SEX RATIOS OF BRAZILIAN FREE-TAILED BAT (*TADARIDA BRASILIENSIS*) FATALITIES AT WIND ENERGY FACILITIES IN CALIFORNIA AND TEXAS

by

SARAH TOYE LICARI

Bachelor of Science, 2020
East Stroudsburg University
East Stroudsburg, Pennsylvania

Submitted to the Graduate Faculty of the
College of Science and Engineering
Texas Christian University
in partial fulfillment of the requirements
for the degree of

Master of Science

May 2023

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by

Sarah LiCari

Dissertation approved:

Dean Williams

Major Professor

Amanda Hale

Sarah Fritts

Sara Weaver

For The College of Science and Engineering

ACKNOWLEDGMENTS

This endeavor would not have been possible without the help of my graduate advisors, Dr. Amanda Hale and Dr. Dean Williams. I also could not have taken this journey without the aid of Dr. Sarah Fritts and Dr. Sara Weaver, who offered their time and expertise throughout this project as members of my thesis advisory committee. Additionally, I would like to thank the Renewable Energy Wildlife Research Fund and the Adkins Fellowship for the financial support of this project.

I thank my graduate student cohort for their feedback and moral support. It was a joy working and growing with them. I am also thankful for my fellow lab mates, who made the long days in the lab much more enjoyable.

Lastly, I would be remiss in not mentioning my mother, significant other, and close friends. Their constant support and belief in me helped me to stay motivated throughout this process. I would also like to thank my dog for providing endless entertainment and emotional support.

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1. INTRODUCTION

Renewable energy has expanded in recent decades to increase energy production and decrease dependence on fossil fuels, which are often supplied by unstable parts of the world and are a significant source of carbon emissions (Saidur et al. 2011; Barthelmie and Pryor 2014; IEA 2022). Wind energy is one of the fastest-growing renewable energy industries in the world (IEA 2022). While there are many recognized benefits of wind energy (Wiser et al. 2015), such as the potential to reduce the need for fossil fuels for energy production (Barthelmie and Pryor 2014), an unintended consequence has been the negative impacts on birds and bats (Kuvlesky et al. 2007; Arnett and May 2016; Allison et al. 2019; AWWI 2020). These impacts include displacement, habitat fragmentation, and collision mortality (Drewitt and Langston 2006; Arnett and May 2016; Allison et al. 2019; Diffendorfer et al. 2019). For bat-wind turbine collisions in North America, migratory tree-roosting bat species such as the hoary bat (*Lasiurus cinereus*), silver-haired bat (*Lasionycteris noctivagans*), and eastern red bat (*Lasiurus borealis*) are the most affected with hundreds of thousands of total fatalities occurring on an annual basis in the United States and Canada (Arnett and Baerwald 2013; Zimmerling and Francis 2016; AWWI 2020). As bats are long-lived and have slow reproductive rates, they are susceptible to population declines and recover slowly (Barclay and Fleming 2020). Because of this, there are concerns that collision mortality could lead to significant population declines and even extinction for some bat species, such as the hoary bat (Frick et al. 2017; Friedenberg and Frick 2021).

Another species of concern with respect to wind energy is the Brazilian free-tailed bat (*Tadarida brasiliensis*). The Brazilian free-tailed bat is the fourth most common bat species found in post-construction fatality monitoring (PCM) studies (AWWI 2020), with most fatalities occurring in the early fall (Arnett et al. 2008; Allison et al. 2019; AWWI 2020). By region, the Brazilian free-tailed bat is the most common bat species found during PCM studies in the Southwest and Pacific Southwest of the United States, at 33.1% and 52.6%, respectively (AWWI 2020). This species is a migratory cave bat that lives in colonies that number in the millions (Glass 1958; Bernardo and Cockrum 1962; Davis et al. 1962). These large populations are a source of agricultural pest control (Federico et al. 2008; Boyles et al. 2011) and ecotourism (Bagstad and Wiederholt 2013). The Brazilian free-tailed bats' pest control services are estimated to reduce cotton crop damage by up to 43% in south-central Texas (Federico et al. 2008). Along with providing agricultural pest control services, these large colonies are a significant source of ecotourism, annually contributing over $6.6 million in tourism to the Southwestern US (Bagstad and Wiederholt 2013).

Considering the importance of the Brazilian free-tailed bat, there is a need to conserve their populations, which have already experienced population declines in the past due to pesticide use (Geluso et al. 1976; 1981) and human disturbances to maternal roosts (Furey and Racey 2016). These declines permanently impacted the population sizes of some cave colonies, such as Carlsbad Cavern in New Mexico (Clark 2001) and Eagle Creek Cave in Arizona (Cockrum 1970). At Eagle Creek Cave, the Brazilian free-tailed bat experienced a nearly 99.9% population decline between 1963 (estimated population size of 25 million) and 1969 (estimated population size of 30,000) (Cockrum 1970). To better conserve these populations, it is essential to understand the population structure and any possible threats to the species. One potential threat to Brazilian free-tailed bat populations' continued growth and stability is fatalities at wind energy facilities. Due to the high proportion of Brazilian free-tailed bats fatalities at wind energy facilities in the Southwest and Pacific Southwest regions of the United States, it is crucial to understand the sex ratio of fatalities to better conserve this migratory cave species.

In this study, we determined the proportion of female fatalities of Brazilian free-tailed bats at wind energy facilities in California and Texas during year-long PCM studies. Sex was determined using molecular methods, which are more accurate than in-field morphological sex determination (Korstian et al. 2013). To date, the most effective and widely adopted strategy to reduce bat fatalities at wind turbines is to prevent the wind turbines blades from spinning faster than 1–2 revolutions per minute (RPM) when bats are most at risk of collision (reviewed in Adams et al. (2021), Whitby et al. (2021)). This strategy is called curtailment and early implementation focused on low wind speed nights during late summer and early autumn (e.g., Baerwald et al. (2009), Arnett et al. (2011)). Several studies have tested improvements to "blanket curtailment" strategies that are based solely on time of year, time of night, and wind speed, to include other meteorological variables that could be important predictors of risk, such as temperature (e.g., Martin et al. (2017)) as well as real-time acoustic bat activity (e.g., Hayes et al. (2019), Rabie et al. (2022)). These refined strategies are often called "smart curtailment" or "informed curtailment" and are designed to be more efficient (i.e., reduced power loss) compared to blanket curtailment. Data on the proportion of female fatalities over time could further inform smart curtailment strategies by focusing minimization efforts during periods of peak female fatalities, since females are the limiting factor for population growth and stability in bats (Grüebler et al. 2008; Wedekind 2012).

The California population of Brazilian free-tailed bats is non-migratory (Krutzsch 1955; Davis et al. 1962). Although the sex ratio is unknown, it may be assumed to be 50:50 (F:M), as seen in other non-migratory bat species (Davis 1969; Cheng et al. 2019). Since this population does not partake in a sex-specific migration and has an assumed sex ratio of 50:50 (F:M), we predict that the proportion of female fatalities should not differ from 0.5 across time. If the proportion of female fatalities does differ from 0.5, then this could indicate that female and male bats in the Californian population interact differently with wind turbines. In contrast, the Texas population of Brazilian free-tailed bats migrates annually, with the migrant population being female-skewed (9:1; F:M) (Davis et al. 1962). There is also evidence of asynchronous movement between males and females during this migration, with a small number of males migrating north ahead of females to obtain breeding opportunities as females arrive at their large maternity caves (Davis et al. 1962).

As these bats migrate to and from their roosting locations, we predict that the proportion of female fatalities will depend on which roost type is closest to the wind energy facility, as cave roosts are female-skewed whereas bridge roosts are male-skewed. Nonetheless, we still expect to find an overall female bias in fatalities in the Texas population because the female-skewed cave roosting populations are much larger than the male-skewed bridge roosting populations (e.g., cave population estimates Davis et al. (1962); Cockrum (1970); USGS (2003), e.g. bridge population estimates Fraze and Wilkins (1990); Kasper and Yancey (2018)). Furthermore, in both the California and Texas populations, we predict that fatalities will peak in September and October due to the addition of pups to the population (Bernardo and Cockrum 1962). For the Texas population, this period also coincides with the return migration to Mexico to overwinter (Glass 1958; Bernardo and Cockrum 1962).

In summary, our goal is to determine the sex ratios of fatalities for the Brazilian free-tailed bats at wind energy facilities in California and Texas and to determine if the sex ratio of fatalities is stable over time. Given the differences in the proportion of females and migratory patterns between the two populations of Brazilian free-tailed bats, we predict that: 1) the proportion of female fatalities in the California population will not differ significantly from 0.5, and 2) the proportion of female fatalities in the Texas population will be > 0.5.

