

The effectiveness of katydid (*Neoconocephalus ensiger*) song cessation as antipredator defence against the gleaning bat *Myotis septentrionalis*

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Abstract Many nocturnal katydids (Orthoptera: Tettigoniidae) produce intense calling songs, and some bat species use these songs to detect and locate prey. One Nearctic katydid species, *Neoconocephalus ensiger*, ceases or pauses singing in response to bat echolocation calls. We tested the hypothesis that song cessation is an effective defence against gleaning bats (i.e., bats that take prey from surfaces). We observed *Myotis septentrionalis*, a sympatric bat species that uses prey-generated sounds when gleaning, attack and feed on singing *N. ensiger* in an outdoor flight room. These bats demonstrated a preference for the calling song of *N. ensiger* over a novel cricket calling song when they were broadcast from a speaker in the flight room. Bats attacked speakers broadcasting *N. ensiger* calling song as long as the song was continuous and aborted their attack if the sound stopped as they approached, regardless of whether a katydid was present as a physical target on the speaker. Echolocation calls were recorded during attacks and no significant differences were found between continuous and interrupted song approaches for four call parameters, suggesting that *M. septentrionalis* may not use echolocation to locate silent prey. Therefore, song

cessation by katydids in response to ultrasound is an effective defence against gleaning bats.

Keywords Antipredator behaviour · Echolocation · Gleaning · Tettigoniidae · Chiroptera

Introduction

Effective defence against predation depends on the sensory ecology of both predator and prey (Kavaliers and Choleris 2001). Prey species that cannot detect predators rely on primary defences that prevent encounters, whereas those that are sensitive to predator cues may use secondary defences in response to a perceived predator, as observed in moths (Soutar and Fullard 2004). The optimal defence strategy also depends on the cues used by the predator for prey detection and localisation. Many predators appear to rely on several senses for prey detection (Montgomerie and Weatherhead 1997; von der Emde and Bleckmann 1998). Electric fish can use active electrolocation, passive electrolocation, vision, and water movement to locate prey and they switch among these senses when one is impaired (von der Emde and Bleckmann 1998). Rattlesnakes, however, are compromised in their ability to detect prey if radiation cues (visual and infrared) are not available (Kardong and Berkhoudt 1999). If a predator is predominantly dependent on a single sensory modality or cue, intended prey could thwart an attack by depriving the predator of this stimulus during an encounter.

Insectivorous bats detect prey primarily through a single modality: hearing. The most common foraging strategy observed in insectivorous bats is aerial hawking (Wilson 1973; Findley 1993), during which prey are located in flight using echolocation, but some species are known to

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glean, i.e. capture prey from surfaces such as vegetation and the ground. For most gleaning bats studied, prey-generated sounds are necessary and sufficient to detect and locate prey (Faure and Barclay 1992; Anderson and Racey 1993; Arlettaz et al. 2001). These bats produce frequency-modulated downward-sweeping echolocation calls during gleaning that are shorter, of greater spectral bandwidth, and less intense and frequent compared to those produced during aerial hawking (Norberg and Rayner 1987; Neuweiler 1989). *Megaderma lyra* responds to prey sounds (Hübner and Wiegrebe 2003) but is also known to use multiple harmonic, high frequency echolocation calls, which allows for the separation of motionless prey from background (Schmidt 1988; Schmidt et al. 2000). Therefore, within a single sensory modality, gleaning bats have the potential to use echolocation, listen to prey-generated sound, use both of these strategies concurrently (but see Barber et al. (2003)), or switch between these two strategies to locate their prey.

Many groups of insects have bat-detecting ears and initiate evasive flight manoeuvres in response to ultrasound (Hoy 1992; Miller and Surlykke 2001), but less is known about the ability of perched insects to effect a secondary defence against a gleaning bat. For gleaning bats that use prey-generated sounds to locate prey, an insect could potentially thwart a gleaning attack by becoming silent upon hearing the bat. Male katydids (Orthoptera: Tettigoniidae) produce intense calling songs to attract females. Acoustic signals such as these are easily located and can be detected at long distances, making them excellent for mate attraction, but also vulnerable to eavesdropping by predators (Sakaluk and Belwood 1984; Zuk and Kolluru 1998). Several gleaning bat species are known to use orthopteran calling songs to detect and locate these insects (Fenton et al. 1983; Tuttle et al. 1985; Belwood and Morris 1987; Bailey and Haythornthwaite 1998). Katydids have ears that are sensitive to ultrasound (Hoy and Robert 1996) and some species exhibit an in-flight response to ultrasound (Libersat and Hoy 1991; Schulze and Schul 2001). Faure and Hoy (2000a) first reported that the katydid *Neoconocephalus ensiger* Harris will startle (cease or pause singing) in response to pulses of ultrasound and suggested that this may be a defence against gleaning bats. When tested for their ability to respond to gleaning attack echolocation sequences of a sympatric bat species (*Myotis septentrionalis* Trouessart), *N. ensiger* ceased singing in response to these sequences at realistic intensities (ter Hofstede and Fullard 2008).

