ASSESSING GENETIC DIVERSITY IN NORTHERN YELLOW BATS KILLED AT WIND

ENERGY FACILITIES

By

Jack Joyce

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Project Approved:

Supervising Professor: Amanda Hale, PhD

Department of Biology

Dean Williams, PhD

Department of Biology

Darren Middleton, PhD

Department of Religion

ABSTRACT

Although wind energy facilities are a growing source of renewable, clean energy, they have been shown to contribute to increasing bat mortalities which could threaten the persistence of bat populations. This study aims to expand what we know about the biology and behavior of bat species impacted by wind energy development. Recent research has indicated that yellow bats (*Lasiurus* spp.) are killed at wind energy facilities in the Rio Grande Valley of south Texas. We have limited understanding of the population biology or movement patterns in these species, so the extent to which wind turbine mortality may impact these bats is currently unknown. As part of ongoing research in our labs, I extracted DNA from 18 tissue samples collected from northern yellow bats (*Lasiurus intermedius*) at a wind energy facility in Willacy County, Texas in 2015. I amplified a region of the mtDNA, the COI locus, and compared genetic diversity of these samples to a larger data set from wind energy facilities in nearby Starr and Hidalgo Counties that were studied in 2016 and 2017. Both subspecies of northern yellow bats were detected in Willacy County, with estimates of genetic diversity being much higher than in the previous study with a larger sample size. Together, these datasets will improve our understanding of *Lasiurus intermedius* genetic diversity and population structure and have the potential to provide much needed insights into the impacts of wind energy development on bats in southern North America.

<u>INTRODUCTION</u>

Wind energy facilities are a growing source of renewable, clean energy with the potential to reduce greenhouse gas emissions and slow the rate of climate change. Wind power capacity in the United States totals 111,808 MW, making it the third largest source of electricity generation and the largest source of renewable energy in the country (American Clean Power Association [ACPA], 2020). Currently, wind energy is responsible for nearly 25% of all electricity generated in six U.S. states and is growing (ACPA, 2020). In fact, the United States Office of Energy Efficiency and Renewable Energy has visions to expand wind energy to provide as much as 20% of national energy demand from wind power by 2030 and 35% by 2050 (*Wind Vision*, 2015).

Nonetheless, wind energy development has the unintended consequence of causing bat fatalities, most notably through blunt force trauma due to collisions with the rotating blades or barotrauma (Arnett et al. 2016). It is widely believed that the continued expansion of wind energy development threatens the persistence of bat populations (O'Shea et al. 2016, Frick et al. 2017, Frick et al. 2019). The long lifespan and slow reproductive rates observed in bats (Barclay and Harder 2003) make the concern for population persistence even greater. Unfortunately, however, we lack much needed information about population sizes and trends for most species of bats which makes establishing policy and mitigation strategies to reduce impacts exceedingly difficult (Hein et al. 2021). Within North America, bat fatality is found to be highest at wind energy facilities in the Great Plains region for migrating tree-roosting species including hoary bats (*Lasiurus cinereus*), eastern red bats (*Lasiurus borealis*), and silver haired bats (*Lasionycterus noctivagans*), and peaks at the end of summer (Arnett et al. 2016). Furthermore, nearly half of the 47 species of bats found in North America are known to be killed at wind energy facilities (Arnett et al. 2016). Interestingly, it seems plausible that the yellow bats found

in south Texas may participate in short- or long- distance migration, and populations located here are at the edges of their respective ranges (Chipps et al. 2020a). However, data has yet to confirm whether yellow bats should be classified among migratory bats.

Concerns about wind energy impacts to bats are especially pronounced in the state of Texas, which has installed more wind farms than any other state (Smallwood 2013) and hosts the greatest bat diversity in the country (Ammerman et al. 2012). Despite Texas leading the country in energy production from wind power, the region has seen the least number of published, peer-reviewed papers about bat mortality patterns (but see Weaver et al. 2020a). As a result, very little is known about which species are most impacted and how they might respond to this anthropogenic source of mortality. Bats make up approximately one-fifth of mammalian diversity and provide numerous ecological services including pollination and the consumption of many agricultural pests, making their conservation of great human interest (Boyles et al. 2011, Ghanem and Voigt 2012).

