

BAT AVOIDANCE IN THE KATYDID GENUS *NEOCONOCEPHALUS*

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by  
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The undersigned, appointed by the dean of the Graduate School, have examined the thesis entitled

BAT-AVOIDANCE IN THE KATYDID GENUS *NEOCONOCEPHALUS*

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And hereby certify that, in their opinion, it is worthy of acceptance

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.....dedicated to all those who have helped me along the way, including friends and  
family and especially Mom

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## ABSTRACT

The hearing system of the katydid genus *Neoconocephalus* functions in two contexts: intraspecific communication and predator (bat) avoidance. Male calls and bat cries differ spectrally and temporally. To respond appropriately, katydids must recognize and discriminate between signal types. We categorized bat avoidance behaviors and examined temporal and spectral recognition of bat cries behaviorally during tethered flight in several species of *Neoconocephalus*.

In response to simulated bat approaches, *Neoconocephalus* katydids performed four behaviors; steering, wingbeat interruption, dives and leg kicks. The first three were amplitude dependent and were elicited by a single pulse of ultrasound while the final behavior was pulse-rate dependent. Response probabilities of specific behaviors varied. Steering and leg kicks were performed consistently in all species but wingstop (wingbeat interruption or dive) occurred significantly less often in *N. robustus*, *N. bivocatus* and *N. exciliscanorus*, all larger species of *Neoconocephalus*.

We also tested how katydids recognize model bat cries. Single pulse experiments showed that katydids respond best to pulses with relatively short rise times and a minimum duration. Spectral experiments showed that both *N. exciliscanorus* and *N. bivocatus* were relatively insensitive to higher frequencies (>30 kHz) while *N. robustus*, *N. retusus* and *N. ensiger* were sensitive. Among these three species, spectral selectivity differed, with *N. ensiger* being very insensitive at 13 kHz while *N. retusus* and *N. robustus* remained sensitive. We discuss how species-specific differences such as call type, habitat and body size might account for the differences in bat avoidance behaviors.

ULTRASOUND AVOIDANCE BEHAVIORS IN TWO SPECIES OF  
*NEOCONCOCEPHALUS*

**Introduction**

Diverse groups of nocturnally flying insects use their ears to monitor for echolocation cries of foraging bats and take evasive action when they detect such cries (Roeder 1967, Miller & Oleson 1979, Hoy *et al.* 1989, Miller & Surlykke 2001, Yager *et al.* 2000). In several of these groups (e.g. Lepidoptera), hearing probably evolved for the purpose of bat detection (see Miller & Surlykke 2001). However, in Ensifera (Gryllidae, Tettigoniidae and allies) the ear evolved in the context of intraspecific communication with bat avoidance likely being a secondary function of the hearing system (Stumpner & Helversen 2001).

Concomitantly with their separate evolutionary origins, positive phonotaxis to male calls and bat evasive responses are separate behaviors in ensiferans (Pollack & El-Feghaly 1993), controlled by independent neural circuits (Nolen & Hoy 1986a, Schildberger & Hörner 1988). The need to attend to bats and males with different responses requires that the two signal classes are reliably discriminated from each other. In crickets, this discrimination is based on the carrier frequency of the signals with bat evasive responses largely limited to ultrasonic signals (Moiseff *et al.* 1978, Nolen & Hoy 1986a). In katydids, however, discrimination based on carrier frequency appears less efficient, as male calls in many groups contain major ultrasonic components or are even

limited to ultrasound (Heller 1988, Morris *et al.* 1994). Differences in temporal pattern likely contribute to the detection of bats within the background of male calls (Schul & Sheridan 2006, Faure & Hoy 2000a)

The need to reliably detect and avoid bats may impose constraints on the acoustic communication system of katydids. For example, the need to detect bats may lead to reduced female selectivity during flight compared to selectivity in walking females (Schul & Schulze 2001). Also, it may limit the potential parameter space for the evolution of communication signals. Therefore, knowledge about bat avoidance behaviors is important to consider when studying the function and evolution of acoustic communication systems.

The katydid genus *Neoconocephalus* is a large group (25+ species) with a wide geographical range and high diversity in male calls (Greenfield 1990). Recently, this group has been extensively studied with regard to the co-evolution of male calls and female preferences (e.g. Deily & Schul 2004, 2006, Bush *et al.* 2009, Beckers & Schul 2008) and the neuroethology of female phonotaxis and bat avoidance (Faure & Hoy 2000a, 2000b, Schul & Sheridan 2006, Höbel & Schul 2007, Triplehorn & Schul 2009). A robust reconstruction of the phylogenetic relationship in this genus is available (Snyder *et al.* 2009), allowing powerful comparative studies in this group.

Bat avoidance behaviors in this group have only been cursorily studied (Libersat & Hoy 1991, Faure & Hoy 2000c). Data for bat avoidance during flight is available for only one species (*N. ensiger*, Libersat & Hoy 1991), which differed significantly in its behavioral repertoire and sensitivity from another katydid species (Schulze & Schul 2000) and from crickets (Moiseff *et al.* 1978, Nolen & Hoy 1986a) However, the

methods used differed considerably among these studies, and thus it is unclear whether the differences among species are real or the result of methodological variation.

Here I study the bat avoidance behaviors of two *Neoconocephalus* species, *N. ensiger* and *N. retusus*, both of which are relatively small in size. Male calls of *N. retusus* have the fast pulse rate (approximately 150 pulses/s at 25°C) typical of this genus, while *N. ensiger* has a much slower pulse rate (12p/s at 25°C) (Greenfield 1990). The ranges of the two species overlap in the northeastern United States, with *N. ensiger* extending into Canada, while the range of *N. retusus* includes subtropical habitats in the southeastern United States (Walker 2008). The two species are at disparate positions in the phylogeny (Snyder *et al.* 2009).

I test the responses during tethered flight to stimulation with models of echolocation cries during the different phases of an attack by an aerially hunting bat. I quantify the temporal parameters of the signals that elicit bat (ultrasound) avoidance behaviors. The results were similar between the two species and largely agree with those described for other katydids.

## **Materials and Methods**

I collected adult males and females of *N. retusus* (Scudder) and *N. ensiger* (Harris) from wild populations near Columbia, Missouri. The insects were kept in the laboratory under a photoperiod of 14h:10h L:D cycle at 20-25°C. Prairie fescue, apples and water were provided *ad libitum*.

Experiments were performed at 25°C in a temperature controlled chamber (2m x 3m x 5m) lined with 10 cm anechoic foam. Insects were tethered at the pronotum and

placed dorsal side up 20 cm in front of a fan producing a wind speed of  $\sim 2.5$  m/s. Under these conditions, the animal assumed a flight posture (Fig. 1) similar to that described by Libersat and Hoy (1991) and Schulze and Schul (2001). Stimuli were only presented if the insect was in stable flight position with all wings beating for at least 15s before stimulation. I monitored insect activity through an infrared sensitive video camera placed underneath the insect and a Sony GV-D1000 digital video recorder. The experiments took place in total darkness with the exception of an infrared light source for the video camera.

### *Acoustic Stimulation*

Acoustic stimuli were delivered through one of two Technics EAS 10TH400C speakers placed 50 cm from the animal and perpendicular to its longitudinal axis on either side of the insect. I generated ultrasonic stimuli using a custom developed DA-converter/amplifier system with 16-bit resolution and a 250 kHz sampling rate. The amplitude of the signals was controlled manually using an attenuator with a resolution of 0.375 dB. The amplitude of the signals was calibrated using a  $\frac{1}{4}$ " condense microphone (G.R.A.S. 40BF) and a Bruel and Kjaer sound level meter (B & K 2231) using its "peak amplitude" function. Sound measurements were obtained at the level of the animal without the animal present. Sound levels are given as dB SPL (re 20  $\mu$ Pa).

Simultaneously with the playback of a bat call model an infrared LED flashed, to mark the stimulus timing on the video recording. This LED was invisible for the insect.

### *Stimuli*

I generated a model of a bat echolocation call of the frequency-modulated (FM) type, resembling search phase calls of aerially hunting bats occurring in the habitats of

*Neconocephalus* katydids (e.g. *Eptesicus fuscus*). The carrier frequency was linearly modulated from 80 to 45 kHz during the first 50% of the pulse duration, and from 45 kHz down to 35 kHz during the remainder of the pulse. The main energy of these pulses was focused around 40 kHz. Duration of the model bat pulses was 10 ms, if not stated otherwise, and had rise and fall times of 0.5 ms. In the experimental series where pulse duration and pulse rise time were manipulated (see below), the pulses had a constant carrier frequency of 40 kHz. The model bat pulses were presented in various combinations during the different experimental series of this study.

I first simulated the approach of an echolocating bat flying at a speed of  $6.3 \text{ m s}^{-1}$ , assuming a call amplitude of 125 dB SPL at a distance of 10 cm (Jensen & Miller 1999, Holderied & von Helversen 2003), a spreading loss of 6 dB per double distance and atmospheric attenuation of  $1 \text{ dB m}^{-1}$  (Lawrence & Simmons 1982, Sivian 1947). The search and approach phase of the simulated bat approach had pulse rate of approximately 7 Hz (period = 140 ms) and 4 s duration. The amplitude of the bat pulses increased from 43 dB SPL (approx. 31 m distance) to 83 dB SPL (6.3 m distance). The final second of the simulated bat approach mimicked the terminal phase (Moss *et al.* 2006). It consisted of three sections with increasing pulse rate (18, 45, 111 Hz) and decreasing pulse durations (4, 3, 2 ms, respectively). Pulse amplitude was held constant at 84 dB SPL during this phase. I presented the approach stimulus four times to each animal, twice from each side.