We had three objectives for this study. The first was to determine if the Northern long-eared bat, *M. septentrionalis*, gleans katydids, and if so, to describe their feeding behaviour. We investigated *M. septentrionalis* because it is a documented gleaner and has a similar geographic range to

N. ensiger (Caceres and Barclay 2000; Walker and Moore 2007). The second objective was to determine the cues used by these bats to locate katydids. *M. septentrionalis* is known to rely on prey-generated sounds when gleaning (Faure et al. 1993; Ratcliffe and Dawson 2003) so we predicted that calling song would be sufficient. The third objective was to determine if song cessation is an effective defence against gleaning bats. We tested the hypothesis that *M. septentrionalis* requires continuous prey-generated sounds (i.e. calling song) to locate *N. ensiger*, and we predicted that bats would not succeed in locating their target if the calling song stopped during the bat's approach.

Materials and methods

Study area and animals

We conducted these experiments in July and August of 2005 to 2007 at the Queen's University Biological Station, near Kingston, Ontario, Canada. Bats (*M. septentrionalis*) were captured in modified harp traps (Tuttle 1974) positioned at the entrances of local abandoned mines, and they were housed indoors in screened cages (H 60 cm×W 40 cm×D 40 cm). They were provided with water ad libitum and fed with mealworms (*Tenebrio molitor* Linnaeus) after trials. A total of 75 individuals were used in the following experiments. Katydids were collected in local fields by following the sound of their song and picking them off grass stems and shrubs. They were housed in plastic and metal mesh cages with water, cat food, grass, and pieces of apple. For experiments requiring dead katydids, we froze and thawed the katydids before presenting them to the bats, and bats ate these as readily as live katydids. For experiments requiring bats in flight, we used a large outdoor flight room with an earthen floor consisting of a wooden frame with fibreglass mesh panels for walls and ceiling and measuring 9.14 m long, 3.66 m wide, and 3.66 m high, as described in Ratcliffe and Dawson (2003). All experiments were conducted in darkness and behavioural responses were either monitored with a night vision scope or near-infrared light sources and video camera.

We recorded the songs of one wild male *N. ensiger* and one lab colony male Australian field cricket, *Teleogryllus oceanicus* Le Guillou (Orthoptera: Gryllidae) (a Pacific species (Simmons 2004) with which this bat would not have had previous experience), within a sound attenuating chamber using a 6.35 mm microphone (2200C, Larson Davies, New York, USA), anti-aliasing filter (150 kHz, Pettersson Elektronik AB, Uppsala, Sweden), data acquisition card (DAQCard 6062E, National Instruments, Austin, TX, USA), and laptop running BatSound Pro (Pettersson Elektronik AB) at a sampling rate of 500 kHz. We created

files consisting of a single call (30 ms duration, 12.6 kHz peak frequency) and 42 ms of silence for *N. ensiger* and one long chirp (five pulses) followed by nine short chirps (two pulses each) for *T. oceanicus* (31 ms mean duration of pulses, 5.0 kHz peak frequency). A custom built MATLAB (Version R2006b, The MathWorks, Natick, MA, USA) application looped these files to produce continuous calling song that was typical for each species (*N. ensiger*, Faure and Hoy (2000a); *T. oceanicus*, Simmons (2004)) and similar in duty cycle, the percentage of time occupied by sound (*N. ensiger*, 42%; *T. oceanicus*, 48%). Although we only used the calling song of one individual for each species, the calling songs for these species have little variability and by comparing our playback recordings with recordings of other individuals and published accounts, the songs we used can be considered representative for each species. During playback experiments, calling songs were broadcast to the bats at the same intensity, 93 dB peak equivalent SPL (peSPL) at 10 cm as compared to a continual pure tone of 13 kHz for *N. ensiger* and 5 kHz for *T. oceanicus* (Stapells et al. 1982) from a laptop via data acquisition card, ultrasonic amplifier (70101, Avisoft Bioacoustics, Berlin, Germany), and speaker (ScanSpeak 60102, Avisoft Bioacoustics) at a sampling rate of 500 kHz. This is the intensity recorded for *N. ensiger* in Faure and Hoy (2000a).

