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# Threshold and suprathreshold responses of the auditory receptors in an arctiid moth

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*Received 28 July 1992; accepted 7 January 1993*

*Abstract.* Considering the responses of both auditory receptors in *Empyreuma pugione* (Arctiidae, Ctenuchinae), it was found that this auditory organ maintains its frequency tuning and directionality over an intensity range of more than 60 dB. For the first time in the Lepidoptera we demonstrate a close frequency match between auditory sensitivity and the power spectrum of the sound emitted by the same species.
*Key words.* Moth audition; moth sound emission; Arctiidae; Ctenuchinae; sensory coding.

The tympanic organ of Noctuoidea has two auditory receptor cells[1-4], except in the Notodontidae, in which it has only one[5]. Most existing data on the spectral characteristics of the two-celled auditory organs refer only to the most sensitive A1 cell[6-14]; only three papers[15-17] deal with both receptors, A1 and A2 cells. All the above-mentioned results were obtained at threshold intensities; only Boyan and Fullard[18] showed a suprathreshold A1-cell response curve at 70 dB SPL up to 30 kHz in *Heliothis virescens* (Noctuidae). In reports of directional responses only the A1-cell threshold responses of some Noctuidae were considered[19,20]. Roeder[21] states that the directionality characteristics of the Arctiidae are unknown.

When considering the possible behavioral role of audition in moths, it should be kept in mind that in prey-predator (moth-bat) relationships the speed of reaction is the most important feature, thus the information coding of stimulus characteristics such as frequency and position is not that important at high intensities (close range), when erratic maneuvers become decisive for the moth in evading the rapidly-approaching insectivorous bat[22]. On the other hand, in intraspecific relations, the information coding of these same stimulus features becomes of paramount importance at close range, when intensity is high, for locating the conspecific insect precisely.

In this study we describe the frequency response and directional sensitivity of the A1 and A2 cells at threshold and suprathreshold intensities in the noctuoid *Empyreuma pugione* (L.) (Arctiidae, Ctenuchinae). We also describe some characteristics of the sound pulses emitted by its microtymbalic organ, and demonstrate for the first time in the Lepidoptera a close frequency match between auditory sensitivity and the power spectrum of the sound emitted in the same species.

## *Material and methods*

Moths of both sexes were collected in the field as imagines or as larvae, which hatched in the laboratory (for details of the rearing method, see Coro[23]). For the electrophysiological recordings we mounted the animal ventral side up, as described by Roeder[24]. *E. pugione* adopts this position naturally in the field, while perching on the lower surfaces of the leaves of *Nerium oleander* and plants, and also during copulation and oviposition.

Spike activity of the auditory receptors was recorded with a stainless steel hook electrode from the tympanic nerve at the position where it joins the alar nerve in the mesothorax. Nerve activity was amplified, filtered (200 Hz high-pass, i.e., only frequencies higher than 200 Hz were analysed; 6 dB per octave) and stored, together with a signal synchronized with the acoustic stimulus, on an AM tape-recorder. Both signals were fed off-line to an 8-bit analog-to-digital converter (type APS-01, designed and constructed by EICI-SOFT, La Habana, Cuba, for NEC PC-9801 series). The nerve signals were sampled every 65 µs during 66.6 ms, starting with the beginning of the stimulus; i.e., sampling was done both during and after the acoustic stimulus, which lasted 45 ms. The A1-cell spikes were discriminated by their amplitude and counted automatically by the computer, whereas those of the A2 cell were counted visually from the computer's video screen. The responses were analysed as the number of action potentials per pulse (AP/p) for each receptor cell.

Acoustic stimuli consisted of 45-ms pulses with 2.5 ms rise and fall time. They were presented at a rate of 1/s during 10 s for each stimulus condition (intensity, frequency, direction of sound incidence). The sound source was placed at a distance of 25 cm from the preparation. Responses of the auditory organ to different acoustic frequencies were measured with sound presented from a direction 135° ipsilateral to the recorded ear (0° is in front and 180° at the rear of the animal), which is approximately normal to the surface of the tympanic membrane. For investigation of the influence of sound-source position, the preparation was turned about its vertical axis in steps of 45° in the horizontal plane. The

acoustic stimuli were generated as described in Coro and Pérez[23], and emitted by a Supertweeter, whose output was calibrated prior to each experiment in 5-kHz steps from 10 to 50 kHz and at 70 kHz, with appropriate equipment for these frequencies. The acoustic field at the recording site varied less than ±2 dB at 35 kHz when the preparation was turned through 360° in the horizontal plane.

