Final Technical Report

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Executive Summary

Documented fatalities of bats at wind turbines have raised serious concerns about the future impacts of increased wind power development on populations of migratory bat species. These mortalities have been recorded for 11 of the 45 bat species found in North America, but wind turbines predominantly affect tree roosting migratory bats, including eastern red bats (*Lasiurus borealis*), hoary bats (*Lasiurus cinereus*), and silver-haired bats (*Lasionycteris noctivagans*), which together represent 72.8% of the annual bat fatalities reported for wind energy facilities in the United States. Although mortalities may occur throughout April to November, most turbine-associated bat fatalities in North America have been reported in late summer and early autumn and appear to be concentrated during the fall migration of the affected migratory tree roosting species. To date, much of the research on bat-wind power interactions has focused on examining patterns and predictors of mortality at wind farms, operational mitigation, and possible deterrents; such studies have provided valuable information to prevent or mitigate bat mortalities on a local scale. However, we need to have a greater understanding of current population sizes, trends in population growth or decline, and patterns of population differentiation across the landscape for affected species to understand whether mortalities at wind power developments pose a serious risk to bat populations and whether the risks encountered by these populations are differentiated geographically. Bat fatalities may thus represent one of the major biological impacts of wind energy development, yet we lack the necessary information to place levels of mortality in context with respect to baseline population estimates and demographic trends. For most bat species we have no knowledge of the size of populations and their demographic trends, the degree of structuring into discrete subpopulations, and whether different subpopulations use spatially segregated migratory routes. Given the difficulties associated with applying traditional demographic methods such as capture-mark-recapture to bats, we require other approaches to estimating population sizes and demographic trends within bat populations.

Genetic approaches provide an alternative to traditional demographic methods of population estimation, and allow us to estimate the degree of population structuring, demographic trends within subpopulations, and effective population size (N_e) using data on allele frequencies or the base composition of DNA sequences. Here, we describe the results of our study using genetic data to (1) estimate the effective population size for eastern red bats (*Lasiurus borealis*), (2) assess signals of population growth or decline in that species, and (3) evaluate patterns of population structure across the landscape. One of the great values of N_e estimation is that by comparing current and historical estimates we can gain valuable information on population trajectories over time. Estimations of N_e have been commonly applied to small populations where high levels of genetic drift are expected; the responsiveness of these methods to declines in very large populations are not as well understood. Therefore, a further goal of our study was to use a simulation approach to explore the influence of initial population size, mortality rate, data type, and analytical method on our power to detect population declines over short time intervals.

Using a large dataset of both nuclear and mitochondrial DNA variation for eastern red bats, we demonstrated that: 1) this species forms a single, panmictic population across their range with no evidence for the historical use of divergent migratory pathways by any portion of the population; 2) the effective size of this population is in the hundreds of thousands to millions; and 3) for large populations, genetic diversity measures and at least one coalescent method are insensitive to even very high rates of population decline over long time scales and until population size has become very small. Our data and analyses suggest that genetic approaches are not an effective tool for assessing potential short-term population declines in large

populations of migratory wildlife affected by wind power development. That said, genetic markers can be used in a wide variety of other contexts to provide valuable information on species identification, patterns of connectivity and gene flow across the landscape, local adaptation, genomic responses to environmental stressors, the evolutionary potential of populations, local population size in capture-mark-recapture studies, and patterns of dispersal and individual movements.

Introduction

As concerns about anthropogenic climate change and the long-term environmental impacts of the burning of fossil fuels on biological and human systems have heightened, there is increasing motivation to develop alternative sources of energy that will reduce the production of greenhouse gasses. Wind power has become an increasingly important sector of the energy industry and is one of the fastest growing sources of renewable energy. Despite the many positive aspects of wind power development, there have been some unexpected environmental costs. In particular, fatalities of bats at wind power installations have emerged as a major environmental impact of wind power development, and large mortality events have been reported at a number of wind energy facilities in the United States and abroad (Erickson et al. 2001, 2005, Kunz et al. 2007, Arnett et al. 2008). The bat species most affected by wind power in North America are migratory, tree-roosting species such as hoary bats (*Lasiurus cinereus*), eastern red bats (*Lasiurus borealis*), and silver-haired bats (*Lasionycteris noctivagans*), which together constitute almost three-quarters of the bat carcasses found at wind turbines (Arnett et al. 2008).

The observed high levels of mortality for these species at wind power developments raise concerns about the long-term impacts on their populations, yet we lack the necessary information to place this mortality in context with respect to baseline population estimates and demographic trends of the affected species. For most bat species we have no knowledge of the size of populations and their demographic trends, the degree of structuring into discrete subpopulations, and whether different subpopulations use spatially segregated migratory routes. While estimates of local population sizes within particular roosts may be feasible using traditional capture-mark-recapture (CMR) methodology or survey techniques (O'Shea and Bogan 2003, Kunz et al. 2009), only one study (Vonhof and Fenton 2004) has estimated population size in a known area (outside of a single roost) using CMR methodology, and no reliable range-wide population estimates exist for any bat species. Traditional demographic approaches have limitations when applied to bats, as they are nocturnal, exhibit cryptic behavior, and are difficult to follow over time during extensive seasonal movements between summer breeding areas and overwintering sites (Cryan 2003, Rivers et al. 2006). The tree-roosting migratory bat species that are killed in high numbers at wind turbines are especially inaccessible for traditional CMR studies, given their solitary nature and restriction to forested habitats (Kunz 1982, Shump and Shump 1982a,b). Large-scale banding studies typically experience extremely low recapture rates (e.g., Glass 1982; reviewed in O'Shea and Bogan 2003), and there are serious data deficiencies with respect to sex- and age-specific survival and reproductive rates that hamper our ability to apply demographic models to bat populations. Given these difficulties, we require other approaches to estimating population sizes and demographic trends within bat populations.

Genetic approaches provide an alternative to traditional demographic methods of population estimation, and allow us to estimate the degree of population structuring, demographic trends within subpopulations, and effective population size (N_e) using data on allele frequencies or the base composition of DNA sequences. Fewer individuals need to be sampled

relative to CMR approaches, and individuals need only be sampled a single time for many analyses. In addition, no accessory data, such as age or sex, is required (although such information is useful if available), and population parameters can be estimated directly from the observed patterns of genetic variation. Molecular markers can be used to examine levels of population differentiation within a species and to geographically delimit populations or groups of populations based on the observed distribution of genetic variation. Importantly, such analyses can be used to define the relevant unit for population monitoring, and highlight demographic connections among populations that may not be obvious from behavioral data alone. As mating is likely to take place during migration in bats (Dodd and Adkins 2007, Cryan 2008), gene flow should occur among populations that interact during migration; therefore it is likely that any genetically distinct populations, if they exist, will be using different migratory pathways. The analysis of genetic population structure is therefore highly relevant to our understanding of bat – wind power interactions.