To measure the importance of pulse duration and pulse rise time duration, I used sound pulses with a constant carrier frequency of 40 kHz instead of the frequency modulated carrier described above. This was necessary, as changes in pulse durations

would have changed the modulation rate in the FM stimuli and would have introduced an additional independent variable. In the pulse durations series, rise and fall time were kept constant at 0.5 ms. The influence of pulse rise time was tested in two series. In the first series, the plateau time of the pulses was kept constant at 9 ms while rise and fall time were varied between 0.5 to 1000 ms; i.e. total pulse duration varied between 10 and 2009 ms. In the second series, the total pulse duration including rise and fall times was kept constant at 2000 ms and rise and fall time varied between 0.5 and 1000 ms. The amplitude during the rise time experiments was set at 12 dB above the threshold for the stimulus with 0.5 ms rise time, which was determined for each individual prior to the experiment (see below).

In the final experiment, we tested the importance of the pulse rate during the terminal phase of the simulated bat approach (see above). I kept the search/approach part of this stimulus as described above (i.e. 28 model bat pulses of increasing amplitude at 7 Hz), and held the pulse rate constant during the terminal phase. I used pulse rates between 7 Hz and 100 Hz. Pulse duration and amplitude of the terminal phase was held constant at 4 ms and 84 dB SPL.

### *Experimental Protocol*

The sequence of the stimuli presented during each experimental series was pseudo-randomly varied among the individuals tested in each series. I kept a silent period of at least 2 minutes between successive stimulus presentations. I could not detect changes of behavioral sensitivity in the course of our experiments. Individuals were tested as long as they maintained consistent flight, for up to 60 minutes.

If not stated otherwise, each experimental series was tested in a repeated measures approach, i.e. each individual contributed a data point for all values of the independent variable tested. In the experimental series during which I scored response probability, each stimulus was presented four times, two times from each side. Here, data are given as 'grand-mean', i.e. as the mean of the mean values of all individuals tested.

To determine the amplitude dependence of three of the ultrasound avoidance behaviors, I tested each individual with all stimulus amplitudes (40 – 70 dB SPL) in 3 dB steps for series of 7 model bat pulses and for single bat pulses. Each amplitude was presented twice. I defined the threshold for each behavior as the lowest stimulus amplitude at which the insect consistently responded to both stimulus presentations.

To determine thresholds as functions of pulse duration, I used a 1/2 up, 0/2 down paradigm (Levitt 1971). In short, if at least one response occurred to two presentations of a given amplitude, the amplitude was lowered, if no response occurred, then the amplitude was increased. At each reversal of direction the step size was halved. I started each experiment at 84 dB SPL with a step size of 12 dB. Threshold determination was stopped after a final step size of 1.5 dB. The lowest stimulus amplitude that elicited a response was defined as threshold.

Threshold data are presented as median in dB. Error bars give the range excluding the extreme values (i.e. from second highest to second lowest threshold).

### *Analysis*

Response probabilities were determined offline from video recordings, using single frame analysis. I used the criteria described in the results to decide when the

behaviors occurred. I confirmed the reliability of these decisions by blind re-scoring a subset of the data through an independent observer.

For a subset of the experiments, the video recordings were digitized and position traces for select body parts generated using motion analysis software (MaxTRAQ Lite v 2.20, Innovision Systems). This analysis was used to determine the nature and timing of behavioral responses.

## **Results**

During tethered flight, both *N. retusus* and *N. ensiger* assumed a flight posture with the antennae and forelegs pointing forward. Mid- and hindlegs were extended straight back, parallel to the abdomen (Fig. 1). The tarsi of the forelegs were often crossed. Both fore- and hind wings were fully opened and beating, although forewings had a much smaller range of movement than the hindwings.

In response to the simulated bat approach, the insects displayed four distinct behaviors. The first behavior, ‘steering’, consisted of swinging the hind leg contralateral to the sound source away from the abdomen (Fig 2). Occasionally, the other legs moved in the same direction. However, I could not detect directional movement of the abdomen during steering. This behavior was clearly observable in the position traces of the hind legs (Fig. 2, label S). The second behavior, ‘wingbeat interruption’, consisted of closing the hindwings (i.e. aligning with the abdomen) while the legs remained in flight posture (Fig 2, label W). Forewings either remained open, or were partially or totally closed during this behavior. The third behavior, ‘dive’, consisted of folding all four wings and rapidly aligning the forelegs with the abdomen (Fig 2, label D). The position traces of the

forelegs show a characteristic movement away from, and back to the body axis, as the forelegs were swung backward. The fourth behavior ‘leg kick’, was a rapid swinging of all 6 legs laterally away from the abdomen (Fig 2. label K). In many cases, a short burst of wingbeats occurred in conjunction with the leg kick. I discuss the function and the reason for the assigned names below.

Considerable behavioral variability existed during stimulation with the simulated bat approaches. Not every individual showed all four behaviors (e.g. Fig. 2A); for example, the ‘wingbeat interruption’ was often skipped, especially in *N. ensiger*. Also, responses of one individual could vary both in which behaviors occurred (Fig. 2B) and in the timing of the behaviors during the approach. Table 1 gives the response probabilities for each behavior.

The timing during the approaches differed distinctly for the four behaviors (Fig 3). Steering occurred typically during the first second of the stimulus in both species. Wingbeat interrupts and dives occurred later during the stimuli, typically during the search/approach phase with 7/s pulse rate. I never observed leg kicks during the first 4 seconds of the stimulus, but only during the terminal phase with pulse rates  $\geq 18/s$  (Fig. 3).

In the previous experiment the first three behaviors occurred during the part of the approach with increasing amplitude of the bat cry models. To quantify the importance of the stimulus amplitude for these behaviors, I measured their behavioral thresholds with constant amplitude stimuli. In response to a series of 7 bat pulses (at 7/s) median thresholds for leg steering was 49 dB SPL (n=8 for all measurements); median thresholds for wingbeat interruption were at 53 dB SPL and the diving response occurred at 63 dB

SPL (Fig. 4A). When I used a single bat pulse as stimulus, median response thresholds were two to five dB higher (54, 55, 66 dB SPL, respectively; Fig 4B). This indicates that single pulses are sufficient to trigger these behaviors and that specific pulse rates were not necessary.

In the next sets of experiments, I quantified the importance of the temporal parameters of a single pulse for the bat avoidance. Because of the response variability of the first three behaviors, I scored both wingbeat interrupt and dive as a single response (see methods). In the first series, I determined relative behavioral thresholds as a function of pulse duration (Fig. 5). In *N.retusus*, thresholds decreased by approximately 6 dB for each doubling of the pulse duration. In *N. ensiger*, the function was steeper for pulses shorter than 10 ms (8 dB/doubling duration), while from 10 to 20 ms thresholds decreased by only 4.5 dB. Thus, thresholds in both species changed significantly more than the -3 dB/double duration (Fig. 5, dashed line) predicted by an energy integrator (Surlykke 1988, Tougaard 1998).

I next tested the importance of the pulse rise time in two experimental series, one keeping plateau duration constant, and the other pulse duration constant. Insects responded best to short pulse rise times, with response probabilities dropping sharply as rise time increased beyond 40 ms. Response probabilities were low for rise times of 250 ms and longer (Fig. 6). In both species, response probabilities were somewhat higher for the constant plateau stimuli.

Leg kicks were never observed in response to single pulses or slow pulse rates (7 pulses/s), even at amplitudes exceeding the maximum amplitude of the simulated approaches. This suggests that a higher pulse repetition rate may be necessary to trigger

this behavior. Correspondingly, I observed leg kicks only during the terminal phase of the simulated approaches, i.e. after pulse rates increased above 7 Hz (Fig. 3). To test the threshold pulse rate required to trigger leg kick behavior I presented the insects with approach stimuli with the terminal phase held at a constant pulse rate between 7 and 100 Hz.

In *N. retusus*, response probabilities were low for pulse rates up to 25 Hz and increased sharply towards higher pulse rates (Fig. 7). In *N. ensiger*, response probabilities increased sharply between 50 and 75 Hz, while no responses occurred up to 25 Hz. The movement amplitude of the leg kicks increased with increasing pulse rate in both species (Fig. 8).

## **Discussion**

I studied behavioral responses to ultrasonic stimulation during tethered flight. Three behaviors were elicited by single sound pulses and occurred during the model search/approach phase. A fourth behavior was triggered only by stimulation with fast pulse rates and consequently was only seen in response to a terminal phase mimic. I could not detect significant differences between the species for the stimuli tested here.

At low pulse amplitudes the hindleg contralateral to the sound source swung away from the body where it impaired the hind wingbeat, which should cause turning away from the sound source during free flight (May & Hoy 1990). Accordingly, I refer to this behavior as steering. At higher pulse amplitudes, the insect stopped wingbeat but remained in flight posture, which should result in a sudden drop of altitude. During experiments with single pulses or short series (Fig. 4) the insects resumed normal

wingbeat within one second, thus I refer to this behavior as wingbeat interruption. At even higher pulse amplitudes, all wings were folded parallel to the body and the animal moved out of flight position ('dive'). During stimulation with single pulses and series of seven pulses (Fig. 4), flight did not resume for several seconds after the dive, so that a free flying insect would likely fall to the ground. Note that during the stimulation with the approach stimuli (Fig. 2) the leg kick behavior often coincided with a short burst of wingbeats. All of these behaviors increase the likelihood that the insect will move away from the echolocation cone (Hartley & Suthers 1989) of the bat, thus I interpret them as bat-avoidance behaviors.

Our approach stimulus assumed a constant bat flight speed of 6.3 m/s (see methods). In both species tested, steering occurred at amplitudes equivalent to a distance of ~26 m between bat and insect (Fig 3). In *N. retusus*, wingbeat interruption and dive occurred at distances of approximately 20 and 12 m (i.e. stimulus amplitudes of 56 and 69 dB SPL; Fig 3). Responses occurred at similar amplitudes during the threshold measurements (Fig 4). In *N. ensiger*, the latter two responses occurred later during the simulated approach, equivalent to distances of 10 and 7 m between the bat and the insect (Fig 3).

