmo, C.G., Mackenzie, S.A. Taylor, P.D., 2012. Migratory stopover in the long-distance migrant silverhaired bat, Lasionycteris noctivagans. Journal of Animal Ecology 81, 377–385.

Miller, A., 2008. Patterns of avian and bat mortality at a utility scaled wind farm on the southern high plains. M.S. Thesis, Texas Tech University, Lubbock, Texas.

Morrison, M., 2002. Searcher bias and scavenging rates in bird/wind energy studies. Technical Report NREL/SR-500-30876, National Renewable Energy Lab, Golden, Colorado. URL http://www.batsandwind.org/pdf/Searcher%20bias%20and%20scavenging%20in%20bird%20wind%20energy%20studies.pdf.

National Research Council, 2007. Environmental impacts of wind-energy projects. National Academies Press, Washington, D.C. 394 p.

Nicholson, C.P., 2003. Buffalo Mountain Windfarm bird and bat mortality monitoring report: October 2001 - September 2002. Tennessee Valley Authority, Knoxville, Tennessee.

Nicholson, C.P., Tankersley, R.D. Jr., Fiedler, J.K., Nicholas, N.S., 2005. Assessment and prediction of bird and bat mortality at wind energy facilities in the southeastern United States: final report, 2005. Tennessee Valley Authority, Knoxville, Tennessee. URL http://docs.wind-watch.org/nicholson2005-bird_bat_mortality.pdf.

Osborn R.G., Higgins, K.F, Usgaard, R.E., Dieter, C.D., Neiger, R.D., 2000. Bird mortality associated with wind turbines at the Buffalo Ridge Wind Resource Area, Minnesota. American Midland Naturalist 143, 41–52.

Piorkowski, M., O'Connell, T.J., 2010. Spatial pattern of summer bat morality and collision with wind turbines in mixed-grass prairie. American Midland Naturalist 164,260-269.

Shoenfeld, P., 2004. Suggestions regarding avian mortality extrapolation. Prepared for Mountaineer Wind Energy Center Technical Review Committee. URL http://

www.wvhighlands.org/Birds/SuggestionsRegardingAvianMortalityExtrapolation.pdf.

Tierney R., 2007. Buffalo Gap I Wind Farm avian mortality study February 2006–January 2007, Final survey report. Technical Report, AES SeaWest, Inc., Albequerque, New Mexico.

Tierney, R., 2009. Buffalo Gap 2 Wind Farm avian mortality study July 2007–December 2008 final study report. Prepared for Buffalo Gap Wind Farm 2, LLC. TRC, Albuquerque, New Mexico. URL http://www.batsandwind.org/pdf/Buffalo%20Gap%202%20Final%20Report.pdf.

Ward, M.R., Stallknecht, D.E.,Willis, J., Conroy, M.J., Davidson, W.R., 2006. Wild bird mortality and West Nile virus surveillance: biases associated with detection, reporting, and carcass persistence. Journal of Wildlife Diseases 42, 92–106.

Wobeser G.,Wobeser, A.G., 1992. Carcass disappearance and estimation of mortality in a simulated die-off of small birds. Journal of Wildlife Diseases 28, 548–554.

Wolfram Mathematica Online Integrator, 2012. Wolfram Research, Inc. URL http://integrals.wolfram.com.

Table 1

Comparison of the percent difference between the simple-uniform model and each perturbed model for various combinations of p and T_S/T_R ratios.

			Perturbed-migration model		
T_S/T_R	p	Perturbed-uniform model	FWHM = $4T_S$	FWHM = $1T_S$	FWHM = $0.5T_S$
* 0.2	0.2	-38.0	-48.0	-48.0	-48.0
* 0.2	0.4	-56.0	-64.0	-64.0	-64.0
* 0.2	0.6	-65.8	-73.0	-73.0	-73.0
* 0.2	0.8	-72.1	-78.0	-78.0	-78.0
0.5	0.2	-20.5	-23.8	-23.6	-23.0
0.5	0.4	-34.1	-38.0	-38.0	-37.0
0.5	0.6	-43.8	-48.0	-48.0	-48.0
0.5	0.8	-51.0	-55.3	-55.2	-55.0
1.0	0.2	-9.4	-10.0	-10.3	-8.6
1.0	0.4	-17.3	-19.0	-18.8	-17.2
1.0	0.6	-23.9	-26.0	-25.8	-24.3
1.0	0.8	-29.5	-31.8	-31.7	-30.3
1.5	0.2	-5.0	-5.6	-5.0	-1.1
1.5	0.4	-9.5	-10.5	-10.0	-6.0
1.5	0.6	-13.6	-14.9	-14.4	-10.8
1.5	0.8	-17.4	-18.8	-18.4	-15.0
* 2.0	0.2	-2.8	-3.2	-2.5	4.4
* 2.0	0.4	-5.5	-6.1	-5.4	1.4
* 2.0	0.6	-8.0	-8.8	-8.1	-1.5
* 2.0	0.8	-10.3	-11.0	-10.6	-4.2

* Indicates conditions in which discrepancy is > 5% between the perturbed-uniform model and at least one of the perturbed-migration models.

Figure Captions

Fig. 1. Representations of each model at $n = 10$ for (A) simple-uniform model, (B) perturbed-uniform model, (C) simple-migration model, and (D) perturbed-migration model.

Fig. 2. Percent-difference contour plots between simple-uniform and perturbed-uniform models at various combinations of p, T_S, and T_R. Contours constructed from 651 simulations assuming constant $n = 30$.

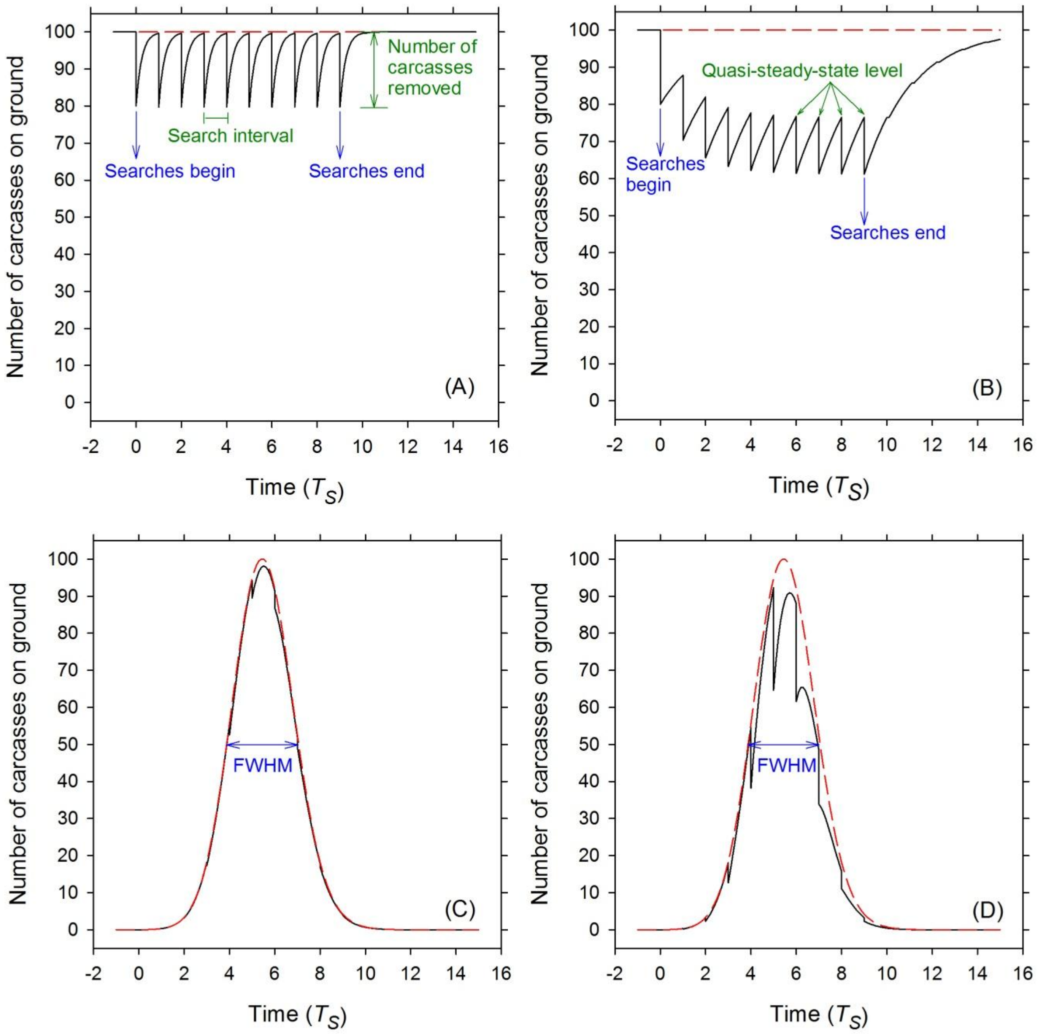
Fig. 3. Percent-difference contour plots between simple-uniform and simple-migration models for various combinations of FWHM, T_S, and T_R. Contours constructed from 840 simulations assuming constant $n = 30$.

Fig. 4. Percent-difference contour plots between simple-uniform and perturbed-migration models for various combinations of p, T_S, and T_R at a FWHM of (A) 4.0 T_S, (B) 1.0 T_S, (C) 0.5 T_S, and (D) 0.125 T_S. Contours constructed from 651 simulations in each sub-plot.

Fig. 5. A non-linear curve fit (solid line) using Eq. (A.19) for bat carcasses reported by Johnson et al. (2003). Dots indicate values from Johnson et al. (2003). Corresponding Gaussian distribution depicting migration profile is shown as dashed line.

Fig. 6. Simulated data illustrating how reported methods may be used to de-convolute animal migration waves (panel B) from carcass counts (panel A). Dashed lines are provided to show two separate migration waves (FWHM = $5T_S$ with $t_o = 8T_S$ and FWHM = $5T_S$ with $t_o = 15T_S$), and solid lines represent the sum of the two waves.

Fig. 7. Flow chart to aid in model selection given site-specific parameters of p, T_S, T_R, and FWHM.



Number of carcasses on ground
Time (T_S)
Number of carcasses removed
Search interval
Searches begin
Searches end
(A)
Quasi-steady-state level
Searches begin
Searches end
(B)
FWHM
(C)
FWHM
(D)
0
10
20
30
40
50
60
70
80
90
100
-2
0
2
4
6
8
10
12
14
16

Figure 1

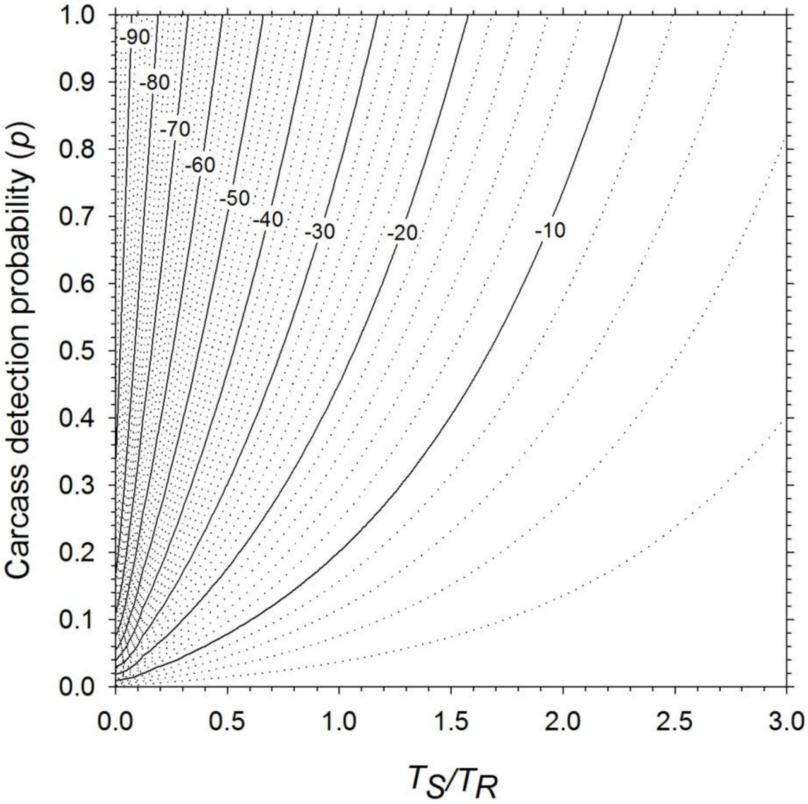

1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
Carcass detection probability (p)
-90
-80
-70
-60
-50
-40
-30
-20
-10
0.0
0.5
1.0
1.5
2.0
2.5
3.0
TS/TR

Figure 2

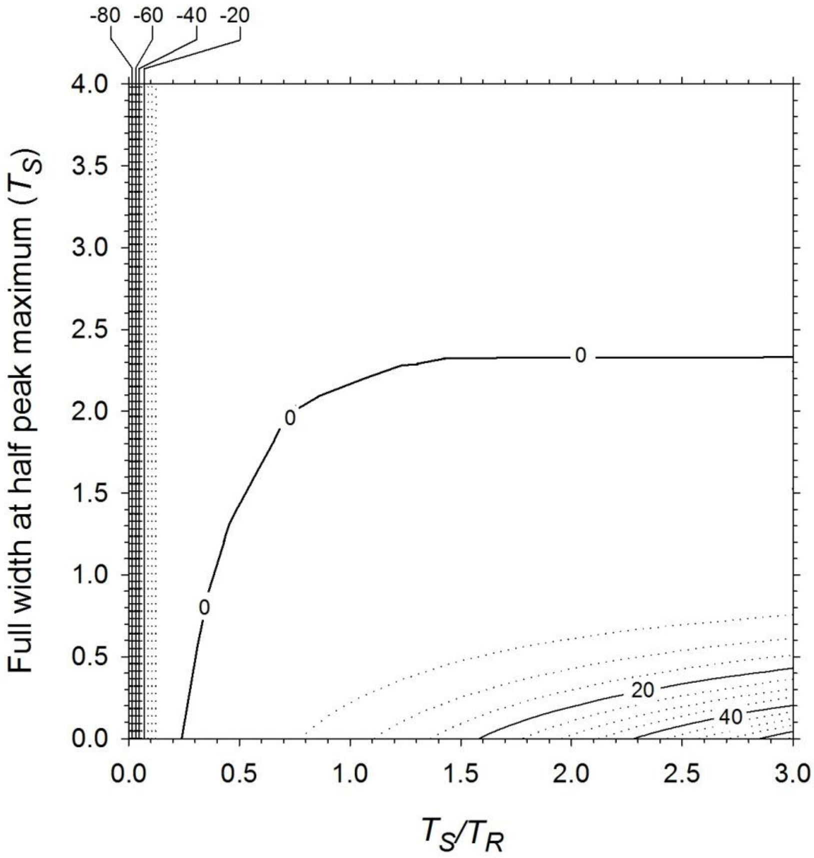

-80
-60
-40
-20
Full width at half peak maximum (T_S)
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0
0
0
20
40
0.0
0.5
1.0
1.5
2.0
2.5
3.0
T_S/T_R

Figure 3

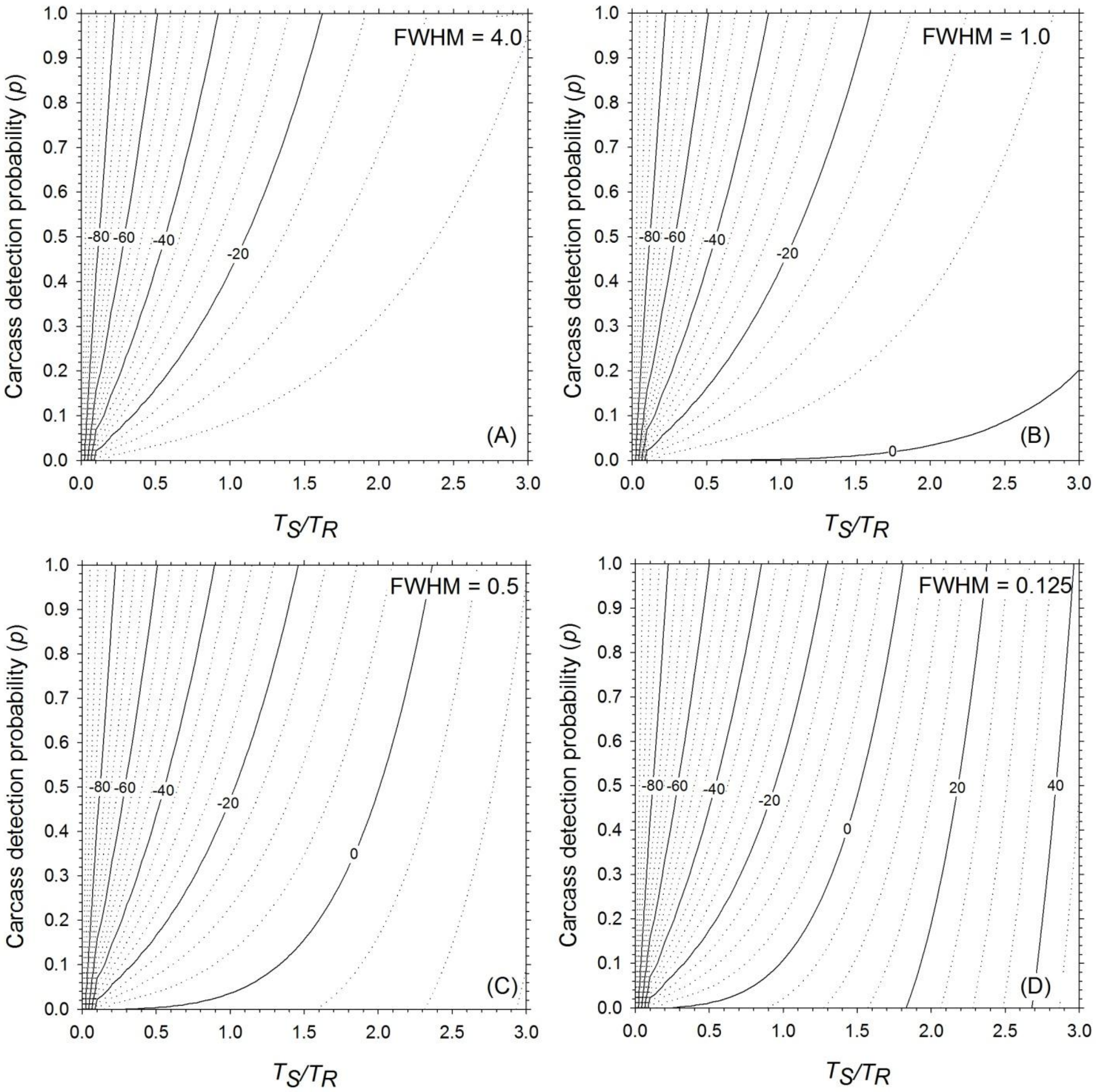

FWHM = 4.0
(A)
-80
-60
-40
-20
FWHM = 1.0
(B)
-80
-60
-40
-20
0
FWHM = 0.5
(C)
-80
-60
-40
-20
0
FWHM = 0.125
(D)
-80
-60
-40
-20
0
20
40
Carcass detection probability (p)
T_S/T_R
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
0.0
0.5
1.0
1.5
2.0
2.5
3.0

Figure 4

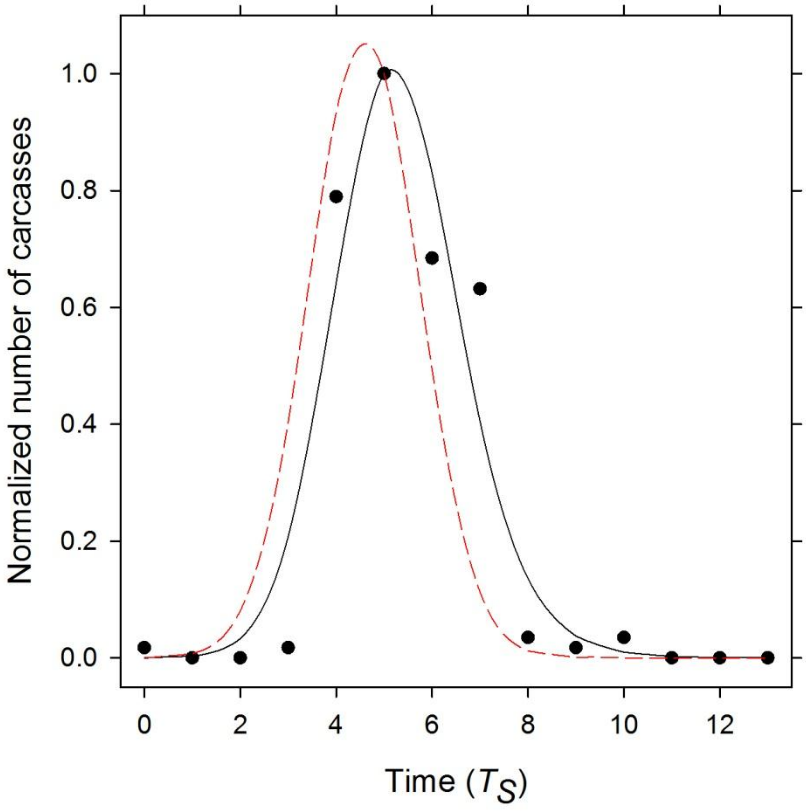

Normalized number of carcasses
1.0
0.8
0.6
0.4
0.2
0.0
0
2
4
6
8
10
12
Time (T_S)

