Received Date: 26-Mar-2015

Revised Date: 09-Nov-2015

Accepted Date: 01-Dec-2015

Article Type: Original Article

Geographic origins and population genetics of bats killed at wind-energy facilities

Cortney L. Pylant[1,2], David M. Nelson[1,*], Matthew C. Fitzpatrick[1], J. Edward Gates[1], Stephen R. Keller[3,*]

[1]University of Maryland Center for Environmental Science, Appalachian Laboratory, 301 Braddock Road, Frostburg, MD 21532 USA

[2]Department of Biology, Frostburg State University, 101 Midlothian Road, Frostburg, MD 21532, USA

[3]Department of Plant Biology, University of Vermont, 111 Jeffords Hall, Burlington, VT 05405

[*]Corresponding authors: DMN: 301-689-7171 (p), 301-689-7200 (f), dnelson@umces.edu; SRK: 802-656-5121 (p), 802-656-0440 (f), srkeller@uvm.edu

Abstract

An unanticipated impact of wind-energy development has been large-scale mortality of insectivorous bats. In eastern North America, where mortality rates are

among the highest in the world, the hoary bat (*Lasiurus cinereus*) and the eastern red bat (*L. borealis*) comprise the majority of turbine-associated bat mortality. Both species are migratory tree bats with widespread distributions; however, little is known regarding the geographic origins of bats killed at wind-energy facilities or the diversity and population structure of affected species. We addressed these unknowns by measuring stable hydrogen isotope ratios (δ^2H) and conducting population genetic analyses of bats killed at wind-energy facilities in the central Appalachian Mountains (USA) to determine the summering origins, effective size, structure, and temporal stability of populations. Our results indicate that ~1% of hoary bat mortalities and ~57% of red bat mortalities derive from non-local sources, with no relationship between the proportion of non-local bats and sex, location of mortality, or month of mortality. Additionally, our data indicate that hoary bats in our sample consist of an unstructured population with a small effective size (N_e) and either a stable or declining history. Red bats also showed no evidence of population genetic structure, but in contrast to hoary bats, the diversity contained in our red bat samples is consistent with a much larger N_e, that reflects a demographic expansion after a bottleneck. These results suggest that the impacts of mortality associated with intensive wind-energy development may affect bat species dissimilarly, with red bats potentially better able to absorb sustained mortality than hoary bats because of their larger N_e. Our results provide important baseline data and also illustrate the utility of stable isotopes and population genetics for monitoring bat populations affected by wind-energy development.

Keywords: eastern red bat, hoary bat, *Lasiurus cinereus*, *Lasiurus borealis*, migration, stable isotopes, population genetics, wind turbine

Introduction

Growing concern over greenhouse gas emissions associated with energy production from the combustion of fossil fuels (IPCC 2014) has prompted a proliferation of renewable-energy production in the United States and abroad (Tabassum-Abbasi et al. 2014). Despite the environmental benefits associated with renewable-energy development (Jaber 2013), recent studies also highlight potential negative impacts on wildlife and the environment (e.g., Saidur et al. 2011, Armstrong et al. 2014). A largely unanticipated impact of utility-scale wind-energy development has been widespread mortality of bats (Kunz et al. 2007, Kuvlesky et al. 2007, Cryan and Barclay 2009). Survey data suggest mortality rates of up to 41 bats/turbine/year in Europe (Rydell et al. 2010) and 70 bats/turbine/year in North America (Arnett et al. 2008), with the highest rates along forested ridgelines. Collectively, estimates are that ~600,000 bats were killed by interactions with wind turbines at utility-scale wind farms in the United States in 2012 (Hayes 2013), which is similar to fatality estimates in Europe on the basis of number of fatalities per MW of installed net energy production (Voigt et al. 2015). As the primary predators of night-flying insects, bats limit the spread of insect-borne plant and animal pathogens and prevent billions of dollars of crop damage each year (Feldhamer et al. 2004, Boyles et al. 2011). Thus, understanding the potential impact of wind-energy

development on bat populations is a high priority for conservation and ecosystem services.

Whereas survey data demonstrate the capacity of wind turbines to kill bats, the population-level impacts of such mortality are less clear. Bats are long-lived and exhibit low annual reproductive rates (Barclay and Harder 2003), which may make it difficult for their populations to recover from sustained mortality. Understanding the impacts of turbine mortality on bats requires detailed information on their population biology where turbine development is occurring, such as migration pathways and geographic extent of regional habitat use, and the genetic diversity and demographic history of populations. Currently, these data are lacking for many affected species (Kunz et al. 2007), yet such information may be useful to policy, conservation, and resource-management decisions.

Stable hydrogen isotope ratios (δ^2H) are an excellent tool for inferring the geographic origins of migratory wildlife (Rubenstein and Hobson 2004) that may not be residents of the region in which they are sampled and that are not amenable to traditional methods of population monitoring (Holland and Wikelski 2009, Taylor et al. 2011). For example, positive relationships exist between δ^2H values of non-exchangeable hydrogen in hair (δ^2H_{hair}) and amount-weighted δ^2H values of precipitation for various species of bats (Cryan et al. 2004, Britzke et al. 2009, Fraser et al. 2012, Popa-Lisseanu et al. 2012, Sullivan et al. 2012, Pylant et al. 2014), which provides a solid basis for using δ^2H_{hair} values to identify potential summering regions of individuals of unknown geographic origin. If turbine-killed bats are primarily from populations that summer around wind-energy sites, then deceased individuals should have δ^2H values characteristic of local precipitation. However, because the most common species of bats killed by wind turbines

in North America (*Lasiurus cinereus*, *L. borealis*, and *Lasionycteris noctivagans*) are migratory, prior studies suggest that turbines may be killing large numbers of individuals during long-distance annual migrations (e.g., Johnson et al. 2003, Baerwald et al. 2014). If turbine-killed bats summer far from the site of mortality then they should have δ^2H values distinct from those of the site of mortality (Voigt et al. 2012, Lehnert et al. 2014). Although δ^2H-based assignments are often coarse, differences in the ratios of local to non-local bats across wind-energy facilities or adjacent regions may help to elucidate important migratory pathways and corridors and advance understanding of population dynamics (Voigt et al. 2012, Lehnert et al. 2014), thus aiding conservation of migratory bats (Roscioni et al. 2013).

The genetic diversity and population structure of impacted bat species may affect how resilient their populations are to sustained mortality, and their capacity for long-term adaptation. For example, species with small effective population sizes (N_e) contain low levels of genetic diversity that may limit their evolutionary potential to respond to selection and avoid inbreeding. Thus, increased mortality on such species by wind turbines or other factors may represent a greater conservation concern to environmental managers and decision makers compared to species with larger N_e. Population genetic analysis can also elucidate historical trends in N_e of impacted species, with those exhibiting historical or contemporary declines in N_e more at risk from sustained mortality compared to species for which N_e has been stable or increasing. Additionally, the existence of population genetic structure may create conservation concerns if genetically distinct subpopulations vary in their exposure to wind-turbine mortality (e.g., depending

on geographic location, use of certain migration routes, etc.). This could have the effect of removing unique diversity (rare or private alleles) from the species-wide gene pool.

Here we report the combined application of stable hydrogen isotope and population genetic analyses to assess the biological impacts of wind-energy development on two species of lasiurine bats, *L. cinereus* (hoary bat) and *L. borealis* (eastern red bat), that collectively comprise nearly 75% of mortalities at wind-energy facilities in North America (Arnett et al. 2008). We address two key questions: 1) Is turbine mortality impacting bats summering locally or individuals from a broader geographic extent? 2) What is the effective size and temporal stability of bat populations experiencing mortality and do these populations exhibit subpopulation structure? We address these questions, and their implications, using samples obtained from utility-scale wind-energy facilities in the central Appalachian Mountains of the eastern United States, a region exhibiting some of the highest rates of turbine-associated bat mortality in the world (Arnett et al. 2008).

