

Characteristics of the Auditory Brainstem Evoked Potential of a Pacific White-Sided Dolphin (*Lagenorhynchus obliquidens*)

Whitlow W. L. Au,¹ Jeanette A. Thomas,² and Kenneth T. Ramirez³

¹Marine Mammal Research Program, Hawaii Institute of Marine Biology, P.O. Box 1106, Kailua, HI 96734, USA; E-mail: wau@hawaii.edu

²Western Illinois University-Quad Cities, 3561 60th Street, Moline, IL 61265, USA

³Marine Mammal Department, John G. Shedd Aquarium, 1200 S. Lake Shore Drive, Chicago, IL 60605, USA

Abstract

Auditory brainstem responses (ABRs) of a Pacific white-sided dolphin (*Lagenorhynchus obliquidens*) in the presence of masking noise were measured at John G. Shedd Aquarium in Chicago. The dolphin was trained to wear suction cups with 1-cm diameter, gold-plated metallic electrodes typically used for human EEG measurements embedded in the cups. The animal was trained to station in a hoop, facing a sound projector 5 m away. ABR thresholds were obtained by progressively reducing the level of click stimuli, having peak frequencies of 8, 16, 32, 64, 80, and 100 kHz. The thresholds were obtained in the presence of broadband masking noise. The ABR waveforms were slightly different than for other odontocetes, having 7 to 8 waves present—the most for any odontocetes measured so far. The response latency of 1.3 to 1.5 ms is similar to those of other dolphins of approximately the same size. The peaks in the Fourier transform of the ABR waveform occurred at 650 and 1,200 Hz, very similar to the 600 to 650 and 1,100 to 1,200 Hz for *Tursiops truncatus*. The deepest null in the spectrum, which occurred at about 950 Hz, was much deeper than for the bottlenose dolphin. Masked ABR thresholds expressed in peak-to-peak values were between 38 and 56 dB above the rms values of the masking noise.

Key Words: auditory brainstem response, masked threshold, click evoked potentials, ABR masked threshold, Pacific white-sided dolphin, *Lagenorhynchus obliquidens*

Introduction

An expedient technique to access the hearing sensitivity of a dolphin is to measure the animal's auditory brainstem response (ABR) to acoustic stimuli. Bullock et al. (1968) first used the ABR technique to examine the auditory processing of

acoustic signals by spinner dolphins (*Stenella longirostris*). They inserted electrodes in the brain stem of their subjects and measured the evoked potentials produced by various types of acoustic stimuli. Since the invasive experiments of Bullock et al. and McCormick et al. (1970), ABR experiments have been non-invasive.

ABR can be measured by placing electrodes on the surface of an odontocete's head. The electrodes can be thin, needle-shaped wires on the order of 0.1 to 0.3 mm in diameter inserted subdermally 3 to 5 mm into the skin as used by Supin & Popov (1993) and Supin et al. (1993). A dolphin's skin is relatively insensitive to these small, subdermally inserted wires. Surface electrodes in the form of a 1-cm cup secured by adhesive tape were used by Supin et al. (1994). Dolphin et al. (1995) used a 2.4-cm silvered disc embedded in 4-cm latex suction cups that were filled with conductive electrode gel as electrodes. Small 1-cm gold-plated metallic cups used for human EEG measurements were embedded in latex suction cups and used as electrodes to measure auditory evoked potentials (AEPs) from dolphins and small whales (Supin et al., 2003).

The ABR technique has been applied to a small number of dolphins, among them are the Atlantic bottlenose dolphin (*Tursiops truncatus*), false killer whale (*Pseudorca crassidens*), beluga whale (*Delphinapterus leucas*), common dolphin (*Delphinus delphis*), Amazon river dolphin (*Inia geoffrensis*), killer whale (*Orcinus orca*), and the harbor porpoise (*Phocoena phocoena*) (see Supin et al., 2001). Most of these measurements were made with restrained animals in a stretcher, a constraining harness, or while in a small bath with the animal's lower jaws submerged and the top of the head out of the water, resulting in an acoustic environment that is difficult to quantify.

Dolphin (1995) and Dolphin et al. (1995) were the first to use trained dolphins in an ABR experiment. The animals were trained to wear suction-cup electrodes and station themselves within an underwater hoop or on a biteplate at a depth of 1 m.