Figure 5

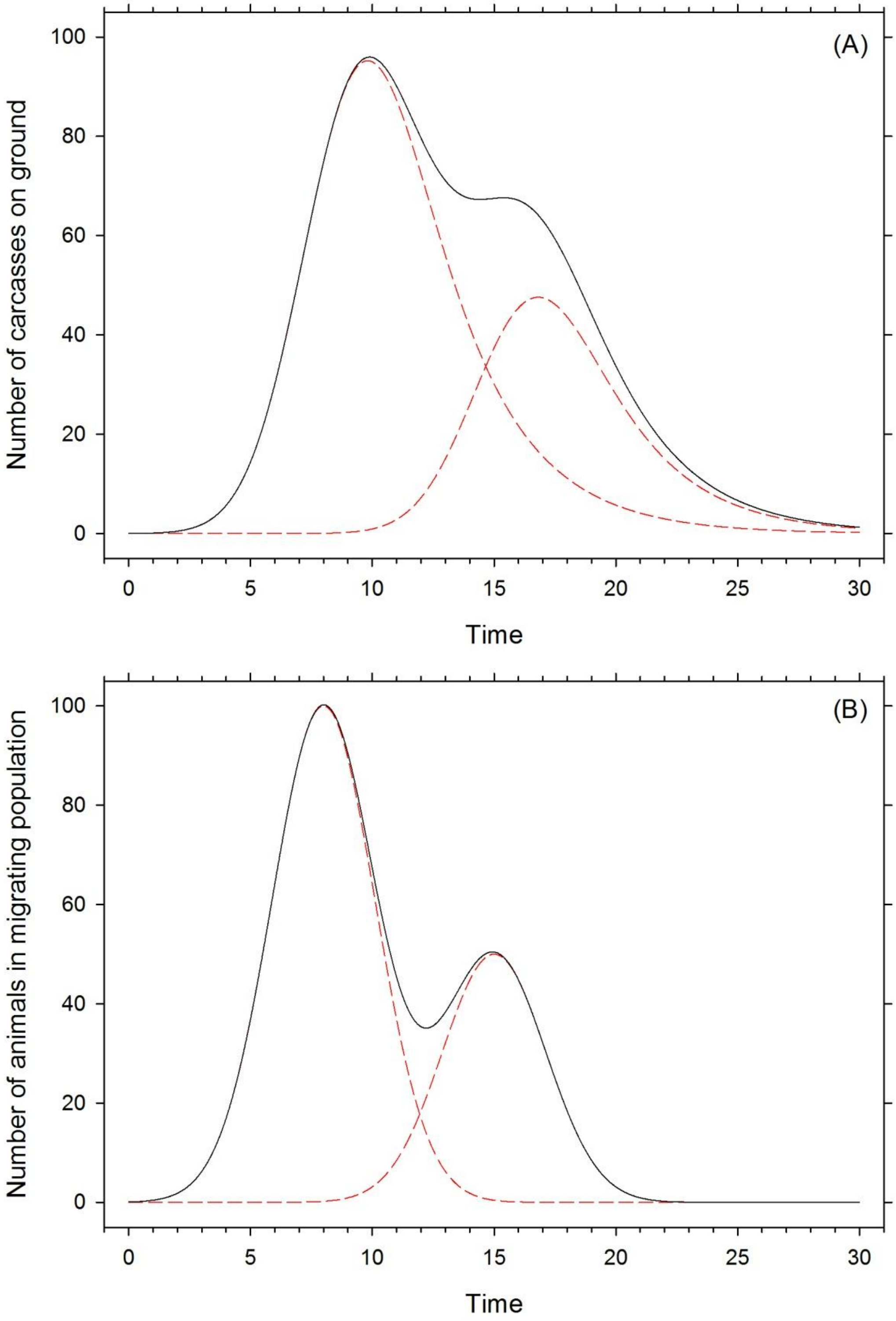

(A)
Number of carcasses on ground
0
20
40
60
80
100
0
5
10
15
20
25
30
Time
(B)
Number of animals in migrating population
0
20
40
60
80
100
0
5
10
15
20
25
30
Time

Figure 6

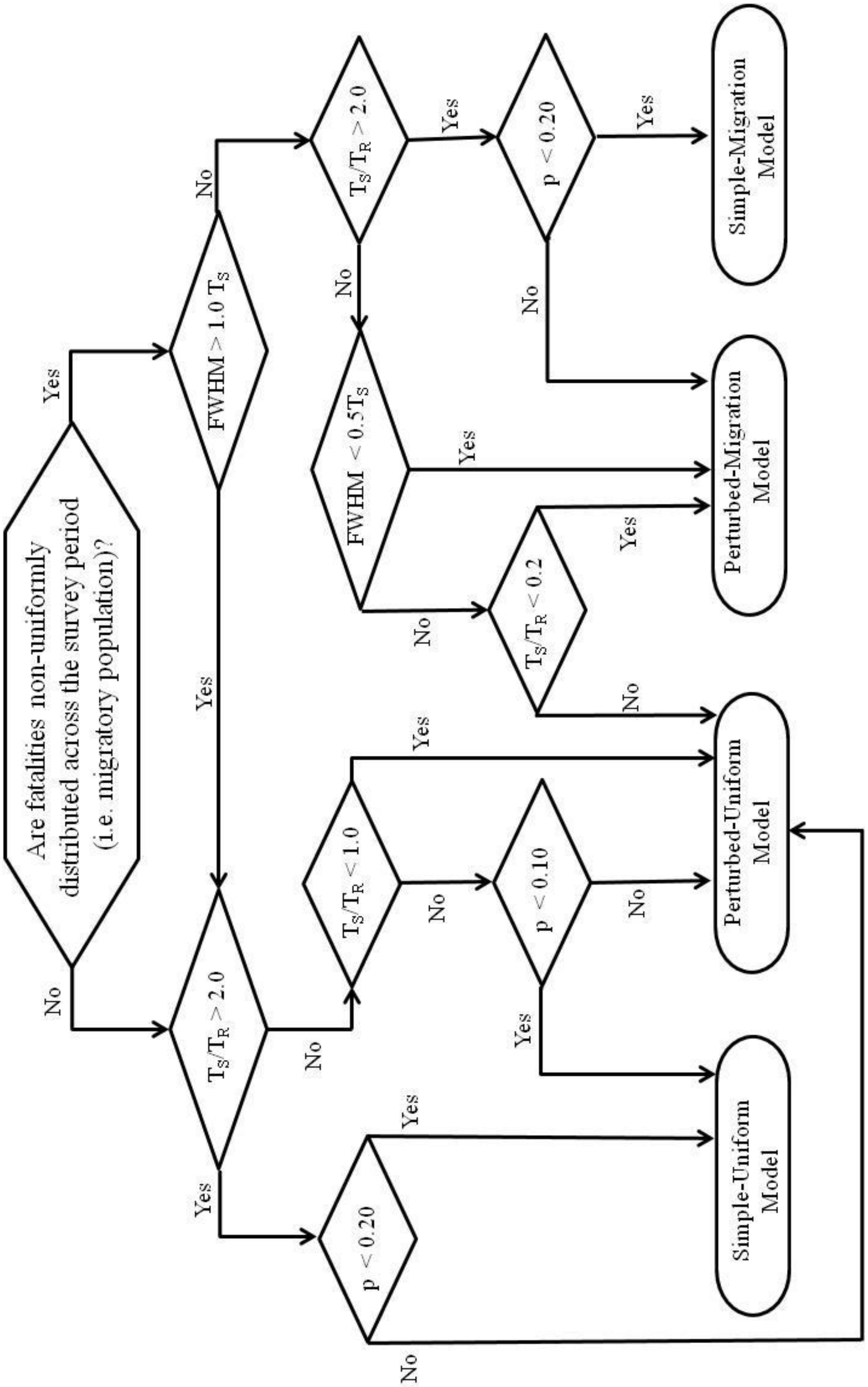

Are fatalities non-uniformly distributed across the survey period (i.e. migratory population)?
Yes
No
FWHM > 1.0 T_S
No
Yes
$T_S/T_R > 2.0$
Yes
No
$p < 0.20$
Yes
No
Simple-Migration Model
FWHM < 0.5T_S
Yes
No
$T_S/T_R < 0.2$
Yes
No
Perturbed-Migration Model
$T_S/T_R > 2.0$
Yes
No
$T_S/T_R < 1.0$
Yes
No
$p < 0.10$
No
Yes
Perturbed-Uniform Model
$p < 0.20$
Yes
No
Simple-Uniform Model

Figure 7

Appendix

A.1 Fatality Models Involving Uniform Population Distributions

The total number of animals killed by wind turbines may be distributed among three categories: carcasses on the ground, G; carcasses that have been recovered by searchers, F; and carcasses that have been scavenged or are fully decayed, R. Animal deaths are assumed to occur at a steady rate of λ. Thus,

$$G(t) + F(t) + R(t) = \lambda t \tag{A.1}$$

The rate animals that are killed by the wind turbines is found by taking the first derivative of Eq. (A.1) with respect to time. The rate that animals are removed either by scavenging or decaying is assumed to occur with a mean removal time of T_R (i.e., $dR(t)/dt = G(t)/T_R$). In the absence of any searches, Eq. (A.1) may then be written as:

$$\frac{dG(t)}{dt} + \frac{G(t)}{T_r} = \lambda. \tag{A.2}$$

The solution of this differential equation is:

$$G(t) = (\lambda T_R C)e^{-\frac{t}{T_r}} + \lambda T_r. \tag{A.3}$$

The integration constant is explicitly written as $\lambda T_R C$ to aid in the analysis of the models. Equation (A.3) predicts total number of animal carcasses on the ground will eventually reach a steady-state value, which is directly related to the mean removal time of the carcasses. The steady-state value may be determined by taking the limit of equation (A.3) as $t \rightarrow \infty$:

$$\lim_{t\to\infty} G(t) = \lim_{t\to\infty} \left[(\lambda T_R C) e^{-\frac{t}{T_r}} + \lambda T_r \right] = \lambda T_R. \tag{A.4}$$

A.1.1 Simple-uniform model

This fatality model focuses on animals whose population density is temporally uniform in the vicinity of a wind farm. If steady-state conditions are established before searches begin ($t < 0$), then number of animals on the ground at the beginning of the survey is simply λT_R. Fatality searches occur at the beginning of each survey period, with the first search ($i = 1$) commencing at $t = T_{S,1} = 0$. The second search ($i = 2$) occurs at $t = T_{S,2}$. Number of carcasses collected for a specific carcass class during the i^{th} search is denoted as F_i. Number of animals found depends on searcher efficiency for that survey, p_i, and number of carcasses on the ground from the prior search period, $G_{i\text{-}1}$, that are available to be collected at the time of the i^{th} search. Therefore, number of carcasses found for a given search event is $F_i = p_i G_{i-1}$. If total number of carcasses collected during fatality searches is relatively small, the act of searching will not significantly perturb number of carcasses on the ground from one search to another. In this case, $G(t)$ can recover its steady-state value during the interval of time between each search.

It is possible that carcass removal rates may change during the course of the fatality survey (e.g., seasonal variations). Therefore, the steady-state value is modeled as $\lambda T_{R,i}$. An individual fatality search is expected to yield:

$$F_i(T_{S,i}) = p_i G_{i-1}(T_{S,i}) = p_i \lambda T_{R,i-1} \tag{A.5}$$

The number of carcasses found depends on the search detection probability for a particular search and the steady-state number of carcasses on ground from the previous search interval. Total number of carcasses collected over the fatality survey is

$$N_F = \sum_{i=1}^{n} F_i(T_{S,i}) = \lambda \sum_{i=1}^{n} p_i T_{R,i-1}, \tag{A.6}$$

where n is the number of fatality searches conducted during the survey. Total number of fatalities, N_K, occurring over the same period of time (i.e., $0 \leq t \leq T_{S,n}$) is

$$N_K = \int_0^{T_{S,n}} \lambda dt = \lambda T_{s,n}. \tag{A.7}$$

Solving Eq. (A.7) for λ and substituting the result into Eq. (A.6) gives an estimate for number of fatalities that occurred during the survey period:

$$N_K = \frac{N_F T_{S,n}}{\sum_{i=1}^{n} p_i T_{R,i-1}} \tag{A.8}$$

Equation (A.8) reduces to

$$N_K = \left(\frac{n-1}{n}\right) \frac{N_F T_S}{p T_R} \tag{A.9}$$

if searches occur in periodic intervals of equal duration (i.e., $T_{S,i} = (i-1)T_S$ for the i^{th} search) and if p and T_R are constant over the course of the fatality survey.

The (n–1)/n factor in Eq. (A.9) appears because the number of search intervals used to evaluate the integral in Eq. (A.7) is one less than the number of searches being conducted. The simple-uniform model assumes there is one search occurring during each search period. Thus, duration of the entire survey is not merely the time elapsed between the first and last survey as this would require two searches to occur in one of the search periods. We defined the first search at $t = 0$, which is the beginning of the first search interval. This means the last search interval occurs immediately following the last search at $t = T_{S,n}$. Duration of this search period is not clearly defined if the survey employed unequal search intervals. In the context of periodic searching, however, the amount of time that should be assigned following the n^{th} search is simply T_S. Therefore, the value of $T_{S,n}$ in Eq. (A.8) is adjusted to $(n - 1)T_S + T_S = nT_S$. This produces the "naïve" estimator that was frequently employed in many early bird and bat fatality surveys at wind power plants, with the result being

$$N_K = \frac{N_F T_S}{p T_R}. \qquad \text{(A.10 or Eq. (3))}$$

The only difference between Eq. (A.9) and Eq. (A.10) is the amount of time counted in the total survey period; fatality rates calculated from these estimators will be identical.

A.1.2 Perturbed-uniform model

If searcher efficiency is relatively high or search intervals short, then $G(t)$ may not have sufficient time to recover the steady-state value between searches. In these cases, the act of searching may exert significant perturbations on $G(t)$ from one search to the next that may influence the total number of carcasses found. The overall strategy for developing a fatality estimator that accounts for these effects is to divide the search interval into discrete sub-intervals demarcated by the searching event. Number of carcasses found for each search is merely the number of animals present on the ground when searching is conducted multiplied by the searcher-detection probability, $F_i(T_{S,i}) = p_i G_{i-1}(T_{S,i})$. As before, searches occur at $t = T_{S,i}$, with the first search ($i = 1$) being at $t = 0$, and the period of time between each search does not have to be equal. The function $G(t)$ is discontinuous at $t = T_{S,i}$. Equation (A.3) is valid for each of the $G_i(t)$ segments:

$$G_i(t) = \lambda T_{R,i} C_i e^{-\frac{t}{T_{R,i}}} + \lambda T_{R,i}. \tag{A.11}$$

The integration constants, C_i, may be determined by number of carcasses on the ground immediately following the previous search:

$$G_i\left(T_{S,i}\right) = (1 - p_i) G_{i-1}\left(T_{S,i}\right). \tag{A.12}$$

Equation (A.12) allows a recursion relationship to be written for the integration constants,

$$\begin{aligned} C_i &= (1 - p_i) C_{i-1} \left(\frac{T_{R,i-1}}{T_{R,i}}\right) \exp\left(\frac{T_{S,i}}{T_{R,i}} - \frac{T_{S,i}}{T_{R,i-1}}\right) \\ &\quad + \left[(1 - p_i) \frac{T_{R,i-1}}{T_{R,i}} - 1\right] \exp\left(\frac{T_{S,i}}{T_{R,i}}\right). \end{aligned} \tag{A.13}$$

This enables each of the previously unknown integration constants to be determined from knowledge of initial conditions of the wind farm before searches began. Times before the first search ($t < 0$) have $G_0(t) = \lambda T_{R,0}$ since steady-state conditions are assumed to be in place before the fatality survey is initiated; C_0 must be zero to be consistent with this requirement. Total number of animals collected for all searches conducted during the fatality survey is found by simply summing the number of carcasses found for all searches,

$$N_F = \sum_{i=1}^{n} p_i G_{i-1}\left(T_{S,i}\right). \tag{A.14}$$

Solving Eq. (A.12) for $G_{i\text{-}1}(T_{S,i})$ and substituting the result into Eq. (A.14) gives:

$$N_F = \lambda \sum_{i=1}^{n} \left\{ T_{R,i} \left[\frac{p_i}{1-p_i} + \frac{p_i}{1-p_i} C_i \exp\left(-\frac{T_{S,i}}{T_{R,i}}\right)\right]\right\}. \tag{A.15}$$

Equation (A.15) may be combined with Eq. (A.7) to produce an estimator for total number of fatalities occurring during the survey period that explicitly takes into account the effect of searcher perturbations on $G(t)$,

$$N_K = \frac{N_F T_{S,n}}{\sum_{i=1}^{n}\left\{T_{R,i}\left[\frac{p_i}{1-p_i}+\frac{p_i}{1-p_i}C_i \exp\left(-\frac{T_{S,i}}{T_{R,i}}\right)\right]\right\}}. \tag{A.16}$$

For periodic search intervals that have constant p and T_R, Eq. (A.16) simplifies to

$$N_K = \frac{N_F T_S}{p T_R}\left(\frac{1}{\frac{1}{1-p}+\frac{1}{n(1-p)}\sum_{i=1}^{n} C_i \exp\left(-\frac{(i-1)T_S}{T_R}\right)}\right). \qquad \text{(A.17) or Eq. (4)}$$

The term in the parentheses modifies the simple-uniform model to account for searcher perturbation on $G(t)$. The recursion relation in Eq. (A.13) is simplified under these conditions, $C_i = (1-p)C_{i-1} - p\exp\left(\frac{(i-1)T_S}{T_R}\right)$.

Equation (A.17) defines the survey period from the first search to the end of the last search period, including the time after the last search. A correction factor of $(n–1)/n$ may be applied if researchers wish to define the survey time period from the time of the first search to the time at which the last search occurs.

A.2 Fatality Models Involving Migratory Population Distributions

Unlike models above, these seek to address fatality estimates for animals whose population density is not uniform over time. The most obvious examples are migratory species traveling through a geographic region containing wind turbines. In this case, the number of animals in the vicinity of the wind turbines may change as migratory species move through the area. A reasonable starting point is to model the temporal population size of migrating animals with a Gaussian distribution. That is, there will be times before and after migration occurs when essentially no migratory animals are found at the wind-energy facility. Animal deaths are assumed to be proportional to the local population size of the animals at a given time. Therefore, Eq. (A.2) is modified accordingly:

$$\frac{dG(t)}{dt} + \frac{G(t)}{T_R} = \lambda \exp\left(-\frac{(t-t_o)^2}{2a^2}\right). \tag{A.18}$$

The parameter a controls the width of the Gaussian function, while the lag time, t_o, is used to define initial value conditions in the following equations. This differential equation is solved using the method of integrating factors and the aid of the Wolfram Mathematica Online Integrator (2012). The solution is given as

$$G(t) = a\lambda\sqrt{\frac{\pi}{2}}\left[C\exp\left(-\frac{t}{t_R}\right) - W(t)\right], \tag{A.19}$$

where $W(t) = \exp\left[\frac{a^2}{2T_R^2} + \frac{(t_o-t)}{T_R}\right]\mathrm{erf}\left[\frac{a}{\sqrt{2}T_R} + \frac{(t_o-t)}{\sqrt{2}a}\right]$. The integration constant, C, may be evaluated by choosing a lag time such that $G(0) \cong 0$:

$$C = W(0). \tag{A.20}$$

A.2.1 Temporally non-uniform fatality rates with negligible searcher-induced perturbations

In the context of negligible searcher-induced perturbations on $G(t)$, number of carcasses found is $F_i(t) = p_i G_{i-1}(t)$; the notation holds the same meaning for the i^{th} search as described in the earlier section. Summing over all of the searches conducted during the fatality survey gives the total number of carcasses,

$$N_F = \sum_{i=1}^{n} F_i(T_{S,i}) = a\lambda\sqrt{\frac{\pi}{2}}\sum_{i=1}^{n}\left\{p_i W_{i-1}(0)\exp\left(-\frac{T_{S,i}}{T_{R,i-1}}\right) - p_i W_{i-1}(T_{S,i})\right\}. \tag{A.21}$$

Total number of animals killed during the migration is

$$N_K = \int_{-\infty}^{\infty} \lambda \exp\left(-\frac{(t-t_o)^2}{2a^2}\right) dt = \lambda a\sqrt{2\pi}. \tag{A.22}$$

Equation (A.22) requires searches to exceed the duration of the animal migration, for times outside of the survey period will not contribute significantly to the total number of animals killed. If the survey period covers a smaller interval of time than the animal migration, limits of integration in Eq. (A.22) must be modified to reflect the actual time period surveyed. Equation (A.22) may be used to eliminate the unknown parameter λ in Eq. (A.21), allowing number of animals killed to be estimated from number of carcasses recovered during searches,

$$N_K = \frac{2N_F}{\sum_{i=1}^{n}\left\{p_i W_{i-1}(0)\exp\left(-\frac{T_{S,i}}{T_{R,i-1}}\right) - p_i W_{i-1}(T_{S,i})\right\}}. \tag{A.23}$$

Periodic searches with constant p and T_R modify Eq. (A.23) to give

$$N_K = \frac{2N_F}{p}\left(\frac{1}{\sum_{i=1}^{n}\left\{W(0)\exp\left(-\frac{(i-1)T_S}{T_R}\right)-W\left((i-1)T_S\right)\right\}}\right). \qquad \text{(A.24) or Eq. (5)}$$

A.2.2 Temporally non-uniform fatality rates with non-negligible searcher-induced perturbations

The general strategy for incorporating searcher-induced perturbations on $G(t)$ is identical to that used for the uniform models. The function $G(t)$ is divided into a series of segments that exhibit discontinuities at each of the searches. Equation (A.3) is valid for each of the $G_i(t)$ segments. The initial condition for each of the segments is defined by $G_i\left(T_{S,i}\right) = (1-p_i)G_{i-1}\left(T_{S,i}\right)$; this allows a recursion relationship to be written for the i^{th} integration constant:

$$C_i = (1-p_i)C_{i-1}\exp\left(\frac{T_{S,i}}{T_{R,i}} - \frac{T_{S,i}}{T_{R,i-1}}\right)$$

$$+\left[p_i W_{i-1}\left(T_{S,i}\right) + W_i\left(T_{S,i}\right) - W_{i-1}\left(T_{S,i}\right)\right]\exp\left(\frac{T_{S,i}}{T_{R,i}}\right). \qquad \text{(A.25)}$$

Number of animals found during the survey period is determined by summing $F_i\left(T_{S,i}\right) = p_i G_{i-1}\left(T_{S,i}\right)$,

$$N_F = \lambda a\sqrt{\frac{\pi}{2}}\sum_{i=1}^{n}\left\{\frac{p_i}{1-p_i}\left[C_i\exp\left(-\frac{T_{S,i}}{T_{R,i}}\right) - W_i\left(T_{S,i}\right)\right]\right\}. \qquad \text{(A.26)}$$

Total number of animals killed over the survey period—and total number killed over the entire migration event, assuming the survey period covers the entire migration event—may be found by using Eq. (A.22) to eliminate λ in Eq. (A.26):

$$N_K = \frac{2N_F}{\sum_{i=1}^{n}\left\{\frac{p_i}{1-p_i}\left[C_i \exp\left(-\frac{T_{S,i}}{T_{R,i}}\right) - W_i\left(T_{S,i}\right)\right]\right\}}. \tag{A.27}$$

Applying conditions of periodic searching and constant values for p and T_R yield,

$$N_K = \frac{2N_F(1-p)}{p}\left(\frac{1}{\sum_{i=1}^{n}\left\{C_i \exp\left(-\frac{(i-1)T_S}{T_R}\right) - W\left((i-1)T_S\right)\right\}}\right). \qquad \text{(A.28) or Eq. (6)}$$

The recursion relationship is simplified to

$C_i = (1-p)C_{i-1} + pW\left((i-1)T_S\right) \exp\left(\frac{(i-1)T_S}{T_R}\right)$ with $C_0 = W(0)$ under these conditions.

