

## Hearing measurements from a stranded infant Risso's dolphin, *Grampus griseus*

Paul E. Nachtigall, Michelle M. L. Yuen\*, T. Aran Mooney and Kristen A. Taylor

Marine Mammal Research Program, Hawaii Institute of Marine Biology, University of Hawaii, PO Box 1106, Kailua, HI 96734, USA

\*Author for correspondence (e-mail: myuen@hawaii.edu)

Accepted 8 September 2005

### Summary

An infant Risso's dolphin (*Grampus griseus*) was rescued from the beach in Southern Portugal, and an audiogram was measured using auditory evoked potentials (AEP) and envelope following response (EFR) techniques for frequencies from 4 to 150 kHz. The stimuli used were custom sinusoidally amplitude-modulated (SAM) tone-bursts, and the AEP responses were collected, averaged and analyzed to quantify the animal's physiological response and, thereby, hearing thresholds. The infant animal showed a wide range of best sensitivity, with the lowest threshold of 49.5 dB re. 1  $\mu$ Pa at 90 kHz. The audiogram showed a typical mammalian U-shape with a gradual, low-frequency slope of 16.4 dB octave<sup>-1</sup> and a sharp high-frequency increase of 95 dB octave<sup>-1</sup>.

When compared with an audiogram of an older Risso's dolphin obtained using behavioral methods, the threshold values at upper frequencies were much lower for this infant animal, and this infant heard higher frequencies. These results redefine the hearing capabilities of Risso's dolphins by demonstrating very high-frequency sensitivity.

Key words: Risso's dolphin, *Grampus griseus*, hearing, audiogram, auditory threshold, auditory evoked potential, AEP, envelope following response, EFR, sinusoidally amplitude-modulated tone-burst, SAM.

### Introduction

Risso's dolphins [*Grampus griseus* (Cuvier 1812)] are a pelagic species of squid-eating odontocetes that are typically found in deep, temperate and tropical waters near continental shelf edges and submarine canyons (Leatherwood et al., 1980). Young animals have dark grey bodies while older animals appear nearly white due to accumulated scratches. Each time a Risso's dolphin receives a scratch or a wound, the dark grey pigment does not return and the healed wound leaves a whitish mark. As a result, adult animals appear scratched and mottled. In contrast to the rounded melon of most delphinids, Risso's dolphins have distinctive melons that are broad, squarish in profile, and creased by a characteristic longitudinal furrow or indentation extending down the melon to the top of the upper jaw. Unlike other members of the subfamily Globicephalinae, such as false killer whales, pilot whales, melon-headed whales and pygmy killer whales, Risso's dolphins usually have only four to six teeth and they are all found in the lower jaw. Some of the members of this subfamily are known to strand in groups, some are hunted for food, and all are taken as bycatch. From the perspective of human impacts and foraging ecology, it is important to learn more about their hearing abilities. With respect to studies of hearing sensitivity in globicephalinid odontocetes, there is one published audiogram for a Risso's dolphin (Nachtigall, 1995) and two (Thomas et al., 1988; Yuen et al., 2005) for false killer whales.

Given the importance of the melon for echolocation and

acoustic propagation (Cranford et al., 1996; Norris, 1968), the furrow has been thought to be important for directional propagation of outgoing echolocation pulses (Philips et al., 2003). Although the initial attempts to demonstrate echolocation behaviorally with a stranded and rehabilitated Risso's dolphin were met with some difficulty (Nachtigall et al., 1995; Philips et al., 2003), another Risso's dolphin was eventually trained to successfully discriminate an aluminum cylinder from a nylon sphere while wearing blindfolding eyecups (Philips et al., 2003). In the previous audiometric work, the clicks emitted by the dolphin were acquired at mean amplitudes of 193 dB (re. 1  $\mu$ Pa) with estimated sources levels up to 216 dB (re. 1  $\mu$ Pa; 1 m). Clicks were predominantly double-peaked with substantial energy at higher frequencies, some as high as 105 kHz. These findings were particularly interesting given that previous work which behaviorally measured hearing thresholds with this same animal subject had shown that the animal could only hear tones with frequencies higher than 80 kHz when projected at high amplitudes (Nachtigall et al., 1995).

The echolocating Risso's dolphin in the 2003 study was a wild-captured older animal (Philips et al., 2003). Her lower teeth had been naturally worn down before she was brought into the laboratory and she was estimated to be over 30 years old. The range of her high-frequency hearing did not reach to

the 150 kHz region, as seen in other odontocetes (Nachtigall et al., 2000), and it was assumed at that time that the species *G. griseus* did not hear high frequencies as well as other dolphins. However, Ridgway and Carder (1997) have demonstrated individual differences in high-frequency hearing in bottlenose dolphins. Four of eight bottlenose dolphins they studied showed high-frequency hearing deficits and most appeared to be age related. Older bottlenose dolphins appear to lose their high-frequency hearing in a manner similar to other mammals, including humans. Given that the one Risso's dolphin originally tested by Nachtigall et al. (1995) was an older animal, it seems likely that the demonstrated hearing capability of this individual may not be representative of the species' hearing range.