2. MATERIALS AND METHODS

Focal Species: the Brazilian Free-tailed Bat

In the United States, there are four Brazilian free-tailed bat populations that display distinct migratory behaviors (i.e., local, short-range, or long-range) (Bernardo and Cockrum 1962) (Figure 1). The westernmost population in California and southern Oregon is characterized by local seasonal movements and hibernation during the winter (Krutzsch 1955; Bernardo and Cockrum 1962). From this point on, we will refer to this population as the non-migratory population. The second population is a short-range migratory group in southeastern California, southern Nevada, and western Arizona. This population migrates relatively short distances south into the low interior valleys of southern California or Baja California during winter (Bernardo and Cockrum 1962; Russell et al. 2005). The last two populations exhibit long-range migratory behavior between the United States and Mexico to form maternal colonies. The two populations are reported to be skewed at 9:1 (Female:Male) because most males remain in Mexico while the females migrate to and form large maternal colonies (Davis et al. 1962). One of these populations is in western New Mexico and eastern and central Arizona, and the other is in Texas, Oklahoma, Kansas, and eastern New Mexico. These populations migrate to the United States from February to March and form maternal colonies from June to July, where they give birth and raise their pups before leaving from September to October to overwinter in Mexico (Glass 1958; Bernardo and Cockrum 1962). While both populations migrate simultaneously, they are distinguished by which flyway they use for their migration. The westernmost migratory population utilizes the flyway on the west side of the Sierra Madre Mountains, whereas the more eastern migratory population utilizes the flyway on the east side of the Sierra Madre Mountains (Bernardo and Cockrum 1962).

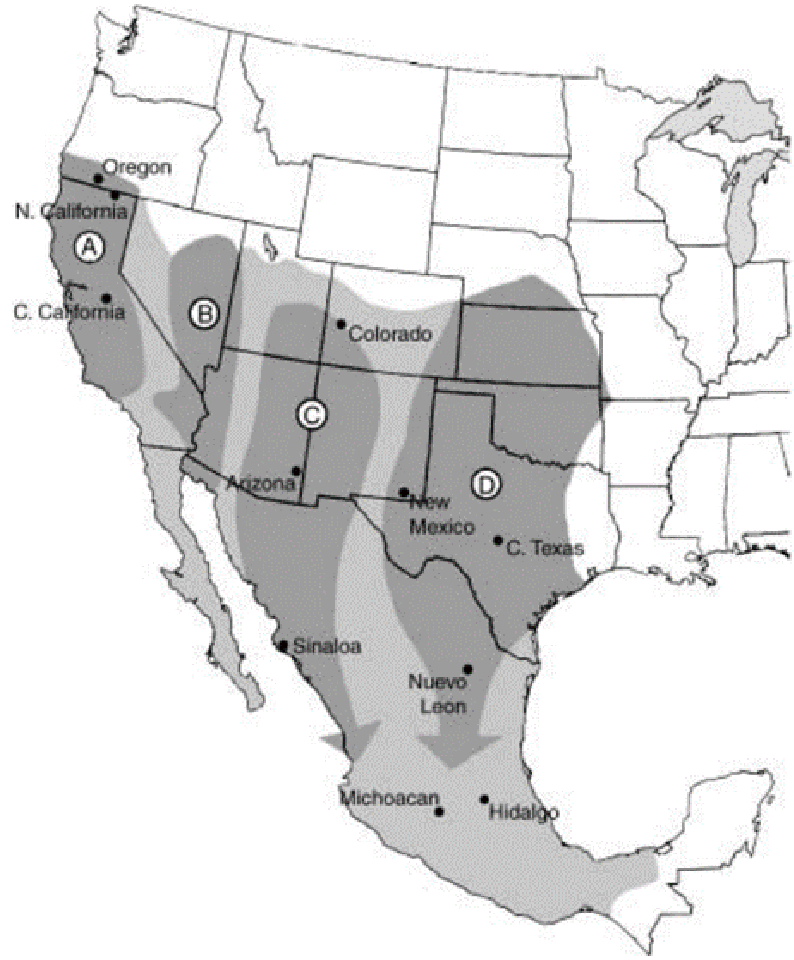

Figure 1. The range of the Brazilian free-tailed bat (*Tadarida brasiliensis*) in Mexico and the United States shaded in light gray, with the distinct non-migratory/migratory populations shaded in dark gray (Figure from Russell et al. (2005)).

The two migratory populations have a reported sex ratio of 9:1 (F:M) (Davis et al. 1962). However, this ratio may not be uniform across the landscape. A literature review of the sex ratios of Brazilian free-tailed bats by roost type (bridges and caves) within the migratory ranges in the United States showed a difference between the proportion of females present in bridge roosts and cave roosts (Table 1). For bridge roosts, there were five studies that reported the sex ratios of bridge roost populations (Table 1). Three of the five (60%) sex ratios reported at bridge roosts were significantly male-skewed (Table 1). The average proportion of females between the five bridge roosts was 0.44. In contrast, most cave populations were significantly female-skewed. Literature reports estimates of the sex ratios for 11 different caves, with most caves being studied for multiple years across various studies. Combining these years and studies, we compiled 35 estimates of sex ratios for the cave studies. Thirty-three of the 35 (94%) estimates were significantly female-skewed (Table 1). The average proportion of females in cave roosts was 0.78. For geographic reference, we have included known Brazilian free-tailed roost locations (bridges and caves) and known bat roosts with assumed Brazilian free-tailed bats within the Texas range of the migratory population (Figure 2).

Table 1. Estimates of the proportion of female Brazilian free-tailed bats at bridge roosts and cave roosts were obtained from the literature. All roosts are within the range of the two US migratory populations and occur during peak population abundance in the United States. The average proportion of females by roost type was calculated by averaging the proportion of female fatalities for each site in the category. Counts were not used to avoid introducing biases from sites with larger numbers of bats. Significance was calculated using a one-proportion z-test (p0 = 0.5, α = 0.05). Symbols: * = significantly male-skewed, ** = significantly female-skewed.

STATE	COUNTY	LOCATION	DATE	FEMALE COUNT	MALE COUNT	TOTAL	PROPORTION FEMALE	REFERENCE
BRIDGE ROOST								
OK	Grady	East Elm Creek Bridge	May-Oct. (2005)	24	48	72	0.33*	Turmelle et al. (2010)
TX	Tom Greem	Highway overpass at Foster Road and Highway Loop 306	Mar.-Oct. (2014)	442	319	763	0.58**	Martinez (2015)
	Bell	Highway overpass at the intersection of Interstate 35 and Farm-to-Market Road (FM) 436	Jun.-Nov. (1996)	453	806	1259	0.36*	Sgro and Wilkins (2003)
	Rusk	McNeail Bridge	May-Oct. (2005)	54	27	81	0.67**	Turmelle et al. (2010)
	Medina	Seco Creek Bridge	May-Oct. (2005)	24	63	87	0.28*	
Average Proportion of Females (Bridge Roost)								**0.44**

CAVE ROOST								
NV	White Pine	Snake Range	Jun.–Oct. (2015-2019)	135	203	338	0.40*	Danielson et al. (2022)
OK	Major	Connor's Cave	Apr.–Sep. (1965)	647	61	708	0.91**	Rogers (1972)
			Apr.–Aug. (1966)	306	48	354	0.87**	
			May–Aug. (1967)	450	40	490	0.92**	
	Woods	Merrihew Cave	Apr.–Sep. (1965)	340	176	516	0.66**	
			May–Aug. (1966)	107	59	166	0.65**	
			May–Aug. (1967)	194	121	315	0.61**	
			Apr.–Oct. (1952;1953)	466	147	613	0.76**	Twente (1956)
	Greer	Reed Cave	Jun.–Aug. (1965)	371	38	409	0.91**	Rogers (1972)
			Jun.–Aug. (1966)	312	39	351	0.89**	
			Jun.–Aug. (1967)	521	119	640	0.81**	
	Woodward	Selman's Cave	Apr.–Oct. (1965)	392	97	489	0.80**	
			May–Sep. (1966)	280	55	335	0.84**	

OK	Woodward	Selman's Cave	May–Sep. (1967)	334	60	394	0.85**	Rogers (1972)
	Major	Vickery Cave	Apr.–Oct. (1965)	586	123	709	0.83**	
			May–Sep. (1966)	242	64	306	0.80**	
			May–Sep. (1967)	374	117	491	0.76**	
TX	Comal	Braken Cave	Mar.–Aug. (1957)	10054	1502	11556	0.87**	Davis et al. (1962)
			Jun.,Sep. (1965)	260	52	312	0.83**	Rogers (1972)
			Jun., Sep. (1966)	188	31	219	0.86**	
			Jun., Sep. (1967)	154	53	207	0.74**	
	Blanco	Davis Cave	Mar.–Aug. (1957)	7962	1516	9478	0.84**	Davis et al. (1962)
			Mar.–Sep. (1958)	6493	4899	11392	0.57**	
			Jun., Sep. (1965)	375	72	447	0.84**	Rogers (1972)
			Jun., Sep. (1966)	156	26	182	0.85**	
			Jun., Sep. (1967)	47	25	72	0.65**	
			May–Oct. (2005)	63	7	70	0.90**	Turmelle et al. (2010)
	Uvalde	Frio Cave	Jun., Sep. (1965)	426	135	561	0.80**	Rogers (1972)