Implications for assessing migratory origins using stable-hydrogen isotopes from claws of hoary bats, *Lasiurus cinereus*

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Running title: *Hydrogen-isotope analysis of bat claws*

Summary

1. Interest in bat migration is increasing as development of wind energy has resulted in bat mortality. Hoary bats, *Lasiurus cinereus*, are highly susceptible to wind-turbine fatalities. A better understanding of their migratory behavior may help mitigate fatality risk.

2. We develop techniques for sampling stable-hydrogen isotopes in mammalian claws to address migratory patterns. Claws may be preferable to fur samples especially for species with unknown molting times or differential molts among individuals.

3. Only the unguis of the claw should be used in isotope analysis. Any claw is appropriate for sampling as there were no differences in isotope values among claws from the same individual. Sampling consecutive cross-sections along the entire claw length revealed expected δD (‰) patterns consistent with southward fall migration, but patterns in claws of spring-killed individuals did not match predicted trends.

4. There was no difference between δD_{fur} and δD_{claw} in females, consistent with a seasonal molt prior to fall migration. In males, δD_{claw} was significantly more depleted than δD_{fur}, suggesting molt does not occur at location of summer residency. Thus, claw samples may be more appropriate than fur for assigning migratory origins.

5. This technique is applicable to other claw-bearing mammals to evaluate migration or other ecological questions. It may also be appropriate for other taxa, but differences in claw morphology should be considered before applying presented method.

Key-words: bat claws, deuterium, migration, stable isotopes, wind energy, δD

Introduction

Hoary bats, *Lasiurus cinereus*, are the most widespread North American bat, occurring throughout the contiguous United States, into northern Canada, and as far south as Argentina (Shump & Shump 1982). This solitary, foliage-roosting bat appears to have sex-biased geographic segregation, except during the mating season, with females concentrated in the eastern United States and males in the west (Findley and Jones 1964; Cryan 2003). The hoary bat migrates, but routes and specific wintering grounds have not been firmly established. Collection of hoary bats in Mexico during winter suggests wintering grounds south of the United States (Cryan 2003). However, some individuals have over-wintered as far north as Michigan (Findley & Jones 1964; Whitaker 1967). Cryan *et al.* (2004) indicated that hoary bats are long-distance latitudinal migrants, possibly migrating >2000 km between summer and winter locations.

Information on bat migration has gained particular relevance because several migratory species have been documented as wind-turbine fatalities. Migratory, tree-roosting bats seem particularly vulnerable, with hoary bats often representing the highest number of fatalities at wind-energy installations (Arnett *et al.* 2008). Fatalities are concentrated in early fall (Arnett *et al.* 2008) coincident with the timing of migration (Cryan & Brown 2007); our lack of knowledge about bat migration hinders our ability to determine why fall migrants of tree-roosting bats are most susceptible. Field techniques typically used to understand bat ecology (i.e. mark/recapture, radiotelemetry) have not been particularly helpful for studying bat migration. However, stable-hydrogen isotopes have been employed to address migratory questions for birds (e.g. Kelly 2006; Lott & Smith 2006; Hobson *et al.* 2007) and may be applicable for bats (Cryan *et al.* 2004; Fraser *et al.* 2010; Fraser 2011).

Mass differences between isotopes of hydrogen (H) result in lighter isotopes (i.e. protium, ^{1}H) reacting more quickly than the heavy isotopes (i.e. deuterium, ^{2}H) in kinetic reactions. There is systematic H isotope-ratio variation associated with key processes in the water cycle, with precipitation, evaporation, and temperature strongly influencing the degree of isotope fractionation (Dansgaard 1964, Bowen and Revenaugh 2003). Climatic processes characteristic of different geographic regions have resulted in systematic variation in ratios of ^{2}H:^{1}H (mapped by Bowen *et al.* 2005); in general, there is a latitudinal gradient of deuterium depletion in local precipitation from south to north across much of the United States (Dansgaard 1964), with hydrogen-isotopic signatures of particular geographic regions being subsequently reflected in trophic webs (e.g. Cormie et al. 1994). As food and water are ingested, their chemical composition is metabolized into organisms' tissues. The majority of H in keratins (e.g. hair and claws) is carbon bound once formed (Chamberlain et al. 1997) and will retain H-isotope signatures that are characteristic of where the tissues grew; thus, H-isotope ratios can be used to infer large-scale movement patterns of individuals.

Reliability of using H-isotope ratios for detecting long-distance movements is influenced by tissue growth patterns, such as molting time, as well as fractionation that may occur between the H-isotope source (food or water) and the tissue being metabolized. Bats typically molt once per year (Quay 1970) during summer in temperate localities (Constantine 1957, 1958; Tiunov & Makarikova 2007); however, the timing and location of molt is inadequately known for most bat species. Using hydrogen isotopes, Cryan *et al.* (2004) inferred that hoary bats likely molt prior to fall migration, with no sex-based differences in molting dates. Thus, hair samples may provide a reference to the geographic location of summer residency. Fur samples collected prior to fall migration should contain isotope ratios similar to local meteoric water, except for an expected diet-tissue discrimination factor. However, studies of other bat species during summer residency, including the eastern red bat, *Laiurus borealis*, have experimentally evaluated congruency of observed and expected hydrogen-isotope values, with mixed results (Britzke *et al.* 2009; Fraser 2011). Isotope ratios of hair samples have not always matched expected values. Such discrepancies could be due to molting times that may vary by sex, age classes, or geographic locations (Britzke *et al.* 2009).

Unlike bat fur that is molted periodically, claws grow continually, and isotopic values contained within the claws will change sequentially as the animal migrates. The use of claw tissue may overcome potential uncertainty of interpreting isotope values found in hair samples when molting time is unknown. However, the use of claw tissue also has inherent challenges. Mammalian cornified-claw tissue encapsulates the distal phalanx (bone), which is covered with a terminal matrix that gives rise to the claw sheath (Fig. 1c; Homberger *et al.* 2009). Traditionally, mammalian claw sheaths are divided into two layers: wall horn (or unguis) and sole horn (or subunguis; Mercer 1961). The unguis, composed of hard keratin, forms along the dorsal crest and lateral sides of the phalanx, and the subunguis, composed of soft keratin, forms on the ventral side of the unguicular process (Fig. 1d; Mercer 1961; Ethier *et al.* 2010).

Homberger *et al.* (2009) further separated the cornified claw into nine layers based on location in the claw and developmental origin. The ungual lateral wall originates at the claw root along the coronary band and is pushed distally by newly generated tissue (Fig. 1e); thus, sides of the claw, the “blade horn”, represent time-series data (Fig. 1b; Ethier *et al.* 2010). New keratinizing cells are generated continuously along the entire dorsal-crest length of the phalanx causing the claw to thicken from root to tip as claw layers merge along the dorsal ridge (Hamrick 1999). Dorsal-crest layers are collectively called the “cone horn” (Fig. 1c; Homberger *et al.* 2009). At the claw tip, several layers are present representing a compilation of tissue generated at different time intervals (Homberger *et al.* 2009; Ethier *et al.* 2010).

Claw growth rates are not known for many species and, within species, may vary with diet (Godwin 1959; Mulling *et al.* 1999), reproductive status (Mulling *et al.* 1999, MacCallum *et al.* 2002), age (Prentice 1973), and season (MacCallum *et al.* 2002). For example, growth rates of human nails are not uniform. Fingernails grow faster than toenails, and rates vary among digits (Yaensiri *et al.* 2010). Growth rates for bat claws have not been reported. Isotopic analysis of mammalian claws/hooves has been employed to investigate diet (Kielland 2001), resource partitioning (Belant *et al.* 2006), altitudinal movements (Fraser *et al.* 2010) and dispersal (Henaux *et al.* 2011). Claws have only been used to address migration in birds, but not mammals (e.g. Bearhop *et al.* 2005; Clark *et al.* 2006; Fraser *et al.* 2008; Oppel & Powell 2008).

Our purpose was to evaluate methodology of stable-hydrogen isotopes as applied to mammals, especially bats, using *L. cinereus* as a model. We gathered time-series data (i.e. along length of claw) to assess the range of isotopic values within a single claw and develop methods to use claws when addressing migratory questions. Because mammalian claws have multiple layers, we investigated isotopic differences between layers to determine potential biases when sampling multiple layers at once. We also compared isotope heterogeneity of fur and claw from the same individuals to address whether there is an optimal-tissue sample for isotope analysis. Guidelines for tissue sampling as it relates to isotopic characteristics and their potential biological meaning are discussed.

Methods

SPECIMEN AND TISSUE COLLECTION

We collected 73 adult hoary bat carcasses at the Blue Canyon II Wind Power Project along the border of Caddo and Kiowa counties of western Oklahoma from August 2006 through November 2008. Carcasses were a result of wind-turbine fatalities, and the physical condition varied (i.e. degree of scavenging, decay, and/or trauma). Specimens are archived in the Sam Noble Oklahoma Museum of Natural History. Sex, based on examination for reproductive organs, was recorded for 36 males and 12 females. When sex could not be determined through visual inspection, morphometric measurements were taken to determine probable sex. Hoary bats are sexually dimorphic in size with females being larger (Williams & Findley 1979). For most specimens, we measured (to nearest 0.01 mm) forearm length, condylocanine length, and maxillary tooth row length. Means ± standard deviations (SD) for each measurement were calculated for males and females and used to assign unknown specimens to probable sex (see supplemental material). Based on size, we sexed nine males and nine females.

Fur typically was collected from the scapular region using surgical scissors to clip at the base of hair shafts. Carcass condition required an alternative sample site for 19 bats. For nine specimens, we sampled the scapular region and also the lower dorsal region on or near the uropatagium. For one bat, we took a fur sample from every bodily region that was previously sampled from other specimens. There were no significant differences between fur from different body regions (see supplemental material).

The phalanx from which claw samples were taken (n = 32 males, 16 females) could not be standardized because availability varied due to scavenging or disarticulation during specimen processing. Thus, claws were selected on biological condition, not anatomical location. For one specimen, every claw, including the pollex (thumb), was taken from the left side of the carcass to assess isotope heterogeneity among claws. Additionally, the tip of every claw was sampled from another specimen, as well as the pollex and a single pedal claw from a third specimen (see supplemental material).

STABLE ISOTOPE PREPARATION AND PROCESSING

An ungual skin fold covers the claw root (Fig. 1a), so claws were soaked in deionized water to soften dermal tissue, and skin was removed from the claw with forceps to expose the entire claw (Fig. 1b). Fur and claw samples were placed in 1-mL eppendorf vials, with cleaning procedures following recommendations of Paritte & Kelly (2009). Tissue samples were washed in a 1:30 (v/v) solution of Fisher Versa-Clean detergent to deionized water. Each vial was filled with detergent solution, sealed, and shaken for 30 s. The solution was decanted with disposable pipettes and replaced with a deionized water rinse (again being sealed, shaken for 30 s, and decanted) for 10 repetitions to rinse detergent solution and oils from samples. A secondary treatment of 2:1 (v/v) chloroform:methanol solution was added to each vial. They were sealed, shaken for 30 s, and decanted, then left open to air dry in a fume hood for 24 h.

Methods for claw cross-sectioning varied slightly among different aspects of the study. To address isotopic difference between claw layers, claws of five males and five females were sectioned as follows. Cross-sections began at the claw root and continued distally for three consecutive cross-sections. Cross-sections were standardized by mass to obtain a 100 ± 10 µg sample from both the unguis and subunguis of each cross-section (Fig. 1f shows a typical cross-section). A fine-needle probe was inserted between the phalanx and unguis, moving between structures to mechanically separate white internal structures from black hard-keratinized unguis. For simplicity, white internal structures, which do not extend into the claw tip, are referred to as the subunguis to indicate the tissue beneath the unguis, but the white tissue actually includes the subunguis, phalanx, and terminal matrix (Fig. 1f). A second claw from each individual was cross-sectioned in the same manner, but the two layers were not separated. Given the mass would be at least twice that of separated sections, nonseparated sections were trimmed both anteriorly and posteriorly until a single 100 ± 10 µg sample of combined layers was obtained.

For studies requiring entire claws to be cross-sectioned, sectioning began at the tip, continuing toward the root. Sections were standardized by sample mass (100 ± 10 µg). If the initial section was >100 µg, the anterior portion was trimmed with the scalpel. Excess was then included in the next cross-section so that the entire claw was sampled to provide consecutive-time-series data. Cross-sections with subunguis, phalanx, and/or terminal matrix present were separated mechanically as described above so that all samples only represented the unguis.

Only the claw cross-section most depleted in deuterium was of interest for comparison with fur samples (location was known from experiments examining the entire claw). Thus, we measured 1 mm from the extensor process of the phalanx and made a lateral cross-section through the point of the flexor tubercle (Fig. 1e). We then continued to cross-section distally to obtain a single, thin cross-section (mechanically separating the unguis with probe and forceps) until a single 100 ± 10 μg sample was obtained.

Fur samples of 150 ± 10 μg (the standard hydrogen-sample mass in our laboratory) were collected. The reduced mass of claw samples was selected to allow smaller cross-sections for better isotopic resolution, as claws are continually integrating new tissue. All fur and claw samples were packed in 3.5 mm × 5 mm silver-foil capsules, as were standards of the same mass (100 ± 10 μg for claws; 150 ± 10 μg for fur). Standards were chicken feathers (CFS), cow hooves (CHS), bowhead whale (*Balaena mysticetus*) baleen (BWB), and brown-headed cowbird (*Molothrus ater*) feathers (BHCO). The first three were supplied by the Environment Canada Stable Isotope Hydrology and Ecology Research Laboratory (Saskatoon, Saskatchewan) as developed by Wassenaar & Hobson (2003). BHCO was developed at the University of Oklahoma (see Kelly *et al.* 2009). Given different sample masses for fur and claws, tissue types were analyzed in separate runs. All isotope-ratio data were collected at the University of Oklahoma with a ThermoFinnigan Delta V isotope-ratio mass spectrometer connected to a high-temperature pyrolysis elemental analyzer (TC/EA, ThermoFinnigan, Bremen, Germany). Samples automatically were dropped from a 50-position zero-blank autosampler with standards at the beginning, middle, and end of each autorun to correct for instrumental drift. All values are reported as the non-exchangeable fraction of hydrogen in each tissue. Stable-isotope ratios are expressed in standard δ notation, where $\delta D = 1000\left[\frac{isotope\ ratio\ of sample}{isotope\ ratio\ of\ standard} - 1\right]$. Thus, δD is expressed in parts per thousand (‰) deviation from Vienna Standard Mean Ocean Water (VSMOW). For more methodological details, see Kelly *et al.* (2009).

STATISTICAL ANALYSIS AND PREDICTIONS

Isotope differences between claw layers and heterogeneity within individuals

To test for an effect of removing internal tissue of claw cross-sections prior to isotope analysis, we used a three-way ANOVA. This analysis evaluated contributions of sex, claw cross-section, and claw layer to the variation in H-isotope ratios. We also pooled data for sexes, performing a two-way repeated-measure ANOVA with two-factor repetition to ensure claw cross-section and claw layer were paired for the same individual.

Because the digit from which claws were collected could not be standardized, we assessed heterogeneity among claws from the same individual. The entire unguis of each claw was sampled in consecutive cross-sections. We measured length, height, and diagonal of each claw (Fig. 1b and 1c; see supplemental information). We compared variation among claws and cross-sections (two-way ANOVA). If claw growth rates varied among digits of an individual, we expected differences in δD values among claws.

Time-series claw sampling

If claws are appropriate tissues for detecting migratory movements, differences in δD should be evident along the claw length. The entire unguis was cross-sectioned for five males and five females collected during fall migration. Spring fatalities were rare; two male and two female specimens were available to provide time-series data. For specimens collected in Oklahoma during fall, many individuals likely had begun southward migration and begun incorporating more deuterium-enriched isotopic signatures in the most recently grown claw portion. Sampling distally was expected to show a temporal decrease (become more negative) in δD, retracing individuals to the location of their northern summer residency. However, as sampling continues toward the tip, we expected δD to begin increasing again, indicating isotopic ratios that were incorporated during the previous spring migration. A different trend was expected for individuals collected during spring migration; individuals likely were migrating northward so the sections most recently grown should be the most depleted in deuterium. As sampling continued distally, δD was expected to increase (become less negative) as the animal’s movement was traced southward toward wintering grounds.

Because the number of cross-sections obtained per claw varied among individuals, we adjusted each cross-section to the proportion of relative mass (e.g. for a claw producing five cross-sections, each cross-section represented 20% of the claw). Data were fitted with a non-linear, second-order regression to reveal patterns of δD changes along the claw.

Comparison of δD_{fur} and δD_{claw}

A two-way repeated-measure ANOVA with one factor repetition paired δD_{fur} and δD_{claw} from the same individual. Sex was the second, unpaired factor. If bats molt prior to fall migration, δD_{fur} should correspond to northern limits of summer residency. Because claws continuously incorporate new tissues, some averaging of isotopic values was expected within each cross-section. Thus, the δD_{claw} was predicted to be $\geq \delta D_{fur}$. If $\delta D_{claw} < \delta D_{fur}$, this may indicate that molting did not occur at the location of northern-summer residency, and that fur should not be used to assess migratory origins.

General statistical procedures

Statistical tests were performed with Sigma Plot 11.0 (significance level $P < 0.05$). Values are reported as mean ± 1 standard error of mean (SEM). For ANOVA analyses, Shaprio-Wilk normality and equal-variance tests were both passed before proceeding. Pairwise comparisons within two- and three-way ANOVAs were evaluated with Holm-Sidak multiple-comparison procedures.

Results

ISOTOPE DIFFERENCES BETWEEN CLAW LAYERS

Table 1 provides δD values obtained for each specimen, cross-section, and claw layer. For three males, the third cross-section did not contain sufficient subunguis tissue to obtain a comparable 100 μg sample. We analyzed effect of sex, claw layer, and claw cross-section on δD variation. There was no significant difference in δD values between sexes ($F = 2.80$, $P = 0.102$) or among cross-sections ($F = 0.01$, $P = 0.988$). However, difference in δD between the unguis and subunguis was highly significant (F

= 36.07, P < 0.001). None of the interactions between any of the three variables were significant.

