ARE TREE BATS FORAGING AT WIND TURBINES IN THE SOUTHERN GREAT PLAINS?

By

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ABSTRACT

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I. INTRODUCTION

Unlike conventional sources of energy such as oil, gas, and coal, utility-scale wind farms require no fuel, do not consume water, and produce no greenhouse gas emissions or other pollutants during the energy production phase. In 2013, wind power supplied 4.5% of the electrical energy consumed in the U.S. and the U.S. Department of Energy intends for this to increase to at least 20% by 2030, which will provide environmental and economic benefits as a sustainable, domestic energy source (USDOE 2015). Despite these benefits, wind energy development has drawbacks; for example, annual wind-related bat fatality is estimated in the hundreds of thousands of bats (Kuvlesky et al. 2007, Piorkowski and O'Connell 2010, Arnett and Baerwald 2013). Consequently, wildlife conservation has become an important consideration in the expansion of the wind energy industry.

Migratory tree bats, particularly lasiurine species, have the highest mortality rates at wind facilities in North America, which peak from midsummer to early fall and coincide with the bats' seasonal migration (Kunz et al. 2007, Arnett et al. 2008, Arnett and Baerwald 2013). More than 75% of wind-related bat fatalities are comprised of 3 species: hoary (*Lasiurus cinereus*), eastern red (*Lasiurus borealis*), and silver-haired (*Lasionycteris noctivagans*) bats. We know little about the migratory behavior or the population status of these tree bats, but there is increasing concern that high fatality rates at wind turbines could have long-term effects on populations (Kunz 2007, Arnett et al. 2008, Cryan and Barclay 2009, Arnett and Baerwald 2013, Jameson and Willis 2014).

Causes of wind-related bat fatalities can be split into two categories: proximate and ultimate causes (Cryan and Barclay 2009). Proximate causes of fatality describe how bats die, i.e., collision with a wind turbine tower or blade, whereas ultimate causes of fatality seek to explain why bats come into contact with wind turbines (Cryan and Barclay 2009). Nevertheless, despite understanding the proximate causes of bat fatality at wind turbines, the ultimate causes are still unclear (Barclay et al. 2007, Kunz 2007, Arnett et al. 2008). Cryan and Barclay (2009) proposed 3 broad ultimate causes of bat mortality at wind farms: 1) collisions occur by chance and the number of bat fatalities is proportional to the number of bats in the area; 2) collisions are coincidental, for example, when a wind farm intersects a bat migratory route; and 3) some aspect of the wind turbine attracts bats, which in turn results in collisions (known as the attraction hypothesis; Cryan and Barclay 2009).

But why would bats be attracted to wind turbines? A number of specific hypotheses have been proposed to further explain bat attraction to wind turbines. One possibility is that bats find something about the turbines themselves to be interesting (Cryan and Barclay 2009). For example, red aviation lights on top of turbine towers have been considered to be a potential source of interest to bats; however, studies have shown that mortality at towers with aviation lights is similar to or even less than mortality at towers without aviation lights (Arnett et al. 2008, Baerwald 2008, Bennett and Hale 2014). Alternatively, bats may misperceive wind turbines to be a resource. For example, a study by Cryan et al. (2014) suggested that tree bats, in particular, may misperceive turbines to be trees and are therefore attracted to the turbines to potentially seek roosting and mating opportunities. Another study hypothesized that bats may misperceive wind turbines to be water, as echolocating bats cannot differentiate between smooth turbine tower surfaces and water (McAlexander 2013). Another possible explanation is that wind turbines may actually provide bats with resources, such as water (as condensation on the tower) and roosting and foraging opportunities. To date, no published study has explored whether wind turbines could provide bats with water or roosting opportunities, but evidence from recent studies based on stomach content analyses (Valdez and Cryan 2013), acoustic monitoring (e.g., McAlexander 2013), and thermal imagery (e.g., McAlexander 2013, Cryan et al. 2014), indicate that bats are actively foraging near wind turbines.

For the foraging attraction hypothesis to be plausible, Cryan and Barclay (2009) outlined 6 predictions that, if confirmed, would demonstrate that bats are using wind turbines as a foraging resource. First, if bats are successfully foraging around wind turbines, then feeding buzzes, a series of echolocation calls produced when a bat is just about to capture its prey, should be detected near turbines. This prediction has been supported by at least 2 studies that have recorded feeding buzzes in the immediate vicinity of turbine towers (McAlexander 2013, Bennett and Hale, unpubl. data). The second prediction is that there should be an abundance of insect prey on and around wind turbines, thereby providing foraging opportunities for bats. This prediction has also been supported by studies that have shown that insects aggregate on the surfaces of the wind turbines (e.g., Cryan and Barclay 2009, Long et al. 2011). Long et al. (2011) found that insects are drawn to light-colored turbines in particular; and as turbines are commonly painted with light colors, bats may be attracted to wind farms as a result of insect aggregations on and around the turbine towers. The third prediction is that insects should be consistently present and active at wind turbines. A study by Cochran (2013) revealed an abundance and diversity of insects at wind turbine towers from April through October at a wind farm in the southern Great Plains (Bennett and Hale, unpubl. data). The fourth prediction is that bats found in fatality searches at wind turbines should have full stomachs if they were killed while foraging. This prediction has been supported by a morphological analysis of prey items collected from the full stomachs of hoary bat carcasses salvaged during wind farm carcass searches (Valdez and Cryan 2013). The fifth prediction is that the types of insects found in bat stomachs should vary with seasonal changes in insect communities at wind turbines. To our knowledge, no study has yet tested this prediction. Finally, the sixth prediction is that bat carcasses found in fatality searches at wind turbines should have insects in their mouths if the bats were actively foraging when killed; however, Valdez and Cryan (2013) have suggested that prey items are likely to get expelled when a bat has a fatal interaction with a turbine.

Despite the emerging evidence in support of the foraging attraction hypothesis, we still do not know if bats are consistently feeding on insects that are present on and around wind turbine towers. To address this need, we conducted a multifaceted study that utilized bat carcasses collected during fatality searches at a wind facility in the southern Great Plains. Our specific objectives were to: 1) determine if bats killed by wind turbines had full stomachs; 2) identify the insect species present in the bat stomachs using genetic sequencing; and 3) determine if the insect species in the bat stomachs were similar to the insects found on the wind turbines where fatality searches were conducted within the late summer season (July – August) (i.e., we tested predictions 4 and 5 outlined above). We did not investigate the seasonal variability of insects in bat stomachs and wind turbines, and instead tested prediction 5 by comparing the insects found in the bats' stomachs to the insects found at the turbines from data collected between July and August. Recent studies have shown that aerial-hawking bats, such as the eastern red and hoary, do not fly far from their foraging site to find a suitable roost to digest their food when their stomachs are full (Knight and Jones 2009, Lison et al. 2013, Montero and Gillam 2015). Thus, if the stomachs of bat carcasses were full, this would suggest that the bats were likely foraging in the vicinity of the wind turbines prior to death. If we can confirm that the insect species in the bat stomachs are found in the insect assemblage on the wind turbines, this finding would provide convincing support for the foraging attraction hypothesis. If we demonstrate that wind turbines provide foraging opportunities for bats, steps may then be taken to ascertain if there are practical and effective methods to deter bats from foraging in the immediate vicinity of wind turbines. Subsequently, the implementation of such methods may help reduce bat fatalities at wind turbines globally.

II. METHODS

Study site

We conducted our study in north-central Texas at Wolf Ridge Wind, LLC, a utility-scale wind farm owned and operated by NextEra Energy Resources (N 33° 44’ 01.19”, W 97° 24’ 57.26”; Fig. 1). The 48-km^2 wind resource area comprises 75 1.5-MW General Electric wind turbines (turbine specifications: 80-m tower, three 42-m blades attached to the front of the nacelle, 84-m diameter rotor swept zone that reaches 122-m above ground) sited in a matrix of cattle-grazed pastures, hayfields and some agricultural lands, and shrub-woodland habitat extending from the riverine valleys of the Red River escarpment. Wolf Ridge has been operational since October 2008, and more than 1,500 bats representing 6 species (hoary, eastern red, silver-haired, tri-colored (*Perimyotis subflavus*), evening (*Nycticeius humeralis*), and Mexican free-tailed (*Tadarida brasiliensis*) bats) have been found in carcass searches at Wolf Ridge since 2009.

