Detection of low frequency tones and whale predator sounds by the American sand lance *Ammodytes americanus*

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Hearing of *A. americanus*

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Auditory evoked potentials (AEPs) were used to measure the hearing range and auditory sensitivity of the American sand lance *Ammodytes americanus*. Responses to amplitude-modulated tone pips indicated that the hearing range extended from 50 to 400 Hz. Sound pressure thresholds were lowest between 200 and 400 Hz. Particle acceleration thresholds showed an improved sensitivity notch at 200 Hz but not substantial differences between frequencies and only a slight improvement in hearing abilities at lower frequencies. The hearing range was similar to Pacific sand lance *A. personatus* and variations between species may be due to differences in threshold evaluation methods. AEPs were also recorded in response to pulsed sounds simulating humpback whale *Megaptera novaeangliae* foraging vocalizations termed 'megapclicks'. Responses were generated with pulses containing significant energy below 400 Hz. No responses were recorded using pulses with peak energy above 400 Hz. These results show that *A. americanus* can detect the particle motion component of low frequency tones and pulse sounds, including those similar to the low frequency components of megapclicks. *Ammodytes americanus* hearing may be used to detect environmental cues and the pulsed signals of mysticete predators.

Key Words: auditory brainstem response ABR; communication; feeding; noise; sand eel; sensory ecology.

INTRODUCTION

Low frequency sounds are generated by biotic and abiotic sources and can be propagated relatively efficiently underwater (Urick, 1983). These acoustic cues and signals are often available for marine organisms (Webster *et al.*, 1992) and many fishes use sound as part of important biological activities, including attracting mates, defending territories and spawning activities (Myrberg, 1986; Lobel, 1992; Myrberg, 1997; Mann & Lobel, 1998). Depending on the associated anatomical structures, fishes detect sound with or without auditory specializations (Popper & Fay, 2011). While both sound pressure and particle motion components of sound are often available to fish (Kalmijn, 1988), species without swim bladders are considered to detect particle motion as the primary stimulus (Enger & Andersen, 1967; Chapman & Sand, 1974). Responses to sound stimuli have been measured in a variety of ways including natural behavioural reactions (Nelson & Gruber, 1963), classical conditioning (Fay, 1969) and physiological measures (Corwin *et al.*, 1982; Kenyon *et al.*, 1998).

While fishes are clearly adapted to detect and avoid predators, specific responses to predator sounds are far less documented. Yet, evaluating an auditory scene, including detecting predators, has been suggested as a primary adaptive force for developing hearing abilities in fishes (Bregman, 1990; Fay, 1992; Fay & Popper, 2000). Presumed predator escape responses have been elicited by exposing Atlantic salmon *Salmo salar* L. 1758 to 10 Hz tones (Knudsen *et al.*, 1992). Alewives *Alosa pseudoharengus* Wilson 1811 and shad *Alosa sapidissima* Wilson , 1811 also avoid ultrasonic sounds suggesting responses to odontocete echolocation clicks (Dunning *et al.*, 1992; Mann *et al.*, 1997). Pacific herring *Clupea pallasii* Valenciennes 1847 display a context-dependent startle and avoidance response to simulated odontocete clicks, both in tank and free-field tests (Wilson & Dill, 2002). Gulf toadfish *Opsanus beta* Goode & Bean 1880 reduce calling rates in the presence of certain marine mammal sounds (Remage-Healey *et al.*, 2006). However, the adaptation for marine predator detection can lead to disadvantages for fish species. Nowacek (2005) suggested that bottlenose dolphin *Tursiops truncatus* Montagu 1821 may take advantage of their prey's hearing range, emitting broadband, low frequency signals called 'pops' to startle prey from seagrass beds into the water column.

Two species of Ammodytidae are abundant in the north-western Atlantic: *A. americanus* DeKay 1842 and *A. dubius* Reinhardt 1837. Both species range from West Greenland to as far south as North Carolina, with *A. americanus* as the slender-bodied inshore species and *A. dubius* as the deep-bodied offshore species (Robards *et al.*, 1999). Due to overlapping meristic characteristics, the two species have been considered both a consolidated population (Robards *et al.*, 1999) and reproductively isolated sympatric populations (Winters & Dalley, 1988). Population distribution varies seasonally, with winter as a dormant period and spring to late summer as a period of high abundance in the water column (O'Connell & Fives, 1995). During seasonal dormancy and periods of low light, *A. americanus* has a tendency to bury into the substrate, even when food is available in the water column (Winslade, 1974*a*, *b*). *Ammodytes americanus* is a visual, water-column predator, which feeds primarily on copepods and other invertebrates, and also fish larvae (Robards *et al.*, 1999). When not concealed in the substrate, *A.*

americanus shows a strong tendency to school and respond collectively to perceived threats (Pitcher & Wyche, 1983).

Ammodytes spp. serve as essential prey to over 100 consumer species, including birds, marine mammals, fishes, and invertebrates (Robards *et al.*, 1999; Willson *et al.*, 1999). They are one of the most important forage fishes in north Atlantic ecosystems because the population's overall health can be closely linked to the reproductive fitness of its seabird predators (Martin, 1989; Monaghan, 1992) as well as the distribution and abundance of cetacean predators (Payne *et al.*, 1986; Weinrich *et al.*, 1997). Stimpert *et al.* (2007) recorded one of these predator species, the humpback whale *Megaptera novaeangliae* Borowski 1781, emitting broadband, low-frequency, short-pulsed signals while foraging at night in Jeffreys Ledge, in an area and during a season in the Gulf of Maine in which *A. americanus* are abundant (Fiedler, 2002). *Ammodytes americanus*, along with the related species *A. dubius,* are large components of the *M. novaeangliae* diet in the northwest *M. novaeangliae* Atlantic population (Kenney *et al.*, 1985). The recorded sounds, termed 'megapclicks', were associated with sharp body rolls corresponding to underwater lunge feeding that concluded with 'buzzes' of short inter-pulse intervals, similar to those emitted by odontocetes or bats immediately prior to prey capture (Griffin *et al.*, 1960; Johnson *et al.*, 2004). The authors suggested that these signals may either serve a biosonar purpose or to manipulate the movements of prey.

Only one audiogram exists for the family Ammodytidae: the Pacific sand lance *A. personatus* Girard 1856, found in the western Pacific along the Japanese coast (Suga *et al.*, 2005). The study focused only on juveniles and did not address the particle velocity component of sound stimuli. The hearing abilities of adult sand lance or any Atlantic *Ammodytes* species are unknown. Hearing sensitivities have been shown to differ between cross-oceanic species, such as *C. pallasii* and Atlantic herring *C. harengus* L. 1758 (Mann *et al.*, 2005). This study investigated the hearing of *A. americanus* with the goal of determining the audiogram of a north Atlantic *Ammodytes* (sand lance) species and their potential detection of pulsed sounds similar to megapclicks. Results are provided in both sound pressure and acceleration. The implications of these data are discussed in reference to the behaviour of both predators and prey of *A. americanus*.