Because sex was independent of trends between claw layers, data were pooled for a two-way repeated-measure ANOVA with two-factor repetition. The missing cross-section for three male specimens precluded their inclusion for this test. There was significant difference in δD among specimens (F = 28.79, P = 0.020), suggesting individuals were from a broad range of latitudes. Differences in δD among the three cross-sections (consecutive sections from claw root) were not significant (F = 3.27, P = 0.077). Consistent with the three-way ANOVA, there was a highly significant difference between the δD of the two claw layers (F = 115.71, P < 0.001), with the subunguis always more depleted in deuterium for all cross-sections, regardless of the range of δD values within each claw. There was significant interaction between cross-section and claw layer (F = 7.58, P = 0.007), with deuterium enrichment in the unguis and depletion in the subunguis with each subsequent cross-section. In all cases, the unseparated cross-section had a δD intermediate between unguis and subunguis values (Table 1).

ISOTOPE HETEROGENEITY AMONG CLAWS

The pollex claw is larger than others and resulted in 10 cross-sections, whereas pedal claws produced six. The pollex was excluded from the mean δD values within each cross-section, as well as statistical analysis in the two-way ANOVA. A significant depletion of deuterium occurred in cross-section samples from claw tip to root (F = 23.00, P < 0.001). With pairwise comparisons, there was no significant difference in δD of the three cross-sections nearest the claw root (same findings as in claw-layer analysis). However, tip cross-sections were significantly different from root cross-sections ($t = 8.71$, $P < 0.001$). Within each respective cross-section, there was no difference in δD among the five pedal claws ($F = 0.99$, $P = 0.435$; Table 2). The greatest disparity was cross-section D, with a difference of 13.9‰, while the root and tip differed by 9.6‰ and 8.7‰, respectively. Despite the pollex being a larger claw, the range of isotopic values appears no greater than for pedal claws. The δD of the pollex for root and tip were similar to mean δD of pedal claws (Table 2).

TIME-SERIES CLAW SAMPLING

The δD of claw cross-sections for spring and fall migrants are presented in Fig. 2 (one fall female is excluded due to auto-sampler malfunction). Mean difference between maximum and minimum δD values within each claw was -27.2 ± 6.52‰ for spring migrants and -36.4±11.61‰ for fall migrants. The most-enriched δD values were for the distal tip in both fall and spring migrants (Fig. 2). The lowest δD for spring migrants typically was located at 60-70% of claw length, whereas it was located usually at 80% of claw length for fall migrants (i.e. second cross-section from claw root). The resulting best-fit curve meets expected trends for fall migrants, showing a decreasing δD from tip to root. However, expected increases of δD from tip to root for spring migrants were not found.

COMPARISON OF δD_{fur} AND δD_{claw}

Among fall migrants, there was a significant difference between male and female δD values ($F = 6.11$, $P = 0.017$) with females having more depleted δD (Table 3). The δD_{claw} was significantly more depleted than δD_{fur} ($F = 33.97$, $P < 0.001$) with a highly significant interaction between sex and tissue ($F = 8.11$, $P = 0.007$). Within females, there was not a significant difference between δD_{fur} ($\bar{X}$ = -8.3‰) and δD_{claw} ($\bar{X}$ = -103.2‰; t = 1.83, P = 0.074); however, within males, δD_{claw} ($\bar{X}$ = 93.8‰) was significantly more depleted than δD_{fur} ($\bar{X}$ = -79.7‰; t = 7.51, P < 0.001). Females exhibited significantly lower δD_{fur} values than males ($\bar{X}$ = -98.3 and -79.7‰, respectively; t = 3.16, P = 0.003), but this was not true for δD_{claws} ($\bar{X}$ = -93.8 and -103.2‰, respectively; t = 1.59, P = 0.118). Congruency between δD_{fur} and δD_{claw} among females paralleled expected results. In contrast, results for males were unexpected, suggesting males do not molt at location of summer residency.

Discussion

When comparing claw layers, it was apparent that separating internal claw structures from the unguis was necessary to avoid significant bias. It is not apparent why the internal claw had more depleted δD than the unguis. While the terminal matrix and subunguis are both keratin-based tissues (Homberger et al. 2009), the phalanx is not. Metabolic processes that form bone may discriminate more strongly against deuterium than those that form keratin. Moreover, bone has low turnover rates and would not strongly reflect recent changes in animal movements; rather, the isotopic signature would likely reflect the geographic location of bone development during nursing and early postnatal ontogeny. The difference in δD between the unguis and internal claw becomes more disparate across cross-sections, with the unguis having a more enriched δD as expected, yet the internal claw becomes more depleted. Toward the tip of the unguicular process, the terminal matrix and subunguis become more pronounced while the phalanx size diminishes. The proportion change of bone to keratin may cause shifts in δD. Regardless of the biological explanation, it is important to remove internal claw layers from the unguis for isotope analysis. Otherwise, samples will be more depleted in δD, biasing assignments of migratory origins to more northern localities.

There were no significant differences between δD values among claw cross-sections for different claws from the same individual. Minimum and maximum differences among δD values within each claw's cross-sections were 6.4‰ and 13.9‰, respectively. Highest discrepancy was in the claw middle. However, the root and tip are naturally more standardized in identical sample position. Sampling across the claw can introduce small deviations in the precise location of cross-sections because samples are standardized by mass, not by cross-section width. We suspect higher variability in the claw middle was due to higher sampling error rather than growth-rate differences. The root and tip both displayed little variation among claws. Claws, including the pollex, produced similar isotope results. If growth rates vary among claws, such differences were not detected isotopically.

Metabolically, there is about a ±3‰ error in deuterium incorporation into tissues and instrumental error is expected to be ±2‰ (Wassenaar & Hobson 2006). Thus, variation within 10‰ is expected and almost all differences between claws or fur of the same individual were within expected error ranges. Analytical error rather than biological explanations may account for much of the variation exhibited among our samples. While we advocate consistently taking keratin samples from the same morphological location when possible, not being able to do so did not produce sufficient error to warrant exclusion of specimens from analyses.

Significant differences in δD_{claw} among individuals indicate that our specimens came from varying latitudes. All individuals exhibited the same directional change in δD along the claw, regardless of summer-residency location. For fall migrants, changes in δD along the claw length reflected expected patterns. Most-recently-grown tissue typically was not the most depleted in deuterium, indicating that isotopic signatures had begun incorporating into tissue during southward movements. As sampling continued toward the claw tip, sections became more depleted before becoming more enriched again. However, δD was not significantly different among the first three cross-sections from the claw root; these sections likely represented months spent in summer residency. As expected, the claw tip was the most enriched cross-section and differed from root values, likely representing isotopic values that were incorporated during spring migration. Because the claw tip has layers assimilated at different times, the use of the tip to assign migratory origins may not be optimal.

In spring-killed individuals, changes in δD along the claw did not fit expected trends if strictly south to north, spring migrations are assumed. The newly grown section was not the most-depleted sample, and cross-sections did not become consecutively more enriched as expected. Data are similar to that of fall migrants, suggesting a possible reverse-spring migration. However, two possible scenarios may explain this result. First, individuals may have been bats that over-wintered in more northern localities. Local $\delta D_{precipitation}$ varies by season and becomes more depleted in winter months; thus, changes in δD_{claw} may reflect natural changes in local water supply rather than the animal's movements. Moreover, hibernation likely affects claw growth rates because food is not actively metabolized during hibernation. For example, Godwin (1959) found a cessation of rat claw growth with poor diet, as well as a near cessation of growth in 6°C chambers. Bats over-wintering in northern localities rather than migrating southward may exhibit reduction or cessation of claw growth, and values sampled may represent retention of isotopic values grown during the previous summer/fall. Furthermore, little is known about partial migration in hoary bats, the proportion of the population adopting a hibernation strategy, and the characteristics that lead to over-wintering in northern latitudes. Additionally, individuals may not hibernate at location of summer residency, but may migrate to another location with more optimal conditions for over-wintering, a strategy common in cave species that can include locations north of summer-residency (Fleming & Eby 2003). Second, the high elevation of the Rocky Mountains and the Sierra Madre Occidental disrupt latitudinal δD patterns and cause southward bending of bands, such that $\delta D_{\mathrm{precipitation}}$ of western Mexico mimics values of the northern United States (Dansgaard 1964; Bowen *et al.* 2005). Thus, results are also consistent with a northeast migration from the western, montane regions of Mexico and could support the hypothesis of Finley & Jones (1964) that an extensive, undetected winter population exists south of the United States. Further research is needed on spring specimens to determine the cause of unexpected δD spring pattern and to determine whether such trends broadly represent the species.

We ascertained the morphological location of the lowest δD values (i.e. generally the second cross-section from the claw root for fall migrants) by sampling entire claws of several specimens. This cross-section is composed primarily of the blade horn, a portion of the claw formed primarily from the coronary band, but also includes a section of the cone horn, which is known to assimilate multiple layers grown at different times. However, claw layers along the dorsal ridge increase in thickness distally along the claw. A cross-section close to the claw root will have had little merging between layers. Given the cone horn's (1) small contribution to sample mass and (2) minor integration of claw layers, we judged cross-sections near the claw root to be best for assigning migratory origin of fall-collected individuals. This deduction was further supported by the congruency in δD_{fur} and δD_{claw} for female hoary bats.

Data for females were consistent with molt occurring during summer residency as there was no difference between δD_{fur} and δD_{claw}. Disparity in δD_{fur} and δD_{claw} of male hoary bats was unexpected. If molting occurs at the northern extent of summer residency, δD_{claw} would not be expected to be less than δD_{fur}, as was found. While isotope discrimination differences between fur and claw tissue could produce this pattern, a similar discrimination in claws of females would also be expected, a pattern not detected. Thus, our data suggest that males do not molt at locations of summer residency. Either molting occurs prior to reaching summer grounds or after fall migration has begun.

The suggestion that male hoary bats molt earlier or later than females is incongruent with results of Cryan *et al.* (2004), who found no difference in timing of molt between sexes. Our findings are comparable to results of Britzke *et al.* (2009) for the closely related eastern red bat (*Lasiurus borealis*). Female eastern red bats displayed the expected correspondence of δD_{fur} and $\delta D_{precipitation}$ during summer residency. However, for males the $\delta D_{precipitation}$ of the summer-residency location was less than δD_{fur}. The apparent explanation is that these males moved northward after molting had occurred.

Several researchers have used claws for H-isotope analysis and noted problems with assigning migratory origins (Oppel & Powell 2008; Fraser *et al.* 2010; Ethier *et al.*

2010). Unexpected results may have stemmed from the method of sampling claws (i.e. sampling only the tip and/or sampling multiple layers). Isotope analysis of claws shows promise in the study of animal migration, but one must consider issues of claw structure and growth patterns. While we analyzed only bat claws, methods we developed may be applicable to other claw-bearing mammals, as well as birds and reptiles; hooves and nails could also be sampled with modified procedures. However, differences in claw morphology and growth patterns in these other taxonomic groups must be considered before adopting our procedures (for taxonomic comparisons, see Ethier *et al.* 2010). For mammals, claws may be more appropriate than fur for use in migration studies, especially for species with unknown or poorly known molt characteristics. Concerns regarding different molt patterns between sexes, age classes, and geographic locations would be eliminated. Alternatively, in some species of mammals claws used in conjunction with fur may delineate migration patterns more accurately than either used alone.

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References

Arnett, E.B., Brown, W.K., Erickson, W.P., Fiedler, J.K., Hamilton, B.L., Henry, T.H., Jain, A. Johnson, G.D., Kerns, J., Koford, R.R., Nicholson, C.P., O’Connell, T.J., Piorkowski, M.D. & Tankersley, Jr. R.D. (2008) Patterns of bat fatalities at wind energy facilities in North America. *Journal of Wildlife Management*, **72**, 61–78.

Bearhop, S., Fiedler, W., Furness, R.W., Votier, S.C., Waldron, S., Newton, J., Bowen, G.J., Berthold, P. & Farnsworth, K. (2005) Assortative mating as a mechanism for rapid evolution of a migratory divide. *Science*, **310,** 502–504.

Belant, J.L., Kielland, K., Follmann, E.H. & Adams, L.G. (2006) Interspecific resource partitioning in sympatric ursids. *Ecological Applications*, **16**, 2333–2343.

Bowen G. J. & Revenaugh J. (2003) Interpolating the isotopic composition of modern meteoric precipitation. *Water Resources Research,* **39**, 1299. doi:10.129/2003WR002086.

Bowen, G J., Wassenaar, L.I. & Hobson, K.A. (2005) Global application of stable hydrogen and oxygen isotopes to wildlife forensics. *Oecologia*, **143**, 337–348.

Britzke, E.R., Loeb, S.C., Hobson, K.A., Romanek, C.S. & Vonhof, M.J. (2009) Using hydrogen isotopes to assign origins of bats in the eastern United States. *Journal of Mammalogy*, **90**, 743–751.

Chamberlain, C.P., Blum, J.D., Holmes, R.T., Feng, X., Sherry, T.W. & Graves, G.R. 1997. The use of stable isotope tracers for identifying populations of migratory birds. *Oecologia* 109, 32–141.

Clark, R.G., Hobson, K.A. & Wassenaar, L.I. (2006) Geographic variation in the isotopic (dD, d13C, d15N, d34S) composition of feathers and claws from lesser scaup and northern pintail: implications for studies of migratory connectivity. *Canadian Journal of Zoology*, **84,** 1395–1401.

Constantine, D.G. (1957) Color variation and molt in *Tadarida brasiliensis* and *Myotis velifer*. *Journal of Mammalogy,* **38**, 461–466.

Constantine, D.G. (1958) Color variation and molt in *Mormoops megalophylla*. *Journal of Mammalogy,* **39**, 344–347.

Cormie, A.B., Luz, B. & Schwarcz, H.P. (1994) Relationship between the hydrogen and oxygen isotopes of deer bone and their use in the estimation of relative humidity. *Geochimica et Cosmochimica Acta* **58**, 3439-3449.

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., Bogan, M.A., Rye, R.O., Landis, G.P. & Kester, C.L. (2004) Stable hydrogen isotope analysis of bat hair as evidence for seasonal molt and long-distance migration. *Journal of Mammalogy,* **85**, 995–1001.

Cryan, P.M. & Brown, A.C. (2007) Migration of bats past a remote island offers clues toward the problem of bat fatalities at wind turbines. *Biological Conservation,***139**, 1–11.

Dansgaard, W. (1964) Stable isotopes in precipitation. *Tellus* **16**, 436-468.

Ethier, D.M., Kyle, C.J., Kyser, T.K. & Nocera, J.J. (2010) Variability in the growth patterns of the cornified claw sheath among vertebrates: implication for using biogeochemistry to study animal movement. *Canadian Journal of Zoology*, **88**, 1043–1051.

Findley, J.S. & Jones, C. (1964) Seasonal distribution of the hoary bat. *Journal of Mammalogy*, **45**, 461–470.

Fleming, T.H. & Eby, P. (2003) Ecology of bat migration. *Bat ecology* (eds T.H. Kunz & M.B. Fenton), pp. 156-208. University of Chicago Press, Chicago.

Fraser, E.E. (2011) *Stable isotope analyses of bat fur: applications for investigating North American bat migration.* PhD thesis, University of Western Ontario London, Ontario, Canada.

Fraser, K.C., Kyser, T.K., Robertson, R.J. & Ratcliffe, L.M. (2008) Seasonal patterns in hydrogen isotopes of claws from breeding wood-warblers (Parulidae): utility for estimating migratory origins. *Avian Conservation Ecology*, **3**, 2.

Fraser, K.C., McKinnon,E.A. & Diamond, A.W. (2010) Migration, diet, or molt? Interpreting stable-hydrogen isotope values in Neotropical bats. *Biotropica*, **42**, 512–517.

Godwin, K.O. (1959) Experimental study of nail growth. *Journal of Nutrition*, **69**, 121–127.

Hamrick, M.W. (1999) Pattern and process in the evolution of primate nails and claws. *Journal of Human Evolution*, **37,** 293–297.

Hobson, K.A., Wassenaar, L.I., Moore,F., Farrington, J. & Wilgenburg, S.V. (2007) Estimating origins of three species of Neotropical migrant songbirds at a gulf coast stopover site: combining stable isotope and GIS tools. *Condor*, **109**, 256–267.

Henaux, V, Powell, L.A., Hobson, K.A., Nielsen, C.K. & LaRue, M.A. (2011) Tracking large carnivore dispersal using isotopic clues in claws: an application to cougars across the Great Plains. *Methods in Ecology and Evolution*, **2**, 489–499.

Homberger, D.G., Ham, K., Ogunbakin, T., Bonin, J.A., Hopkins, B.A., Osborn, M.L., Hossain, I., Barnett, H.A., Matthews, K.L., II, Butler, L.G. & Bragulla, H.H.

(2009) The structure of the cornified claw sheath in the domesticated cat (*Felis catus*): implications for the claw-shedding mechanism and the evolution of cornified digital end organs. *Journal of Anatomy*, **214**, 620–643.

Kelly, J.F. (2006) Stable isotope evidence links breeding geography and migration timing in wood warblers (Parulidae). *Auk*, **123**, 431–437.

Kelly, J.F., Bridge, E.S., Fudickar, A.M. & Wassenaar, L.I. (2009) A test of comparative equilibration for determining non-exchangeable stable hydrogen isotope values in complex organic materials. *Rapid Communications in Mass Spectrometry*, **23**, 2316–2320.

Kielland, K. (2001) Stable isotope signatures of moose in relation to seasonal forage composition: a hypothesis. *Alces*, **37,** 329–337.

Lott, C.A., & Smith, J.P. (2006) A geographic-information-system approach to estimating the origin of migratory raptors in North America using stable hydrogen isotope ratios in feathers. *Auk*, **123**, 822–835.

MacCallum, A.J., Knight, C.H., Hendry, K.A.K., Wilde, C.J., Logue, D.N. & Offer, J.E. (2002) Effects of time of year and reproductive state on the proliferation and keratinisation of bovine hoof cells. *Veterinary Record*, **151**, 285–289

Mercer, E.H. (1961). *Keratin and keratinization*. Pergamon Press, Oxford.

Mulling, C.K., Bragulla, H.H., Reese, S., Budras, K.D. & Steinberg, W. (1999) How structures in bovine hoof epidermis are influenced by nutritional factors. *Anatomia, Histologia, Embryologia*, **28**, 103–108.

Oppel, S. & Powell, A.N. (2008) Assigning king eiders to wintering regions in the Bering Sea using stable isotopes of feathers and claws. *Marine Ecology Progress Series*, **373**, 149–156.

Paritte, J.M. & Kelly, J.F. (2009) Effect of cleaning regime on stable-isotope ratios of feathers in Japanese quail (*Coturnix japonica*). *Auk*, **126**, 165–174.

Prentice, D.E. (1973) Growth and wear rates of hoof horn in Ayrshire cattle. *Research in Veterinary Science*, **14**, 285–290.

Quay, W.B. (1970) Integument and derivatives. *Biology of Bats* (ed W.A. Wimsatt), pp. 1–56. Academic Press, New York.

Shump, K.A. & Shump, A.U. (1982) *Lasiurus cinereus*. *Mammalian Species*, **183**, 1–6.

Tiunov, M.P. & Makarikova, T.A. (2007) Seasonal molting in *Myotis petax* (Chiroptera) in the Russian Far East. *Acta Chiropterologica*, **9**,538–541.

Wassenaar, L. I. & Hobson, K.A. (2003) Comparative equilibration and online technique for determination of non-exchangeable hydrogen of keratins for use in animal migration studies. *Isotopes in Environmental and Health Studies*, **39**, 211–217.

Wassenaar, L.I. & Hobson, K.A. (2006). Stable-hydrogen isotope heterogeneity in keratinous materials: Mass spectrometry and migratory wildlife tissue subsampling strategies. *Rapid Communications in Mass Spectrometry*, **20**, 2505–2510.

Williams, D.F. & Findley, J.S. (1979) Sexual size dimorphism in vespertilionid bats. *American Midland Naturalist*, **102**, 113–127.

Whitaker, J O., Jr. (1967) Hoary bat apparently hibernating in Indiana. *Journal of Mammalogy*, **48**, 663.

Yaensiri, S., Hou, N., Slining, M.M. & He, K. (2010) Growth rate of human fingernails and toenails in healthy American young adults. *Journal of European Academy of Dermatology and Venereology*, **24**, 420–423.

Table 1. Comparison of δD (‰) for unguis and subunguis claw layers, as well as for these same layers combined

Specimen	Unguis cross-section*			Subunguis cross-section*			Both cross-section*		
no.	A	B	C	A	B	C	A	B	C
				Males					
23	-69.2	-74.4	–	-107.9	-110.6	–	-96.6	-94.7	–
24	-90.7	-77.8	-74.3	-111.6	-97.3	-105.2	-100.3	-89.5	-85.7
20	-89.4	-99.1	–	-126.4	-151.9	–	-89.7	-110.8	–
48	-83.5	-78.7	-70.5	-97.0	-93.0	-135.5	-103.7	-99.4	-84.7
27	-94.8	-82.9	–	-167.3	-175.7	–	-109.7	-110.5	–
Mean	-85.5	-82.6	-72.4	-122.1	-125.7	-120.4	-100.0	-101.0	-85.2
				Females					
114	-139.5	-134.8	-120.3	-146.9	-151.6	-173.6	-142.8	-139.1	-136.0
21	-80.6	-83.8	-84.9	-118.3	-120.4	-126.1	-93.0	-96.0	-94.6
25	-82.1	-83.0	-80.4	-105.5	-110.8	-131.7	-99.1	-95.3	-93.2
106	-86.8	-95.9	-91.1	-117.2	-107.3	-110.0	-89.5	-100.7	-96.6
63	-89.8	-91.5	-94.5	-122.1	-124.9	-148.4	-115.9	-105.9	-106.8
Mean	-95.8	-97.8	-94.2	-122.0	-123.0	-138.0	-108.1	-107.4	-105.4

*Sectioning began at claw root, continuing distally for three-consecutive cross-sections.