Audiograms of marine mammals, particularly odontocetes, have most often been estimated from single animals (Nachtigall et al., 2000). There is a general difficulty with estimating a species or population's hearing capabilities from thresholds obtained from a single animal. For example, the first measured auditory thresholds of a killer whale, *Orcinus orca* (Hall and Johnson, 1972), found that the animal did not hear above 32 kHz, which would be very unusual for an echolocating odontocete, and yet that measure stood as the estimate for the species until many years later when another audiogram was obtained for different individuals, which showed high-frequency hearing extending to above 100 kHz (Szymanski et al., 1999). Clearly, there is value in increasing the number of animals within a species measured for hearing capabilities whenever possible.

Most odontocete audiograms measured to date have been collected using behavioral psychophysical procedures in which the animal was kept within a laboratory setting and trained to respond to the presence or absence of pure tone stimuli. These measurements are ideally made in quiet laboratory tank environments but are occasionally made at oceanaria, in sea pens or in concrete or above-ground tanks. While the quiet laboratory environment is the ideal baseline setting for determining hearing thresholds, the normal psychophysical behavioral procedures are expensive and time consuming. This tends to limit the number of individuals for which audiograms may be obtained. Auditory evoked potential (AEP) experiments, in which the animal's hearing is measured by passively receiving the animal's electric potential responses from the surface of the skin over its head when in the presence of sound stimuli, provides an opportunity to increase the number of odontocete audiometric measurements. AEP measurements have been made with stranded dolphins in rehabilitation facilities (Andre et al., 2003; Popov and Klishin, 1998) without prior animal training. A stranded animal rehabilitation facility also provides an appropriate setting for identifying hearing deficits that may be caused by overexposure to anthropogenic sound. Recent increased concern about animals and ocean noise provides ample motivation for testing the hearing of stranded marine mammals, especially if the stranded animals are suspected of having been overexposed to noise.

The current study involved an infant Risso's dolphin (*G. griseus*) that was rescued on the Algarve coast in Southern Portugal. The animal was taken to the Mundo Aquatico Rehabilitation Facility of ZooMarine in Guia, Albufeira. During rehabilitation, the animal's audiogram was measured using the AEP envelope following response (EFR) procedure to estimate hearing thresholds between 4 and 150 kHz.

## Materials and methods

### Subject

The subject of this study was a stranded, infant male Risso's dolphin. Its exact age was unknown due to the fact that it stranded from the wild; however, it was known to be an infant based on its size and its fetal fold markings (Fig. 1). It was fed from a bottle with an infant dolphin formula and gained weight during the period of audiometric testing. This animal stranded during a two-week period, during which three other individual odontocetes of unknown species also stranded. This Risso's dolphin was the only animal that survived, and was transported to the rehabilitation facility at ZooMarine in Guia, Albufeira, Portugal. It was 147 cm long and weighed 47 kg. The animal received constant care from the veterinary staff, and data were collected when the animal was available between feedings and medical care procedures. Sessions were conducted for four continuous days in May 2004. While it appeared to be recovering and doing well during the time of the audiometric measurements, two weeks after the testing, the Risso's dolphin calf died of pneumonia, possibly secondary to a viral infection diagnosed at the time of its stranding.

### Tank and background noise levels

Under the care of the veterinary staff, the Risso's dolphin was housed in a rehabilitation holding tank. The concrete tank was approximately 3 m deep with a 5 m inside diameter, and it contained an artificial seawater to a depth of 1.1 m. The tank

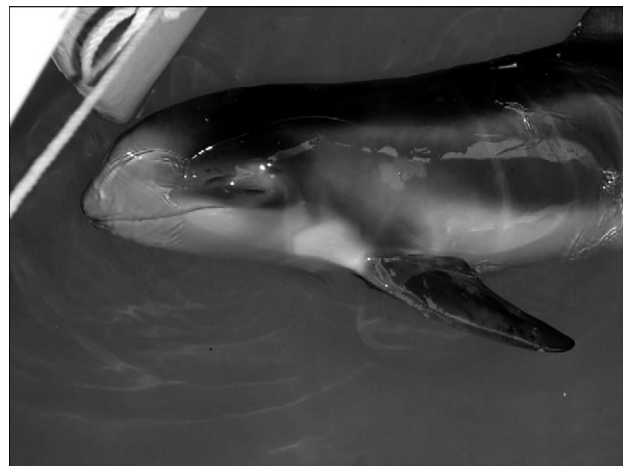


Fig. 1. The infant Risso's dolphin that was rescued and rehabilitated at ZooMarine in Albufeira, Portugal. Note the natal folds and the lack of white scarring on the head and back.

pumps and filters were turned off at least 15 min before each session to eliminate air bubbles and to reduce background noise. The tank was lined with canvas-covered foam pads that prevented the calf from bumping into the concrete sides and also served to dampen spurious reflections. Ambient noise level measurements were performed within the pool to determine background noise levels. However, ambient noise proved to be exceedingly low, below the sensitivity of the acoustic recording equipment (less than 65 dB re.  $1 \mu\text{Pa}^2 \text{Hz}^{-1}$  measured across a 40 kHz bandwidth). Similar low noise situations are a valuable situation for conducting absolute hearing thresholds (*sensu* Au et al., 2002).

