

1 Detection of low frequency tones and whale predator sounds by the American sand lance

2 *Ammodytes americanus*

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13 Hearing of *A. americanus*

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

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

INTRODUCTION

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

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57 While fishes are clearly adapted to detect and avoid predators, specific responses to
58 predator sounds are far less documented. Yet, evaluating an auditory scene, including detecting
59 predators, has been suggested as a primary adaptive force for developing hearing abilities in
60 fishes (Bregman, 1990; Fay, 1992; Fay & Popper, 2000). Presumed predator escape responses
61 have been elicited by exposing Atlantic salmon *Salmo salar* L. 1758 to 10 Hz tones (Knudsen *et*
62 *al.*, 1992). Alewives *Alosa pseudoharengus* Wilson 1811 and shad *Alosa sapidissima* Wilson ,

63 1811 also avoid ultrasonic sounds suggesting responses to odontocete echolocation clicks
64 (Dunning *et al.*, 1992; Mann *et al.*, 1997). Pacific herring *Clupea pallasii* Valenciennes 1847
65 display a context-dependent startle and avoidance response to simulated odontocete clicks, both
66 in tank and free-field tests (Wilson & Dill, 2002). Gulf toadfish *Opsanus beta* Goode & Bean
67 1880 reduce calling rates in the presence of certain marine mammal sounds (Remage-Healey *et*
68 *al.*, 2006). However, the adaptation for marine predator detection can lead to disadvantages for
69 fish species. Nowacek (2005) suggested that bottlenose dolphin *Tursiops truncatus* Montagu
70 1821 may take advantage of their prey's hearing range, emitting broadband, low frequency
71 signals called 'pops' to startle prey from seagrass beds into the water column.

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

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

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90 *Ammodytes* spp. serve as essential prey to over 100 consumer species, including birds,
91 marine mammals, fishes, and invertebrates (Robards *et al.*, 1999; Willson *et al.*, 1999). They are
92 one of the most important forage fishes in north Atlantic ecosystems because the population's
93 overall health can be closely linked to the reproductive fitness of its seabird predators (Martin,
94 1989; Monaghan, 1992) as well as the distribution and abundance of cetacean predators (Payne
95 *et al.*, 1986; Weinrich *et al.*, 1997). Stimpert *et al.* (2007) recorded one of these predator species,
96 the humpback whale *Megaptera novaeangliae* Borowski 1781, emitting broadband, low-

97 frequency, short-pulsed signals while foraging at night in Jeffreys Ledge, in an area and during a
98 season in the Gulf of Maine in which *A. americanus* are abundant (Fiedler, 2002). *Ammodytes*
99 *americanus*, along with the related species *A. dubius*, are large components of the *M.*

100 *novaeangliae* diet in the northwest *M. novaeangliae* Atlantic population (Kenney *et al.*, 1985).

101 The recorded sounds, termed 'megapclicks', were associated with sharp body rolls
102 corresponding to underwater lunge feeding that concluded with 'buzzes' of short inter-pulse

103 intervals, similar to those emitted by odontocetes or bats immediately prior to prey capture

104 (Griffin *et al.*, 1960; Johnson *et al.*, 2004). The authors suggested that these signals may either

105 serve a biosonar purpose or to manipulate the movements of prey.

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108 Only one audiogram exists for the family Ammodytidae: the Pacific sand lance *A.*
109 *personatus* Girard 1856, found in the western Pacific along the Japanese coast (Suga *et al.*,
110 2005). The study focused only on juveniles and did not address the particle velocity component
111 of sound stimuli. The hearing abilities of adult sand lance or any Atlantic *Ammodytes* species are
112 unknown. Hearing sensitivities have been shown to differ between cross-oceanic species, such as
113 *C. pallasii* and Atlantic herring *C. harengus* L. 1758 (Mann *et al.*, 2005). This study investigated
114 the hearing of *A. americanus* with the goal of determining the audiogram of a north Atlantic
115 *Ammodytes* (sand lance) species and their potential detection of pulsed sounds similar to
116 megapclicks. Results are provided in both sound pressure and acceleration. The implications of
117 these data are discussed in reference to the behaviour of both predators and prey of *A.*
118 *americanus*.

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MATERIALS AND METHODS

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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 $23.5 \pm 0.2^\circ$
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

131 behaviours (Robards *et al.*, 1999). Hearing was measured using auditory evoked potential (AEP)
132 methods. The AEP technique involves measuring neurophysiological activity in response to short
133 acoustic stimuli (Hall, 2007). The technique has been used for hearing tests in both invertebrates
134 (Lovell *et al.*, 2005; Mooney *et al.*, 2010) and vertebrates (Jewett, 1970), including a wide range
135 of fish species (Corwin *et al.*, 1982; Kenyon *et al.*, 1998; Yan *et al.*, 2000; Wilson *et al.*, 2009).
136 Twenty-one *Ammodytes americanus* were examined using short tone pips to establish an AEP
137 audiogram [10.82 cm mean total length (L_T), S.D. 1.84 cm, 3.24 ± 0.40 g mean mass]. Not every
138 frequency was tested with every subject; respective sample sizes are listed in Table 1. Twelve
139 animals were tested for megapclick responses [9.84 cm (L_T), S.D. 1.41 cm, 2.38 ± 1.20 g mean
140 mass].