Table 2. δD_{claw} (‰) heterogeneity of samples taken from an individual hoary bat

Location	Cross-section (from tip to root)					
	A	B	C	D	E	F
Pollex*	-60.5	-65.4	-84.1	-84.0	-90.0	-79.1
Digit I	-59.1	-65.3	-72.8	-88.6	-89.2	-90.7
Digit II	-59.0	-73.8	-75.2	-75.2	-91.7	-81.1
Digit III	-66.1	-68.3	-84.1	-80.2	-82.6	-81.9
Digit IV	-62.5	-67.3	-79.0	-85.1	-85.3	-85.8
Digit V	-67.7	-73.6	-83.9	-89.1	-89.1	-81.8
Mean ± SEM	-62.9 ± 1.77	-69.7 ± 1.72	-80.0 ± 2.27	-83.6 ± 2.64	-87.6 ± 1.61	-84.3 ± 1.80

*δD values for cross-sections G-J were: -91.8; -83.2; -73.5; -82.0.

Table 3. Comparison of δD_{fur} and δD_{claw} (‰) in samples taken from same individual hoary bats, separated by gender

Specimen no.	δD_{fur}	δD_{claw}
Males		
20	-79.83	-99.13
23	-67.29	-78.51
24	-93.72	-90.66
33	-79.02	-83.44
43*	-71.25	-87.54
48	-65.44	-83.50
50	-88.02	-100.97
62	-53.69	-73.55
65	-77.76	-112.17
66	-111.49	-125.83
67	-89.95	-99.20
69	-107.42	-123.17
71	-78.83	-90.74
75	-54.30	-75.14
78	-73.57	-91.28
88*	-65.77	-86.29
89	-91.72	-81.65
91	-87.89	-98.81
92	-62.11	-78.47
93	-78.22	-88.17
101*	-61.18	-72.80
103	-74.40	-96.51
105	-113.51	-122.91
107	-67.14	-69.74
115	-62.40	-100.89
118	-121.75	-145.44
120	-100.80	-95.72
126	-86.30	-112.66
130	-70.44	-78.24
135	-57.05	-59.17
136	-86.43	-103.70
137	-70.72	-94.97
Mean ± SEM	-79.67 ± 3.11	-93.78 ± 3.27

Table 3 continued.

Specimen no.	δD_{fur}	δD_{claw}
Females		
21	-83.11	-84.94
25	-105.09	-83.02
26*	-100.87	-115.41
47	-118.19	-113.88
63	-89.38	-100.04
64*	-81.84	-95.16
68	-115.19	-125.15
70*	-72.06	-85.89
73*	-73.34	-93.92
87*	-73.21	-77.91
97*	-78.39	-93.63
106	-95.07	-95.88
111*	-117.85	-108.77
113	-119.97	-127.10
114	-158.16	-151.75
116	-91.14	-97.97
Mean ± SEM	-98.30 ± 5.83	-103.15 ± 4.88

*Sex was based on morphometric measurements.

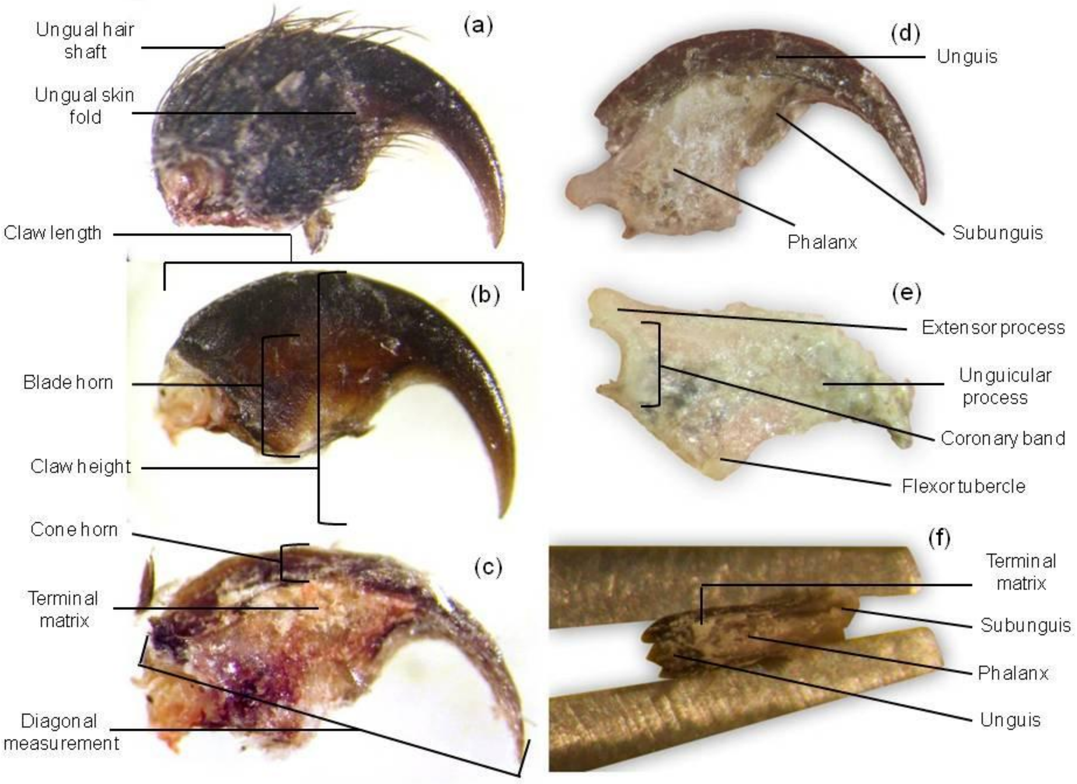

Fig. 1. Gross morphological characteristics and terminology for hoary bat claws. Bat-claw representations for (a) unmodified claw with skin fold still present, (b) claw with skin fold removed, (c) claw with blade horn removed revealing terminal matrix, (d) claw with terminal matrix removed revealing position of unguis and subunguis on phalanx, (e) phalanx with all keratinous tissues removed, and (f) lateral cross-section of claw (held by forceps).

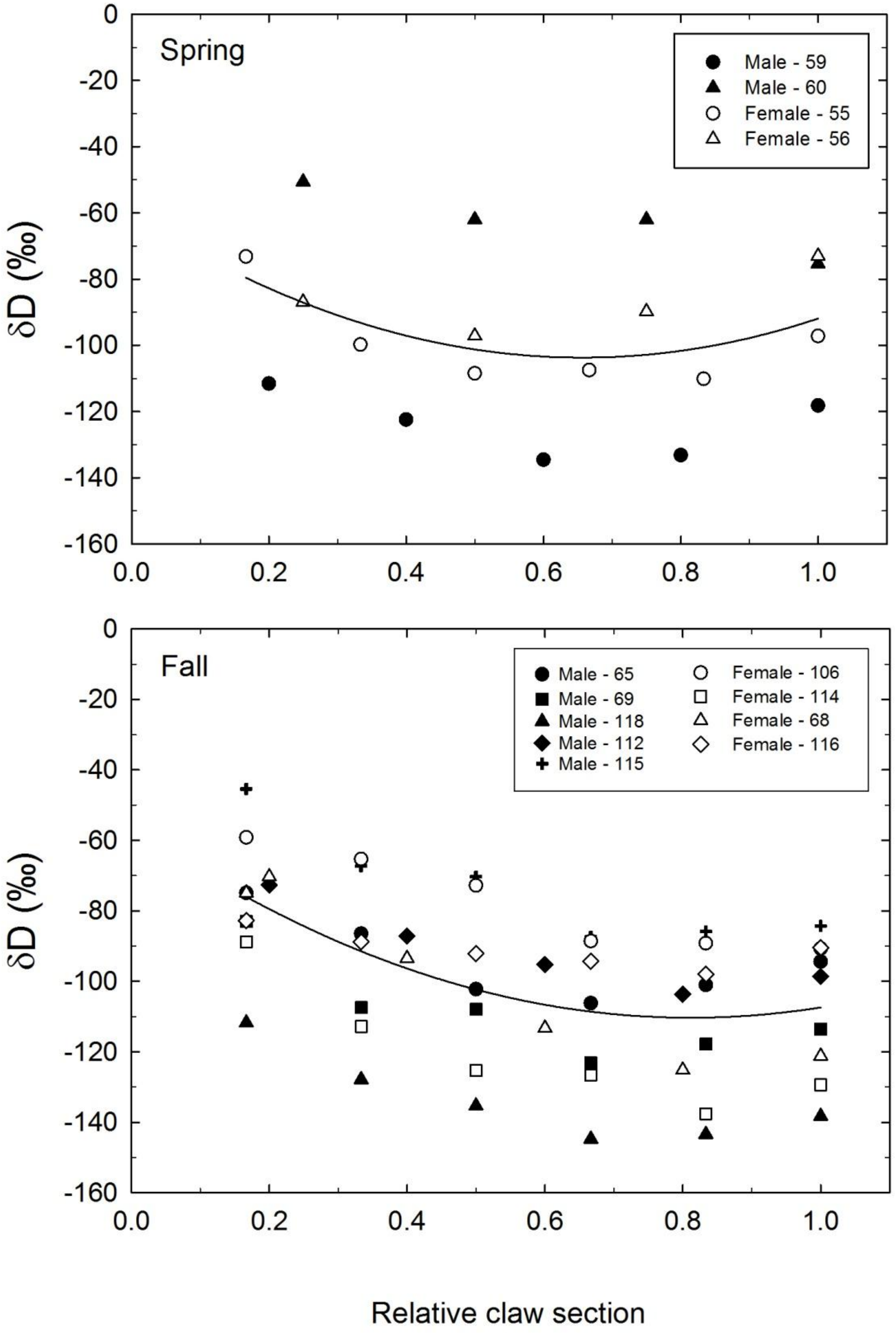

Fig. 2. Non-linear curve fit of δD for claw cross-sections from claw tip to root of spring-killed ($y = 60.45 – 131.70x + 100.22x^2$; $r^2 = 0.094$) and fall-killed ($y = -56.01 - 1.33.91x + 82.51x^2$; $r^2 = 0.277$) hoary bats. Low r^2 values due primarily to variation among individuals.

Supplemental Information

SEX DETERMINATION

For bats in which sex could not be determined by visual inspection, morphometric measurements were taken after specimen processing in order to determine the probable sex. Hoary bats are sexually dimorphic in size with females being larger than males, as first described by Williams and Findley (1979). We measured the forearm length (FAL), condylocanine length (CCL), and maxillary tooth row length (MTL). Measurements were made to the nearest 0.01 millimeter with digital calipers as follows: FAL – entire length of the humerus, CCL – distance from the occipital condyle to the anterior edge of the upper canine, and MTL – distance from the posterior edge of the third upper molar to the anterior edge of the upper canine. Total body length is a standard measurement but bats were typically stiffened in a curled mass upon collection, preventing reliable body length measurement. For some specimens, the skull and/or forearm was broken resulting in missing measurements.

We calculated the mean ± standard deviation (SD) for the FAL, CCL, and MTL for all known males and females. Our measurements were compared with those of Williams and Findley (1979) to ensure congruency between our study and theirs (Table 1). Values obtained in this study were used to assign unidentified specimens to the probable sex. The measurement length for FAL, CCL, and MTL between males and females overlap within two SD of the mean. Therefore, specimens were only considered a female if at least one measurement value was greater than +2 SD of mean male measurements. Likewise, specimens were only considered males if at least one measurement was less than -2 SD of mean female measurements. If all measurements consistently fell within the overlap of two SD between males and females the carcass was still considered unidentifiable.

ISOTOPE HETEROGENEITY AMONG FUR SAMPLES

For most seasonally molting species, the molt often begins in one region of the body and forms bands that progressively wave across the body, but the direction in which they progress may vary among species (Chase and Eaton 1959). For some bat species, molt begins in the lower dorsal region and progresses anteriorly in waves across the dorsum (Constantine 1957; Dwyer 1963; Tiunov and Makarikova 2007). Because the pattern of fur replacement in bats appears to proceed from the posterior to the anterior dorsum, we compare fur samples from the lumbar and scapular region of the same individual. Because additional fur samples for this experiment were collected after museum preparation, only bats in which the skin could be salvaged (n=9) were available for analysis. If molting is not complete prior to migration, we expected the lumbar region to have more depleted δD values than the scapular region. If the entire molting process occurs prior to migration, we expected that there will be no difference in δD between fur samples from the same individual. A molt completed prior to migration would enable the collecting of tissue samples from different bodily locations without introducing bias. A paired t-test was used to assess the heterogeneity among the two fur samples.

The difference exhibited among fur samples from the same individual ranged between -12.9 and 9.2‰ (mean -2.1‰ ±7.79; Table 2). There was no significant difference found between samples taken from the anterior (scapular region) and posterior (lumbar region) dorsum ($t = -0.824$, $df = 8$, $P = 0.434$) as one isotopic value was never consistently higher or lower than the other. This is not consistent with the disparity caused by an incomplete molt prior migration, but rather is more indicative of analytical error. Expected analytical error ranges within 10‰ (Wassenaar & Hobson 2006); thus differences are similar to expected values. This result suggests that molting has occurred prior to migration and that collecting fur samples from different areas of the dorsum will not substantially bias results.

ISOTOPE HETEROGENITY AMONG CLAWS FROM THE SAME INDIVIDUAL

All claws, including that of the pollex (thumb), were collected from the left side of specimen 106. Size dimensions (claw length, height, and diagonal; see main text Fig. 1) of each claw are provided in Table 3. Excluding the pollex, diagonal measurements for pedal claws were similar, but length and height measurements varied. For example, digits II and IV have relatively opposite dimensions, with digit II being shorter in claw length with a greater claw height, and digit IV having a greater claw length with a shorter claw height. Despite these size differences, δD values of claw cross-sections exhibited little variation (Table 4 – modified version of Table 2 in text of article to include additional data). In addition to all claws of specimen 106 being cross-sectioned, the pollex and a single pedal claw also were compared from specimen 114 as well as the distal tip of specimen 137. If claw growth rates vary, we surmised that δD differences may be most pronounced at the distal tip of the claw. However, no meaningful differences were found between either specimen 106 or 137. The disparity of δD between claw tips was 8.7‰ and 8.5‰, respectively.

Despite the pollex bearing a larger claw, the range of δD values was very similar to those of pedal claws (Table 4). One advantage of the larger size is that thinner cross-sections may be obtained, providing better isotopic resolution because less integration and isotopic averaging per section has occurred. This is circumstantially supported by the pollex cross-sections of specimen 114, which was the most depleted in deuterium, showing a difference of 14.1‰ compared to pedal claws. However, no disparity was detected between thumb and foot claws in specimen 106, with the most depleted thumb cross-section being nearly identical to that of all pedal claws (Table 4). An analysis of additional specimens would be required to confirm this supposition. If such a benefit does exist, the location of the most depleted section in the pollex claw would differ from pedal claws (i.e. the most-depleted cross-section in the thumb claw is not likely to be the second cross-section from the claw root) and experiments similar to those presented in the article would be necessary to ascertain the ideal claw cross-section location.

Overall, given that isotopic averaging occurs in claws, as well as the differences in size dimensions among claws, δD values among claws of the same individual are remarkably similar. If differences in claw growth rates exist, growth rate does not appear to influence isotope ratios of cross-sections to a detectable limit.

Table 1: Mean (mm) ± SD measurements for hoary bat specimens of identifiable sex

Sex	FAL	CCL	MTL
Male	51.96 ± 0.990 (n=32)	16.46 ± 0.332 (n=29)	6.00 ± 0.142 (n=31)
Mean + 2 SD*	53.94	17.12	6.28
Williams and Findley 1979	52.64 ±1.375 (n=30)	16.69 ± 0.367 (n=30)	6.13 ± 0.137 (n=30)
Female	54.94 ± 0.990 (n=12)	17.33 ± 0.371 (n=6)	6.46 ± 0.181 (n=11)
Mean - 2SD *	52.96	16.59	6.10
Williams and Findley 1979	54.22 ± 1.462 (n=30)	17.34 ± 0.361 (n=30)	6.47 ± 0.148 (n=30)

* a measurement of an unknown specimen that fell within the overlap of 2 SD (FAL: 52.96-53.94, CCL: 16.59-17.12 , MTL: 6.10-6.28) was considered inconclusive for sex determination

Table 2: Difference in δD between scapular and lumbar regions of the same individual hoary bat

Specimen	Sex	Scapular	Lumbar	δD difference
47	Female	-118.19	-105.27	-12.92
106	Female	-95.07	-85.54	-9.53
51	Male	-88.02	-90.79	2.76
65 *	Male	-77.76	-74.22	-3.54
81	Male	-86.18	-89.88	3.70
91	Male	-87.89	-77.06	-10.83
107	Male	-67.14	-64.14	-3.00
135	Male	-48.21	-53.12	4.91
136	Male	-69.04	-78.24	9.20

* Additional fur samples were taken from specimen 65 from the top of the head (δD = -83.24), mid-dorsum (δD = -81.36), and the ventral side (δD = -86.56) for a maximum δD difference of -12.34

Table 3. Size comparison (mm) among claws of manual digit I and all pedal digits from an individual hoary bat

Measurement	Pollex	Pedal digit				
		I	II	III	IV	V
Length	2.72	2.37	2.30	2.62	2.64	2.28
Height	3.32	2.31	2.93	2.74	2.29	2.61
Diagonal	3.98	3.41	3.40	3.43	3.44	3.47

Table 4. Heterogeneity of δD_{claw} values between the thumb (pollex) claw and pedal claws (digits I-V) in each of three individual[a] hoary bats

Location	Cross-section (from claw tip to claw root)					
	A	B	C	D	E	F
			Specimen 106			
Pollex[b]	-60.5	-65.4	-84.1	-84.0	-90.0	-79.1
Digit I	-59.1	-65.3	-72.8	-88.6	-89.2	-90.7
Digit II	-59.0	-73.8	-75.2	-75.2	-91.7	-81.1
Digit III	-66.1	-68.3	-84.1	-80.2	-82.6	-81.9
Digit IV	-62.5	-67.3	-79.0	-85.1	-85.3	-85.8
Digit V	-67.7	-73.6	-83.9	-89.1	-89.1	-81.8
Mean ± SEM	-62.9 ± 1.77	-69.7 ± 1.72	-80.0 ± 2.27	-83.6 ± 2.64	-87.6 ± 1.61	-84.3 ± 1.80
			Specimen 114			
Pollex[c]	-91.6	-111.5	-123.8	-135.1	-144.4	-151.8
Digit III	-88.8	-112.8	-125.3	-126.6	-137.6	-129.4

[a] Cross-section A also analyzed for specimen 137. δD values for pollex, digits I-V and mean ± SEM, respectively, were: -79.7; -78.2; -69.7; -76.5; -77.0; -71.7; -75.5 ± 3.92.

[b] δD values for cross-sections G-J were: -91.8; -83.2; -73.5; -82.0.

[c] δD values for cross-sections G-J were: -141.2; -143.4; -144.9; -136.1.

References

Chase, H.B. & Eaton, G.J. (1959) The growth of hair follicles in waves. *Annual New York Acadamy of Science*, **83**, 365-368.

Constantine, D.G. (1957) Color variation and molt in *Tadarida brasiliensis* and *Myotis velifer*. *Journal of Mammalogy,* **38**, 461–466.

Constantine, D.G. (1958) Color variation and molt in *Mormoops megalophylla*. *Journal of Mammalogy*, **39**, 344–347.

Dwyer, P.D. (1963) Seasonal changes in pelage of *Miniopterus schreibersi blepotis* (Chiroptera) in north-eastern New South Wales. *Australian Journal of Zooogy,* **11,** 290-300.

Tiunov, M.P. & Makarikova, T.A. (2007) Seasonal molting in *Myotis petax* (Chiroptera) in the Russian Far East. *Acta Chiropterologica*, **9**,538–541.

Wassenaar, L.I. & Hobson, K.A. (2006). Stable-hydrogen isotope heterogeneity in keratinous materials: Mass spectrometry and migratory wildlife tissue subsampling strategies. *Rapid Communications in Mass Spectrometry*, **20**, 2505–2510.

Williams, D.F. & Findley, J.S. (1979) Sexual size dimorphism in vespertilionid bats. *American Midland Naturalist*, **102**, 113-127.