Although it is not possible to directly estimate adult census population size (N_c) using molecular data (although genetic markers can be used to identify individuals for traditional CMR analyses; Luikart et al. 2010), it is possible to estimate N_e, which provides information on how quickly genetic variation is being lost, or relatedness is increasing, in a population of interest. It is defined as the number of individuals in an ideal population (a large, constant-sized, randomly-mating, hermaphroditic population with discrete generations) that would lose genetic variation at the same rate as the actual population (Crow and Denniston 1988). Essentially it is the size of the population that is experiencing genetic drift, and hence estimates the number of individuals actually contributing genes to the next generation. The estimation of N_e has seen wide application in studies of threatened or isolated populations, as the extent of genetic drift, and hence loss of genetic variation, is inversely proportional to N_e. Current estimates of N_e can be used to assess the 'genetic health' of populations and their capability to respond to future environmental change or anthropogenic changes via selection (Frankham et al. 2002).

The purpose of our project was to apply population genetic approaches to assess levels of genetic differentiation among populations and provide estimates of N_e using multiple techniques for the eastern red bat (*Lasiurus borealis*). This bat species was chosen because it is one of the three bat species of greatest concern with regard to the biodiversity impacts of wind energy, and has the highest fatality rate at a number of wind power installations in the eastern United States. Although estimates of total population size (actual numbers of individuals) would have been preferable, from the perspective of understanding the size of bat populations and the potential impact of fatalities at wind power installations, we wanted to test whether N_e estimates could provide us with information on the size of the evolutionarily-relevant portion of the population (that portion contributing genes to the next generation). We further assessed the potential to use estimates of N_e as a population monitoring tool through sensitivity analyses, where we modeled various starting population sizes, mortality rates, and time between measurements, and asked how much of a decline over what time scale is necessary to detect a significant change in N_e.

Background

We proposed that genetic estimates of effective population size (N_e) may be the best approach to understand the importance of fatalities at wind power installations for bat populations, as we cannot currently estimate total population size or population trends using traditional demographic methods. Our proposed research had two main objectives. First, using existing tissue samples taken from wild-captured and turbine-killed individuals from across their range, we set out to provide the best possible estimate of the N_e of eastern red bats. Multiple

analytical methods were used to estimate N_e to ensure that various aspects of the data were utilized and that analyses were not overly biased by the assumptions of any single method. Second, we performed sensitivity analyses to assess the utility of temporally-spaced estimates of N_e as a population monitoring tool. By using this current estimate of N_e as a baseline and modeling genetic diversity under defined scenarios of population decline, we determined whether population declines consistent with the magnitude of bat fatalities observed at wind turbines are detectable over relatively short time spans, and thus whether genetic monitoring is a potentially useful tool for managing these populations. In addition, we examined the influence of varying the number and diversity of molecular markers and the number of individuals sampled on our ability to detect demographic trends.

Our proposed research specifically applied to **Topic Area 3 – Environmental Impacts: Genetic studies to better estimate effective population sizes of affected bat species and potential population impacts that may be associated with wind development** in the 20% Wind by 2020 program. Our estimates of N_e will provide valuable information on the number of individuals across the range of eastern red bats, and thus provide the necessary context that has been lacking to understand the potential biological impact of increased wind power development on populations of eastern red bats.

Fatalities of bats at wind power installations represent one of the key environmental issues faced by the expanding wind energy industry. Genetic estimates of N_e may be our best option with respect to understanding the importance of fatalities at wind power installations for bat populations, as we cannot currently estimate total population size or population trends using traditional demographic methods. Our specific objectives and the approach we used to address them were as follows:

Objective 1. To use genetic approaches to estimate the effective population size of eastern red bats across their range.

Using existing tissue samples taken from wild-captured and turbine-killed individuals from across the range of eastern red bats, we used multiple analytical methods to provide the best possible estimate of the current N_e of eastern red bats.

Estimating Effective Population Size

There are two commonly estimated measures of N_e. Variance effective size (N_{eV}) is the size of an ideal population that would lose genetic variation via genetic drift at the same rate as the actual population, while inbreeding effective size (N_{eI}) is the size of an ideal population losing heterozygosity, due to increased relatedness, at the same rate as the actual population. These two measures may differ dramatically when a population is not stable (Leberg 2005). A rapid decrease in N_c is likely to cause a concurrent decrease in N_{eV}, via the immediate loss of alleles (and hence genetic variation) from the population, while N_{eI} may not decrease as rapidly because heterozygosity may decline much more slowly than the number of alleles. Similarly, during population growth, accumulated relatedness in a small population will not be reduced quickly by a rapid increase in N_c, but additional drift will be minimized and new alleles created via mutation are likely to be retained during population expansion. The differential sensitivity of these different measures of N_e make them applicable to different temporal scales, with N_{eV} typically used to estimate short-term or contemporary N_e, while N_{eI} is used to estimate long-term or historical N_e (Crandall et al. 1999). Most approaches to estimating N_{eV} make a number of assumptions, including that the population is sampled at random, the genetic markers are selectively neutral, unlinked, and do not undergo mutation between samples, no population

subdivision, no immigration, stable population size, and no overlapping generations (Leberg 2005, Wang 2005, Luikart et al. 2010).

The two most common methods used to estimate short-term N_{eV} are: 1) the temporal method, wherein multiple samples separated by several generations are collected from a population, and changes in allele frequencies between samples, which reflect genetic drift and are proportional to population size, are used to calculate N_{eV} (Waples 1989); and 2) the linkage disequilibrium method, which utilizes information on linkage disequilibrium to estimate the degree of genetic drift experienced by a population and hence N_{eV} (Waples and Do 2009, 2010). Assumptions about genetic markers (neutrality, no linkage, and no mutation) are likely met in most genetic surveys, and various maximum-likelihood and Bayesian methods have been developed that allow the other assumptions (no population subdivision, immigration, or overlapping generations and stable population size) to be relaxed (Williamson and Slatkin 1999, Anderson et al. 2000, Berthier et al. 2002, Wang and Whitlock 2003, Tallmon et al. 2004, Waples and Yokota 2007, Waples and England 2011, Robinson and Moyer 2013). Because these short-term estimates are based on changes in allele frequencies or allele frequency distributions, highly variable markers such as microsatellites (tandem repeats of 2-7 base pairs; Tautz 1989) are required. Precision of estimates benefits from increasing (1) sample size, (2) number of marker loci, and (3) number of generations between samples (Waples 1989, Luikart et al. 1998, Anderson et al. 2000, Berthier et al. 2002, Wang and Whitlock 2003), although marker diversity may be even more important than these other factors (Palstra and Ruzzante 2008).