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143 EVOKED POTENTIAL RECORDINGS

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146 *Ammodytes americanus* were transferred to a rectangular plastic experimental tank (49.5
147 x 45.7 x 35.6 cm) containing gently flowing, unfiltered seawater ($23.2 \pm 0.1^\circ$ C). To reduce the
148 influence of outside vibrations, the plastic tank was placed in a foam-lined wooden box (58.7 x
149 57.8 x 86.4 cm) elevated 22.3 cm above the concrete floor on cinderblocks and rubber gaskets.
150 Each *A. americanus* was wrapped in acoustically transparent mesh fabric, held closed with
151 plastic paper clips, and completely submerged in the water [Fig. 1(b)]. The fabric, suspended
152 with nylon monofilament between two pieces of PVC pipe, created a tight hammock-like
153 arrangement to keep the *A. americanus* immobile [Fig. 1(c)]. Each *A. americanus* was positioned

154 in the centre of the tank, directly above the speaker presenting the stimuli. A wooden desk next
155 to the tank held the experimental equipment. Overall, the AEP procedures follow standard
156 techniques used for fish evoked potentials (e.g., Corwin *et al.*, 1982; Kenyon *et al.*, 1998; Yan *et*
157 *al.*, 2000; Wilson *et al.*, 2009) and followed approved Institute for Animal Care and Use
158 protocols. The subjects fully recovered from these procedures, swimming and borrowing
159 normally when returned to their holding tank.

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

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

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193 Stimuli consisted of amplitude modulated tone pips from 50–2000 Hz (50, 100, 150, 200,
194 300, 400, 500, 1000 and 2000) and pulsed sounds of simulated megapclicks. The 50 Hz tone
195 could drift in frequency by ± 5 Hz. The update rate for all stimuli was 16 kHz. Tone signals were
196 at least six cycles in duration, thus signal length varied relative to frequency but was never more
197 than 120 ms (50 Hz) and was as short as 20 ms (for stimuli ≥ 350 Hz). Sound presentations
198 digitally triggered AEP recordings; thus, stimuli and evoked potential records were

199 synchronized. For an individual response record, the test tone was presented 1000 times, with
200 1000 concurrent averaged AEP records. Measurements typically started at maximum sound
201 pressure levels (SPLs) for each frequency (133–167 dB re. 1 μ Pa depending on the frequency).
202 Within each frequency presentation, the SPL was decreased in 10-dB steps until the production
203 of recognizable and repeatable AEP waveforms weakened [Fig. 2(a)]. The SPL was then
204 decreased in 5-dB steps until the waveform disappeared. Two-to-three more measurements were
205 made at 10 to 15 dB below this apparent ‘threshold’ in order to ensure weak responses were not
206 overlooked. Each test began and concluded with a frequency known to produce a strong
207 response, usually 150 Hz.

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

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

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

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$$230 \quad \alpha = - \Delta sig / (\rho \Delta r)$$

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232 where Δsig is the magnitude of the difference between the waveforms of the two hydrophones
 233 (in Pa), ρ is the density of the medium and r is the distance between the hydrophones (Wahlberg
 234 *et al.*, 2008). The particle motion was measured in three dimensions by positioning the two
 235 hydrophones along three orthogonal axes (Kalmijn, 1988; Wahlberg *et al.*, 2008). Subsequently,
 236 particle acceleration values for the pressure-derived AEP thresholds were determined by relating
 237 the measured pressure at threshold with the corresponding particle acceleration at the head of the
 238 fish. Although a fish acts as a rigid body in the acoustic near field (Denton & Gray, 1982;
 239 Coombs *et al.*, 1992), measurements at the head were compared with additional measurements \pm
 240 5 cm along the anterior–posterior axis to confirm the sound acceleration field. These
 241 measurements were similar (± 2 dB) to those at the head.

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

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263 Control experiments included recordings with naturally deceased *A. americanus*,
264 electrodes in the water without a subject and from subjects with the recording electrode placed in
265 the posterior muscle [Fig. 2(b)] (Ramcharitar & Popper, 2004; Ramcharitar *et al.*, 2004; Mooney
266 *et al.*, 2010). In this case the recording electrode was inserted into the left posterior-lateral trunk

267 musculature, ~ 2 cm from the tip of the caudal fin, and the reference electrode in the left
268 mediolateral muscle, ~ 5 cm from the tip of the caudal fin.

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271 DATA ANALYSIS

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

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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.