The original electrodes were silver discs 2.4 cm in diameter embedded in custom-designed latex suction cups. Evoked responses were recorded differentially from the scalp source between the parietal (non-inverting) just posterior to the blowhole and the mastoid (inverting) bones of the head. A ground electrode was placed either on the melon or trunk region. The advantage of using a trained subject consists of obtaining a good free-field acoustic environment with a minimum amount of boundary reflections of the acoustic signal. The sound pressure level (SPL) that the subject is exposed to can be determined accurately, especially if a directional source is used and the separation distance between the animal and transducer is greater than 1 m. The goals of this study were to determine the characteristics of ABR signals obtained from a Pacific white-sided dolphin (*Lagenorhynchus obliquidens*) to compare the ABR signals with other species of dolphins and to determine the masked hearing threshold of the subject to click signals. This is the first ABR study with this species of dolphin. A basic behavioral audiogram was conducted by Tremel et al. (1998) on the same study animal.

Materials and Methods

The experiment was conducted in the off exhibit medical pool of the John G. Shedd Aquarium in Chicago, Illinois. The subject was a female Pacific white-sided dolphin by the name of Kri, who weighed 444 kg and was 2 m in length. She was trained to wear rubber suction cups on various parts of her body. Embedded in each suction cup was a 1-cm, gold-plated metallic electrode cup typically used for human EEG measurements. One surface electrode was normally placed within

6 cm posterior to the blowhole close to the mid-dorsal line and the other electrode was placed near the dorsal fin. The electrode leads, approximately 7.5 m in length, connected to a Tucker Davis (TD) HS2 battery-operated differential amplifier with the ground lead of the amplifier in contact with the water. A fiber-optic link connected the differential amplifier assembly to a TD-DB4 Bioamp Controller with an adjustable low-pass filter, which was in turn connected to a TD-AD2, 16-bit data acquisition unit that was controlled by the TD-AP2 controller housed in a lunch-box computer. Communication between the AD2 and the AP2 was via another fiber-optic link.

The dolphin was trained to swim into a hoop that had its center at 1-m depth as depicted in Figure 1. While in the hoop, the dolphin faced a sound projector located 5 m from the hoop. Different sound projectors were used for different frequency ranges. A J-9 electrodynamic transducer was used for frequencies between 8 and 32 kHz. An F-30 piezoelectric transducer was used for frequencies of 64 and 80 kHz. Both the J-9 and F-30 transducers are standard transducers from the U.S. Navy's Underwater Sound Reference Detachment (USRD). The WAU-7, a transducer made of 1-3 composite piezoelectric element from Material Systems Inc. was used at 100 kHz. A Qua-Tech A-100 function generator board housed in the lunch-box computer was used to generate the signal used by the transducers. The signal consisted of a single cycle sine pulse at the frequency of interest. The function generator also produced a sync pulse in concert with the sine pulse that triggered the data acquisition system. The ABR signal was digitized at a 20-kHz rate in blocks of 320 points, representing a 16-ms time span. The results were stored on the TD-AP2 board. Each block of 320

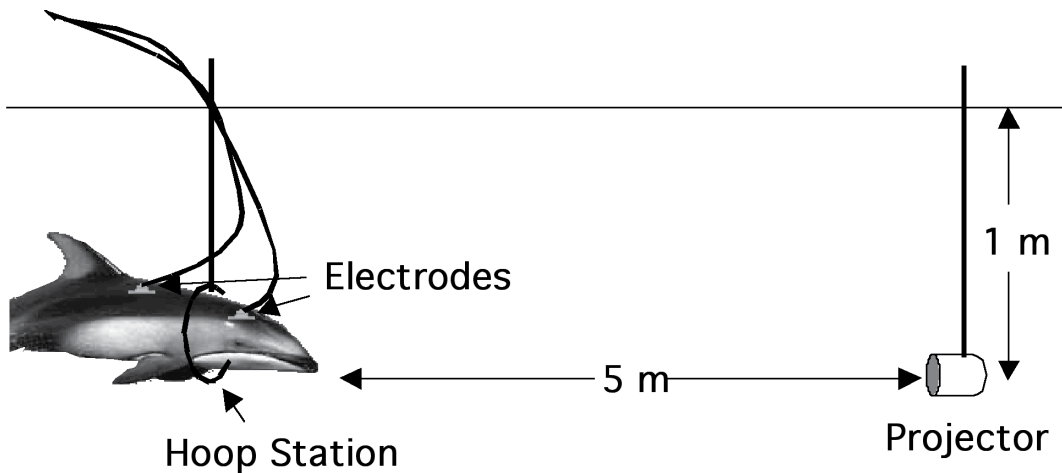


Figure 1. Experimental configuration for the ABR measurements of underwater hearing in a Pacific white-sided dolphin

digitized points were averaged with the previous block of 320 points. The process continued until 500 averages were obtained. The noise level of the medical pool was relatively high because of pump and filter noise and the presence of five beluga whales in an adjacent large exhibition pool. Only a net gate separated the animals in both pools, so the two pools were not acoustically isolated. Therefore, it was advantageous to use a relatively stable masking noise, which was at levels above the ambient noise of the medical pool.