All the electrophysiological recordings were made at 22–24 °C in a sound-proof air-conditioned chamber in the Faculty of Biology, Havana University. At the end of each experiment, recording conditions were tested for stability at the initial stimulus conditions (20 or 35 kHz for frequency responses and 90° for directional sensitivity). A recording was discarded if the spike-count function differed from the first one obtained at at least 2 of the intensities used (see 'Results' for further details).

Sound emission was studied at the Max-Planck-Institut für Verhaltensphysiologie in Seewiesen, Germany, in live moths brought from La Habana, Cuba. The sound emission of 3 males and 2 females was elicited by gently pressing and releasing the dorsal part of the thorax, which caused buckling and unbuckling of the microtymbalic organ. Sound recordings were made with a 6-mm condenser microphone (Bruel & Kjaer type 4135) coupled to a measuring amplifier (Bruel & Kjaer type 2806), and stored on a tape-recorder (Racal model DS4) at a speed of 1.5 m/s. The equipment allowed recordings up to 100 kHz (±2 dB). The moths were positioned at 1–2 cm from the microphone, and the best recordings were obtained when one microtymbalic organ was destroyed and the remaining one faced the microphone. Recordings were replayed at a speed of 35–40 cm/s and digitized (400-µs sampling interval). The temporal structure and the power spectrum of individual pulses were analysed (Macintosh SE and MacAdios). The spectral range studied by FFT analysis on 1024 data points extended up to 80 kHz.

*Results*

For studying the threshold responses of the auditory receptors at different frequencies we used the following method: at each test frequency we stimulated the auditory organ from approximately 10 dB below the A1-cell threshold (determined by aural and visual monitoring of the spike activity) up to 90 dB SPL in 10 dB steps. For isoresponse curves (fig. 1) we used the spike count functions in the whole intensity range at each frequency to determine from the graphs the acoustic intensity needed to evoke the threshold response in each auditory receptor. Threshold criteria are 7 AP/p for the A1 cell and 3 Ap/p for the A2 receptor. These different criteria are the consequence of the difference in the spontaneous activity of the A1 and A2 cells in this species (36 spikes/s for A1 and 6 spikes/s for A2 cell as mean values at 22–24 °C, as reported previously[26,27]). The A1-cell threshold response represents a spike activity of approximately 100 spikes/s during the time of analysis, which corresponds to a threefold increase with respect to its spontaneous activity. The 3 AP/p threshold criterion also considers the fact that the A2-cell threshold intensities coincide with intensities of maximal responses of the A1 cell, and consequently the A2-cell threshold response corresponds to approximately a seven-fold increase (42 spikes/s) with respect to its spontaneous activity.

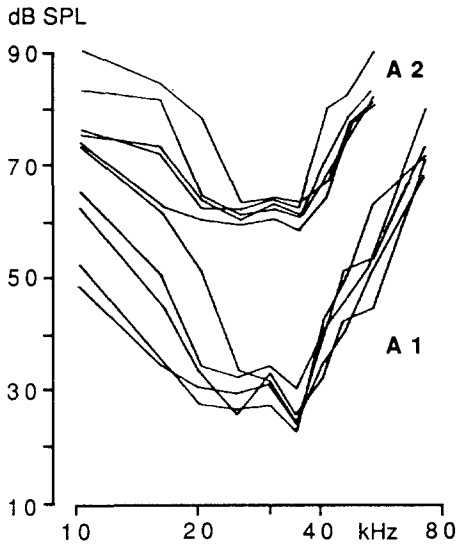


Figure 1. Audiograms of the two auditory receptors (A1 and A2 cells) in 5 specimens (3 males and 2 females) of *E. pugione*. Acoustic stimuli were 45-ms pulses presented at a rate of 1/s, with the sound source placed at 135° ipsilateral to the recorded ear (re. 0° frontal). Threshold response criteria were 7 action potentials per pulse (AP/p) for the A1 cell and 3 AP/p for the A2 receptor.