Methods and materials

Sample collection and sites

Hair and tissue samples from hoary (n = 246) and red (n = 144) bats were obtained from carcasses collected at wind-energy facilities in the central Appalachian Mountains between April and October in 2003 and 2009-2011 (Fig. 1, Table 1). These facilities are located in the Appalachian and Ridge and Valley physiographic provinces, which are regions dominated by forested plateaus, ridges, and agricultural activity in lowlands, with elevations across the provinces ranging from 152 to 1,402 m a.s.l. (Woods et al. 1999). Samples from the Criterion Wind Project (28 turbines) in Maryland were

obtained from the environmental consulting firm Western Ecosystems Technology, Inc. and represent mortalities from 2011. Samples from two West Virginia facilities, the Mountaineer Wind Energy Center (44 turbines) and the Mount Storm Wind Farm (132 turbines) were obtained from the West Virginia Department of Natural Resources and represent mortalities from 2003 and 2009–2011, respectively. For samples from bats killed in Maryland and West Virginia we determined the centroid of the wind turbines at each facility for geospatial analyses (see below). Samples from wind-energy facilities throughout Pennsylvania were obtained from the Pennsylvania Game Commission (PAGC) and represent mortalities from 2011. In accordance with PAGC's Wind Energy Voluntary Cooperative Agreement (http://www.portal.state.pa.us/portal/server.pt?open=514&objID=613068&mode=2), individual wind-energy facilities are not identified as sites of mortality; rather, samples are pooled across regional game units of Pennsylvania. Samples from the northeastern, southcentral, and southwestern game units were included in this study. For samples from bats killed in Pennsylvania we determined the centroid of the wind turbines in operation at each facility within each of the three game units at the time of sample collection. We calculated a probability of origin for each of the centroid values and then averaged these values to produce a single probability-of-origin value for the game unit from which each bat originated (see below). All samples were stored at -20° C prior to analysis, with tissue samples preserved in 95% ethanol.

Isotope and geospatial analysis

Hair samples were cleaned as in Coplen and Qi (2012) using 1:200 Triton X-100 detergent, nano-pure water, and 100% ethanol. They were then air dried. We followed the Coplen and Qi (2012) protocol, so that our samples were cleaned in the same fashion as our standards (see below) based on the principle of identical treatment (Meier-Augenstein et al. 2013). We measured δ^2H values of non-exchangeable hydrogen in hair keratin using a comparative equilibration approach (Wassenaar and Hobson 2003). Approximately 0.3 mg of cleaned, homogenized hair from each sample, as well as international standards (USGS42, Tibetan hair, and USGS43, Indian hair; Coplen and Qi 2012) and an internal keratin standard (porcine hair and skin, Spectrum Chemical product # K3030), was exposed to ambient air for >72 hrs prior to analysis to allow for equilibration of exchangeable hydrogen in keratin. The capsules were sealed and placed in a zero-blank autosampler (Costech Analytical, Valencia, CA). The autosampler was purged with helium and the samples and standards analyzed for δ^2H using a ThermoFisher high temperature conversion/elemental analyzer (TC/EA) pyrolysis unit interfaced with a ThermoFisher Delta V+ isotope ratio mass spectrometer (ThermoFisher Scientific, Bremen, Germany) at the Central Appalachians Stable Isotope Facility (CASIF) at the Appalachian Laboratory (Frostburg, MD). Values of δ^2H are reported in parts per mil (‰) and were normalized to the Vienna Standard Mean Ocean Water-Standard Light Antarctic Precipitation (VSMOW-SLAP) scale using a two-point normalization curve with USGS42 and USGS43, whose δ^2H values of non-exchangeable hydrogen are -78.5 and -50.3‰, respectively. The long-term analytical precision (1σ) of these standards at CASIF is 2.1 and 2.0‰, respectively. Our δ^2H_{hair} values span a larger range (-122.7 to -8.8‰) than do USGS42 and USGS43, but prior studies suggest that linear extrapolation

of normalization relationships for δ^2H values has minimal influence on values within ~100‰ of the range of the standards used for normalization, provided that at least two standards are analyzed (Kelly et al. 2009, Wiley et al. 2012). The long-term accepted δ^2H value of the internal keratin standard at CASIF is -59.5 $\pm$ 2.3‰.

Prior studies have typically used amount-weighted δ^2H values of growing-season precipitation or mean annual precipitation when developing transfer functions between δ^2H values of bat hair and precipitation for geospatial analysis (e.g., Fraser et al. 2012, Popa-Lisseanu et al. 2012, Cryan et al. 2014, Lehnert et al. 2014). Although hoary and red bats are thought to molt during June-August (Cryan et al. 2004, Cryan et al. 2014, Pylant et al. 2014), the specific months of precipitation that most strongly influence the foodwebs supporting them during the period of molt are unknown. Thus, we developed geostatistical models and maps of June-August δ^2H-precipitation (δ^2H_{JJA}) values and mean annual δ^2H-precipitation (δ^2H_p) values for North America using the online workspace IsoMAP (Bowen et al. 2014). The input precipitation isotope data used to build these isoscapes in IsoMAP come from the years 1980-2009 and include data from the Global Network of Isotopes in Precipitation (http://www-naweb.iaea.org/napc/ih/index.html), as well as other published and unpublished time series of precipitation isotope data. Elevation, latitude, and latitude^2 were used as independent variables in the models because elevation and latitude are strong predictors of δ^2H values of precipitation in North America (Bowen 2010). Because the isotopic composition of precipitation may also vary with longitude, for example due to orographic influences, we also included longitude as a predictor in initial models and found it did not contribute significantly to predicting spatial patterns of δ^2H in our data. Bowen (2010)

also found little influence of longitude on spatial patterns of δ^2H values of precipitation in North America. The June-August model and map are available as IsoMAP jobs 41780 and 41783 (http://www.isomap.org), and the mean annual precipitation model and map are available as jobs 46912 and 46922. We used these isoscapes to determine δ^2H_{JJA} and δ^2H_p values for museum specimens of 117 hoary bats (Cryan et al. 2014) and 64 red bats (Pylant et al. 2014) that were collected during the presumed period of summer molt (i.e., they have known summering locations) for which δ^2H_{hair} values were also available. This was done to establish transfer functions that relate δ^2H_{JJA} and δ^2H_{hair} values, as well as δ^2H_p and δ^2H_{hair} values; these functions can then be used to predict δ^2H values of precipitation (and thus infer the geographic location of summering grounds) from δ^2H_{hair} values of turbine-killed bats of unknown origin. In producing these transfer functions, we used reduced major axis regression because of symmetry between the dependent and independent variables (Smith 2009) and because both variables contain measurement uncertainty (McArdle 1988). During initial analyses we found that δ^2H_{hair} values of the 117 hoary bats in Cryan et al. (2014) were more strongly correlated with δ^2H_{JJA} (r^2=0.49, $p<0.001$) than δ^2H_p (r^2=0.44, $p<0.001$), whereas the δ^2H_{hair} values of the 64 red bats in Pylant et al. (2014) were more strongly correlated with δ^2H_p (r^2=0.41, $p<0.001$) than δ^2H_{JJA} (r^2=0.36, $p<0.001$). Thus, we used the relationship between δ^2H_{hair} and δ^2H_{JJA} values [δ^2H_{hair} =0.874 (δ^2H_{JJA}) - 41.8] as a transfer function for hoary bats and the relationship between δ^2H_{hair} and δ^2H_p values [δ^2H_{hair} =1.00(δ^2H_p) + 8.17] as a transfer function for red bats.