ABR thresholds were determined for click stimuli having peak frequencies of 8, 16, 32, 64, 80, and 100 kHz. For each threshold determination, the level of the stimulus was continually decreased by 10 dB or 5 dB until an ABR signal could not be detected.

Results

An example of an ABR signal for a click stimulus with a peak frequency of 64 kHz and a peak-to-peak SPL of 130 dB is shown in Figure 2, along with an ABR waveform obtained with a bottlenose dolphin (Supin et al., 2001). Since the projector was approximately 5 m from the hoop station, there was a propagation delay of 3.4 ms along with a 1-ms delay from the low-pass filter. The Pacific white-sided dolphin's ABR waveform had eight

different sequences of waves compared to the six for the bottlenose dolphin. The number of waves was highest among odontocetes examined to date. The horizontal scales of both ABRs in Figure 3 have the same scale, so the relative difference in duration can be seen. Because of the presence of additional waves, the ABR for the Pacific white-sided dolphin had about a 1.5-ms longer duration than that of the bottlenose dolphin. The response latency of 1.3 to 1.5 ms for Wave I is similar to those of other dolphins of approximately the same size.

The frequency spectrum of the ABR signal shown in the left graph of Figure 3 had a major peak at approximately 1,220 Hz, a secondary peak at 667 Hz, and a deep null at 960 Hz. The spectrum resembles that shown in Supin et al. (2001) for *T. truncatus*, which typically has a major peak between 1,000 and 1,400 Hz, a secondary peak between 600 and 650, and a null at about 900 Hz. There are also many minor peaks and valleys in the spectrum of the ABR signal from both species.

The ABR waveforms for different peak frequencies for supra-threshold stimuli are shown in Figure 4. The shape of the various ABR waveforms generated by different click stimuli were similar to each other as they should be. Each ABR waveform had seven to eight waves and were

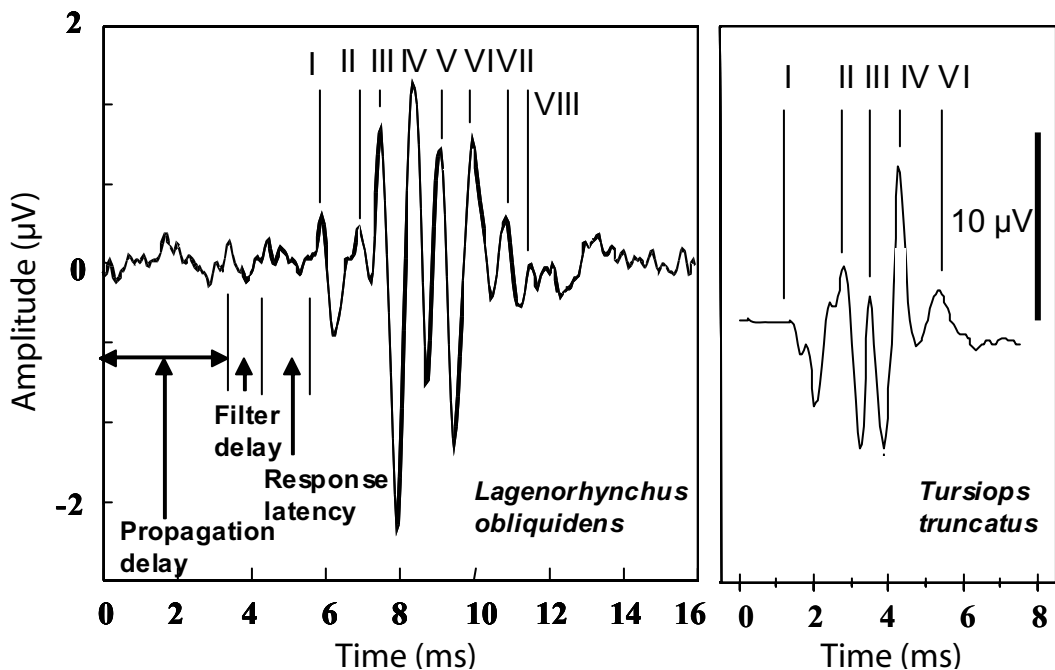


Figure 2. Typical ABR signal for the *Lagenorhynchus obliquidens* in a supra-threshold condition generated by a click with a peak frequency of 64 kHz (left panel), and a typical ABR signal for a *Tursiops truncatus* (adapted from Supin et al., 2001)