Long-term estimates of N_{el} are typically estimated using a coalescent approach (Leberg 2005, Wang 2005). Coalescent theory uses relationships between genes, as influenced by mutation and genealogy, to understand evolutionary phenomena (Kingman 1982). Using a sample of genotypes (typically sequences from mitochondrial or nuclear genes), coalescent methods trace backward in time to infer events that occurred since the individuals in the sample shared a common ancestor (Fu and Li 1999). Current patterns of genetic variation in a population reflect both past and ongoing events, and hence we can learn a great deal about current and former population size and demographic trends using coalescent analyses. Because the ancestry of sampled alleles (i.e., the coalescent process) can only be inferred when mutations differentiate those alleles, coalescent methods estimate the scaled parameter θ, which is defined as $4N_e\mu$ when based on biparentally inherited markers (such as microsatellites), or $2N_e\mu$ when based on markers with uniparental inheritance (such as mitochondrial DNA or Y chromosome markers), where μ is the mutation rate of the marker used. This approach assumes that the correct mutation model and rate are known. Modern coalescent methods allow the simultaneous estimation of θ, migration (gene flow), recombination rates, and population growth rate, relaxing some of the assumptions of N_e estimation (Hey and Nielsen 2004, Kuhner 2006). In addition, because population growth (positive or negative) can simultaneously be taken into account, contemporary N_e estimates can be obtained, within the limits imposed by the mutation rate of the marker(s) used. However, these estimates still represent longer-term averages relative to estimates based on more rapidly evolving markers such as microsatellites. Sequence-based coalescent methods have much lower sample size requirements than do temporal methods, and in fact very large samples are not computationally feasible.

We used a number of approaches to estimate N_e for eastern red bats. Although we originally set out to estimate short-term N_{eV}, it quickly became apparent that N_e was large, precluding the use of single sample estimators based on linkage disequilibrium or summary statistics (Waples and Do 2009, 2010, Tallmon et al. 2004, 2008) which are only effective for N_e <1,000, and temporal methods such as the Jorde and Ryman (1995) method which are based on

changes in allele frequencies due to genetic drift between time points (as drift is negligible and undetectable with large N_e). Further, the cohort-based demographic data required for the latter method were simply not available for any bat species. Therefore, we focused on coalescent analyses of long-term N_{eI}.

Sampling

Tissue samples from across the range of eastern red bats were collected by researchers working in the field. From these samples we generated three data sets:

1) A sample of individuals (N = 277) from known populations collected in the summer months (June to mid-August when bats are likely to be resident) primarily between 2000-2006 for the purpose of assessing levels of genetic population structure and estimating N_e (Table 1). We received tissue samples for between 1 – 39 bats from any given location. We had sufficient samples (N>15) for 12 localities with which to carry out population genetic analyses (Figure 1, Table 1). Unlike colonial bats roosting in buildings or trees where bats can be captured in numbers from a single site during a single sampling session, tree-roosting bats such as eastern red bats are solitary, and sampling therefore must involve the capture of foraging individuals and may encompass individuals from a wider area over a longer time scale. For six of our sampled populations, bats were captured within a single county or location (AR, GA, MO, ON, TX, WV-Mason), while the other six populations consisted of individuals captured in several adjacent counties within a given state (IL, MD, MI, NC, TN, WV-Pend). Sixteen highly-variable microsatellite loci were genotyped for all individuals used in population level analyses (N = 285). A 408 bp fragment of the hypervariable 2 portion of the mitochondrial DNA control region (hereafter HV2) was sequenced for 218 individuals used in population-level analyses (not all individuals in each population were sequenced), as well as 77 bats from 30 additional locations that were not included in population-level analyses, for a total of 295 individuals sequenced. We further sequenced a 651 bp fragment of the Chymase Intron 1 (CHY) for a random subset of 103 individuals.

2) A sample of individuals from 2002 only (N = 353, including 109 individuals used in the first dataset) for estimating N_e at a single time point.

3) A sample of individuals from 2010 only (N = 226) for estimating N_e at a second time point. Data sets 2 and 3 were comprised of a mixture of individuals of known summer origin, as well as bats of unknown origin killed at wind power developments during fall migration. We performed microsatellite genotyping for individuals in data sets 2 and 3 but did not sequence HV2 or CHY.

Laboratory Methods

DNA was extracted from samples using a DNEasy Tissue Extraction Kit (Qiagen). All individuals in all three data sets were genotyped at 16 microsatellite loci using primers developed specifically for eastern red bats (primers Lbo-B06, C07, D08, D200, D202, D203, D204, D226, D240, D245, and D248; Eackles and King, pers. comm.), as well as primers originally developed for other bat species (MS3E10 and MS1C01, Trujillo and Amelon 2009; IBat-Ca22 Oyler-McCance and Fike 2011; Cora_F11_C04, Piaggio et al. 2009a; and Coto_G12F_B11R, Piaggio et al. 2009b). Loci were multiplexed whenever possible, and PCR reactions combined 10 ng of each primer and 2 μL template DNA, with an illustra PuReTaq ready-to-go PCR bead (GE Health Care) to a total volume of 25 μL. Cycling conditions followed those in Vonhof et al. (2002) with the exception of a 10s (rather than a 1s) extension step at 72 °C. Multiple PCR reactions were subsequently pooled for loading on an ABI3130 Sequencer at the Vanderbilt University DNA Sequencing Facility for fragment analysis, and visualized and scored using

GeneMarker software (SoftGenetics).

Amplification of the 408 bp fragment of the mitochondrial HV2 was initially carried out using the reverse complement of primer F from Wilkinson and Chapman (1991; RevF: CTA CCT CCG TGA AAC CAG CAA C) sitting in the central conserved sequence block and the primer sH651 located in the $tRNA_{Pro}$ gene (Castella et al. 2001). However, these primers span a region containing a large stretch of 6 bp repeats, resulting in a large amplicon of 1500-2000 bp. We therefore designed a new primer (HV2R2: TCC TGT WAC CAT TAA YTA ATA TGT CCC) to sit in the 3' tail of the HV2 before the repeats that amplified a 408 bp fragment. Amplification was carried out using the above reaction conditions and the cycling conditions in Castella et al. 2001. PCR reactions were cleaned using ExoSAP-IT (PCR Product Pre-Sequencing Kit, Affymetrix) and sent to the University of Arizona Genetics Core for bi-directional sequencing. Sequences were edited using CodonCode Aligner software (CodonCode Corporation).