#### *The animal's position and sounds received*

The animal was held so that its head was 0.5 m from the center of the tank, 1 m from the projecting hydrophone, and held in place by the experimenter (Fig. 2). By placing a calibrated Biomon 8261 (sensitivity 182–185 dB and frequency response up to 200 kHz) hydrophone (Santa Barbara, CA, USA) near the dolphin's lower jaw while the dolphin was in the correct position, both of the projecting hydrophones were calibrated to determine the received levels before the data were collected. The size of the infant's head was very small, and when the calibrated hydrophone was moved around the animal, there was no measurable variation in received levels around the subject's head. Pure-tones were created with a Wavetek function generator for the following frequencies: 4, 5.6, 8, 11.2, 16, 22.5, 32, 40, 50, 64, 70, 80, 90, 100, 110, 120 and 150 kHz. Each of these pure-tone sine waves was transmitted in the tank and the received peak-to-peak voltage ( $V_{p-p}$ ) was measured with the calibrated hydrophone. This  $V_{p-p}$  was converted to peak-equivalent root-mean-square voltage (perRMS) by subtracting 9 dB. The perRMS was taken as the RMS voltage and used to calculate the sound pressure level (SPL) for that frequency. The spectrum of the received



Fig. 2. Experimental design in the rehabilitation pool. The infant Risso's dolphin was held by the experimenter in its position 0.5 m from the center of the pool and 1 m from the suspended hydrophone.

signals was viewed with a Techtronix TDS 1002 oscilloscope (Beaverton, OR, USA), ensuring that there were no competing reflections produced from other signals or reflections in the tank. In this environment, there were no constructing or destructing interferences observed with the transmitted signal. Had these sorts of interferences been present in the tank, they would have been apparent in the pure-tone case, but, even if they had been present, these sorts of interferences would have been countered by the use of short sinusoidally amplitude modulated (SAM) tone-bursts.

#### *Sounds presented to the animal*

The acoustic stimuli were SAM tone-bursts created from each pure-tone carrier frequency for which a threshold was desired. SAM tone-bursts were presented rather than pure tones because they are optimal for producing clear AEPs as EFR. The tone-bursts were digitally synthesized with a customized LabView data acquisition program that was created with a National Instruments PCI-MIO-16E-1 DAQ card (Austin, TX, USA) implemented into a desktop computer (Fig. 3). Each of 1000 SAM tone-bursts was 20 ms long, with an update rate of 200 kHz for carrier tones less than 70 kHz, and 500 kHz for carrier frequencies equal to or above 70 kHz. The carrier frequencies were modulated at a rate of 1000 Hz, with a modulation depth of 100%. This modulation rate was chosen based on ideal measurement modulation rates for similar odontocetes (Dolphin et al., 1995). A 30 ms break of no sound was alternated between the 20 ms stimulus presentations. The stimuli were sent from the computer to a custom-built signal shaping box that could attenuate the tone-bursts in 1 dB steps. A Techtronix TDS 1002 oscilloscope was used to monitor the outgoing stimuli from the signal shaping box to the projecting hydrophones.

Two hydrophones were required to project the underwater stimuli, one for low-frequency tones and another for high-frequency tones. The first, an ITC-1032 (Santa Barbara, CA, USA) with a resonance frequency of 38 kHz, was used to project stimuli from 4 to 40 kHz. The second hydrophone was a Clevite CH 17 (Beford, OH, USA) that projected tones from 40 to 150 kHz. When one of the two hydrophones was in use, it was suspended from an overhead line that stretched across the tank and secured at a position that was 0.5 m from the center of the circular tank and also 1 m from the animal. From the overhead line, the hydrophone was also adjusted to hang 30 cm below the surface of the water.

#### *AEP measurements taken from the animal when sounds were produced*

Rubber suction cups containing gold sensors to pick up the evoked potentials were easily placed on the animal at the beginning of each session by the experimenter. Standard conductive gel was used to assure a good connection between the animal's skin and the 10 mm EEG gold electrodes (West Warwick, RI, USA). The active electrode was attached ~3–4 cm behind the blowhole, slightly off to the right and over the brain, while the reference electrode was attached on the

