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Original Investigation

Bats may eat diurnal flies that rest on wind turbines

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ABSTRACT

Bats are currently killed in large numbers at wind turbines worldwide, but the ultimate reason why this happens remains poorly understood. One hypothesis is that bats visit wind turbines to feed on insects exposed at the turbine towers. We used single molecule next generation DNA sequencing to identify stomach contents of 18 bats of four species (*Pipistrellus pygmaeus*, *Nyctalus noctula*, *Eptesicus nilssonii* and *Vespertilio murinus*) found dead under wind turbines in southern Sweden. Stomach contents were diverse but included typically diurnal flies, e.g. blow-flies (Calliphoridae), flesh-flies (Sarcophagidae) and houseflies (Muscidae) and also several flightless taxa. Such prey items were eaten by all bat species and at all wind turbine localities and it seems possible that they had been captured at or near the surface of the turbines at night. Using sticky traps, we documented an abundance of swarming (diurnal) ants (*Myrmica* spp.) and sometimes blow-flies and houseflies at the nacelle house. Near the base of the tower the catches were more diverse and corresponded better with the taxa found in the bat stomachs, including various diurnal flies. To evaluate if flies and other insects resting on the surface of a wind turbine are available to bats, we ensonified a house fly (*Musca*) on a smooth (plastic) surface with synthetic ultrasonic pulses of the frequencies used by the bat species that we had sampled. The experiment revealed potentially useful echoes, provided the attack angle was low and the frequency high (50–75 kHz). Hence resting flies and other arthropods can probably be detected by echolocating bats on the surface of a wind turbine. Our findings are consistent with published observations of the behavior of bats at wind turbines and may actually explain the function of some of these behaviors.

Introduction

The increasing demand for wind energy worldwide results in large-scale killing of bats, particularly of species that fly and feed in the open air, some of which are also migratory (Arnett et al., 2008; Rydell et al., 2010a; Voigt et al., 2012; Lehnert et al., 2014). The bats die as they are hit by the moving rotor or following ruptures of lungs and blood vessels caused by the rapidly falling air pressure behind the peripheral parts of the blades (Baerwald et al., 2008). The cumulative effect on bat populations is considered to be serious and doubts are raised about the continued welfare of some populations (Kunz et al., 2007; Voigt et al., 2015). Action to minimize or halt the killing of bats at wind turbines may therefore be urgent. Efficient and economically feasible mitigation methods exist (e.g. Arnett et al., 2011) but need to be implemented consistently (Arnett et al., 2015).

Unlike birds, bats seem to be attracted to wind turbines (Cryan et al., 2014) and this makes the problem more serious. There are several hypotheses that may explain why this happens (reviewed by Kunz et al., 2007; Cryan and Barclay, 2009; Arnett et al., 2015). One of them is that insects accumulating around the turbine tower may provide feeding opportunities (Kunz et al., 2007; Rydell et al., 2010b). Heat-image camera observations of bats near turbines have revealed behaviors reminiscent of insect gleaning as well as of aerial insect pursuits (e.g. Ahlén et al., 2007; Horn et al., 2008; Hale et al., 2013; Cryan et al., 2014). However, conclusive evidence that these manoeuvers actually involve insect capture is missing.

While sampling the ground under wind turbines in southern Sweden for bat carcasses, we noticed that large calyptrate flies, such as blow-flies, flesh-flies and houseflies frequently rest or bask on wind turbine towers, sometimes in large numbers, perhaps attracted by the relatively high temperature at the surface. Some