312 The sound pressure audiograms were somewhat irregular in shape. Thresholds were
313 lowest at 300 Hz and increased slightly at 200 and 400 Hz. Responses were not detected above
314 400 Hz. A substantial audiogram notch was found at 150 Hz, at which sensitivity decreased
315 sharply in relation to other frequencies (Fig. 4). Thresholds then decreased and sensitivities
316 improved at 100 Hz and below. While most thresholds did not vary widely among individuals,
317 some frequencies showed greater variation. This seemed partially, but not always, due to
318 differences in the number of samples per frequency (Table I). For example, 200 Hz had the
319 greatest S.D. values despite a relatively high ($n=18$) sample size. While the sound pressure
320 audiogram shape did not differ substantially between the visual method and the FFT methods,
321 visual thresholds were elevated about 20-30 dB (Fig. 4).

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

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332 Particle velocity sensitivities were lowest at 200 Hz (Fig. 5). While 400 Hz was the
333 highest overall threshold, there were not always substantial differences between the frequencies.
334 The visual thresholds showed a general trend of improved sensitivity from high to lower

335 frequencies. This trend was not apparent in the FFT measurements. For example, while the
336 highest FFT thresholds were at 400 Hz, they were only significantly greater than thresholds at
337 200 and 300 Hz (one-way ANOVA, $F_{7,93}=19.12$, $P \leq 0.001$; subsequent Tukey's pairwise
338 comparison). FFT thresholds at 50 Hz were only significantly different from 200 Hz. Thus, the
339 FFT-determined particle velocity thresholds were essentially flat.

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342 PULSED STIMULI

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345 Responses were generated using pulsed sounds that contained high spectral energy from
346 75 to 350 Hz and peak frequencies at or below 178 Hz [Table II; Fig. 6(a)]. Evoked potential
347 waveforms were similar to those for tone pips, consisting of a repeatable sinusoid-like
348 waveform. This was likely due to a ringing of the speaker, which is expected with impulse
349 sounds. Response delays were detectable but less than those of the tonal stimuli (~ 5 ms).
350 Response amplitudes decreased correspondingly with SPL attenuation. Again, controls
351 (deceased *A. americanus*, no *A. americanus* and electrodes in the posterior musculature) did not
352 generate responses. At very high SPLs (> 160 dB), the short-duration stimulus waveform was
353 visible in the AEP record, potentially masking the first few ms of recording. Also notably, while
354 responses were consistently generated using pulses with lower frequency peaks, these responses
355 were not generated in every *A. americanus*. There appeared to be some variation with these brief
356 signals and successful AEP recordings compared to the longer duration tonal stimuli.

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

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DISCUSSION

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367 At regions of best sensitivity, 50-350 Hz, responses were clear and easily distinguishable
368 from the background noise. All responses showed a standard physiological time delay before
369 they were observed. This delay was shorter for the pulsed sounds likely because their onset was
370 rapid (*e.g.* Wysocki & Ladich, 2002). Tone pip stimuli were ramped up to reduce frequency
371 spreading but this increased the latency until a response was observable, likely due to an
372 increased time until sufficient stimulus amplitudes were received by the *A. americanus*. Thus,
373 lower frequencies, with longer ramp-up times (due to larger wavelengths), had slightly longer
374 physiological delays (Wysocki & Ladich, 2001). The AEP waveforms measured also had
375 significant energy at twice the stimulus frequency, making them easily identifiable using FFTs
376 (Casper & Mann, 2007). This suggests that otolith hair cells of *A. americanus* are arranged in
377 opposite directions and are alternately stimulated *via* the sound stimuli (Fay & Edds-Walton,
378 1997). Finally, and as expected, responses decreased with stimulus level [Figs 2(a),3] and
379 comparatively, no responses were generated during various control experiments. These *A.*
380 *americanus* tone-generated AEP characteristics of a delayed response, decreases with stimulus

381 level and doubling for frequency general were similar to other fishes (*e.g.* Kenyon *et al.*, 1998;
382 Egner & Mann, 2005) and some invertebrates (Mooney *et al.*, 2010). Overall, thresholds levels
383 shown here are also similar to those in other studies of fishes without auditory specializations
384 (Wysocki *et al.*, 2009; Anderson & Mann, 2011) showing *A. americanus* are not hypersensitive,
385 nor insensitive, to sound. Pulsed stimuli with significant low frequency energy generated
386 responses that were much shorter in delay but overall still exemplified the doubling-of-frequency
387 following response [Fig. 6(a)]. These reflected novel fish AEP responses to a predator-like
388 sound.