To prepare δ^2H_{hair} values of turbine-killed bats for analysis in IsoMAP, they were rescaled to δ^2H_{JJA} (hoary bats) or δ^2H_p (red bats) values by rearranging the previous

equations. We then used the IsoMAP assignment tool, which is based on a Bayesian probabilistic framework, to produce a likelihood-of-origin map for each bat using its δ^2H value of precipitation and a standard deviation of that value. The standard deviation of δ^2H_{JJA} and δ^2H_p values used for these assignments was set at 16‰ and 10‰ for hoary and red bats, respectively. These values are the standard deviations of the residuals of δ^2H values of precipitation in the above equations relating δ^2H_{hair} and δ^2H_{JJA} or δ^2H_p values for these species. Unlike Baerwald et al. (2014), we generated a likelihood-of-origin map for each bat rather than using the IsoMAP batch function to create a map of joint probability-of-origin values. We did not use the batch function, because such an analysis assumes that the sampled individuals represent one population with a shared geographic origin (Wunder 2012, Bowen et al. 2014), which may not be the case for migratory bats of potentially diverse geographic origins.

To quantify the accuracy of the likelihood-of-origin maps produced by IsoMAP (e.g. Fig. A1), we first generated such maps for each of the 117 specimens of hoary bats (Cryan et al. 2004, Cryan et al. 2014) and 64 specimens of red bats (Pylant et al. 2014) that were collected during the period of summer molt. These specimens served as training samples with known geographic origins that we used to determine the sensitivity of the assignment model. For each of these specimens, we calculated probability of origin maps (contour range = 0.1 to 0.95, at intervals of 0.05) using the Geospatial Modelling Environment (http://www.spatialecology.com/gme/). These maps represent continuous probability surfaces, with each contour interval ("isopleth") containing all pixels up to a given probability that are predicted to contain the location of origin for an individual. For each individual in our training dataset, we recorded the minimum isopleth contour that

contained the known sampling location (e.g. Fig. A2). In other words, we asked: what is the smallest likelihood of origin that contains the sampling location? We then fit multiple candidate functions to the distributions of these minimum isopleth values for hoary and red bats by numerically minimizing the negative log-likelihood using the 'mle2' function in the bbmle library (Bolker and Team 2014) in R 3.1.0 (Team 2014). We used Akaike's Information Criterion (AIC) to determine the model best supported by the data (Burnham and Anderson 2002). The distributions of these minimum isopleth values were best approximated by a beta distribution (AIC weight = 1.00) for hoary bats and a log-normal distribution (AIC weight = 1.00) for red bats (Fig. 2).

Using these distributions, we implemented a Monte Carlo simulation to estimate the probability that a turbine-killed bat was of local or non-local origin, as follows. For each turbine-killed bat of unknown origin, we randomly selected a value from the applicable distribution (beta or log-normal) of the minimum isopleth values for each species, and plotted that isopleth on a likelihood-of-origin map. All pixels within the selected isopleth were assigned a probability of origin value of 1, and those outside of the isopleth were assigned a value of 0. This procedure was repeated 10,000 times per bat and the average value of each pixel was calculated across bootstrapped replicates to create a continuous probability surface identifying that individual's most likely area of origin. We then extracted the average probability value for the location at which each bat was killed (i.e., the turbine site where the sample was collected). A value of 1 indicates that the minimum isopleth contour contained the location of mortality all 10,000 times, whereas a value of 0 indicates that the minimum isopleth contour never contained the location of mortality. We applied a majority rule criterion, whereby bats with probability

values >0.5 at the site of turbine mortality were considered to be summering locally (i.e., we cannot exclude the possibility that these bats summered near the site of their death; hereafter termed 'local'), whereas bats with probability values ≤0.5 were considered to have summered elsewhere prior to being killed at a given turbine site (hereafter 'non-local'). This procedure thus provides an estimate of probability of local origin that incorporates the uncertainty associated with estimating the geographic provenance for turbine-killed samples of unknown origin, and is based on the empirical sampling distribution of assignment probabilities determined from hoary and red bats with known summering origins. We also explored using more restrictive threshold probability values of <0.3 and >0.7 for distinguishing non-local versus local bats (thus leaving bats with values of 0.3-0.7 as unclassified in regards to being non-local versus local), respectively. Both threshold options yielded similar percentages of local and non-local bats and thus we only discuss results based on the 0.5 threshold. Further, we also explored sampling from the actual distributions of data instead of functions fit to the data, but this process had minimal influence on classification of bats as local versus non-local (data not shown).

Statistical analysis of isotope data

Analysis of variance (ANOVA) on the derived probability values (i.e., of bats being local versus non-local) was performed in R 3.1.0 (Team 2014). We used the probability of origin values as the response variable to incorporate uncertainty in the correspondence between δ^2H_{JJA} (hoary bats) and δ^2H_p (red bats) values and likelihood of origin. We

modeled the probability of local origin as a function of site ID (i.e., the location of mortality), the month of mortality, sex, and 2-way interactions.

DNA extraction and PCR amplification

Total genomic DNA was extracted from 1-2 wing punches per bat carcass using Qiagen DNeasy Blood and Tissue kits and normalized to approximately 20 ng/µl. Templates were used to amplify fourteen microsatellite loci developed by Keller et al. (2014): LAS4206AC, LAS6266AG, LAS7831AC, LAS8539AC, LAS8830AC, LAS8843AC, LAS8953AC, LAS9084AC, LAS9141AC, LAS9290AC, LAS9524AC, LAS9555AG, LAS9613AC, and LAS9618AC, as well as one mitochondrial locus (cytochrome b, *cytb*). Primer sequences for *cytb* were obtained from Smith and Patton (1993). Microsatellite PCR reactions were carried out in 10 µl volumes using Qiagen Multiplex PCR kits following the methods described in Keller et al. (2014). Microsatellite PCR reactions were sent to either the Penn State Huck Genomics Core Facility or the University of West Virginia Genomics Core Facility for genotyping with an Applied Biosystems (ABI 3730XL). Fragments were sized against the LIZ500 size standard using Peak Scanner v1.0 software. We standardized genotypes from the different sequencing facilities by sending a subset of samples to both facilities. The scores from these samples were within 1 bp or less of each other.

Mitochondrial PCR reactions were carried out in 15 µl volumes with reagent concentrations as follows: 1X PCR buffer solution (with 2.0 mM $MgCl_2$, 4.5 µmol forward primer, 4.5 µmol reverse primer, 1.2 mM each dNTPs, 3 units of Taq polymerase, 1.2 µl template DNA (approximately 20-30 ng) and brought up to volume

with water. PCR reactions were run on an Eppendorf Mastercycler Pro under the following conditions: 94° C for 4 min; followed by 35 cycles of 94° C for 40s; 50° C for 40s; 72° C for 1 min; a final extension of 72° C for 10 min; then a 4°C hold. PCR reactions were Exosap cleaned and sent to the Penn State Huck Genomics Core Facility for bidirectional Sanger sequencing with an ABI 3730XL. Mitochondrial sequences were trimmed, contigs made from overlapping reads, and aligned using Geneious v5.5.5.

Population genetic summary statistics

Microsatellite genotype scores were binned using Tandem (Matschiner and Salzburger 2009) and formatted for subsequent analyses using CONVERT (Glaubitz 2004). Summary statistics (e.g., number of alleles, allelic richness, and expected heterozygosity) were calculated using FSTAT v2.9.3.2 (Goudet 1995) and Arlequin v3.5.1.3 (Excoffier and Lischer 2010). Mitochondrial DNA diversity was estimated from the number of haplotypes, haplotype diversity, Watterson's Θ, per-site nucleotide diversity (π), and Tajima's *D* (Tajima 1989) using DnaSP (Librado and Rozas 2009).

During preliminary analyses, we discovered one potential hoary × red bat hybrid, which was identified in the field as a hoary bat, yet clearly carried a red bat mtDNA haplotype. This individual also possessed intermediate ancestry coefficients from a STRUCTURE analysis of the nuclear microsatellite data (see below) conducted on a combined two-species dataset, with roughly equal assignment of its ancestry to each species (average assignment value per cluster: 0.536 and 0.464). Therefore, this individual likely represents a rare interspecific hybrid, and was removed from all subsequent analyses.