Bat feeding behaviour

Individual *M. septentrionalis* were released into the flight room with several *N. ensiger*, and singing katydids were observed with a night vision scope. In this situation, we observed three *M. septentrionalis* capturing singing *N. ensiger* from the walls of the flight room. We pursued three lines of evidence to determine if and how *M. septentrionalis* consumes katydids. First we wished to determine if these bats are capable of removing the katydids from their natural perches, tall grasses. We exposed six of the 25 bats we captured in 2005 to katydids singing in local species of potted grasses the first time they were released into the flight room and observed their behaviour. Second, we observed prey handling behaviour. Seven bats were placed individually with two or three live *N. ensiger* in the bat housing cages described above and videotaped in a dimly lit room. Third, we conducted fecal analysis. Six bats were placed individually in plastic feeding boxes with two to five dead katydids for 2 h and both the culled parts and feces were collected. We conducted fecal analysis on both the feces collected from these six bats that had only eaten katydids for one night and 39 bats at the time of capture. Feces were stored in 70% ethanol and teased apart in a petri dish under a dissecting scope. Insect pieces were compared to a representative insect collection for the area. We looked

specifically for identifiable katydid parts and did not attempt to identify other insects in the diet.

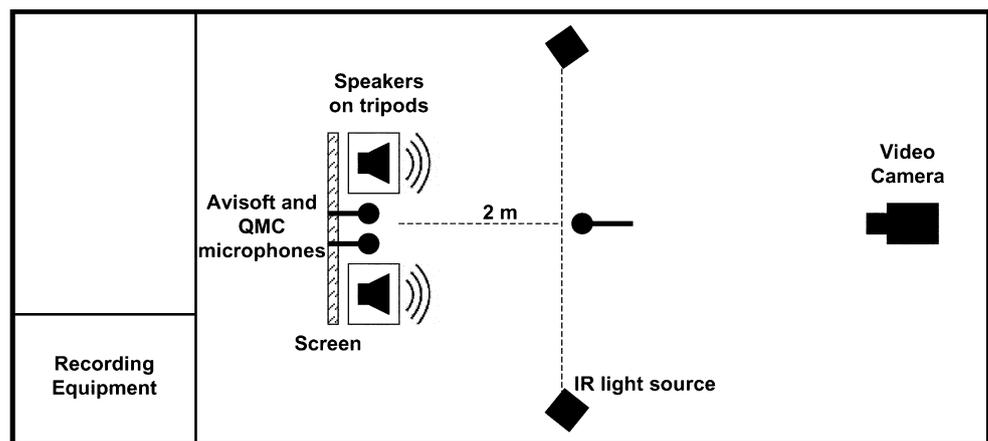
Bat responses to orthopteran calling songs

In 2005, 25 naive bats were exposed to either live singing katydids (nine bats total, six to katydids in potted grasses (see above) and three to katydids singing on the walls of the flight room) or a speaker broadcasting the calling song (16 bats) for 15 min after being released individually into the flight room within 48 h after capture. We scored whether the bats exhibited a response to the calling song during this time, defined here as landing on or hovering within 20 cm of the katydid or speaker. Next we tested if this response was specific to *N. ensiger* calling song. In 2006, 50 naive bats were exposed to 15 min of *N. ensiger* calling song and 15 min of *T. oceanicus* calling song, a novel acoustic stimulus for these bats. This second treatment provided a control to test if these bats investigate orthopteran calling songs in general, or if the response is specific to *N. ensiger*. We chose *T. oceanicus* as our control because an Australian vespertilionid gleaning bat (*Nyctophilus geoffroyi*) sympatric with *T. oceanicus* and of similar size to *M. septentrionalis* can locate these crickets using their calling song (Bailey and Haythornthwaite 1998). These treatments were played consecutively and the treatment presented first was alternated between bats. In August, 2007, we compared the responses of 12 *M. septentrionalis* to the calling song of *N. ensiger* and *Amblycorypha oblongifolia* De Geer, the latter being a larger local katydid species with a lower duty cycle calling song (see ter Hofstede and Fullard (2008) for details on the calling song of this species). We directly measured sound intensity for ten calls per individual as SPL using the hold function on the measuring amplifier. The average for each species (*N. ensiger*, 98 dB SPL at 10 cm; *A. oblongifolia*, 95.5 dB SPL at 10 cm) was used for playbacks to bats. We used the same playback set up as described above for comparison with *T. oceanicus* calls, but in this case we also recorded the latency to respond to the playback.

Bat response to song cessation

In 2006, 14 of 50 bats tested demonstrated a response to *N. ensiger* calling song (see “Results” for details). Three of these bats stopped responding to the song during the experiment reducing our effective sample size to 11 bats. Figure 1 is a schematic of the physical layout of this experiment. Two Avisoft speakers were placed in individual speaker boxes composed of five sides wood and one side fibreglass mesh allowing the sound to transmit unimpeded while providing a landing place for the bat. For each trial, one of the speakers produced sound while the other was