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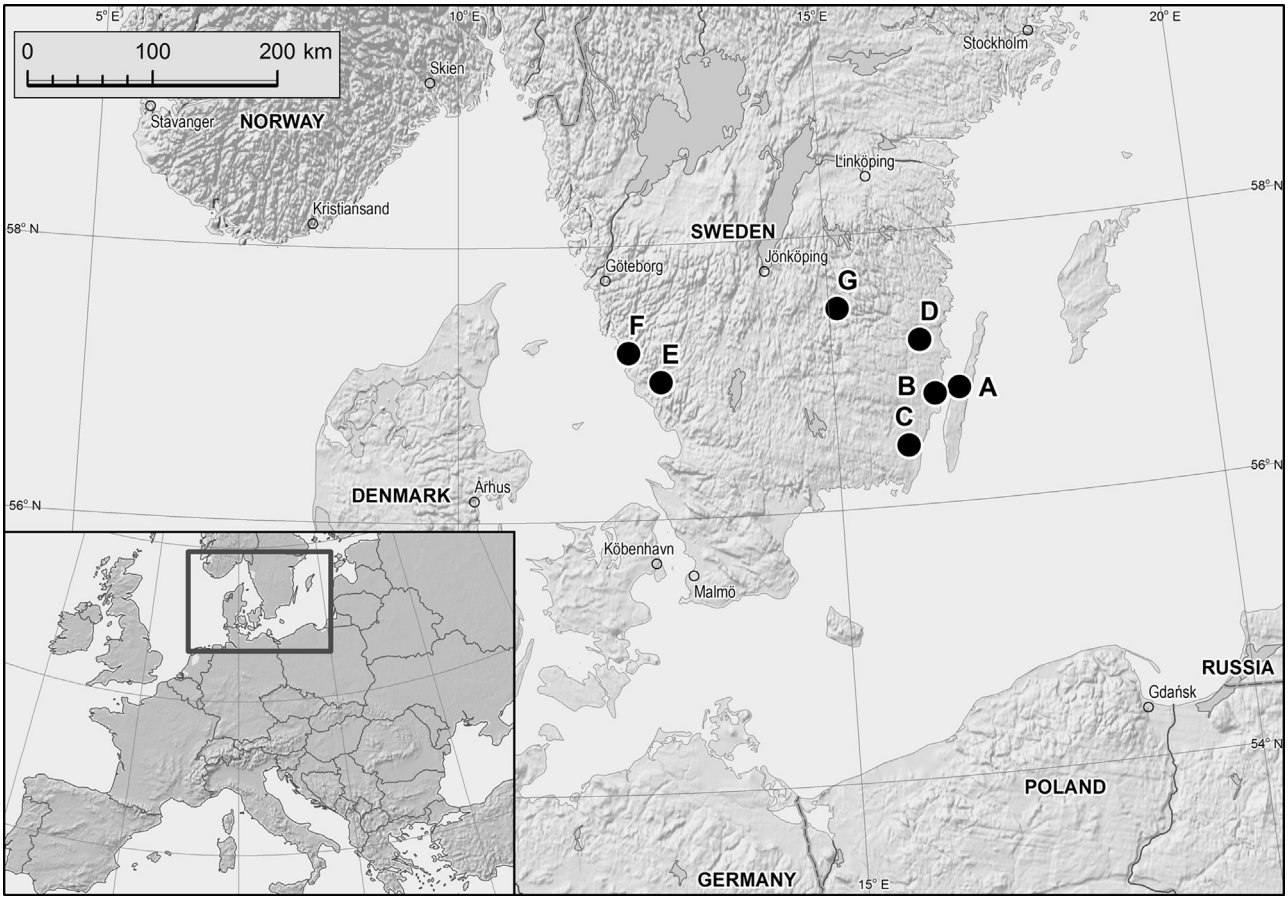

Fig. 1. Map of southern Sweden showing the location of the wind farms sampled in this study. The letters refer to Table 1 and the running text.

of them remain after dusk and there are even insect species that overwinter at the surface of wind turbines (Dudek et al., 2015). Calyptrate flies typically have high wing loading and require high body temperature (T_b) in order to fly. A pre-flight T_b of 30–40 °C is achieved by basking, facilitated by the black bodies and its lack of insulation, or by absorption of heat from the substrate, and the flies are therefore diurnal (Willmer, 1982; Chappell and Morgan, 1987). We hypothesized that roosting insects may constitute targets for bats when exposed on the smooth surface of a wind turbine.

Materials and methods

Study sites and bat sampling

Bat carcasses were sampled at seven different wind farms (A–G) in southern Sweden (Fig. 1) but in two of them (F and G) no carcasses were found. All sites except A were located in the boreal or hemiboreal forest on the mainland at 100–200 m altitude. Site A was in an agricultural area near sea level on the island of Öland in the Baltic Sea. The turbines sampled are all parts of small wind farms (5 turbines in A and B, 6 in C, F and G, 8 in D and 10 in E), of modern construction with high towers (90–125 m) and with total heights of 150–200 m. The carcasses were collected during post-construction surveys in the summers 2012–2014 either as a private initiative (sites A and B) or for the respective wind companies (sites C–G). The carcasses were kept frozen until they were sent to the Museum and Institute of Zoology PAS in Warszawa for analysis of the stomachs in autumn 2014.

Insect sampling

In summer 2014 we sampled insects at four of the six wind farms (C and E–G), using sticky traps. We placed 2 traps (10 × 20 cm; Anticimex AB, Sweden) on the surface of the nacelle house of each turbine. The traps were replaced after about two weeks. We did not have access to the towers at wind farms A, B and D, so we could not trap insects there. In 2015 we sampled insects at four turbines of wind farm E. At two of them we sampled simultaneously at the nacelle and near the base of the tower (at the door 5–10 m above the ground), using the same method as in 2014.

Insects and spiders sampled using sticky traps were counted and identified by visual inspection under a binocular microscope. For identification we used various insect guides such as e.g. Rognes (1991) for blow-flies, Pape (1987) for flesh-flies and Douwes et al. (2012) for ants and wasps. We omitted flies, thrips, beetles and some other insects with wingspan <4 mm (roughly the size of biting midges and small aphids), since this appears to be the lower size limit of prey eaten by any of the bat species examined here (Swift et al., 1985; Barlow 1997; Bartonička et al., 2008).

