

## RESEARCH ARTICLE

# Ultrasound avoidance by flying antlions (Myrmeleontidae)

Marc W. Holderied<sup>1,\*</sup>, Liam A. Thomas<sup>1</sup> and Carmi Korine<sup>2</sup>

## ABSTRACT

The acoustic arms race between insectivorous bats and their invertebrate prey has led to the convergent evolution of ultrasound hearing in seven orders of nocturnal insects. Upon hearing the echolocation calls of an approaching bat, such insects take defensive action. Here, we document a previously unknown sense of ultrasound hearing and phonotactic flight behaviour in the neuropteran family Myrmeleontidae (antlions). The antlion *Myrmeleon hyalinus* was presented with sound pulses at ultrasonic frequencies used by echolocating bats and its response thresholds in tethered flight determined. Behaviours included abdominal twitches, wing flicks, brief pauses in flight and flight cessation. Such behaviours create erratic evasive flight manoeuvres in other eared insects, particularly mantids and lacewings. Antlions responded best to ultrasound between 60 and 80 kHz (75 dB peSPL at 80 kHz), showing response thresholds similar to those of the related lacewings (Neuroptera, Chrysopidae). Yet, at lower ultrasonic frequencies (20–50 kHz), antlions were far less sensitive than lacewings. Based on calculated response distances, we conclude that antlions respond only after having been detected by bats rather than using early evasive flights. We argue that the high response threshold for low-frequency ultrasound is adaptive for an insect that is mainly active close to and within vegetation, because a behavioural response to the lower ultrasonic frequencies used by high-flying bats would result in evasive action in the absence of actual predation risk.

**KEY WORDS:** Neuroptera, Insect hearing, Bioacoustics, Bat echolocation, Desert

## INTRODUCTION

The ability to hear airborne sound has evolved independently at least 18–20 times (see Yager, 1999; Yack, 2004; Strauß and Lakes-Harlan, 2014) across seven insect orders (Lepidoptera, Hemiptera, Orthoptera, Coleoptera, Diptera, Mantodea and Neuroptera). Insects use hearing either for interspecific/intraspecific communication (Haskell, 1961) or, more commonly, for the detection of predators, i.e. echolocating insectivorous bats (Hoy, 1992).

Bats echolocate by producing high-frequency (mainly above human hearing) sound waves and interpret the returning echoes. This allows them to orient in their environment and to detect and capture insect prey. With bats being the most widely distributed and second-most speciose mammalian order (Simmons, 2005), they exert substantial predation pressure on nocturnal invertebrates.

This applies to prey they detect by their walking noises or calls (e.g. Römer et al., 2010; Holderied et al., 2011), but even more so to nocturnally flying insects that bats detect by their echoes (e.g. Miller and Surlykke, 2001; ter Hofstede et al., 2013).

The first flight response of insects to an ultrasound stimulus simulating an approaching bat is to steer away from the ultrasound source. This has been shown in beetles (Yager and Spangler, 1997), mantids (Yager et al., 1990), crickets (Moiseff et al., 1978), bush-crickets (Libersat and Hoy, 1991) and moths (Conner and Corcoran, 2012). A more drastic ‘last ditch’ evasive behaviour just before capture is a nose dive or free fall exhibited by moths, mantids, bush crickets and lacewings (Conner and Corcoran, 2012). Such evasive flight responses have long been utilised for characterisation of insect hearing by behavioural audiograms (Treat, 1955). The resulting behavioural response thresholds typically are around 20 dB higher than the neuronal response threshold of the respective hearing organ (Miller and Surlykke, 2001). Spectrally, insect ears are tuned to the search call frequencies used by their predators, with different populations of some moth species even tuned to different best frequencies, correlating with the call frequencies of the local insectivorous bat fauna (ter Hofstede et al., 2013).

Roeder (1962) observed that green lacewings (Chrysopidae) presented with ultrasound cease flight. Miller and Olesen (1979) documented unpredictable, varied flight responses of green lacewings to ultrasound, including steering and ‘last ditch’ diving responses. The hearing organ in lacewings was identified as a swelling along the radial vein of the forewing (Miller, 1970; Miller and Macleod, 1966). Chrysopids are the only known family with ultrasound hearing ability in the order Neuroptera (comprising lacewings, antlions and 13 less speciose families).

We investigated adult antlions (Neuroptera, Myrmeleontidae), winged insects with a wide tropical and temperate distribution (Stange, 2004) that superficially resemble damselflies. Their nocturnal lifestyle makes antlions a likely prey and they are indeed encountered occasionally in the diets of insectivorous bats (Johnston and Fenton, 2011; Bayefsky-Anand, 2005). Here, we document that antlions respond to ultrasound and we measured their behavioural audiogram. We hypothesised that antlions possess a biologically relevant ability to hear and respond to ultrasound, and that this ability has evolved in defence against echolocating bats. Therefore, we predicted flight response behaviours similar to those exhibited by other nocturnal insects with ultrasound hearing capabilities. Further, we predicted that their hearing should be tuned to the frequencies of the search calls of the insectivorous bat community, and that their hearing is sensitive enough at these frequencies to allow timely evasive actions.