MATERIALS AND METHODS

Ammodytes americanus were captured using a seine net in the sand flats of Cape Cod Bay (East Dennis, MA, 41°45’ N, 70°07’ W) from June to August 2010. *Ammodytes americanus* were immediately transported in aerated coolers with sand and local seawater to the Woods Hole Oceanographic Institution, Woods Hole, MA. There they were held in an aerated fibreglass holding tank (61.6 x 43.8 x 92.1 cm) filled with constantly flowing ambient seawater 23.5 ± 0.2˚ C and fed live *Artemia* spp. daily. The tank bottom contained a layer of sand to provide burying opportunities. *Ammodytes americanus* appeared to exhibit normal swimming and burrowing behaviours (Robards *et al.*, 1999). Hearing was measured using auditory evoked potential (AEP) methods. The AEP technique involves measuring neurophysiological activity in response to short acoustic stimuli (Hall, 2007). The technique has been used for hearing tests in both invertebrates (Lovell *et al.*, 2005; Mooney *et al.*, 2010) and vertebrates (Jewett, 1970), including a wide range of fish species (Corwin *et al.*, 1982; Kenyon *et al.*, 1998; Yan *et al.*, 2000; Wilson *et al.*, 2009). Twenty-one *Ammodytes americanus* were examined using short tone pips to establish an AEP audiogram [10.82 cm mean total length (L_T), S.D. 1.84 cm, 3.24 ± 0.40 g mean mass]. Not every frequency was tested with ever subject; respective sample sizes are listed in Table 1. Twelve animals were tested for megapclick responses [9.84 cm (L_T), S.D. 1.41 cm, 2.38 ± 1.20 g mean mass].

EVOKED POTENTIAL RECORDINGS

Ammodytes americanus were transferred to a rectangular plastic experimental tank (49.5 x 45.7 x 35.6 cm) containing gently flowing, unfiltered seawater (23.2 ± 0.1˚ C). To reduce the influence of outside vibrations, the plastic tank was placed in a foam-lined wooden box (58.7 x 57.8 x 86.4 cm) elevated 22.3 cm above the concrete floor on cinderblocks and rubber gaskets. Each *A. americanus* was wrapped in acoustically transparent mesh fabric, held closed with plastic paper clips, and completely submerged in the water [Fig. 1(b)]. The fabric, suspended with nylon monofilament between two pieces of PVC pipe, created a tight hammock-like arrangement to keep the *A. americanus* immobile [Fig. 1(c)]. Each *A. americanus* was positioned in the centre of the tank, directly above the speaker presenting the stimuli. A wooden desk next to the tank held the experimental equipment. Overall, the AEP procedures follow standard techniques used for fish evoked potentials (e.g., Corwin *et al.*, 1982; Kenyon *et al.*, 1998; Yan *et al.*, 2000; Wilson *et al.*, 2009) and followed approved Institute for Animal Care and Use protocols. The subjects fully recovered from these procedures, swimming and borrowing normally when returned to their holding tank.

Stimuli were digitally generated using custom Labview software (National Instruments, Austin, TX, http://www.ni.com/) implemented on a personal laptop computer (Panasonic CF-52 Toughbook, Secaucus, NJ, http://www.panasonic.com/business/toughbook/laptop-computers.asp). Signal polarity was alternated by this program and sounds were then converted from digital to analog using a 6062E PCMCIA data acquisition card (National Instruments) in the laptop. This card connected to a BNC connector box (National Instruments) and then to a Hewlett-Packard 350D attenuator that controlled the sound pressure levels in 1 dB steps. Signals were relayed to a battery-powered amplifier (PLA-2210, PYLE Chopper Series, Pyle Audio, Brooklyn N.Y., http://www.pyleaudio.com/sku/PLA2210) and then to an underwater speaker (UW-30, Lubell Labs Inc., Columbus, OH, http://www.lubell.com) to play the outgoing sounds. All sounds were concurrently monitored on a digital oscilloscope (Tektronix TPS 2014, Beaverton, OR, http://www.tek.com).

The response of each *A. americanus* was recorded using the same laptop, program, and data acquisition card. A recording (non-inverting) electrode was superficially inserted above the medulla at the skull's midline [Fig. 1(a)]. A reference (inverting) electrode was inserted into the left posterior-lateral trunk musculature about 2 cm from the tip of the caudal fin. The electrodes were reusable, stainless steel, 27 Ga, 12 mm length (Rochester Electro-Medical, Inc., Lutz, FL, http://rochestermed.com), and were coated with Por-15 (Morristown, NJ, http://www.por15.com) except at the tips to reduce non-response related electrical noise. Wire connections were coated with insulating epoxy to prevent corrosion. Both electrodes and a carbon-rod ground were connected to a Grass CP-511 bio-amplifier (Astro-Med Inc., West Warwick, RI, http://www.astro-med.com), which amplified (10 000 fold) and filtered (10-3000 Hz) the response of the *A. americanus*. An additional copper wire grounded the stimulus amplifier. The responses were filtered again (30-3000 Hz; Krohn-Hite 3362, Brockton, MA, http://www.krohn-hite.com) and connected simultaneously to the oscilloscope and the laptop in order to observe the recordings in real-time. All equipment ran on battery to reduce electrical noise and was fully charged every day.

Stimuli consisted of amplitude modulated tone pips from 50–2000 Hz (50, 100, 150, 200, 300, 400, 500, 1000 and 2000) and pulsed sounds of simulated megapclicks. The 50 Hz tone could drift in frequency by ±5 Hz. The update rate for all stimuli was 16 kHz. Tone signals were at least six cycles in duration, thus signal length varied relative to frequency but was never more than 120 ms (50 Hz) and was as short as 20 ms (for stimuli $\geq$ 350 Hz). Sound presentations digitally triggered AEP recordings; thus, stimuli and evoked potential records were synchronized. For an individual response record, the test tone was presented 1000 times, with 1000 concurrent averaged AEP records. Measurements typically started at maximum sound pressure levels (SPLs) for each frequency (133–167 dB re. 1 μPa depending on the frequency). Within each frequency presentation, the SPL was decreased in 10-dB steps until the production of recognizable and repeatable AEP waveforms weakened [Fig. 2(a)]. The SPL was then decreased in 5-dB steps until the waveform disappeared. Two-to-three more measurements were made at 10 to 15 dB below this apparent 'threshold' in order to ensure weak responses were not overlooked. Each test began and concluded with a frequency known to produce a strong response, usually 150 Hz.

Stimuli were calibrated for both sound pressure and particle motion components. Sound pressure was calibrated four times during the experiment using a Reson 4014 hydrophone placed directly above the speaker in the same position as the head of the *A. americanus* (± 2 cm). The same test stimuli presented in the tank hearing experiments were presented *via* the UW-30. The received peak-to-peak voltage (Vp–p) at each location was measured on the oscilloscope and converted to peak-equivalent root-mean square voltage (peRMS) by subtracting 9 dB. Stimuli were also digitally recorded for reference to an Olympus LS-10 recorder (96 kHz sample rate) and assessed later to ensure UW-30 stimuli were the proper frequency.