DNA sequencing and statistics

The bat carcasses with intact stomachs ($N = 18$) were dissected and the stomachs opened immediately before analysis. DNA was then isolated from samples with negative control extractions to monitor for contamination. NucleoSpin Soil (MACHEREY-NAGEL) was used for around 750 μl of the starting material. The procedure

was conducted according to manufactures' protocol with use of SL1 buffer, Enhancer XS and elution in 30 μl.

For identification of insects the barcoding fragment of COI (cytochrome oxidase 1) was used following Zeale et al. (2011). Primers ZBJ-ArtF1c and ZBJ-ArtR1c amplify product 157 bp long, located within and at the 5′ end of the standard 658 bp COI barcode region. PCR reaction was performed in 25 μl volume, with 12.5 μl Multiplex PCR Master Mix (QIAGEN), 0.6 μl of each 10 μM primer, 6 μl of sample DNA and H_2O to the final volume. The PCR thermal cycling conditions were as follows: 95 °C for 15 min, then 45 cycles of: 94 °C for 30 s, 47,5 °C for 90 s, 72 °C for 90 s, with final elongation of 60 °C for 30 min. Amplicons were then used for library preparation for sequencing on the single molecule next generation sequencer PacBio RS II (Pacific Biosciences). This procedure was performed according to the manufacture's protocol for short fragments with the chemistry C2/P4. Each DNA sample was sequenced in one SMRTcell.

The sequences from each sample were derived with a use of RS ReadofInsert Protocol. Data preparation was then conducted in PRINSEQ Software (Schmieder and Edwards 2011). Primers from both ends were removed and sequence with minimum average quality of 20 (Phred score) were taken. In order to eliminate possible spurious PCR amplicons, only sequences of c. 157 bp were used for further analyses. All the sequences were then collapsed to unique haplotypes. Perl script was used to compare the obtained sequences against the NCBI nt nucleotide sequence database (ncbi.nlm.nih.gov), using the software BLAST 2.2.28+ (Camacho et al., 2009), followed by visualization of the results using MEGANv5.10.0 (Huson et al., 2011). All taxonomic matches of each read were evaluated and the lowest common taxonomic level assigned.

MEGAN processed the BLAST data of each sequence to determine all the hits. In the analysis, hits were discarded due to the threshold for the maximum percentage (top percentage, standard setting = 10). Based on the hits MEGAN finds the lowest node that encompassed all these hits using the LCA (Lowest Common Ancestor)—assignment algorithm to assign sequences to taxa. Minimum support was set to 1, implying that only one sequence had to be assigned to a taxon in order for the taxon to appear in the resulting cladogram. Min Score was set to 100 and Min Complexity to 0. Also hits that exceeded e-value 1E-20 were discarded.

A sequence was identified as belonging to a specific species when it met previous criteria and showed at least the 98% identity with a record in a database (Clare et al., 2009, 2011; Vesterinen et al., 2013). Each hit was then examined one by one to see, if the read was matching more than one species in the database. In fact, all matches at the specific level presented in Table 4 had at least 99% identity except for noctuid moth *Lithopane lambda* (98% identity).

A sequence was assigned to a family level based on the previous criteria used in MEGAN and when the identity with a record in a database was >95%. This rather conservative threshold for the family rank was based on the average congeneric divergence (i.e. at the lower level than the family) observed in COI sequences in Insecta, ranging from 4.5% in Bethylidae (Hymenoptera; Jiang et al., 2015), 4.7% in Muscidae (Diptera; Renaud et al., 2012), 5.1% in Lycaenidae (Lepidoptera; Wiemers and Fiedler, 2007), 6.4% in Aphidae (Hemiptera; Couer d'acier et al., 2014), 6.7% in Sacrophagidae (Diptera; authors' unpublished data), 7.3% in Drosophilidae (Diptera; authors' unpublished data) and up to 12.8% in Elateridae (Coleoptera; Oba et al., 2015) and 14.9% in Simuliidae (Diptera; Rivera and Currie, 2009).

Acoustic experiment

We used the Avisoft Vifa speaker to ensonify an insect (a housefly, Muscidae) on a perfectly smooth and flat surface that was bent to increasing degree to mimic the curved cylindrical surface of a wind turbine tower. The speaker was mounted such that the insect target sitting on the top of the curved surface was in the center of the speaker cone at 80 cm distance, thus being ensonified from the side. We used 3 different flies, each with a body length of ca 5 mm. Each one was ensonified 3 times. Fig. 2 represents the resulting spectrogram of the ensonification experiment for a tower diameter of 2.4 m.

Three linearly modulated sweeps were emitted consecutively with intervals of 10 ms (the intervals were shortened in the figure for display purposes). The emitted pulses swept from 75 to 50 kHz, from 50 to 25 kHz and from 25 to 0 kHz (8 kHz effectively), respectively, each sweep lasting 3 ms. The emitted pulses were sometimes received distorted or strongly amplitude modulated because the microphone (Avisoft CM16/CMPA [+/−3 dB; 8–75 kHz]) was directed to the same target-point as the loudspeaker, but only 4 cm from its center. Recordings were made with a sampling rate of 375 kHz and 16 bits.