The nuclear chymase sequence data were generated via cloning of PCR amplicons from a randomly-selected subset of individuals. First, a region of the chymase gene spanning intron 1 was amplified through PCR using the primers Chy-F (5'-GTC CCA CCT GGG AGA ATG TG-3') and Chy-R (5'-TGG GAG ATT CGG GTG AAG-3'; Venta et al. 1996). In this case the PCR reaction utilized 1 μL of template, but otherwise reaction conditions were identical to the above. The temperature profile included an initial extended denaturation of 95°C for 5 minutes, followed by 40 cycles of 95°C for 1 minute, 52°C for 1 minute and 72°C for 1.5 minutes, with a final extension step at 72°C for 4 minutes. This PCR reaction was cleaned using a PCR purification kit (Qiagen) and sent to the University of Arizona Genetics Core for bi-directional sequencing using the Chy-F and Chy-R primers. These diplotypes were edited and heterozygous sites called using Sequencher v.4.8 (GeneCodes). Individuals found to contain two or more heterozygous sites were targeted for cloning.

Cloning and transformation was performed using the TOPO TA cloning kit (Life Technologies). The cloning step combined 4 μL of fresh PCR product, 1 μL of salt solution, and 0.75 μL pCR®2.1-TOPO® TA vector in a total reaction volume of 6 μL. This cloning reaction was incubated at room temperature for 15 minutes, then stored overnight at –20°C. The prepared vector was then transformed into TOP10 ultracompetent *E. coli* cells using a 30 second heat shock at 42°C. Following the heat shock and hour-long recovery at 37°C in SOC media, 50 μL of the transformed cells were plated and grown overnight at 37°C on selective plates containing ampicillin and blue-white screening reagent (Sigma).

On the following day, 6-8 distinct white colonies were picked per plate, and used in PCR. The picked colonies were each suspended in 10 μL dH_2O and heated to 95°C for 10 minutes to lyse the cells. The cell lysate was then used directly as template DNA for colony screening through PCR. The PCR reaction combined 10 ng of each primer and 10 μL template DNA (cell lysate), with an illustra PuReTaq ready-to-go PCR bead (GE Health Care) to a total volume of 25 μL. The temperature profile followed that described above for the initial PCR. PCR reactions yielding clean amplicons of the expected size (~650 bp) were cleaned using ExoSAP-IT (Affymetrix) following the manufacturer's instructions. Cleaned PCR amplicons were then sent to the University of Arizona Genetics Core for bi-directional sequencing using the Chy-F and Chy-R primers.

CHY sequences were edited using Sequencher v.4.8 (GeneCodes). Heterozygous sites identified during the initial sequencing of diplotypes were resolved into continguous haplotypic alleles (i.e., phased alleles). Thirty-six individuals were experimentally phased through cloning and sequencing. The other 67 individuals with ambiguous diplotypes were computationally phased using Phase v.2.1.1 (Stephens et al. 2001; Stephens and Donnelly 2003) with a

confidence threshold of 0.95.

Analysis

Population Structure

Mitochondrial DNA analysis

To describe overall levels of mtDNA diversity within populations, we calculated haplotype (*h*) and nucleotide (π) diversities in DNASP 5.10.1 (Librado and Rozas 2009). We calculated pairwise F_{ST} values between populations and tested for significance with permutations in Arlequin 3.11 (Excoffier et al. 2005) to identify pairs that were genetically distinct. We also performed an analysis of molecular variance (AMOVA; Excoffier et al. 1992) to describe the relative amount of genetic variation within and among populations.

Microsatellite DNA analysis

Deviations from Hardy-Weinberg equilibrium (HWE) for each locus were estimated and loci were confirmed to be in linkage equilibrium using FSTAT v2.9.3 (Goudet 1995). To test for differences among sampling sites in levels of genetic diversity, several indices of nuclear genetic diversity were estimated, including number of alleles per locus, allelic richness, and the inbreeding coefficient (F_{IS}) using FSTAT, private allelic richness using HP-RARE 1.0 (Kalinowski 2005), and observed and expected heterozygosity using GENODIVE (Meirmans 2012). We then tested for differences among populations (or groups of populations) in allelic richness, and F_{IS} in FSTAT, and expected heterozygosity in GENODIVE, using 10,000 permutations.

We applied two different approaches to determine the most likely number of distinct genetic clusters independent of original sampling locations, as different clustering algorithms can produce different solutions and concordance among multiple techniques is suggestive of the presence of a strong genetic signal (Guillot et al. 2009). First, we utilized the model-based Bayesian clustering approach in STRUCTURE 2.3.3 software (Pritchard et al. 2000, Falush et al. 2003) with population membership as a prior (Hubisz et al. 2009). To determine the optimal number of clusters (K), we ran 10 runs per K, for K = 1–10, with an MCMC search consisting of an initial 100,000-step burn-in followed by 400,000 steps using the admixture model with correlated allele frequencies. The most likely number of clusters was determined using the Evanno et al. (2005) method implemented in the program STRUCTURE HARVESTER (Earl and vonHoldt 2012). The Evanno et al. (2005) method is not informative for the highest and lowest K values, therefore if the highest log likelihood value was observed for K=1 or 10 across all replicates, we accepted that as the K with the highest probability.

Second, we applied the repeated allocation approach of Duchesne and Turgeon (2012) implemented in the software FLOCK. In this method, samples are initially randomly partitioned into K clusters ($\geq$2), allele frequencies are estimated for each of the K clusters, and each genotype is then reallocated to the cluster with the highest likelihood score. Repeated reallocation based on likelihood scores (20 iterations per run) resulted in genetically homogeneous clusters within a run. Fifty runs were carried out for each K, and at the end of each run the software calculated the log likelihood difference (LLOD) score for each genotype (the difference between the log likelihood of the most likely cluster for the genotype and that of its second most likely cluster) and the mean LLOD over all genotypes. Strong consistency among

runs (resulting in 'plateaus' of identical mean LLOD scores) is used to indicate the most likely number of clusters (Duchesne and Turgeon 2012).

The level of genetic differentiation among pre-defined populations was determined by calculating pairwise distance measures, including F_{ST} (Weir and Cockerham 1984) and a measure independent of the amount of within population diversity (Jost's D; Jost 2008). As with mtDNA, we performed an AMOVA on microsatellite genotypes using ARLEQUIN.

N_e Estimation

We used three primary methods to estimate N_e, all of which are coalescent-based approaches.