Fig. 1 Schematic of the physical layout of the flight room experiment



silent, and the location of the active speaker was randomised between trials. The speakers were placed at one end of the flight room on tripods 0.7 m apart facing the centre of the room. A fabric screen behind the tripods shielded the bats from the equipment and observers located at this end of the room. Behaviour was monitored and recorded using an infrared-sensitive CCD camera (VCB-3524, Sanyo, San Diego, CA, USA) situated at the rear of the room facing the speakers, two near-infrared light sources, a digital video converter (ConvertX PX-M402U, Plextor, Fremont, CA, USA), and a laptop running video capture software (InterVideo WinDVD Creator, Corel Corp., Ottawa, Canada). A QMC 200S (UltraSound Advice, London, UK) and an Avisoft CM16 condenser microphone were placed between the speakers, and a third microphone (Avisoft CM16) was placed in the centre of the room facing the speakers to record the katydid calling song. The QMC microphone was set to frequency division heterodyne output and connected to the audio input on the digital video converter producing an audible trace on the video. Both Avisoft microphones were recorded via a USB ultrasound acquisition board (Avisoft Ultrasound Gate 416) connected to a laptop running Avisoft Recorder at a sampling rate of 300 kHz.

Each of the 11 bats was released into the flight room and exposed once to each of four treatments: continuous song with a physical target (dead katydid on active speaker), continuous song without a physical target (dead katydid on silent, i.e. mismatched, speaker), interrupted song with a physical target, and interrupted song with a mismatched target. These treatments were presented in random order to each bat individually. When the song started playing for each treatment, the behaviour of the bat was observed on a monitor, and for the treatments in which the song was interrupted, we turned off the sound when the bat was approximately 1 m away from the speaker and clearly approaching it. For all trials, we documented whether the bat landed on a speaker box and recorded the echolocation calls.

We isolated the sections of the echolocation call recordings when bats were within approximately 1 m of the speaker using Studio (v. 10, Pinnacle Systems, Mountain View, CA, USA). As we were only interested in potential differences between the echolocation calls produced when the calling song was continuous vs. interrupted, we only compared the sequences from 1 m away from the speaker to landing, or 1 m away from the speaker to the point where the bat turned and flew away from the area of the speaker. We bandpass filtered the call recordings between 25 and 130 kHz to remove katydid song and noise. For each call with a good signal to noise ratio (i.e. FFT spectral peak 15 dB above background noise) we measured duration (ms) and instantaneous call rate (calls/s, calculated as the inverse of the time from the beginning of one call to the beginning of the next call) from the oscillogram and peak frequency (kHz, frequency with the most energy) and bandwidth (kHz, the difference between lowest and highest frequencies 15 dB less intense than the peak frequency) from the power spectrum (1024-point fast Fourier transform, Hamming window). For statistical analyses, each data point was the mean of the call data for each bat per trial ($n=2-31$ calls).

Results

Bat feeding behaviour

We observed seven bats feeding on live *N. ensiger* in cages. Three of the bats captured katydids on the floor of the cage, and four captured them from the walls. All seven of the bats captured and ate katydids in a stereotypical manner. They landed on top of the katydid, covered it with their wings, and bit down repeatedly on the anterior thorax. They then hung from the wall of the cage in a head-upright position, holding the screening with their thumbs and hind feet, forming a pouch with their tail membrane over which they ate. By comparison, these bats have been observed to eat

Table 1 Number of bats demonstrating a response to orthopteran calling song

	Mid-July to early August			Mid-August			Late August to early September		
	2005	2006		2005	2006		2005	2006	
	<i>N.e.</i>	<i>N.e.</i>	<i>T.o.</i>	<i>N.e.</i>	<i>N.e.</i>	<i>T.o.</i>	<i>N.e.</i>	<i>N.e.</i>	<i>T.o.</i>
Response	1	1	1	6	0	0	6	13	4
No Response	6	9	9	0	14	14	6	13	22

Landing on or hovering within 20 cm of a singing katydid or a speaker broadcasting the calling song was scored as a response; 15 min without a response was scored as no response

N.e. *Neconocephalus ensiger*, *T.o.* *Teleogryllus oceanicus*

moths in a head-up position, head-down position, or in flight (J. M. Ratcliffe, unpublished). They always consumed katydids from the anterior to the posterior end, allowing the harder parts, such as mouthparts, wings, and legs, to fall into the pouch. When they had finished consuming the body, they picked through the pieces remaining in the pouch, chewing on the meatier parts and discarding the rest. Handling time was 8.1 ± 0.7 min (mean \pm SEM). The culled parts dropped by the bats were quantified as the percentage of the total number of parts present on the katydids provided for feeding (two to five katydids per bat, 18 katydids total). All of the fore- and hindwings, $70 \pm 4\%$ of the legs, $77 \pm 17\%$ of the antennae, and $79 \pm 12\%$ of the mandibles were recovered (i.e. were not eaten by the bats).