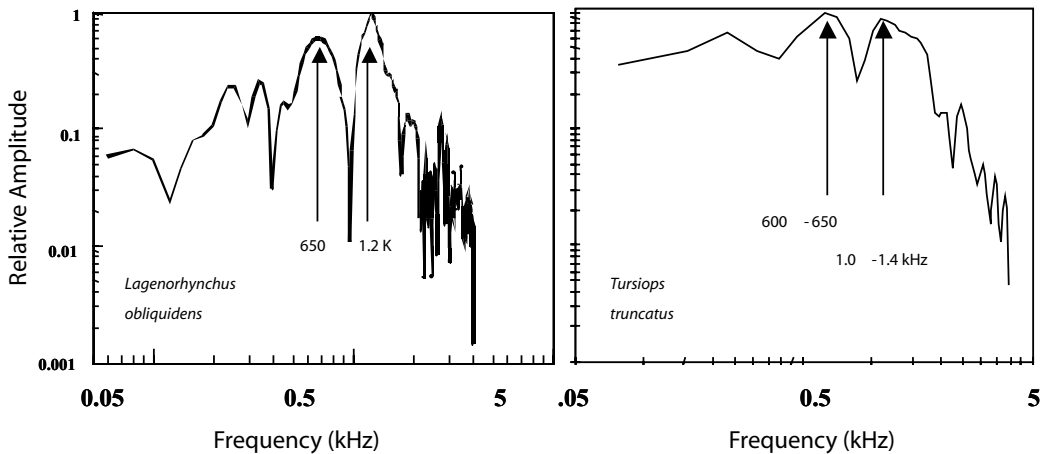


Figure 3. Frequency spectra of the ABR signals shown in Figure 2

approximately 6.5 ms in duration. The response latencies for Wave I were also similar for the different ABR signals. The major difference in the ABR for this species was in the peak-to-peak amplitude. The largest ABR occurred when the stimulus had a peak frequency of 64 kHz. This is the same frequency in which the dolphin's hearing sensitivity is most acute (Tremel et al., 1998).

As the amplitude of the acoustic stimulus decreased, the amplitude of the ABR signal also decreased. A simple way to determine the ABR threshold is to reduce the amplitude of the stimulus by a set increment until the ABR signal cannot be detected. An example of such an ABR threshold determination is shown in Figure 5. When the peak-to-peak stimulus amplitude was greater than 115 dB re 1 μ Pa, the presence of an ABR is rather obvious. When the stimulus amplitude decreased to 110 dB, however, the ABR signal could not be found in the recorded data. Therefore, one could specify that the ABR threshold occurred for a stimulus amplitude of 110 dB. An extrapolation technique also was used to determine more accurately the ABR threshold of the subject. This technique relies on the fact that near the threshold the amplitude of the ABR signal varies almost linearly with the amplitude of the stimulus (Supin et al., 2001). Examples of this extrapolation process can be found in Figure 6. The solid dots represent the relative spectral amplitude of the ABR signals as a function of the stimulus level. The solid lines are the linear regression lines along with their R^2 values.

The ABR threshold is plotted against the peak frequency of the click stimulus in Figure 7. The ABR threshold is described in terms of the peak-to-peak level of the click stimuli. The masking noises from the two transducers are also plotted in terms of the noise spectral density, which is the

rms values of the noise in a 1-Hz band for the different frequencies. The noise spectral density was actually measured with an rms meter in $1/3$ -octave bands, and the results were converted to a density measure. Masked ABR thresholds expressed in peak-to-peak values were between 38 and 56 dB above the masking noise expressed in terms of its rms spectral density.

Discussion

This is the first reported measurement of ABR for the Pacific white-sided dolphin, or for any dolphin in the genus *Lagenorhynchus*. The general overall ABR waveform is slightly different than other odontocetes in that seven to eight waves were present compared to six waves normally associated with *T. truncatus* and *Delphinapterus leucas* (Supin et al., 2001). There is some subjectivity in determining the number of waves in an ABR, however. For example, Ridgway (1983) reported seven waves for *T. truncatus*, whereas the example in Figure 2 clearly shows six waves. Nevertheless, the differences in ABR waveforms between the Pacific white-sided dolphin and the Atlantic bottlenose dolphin can be clearly seen in Figure 2.

