Increasing evidence that bats actively forage at wind turbines

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Although the ultimate causes of high bat fatalities at wind farms are not well understood, several lines of evidence suggest that bats are attracted to wind turbines. One hypothesis is that bats would be attracted to turbines as a foraging resource if the insects that bats prey upon are commonly present on and around the turbine towers. To investigate the role that foraging activity may play in bat fatalities, we conducted a series of surveys at a wind farm in the southern Great Plains of the U.S. from 2011-2016. From acoustic monitoring we recorded foraging activity, including feeding buzzes indicative of prey capture, in the immediate vicinity of turbine towers from all 6 bat species known to be present at this site. From insect surveys we found Lepidoptera, Coleoptera, and Orthoptera in consistently high proportions over several years suggesting that food resources for bats were consistently available at wind turbines. We used DNA barcoding techniques to assess bat diet composition of 1) stomach contents from 47 eastern red bat (*Lasiurus borealis*) and 24 hoary bat (*Lasiurus cinereus*) carcasses collected in fatality searches, and 2) fecal pellets from 23 eastern red bats that were found on turbine towers, transformers, and tower doors at the site. We found that the majority of the eastern red bat and hoary bat stomachs, the 2 bat species most commonly found in fatality searches at this site, were full or partially full, indicating that the bats were likely killed while foraging. Although Lepidoptera and Orthoptera dominated the diets of these 2 bat species, both consumed a range of prey items with individual bats having from 1 to 6 insect species in their stomachs at the time of death. The prey items identified from eastern red bat fecal pellets showed similar results. A comparison of the turbine insect community to the diet analysis results revealed that the most abundant insects at wind turbines, including terrestrial insects such as crickets and several important crop pests, were also commonly eaten by eastern red and hoary bats. Collectively these findings indicate that bats are actively foraging around wind turbines and that measures to minimize bat fatalities should be broadly implemented at wind facilities.

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14 **Abstract**

15 Although the ultimate causes of high bat fatalities at wind farms are not well understood, several

16 lines of evidence suggest that bats are attracted to wind turbines. One hypothesis is that bats

17 would be attracted to turbines as a foraging resource if the insects that bats prey upon are

18 commonly present on and around the turbine towers. To investigate the role that foraging activity

19 may play in bat fatalities, we conducted a series of surveys at a wind farm in the southern Great

20 Plains of the U.S. from 2011-2016. From acoustic monitoring we recorded foraging activity,

21 including feeding buzzes indicative of prey capture, in the immediate vicinity of turbine towers

22 from all 6 bat species known to be present at this site. From insect surveys we found

23 Lepidoptera, Coleoptera, and Orthoptera in consistently high proportions over several years

24 suggesting that food resources for bats were consistently available at wind turbines. We used

25 DNA barcoding techniques to assess bat diet composition of 1) stomach contents from 47 eastern

26 red bat (*Lasiurus borealis*) and 24 hoary bat (*Lasiurus cinereus*) carcasses collected in fatality

27 searches, and 2) fecal pellets from 23 eastern red bats that were found on turbine towers,

28 transformers, and tower doors at the site. We found that the majority of the eastern red bat and

29 hoary bat stomachs, the 2 bat species most commonly found in fatality searches at this site, were

30 full or partially full, indicating that the bats were likely killed while foraging. Although

31 Lepidoptera and Orthoptera dominated the diets of these 2 bat species, both consumed a range of

32 prey items with individual bats having from 1 to 6 insect species in their stomachs at the time of

33 death. The prey items identified from eastern red bat fecal pellets showed similar results. A

34 comparison of the turbine insect community to the diet analysis results revealed that the most

35 abundant insects at wind turbines, including terrestrial insects such as crickets and several

36 important crop pests, were also commonly eaten by eastern red and hoary bats. Collectively these

37   findings indicate that bats are actively foraging around wind turbines and that measures to

38   minimize bat fatalities should be broadly implemented at wind facilities.

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Introduction

40

41 Unlike conventional sources of energy such as oil, gas, and coal, utility-scale wind farms require

42 no fuel, do not consume water, and produce no greenhouse gas emissions or other pollutants

43 during the energy production phase. In 2013, wind power supplied 4.5% of the electrical energy

44 consumed in the United States (U.S.) and the U.S. Department of Energy's goal is to increase

45 this percentage to at least 20% by 2030, providing substantial environmental and economic

46 benefits from a sustainable, domestic energy source (USDOE 2015). Despite these recognized

47 benefits, wind energy development has drawbacks; for example, annual wind-related bat fatality

48 is estimated in the hundreds of thousands of bats (Cryan, 2011; Arnett & Baerwald, 2013;

49 Smallwood, 2013) with projected increases as wind energy development continues (e.g.,

50 Zimmerling & Francis, 2016). Consequently, wildlife conservation has become an important

51 consideration in the expansion of wind power.

52   Migratory tree bats, particularly lasiurine species, have the highest mortality rates at wind

53 facilities in North America, which peak from midsummer to early fall and coincide with the bats'

54 seasonal migration (Kunz et al., 2007; Arnett et al., 2008; Arnett & Baerwald, 2013; Hein &

55 Schirmacher, 2016). More than 75% of wind-related bat fatalities are comprised of three species:

56 hoary (*Lasiurus cinereus*), eastern red (*Lasiurus borealis*), and silver-haired (*Lasionycteris*

57 *noctivagans*) bats. We know little about the migratory behavior or the population status of these

58 tree bats, but there is increasing concern that high fatality rates at wind turbines could have long-

59 term effects on bat populations (Kunz et al., 2007; Arnett et al., 2008; Cryan & Barclay, 2009;

60 Arnett & Baerwald, 2013; Jameson & Willis, 2014; Frick et al., 2017).

61   Although the proximate causes of bat fatality at wind turbines are relatively well

62 understood (i.e., bats may die from barotrauma (Baerwald et al., 2008; Grodsky et al., 2011; but

63   see Rollins et al., 2012), collision with the rotating blades (Horn, Arnett & Kunz, 2008), or a

64   combination of the two, the ultimate causes are still unclear (Kunz et al., 2007; Arnett et al.,

65   2008; Cryan & Barclay, 2009). Nevertheless several lines of evidence suggest that bats may be

66   attracted to wind turbines and a number of specific hypotheses have been proposed to explain

67   this phenomenon. One possibility is that bats find something about the turbines themselves to be

68   interesting (Cryan & Barclay, 2009). For example, red aviation lights on top of turbine towers

69   have been considered to be a potential source of interest to bats; however, studies have shown

70   that mortality at towers with aviation lights is similar to or even less than mortality at towers

71   without aviation lights (Arnett et al., 2008; Baerwald, 2008; Bennett & Hale, 2014).

72   Alternatively, bats may misperceive wind turbines to be a resource. For example, a study by

73   Cryan et al. (2014) suggested that tree bats, in particular, may misperceive turbines to be trees

74   and are therefore attracted to the turbines to seek roosting and mating opportunities. Another

75   study hypothesized that bats may misperceive wind turbine towers as water (McAlexander

76   2013), as previous research has shown that echolocating bats misidentify artificial smooth

77   surfaces to be water (Greif & Siemers, 2010; Russo, Cistrone & Jones, 2012). Another possible

78   explanation is that wind turbines may actually provide bats with resources, such as water (as

79   condensation on the tower), roosting, and foraging opportunities. To date, no published study has

80   demonstrated that wind turbines provide bats with water or roosting opportunities, but evidence

81   from recent studies based on stomach content analyses (e.g., Valdez & Cryan, 2013; Rydell et

82   al., 2016), acoustic monitoring (e.g., McAlexander, 2013), and thermal imagery (e.g., Horn,

83   Arnett & Kunz, 2008; McAlexander, 2013; Cryan et al., 2014), indicate that bats are actively

84   foraging near wind turbines. It is also important to recognize that these various attractors are not

85   mutually exclusive and their relative importance likely varies by species, time of year, and

86   geographic location.