TX	Uvalde	Frio Cave	Jun., Sep. (1966)	225	214	439	0.51	Rogers (1972)
			Jun., Sep. (1967)	140	70	210	0.67**	
	Uvalde	Frio Cave	May–Oct. (2005)	66	19	85	0.78**	Turmelle et al. (2010)
	Mason	James River Cave	Mar.–Aug. (1957)	7591	2141	9732	0.85**	Davis et al. (1962)
			May–Oct. (2005)	78	3	81	0.96**	Turmelle et al. (2010)
	Medina	Ney Cave	Jun.–Aug. (1949)	472	208	680	0.69**	Cagle (1950)
			Mar.–Aug. (1957)	1667	12059	13726	0.78**	Davis et al. (1962)
Average Proportion of Females (Cave Roosts)								**0.78**

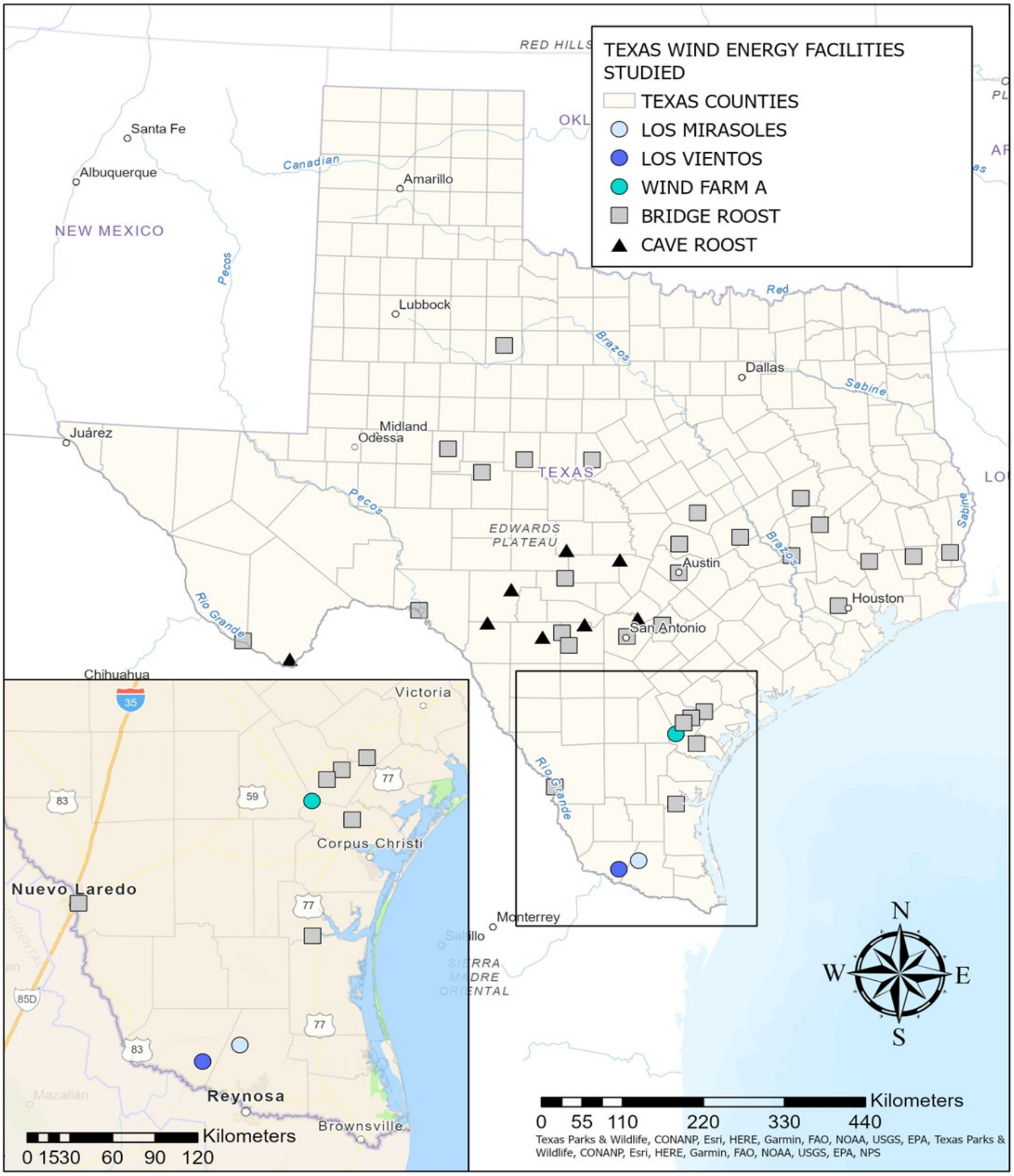

Figure 2. The locations of wind energy facilities in Texas included in this study (Los Mirasoles, Los Vientos, and Wind Farm A), along with known Brazilian free-tailed bat roosts and known bat roosts with assumed Brazilian free-tailed bats by type (bridge roost or cave roost).

Collection of Wing Tissue Samples

The wing tissue samples used in this project were obtained from bat carcasses collected during PCM surveys (n = 1,744). For the non-migratory population, we received samples from two locations within the Altamont Pass Wind Resource Area in Alameda County, California: Golden Hills (GH) (19 September 2016–15 September 2019, n = 217) and Golden Hills North (GHN) (8 October 2018–31 December 2020, n = 317) (Figure 3). For the migratory population, we received samples from three wind energy facilities in Texas: Los Mirasoles (Hidalgo County and Starr County) (3 August 2017–31 July 2018, n = 252), Los Vientos (Starr County) (24 March 2017–23 March 2018, 31 July 2018–30 October 2018, n = 568), and Wind Farm A (San Patricio County and Bee County) (2 November 2020January 2021–27 October 2021, 18 July 2022–26 October 2022, n = 391) (Figure 2). Texas bat carcasses were collected in accordance with Texas Parks and Wildlife Department (TPWD: permit number SPR-1120-189, to S. Weaver). Wing tissue samples were stored in NaCl/DMSO preservative solution (6M NaCl and 20% DMSO) in 2 ml screw-cap plastic tubes. With each tissue sample, we also received information on the collection date and location, estimated time since death, species identification in the field, and sex identification in the field (male, female, unknown).

Figure 3. The locations of wind energy facilities from California that were included in this study: Golden Hills (GH) and Golden Hills North (GHN).

DNA Extraction

We extracted DNA using 96-well PCR plates, following the methods detailed in Ivanova et al. (2006), with an extra wash step. We placed a small piece of each wing tissue sample (approx. 2-3 mm^2) in an individual well in a 96-well PCR plate. Each well contained 50 µl of Vertebrate Lysis Buffer (VLB) (100 mM NaCl, 50 mM Tris-HCL pH 8.0, 10 mM EDTA pH 8.0, and 0.5% SDS) and 5 µl of Proteinase K (20 mg/ml). We then covered the 96-well PCR plate with a PCR mat and incubated it at 56 °C overnight to allow digestion. After the wing tissue had been digested, we centrifuged the 96-well PCR plate at 1500 g for 10 sec to remove condensation from the PCR mat. Next, we added 100 µl of Binding Mix (BM), made from equal volumes of EtOH 96% and Binding Buffer (BB) (6 M GuSCN, 20 mM EDTA pH 8.0, 10 mM Tris-HCL pH 6.4, 4% Triton X-100) to each sample. We then covered the 96-well PCR plate with a new PCR mat, vortexed the plate vigorously for 15 sec, and centrifuged the 96-well PCR plate at 1000 g for 20 sec. We transferred the lysate (about 150 µl) from the wells of the 96-well PCR plate into the wells of a Glass Fiber (GF) Plate (PALL1) placed on top of a square-well block. The GF Plate was sealed with a self-adhering cover. The covered GF Plate and square-well block were centrifuged at 5000 g for 5 min to bind the DNA to the GF membrane. Three wash steps followed. For the first wash step, we added 180µl of Protein Wash Buffer (PWB) (46.8 µl BB, 126 µl EtOH 96%, and 7.2 µl autoclaved dH_2O) to each well of the GF Plate, sealed the GF Plate with a new self-adhering cover, and centrifuged the GF Plate and square-well block at 5000 g for 2 min. For the second wash step, we added 750 µl of Wash Buffer (WB) (60% EtOH 96%, 50 mM NaCl, 10 mM Tris-HCL pH 7.4, and 0.5 mM EDTA pH 8.0) to each well of the GF Plate, sealed the plate with a new self-adhering cover, and centrifuged the GF Plate and square-well block at 5000 g for 5 min. For the third wash step, we added 500 µl of 70% ethanol to each well of the GF Plate, sealed the plate with a new self-adhering cover, and centrifuged the GF Plate and square-well block at 5000 g for 3 min. After the wash steps, we centrifuged the GF Plate and square-well block at 5000 g for 5 min to remove excess moisture from the samples. We then placed the GF Plate on top of a microplate equipped with a PALL collar and dispensed 70 µl of 10 mM Tris-HCL, pH 8.5 (pre-warmed to 56 °C) directly onto the membrane in each well of the GF Plate. The GF Plate on top of a microplate equipped with a PALL collar was incubated at room temperature for 1 min. After incubating at room temperature, we covered the GF Plate with a new self-adhering cover. We then centrifuged the assembled plate at 5000 g for 5 min to collect the DNA eluate into the bottom microplate. We then sealed the DNA plate with a new self-adhering cover and stored the extracted samples at -20 °C.