Few identifiable katydid remains were recovered in fecal samples from the bats that had only eaten katydids. The most abundant parts found were small antennal segments, found in all the samples, averaging three pieces per katydid consumed. The six bats ate 23 katydids in total (this is greater than the number included in the culling analysis because some individuals ate katydids prior to being placed in plastic feeding boxes where cullings were collected), and we recovered six of the 46 maxillas (13%) and four of the 138 leg ends including tarsi and claws (3%). Fecal analysis was also conducted on 39 bats at capture, and only one katydid part was found (a maxilla of *A. oblongifolia*).

Bat responses to orthopteran calling songs

The number of bats tested and their responses to *N. ensiger* calling song is given in Table 1. In 2005, nine bats were tested with live singing *N. ensiger* and 16 were tested with a speaker broadcasting the calling song, and in 2006 all bats were tested using the speaker. In general, there was an increase in the proportion of bats that responded to the calling song over the summer, as measured from mid-July to early September (Fig. 2). Two bats attempted to capture, and one bat successfully captured, katydids from potted grasses in the flight room. When bats were tested for their

responses to both *N. ensiger* and *T. oceanicus* calling song in 2006, they only landed on speakers broadcasting *N. ensiger* song (14 of 50 bats tested). Five of these bats (the same individuals responding to *N. ensiger* song), however, hovered within 20 cm of the speaker when it broadcast *T. oceanicus* calling song, but this was still statistically less than the number responding to *N. ensiger* song (McNemar test, $\chi^2=22.0$, $P<0.001$). When bats were tested for their responses to both *N. ensiger* and *A. oblongifolia* calling song in 2007, there were no statistical differences in the number of bats that landed on the speaker (five of 12 bats for *N. ensiger* song, three of 12 bats for *A. oblongifolia* song, $\chi^2=0.9$, $P>0.05$) or responded to the songs (eight of 12 bats for *N. ensiger* song, six of 12 bats for *A. oblongifolia* song, $\chi^2=0.1$, $P>0.05$). The latency to land (108 ± 45 s for *N. ensiger* song, 322 ± 174 s for *A. oblongifolia* song) or respond (144 ± 35 s for *N. ensiger* song, 252 ± 88 s for *A. oblongifolia* song), however, was less for *N. ensiger* than *A. oblongifolia* song. For those bats that landed, the number of times the bat hovered in front of the speaker prior to landing was fewer for *N. ensiger* (four of five bats never hovered, i.e. they landed on the katydid during their initial response, and one individual hovered

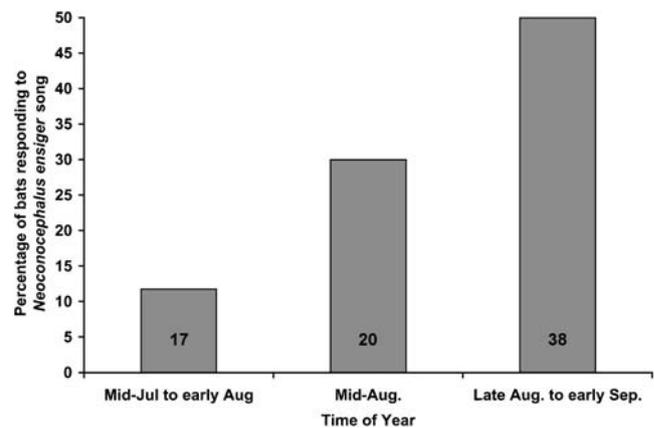


Fig. 2 Percentage of *Myotis septentrionalis* captured during 2005 and 2006 that responded to the calling song of *Neoconocephalus ensiger* in the flight room (see “Materials and methods” for definition of a response). Sample sizes for each time of year are given in the bars

once before landing) than *A. oblongifolia* (one bat did not hover, one bat hovered twice, and one bat hovered three times before landing).

Bat response to song cessation

Significantly more bats landed on the speaker when the song was continuous than when interrupted (Cochran's Q test, $Q=28.6$, $df=3$, $P<0.001$). All 11 bats tested landed on the speaker when the calling song was continuous, regardless of the presence of a physical target (dead katydid). Only one bat in each of the two treatments with interrupted song landed on the speaker (one with and one without a physical target), and this was not the same bat. We were confident that we stopped the calling song for the interrupted treatments when the bat was mid-attack (not just flying by) because they always investigated the speaker after it became silent, either by hovering near it or flying in circles around it before flying away, behaviours that preceded successful attacks in the other treatments. The mean duration (\pm SD) of interest in the speaker after the song stopped (measured from videos as the frame in which the katydid song stops to the frame in which the bat starts to leave the area of the speaker for the last time) was 4.7 ± 1.6 s. In all cases, when bats landed they consumed the dead katydid if it was present on the speaker.