87   Despite the emerging evidence in support of the foraging attraction hypothesis, we still

88   do not know the extent to which bats are feeding on insects that are present on and around wind

89   turbine towers. To address this need, we conducted a multifaceted study to investigate the

90   foraging attraction hypothesis at a wind facility in the southern Great Plains, U.S. This region has

91   some of the highest installed wind capacity in the continental U.S. (Wiser & Bolinger, 2016),

92   and yet remains relatively understudied with respect to a wide range of wind-wildlife issues.

93   Long, Flint & Lepper (2011) found that insects are drawn to light-colored turbines in particular;

94   and as turbines are commonly painted with light colors, bats may be attracted to wind farms as a

95   result of insect aggregations on and around the turbine towers. Herein, we examine multiple lines

96   of evidence with respect to bats foraging at wind turbines, specifically addressing several

97   predictions of the "attraction to insect aggregations" hypothesis as outlined by Cryan & Barclay

98   (2009). First, we used acoustic monitoring to determine if bats were successfully foraging near

99   turbine towers, as evidenced by feeding buzzes (listed as the first prediction in Cryan & Barclay,

100   2009). Second, we used insect surveys to ascertain if there were foraging opportunities for bats

101   near turbine towers (listed as the third prediction in Cryan & Barclay, 2009). Third, we utilized

102   bat carcasses collected during fatality searches to determine if the bats had full stomachs at the

103   time of death (listed as the fourth prediction in Cryan & Barclay, 2009) and to identify (using

104   DNA barcoding) what the bats had been eating prior to being killed. Fourth, we utilized bat fecal

105   pellets collected during turbine searches as a second source of material to identify (using DNA

106   barcoding) what the bats had been eating. And fifth, we compared insects found in the bats'

107   stomachs and fecal pellets to the insects found at the turbines (listed as the fifth prediction, Cryan

108 & Barclay, 2009). Recent studies have shown that when aerial-hawking bats, such as the eastern

109 red and hoary, have full stomachs, they do not fly far from their foraging sites to find a suitable

110 roost to digest their food (Knight & Jones, 2009; Lison, Palazon & Calvo, 2013; Montero &

111 Gillam, 2015). Thus, if the stomachs of bat carcasses were full and the prey species were also

112 found near turbine towers, this would suggest that the bats were likely foraging in the vicinity of

113 the wind turbines prior to death.

114

115 **Materials & Methods**

116 **Study site**

117 We conducted our study at Wolf Ridge Wind, LLC (hereafter Wolf Ridge), a utility-scale wind

118 farm owned and operated by NextEra Energy Resources in the southern Great Plains of the U.S.

119 (N 33° 44' 01.19", W 97° 24' 57.26"). The 48-km^2 wind resource area comprises 75 1.5-MW

120 General Electric wind turbines (GE 1.5xle specifications: 80-m tower, three 42-m blades

121 attached to the front of the nacelle, 84-m diameter rotor swept zone that reaches 122-m above

122 ground) situated in a matrix of cattle-grazed pastures, hayfields, and some agricultural lands,

123 with shrub-woodland habitat extending from the riverine valleys of the Red River escarpment to

124 the north. Wolf Ridge has been operational since October 2008, and based on fatality monitoring

125 surveys and acoustic surveys conducted from 2009 to 2014, six bat species are known to be

126 present at this site: eastern red bat, hoary bat, silver-haired bat, tri-colored bat (*Perimyotis*

127 *subflavus*), evening bat (*Nycticeius humeralis*), and Mexican free-tailed bat (*Tadarida*

128 *brasiliensis*) (Bennett & Hale, 2014).

129 **Acoustic monitoring**

130   We conducted acoustic surveys at wind turbines to determine which bat species were in close

131   proximity to wind turbine tower surfaces and to characterize bat activity at wind turbines. Over a

132   seven year period in conjunction with other surveys (e.g., fatality searches or thermal camera

133   studies), we recorded bat activity using two different acoustic monitoring systems in close

134   proximity to wind turbine tower surfaces. Between 28 July 2010 and 22 July 2011, we deployed

135   two ReBAT™ acoustic monitoring systems ~85 m above ground on the rear of the nacelle of

136   two operational wind turbines. Each ReBAT™ system comprised two acoustic detectors, one

137   facing upward from the nacelle into the upper half of the rotor swept zone (RSZ) and the other

138   facing downward into the lower half of the RSZ. From 14 May to 1 October 2012, 5 April to 24

139   October 2013, 9 July to 28 September 2015, and 1 July to 10 August 2016, we used Binary

140   Acoustic Technology (BAT, Tucson, AZ) AR125-EXT ultrasonic receivers and BAT FR125

141   recorders mounted to tripods at the base of the wind turbines to record bat activity. We

142   positioned this latter acoustic detector assembly on the gravel pad approximately 2 m from the

143   turbine base with the AR125 receiver pointing 45° toward the tower surface to record bat activity

144   within close proximity of the wind turbine tower surface.

145   Both types of acoustic detector assemblies recorded sound files as a standard .wav file.

146   Furthermore, from 2010 to 2012 detectors were set up to begin recording from dusk until dawn,

147   whereas in 2013, 2015, and 2016 detectors recorded data starting at dusk and continued

148   recording for three hours, a time interval considered to be a primary foraging activity period of

149   bats (Baerwald & Barclay, 2011).

150   We used SonoBat bat call analysis software (version 3.04) to analyze the recorded sound

151   files. Each file containing a bat call was counted as one bat pass (Miller, 2001). Using full-

152   spectrum spectrograms generated in Sonobat we manually determined each call to species and

153   activity (where possible) using available call libraries. For the latter, we identified the following

154   four distinct activities: *commuting* – consecutive calls (i.e., individual chirps) were synchronized

155   with wing beats (Altringham, 2011) and were either constant, steadily decreasing, or steadily

156   increasing in call strength (in addition any sound file with <2 calls was also categorized as

157   commuting; e.g., a bat moving through the area from one foraging site to another); *searching* –

158   consecutive calls were synchronized with wing beats, but varied in strength due to the bat turning

159   its head from side to side while echolocating (Altringham, 2011); *foraging or approach phase*–

160   call interval varied with multiple calls occurring in succession within a single wing beat, and call

161   strength was constant, steadily decreasing, or steadily increasing (Altringham, 2011); and *feeding*

162   *or terminal buzz* – interval between successive calls decreased rapidly and the frequency of these

163   calls was higher or lower (depending on species) than calls representing the other three activities

164   (Altringham, 2011). The latter two acoustic activities are generally associated with foraging

165   behavior, as the *foraging or approach phase* of echolocation calls is an indication that bats are in

166   pursuit of prey, and *feeding or terminal buzzes* suggest that bats are successfully capturing prey.

167   **Insect surveys**

168   We used light traps and malaise traps to survey insects at three pairs of turbines at Wolf Ridge in

169   2012 (Cochran, 2013), 2013, and 2015. Light traps use a UV light that attracts nearby insects and

170   typically captures a large variety of species, whereas malaise traps have no attractant and

171   typically catch a more limited sample with fewer individuals and fewer species represented

172   (Hosking, 1979). Light trapping therefore provided us with a general characterization of the

173   insect community at the wind turbines, whereas malaise trapping functioned as a passive control

174   that would inform us if we were missing species with the light traps that were otherwise present

175   at the study site.

176   We conducted insect surveys at two turbines a night, three nights a week, over a six week

177   period in July and August during each year. We selected this time period as it coincides with

178   peak bat fatality at our site (Bennett & Hale, 2014). We were able to light trap as long as wind

179   speeds were <15 mph and there was no precipitation. We were able to employ malaise traps as

180   long as wind speeds were <10 mph and there was no precipitation.

181   In 2012, surveys took place in two 3-hour periods, the first beginning at dusk and the

182   second beginning 3 hours before dawn, as these times have been shown to coincide with peak bat

183   foraging activity (Baerwald & Barclay, 2011). In 2013, surveys began at dusk and ran

184   continuously through the night until dawn. While in 2015, we streamlined the survey method and

185   only sampled insects for a 3-hour period beginning at dusk.