Assessing migratory patterns of hoary bats (*Lasiurus cinereus*) from wind-farm fatalities using stable-hydrogen isotopes

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Rapid development of wind energy has caused bat fatalities and generated concern for population sustainability of affected species. Hoary bats (*Lasiurus cinereus*) are among the bat species most susceptible to wind-turbine fatalities. Our limited knowledge of bat migration makes it difficult to predict risk of fatality and long-term demographic impacts of wind-energy development. We used stable-hydrogen isotope ratios from fur (δD_{fur}) and claws ($\delta D_{claw)}$ of hoary bats killed at a wind-energy installation in western Oklahoma to assess migratory patterns of the species. Usefulness of this sampling approach is influenced by our understanding of patterns of fur and claw growth. Among male hoary bats, δD_{claw} was significantly depleted relative to δD_{fur} indicating that these bats most likely did not molt at their location of summer residency; this pattern was not evident for females. Based on δD_{claw}, we conclude that all females and 87.8% of males recovered at the wind farm were not summer residents, but migrants from other geographic locations. We estimated that locations of summer residency for the bats we sampled were from a broad range of latitudes that extended from Oklahoma into northern Canada (δD from -43.6‰ to -153.0‰). We cannot be certain what portion of the claw was grown during spring migration and/or at wintering grounds, but

$\delta D_{\text{claw tip}}$ for most specimens suggests that the bats we sampled wintered at localities south of Oklahoma (δD from -22.3‰ to -103.6‰). The difference between the $\delta D_{\text{claw root}}$ and $\delta D_{\text{claw tip}}$ within an individual significantly increased with increased latitudinal assignments (based on $\delta D_{\text{claw root}}$), indicating that a differential migration occurs with some individuals migrating greater distances than others. Differential migration was not explained by sex, because there was no difference in latitudinal distributions of males and females. There was a significant relationship between the arrival date at the study site and $\delta D_{\text{claw root,}}$ with individuals from lower latitudes arriving before those farther north. Our study highlights the utility of wind-farm fatalities as a source of information on bat migratory patterns and, in turn, insights to the development of mitigation strategies for lessening turbine fatalities.

Key Words: bat claws, bat migration, deuterium, hoary bat, hydrogen isotopes, *Lasiurus cinereus*, stable isotopes, wind energy, wind-turbine fatalities

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The hoary bat (*Lasiurus cinereus*) is the most wide-spread American bat, occurring throughout the length of South America, across Mexico and the contiguous United States, and into central Canada (Shump and Shump 1982). They are solitary, foliage-roosting bats and appear to have sex-biased geographic segregation, except during mating season, with females more concentrated in the eastern United States and males in the western United States (Cryan 2003; Findley and Jones 1964). Sex segregation along elevation gradients has also been documented, with males selectively occupying higher elevations (Cryan et al. 2000; Findley and Jones 1964). Hoary bats migrate, but migration routes and specific wintering grounds have not been firmly established. Museum records during winter months mostly are from localities in southern California and Mexico, so these regions may represent over-wintering sites (Cryan 2003). However, specimens collected in winter as far north as Michigan suggests a partial migration with some individuals hibernating instead of migrating (Findley and Jones 1964; Harvey et al. 2011; Whitaker 1967).

Understanding of bat migration has become increasingly important as several migratory, tree-roosting species are being killed at wind-turbine facilities; hoary bats often represent the highest number of fatalities (Arnett et al. 2008). Fatalities of hoary bats at these facilities have been highly concentrated during late summer and early fall (August-September; Arnett et al. 2008) and likely are associated with fall migratory behavior (Cryan and Brown 2007). Our relatively meager knowledge of migratory patterns limits understanding of causes and implications of such fatalities. Additionally, even in the eastern United States, where females are disproportionately represented, fatalities of hoary bats often are male biased (Arnett et. al 2008).

Our knowledge concerning bat migration, especially for solitary, tree-roosting species like the hoary bat, is limited, in part, due to difficulty in tracking individuals with traditional field techniques (i.e., mark/recapture, radiotelemetry). Development of stable-isotope techniques has provided new opportunities to investigate questions regarding migration. Specifically, stable-hydrogen isotopes are useful because regional differences in isotope ratios roughly correspond to latitudinal bands across the eastern United States and much of Canada (see example in Fig. 1; Bowen et al. 2005; Dansgaard 1964). Furthermore, the hydrogen-isotopic signature of a particular geographic region is reflected in the local meteoric water and trophic webs of the area (e.g., Cormie et al. 1994). As food and water are ingested, the isotopic ratio of the consumer arrives at an equilibrium with the isotope ratio characteristic of the geographic region. Most of the hydrogen in keratin-based tissues (e.g., hair and claws) is inert (i.e, carbon bound) once formed and therefore these tissues retain the isotopic signatures of the geographic region in which they were formed for some time after migration (Chamberlain et al. 1997) or until molting occurs. Thus, researchers can assess migratory patterns by analyzing the isotopic composition of an animal's tissues. Such techniques have been employed to address migratory questions for various bird species (e.g., Hobson et al. 2007; Kelly et al. 2002; Rubenstein et al. 2002) and more recently in bats (Britzke et al. 2009; Cryan et al. 2004; Fraser et al. 2010; Fraser 2011).

Reliability of this method is influenced by knowing the patterns of tissue growth, such as the timing of molt and claw growth rates, as well as any isotope discrimination that may occur between the isotope ratio of the animal's diet and the tissue in which it is metabolized. Bats typically molt once per year (Quay 1970) and, in temperate regions, this often occurs during the summer (Constantine 1957, 1958; Tiunov and Makarikova 2007). Cryan et al. (2004) inferred that hoary bats likely molt in summer prior to fall migration; if this is true, then isotopic values in hair samples should reflect the geographic region of summer residency and migratory origin. However, studies of hair samples of other bat species collected during summer residency, including the eastern red bat (*Lasiurus borealis*), which is closely related to the hoary bat, have given mixed results when used to evaluate the congruency of observed and expected hydrogen-isotope values (Britzke et al. 2009; Fraser 2011). Isotope ratios of hair samples have not always matched the expected isotopic values from the location in which they presumably were grown. Differential molting times may occur among sexes, age classes, and geographic locations (Britzke et al. 2009; Hobson et al. 2012) and could influence hydrogen-isotope values obtained from hair samples.

Unlike fur, claws grow continually and their isotopic values do not represent a single geographic location but change sequentially as an animal migrates. Since claw growth is not dependent on the timing of a molt, claw isotopes may be useful in reducing uncertainty in the interpretation of isotope values found in hair samples. For claws of fall migrants, cross-sections near the root of the claw likely grew during summer residency, but the tip may have been grown in winter (Burba 2013); as a result, the magnitude of spring migration would be reflected as the difference in hydrogen-isotope ratios between the claw root and the claw tip. We sampled bat claws as a means of investigating migratory patterns. Properly sampled, claw tissue may be a better indicator of migratory origins than that of fur, especially given the evidence that male hoary bats may not molt at location of summer residency (Burba 2013). Additionally, ability to infer multiple geographic locations for each individual also may provide more power in discerning migratory patterns.

Migratory connectivity describes the degree to which individuals or populations are geographically linked throughout periods of their annual migratory cycles (Marra et al. 2006). In the case of fatalities at specific wind-energy installation, all individuals share at least one geographic point in their fall migration. However, it is unknown whether other geographic locations (e.g., wintering and summer grounds) are shared by the individuals migrating contemporaneously in fall.

While understanding migratory patterns may be helpful in developing approaches to mitigate wind-farm fatality risks, locations of migratory origins are important considerations for the potential impact of wind energy on long-term population sustainability. The geographic distribution of fatalities (i.e., individuals being from the same geographic region vs. individuals from many different geographic regions) can influence the degree of population impacts. This is especially true if hoary bats are philopatric, as is typical for temperate vespertilionid bats and has been anecdotally reported for hoary bats (Arnold 2007; Willis 2003). Philopatric populations likely possess low interannual variation in migratory pathways. Wind-energy facilities constructed within migratory corridors may cause localized population reduction if a specific population is exposed to repeated, annual fatality events. Furthermore, recolonization of these areas may not readily occur if individuals typically return to natal localities, especially given the sex-segregation of this species. Thus, fatalities occurring at a specific wind farm may be impacting populations and long-term community composition of other geographic regions.

We investigated migratory patterns through stable-hydrogen isotopes of hoary bats using fur and claw samples taken from specimens collected during fall from a wind-turbine facility in Oklahoma. Hoary bats may be summer residents in Oklahoma; therefore, we assessed isotopic values of individuals killed at the wind-turbine facility to determine whether fatalities represented local summer-residents or fall migrants from other geographic regions. For those from other regions, we investigated the distribution of geographic regions from which individuals were migrating, as well as patterns in their arrival time at the wind-energy facility. Such information may provide new insights into bat migratory behavior, which in turn, may help to better understand the population structure of hoary bats and aid in management decisions to minimize wind-turbine fatalities.

MATERIALS AND METHODS

Specimen and tissue collection.—We collected 41 adult male and 18 adult female hoary bats during August-October of 2006-2008 as part of a wind-turbine fatality study at Blue Canyon II Windpower Project (hereafter referred to as the study site) located along the border of Caddo and Kiowa counties of western Oklahoma (coordinates 34°52.41’ N, 98°39.71’ W; elevation ca. 680 m). For 18 of the specimens (eventually determined to be 9 males and 9 females), carcass condition prevented sex identification by visual examination of sex organs, so sex was determined through morphometric measurements (see Burba 2013). We performed carcass searches weekly during the survey period, but occasionally intervals up to 2 weeks occurred.

To estimate the number of days since death, we assessed carcass decay. To aid in these estimates, larvae and pupae of blowflies (family Calliphoridae) were collected, measured, and assessed for current developmental stage. We did not identify blowfly species, but used a mean development time for all species studied by Anderson (2000). Given that most bats were collected in August and September, we assumed that temperatures were within the optimal range for flies to complete their lifecycle in about 19 days (Anderson 2000). Accordingly, the collection date was adjusted to more accurately reflect mortality date of individuals at the study site.

Carcasses were prepared for archival storage at the Sam Noble Oklahoma Museum of Natural History. During preparation, fur samples for most specimens were clipped from the scapular region with surgical scissors. Carcass deterioration or damage in the scapular region of 19 bats required us to take a fur sample from an alternative morphological site. Burba (2013) evaluated differences in isotope values of fur among the morphological sites used in this study: scapular, lumbar, mid-dorsal, ventral, and head region, as well as comparison between the scapular and lumbar region for 9 bats, and found isotope heterogeneity among morphological sites was low and within expected error ranges. The use of multiple sample locations will likely result in little, if any, bias (Burba 2013).

Most specimens were not suitable for preparation as museum skins, therefore, carcasses were skeletonized and cleaned in a captive dermestid beetle colony. Claws were collected from all specimens that had them intact (n = 50) by disarticulating the distal phalanx from the intermediate phalanx. Claws could not consistently be collected from the same phalanx as some were damaged or disarticulated during dermestid processing. For two specimens, every claw (5 pedal and 1 manual) was taken from the left side of the individual to assess isotope heterogeneity among claws. For another specimen, only 1 pedal and 1 manual claw were compared. Isotope values did not differ among claws of the same individual (Burba 2013); therefore, use of claws from various pedal digits should not bias results.

Stable-isotope processing.—Claws were inspected to determine the absence of the ungual skin fold, which often is removed during cleaning by dermestid beetles. Claws with the ungual skin still present were soaked in deionized water to soften the dermal tissue, and skin was removed from the claw with forceps to expose the entire claw. Claw and fur samples were placed in 1-mL eppendorf vials. Cleaning procedures followed recommendations of Paritte and Kelly (2009). Tissue samples were first washed in a 1:30 (v/v) solution of Fisher Versa-Clean detergent and deionized water to remove dirt and surface oils. Each vial was filled with detergent solution, sealed, and shaken for 30 s. The solution was then decanted with disposable pipettes to remove liquid and then the vial was refilled with deionized water. Vials were sealed, shaken for 30 s, and decanted of deionized water for 10 repetitions to rinse detergent solution from tissue samples. After washing, a secondary treatment of 2:1 (v/v) chloroform:methanol solution was added to each vial. Vials were sealed, shaken for 30 s, and decanted. The vials were left open and allowed to air dry in a fume hood for 24 h.

Claws of hoary bats killed in fall showed an enrichment of deuterium from the claw root to the claw tip (Burba 2013). The claw root, the most recently grown portion of the claw, is the most depleted in deuterium and represents tissue likely grown during summer residency. Enrichment of deuterium toward the tip of the claw suggests that isotopic remnants of spring migration are retained in the claw and the location of wintering grounds also may be detectable. Thus, we cross-sectioned claws of hoary bats to obtain the tissues most depleted and most enriched in deuterium. See methods developed by Burba (2013) for more details and justification for the following sample procedure. Under a dissecting microscope, we made a lateral cut, vertically and ventrally, with a scalpel 1 mm from the extensor process of the phalanx through the point of the flexor tubercle. We made another lateral cut distally to obtain a single, thin cross-section from the center of the blade horn (side wall) of the claw; this portion of the claw is typically the most depleted in deuterium and thought to represent tissue grown at location of summer residency. We inserted a fine-needle probe between the phalanx (bone) and the unguis (keratinous sheath), moving between the claw layers to mechanically separate the unguis from the underlying claw structures. We obtained a 100 ± 10 µg sample of unguis keratin from each cross-section near the claw root. Similarly, a 100 ± 10 µg sample was taken from the most distal portion of the claw tip, the portion of the claw most enriched in deuterium, which may represent location of winter residency. The various tissues present at the base of the claw (phalanx, terminal matrix, and subunguis) do not extend into the tip of the claw, which is composed entirely of keratin, eliminating the need to separate claw layers from the claw-tip samples.

After the fur was cleaned using the procedures described above, we also collected fur samples of 150 ± 10 µg from each specimen. For claw samples, we selected the reduced mass to allow thinner cross-sections for better isotopic resolution, because claws are continually integrating new tissue. All fur and claw samples were packed in 3.5 mm × 5 mm silver-foil capsules, as were isotope standards of the same mass (100 ± 10 µg for claws and 150 ± 10 µg for fur). Standards used were chicken feathers (CFS), cow hooves (CHS), bowhead whale (*Balaena mysticetus*) baleen (BWB), and brown-headed cowbird (*Molothrus ater*) feathers (BHCO). The first 3 standards were supplied by the Environment Canada Stable Isotope Hydrology and Ecology Research Laboratory in Saskatoon, Saskatchewan, as developed by Wassenaar and Hobson (2003). The last (BHCO) was developed at the University of Oklahoma (for details of its development, see Kelly et al. 2009).

Given the different sample mass between fur and claws, we analyzed these two tissue types in separate runs. All isotope-ratio data were collected at the University of Oklahoma with a ThermoFinnigan Delta V isotope ratio mass spectrometer connected to a high-temperature pyrolysis elemental analyzer (TC/EA, ThermoFinnigan, Bremen, Germany). Samples were automatically dropped from a 50-position zero-blank autosampler. Standards were placed at the beginning, middle, and end of each autorun to correct for instrumental drift and exchangeable hydrogen. All values are reported as the non-exchangeable fraction of hydrogen in each tissue. Stable-isotope ratios are expressed in standard δ notation, where

$$\delta D = 1000\left[\frac{isotope\ ratio\ of sample}{isotope\ ratio\ of\ standard} - 1\right].$$

Thus, δD is expressed in parts-per-thousand (‰) deviation from Vienna Standard Mean Ocean Water (VSMOW). For more specific details of methods, refer to Kelly et al. (2009). We denote the different δD measurements as follows: hair samples as δD_{fur}, claw cross-sections 1mm from extensor process as $\delta D_{claw\ root}$, claw cross-sections from the distal tip of the claw as $\delta D_{claw\ tip}$, and precipitation values as δD_{precip}.

Cryan et al. (2004) estimated an isotope discrimination factor for hoary bats as follows: $\delta D_{fur} = -24.81 + 0.7884(\delta D_{precip})$. Using this equation, δD_{fur} and δD_{claw} of our specimens were converted to the δD_{precip} to adjust tissue samples to the geographic location where the tissue grew. All δD values are reported accordingly. Expected δD_{precip} for the study site was determined using geospatial data available from www.waterisotopes.org (Bowen 2012a). Because there are no water-isotope monitoring stations in western Oklahoma, precipitation values were interpolated (Bowen et al. 2005; Bowen 2012b). Furthermore, there have been no studies in bats that evaluate the congruency of observed δD in claw keratin to expected values based on δD_{precip}. To better support the reliability of our methods, cross-sections from the claw root were taken from 1 cave myotis (*Myotis velifer*) and 3 tri-colored bats (*Perimyotis subflavus*) to represent resident species taken at the study site in late August and early September. Both species are summer residents and also thought to be regional migrants between summer roosting sites and winter hibernacula (Fitch et al. 1981; Fujita and Kunz 1983). While it is unknown if individuals were in the process of migrating between roost sites at the time of death, we still expect recently grown claw tissue to be from the same geographic region as the study site. Because claws of these species are smaller than those of hoary bats, we did not measure 1 mm from the extensor process but rather sampled the most-anterior section of the blade horn (i.e., most recently grown) that resulted in a sample of 100 ± 10 µg. For both cave myotis and tri-colored bats, $\delta D_{claw\ root}$ was adjusted to δD_{precip} using discrimination factors developed for the tri-colored bat by Fraser (2010): $\delta D_{fur} = -0.036(\delta D_{precip})^2 - 1.789(\delta D_{precip}) - 45.607$. We compared

$\delta D_{\text{claw root}}$ of local bats to the interpolated δD_{precip} for the study site to evaluate congruency between observed and expected values.

Statistical methods.—Statistical tests were performed with Biomstat 3.30 or SigmaPlot 11.0. Isotope values are given as mean ± 1 standard deviation (SD) with statistical significance set at $P < 0.05$. To evaluate whether a bat killed at the site was a summer resident or migrant, we used predicted δD_{precip} values for the growing season at the study site (Bowen et al. 2005; Bowen 2012a). Cryan et al. (2004) determined that hoary bats probably molt in late summer (June-August) when mean δD_{precip} for the study site is expected to be -42‰. Thus, $\delta D_{\text{claw root}}$ and δD_{fur} corrected for isotope discrimination for summer-resident bats at the study site would be expected to have approximately the same value; Migrant hoary bats are expected to have tissues more depleted than the δD_{precip} of the study site. We used Fisher's exact test to compare the proportion of males and females that were resident vs. migrant.

Spring migration is thought to occur mostly in April (Kurta 2010; Zinn and Baker 1979), and the mean interpolated δD_{precip} at the study site prior to spring migration (January – March) is -66‰. If δD_{precip} of wintering grounds are isotopically retained in the claw tip, we expect $\delta D_{\text{claw tip}}$ to be greater (i.e., less negative) than -66‰.

The resulting $\delta D_{\text{claw root}}$ values for hoary bats were partitioned into 5 bins to cover the range of isotopic values found in tissue samples: -50 ± 10‰ -70 ± 10‰, -90±10‰, -110 ± 10‰, and < -120‰ (± values in this context indicate extent of bin, not SD). Similarly, we also established categories for $\delta D_{\text{claw tip}}$ values: -30 ± 10‰, -50 ± 10‰, -70 ± 10‰, and -90 ± 10‰, -110 ± 10‰. We determined geographic regions for δD values with interpolated North American hydrogen-isotope maps (www.waterisotopes.org; Bowen 2012b). We analyzed the numbers of individuals in each category according to sex with a row by column test for independence.

To evaluate congruency of δD_{fur} and $\delta D_{claw\ root}$, we compared the difference between males and females within each tissue type with a 2-way repeated-measure analysis of variance (ANOVA) with one-factor repetition (i.e., the δD_{fur} and $\delta D_{claw\ root}$ were paired within the same individual). If hoary bats molt at location of summer residency, δD_{fur} is expected to be less than or equal to all other tissues sampled. If $\delta D_{claw\ root}$ is more depleted than δD_{fur}, it suggests that the molt did not occur at the most northern limit of summer residency. Within claw samples, we also compared the difference between $\delta D_{claw\ root}$ and $\delta D_{claw\ tip}$ ($\Delta\delta D$) to assess the amount of variation within individual claws. We used 1-way ANOVA to evaluate the variance in $\Delta\delta D$ between sexes as well as among geographic regions. A 2-way ANOVA without repeated measures was also used to evaluate interactions between sex and geographic regions. Both procedures were necessary because missing values required a simplified data set for the 2-way ANOVA. For all ANOVA analyses, we assessed departures from normality using a Shaprio-Wilk test as well as equality of variances. For all 2-way ANOVA analyses, we also evaluated each pairwise comparison in the absence of any interactions with Holm-Sidak multiple comparison procedures.

For 45 hoary bats, we assessed migratory patterns with linear regression between the $\delta D_{claw\ root}$ and the corresponding arrival time at the study site. We estimated arrival time as the estimated day of death. Specimens that were determined to be summer residents (i.e., those having $\delta D_{claw\ root}$ similar to local δD_{precip}) were excluded from migration analysis. The timing of migration was very similar among the 3 years of study (Burba 2013), so we combined all years for analysis. We analyzed males and females with separate linear regressions and used an analysis of covariance (ANCOVA) to evaluate whether slopes or means were different between sexes.