Particle acceleration values at the position of the auditory pathway of the *A. americanus* were obtained by measuring the pressure gradient over two closely spaced sound receivers

(Gade, 1982). Two Brüel and Kjær 8103 hydrophones, vertically spaced 2 cm apart, were fixed directly above and below the location of the head of the *A. americanus* (3 cm depth). Thus, the hydrophones were at 2 and 4 cm depth (Fig 1(d,e)). Each hydrophone was connected to a charge amplifier (Brüel and Kjær 2635) that was connected to an analog-to-digital preamplifier (RA8GA; TDT, http://www.tdt.com) and a digital signal processor (RM2; TDT). As a stimulus was played, particle acceleration was computed from the pressure gradient across the two hydrophones:

$$\alpha = - \Delta sig/(\rho \, \Delta r)$$

where Δsig is the magnitude of the difference between the waveforms of the two hydrophones (in Pa), ρ is the density of the medium and r is the distance between the hydrophones (Wahlberg *et al.*, 2008). The particle motion was measured in three dimensions by positioning the two hydrophones along three orthogonal axes (Kalmijn, 1988; Wahlberg *et al.*, 2008). Subsequently, particle acceleration values for the pressure-derived AEP thresholds were determined by relating the measured pressure at threshold with the corresponding particle acceleration at the head of the fish. Although a fish acts as a rigid body in the acoustic near field (Denton & Gray, 1982; Coombs *et al.*, 1992), measurements at the head were compared with additional measurements ± 5 cm along the anterior–posterior axis to confirm the sound acceleration field. These measurements were similar (± 2 dB) to those at the head.

Responses were also measured using the pulsed, broadband sounds of simulated megapclicks. These pulses were created using the Labview program to initiate a short-duration waveform and frequency spectrum reflective of megapclicks recorded by Stimpert *et al.* (2007). Low frequency pulses were varied by duration and centre frequency, and in-water recordings were made of each potential stimulus at the location of the *A. americanus*. The spectrum of each recorded sound was then viewed using Cool Edit software to compare the pulse spectrum and duration to that of published megapclicks. Stimpert *et al.* (2007) high-pass filtered original megapclick data at 400 Hz during analysis, eliminating the low-frequency energy in the published recordings. However, the harmonic structure (peaks at ~ 800 and 1600 Hz) suggests substantial lower frequency energy with peaks likely near 200 and 400 Hz. Because of this uncertainty of the actual megapclick spectrum, multiple pulses were examined based on their peak frequency, which suggested the prevalence of the dominant frequency. An arbitrary label of simulated megaplick 1-8 (*e.g.* MC1, MC2…) was given to each pulse. These pulses were calibrated in the manner as described above, however pulses are presented in dB peak-to-peak. Each AEP session using pulsed sounds began and concluded by collecting thresholds to 150 Hz tone pips. This was to determine baseline auditory capabilities of each *A. americanus* and to ensure that no temporary threshold shifts occurred.

Control experiments included recordings with naturally deceased *A. americanus*, electrodes in the water without a subject and from subjects with the recording electrode placed in the posterior muscle [Fig. 2(b)] (Ramcharitar & Popper, 2004; Ramcharitar *et al.*, 2004; Mooney *et al.*, 2010). In this case the recording electrode was inserted into the left posterior-lateral trunk musculature, ~ 2 cm from the tip of the caudal fin, and the reference electrode in the left mediolateral muscle, ~ 5 cm from the tip of the caudal fin.

DATA ANALYSIS

Both visual determination and a custom Matlab program were used to assess the auditory threshold of each subject for each tested frequency. For the visual method, the threshold was determined as the last SPL producing a clear, repeatable waveform (Kenyon *et al.*, 1998). The Matlab program graphs the amplitude of an evoked response for its respective attenuation records against the SPL at the peaks of the fast Fourier transformed waveform. The script then performs a best-fit linear regression. The threshold was considered the point at which the regression line crosses the horizontal axis, *i.e.* the SPL at which the amplitude of the response equals 0 μV (Nachtigall *et al.*, 2007; Mooney *et al.*, 2010). Five to 10 attenuation records were used per frequency (mean = 6.13), and the points producing the highest r^2 value were used to plot the regression line (Fig. 3). Threshold values procured from all *A. americanus* were averaged to produce an audiogram for the species based on each method (Kenyon *et al.*, 1998). Megapclick detection was determined using a similar method of examining the fast Fourier transformed waveform to determine if high peak activity occurred at twice the frequency around which each megapclick was centred. The waveforms were also visually scanned for potential responses (*e.g.* Kenyon *et al.*, 1998; Mooney *et al.*, 2010). Statistical tests were performed using JMP 9.0.0. All measurements are reported as mean ± standard error unless otherwise specified.

RESULTS

TONAL AUDIOGRAMS

Responses were found from 50-400 Hz. At higher amplitudes, response waveforms were clearly visible [Figs 2(a), 3]. Tone pip stimuli generated response waves that oscillated at twice the stimulus frequency, consistent with previous studies of fish evoked potentials (Fay & Popper, 1974; Egner & Mann, 2005; Casper & Mann, 2007). A typical response consisted of a clear, repeatable waveform slightly delayed (10-15 ms) from the stimulus onset. Responses were clear and consistent at 350 Hz and below. Response amplitudes decreased with stimulus attenuation [Figs 2(a),3]. Only three of 10 *A. americanus* showed responses at 400 Hz and responses were not elicited at higher frequencies. No responses were found in the controls, including when electrodes were placed in the water without the *A. americanus*, in locations posterior and away from the otoliths or when electrodes were placed properly but the *A. americanus* was deceased [Fig. 2(b)]. Thresholds were at least 40 dB above the background noise present in the tank, which remained under 90 dB for all frequencies.

The sound pressure audiograms were somewhat irregular in shape. Thresholds were lowest at 300 Hz and increased slightly at 200 and 400 Hz. Responses were not detected above 400 Hz. A substantial audiogram notch was found at 150 Hz, at which sensitivity decreased sharply in relation to other frequencies (Fig. 4). Thresholds then decreased and sensitivities improved at 100 Hz and below. While most thresholds did not vary widely among individuals, some frequencies showed greater variation. This seemed partially, but not always, due to differences in the number of samples per frequency (Table I). For example, 200 Hz had the greatest S.D. values despite a relatively high (*n*=18) sample size. While the sound pressure audiogram shape did not differ substantially between the visual method and the FFT methods, visual thresholds were elevated about 20-30 dB (Fig. 4).

In contrast to the audiogram of *A. personatus* obtained by Suga *et al.* (2005), the audiogram of *A. americanus* showed no responses at 500 Hz. Sensitivities of *A. americanus* were within the same general range as those of *A. personatus*, although the audiogram of *A. personatus* did not display an audiogram notch. Visual thresholds for *A. americanus* were elevated relative to *A. personatus*, but *A. americanus* generated FFT thresholds that were below those of *A. personatus*.

Particle velocity sensitivities were lowest at 200 Hz (Fig. 5). While 400 Hz was the highest overall threshold, there were not always substantial differences between the frequencies. The visual thresholds showed a general trend of improved sensitivity from high to lower frequencies. This trend was not apparent in the FFT measurements. For example, while the highest FFT thresholds were at 400 Hz, they were only significantly greater than thresholds at 200 and 300 Hz (one-way ANOVA, $F_{7,93}$=19.12, $P \leq 0.001$; subsequent Tukey's pairwise comparison). FFT thresholds at 50 Hz were only significantly different from 200 Hz. Thus, the FFT-determined particle velocity thresholds were essentially flat.