The A1 and A2 cells in *E. pugione* differ in their sensitivity by about 30–35 dB in the frequency range between 16 and 52 kHz, when the median values at each frequency are compared. In all the 5 audiograms (fig. 1) the A1 cell showed its highest sensitivity (=best frequency, BF) at about 35 kHz, with a median threshold at BF of 24 dB SPL (range from 22 to 33 dB), and a mean Q(10 dB) value of 1.8. The Q(10 dB) value is a measure of the frequency selectivity (=tuning) of the acoustic system; the higher this value, the higher the frequency selectivity of the system. The A2 cell responded best to frequencies between 25 and 35 kHz, with a median threshold of 62 dB SPL (range from 58 to 65 dB). Both cells show a stronger loss of sensitivity towards the high frequencies (A1 cell: 47 dB/octave; A2 cell: 36 dB/octave) than towards the lower ones (A1 cell: 16 dB/octave; A2 cell: 6 dB/octave). No obvious difference was found between audiograms of males and females.

To study suprathreshold responses, we analysed the intensity-response functions over their whole range of each frequency used, thus obtaining the isointensity

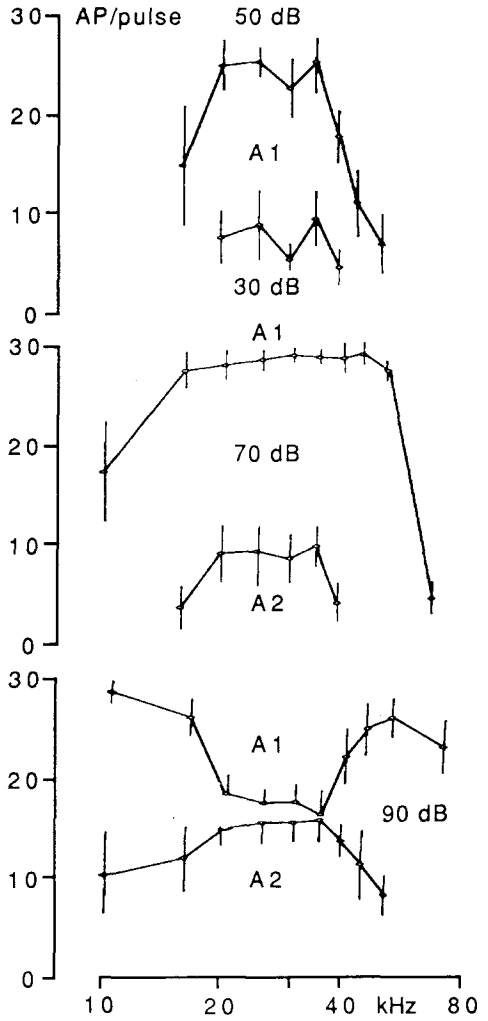


Figure 2. Isointensity functions for the two auditory receptors at different stimulus intensities, expressed in dB SPL. Each point represents the mean value ± 1 SD of 30 responses (pooled data from 3 different specimens, in each of which 10 responses were analysed).

functions shown in figure 2. These functions show that at 30 dB SPL (near threshold) and at 50 dB the A1-cell response is strongest between 20 and 35 kHz, while at 70 dB there is an isoresponse plateau for a wider spectral range (from 16 to 52 kHz). At the latter intensity, the A2 cell shows the same near-threshold responses as the A1 cell at 30 dB SPL. At 90 dB there is a mirror image of the response characteristics of the two auditory receptors. These results show that the tympanic organ in *E. pugione* maintains its tuning to the frequency range of 20–35 kHz in the whole intensity range tested (30–90 dB SPL), when the combination of the responses of both auditory receptors is considered.

The analysis of the influence of sound-source position in the horizontal plane on the threshold and suprathreshold response of the auditory receptors was done at 35 kHz, since this is their BF. At 35 kHz both auditory receptors show an evident directional sensitivity (best position 135°; worst 225°) at near-threshold intensities (35 dB for the A1 cell and 75 dB for the A2 cell in the specimen shown in fig. 3). As intensity is increased, there are isoresponses for ipsilateral sound presentation, but a minimum of sound reception sensitivity at 225° is still observed (55 dB for the A1 cell; 95 dB for the A2 cell). At 75 dB the A1 cell shows omnidirectional sensitivity due to its saturation response, but this is a near-threshold intensity for the A2 cell, thus the latter receptor shows evident directional sensitivity. Similar results were obtained in two more specimens. If the responses of the two auditory receptors are combined, directional information is conveyed to the central nervous system (CNS) over an intensity range of at least 60 dB, rendering it possible to maintain sensory information on sound-source position even at near ranges.