We were able to match echolocation sequences to video for five of the 11 bats. We only compared the two treatments that had a dead katydid present on the active speaker as a target (Fig. 3). Table 2 provides mean values for each bat for the four echolocation call parameters measured (call duration, instantaneous rate, bandwidth, and peak frequency), none of which demonstrated a significant difference between approaches when the sound was continuous vs. interrupted (two-way paired t -tests). The mean values also did not show any trends, with some bats increasing and others decreasing the values of each parameter between treatments.

Discussion

Our study shows that *M. septentrionalis* preys on the katydid *N. ensiger* and that song cessation is an effective defence against the gleaning attacks of this bat species: 20 of the 22 dead katydids that “stopped singing” in our experiment would have survived compared to none of the continuous singers. We present three lines of evidence suggesting that *M. septentrionalis* is a natural predator of *N. ensiger*. First, wild-collected bats attack singing *N. ensiger*, or a speaker broadcasting their calling song, within 30 min of release into the flight cage and can remove them from tall grasses, their natural habitat (Gwynne 1977). Second, *M. septentrionalis* attacks speakers playing *N. ensiger*

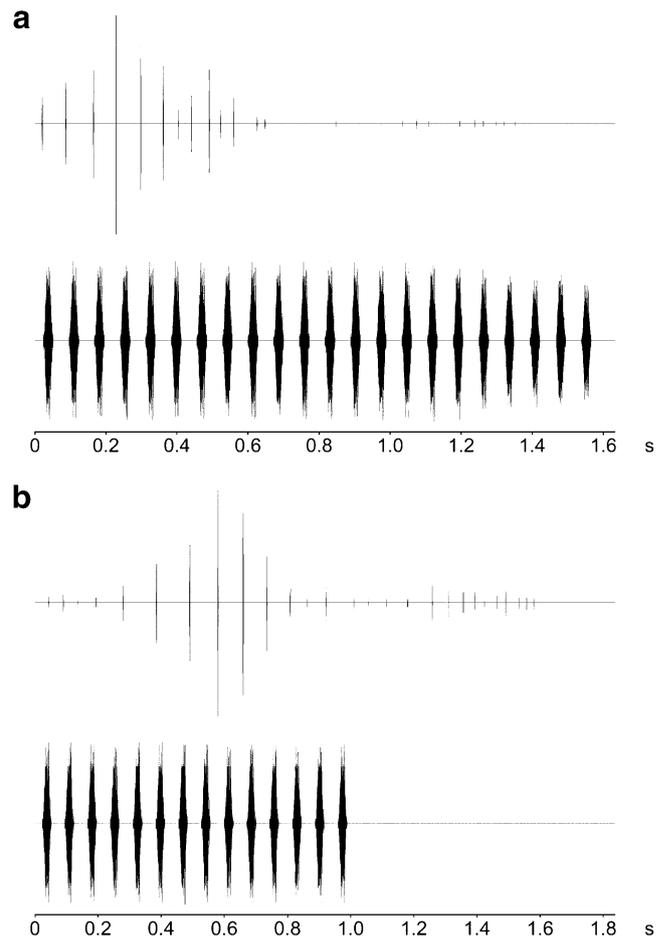


Fig. 3 Synchronised oscillograms of *Myotis septentrionalis* echolocation calls (upper trace) and *Neoconocephalus ensiger* calling song (lower trace) during two playback trials: **a** a gleaning attack with continuous *N. ensiger* calling song (the bat lands at the end of this trace), **b** an aborted gleaning attack with interrupted *N. ensiger* calling song (same time scale as above for comparison)

calling song, but not a novel orthopteran calling song with similar duty cycle (*T. oceanicus*), suggesting that this is prey recognition, not just the interest of a captive animal in prey-like sound. That the proportion of bats demonstrating a response increases as the season progresses suggests increasing familiarity with this prey item following its emergence as a singing adult. Third, the bats consume few of the harder parts used to identify insects during fecal analysis, and this could explain why diet analyses for *M. septentrionalis* have not previously detected katydids as prey (Whitaker 1972; Griffith and Gates 1985; Brack and Whitaker 2001; Carter et al. 2003; Lee and McCracken 2004; Whitaker 2004). One katydid part, however, was recovered from fecal analysis on a wild-caught *M. septentrionalis*, although it was a different species from *N. ensiger*. Katydid parts are large prey items for a small bat (*N. ensiger*, 0.458 ± 0.060 g, $n=5$; *A. oblongifolia*, 0.752 ± 0.034 g, $n=5$; *M. septentrionalis*, 5.75 ± 0.212 g, $n=8$; H.