1. msvar

The first approach we used was the coalescent-based software msvar v.1.3 (Beaumont 1999), which estimates effective population size and demographic trends from microsatellite genotype data. This analysis considers a model in which a single ancestral population of size N_A experiences exponential population size change beginning at time t until the population reaches the current size N_1. Bats sampled at two different time points (2002 and 2010; datasets 2 and 3, respectively) were analyzed separately to determine whether increases in mortality over that time interval had a measurable effect on genetic diversity. To make the analysis computationally feasible, we randomly subsampled 100 diploid individuals from each dataset. Subsampling was performed twice for each time point, yielding datasets A and B, to ensure that no bias was introduced. Each analyzed dataset thus included 100 genotypes (= 200 chromosomes) for each of 16 autosomal microsatellite loci.

The msvar analysis requires the specification of hyperpriors for each of the four demographic parameters, N_1, N_A, t, and the mutation rate μ. These hyperpriors describe distributions from which the locus-specific initial parameter values are drawn, and are given here as [$\log_{10}(N_1)$, $\log_{10}(N_A)$, $\log_{10}(\mu)$, $\log_{10}(t)$]. The parameter means were assumed to be normally distributed with means (7, 7, -3.5, 4.3) and standard deviations (3.5, 4, 0.5, 2). We chose these values for (1) N_1 based on estimates of N_e for eastern red bats from our own Lamarc analyses with a relatively large standard deviation to reflect our own uncertainty regarding this parameter, (2) N_A based on a null hypothesis of no change in population size with a slightly larger standard deviation to accommodate increased uncertainty in historical parameters, (3) μ based on Storz and Beaumont's (2002) msvar analysis of microsatellite variation in *Cynopterus* fruit bats, and (4) t based on a hypothesis of population size change due to ecological change associated with deglaciation with a relatively large standard deviation to reflect our own uncertainty regarding this parameter. The parameter standard deviations were assumed to be normally distributed with means (0, 0, 0, 0) and standard deviations (0.5, 0.5, 2, 0.5). The means of the parameter standard deviations were set to 0 to start the search algorithm with no inter-locus variation; the standard deviations of the parameter standard deviations followed recommendations of Storz and Beaumont (2002). Each of the four datasets (2 time points, with 2 subsamples each) were analyzed 2-3 times, with each run lasting ~74-200 million steps.

2. IMa2

Second, we used the coalescent-based software IMa2 (release date 27 August 2012; Hey 2010a, b) to estimate the effective size of the panmictic eastern red bat population using dataset 1. The analysis included the CHY and HV2 sequences and 16-locus microsatellite genotypes. One hundred microsatellite genotypes (= 200 chromosomes) for each locus were subsampled at random out of the full dataset in order to reduce the computational time of the analysis. The

DNA sequence data (CHY and HV2) were edited to conform to an infinite sites model of mutation; microsatellite data were analyzed assuming a single-step model of mutation.

In the IMa2 analysis, we modified the underlying population model to consider only a single population, with a uniform prior on the size of that population varying from $\theta = 0.05$ to 99.95. We ran 40 heated chains for an initial burn-in of ~3.6 million steps, followed by an MCMC search of ~10.2 million steps. Stationarity of the search chains was validated by monitoring ESS values.

3. Lamarc

Third, we used the software package Lamarc v.2.1.8 (Kuhner 2006) to estimate effective population size and population growth rates independently from the nuclear CHY and the mitochondrial HV2 sequence data from dataset 1. As implemented here, we considered a model of a single panmictic population that undergoes population size change (growth or decline) until it reaches the current population size. We implemented a Bayesian analysis in Lamarc with priors on θ ranging from 10^{-5} to 50 and on the population size change parameter (g) ranging from -500 to 2000. Each sequence dataset was analyzed in three independent runs, with each run consisting of an MCMC search that was 20 million steps long and sampled every 200 steps. The first 2 million steps were discarded as a burn-in. Each MCMC search was run as 3 chains, heated to 1, 1.5, and 3, and each search was replicated three times internally within each of the independent runs. Posterior distributions for each independent run and for overall results per locus were visualized using Tracer v.1.5 (Rambaut and Drummond, unpublished software), and results are reported as median point estimates with 95% confidence intervals. All parameter estimates were well supported, with ESS values exceeding 100 in all cases. Effective population size was calculated from the estimated coalescent-scaled parameter θ using the equations

$$\theta = 2N_{e\cdot f}\mu$$

for mitochondrial data and

$$\theta = 4N_{e}\mu$$

for autosomal data, where N_{ef} is the effective population size of females and N_e is the effective size of the entire population. This software uses mutation rates in units of substitutions per site per generation; based on the relative mutation rates estimated for the same data in the IMa2 analysis, we used a mutation rate of 4.29×10^{-8} per site per generation for the HV2 dataset and 7.76×10^{-9} per site per generation for the CHY dataset.

Objective 2. To perform sensitivity analyses to assess the utility of temporally-spaced estimates of effective population size as a population monitoring tool.

If we take current estimates of N_e as a baseline and make several temporally-spaced estimates of N_e in the future, can we detect population trajectories and document the extent of any population-level impact of bat fatalities at wind turbines that may be occurring? We tested the utility of various N_e estimators and different types of data as monitoring tools for bat populations by performing sensitivity analyses in which we modeled various starting population sizes, mortality rates, and time between estimates, and asked how much of a decline over what time scale is necessary to detect a significant change in genetic diversity. In addition, we examined the influence of varying the number and diversity of molecular markers and the

number of individuals sampled on our ability to detect demographic trends. Initially we focused on simple diversity measures, with the idea that if we observe insensitivity of these simple measures to the range of conditions we simulated, then the use of more computationally intensive methods used to estimate N_e that ultimately rely on patterns of genetic variation would not be warranted.

Simulations

We used coalescent simulations of DNA sequence and microsatellite genotype data to evaluate the power of genetic analyses to detect signals of population decline. While similar questions have been addressed for small populations of conservation concern ($N = 10^1$–10^3; Garza and Williamson 2001, Girod et al. 2011), our study is unique in examining the effects of decline in large populations ($N = 10^3$–10^7). To produce datasets comparable to that observed for eastern red bats, our demographic model (Figure 2) considered only a single panmictic population experiencing rates of decline not higher than –10% per generation. Simulations were performed using the program *ms* (Hudson 2002). For these simulations, N_A varied incrementally on the $\log_{10}$ scale from 3-7; the rate of decline (α) was set to -1%, -5%, or -10%; and the time of onset of decline (t) took a value of 1, 5, 10, 50, 100, 500, or 1000 generations. We explored all possible combinations of these parameters (5 values of N_A x 3 values of α x 7 values of time), for a total of 105 demographic scenarios. The current population size (N_0) is easily calculated from the other three parameters as

$$N_A = N_0 e^{-\alpha t}$$

DNA sequence datasets were simulated assuming basic molecular genetic parameters consistent with the mitochondrial HV2 locus, including a mutation rate $\mu = 4 \times 10^{-8}$ substitutions per site per year, a generation time of 1 year per generation, and a sample size $n = 30$ haploid individuals. Output from the *ms* simulations were recorded as 10,000 replicate binary datasets per demographic scenario. Variation in each dataset was then summarized using the *ms_stats_v3.1* script (Thornton 2003).