Estimates for the detection ranges of bats for medium to large insects range between 5 m (Kick 1982) and 10-15 m (Holderied & Helversen 2003, Surlyyke & Kalko 2008). Thus, these three avoidance behaviors would occur either before or close to the time when the bat would detect the insect. Therefore, these three behaviors are early warning behaviors, which reduce the likelihood of being detected by the bat (Miller & Oleson 1979). However, early warning behaviors also have potential costs for the insect.

Any deviation from the original flight path should cause a loss in time or energy for the insect. A dive would also increase the chance that the insect is exposed to terrestrial predators (e.g. spiders, mantises). Seemingly, as the risk of bat predation increases (decreasing distance) the insect employs more costly behaviors (Fig 3).

The consistency with which the leg kick occurs (Table 1), and the low variability in time of occurrence (Fig 3) support its function as bat avoidance behavior. The leg kick never occurred to single pulses, or stimuli with slow pulse rates. It was triggered only by fast pulse rates (Fig. 7), as they occur during the terminal phase of a bat approach ("feeding buzz", Griffen *et al.* 1960, Schnitzler & Kalko 2001). I therefore consider the leg kick a 'last chance behavior' (Miller & Oleson 1979). Last chance behaviors triggered by fast pulse rates have been described in several other insects including green lacewings (Miller 1975), dogbane tiger moths (Fullard *et al.* 1994) and praying mantids (Tribblehorn & Yager 2005).

The effect of this behavior during free flight is not obvious. Potentially, the leg kicks could cause a sudden change in the flight path of the insect. The burst of wingbeats, that often occur in conjunction with this behavior (Fig 2), supports this interpretation. Alternatively, the leg kick might make the insect more difficult for the bat to capture. The species studied here (body length 37-55 cm) are probably at the upper end of the size range of the prey spectrum captured by larger bats (e.g. *Eptesicus fuscus*, Agosta *et al.* 2003). Thus, the leg kick might be a successful strategy used to avoid capture. A third alternative is that the leg kick behavior could change the echo signature received by the bat, potentially confusing or startling the bat.

The leg kick bears a striking resemblance to a terrestrial startle behavior described in other Copiphorinae. In response to terrestrial predators, *Mygalopsis spec.* spread forelegs, hind legs, and mandibles rapidly, while keeping the midlegs on the substrate (Sandow & Bailey 1978). In Saginae, such startle behavior was accompanied by a flaring of the wings (Kaltenbach 1990).

The similarities of these terrestrial startle behaviors to the in-flight leg kick leads us to hypothesize that the leg kick is an aerial manifestation of the terrestrial startle response. The change in behavioral context (sitting vs. flying insect) is accompanied by a change in sensory modality (optical to acoustic stimulation). A similar behavioral transfer has been reported in praying mantids (*Parasphendale agrionina*), which have incorporated an optically/tactilely-induced, terrestrial startle response into their flight repertoire (Yager & May 1990). When performed in flight, the behavior can only be elicited by acoustic stimulation (Yager & May 1990).

Previous bat-avoidance studies with flying *Neoconocephalus* reported only one behavior with very high thresholds (*N. ensiger*, Libersat & Hoy 1991). However, these authors used very different experimental methods than our own, as well as insects from geographically different populations. Either of these factors might account for the differences in observed behavioral repertoires.

The behavioral repertoire and sensitivities that I found in *Neoconocephalus* were similar to those described for another katydid, *Tettigonia viridissima* (Schulze & Schul, 2001), tested with methods similar to our own. Only minor differences were detected between the behavioral repertoires of *T. viridissima* and *Neoconocephalus*, namely a lack of abdominal steering in *Neoconocephalus*. The consistency in behavioral repertoires

and behavioral sensitivities in these two distant genera of Tettigoniidae suggest that bat-avoidance strategies are relatively well conserved within the family. While the leg kick has not been described in previous studies of in-flight, bat-avoidance behaviors (Libersat & Hoy 1991, Schulze & Schul 2001), the stimulation used by these authors would not have revealed this behavior, as no fast pulse rates were used.

The three early warning behaviors were all elicited by a single pulse of ultrasound, indicating that pulse repetition rate played no role in the recognition of the bat pulses. Therefore, some feature of a single pulse must be used to recognize the signal as a bat cry. In order to narrow down the necessary parameters, I tested two fine-scale temporal parameters, pulse rise time and duration.

Both *N. retusus* and *N. ensiger* had the highest response probabilities to pulses with short rise time durations, regardless of the overall duration of the plateau or the total pulse duration. Total length of the stimuli, which reached nearly 2 s in the constant length stimuli, did not negatively affect response probabilities, indicating that maximum pulse duration is probably not a critical value. I confirmed this by measuring behavioral thresholds for pulses of varying duration. Although long duration pulses do not negatively affect thresholds, decreasing the duration did have an effect.

I measured shifts in the slope of behavioral thresholds at 6-8 dB per double duration (dB/dd), much higher than expected from the usage of an energy detector alone (3dB/dd; Au 1988). This difference indicates that some parameter other than signal energy determines behavioral thresholds for signals of varying duration. A similar conclusion was reached for the greater wax moth (*Galleria mellonella*), which had a slope in behavioral threshold shift of 7.2 dB/dd (Skals & Surlykke 2000).

The effect of variations in rise time and the relative unimportance of lengthening pulse duration indicate that the signal onset is critical for recognition. In order to be recognized a signal must have a minimum duration ( $>2$  ms) and have a short rise time. I hypothesize that very short duration signals are not recognized as aversive because of their similarity to sounds produced by other katydids (e.g. Phaneropterinae; Heller 1988, Walker 2008).

The increase in behavioral thresholds as a function of decreasing pulse duration suggests a potentially useful strategy for bats. If a bat could decrease the duration of its pulses (and thus the total energy), its prey detection range would inevitably decrease. However, the detection range of the katydid for bat echolocation would decrease by a greater amount, because of the greater effect of pulse duration on behavioral thresholds in katydids. Thus, decreasing the duration of echolocation cries might be a successful hunting strategy for bats.

One additional problem that katydids face, beyond simple recognition of signals, is that bats must be detected in the midst of the background noise produced by other insects, including conspecific calls. One solution proposed for *Neoconocephalus* katydids with 'typical' calls (fast pulse rate), such as *N. retusus*, is that signals are discriminated based on differences in pulse rate, via the TN-1 neuron. TN-1 responds to stimuli with slow pulse rates ( $<20$  Hz), while no responses occur to faster pulse rates. TN-1 detects slow pulse rates even in the presence of fast rates, if the two signals differ sufficiently in carrier frequency (Schul and Sheridan 2006). In *N. retusus*, this interneuron is broadband, responding to frequencies associated with both bats and male calls.

This solution would not work for *N. ensiger*, which has a call with slow pulse rates.. *N. ensiger* may have solved this problem by shifting the tuning of TN-1 to higher frequencies (Faure & Hoy 2000b). I hypothesize that *N. ensiger*, unable to discriminate signals based on pulse rate, instead relies on spectral differences between signals for discrimination.

INTERSPECIFIC VARIATION OF BAT AVOIDANCE BEHAVIORS IN  
*NEOCONOCEPHALUS*

**Introduction**

In the katydid genus *Neoconocephalus*, the hearing system functions in two behavioral contexts; intraspecific communication, and the detection of acoustically hunting predators (bats). Thus, katydids need to be able to detect bat cries in the presence of male calls. Male calls of most *Neoconocephalus* species are characterized by a fast pulse rate (>100 Hz, Greenfield 1990). In contrast, bat echolocation calls during search and approach phase have pulse rates an order of magnitude lower (7-15 Hz) with low duty cycles.

In a species with fast pulse rates, an auditory interneuron (TN-1) responded selectively to bat cries in the presence of male calls (Schul & Sheridan 2006), even when the male call models were more than 12 dB louder than the bat signals. While TN-1 responses to fast pulse-rate signals were quickly suppressed, the neuron remained sensitive to slow pulse rates, as long as they differed sufficiently in carrier frequency (Schul & Sheridan 2006). This neuronal mechanism should allow a katydid to reliably detect bat cries when flying above a dense male chorus.

Several *Neoconocephalus* species have evolved calls with lower amplitude modulation rates (3-15 Hz), which are similar to those of bat cries during the search and approach phases. These rates are achieved either by producing pulses at a slow rate (e.g. *N. ensiger*) or by grouping few high-pulse rate pulses into rhythmically repeated chirps,

repeated at rates of 3-7 Hz (e.g. *N. exciliscanorus*, *N. spiza*). In such species, the neuronal mechanisms to segregate bat and male signals based on pulse rate (Schul & Sheridan 2006) would not allow for reliable detection of bat cries.

Spectral content may play a role in signal discrimination for these species. While ‘typical’ katydid calls contain broadband ultrasonic components (Heller 1988), *Neoconocephalus* calls have most energy concentrated in a narrow, low-frequency band (7-15 kHz) with ultrasound components strongly reduced in amplitude (Greenfield 1990, Schul and Patterson 2003). This call spectrum of *Neoconocephalus* should, in principle, allow for the categorization of male calls and bat cries based on spectrum alone, as observed in crickets (Wytenbach *et al.* 1996)

Here I test whether the responsiveness and selectivity of bat avoidance behaviors differs between species with calls with fast and slow AM-rates. I chose three species with fast pulse rates (*N. retusus*, *N. robustus*, *N. bivocatus*), that differ in body size and habitat. I compare them to one species with slow pulse rate (*N. ensiger*), and one species with calls grouped in chirps (*N. exciliscanorus*).