Northern yellow bats (*Lasiurus intermedius*) are the primary subject of interest in this research in part because of the high mortality observed from wind energy development in South Texas (Weaver 2019). Northern yellow bats are a tree-roosting, insectivorous species found in the southeastern United States, throughout the gulf coast region, and in parts of Mexico and Central America. Two sub-species are recognized for northern yellow bats: *Lasiurus intermedius floridanus* and *Lasiurus intermedius intermedius*, both of which can be found in south Texas where this study was conducted (Chipps et al. 2020a, b). Interestingly, southern yellow bats (*Lasiurus ega*) share part of their range in south Texas where this study was conducted. Therefore, it is possible that among the samples collected we could find a combination of northern and southern yellow bats. Correctly identifying the species of bats killed by wind

turbines is essential for assessing which species is more impacted by wind energy development (Chipps et al. 2020b). These tree-roosting species of yellow bats are considered cryptic species due to their solitary lifestyles (Hein et al. 2021). Therefore, while we are able to estimate annual bat fatality for a region of interest, it is quite difficult to assess whether these numbers represent a substantial portion of the population as actual population sizes are challenging to quantify.

As a result, estimates of population genetic diversity and population structure are one of the best tools available to understand the degree to which these populations are being impacted by wind energy development. Due to the fact that migratory tree roosting species do not aggregate in conspicuous locations like their cave dwelling counterparts, but rather roost individually, estimating population sizes through traditional census methods is impractical (Hein et al. 2021). Estimating effective population sizes remains one of the most consistent, long-term data collection strategies to gain information about population trends for tree-roosting bats (Hein et al. 2021). Genetic approaches are also useful to reduce uncertainty with population status and trends, seasonal movements, and cryptic species (Hein et al. 2021). Each of these aspects represent many questions that need to be answered about bat populations to better understand mortality trends. This type of genetic monitoring can reveal changes in genetic diversity that reflect changes in population size (Hein et al. 2021). Together, these approaches are useful in expanding our understanding of mortality trends and for assessing the degree to which bat populations are sensitive to wind energy caused mortality.

Previous studies in collaboration with SP Weaver (Texas State University, Texas A&M University San Antonio, and Bowman Consulting) have provided TCU researchers with access to tissue samples from bat carcasses killed at wind energy facilities in south Texas. A recent study by Chipps et al. (2020a) revealed that four species of *Lasiurus* bats are killed at wind

turbines in this region. In these studies, mtDNA sequencing and microsatellite genotyping were used to characterize genetic diversity and estimate effective population sizes in two species, *Lasiurus ega* and *Lasiurus intermedius,* from samples collected at Starr and Hidalgo Counties (Chipps et al. 2020b). From a study conducted in Starr County, average annual bat mortality for all species was estimated to be about 8,000, bats per year with northern yellow bats being the second most affected species (Weaver et al. 2019). Some mitigation strategies to reduce mortality have been employed in the Rio Grande Valley region of South Texas with varying success. The use of ultrasonic acoustic deterrents (UADs) on operational wind turbines showed significant reductions in fatalities for hoary bats (*Lasiurus cinereus*) and Mexican free-tailed bats (*Tadarida brasiliensis*), but not for northern yellow bats (Weaver et al. 2020b).

This study aims to expand on research conducted at south Texas wind energy facilities to include 18 wing tissue samples from northern yellow bats (*Lasiurus intermedius*) collected from Willacy County, TX in 2015. Using DNA extracted from these tissue samples, I amplified and sequenced the Cytochrome c Oxidase I (COI) locus of the mitochondria. I used DNA barcoding techniques to confirm or correct species identification from the bat carcass study (e.g., Korstian et al. 2016, Chipps et al. 2020b). I then used these sequence data to estimate genetic diversity in my samples. Finally, I compared my results to those obtained by Chipps et al. (2020a) from a collection of northern yellow bats recovered at nearby Starr and Hidalgo Counties in 2016 and 2017 to gain greater insight into genetic diversity and population structure in this species. Considering that the population at Willacy County has never been studied before, northern yellow bats are a major species impacted by wind energy facilities, and successful mitigation strategies have not been found for northern yellow bats all highlight the importance for this research. These data can help fill the knowledge gap between wind farm development and bat

mortality patterns, as well as expand our knowledge of the biology and behavior of northern yellow bats by providing conservation biologists, wind energy developers, and regulatory agencies with information useful to guide future wind energy development. Furthermore, this research paves the way for future collaboration with Texas scientists, Texas Parks and Wildlife Department, and wind energy companies contributing to the development of new projects in the state of Texas.