Datasets for microsatellite analysis were simulated assuming a mutation rate of 10^{-5} substitutions per locus per year, a generation time of 1 year per generation, and a sample size of 30 diploid individuals. We simulated 10,000 replicate datasets per demographic scenario. Binary output from the *ms* simulations was converted into 20-locus microsatellite genotype data using the perl script *ms2ms* (Pidugu and Schlötterer 2006). The simulated genotype data were then summarized using the *MicroStat* script (M. Cox, unpublished software).

We also assessed whether we could detect changes in population size using sequential N_e estimates (remember that using coalescent approaches we estimate θ, and then use that estimate to calculate N_e) in the program Lamarc v.2.1.3 (Kuhner 2006) using sequence data, which is inherently less variable than microsatellite genotype data and therefore more likely to lose variation under a scenario of population decline (we tested a single scenario of 1% mortality per generation). Molecular genetic parameters (mutation rate, generation time, and sample size) follow those described above for simulated HV2 data. Five replicate datasets were simulated for coalescent analysis using a demographic scenario of –1% decline from an ancestral population with $N_A = 10^6$, with *ms* output recorded as coalescent genealogies. DNA sequence data were then simulated across these genealogies using the program Seq-Gen (Rambaut and Grassly 1997). The five replicate datasets per timepoint were then analyzed together as independent loci in Lamarc to estimate the parameter θ ($= 2N_e\mu$ for haploid data). These replicate datasets were

pooled in this manner to add power to the Lamarc analysis (Wakeley 2006). To make the Lamarc model more appropriate to the known evolutionary history of the simulated data, we included the population size change parameter (*g*) in the analysis, although we did not focus on the estimation of this parameter as that was not the point of the analysis. Each Lamarc run utilized a Bayesian analysis with one long run of one million steps in which trees were sampled every 100 steps, yielding a final sample of 10,000 trees. The initial 1,000 trees were discarded as the burn-in. This long run included three heated chains with relative temperatures of 1, 1.5, and 3. Results were reported as maximum probability estimates of θ with 95% confidence intervals and compared with known values of N_0 and N_A as specified in each simulation.

Results and Discussion

Population Structure

We observed 167 unique haplotypes among the 295 individuals sequenced at the mitochondrial HV2 gene representing 84 segregating sites. The number of haplotypes per sampled population ranged from 13-23 (mean: 18.6), and haplotype diversity (h, mean: 0.986, range: 0.961 – 1) was high in all populations (Table 1). However, nucleotide diversity (π, mean: 0.011, range: 0.009 – 0.016) was relatively low in all populations (Table 1). Such high levels of haplotype diversity and low nucleotide diversity are often indicative of historical population size increases, and are consistent with our findings below.

AMOVA analysis indicated very low levels of mitochondrial differentiation among sampling sites (F_{ST} = 0.0113, meaning that 1.13% of the variation is explained by differences among sampling sites, and 98.87% of the variation occurs within sites). Accordingly, pairwise F_{ST} values among sites were consistently low (Table 2) and range from -0.03 – 0.049 (Table 2).

All microsatellite loci were unlinked and 11 of the 16 loci met HWE expectations. Four loci (MS3E10, MS1C01, D202, D226) had heterozygote deficits, but removing them from the analysis made no difference in our conclusions, and therefore we present analyses with all loci included. Mean observed and expected heterozygosities were high (0.82 and 0.88, respectively), as was the mean number of alleles per locus (14.77) and allelic richness (12.9), although private allelic richness was low (0.78; Table 1).

Both clustering methods employed [Bayesian clustering (STRUCTURE) and repeated reallocation (FLOCK),] identified $K = 1$ as the most likely number of genetic clusters, indicating a lack of population differentiation. Similarly, AMOVA analysis on microsatellite genotypes indicated an almost complete lack of structure (F_{ST} = 0.0044, meaning that 0.44% of the variation occurs between sites, and 99.56% of the variation occurs within sites), with pairwise F_{ST} and Jost's *D* values between populations consistently low (Table 3; F_{ST} range: -0.005 – 0.009; Jost's *D* range: -0.036 – 0.068).

The picture that emerges from these data is one of extremely low levels of population structure and effective panmixia across the sampled populations of eastern red bats. Further, there is no evidence for the historical use of different migratory pathways and no evidence for any barriers to gene flow among any of the sampled populations. Few geographic barriers to the movement of vagile organisms such as bats exist east of the Rocky Mountains, and therefore there are likely few impediments to the movement of individuals across the landscape. Phylogeographic studies of widespread bats and birds have shown low levels of genetic differentiation among eastern North American populations, and, when present, genetic structure is often restricted to broad-scale differentiation between eastern and western populations on either side of the Rocky Mountains (Gibbs et al. 2000, Kimura et al. 2002, Jones et al. 2005, Turmelle et al. 2011, Irwin et al. 2011). In the case of eastern red bats, evidence from museum

records indicates that they most likely migrate from northern parts of their range to the southeastern United States (Cryan 2003) where they roost in trees during warmer temperatures and may hibernate beneath leaf litter for short durations during colder temperatures (Saugey et al. 1998, Moorman et al. 1999, Mormann et al. 2007). There are resident populations in the southeastern United States that likely do not migrate, and we might have hypothesized differentiation between migratory northern populations and non-migratory populations in the south. However, given that mating likely takes place before or during migration in eastern red bats (Cryan et al. 2012) and can take place before bats hibernate or during warm periods on the wintering grounds, the potential for gene flow among bats spending their summers in geographically disparate areas is likely very high.

In most colonial temperate bat species females are philopatric to natal nursery colonies or exhibit short dispersal distances to nearby colonies, while mating takes place during swarming and/or hibernation at distant sites that act as hotspots of gene flow between bats occupying distant roosts during the summer (Kerth et al. 2003, Veith et al. 2004, Furmankiewicz and Altringham 2007). As a consequence, levels of mitochondrial differentiation (indicative of female movements) are often quite high among summer maternity colonies while levels of nuclear differentiation (indicative of gene flow through mating) are typically low (Castella et al. 2001, Bilgin et al. 2008, Kerth et al. 2008, Vonhof et al. 2008, Bryja et al. 2009, Lack et al. 2010, Turmelle et al. 2011). Eastern red bats and other members of the genus *Lasiurus* roost solitarily in foliage during the summer (Shump and Shump 1982a,b), and if they exhibited philopatry it would likely occur within broader landscape units such as forest patches or stands rather than a single roost,. The absence of any mitochondrial differentiation among populations of eastern red bats suggests that females may be exhibiting similar levels of dispersal and/or dispersal distances to males, and that gene flow likely take place through both female movements and mating (e.g., Russell et al. 2005).