RESULTS

Variation in δD_{fur} *and* δD_{claw}.—The mean $\delta \mathrm{D}_{\mathrm{fur}}$ of males and females was $\bar{X}$ = -70.5 ± 23.65‰ and $\bar{X}$ = -92.2 ± 30.93‰, respectively. The mean $\delta \mathrm{D}_{\mathrm{claw\ root}}$ for males was $\bar{X}$ = -85.0 ± 22.40‰ and $\bar{X}$ = -95.9 ± 23.40‰ for females. The 2-way repeated-measures ANOVA revealed a highly significant interaction between sex and tissue type ($F_{1,47} = 9.61$, $P = 0.003$; Table 1). For females, there was no significant difference between $\delta \mathrm{D}_{\mathrm{fur}}$ and $\delta \mathrm{D}_{\mathrm{claw\ root}}$ ($t = 0.58$, $P = 0.567$), but the difference was highly significant for males ($t = 6.25$, $P < 0.001$). The equivalence between $\delta \mathrm{D}_{\mathrm{fur}}$ and $\delta \mathrm{D}_{\mathrm{claw\ root}}$ within females is consistent with molt occurring at the location of summer residency, while the disparity of these measurements in males suggests the opposite. The $\delta \mathrm{D}_{\mathrm{fur}}$ of females was significantly more depleted compared to that of males ($t = 3.31$, $P = 0.002$), indicating that females may spend summer residency in more northern localities compared to males. However, there was no difference in $\delta \mathrm{D}_{\mathrm{claw\ root}}$ between sexes ($t = 1.48$, $P = 0.144$). Given that $\delta \mathrm{D}_{\mathrm{claw\ root}} < \delta \mathrm{D}_{\mathrm{fur}}$ in males, claw data may be more reliable for assigning migratory origins. Thus, data indicate that there is no significant latitudinal difference in location of summer residency between males and females. Given the possibility that fur tissues may be unreliable for assigning migratory origins, only $\delta \mathrm{D}_{\mathrm{claw}}$ is used for subsequent analysis.

Migratory status.—The adjusted values of $\delta D_{\text{claw root}}$ for the local species were -48.4‰ for the cave myotis, and -63.7‰, -54.3‰, and -48.2‰ for the tri-colored bats ($\bar{X}$= -53.7 ± 7.32‰). These bats were collected in late August and early September, but the majority of the claw keratin sampled likely grew throughout the earlier part of the same summer (June-August). The average interpolated values of δD_{precip} for the study site during these months is -42‰ (Bowen 2012b). The mean $\delta D_{\text{claw root}}$ of -53.7‰ for these "permanent" resident bat species is more depleted than expected if tissue grew at the study site. However, these species are regional migrants and movements between summer and winter roosts within the region may contribute to this variation. Furthermore, instrumental and/or experimental error can also account for small differences of approximately ±5‰ in δD measurements (Wassenaar and Hobson 2006). Thus, the δD_{claw} measurements of these other local species provide slightly higher than expected values, but these results are reasonable approximations of expected values based on δD_{precip} for the study site, supporting the general reliability of interpolated δD_{precip} values to assign migratory origins of hoary bats. To account for these possible errors, we selected an 18‰ buffer from δD_{precip} (-42‰) to evaluate whether individual hoary bats are likely migrant or summer-residents; thus, individuals with $\delta D < –60‰$ were considered migratory through the study area, while those with $\delta D > –60‰$ were potential summer residents near the study site. We conclude that 100% of female (15 of 15) and 87.8% of male (36 of 41) hoary bats were likely migrants. There was no significant difference between the proportion of individuals that were migrant between sexes ($P = 0.309$). Thus, only five hoary bat fatalities were of individuals that potentially spent summer residency near the study site.

Fig. 1 illustrates the isoclines in δD_{precip} values across North America based on data provided by Bowen (2012b). These isoclines correspond to the respective geographic regions that are represented by each $\delta D_{claw\ root}$ bin: -50 ± 10‰, -70 ± 10‰, -90±10‰, -110 ± 10‰, and < -120‰. All individuals encompassed by -50 ± 10‰ were those classified as potential summer residents, whereas all others were classified as migrants. When individual hoary bats were placed into the five δD bins, the proportion of individuals in each bin was not significantly different between the $\delta D_{claw\ root}$ of males and females (G = 3.44, P = 0.486). For both sexes combined, $\delta D_{claw\ root}$ ranged from -43.6‰ to -153.0‰ ($\bar{X}$= -88.0 ± 22.99‰) with the highest proportion being within -90 ± 10‰ (Fig. 2).

Based on $\delta D_{claw\ tip}$, the number of individuals in each δD bin is depicted in Fig. 2, showing the shift in δD values between the claw root and tip. Most bats, 60.0% of females (9 of 15) and 78.8% of males (26 of 33), had $\delta D_{claw\ tip}$ values that were > -66‰, which corresponds to winter δD_{precip} from localities south of the study site. However, for many individuals, the $\delta D_{claw\ tip}$ was < -66‰, indicating the isotopic values of the entire spring migration may not be retained for some, if any, individuals. Alternatively, the lower values could reflect individuals that over-wintered north or northwest of the study site or at higher elevations the previous winter. Like the claw root, there was no difference in the distribution of $\delta D_{claw\ tip}$ values between sexes (G = 1.50, P = 0.826; Fig 2) with most individuals ranging between -50 ± 10‰. For both sexes combined, $\delta D_{claw\ tip}$ ranged from –22.3‰ to -103.6‰ ($\bar{X}$= -55.8 ± 17.64‰).

Variation in δD between claw cross-sections.—The extent of variation in δD values along the length of the claw differed among individuals. The ΔδD (i.e., difference between $\delta D_{\text{claw root}}$ and $\delta D_{\text{claw tip}}$) ranged from 12.1‰ to 70.1‰ within females ($\bar{X}$=38.5 ±19.73‰) and 4.5 to 53.23‰ within males ($\bar{X}$=29.7 ± 13.56‰). Although the mean ΔδD of females was greater than males, this difference did not reach significant ($F_{1,45}$ = 3.24, P = 0.079). The ΔδD for individuals among different δD bins was highly significant ($F_{4,42}$ = 9.41, P < 0.001); the ordering of these differences among δD bins indicates that specimens with migratory origins from areas of lower δD_{precip} (and therefore probably higher latitude) exhibit greater ΔδD values (Fig. 3). The influence of sex and δD bin on the variation in ΔδD were analyzed independently because the only individuals represented in the -50 ± 10‰ bin were males, precluding comparison with females in a 2-way ANOVA. With this bin excluded, the 2-way ANOVA revealed a significant interaction between sex and δD bin ($F_{1,3}$ = 3.06, P = 0.042). The 2-way ANOVA indicated that only the geographic region (i.e., δD bin) accounted for a significant portion of the variation in ΔδD ($F_{3,37}$ = 9.62, P < 0.001), and sex was no longer near significance ($F_{1,37}$ = 0.73, P =0.400). When comparing the results between the 1-way and 2-way ANOVA, the difference in the resulting probabilities for sex explaining the variation in ΔδD should consider that data sets were not identical. Individuals with the least amount of variation, all males, were omitted in the latter analysis. Furthermore, the interaction is most likely caused by the significant pair-wise comparisons for sex within the < -120‰ bin, with females displaying greater ΔδD than males (t = 2.69, P = 0.011).

Migratory patterns.—The mean Julian date of arrival was 240.5 ± 8.74 days for females (approximately 28 August) and 245.8 ±13.99 days for males (approximately 03 September); these differences were not significant ($F_{1,43}$= 1.746, p = 0.193). Linear regression of $\delta D_{\text{claw root}}$ on Julian date of arrival was significant for females ($F_{1,13} = 6.16$, $P = 0.028$), but not for males ($F_{1,28} = 2.19$, $P = 0.150$; Figure 4). Additionally, neither the slopes ($F_{1,41} = 2.79$, $P = 0.102$) nor the means ($F_{1,42} = 2.00$, $P = 0.165$) were significantly different between sexes; thus, data were pooled for a single linear regression resulting in an overall significant relationship between $\delta D_{\text{claw root}}$ and Julian date of arrival ($F_{1,43} = 4.14$, $P = 0.048$). Data indicate that individuals with more depleted $\delta D_{\text{claw root}}$ (i.e., those from more northern localities) arrive at the study site later than individuals with more enriched values. Thus, arrival time at the study site appears to be dependent on the geographic location from which migration began.

DISCUSSION

The result of $\delta D_{\text{claw root}}$ being more depleted than δD_{fur} in male hoary bats was expected given the similar analysis by Burba (2013). However, the data she reported had not been adjusted for isotope discrimination to reflect the corresponding δD_{precip} for the geographic region where the tissue likely grew. The correction factor is not a single value, but rather depends on the initial values of tissues (Cryan et al. 2004). Tissues with more enriched δD will require greater adjustments than tissues that are more depleted, so we evaluated whether previously reported patterns hold for adjusted data, and the results are consistent (Burba 2013). However, Cryan et al. (2004) evaluated only fur tissue and the reliability of these corrections in our work depends on isotope discrimination also being similar for claw tissue. The disparity between male δD_{fur} and

$\delta D_{\text{claw root}}$ could be explained by claw keratin discriminating against deuterium more than fur; however, if this were the case, we would expect the same disparity in females, a pattern not detected. The congruency between δD_{fur} and $\delta D_{\text{claw root}}$ in females supports discrimination factors being the same between the tissue types.

The δD_{fur} of males being more enriched than $\delta D_{\text{claw root}}$ indicates that fur was grown at lower latitudes than was claw tissue. In some females, δD_{fur} was also more enriched than $\delta D_{\text{claw root}}$, but the opposite scenario was equally common so that the mean δD_{fur} and $\delta D_{\text{claw root}}$ in females was very similar. In general, claw data are consistent with the conclusion that females molt at the location of their summer residency prior to migration. However, the $\delta D_{\text{claw root}}$ was consistently more depleted than δD_{fur} in males and suggests that males molt prior to reaching the location of summer residency or after fall migration has begun. The only other explanation would be a reverse migration, moving south to north in fall (as could occur in those hoary bats that hibernate), but the $\Delta\delta D_{\text{claw}}$ from root to tip is consistent with a southward fall migration. Furthermore, the congruency between δD_{fur} and $\delta D_{\text{claw root}}$ in females supports the supposition that the keratin region sampled in claws is indicative of the location of summer residency. Therefore, the use of fur samples to assign migratory origins may be unreliable, at least for male hoary bats, but properly sampled claw tissue may be used to assign migratory origins.

The suggestion of male hoary bats having an early molt is incongruent with findings of Cryan et al. (2004), who found no significant difference in molting times of males and females. However, our results are concordant with those of Britzke et al. (2009), who studied the closely related eastern red bat. In female eastern red bats, δD_{fur} was positively correlated with the δD_{precip} for the location of summer residency. However, a negative correlation was found in males with the δD_{fur} being more enriched than expected given the δD_{precip} for the location of summer residency. As with our results, the apparent explanation for such a trend is a northward movement after molting has occurred. Seasonal shifts in sex ratios, with males increasing in July, have also led to the suggestion of a late northward migration of male eastern red bats (Kurta 2010) and could also explain the disparity in results from fur and claws of male hoary bats.

Variation in the stable-isotope ratios of tissues are not direct recorders of long-term average isotope ratios among geographic regions. In particular, they reflect the diet, water source, and physiology of individual consumers; Variation in these factors can create large δD variation among individuals within a geographic region. For example, hoary bats collected from the same locality over two summers had δD variation of 59‰ (Fraser 2011). In controlled-diet studies of Japanese quail (*Coturnix japonica*), Wolf (2012) found considerable variation in δD diet incorporation rates and isotope discrimination factors among individuals. Therefore, animals that feed on naturally varied diets are likely to exhibit even greater variation in δD values than diet-controlled individuals, even among tissues grown in the same location. However, some of the variation in hoary bats found by Fraser (2011) could have been due in part to considering the variation of both sexes together. As our data suggest, males may molt prior to reaching the location of their summer residency; thus, the hair samples Fraser took may not have grown in the assumed location in her study, potentially adding substantial unexplained variation in the data. Thus, the use of claw keratin in isotope analysis is independent of variation in molting patterns and may help in delineating migratory patterns.

Most bats killed at our study site were judged to be migrant individuals and not summer residents. To further support the conclusion that tissues of hoary bats were grown in other geographic regions, we evaluated the $\delta D_{\text{claw root}}$ for other species that were likely local individuals or at least regional migrants. The $\delta D_{\text{claw root}}$ values of these more sedentary species (cave myotis and tri-colored bat) were slightly more depleted than would be expected if grown at study site. However, recent stable-isotope studies of the tri-colored bat suggest this species may migrate greater distances than previously thought (Fraser et al. 2012), potentially contributing to this disparity. Even though our δD limit for migrant classification (-60‰) was corrected for this potential variation, as well as for the typical limits of experimental error, most of the bats' keratin tissues still could not have grown in Oklahoma. Summer residents could easily be mistakenly categorized because all of the eastern United States is characterized by δD > -60‰ in July (see Fig. 1). An individual classified as a resident could have migrated from the eastern United States. However, the Rocky Mountains disrupt latitudinal δD bands, causing complex patterns in δD isoclines. As a result, geographic regions with δD_{precip} = -60‰ are much nearer the study site in the western United States. Given potential errors in estimated discrimination factors, interpolated δD_{precip} values, and experimental methods, a buffer was necessary to confidently identify individuals as migrants. Given that so much of the United States is encompassed within the δD limit of -60‰ and all but five individuals were still classified as migrants, lead us to conclude that the wind farm's primary impact is on migratory individuals coming from other geographic regions.

Data suggest that hoary bats were summer residents in a broad range of latitudes with migratory origins ranging from geographic regions with δD_{precip} of -42.7‰ to -150.6‰. The majority of bats migrating through the study site came from latitudes corresponding to $\delta D_{precip} = -90 \pm 10‰$, isotopic signatures that are characteristic of the northwest United States (excluding Alaska) and parts of Canada. However, the distribution extends into northern Canada for individuals with $\delta D < -120‰$. There was no apparent latitudinal sex segregation among geographic regions, as males and females were proportionally distributed among δD bins.

Isotopic analysis of time-series data across the entire bat claw (Burba 2013) indicates that northward movements during spring migration are retained in the tip of the claw of fall migrants. Thus, we assessed the claw tip to determine if wintering grounds could also be detected. Claw-tip data did not converge to δD values that would indicate shared wintering grounds among the individuals sampled. Like $\delta D_{claw\ root}$, $\delta D_{claw\ tip}$ represented a broad range of values (-22.8‰ to -103.6‰) with the highest proportion in the -50 ± 10‰ bin, measurements indicative of eastern Mexico and southeastern United States during late winter. In January through March, the expected δD_{precip} of the study site is about -66‰. The majority of bats had values less than this limit, but many did not. This could indicate that isotopic remnants of spring migration are not sufficient to cover the entire migratory event, because some $\delta D_{claw\ tip}$ values correspond to locations farther north than the study site. However, interpretation of claw tip data is difficult without knowledge of claw growth rates. Local δD_{precip} values can vary greatly between winter and summer months, with spring months being transitional between the two periods. Without knowing the time at which a tissue was grown, the expected δD_{precip} values cannot be confidently established to properly assign individuals to geographic regions. Furthermore, winter δD bands dip into Mexico similar to the summer values shown in Fig. 1 (but are more depleted in deuterium in winter) such that δD_{precip} values in western Mexico, within the Sierra Madre Occidental, are the same as localities north of the study site. In February, portions of the Sierra Madre Occidental have expected $\delta D_{precip} < -100‰$. Thus, the most depleted $\delta D_{claw\ tip}$ value in our data, -103.6‰, could be from western Mexico or localities substantially further north than the study site. The most probable assignment depends on the month in which the tissue grew (i.e., before or after spring migration was complete). Therefore, it is possible that $\delta D_{claw\ tip} > -66‰$ were from individuals wintering in western Mexico. Comparatively, spring migratory origins from western Mexico were a possible explanation for the trends found in claw analysis of spring migrants (Burba 2013).

Little is known about winter hibernation in hoary bats and the characteristics that lead to a partial migration. It is unknown why an individual and what proportion of a population adopts a hibernation strategy and if the behavior varies by year. Perhaps an individual may hibernate one winter and migrate the next year. It is possible that $\delta D_{claw\ tip}$ values more depleted than the δD_{precip} of the study site could be of individuals that hibernated in northern latitudes the winter prior to the fall migration in which they were killed. Additionally, individuals may not hibernate at location of summer residency, but may migrate to another location with more optimal conditions for hibernation, a strategy common in cave species, which can include locations north of summer residency (Fleming & Eby 2003). Moreover, hibernation likely affects claw-growth rates when food is not actively metabolized. For example, Godwin (1959) found a near cessation of claw growth in rats with poor diet or for those housed in 6°C chambers. Bats over-wintering in northern localities rather than migrating southward may exhibit reduction or cessation of claw growth, which would impact interpretation if isotope values in the claw tip. Thus, future studies measuring claw growth rates, as well as analyses of the trends in δD patterns in the claws of spring migrants, are needed to determine when claw tip samples likely grew, if a single claw could be used to evaluate both summer and winter residency, and if winter-survival strategies (i.e., migration vs. hibernation) can be isotopically distinguished.

It is common for bats to experience differential migrations with females typically migrating farther than males (Fleming and Eby 2003). This supposition is supported in hoary bats based on data for δD_{fur} values, which indicate that females migrate to more northern localities. However, the supposition is not consistent with results from $\delta D_{claw\ root,}$ which we propose are more reliable. There were no significant differences between the $\delta D_{claw\ root}$ of males and females, nor significant differences in the distribution of sex within δD bins, which is related to location of migratory origin. However, our data suggest that females have greater ΔδD, but that the overall difference between males and females was not significant ($P = 0.079$). The relationship was only significant in the pairwise comparison of males and females within the < -120‰ bin, in which ΔδD of females was greater than that of males.

The $\Delta\delta D$ is indicative of a differential migration (i.e., some individuals migrating farther than others), but it is unclear what roles, if any, sex and hibernation have in contributing to the variation in $\Delta\delta D$. Analysis of $\Delta\delta D$ within each $\delta D_{\text{claw root}}$ bin clearly demonstrated that individuals with summer residency in the more northern latitudes had significantly greater $\Delta\delta D$ values, suggesting these individuals migrated greater distances. For example, with longitudinal-chain migration in birds (Boulet and Norris 2006) a sub-population may reside farther north than another sub-population, but both may migrate the same distance southward so that the wintering grounds are still equivalently separated at different latitudes. If this scenario applies to hoary bats, we would expect the $\Delta\delta D$ to be the same, even though the $\delta D_{\text{claw root}}$ would be more depleted in some individuals. Given the variation within $\Delta\delta D$, from 4.5 to 70.1‰, and the variation being highly related to the location of migratory origin, data strongly suggest a differential migration among individuals with those residing in lower latitudes migrating the shortest distances.

Because no difference was detected in the proportion of males and females within each δD bin, data do not support the notion that sex is a contributing factor in how far individuals migrate; rather, some other biological characteristic, such as body condition or resource availability, may provide a better explanation. However, sex segregation may mask any differences between the latitude of summer residency between males and females. There is evidence of altitudinal segregation of this species, with males more likely to occupy higher elevations in montane regions (Cryan et al. 2000; Findley and Jones 1964). Male hoary bats have also been reported to dominate western United States localities (Cryan 2003). Although studies of sex ratios of hoary bats in Michigan indicate male, not female, bias (Kurta 2010), as do wind-farm fatalities in the eastern United States (Arnett et al. 2008). These data bring uncertainty to the pattern and degree of sex segregation for this species. However, if males disproportionally reside in the western United States and at higher elevations compared to females, δD interpretation may be skewed. The high elevation of the Rocky Mountains disrupts latitudinal δD bands in the western United States (Fig. 1), and the resulting δD_{precip} values can be indistinguishable from those of more northern latitudes. Thus, it may appear, isotopically-speaking, that males are residing in more northern localities, and thus, obscuring any true latitudinal differences with females. Furthermore, this may also explain why there was no significant relationship between the arrival date at the study site and the $\delta D_{claw\ root}$ in males. The δD is an indirect measure of the relative distance traveled. If males are arriving from both the Rocky Mountains and localities north of the study site, these latitudinal differences cannot be detected, blurring any pattern between the arrival date and the assumed distance traveled.

Findley and Jones (1964) suggested females may begin spring migration earlier than males, but we did not detect any differences in arrival time at the study site during fall migration between sexes. Within females, individuals with summer residency at lower latitudes (i.e., more enriched $\delta D_{claw\ root}$) arrive at the study site earlier than individuals from more northern latitudes. Overall, this relationship was still significant with both sexes combined, despite the insignificance of the relationship within males alone. One type of differential migration is a “leap-frog” pattern, where individuals from more northern, summer localities also are those that winter in the most southern localities, surpassing (i.e., “leap-frogging”) other populations that migrate shorter distances. Our data is consistent with a leap-frog migration. An explanation for such a pattern is individuals arriving to the best summer and/or wintering grounds first, force later-arriving individuals to continue migrating to more distant localities through competition (Alerstam 1990). Yet, the $\Delta\delta D$ and arrival patterns in our data could be consistent with shared wintering grounds and segregation only occurring in the northern, summer range. Our data do not support any specific wintering region for the species as a whole (i.e., $\delta D_{\text{claw tip}}$ does not converge to the same value). However, more research is needed to determine claw-growth rates and if the $\delta D_{\text{claw tip}}$ of fall migrants is representative of wintering grounds before the specific type of migration can be established. More research is also needed to determine why some hoary bats hibernate rather than migrate, where each sex spends the winters, and what effect hibernation might have on isotope sequestration in their tissues.