## **Materials and Methods**

I tested bat avoidance behaviors of males of five species. *N. retusus* (Scudder), *N. robustus* (Scudder) and *N. bivocatus* (Walker, Whitesell, Alexander) have fast pulse-rate calls (>150 Hz) typical of this genus (Greenfield, 1990). *N. ensiger* (Harris) and *N. exciliscanorus* (Davis) both have low AM-rate calls, but generate them in different ways. *N. ensiger* produces pulses at a slow pulse rate (5-15 Hz, depending on ambient temperature, Frings & Frings 1957). The calls of *N. exciliscanorus* consist of

rhythmically repeated chirps (chirp rate 3-7 Hz); each chirp comprises 5-7 pulses at a pulse rate of 80 Hz (21-27° C, Walker 1975).

Three of the species (*N. retusus*, *N. robustus*, *N. ensiger*), occur in wide range of grasslands varying in humidity and vegetation (Whitesell 1969, Walker 2008). The other two species are limited to specialized habitats: *N. bivocatus* to dry grasslands (e.g. tallgrass prairies; Walker *et al.* 1974), and *N. exciliscanorus* to marshes and similarly wet habitats (Whitesell 1969).

I collected adult males of *N. retusus*, *N. bivocatus*, *N. ensiger*, and *N. robustus* from wild populations in Boone and Macon Co., Missouri. *N. exciliscanorus* were collected from marshes in Wayne Co., Missouri. The insects were kept in the laboratory under a photoperiod of 14h:10h L:D cycle at 20-25°C. Prairie fescue, apples and water were provided *ad libitum*. I measured hind femur and pronotum length of the insects used in the experiments.

Experiments were performed at 25°C in a temperature controlled chamber (2m x 3m x 5m) lined with 10 cm anechoic foam. Insects were tethered at the pronotum and placed dorsal side up 20 cm in front of a fan producing a wind speed of ~2.5 m/s. Under these conditions, the animal assumed a flight posture described in the previous chapter. Stimuli were only presented if the insect was in stable flight position with all wings beating for at least 15s before stimulation. I monitored insect activity through an infrared sensitive video camera placed underneath the insect and a Sony GV-D1000 digital video recorder. The experiments took place in total darkness with the exception of an infrared light source for the video camera.

### *Acoustic Stimulation*

Acoustic stimuli were delivered through one of two Technics EAS 10TH400C speakers placed 50 cm from the animal and perpendicular to its longitudinal axis on either side of the insect. I generated ultrasonic stimuli using a custom developed DA-converter/amplifier system with 16-bit resolution and a 250 kHz sampling rate. The amplitude of the signals was controlled manually using an attenuator with a resolution of 0.375 dB. The amplitude of the signals was calibrated using a ¼” condense microphone (G.R.A.S. 40BF) and a Bruel and Kjaer sound level meter (B & K 2231) using its “peak amplitude” function. Sound measurements were obtained at the level of the animal without the animal present. Sound levels are given as dB SPL (re 20 µPa).

To mark the stimulus timing on the video recording, an infrared LED flashed simultaneously with the playback of each stimulus.. This LED was invisible for the insect.

I kept a silent period of at least 2 minutes between successive stimulus presentations. I could not detect changes of behavioral sensitivity in the course of my experiments. Individuals were tested while they maintained consistent flight, for up to 60 minutes.

### *Experiments*

I tested insects with two experimental series. I first tested insects with a stimulus that simulated the approach of an echolocating bat. I generated a model of a bat echolocation call of the frequency-modulated (FM) type, resembling search phase calls of aerially hunting bats occurring in the habitats of *Neoconocephalus* katydids (e.g. big brown bat, *Eptesicus fuscus*). The carrier frequency was linearly modulated from 80 to

45 kHz during the first 50% of the pulse duration, and from 45 kHz down to 35 kHz during the remainder of the pulse. The main energy of these pulses was concentrated around 40 kHz. Duration of the model bat pulses was 10 ms, and had rise and fall times of 0.5 ms.

I first simulated the approach of an echolocating bat flying at a speed of  $6.3 \text{ m/s}^{-1}$ , assuming a call amplitude of 125 dB SPL at a distance of 10 cm (Jensen & Miller 1999, Holderied & Helversen 2003), a spreading loss of 6 dB per double distance and atmospheric attenuation of  $1 \text{ dB m}^{-1}$  (Lawrence & Simmons 1982, Sivian 1947). The search and approach phase of the simulated bat approach had pulse rate of approximately 7 Hz (period = 140 ms) and 4 s duration. The amplitude of the bat pulses increased from 43 dB SPL (approx. 31 m distance) to 83 dB SPL (6.3 m distance). The final second of the simulated bat approach mimicked the terminal phase (Moss *et al.* 2006) and consisted of three sections with increasing pulse rate (18, 45, 111 Hz) and decreasing pulse durations (4, 3, 2 ms, respectively). Pulse amplitude was held constant at 84 dB SPL during this phase. I presented the approach stimulus four times to each insect, twice from each side.

In the previous chapter, I described four avoidance behaviors for katydids in genus *Neoconcephalus* in response to simulated bat approaches. I calculated the response probabilities for individual behaviors in response to the same approach stimuli. Because wingbeat interruption and dive often occurred simultaneously and were frequently difficult to distinguish, here I give their combined probability, and refer to them as wingstop. Response probabilities were determined offline from video recordings, using single frame analysis. I used the criteria described in the previous chapter to determine

behavioral responses. Data are presented as 'grand-mean', i.e. as the mean of the mean values of all individuals tested.

A second experimental series was used to test the sensitivity and the spectral selectivity of each species. The stimuli consisted of a series of 7 pure-tone pulses presented at a rate of 7 pulses/s. Each pulse was 10 ms in duration with a rise/fall time of 0.5 ms. Carrier frequency ranged from 10 to 60 kHz.

The sequence of the stimuli presented was pseudo-randomly varied among the individuals tested in each series. I measured behavioral thresholds as function of carrier frequency for the 'wingstop' behavior (see above). This behavior could be reliably detected at the video screen while conducting the experiments. Thresholds were determined using a 1/2 up, 0/2 down paradigm (Levitt 1971). If at least one response occurred to two presentations of a given amplitude, the amplitude was lowered, if no response occurred, then the amplitude was increased. At each reversal of direction, the step size was halved. I started each experiment at 84 dB SPL with a step size of 12 dB. Threshold determination was stopped after a final step size of 1.5 dB. The lowest stimulus amplitude that elicited a response was defined as threshold. In preliminary experiments, repeated measurements of the threshold to the same stimulus resulted in a median difference of -1.5 dB (range -10.5 to +6 dB,  $n = 9$ ) between first and second measurement.

I measured behavioral tuning curves for each species between 10 and 60 kHz. It was not possible, to determine the complete threshold curve in most individuals. Each individual contributed thresholds for 2-7 frequencies.

To compare the overall sensitivity of each species for frequencies similar to those produced by echolocating bats, I compared median thresholds for the most sensitive frequency for each species (*N. retusus*: 40 kHz; *N. bivocatus*, *N. ensiger*: 50 kHz; *N. exciliscanorus*, *N. robustus*: 60 kHz).

To compare spectral sensitivity among species independent of absolute sensitivity, I present individual threshold values relative to the most sensitive frequency. Relative median thresholds were calculated from individual values and compared statistically.

A final experimental series examined how behavioral thresholds for a higher frequency (40 kHz) were affected by the addition of low frequency (13 kHz) signals, presented simultaneously. This experimental series was tested in *N. retusus* and *N. ensiger*. Thresholds were measured with a 1/2 down, 0/2 up threshold paradigm as described above. Thresholds were determined for a 40 kHz signal and compared to thresholds determined for a signal that was composed of a simultaneous playback of a 13kHz tone and a 40 kHz tone, with the 13 kHz tone presented at +12 dB, relative to the 40 kHz tone. Experiments with similar two-tone stimulation in crickets showed that addition of a low frequency to a high frequency resulted in a reduction in avoidance behaviors, subsequently termed two-tone suppression (Nolen & Hoy 1986).

For comparison, we also measured the typical shift in behavioral thresholds in *N. ensiger*. Behavioral thresholds for 40 kHz were measured twice with second measures occurring on either the same day or on a different day from the initial measurement.

## *Statistics*

Using Microsoft Excel (2003), I compared the two measures for body size among the five species using ANOVA. A Scheffe's post-hoc analysis was performed to determine significant differences between pairs of species

I compared absolute sensitivity and relative thresholds at 13 kHz among species with Kruskal Wallis tests (STATEXT v1.0). Post-hoc tests were calculated using nonparametric multiple comparisons for unequal sample sizes and tied ranks (Dunn's test; Zar 1984). I chose this test, instead of an ANOVA across the complete thresholds, because I was neither able to collect complete threshold curves for individual insects, nor collect enough individuals for each species to collect all data independently. Individual females contribute unequal numbers of data points to the threshold curves.

Error bars indicate the range of data, excluding extreme values (i.e. from second highest to second lowest threshold).

## **Results**

The species differed significantly in both measures for body size (Fig. 9; ANOVA; hind femur:  $F_{4,54} = 39.9386$ ,  $P = 5.412E^{-15}$ ; pronotum:  $F_{4,57} = 58.3863$ ,  $P = 1.65727E^{-19}$ ). Post-hoc analyses indicate that *N. robustus*, *N. bivocatus* and *N. exciliscanorus* were always significantly larger than *N. ensiger* and *N. retusus* (Table 2).

I measured the response probabilities of the three avoidance behaviors to simulated bat approaches (Fig. 10). Steering occurred with similar probabilities across the five species (51% to 66%, Fig 10). The wingstop behavior occurred within 92% of trials with *N. retusus* and had a somewhat lower probability in *N. ensiger* (61%). In the three

other species, this behavior occurred with probabilities below 15%. Leg kicks occurred in 97% of trials with *N. robustus*; the other four species had similar response probabilities for this behavior (means between 60 and 75%).