The ABR waveforms for six other odontocete species are shown in Figure 8, along with the ABR waveform from the *L. obliquidens* used in this study in order to see the similarities and differences for the different species. Figure 8 clearly suggests that species-specific differences exist, which should not be surprising. The ABR of the Pacific white-sided dolphin is longer in duration than the others, and this is because more waves are present. It is beyond the scope of this study to delve into the reasons for the extra waves in the ABR of the Pacific white-sided dolphin, so this will be left for future investigations.

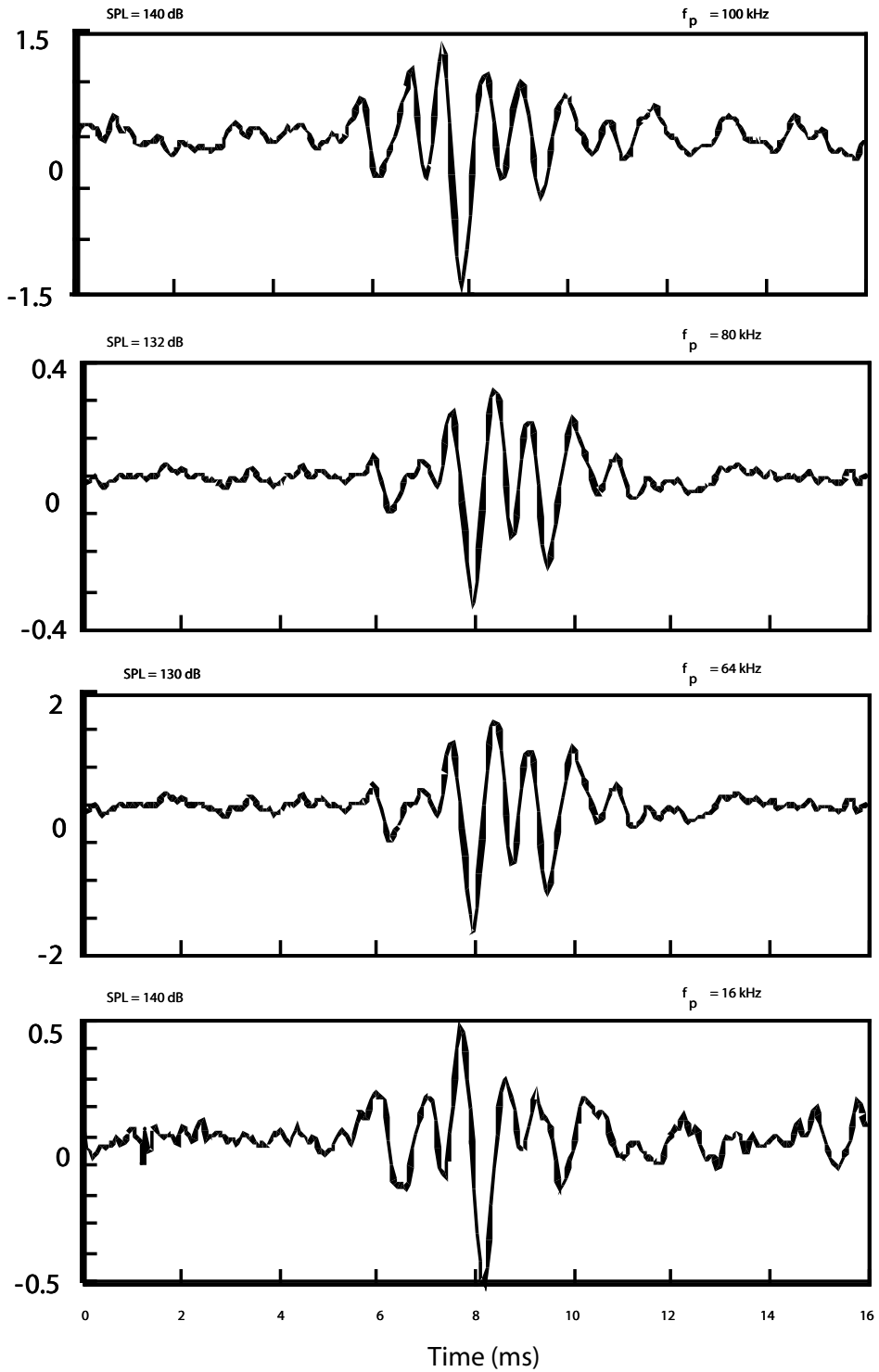


Figure 4. ABR signals for supra-threshold click stimuli having different peak frequencies in the Pacific white-sided dolphin