186   We assembled our light traps on the gravel pad surrounding the turbine tower. Light traps

187   consisted of a fluorescent black bulb shielded by opaque plastic on three sides: the side facing

188   the turbine and the top were not shielded, illuminating the turbine tower. The light trap was

189   placed on a white sheet on the gravel pad, which allowed us to see and collect insects more

190   easily. We assembled malaise traps on the ground next to the gravel pad (≤5 m from the turbine)

191   on the opposite site of the turbine from the light trap, where they would be shielded from the UV

192   light. Because we left the traps out for different lengths of time and checked them at different

193   time intervals each year, we cannot directly compare insect abundance and diversity among

194   years. Instead, we use the results of the insect surveys to characterize the insect community at

195   wind turbines at the time of year when bat fatality rates are the highest.

196   We collected and tallied each insect during the survey period by morpho-species in the

197   field. In order to avoid counting individual insects multiple times, we collected the insects as we

198   counted them and released them at the end of the survey period each night. For each survey

199   night, unique specimens were photographed, identified to order, and preserved in either glycine

200   bags or 100% ethanol.

201   **Bat carcasses and assessment of bat stomach fullness**

202   Although six bat species have been found in fatality monitoring surveys, we only included

203   eastern red bat and hoary bat carcasses in this study because these species had the highest fatality

204   rates at Wolf Ridge (Bennett & Hale, 2014), and experience high levels of fatality at wind

205   facilities across North America (e.g., Arnett & Baerwald, 2013; Zimmerling & Francis, 2016).

206   To select carcasses for analysis, we prioritized those in best overall condition (i.e. no obvious

207   decay or damage by insect scavengers), including adult and juvenile males and females of both

208   species that were collected between July and August of 2013 and 2014 and subsequently stored

209   at -4°C. Thus, we dissected 45 eastern red bat (27 females, 18 males; 40 adults, 5 juveniles) and

210   23 hoary bat carcasses (11 females, 12 males; 19 adults, 4 juveniles), removing their digestive

211   systems (esophagus, stomach, and intestines) and storing them with their contents intact in 70%

212   ethanol.

213   Before beginning the genetic analyses, we separated the bat stomachs from the esophagi

214   and intestines and visually determined if the stomachs were full using the following definitions.

215   A stomach was considered "full," if it appeared taut from the outside (i.e. not folded or

216   wrinkled), whereas a stomach with obvious extra space or folded membrane was considered "not

217   full". The stomach contents from each bat were then homogenized using a mortar and pestle and

218   weighed. In some instances the stomach membrane was perforated and the contents had been

219   exposed to ethanol, so we allowed the ethanol to evaporate for up to an hour in the extraction

220   hood (described below) prior to homogenization and weighing. We conducted a two-sample t-

221   test to determine if stomachs classified as "full" were significantly heavier than "not full"

222   stomachs.

223   Genetic analysis of bat stomach contents

224   We extracted DNA from the homogenized stomach contents using DNeasy® mericon food kits

225   (QIAGEN; Zarzoso-Lacoste, Corse &Vidal, 2013). We included a negative control with each

226   round of extraction (3 to 7 bat stomach samples) to ensure non-contamination of reagents. All

227   extractions were completed in a dedicated extraction AirClean® 600 PCR workstation to

228   minimize contamination.

229   Samples were then amplified using arthropod-specific primers ZBJ-ArtF1c and ZBJ-

230   ArtR2c developed by Zeale et al. (2011). We set up the polymerase chain reactions (PCR) in a

231   dedicated PCR AirClean® 600 PCR workstation in a different room from where the DNA

232   extractions took place. Again, we included negative controls in our PCR reaction batches. PCR

233   (10µL) contained 2 µl DNA, 0.5 µM of each primer, 1X Qiagen Multiplex PCR Master Mix with

234   HotStarTaq, Multiplex PCR buffer with 3mM $MgCl_2$ pH 8.7, and dNTPs. Reactions were cycled

235   in an ABI 2720 thermal cycler. PCR ran for one cycle at 95°C for 15 min, followed by 40 cycles

236   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

237   purified the products on a gel, ligated them into pGEM-T vectors (Promega), and then

238   transformed them into JM109 competent cells. We plated the transformed cells on ampicillin

239   plates and left them in a 37°C incubator overnight. The following day we selected colonies that

240   had been successfully transformed (i.e., those in which the PCR product had been inserted) based

241   on color (white colonies had been transformed, whereas blue colonies had not). Each clone was

242   amplified using vector-specific primers (F: CGACTCACTATAGGGCGAATTG, R:

243   CTCAAGCTATGCATCCAAGG). Unincorporated nucleotides and excess primers were

244   removed from PCR products using *ExoI* and *rSAP* (New England Biolabs) according to

245   manufacturer protocols. PCR products were then unidirectionally sequenced using the forward

246   vector primers and ABI Big Dye Terminator Cycle Sequencing v3.1 Chemistry (Life

247   Technologies). We electrophoresed sequences on an ABI 3130XL Genetic Analyzer (Life

248   Technologies), edited and trimmed the sequences using Sequencher v5.0 (Gene Codes USA),

249   and then aligned the sequences in MEGA 6.0 using Muscle (Edgar, 2004; Tamura et al., 2013).

250   We used the Barcode of Life Data System (BOLD), an online index of known DNA sequences,

251   to identify sequences (Ratnasingham & Hebert, 2007; www.boldsystems.org). We assigned

252   species for a 99-100% matched sequence, we assigned genus for a 95-98% match, assigned

253   family for a 90-94% match, and assigned order for an 85-90% match according to the methods in

254   Clare et al. (2009) and Zeale et al. (2011). From each stomach sample, we picked and sequenced

255   at least 12 colonies containing recombinant clones since a preliminary study indicated that 10

256   clones was sufficient to detect all insect species present. In two stomachs, we were only able to

257   sequence nine clones and in one stomach we were only able to sequence 8 clones because fewer

258   than 10 recombinant clones were present after cloning or some clones gave low quality

259   sequence.

260   We created neighbor-joining trees using the Kimura Two-Parameter distance in MEGA

261   to determine the number of OTUs (operational taxonomic units) that were present in the samples

262   for which there was less than a 99% match in BOLD. We created separate trees for each order

263   and classified samples as belonging to different species if they were >2% different and clearly

264   clustered separately from other known species identified in BOLD. We used letters to distinguish

265   unidentified species from one another (e.g., Lepidoptera A and Lepidoptera B).

266   **Fecal collection, DNA extraction, amplification, and sequencing**

267   From July to November 2011 and April to October 2012, we conducted weekly systematic

268   searches for bat feces at all 75 wind turbines at Wolf Ridge (Bennett, Hale & Williams, 2017).

269   Single fecal samples were collected from between the upper slats of the turbine door, between

270   the gills of the transformer, on the frame beneath the gills of the transformer, and beneath the

271   stairwell. Once found, we placed each fecal pellet in a 1.5 ml plastic tube and stored them at

272   room temperature.

273   DNA extraction followed the protocol outlined in the QIAamp DNA Stool Mini-kit

274   (Qiagen Genomics, Valencia, CA). A negative control (i.e., a tube with no fecal sample in it) was

275   included with each round of extraction to ensure that the extraction reagents used were not

276   contaminated. Two successive PCR procedures were then undertaken. We first used PCR to

277   identify fecal samples to bat species following the methods described in Korstian et al. (2015).

278   As most of the fecal pellets (59% of 56 pellets) were from eastern red bats (<5 were from hoary

279   bats; Bennett, Hale & Williams, 2017), we limited diet analysis from fecal pellets to eastern red

280   bats only. Following bat species identification, we then used a second PCR to amplify the

281   remnants of prey items in the fecal pellets from eastern red bats. PCR, cloning, sequencing, and

282   arthropod identification protocols were identical to the ones used for bat stomach contents. For

283   each fecal sample, we picked and sequenced at least 12 colonies containing recombinant clones

284   since a preliminary study indicated that 10 clones was sufficient to detect all insect species

285   present. For two fecal pellets, we were only able to sequence seven and nine clones because less

286   than 10 recombinant clones were present after cloning or some clones gave low quality

287   sequence.