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391 The shape of the audiograms differed when plotted in sound pressure and particle
392 velocity. The sound pressure thresholds of *A. americanus* were irregular, with peaks and valleys
393 and displayed greatest sensitivity from 300 Hz to 400 Hz. Acceleration thresholds were lowest at
394 200 Hz. Even excluding this 200 Hz point, the acceleration thresholds slightly improved in
395 sensitivity as frequency decreased. Thresholds plotted in sound pressure were much more
396 uneven in shape. These shape differences are a little surprising because in many instances, fish
397 sound pressure and particle velocity hearing curves have similar curves (Horodysky *et al.*, 2008;
398 Wysocki *et al.*, 2009). Yet, this is not always the case (Anderson & Mann, 2011). The
399 differences between the two audiograms' shapes seen here and elsewhere may be partially due to
400 the complex interaction of acoustic stimuli in small experimental tanks. While free-field
401 calculations between sound pressure and particle motion are a direct theoretical relationship, this
402 is not true in a small tank with reflections and reverberation. Thus, sound pressure and
403 acceleration may not follow the same trends in certain situations.

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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.

427 The range and shape of the acceleration audiogram reflect hearing abilities of some other
428 fishes (Casper & Mann, 2006; Horodysky *et al.*, 2008; Wysocki *et al.*, 2009; Belanger *et al.*,
429 2010; Anderson & Mann, 2011). Acceleration audiograms typically have a flattened ‘J’ shape,
430 with most sensitive thresholds at lower frequencies (often below 200 Hz) and thresholds
431 substantially increasing at higher frequencies (*e.g.* Karlsen, 1992). This is quite different than
432 the ‘U’ shape of sound pressure mammalian curves (*e.g.* Johnson, 1967). The acceleration
433 thresholds here somewhat reflect the ‘J’ shape, but only start the expected increase at the upper
434 limit of hearing, perhaps suggesting that these results were near, but not reaching the true hearing
435 limit of *A. americanus*. Fish thresholds are most sensitive at lower frequencies in the optimal
436 range of otolith hair cells and as frequencies increase, hair cell response efficiency can decrease
437 (Sand *et al.*, 2001). This appears to limit the frequency range of responses (Sand & Karlsen,
438 2000) and consequently many particle motion audiograms are limited to low frequencies
439 (Karlsen, 1992; Casper & Mann, 2006; these data). Particle velocity also appears to be the most
440 relevant stimulus for fish without swim bladders (Enger & Andersen, 1967; Chapman & Sand,
441 1974). The lack of a swim bladder (Robards *et al.*, 1999) and the presence of dense otolith
442 structures suggest that *A. americanus* are without auditory specializations (Popper & Fay, 2011),
443 and particle velocity is the likely acoustic stimulus. This further suggests that acceleration
444 thresholds, which are increasingly becoming standard in current studies (see references above),
445 are experimentally necessary because they are biologically relevant to these and other fishes.

446

447

448 Relatively few responses were generated at 350 Hz and 400 Hz. The starting stimulus
449 levels at these frequencies were limited by the transmission response curve of the underwater

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

460
461
462 *Ammodytes americanus* and *A. personatus* had somewhat different thresholds, although
463 frequency response ranges were similar (Suga *et al.*, 2005). The lack of responses at 500 Hz in
464 this study may be a result of the 140 dB starting SPL, which is very close to the threshold
465 observed in *A. personatus* by Suga *et al.* (2005) at 512 Hz (~ 133 dB). Levels of 140 dB are
466 significantly above threshold for many, but not all fishes without hearing specializations
467 (Wysocki *et al.*, 2009; Belanger *et al.*, 2010). The differences could be an artefact of the maturity
468 of *A. americanus* compared to the juveniles used in Suga *et al.* (2005), although threshold
469 differences were not detected across this study's size spectrum or in similar studies (Belanger *et*
470 *al.*, 2010). Population, and thus genetic or subtle morphological differences, have also been
471 suggested to impact thresholds (Ladich & Wysocki, 2009; Wysocki *et al.*, 2009). The observed
472 differences may also be a remnant of different methods used. Suga *et al.* (2005) placed the

473 speaker out of the water and kept the heads of *A. personatus* at the water surface. While animal
474 placement and speaker alone may not impact the sound pressure thresholds of otophysans
475 (Ladich & Wysocki, 2009), calibration errors, among other possibilities, may impact threshold
476 levels (Ladich & Wysocki, 2009). Sound measurements are particularly difficult at the air-water
477 boundary due to reflective and refractive effects (Urlick, 1983; Au & Hastings, 2009). Thus
478 placing the animal at the surface may lead to unquantified particle velocities to which *A.*
479 *personatus* actually responded.