These results suggested that the tympanic organ in *E. pugione* is able to maintain cues on stimulus position and frequency also at high intensities, which could have significance in intraspecific interactions. As in other Ctenuchinae[28,29], *E. pugione* has sound-emitting (microtymbalic) organs in the upper part of the metathorax, the morphology and structure of which have been described[30]. These organs emit brief pulses (0.3–0.5 ms duration), the power spectrum of which shows the following characteristics (fig. 4): the emitted sound intensity rises sharply above ca 16 kHz, with no spectral components below 14 kHz. A first intensity peak occurs at 20 ± 2 kHz (n = 14). The intensity optimum is found at 34 ± 2 kHz (n = 15) with a Q(10 dB) value of 2.8 ± 0.4. A third intensity optimum may be present at 47 ± 4 kHz (n = 8) in individual pulses resulting from the same actions of buckling and unbuckling of the microtymbalic organ. No spectral components above 64 kHz were observed in the 15 pulses analysed from 5 different specimens. In the animals analysed we found no evidence of sexual dimorphism in these characteristics.

When comparing sound emission and reception, we found that hearing in *E. pugione* is tuned to the frequency of its own sound emitting structure (fig. 4): the BF is about 35 kHz and the dominant emission optimum is at ca 34 kHz. This match is also evident in the Q(10 dB) bandwidth, which for the A1-cell responses extends from 16 to 54 kHz, and covers the range between 22 and 46 kHz found in sound emission.

*Discussion*

Today there exists very strong evidence that in an increasing number of Arctiidae intraspecific acoustic communication plays an important role in mating behavior. This idea was proposed more than 120 years ago by Laboulbene[31], and Peter[32] has described acoustic communication between male and female in one Alpine diurnal species, but only recently have such interactions been confirmed with sufficiently sensitive equipment for recording ultrasonic signals[33–35]. It has also been observed in 4 species of the genus *Endrosa* (including