PULSED STIMULI

Responses were generated using pulsed sounds that contained high spectral energy from 75 to 350 Hz and peak frequencies at or below 178 Hz [Table II; Fig. 6(a)]. Evoked potential waveforms were similar to those for tone pips, consisting of a repeatable sinusoid-like waveform. This was likely due to a ringing of the speaker, which is expected with impulse sounds. Response delays were detectable but less than those of the tonal stimuli (~ 5 ms). Response amplitudes decreased correspondingly with SPL attenuation. Again, controls (deceased *A. americanus*, no *A. americanus* and electrodes in the posterior musculature) did not generate responses. At very high SPLs (> 160 dB), the short-duration stimulus waveform was visible in the AEP record, potentially masking the first few ms of recording. Also notably, while responses were consistently generated using pulses with lower frequency peaks, these responses were not generated in every *A. americanus*. There appeared to be some variation with these brief signals and successful AEP recordings compared to the longer duration tonal stimuli.

Ammodytes americanus did not respond to pulses containing peak energy above 178 Hz [Fig. 6(b)]. Unfortunately, the irregularities and inefficiencies of the UW-30 made it difficult to produce pulsed stimuli with peak energy between about 200-700 Hz.

DISCUSSION

At regions of best sensitivity, 50-350 Hz, responses were clear and easily distinguishable from the background noise. All responses showed a standard physiological time delay before they were observed. This delay was shorter for the pulsed sounds likely because their onset was rapid (*e.g.* Wysocki & Ladich, 2002). Tone pip stimuli were ramped up to reduce frequency spreading but this increased the latency until a response was observable, likely due to an increased time until sufficient stimulus amplitudes were received by the *A. americanus*. Thus, lower frequencies, with longer ramp-up times (due to larger wavelengths), had slightly longer physiological delays (Wysocki & Ladich, 2001). The AEP waveforms measured also had significant energy at twice the stimulus frequency, making them easily identifiable using FFTs (Casper & Mann, 2007). This suggests that otolith hair cells of *A. americanus* are arranged in opposite directions and are alternately stimulated *via* the sound stimuli (Fay & Edds-Walton, 1997). Finally, and as expected, responses decreased with stimulus level [Figs 2(a),3] and comparatively, no responses were generated during various control experiments. These *A. americanus* tone-generated AEP characteristics of a delayed response, decreases with stimulus level and doubling for frequency general were similar to other fishes (*e.g.* Kenyon *et al.*, 1998; Egner & Mann, 2005) and some invertebrates (Mooney *et al.*, 2010). Overall, thresholds levels shown here are also similar to those in other studies of fishes without auditory specializations (Wysocki *et al.*, 2009; Anderson & Mann, 2011) showing *A. americanus* are not hypersensitive, nor insensitive, to sound. Pulsed stimuli with significant low frequency energy generated responses that were much shorter in delay but overall still exemplified the doubling-of-frequency following response [Fig. 6(a)]. These reflected novel fish AEP responses to a predator-like sound.

The shape of the audiograms differed when plotted in sound pressure and particle velocity. The sound pressure thresholds of *A. americanus* were irregular, with peaks and valleys and displayed greatest sensitivity from 300 Hz to 400 Hz. Acceleration thresholds were lowest at 200 Hz. Even excluding this 200 Hz point, the acceleration thresholds slightly improved in sensitivity as frequency decreased. Thresholds plotted in sound pressure were much more uneven in shape. These shape differences are a little surprising because in many instances, fish sound pressure and particle velocity hearing curves have similar curves (Horodysky *et al.*, 2008; Wysocki *et al.*, 2009). Yet, this is not always the case (Anderson & Mann, 2011). The differences between the two audiograms' shapes seen here and elsewhere may be partially due to the complex interaction of acoustic stimuli in small experimental tanks. While free-field calculations between sound pressure and particle motion are a direct theoretical relationship, this is not true in a small tank with reflections and reverberation. Thus, sound pressure and acceleration may not follow the same trends in certain situations.

Particle velocities are often the relevant stimuli for many fishes, but fish hearing with respect to pressure and acceleration is now seen as a continuum between species (Popper & Fay, 2011). Unfortunately, it can be difficult to predict the details of audiograms and it is suggested that morphological variations do not always coincide with hearing estimations (Wysocki *et al.*, 2009; Anderson & Mann, 2011). The sensory mechanism of fishes (Popper & Fay, 2011) and the differences between pressure and acceleration audiograms, as seen here, support substantial consideration of acceleration values, not simply sound pressure (Suga *et al.*, 2005). Conversion metrics and laboratory settings used here provide an estimate of natural setting data. *In situ* acceleration measurement devices are now more readily available (McConnell, 2003; Wysocki *et al.*, 2009) and increase the scope of potential understandings of fish pressure and particle motion detection. Additional tests using isolated pressure and particle motion stimuli (Packard *et al.*, 1990; Mooney *et al.*, 2010) will improve understanding of the relative importance of particular stimuli. Notably, in these experiments *A. americanus* essentially rested directly above the speaker and responses were likely dominated by the acceleration component of the sound wave (Kalmijn, 1988). Both the ear and lateral line may have been highly stimulated (Coombs *et al.*, 1992; Wilson *et al.*, 2009). Given that the AEP is a whole brain response, responses were potentially recorded from both auditory and lateral line inputs. The relative contributions of these two systems may have also influenced the acceleration (but not the pressure) audiogram shape, reflecting in differences between the two stimulus types.

The range and shape of the acceleration audiogram reflect hearing abilities of some other fishes (Casper & Mann, 2006; Horodysky *et al.*, 2008; Wysocki *et al.*, 2009; Belanger *et al.*, 2010; Anderson & Mann, 2011). Acceleration audiograms typically have a flattened 'J' shape, with most sensitive thresholds at lower frequencies (often below 200 Hz) and thresholds substantially increasing at higher frequencies (*e.g.* Karlsen, 1992). This is quite different than the 'U' shape of sound pressure mammalian curves (*e.g.* Johnson, 1967). The acceleration thresholds here somewhat reflect the 'J' shape, but only start the expected increase at the upper limit of hearing, perhaps suggesting that these results were near, but not reaching the true hearing limit of *A. americanus*. Fish thresholds are most sensitive at lower frequencies in the optimal range of otolith hair cells and as frequencies increase, hair cell response efficiency can decrease (Sand *et al.*, 2001). This appears to limit the frequency range of responses (Sand & Karlsen, 2000) and consequently many particle motion audiograms are limited to low frequencies (Karlsen, 1992; Casper & Mann, 2006; these data). Particle velocity also appears to be the most relevant stimulus for fish without swim bladders (Enger & Andersen, 1967; Chapman & Sand, 1974). The lack of a swim bladder (Robards *et al.*, 1999) and the presence of dense otolith structures suggest that *A. americanus* are without auditory specializations (Popper & Fay, 2011), and particle velocity is the likely acoustic stimulus. This further suggests that acceleration thresholds, which are increasingly becoming standard in current studies (see references above), are experimentally necessary because they are biologically relevant to these and other fishes.