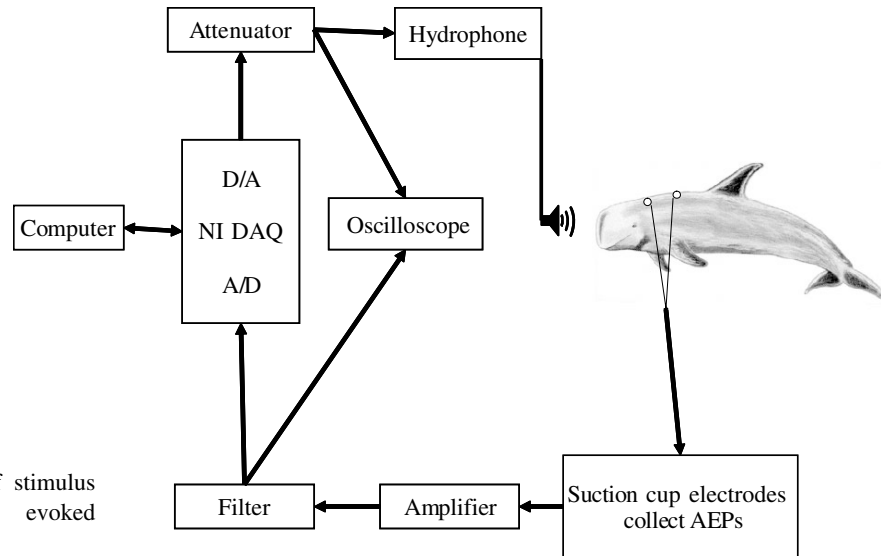


Fig. 3. Experimental layout of stimulus presentation and auditory evoked potential (AEP) collection.

back near the dorsal fin. The animal was easily held at the surface with most of its head underwater (Fig. 2) to receive sound input through the major tissue routes to the ears (Mohr et al., 1999; Ketten, 1997) while the suction cups, with the embedded electrodes, remained in the air.

An Iso-Dam Isolated Biological Amplifier (Sarasota, FL, USA) amplified the AEP responses from the electrodes by 10 000. The Iso-Dam, as well as a Krohn-Hite Filter Model 3103 (Brockton, MA, USA) with a band pass of 300–3000 Hz, filtered the responses for anti-aliasing protection. The amplified and filtered responses were transferred to an analog input of the same DAQ card in the same desktop computer. The received signal was digitized at a rate of 16 kHz in order to extract the recorded AEP from noise, and the entire trial for each stimulus presentation lasted about 1 min.

#### Threshold determination

The general procedure used to estimate a hearing threshold for each frequency was to select a carrier frequency to be modulated, determine the initial intensity level to be used for that frequency and then present a series of trials with progressively decreasing stimulus amplitudes. Because this was the first audiogram of a neonate Risso's dolphin, stimulus presentation levels were based on a previously published audiogram of an adult Risso's dolphin (Nachtigall et al., 1995) and a bottlenose dolphin (Johnson, 1966). Stimulus levels began 20–30 dB above the lowest threshold levels of the preceding audiograms. Eighteen carrier frequencies were tested ranging from 4 to 150 kHz. The following frequencies were first tested based on the previous Risso's audiogram: 4, 5.6, 8, 11.2, 16, 22.5, 32, 40, 50, 64, 76, 80, 90, 100, 110 kHz, however it became immediately obvious that the animal heard relatively well at the highest of these frequencies and therefore an additional three frequencies, 108, 128 and 150 kHz, were successively added. The initial levels for these three frequencies were determined by starting 20–30 dB above the previously obtained threshold. The amplitudes of the

transmitted SAM tone-bursts were reduced in 5–10 dB steps until the amplified evoked potential responses to the SAM bursts could no longer be distinguished from the background noise. Step size was based on the intensity of the signal and the animal's neurological response. An average of nine stimulus intensity levels was presented for each of the 18 different frequencies. The stimuli were initially calibrated at each frequency tested using continuous pure-tones at the position of the animal's head. The received peak-to-peak levels (V) of the stimuli were measured and used to calculate perRMS (V) and received SPL. These values were taken as the received level of each stimulus frequency.

When presenting the SAM stimuli, the values were converted to RMS (V) to determine the equivalent received level when presented in SAM tones.

#### Data analysis

The data obtained for each frequency for each intensity level was an evoked potential record comprised of at least 1000 averaged evoked responses to the 20 ms SAM and the first 10 ms of the 30 ms quiet interval as depicted in Fig. 4. Fourier transforms were calculated for a 16 ms window (shown in Fig. 4) of the average evoked response recorded at each intensity level for each frequency in order to quantitatively estimate the animal's hearing threshold (Fig. 5). This window contained a whole number of response cycles to the stimulus. The 256-point Fast Fourier transforms (FFT) provided response frequency spectra of the data where a peak reflected energy received, or the animal's physiological following response, to the 1000 Hz modulation rate. Thus, a larger EFR response was reflected as a higher peak value. The peak FFT amplitude at the modulation rate was used to estimate the magnitude of the response evoked by the SAM stimulus.