Table 1
The bat carcasses analyzed and their origin. Asterisk indicates an individual with empty stomach, which was not analyzed. The wind farms are located as shown in Fig. 2.

Sample Id	Species	Wind farm	Date of carcass collecting
8224	*E. nilssonii*	A	3.9.2013
8227	*E. nilssonii*	A	29.8.2013
8225	*P. pygmaeus*	A	18.8.2013
8941	*P. pygmaeus*	A	7.9.2013
8223	*N. noctula*	B	16.8.2013
8226	*N. noctula*	B	16.8.2013
8943*	*P. pygmaeus*	B	16.8.2014
8944	*N. noctula*	B	16.8.2014
8945	*N. noctula*	B	16.8.2014
8946	*P. pygmaeus*	B	29.8.2014
8947	*P. pygmaeus*	B	29.8.2014
8949	*N. noctula*	C	8.9.2014
8951	*N. noctula*	C	8.9.2014
8952	*N. noctula*	C	8.9.2014
8953	*N. noctula*	C	1.9.2014
8950	*P. pygmaeus*	C	8.9.2014
8954	*V. murinus*	D	7.8.2012
8955	*N. noctula*	D	21.8.2012
8956	*E. nilssonii*	E	31.7.2014

Table 2
Result of the sticky trap sampling at nacelle level with respect to flies (Diptera). The sampling periods were two weeks, ending on the day given in the column. Non-dipteran insects were also recovered, but, with the exception of abundant swarming red fire-ants (*Myrmica* sp.), they were nearly always in poor shape and could not be identified.

Wind farm	Date	Number of Diptera	Taxon
C	14.8.2014	0	
E	3.9.2014	0	
E	9.7.2014	0	
E	20.6.2014	10	*Pollenia*
E	18.8.2014	0	
E	3.9.2014	1	undetermined
F	28.7.2014	0	
F	28.7.2014	1	undetermined
F	28.7.2014	0	
G	13.9.2014	0	
G	13.9.2014	1	*Calliphora*
G	13.9.2014	3	*Calliphora*, *Pollenia*
G	13.9.2014	12	*Calliphora*, *Pollenia*, undetermined Muscidae
G	13.9.2014	11	*Calliphora*, *Pollenia*, *Lucilia*, undetermined Muscidae

Table 3
Summary of sticky trap catches at four turbines at wind farm E in 2015, pooled across the sampling period (25 June–27 September). The number of trap days are in brackets. Insects with <4 mm wing span were omitted, as they are seldom caught by the bat species sampled in this study (see text). Asterisks indicate that the taxon was also recovered from the bat stomachs.

Taxon		Turbine no.					
Class/Order	Family	E5		E10		E3	E6
		Base (217)	Nacelle (168)	Base (41)	Nacelle (85)	Base (42)	Base (84)
Araneae*			3	1		2	
Hexapoda							
Heteroptera	Cercophidae	1					
	Aphidae*		2	1	1	2	
Psocoptera*		9					
Neuroptera*		1					
Trichoptera*		1					
Lepidoptera*		11					
Hymenoptera	Formicidae	100+	100+		100+	100+	40
	Vespidae						1
	Ichneumonidae*	1				1	3
	Others	1		1		1	3
Coleoptera	Carabidae*	1					
	Chrysomelidae	3				1	
	Coccinellidae*	2				1	5
	Cerambycidae					1	2
	Elateridae						1
	Curculionidae*	2		1			
	Others	4					
Diptera	Chironomidae*	2	1				1
	Asilidae			1			
	Dolichopodidae*					1	1
	Empididae?	2					
	Hybotidae	13				1	1
	Sciaridae*	1					
	Syrphidae*	1					
	Chlorophidae	20					1
	Anthomyidae*	3				3	3
	Muscidae*	6	1				1
	Sarcophagidae*	1					3
	Calliphoridae*	7	3				
	Others	3					

Results

Bats sampled

We collected 22 specimens belonging to four species of aerial-hawking bats. Nineteen of the carcasses had intact stomachs but in one case the stomach was empty. Hence 18 specimens were suitable for diet analysis. The carcasses were collected between late July and early September 2012–2014 at five wind farms in southern Sweden, with the numbers for each wind farm shown in Table 1. For site A the ground beneath turbines were searched daily throughout the summers (June–September) and in this case the carcasses were probably found the day after death. For the other sites, searches were made less frequently and less regularly and the time between deaths of the bats and collection of the carcasses are unknown. One specimen (no. 8956) was noticeably rotten, but the others appeared fresh.