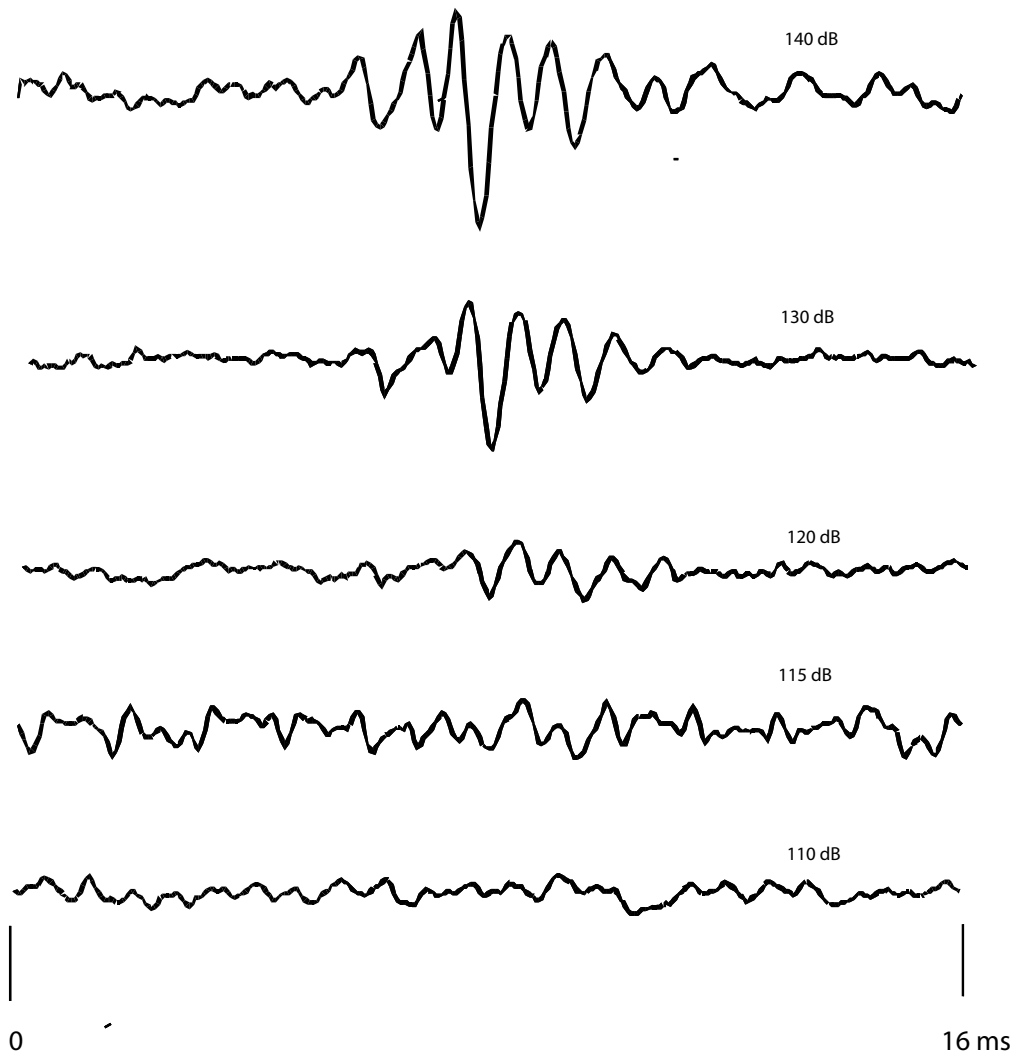


Figure 5. ABR waveforms for different stimulus levels in the Pacific white-sided dolphin, varying from a supra-threshold condition to a threshold condition for a click signal with a peak frequency of 64 kHz

The peak-to-peak amplitude of approximately $2.9 \mu\text{V}$ is lower than the $8 \mu\text{V}$ that Dolphin (2000) measured for a fully submerged false killer whale and the 10 to $15 \mu\text{V}$ that is obtained for a semisubmerged *T. truncatus* with the surface electrodes above the water surface. These differences in ABR amplitudes can be attributed to many different possibilities such as the shorting effect of the water with a fully submerged animal, difference in electrode size and design, difference in the amount of and kinds of tissues between the brain stem and the electrodes, difference in subject brain size, and even differences in electrode placement.