480
481
482 The absence of low frequency energy in field-recorded megapclicks is an important
483 factor to consider in interpreting responses to pulses and simulated megapclicks. The methods
484 used by Stimpert *et al.* (2007) involved applying a high-pass filter to the acoustic tag recordings
485 that effectively removed the majority of energy below 400 Hz. Although used as an analysis
486 method to reduce water-flow noise on the recordings, this most likely removed the low-
487 frequency energy that falls within the auditory range of *A. americanus*. Recorded megapclicks
488 had a source level at the tag on the animal's back of 143 ± 5 dB and 154 ± 5 dB re 1 μ Pa pp.
489 These levels are probably higher in front of the *A. americanus* and are also above *A. americanus*
490 hearing thresholds for all tested frequencies. Unfortunately, only sound pressure values are
491 available for the megapclicks. As noted above, acceleration is likely to be the primary stimulus for
492 *A. americanus*. However, given that both pressure and particle motion are generated with all
493 sounds (Urlick, 1983; Kalmijn, 1988), these megapclicks probably have significant particle
494 motion components that would be available to *A. americanus*. By modelling the available
495 pressure stimuli and calibrating both components, this study assumed that natural megapclicks

496 would have similar acceleration components to which *A. americanus* may respond. The results
497 also show pulses with a variety of low frequency spectra can generate responses. Detection
498 ranges are not predicted without the actual source levels. Based on the published source levels
499 and assuming similar energy below 400 Hz, it seems likely that megapclicks are detectable by *A.*
500 *americanus*.

501
502
503 A prey's ability to hear approaching predators is not a new strategy for predator
504 avoidance, and predator detection is considered one of the primary drivers of hearing evolution
505 (Gans, 1992; Fay & Popper, 2000). *Ammodytes* spp. often show a tightly compacted schooling
506 response when a threat is perceived (Girsa & Danilov, 1976; Pitcher & Wyche, 1983). This
507 'selfish herd' response (Hamilton, 1971) may reduce fish and bird predation for many
508 individuals. Fishes constitute the majority of *Ammodytes* spp. predators and much of their
509 avoidance behaviour is considered to be adapted to reduce fish predation (Girsa & Danilov,
510 1976; Willson *et al.*, 1999). For *M. novaeangliae*, this behaviour may result in a higher density
511 prey patch. The acoustic signals produced by *M. novaeangliae* may serve to exploit *A.*
512 *americanus* behavioural responses and manipulate the fish into a denser school. Similar
513 *Ammodytes* spp. 'balling up' has been observed as a reaction to nets (Girsa & Danilov, 1976).
514 Perhaps less likely, the sounds could serve to startle *A. americanus* out of the substrate (*e.g.*
515 Nowacek, 2005). Behavioural tests with *A. americanus* in the water column would help evaluate
516 this hypothesis. *A. americanus* might also use this sensory modality to navigate or to 'eavesdrop'
517 on sounds produced by predators (Fay & Popper, 2000). The acoustic detection of predators may

518 also facilitate prey escape, whether or not the detected sounds correlate with predators' foraging
519 strategies.

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521

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

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531

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532

533

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589 Table I. Thresholds (dB re 1 uPa and dB re m s²), standard deviations, and sample sizes
 590 for the tone-pip stimuli.
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Freq (Hz)	SPL				Acceleration				<i>n</i>
	FFT	S.D.	Visual	S.D.	FFT	S.D.	Visual	S.D.	
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

589 Table II. Simulated megapclick (MC) sound settings.

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Simulated megapclick pulse no.	Peak Freq. (Hz)	Duration (ms)	Starting SPL (dB re 1 μ Pa)	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

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Figure 1.