Sticky trap sampling

Insect taxa and individuals on the traps at nacelle level were few, reflecting low diversity of arthropods at such height. The samples were dominated by fire ants (*Myrmica* spp.), which swarm during the day (Douwes et al., 2012), and in some cases almost covered the trap. There were also several species of common calyptrate flies, particularly blow-flies (Calliphoridae) and houseflies (Muscidae) as well as winged aphids. The traps also contained occasional small beetles, parasitic wasps and the like, but these insects were few and usually in bad shape and thus hard to recognize (Tables 2 and 3).

In the 2015 samples from wind farm E, the traps from the base of the towers showed a much higher abundance and diversity of arthropods than the traps from the nacelle level (Table 3). The catches included nocturnal as well as diurnal forms and were probably representative of what occurs below tree-top level. Interestingly, the traps from the tower bases contained very few crepuscular or nocturnal, thin-bodied and swarming flies (Nematocera; e.g. Chiromidae), although such insects usually are very abundant in the air nearby. In contrast, several diurnal thick-bodied and more or less warm-blooded forms (Brachycera, including the calyptrate taxa) were captured. This suggests that the latter but not the former tended to rest of the surface of the tower, and this may have implications for their vulnerability to bats (see below).

Bat diet

Generally the diets of the bats were very diverse in terms of number of taxa eaten (Tables 4 and 5). However, diurnal Diptera of one kind or another, e.g. flesh-flies (Sarcophagidae), blow-flies (Calliphoridae) and houseflies (Muscidae) among the Calyptrata and hover-flies (Syrphidae) and fruit-flies (Drosophilidae) appeared in most (12 of 18) stomachs (5/5 *P. pygmeaus*, 3/9 *Nyctalus noctula*, 3/3 *Eptesicus nilssonii* and the 1/1 *Vespertilio murinus*; Table 4). Several almost or entirely flightless and/or diurnal beetles, such as ground beetles (Carabidae), true weevils (Curculionidae) and lady-beetles (Coccinellidae) were also recovered. Hence diurnal and/or flightless insect taxa occurred consistently in three of the four bat species, but less frequently in *N. noctula* (9/9 vs. 3/9).

However, most identified prey taxa were not diurnal or flightless, but included more typical prey for aerial-hawking bats such

Table 4
The number of sequence reads of arachnids and insects in the diet of four bat species assigned to a family level for each individual bat. Names in bold represent families of diurnal flies. Families which were also identified at the species level are indicated by asterisks (see Table 5).

Order	Family	*P. pygmaeus*					*N. noctula*									*E. nilssoni*			*V.murinus*
		8225	8941	8950	8946	8947	8223	8226	8944	8945	8949	8951	8952	8953	8955	8227	8224	8956	8954
Araneae	Theridiidae*				14														
Coleoptera	Carabidae*																154		425
	Coccinellidae*									22									
	Curculionidae	132																	
	Dytiscidae*	5														385			556
	Hydrophilidae*																		7
	Nitidulidae*														27				
Diptera	Anthomyiidae*	**4**															**116**		
	Calliphoridae															**7**			
	Ceratopogonidae*	32																	
	Chironomidae*	8906	484	138	7641	11724			734	11	12	6	1185			343	8007	4	30
	Culicidae*	303	8	1663					2	93	21	666	355		11		31	434	125
	Dolichopodidae														3				
	Drosophilidae*	**67**	**12**	**26**	**39**	**31**						**5**						**96**	
	Fanniidae														**16**				
	Psychodidae*				570														
	Sarcophagidae	**799**		**444**	**231**		**5**					**511**			**4**		**304**	**12**	**30**
	Sciaridae*		9	53	3														
	Syrphidae					**4**													
	Tephritidae														**6**				
	Tipulidae*			11			174	8		6			75		4			777	169
Ephemeroptera	Baetidae										3891								
	Caenidae	8				191													
Hemiptera	Acanthosomatidae					7			4	43			170	130	4411				
	Aphididae*	219				15												38	
	Corixidae*	9								15						5			13
	Drepanosiphidae					27					4							389	16
	Lachnidae		22																
	Miridae*																747	84	3
	Nabidae*	4													4				22
Hymenoptera	Ichneumonidae*			13															
Lepidoptera	Crambidae*																	12	3
	Geometridae*							2								237		7	
	Gracillariidae*			20		17			2		8	4						59	
	Hepialidae	432																	
	Momphidae*	58																	
	Noctuidae*							5129				121			3	7902	35	6	
	Plutellidae*	81		5											13	13		15	
	Pyralidae*							63			28		55						
	Tortricidae*		127							68		56			59			226	8
	Yponomeutidae*		16															7	138
Neuroptera	Chrysopidae*		449	145		243			3	79	207	163	255	203	193			126	103
	Hemerobiidae*		1883	1359		107	9		2	13			33	20	218		12	92	1118
Trichoptera	Leptoceridae				24				4423	34	75		585						
	Limnephilidae*		1825	451	12		110	3375	155	1376	59	59	257	101		597	5576		
	Polycentropodidae*			3														87	
	Psychomyiidae*			10															
Number of reads		11,059	4835	4341	8509	12,366	298	8577	5325	1760	4305	1591	2970	454	4972	9489	14,982	2471	2766

Table 5
List of arthropods identified to the species level (identity ≥ 98%) in the stomachs of different species of bats and the number of reads for each bat species. In a few cases only matching sequences were present (without species Id) and then BOLD System Numbers are shown.