Fatality monitoring surveys, with increased sampling effort during the fall migratory season of tree bats, were conducted at the site from 2009 to 2014 as part of the TCU-NextEra Wind Research Initiative. Insect surveys conducted at this site in 2012 (Cochran 2013) and 2013 (Bennett and Hale, unpubl. data) show that potential bat prey items are abundant and diverse at wind turbines.

We only included eastern red bat and hoary bat carcasses in this study because these species have the highest fatality rates at Wolf Ridge (Bennett and Hale 2014), and experience high levels of fatality at wind facilities across North America (e.g. Arnett and Baerwald 2013). To select carcasses for analysis, we prioritized those in best overall condition, including adult and juvenile males and females of both species, collected between July and August of 2013 and 2014. Thus, we dissected 47 eastern red bat and 24 hoary bat carcasses, removing their digestive systems (esophagus, stomach, and intestines) and storing the digestive systems with their contents intact in 70% ethanol.

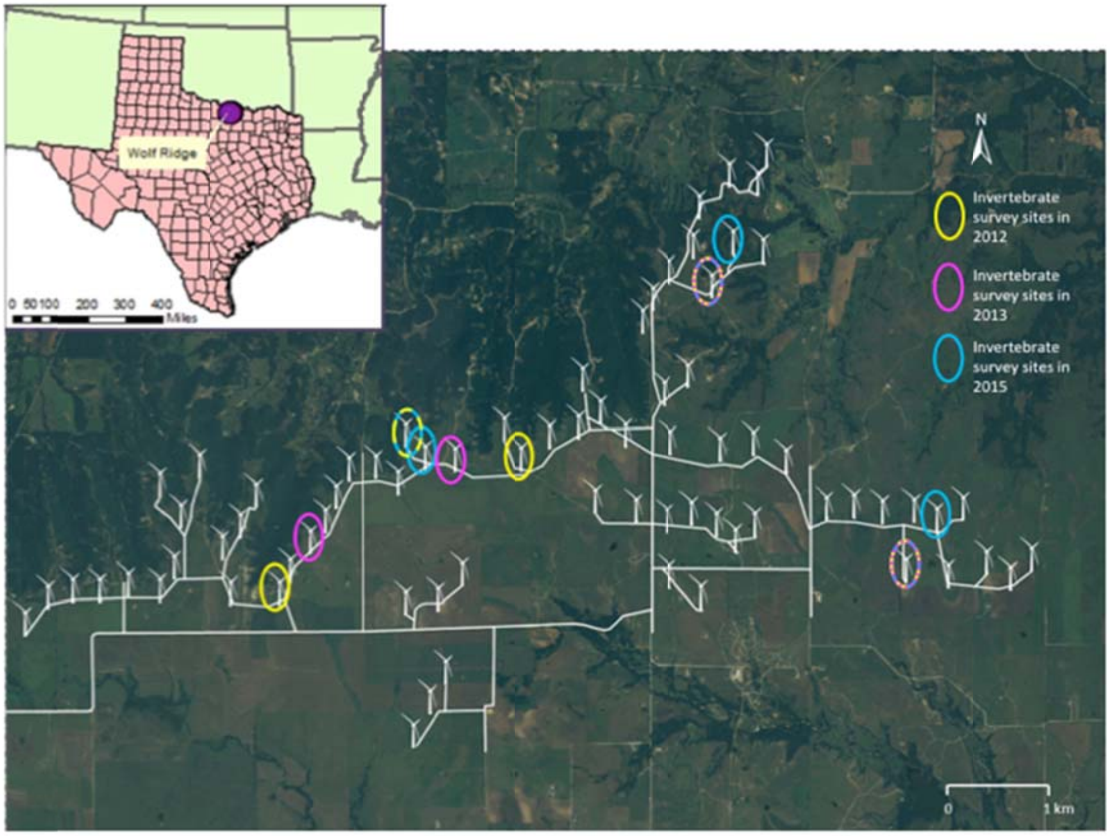

Figure 1. Wolf Ridge wind energy facility. Wolf Ridge is located in Cooke County in north-central Texas. Insects were surveyed at 5 turbines in 2012 (yellow), 4 turbines in 2013 (pink), and 6 turbines in 2015 (blue).

Assessment of bat stomach fullness

Before beginning the genetic analyses, we separated the bat stomachs from the esophagi and intestines and visually determined if the stomachs were full using the following definitions. A taut stomach with no extraneous or folded membrane was considered “full,” whereas a stomach with obvious extra space or folded membrane was considered “not full”. The stomach contents from each bat were then homogenized using a mortar and pestle and weighed. In some instances the stomach membrane was perforated and the contents had been exposed to ethanol, so we allowed the ethanol to evaporate for up to an hour in the extraction hood (described below) prior to homogenization and weighing. We conducted a two-sample t-test to determine if stomachs classified as “full” were significantly heavier than “not full” stomachs. We used these data to test Cryan and Barclay’s prediction that if bats are killed while foraging at wind farms, then they should have full stomachs at the time of death.

Genetic analysis of bat stomach contents

We extracted DNA from the homogenized stomach contents using DNeasy® mericon food kits (QIAGEN; Zarzoso-Lacoste et al. 2013). We included a negative control with each round of extraction (3 to 7 bat stomach samples) to ensure non-contamination of reagents. All extractions were completed in a dedicated extraction AirClean® 600 PCR workstation to minimize contamination.

Samples were then amplified using arthropod-specific primers ZBJ-ArtF1c and ZBJ-ArtR2c developed by Zeale et al. (2011). We set up the polymerase chain reactions (PCR) in a dedicated PCR AirClean® 600 PCR workstation in a different room from where the DNA extractions took place. Again, we included negative controls in our PCR reaction batches. PCR (10µL) contained 2 µl DNA, 0.5 µM of each primer, 1X Qiagen Multiplex PCR Master Mix with HotStarTaq, Multiplex PCR buffer with 3mM $MgCl_2$ pH

8.7, and dNTPs. Reactions were cycled in an ABI 2720 thermal cycler. PCR ran for one cycle at 95°C for 15 min, followed by 40 cycles of 30s at 94°C, 90s at 55°C, 90s at 72°C, and then a final extension at 72°C for 5 minutes. We purified the products on a gel, ligated them into pGEM-T vectors (Promega), and then transformed them into JM109 competent cells. We plated the transformed cells on ampicillin plates and left them in a 37°C incubator overnight. The following day we selected colonies that had been successfully transformed (i.e., those in which the PCR product had been inserted) based on color (white colonies had been transformed, whereas blue colonies had not).

From each stomach sample, we picked at least 9 colonies containing recombinant clones and then amplified each one directly using vector-specific primers (F: CGACTCACTATAGGGCGAATTG, R: CTCAAGCTATGCATCCAAGG). Unincorporated nucleotides and excess primers were removed from PCR products using *ExoI* and *rSAP* (New England Biolabs) according to manufacturer protocols. PCR products were then unidirectionally sequenced using the forward vector primers and ABI Big Dye Terminator Cycle Sequencing v3.1 Chemistry (Life Technologies). We electrophoresed sequences on an ABI 3130XL Genetic Analyzer (Life Technologies), edited and trimmed the sequences using Sequencher v5.0 (Gene Codes USA), and then aligned the sequences in MEGA 6.0 using Muscle (Edgar 2004, Tamura et al. 2013). We used the Barcode of Life Data System (BOLD), an online index of known DNA sequences, to identify sequences (Ratnasingham and Hebert 2007, www.boldsystems.org). We assigned species for a 99-100% matched sequence, we assigned genus for a 95-98% match, assigned family for a 90-94% match, and assigned order for an 85-90% match according to the methods in Clare et al. (2009) and Zeale et al. (2011).