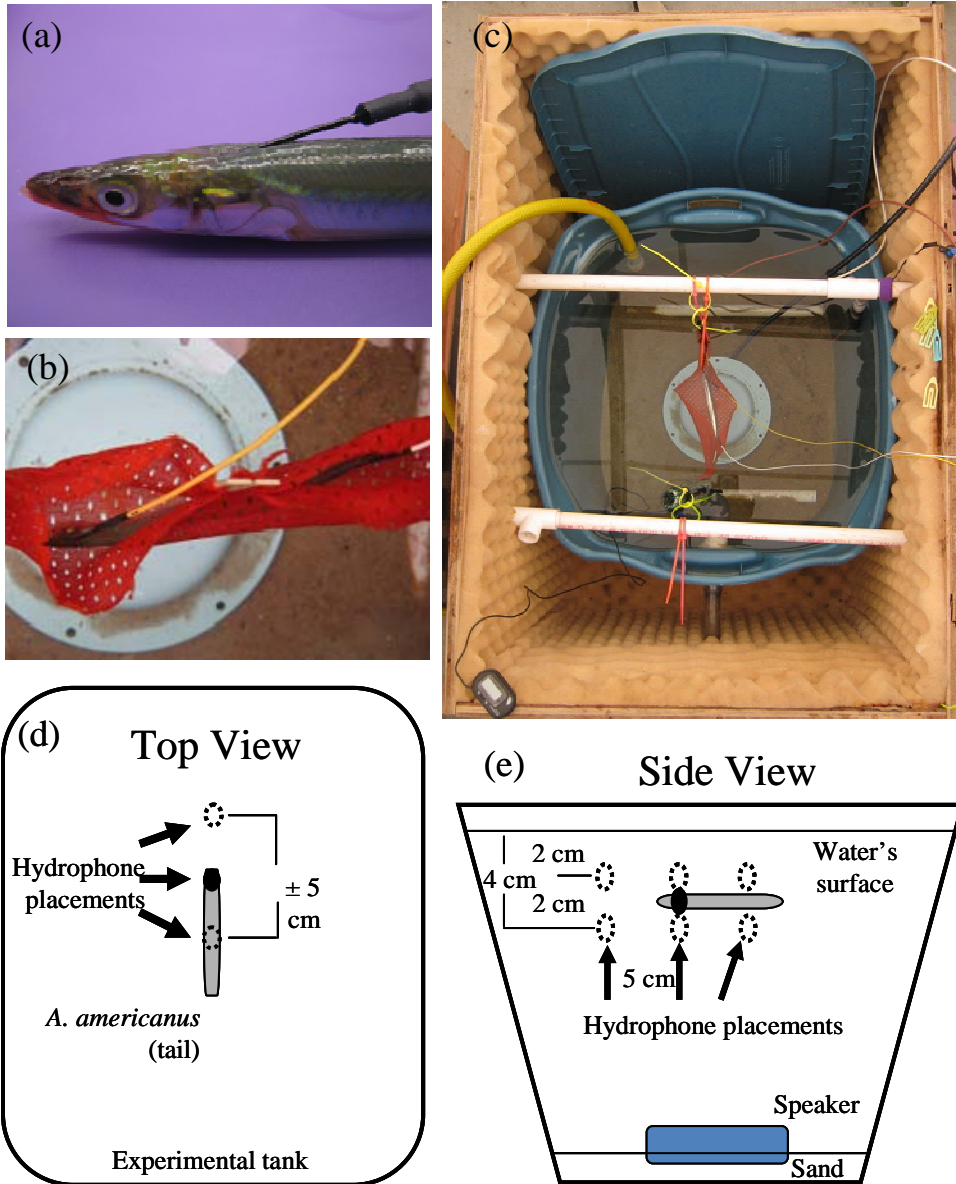


Figure 2.