Order	Family	Species	*P. pygmaeus*	*N. noctula*	*E. nilssoni*	*V. murinus*
Araneae	Theridiidae	*Platnickinia tincta*	11			
Coleoptera	Carabidae	*Amara apricaria*				68
		Trechus quadristriatus				73
	Coccinellidae	*Harmonia axyridis*		13		
	Dytiscidae	*Ilybius ater*				367
		Rhantus suturalis			193	
	Hydrophilidae	*Hydrobius fuscipes*				4
	Nitidulidae	*Cychramus variegatus*		13		
	Scirtidae	*Contacyphon padi*	1			
Diptera		Diptera sp. BOLD:AAG4872		70		1
	Anthomyiidae	*Delia platura*			1	
	Ceratopogonidae	*Culicoides grisescens*			1	
	Chironomidae	*Ablabesmyia aspera*	44			
		Ablabesmyia monilis	3			
		Chironomus aprilinus	4980	616	4744	
		Dicrotendipes nervosus	1,223		1	
		Glyptotendipes barbipes	24	2	30	
		Halocladius variabilis	3			
		Macropelopia notata	3			
		Procladius furrugineus	169			
		Tanytarsus mendax	1567			
	Culicidae	*Aedes vexans*	102	77		
		Anopheles claviger	3			
		Culex pipiens complex	1374	148		
		Culex torrentium/vagans	47	2	7	
		Culiseta morsitans	7	275		
		Ochlerotatus detritus		11		
	Drosophilidae	Drosophilidae sp. BOLD:AAG8493	24		53	
		Scaptomyza flava	28			
	Muscidae	*Hydrotaea irritans*		1		
	Psychodidae	*Psychoda phalaenoides*	407			
	Scathophagidae	*Scathophaga stercoraria*		101		
	Sciaridae	Sciaridae sp. BOLD:AAM9252	34			
	Syrphidae	*Eupeodes corollae/bucculatus*	3			
	Tipulidae	*Tipula paludosa*				18
Hemiptera	Aphididae	*Euceraphis betulae*	14	4	264	13
		Hyalopterus pruni	156			
		Rhopalomyzus lonicerae	2			
		Tuberolachnus salignus	19			
	Cicadellidae	*Empoasca decipiens*	1			
	Corixidae	*Sigara falleni*		8		
	Mirinae	*Phytocoris tiliae*			66	
	Nabidae	*Nabis ferus*		2		18
Hymenoptera	Ichneumonidae	*Gelis* sp. BOLD:AAI5646	310			
Lepidoptera	Crambidae	*Agriphila straminella*			5	2
	Crambidae	*Eudonia lacustrata*			5	
	Geometridae	*Pelurga comitata*			4	
	Gracillariidae	*Caloptilia strictella*	1			
		Phyllonorycter apparella				
	Momphidae	*Mompha epilobiella*	28			
	Noctuidae	*Lithopane lambda*		2		
	Plutellidae	*Plutella xylostella/karsholtella*	34	7	15	
	Pyralidae	*Dioryctria abietella*		30		
	Tortricidae	*Cydia splendana*				3
		Epinotia nisella	103	136	181	
		Epinotia solandriana				3
		Eudemis profundana		1		
		Hedya nubiferana			1	
	Yponomeutidae	*Argyresthia goedartella*	9		3	67
Neuroptera	Chrysopidae	*Nineta vittata/flava*				19
		Nothochrysa fulviceps			3	
		Peyerimhoffina gracilis	187	457	46	30
	Hemerobiidae	*Hemerobius lutescens*			8	
		Hemerobius pini	6			
		Micromus angulatus		1		
		Wesmaelius subnebulosus		1		1
Psocoptera		Psocoptera sp. BOLD:AAN8447	4	8		
Trichoptera	Limnephilidae	*Limnephilus indivisus*		5		
		Limnephilus sansoni		1		
	Polycentropodidae	*Polycentropus flavomaculatus*	2		69	
	Psychomyiidae	*Lype phaeopa*	7			