Although wingstop behavior occurred with low probability in three species during the approach stimuli (i.e. with increasing amplitude between bat pulses), all five species showed this behavior reliably to stimuli with 7 pulses of equal amplitude. Using such stimuli, I determined behavioral thresholds for carrier frequencies between 10 and 60 kHz.

All five species were most sensitive between 40 and 60 kHz, however, significant differences in absolute sensitivity for the most sensitive frequency occurred (Kruskal-Wallis:  $H_{0.05, 17, 10, 7, 8, 8} = 11.783$ ,  $P < 0.01$ ). Median thresholds at the most sensitive frequency were greater than 70 dB SPL for *N. bivocatus* and *N. exciliscanorus* and less than 60 dB SPL for *N. robustus*, *N. retusus* and *N. ensiger* (Fig. 11). Thresholds in *N. bivocatus* and *N. exciliscanorus* were significantly higher than in *N. ensiger*, *N. robustus* and *N. retusus*, as revealed by pairwise comparisons (Table 3).

Spectral tuning was compared for the three most sensitive species. Because of their low overall sensitivity, I excluded *N. exciliscanorus* and *N. bivocatus* from this analysis. Figure 12 shows thresholds relative to lowest median threshold of each species, allowing us to compare spectral tuning independently of absolute sensitivity. All three species were most sensitive between 40 to 60 kHz, and thresholds increased by 12 to 24 dB between 40 and 20 kHz (Fig. 12). In *N. retusus* and *N. robustus*, thresholds remained in this range at 13 kHz and increased steeply at 10 kHz. In *N. ensiger*, the steep increase of thresholds was shifted towards higher frequencies: already at 13 kHz the relative

threshold was at 41 dB and remained that high at 10 kHz (Fig 12). There was a significant difference among the species in relative threshold at 13 kHz (Kruskal-Wallis:  $H_{0.05, 13, 12, 8} = 11.783$ ,  $P = 0.0028$ ), with *N. ensiger* significantly less sensitive than *N. retusus* (Dunn's test  $Q = 2.85$ ,  $p < 0.01$ ) and *N. robustus* ( $Q = 3.06$ ,  $p < 0.01$ ) (Fig 13).

Two-tone experiments showed very little shift between behavioral thresholds for the stimuli used. The shift in thresholds between the 13 kHz/40 kHz and the 40 kHz alone signal indicated a median threshold shift of -0.75 dB in *N. ensiger* and -4.5 dB in *N. retusus*. In other words, addition of a low frequency tone seemed to make thresholds slightly lower (more sensitive). However, the range of threshold shifts was broad with thresholds either increasing or decreasing,  $\sim 15$  dB in *N. retusus* and  $\sim 25$  dB in *N. ensiger*. No consistent directional shift was observed (Fig 14). Repeated measurements of behavioral thresholds in *N. ensiger* to a 40 kHz stimulus showed a similar pattern. While the median threshold shift was small (-1.5 dB), thresholds shifted in either direction by a much greater amount ( $\sim 10$  dB, Fig 14).

## Discussion

I tested the bat avoidance behaviors comparatively among five *Neoconocephalus* species with diverse call patterns. The five species differed in the response probability of wingstops as well as in their absolute sensitivity to bat signals. Also, significant differences occurred in their spectral selectivity. I discuss potential correlates of the behavioral differences with differences in call patterns, habitats, and body sizes.

## *Responsiveness*

The responsiveness of the five species differed as indicated by the differences in response probability of the wingstop behavior and in absolute sensitivity (Figs. 11, 12). Two species, *N. retusus* and *N. ensiger*, had high response probabilities for the wingstop behavior (> 50%, Fig. 10) as well as high sensitivity in the frequency range between 40 and 60 kHz (thresholds below 55 dB SPL, Fig. 11). This responsiveness was comparable to that described in other tettigoniids (Schulze & Schul 2001) and other nocturnally flying insects (Roeder 1967; Miller & Oleson 1979, Hoy *et al.* 1989, Miller & Surlykke 2001, Yager *et al.* 2000). This suggests considerable selection pressure by aerially hawking bats on these two species, which maintain such responsive and sensitive bat avoidance behaviors.

In the three other species, *N. robustus*, *N. exciliscanorus* and *N. bivocatus*, response probability of the wingstop behavior was greatly reduced compared to *N. retusus* and *N. ensiger* (below 15 %, Fig. 10), while the other two behaviors (steering, leg-kick) occur at similar rates across all species. The latter two behaviors likely have relatively small costs for the flying insects, as they should lead only to small deviations from the intended flight path. The wingstop behavior, however, likely causes the flying insect to drop to the ground, thus exposing it to terrestrial predators (e.g. praying mantids, spiders). Thus, wingstops elicited by false alarms likely have a considerable cost to the flying insect (see previous chapter). The three species with reduced probability of the wingstops were significantly larger than the two more responsive species. While bats prey on tettigoniids (Arlettaz 1996, Agosta *et al.* 2003), the typical prey size (6-27 mm body length, Agosta *et al.* 2003) is smaller than most *Neoconocephalus* species (body

length > 35 mm, Walker 2008) and especially the three large species tested here (body length > 49 mm, Walker 2008). I hypothesize that the three large species here are protected against most bat species through their body size and thus have a much reduced predation risk. The cost of the wingstop behavior therefore might select for a reduced response probability of this behavior in the large species. Large body size as a defense against predation has been suggested for other large insects (Whitman & Vincent 2008).

Within the three large species, I detected significant differences in their sensitivity to bat cries. *N. robustus* was similar in sensitivity to the two small species (lowest threshold of 53 dB SPL, Fig. 3). Both *N. bivocatus* and *N. exciliscanorus* were significantly less sensitive (lowest thresholds of 72 and 74 dB SPL, respectively; Fig. 11). These differences in absolute sensitivity coincide with habitat differences among the species.

*N. robustus*, *N. retusus* and *N. ensiger* are grassland generalists, typically found in a wide range of grassland habitats (Walker 2008), while *N. bivocatus* and *N. exciliscanorus* are grassland specialists, typically found in open habitats (prairies, marshes respectively) with very little vertical structure (e.g. trees). Bats rely largely on such landmarks for spatial orientation (Jensen *et al.* 2005, Schnitzler *et al.* 2003). Very low levels of bat activity have been found in open habitats (open prairies, Holloway & Barclay 2000). Thus, these two species might be less likely to encounter foraging bats, further reducing the risk of bat predation.

Insects inhabiting geographically or temporally bat-free spaces (e.g. bat-free islands) demonstrate a loss of sensitivity to frequencies associated with hunting bats and a reduction in avoidance responses (Fullard *et al.* 1997, 2004, 2007) Based on my results,

I suggest that *N. bivocatus* and *N. exciliscanorus* have essentially moved into a niche with strongly reduced rates of predation by bats, through their body size and habitat preference, where high sensitivity of bat detection was not maintained by selection.

#### *Spectral selectivity among sensitive species*

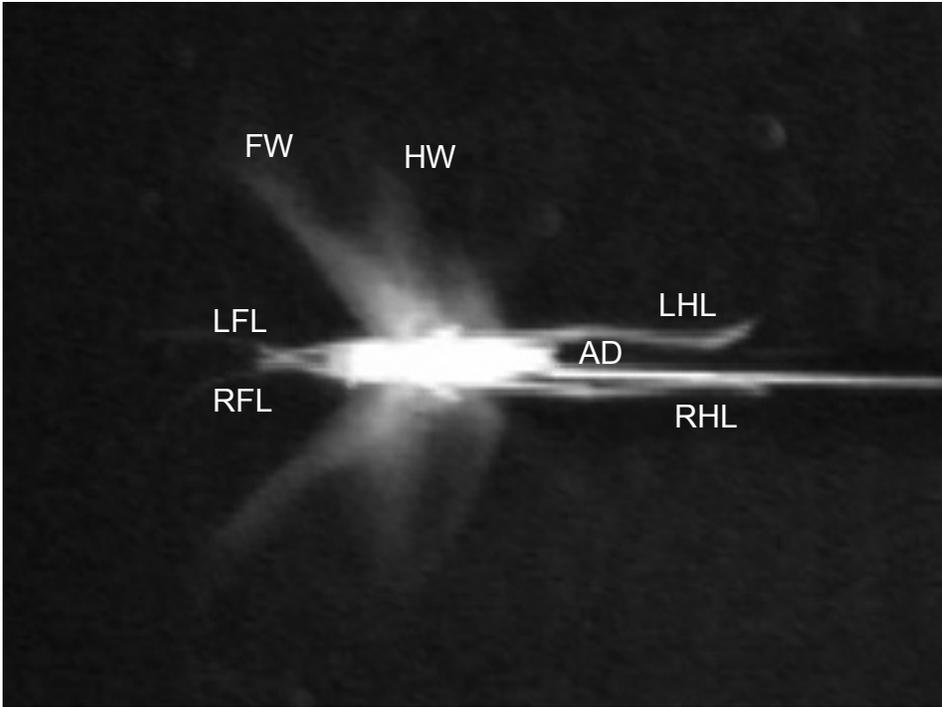
Insects that rely on intraspecific communication, such as katydids need to be able to detect bats against a background of male calls. In most *Neoconocephalus*, this problem seems to have been solved via TN-1, the presumed bat-detecting neuron. This neuron responds selectively to pulses presented at a slow rate (bat cries), and adapts to pulses presented at a fast rate (conspecific calls) (Schul & Sheridan 2006). TN-1 is broadly tuned in *N. retusus*, a species with the ancestral call type of the genus (Snyder 2008), and readily responds to a range of frequencies including those typical of bats as well as the lower frequencies used by conspecifics (Schul & Sheridan 2006). This broad tuning of TN-1 corresponds with the behavioral tuning for bat avoidance in both *N. retusus* and *N. robustus*. Both *N. retusus* and *N. robustus* had relatively low thresholds for bat avoidance behaviors for a wide range of frequencies (Fig 12)

The pulse rate selectivity of TN-1 would work well for species with the fast rate ancestral call (Snyder 2008), such as *N. retusus* and *N. robustus*. However, some species have evolved calls with slow pulse repetition rates (e.g. *N. ensiger*). In these species, the TN-1 rate mechanism would be ineffective, as TN-1 would respond to male calls (Faure & Hoy 2000b). These species would need to rely on some other signal parameter, such as spectrum.