## MATERIALS AND METHODS

### Field observations

In June and July 2015, unidentified flying antlions encountered at the Sede Boqer campus (SBC) of the Ben-Gurion University of the Negev (30°52'N, 34°47'E) and at Golda Meir park (31°01'N, 34°76'E), Israel, were ensounded opportunistically with ultrasound

<sup>1</sup>School of Biological Sciences, University of Bristol, Life Sciences Building, 24 Tyndall Avenue, Bristol BS8 1TH, UK. <sup>2</sup>The Mitrani Department of Desert Ecology, Jacob Blaustein Institutes for Desert Research, Ben-Gurion University of the Negev, Midreshet Ben-Gurion, 8499000, Israel.

\*Author for correspondence (marc.holderied@bristol.ac.uk)

© M.W.H., 0000-0002-1573-7908; L.A.T., 0000-0002-2465-0441; C.K., 0000-0002-3038-6464

from distances between 0.5 and 2 m using a Dazer II [Dazer International, London, UK; 115 dB sound pressure level (SPL) at 25 kHz from 0.5 m, overtones present], and changes to their flight behaviour were noted. In April 2018, no antlions were found at SBC so, as an alternative, 23 individuals from a large population of the antlion *Macronemurus linearis* (Klug 1834) found near the water reservoir just south of Tse'elim (31°11'N, 34°32'E) in the western Negev were tested for their flight response.

### Specimens for flight experiments

Antlions for behavioural experiments [*Myrmeleon hyalinus* Olivier 1811, *Myrmeleon formicarius* Linnaeus 1767 and *Scotoleon carrizonus* (Hagen 1888) as available] were caught on SBC and the surrounding area of Midreshet Ben-Gurion or at Golda Meir park. Specimens were found in areas with dense shrubbery. Antlions were attracted using a portable light source and collected by hand-netting during June and July 2015. Immediately after capture, antlions were placed in a cage under a cloth cover, keeping them in a dark environment. All specimens were tested between 21:00 h and 04:00 h on the night of their capture as individuals kept for a day and flown the following night showed drastically reduced flight performance.

### Playback setup

Experiments took place in an 8×4×3 m indoor laboratory with an average temperature of 31°C during behavioural testing. The setup consisted of an ultrasound speaker (S56) and amplifier (S55, both Ultra Sound Advice, London, UK), with its acoustic axis 38 cm above and parallel to a table surface and pointing at the tip of a 1 mm diameter brass tube at a distance of 65 cm from the speaker, which served as a mount for tethered insects. A 0.91×0.51 m panel of ultrasound absorbing foam (Studiofoam 4 inch pyramids, Auralex Acoustics Inc., Indianapolis, IN, USA) fully covered the table around and between the speaker and tether to reduce sound reflections off the table surface. The speaker amplifier was connected to a soundcard (USB-6251, National Instruments, Austin, TX, USA) and operated by RECORDER NI-DAQmx software (Avisoft Bioacoustics, Berlin, Germany). The experimental room was lit indirectly from a single, wall-mounted fluorescent light 4 m away from the setup. It was largely covered with opaque foam to reduce overall ambient light levels, with additional foam panels used to block any direct light on the specimen. Adjusting light levels this way allowed safe operation for the experimenter while tethered antlions flew well under these conditions. No alternative lighting options were tested. Responses in flight behaviour were observed through a webcam (AWC213, Advent Computers, London, UK) connected to a laptop, which fed a live view of the tethered specimen alongside the playback controls. This allowed for simultaneous observation of the specimen and control of the playback frequencies and amplitude manipulation. The laptop used for playback control and specimen observation was placed 0.5 m behind the ultrasound speaker.

### Tethering

For attaching the tether, the antlion was held firmly by the wings using forceps. A small amount of beeswax and pine resin adhesive was melted onto the head of a thin insect pin (size 000) using a lighter; the pin was then allowed to cool until the adhesive was close to solidifying, before it was manually placed onto the mesothorax of the specimen, with the length of the pin running above the antennae. It was held in place until the adhesive had turned solid and the tether attached firmly. The specimen was then offered a small amount of

sugar water and mounted onto the brass tether in a horizontal upside-down posture with its head facing away from the speaker, as this prompted the most consistent and lasting flight behaviour. Once in position, the antlion was given a mount, consisting of a small piece of folded paper, to avoid flight before playback. This mount was chosen as it was light, easily replaceable and simple for the antlion to grasp without providing enough purchase to hinder removal. The mount was removed from the antlion to induce flight, which was considered steady when the antlion showed consistent, continued wingbeats for at least 2–3 s without signs of struggle. Experiments commenced once the antlion was in steady flight.

### Stimulus generation

Stimuli were created using SASlab Pro software (Avisoft Bioacoustics) for a sampling rate of 250,000 samples s<sup>-1</sup> and 16-bit resolution. Each stimulus consisted of a series of five constant frequency pulses each of 24 ms duration, with 2 ms linear ramps and 20 ms plateau, alternating with pauses of 76 ms. This is shorter than the 30 ms pulses used by Miller (1975) for green lacewings. The resulting 500 ms file had a 100 ms repetition interval, roughly resembling a search call sequence from an echolocating bat (Hackett et al., 2017). Separate stimuli were created for 20–100 kHz in steps of 10 kHz.