Analysis of population structure

We used Bayesian clustering analysis to test for the presence of more than one genetic subpopulation among turbine-killed bats using STRUCTURE version 2.3.4 (Pritchard et al. 2000). STRUCTURE estimates K, the number of genetic clusters, by imposing population structure on sample data while minimizing Hardy-Weinberg and linkage disequilibrium within each group. Because this approach does not require *a priori* knowledge of geographic populations, it is ideal for testing for the presence of genetic structure within the sample of bats killed by turbines that otherwise lack information on the geographic origin of their mating populations. We specified an admixture model with correlated allele frequencies, and inferred the admixture parameter, α, from the data. We evaluated a range of potential K values from 1-10 using 10 iterations per K, with a burn-in period of 50,000 steps of the MCMC chain, followed by analysis over the next 200,000 steps. STRUCTURE output was processed using STRUCTURE HARVESTER (Earl and Vonholdt 2012) to compute ΔK, an *ad hoc* estimate of the most probable number of K-clusters based on the rate of change in the log probability of data between consecutive K values (Evanno et al. 2005). Ancestry coefficients among replicate runs for a given K were permuted using CLUMPP v1.1.2 (Jakobsson and Rosenberg 2007) and visualized using DISTRUCT v1.1 (Rosenberg 2004).

We tested for geographic structure in mtDNA sequence diversity and microsatellite allele frequencies using analysis of molecular variance (AMOVA; Excoffier et al. 1992) implemented in Arlequin v3.5.1.3 (Excoffier and Lischer 2010).

We used AMOVA to test two hypotheses for genetic differentiation: (1) among wind-energy sites where bats were killed, and (2) between local versus non-local bats as inferred from analysis of the δ^2H values of precipitation (derived from δ^2H_{hair} values). Significance was assessed by 1,000 permutations of the data.

Analysis of historical and contemporary effective population size

We modeled the historical demography and effective population sizes (N_e) for each species using approximate Bayesian computation (ABC) on a combined dataset of microsatellite genotypes and mitochondrial sequence data. All ABC calculations were performed using DIYABC v1.0.4.43 (Cornuet et al. 2008). For both species, we evaluated three historical demographic scenarios that described the history of the sample: 1) a historically stable population (i.e., a population that exhibited no discrete change in size); 2) a population that exhibited one discrete change in size, such as a bottleneck or population expansion; and 3) a population that exhibited two discrete changes in population size. Bayesian prior values for all parameters are provided in Table A1. Upper and lower bounds on the uniform prior for N_e were determined by first conducting a series of exploratory analyses to determine approximate prior values for each species that contained the full posterior probability. These prior values were then used in final analyses based on long runs.

We chose 9 summary statistics to evaluate for ABC analysis, including 4 statistics for the microsatellite genotypes (mean number of alleles, mean genetic diversity, mean size variance, and mean M; Garza and Williamson 2001) and 5 statistics for the mtDNA sequences (number of haplotypes, number of segregating sites, mean pairwise distance,

Tajima's *D* (Tajima 1989), and mean minor allele frequency). Nuclear microsatellites were simulated using a generalized stepwise mutation model. The mtDNA sequences were simulated using a Kimura 2-parameter model with 10% invariant sites and a gamma distribution of mutation rate heterogeneity among sites (Table A1). We performed coalescent simulations of >7 million genealogies for each species (10 million for red bats and 7.3 million for hoary bats; numbers of simulations varied according to the size of the empirical datasets) by drawing values from the prior distributions for each parameter, and used the logistic regression method to estimate the posterior probability of each scenario (Cornuet et al. 2008). We then retained the top 1,000 genealogies closest to the observed summary statistics and computed estimates of the demographic parameters from the posterior distributions.

While DIYABC attempts to estimate N_e by integrating information from both genomic datasets (nuclear microsatellites and mtDNA sequences), it also provides separate estimates per genome of the composite parameter, Θ (= $XN_e\mu$, where μ is the per-generation mutation rate, and X is an inheritance scalar). To investigate overall agreement between the microsatellite and mtDNA datasets in their assessments of effective population size, we estimated N_e separately for nuclear and mtDNA loci using the genome-specific values of Θ from the ABC posterior distributions. We transformed Θ to N_e based on the corresponding inheritance scalar for each dataset (4 for nuclear microsatellites; 1 for haploid maternally inherited mtDNA), and the posterior estimate of the mutation rate from ABC. We then applied kernel density estimation using the 'density' function in R, and took the modal value as our best estimate of N_e and the values that summed 95% of the probability distribution as Bayesian posterior credible intervals.

While none of the microsatellite loci showed the presence of null alleles in hoary bats, six loci indicated the presence of null alleles in red bats (Keller et al. 2014). Therefore, we also repeated the ABC analysis for red bats using a reduced dataset of 8 loci for which there was no evidence of null alleles.

Results

Isotope data

Our dataset included a small number (n = 4) of carcasses of juvenile hoary bats collected during July prior to the beginning of the fall migration period. Thus, these individuals were likely killed on their natal grounds within close proximity to the wind-energy facilities at which their carcasses were collected. In each instance, these juveniles were correctly classified as local using the threshold of 0.5 (each had probability values >0.62). These results, along with prior studies based on museum specimens of hoary (Cryan et al. 2014) and red (Pylant et al. 2014) bats collected on their presumed summering grounds, provide confidence for using δ^2H data to infer whether bats killed at wind-energy facilities summer near or far from where they are killed.

δ^2H_{hair} values ranged between -122.7 and -27.7‰ for hoary bats and between -76.2 and -8.8‰ for red bats (Fig. A3). δ^2H_{JJA} values ranged between -92.7 and 0‰ for hoary bats and δ^2H_p values ranged between -84.3 and -17.0‰ for red bats. The highest frequency of δ^2H_{JJA} values for hoary bats occurred around -25‰ and the highest frequency of δ^2H_p values for red bats occurred at -30‰ (Fig. 3). Using our Monte Carlo

method to determine probability of local origin, we found that 243 (98.8%) of hoary bat samples and 62 (43.1%) of red bat samples had probability values of >0.5 and were thus considered local (Fig. 4). Inferences of the proportions of local bats were similar when using the more restrictive thresholds of <0.3 for non-local bats and >0.7 for local bats: 88 of 89 (98.9%) of hoary bats and 45 of 104 (43.3%) of red bats were classified as local. Based on their relatively negative δ^2H_{JJA} values, the three non-local hoary bats (using a threshold of 0.5) likely summered to the north of the wind-energy site at which they were killed. The relatively positive δ^2H_p values for 81 of the non-local red bats (using a threshold of 0.5) suggest that the vast majority of non-local red bats likely summered to the south or west of our study sites or in coastal areas (Figs. 3 and A1). δ^2H_p data suggest that only one of the non-local red bats likely summered to the north of where it was killed. Neither sex, site ID, or month of mortality influenced the probability of being local for either species (Table A2).

Genetic diversity

Overall, 13 of the 14 nuclear microsatellite loci were polymorphic for both species (locus LAS9084AC was monomorphic in hoary bats), with the number of alleles per locus ranging from 1 to 35 for hoary bats and 2 to 46 for red bats (Table A3). The average number of alleles and allelic richness were higher overall in red bats, but these differences were not statistically significant (Mann-Whitney test; z = -1.14; $P = 0.250$). Mitochondrial diversity was also higher in red bats, which exhibited a greater number of haplotypes (hoary bat: 58; red bat: 104), higher haplotype diversity (hoary bat: 0.871 ± 0.015; red bat: 0.991 ± 0.0032), and higher nucleotide diversity (π) (hoary bat: 0.00693 ±

0.00032; red bat: 0.00747 ± 0.00053). In addition, Tajima's *D* was negative for both species (hoary bat: *D* = -1.553; red bat: *D* = -2.454; Table 2), but only deviated significantly from neutral equilibrium for red bats, indicating an excess of low frequency mutations typically associated with a history of population expansion.