288   For the clones generated from any given bat stomach or fecal sample, each insect species

289   detected was counted only one time. We calculated the Simpson's diversity index to summarize

290   the number and abundance of prey items separately for eastern red bat stomachs, hoary bat

291   stomachs, and eastern red bat fecal pellets. We present sampling curves for both the stomach and

292   fecal samples using the number of clones successfully sequenced for all samples and the number

293   of insects identified.

294   **Comparing stomach and fecal contents to insect surveys**

295   To determine whether insect species consistently found in bat stomachs and fecal pellets were

296   also present near turbine towers, we compared all of the insect species identified in BOLD that

297   were found in ≥5 stomach samples to the insect specimens that were collected during surveys in

298   July and August of 2013 and 2015 and identified these species using the voucher specimens we

299   collected. In all cases, the most common species found in bat stomachs were easy to identify

300   morphologically from the insects we collected. If we found that bats were consistently eating

301   insects that were not captured at turbines, it would suggest that bats were foraging elsewhere and

302   not using the turbines as a foraging resource. Conversely, if the species that we consistently

303   found in bat stomachs were present in insect surveys conducted at turbines, then this would

304   provide support for the foraging attraction hypothesis.

305

306   **Results**

307   **Acoustic bat activity at wind turbines**

308   Acoustic data were collected at wind turbine towers on 284 nights (93 in 2010, 42 in 2012, 90 in

309   2013, 38 in 2015, and 21 in 2016). We recorded a total of 3,606 bat passes and identified calls

310   from all six bat species known to be in the study area (Fig. 1). Within this dataset, foraging and

311   approach phase activity were recorded in 23% of the bat passes (n = 836) and feeding or terminal

312   buzzes were recorded in 3.1% of the bat passes (n = 113). Of these feeding or terminal buzzes,

313   56% were recorded at detectors placed at the turbine nacelles. All six bat species exhibited

314   foraging behavior at wind turbines (Fig. 1).

Insect surveys

316   We confirmed that the majority of the insect orders caught in the malaise traps did not differ

317   from the insect orders collected with light trapping (Fig. S1, Fig. 2), therefore we only

318   summarized the light trapping results to characterize the insect community near the turbine

319   towers. In 2012, we light trapped a total of 17 nights between July and August, collecting 1,238

320   invertebrates belonging to nine orders. The three most abundant orders were Coleoptera (37.2%),

321   Orthoptera (23.7%), and Lepidoptera (20.0%). In 2013, we light trapped a total of 13 nights

322   between July and August, collecting 1,937 invertebrates belonging to 11 orders. The three most

323   abundant orders were Lepidoptera (42.8%), Coleoptera (38.0%), and Hemiptera (9.1%). In 2015,

324   we light trapped for 16 nights between July and August, collecting 7,479 invertebrates belonging

325   to 13 orders. The three most abundant orders were Coleoptera (54.9%), Lepidoptera (14.7%),

326   and Hemiptera (13.2%).

327   Due to differences in survey methods among years, we could not statistically compare the

328   three years of insect surveys to determine if the insect community at Wolf Ridge changed over

329   time. However, an informal comparison based on the average biweekly proportions of each order

330   suggests that the insect community has remained relatively consistent between July and August

331   of 2012, 2013, and 2015 (Fig. 2). Note that the confidence intervals are wide due to nightly

332   variation in insect abundance among survey periods. For example, on one night we might have

333   not caught any water beetles, whereas the next survey night might have coincided with an

334   emergence of water beetles. Additionally, some species of insects were only captured on a single

335   survey night during the season.

Assessment of bat stomach fullness

337   Of the 45 eastern red bats included in this study, 22 had full stomachs (three stomachs were not

338   weighed). The mean ± SD weight of full stomach contents was 0.139 ± 0.076 g, whereas not full

339   stomach contents weighed 0.043 ± 0.039 g (t = 5.04, df = 27, *P* < 0.001, n = 20 and 22 stomachs,

340   respectively). Of the 23 hoary bats included in this study, 16 had full stomachs (1 stomach was

341   not weighed). The mean ± SD weight of full stomach contents was 0.458 ± 0.252 g, whereas not

342   full stomach contents weighed 0.131 ± 0.114 g (t = 4.19, df = 19, *P* < 0.001, n = 15 and 7

343   stomachs, respectively). For both eastern red bats and hoary bats, the body masses of juveniles

344   and adults were not significantly different (see Supplemental File: Data.xlsx), so we did not

345   separate our analysis by age group.

346   **Genetic analysis of bat stomach contents**

347   Insect DNA was successfully extracted and amplified from all of the 68 bat stomachs included in

348   this study. The average number of clones for the 68 bat stomachs was 13.4 (range: 8-21 clones).

349   The sampling curve peaked at 10 clones indicating that 10 clones was sufficient to detect all prey

350   species within a single stomach sample (Fig. S2A).

351   Collectively, the results of our stomach analysis yielded 153 insects representing 60

352   genetically distinct species. Based on the percentage match to known sequences in BOLD, 38

353   insects were identified to species, 10 were identified to genus, three were identified to family,

354   and nine were identified to order (Table S1 and S2). Individual bats in our study had a mean (±

355   SE) of 2.26 ± 0.11 prey species in their stomachs (range: 1-6 species; Fig. 3). The majority of our

356   samples consisted of only one or two species of insects (28 bats and 15 bats, respectively).

357   Eastern red bats (n = 45) had a mean (± SE) of 2.2 ± 0.19 individual prey species in their

358   stomachs (range: 1-5 species; Fig. 3). We found 43 different species of insects from seven orders

359   in eastern red bat stomachs (Fig. 4A; Table S1). Thirty-one of these species were detected in

360   only one stomach (Fig. S3). We detected one species of moth (*Spodoptera frugiperda*) in 11

361   different stomachs and one species of cricket (*Gryllus spp.*) in 29 stomachs (Table S1).

362   Lepidoptera comprised 55.1% and Orthoptera comprised 32.7% of the insect species identified in

363   eastern red bat stomachs. The remainder belonged to Blattodea, Coleoptera, Diptera, Hemiptera,

364   and Neuroptera. In addition to the species mentioned above, the following insects were detected

365   in bats from both years: *Parcoblatta spp.*, *Achyra rantalis*, *Euchromius ocelleus*, and *Bleptina*

366   *caradrinalis*.

367   Hoary bats (n = 23) had a mean (± SE) of 2.4 ± 0.34 individual prey species in their

368   stomachs (range: 1-6 species; Fig. 3). We found 25 different species of insects from three orders

369   in hoary bat stomachs (Fig. 4B; Table S2). Eighteen of these species were found in only one

370   stomach (Fig. S3). Similar to our eastern red bat stomach analysis, we found that Lepidoptera

371   was the most abundant and diverse order in the stomachs; the most frequently detected moth, *S.*

372   *frugiperda*, was found in seven stomachs. *Gryllus spp.* were the most frequently detected species

373   and were found in 18 stomachs. Lepidoptera comprised 60.7% and Orthoptera comprised 32.1%

374   of the species identified in hoary bat stomachs. The remainder consisted of four species of

375   Coleoptera, each only detected once. In addition to the species mentioned above, only two other

376   species of moths were detected in both years: *E. ocelleus* and *Helicoverpa zea*.

377   **Genetic analysis of bat fecal pellets**

378   Insect DNA was successfully extracted and amplified from 23 of the 33 eastern red bat fecal

379   pellets collected from wind turbines (Bennett, Hale & Williams, 2017). The average number of

380   clones for the fecal pellets was 13.5 (range: 7-18 clones), and the sampling curve appeared to

381   level off at approximately 10 clones; however, a few samples were highly diverse causing the

382   curve to continue to increase after 14 clones (Fig. S2B).