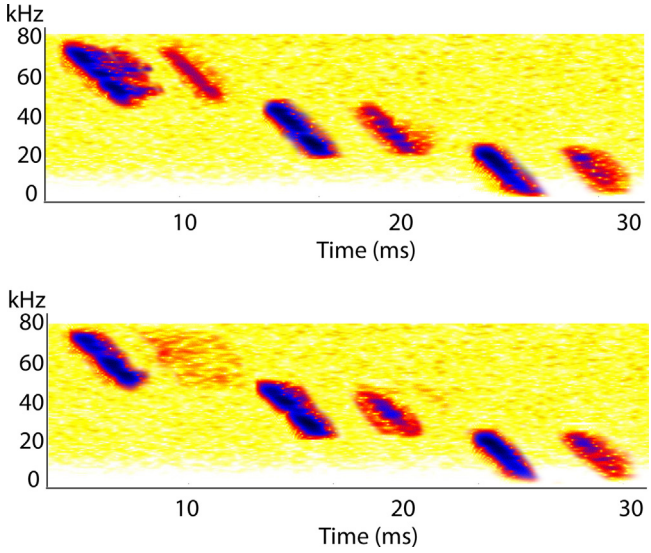

Fig. 2. Spectrogram showing echoes from a housefly on a cylinder 2.4 m in diameter, mimicking a wind turbine tower (above), and the same setup without the fly (below).

as midges (Chironomidae), crane flies (Tipulidae), mosquitoes (Culicidae) and other small and mostly crepuscular or nocturnal flies, but also caddis-flies (Trichoptera), mayflies (Ephemeroptera), and moths (Lepidoptera). The latter category included migrating species such as the cabbage moth *Plutella xylostella* (Plutellidae; Table 5).

Acoustic experiment

The plastic board mimicking the turbine tower produced a great deal of clutter, particularly when approached horizontally and against the curvature. Moreover, the lower frequencies (<50 kHz) did not pick up the echo from the fly very efficiently. Hence, the resulting sonograms showed clear echoes from the fly only when the high frequency sweep (75–50 kHz) was used. The two lower frequency sweeps show about equal echo intensities for cylinder with and without the fly (Fig. 2). Hence, both the short wavelength and the narrow beam-width of the high-frequency pulse facilitate detection of small targets sitting on the surface and, in addition, the surface must be approached at low angle to minimize the clutter.

Discussion

Calyptrate Diptera and diurnal or flightless insects do not normally appear in the diet of any of the bat species studied here (e.g. Swift et al., 1985; Rydell, 1989, 1992), although remains of muscid flies sometimes appear in droppings of *Pipistrellus pygmaeus* and its sibling species *Pipistrellus pipistrellus* (Barlow, 1997; Bartonička et al., 2008). Nevertheless we found such insects quite frequently both on the turbine surfaces and in the bat stomachs.

However, the correspondence between the trap catches at nacelle height and the bat stomachs was poor, suggesting that bats did not forage extensively at nacelle height during the time of sampling. Instead, the trap catches from the tower base were more similar to the stomach contents in terms of insect diversity and taxa. Indeed, 17 of 28 (61%) taxa, recognized at the Family level, from the sticky traps at the tower bases were also recovered from the bat stomachs (Table 3). Assuming that bats had fed from the wind turbines, the data indicate that they had fed more frequently on the lower part of the turbine towers than from the top.

As an example, the Sarcophagidae (flesh-flies), which are mostly large calyptrate flies, occurred both on the traps from the tower base and in the stomachs of each bat species in this study (Tables 3 and 4). They have not been found in any of these bat species before. The Sarcophagidae are hot-blooded and diurnal (Willmer, 1982) and we believe that their basking or roosting behavior on the smooth surface of the turbines made them particularly detectable and vulnerable to bats. This may also apply to e.g. the other calyptrate flies such Anthomyidae, Calliphoridae and Muscidae and some other diurnal flies as well (e.g. Drosophilidae, Syrphidae).

The typical food of all the bat species we examined consists of nocturnal or crepuscular nematoceran Diptera, and other insects that occur near water (Swift et al., 1985; Rydell, 1989, 1992; Barlow, 1997; Rydell and Petersons, 1998) and the majority of the prey species recovered from the bat stomachs in this study were of this category. Hence, in contrast to the calyptrate dipterans, it seems most likely that these insects usually were captured in flight. This is corroborated by their near absence on the stick traps from both tower bases and nacelles.

Insect remains in stomachs and intestines of the North American bats *Lasiurus cinereus* and *Lasionycteris noctivagans* killed at wind turbines consisted mostly of moths, the normal diet of these species, but there were also small numbers of diurnal and/or non-flying taxa, including calyptrate flies (Calliphoridae and Muscidae; Reimer et al., 2010; Valdez and Cryan, 2013). Hence the situation in *Lasiurus* seems to be similar to that in *N. noctula* in our study, with some prey items that could have been captured at or near the surface of the towers. *Lasiurus* and *Nyctalus* in particular are ecologically similar, both being relatively large and fast flying bats that normally feed in the open air. The moths that constituted the bulk of the prey of *L. cinereus* may of course have been captured far away from the turbines (Valdez and Cryan, 2013), but on the other hand, we have observed that moths, like flies, sometimes rest on turbine towers (Table 3 and unpublished observations). Therefore, we would not exclude the possibility that some of the moths were also taken at or near the tower surface.