Table 2 Mean values of four echolocation call parameters for each bat when they were within 1 m of the target speaker

Bat	Call duration (ms)		Instantaneous rate (calls/s)		Bandwidth (kHz)		Peak frequency (kHz)	
	Continuous song	Interrupted song	Continuous song	Interrupted song	Continuous song	Interrupted song	Continuous song	Interrupted song
1	0.6 (5)	1.3 (17)	20.1 (5)	17.2 (13)	N/A	N/A	N/A	N/A
2	0.4 (10)	0.9 (20)	34.3 (10)	16.2 (19)	24.8 (2)	36.1 (13)	97.6 (2)	82.0 (13)
3	0.9 (8)	1.1 (18)	18.7 (8)	16.9 (18)	34.1 (3)	41.2 (8)	89.8 (3)	94.3 (8)
4	1.3 (12)	0.9 (10)	21.8 (26)	22.2 (15)	41.7 (7)	35.1 (10)	80.7 (7)	78.7 (10)
5	1.3 (23)	0.9 (29)	23.4 (27)	25.7 (31)	43.6 (15)	39.6 (21)	85.4 (15)	86.9 (21)
Mean±SEM	0.9±0.2	1.0±0.1	23.7±2.8	19.6±1.9	36.1±4.3	38.0±1.4	88.4±3.6	85.5±3.4
Paired <i>t</i> -test	$t_{0.05,4}=-0.53, p=0.62$		$t_{0.05,4}=1.11, p=0.33$		$t_{0.05,3}=-0.45, p=0.68$		$t_{0.05,3}=0.65, p=0.56$	

Group means for each treatment, and two-way paired *t*-test results for each parameter (sample sizes in brackets)

M. ter Hofstede, personal observation). A sympatric and similar-sized bat, *Myotis lucifugus*, is known to consume between 2 and 3.7 g of insects per night, depending on age and reproductive status (Anthony and Kunz 1977). If *M. septentrionalis* consumes a similar amount of insects nightly, then four to eight katydids could supply their entire energy requirements for one night, and a single katydid would be a significant contribution. Late summer and early autumn, when the proportion of *M. septentrionalis* responding to the calling song increased, is also an important time for weight gain in preparation for hibernation (Ewing et al. 1970; Caire et al. 1979).

M. septentrionalis also attacked a speaker broadcasting *A. oblongifolia* calling song, which has a lower duty cycle than *N. ensiger* song. It is possible that this low duty cycle is responsible for the increased time required by *M. septentrionalis* to locate a speaker broadcasting *A. oblongifolia* calls compared to *N. ensiger* calls and thus could represent a primary defence against gleaning bats. Although we did not test this difference statistically, a difference of several minutes on average to locate a prey item may cause a bat in the wild to abandon the search. The bats responding to *A. oblongifolia* calling song also hovered in front of the speaker more often than *N. ensiger*, increasing the probability that the katydid could have detected the bat through other means, and possibly indicating uncertainty of the location on the part of the bat. This agrees with previous studies suggesting that low duty cycle calling song can have protective benefits against acoustically orienting predators such as gleaning bats (Belwood and Morris 1987; Hosken et al. 1994; Bailey and Haythornthwaite 1998).

One potential criticism of our experimental design is that there may be differences in the auditory thresholds of the bats for the frequency of the cricket calls (5 kHz) versus the katydid calls (13 kHz) resulting in bats not hearing the crickets well enough to locate them. Although audiograms

of some gleaning bats reveal that they are less sensitive to 5 than 13 kHz (Neuweiler 1990), this should presumably only pose a significant issue for bats at lower intensities. The range of thresholds for hearing 5 kHz is 5–40 dB SPL, depending on the species (Neuweiler 1990), whereas we presented these stimuli at 93 dB peSPL. At an intensity 50–80 dB greater than threshold, we would not expect preference based on perceived intensity; for example, the gleaning bat *Nyctophilus geoffroyi* does not differ in its response to katydid calling song at 82 versus 89 dB SPL (Hosken et al. 1994).

Both behavioural and echolocation call data support the hypothesis that *M. septentrionalis* requires continuous prey-generated sound to locate intended prey during gleaning attacks. Whereas the gleaning bat *Cardioderma cor* will land in the location from which it last heard prey sounds (Ryan and Tuttle 1987), suggesting that this bat remembers prey location, *M. septentrionalis* does not use this potential counter-strategy against song cessation. Similarly, *M. septentrionalis* does not appear to use echoic cues during gleaning attacks since they landed on speakers lacking a katydid when the song continued, and did not locate a katydid on the speaker when the song stopped. *M. septentrionalis* always hovered and flew around the speaker after the signal stopped, during which time they may have been listening for the insect to move or sing again. Two other studies reported observations that bats aborted gleaning attacks when fluttering moths stop fluttering (*Myotis evotis*, Faure and Barclay (1992); *M. septentrionalis*, Ratcliffe and Dawson (2003)). Our study confirms and quantifies this observation experimentally, demonstrating that for this bat species, insects essentially “vanish” when prey-generated sound stops.