Relatively few responses were generated at 350 Hz and 400 Hz. The starting stimulus levels at these frequencies were limited by the transmission response curve of the underwater speaker. It is likely that the starting sound levels at 350 Hz and 400 Hz were not sufficiently high to induce reliable responses. Further, at least four reliable response records were required for the FFT threshold determinations. These were difficult to obtain if stimuli started near threshold. In any case, comparing these data to Suga *et al.*'s work (2005) and audiograms of similar fishes without gas bladders or auditory specializations (Karlsen, 1992; Sand & Karlsen, 2000; Sand *et al.*, 2001; Wysocki *et al.*, 2009; Popper & Fay, 2011), similar results are found – that 400 Hz is probably close to the upper limit of *A. americanus* hearing range. Predominant sources of ocean sounds are also in this low frequency range (Urick, 1983; Au & Hastings, 2009). In these respects, the 'auditory scene' (Bregman, 1990) of *A. americanus* is likely in the frequencies measured and thus these data provide sensitivities in the primary sensory range of *A. americanus*.

Ammodytes americanus and *A. personatus* had somewhat different thresholds, although frequency response ranges were similar (Suga *et al.*, 2005). The lack of responses at 500 Hz in this study may be a result of the 140 dB starting SPL, which is very close to the threshold observed in *A. personatus* by Suga *et al.* (2005) at 512 Hz (~ 133 dB). Levels of 140 dB are significantly above threshold for many, but not all fishes without hearing specializations (Wysocki *et al.*, 2009; Belanger *et al.*, 2010). The differences could be an artefact of the maturity of *A. americanus* compared to the juveniles used in Suga *et al.* (2005), although threshold differences were not detected across this study's size spectrum or in similar studies (Belanger *et al.*, 2010). Population, and thus genetic or subtle morphological differences, have also been suggested to impact thresholds (Ladich & Wysocki, 2009; Wysocki *et al.*, 2009). The observed differences may also be a remnant of different methods used. Suga *et al.* (2005) placed the speaker out of the water and kept the heads of *A. personatus* at the water surface. While animal placement and speaker alone may not impact the sound pressure thresholds of otophysans (Ladich & Wysocki, 2009), calibration errors, among other possibilities, may impact threshold levels (Ladich & Wysocki, 2009). Sound measurements are particularly difficult at the air-water boundary due to reflective and refractive effects (Urick, 1983; Au & Hastings, 2009). Thus placing the animal at the surface may lead to unquantified particle velocities to which *A. personatus* actually responded.

The absence of low frequency energy in field-recorded megapclicks is an important factor to consider in interpreting responses to pulses and simulated megapclicks. The methods used by Stimpert *et al.* (2007) involved applying a high-pass filter to the acoustic tag recordings that effectively removed the majority of energy below 400 Hz. Although used as an analysis method to reduce water-flow noise on the recordings, this most likely removed the low-frequency energy that falls within the auditory range of *A. americanus*. Recorded megapclicks had a source level at the tag on the animal’s back of 143 ± 5 dB and 154 ± 5 dB re 1 μPa pp. These levels are probably higher in front of the *A. americanus* and are also above *A. americanus* hearing thresholds for all tested frequencies. Unfortunately, only sound pressure values are available for the megaplicks. As noted above, acceleration is likely to be the primary stimulus for *A. americanus*. However, given that both pressure and particle motion are generated with all sounds (Urick, 1983; Kalmijn, 1988), these megapclicks probably have significant particle motion components that would be available to *A. americanus*. By modelling the available pressure stimuli and calibrating both components, this study assumed that natural megapclicks would have similar acceleration components to which *A. americanus* may respond. The results also show pulses with a variety of low frequency spectra can generate responses. Detection ranges are not predicted without the actual source levels. Based on the published source levels and assuming similar energy below 400 Hz, it seems likely that megapclicks are detectable by *A. americanus*.

A prey's ability to hear approaching predators is not a new strategy for predator avoidance, and predator detection is considered one of the primary drivers of hearing evolution (Gans, 1992; Fay & Popper, 2000). *Ammodytes* spp. often show a tightly compacted schooling response when a threat is perceived (Girsa & Danilov, 1976; Pitcher & Wyche, 1983). This 'selfish herd' response (Hamilton, 1971) may reduce fish and bird predation for many individuals. Fishes constitute the majority of *Ammodytes* spp. predators and much of their avoidance behaviour is considered to be adapted to reduce fish predation (Girsa & Danilov, 1976; Willson *et al*., 1999). For *M. novaeangliae*, this behaviour may result in a higher density prey patch. The acoustic signals produced by *M. novaeangliae* may serve to exploit *A. americanus* behavioural responses and manipulate the fish into a denser school. Similar *Ammodytes* spp. 'balling up' has been observed as a reaction to nets (Girsa & Danilov, 1976). Perhaps less likely, the sounds could serve to startle *A. americanus* out of the substrate (*e.g.* Nowacek, 2005). Behavioural tests with *A. americanus* in the water column would help evaluate this hypothesis. *A. americanus* might also use this sensory modality to navigate or to 'eavesdrop' on sounds produced by predators (Fay & Popper, 2000). The acoustic detection of predators may also facilitate prey escape, whether or not the detected sounds correlate with predators' foraging strategies.

The results of this study demonstrate that *A. americanus* detect low frequency sound and potentially the low frequency components of megapclicks. Particle velocity is likely to be the stimulus for *A. americanus.* Their hearing range encompasses the frequencies of many potential predators, including cetaceans and soniferous fishes but also many more general ambient sounds such as reef, rain, wave and anthropogenic noise (Urick 1983; Robards *et al.*, 1999). The functional uses of sound in the species, as well as their behavioural reactions and directional responses to sound have yet to be determined.

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Table I. Thresholds (dB re 1 uPa and dB re m s^2), standard deviations, and sample sizes for the tone-pip stimuli.

	SPL				Acceleration				
Freq (Hz)	FFT	S.D.	Visual	S.D.	FFT	S.D.	Visual	S.D.	*n*
2000	no response								4
1000	no response								9
500	no response								11
400	100.1	2.9	130.6	6.3	-8.1	2.9	22.4	6.3	10
350	96.3	2.8	130.0	5.0	-17.7	2.8	16.0	5.0	4
300	90.6	4.2	124.7	3.4	-33.1	4.2	8.8	3.4	19
250	96.8		128.0		-19.7		11.6		2
200	100.3	7.6	130.7	7.8	-42.8	7.6	-12.1	7.8	18
150	124.3	3.3	141.9	2.9	-9.3	3.3	8.7	2.9	21
100	114.9	2.8	132.4	3.2	-28.3	2.8	-0.3	3.2	21
50	99.2	3.1	121.9	5.1	-13.9	3.1	6.7	5.1	12

Table II. Simulated megapclick (MC) sound settings.

Simulated megapclick pulse no.	Peak Freq. (Hz)	Duration (ms)	Starting SPL (dB re 1 μPa)	Responses/fish tested
MC1	123	43	159.5	3/3
MC2	136	50	169.4	4/6
MC3	137	51	169.9	3/7
MC4	139	44	160.0	2/3
MC5	178	52	169.6	6/6
MC6	763	3	157.7	0/4
MC7	1323	2	154.1	0/4
MC8	2153	4	152.5	0/5

Figure 1.