The spectra of the ABR signals showed a closer similarity to the ABR spectra of *T. truncatus* than the waveforms. The peaks in the spectrum occurred at 650 and 1,200 Hz, which compares well with 600 to 650 Hz and 1,100 and 1,200 Hz for *T. truncatus* (Supin et al., 2001). This is rather strange since the time- and frequency-domain signals are merely different representations of the same signals, perhaps suggesting that similarities in patterns are easier to notice in the frequency domain. The main null in the ABR spectrum for *L. obliquidens* at about 950 Hz was much deeper than the spectrum for *T. truncatus* shown in Figure 2.3 of Supin et al. (2001). It is not clear whether

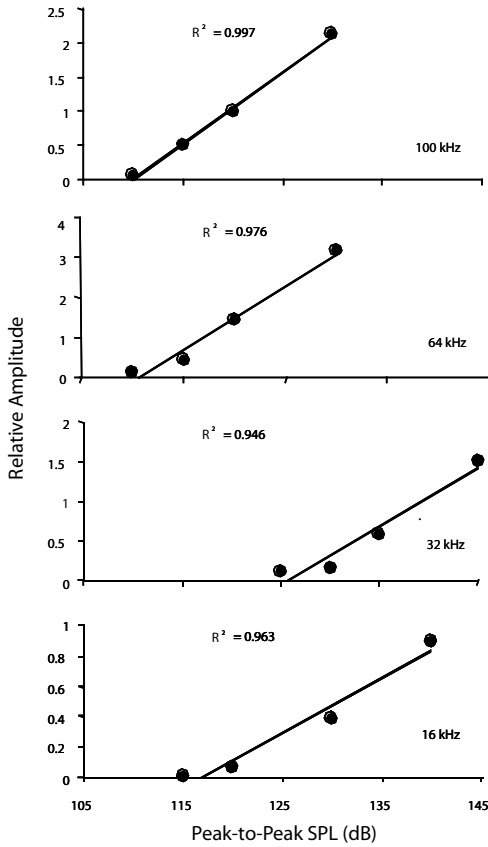


Figure 6. Threshold determination using linear interpolation results from ABR measurements in the Pacific white-sided dolphin

the differences in the depth of the null is due to differences in measurement procedures (16-bit A/D vs 12-bit A/D conversions) or to differences in the ABR responses between both species.

Similarity in the Wave I response latency of 1.3 to 1.5 ms for the *L. obliquidens* and about 1.2 ms for *T. truncatus* (Ridgway, 1983), stimulated by a 60-kHz click signal, suggests that both species process acoustic information at similar speeds. This response latency is on the same order of magnitude of response latency for much smaller mammals such as cats, rats, and monkeys (Ridgway, 1983).

The technique of using a linear regression to estimate the threshold of sensitivity worked well with our subject. The ABR response is almost linear near the threshold of hearing as indicated by the R² value being so high, varying from a low of 0.946 to a high of 0.997. Unfortunately, the masked threshold results were difficult to interpret since the projected stimulus was broadband instead of a tonal stimuli. With tonal stimuli, in such a curve as shown in Figure 7, we could have calculated the critical ratio of the animal's peripheral auditory system. As it is, the results shown in Figure 7 will only be useful in comparing masked ABR results from other species. To date, there do not seem to be any masked ABR data reported for other species of odontocetes.

Whether or not any kind of inference on the auditory bandwidth can be extracted from Figure 7 is questionable, and so we will not do so here. Normally, critical ratio estimates are conducted with pure-tone burst stimuli and not broadband clicks as done in this study. It should be kept in