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METHODS

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We obtained wing tissue samples from *L. intermedius* carcasses collected during post-construction fatality surveys at wind energy facilities in Willacy County, TX during 2015. Wing tissue samples were stored in vials containing 95% ethanol and were provided by SP Weaver from Texas State University to AM Hale at TCU in 2018. I extracted DNA from the preserved tissue samples following the ammonium acetate/isopropanol precipitation method detailed in Korstian et al. (2013).

I then sequenced the DNA samples at a 550 bp section of the mitochondrial cytochrome c oxidase I (COI) gene. To amplify the COI gene using polymerase chain reaction (PCR), I used an M13-tailed primer cocktail (cocktail 2 in Clare et al. 2007). PCR reactions (10 µL) contained 10-50 ng DNA, 0.2 µM of the primer cocktail, 1X BSA, and 1X AccuStart II PCR SuperMix. PCR reactions were completed using an ABI 2720 thermal cycler with parameters: one cycle at 94°C for 10 min, followed by 30 cycles of 15 s at 94°C, 15 s at 50°C, 1 min at 72°C, and then a final extension of 5 min at 72°C. Products were sequenced using ABI Big Dye Terminator Cycle Sequencing v3.1 Chemistry (Applied Biosystems, USA) with the PCR Primers. DNA sequences were analyzed on an ABI 3130XL Genetic Analyzer (Applied Biosystems, USA); trimmed,

edited, and assembled into contigs using Sequencher v5.1 (Gene Codes, USA); and then aligned in MEGA v10 (Kumar et al. 2018). Aligned sequences were translated to verify the absence of stop codons, after which they were compared to GenBank voucher sequences to generate a species ID. Only sequences >400 bp in length were used and the criterion to accept a molecular species identification required an identity value >98% in BLAST. I identified unique sequence haplotypes using GenAlEx v6.5 (Peakall and Smouse 2006, 2012). I used the mitochondrial DNA sequences to estimate genetic diversity of the samples, calculating haplotype diversity (h) in GenAlEx v6.5 and nucleotide diversity of mitochondrial haplotypes (π) in DnaSP v6 (Rozas at al. 2017).

RESULTS

Seventeen of the 18 samples collected from Willacy County were able to be identified with a species ID using the COI gene. All 17 samples were northern yellow bats with 16 belonging to the *L. i. intermedius* sub-species and one belonging to the *L. i. floridanus* sub-species (Table 1). In total, I found 11 haplotypes in this sample with a haplotype diversity of 0.85 and a nucleotide diversity of 0.0195 (Table 1). Specifically, 10 haplotypes were found in the *L. i. intermedius* and one was found in *L. i. floridanus*. Among the *L. i. intermedius*, haplotype diversity was 0.83 and nucleotide diversity was 0.0074 (Table 1). A maximum likelihood tree using the 11 unique haplotypes from this study and unique haplotypes from the *L. intermedius* samples from Chipps et al. (2020a) revealed two well-supported clades, each representing one of the two sub-species (Figure 1). Sample #9 from my study shared a haplotype with Dini 1 and 5, sample #4 shared a haplotype with Dini 7, samples #1, 8, 11, 12, 13, 17, and 18 shared a haplotype with Dini 6, and sample #16 shared a haplotype with Dinf 7 (Figure 1; where Dini

refers to the *L. i. intermedius* sub-species and Dinf refers to the *L. i. floridanus* sub-species from Chipps et al. 2020a).

Table 1. Characterization of mitochondrial diversity (COI locus) for each *Lasiurus* taxon examined in the collection of samples from Willacy County, TX in 2015 (n = 17 bats).