Molecular Sex Determination

We determined sex by amplifying the *zfx* and *zfy* introns found on the sex chromosomes using the Brazilian free-tailed primers in Korstian et al. (2013). All PCRs (10 µl) contained between 2 ng/µl to 50 ng/µl of template DNA, 0.5 µM of each X-primer, 0.35 µM of each Y-primer, and 1X AccuStart ™ IWEPCR SuperMix. The cycling parameters for the PCRs were one cycle at 95 °C for 15 min, followed by 35 cycles of 30 sec at 94 °C, 1 min at 57 °C, and 30 sec at 72 °C. We stained PCR products using Gel Green (Biotium), electrophoresed at 200 V in 1% agarose gel for 35 min and visualized the bands using blue-green LED light.

Sex was determined based on the number of bands present in the sample. Females produce a single band corresponding to the X-chromosome intron (245 bp). Males produce two bands, one corresponding to the X-chromosome intron (245 bp) and one corresponding to the Y-chromosome intron (80 bp) (Figure 4). Bands larger than ~300 bp were disregarded when determining sex.

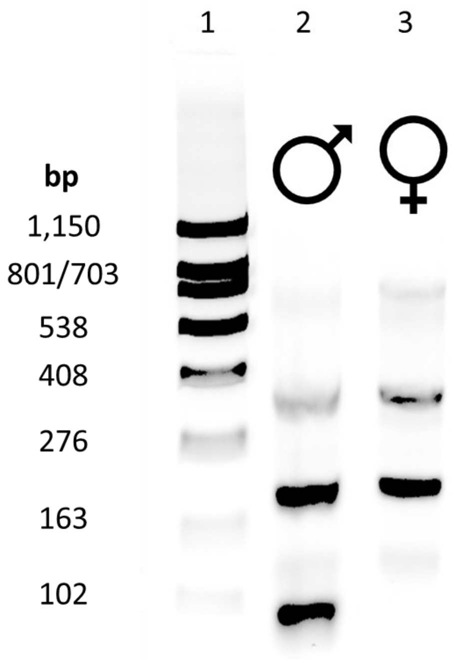

Figure 4. Amplification of the sex chromosome-specific PCR products for a known male and female Brazilian free-tailed bat. (Lanes: 1. Size standard ladder; 2. PCR product from a male bat; 3. PCR product from a female bat).

California Data Analysis

The groups used for analysis were year by location and month by location. For year analysis, we analyzed data only for years with searches conducted from January–December. With these conditions, there was no overlap in data between the two locations for the by-year analysis (GH 2017, GH 2019, and GHN 2018 were excluded due to partial search years). We separated month analysis by location and only analyzed months with combined totals of ≥ 30 bats pooled across years.

For all groupings, we used a one-proportion z-test (α = 0.05) to determine whether the proportion of female fatalities differed significantly from 0.5. Pairwise comparisons were conducted using multiple two-proportion z-tests, with alphas corrected using Bonferroni correction, to determine if the proportion of female fatalities differed significantly among years by location (α = 0.008) and months by location (GH, α = 0.05; GHN, α = 0.003).

Texas Data Analysis

Because search efforts varied across sites and years, we analyzed bat fatalities only from August to October, as most facilities were searched during those months. This time frame also coincided with peak bat fatalities. We did not investigate the overall proportion of female fatalities by month due to differences in survey efforts across sites and seasons. The groups analyzed were location (Los Vientos, Los Mirasoles, and Wind Farm A) and year by location (Los Vientos, 2017–2018; Los Mirasoles, 2017; Wind Farm A, 2021–2022).

For all groupings, we used a one-proportion z-test (α = 0.05) to determine whether the proportion of female fatalities differed significantly from 0.5. Pairwise comparisons were conducted using multiple two-proportion z-tests (α = 0.05), with alphas corrected using Bonferroni correction to determine if the proportion of female fatalities differed significantly between locations (α = 0.017). For locations with two years of data, a two-proportion z-test was used to determine if the sex ratio significantly changed (α = 0.05) between years at the location.

3. RESULTS

Using molecular methods, we successfully determined sex for 1,656 of the 1,744 bat tissue samples received (95%). We were unable to determine sex for 88 bat tissue samples due to poor DNA quality. For the samples that amplified successfully, the amplification produced a product specific to the X-chromosome (245 bp), and males produced another product specific to the Y-chromosome (80 bp) (Figure 4).

California

For the non-migratory California population, the proportion of female fatalities did not differ from 0.5 for locations by year (Figure 5) or month by location, except for June at GHN ($p_{female} = 0.34$, $n = 56$, $z = -2.41$, $p = 0.008$) (Figure 6). There were also no significant differences among groupings (years by location ($\alpha = 0.008$) and months by location (GH, $\alpha = 0.05$; GHN, $\alpha = 0.003$)).

One unexpected finding from the analysis was that the California population experienced a shift in the timing of peak fatalities across the facilities surveyed. For the first two complete years of surveys at GH (2017 and 2018), fatalities peaked between September and October, as expected (AWWI 2020) (Figure 7, B-C). However, for GHN, fatalities peaked between May and June in 2019 and 2020 (Figure 7, F-G).

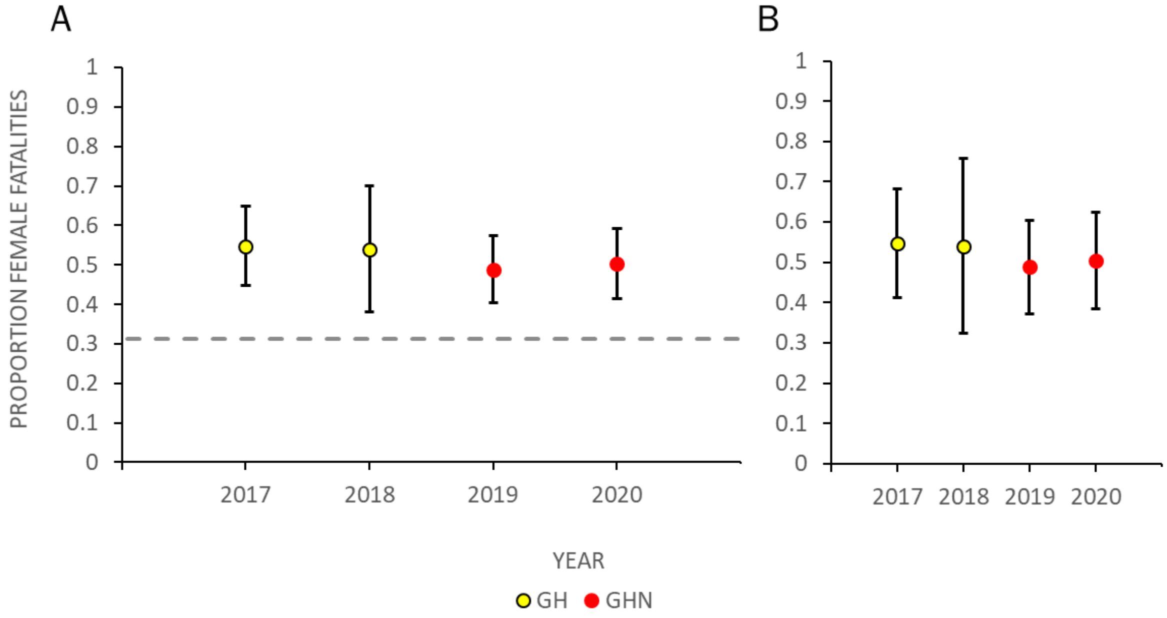

Figure 5. The proportion of female fatalities in CA by location and year (GH, 2017–2018; GHN, 2019–2020), with 95% confidence intervals. The dotted line represents a 0.5 proportion of female fatalities. Confidence intervals overlapping with the dotted line are not significantly different from 0.5. (GH, 2017 (pfemale = 0.55, n = 95); GH, 2018 (pfemale = 0.54, n = 37); GHN, 2019 (pfemale = 0.49, n = 131); GHN, 2020 (pfemale = 0.52, n = 122)). The proportion of female fatalities in CA by location and year (GH, 2017–2018; GHN, 2019–2020). Data are shown with 99.2% confidence intervals, corrected for multiple comparisons. Confidence intervals overlapping with the mean of the other data points are not significantly different. Asterisks have been included in figures with significant differences between data points. (GH, 2017 (pfemale = 0.55, n = 95); GH, 2018 (pfemale = 0.54, n = 37); GHN, 2019 (pfemale = 0.49, n = 131); GHN, 2020 (pfemale = 0.52, n = 122)).