383   Collectively, the results of our fecal analysis yielded 57 insects representing 39

384   genetically distinct species. Based on the percentage match to known sequences in BOLD, 18

385   insects were identified to species, 10 were identified to genus, five were identified to family, and

386   six were identified to order (Table S3). Individual fecal pellets in our study contained a mean (±

387   SE) of 2.4 ± 0.34 prey species (range: 1-6 species; Fig. 3), with the majority (61%) of the fecal

388   pellets containing only one or two species of insects. The 39 species of insects detected in fecal

389   pellets belonged to nine insect orders (Fig. 4C; Table S3). Thirty-one of these species had a

390   detection frequency of 1 (Fig. S3). We detected one species of cricket (*Gryllus rubens*) in 35% of

391   the fecal samples (n = 8 pellets). The remaining species were detected in fewer than five pellets

392   each. Lepidoptera comprised 32.1%, Orthoptera 17.8%, Diptera 21.4%, and Coleoptera 17.9% of

393   the insect species identified in fecal pellets. The remainder belonged to Blattodea,

394   Ephemeroptera, Hemiptera, Neuroptera, and Isopoda. The single fecal sample that contained

395   Isopoda (*Armadillium* sp.) was probably due to environmental contamination since these species

396   are terrestrial and have not been observed on the surface of the turbine tower where they might

397   be exposed to bat predation.

398   **Comparing diversity between species and sample types**

399   Eastern red bat stomachs had a Simpson's diversity index of 0.898, hoary bats 0.878, and eastern

400   red bat fecal pellets 0.973, indicating that both species have similar diversity of insects in their

401   stomach contents; however, fecal samples had slightly higher diversity than the stomach

402   samples.

403   As we analyzed more bat stomachs and fecal pellets, we continued to identify insect

404   species that we had not previously found in our study (Fig. 5). Our discovery rate suggests that

405   we would have continued to discover more species of insects with larger sample sizes of bat

406 stomachs and fecal pellets, suggesting that our analysis may only reveal a fraction of the insect

407 species that bats are eating in a night at our study site.

408 **Comparing stomach and fecal contents to insect surveys**

409 To determine whether insects frequently found in bat stomachs were present at wind turbines, we

410 compared the insect species detected most frequently in the stomach contents to the insect

411 surveys. Because 2012 insect surveys did not incorporate species identification, we only

412 compared our stomach content results to 2013 and 2015 insect surveys. We omitted any insects

413 that were found in <5 stomachs because so many species from our genetic analysis were single-

414 stomach detections. This left us with four species of moths (*E. ocelleus*, *B. caradrinalis*, *H. zea*,

415 and *S. frugiperda*) and two unidentified species of cricket (*Gryllus spp.*). We found these insects

416 in the stomachs of both eastern red bats and hoary bats (Table 1). Of these commonly eaten

417 insect species, we documented most at wind turbines in 2013 and all of them at wind turbines in

418 2015 (Table 1). Furthermore, all of these species were found at wind turbines on multiple nights

419 throughout the survey period in both 2013 and 2015 (Table 1).

420 Fecal analysis also had a high number of single-pellet detections, so we only compared

421 insect species detected in ≥5 eastern red bat fecal pellets to the results of our insect surveys.

422 Crickets (*Gryllus spp.*) were the only species of insect consistently detected in eastern red bat

423 fecal pellets; crickets were also collected on multiple nights throughout the insect survey period

424 in both 2013 and 2015.

425 **Discussion**

426 Our study provides strong support for the hypothesis that bats are using wind turbines as a

427 foraging resource. We recorded acoustic foraging activity, including feeding buzzes, in the

428 immediate vicinity of wind turbines for all six bat species known to be present at this site (listed

429   as prediction 1 in Cryan & Barclay, 2009). We know that light-colored turbines attract

430   aggregations of insects (Long, Flint & Lepper, 2011), and found that the orders of insects present

431   at turbines were in relatively consistent proportions from one year to the next (listed as

432   prediction 3 in Cryan & Barclay, 2009). Furthermore, for the two bat species most commonly

433   found in fatality searches at this site, we demonstrated that they had full or partially full

434   stomachs, indicating that they were foraging just prior to their deaths (listed as prediction 4 in

435   Cryan & Barclay, 2009). We collected bat fecal pellets from turbine structures (e.g., between

436   door slats), which indicates that bats were likely using the turbines as night roosts between

437   successive foraging bouts (Bennett, Hale & Williams, 2017). We also demonstrated that the

438   insect species eastern red and hoary bats consistently preyed upon were also present at wind

439   turbines (listed as prediction 5 in Cryan & Barclay, 2009).

440   The presence of foraging or approach phase calls and feeding buzzes from all six species

441   known to be in the study area demonstrates that bats are actively foraging in close proximity to

442   the wind turbines. More specifically, feeding buzzes recorded from all six species at acoustic

443   detectors placed on the nacelle (63 of the 113 feeding buzzes recorded) provides evidence that

444   bats are successfully capturing prey items in the rotor swept zone. This latter observation is

445   important as it indicates that bats are foraging at heights at which they are susceptible to collision

446   with rotating turbine blades. It is also possible that we overestimated foraging activity based on

447   feeding buzzes as these types of acoustic signals are also indicative of bats attempting to locate

448   landing sites (e.g., Melcón, Denzinger & Schnitzler, 2017) or drink water (Griffiths, 2013). The

449   relative frequency of these activities may warrant additional research as it has been shown that

450   bats will roost on wind turbines (Bennett, Hale & Williams, 2017) and it has been hypothesized

451   that bats may misperceive the smooth surfaces of wind turbine tower monopoles to be water

452   (McAlexander, 2013).

453   We found Lepidoptera and Coleoptera in consistently high proportions at turbines at

454   Wolf Ridge in July and August over three years (2012, 2013, and 2015), which suggests that

455   food resources for insectivorous bats were consistently available. Overall, the patterns of

456   abundance in the three survey years remained consistent, despite differences in survey methods.

457   The proportions of Lepidoptera in 2013 and the proportions of Hemiptera in 2015 had much

458   wider confidence intervals than those orders in other years, which could be due to survey

459   methods or differences in other variables that contribute to insect emergence patterns or

460   abundances (e.g., moonlight and weather). While Orthoptera were not as abundant as

461   Lepidoptera or Coleoptera, we consistently caught *Gryllus spp.* each year.

462   The majority of the bat stomachs in our study were full, or partially full, also indicating

463   that the bats were likely killed while they were foraging. Stomach fullness is a good indicator of

464   recent foraging, because insectivorous bats typically forage until they have consumed

465   somewhere between one-quarter of their body weight to their full body weight in insects, after

466   which they go to a nearby night roost to digest (Barclay, Dolan & Dyck, 1991; Kunz, Whitaker

467   & Wadanoli, 1995; Knight & Jones, 2009; Ammerman, Hice & Schmidly, 2012; Gonsalves et

468   al., 2013; Lison, Palazon & Calvo, 2013; Montero & Gillam, 2015). The fecal pellets included in

469   our study were collected from within structures associated with the turbine towers, providing

470   evidence that eastern red bats were roosting on the turbine structures, likely between successive

471   foraging bouts at night (Bennett, Hale & Williams, 2017).

472   Genetic analysis of dietary habits for insectivorous bats potentially allows for better prey

473   identification, often to species level, compared to the morphological analysis methods used in

474   previous investigations (Clare et al., 2009; Valdez & Cryan, 2013). For both eastern red and

475   hoary bats, we found between one and six species of insects in their stomachs and fecal pellets,

476   which is consistent with other studies (Clare et al., 2009; Whitaker et al., 2009). If bats consume

477   up to their own body weight in insects per night, the results of our study (and other studies of bat

478   stomach contents and feces) probably represent only a fraction of the bats' nightly diets (Barclay,

479   Dolan & Dyck, 1991). This could explain why so many of the insect species we identified were

480   only detected once. We expect that if we had included more bat stomachs and fecal pellets in our

481   analysis, we would have continued to identify additional insect species in the diets of these bats.

482   Lepidoptera dominated the diets of both eastern red bats and hoary bats, adding to the

483   body of research showing that moths make up a large part of the diet of insectivorous bats (e.g.,

484   Carter et al., 2003; Valdez & Cryan, 2009; Clare et al., 2009; Reimer, Baerwald & Barclay,

485   2010; Zeale et al., 2011; Valdez & Cryan, 2013). Bats digest moths more efficiently than other

486   types of prey, which could explain this abundance in their diets (Barclay, Dolan & Dyck, 1991).