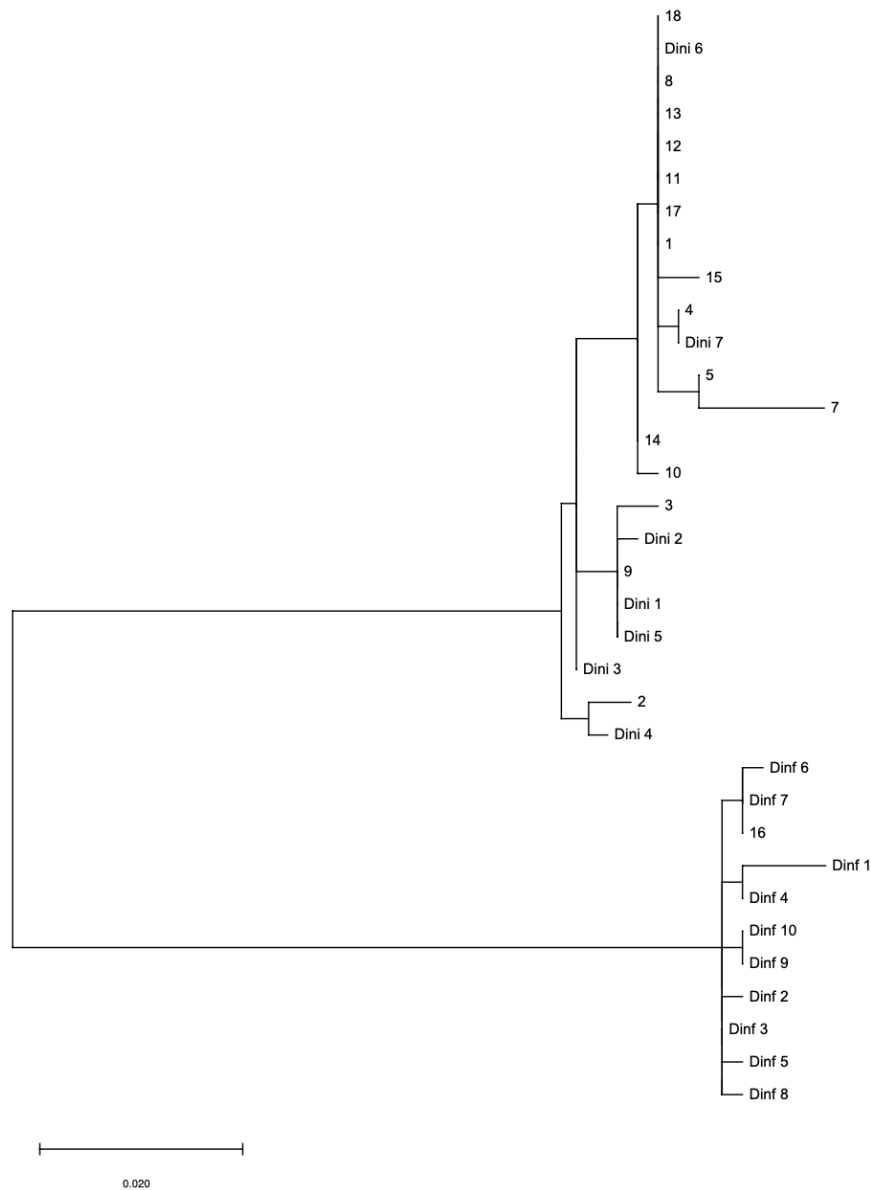| Taxon	n	H	h	π
L. intermedius	17	11	0.85	0.0195
L. i. intermedius	16	10	0.83	0.0074
L. i. floridanus	1	1	-	-

Figure 1. Maximum likelihood tree of unique COI sequences (i.e., haplotypes) from *Lasiurus intermedius* collected from Willacy County, TX in 2015 (n = 17 bats in total, this study) and from *Lasiurus intermedius* collected from Starr and Hidalgo Counties, TX in 2016 and 2017 (n = 212 bats; Chipps et al. 2020a). Branch length represents genetic distance. Numbers at branch tips represent the number of individuals with that unique haplotype.

DISCUSSION

This study revealed high genetic diversity in a small sample of northern yellow bats killed at a wind farm in Willacy County, TX. In contrast to the results from Chipps et al. (2020a) presented in Table 2, I found higher haplotype diversity (h) and higher nucleotide diversity of mitochondrial haplotypes (π) in my sample of northern yellow bats.

Table 2. Characterization of mitochondrial diversity (COI locus) for each *Lasiurus* taxon examined in the collection of samples from Starr and Hidalgo Counties, TX in 2016 and 2017 (n = 365 bats; from Chipps et al. 2020a).