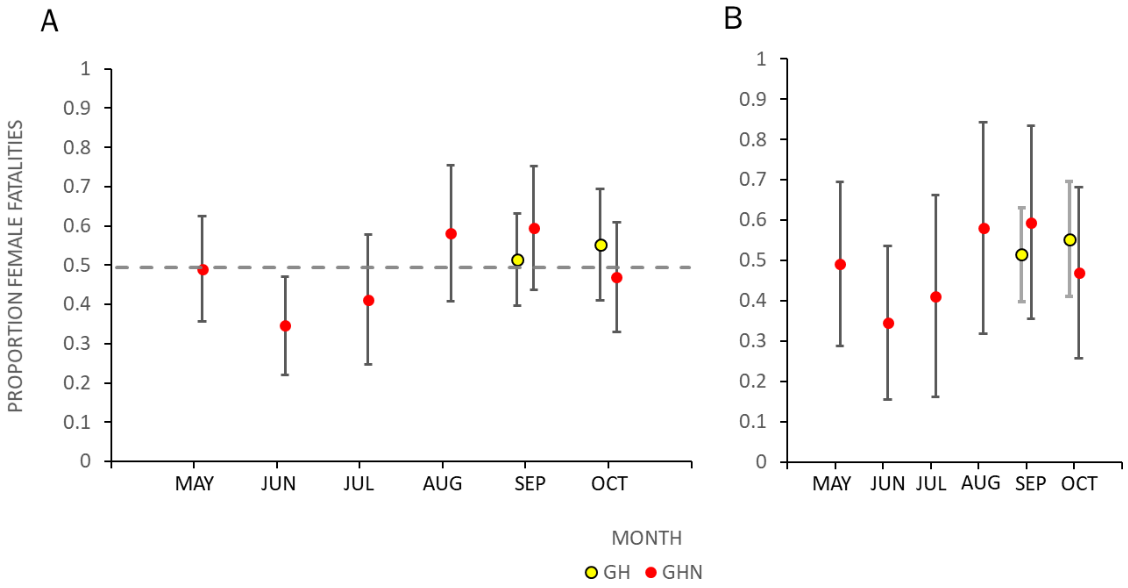

Figure 6. A) The proportion of female fatalities in CA at GH and GHN by month (GH, September–October; GHN, May–October), with 95% confidence intervals. The dotted line represents a 0.5 proportion of female fatalities. Confidence intervals overlapping with the dotted line are not significantly different from 0.5. (GH, September (pfemale = 0.51, n = 70); GH, October (pfemale = 0.55, n = 47); GHN, May (pfemale = 0.49, n = 53); GHN, June (pfemale = 0.34, n = 55); GHN, July (pfemale = 0.41, n = 34); GHN, August (pfemale = 0.58, n = 31); GHN, September (pfemale = 0.59, n = 37); GHN, October (pfemale = 0.47, n = 49)). B) The proportion of female fatalities in CA at GH and GHN by month (GH, September–October; GHN, May–October), with 95% confidence intervals for GH. Data for GHN are shown with 99.7% confidence intervals, corrected for multiple comparisons at GHN. Confidence intervals overlapping with the mean of the other data points are not significantly different within the group (GH by month; GHN by month). Asterisks have been included in figures with significant differences between data points. (GH, September (pfemale = 0.51, n = 70); GH, October (pfemale = 0.55, n = 47); GHN, May (pfemale = 0.49, n = 53); GHN, June (pfemale = 0.34, n = 55); GHN, July (pfemale = 0.41, n = 34); GHN, August (pfemale = 0.58, n = 31); GHN, September (pfemale = 0.59, n = 37); GHN, October (pfemale = 0.47, n = 49).

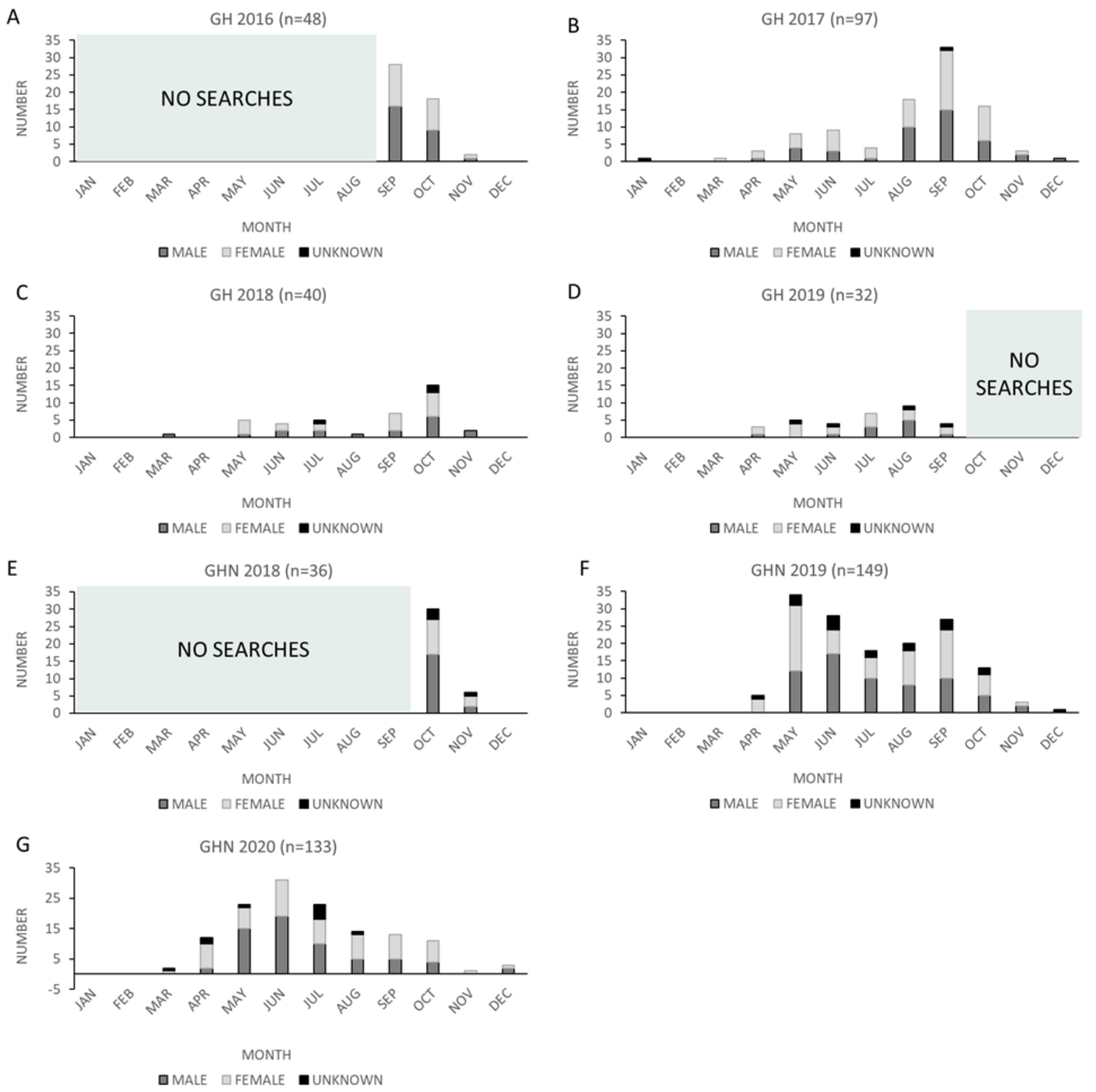

Figure 7. Monthly counts of Brazilian free-tailed bat carcasses by sex (Male:Female:Unknown) found at California wind energy facilities Golden Hills (GH) (2016–2019, A-D) and Golden Hills North (GHN) (2018–2020, E-G).