### Calibration of sound field

We used a CO-100K microphone (Sanken, Tokyo, Japan) with Quadmic amplifier (RME, Haimhausen, Germany) at the tethering position facing the loudspeaker for calibration of the sound field. The microphone signal was recorded using a USB-6251 soundcard (National Instruments) with RECORDER NI-DAQmx software (Avisoft Bioacoustics). The microphone frequency response is flat up to 10 kHz and becomes more sensitive between 10 kHz and 100 kHz. The microphone was amplitude calibrated using a D-1411E calibrator (DAWE Instruments Ltd, London, UK; 114 dB SPL at 1 kHz). Each stimulus was played back at maximum output amplitude (0 dB attenuation), recorded with the microphone, and the peak-to-peak voltage measured ( $V_{\text{meas}}$ ). SPL of each stimulus at the microphone/insect tether was then calculated using:

$$\text{dB peSPL} = 114 + 20\log_{10}(V_{\text{meas}}/V_{\text{ref}}) + S(f), \quad (1)$$

where peSPL is peak equivalent SPL;  $V_{\text{ref}}$  is the peak-to-peak voltage of the calibration tone; and  $S(f)$  is the frequency-dependent microphone sensitivity difference between 1 kHz and the respective stimulus (20–100 kHz) as read from the individual microphone calibration chart, rounded to the nearest full dB. This gave the maximum SPL (dB peSPL re. 20 μPa, henceforth abbreviated to dB peSPL) for each of the frequencies from 20 to 100 kHz. The maximum SPLs achieved were: 20 kHz, 102 dB peSPL; 30 kHz, 97 dB peSPL; 40 kHz, 105 dB peSPL; 50 kHz, 105 dB peSPL; 60 kHz, 97 dB peSPL; 70 kHz, 99 dB peSPL; 80 kHz, 100 dB peSPL; 90 kHz, 99 dB peSPL; and 100 kHz, 86 dB peSPL. This calibration procedure was repeated daily before experiments, and the recorded peSPL changed by less than 1 dB.

### Threshold measurement

Antlions in tethered flight showed three different responses to playback of ultrasound (see Results): either complete or temporary cessation of flight (with or without abdominal flexion) or abdominal flexion but with the wings continuing to beat. All three behaviours were counted as responses, while continued flight was not. One full trial meant that the respective stimulus (combination of frequency

and amplitude) was tested five times (requiring steady flight before each stimulus presentation), and any behavioural responses of the flying specimen noted. A response rate of at least 60% (three out of five repeats) signified a behavioural response to that stimulus. The playback amplitude was adjusted manually by setting an attenuation (in dB) in the RECORDER software. We then determined the behavioural threshold by adjusting this attenuation value using a sequential approach, starting at our chosen maximum attenuation of 40 dB below the maximum amplitude for each respective frequency. For example, for a stimulus frequency of 50 kHz, the maximum amplitude is 105 dB peSPL minus the maximum attenuation of 40 dB, which gives a minimum amplitude of 65 dB peSPL. Amplitude was then increased in steps of 12 dB until a response was observed. Then, amplitude was reduced in steps of 6 dB until the response faded, and finally it was increased again in 3 dB steps to find the threshold amplitude of the antlion hearing at the tested frequency. A desirable resolution of 1 dB proved impracticable for most specimens, as antlions are not very persistent fliers, offering only a limited number of trials. The 3 dB resolution was established as a workable compromise, allowing testing of all frequencies before the test specimens ceased adequate flight activity. Threshold amplitude was taken as the lowest amplitude at and above which there was a consistent behavioural response. A full test series included successive threshold measurements for all frequencies. To avoid bias by order of presentation, e.g. through potential habituation, frequencies were tested in a pre-randomised order of 50, 90, 30, 70, 40, 80, 20, 60 and 100 kHz. If a response was not elicited at the maximum amplitude of a certain frequency (often at 20 and 30 kHz), the threshold for that frequency was taken as 1 dB above that maximum amplitude.

If a test specimen had not responded even when the first five frequencies had been played at maximum amplitude, this specimen was classified as non-responsive and no further frequencies were tested. A test series was also ended when the test specimen showed any of the following signs of fatigue or habituation: (i) it required multiple mount removals to induce a single flight, (ii) it no longer reached steady flight of 2–3 s before playback, or (iii) its responses became erratic, with the threshold measurement becoming inconclusive.