We created neighbor-joining trees using the Kimura Two-Parameter distance in MEGA to determine the number of OTUs (operational taxonomic units) that were present in the samples for which there was less than a 99% match in BOLD. We created separate trees for each order and classified samples as belonging to different species if they were >2% different and clearly clustered separately from other known species identified in BOLD. We used letters to distinguish unidentified species from one another (e.g., Lepidoptera A and Lepidoptera B). To determine the minimum number of clones necessary to discover all of the insect species present in the bat stomachs, we created sampling curves using the number of clones successfully sequenced and the number of insects identified.

For the clones generated from any given bat stomach sample, each insect species detected was counted only one time. For example, if all 10 clones from a stomach were identified as Species 1, then that bat was recorded as having eaten a single species of prey. If however, 5 clones were identified as Species 1 and 5 clones were identified as Species 2 from a single stomach, then that bat was recorded as having eaten 2 species of prey.

For eastern red and hoary bats separately, we calculated the Simpson's diversity index to summarize the number and abundance of prey items within the stomach contents of each species.

Insect surveys

Insect surveys using light traps and malaise traps were conducted at Wolf Ridge in 2012 and 2013 (Cochran 2013, Hale and Bennett, unpubl. data), and 2015.

Light traps use a UV light that attracts nearby insects and typically captures a large variety of species, whereas malaise traps have no attractant and typically catch a more limited sample with fewer individuals and fewer species represented (Hosking 1979). We therefore used light trapping to provide a general characterization of the insect community at the wind turbines. In comparison, malaise trapping functioned as a passive control that would inform us if we missed species with the light traps that were present at Wolf Ridge.

In 2012, we had 2 sampling sessions per survey night that coincided with bat foraging periods: one 3-hour period between sunset and midnight and one 3-hour period between 3:00am and 7:00am (Arnett 2005). In 2013, we left traps out all night and improved our methods in order to avoid recapture and better estimate abundances of each order. In 2015, we only collected insects for three hours after sunset when bats were most likely to be foraging; we checked the light traps continuously throughout the 3-hour period and checked the malaise trap at the end of the 3-hour session. Because we left the traps out for different lengths of time and checked them at different intervals each year, this could introduce biases in the insect trend and abundance data. Instead of analyzing the differences between survey years, we used the results to characterize the insect community at Wolf Ridge during that time.

In 2015 we also tested the utility of an additional survey method, insecticide fogging of turbine towers, to see if the insect community sampled using this method differed from what we found with light traps and malaise traps. Because light trap surveys and malaise trapping are both conducted on the ground, we used insecticide fogging to attempt to capture insects farther up the turbine tower. Insecticide fogging has been used to survey invertebrates in the canopy of rainforests (Stork 1991, Kitching et al. 1993).

Light trapping – We conducted insect surveys at 2 turbines a night, 3 nights a week over a 6-week period in July and August 2015. Our survey included 3 pairs of turbines that were surveyed on a rotating basis (Fig. 1). We were able to light trap as long as wind speeds were <15 mph and there was no precipitation. Additionally, in accordance with Wolf Ridge safety protocol, we could not be on the wind farm if there was lightning within 50 miles of the site.

We assembled our light traps on the gravel pad surrounding the turbine tower (Fig. 2). Light traps consisted of a Feit Electric BPEFL15T/BLB 13-Watt compact fluorescent black bulb in a ceramic light fixture that attached to the top of a 5-gallon bucket. A 12V 35 Amp Hour car battery with a 200-Watt power inverter inside the bucket powered the light. The bucket was placed inside of a large opaque plastic tote on its side, facing the turbine. This tote had a portion of the top removed to allow the black light to shine up the turbine tower. The sides of the tote shielded the light in other directions to minimize attraction of insects from the surrounding area. Next to the light trap, we placed two transparent plastic totes with empty egg cartons on the bottoms that the insects attracted to the light used as a shelter. This set-up was placed on a white sheet on the gravel pad, which allowed us to see and collect insects more easily.

Light trapping surveys were conducted by 2 technicians for a 3-hour period beginning at dusk, which coincided with peak bat foraging activity in the evening (Baerwald and Barclay 2011). Technician 1 continuously collected all of the invertebrates on the sheet, turbine, totes, and bucket in small plastic containers of varying sizes. Technician 2 recorded and labeled the containers that held unique insect specimens (vouchers), which were then placed in a cooler. Technician 2 also tallied duplicates when a voucher had already been collected in order to have a record of the number of individuals collected during each survey period. These duplicates were kept in their containers and released after the survey period was finished. Technicians estimated counts of small invertebrates (< 2 mm in total length) visually at the end of the survey session and collected one voucher specimen each. We kept the voucher specimens in their containers and placed them in a -4 °C freezer at the end of the night. See below for processing methods.

Figure 2. a) Light trap placement relative to turbine tower, and **b)** representative light trap set-up at a turbine tower at Wolf Ridge in 2015.

Malaise traps – We assembled malaise traps on the ground next to the gravel pad ($\leq$ 5 m from the turbine) on the opposite site of the turbine from the light trap, where they would be shielded from the UV light (Fig. 3). Technicians set up malaise traps at dusk and left them up for the duration of the 3-hour light trapping survey (Preisser et al. 1998). Malaise traps passively collected flying insects in a fine net; these insects flew into the net and then fell into a tray of soapy water or flew or climbed up the net until they were funneled into a collection bottle of soapy water at the top of the trap (Fig. 3). We were able to employ malaise traps as long as wind speeds were <10 mph and there was no precipitation. Technicians checked the trays beneath the trap and the collection bottle, and transferred the contents of the tray and the bottle into a collecting jar at the end of the light trapping session each night. The following day, technicians sorted, identified to order, and identified new vouchers by comparing these samples to those already collected from previous insect surveys that year.

Figure 3. Representative malaise trap set-up at a turbine tower at Wolf Ridge in 2015.

Insecticide fogging – Prior to beginning a fogging survey, 4 technicians laid a modified parachute (radius ~9 m, area ~262 m^2) around the base of the turbine tower to collect insects paralyzed by the fog. One technician, in full personal protective equipment (PPE) consisting of a hazmat suit, respirator, and goggles, operated the Dyna-Fog BlackHawk Model 2620 insecticide fogger (with ULD BP-100 contact insecticide) and walked around the base of the turbine tower (staying upwind) directing fog up the turbine tower. A second person, also in full PPE, guided the technician carrying the fogger. The fogger vaporized a liquid insecticide and released fog that slowly rose up the turbine tower, paralyzing any insects, which then fell down onto the parachute (Fig.4). After the turbine had been circled once, the technicians turned the fogger off and waited approximately 20 minutes for the fog to clear and begin insect collection (Fig. 5). We collected invertebrates in plastic containers and then transferred them to kill jars (Stork 1991). Because the insecticidal fog can easily blow away, insect fogging could only be conducted on completely still nights (wind speed < 0.1 mph).

Figure 4. Fogging a wind turbine tower at Wolf Ridge in 2015.

Figure 5. Collecting insects after fogging a turbine tower at Wolf Ridge in 2015.

Insect processing - The next day, technicians used field guides to sort and identify insects to order. These were compared to vouchers collected from previous insect surveys in 2015 to identify previously unsampled insects. Vouchers were separated by species where possible; however, some species were not easily distinguished and were instead sorted to genus (e.g., *Gryllus rubens* and *Gryllus veletis*, 2 species of field crickets were classified as *Gryllus* spp.). Due to high numbers of insect species collected, most of our insect samples were only identified to order due to limited time and resources. Unique voucher specimens were photographed and given a unique identification number based on the turbine number, the date, trap type, and the order in which they were collected during the survey night (e.g., voucher 34T24LT04Jul15 was the thirty-fourth insect caught at turbine 24 during the light trapping survey on July 4, 2015). We preserved one example of each unique voucher specimen in glycine bags that were stored at -4°C (for Lepidoptera and Trichoptera) or 100% ethanol (for all other orders).

Comparing stomach contents to insect surveys

In order to test Cryan and Barclay's (2009) prediction that if bats are foraging at wind farms, then the insects in the bats' stomachs should also be present at wind turbines, we compared the insects present in the stomach contents of bats collected in July and August to insect surveys conducted in July and August.