Texas

For the migratory Texas population, the proportion of female fatalities varied among the three sampled wind energy facilities. The proportion of female fatalities collected at Los Mirasoles did not differ from 0.5 ($p_{female} = 0.56$, $n = 124$, $z = 1.33$, $p = 0.09$; Figure 8). In contrast, fatalities collected at Los Vientos were female-skewed ($p_{female} = 0.55$, $n = 516$, $z = 2.20$ $p = 0.027$; Figure 8).

When comparing the proportion of female fatalities between the three locations, the proportion of female fatalities at WFA was significantly lower than the proportion of female fatalities at Los Mirasoles ($z = -2.92$, $p < 0.004$) and Los Vientos ($z = -3.91$, $p = 0.001$).

The proportion of female fatalities varied between years at sampled wind energy facilities in Texas (Figure 9). At Los Vientos, fatalities were female-skewed in 2017 ($p_{female} = 0.60$, $n = 286$, $z = 3.43$, $p < 0.001$) but not in 2018 ($p_{female} = 0.48$, $n = 230$, $z = -0.61$, $p = 0.54$). The proportion of female fatalities at Los Vientos decreased significantly between 2017 and 2018 ($z = -2.69$, $p = 0.007$). Wind Farm A also experienced a change in the proportion of female fatalities over time. In 2021, the proportion of female fatalities did not differ from 0.5 ($p_{female} = 0.44$, $n = 102$, $z = -1.21$, $p = 0.23$), but the ratio became male-skewed in 2022 ($p_{female} = 0.40$, $n = 240$, $z = -3.10$, $p < 0.002$).

Lastly, the peak in Brazilian free-tailed bat fatalities consistently occurred in late summer and early fall at the Texas wind energy facilities included in this study (Figure 10).

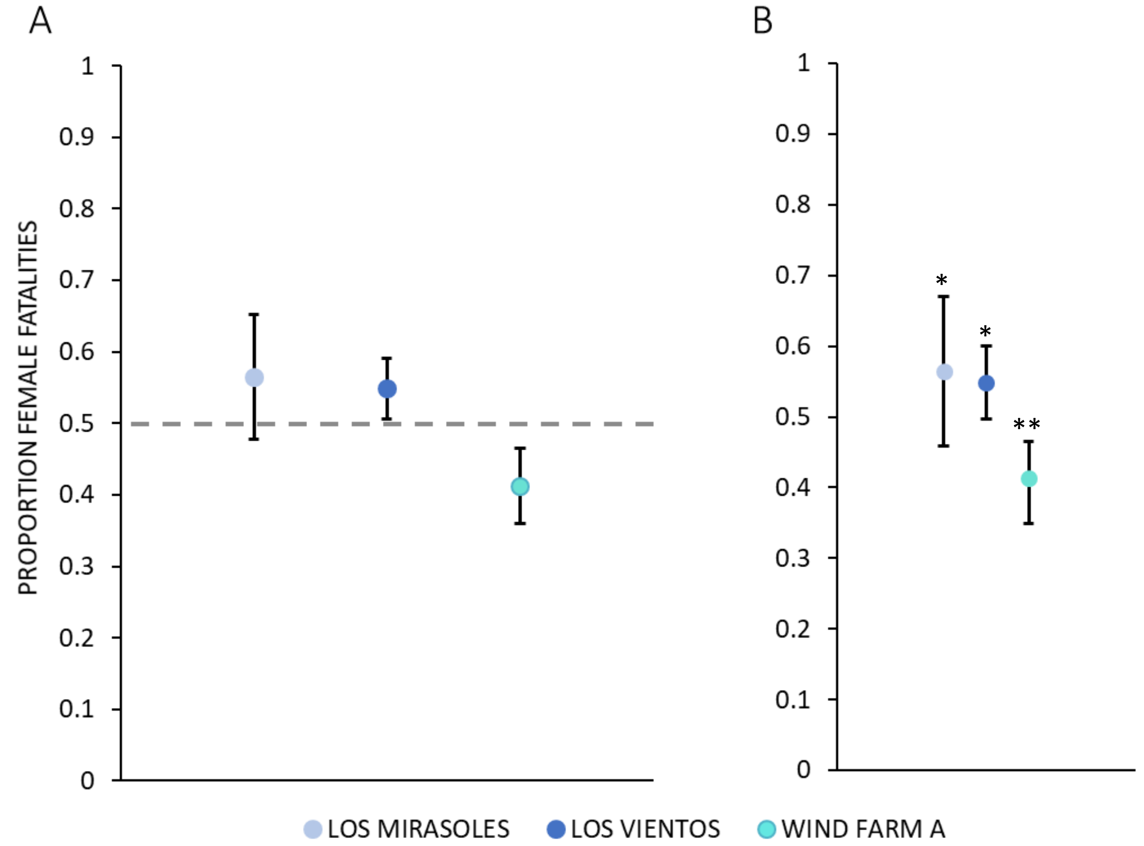

Figure 8. A) The proportion of female fatalities in TX by location (Los Mirasoles, Los Vientos, and Wind Farm A), with 95% confidence intervals. The dotted line represents a 0.5 proportion of female fatalities. Confidence intervals overlapping with the dotted line are not significantly different from 0.5. (Los Mirasoles (pfemale = 0.56, n = 124); Los Vientos (pfemale = 0.55, n = 516); Wind Farm A (pfemale = 0.41, n = 342)). B) The proportion of female fatalities in TX by location (Los Mirasoles, Los Vientos, and Wind Farm A). Data are shown with 98.3% confidence intervals, corrected for multiple comparisons. Confidence intervals overlapping with the mean of the other data points are not significantly different. Asterisks have been included in figures with significant differences between data points. (Los Mirasoles (pfemale = 0.56, n = 124); Los Vientos (pfemale = 0.55, n = 516); Wind Farm A (pfemale = 0.41, n = 342)).

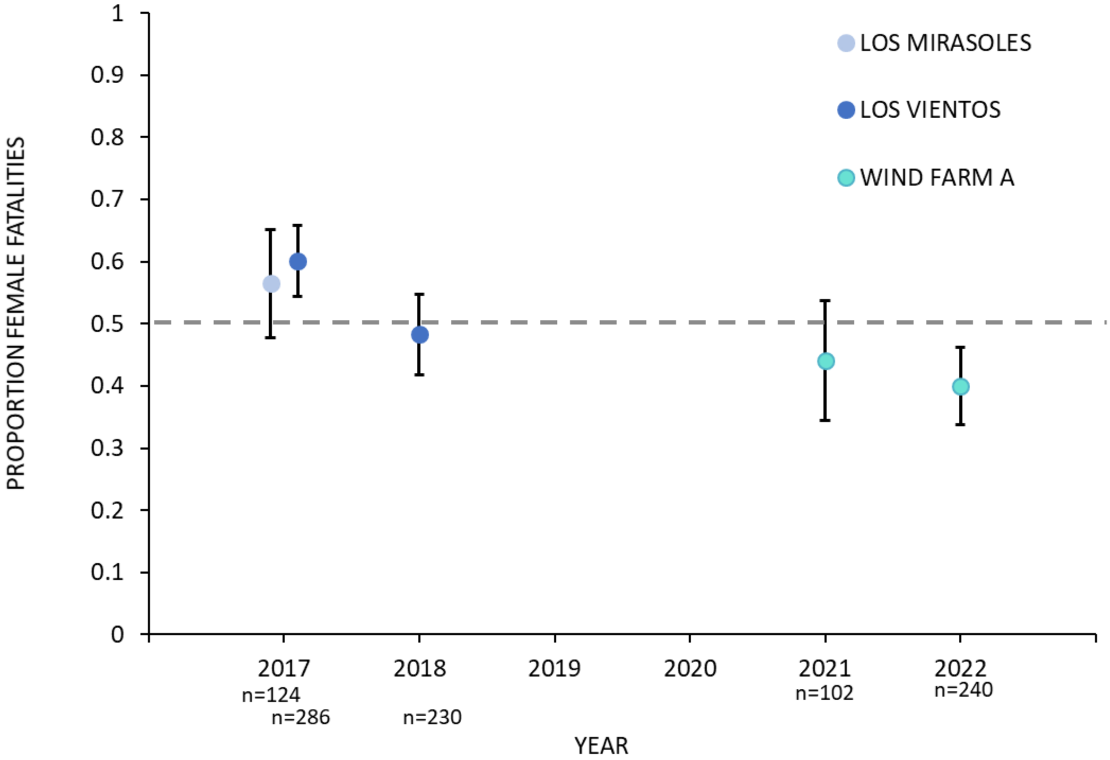

Figure 9. The proportion of female fatalities in TX by location and year (Los Mirasoles 2017, Los Vientos 2017–2018, and Wind Farm A 2021–2022), with 95% confidence intervals. The dotted line represents a 0.5 proportion of female fatalities. Confidence intervals overlapping with the dotted line are not significantly different from 0.5. (Los Mirasoles, 2017 (pfemale = 0.56); Los Vientos, 2017 (pfemale = 0.60); Los Vientos, 2018 (pfemale = 0.48); Wind Farm A, 2021 (pfemale = 0.44); Wind Farm A, 2022 (pfemale = 0.41)).