### Hearing range calculations

Behavioural audiogram levels were compared with echolocation call frequencies used by the local bat fauna (Hackett et al., 2017). For each frequency in the behavioural audiogram, we calculated the distance at which an antlion would first respond to ultrasound emitted by an approaching bat calling at that frequency, with a source level as used by foraging bats in the field:

$$T = SL - 20 \times \log_{10} \left( \frac{r}{r_{\text{ref}}} \right) - r \times A, \quad (2)$$

where  $r$  is the response distance,  $r_{\text{ref}}$  is the reference distance,  $SL$  is the emitted call source level (in dB peSPL) at the reference distance,  $T$  is the antlion behavioural threshold (in dB peSPL) and  $A$  is the frequency-dependent absorption in  $\text{dB m}^{-1}$ . The maximum response distance was calculated using a reference bat call with a  $SL$  of 125 dB at a  $r_{\text{ref}}$  of 0.1 m (Holderied and von Helversen, 2003).  $T$  was taken as the average per frequency from the *M. hyalinus* behavioural audiogram. The absorption  $A$  for each tested frequency was calculated using Matlab (R2016a, The Mathworks, Natick, MA, USA) following the equations set out in Bazley (1976) for an average temperature of 22°C and relative humidity of 70% (taken from weather records for SBC during June–July 2015). In the following,

we will refer to frequencies of 20–50 kHz as ‘lower’ ultrasonic range and frequencies above that as ‘higher’ ultrasonic range.

## RESULTS

### Field observations

We found that adult antlions are weak and slow fliers in the field, and we only encountered them flying within 2–3 m of the ground and/or vegetation and often below tree cover. Upon ensonification, all 23 free-flying *Macronemurus* tested individually responded with a sudden (and often brief) change in flight direction and a drop in flight height, regularly accompanied by an increase in speed; 17 of the 23 individuals fled towards or into vegetation cover but maintained flight, while the rest actively spiralled to the ground, where they often closed their wings upon landing. None dropped to the ground passively with closed wings.

### Behavioural testing

Tethered antlions exhibited three different behaviours in response to ultrasound stimulation. Most commonly, they ceased beating their wings completely at stimulus onset and held the wings still in a horizontal position. The antlions would sometimes also flex their abdomen, bringing the posterior abdomen into contact with the thorax. Once in that position, they would not show any further responses to ultrasound playbacks until steady flight was re-induced manually. Alternatively, they showed the same abrupt flight cessation with the wings being moved to a horizontal position, but resumed full flight autonomously after some time. Lastly, antlions would show the same abdominal movement as above but their wings continued beating.

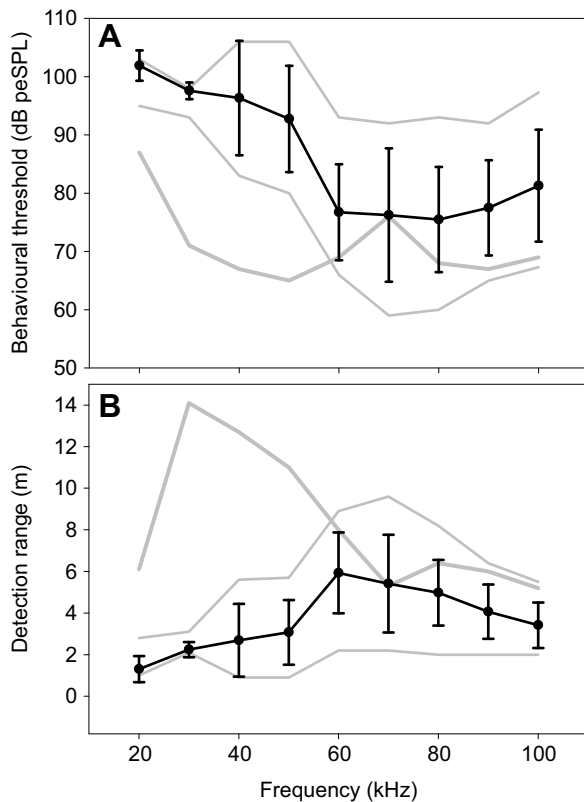
Mirroring their flight behaviour in the field, tethered antlions were poor fliers. Of over 200 tested individuals, only 33 flew long enough to enable us to measure behavioural thresholds for at least the first half of the tested frequencies and only 20 completed all frequencies. Complete behavioural audiograms were measured for 13 *M. hyalinus* (Fig. 1A), five *M. formicarius* and two *S. carrizonus*. *Myrmeleon hyalinus* were the most consistent fliers and exhibited a stable behavioural response between 40 and 100 kHz, peaking between 70 and 80 kHz. At 40 kHz, antlion behavioural responses began at around 90 dB peSPL with the sensitivity reaching a low of 75 dB peSPL at its peak frequency of 80 kHz and rising again to 80 dB for frequencies approaching 100 kHz (Fig. 1A). Responses were also measured at 20–30 kHz but these were often inconsistent, with individuals regularly failing to respond to higher SPLs than those they had previously responded to. From just five individual *M. formicarius*, we obtained a very similar audiogram ranging between 95 and 105 dB peSPL from 20 to 40 kHz and between 75 and 85 dB peSPL from 50 to 100 kHz, with peak average sensitivity of 75.5 dB peSPL at 70 kHz. The two individual *S. carrizonus* showed mean thresholds of around 100 dB peSPL at 20–30 kHz, 85 dB peSPL at 40 kHz and between 78 and 80 dB peSPL across 50–100 kHz.