First, we determined whether or not the species commonly found in bat stomachs were present at Wolf Ridge during insect surveys. In order to determine whether insect species consistently found in bat stomachs were at the wind farm, we compared all of the insect species identified in BOLD that were found in $\geq$5 stomach samples to the photographs and vouchers preserved during insect surveys in 2013 and 2015. If we found that bats were consistently eating insects that were not captured at turbines, it could indicate that bats were foraging elsewhere and not using the turbines as a foraging resource. Conversely, if the species that we consistently found in bat stomachs were present in insect surveys conducted at turbines at Wolf Ridge, this would provide support the foraging attraction hypothesis.

III. RESULTS

Assessment of bat stomach fullness

Of the 47 eastern red bats included in this study, 23 had full stomachs. The mean ± SD weight of full stomach contents was 0.138 ± 0.074 g (n = 23 stomachs), whereas not full stomach contents weighed 0.0416 ± 0.039 g (n = 22 stomachs; Fig. 6). Three stomachs were not weighed.

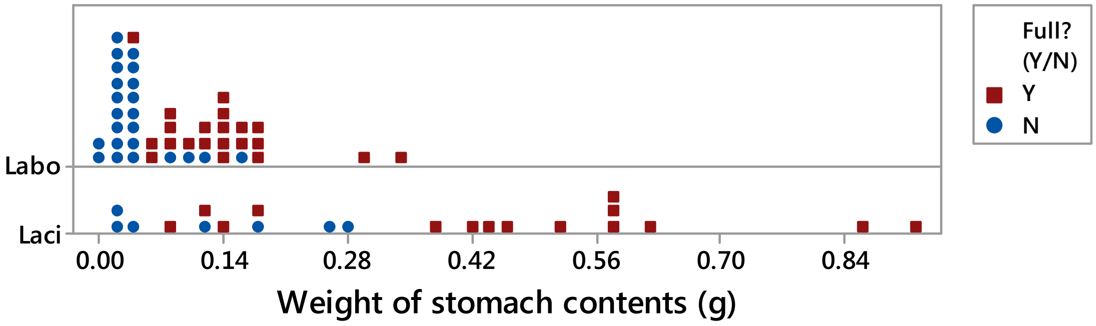

Figure 6. Weight of stomach contents of eastern red (Labo) and hoary (Laci) bats. Based on visual classification, stomachs were described as either “full” or “not full”.

Of the 24 hoary bats included in this study, 17 had full stomachs. The mean ± SD weight of full stomach contents was 0.458 ± 0.252 g (n = 17 stomachs), whereas not full stomach contents weighed 0.131 ± 0.114 g (n = 5 stomachs; Fig. 6). Two stomachs were not weighed.

Stomachs classified as “full” and “not full” had significantly different masses for both species of bats (eastern red bats: t = -5.48, df = 42, p-value < 0.001; hoary bats: t = -3.25, df = 20, p-value = 0.004). For both eastern red and hoary bats, the body masses of juveniles and adults were not significantly different (data not shown), so we did not separate our analysis by age group.

Genetic analysis of bat stomach contents

Insect DNA was successfully extracted and amplified from 69 of the 71 bat stomachs processed in this study. The average number of clones for the 69 bat stomach was 13.5 (range: 9-21 clones). The sampling curve peaked at 10 clones indicating that 10 clones was sufficient to detect all prey species within a single stomach sample (Fig. 7). We had ≥10 clones for 67 stomachs; in the remaining 2 stomachs, we were only able to sequence insect DNA from 9 clones.

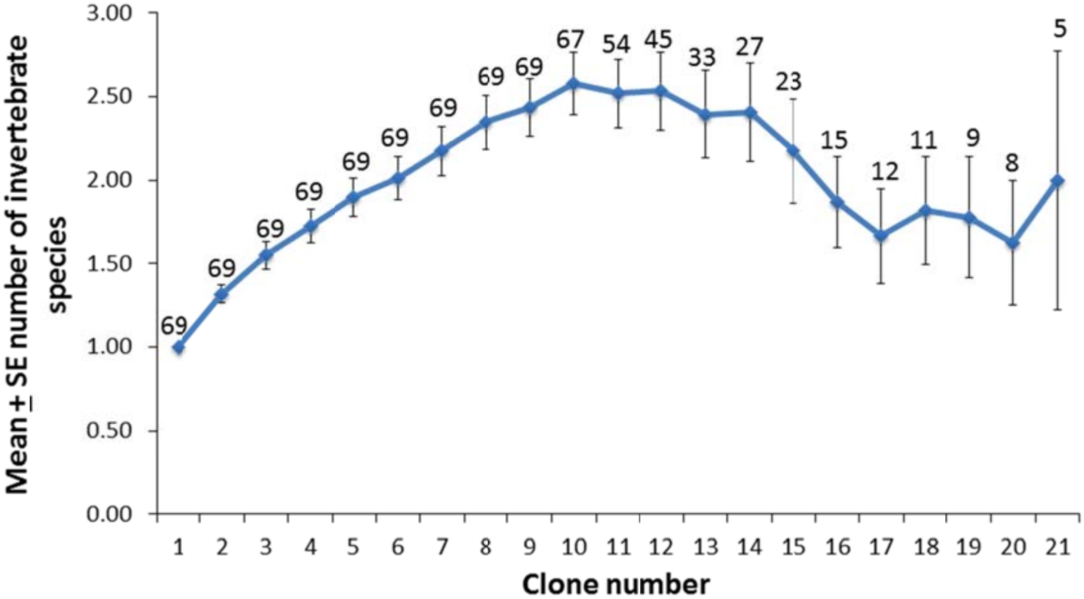

Figure 7. Sampling curve used to determine how many clones (+ SE) were needed to detect the insects in the bat stomach samples (n = 69 stomachs). The number above each data point indicates the number of stomach samples.

Collectively, the results of our stomach analysis yielded 192 insects representing 83 genetically distinct species. Based on the percentage match to known sequences in BOLD, 38 insects were identified to species, 24 were identified to genus, 7 were identified to family, and 14 were identified to order.

Individual bats in our study had a mean (± SE) of 2.8 + 0.19 prey species in their stomachs (range: 1-7 species). The majority of our samples consisted of only 1 or 2 species of insects (18 bats and 17 bats, respectively); however, 2 bats had the highest observed number of 7 species of insects in their stomachs (Fig. 8).

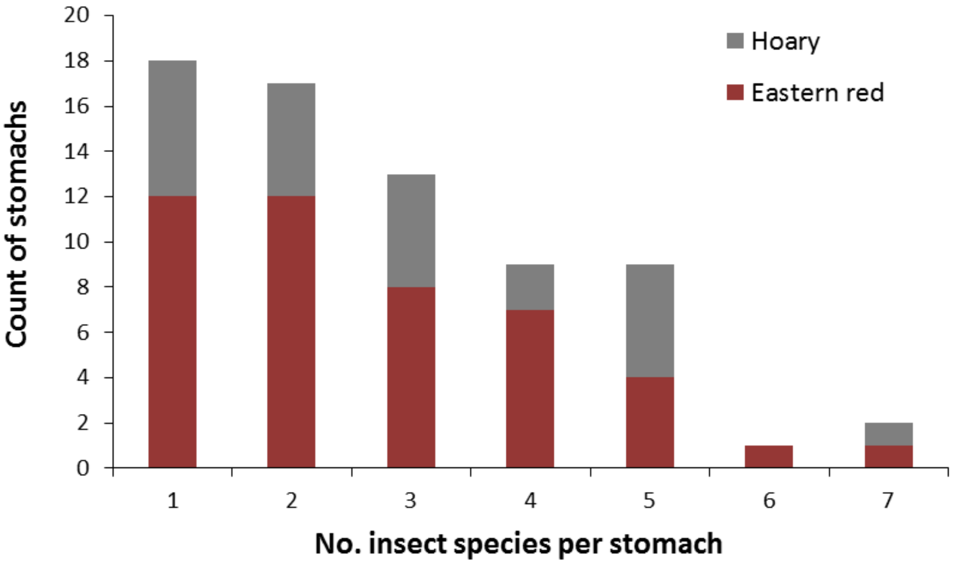

Figure 8. Number of different insects found in eastern red (n= 45) and hoary bat (n = 24) stomach samples.