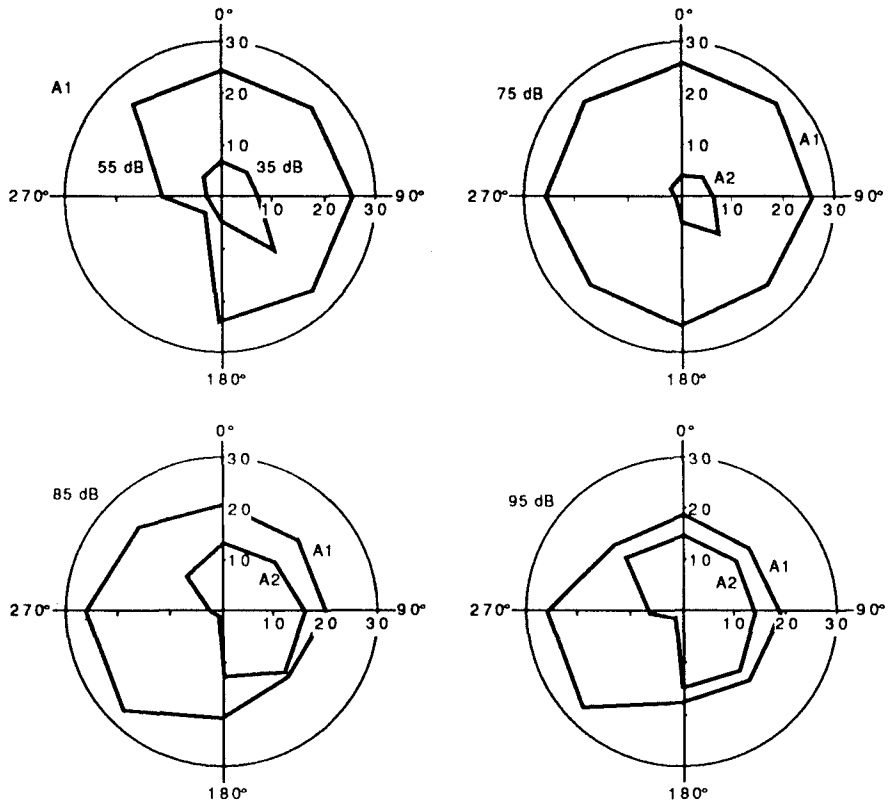


Figure 3. Polar graphs showing the directional characteristics of the two auditory receptors in one and the same specimen at intensities between 35 and 95 dB SPL. The stimulus frequency was 35 kHz and the preparation was turned in steps of 45° in the horizontal plane. 0° is in front of the animal, 180° in the rear, and 90° is ipsilateral to the recorded ear. The numbers close to the axes represent the number of AP/p. For each directional sound incidence and each stimulus intensity 10 responses of the auditory receptors to consecutive acoustic pulses were analysed.

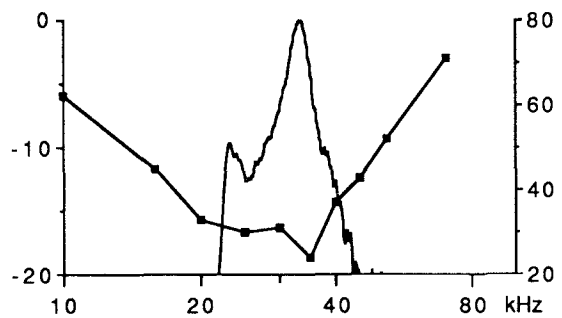


Figure 4. Overlay of a representative power spectrum (—— rel. DB) with the median A1-cell threshold values (—■— dB SPL) of 5 animals, showing the close match between auditory sensitivity and tuning of the sound-emitting organ in *E. pugione*. The power spectrum is from one pulse, and shows the main energy peak at 33.0 kHz with a Q(10 dB) value of 3.5; it also shows a minor peak at 23.1 kHz, but no other peak frequencies higher than that of the maximal emission.

*v. ramosa*) from the Alps, and in *Cymbalophora haroldi* (Arctiidae) from North Africa, that sound emission in females is elicited by sound emissions of males as part of their mating behavior (Michael Eckrich, personal communication). This sound emission is at present being investigated further. In the only studies of Ctenuchinae carried out with similar equipment, it has been described that *Syntomeida epilais* uses dimorphic sounds during courtship, with clicks that have a peak at 32 kHz. The sounds emitted by males and females differ in their temporal structure. On the other hand, in *E. pugione* it was shown[36] that deafened moths of either sex were unable to mate with a non-deafened normal partner, thus the use of acoustic signals as part of mating behavior was suggested. Except for *E. pugione*[23], there is almost no information reported on the characteristics of the auditory receptors in arctiid species.

One step in understanding the morpho-functional basis of intraspecific acoustic interactions is to demonstrate that the sound emitting and sound-receiving organs are closely tuned to one another, as in *E. pugione*. Another feature is a high sensitivity of the A1 cell, which in the latter species has its threshold at ca 25 dB SPL. This sensitivity is as high as that reported for the most sensitive moth species, such as *Ascalpha odorata* (Noctuidae)[9] and *Hecatesia thyridion* (Agaristidae)[37]. Also the Q(10 dB) value of the A1 cell in *E. pugione* is similar to that obtained in the latter two species[9,11], which is considered to be sharply tuned as compared to noctuids, with values around 1.0. This combination of sharp tuning, which filters out other frequencies present in the biotope, and high sensitivity should increase the communication

distance. No differences between males and females were observed in any of these features. This agrees well with the observations on mating behavior[35] in *S. epilais* (a species closely related to *E. pugione* in its taxonomy and ecology), in which both sexes emit acoustic signals. Another feature of the acoustic organs in *E. pugione*, which may favor their role in intraspecific interactions, is the high Q(10 dB) value of their sound emissions, compared to those in other Arctiidae[38]. It is also important that in *E. pugione* the Q(10 dB) band-width of the A1 cell covers completely that of the sound emission, and that at suprathreshold intensities (up to 90 dB SPL) the responses of both auditory receptors combined maintain a tuning between 20 and 35 kHz. In other noctuoids, in which hearing plays a role mainly in the evasion of insectivorous bats, at suprathreshold intensities there is a wide spectral range of A1-cell isoresponses, i.e., this receptor loses its tuning characteristics.