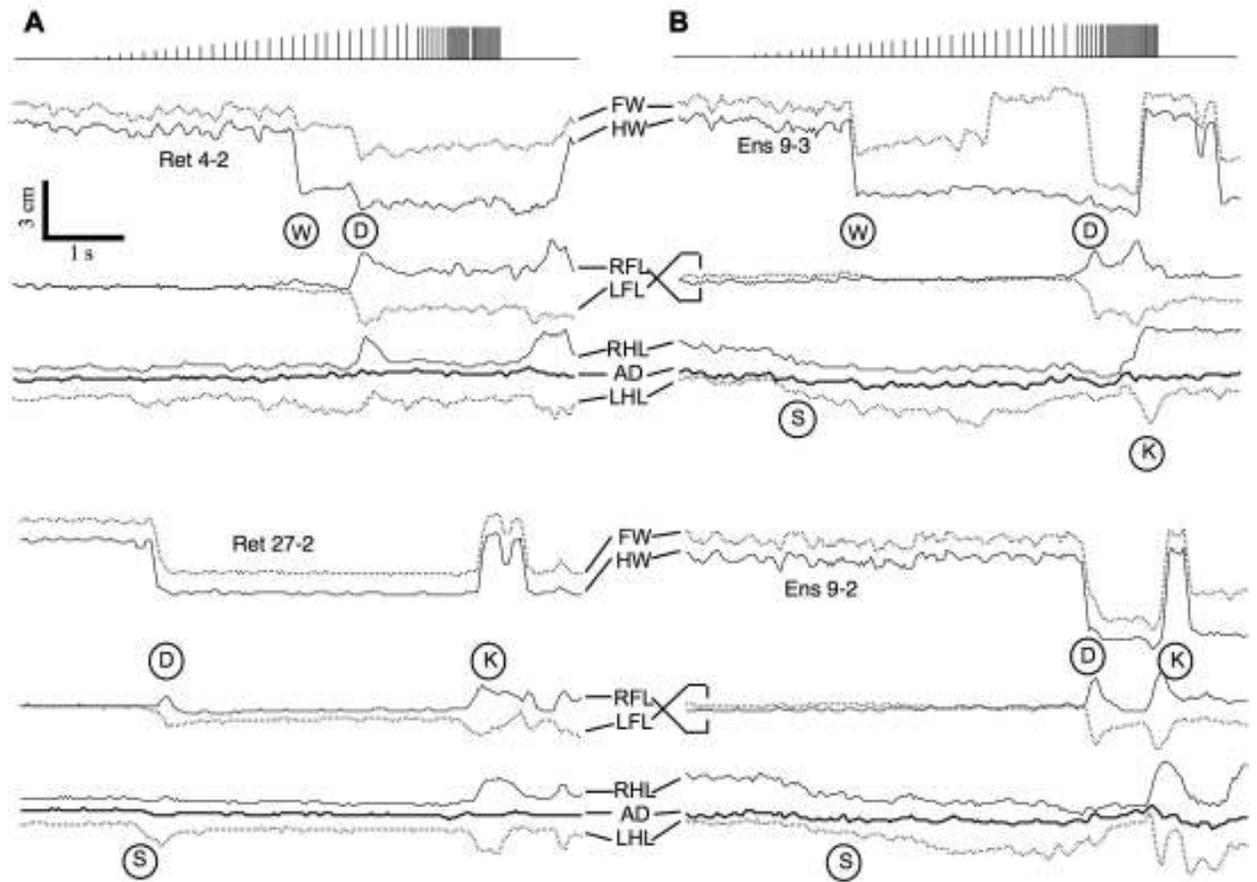
Unlike most katydids (Heller 1988, Morris *et al.* 1994), the calls of *Neoconocephalus* are relatively narrowband and have most energy concentrated at low

frequencies (7-15 kHz, Schul & Patterson 2003, Walker 2008). Bat cries are generally composed of higher frequencies. Species with derived calls (slow pulse rate), could potentially use spectral differences to detect bats against a background of calling males. I see this spectral selectivity in *N. ensiger*, which does not produce bat-avoidance behaviors in response to lower frequencies (13 kHz). This behavioral spectral selectivity matches the tuning of TN-1 in this species, which although still broadly tuned, is shifted to higher frequencies, with low responsiveness to frequencies under 20 kHz (Faure & Hoy 2000b). In *Neoconocephalus* species with an ancestral call type, pulse repetition rate seems to be the primary cue for triggering bat avoidance behaviors, with spectrum seemingly of much less importance.

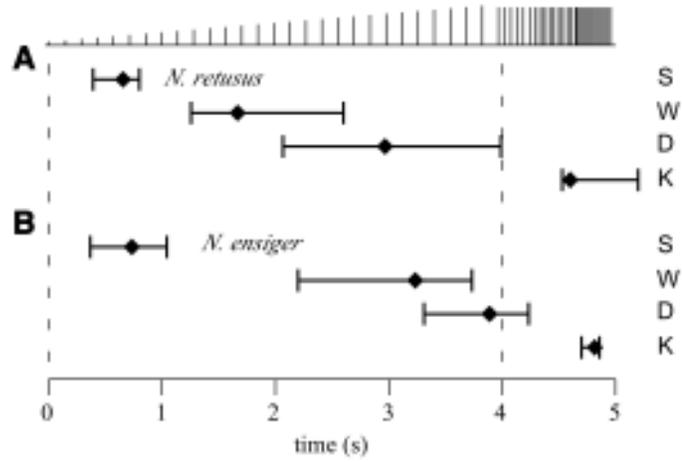
Distinct differences in bat avoidance exist among *Neoconocephalus* species. Species differ in probability of response of different bat avoidance behaviors, spectral sensitivity, and spectral selectivity. These differences seem to coincide with differences in habitat and body size, which could affect rates of predation, as well as with differences in call type, which seemingly affect signal recognition.



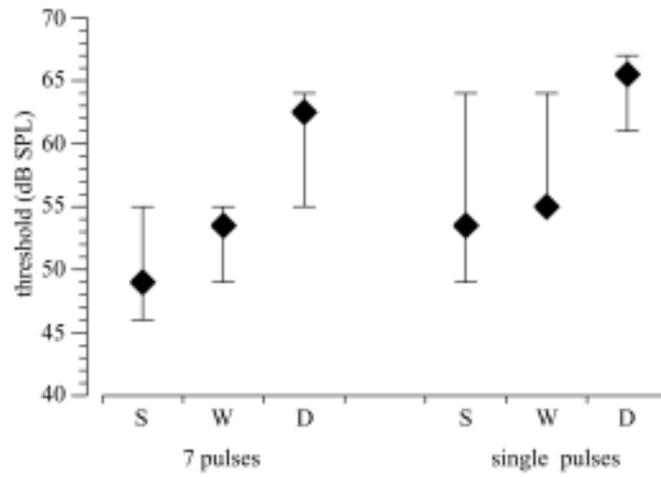
**Fig 1.** Ventral view of a male *Neoconocephalus ensiger* during tethered flight. On the right, the tether extends behind the insect to the edge of the frame. The points used in the position analysis (Fig 2) are indicated (RFL, LFL right/left fore wing; RHL, LHL right/left hindleg; AD abdomen; FW, HW fore/hindwing)



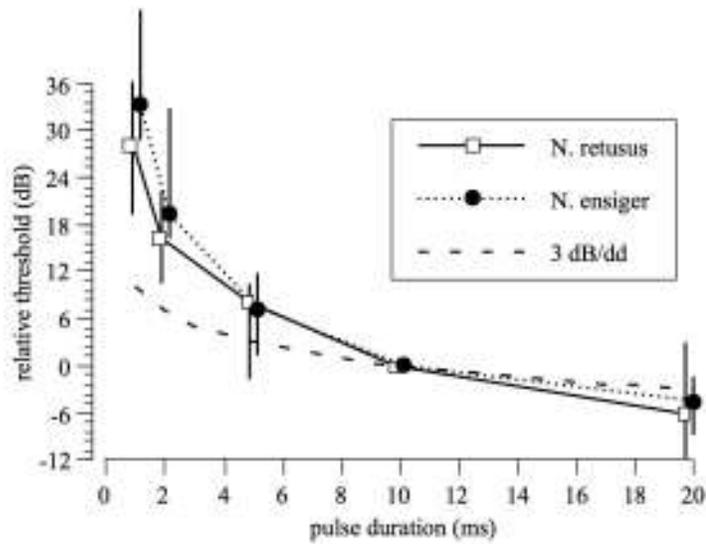
**Fig 2.** Relative position traces of various body parts of *Neoconocephalus retusus* (left) and *N. ensiger* (right) in response to the simulated bat approach during tethered flight. Top trace indicates the timing of the stimulus; amplitudes not shown to scale. The stimulus presentation was from the right (from the top in the figure). Two examples are shown for each species. For *N. retusus* (left) the two examples are from two different individuals; for *N. ensiger* (right) the two examples are from the same individual. For clarity, traces of wings, forelegs, and hindlegs have been shifted relative to each other. Positions are shown only for the right fore and hindwing (FW, HW). RFL, LFL right/left foreleg, RHL, LHL right/left hindleg, AD abdomen tip. The measuring points are indicated in Fig. 1. The circled labels indicate the timing of behavioral responses; S steering, W wingbeat interruption, D dive, K leg kick. Note that in the examples of *N. ensiger*, the forelegs were crossed at the beginning of the trial.