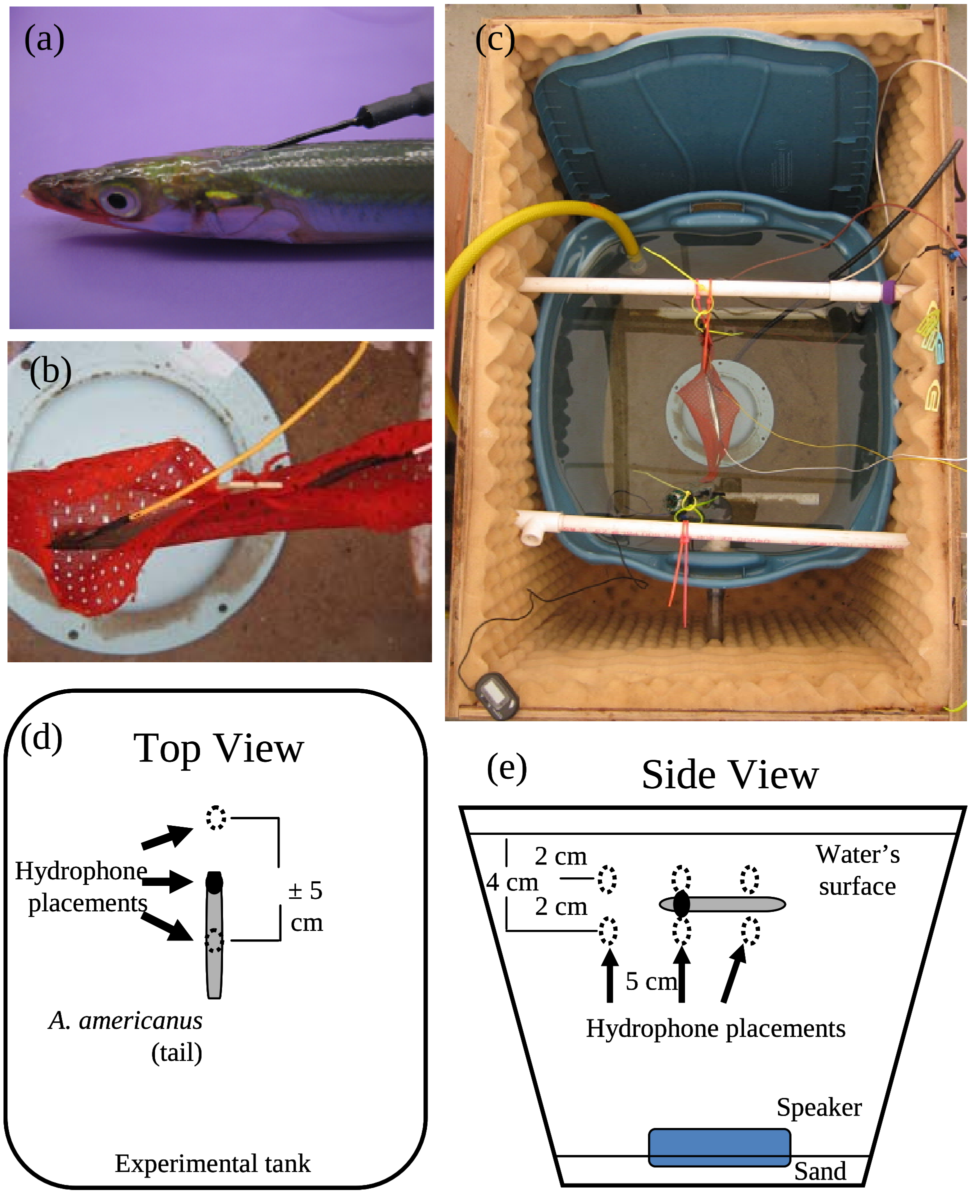

(a)
(b)
(c)
(d)
Top View
Hydrophone placements
± 5 cm
A. americanus (tail)
Experimental tank
(e)
Side View
2 cm
4 cm
2 cm
Water's surface
5 cm
Hydrophone placements
Speaker
Sand

Figure 2.

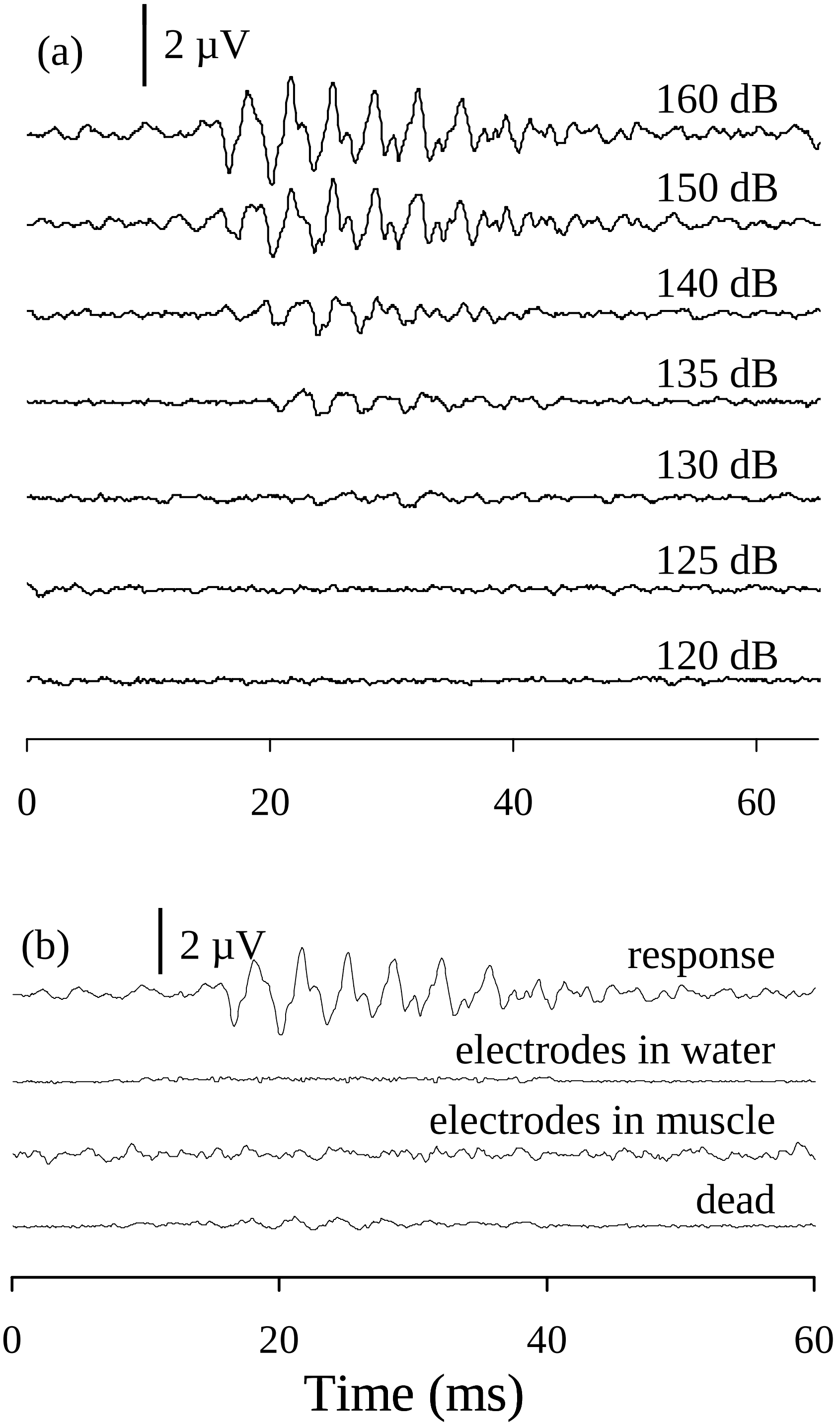

(a)
2 µV
160 dB
150 dB
140 dB
135 dB
130 dB
125 dB
120 dB
0
20
40
60
(b)
2 µV
response
electrodes in water
electrodes in muscle
dead
0
20
40
60
Time (ms)

Figure 3.