Calculated response distances of *M. hyalinus* mirror the threshold SPLs as expected (Fig. 1B). Increased ultrasound absorption at higher frequencies meant that the highest average response distance occurred at 60 kHz, reaching  $5.8 \pm 2.0$  m. Response distance increased gradually with frequency up to 50 kHz, with the sharpest increase between 50 and 60 kHz (+2.2 m). Response distance then showed a slow and steady decline from 60 kHz upwards. The individual response distances ranged from 2.0 to 9.6 m.

## DISCUSSION

We document that free-flying antlions respond to ultrasound with sudden changes in flight direction or powered spiralling dives, and





**Fig. 1. Behavioural responses of antlions to ultrasound.** (A) Behavioural audiogram of *Myrmeleon hyalinus* ( $N=13$ ). dB peSPL is peak equivalent sound pressure level re.  $20 \mu\text{Pa}$ . (B) Response distances of *M. hyalinus* calculated from the behavioural audiogram. Data are means  $\pm$  s.d.; thin grey lines are maximum and minimum values. Thick grey lines show mean values for lacewings (modified after Miller, 1975).

that tethered antlions flex their abdomen and/or stop flight (temporarily). Abdominal flexion is used for evasive steering by orthopterans (Moiseff et al., 1978; Huber et al., 1989), hawkmoths (Dyher et al., 2012) and mantids (Yager and Hoy, 1986; Yager and May, 1990), and it occurs during evasive dives of bush-crickets (Libersat and Hoy, 1991) and in spiral dives of mantids (Yager and Hoy, 1986; Yager and May, 1990). The rapid abdominal downward plantarflexion of antlions could thus cause abrupt changes in direction just like a rear rudder can bring an aircraft's nose down to initiate a dive. Flight cessation followed by a passive dive is a common evasive response in several insects. It was the most common behaviour of tethered antlions in the present study, yet curiously absent in the observed population of free-flying antlions. Lacewings pause beating their wings briefly when they change flight direction (Miller and Olesen, 1979), and the same might be the case in antlions. In summary, antlion response behaviours resemble last-chance avoidance tactics known from other insects.

### Threshold amplitude

*Myrmeleon hyalinus* response threshold was 75–80 dB peSPL over 60–100 kHz and could well extend to higher frequencies (compare with lacewings: Miller, 1975). The adaptiveness of these response thresholds can be judged by comparison with ultrasound avoidance behaviours of other insects, and also with respect to different hunting strategies of echolocating bats.

The 75 dB peSPL best threshold of antlions was within the 70–80 dB peSPL at which many other insects initiate last-ditch

evasion of attacking aerial-hawking bats (moths: e.g. Skals and Surlykke, 2000; mantids: Yager et al., 1990; tiger beetles: Yager and Spangler, 1997; and green lacewings: Miller, 1975). This close agreement across several insect orders corroborates the interpretation that the observed antlion behaviours serve as last-chance responses against aerial-hawking bats with their typical high-amplitude calls (Holderied and von Helversen, 2003). But note that cricket steering rather than last-ditch responses is also triggered at 75–80 dB peSPL (Moiseff et al., 1978).

In contrast, male bush-crickets stop singing when hearing bat calls of just 25–35 dB peSPL (ter Hofstede et al., 2010), which is 40–50 dB more sensitive than the abovementioned last-chance responses. The high sensitivity of these bush-crickets is shown in defence against gleaner bats, which hunt using the sounds their prey generates (e.g. songs, rustling noises). Such gleaners use whispering echolocation at just 75 dB peSPL (Holderied et al., 2011). Insect defence thresholds against both bat foraging strategies – gleaners and aerial hawkers – are about 40–50 dB below the respective echolocation call levels: 70–80 dB peSPL responses in many insects against the 125–130 dB peSPL calls of aerial-hawking bats and 25–35 dB peSPL responses of bush-crickets against the 75 dB peSPL calls of whispering gleaner bats. This similarity in level difference might relate to a similar relative predation risk at the response threshold.