To calculate a threshold for each frequency tested, the FFT peak at each stimulus intensity level was plotted as response intensity against SPL of the stimulus (Fig. 6). A linear regression addressing the data points obtained was

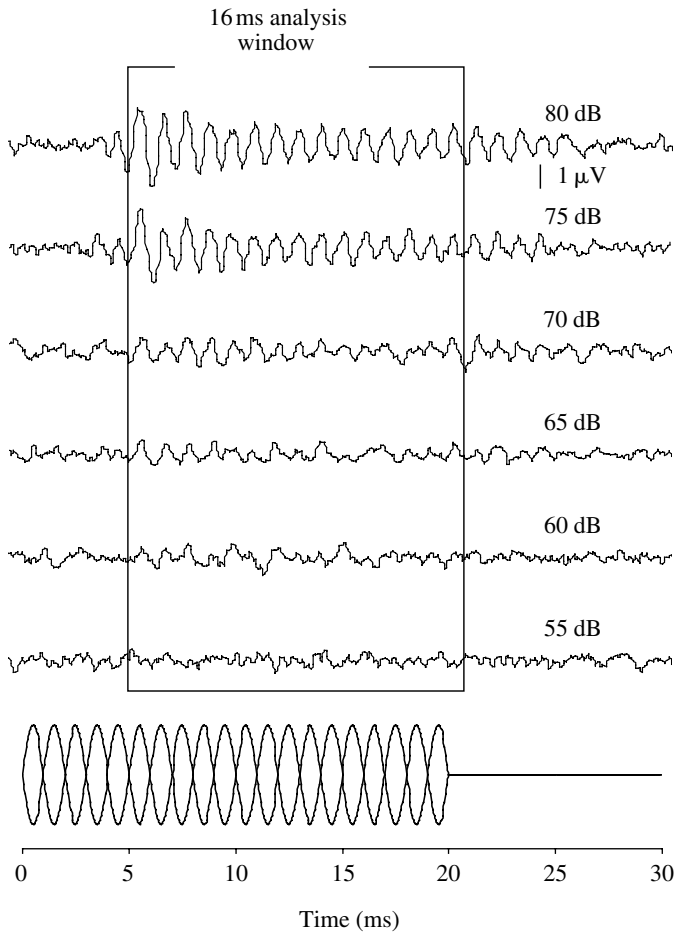


Fig. 4. Sinusoidal envelope of stimulus (lowest trace) and envelope following response to threshold of a 76 kHz tone from 80 to 55 dB re. 1  $\mu$ Pa.

hypothetically extended to zero, the theoretical point where there would be no response to the stimulus. With the stimulus SPL value at the zero response, it was possible to estimate the threshold for each of the frequencies presented to the animal as described in Supin et al. (2001). Analysis was conducted using Excel, Matlab and Minitab software.

### Results

The quiet environment of the concrete tank, without the extraneous noises normally found in a natural environment (e.g. Au et al., 2002) provided an excellent opportunity to obtain threshold values without background noise interference. The animal's EFR above threshold values were clearly observed as the data were collected (Fig. 4). The AEP response showed a temporal lag of ~4–5 ms compared with both the onset and offset of the tone-burst stimulus. This lag was the result of the latency of the evoked potential following the presentation of the stimulus. It was not an artifact, but rather it served as a predictable electrophysiological feature demonstrating that the brainwave recording occurred in direct response to the SAM acoustic stimulus. When stimulus

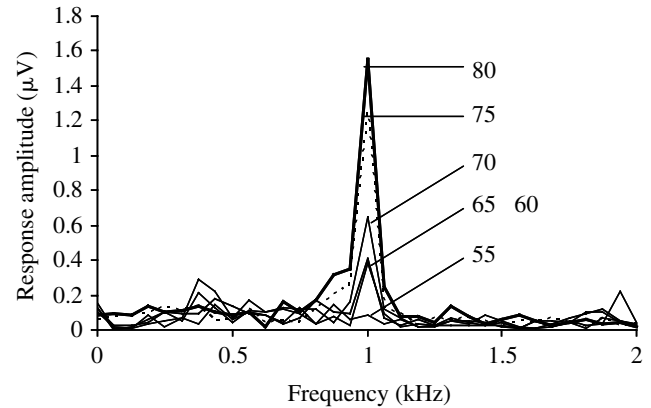


Fig. 5. Fourier transform of the envelope following response amplitudes of a Risso's dolphin. Response is to a SAM tone presented at a 76 kHz carrier frequency, a 1000 Hz modulation rate and stimulus intensities from 80 to 55 dB re. 1  $\mu$ Pa. Intensities are listed on the right side of the graph.

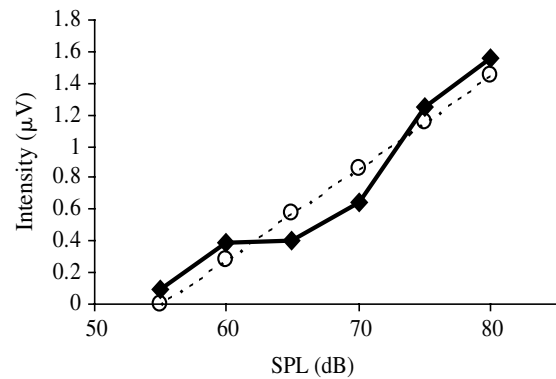


Fig. 6. Intensity of spectrum peaks (solid line, diamonds) versus sound pressure level (SPL) using a 76 kHz tone where SPL is in dB re. 1  $\mu$ Pa. The regression of the spectrum peaks (broken line, open circles) is concurrently graphed. The threshold for the 76 kHz stimulus is measured as the point at which the regression line crosses zero on the response amplitude scale, in this case 55 dB re. 1  $\mu$ Pa.

intensities were high relative to threshold, such as 80 dB for a 76 kHz tone, EFRs were discernible well above the noise level (Fig. 4). As the measurements approached the auditory threshold levels, the decreasing EFR magnitudes reflected the synchronously decreasing SPL of the stimuli.