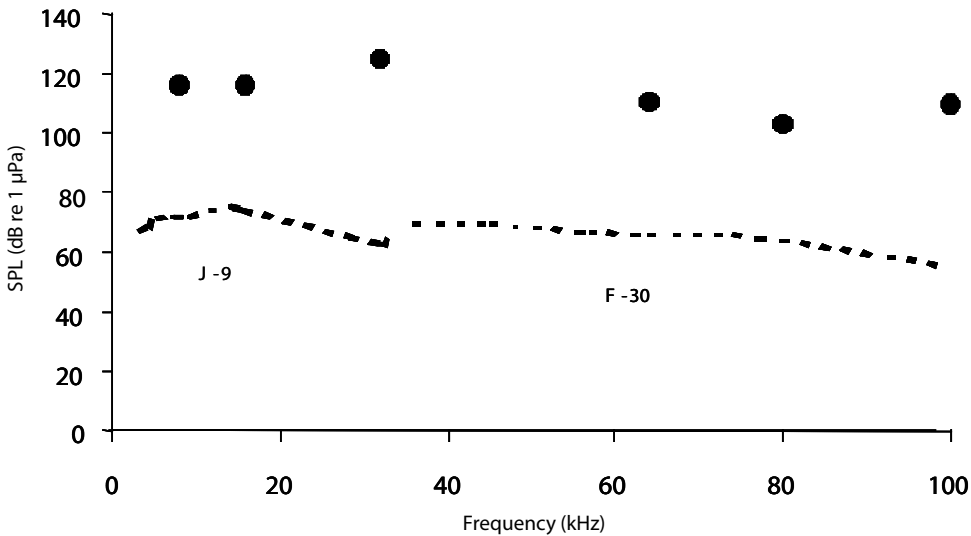


Figure 7. ABR thresholds for click stimuli with different peak frequencies in the Pacific white-sided dolphin where the level of the click stimuli is given by their peak-to-peak values; the noise spectral density curves are presented in terms of their rms values.

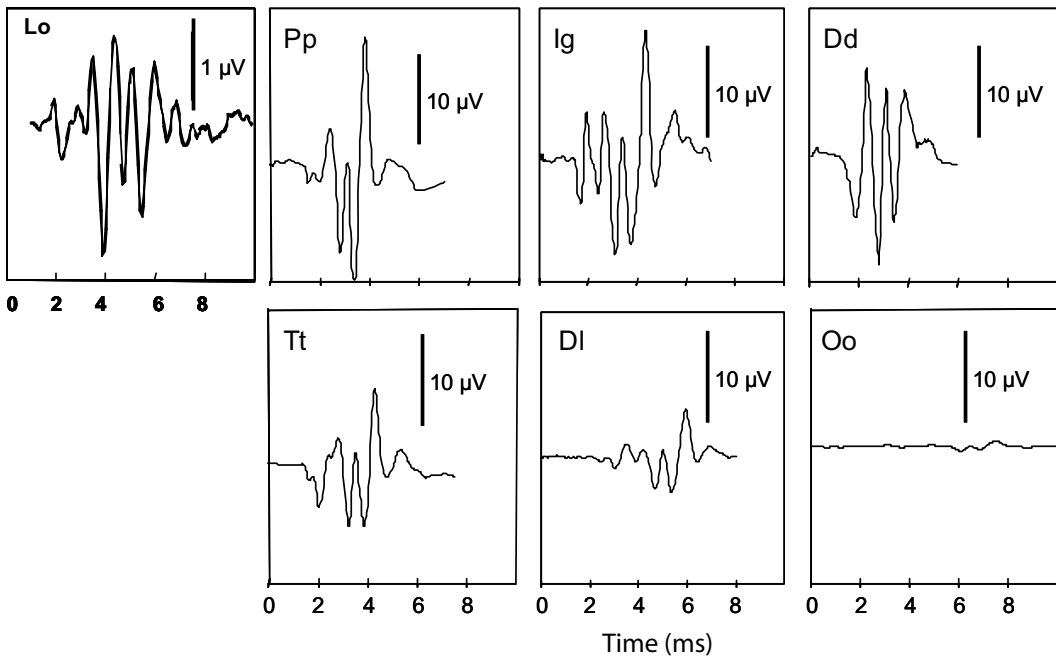


Figure 8. ABR waveforms for different odontocete species: Lo - *Lagenorhynchus obliquidens*, Ig - *Inia geoffrensis*, Pp - *Phocoena phocoena*, Dd - *Delphinus delphis*, Tt - *Tursiops truncatus*, Dl - *Delphinapterus leucas*, Oo - *Orcinus orca* (except for the Lo waveform, all others are from Supin et al., 2001)

mind that broadband click signals are not very frequency-specific, with energy spread throughout a large portion of the animal's hearing range, even though stimuli with different peak frequencies were used. Data shown in Figure 7 should be comparable to echolocation detection thresholds for frequencies about the peak frequency of the *Lagenorhynchus* echolocation signals as was done by Au (1993) for *T. truncatus*.

In order to determine the frequency selectivity and sensitivity of a dolphin's auditory system, sinusoidally amplitude-modulated (SAM) waveforms that have very specific frequencies should be used. Time and budgetary limitations prevented this, however. Nevertheless, the spectrum of the ABR signal for the *Lagenorhynchus* shown in Figure 3 suggests that a good modulation frequency would be 1.3 kHz. The results of this study also indicated that such an approach using SAM signals should not be too difficult.

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