When acoustic communication is used in intraspecific interactions, it would be expected to be important that the auditory organ is able to convey directional information to the CNS, from low intensities when the animals are far apart, up to high intensities produced when they come closer. The A1 cell in *E. pugione* shows a significant response decrease with increasing intensity above about 70 dB SPL at 34 kHz[23]. The changes in directionality described for the A1 cell at 35 kHz in figure 3 may be explained on this same basis. We assume that the biophysical aspects of the directionality characteristics of the auditory organ do not depend on stimulus intensity in the range tested (30–95 dB SPL), thus we consider that at all intensities the ear receives the strongest stimulus from 135° on the ipsilateral side. At low to medium intensities (35–55 dB SPL in fig. 3) this is directly seen in the polar diagrams of the A1 cell. At 75 dB the A1 cell shows omnidirectional sensitivity due to its response saturation. At higher intensities (85 and 95 dB in fig. 3) the saturated response shows up in the directionality plots for contralateral sound incidence (225°–270°) only, whereas for ipsilateral sound the effective stimulus is so strong that the A1 cell shows a response decrease. This could explain the inversion of the directionality of the response of this receptor.

Considering the responses of both receptors combined, the auditory organ in *E. pugione* is able to convey directional information up to the highest intensity tested, i.e., in a range of about 60 dB. This result contrasts with that described in noctuid moths with binaural recordings and bat echolocation pulses as acoustic stimuli: the difference in the responses of each tympanic organ is greatest at low intensities, decreases with increasing intensity, and disappears above a certain stimulus strength, i.e., the ears no longer convey directional information to the CNS at intensities about 40 dB above the A1-cell threshold, when the insectivorous bat is close to the moth[2].

Intraspecific acoustic interactions would be expected to require central neurons with characteristics similar to those described in the pterothoracic ganglion-complex in *E. pugione*[39]. There is a repeater neuron (named RA) that seems to receive convergent excitatory input from both auditory receptors, while the suppression-type neuron decreases its response as that of the A2 cell increases. The presence of neurons with binaural summation could be useful during this information processing. There is also divergence of the auditory afferent input in the pterothoracic ganglion, as in other noctuoid species[18,40], which could provide the basis for a parallel processing of the auditory information. The facts that in *E. pugione* the auditory information flows caudally to the last abdominal ganglion[36], and that acoustic stimuli evoke spikes from this ganglion[41], support the view that hearing might be involved in intraspecific interactions in this species.

On the other hand, as stated in Fullard[38], the acoustic behavior in the Arctiidae probably has a number of inter-related functions; thus interactions between moths and bats should also be analysed in *E. pugione*. This moth is highly unpalatable (at least to monkeys, spiders and scorpions), and its main flight activity is at sunrise (while mating) and sunset (during oviposition)[25]. The only Cuban bat species in which remains of Ctenuchinae (not identified to genus or species levels) have been found in the stomach is *Mormoops blainvillei*[42]. Recently the echolocating cries of this bat have been characterized[43]. These signals contain 3–4 harmonics with most energy in the 2nd or 3rd harmonic. The first harmonic is at about 30 kHz; the 2nd covers a range of about 68–49 kHz; while the 3rd is above 80 kHz. Considering the threshold responses of both auditory receptors in *E. pugione* (fig. 1) it is seen that the low frequencies (below 40 kHz) of these echolocating signals are better detected than the higher ones. It could also be possible that *E. pugione* interacts with other bat species that emit acoustic signals with other spectral characteristics. In any case, the auditory system of this moth species probably detects, and discriminates between, acoustic signals emitted by bats and by conspecific individuals of both sexes.

The analysis of the morpho-functional bases of such acoustic interactions in animals with two-celled auditory organs and probably not many auditory central neurons should be fruitful for a better understanding of sensory information processing.

Acknowledgments. This research work was partially done during two stays of F. Coro at the Max-Planck-Institut für Verhaltensphysiologie at Seewiesen, one due to a kind invitation from Prof. F. Huber, and the other under a DAAD Fellowship. Dr H.-U. Kleindienst is gratefully acknowledged for technical assistance in the measurement and processing of acoustic signals. Prof. H.-U. Schnitzler is thanked for the information on the echolocating cries of *M. blainvillei*. Dr H. Ghiradella and Dr A. E. Treat made invaluable comments on the results, for which the authors express their thanks.

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