We found that eastern red bats (n = 45) had a mean (± SE) of 2.7 + 0.22 individual prey species in their stomachs (range: 1-7 species). We found 59 different species of insects from 7 orders in eastern red bat stomachs (Fig. 9). Forty-six of these species had a detection frequency of 1 (Fig. 10a). We detected one species of moth (*Spodoptera frugiperda*) in 11 different stomachs, one species of cricket (*Gryllus rubens*) 29 times, and another species of cricket (*G. veletis*) 7 times (Table 1). Lepidoptera comprised 56.2% of the species identified in eastern red bat stomachs; Orthoptera comprised 32.2% of the insect species identified. The remainder belonged to Blattodea, Coleoptera, Hemiptera, and Neuroptera. In addition to the species mentioned above, the following species were detected in bats from both years: Parcoblatta A, *Achyra rantalis*, *Euchromius ocelleus*, *Bleptina caradrinalis*, and Lepidoptera L (Table 1).

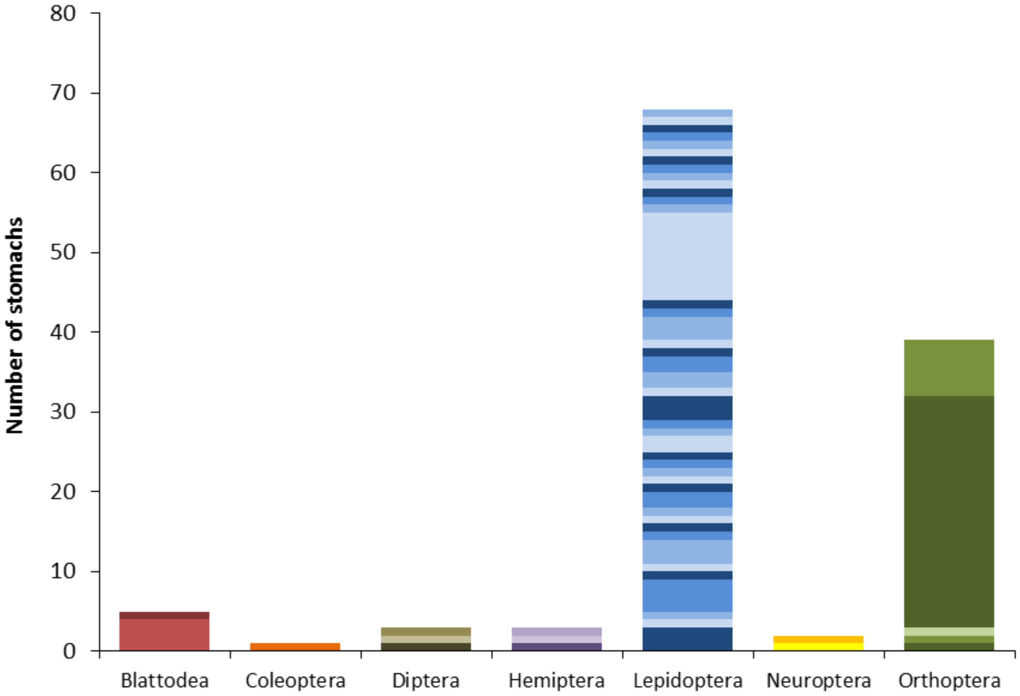

Figure 9. Insect orders found in eastern red bat stomach contents from fatality searches at Wolf Ridge in 2013 and 2014. Each band in the bar represents a different insect species.

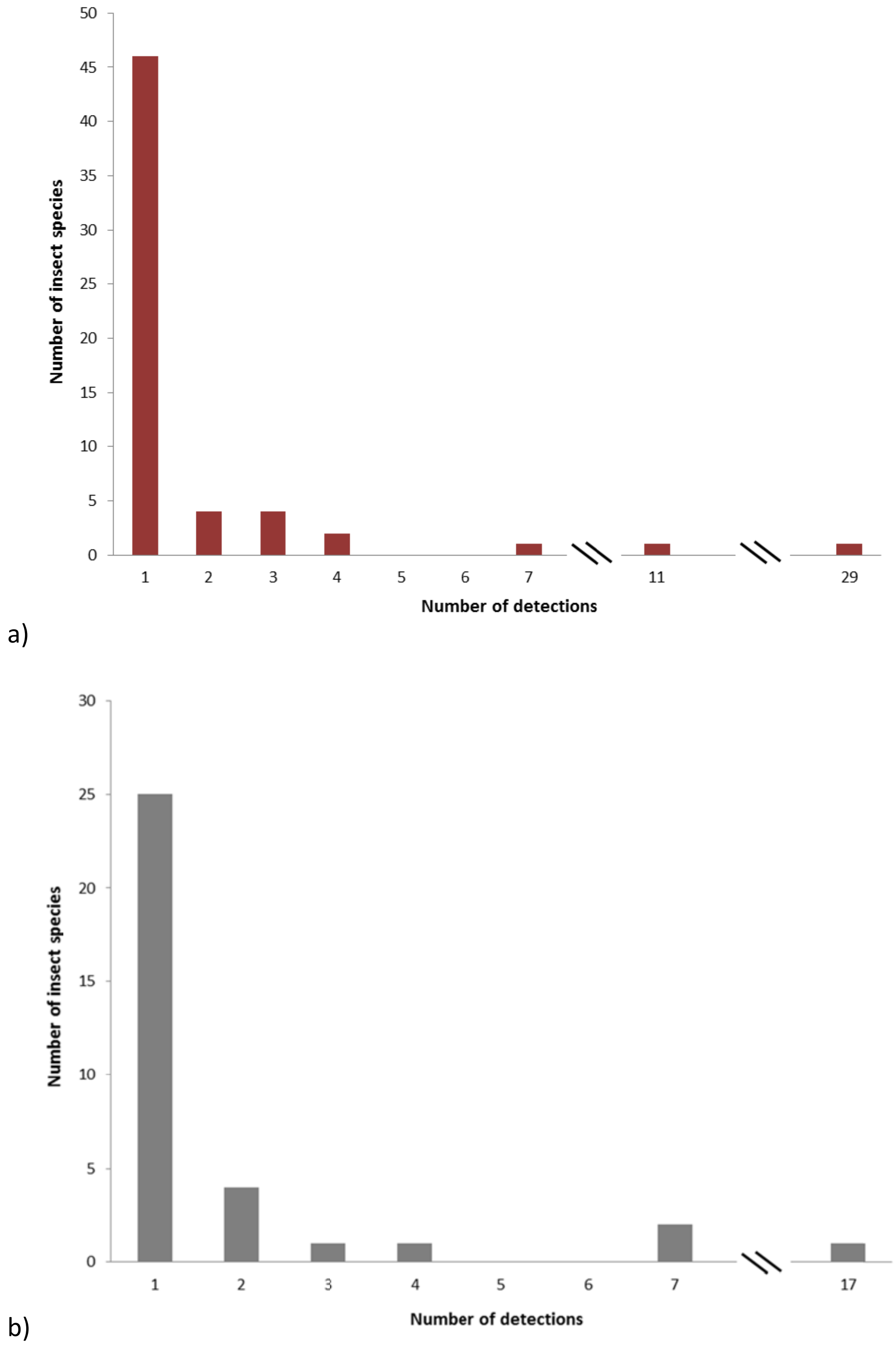

Figure 7. Detection frequency of each insect species in **a)** eastern red (n = 45) and **b)** hoary bat (n = 24) stomach samples.

Table 1. Insects identified in eastern red bat stomachs. Species identification is based on the percentage match in BOLD. Insects not identified to species in BOLD are differentiated by letters. Insects identified in eastern red bats collected in both 2013 and 2014 are indicated by (*).