487   Despite the differences in the orders found in the stomach contents of eastern red and hoary bats

488   at Wolf Ridge, the two species had similarly high Simpsons' indexes of diversity, indicating that

489   both species eat a wide range of prey. On the other hand, eastern red bat fecal pellets had a

490   higher Simpson's index of diversity than the stomach contents of either species, perhaps because

491   fecal pellets contain a mix of insects from ≥ 1 foraging bouts.

492   We found only three orders of insects in the hoary bat stomach contents, which primarily

493   consisted of moths and *Gryllus spp*. These findings are consistent with the results of the hoary

494   bat fecal analysis conducted in Texas by Valdez and Cryan (2013). In that study, there was

495   evidence of Coleoptera in fecal pellets, but Lepidoptera and Orthoptera comprised a larger

496   percentage of the volume of the fecal pellets and had higher detection frequencies overall.

497   We found nine orders of insects in eastern red bat fecal pellets, primarily consisting of

498   Lepidoptera, Coleoptera, Diptera, and Orthoptera. High percentages of Lepidoptera, Coleoptera,

499   and Orthoptera are consistent with the results of other studies mentioned above. While 10

500   different species of Diptera were detected, only one was identifiable to the species-level. The

501   presence of a diversity of Dipteran species in the fecal pellets was different than what we

502   observed in the stomach contents. The Dipterans consumed by bats included several families that

503   feed on plant tissue, including Agrommyzidae (leaf miner flies), Ephydridae (shore flies), and

504   Tephritidae (fruit flies).

505   Five species of insects met our criteria for consistent detection in bat stomachs (≥5 bat

506   stomachs) and we documented all five in our insect surveys at the turbines at Wolf Ridge, which

507   provides further support for the foraging attraction hypothesis. We found four of the five insect

508   species in both 2013 and 2015, and all species were detected on multiple survey nights in any

509   given year. In contrast, only one species of insect met our criteria for consistent detection in

510   eastern red bat fecal pellets.

511   We consistently found field crickets, *Gryllus spp.*, in the stomachs of both eastern red and

512   hoary bats as well as in the eastern red fecal pellets, indicating that crickets are an important food

513   source for bats foraging at Wolf Ridge. Ours was not the first study to document *Gryllidae*

514   crickets in bat diets in Texas (Valdez & Cryan, 2013), and several explanations have been

515   posited about how and why bats eat crickets. Field crickets have been observed to be attracted to

516   light, and may therefore concentrate at the white turbine towers that are often illuminated by the

517   moon (Tinkham, 1938; Long, Flint & Lepper, 2011; Thomson, Vincent & Bertram, 2012).

518   Additionally, bats may be able to hear crickets chirp, making them easy prey to target. Eastern

519   red and hoary bats are aerial insectivores, meaning they eat on the wing, but studies have

520   suggested that they may glean crickets from surfaces such as canyon walls (Easterla & Whitaker,

521   1972) and turbine towers (Valdez & Cryan, 2013). Crickets are primarily terrestrial, but within

522   populations some crickets possess a longer-wing mutation that makes them better flyers; perhaps

523   the crickets found in our diet analysis possess this mutation (Olvido, Elvington & Mousseau,

524   2003; Valdez & Cryan, 2013). We do not have direct observations of bats capturing crickets or

525   other insects at turbine towers, but a recent study by Rydell et al. (2016) reported that bats killed

526   at wind turbines in southern Sweden had consumed diurnal flies as well as flightless insect taxa,

527   indicating that bats were able to effectively capture prey resting on the turbine surface or from

528   the air near the surface after being disturbed by the bats. These recent diet analyses (this study,

529   Rydell et al., 2016) in combination with several published observations of bats making close

530   "investigative" approaches of turbine towers (e.g., Horn, Arnett & Kunz, 2008; McAlexander,

531   2013; Cryan et al., 2014), suggest that at least some aerial-hawking bat species may be able to

532   capture insects that rest on wind turbine tower surfaces.

533   We consistently found two species of crop pests in the stomachs of the bats in this study.

534   This result underscores the important pest-management role insectivorous bats play in the

535   ecosystem and in the agriculture industry (e.g., Boyles et al., 2011). The most common moth

536   species we found in the bat stomachs, *S. frugiperda*, or the fall armyworm moth, migrates from

537   South Texas and Mexico to North Texas (Knutson, 2008; Westbrook, 2008). This species is a

538   crop pest, primarily on Bermuda grass (*Cynodon dactylon*), wheat (*Triticum spp.*), and rye grass

539   (*Lolium spp.*), but attacks other crops as well and is most abundant in Texas from August

540   through November (Knutson, 2008). In addition to *S. frugiperda*, we also consistently found the

541   corn earworm moth (*H. zea*; Cole & Jackman, 1981).

542   **Conclusion**

543   The results of this study and others are providing compelling evidence that at least some bat

544   species are foraging at wind turbines, that foraging ecology in a broad sense is likely contributing

545   to bat fatalities at wind turbines worldwide, and that potential attraction to insect aggregations at

546   wind turbines may interfere with the ability of pre-construction activity surveys to predict risk to

547   bats. For example, if turbines reliably attract insects which in turn attract bats, then pre-

548   construction bat activity surveys at potential wind facilities could drastically underestimate post-

549   construction bat fatality rates. If reliable and abundant foraging opportunities are available for

550   migrating bats at wind turbines and the resulting foraging activity increases risk of barotrauma or

551   collision with rotating blades, then future efforts must focus on technological innovations (e.g.,

552   acoustic deterrents) and/or operational changes (e.g., raising the cut-in speed on low wind speed

553   nights) to reduce bat mortality at wind turbines.

554   While the focus of this study was on the foraging attraction hypothesis, bats may be

555   coming into contact with wind turbines for a variety of reasons that include other sources of

556   attraction in addition to coincidental and random explanations (Cryan & Barclay, 2009).

557   Moreover, the attractors likely vary in relative importance by species and over time and

558   geographical space. Thus, this multitude of ultimate causes for bats approaching wind turbines

559   towers, including the aforementioned aggregations of insects, makes devising and implementing

560   effective means to reduce bat fatality without incurring losses in power production a critical

561   element of continued wind power development.