Determining migratory patterns for the hoary bat can also aid in predicting the biological impact wind turbines have on populations. Data suggest that bats were summer residents in a broad range of latitudes and that fatalities are of individuals dispersed across broad geographic regions. Fatality patterns in this scenario disperse the risk among many populations, minimizing effects of a single wind farm on a local population. Additionally, individuals intermixing from several geographic regions during migration, when copulation in hoary bats is thought to occur (Cryan and Brown 2007), would also suggest substantial gene flow among the North American population as a whole. Among other bat species, migratory distance was related to F_{ST} values (measure of genetic differentiation in the population), with long-distance migrants having the lowest amounts of genetic differentiation (Fleming and Eby 2003). We hypothesize that hoary bats would also exhibit low F_{ST} values, which may buffer against any genetic depauperization that could theoretically occur from wind-turbine fatalities. Fatality rates at this study site, like many others, also were male biased (Arnett et al. 2008; Burba 2013). Assuming that hoary bats exhibit some form of a typical mammalian, polygynous breeding system, as most bat species do (McCracken and Wilkinson 2000), the biased removal of males will have less of an impact on the population's reproductive potential than would the removal of females. While the cumulative fatality rates of hoary bats at wind-farms nationwide are of concern, the apparent intermixing of individuals from numerous geographic regions and male-biased fatality rates are at least among the best-case scenarios for minimizing population impacts.

While our study only provides basic understanding of migratory behavior, it is the first to use both claw keratin and wind-farm fatalities to assess migratory origins. Little is known about bat migration and even modest steps are a needed contribution. We advocate the salvage of wind farm fatalities, especially at facilities in the eastern United States where fatalities are high and migratory assignments are less likely to be affected by the disrupted patterns in δD latitudinal bands of the western United States. Specimens collected at numerous facilities across the country could provide sufficient sample sizes, provide opportunities for genetic analysis, create collaborative research efforts, and make meta-analysis possible to better understand bat migratory behavior, how wind-farm fatalities might be mitigated, and the impact wind-energy may have on population sustainability.

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LITERATURE CITED

ALERSTAM, T. 1990. Bird migration. Cambridge University Press, Cambridge.

ANDERSON, G. S. 2000. Minimum and maximum development rates of some forensically important Calliphoridae (Diptera). Journal of Forensic Sciences 45:824-832.

ARNETT, E. B., W. K. BROWN, W. P. ERICKSON, J. K. FIEDLER, B. L. HAMILTON, T. H. HENRY, A. JAIN, G. D. JOHNSON, J. KERNS, R. R. KOFORD, C. P. NICHOLSON, T. J. O'CONNELL, M. D. PIORKOWSKI, AND R. D. TANKERSLEY, JR. 2008. Patterns of bat fatalities at wind energy facilities in North America. Journal of Wildlife Management 72:61-78.

ARNOLD, B. D. 2007. Population structure and sex-biased dispersal in the forest dwelling vespertilionid bat, *Myotis septentrionalis*. American Midland Naturalist 157:374-384.

BOULET, M., AND D. R. NORRIS. 2006. The past and present of migratory connectivity. Ornithological Monographs 61:1-13.

BOWEN, G. J. 2012a. The online isotopes in precipitation calculator (OIPC), version 2.2. http://www.waterisotopes.org.

BOWEN, G. J. 2012b. Global and regional maps of isotope ratios in precipitation. http://wateriso.eas.purdue.edu/waterisotopes/pages/data_access/figures.html

BOWEN G. J., L. I. WASSENAAR, AND K. A. HOBSON. 2005. Global application of stable hydrogen and oxygen isotopes to wildlife forensics. Oecologia 143: 337-348.

BRITZKE, E. R., S. C. LOEB, K. A. HOBSON, C. S. ROMANEK, AND M. J. VONHOF. 2009. Using hydrogen isotopes to assign origins of bats in the eastern United States. Journal of Mammalogy 90:743-751.

BURBA, E. A. 2013. Estimating bird/bat fatality rates and using stable-hydrogen isotopes to asses hoary bat migratory patterns associated with a wind-energy facility in western Oklahoma. Ph.D. dissertation, University of Oklahoma, Norman.

CHAMBERLAIN, C. P., J. D. BLUM, R. T. HOLMES, X. FENG, T.W. SHERRY, AND G. R. GRAVES. 1997. The use of stable isotope tracers for identifying populations of migratory birds. Oecologia 109:32–141.

CONSTANTINE, D. G. 1957. Color variation and molt in *Tadarida brasiliensis* and *Myotis velifer*. Journal of Mammalogy 38:461–466.

CONSTANTINE, D. G. 1958. Color variation and molt in *Mormoops megalophylla*. Journal of Mammalogy 39:344–347.

CORMIE, A. B., B. LUZ, AND H. P. SCHWARCZ. 1994. Relationship between the hydrogen and oxygen isotopes of deer bone and their use in the estimation of relative humidity. Geochimica et Cosmochimica Acta 58:3439-3449.

CRYAN, P. M., M. A. BOGAN, AND J. S. ALTENBACH. 2000. Effect of elevation on distribution of female bats in the Black Hills, South Dakota. Journal of Mammalogy 81:719–725.

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., 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.

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.

DANSGAARD, W. 1964. Stable isotopes in precipitation. Tellus 16:436-468.

FINDLEY, J. S., AND C. JONES. 1964. Seasonal distribution of the hoary bat. Journal of Mammalogy 45:461-470.

FITCH, J.H., K. A SHUMP, AND A.U. SHUMP. 1981. *Myotis velifer*. Mammalian Species 149:1-5.

FLEMING, T. H., AND P. EBY. 2003. Ecology of bat migration. Pp. 156-208 in Bat ecology (T. H. Kunz and M. B. Fenton, eds.). University of Chicago Press, Chicago.

FRASER, E. 2011. Stable isotope analyses of bat fur: applications for investigating North American bat migration. Ph.D. dissertation, University of Western Ontario London, Ontario, Canada.

FRASER, E. E., L. P. MCGUIRE, J. L. EGER, F. J. LONGSTAFFE, AND M. B. FENTON. 2012. Evidence of latitudinal migration in tri-colored bats, *Perimyotis subflavus*. PLoS ONE 7(2):e31419. doi:10.1371/journal.pone.0031419

FRASER, K. C., E. A. MCKINNON, AND A. W. DIAMOND. 2010. Migration, diet, or molt? Interpreting stable-hydrogen isotope values in Neotropical bats. Biotropica 42:512-517.

FUJITA, M.S., AND T.H. KUNZ. 1984. *Pipistrellus subflavus.* Mammalian Species 228:1-6.

GODWIN, K.O. 1959. Experimental study of nail growth. *Journal of Nutrition*, **69**, 121–127.

HARVEY, M. J., J. S. ALTENBACH, AND T. L. BEST. 2011. Bats of the United States and Canada. Johns Hopkins University Press, Baltimore.

HOBSON, K. A., L. I. WASSENAAR, F. MOORE, J. FARRINGTON, AND S. V. WILGENBURG. 2007. Estimating origins of three species of Neotropical migrant songbirds at a gulf coast stopover site: combining stable isotope and GIS tools. Condor 109:256–267.

HOBSON, K.A., S.L VAN WILGENBURG, L.I. WASSENAAR, AND K. LARSON. 2012. Linking hydrogen (δ2H) isotopes in feathers and precipitation: sources of variance and consequences for assignment to isoscapes. PLoS One 7:e35137. doi: 10.1371/journal.pone.0035137

KELLY, J. F., V. ATUDOREI, Z. D. SHARP, AND D. M. FINCH. 2002. Insights into Wilson's warbler migration from analyses of hydrogen stable-isotope ratios. Oecologia 130: 216-221.

KELLY, J. F., E. S. BRIDGE, A. M. FUDICKAR, AND L. I. WASSENAAR. 2009. A test of comparative equilibration for determining non-exchangeable stable hydrogen isotope values in complex organic materials. Rapid Communications in Mass Spectrometry 23:2316-2320.

KURTA, K. 2010. Reproductive timing, distribution, and sex ratios of tree bats in Lower Michigan. Journal of Mammalogy 91:586-592.

MARRA, P. P, D. R NORRIS, S. M. HAIG, M. WEBSTER, AND J. A ROYLE. 2006. Migratory connectivity. Pp. 157-183 in Connectivity conservation (K. Crooks and M. Sanjayan, eds.). Cambridge University Press, New York.

MCCRACKEN, G. F., AND G. S. WILKINSON. 2000. Bat mating systems. Pp. 321-362 in Reproductive biology of bats (E. G. Crichton and P. H. Krutzsh, eds.). Academic Press, New York.

PARITTE , J. M. AND J. F. KELLY. 2009. Effect of cleaning regime on stable-isotope ratios of feathers in Japanese quail (*Coturnix japonica*). *Auk*, **126**, 165–174.

QUAY, W. B. 1970. Integument and derivatives. Pp. 1-56 in Biology of bats (W. A. Wimsatt, ed.). Academic Press, New York.

RUBENSTEIN, D. R., C. P. CHAMBERLAIN, R. T. HOLMES, M. P. AYRES, J. R. WALDBAUER, G. R. GRAVES, AND N. C. TUROSS. 2002. Linking breeding and wintering ranges of a migratory songbird using stable isotopes. Science 295:1062–1065.

SHUMP, K. A. and A. U. SHUMP. 1982. *Lasiurus cinereus*. Mammalian Species 183:1-6.

TIUNOV, M. P., AND T. A. MAKARIKOVA. 2007. Seasonal molting in *Myotis petax* (Chiroptera) in the Russian Far East. Acta Chiropterologica 9:538–541.

WASSENAAR, L. I., AND K. A. HOBSON. 2003. Comparative equilibration and online technique for determination of non-exchangeable hydrogen of keratins for use in animal migration studies. Isotopes in Environmental and Health Studies 39:211-217.

WASSENAAR, L. I., AND K. A. HOBSON. 2006. Stable-hydrogen isotope heterogeneity in keratinous materials: mass spectrometry and migratory wildlife tissue subsampling strategies. Rapid Communications in Mass Spectrometry 20:2505–2510.

WHITAKER, J. O., JR. 1967. Hoary bat apparently hibernating in Indiana. Journal of Mammalogy 48:663.

WILLIS, C. K. R. 2003. Physiological ecology of roost selection in female, forest-living big brown bats (*Eptesicus fuscus*) and hoary bats (*Lasiurus cinereus*). Ph.D. dissertation. University of Regina, Regina, Saskatchewan.

WOLF, N., S. D. NEWSOME, M. L. FOGEL, AND C. MARTÍNEZ DEL RIO. 2012. An experimental exploration of the incorporation of hydrogen isotopes from dietary sources into avian tissues. Journal of Experimental Biology 215:1915-1922.

ZINN, T. L., AND W. W. BAKER. 1979. Seasonal migration of the hoary bat, *Lasiurus cinereus*, through Florida. Journal of Mammalogy 60:634-635.

Table 1.—Mean δD ± SD values in fur and claw tissue of hoary bat carcasses, by tissue type within sex and resulting probability for pairwise comparisons

	Mean δD_{fur}	Mean $\delta D_{claw\ root}$	Pairwise comparison (P)
Male	-70.5 ± 23.65	-85.0 ± 22.40	< 0.001
Female	-92.2 ± 30.93	-95.9 ± 23.40	0.567
Pairwise comparison (*P*)	0.002	0.144	0.003*

* Significance of interaction between sex and tissue type.

FIGURE CAPTIONS

Fig. 1.— Map indicating δD_{precip} isoclines that represent the geographic regions encompassed by the $\delta D_{claw\ root}$ bins selected for data analysis. Black dot indicates the study site. Contours were constructed from July δD_{precip} data reported by Bowen (2012b). Molting most likely occurs in July (Cryan et al. 2004) when δD_{precip} of study site is -37‰.

Fig. 2.— Number of individual hoary bats assigned to each δD bin based on sex and portion of claw sampled. Geographic regions represented by bins of $\delta D_{claw\ root}$ correspond to regions bounded by summer isoclines in Fig. 1 and, thus, reflect locations of migratory origin.

Fig. 3.— Variation is hydrogen-isotope values between $\delta D_{claw\ root}$ and $\delta D_{claw\ tip}$ for individual hoary bats from different geographic regions determined by δD bins. Black dots are outliers and whiskers are 95% confidence limits (CI). Box plots without whiskers had $n < 9$ and CI could not be calculated.

Fig. 4.— Linear regression of $\delta D_{claw\ root}$ and Julian day of arrival for male and female hoary bats. The date scale in Gregorian days is 8 August – 27 October.

Fig. 1.

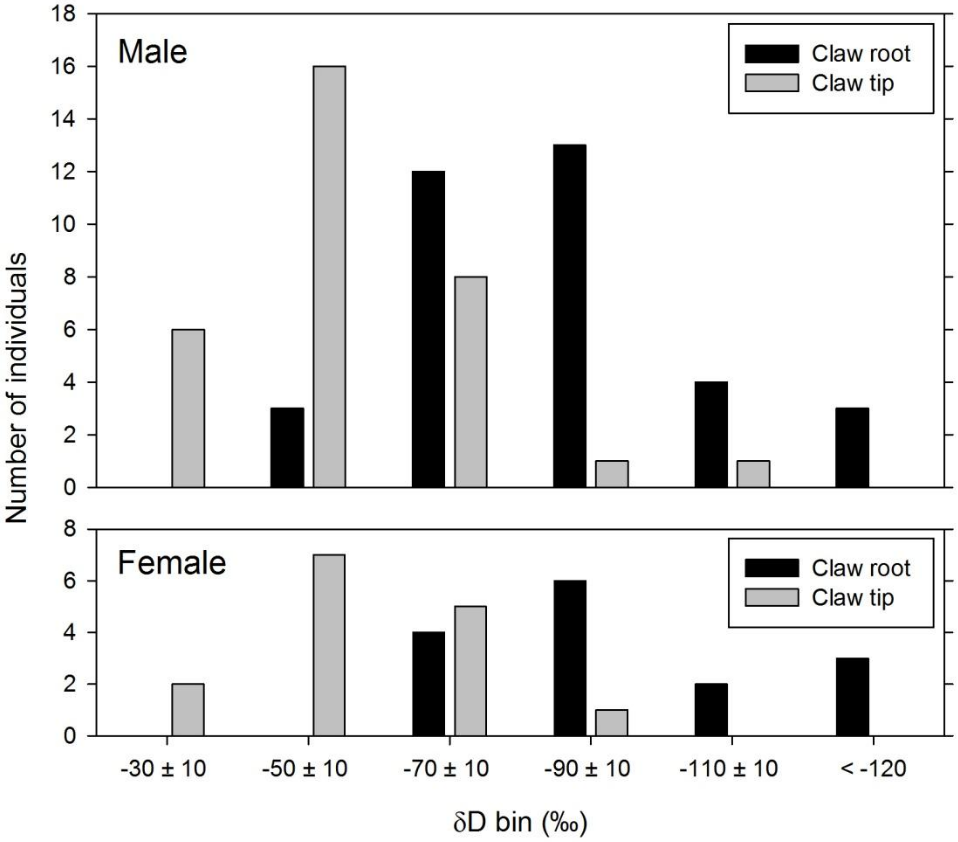

Male
Female
Claw root
Claw tip
Number of individuals
0
2
4
6
8
10
12
14
16
18
-30 ± 10
-50 ± 10
-70 ± 10
-90 ± 10
-110 ± 10
< -120
δD bin (‰)

Fig. 2.

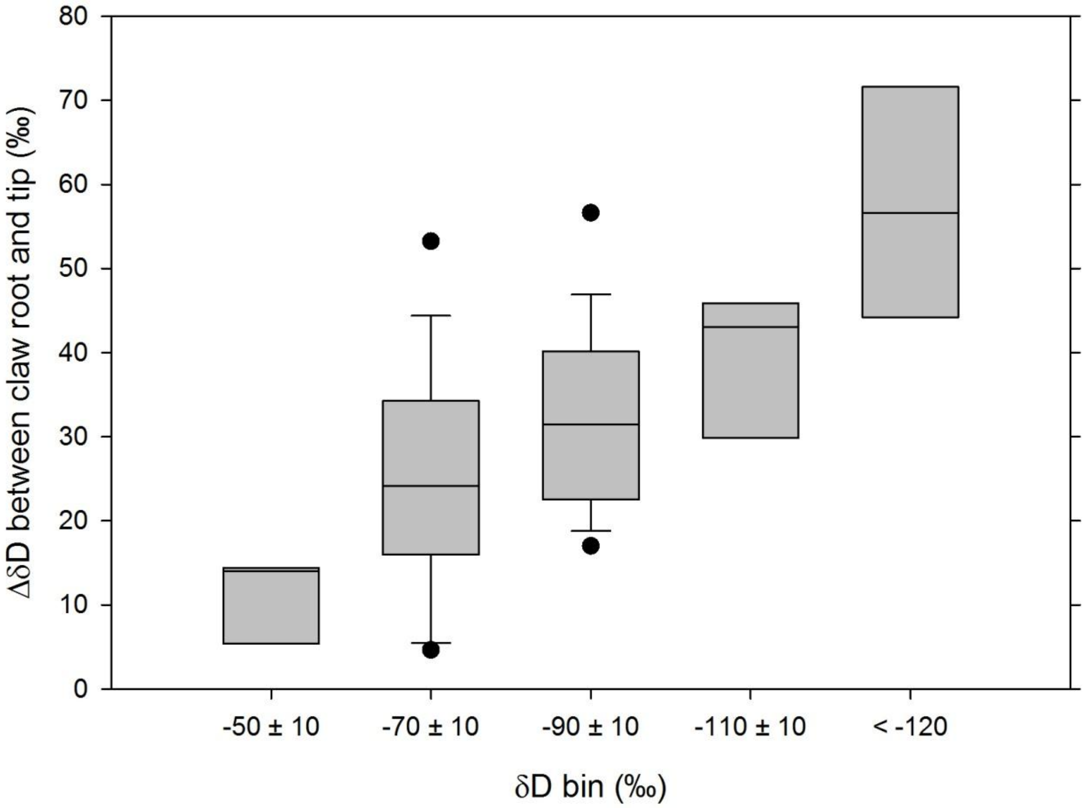
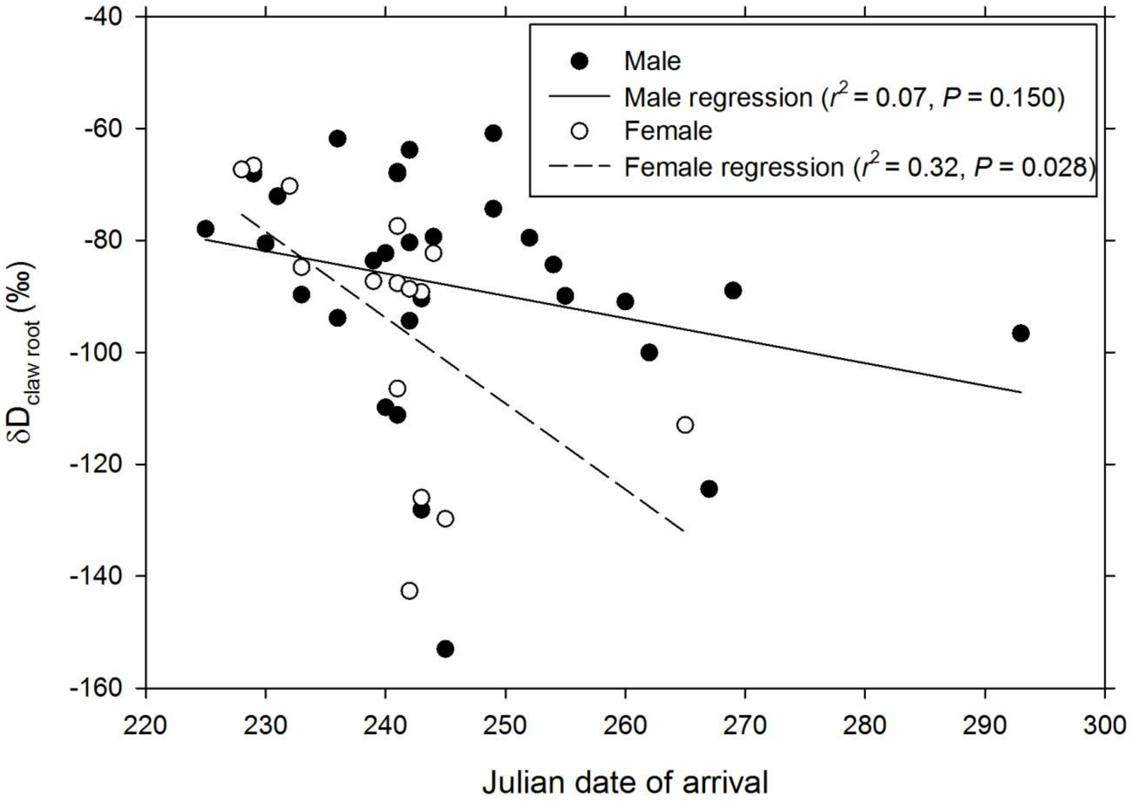

80
70
60
50
40
30
20
10
0
ΔδD between claw root and tip (‰)
-50 ± 10
-70 ± 10
-90 ± 10
-110 ± 10
< -120
δD bin (‰)

Fig. 3



Male
Male regression (r^2 = 0.07, P = 0.150)
Female
Female regression (r^2 = 0.32, P = 0.028)
$\delta D_{\text{claw root}}$ (‰)
-40
-60
-80
-100
-120
-140
-160
220
230
240
250
260
270
280
290
300
Julian date of arrival

Fig. 4.