Taxon	n	H	h	π
L. ega	112	2	0.018	0.00003
L. i. floridanus	50	6	0.588	0.00225
L. i. intermedius	203	7	0.542	0.00177

Chipps et al. (2020a) measured the population diversity of several bats in Hidalgo and Starr Counties. *L. ega* and the two sub-species of *L. intermedius* will be considered here. *L. ega* had two haplotypes, a haplotype diversity of 0.018, and a nucleotide diversity of 0.00003 (Table 2). *L. i. floridanus* was represented by six haplotypes with a haplotype diversity of 0.588 and a nucleotide diversity of 0.00225 (Table 2). Finally, *L. i. intermedius* contained seven haplotypes, had a haplotype diversity of 0.542, and a nucleotide diversity of 0.00177 (Table 2). Haplotype diversity and nucleotide diversity for *L. ega* was significantly lower in comparison to both sub-species of *L. intermedius*. This means that the probability of obtaining two southern yellow bats with unique DNA sequences from these populations is lower in comparison to both sub-species of northern yellow bat. This indicates a much lower population diversity amongst this population

of southern yellow bats compared to the northern. Within the northern yellow bats, *L. i. floridanus* exhibited greater population diversity in comparison to *L. i. intermedius*.

Figure 2 below (Figure 1 from Chipps et al. 2020a) shows the minimum spanning haplotype network which revealed two genetic clusters separated by 56 base pair substitutions corresponding to the two sub-species of *L. intermedius* obtained from Hidalgo and Starr Counties, where Dini refers to the *L. i. intermedius* sub-species and Dinf refers to the *L. i. floridanus* sub-species.
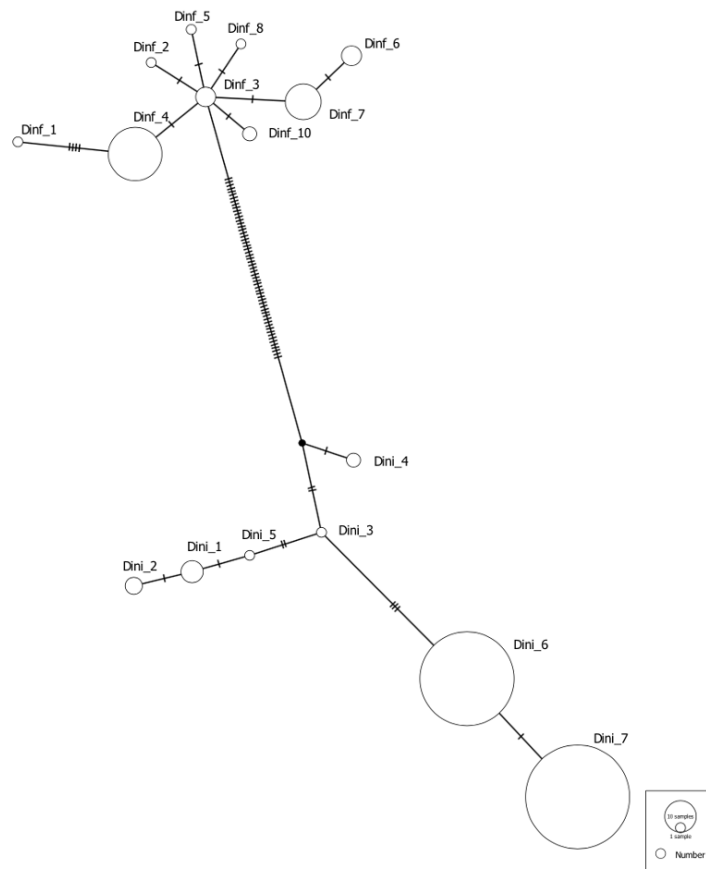

Figure 2. Minimum spanning haplotype network of unique mitochondrial COI sequences. Minimum spanning haplotype network of unique COI sequences from *L. i. floridanus* (Dinf) and *L. i. intermedius* (Dini) individuals from this study and from Genbank. Circles indicate haplotypes and the size of each circle corresponds to the number of individuals having that haplotype. Vertical hatch marks represent the number of nucleotide substitutions between haplotypes.

Results from Willacy County showed only northern yellow bats were collected at this site, while Hidalgo and Starr Counties saw carcasses collected from both southern and northern yellow bats. Additionally, Willacy County strongly favored the *L. i. intermedius* sub-species, unlike the study in Hidalgo and Starr Counties. However, it should be noted that only eighteen samples were collected from Willacy County compared to 365 at Hidalgo and Starr Counties. This difference in the sample size may account for many of the differences observed among these populations. Furthermore, high diversity was observed for the *L. i. intermedius* sub-species at Willacy County. While the Chipps et al. (2020a) study also observed high diversity in this same sub-species, haplotype diversity was significantly greater at Willacy County at 0.83 compared 0.542 at Hidalgo and Starr Counties (Table 1, 2). It is also interesting that Hidalgo and Starr Counties saw greater diversity in the *L. i. floridanus* compared to the *L. i. intermedius*, while the opposite was observed for Willacy County. Again, it should be noted only one carcass of the *L. i. floridanus* was collected at Willacy County so this may not be a full picture of the diversity of this sub-species in this population. Ultimately, this population of northern yellow bats, particularly the *L. i. intermedius* sub-species, showed quite impressive diversity in Willacy County. This is surprising given how many yellow bats we observed to be killed by wind turbines in this region. Therefore, it will be interesting to see if this population remains this diverse if we are to return to this site in the future.