In determining threshold values, these EFRs were Fourier transformed (FFT) to obtain the frequency spectrum of the animal's evoked response (Fig. 5). The consistent peak at 1000 Hz reflected the animal's EFR, and thus neurophysiological 'following' of the carrier tone modulated at an 1000 Hz rate. The strength of the evoked response was reflected in the amplitude of the peak at the modulation frequency; as stimulus level was decreased, the peak amplitude decreased correspondingly. Fig. 5 illustrates a typical peak at 76 kHz carrier frequency that decreases as the stimulus intensity is attenuated. At the lowest stimulus intensity of 55 dB, the peak of the response spectra was no different from

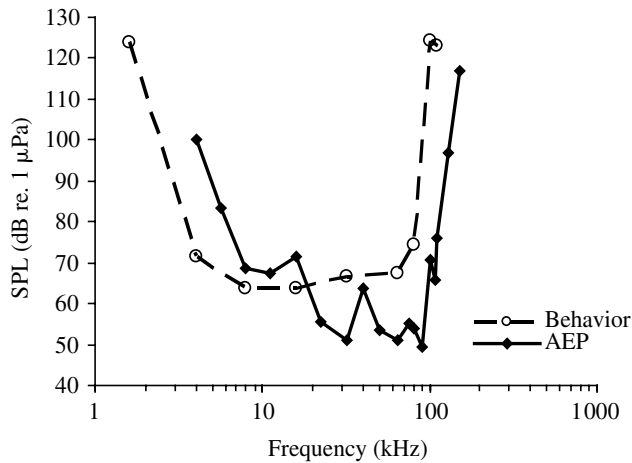


Fig. 7. Comparison of an auditory evoked potential (AEP) and behavioral audiogram of two Risso's dolphins. The behavioral threshold was published by Nachtigall et al. (1995). The y-axis is intensity of stimulus, or sound pressure level (SPL) in dB. Nachtigall et al. (1995) used a pure-tone, 3 s stimulus; the present study used a 20 ms SAM.

the background physiological noise. The intensity of each of the spectrum peaks was plotted as a function of stimulus SPL, and regression lines were drawn to calculate the theoretical zero response value. Therefore, for a stimulus of 76 kHz, the threshold may be seen to be estimated in Fig. 5 to be 55 dB.

The hearing threshold for each of the carrier frequencies was determined in the same manner, with thresholds calculated as the stimulus level predicted to generate a response amplitude of zero. Results of the threshold calculations are depicted as an audiogram in Fig. 7 along with the results of the only other

Table 1. Mean behavioral and auditory evoked potential (AEP) thresholds at each frequency tested

Frequency (kHz)	Behavioral threshold (dB re. 1 $\mu$ Pa)	AEP threshold (dB re. 1 $\mu$ Pa)
1.6	124	
4	71.7	100.3
5.6		83.2
8	63.7	68.8
11.2		67.4
16	63.8	71.5
22.5		55.7
32	66.5	50.9
40		63.9
50		53.4
64	67.3	50.9
76		55.0
80	74.3	54.1
90		49.5
100	124.2	70.5
108		65.6
110	122.9	76.0
128		97.0
150		116.9

audiogram of a Risso's dolphin, which was collected on an older animal (Nachtigall et al., 1995). The infant animal showed a wide range of best sensitivity with hearing thresholds better than 60 dB between 22.5 and 90 kHz (Table 1). The lowest thresholds were 50 dB or lower at three of the measured frequencies (32, 64 and 90 kHz).

The AEP audiogram's general shape was a typical mammalian U-shape (Fig. 7). At high frequencies, the slope of thresholds increased steeply beyond 90 kHz at a rate of 95 dB octave<sup>-1</sup>. Below 32 kHz, the slope of increasing thresholds was more gradual, at 16.4 dB octave<sup>-1</sup>. Poorest sensitivity was measured at the very low and very high frequencies, 100.3 dB at 4 kHz and 116.9 dB at 150 kHz, respectively. There was an apparent notch in the audiogram at 40 kHz.

Compared with the previously determined behavioral audiogram, the AEP audiogram was similar in shape. However, the frequency region with the lowest thresholds, figuratively forming the bottom of the U-shape in the AEP audiogram, was not flat as in the 1995 audiogram, but rather this current audiogram had a saw-toothed up-and-down shape in the areas of best sensitivity, similar to those typically seen in odontocetes (Nachtigall et al., 2000). The current AEP audiogram revealed lower thresholds at high frequencies and conversely higher thresholds at lower frequencies than previously seen in the Risso's dolphin. Note that the best sensitivity measured in the current work was 20 dB lower than reported by Nachtigall et al. (1995).