562

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J

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572

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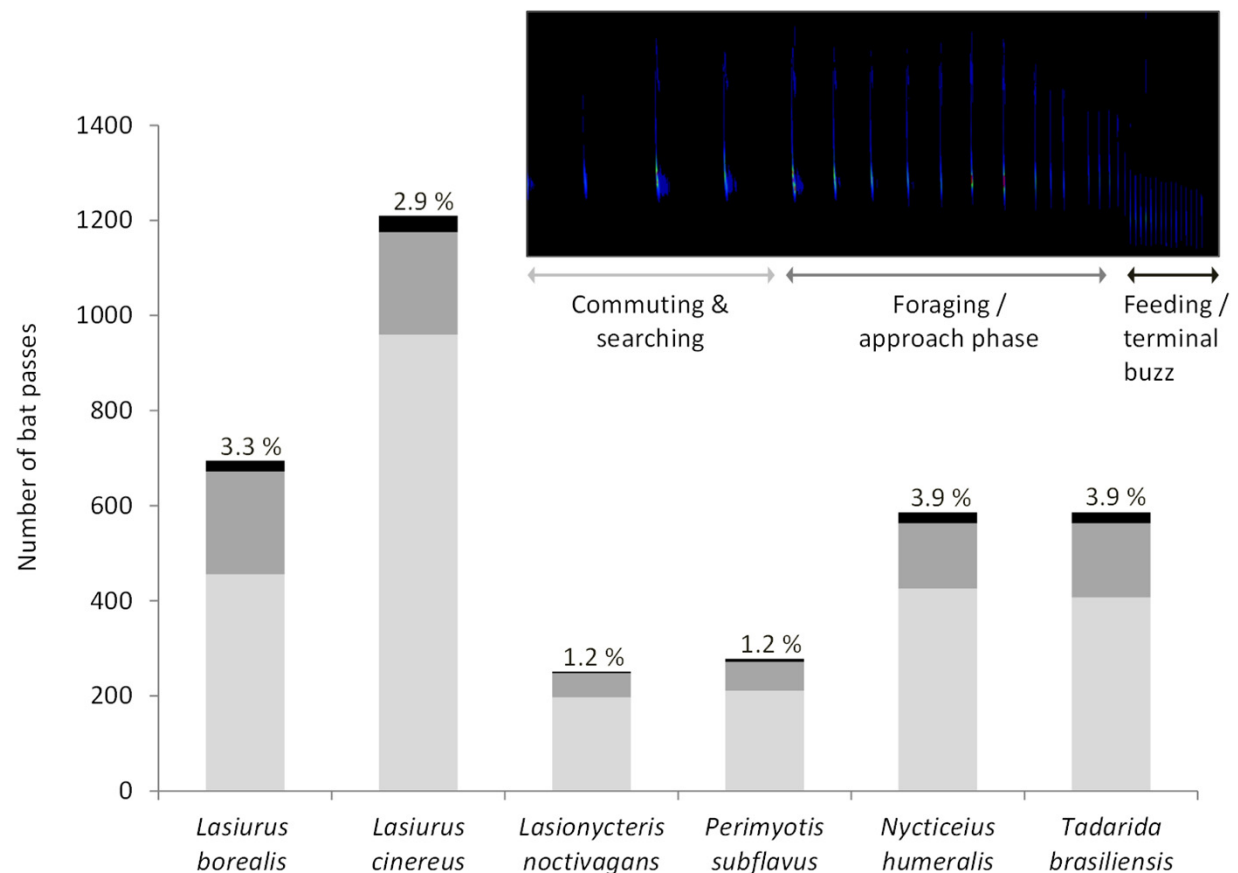
Figure 1. Number of bat passes recorded at turbine towers.
Number of bat passes, separated into 3 activity categories (light grey – commuting & searching; dark grey – foraging/approach phase; black – feeding/terminal buzz), by species recorded in acoustic surveys at wind turbine towers in 2010, 2012, 2013, 2015, and 2016 at the Wolf Ridge wind farm. The percentage of bat passes that included feeding or terminal buzz activity is included above each species' bar.
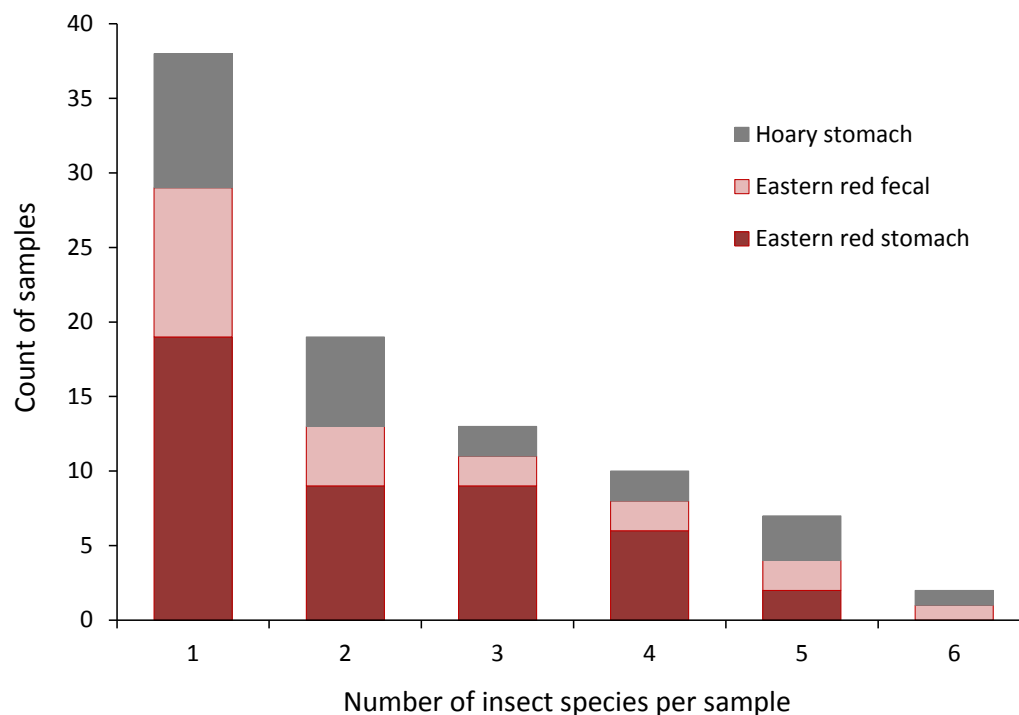
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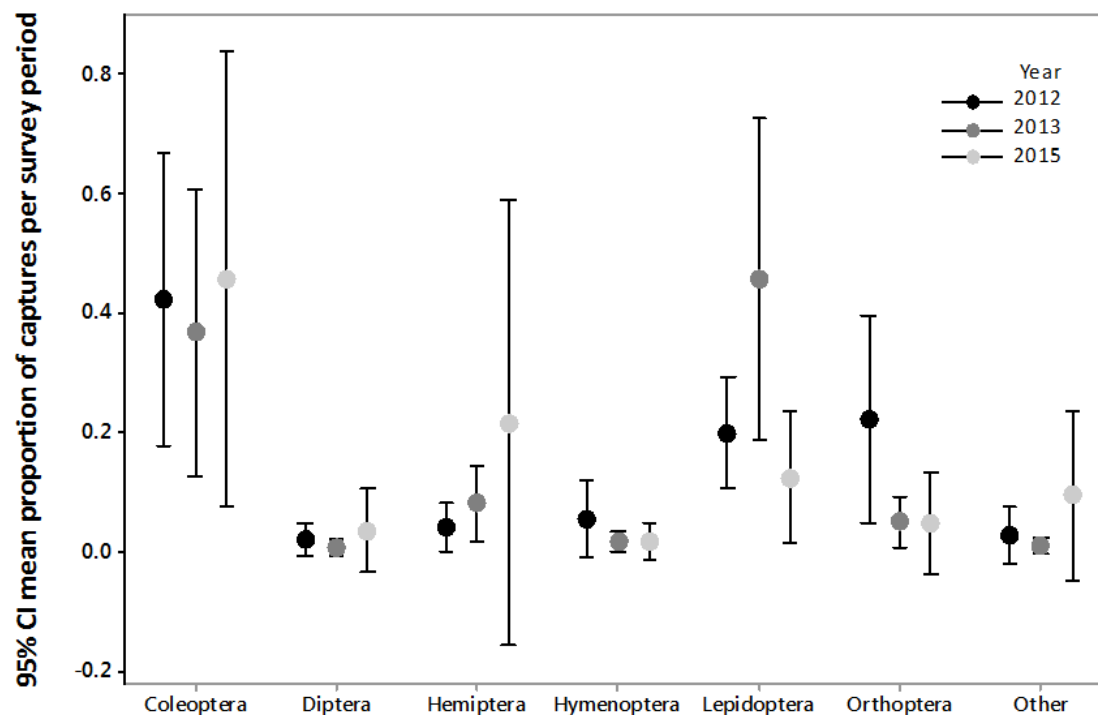
Figure 2. Biweekly proportions of insect orders surveyed at turbine towers.
Biweekly averages and 95% CI of the proportions of each order collected during July-August
light trapping in 2012, 2013, and 2015 at the Wolf Ridge wind farm. The "other" category
includes Blattodea, Ephemeroptera, Neuroptera, Odonata, Plecoptera, Trichoptera, and spiders.