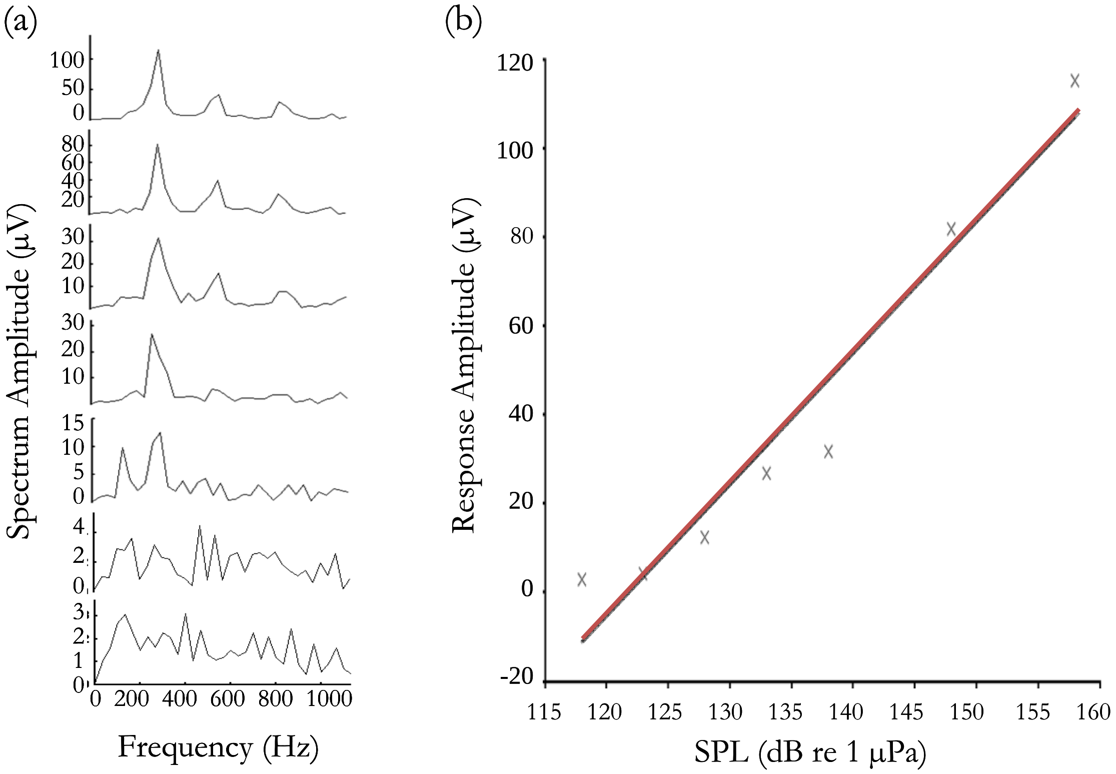
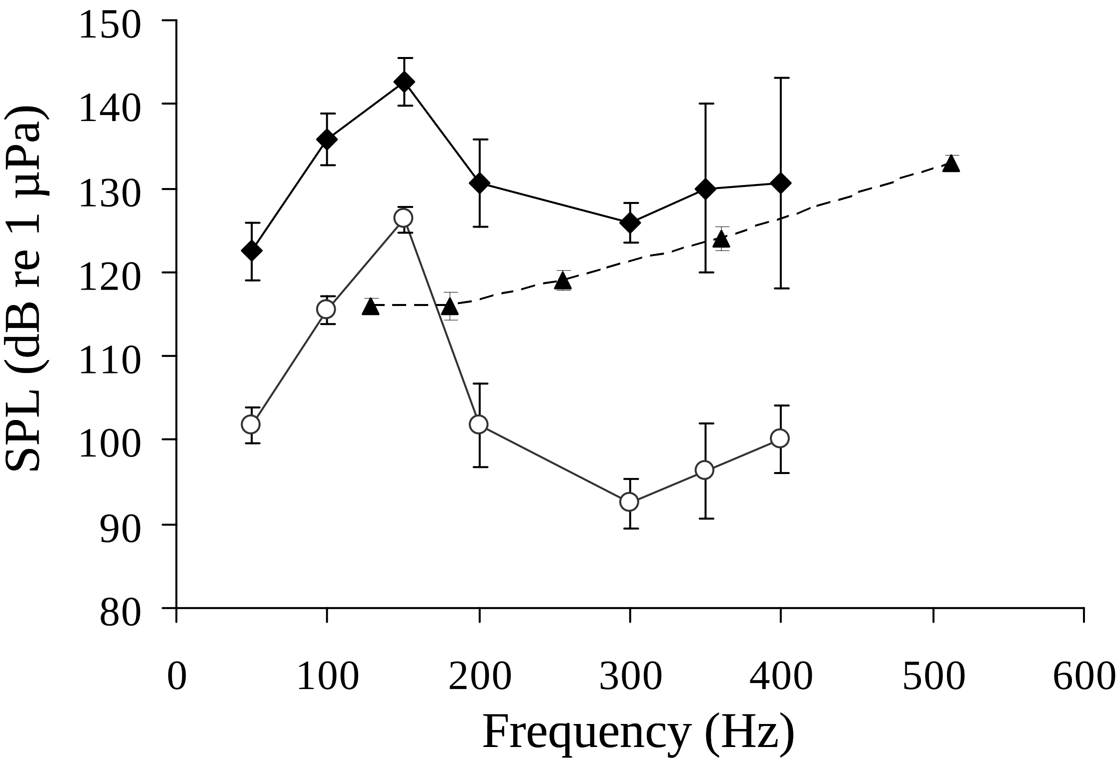
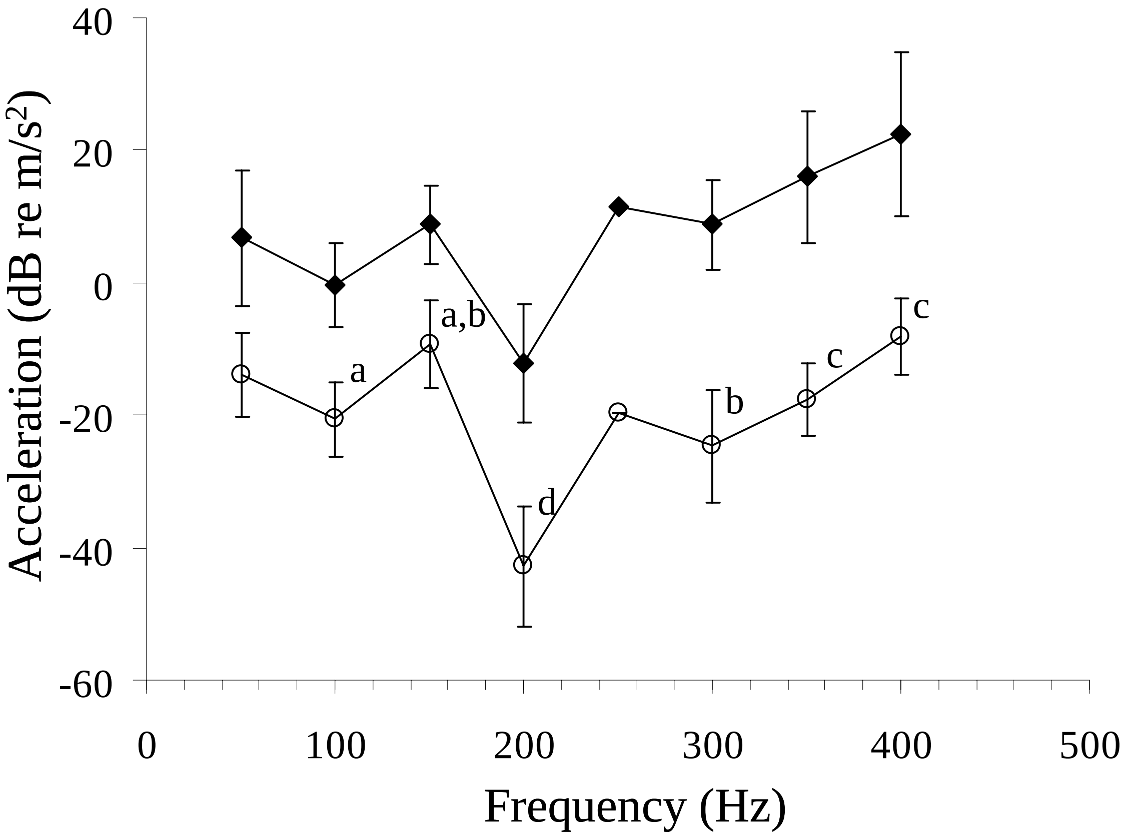