## Discussion

The two most obvious and interesting findings when comparing the 1995 audiogram of an older Risso's dolphin and the current results from an infant of the same species were that: (1) the threshold values were much lower at the interesting high frequencies for the infant animal than for the older animal and (2) the infant Risso's dolphin heard much higher frequencies than the older Risso's dolphin. Given that there were differences in experimental conditions and animal subjects, it was reasonable to examine the role that these differences in the environments and the methodology may have played in producing those hearing threshold discrepancies.

The data collected from the older animal subject were obtained from a wild-caught animal tested in the natural environment of Kaneohe Bay of the island of Oahu, Hawaii. Ambient noise power density plotted in 1 Hz bands (Nachtigall et al., 1995) showed that it was likely that the thresholds from the Risso's dolphin were not absolute thresholds but were instead masked by the ambient noise conditions. The lowest intensity levels detected by the older animal were ~65 dB between 8 and 32 kHz, while the lowest intensity levels heard by the current infant animal were below 50 dB at much higher frequencies. The data from this infant were collected in essentially quiet conditions. It seemed likely that the absolute levels of hearing reported in the two audiograms were not directly comparable because of the differences in the

background noise conditions under which the different data were obtained.

There was also an obvious difference in the methods for hearing measurements used in the two investigations. The 1995 audiogram from the older Risso's dolphin was measured using a standard psychophysical procedure in which the animal was trained to behaviorally report the presence or absence of a pure-tone stimulus. The animal was trained to respond, or 'go', when a 3 s tone was presented and to not respond, or 'not go', when no signal was presented. Conversely, the data from this infant dolphin were collected using AEP procedures that measured the brainstem's EFR to a SAM tone-burst only 20 ms long. Recent work comparing these two procedures (Yuen et al., 2005) indicated that odontocete AEP thresholds may be higher than thresholds gathered with the psychophysical behavioral procedure due to the fact that the AEP stimuli are much shorter than the assumed Risso's dolphin temporal integration time (Johnson, 1966). By contrast, most behavioral audiograms are based on stimuli that are one or two seconds long, providing ample time for temporal integration, and thus provide lower thresholds.

While the AEP procedure usually produces less-sensitive threshold measurements, this threshold and stimulus duration relationship was reversed when comparing the 1995 and present Risso's dolphins' audiograms at the frequencies above 20 kHz. The AEP study with the young animal produced lower thresholds than the previous Risso's dolphin behavioral study with the older animal. The other striking difference between the audiograms of the young and old Risso's dolphins was the difference in the range of high-frequency hearing. While the older Risso's dolphin heard relatively well, for example 74 dB threshold at 80 kHz, it heard quite poorly at the likely unmasked frequency of 100 kHz with a threshold of 124 dB. When those thresholds were compared with the current infant's thresholds of 54 dB at 80 kHz, 50 dB at 90 kHz and 66 dB at 108 kHz, the audiogram of this infant animal included lower, more-sensitive hearing thresholds. This difference might be explained by the age differences and the occurrence of presbycusis in older animals. Presbycusis is the reduction in sensitivity at higher frequencies and the loss of hearing at very high frequencies with increasing age. Ridgway and Carder (1997) found that bottlenose dolphins showed age-related hearing loss, especially with higher frequencies, in a manner similar to many other mammalian species. The difference in the infant Risso's dolphin's ability to hear high frequencies may have resulted from the age difference of the two subjects tested. It is likely that not only was the older animal in the 1995 study masked by the noise of Kaneohe Bay, but it probably also suffered from high-frequency hearing loss associated with age. This finding supports the idea that the previously reported audiogram for a single older Risso's dolphin probably underestimated the best hearing sensitivity for this species.

The older animal did show lower behavioral thresholds than the younger animal's AEP thresholds for frequencies below 20 kHz. Unmasked behavioral hearing measurements of *Pseudorca crassidens*, another globicephalinid odontocete, at

similar frequencies (Thomas et al., 1988) are quite similar to those measured for the older Risso's in Kaneohe Bay. The older Risso's thresholds below 20 kHz are above the Bay noise levels (Nachtigall et al., 1995) and may, in fact, represent typical Risso's hearing for lower frequencies. Thus, the elevated thresholds shown for the younger Risso's, here in a quiet environment for frequencies below 20 kHz, may represent the simple methodological difference between measurements taken with short AEP signals and longer behavioral stimuli (Yuen et al., 2005).

Another interesting feature of the peak sensitivity range of the infant's audiogram is that the shape of the audiogram nicely matches the center frequencies of the Risso's dolphin click structure noted in both laboratory (Philips et al., 2003) and field (Madsen et al., 2004) studies of Risso's echolocation. The center or peak frequencies of the clicks most often fell in the 30–100 kHz range, which is the same range of peak sensitivity shown in the infant Risso's audiogram. The click data from the echolocation studies showed frequencies that were high in comparison with the 1995 older Risso's data but fit very well with the new data. There is, therefore, a very nice match between the frequency emphasis of the biosonar signals in the wild and the frequency range of best hearing in the young animal with presumed normal hearing.