Order	**Species**	**Number of stomachs**
Blattodea	*Parcoblatta A**	4
	Parcoblatta B	1
Coleoptera	*Typhaea haagi*	1
Diptera	*Drosophila A*	1
	Drosophila B	1
	Exechia A	1
Hemiptera	*Hemiptera A*	1
	Lygaeidae A	1
	Charagochilus A	1
Lepidoptera	*Achyra rantalis**	3
	Euchromius B	1
	Euchromius C	1
	*Euchromius ocelleus**	4
	Ostrinia penitalis	1
	Argyrostrotis anilis	1
	*Bleptina caradrinalis**	3
	Bleptina n. sp. 4	1
	Bulia deducta	1
	Caenurgia chloropha	1
	Idia A	1
	Idia suffusalis	2
	Melipotus jucunda	1
	Fascista A	1
	Digrammia pallidata	1
	Lepidoptera A	1
	Lepidoptera C	1
	Lepidoptera D	2
	Lepidoptera E	1
	Lepidoptera F	1
	Lepidoptera I	3
	Lepidoptera J	1
	Lepidoptera K	2
	*Lepidoptera L**	2
	Lepidoptera M	1

	Elaphria grata	1
	Helicoverpa zea	3
	Leucania A	1
	Peridroma saucia	1
	*Spodoptera frugiperda**	11
	Spodoptera A	1
	Tripudia quadrifera	1
	Clostera inclusa	1
	Nymphalidae A	1
	Homoeosoma electella	1
	Peoria tetradella	1
	Pyradlida A	1
	Tylochares A	1
	Saturniidae B	1
	Acrolophus texanella	1
	Cydia latiferreana	1
	Gretchena bolliana	1
	Pelochrista A	1
Neuroptera	*Myrmeleontidae A*	1
	Chrysoperla A	1
Orthoptera	*Syrbula admirabilis*	1
	Allonemobius A	1
	Allonemobius fasciatus	1
	*Gryllus rubens**	29
	*Gryllus veletis**	7

We found that hoary bats (n = 24) had a mean (± SE) of 3.0 ± 0.35 individual prey species in their stomachs (range: 1-7 species). We found 34 different species of invertebrates from 3 orders in hoary bat stomachs (Fig. 11). Twenty-five of these species had a detection frequency of one (Fig. 10b). Similar to our eastern red bat stomach analysis, we found that Lepidoptera was the most abundant and diverse order in the stomachs; the most frequently detected moth, *S. frugiperda*, was found in 7 stomachs (Table 2). Again crickets were the most frequently detected species with *G. rubens* in 17 stomachs and *G. veletis* in 7 stomachs. Lepidoptera comprised 60.6% of the species identified in hoary bat stomachs; Orthoptera comprised 33.8% of the insect species identified. The remainder consisted of four species of Coleoptera, each only detected once. In addition to the species mentioned above, only two other species of moths were detected in both years: *E. ocelleus* and *Helicoverpa zea* (Table 2).

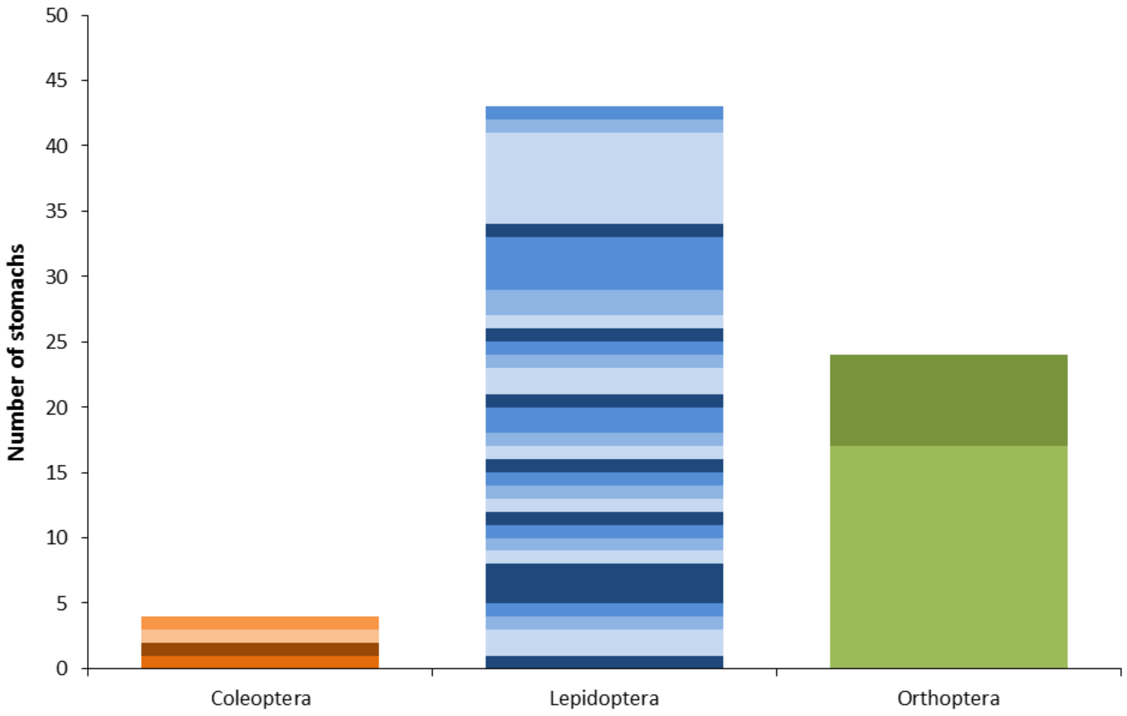

Figure 8. Insect orders found in hoary bat stomach contents. Different species in each order are indicated by different colors.

Table 2. Insects identified in hoary bat stomachs. Resolution of species identification is based on the percentage match in BOLD. Insects not identified to species are differentiated by letters. Insects identified in hoary bats collected in both 2013 and 2014 are indicated by (*).

Order	**Species**	**Number of stomachs**
Coleoptera	*Dytiscidae A*	1
	Hydrophilus triangularis	1
	Digitonthophagus gazelle	1
	Lobopoda A	1
Lepidoptera	*Euchromius A*	1
	*Euchromius ocelleus**	2
	Eudonia A	1
	Depressaria alienella	1
	Bleptina caradrinalis	3
	Caenurgina erechtea	1
	Grammia arge	1
	Melipotis jucunda	1
	Melipotis A	1
	Zale A	1
	Iridopsis A	1
	Iridopsis B	1
	Melanolophia BioLep02	1
	Lepidoptera B	1
	Lepidoptera G	1
	Lepidoptera H	2
	Lepidoptera I	1
	Lepidoptera L	2
	Condica sutorDHJ02	1
	Copitarsia n. sp. 1 RBS-2008	1
	Cucullia laetifica	1
	Galgula partita	1
	*Helicoverpa zea**	2
	Peridroma saucia	4
	Pseudaletia unipuncta	1
	*Spodoptera frugiperda**	7
	Xestia A	1
	Saturniidae A	1
Orthoptera	*Gryllus rubens**	17
	*Gryllus veletis**	7

We calculated the Simpson's index of diversity of eastern red and hoary bat stomach contents. Eastern red bat stomachs had a Simpson's diversity index of 0.930 and hoary bats had a Simpson's diversity index of 0.923, indicating that both species have similar diversity of insects in their stomach contents.

As we analyzed more bat stomachs, we continued to identify insect species that we had not previously found in our study (Fig. 12). Our discovery rate suggests that we would have continued to discover more species of insects with a larger sample size of bat stomachs, suggesting that our analysis may only reveal a fraction of the insect species that bats are eating in a night at our study site (Fig. 12).