(a)
Spectrum Amplitude (µV)
100
50
0
80
60
40
20
30
20
10
30
20
10
15
10
5
0
4.
2.
0.
3.
2.
1
0
0
200
400
600
800
1000
Frequency (Hz)
(b)
Response Amplitude (µV)
120
100
80
60
40
20
0
-20
115
120
125
130
135
140
145
150
155
160
SPL (dB re 1 µPa)

Figure 4.



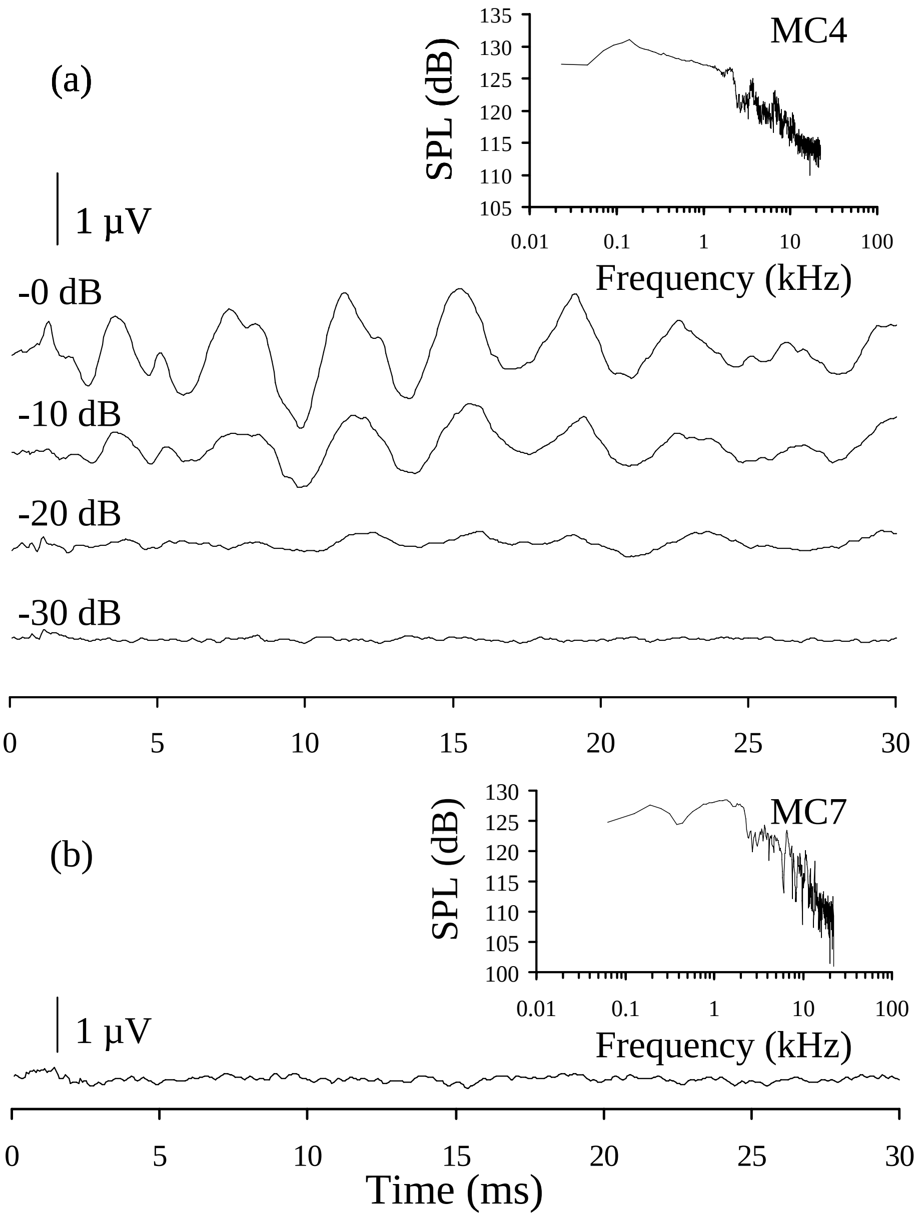
150
140
130
120
110
100
90
80
SPL (dB re 1 µPa)
0
100
200
300
400
500
600
Frequency (Hz)

Figure 5.



40
20
0
-20
-40
-60
Acceleration (dB re m/s^2)
0
100
200
300
400
500
Frequency (Hz)
a
a,b
d
b
c
c

Figure 6.


(a)
MC4
SPL (dB)
135
130
125
120
115
110
105
0.01
0.1
1
10
100
Frequency (kHz)
1 µV
-0 dB
-10 dB
-20 dB
-30 dB
0
5
10
15
20
25
30
(b)
MC7
SPL (dB)
130
125
120
115
110
105
100
0.01
0.1
1
10
100
Frequency (kHz)
1 µV
0
5
10
15
20
25
30
Time (ms)