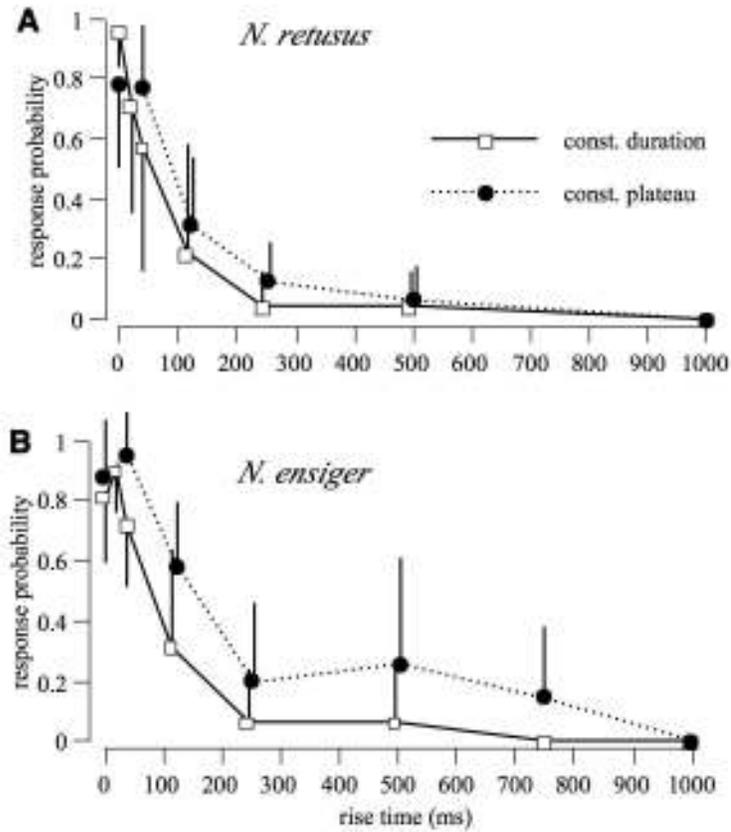
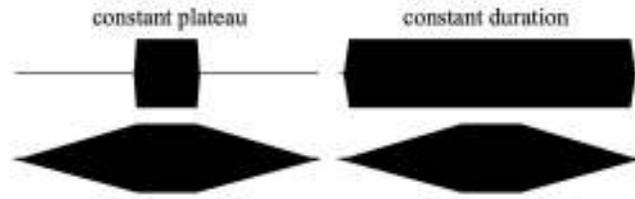
**Fig 3.** Timing of the four bat avoidance behaviors during the simulated bat approach in *N. retusus* (A) and *N. ensiger* (B). Top trace indicates the timing of the stimulus; amplitudes not shown to scale. Dashed lines mark the beginning of the stimulus and beginning of terminal phase. Median response times shown; error bars indicate range of data, excluding the highest and lowest value. N=9 (*N. retusus*) and 11 (*N. ensiger*). S steering, W wingbeat interruption, D dive, K leg kick.



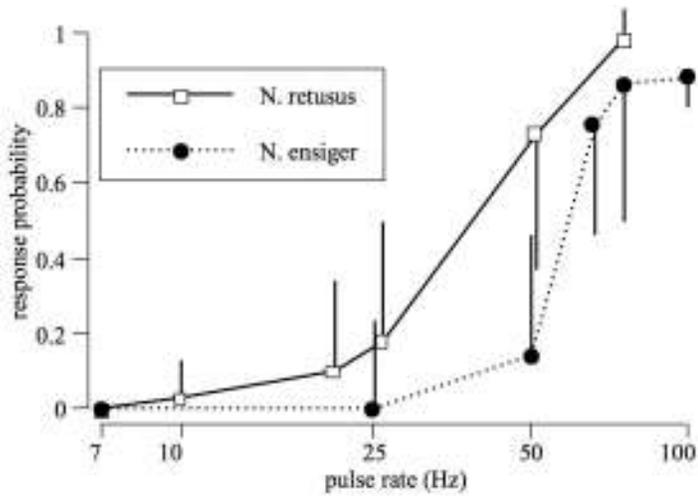
**Fig 4.** Behavioral thresholds of the three early warning behaviors in *N. retusus* in response to a series of 7 model bat cries presented at a rate of 7/s (left) and a single model bat cry (right). Median thresholds (n=8) are shown with error bars showing the range of data, excluding highest and lowest value. S steer, W wingbeat interruption, D dive.



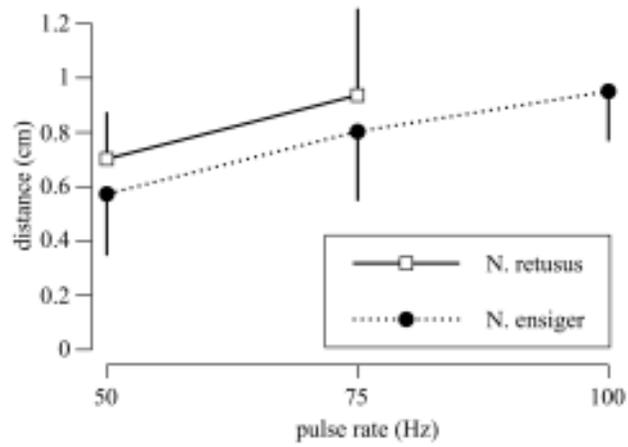
**Fig. 5:** Relative behavioral threshold for wingbeat interruption in response to single sound pulses of varying duration. Thresholds are given relative to the threshold for 10 ms pulse duration. Symbols indicate median thresholds (open squares *N. ensiger*, n=11; filled circles *N. retusus*, n=10); error bars represent range, excluding highest and lowest values. Dashed line indicates the relative thresholds expected for an energy integrator with a slope of -3dB per doubling duration. Stimuli had a constant carrier frequency of 40 kHz.



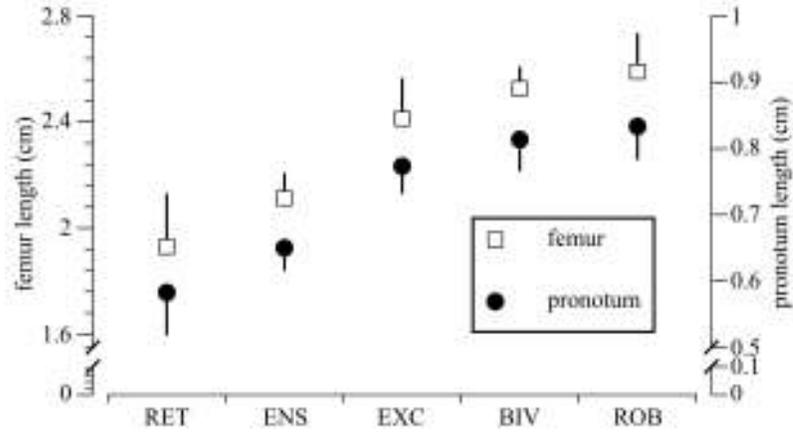
**Fig. 6:** Mean response probabilities of *N. retusus* (A, n=8) and *N. ensiger* (B, n=8) for wingbeat interruption in response to pure tone (40 kHz) pulses with varying rise times. Top: sketches of the stimuli, not drawn to scale: In one experimental series (dotted lines) the plateau duration was held constant at 10 ms, in the other (solid line) the pulse duration (including rise and fall time) was held constant at 2010 ms. Grand means (3-4 trials/individual)  $\pm$  standard deviation are shown.



**Fig. 7:** Response probabilities of *N. ensiger* (closed circles, n=9) and *N. retusus* (open squares, n=11) for leg kicks in response to approaches with varying pulse rate in the terminal phase. Grand mean (3-4 trials/individual)  $\pm$  standard deviation are shown.

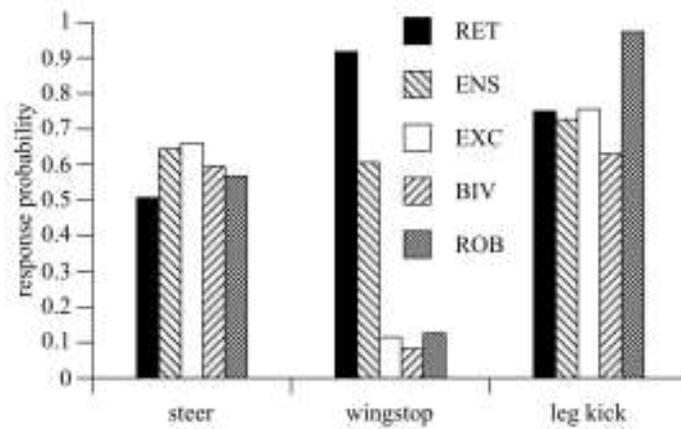


**Fig. 8:** Amplitude of the hindleg movement during the legkick behavior in response to stimuli with varying pulse rates of the terminal phase. Grand mean (3-4 trials/individual)  $\pm$  SD (*N. ensiger*, filled circles, n=9; *N. retusus*, open squares, n=11).



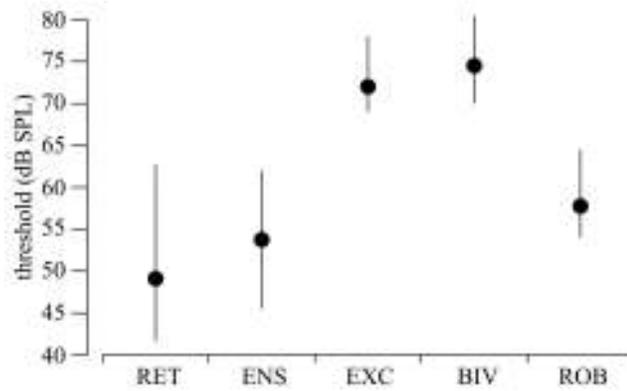
**Fig 9.**

Mean lengths (cm) of hind femur (open square, left y-axis) and pronotum (filled circle, right y-axis) for *N. retusus* (RET, n=11), *N. ensiger* (ENS, n=11), *N. exciliscanorus* (EXC, n=18), *N. bivocatus* (BIV, n=10) and *N. robustus* (ROB, n=12). Error bars are one standard deviation.