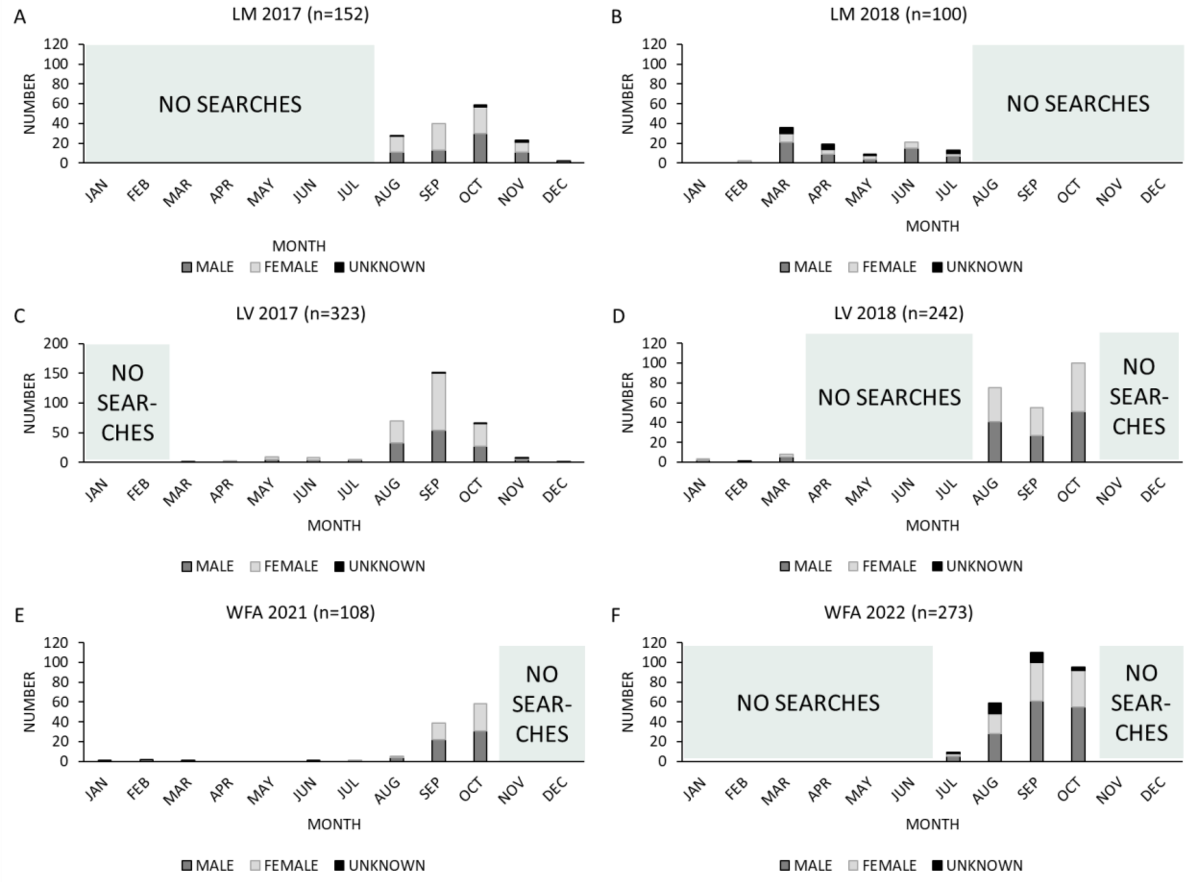

Figure 10. Monthly counts of Brazilian free-tailed bat carcasses by sex (Male:Female:Unknown) found at Texas wind energy facilities Los Mirasoles (LM) (2017–2018, A-B), Los Vientos (LV) (2017–2018, C-D), and Wind Farm A (WFA) (2021–2022, E-F).

4. DISCUSSION

Results suggest an equal sex ratio of Brazilian free-tailed bat fatalities in the non-migratory California population except at the northern location in June, when fatalities were male-skewed. Additionally, the proportion of female fatalities did not differ significantly among groupings. For example, the proportion of female fatalities in June at GHN was not significantly different from that in September at GHN (α = 0.003). Additionally, the proportion of female fatalities was not significantly different between locations by year ($\alpha = 0.008$). This was expected since California's non-migratory Brazilian free-tailed bat population does not segregate by sex over large areas (Krutzsch 1955), so we expected similar proportions of females across time and locations. The time of peak fatalities in California was not consistent across time. In 2017–2018, fatalities peaked between September and October, as we expected from previous studies (AWWI 2020). However, in 2019–2020, fatalities peaked between April and May. Our analysis of the migratory and sex-skewed (9:1 [F:M])) Texas population found that the proportion of female fatalities was significantly different from 0.5, varying temporally and geographically. For all locations with two years of data analysis, the proportion of female fatalities decreased over time. Unlike the California population, the fatalities consistently peaked between September and October, as expected (AWWI 2020).

GenEst was not used in our analysis. GenEst is used to more accurately estimate the total fatalities by accounting for bias created by carcass removal, searcher efficiency, and density-weighted proportion (DWP) of the area searched. We do not expect these biases to greatly impact these sex ratios. Carcass removal by scavengers at wind energy facilities has not been found to be sex-specific, so we would expect each sex to remain on the landscape for equal amounts of time (Villegas-Patraca et al. 2012; DeVault et al. 2017). We also do not expect searcher efficiency to influence the sex ratios. The Brazilian free-tailed bat is not sexually dimorphic (Twente 1956; Davis et al. 1962), so we expect each sex to be equally visible on the landscape. There is a possibility that searcher efficiency could change between seasons due to a change in physical conditions. This change could cause the average sex ratio of fatalities to be biased towards seasons when searches were less difficult and more carcasses were collected. For example, harsh summers in Texas could lead to lower search effort and cause an underrepresentation of the summer sex ratio fatalities when creating a year average with counts. This bias would be an issue for the Texas population, where we expect to see a difference in the sex ratio of fatalities across seasons. However, in Texas, we only analyzed data from August–October, so we did not analyze across all seasons. Along with this, the raw carcass counts we obtained in Texas followed the same pattern as GenEst estimates for fatality counts in the South West region of the United States, so we believe that the counts of our carcasses by season are not biased by searcher efficiency by season. Lastly, we do not expect the sex ratios of fatalities to be biased by DWP of the area searched. There is no evidence that Brazilian free-tailed bats exhibit sex-specific behaviors around turbines, which could cause carcasses to be dispersed at differing distances from the monopole after a collision. Additionally, since the sexes are similar in mass (Twente 1956; Davis et al. 1962), we would not expect to see sex differences in the dispersal of carcasses around the monopole after collisions due to mass differences.

For temporal analysis, the estimated time of death (ETD) was not used as a factor, and instead, the collection date metadata was used for data analysis. A preliminary data analysis was done using the Texas samples to compare the temporal trend when using collections dates versus ETD, with the removal of samples without ETD metadata. There was no significant difference in the proportion of female fatalities over time for the two date types. Since the smallest time frame analyzed was a month, most samples remained in the same month category when comparing the collection date to the ETD corrected date. However, when ETD was used, and samples without ETD metadata were removed, the smaller sample sizes reduced the power of our tests, causing no differences in the average proportion of female fatalities between the three locations in Texas. Because there was no difference in the monthly sex ratio trends between the two date types (collection date or ETD date with removal), the collection dates were used for the data analysis to keep sample sizes large enough to see location differences in the proportion of female fatalities in Texas.

California

When we compared the proportion of female fatalities for GH and GHN, there was no significant difference between locations by year ($p > 0.05$). This result was expected due to the proximity of GH and GHN (less than 5 km apart).

The proportion of female fatalities was not significantly different from 0.5 when analyzed by location (GH and GHN), year, and month by location, except for June at GHN in 2019 and 2020. This high proportion of male fatalities in June at GHN could be due to the local movement of male Brazilian free-tailed bats leaving roosting caves due to overcrowding as females move into caves and prepare for pupping in June (Davis et al. 1962). However, since this pattern is not observed in June for GH, we are not sure this is the cause. Other than this unusual male-skew in June at GHN, the sex ratios did not differ significantly from 0.5. Under the assumption that the Brazilian free-tailed bat population in California is not significantly different from 50:50 (F:M), as seen in other non-migratory bat species (Davis 1969; Cheng et al. 2019), it appears that the proportion of female fatalities reflects the proportion of females in the population. This could indicate no sex-specific bat-wind turbine behaviors in this non-migratory population.