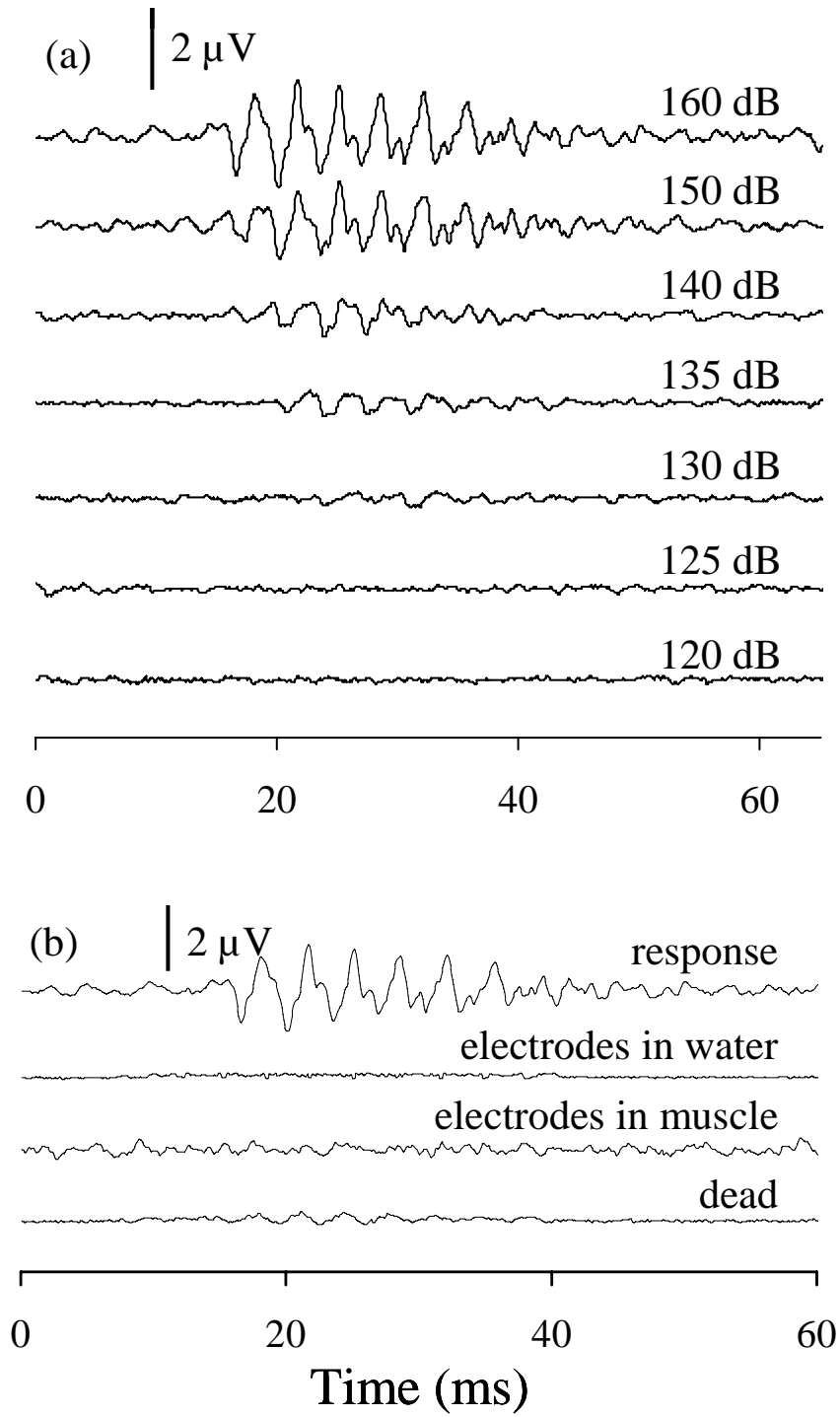


Figure 3.

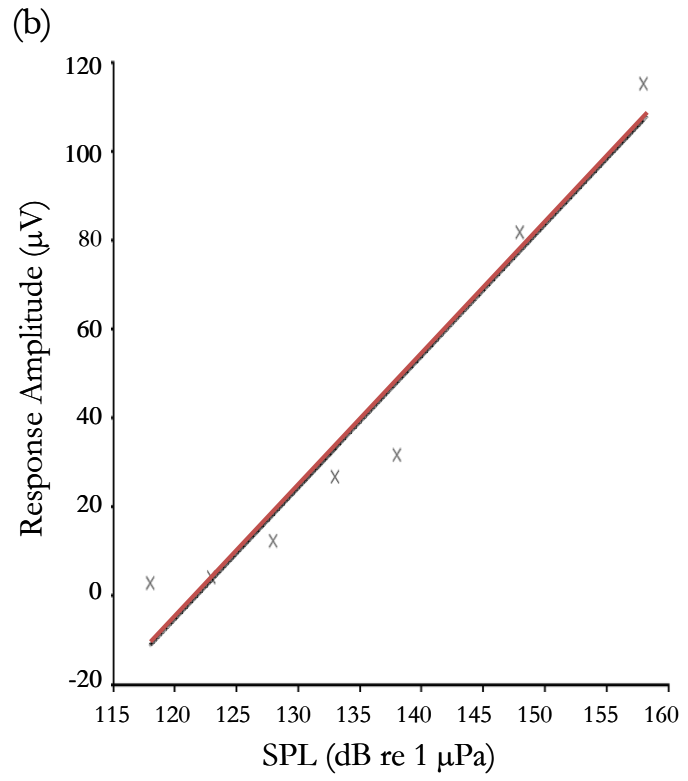
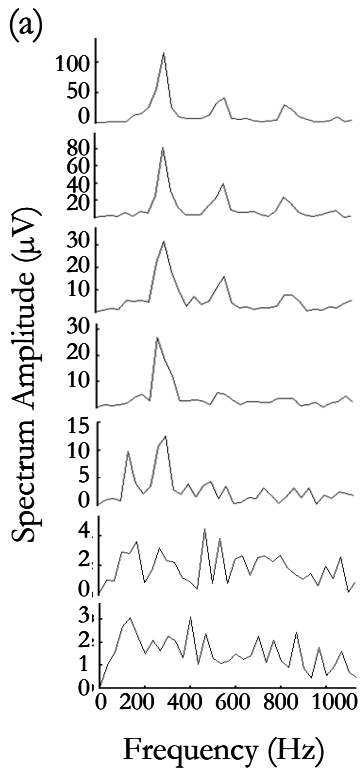


Figure 4.