Population structure

No evidence of population genetic structure was found for turbine-killed bats of either species, as determined by STRUCTURE analysis of the microsatellite genotypes. In both species, mean Ln(probability of data) was highest at K = 1, and generally decreased with increasing K (Fig. 5). A small degree of geographic structure in microsatellite allele frequencies was present among wind-turbine sites for red bats (AMOVA Φ_{ST} = 0.0288, $P < 0.001$), although this structure was not evident for hoary bats, nor in the mitochondrial sequence data of either species (Tables 3 and A4). Red bats exhibited no geographic structuring in either microsatellite or mitochondrial sequence data by isotopic region of origin (local versus non-local).

Demographic scenarios and effective population size (N_e)

Three historical demographic scenarios were simulated in DIYABC using the combined dataset of microsatellite and mitochondrial sequence data: 1) a historically stable population; 2) a population that exhibited one discrete change in size; and 3) a population that exhibited two discrete changes in size. For hoary bats, no one historical scenario had a substantially higher probability than the others even though the observed data fell within the range of the simulations (Fig. A4), indicating that even though the

simulations captured the patterns of polymorphism in the observed data well, they were not able to discriminate among the competing demographic models. Therefore, we chose to use a model-averaging approach to estimate posterior parameters using the 1000 best simulations (i.e., those closest to the observed data) selected from across all three scenarios.

The ABC posterior estimate of the current N_e for hoary bats, which incorporated both the microsatellite and the mtDNA sequence data as well as variability in the mutation rates for both types of loci, was 6,091 (95% Credible Interval: 2,481 – 89,913; Fig. A5, Table 4). We also calculated N_e from the composite Θ parameters separately for microsatellites and mtDNA sequences. For this, we used the corresponding median posterior estimate of the mutation rates for each locus type from DIYABC (μ_{mic} = 5.76 x 10^{-4}, μ_{seq} = 1.19 x 10^{-5}), which were on par with values reported from the literature (Dallas 1992, Crawford and Cuthbertson 1996). The resulting N_e estimates ranged from 1,600 – 6,500, but with credible intervals that do not explicitly incorporate uncertainty in the mutation rates.

For red bats, scenario 3 showed high levels of posterior probability that clearly exceed the other two demographic scenarios (Fig. A4). Scenario 3 described a population whose ancestral size underwent a bottleneck approximately 287,000 generations ago (95% Credible Interval: 33,300–488,000) to less than 10% of the ancestral N_e, followed by a recent demographic expansion roughly 1,580 generations ago that increased the population size dramatically (Fig. A6). The posterior distribution for the current N_e integrated across both microsatellite and mtDNA data showed a modal value of ~3.6 million, suggesting an effective population size that is much larger than for hoary bats,

but also estimated with low precision as evidenced by the wide posterior distribution (95% Credible Interval: ~423,000 – 4.1 million; Fig. A5, Table 4). Using the median posterior estimate of the microsatellite mutation rate from DIYABC (μ_{mic} = 2.37 x 10^{-4}), we calculated an estimated current nuclear N_e of ~335,000 (95% Credible Interval: 55,529 – 2.61 million; Fig. A5, Table 4). Similarly, approximate mitochondrial N_e was calculated using the mtDNA sequence mutation rate from DIYABC (μ_{seq} = 1.19 x 10^{-6}) gave an estimate of ~1.0 million (95% Credible Interval: 0 – 11.6 million; Fig. A5, Table 4). Red bat N_e showed low sensitivity to the presence of null alleles in the microsatellite data, with estimates based on a subset of 8 loci with no null alleles producing very similar N_e estimates compared to the full dataset (Table 4).

Discussion

Hoary and red bats are the bat species most commonly killed at utility-scale wind-energy facilities in North America (Arnett et al. 2008). Overall, our results indicate that red bats killed at wind-energy facilities in the central Appalachians originate from both local and non-local sources, and currently represent a single diverse and unstructured population, with a very large N_e (in the range of 10^5-10^6 individuals). In stark contrast, hoary bat mortalities in the central Appalachians consist of predominantly local bats, originating from a population of substantially smaller N_e (in the range of 10^3-10^4 individuals) with reduced diversity.

For both species, it is important to note that our sampling of bats from central Appalachian wind-energy facilities likely encompassed only a small portion of the total summering range of each species, and thus would not capture the contribution of any larger-scale population structure or diversity that might exist outside of our study region. A recent range-wide study of red bats also concluded no population structure and an N_e of approximately 10^5-10^6 (Vonhof and Russell 2015), which suggests that diversity is well homogenized across the range of this species due to extensive mixing during migration and lack of female philopatry. Similarly, Korstian et al. (2015) also observed no genetic structure for red and hoary bats obtained from wind-energy facilities in Minnesota and Texas, which is similar to our results. However, Korstian et al. (2015) suggested that both red and hoary bats have large present-day N_e. Thus our conclusion a much smaller N_e for hoary than red bats must be conservatively interpreted as pertaining only to the central Appalachian region. A more geographically extensive sample is required to assess N_e across the range of the hoary bat. Nevertheless, within the central Appalachian region where rates of wind-turbine mortality are among the highest reported (Arnett et al. 2008), our results suggest that red bats may be better able to absorb sustained mortality than hoary bats, and that continued mortality of hoary bats as the result of wind-energy development may have long-lasting impacts.

Local versus non-local origins

Little is known about potential pathways and corridors used by migratory bats in North America or how wind-energy development may affect such patterns. Our results indicate that ~57% of red bats killed are non-local (Figs. 4 and A1) and likely represent

individuals that summered at locations far from wind-energy facilities in the central Appalachian Mountains, whereas ~99% of hoary bats summered locally. The variation in the likelihood of local origin for these species may indicate differences in migratory pathways/corridors that they use when traveling to their summering and wintering grounds. Although such a conclusion requires future validation across a larger geographic extent, our δ^2H data suggest that mortality caused by wind-energy development affects red bats from across a broad area. In contrast, although the number of turbine-associated hoary (and red) bat mortalities peak during migratory periods (fall, in particular; Cryan and Barclay 2009), the bulk of hoary bat mortality appears to be composed of local bats originating near the site of mortality. Thus, although the spatial precision is coarse, the estimates of origin from our δ^2H data suggest inter-specific differences in geographic provenance for two closely related species of bats that have similar life-history traits (Shump and Shump 1982b, a). The range of hoary bats is much larger than the range of red bats, yet our inference of origins suggests that hoary bats are more likely to be local than red bats. This might suggest that the bats in our sample from Central Appalachian wind-energy facilities come from a geographically restricted subpopulation of hoary bats, and may not be representative of the larger population. Overall, these results suggest that a given wind-energy facility may impact bats originating across a broad geographic extent, in agreement with recent studies in Germany (Voigt et al. 2012) and southwestern Canada (Baerwald et al. 2014).

Population genetic structure

The lack of genetic structure in our data suggests that wind-turbine mortality is affecting panmictic populations of hoary and red bats, at least within the central Appalachians (Fig. 5). The observed lack of structure suggests high historical gene flow and also that the potential genetic impacts of turbine-associated mortality (e.g., reduced genetic diversity) will largely affect the breeding population as a whole rather than disproportionately affecting specific subpopulations. Previous studies of both species also support an unstructured population (Korstian et al. 2015, Vonhof and Russell 2015),. Unlike colonially roosting temperate bats which tend to exhibit highly structured populations due to restricted gene flow (e.g., Russell et al. 2005, Lack et al. 2010, Dixon 2011), lasiurine bats, although isolated from others for most of the year (Shump and Shump 1982a, b, Cryan 2003), comingle during the fall migration period to mate. Thus gene flow occurs when bats converge during migration and is not necessarily limited to individuals summering within close geographic proximity. This is similar to patterns of nuclear population structure in the migratory noctule bat in Europe, which suggest widespread gene flow as a result of male movement; however, unlike our results, noctule bats showed elevated population structure in mtDNA, likely due to female philopatry (Petit and Mayer 1999a).