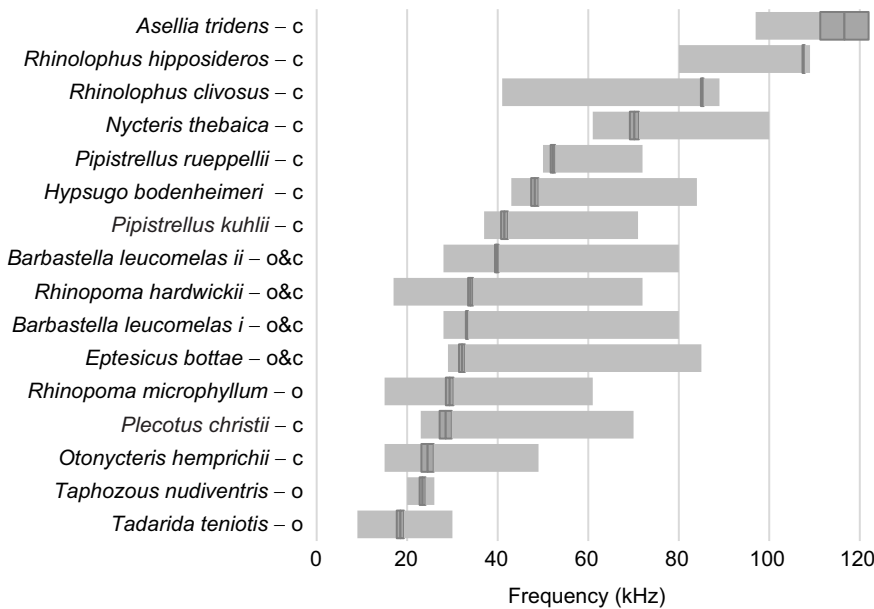
### Response distance and time

Mantids steer away from bats at 10 m distance (early response manoeuvres) but initiate last-ditch behaviours at 3–5 m (Yager et al., 1990), and lacewings respond to bats up to 2 m away (Miller and Olesen, 1979). These last-ditch initiation distances match the calculated antlion response distances of 2–6 m. At the foraging flight speed of *Pipistrellus kuhlii* measured at the study site ( $6.7 \text{ m s}^{-1}$ ; Grodzinski et al., 2009) antlions thus have a time-to-contact of 358 ms at 20 kHz and 866 ms at 60 kHz to complete their evasive action. This is ample time for an evasive response that takes 150 ms from stimulus onset (Miller and Olesen, 1979).

### Neuropteran hearing and bat echolocation

Lacewings have a calculated peak response distance of 14.1 m at 30 kHz, 4.5 m further than the maximum calculated for *M. hyalinus*. This mirrors threshold differences at best frequency (55 dB peSPL in lacewings compared with 75 dB peSPL in antlions), and at lower ultrasonic frequencies (20–50 kHz), where green lacewings are up to 30 dB more sensitive than antlions. We propose this substantial difference at lower ultrasonic frequencies between these two neuropteran families forms an adaptation to their differing ecology and respective exposure to different bat communities: antlions are weak fliers and always stay close to cover, while green lacewings are long-distance migrants and specimens have been caught up to 200 m above ground (Chapman et al., 2003). Thus, green lacewings frequently encounter and have to evade high-flying aerial insectivores that use low-frequency echolocation calls to detect more distant prey (Schnitzler et al., 2003), while antlions are more at risk from bat species flying closer to structures (background cluttered or cluttered space; Schnitzler et al., 2003).

A detailed comparison of the search frequencies used by the local bat fauna (Fig. 2) supports this. The *M. hyalinus* sensitive frequency range starting from 60 kHz covers the frequency range of 87% (13 of 15) of the local insectivorous bat species. Only two high-flying aerial-hawking bat species with low-frequency calls peaking below 30 kHz (*Tadarida teniotis* and *Taphozous nudiventris*; Korine and Pinshow, 2004; Ulanovsky et al., 2004; Hackett et al., 2017) had a



**Fig. 2. Frequencies of echolocation calls of the local bat fauna.** Dark grey indicates peak frequencies (means $\pm$ s.d.), light grey indicates the frequency range from minimum to maximum including all (even faint) harmonics. The main foraging habitat of local bat species is shown: 'o', open space, high above ground and above vegetation – fast fliers; 'c', cluttered and background-cluttered space, flying within a few metres of the ground, often within gaps in vegetation – slower and more manoeuvrable fliers (adapted from Hackett et al., 2017).

range completely below 60 kHz. Their search calls will still reach the ground at considerable amplitude without posing any actual predation risk to insects there. So, the low sensitivity of antlions at these frequencies is possibly adaptive. Six species with peak frequencies from 29 to 52 kHz (*Rhinopoma microphyllum*, *Eptesicus bottae*, *Rhinopoma hardwickii*, *P. kuhlii*, *Pipistrellus rueppellii* and *Hypsugo bodenheimeri*) are increasingly more likely to enter the cluttered habitat of antlions, and the antlion hearing threshold rises gradually over that frequency range, which we interpret as a hearing response to the increasing predation pressure these bat species exert. The relatively high antlion peak sensitivity of 60 kHz supports the idea that antlion hearing is mainly used for last-chance avoidance when under attack by a bat, because most of the abovementioned species will add high frequencies to their echolocation calls when initiating an attack. Of the remaining bat species, at least two use whispering echolocation (*Otonycteris hemprichi* and *Barbastella leucomelas*) and have specialised diets dominated either by ground-dwelling arthropods (often scorpions; Holderied et al., 2011) or eared flying insects (mainly eared moths). The third member of the tribe Plecotini in the local bat fauna, *Plecotus christii*, has not been studied in that respect, but might use similarly low call amplitudes, as might *Nycteris thebaica*. Whispering echolocation would allow them to capture flying antlions as has been shown by Johnston and Fenton (2011) for *Antrozous pallidus*, but at least one of these species (*O. hemprichi*) regularly switches to high-amplitude calls when hunting for flying prey (Hackett et al., 2014), which would reveal its presence to antlions. The remaining three bat species (*Rhinolophus clivosus*, *Rhinolophus hipposideros* and *Asellia tridens*) have calls of very high peak frequencies that are within the (extrapolated beyond the measured values) sensitive antlion hearing range. In summary, their audiogram shows antlions are able to detect and respond to all bat species with last-chance evasive actions, except those high-flying species that do not pose a direct predation risk to them.