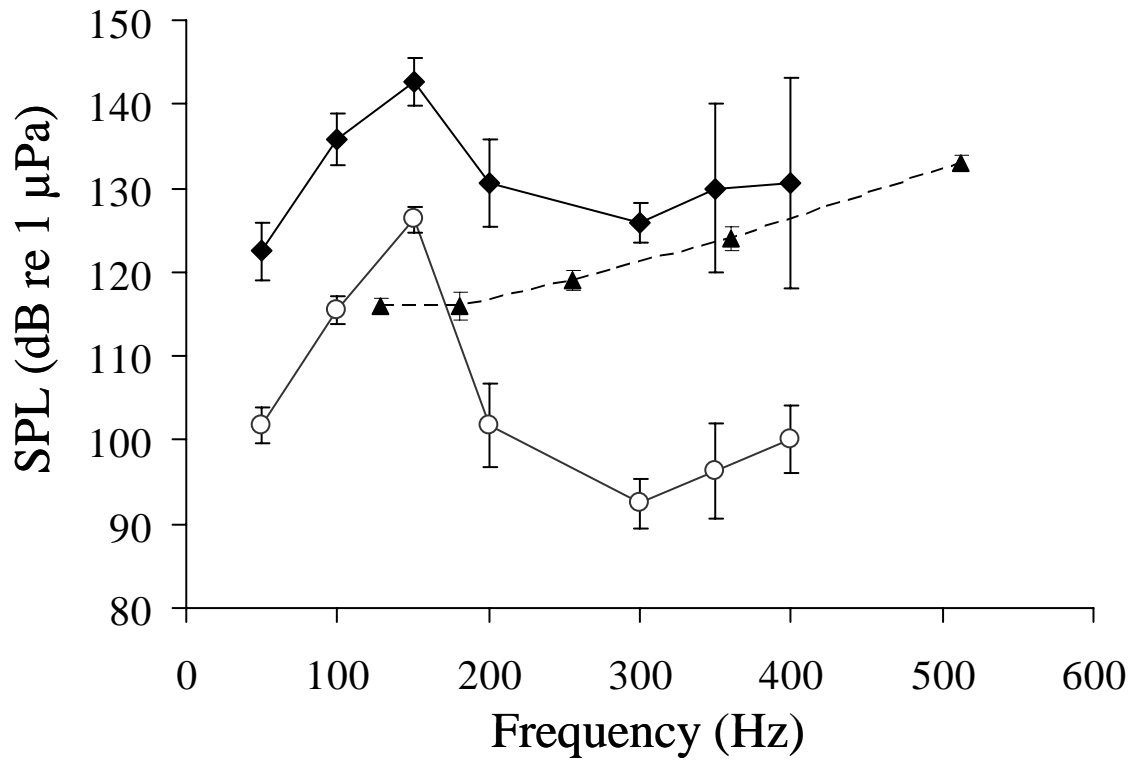


Figure 5.

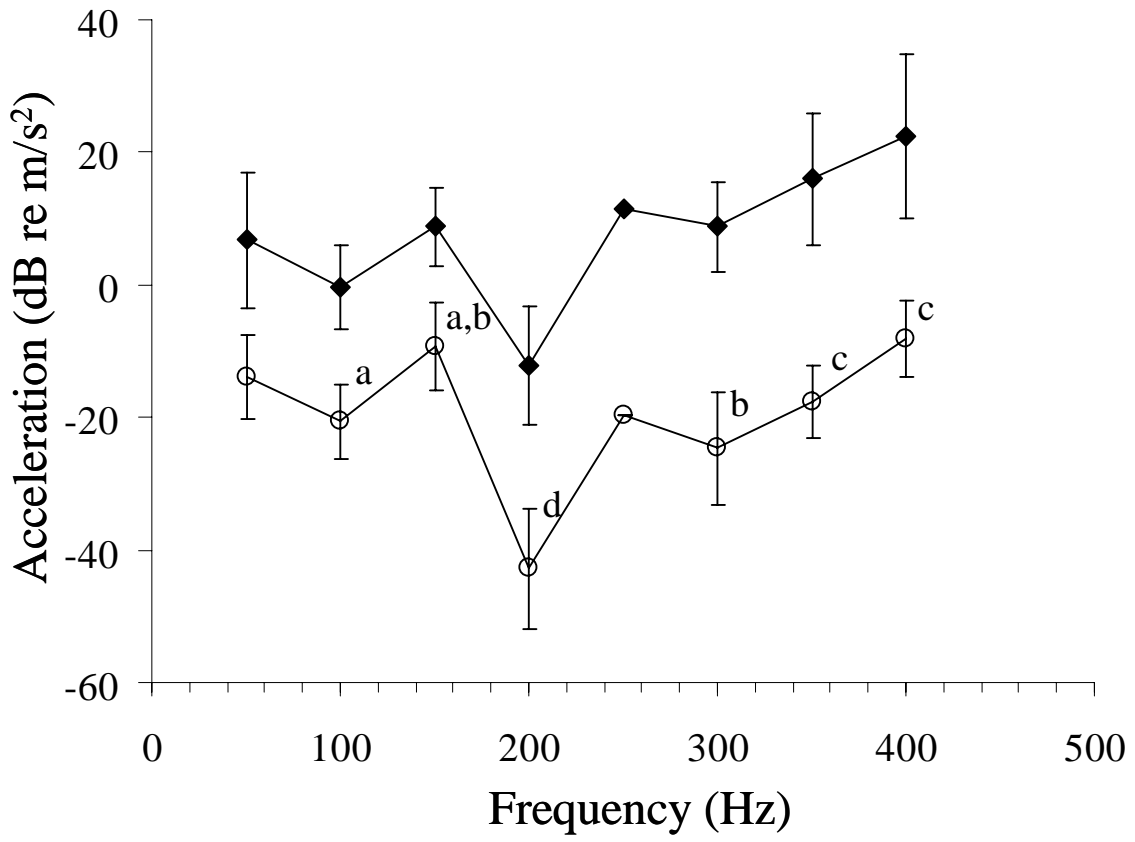


Figure 6.