N_e Estimation

We used three coalescent methods to estimate N_e for eastern red bats: msvar, IMa2, and Lamarc. These methods utilize different suites of data (microsatellites only for msvar, nuclear intron and mitochondrial sequences only for Lamarc, all three data types for IMa2) and therefore were expected to provide complementary estimates based on differences in the mutation rates of the markers used and differences in the underlying models assumed. Here we present the results for each of these estimation approaches as well as results of simulations, and then discuss the interpretation of the combined results.

msvar

Although we found considerable variation from run to run, there were some clear patterns that emerged from these analyses. Importantly, we found no consistent difference between parameter estimates from the 2002 (runs 1-5) vs. 2010 (runs 6-11) time points (Figures 3-5). We also found no consistent difference between independent subsamples of the full dataset (runs 1-3 vs. 4-5 for 2002; runs 6-8 vs. 9-11 for 2010). For the current effective population size N_1, we found generally consistent estimates on the order of 10^5 (average N_1 = 103,610, disregarding outlier run 3). Estimates of ancestral effective population size NA were less consistent among runs, and we have little confidence in any given estimate of this parameter. Due to this difficulty in converging on a consistent estimate of ancestral N_e, these analyses yielded differing signals of population growth vs. decline between runs (Table 4), although a majority of runs (8 of 11) indicate population decline rather than growth. The time of this population size change (t) was

also variable among runs, but generally was on the order of 10^4 years (average t = 50,317 years, disregarding outlier run 3). While the time of population size change is difficult to pinpoint with great accuracy, these analyses clearly do not support a pattern of very recent population size change.

IMa2

The analysis converged on an unambiguous, unimodal posterior distribution for the single population parameter θ (= $4N_e\mu$) for the panmictic eastern red bat population. The most probable value of θ was estimated to be 37.95 with a 95% confidence interval of 32.15 – 45.55 (Table 5). To convert this coalescent-scaled parameter θ to the natural parameter N_e, it is necessary to estimate the mutation rate for our data. The IMa2 analysis provided an estimate of relative per-locus mutation rates, which ranged from a low of 0.1028 for the microsatellite locus D202 to a high of 7.798 for microsatellite locus D248 (Table 6). We used Pesole et al.'s (1999) estimate of mammalian mitochondrial mutation rates (= 2.740×10^{-8} substitutions per site per year) to calculate locus-specific mutation rates for our data. The geometric mean of these rates (= 8.03×10^{-6} substitutions per locus per year = 1.61×10^{-5} substitutions per locus per generation) was used to convert coalescent-scaled estimates of θ into estimates of N_e (Table 5). Our analysis thus supports an effective size of approximately 5.91×10^5 individuals, with a 95% confidence interval of $5.00 – 7.09 \times 10^5$ (Figure 6). Because this estimate includes both appropriately-scaled mitochondrial and autosomal microsatellite data, this estimate reflects the totality of the population (*i.e.*, both males and females).

Lamarc

We used the coalescent-based analyses in Lamarc to provide estimates of θ and population growth independently for the nuclear CHY and mitochondrial HV2 loci. Analyses of both markers provided unambiguous, unimodal posterior probability distributions for θ and population growth (Figures 7-10). Utilizing the mutation rates provided in the previous section, estimates of N_e (based on conversion of θ estimates) were of the same order of magnitude as the estimates generated with msvar and IMa2 for HV2 (females only), where the mean estimate across three runs was 1.29×10^5 (95% confidence limits: $1.06 – 1.80 \times 10^5$; Table 7). The estimate of N_e using CHY (males and females) was considerably larger, with a mean of 1.52×10^6 (95% confidence limits: $1.05 – 2.18 \times 10^6$; Table 7). There was a clear signal of historical positive population growth for both CHY and HV2, although the signal was stronger for the mitochondrial locus (Table 7); however the analysis does not provide the time scale over which growth occurred, and estimates based on sequence data typically integrate growth over 100's to 1000's of generations.

Simulations

Our analyses demonstrate that simple summary statistics such as the number of segregating sites (S) and number of alleles are remarkably unresponsive over short timespans to population declines even at rates of up to –10% (Tables 8-9, Figures 11-16). To relate these simulations more explicitly to eastern red bats with an estimated N_e of 10^5-10^6, we find that it takes 500-1000 generations (1000-2000 years) before a decline of –1% results in a significant loss of genetic diversity at a rapidly-evolving mitochondrial locus (Table 4, Figure 11). Even at a decline of –10%, genetic diversity is maintained in the population through at least 50 generations (100 years; Table 4, Figure 13). Simulated microsatellite genotypes are similarly unresponsive to

population declines in large populations. For effective population sizes of 10^5-10^6, it again takes more than 500 generations before a decline of –1% results in a significant loss of genetic diversity (Table 5, Figure 14), and even declines of –10% require at least 50-100 generations before they result in a significant loss of alleles (Table 5, Figure 16). These declines model selectively neutral mortality; selection on behavioral traits that might be associated with mortality might increase or decrease these times to decline, depending on the type of selection.

As seen in Figure 17, the modeled decline in Lamarc shows a gradual drop in current effective population size relative to the ancestral effective population size. If the Lamarc method were a sensitive tool for detecting very recent population declines, we would expect to see the estimates from Lamarc tracking the known current effective population size. If the Lamarc method were an accurate measure of historical evolutionary effective population size, we would expect to see those estimates being much more consistent across timepoints and more similar to the known ancestral effective population size. Our results indicate more support for the latter interpretation, with estimates from Lamarc being relatively consistent across timepoints until the very end of the simulation, at which point very little variation remains in the population. Interestingly, though, we see that Lamarc consistently underestimates ancestral θ compared to its true value in these analyses, which may be a result of the simulated population decline.

Effective Population Size as a Monitoring Tool?

Estimates of N_e varied considerably (almost an order of magnitude) among the different approaches we used, ranging from 1.93×10^5 based on microsatellite genotypes only (msvar), to 1.5×10^6 for sequence data only (CHY in Lamarc), with an intermediate estimate of 5.91×10^5 using all markers combined (IMa2). This variation is the result of methodological differences among the approaches we used, which all utilize different aspects of the data and make varying assumptions about the underlying historical population processes that may have occurred. Further, the analyses each used different marker data, which vary in their mutation rates, and so are providing estimates across varying time scales. Nevertheless, in combination with the results of population structure analyses, our data indicate that eastern red bats form a single, large, panmictic population across their range and that minimum effective population sizes are likely in the hundreds of thousands or low millions.