In conclusion, we document the existence of ultrasound avoidance behaviour in antlions, and show that their responses are comparable with bat-avoidance behaviours of other eared insects. Comparison between antlion frequency response ranges and bat search call frequencies has shown that antlion ears are more sensitive

to frequencies used by low-flying bats, which they are more likely to encounter, and only gleaning bats have been documented as consuming antlions. The response distances calculated here allow sufficient response time to evade a bat attack with a last-chance avoidance manoeuvre. This shows that antlions have developed the ability to detect ultrasound as a countermeasure against echolocating insectivorous bats. The location of the antlion ear is still unknown, however. The ear of green lacewings, the only other neuropterans showing responses to bat ultrasound, is based in a swelling in the radial vein of the forewing. Its apparent absence in antlions means another ultrasound ear has evolved within the Neuroptera at an as-yet unknown location.

#### Acknowledgements

The authors would like to offer thanks to Evyatar Feingold for assistance in specimen collection. Undergraduate students Laura Brooks, Fiona Messer, Owen Crawshaw and Liam O'Reilly and Dr Hannah ter Hofstede helped in pilot characterisation of antlion acoustic responses as part of the summer school on the Biology of Desert-dwelling Bats. This is paper number 983 of the Mitrani Department of Desert Ecology.

#### Competing interests

The authors declare no competing or financial interests.

#### Author contributions

Conceptualization: M.W.H., L.A.T., C.K.; Methodology: M.W.H., L.A.T., C.K.; Software: M.W.H.; Formal analysis: M.W.H., L.A.T.; Investigation: L.A.T.; Resources: M.W.H., C.K.; Data curation: L.A.T.; Writing - original draft: M.W.H., L.A.T.; Writing - review & editing: M.W.H., C.K.; Supervision: M.W.H., C.K.; Project administration: M.W.H.; Funding acquisition: M.W.H., C.K.

#### Funding

This research was supported by a Leverhulme Research Fellowship to M.W.H. (RF-2017-717) and Biotechnology and Biological Sciences Research Council grant 'Diffraction of Life' (BB/N009991/1) to M.W.H.

#### References

- Bayefsky-Anand, S. (2005). Effect of location and season on the arthropod prey of *Nycteris grandis* (Chiroptera: Nycteridae). *Afr. Zool.* **40**, 93-97.
- Bazley, E. N. (1976). Sound absorption in air at frequencies up to 100 kHz. In *NPL Acoustics Report Ac 74*. Teddington, UK: National Physics Laboratory.
- Chapman, J. W., Reynolds, D. R. and Smith, A. D. (2003). Vertical-looking radar: a new tool for monitoring high-altitude insect migration. *Bioscience* **53**, 503-511.
- Conner, W. E. and Corcoran, A. J. (2012). Sound strategies: the 65-million-year-old battle between bats and insects. *Annu. Rev. Entomol.* **57**, 21-39.