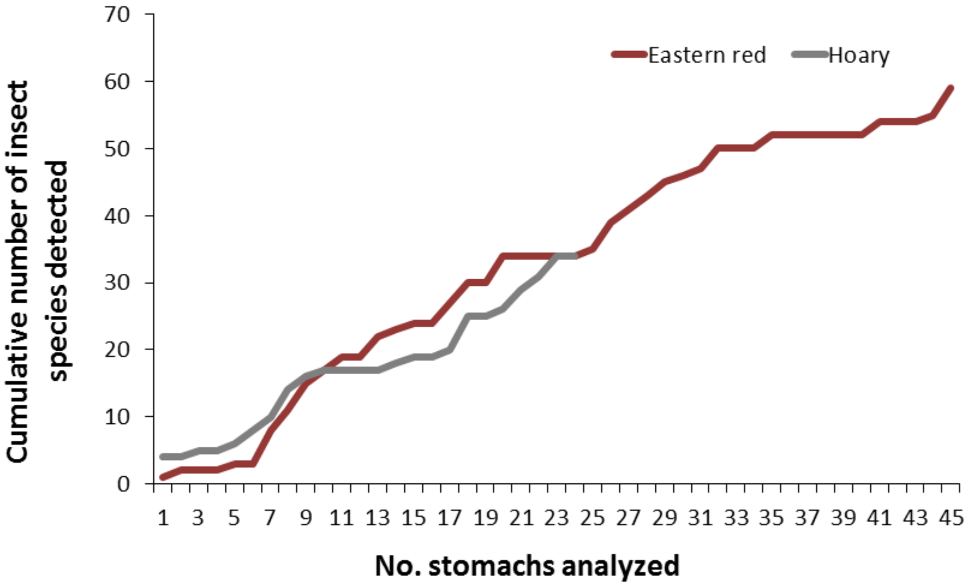

Figure 9. Discovery rate of new insect species by number of bat stomachs analyzed. As our stomach sample size increased, we continued to discover more species of insects.

Insect Surveys

We confirmed that the insect species caught in the malaise traps did not differ from the species collected with light trapping (data not shown), therefore we provide the light trapping results to represent the insect community at Wolf Ridge. We did not include the insects caught with the malaise traps in our analysis.

In 2012, we light trapped a total of 17 nights between July and August. A total of 1,238 invertebrates belonging to 9 orders were collected. The 3 most abundant orders were Coleoptera (37.2% of total), Orthoptera (23.7% of total), and Lepidoptera (20.0% of total). In 2013, we light trapped a total of 13 nights between July and August. A total of 1,937 invertebrates belonging to 11 orders were collected. The three most abundant orders were Lepidoptera (42.8% of total), Coleoptera (38.0% of total), and Hemiptera (9.1% of total). In 2015, we light trapped for 16 nights between July and August. We collected a total of 7,479 invertebrates belonging to 13 orders. The three most abundant orders were Coleoptera (54.9% of total), Lepidoptera (14.7% of total), and Hemiptera (13.2% of total).

Due to differences in survey methods among years, we could not statistically compare the 3 years of insect surveys to determine if the insect community at Wolf Ridge changed over time. However, an informal comparison based on the average biweekly proportions of each order suggests that the insect community has remained relatively consistent between July and August of 2012, 2013, and 2015 (Fig. 13). Note that the confidence intervals are high because there is nightly variation in insect abundance among survey periods. For example, on one night we might not catch any water beetles, whereas the next survey night might coincide with the emergence of water beetles. Additionally, some species of insects were only captured on a single survey night during the season.

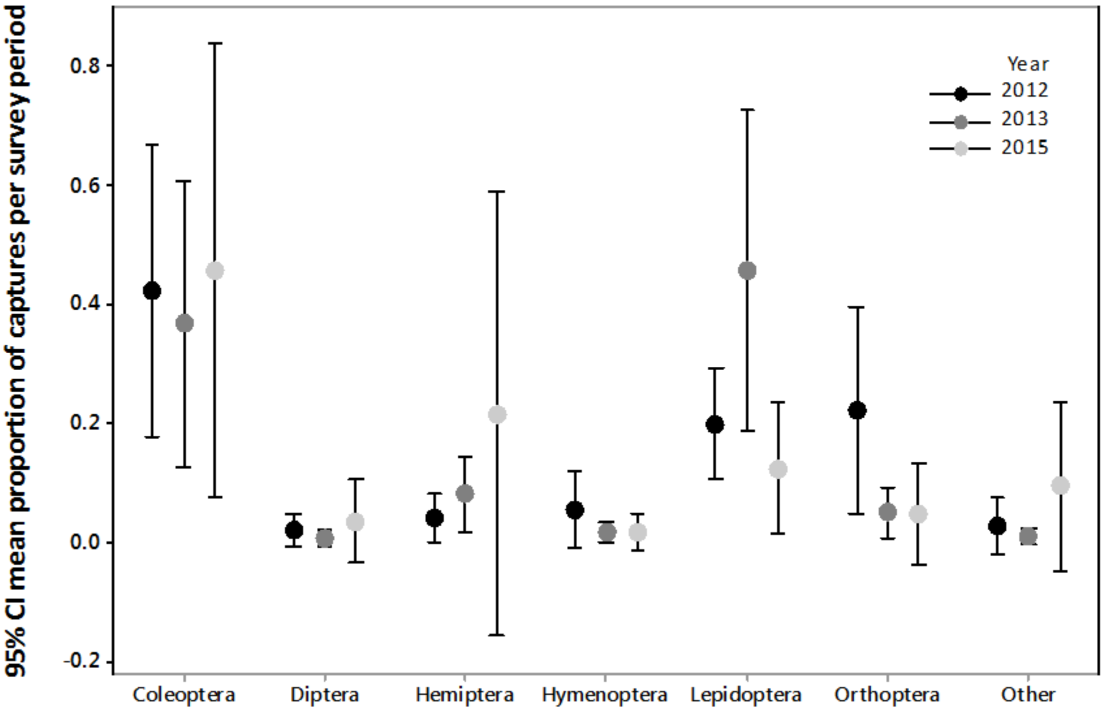

Figure 10. Biweekly averages and 95% CI of the proportions of each order collected during July-August insect trapping in 2012, 2013, and 2015.

Insecticide fogging was also used as a survey method in 2015; however, due to wind, fogging was only conducted on one night at two turbines (one turbine was fogged twice) for a total of 3 fogging sessions. The fogging survey resulted in a total of 35 grasshoppers (plus one parasitic nematode found next to its grasshopper host). The results from fogging were therefore not included in our analysis of the insect community due to the limited number of successful surveys.

Comparing stomach contents to insect surveys

To determine whether insects frequently found in bat stomachs were present at wind turbines, we compared the insect species detected most frequently in the stomach contents to the insect surveys. Because 2012 insect surveys did not incorporate species identification, we only compared our stomach content results to 2013 and 2015 insect surveys. We omitted any insects that were found in $\leq$5 stomachs since so many species from our genetic analysis were single-stomach detections. This left us with 5 species of moths (*E. ocelleus*, *B. caradrinalis*, *H. zea*, *Peridroma saucia*, and *S. frugiperda*) and 2 species of cricket (*Gryllus spp.*). We found these insect species in the stomachs of both eastern red and hoary bats (Table 3).

Of these commonly eaten insect species, we documented most at wind turbines in 2013 and all of them at wind turbines in 2015 (Table 3). Furthermore, most of these species were found at wind turbines on multiple nights throughout the survey period in both 2013 and 2015 (Table 3).

Table 3. Comparison of insect species commonly found in bat stomach contents to relative abundance in insect surveys at wind turbines. Crop pest information comes from Cole and Jackman (2011).

			Found in proportion of stomachs		Present at Wolf Ridge			
					2013		2015	
Common name	Scientific name	Crop pest	Eastern red (n = 45)	Hoary (n = 24)	Proportion of nights (n = 13)	Total count	Proportion of nights (n = 16)	Total count
Field cricket	*Gryllus spp.*	no	0.644	0.792	0.538	26	0.875	191
Fall armyworm moth	*Spodoptera frugiperda*	yes	0.244	0.291	0.538	60	0.625	68
Necklace Veneer moth	*Euchromius ocelleus*	no	0.089	0.083	0.231	14	0.188	25
Bent-winged Owlet moth	*Bleptina caradrinalis*	no	0.067	0.125	0.000	0	0.125	3
Corn earworm moth	*Helicoverpa zea*	yes	0.067	0.083	0.308	9	0.375	29
Pearly underwing moth	*Peridroma saucia*	yes	0.022	0.167	0.077	1	0.063	1

IV. DISCUSSION

Our study provides strong support for the hypothesis that bats are using wind turbines as a foraging resource. We demonstrated that bats killed at wind turbines had full or partially full stomachs, indicating that they were foraging just prior to their deaths (Valdez and Cryan 2013). We know that light-colored turbines attract aggregations of insects (Long et al. 2011), and that the orders of insects were present at turbines in relatively consistent proportions from one year to the next. We also demonstrated that the insect species eastern red and hoary bats consistently prey upon were consistently present at wind turbines.