Although no genetic population structure was apparent in the STRUCTURE analysis, red bats did exhibit a small amount of geographic structure in allele frequencies among sites (Tables 3 and A4). This small yet significant geographic population structure suggests that spatial segregation of red bats into different summering areas imposes temporary genetic differentiation among sites. However, such segregation does not result

in long-term genetic differentiation within the breeding population since individuals converge again to mate.

Effective population size

For red bats, the DIYABC analyses revealed a large effective population with signatures of demographic expansion after a historical decline. The broad confidence intervals mean the timing of the expansion following the bottleneck is not precisely known; however, the range overlaps with the end of the last glacial period, a time when many species of plants and animals underwent similar population expansions (Hewitt 2000), including migratory bats (Petit et al. 1999b). The potential expansion in N_e of red bats was also captured by the negative Tajima's *D* observed for the mtDNA sequences (Table 2).

When coupled with the lack of genetic population structure, our data suggest that red bats currently represents a single, massive breeding population, with an effective population size in the hundreds of thousands to millions of individuals. Since the ratio of N_e to the census population size, N_c, is often substantially less than 1 (often ~ 0.1) (Frankham 1995), the number of red bat individuals is likely to be substantially higher. A similar conclusion was reached independently by Vonhof and Russell (2015), who also used genetics to infer the effective population size of red bats in North America. Using a similar approach of applying nuclear microsatellite markers and mtDNA sequence data to several different coalescent-based estimators, Vonhof and Russell (2015) found N_e values of 10^4-10^6, but with intermediate estimates of ~10^5, similar to our coalescent estimates for red bats. It is important to note that Vonhof and Russell (2015) sampled red bats from

across most of this species range and across a broader geographic extent than ours; however, given the lack of genetic structure reported in both their study and ours, and the approximately similar values of N_e, it seems likely that our more geographically restricted sampling still captured a substantial portion of the diversity in this widely dispersing species.

In contrast to the large, expanded population size of red bats, our results from the central Appalachians suggest that hoary bats in our sample are less diverse, with a substantially smaller N_e (Table 4). Although the ABC analysis could not discriminate among a historical scenario of a temporally stable population versus scenarios that included one or more changes in N_e (Fig. A4), the negative Tajima's *D* in the mtDNA data suggests some support for population expansion (Table 2). Overall, while it should be stressed that there was considerable uncertainty associated with the estimated N_e values, it appears that hoary bats killed at wind-energy facilities in the central Appalachians represent a relatively small effective population.

Given that hoary bats are a very widespread species with summer grounds that extend across the longitudinal breadth of North America, and that large numbers of hoary bats are killed by wind turbines each year, it is perhaps surprising that N_e for hoary bats in our sample is so small, especially relative to red bats. There are several factors that may reduce N_e when in fact a much larger census population size exists. For instance, the presence of overlapping generations, unequal sex ratios, and the occurrence of historical bottlenecks are some of the features that may reduce the N_e relative to the actual population size (Palstra and Ruzzante 2008, Hare et al. 2011, Waples et al. 2014). Some of these concerns may be especially pertinent for long-lived migratory tree bats.

It is also possible that the small N_e value for hoary bats could represent a regional subpopulation derived from a historically larger population that was not represented in our sample. Our sample of both species comes from a geographically restricted number of wind-energy facilities in the central Appalachian region (Fig. 1), and thus might have missed other genetic subpopulations of hoary bats that might exist outside our geographic region of sampling. Subpopulation structure is common among organisms that are widely distributed such as hoary bats, particularly those with expansive east-to-west distributions (e.g., Milot et al. 2000). If our sample of hoary bats is just one of multiple regional subpopulations distributed in different areas of North America, then sustained mortality in each subpopulation could decrease N_e at a faster rate than the same level of mortality occurring in an unstructured population. Consequently, a level of mortality that might not severely impact a panmictic population may be detrimental to the long-term stability of a genetically structured population. Potential support for the idea of regional subpopulations comes from Korstian et al. (2015) who reported similar and large N_e values for red and hoary bats in Minnesota and Texas. Further investigation with more geographically widespread samples is required to assess the species-wide genetic diversity and effective population size of hoary bats across North America, and how wind-turbine mortality may affect the species as a whole.

Although our study focused on bats killed at utility-scale wind-energy facilities in eastern North America, the expansion of wind energy and the potential long-term impacts to bat populations is a global issue. Besides North America and Europe, wind-energy facilities in Australia and South Africa have also reported bat mortality (Aronson et al. 2013, Hull and Cawthen 2013). Understanding the potential biological impacts of

turbine-associated mortality to bats is often complicated by a lack of data. Studies such as ours can help to identify species or even subpopulations at particular risk of wind-turbine mortality. In addition to providing critical genetic data on two of the most commonly killed species in North America, our results provide a much-needed baseline for future monitoring and research. Overall, our results illustrate that stable isotopes and population genetics are powerful tools for assessing the population status of bats affected by wind-energy development.

Acknowledgements

We thank West, Inc., the Pennsylvania Game Commission, the West Virginia DNR, and numerous field technicians for providing samples, Robin Paulman for assistance with stable isotope analysis, Regina Trott for assistance with genetic analysis, and John Sherwell for discussion and support. We thank Robin Waples for helpful comments and discussion on N_e and Christian Voigt and two anonymous reviewers for helpful comments on an earlier version of the manuscript. This research was supported by the Maryland Department of Natural Resources (DNR) Power Plant Research Program and the EPA STAR Fellowship program.

References

Armstrong, A., S. Waldron, J. Whitaker, and N. J. Ostle. 2014. Wind farm and solar park effects on plant-soil carbon cycling: uncertain impacts of changes in ground-level microclimate. Global Change Biology **20**:1699-1706.

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. 2008. Patterns of bat fatalities at wind energy facilities in North America. Journal of Wildlife Management **72**:61-78.

Aronson, J. B., A. J. Thomas, and S. L. Jordaan. 2013. Bat fatality at a wind energy facility in the Western Cape, South Africa. African Bat Conservation News **31**:9-12.

Baerwald, E. F., W. P. Patterson, and R. M. R. Barclay. 2014. Origins and migratory patterns of bats killed by wind turbines in southern Alberta: evidence from stable isotopes. Ecosphere **5**.

Barclay, R. M. R., and L. M. Harder. 2003. Life histories of bats: life in the slow lane.*in* T. H. Kunz, editor. Bat ecology. University of Chicago Press, Chicago.

Beerli, P. 2006. Comparison of Bayesian and maximum-likelihood inference of population genetic parameters. Bioinformatics **22**:341-345.

Bolker, B., and R. D. C. Team. 2014. bbmle: Tools for general maximum likelihood estimation. R package version 1.0.17.

Bowen, G. J. 2010. Isoscapes: Spatial pattern in isotopic biogeochemistry. Annual Review of Earth and Planetary Sciences **38**:161-187.

Bowen, G. J., Z. Liu, H. B. Vander Zanden, L. Zhao, and G. Takahashi. 2014. Geographic assignment with stable isotopes in IsoMAP. Methods in Ecology and Evolution **5**:201-206.

Boyles, J. G., P. M. Cryan, G. F. McCracken, and T. H. Kunz. 2011. Economic importance of bats in agriculture. Science **332**:41-42.

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.

Coplen, T. B., and H. P. Qi. 2012. USGS42 and USGS43: Human-hair stable hydrogen and oxygen isotopic reference materials and analytical methods for forensic science and implications for published measurement results. Forensic Science International **214**:135-141.

Cornuet, J. M., F. Santos, M. A. Beaumont, C. P. Robert, J. M. Marin, D. J. Balding, T. Guillemaud, and A. Estoup. 2008. Inferring population history with DIY ABC: a user-friendly approach to approximate Bayesian computation. Bioinformatics **24**:2713-2719.

Crawford, A. M., and R. P. Cuthbertson. 1996. Mutations in sheep microsatellites. Genome Research **6**:876-879.

Cryan, P. M. 2003. Seasonal distribution of migratory tree bats (Lasiurus and Lasionycteris) in North America. Journal of Mammalogy **84**:579-593.