There are two possible explanations for the shifts in peak fatalities, which occurred in May and June at GHN and in September and October in GH, which was more expected, and both are related to foraging. One possibility proposed in the PCM report (GBBO and HTH 2022) is that GHN is near a reservoir that bats may forage over, as Brazilian free-tailed bats are known to forage over large bodies of fresh lacustrine water during summer and fall (Johnston 2013). Nevertheless, it is interesting that this same shift was not seen at GH in 2017 and 2018 since it is located very close to GHN (less than 5 km apart) (Figure 2), and these bats fly over 56 km from their roosting sites when foraging at night (Best 2009). Another possible factor influencing this shift is changes in moth abundance. Moths comprise a significant proportion of the Brazilian free-tailed bat's diet (Kunz and Robson 1995; Whitaker et al. 1996; Lee and McCracken 2002). They have even been observed changing their foraging behavior in response to moth migrations (Krauel et al. 2018). UC Berkeley's Essig Museum of Entomology noted moth outbreaks in 2019 and 2020 from May to June in the same county as the wind energy facilities in this study (Oboyski 2020). The moth was the California oak moth (*Phryganidia californica*), a species that experience population outbreaks at irregular intervals that last one to two years (Burke and Herbert 1920). It is possible that GH also experienced a change in the time of peak fatalities in 2019, but sample sizes were too small to detect a shift (n = 32). It would be necessary to analyze the stomach contents of the bats from GHN and GH using DNA barcoding to see if there are differences in their diets between locations (waterbody influence) or times (changes in moth abundance across years). These findings could help us better understand what external factors influence fatalities at California's wind energy facilities.

Texas

The differences in sex ratios by location and the changes in the proportion of females over time suggest location may impact the proportion of female fatalities at wind energy facilities depending on their proximity to a roosting location or if they are in a direct flight path between roosting locations. Brazilian free-tailed bats have been found to move between roosting locations during their migration (Davis et al. 1962; Fraze and Wilkins 1990). We possibly observed the effects of roost proximity impacting the proportion of female fatalities at WFA. Wind Farm A is adjacent to four bridge roosts, all within 50 km, which could be causing a high proportion of male fatalities. Additionally, WFA is 112.7 km north of a bridge roost location that males use over the winter (Mink 2012). This could be causing the male-skew we report in September and October, as males travel from their bridge roosts to the north of WFA to this known winter male roost in southern Texas (Figure 3). However, because these sites were surveyed in different years, we cannot dismiss the possibility of temporal trends influencing the differences in the proportion of female fatalities between the southern wind farms (LM and LV) and the northern wind farm (WFA).

Across all of the Texas locations that were studied for multiple years, we observed a decrease in the proportion of female fatalities. This decrease in the proportion of female fatalities over time could be a cause for concern. As we saw in California, the sex ratio of fatalities reflected the assumed sex ratio of the California population. This decrease in the proportion of female fatalities at the Texas locations across the years could indicate a reduction in the proportion of females in the migratory population over time. This decrease in the proportion of female fatalities could be due to mass female fatalities during their migration or a loss of females from bioaccumulation of pesticides (Oliveira et al. 2021; Torquetti et al. 2021). However, more research would need to be done to determine if this is a population-level trend.

With the continued growth of wind energy, it is important for us to have accurate data on the species and sex of the bats impacted at wind energy facilities. DNA Barcoding provides a more accurate understanding of the species impacted than morphological identification based on the remains of partially decomposed and scavenged carcasses (Korstian et al. 2015; Chipps et al. 2020). Molecular methods are also more accurate at determining the sex of bat carcasses compared to morphological characteristics of carcasses that are often partially scavenged and decomposing in the field (Korstian et al. 2013; Nelson et al. 2018; Chipps et al. 2020). This can often cause females to be undercounted since they are harder to identify because they lack external genitalia (Korstian et al. 2013; Nelson et al. 2018; Chipps et al. 2020). Using molecular methods, we identified 95% of the bat carcasses received. Furthermore, to minimize sex identification errors in the field, it is not recommended to use morphological characteristics to determine the sex of carcasses with an estimated time of death greater than one day (Korstian et al. 2013; Nelson et al. 2018; Chipps et al. 2020). These restrictions significantly reduce the number of carcasses that can be accurately sexed during PCMs when using morphological characteristics. Of the 1744 samples we received, only 566 (32%) had an estimated time of death of one day or less. By using molecular methods, we were able to get more accurate data on the sex ratios of fatalities at these wind energy facilities and increase the number of data points by over two-thirds.

5. CONCLUSION

In conclusion, with the expansion of wind energy, it is essential to understand the species and sexes impacted accurately. This knowledge can then be used for management and conservation decisions at wind energy facilities. One management strategy that has effectively mitigated bat fatalities at wind energy facilities is curtailment, slowing wind turbines' speed down to 1-2 RPM during peak fatalities (Adams et al. 2021; Whitby et al. 2021). This curtailment method can be made more efficient by targeting times when females are most at risk, because females are the limiting factor of population growth and stability (Grüebler et al. 2008; Wedekind 2012). Smart curtailment would allow wind energy facilities to minimize energy losses by shortening curtailment times and maximizing conservation outcomes by protecting females in the population.

However, it does not appear that there is a one size fits all approach to curtailment in either location due to inconsistencies in the proportion of female fatalities over time and location. While California had nearly five years of consecutive data at two sister locations, there were no consistent patterns in fatalities across the two locations over time. Unexpectedly, fatalities peaked between the two California locations at different times, possibly due to proximity to foraging habitat between the two locations or changes in moth abundance between years of observation. The Texas migratory population showed no consistent patterns in female fatalities. The proportion of female fatalities appeared site-specific, with the proximity of roost types possibly impacting the proportion of female fatalities, as was seen at Wind Farm A. In Texas, the proportion of female fatalities decreased over time.

There may be a pattern in the proportion of female fatalities over time that is region or location specific. However, more research must be done to better understand these patterns. To elucidate potential differences, monitoring should be conducted continuously for longer than one year, and gaps in searches should be avoided, especially during times of high abundance for the species of interest. Multiple years of data at each location will help to distinguish year(s) of unusual activity for the species of interest that could be caused by external factors such as wildfires, droughts, or outbreaks of prey species.

However, as funding is limited and longer monitering periods may not be feasible, another option would be to obtain more data from the samples already collected in past PCMs. These tissue samples could be sexed using genetic methods. Using genetic sex determination would also aid in improving the accuracy of previously conducted studies that used morphological sex determination in the field. By sexing the bat tissue samples that are already preserved in tissue repositories, we would greatly increase the amount of data on the sex ratios of bat fatalites temporally and geographically at wind energy facilities.

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VITA

Sarah LiCari was born on 3 January, 1998, in Yingtan , Jiangxi, China. She is the daughter of Dawn LiCari. A 2016 graduate of Bethlehem Catholic High School, Bethlehem, Pennsylvania, she received a Bachelor of Science degree with a major in Biology and Minors in Physics and Psychology from East Stroudsburg University, East Stroudsburg, Pennsylvania in 2020.

ABSTRACT

SEX RATIOS OF BRAZILIAN FREE-TAILED BAT (*TADARIDA BRASILIENSIS*) FATALITIES AT WIND ENERGY FACILITIES IN CALIFORNIA AND TEXAS

By Sarah Toye LiCari, M.S., 2023
Department of Biology
Texas Christian University
Thesis Advisor: Dean A. Williams, Professor of Biology

Amanda M. Hale, Research Associate (TCU), Senior Research Biologist, WEST Inc.

Sara P. Weaver, Principal, Bowman Consulting

Sarah R. Fritts, Associate Professor of Biology (TSU)

Fatalities at wind energy facilities may threaten some bat populations more than others. The Brazilian free-tailed bat (*Tadarida brasiliensis*) is the most common wind turbine-bat fatality in the Southern Great Plains and the western United States. In California, the population is considered non-migratory with an even sex ratio, whereas in Texas, the population is migratory and female-skewed. If these underlying differences in population sex ratios are reflected in collision fatalities, measures to reduce fatality rates may benefit the female-skewed migratory population more. We used molecular methods to determine the sex ratio of fatalities at wind energy facilities. In California, the proportion of female fatalities did not differ significantly from 0.5 and was stable over the survey years, but the time of peak fatalities was inconsistent. In Texas, the proportions of female fatalities differed across facilities, perhaps influenced by the proximity of roosts, and became less female-skewed over the years surveyed.