Although there were previous reports of katydids demonstrating song cessation in the presence of bat echolocation calls (Sales and Pye 1974; Spangler 1984), Faure and Hoy (2000a) provided the first experimental

evidence that this was a specific response to ultrasound. *N. ensiger* cease singing in response to echolocation calls of *M. septentrionalis* from 60 to 85 dB peSPL at the katydid (ter Hofstede and Fullard 2008). These are realistic values given the range of call intensity estimates for gleaning bats (Faure et al. 1993; Miller and Treat 1993; Faure and Barclay 1994; Waters and Jones 1995). Several studies on perched moths have demonstrated reduced movement or sound production in response to ultrasound (Werner 1981; Acharya and McNeil 1998; Jones et al. 2002; Greenfield and Baker 2003), suggesting that other insect groups may have also evolved this defence against gleaning bats. Although gleaning is observed less frequently in bats than aerial hawking (Wilson 1973; Findley 1993), recent studies reveal that many bats may be capable of both foraging strategies (Grant 1991; Schumm et al. 1991; Faure and Barclay 1994; Arlettaz 1996; Ratcliffe and Dawson 2003; Ratcliffe et al. 2006), perhaps providing more selection pressure for secondary defences by insects than originally predicted (Faure et al. 1993). Ultimately, the ability to produce a secondary defence against gleaning bats may be related to ultrasound sensitivity; the best hearing threshold for 10 ms pulses in *N. ensiger* is 32 dB peSPL (Faure and Hoy 2000b), whereas it is approximately 52 dB peSPL in *Catocala* moths (Lepidoptera: Noctuidae) with their wings covering their ears, the natural position when perched (Faure et al. 1993). These moths exhibit little neural evidence that they can hear gleaning attacks of *M. septentrionalis* (Faure et al. 1993). It is possible that moths have not experienced the same degree of selection pressure for sensitivity to gleaning bats since they do not provide the conspicuous cues that katydids do (i.e. katydids sing, moths (with few exceptions (Conner 1999) do not). Our study provides the first experimental evidence using naturally realistic sounds that song (and potentially sound) cessation is an effective defence against gleaning bats.

Based on the design of the echolocation calls used during gleaning attacks (i.e., high frequency and broad bandwidth), Ratcliffe and Dawson (2003) predicted that *M. septentrionalis* use echolocation for prey-background resolution. Our data, however, suggest that for the localisation of prey in the absence of prey-generated sounds, *M. septentrionalis* does not use echoic information, in contrast to some species of gleaning bats, e.g. *Megaderma lyra* (Schmidt et al. 2000). This could be the result of sensory constraint (i.e., echoic information is not sufficient for the resolution of prey from background). It is also possible that there has been little selection pressure to overcome this defence over evolutionary time, an individual bat's lifetime, or both. If katydids do not often hear bats approaching, and if it is more profitable for the bat to move on to the next singing katydid than to attempt to locate a silent one, then there may be relatively little impetus for either an evolved

or learned counter-tactic on the part of the bats. In general, examples of coevolution between predators and prey are rare due to the life-dinner principle: a successful defence means life to a prey, but only a lost meal to a predator (Dawkins and Krebs 1979; Endler 1991). We observed one of our bats in the flight room bite down on the seed head of the grass stem on which a singing katydid was perched. A few unsuccessful attempts in the wild could deter bats from trying to locate prey without passive cues, particularly given that gleaning attacks are presumed to put the bats at risk of predation (Jones et al. 2003). Other bats are known to adapt their foraging behaviour after negative experiences (Schmidt et al. 2000).

Unlike some other predators, we found that under conditions similar to those found in nature *M. septentrionalis* does not switch prey detection strategies during an attack, and we suggest that insects such as *N. ensiger* can exploit this limitation using a simple defence strategy (silence). *N. ensiger* demonstrate various antipredator behaviours, such as diving to the ground or flying away (Faure and Hoy 2000a), but we did not observe either response to ultrasound (H. M. ter Hofstede, unpublished). Flight would be ineffective against a bat, and dropping to the ground would be costly due to terrestrial predators and the loss of a singing perch. Many bats, and *M. septentrionalis* in particular (Ratcliffe and Fullard 2005), appear to be restricted to auditory information during foraging, simplifying studies of defensive behaviours in their prey. Predators that use multiple senses for prey detection may select for more complex or primary antipredator defences than simply depriving the predator of a single cue. For example, Templeton and Shriner (2004) investigated antipredator behaviours in Trinidadian guppies (*Poecilia reticulata*) and found that they use different antipredator behaviours more often in response to a simulated avian predator than an aquatic predator; dashing and dropping low in the water were common responses to the avian model, but were rare in response to the fish, possibly because birds may be restricted to visual cues when hunting fish, whereas some fish can use water movements to detect prey (New 2002). Studies of this kind reveal the importance of considering both the prey's sensitivity to predator cues and the flexibility of the predator to gather information about prey location when conducting research on variation in antipredator behaviour and its evolution.

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