Cryan, P. M., and R. M. R. Barclay. 2009. f. Journal of Mammalogy **90**:1330-1340.

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., C. A. Stricker, and M. B. Wunder. 2014. Continental-scale, seasonal movements of a heterothermic migratory tree bat. Ecological Applications **24**:602-616.

Dallas, J. F. 1992. Estimation of microsatellite mutation rates in recombinant inbred strains of mouse. Mammalian Genome **3**:452-456.

Dixon, M. D. 2011. Population genetic structure and natal philopatry in the widespread North American bat *Myotis lucifugus*. Journal of Mammalogy **92**:1343-1351.

Earl, D. A., and B. M. Vonholdt. 2012. STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. Conservation Genetics Resources **4**:359-361.

Evanno, G., S. Regnaut, and J. Goudet. 2005. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Molecular Ecology **14**:2611-2620.

Excoffier, L., and H. E. L. Lischer. 2010. Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. Molecular Ecology Resources **10**:564-567.

Excoffier, L., P. E. Smouse, and J. M. Quattro. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: Application to human mitochondrial-DNA restriction data. Genetics **131**:479-491.

Feldhamer, G., L. Drickhamer, S. Vessey, and J. Merritt. 2004. Chiroptera. Pages 199-218 Mammalogy Adaptations, Diversity, Ecology. McGraw-Hill.

Frankham, R. 1995. Effective population/size adult population size ratios in wildlife: a review. Genetical Research **66**:95-107.

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**.

Garza, J. C., and E. G. Williamson. 2001. Detection of reduction in population size using data from microsatellite loci. Molecular Ecology **10**:305-318.

Glaubitz, J. C. 2004. CONVERT: A user-friendly program to reformat diploid genotypic data for commonly used population genetic software packages. Molecular Ecology Notes **4**:309-310.

Goudet, J. 1995. FSTAT (Version 1.2): A computer program to calculate F-statistics. Journal of Heredity **86**:485-486.

Hare, M. P., L. Nunney, M. K. Schwartz, D. E. Ruzzante, M. Burford, R. S. Waples, K. Ruegg, and F. Palstra. 2011. Understanding and estimating effective population size for practical application in marine species management. Conservation Biology **25**:438-449.

Hayes, M. A. 2013. Bats killed in large numbers at United States wind energy facilities. Bioscience **63**:975-979.

Hewitt, G. 2000. The genetic legacy of the Quaternary ice ages. Nature **405**:907-913.

Holland, R. A., and M. Wikelski. 2009. Studying the migratory behavior of individual bats: Current techniques and future directions. Journal of Mammalogy **90**:1324-1329.

Hull, C. L., and L. Cawthen. 2013. Bat fatalities at two wind farms in Tasmania, Australia: Bat characteristics, and spatial and temporal patterns. New Zealand Journal of Zoology **40**:5-15.

IPCC. 2014. Climate Change 2014: Impacts, Adaptation, and Vulnerability. Contribution of Working Group II to the fifth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge/New York.

Jaber, S. 2013. Environmental impacts of wind energy. Journal of Clean Energy Technologies **1**:251-254.

Jakobsson, M., and N. A. Rosenberg. 2007. CLUMPP: a cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure. Bioinformatics **23**:1801-1806.

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

Keller, S. R., R. Trott, C. Pylant, and D. M. Nelson. 2014. Genome-wide microsatellite marker development from next-generation sequencing of two non-model bat species impacted by wind turbine mortality: *Lasiurus borealis* and *L. cinereus* (Vespertilionidae). Molecular Ecology Resources **14**:435-436.

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.

Korstian, J. M., A. M. Hale, and D. A. Williams. 2015. Genetic diversity, historic population size, and population structure in two North American tree bats. Journal of Mammalogy **in press**.

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. Frontiers in Ecology and the Environment **5**:315-324.

Kuvlesky, W. P., L. A. Brennan, M. L. Morrison, K. K. Boydston, B. M. Ballard, and F. C. Bryant. 2007. Wind energy development and wildlife conservation: Challenges and opportunities. Journal of Wildlife Management **71**:2487-2498.

Lack, J. B., J. E. Wilkinson, and R. A. V. D. Bussche. 2010. Range-wide population genetic structure of the pallid bat (*Antrozous pallidus*) — incongruent results from nuclear and mitochondrial DNA. Acta Chiropterologica **12**:401-413.

Lehnert, L. S., S. Kramer-Schadt, S. Schonborn, O. Lindecke, I. Niermann, and C. C. Voigt. 2014. Wind farm facilities in Germany kill noctule bats from near and far. PLoS One **9**.

Librado, P., and J. Rozas. 2009. DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. Bioinformatics **25**:1451-1452.

Matschiner, M., and W. Salzburger. 2009. TANDEM: integrating automated allele binning into genetics and genomics workflows. Bioinformatics **25**:1982-1983.

McArdle, B. H. 1988. The structural relationship - regression in biology. Canadian Journal of Zoology-Revue Canadienne De Zoologie **66**:2329-2339.

Meier-Augenstein, W., K. A. Hobson, and L. I. Wassenaar. 2013. Critique: measuring hydrogen stable isotope abundance of proteins to infer origins of wildlife, food and people. Bioanalysis **5**:751-767.

Milot, E., H. L. Gibbs, and K. A. Hobson. 2000. Phylogeography and genetic structure of northern populations of the yellow warbler (*Dendroica petechia*). Molecular Ecology **9**:667-681.

Palstra, F. P., and D. E. Ruzzante. 2008. Genetic estimates of contemporary effective population size: what can they tell us about the importance of genetic stochasticity for wild population persistence? Molecular Ecology **17**:3428-3447.

Popa-Lisseanu, A. G., K. Sorgel, A. Luckner, L. I. Wassenaar, C. Ibanez, S. Kramer-Schadt, M. Ciechanowski, T. Gorfol, I. Niermann, G. Beuneux, R. W. Myslajek, J. Juste, J. Fonderflick, D. H. Kelm, and C. C. Voigt. 2012. A triple-isotope approach to predict the breeding origins of European bats. PLoS One **7**.

Pritchard, J. K., M. Stephens, and P. Donnelly. 2000. Inference of population structure using multilocus genotype data. Genetics **155**:945-959.

Pylant, C., D. M. Nelson, and S. R. Keller. 2014. Stable hydrogen isotopes record the summering grounds of eastern red bats (*Lasiurus borealis*). PeerJ **2**:e629.

Roscioni, F., D. Russo, M. Di Febbraro, L. Frate, M. L. Carranza, and A. Loy. 2013. Regional-scale modelling of the cumulative impact of wind farms on bats. Biodiversity and Conservation **22**:1821-1835.

Rosenberg, N. A. 2004. DISTRUCT: a program for the graphical display of population structure. Molecular Ecology Notes **4**:137-138.

Rubenstein, D. R., and K. A. Hobson. 2004. From birds to butterflies: animal movement patterns and stable isotopes. Trends in Ecology & Evolution **19**:256-263.

Russell, A. L., R. A. Medellin, and G. F. McCracken. 2005. Genetic variation and migration in the Mexican free-tailed bat (*Tadarida brasiliensis mexicana*). Molecular Ecology **14**:2207-2222.

Rydell, J., L. Bach, M. J. Dubourg-Savage, M. Green, L. Rodrigues, and A. Hedenstrom. 2010. Bat mortality at wind turbines in northwestern Europe. Acta Chiropterologica **12**:261-274.

Saidur, R., N. A. Rahim, M. R. Islam, and K. H. Solangi. 2011. Environmental impact of wind energy. Renewable & Sustainable Energy Reviews **15**:2423-2430.

Shump, K. A., and A. U. Shump. 1982a. *Lasiurus borealis*. Mammalian Species:1-6.

Shump, K. A., and A. U. Shump. 1982b. *Lasiurus cinereus*. The American Society of Mammalogists **185**:1-5.