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Figure 3. Number of insect species found in bat stomachs and fecal pellets.
Number of bat stomach samples (n = 45 eastern red bats, n = 23 hoary bats) and bat fecal
samples (n = 23 eastern red bats) in which from 1 to 6 different insect species were found.
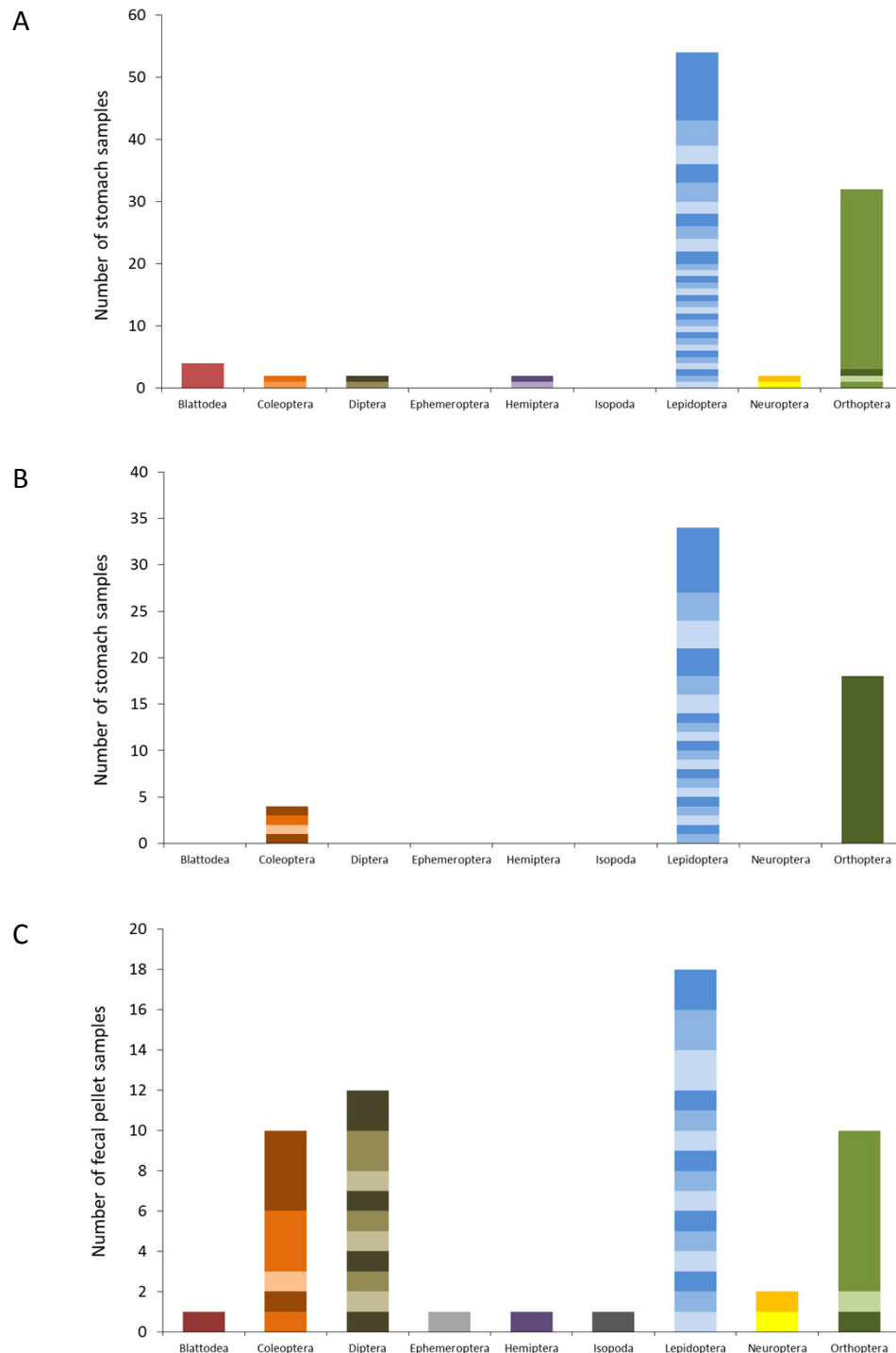
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Figure 4. Insect orders (and species within each) found in bat stomachs and fecal pellets.
Insect orders found in (A) eastern red bat stomach contents (n = 45 bats collected in 2013 and 2014), (B) hoary bat stomach contents (n = 23 bats collected in 2013 and 2014), and (C) eastern red bat fecal samples (n = 23 pellets collected in 2011-2012) from the Wolf Ridge wind farm. Each band in the bar represents a different insect species. Note that the y-axis differs in each of the figure panels.
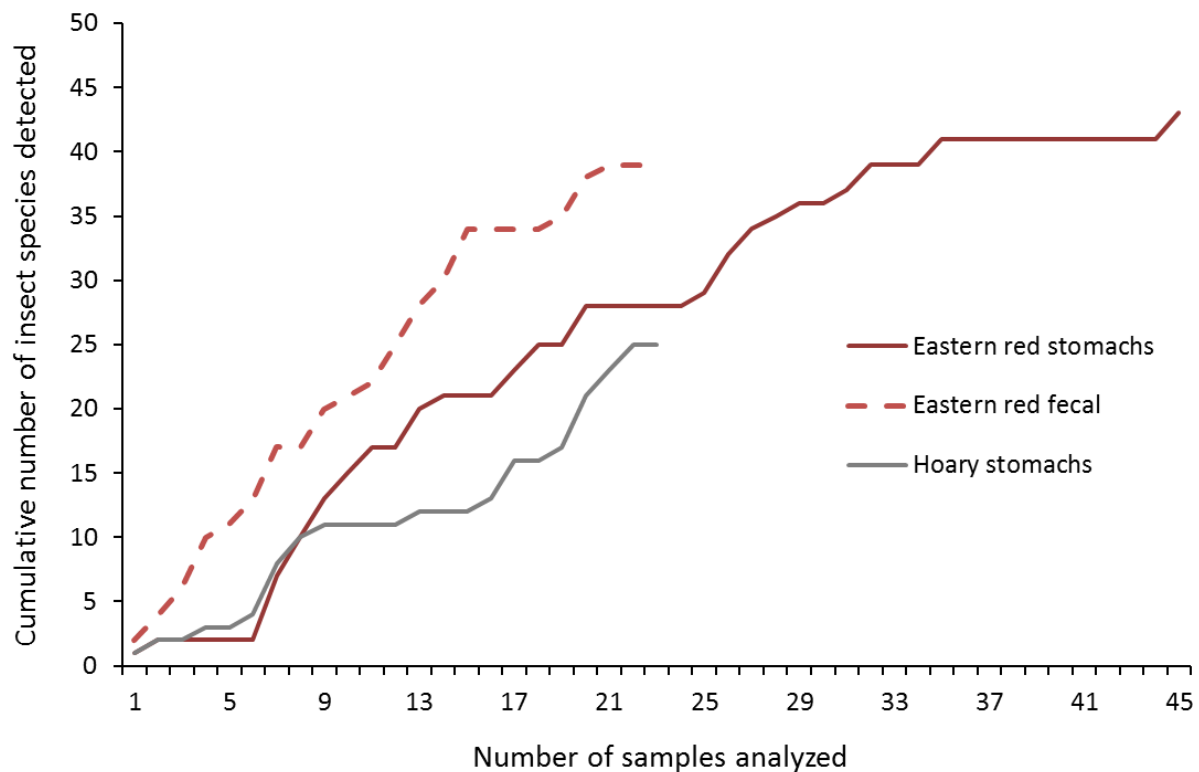
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Figure 5. Discovery rate of new insect species in bat stomachs and fecal pellets.
Discovery rate of new insect species by number of bat stomachs and fecal pellets analyzed from
the Wolf Ridge wind farm.

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751 **Table 1.** The insect species most commonly found in bat stomach and fecal pellet samples compared to their relative abundance in
752 insect surveys at wind turbines at the Wolf Ridge wind farm. Crop pest information comes from Cole and Jackman (1981).

Common name	Scientific name	Crop pest	Found in proportion of samples			Present at Wolf Ridge	
			Eastern red bat stomachs (n = 45)	Eastern red bat fecal pellets (n = 23)	Hoary bat stomachs (n = 23)	Proportion of survey nights (n = 29)	Total count
Field cricket	*Gryllus spp.*	No	0.644	0.348	0.783	0.724	217
Fall armyworm moth	*Spodoptera frugiperda*	Yes	0.244	0.087	0.304	0.586	128
Necklace veneer moth	*Euchromius ocelleus*	No	0.089	0.000	0.130	0.207	39
Bent-winged owlet moth	*Bleptina caradrinalis*	No	0.067	0.000	0.130	0.069	3
Corn earworm moth	*Helicoverpa zea*	Yes	0.067	0.000	0.087	0.345	38

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