We did not make any direct observations of bats that captured flies or other insects at the wind turbines. However, it seems possible that the roosting flies were taken from the surface or, perhaps more likely, in the air near the surface after having been disturbed or touched by the bat. Although e.g. calyptrate flies are generally diurnal, they may not be entirely flightless at night, but will move short distances if disturbed while sitting on the surface of a wind turbine (authors' unpublished observations). Indeed, flesh-flies and blow-flies and some houseflies occasionally visit carcasses at dusk or at night, so they can presumably fly at least short distances (Peng et al., 1992; Stamper and DeBry, 2007). Likewise, dispersal flights occur in some carabid beetles (including *Amara* sp.), which do not fly otherwise (van Huizen, 1977), and we appreciate that our distinction of which insects are diurnal and which are not may not be entirely accurate. Nevertheless, the abundance and diversity of diurnal and/or flightless taxa in the bats' stomachs and the consistency by which they occurred there suggest that they were not all captured in flight.

It seems that the behavior as performed by bats at wind turbines has already been described in some detail, although the observers' interpretations of the behavior were different from ours. For example *L. cinereus* in North America has been observed to circle and approach the same spot of the turbine tower repeatedly from below and at a low angle, as if they "investigated" it for places to roost, and it was argued that they "mistake" wind turbines for trees (Cryan, 2008; Horn et al., 2008; Cryan et al., 2014). Bats that repeatedly approached certain spots on turbine towers have also been interpreted as attempting to drink, presumably "mistaking" the vertical tower for a water surface (Hale et al., 2013). What seems to be the same behavior was described by Ahlén et al. (2007), observing *E. nilssonii* and *P. pygmaeus* at wind turbines, but the

interpretation in this case was that the bats gleaned insects from the surface. To distinguish between the different interpretations of what seems to be the same behavior have not been done, because of the practical difficulties in observing insects over the distances involved in the dark. Interestingly, Cryan et al. (2014) observed that *L. cinereus* approached the surface of turbines predominantly against the wind. This is to be expected if the bats searched for insects, which usually rest on the leeward side of the tower (Dudek et al., 2015; authors' unpublished observations), but likely not if they search for roosts, mates or water.

The bat species examined in this study are described as strictly aerial-hawkers and as far as we know they do not use a gleaning foraging technique. However, our ensonification experiment suggested that the resting insects provided the essential acoustical cues to the bats, and based on the behavioral observations referred to above, it seems possible that the bats actually captured insects from the surface or caught them close to it, probably guided by echolocation. This strategy would only work on smooth surfaces, such as a turbine tower, which can be approached at a low angle to eliminate much of the clutter. In "natural" and acoustically more complex situations, such an approach would be harder to use due to excessive clutter from vegetation and rough or irregular surfaces. However, the behavior may be similar to that of bats taking prey from calm water surfaces (e.g. Britton et al., 1997; Rydell et al., 1999). The noctule is the largest and least manoeuvrable of the bats studied here and therefore probably the one that is least likely to pick insects from a surface. Indeed, while the other three species consistently had fed on diurnal or flightless taxa, this was only the case in one third of the noctules (Tables 4 and 5).

The measurement of acoustic reflections from insects resting on a cylindrical surface mimicking the tower base revealed that flies the size of a housefly can only be detected well when high frequency sound is used (75–50 kHz). The wavelengths at these frequencies approach the size of the fly, which will therefore reflect the sound more strongly than at lower frequencies. Larger flies will reflect echoes also at lower frequencies (20–40 kHz), which dominate the echolocation calls of the species investigated here, with the exception of *P. pygmaeus* (50–60 kHz). However, lower frequencies are less directional than high frequencies and would also include more echoes from the tower surface, as seen in Fig. 2. The loudspeaker beam-width at the frequencies used here narrows down to 10° (−6 dB) so that relatively less of the reflected intensity consists of cluttering echoes. In reality, however, bats are unlikely to achieve such narrow beam-widths, so the noise from the tower as received by bats will be higher than depicted in Fig. 2.

There is a notable absence of feeding-buzzes and associated behaviors, that would have indicated aerial insect pursuit, from some observations of bats at wind turbines (e.g. Arnett et al., 2015; Rydell and Wickman, 2015), although there are also exceptions (e.g. Horn et al., 2008). However, if bats actually pick insects from the surface of the tower or close to it, they would not be expected to use echolocation calls and feeding-buzzes designed for aerial hawking, but rather relatively low-amplitude, short, broad-band pulses, like those used while gleaning or perhaps drinking, i.e. when approaching a surface from a low angle (e.g. Faure et al., 1993; Russo et al., 2007, 2015).

In conclusion, the absence of detectable feeding-buzzes of bats observed at wind turbines in some studies should not be used to argue that they do not feed, only that they do not forage by aerial-hawking in the open. Indeed, the frequently observed approaching ("investigating") behavior of several species of bats at wind turbines is in agreement with our hypothesis and the results of this study. Taken together, the observations suggest that several species of bats sometimes feed on insects that rest on the tower of wind turbines.

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