Smith, M. F., and J. L. Patton. 1993. The diversification of South-American murid rodents - Evidence from mitochondrial-DNA sequence data for the akodontine tribe. Biological Journal of the Linnean Society **50**:149-177.

Smith, R. J. 2009. Use and misuse of the reduced major axis for line-fitting. American Journal of Physical Anthropology **140**:476-486.

Sullivan, A. R., J. K. Bump, L. A. Kruger, and R. O. Peterson. 2012. Bat-cave catchment areas: using stable isotopes (δD) to determine the probable origins of hibernating bats. Ecological Applications **22**:1428-1434.

Tabassum-Abbasi, M. Premalatha, T. Abbasi, and S. A. Abbasi. 2014. Wind energy: Increasing deployment, rising environmental concerns. Renewable & Sustainable Energy Reviews **31**:270-288.

Tajima, F. 1989. Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. Genetics **123**:585-595.

Taylor, P. D., S. A. Mackenzie, B. G. Thurber, A. M. Calvert, A. M. Mills, L. P. McGuire, and C. G. Guglielmo. 2011. Landscape movements of migratory birds and bats reveal an expanded scale of stopover. PLoS One **6**.

Team, R. D. C. 2014. R: a language and environment for statistical computing. Vienna, Austria.

Voigt, C. C., L. S. Lehnert, G. Petersons, F. Adorf, and L. Bach. 2015. Wildlife and renewable energy: German politics across migratory bats. European Journal of Wildlife Research **61**:213-219.

Voigt, C. C., A. G. Popa-Lisseanu, I. Niermann, and S. Kramer-Schadt. 2012. The catchment area of wind farms for European bats: A plea for international regulations. Biological Conservation **153**:80-86.

Vonhof, M. J., and A. L. Russell. 2015. Genetic approaches to the conservation of migratory bats: a study of the eastern red bat (*Lasiurus borealis*). PeerJ **3**:e983.

Waples, R. S., T. Antao, and G. Luikart. 2014. Effects of overlapping generations on linkage disequilibrium estimates of effective population size. Genetics **197**:769-U603.

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.

Wiley, A. E., A. J. Welch, P. H. Ostrom, H. F. James, C. A. Stricker, R. C. Fleischer, H. Gandhi, J. Adams, D. G. Ainley, F. Duvall, N. Holmes, D. C. Hu, S. Judge, J. Penniman, and K. A. Swindle. 2012. Foraging segregation and genetic divergence

between geographically proximate colonies of a highly mobile seabird. Oecologia **168**:119-130.

Woods, A. J., J. M. Omernik, and D. D. Brown. 1999. Level III and IV Ecoregions of Delaware, Maryland, Pennsylvania, Virginia, and West Virginia. U.S. Environmental Protection Agency.

Wunder, M. B. 2012. Determining geographic patterns of migration and dispersal using stable isotopes in keratins. Journal of Mammalogy **93**:360-367.

SUPPORTING INFORMATION

Four supporting tables and six supporting figures are associated with this manuscript.

TABLES

Table 1. Summary of bats collected by year and wind-energy facility.

State	Project	Year	No. *L. cinereus*	No. *L. borealis*
West Virginia	Mountaineer	2003	30	-
West Virginia	Mount Storm	2009	12	6
West Virginia	Mount Storm	2010	13	15
West Virginia	Mount Storm	2011	19	31
Pennsylvania*	Northeast	2011	18	-
Pennsylvania*	Southcentral	2011	29	-
Pennsylvania*	Southwest	2011	5	-
Maryland	Criterion	2011	120	92
			246	144

*Names and locations for individual wind-energy facilities were not provided. Samples were pooled according to regional game unit designations.

Table 2. Summary of mtDNA *cytb* diversity. Values in parentheses are the 95% CI from 1,000 coalescent simulations of a population in neutral demographic equilibrium; thus negative *D* values outside of the CI are evidence for demographic expansion.

	L. cinereus	***L. borealis***
N individuals	240*	141*
Length (bp)	726	765
$N_{haplotypes}$	58	104
Haplotype diversity	0.871	0.991
Θ	0.0146	0.0312
π	0.00693	0.00747
Tajima's *D*	-1.553 (-1.589 , 1.901)	-2.454 (-1.559 , 2.061)

*values differ from those reported in Table 1 due to lack of amplification for some individuals

Table 3. Summary AMOVA results for each species by location of mortality and by isotopic region of origin (i.e., local versus non-local). For this analysis, local bats are those with probability values > 0.7, whereas non-local bats are those with probability values < 0.3. As nearly all *L. cinereus* were found to be of local origin, these data were excluded from an analysis by region of origin.

	By Location of Mortality		**By Isotopic Region**
Microsatellite	***L. cinereus***	***L. borealis***	***L. borealis***
Source of Variation	Percentage of Variation	Percentage of Variation	Percentage of Variation
Among Locations	0	2.88*	0.09
Within Locations	100	97.12	99.91

	By Location of Mortality		**By Isotopic Region**
Mitochondrial	***L. cinereus***	***L. borealis***	***L. borealis***
Source of Variation	Percentage of Variation	Percentage of Variation	Percentage of Variation
Among Locations	-0.80	-0.31	-0.15
Within Locations	100.80	100.31	100.15

*significant result ($p < 0.001$)

Table 4. Population genetic estimates of effective population size (N_e) for bats killed by wind turbines in the Central Appalachians. Reported estimates reflect long-term evolutionary N_e based on rates of gene coalescence from approximate Bayesian computation (ABC). Estimates are given for the combined microsatellite and mtDNA sequence data as well as for each type of genetic data separately. For *L. borealis*, we also report N_e estimates for ABC models that excluded N=6 loci which showed evidence of null alleles.

	Dataset	**N_e Estimate**	**Lower 95% CI**	**Upper 95% CI**
L. cinereus				
All loci:	combined	6,091	2,481	89,913
	mtDNA[a]	6,526	0	63,418
	msats[a]	1,611	662	4,697
L. borealis				
All loci:	combined	3,609,910	422,857	4,090,010
	mtDNA[a]	1,011,553	0	11,635,990
	msats[a]	334,986	55,529	2,605,573
Loci without null alleles:	combined	3,787,759	596,954	3,993,906
	mtDNA[a]	789,041	18,637	14,021,859
	msats[a]	265,324	32,353	4,658,494

[a] N_e estimate derived from transformation of the composite parameter Θ (= $4N_e\mu$ for nuclear microsatellites, $N_e\mu$ for mtDNA), using the μ values for each species estimated by DIYABC.

Figure legends

Figure 1. Locations of wind-energy facilities contributing samples to this study. Pennsylvania samples were pooled according to Pennsylvania Game Commission (PAGC) regional game unit designations (PAGC_NE = Northeast; PAGC_SC = Southcentral; PAGC_SW = Southwest). Maryland: Criterion = Criterion Wind Project. West Virginia: Mtneer = Mountaineer Wind Energy Center; Mt Storm = Mount Storm Wind Farm. Locations shown for PAGC regional game units represent most wind-energy facilities operational at the time of this study and do not necessarily represent individual facilities. The solid line represents the known range of *L. borealis*. The range of *L. cinereus* spans the majority of the extent of North America that is shown.

Figure 2. Best-fitting probability density functions (black curves) versus the observed density distributions of minimum isopleth contour values (grey bars) containing the sampling location for (A) 64 known-location *L. borealis* museum specimens (Pylant et al. 2014) and (B) 117 known-location *L. cinereus* samples (Cryan et al. 2014).

Figure 3. Frequencies of stable hydrogen isotope ratios of precipitation for *L. cinereus* (n = 246) and *L. borealis* (n = 144).

Figure 4. Probability values at the location of mortality for *L. cinereus* and *L. borealis*.

Figure 5. Bayesian clustering analysis of population structure. Top panels represent results for *L. cinereus*, whereas bottom panels represent results for *L. borealis*. Results of

model selection are in the left column, and ancestry assignments to clusters (Q-values) for each individual are displayed in the right column.