The majority of the bat stomachs in our study were full, or partially full, indicating that the bats were killed while they were foraging. Stomach fullness is a good indicator of recent foraging, because insectivorous bats typically forage until they have consumed somewhere between one-quarter of their body weight to their full body weight in insects, after which they go to a nearby night roost to digest (Barclay et al. 1991, Kunz et al. 1995, Knight and Jones 2009, Ammerman et al. 2012, Gonsalves et al. 2013, Lison et al. 2013, Montero and Gillam 2015).

Our use of stomach contents allowed us to identify prey eaten more recently prior to their death and genetic analysis potentially allows for better prey identification, often to species level, compared to the morphological analysis methods used in previous investigations (Clare et al. 2009, Valdez and Cryan 2013). For both species of bats, we found between 1 and 7 species of insects in their stomachs, which provides further evidence that these bats were in the process of foraging when they were killed. This range of prey species is consistent with other studies (Clare et al. 2009, Whitaker et al. 2009). If bats consume up to their own body weight in insects per night, the results of our study (and other studies of bat stomach contents and feces) probably represent only a fraction of their nightly diets (Barclay et al. 1991). This could explain why so many of the insect species we detected were only detected once. We expect that if we had included more bat stomachs in our analysis, we would have continued to identify additional insect species.

Lepidoptera dominated the diets of both eastern red bats and hoary bats. Our results add to the body of research that shows moths make up a large part of the diet of insectivorous bats (e.g., Carter et al. 2003, Valdez and Cryan 2009, Clare et al. 2009, Reimer et al. 2010, Zeale et al. 2011, Valdez and Cryan 2013). Bats digest moths more efficiently than other types of prey, which could explain this abundance (Barclay et al. 1991). Despite the differences in the orders found in the stomach contents of eastern red and hoary bats at Wolf Ridge, the two species had similarly high Simpsons' indexes of diversity. This shows that both species eat a wide range of prey.

Eastern red bats consume primarily Lepidoptera, but eat insects belonging to many different orders. Our results are consistent with other studies that used morphological analysis of fecal samples to study eastern red bat diets (e.g., Brack and Finni 1987, Clare et al. 2009, Feldhamer 2009). We also found high numbers of *Gryllus spp.* crickets in their stomachs (see below). Other studies conducted in the eastern United States found larger proportions of Coleoptera in eastern red bat diets, most likely due to regional differences in insect communities (Carter et al. 2003, Carter et al. 2004, Whitaker Jr. et al. 2009, Ammerman et al. 2012).

We found only 3 orders of insects in the hoary bat stomach contents, which primarily consisted of moths and *Gryllus spp*. Other studies found hoary bats consumed these 3 orders in addition to others (e.g., Rolseth et al. 1994, Reimer et al. 2010, Valdez and Cryan 2013). These findings are consistent with the results of the hoary bat fecal analysis conducted in Texas by Valdez and Cryan (2013); they found evidence of Coleoptera in fecal pellets, but Lepidoptera and Orthoptera comprised a larger percentage of the volume of the fecal pellets and had higher detection frequencies overall. While beetles appeared to be consistently abundant at Wolf Ridge, they did not make up a large part of the diet of either species of bat.

We found Lepidoptera and Coleoptera in consistently high proportions at a rotating set of turbines at Wolf Ridge in July and August over 3 years (2012, 2013, and 2015), which suggests food resources for bats were consistently available. Overall, the patterns of abundance in the 3 survey years remained consistent, despite differences in survey methods. The proportions of Lepidoptera in 2013 and the proportions of Hemiptera in 2015 had much wider confidence intervals than those orders in other years, which could be due to survey methods or differences in other variables that contribute to insect emergence patterns or abundances (e.g., moonlight and weather). While Orthoptera were not as abundant as Lepidoptera or Coleoptera, we consistently caught *Gryllus spp* each year.

Six species of insects met our criteria for consistent detection in bat stomachs ($\geq$5 bat stomachs) and we documented all 6 in our insect surveys at the turbines at Wolf Ridge, which provides further support for the foraging attraction hypothesis. We found 5 of the 6 species in both 2013 and 2015, and all species except for one were detected on multiple survey nights throughout the survey seasons. While the results from the bat stomach contents overlapped with the insect surveys we conducted, our findings most likely underestimate both how often bats eat these insects and the extent to which these insects associate with turbines. Due to time and cost constraints, we limited our study to 69 bat samples and insect surveys at a subset of turbines. We expect that if we had included more bats, more turbines, and more trapping nights, we would have observed these 6 species more often. Additionally, if we had conducted insect surveys in 2014 and bat carcass searches in 2015, we expect we would have strengthened these associations in addition to discovering more species that met our criteria for frequent detection in bat stomachs.

We consistently found field crickets, *Gryllus spp*., in the stomachs of both eastern red and hoary bats. Ours was not the first study to document *Gryllidae* crickets in bat diets in Texas (Valdez and Cryan 2013). Several explanations have been posed about how and why bats eat crickets. Field crickets have been observed to be attracted to light, and may concentrate at the white turbine towers that are often illuminated by the moon (Tinkham 1938, Long et al. 2011, Thomson 2012). Additionally, bats may be able to hear crickets chirp, making them easy prey to target. Eastern red and hoary bats are aerial insectivores, meaning they eat on the wing, but several studied have suggested that they may glean crickets from surfaces such as canyon walls (Easterla and Whitaker 1972) and turbine towers (Valdez and Cryan 2013). Crickets are primarily terrestrial, but within populations some crickets possess a longer-wing mutation that makes them better flyers; perhaps the crickets found in our diet analysis possess this mutation (Olvido et al. 2003, Valdez and Cryan 2013).

We consistently found 3 species of crop pests in the stomachs of the bats in this study. This result underscores the important pest-management role insectivorous bats play in the ecosystem and in the agriculture industry (e.g., Boyles et al. 2011). The most common moth species we found in the bat stomachs, *S. frugiperda*, or the fall armyworm moth, migrates from South Texas and Mexico to North Texas (Knutson 2008, Westbrook 2008). This species is a crop pest, primarily on bermudagrass, wheat, and rye grass, but attacks other crops as well and is most abundant in Texas from August through November (Knutson 2008). In addition to *S. frugiperda*, we consistently found other crop pests in bat stomachs including the corn earworm moth (*H. zea*), and the pearly underwing moth, or variegated cutworm, (*P. saucia*; Cole and Jackman 2011).

Pre-construction bat activity surveys at wind facilities could drastically underestimate bat fatality if, once the turbines are constructed, they attract insects which in turn attract bats. While we conclude that bats are using wind turbines as a foraging resource, bats may be coming into contact with wind turbines for a variety of reasons (Cryan and Barclay 2009). We recommend that, rather than trying to come up with a solution that will deter insects from aggregating at turbines, further research should be devoted to researching and testing technologies to deter bats from coming into contact with wind turbines.

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ABSTRACT

ARE TREE BATS FORAGING AT WIND TURBINES IN THE SOUTHERN GREAT PLAINS?

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Although the ultimate causes of high tree bat fatalities at wind farms are not well understood, several lines of evidence suggest that bats are attracted to wind turbines. One such hypothesis is that bats could be attracted to turbines as a foraging resource if insects that bats prey upon are commonly found on and around turbines. To investigate the foraging attraction hypothesis, we conducted a series of surveys at a wind farm in north-central Texas from 2012-2015 to determine if eastern red (*Lasiurus borealis*) and hoary (*Lasiurus cinereus*) bats forage on insects near wind turbines. First, we conducted light trapping surveys to characterize the insect community. Second, we assessed bat diets using DNA barcoding of the stomach contents of 45 eastern red and 24 hoary bat carcasses collected in fatality searches. Third, we compared the turbine insect community to the diet analysis results. The insects present at wind turbines were similar to what we found in the bat stomach contents, and those same insects were abundant at turbines throughout the survey period. Together these results provide support for the foraging attraction hypothesis.