The parameter that we would most like to know, the actual number of individuals in the population (N_c), is not obtainable from our estimates of N_e. A variety of factors reduce N_e relative to N_c, including fluctuations in population size over time, overlapping generations, and variation among individuals in reproductive success. Attempts have been made to compare estimates of N_e to N_c, and across a wide range of organisms the average N_e / N_c ratio is 0.11 – 0.14 (Frankham 1995, Palstra and Ruzzante 2008); for mammals alone, the average ratio is 0.34 (Frankham 1995). If we applied this latter mean ratio (0.34) to our point estimates of N_e, we would obtain N_c estimates of 567,000 to 4,471,000 individuals, and using the overall mean (0.11) estimates would be 1,750,000 to over 13,818,000 individuals. However, there are a number of serious problems with the use of our estimates in this way. N_e is a theoretical concept that relates the genetic characteristics of a population to those expected of an ideal population under a Wright-Fisher model. We can estimate N_e as a measure of the evolutionary potential of populations, but there is no clear relationship between current or historical demography and changes in genetic variation that influence genetic estimates of N_e. Further, there are a number of methodological concerns. First, N_e has most often been estimated for very small populations of less than 1,000 individuals, and we do not know how the N_e / N_c ratio may vary with the magnitude of N_c. Second, the majority of the ratios provided by Frankham (1995) utilize demographic, rather then genetic,

estimates of N_e, and demographic estimates may differ substantially from genetic estimates even when population sizes are small (Luikart et al. 2010). Third, the majority of estimates in Frankham (1995) come from organisms with very different life histories than bats, and we do not know to what extent the N_e / N_c ratio might vary from the overall mean for bats (or most other organisms). Fourth, the calculation of N_e using coalescent-based methods requires division of estimates of θ by the mutation rate (μ), but mutation rates are extremely difficult to estimate and few good estimates exist for any gene (Ho et al. 2006, Montooth and Rand 2008, Nabholz et al. 2009), much less for any bat species. As a result, any inaccuracy in the mutation rate estimate is amplified arithmetically in the subsequent calculation of N_e (Ovenden et al. 2007, Luikart et al. 2010). Therefore, applying a standard conversion to convert N_e to N_c is highly problematic, and it is best to use our estimates to indicate relative orders of magnitude of bat population sizes rather than to provide any specific population size estimates.

Given these issues, a major focus of our project was to determine whether temporally-spaced estimates of N_e could be used to monitor population declines in bat species affected by wind power development. We initially focused on modeling change in simple diversity measures with the idea that if we observed insensitivity of these simple measures to the range of conditions we simulated, then the use of more complex and computationally intensive methods used to estimate N_e that ultimately rely on patterns of genetic variation would not be warranted. The results of our simulation analyses suggest that patterns of genetic variation are remarkably stable even under high rates of simulated mortality (up to 10% of the population per generation; see Figures 11-16), and detectable changes in diversity were only apparent after 100's of generations and only after population sizes had been dramatically reduced. We also found that coalescent-based approach in Lamarc did a poor job of tracking changes in population size. It consistently underestimated θ at the beginning of population declines, and only registered a change in θ after population size had decreased to extremely small size and almost all variation had been lost from the population. We did not believe that it would be fruitful to apply other coalescent approaches, as they would be affected by the same patterns of change in genetic variation, and were prohibitively computer- and time-intensive (for example, each msvar run took 25-30 days to run, making extensive simulations using this technique simply intractable).

The combined outcome of these simulations is that genetic approaches do a poor job of detecting and tracking population declines before population sizes become very small. Similar findings have been made for approaches to N_{eV} estimation based on linkage disequilibrium (e.g., Waples and Do 2010), suggesting that most if not all methods for estimating N_e may only be successfully applied to very small populations numbering in the 10's or 100's unless genetic data can be combined with demographic data (as in the approach of Jorde and Ryman 1995). Given recent evidence suggesting that the demographic effects of declining population size (such as demographic stochasticity and Allee effects) better predict the risk of extinction than genetic effects such as loss of diversity and inbreeding (Wootton and Pfister 2013), future analyses applying demographic models to the problem of bat mortality at wind power developments may be more fruitful. One approach we are pursuing is to use our estimates as starting points for demographic approaches that model sustainable yield, taking into account demographic characteristics of bats, to understand how many bats can be killed before we expect to see demographic effects associated with population decline.

Accomplishments

Our research was not intended to result in product development, technology transfer, or commercialization activities, but rather it was focused on providing context for one of the major environmental concerns surrounding future wind power development in the United States. Our results will form the basis of a publication that will be submitted to a peer-reviewed scientific journal in coming months.

Conclusions

We set out to provide a proof-of-concept for the use of genetic approaches to assess the population-level effects of bat mortality at wind power developments. Using a large dataset of both nuclear and mitochondrial DNA variation for eastern red bats, we have demonstrated that: 1) this species forms a single, panmictic population across their range with no evidence for the historical use of divergent migratory pathways by any portion of the population; 2) the effective size of this population is in the hundreds of thousands to millions; and 3) for large populations, genetic diversity measures and at least one coalescent method are insensitive to even very high rates of population decline over long time scales and until population size has become very small. Our data and analyses suggest that genetic approaches are not an effective tool for assessing potential short-term population declines in large populations of migratory wildlife affected by wind power development. That said, genetic markers may be used in a wide variety of other contexts to provide valuable information on species identification, patterns of connectivity and gene flow across the landscape, local adaptation, genomic responses to environmental stressors, the evolutionary potential of populations, local population size in capture-mark-recapture studies, and patterns of dispersal and individual movements.

Recommendations

We have no specific recommendation for future work. We do not feel that further simulations (for instance, to test the influence of marker number, population structure, sex-specific mortality) will be fruitful, as it was clear that populations retained genetic variation under all scenarios of population decline until populations became very small. However, it is important to note that our analyses focused on neutral markers, and the influence of population decline on genetic variation in adaptive markers could be quite different. Certainly, analyses of population differentiation and testing for the presence of populations using different migratory pathways should be carried out for the other two species of long-distance migratory bats experiencing mortality at wind power developments (hoary and silver-haired bats). Both species are distributed across North America from east to west, and are more common than eastern red bats in the boreal forest of Canada. Populations east and west of the Rocky Mountains, and populations in the far north and south, could be behaving differently, as has been demonstrated for a number of species of migratory birds.

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