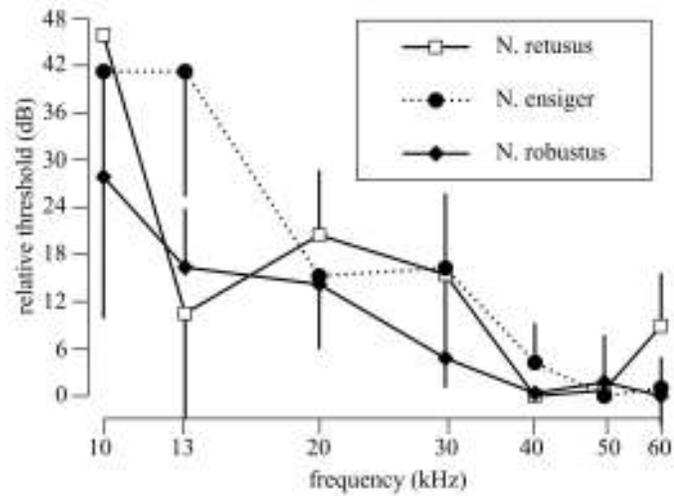


**Fig 10.**

Response probabilities for bat-avoidance behaviors in response to a simulated bat approach in *N. retusus* (RET, n=23), *N. ensiger* (ENS, n=21), *N. exciliscanorus* (EXC, n=13), *N. bivocatus* (BIV, n=7) and *N. robustus* (ROB, n=25).

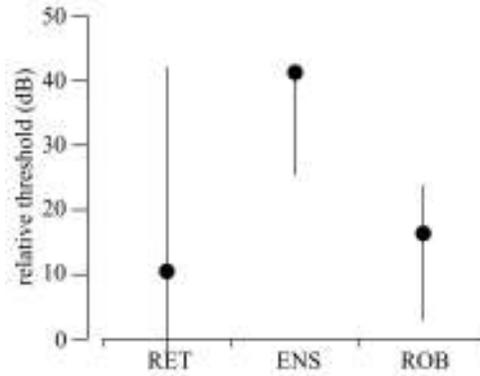


**Fig 11.** Behavioral thresholds (db SPL) for the 'wingstop' at the most sensitive frequency for each species. Median thresholds are shown for *N. retusus* (RET, 40 kHz, n=17), *N. ensiger* (ENS, 50 kHz, n=10), *N. exciliscanorus* (EXC, 60kHz, n=7), *N. bivocatus* (BIV, 50kHz, n=7) and *N. robustus* (ROB, 60kHz, n=8) with error bars showing the range of data, excluding highest and lowest value.



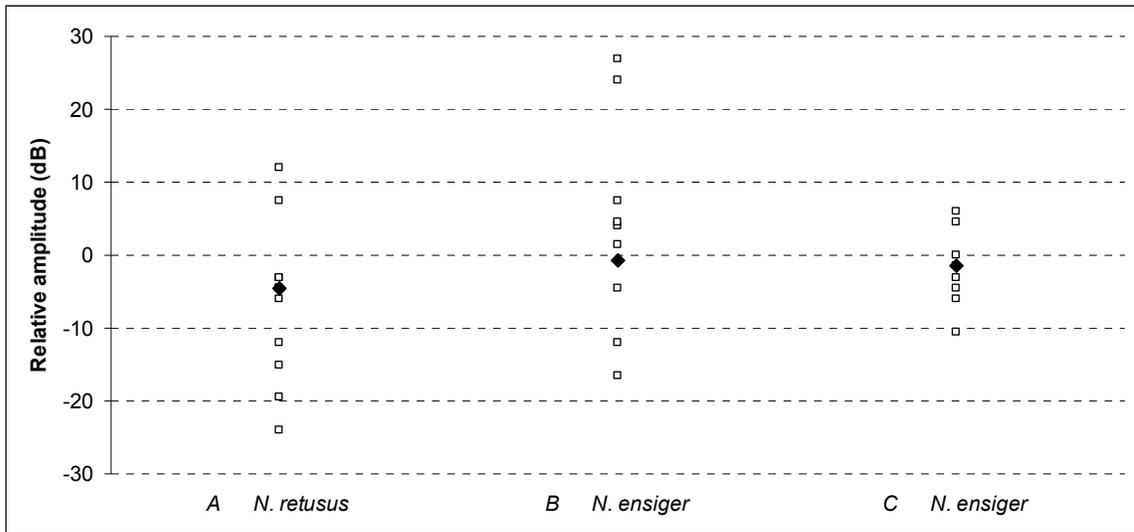
**Fig 12.**

Relative tuning curves for the 'wingstop' in *N. retusus* (open squares), *N. ensiger* (filled circles) and *N. robustus* (filled diamonds) for frequencies ranging from 10-60 kHz. Median thresholds (dB) are shown with error bars showing the range of data, excluding the highest and lowest value.



**Fig 13.**

Relative behavioral thresholds for the wingstop behavior at 13 kHz for *N. retusus* (RET, n=13), *N. robustus* (ROB, n=8) and *N. ensiger* (ENS, n=12). Median relative thresholds are shown with error bars showing the range of data, excluding highest and lowest value. All values are plotted relative to the most sensitive frequency for each species (*N. retusus* 40 kHz, *N. ensiger* 50 kHz, *N. robustus* 60 kHz)



**Fig 14.**

Shift in behavioral thresholds for the wingstop behavior for two-tone stimuli. Shift is the difference between the threshold for the combined (13kHz/40kHz) tone and the threshold for the 40 kHz tone alone (threshold to 13kHz/40kHz – threshold to 40 kHz). A) *N. retusus* (n=8) B) *N. ensiger* (n=12). C) Shifts in behavioral thresholds to repeated measurements of a 40 kHz pure tone stimulus in *N. ensiger* (n=9).

**Table 1:** Response probabilities of bat avoidance behaviors during stimulation with simulated bat approach (2-4 trials/insect)

	Steering	Wingbeat interrupt	Dive	Leg Kick
<i>N. retusus</i> (n=23)	51%	44%	80%	75%
<i>N. ensiger</i> (n=21)	64%	28%	43%	73%

Table 2. Scheffe's Post-Hoc analysis of body size measurements. Asterisks denote significance.

Species comparison	p-value (hind femur)	p-value (pronotum)
<i>N. retusus</i> vs. <i>N. exciliscanorus</i>	0.000000*	0.000000*
<i>N. robustus</i> vs. <i>N. exciliscanorus</i>	0.053161	0.033585*
<i>N. ensiger</i> vs. <i>N. exciliscanorus</i>	0.000169*	0.000010*
<i>N. retusus</i> vs. <i>N. bivocatus</i>	0.000000*	0.000000*
<i>N. robustus</i> vs. <i>N. bivocatus</i>	0.902409	0.920052
<i>N. ensiger</i> vs. <i>N. bivocatus</i>	0.000003*	0.000000*
<i>N. ensiger</i> vs. <i>N. robustus</i>	0.000000*	0.000000*
<i>N. ensiger</i> vs. <i>N. retusus</i>	0.099258	0.039113*
<i>N. retusus</i> vs. <i>N. robustus</i>	0.000000*	0.000000*
<i>N. bivocatus</i> vs. <i>N. exciliscanorus</i>	0.487289	0.351261

Table 3. p-values for comparison of spectral sensitivity using a Mann-Whitney U-test with Bonferroni correction. The necessary p-value for significance was set at 0.005 (0.5/10 comparisons)

Species comparison	p-value
<i>N. retusus</i> vs. <i>N. exciliscanorus</i>	0.000006*
<i>N. robustus</i> vs. <i>N. exciliscanorus</i>	0.001865*
<i>N. ensiger</i> vs. <i>N. exciliscanorus</i>	0.000103*
<i>N. retusus</i> vs. <i>N. bivocatus</i>	0.000003*
<i>N. robustus</i> vs. <i>N. bivocatus</i>	0.001088*
<i>N. ensiger</i> vs. <i>N. bivocatus</i>	0.000051*
<i>N. ensiger</i> vs. <i>N. robustus</i>	0.86407
<i>N. ensiger</i> vs. <i>N. retusus</i>	0.236749
<i>N. retusus</i> vs. <i>N. robustus</i>	0.032847
<i>N. bivocatus</i> vs. <i>N. exciliscanorus</i>	0.5

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## VITA

Mary K. Brueggen (Katie) was born in New Hartford, MO in 1983, the daughter of Leo and Mary Nell Brueggen. She grew up on the family farm and upon graduating from nearby Bowling Green High School in 2001, attended college at College of the Ozarks in Point Lookout, MO. She received her B.S. in Biology in May 2005. Upon graduation, she worked as a research technician at Mote Marine Laboratories, in Sarasota, FL. Her work involved a study of bottlenose dolphin (*Tursiops truncatus*) prey availability in Sarasota Bay, FL as well as an examination of acoustically mediated predator-prey interactions between bottlenose dolphins (*Tursiops truncatus*) and spotted sea trout (*Cynoscion nebulosus*).

In June 2006, Katie returned to Missouri to join the lab of Dr. Johannes Schul at the University of Missouri-Columbia, studying bat avoidance behaviors in the katydid genus *Neoconocephalus*. She married John Kilmer and became Mary K. Kilmer in February 2010 and graduated shortly thereafter, in May 2010. During her time at University of Missouri-Columbia, Katie developed a great interest in teaching and after graduation, planned to pursue a career in teaching science at the secondary or post-secondary level. She also planned to continue with a second interest, teaching fitness classes, especially pilates and yoga.