The additional population genetic data collected from Willacy County, provided in this thesis, have the potential to teach us more about the extent to which bat populations are impacted by wind energy. Specifically, these data in combination with the results from Chipps et al. (2020a) provide baseline estimates of population genetic diversity that can be used in the future should scientists reassess population diversity in five-to-ten-year intervals. This type of long-

term genetic monitoring will provide clues into the population status and trends for the northern yellow bat. For example, should this monitoring reveal a significant reduction in population genetic diversity, this could serve as evidence to support the hypothesis that wind energy facilities are substantially affecting bat populations in adverse ways.

Limited knowledge on the interactions between bat populations and wind energy facilities in South Texas and the lack of successful mitigation strategies for northern yellow bat fatalities illustrate how more information on population structure and diversity is necessary to better understand the degree to which bat populations are being impacted by wind energy facilities. Weaver et al. (2020a) found that the majority of bats found in fatality surveys were the Mexican free tailed bats (*Tadarida brasiliensis*) followed by the yellow bats during the late summer. This differs from the Arnett et al. (2016) study, which found that most species of bats found in mortality surveys include migratory tree roosting bats. This may be explained by the lack of studies performed in Texas and Mexico, which may skew data in favor of those tree roosting species (Weaver et al. 2020a). Finally, these measures of genetic diversity are important to understand the sensitivities of bat populations as reduced diversity can be an indication of shrinking population sizes, increased inbreeding, and less diversity available for natural selection to act on both favorable and deleterious mutations (Hein et al. 2021). Furthermore, these baseline diversity measures can be useful for examining trends in population diversity to assess the kind of impact some kind of stressor is placing on a population. While Chipps et al. (2020a) points out the plausibility of migration amongst yellow bats at the edges of their species range, no statistical evidence was found to support population structure or expansion in the yellow bat populations at Starr and Hidalgo Counties. This is interesting, considering that nation-wide, migratory bats were shown to be found most often during post-construction fatality surveys (Arnett et al. 2016).

However, as pointed out previously from Weaver et al. (2020a), this may be as a result of skewed data due to the lack of studies coming out of south Texas.

Jurisdictional issues with wind energy development are another important aspect to discuss related to bat mortality. Typically, wind turbines are not constructed on federal land, which means development projects fall to state and local planning commissions, zoning boards, and city councils often without the knowledge needed to make informed decisions (Arnett et al. 2016). In fact, concerns about bat fatality may never be addressed in decision making. Furthermore, most species of bats are not protected under Federal Law. The US Fish and Wildlife Service provides only voluntary guidelines to help wind energy project developers reduce impacts to wildlife and there are no consequences to violating or ignoring these guidelines (Arnett et al. 2016).

These failures illustrate the importance of data collaboration amongst Texas scientists to provide a standardized method for assessing at risk bat populations so that pre-construction bat activity surveys can identify at-risk sites prior to wind energy facility construction (Arnett et al. 2016). Furthermore, research into developing and testing mitigation studies must continue to be prioritized as solutions are needed to reduce bat mortality at wind turbines in Texas and beyond. While ultrasonic acoustic deterrents (UADs) have not yet been demonstrated to successfully reduce wind turbine mortality for northern yellow bats, their success at reducing wind turbine mortality in other bat species like the hoary bat (*L. cinereus*) and Mexican free-tailed bat (*T. brasiliensis*) point out the potential for technological solutions to help reduce bat mortality at wind energy facilities (Weaver et al. 2020b). As knowledge of bat populations expands, this provides guidance for conservation biologists, wind energy developers, and regulatory agencies for future development of wind energy. Ultimately, this paves the way for collaboration amongst

Texas scientists, Texas Parks and Wildlife Department, and wind energy companies to continue wind energy development, which is providing such a great resource in renewable energy, but in a responsible way that conserves these threatened species.

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