- Dyher, J. P., Morgansen, K. A., Daniel, T. L. and Cowan, N. J.** (2012). Flexible strategies for flight control: an active role for the abdomen. *J. Exp. Biol.* **216**, 1523-1536.
- Grodzinski, U., Spiegel, O., Korine, C. and Holderied, M. W.** (2009). Context-dependent flight speed: evidence for energetically optimal flight speed in the bat *Pipistrellus kuhlii*? *J. Anim. Ecol.* **78**, 540-548.
- Hackett, T. D., Korine, C. and Holderied, M. W.** (2014). A whispering bat that screams: bimodal switch of foraging guild from gleaning to aerial hawking in the desert long-eared bat. *J. Exp. Biol.* **217**, 3028-3032.
- Hackett, T. D., Holderied, M. W. and Korine, C.** (2017). Echolocation call description of 15 species of Middle-Eastern desert dwelling insectivorous bats. *Bioacoustics* **26**, 217-235.
- Haskell, P. T.** (1961). *Insect Sounds*. London, UK: HF & G Witherby.
- Holderied, M. W. and von Helversen, O.** (2003). Echolocation range and wingbeat period match in aerial-hawking bats. *Proc. R. Soc. Lond. B. Biol.* **270**, 2293-2299.
- Holderied, M. W., Korine, C. and Moritz, T.** (2011). Hemprich's long-eared bat (*Otonycteris hemprichii*) as a predator of scorpions: whispering echolocation, passive gleaning and prey selection. *J. Comp. Physiol. A* **197**, 425-433.
- Hoy, R. R.** (1992). The evolution of hearing in insects as an adaptation to predation from bats. In *Comparative and Evolutionary Biology of Hearing* (ed. D. Webster, R. Fay and A. Popper), pp. 115-130. New York, USA: Springer.
- Huber, F., Moore, T. E. and Loher, W.** (1989). *Crickets: Behaviour and Neurobiology*. London, UK: Cornell University Press.
- Johnston, D. S. and Fenton, M. B.** (2001). Individual and population-level variability in diets of pallid bats (*Antrozous pallidus*). *J. Mammal.* **82**, 362-373.
- Korine, C. and Pinshow, B.** (2004). Guild structure, foraging space use, and distribution in a community of insectivorous bats in the Negev desert. *J. Zool.* **262**, 187-196.
- Libersat, F. and Hoy, R. R.** (1991). Ultrasonic startle behavior in bushcrickets (Orthoptera; Tettigoniidae). *J. Comp. Physiol. A* **169**, 507-514.
- Miller, L. A.** (1970). Structure of the green lacewing tympanal organ (*Chrysopa carnea*, Neuroptera). *J. Morphol.* **131**, 359-382.
- Miller, L. A.** (1975). The behaviour of flying green lacewings, *Chrysopa carnea*, in the presence of ultrasound. *J. Insect Physiol.* **21**, 205-219.
- Miller, L. A. and Macleod, E. G.** (1966). Ultrasonic sensitivity: a tympanal receptor in the green lacewing *Chrysopa carnea*. *Science* **154**, 891-893.
- Miller, L. A. and Olesen, J.** (1979). Avoidance behaviour in green lacewings I: Behaviour of free flying green lacewings to hunting bats and ultrasound. *J. Comp. Physiol.* **131**, 113-120.
- Miller, L. A. and Surlykke, A.** (2001). How some insects detect and avoid being eaten by bats: tactics and counter-tactics of prey and predator. *Bioscience* **51**, 570-581.
- Moiseff, A., Pollack, G. S. and Hoy, R. R.** (1978). Steering responses of flying crickets to sound and ultrasound: mate attraction and predator avoidance. *Proc. Natl. Acad. Sci. USA* **75**, 4052-4056.
- Roeder, K. D.** (1962). The behaviour of free flying moths in the presence of ultrasound pulses. *Anim. Behav.* **10**, 300-304.
- Römer, H., Lang, A. and Hartbauer, M.** (2010). The Signaller's dilemma: a cost-benefit analysis of public and private communication. *PLoS ONE* **5**, e13325.
- Schnitzler, H. U., Moss, C. F. and Denzinger, A.** (2003). From spatial orientation to food acquisition in echolocating bats. *TREE* **18**, 386-394.
- Simmons, N. B.** (2005). Order Chiroptera. In *Mammal Species of the World* (ed. D. E. Wilson and D. M. Reeder), pp. 312-529. Baltimore, USA: Johns Hopkins University Press.
- Skals, N. and Surlykke, A.** (2000). Hearing and evasive behaviour in the greater wax moth, *Galleria mellonella* (Pyralidae). *Physiol. Entomol.* **25**, 354-362.
- Stange, L. A.** (2004). *A Systematic Catalogue, Bibliography and Classification of the World Antlions (Insecta: Neuroptera: Mymeleontidae)*. Gainesville, FL: Memoirs of the American Entomological Institute, American Entomological Institute.
- Strauß, J. and Lakes-Harlan, R.** (2014). Evolutionary and phylogenetic origins of tympanal hearing organs in insects. In *Insect Hearing and Acoustic Communication* (ed. B. Hedwig), pp. 5-26. New York, USA: Springer.
- ter Hofstede, H. M., Kalko, E. K. V. and Fullard, J. H.** (2010). Auditory-based defence against gleaning bats in neotropical katydids (Orthoptera: Tettigoniidae). *J. Comp. Physiol. A* **196**, 349-358.
- ter Hofstede, H. M., Goerlitz, H. R., Ratcliffe, J. M., Holderied, M. W. and Surlykke, A.** (2013). The simple ears of noctuid moths are tuned to the calls of their sympatric bat community. *J. Exp. Biol.* **216**, 3954-3962.
- Treat, A. E.** (1955). The response to sound in certain Lepidoptera. *Ann. Entomol. Soc. Am.* **48**, 272-284.
- Ulanovsky, N., Fenton, M. B., Tsoar, A. and Korine, C.** (2004). Dynamics of jamming avoidance in echolocating bats. *Proc. R. Soc. Lond. Biol.* **271**, 1467-1475.
- Yack, J. E.** (2004). The structure and function of auditory chordotonal organs in insects. *Microsc. Res. Tech.* **63**, 315-337.
- Yager, D. D.** (1999). Structure, development and evolution of insect auditory systems. *Microsc. Res. Tech.* **47**, 380-400.
- Yager, D. D. and Hoy, R. R.** (1986). The cyclopean ear: a new sense for the praying mantis. *Science* **231**, 727-729.
- Yager, D. D. and May, M. L.** (1990). Ultrasound-triggered, flight-gated evasive manoeuvres in the praying mantis *Parasphendale agrionina* II. Tethered flight. *J. Exp. Biol.* **152**, 41-58.
- Yager, D. D. and Spangler, H. G.** (1997). Behavioral response to ultrasound by the tiger beetle *Cicindela marutha* dow combines aerodynamic changes and sound production. *J. Exp. Biol.* **200**, 649-659.
- Yager, D. D., May, M. L. and Fenton, M. B.** (1990). Ultrasound-triggered, flight-gated evasive manoeuvres in the praying mantis *Parasphendale agrionina* I. Free flight. *The J. Exp. Biol.* **152**, 17-39.