It was also notable that this infant Risso's dolphin heard quite well at higher frequencies, with a threshold of 116.9 dB at 150 kHz. When compared with other odontocete species, the auditory sensitivity of this animal at that frequency is comparable with thresholds measured from the harbor porpoise, *Phocoena phocoena* (Kastelein et al., 2002), the striped dolphin, *Stenella coeruleoalba* (Kastelein et al., 2003), and the bottlenose dolphin, *Tursiops truncatus* (Johnson, 1966; Au et al., 2002). However, when this infant Risso's audiogram is compared with the only other audiogram collected from another species representing the same subfamily, the false killer whale measured by Thomas et al. (1988), an interesting difference once again may be noted. The false killer whale's high-frequency cut-off occurred just above 100 kHz, not at 150 kHz as was found with the infant Risso's dolphin. Although Thomas et al. assumed the hearing of their measured adult false killer whale was normal, it seems likely, based on this experience measuring a very young Risso's dolphin, that further measurements of other young false killer whales (and other young members of that subfamily, including pilot whales and melonheaded whales) might also show good high-frequency hearing up to 150 kHz, as has been shown in the other odontocete species.

Given the recent odontocete strandings associated with intense sound, it seems reasonable to diagnostically test the hearing of stranded dolphins and whales that may have been exposed to loud noise. Traditionally, animal psychophysical audiograms have taken years to train and to complete. This audiogram of the infant Risso's dolphin was gathered over 4 days and was partially obtained in order to diagnostically ascertain that this infant Risso's dolphin did not have hearing damage due to possible overexposure to intense sound. This

animal heard high-frequency sounds (above 100 kHz) much better than anticipated. If, however, assumptions about normal hearing had been made by examining the peak frequencies measured from the biosonar of wild Risso's dolphins, the high-frequency hearing would have been predicted. Future diagnostic examinations of the hearing of stranded odontocetes might benefit from considering the center and peak frequencies of the recorded echolocation signals. In this case, they were a better predictor of the high-frequency components of the audiogram of a young Risso's dolphin than was the measured audiogram of an older Risso's dolphin.

Of the >83 species of whales and dolphins in existence (Rice, 1998), audiograms have been measured for only 12 species. The electrophysiological AEP procedure of measuring the hearing thresholds of stranded odontocetes is a reasonable approach for both diagnostically testing individual animals and for expanding our knowledge base and scientific evidence of the hearing characteristics of cetaceans. This work is the first published complete audiogram on a neonate marine mammal, demonstrating that AEP audiograms are particularly efficient in collecting hearing information on untrained animals, notably infants and stranded individuals.

The authors are in grateful appreciation of Pedro Lavia and Elio Vicente of ZooMarine Portugal, acknowledging that none of the data would have been collected without their generosity and scientific interest. Also, the authors thank Alexander Ya. Supin for invaluable continual advice, Darlene R. Ketten for referring the task, Trevor Spradlin and Roger Gentry for advice about permits, and Whitlow Au and our colleagues at the Marine Mammal Research Program of the University of Hawaii. This study was supported by the Office of Naval Research grant N00014-98-1-0687, for which the authors thank Mardi Hastings and Robert Gisiner. This is contribution #1210 from the Hawaii Institute of Marine Biology. We also are grateful to two thorough and helpful anonymous reviewers whose efforts substantially improved the manuscript.

### References

- Andre, M., Supin, A. Y., Delory, E., Kamminga, C. and Degollada, E. (2003). Evidence of deafness in a striped dolphin, *Stenella coeruleoalba*. *Aquat. Mamm.* **29**, 3-8.
- Au, W. W. L., Lemonds, D. W., Vlachos, S., Nachtigall, P. E. and Roitblat, H. L. (2002). Atlantic bottlenosed dolphin (*Tursiops truncatus*) hearing thresholds for brief broadband signals. *J. Comp. Psych.* **116**, 151-157.
- Cranford, T. W., Amundin, M. and Norris, K. S. (1996). Functional morphology and homology in the odontocete nasal complex: implications for sound generation. *J. Morphol.* **228**, 223-285.
- Dolphin, W. F., Au, W. W. L., Nachtigall, P. E. and Pawloski, J. L. (1995). Modulation rate transfer functions to low frequency carriers by three species of cetaceans. *J. Comp. Physiol. A* **177**, 235-245.
- Hall, J. D. and Johnson, C. S. (1972). Auditory thresholds of a killer whale, *Orcinus orca*. *J. Acoust. Soc. Am.* **51**, 515-517.
- Johnson, C. S. (1966). *Auditory thresholds of the bottlenosed porpoise (Tursiops truncatus)*. China Lake, California: U. S. Naval Ordnance Test Station (NOTS) Technical Publication 4178.
- Kastelein, R., Bunskoek, P., Hagedoorn, M., Au, W. W. L. and de-Haan, D. (2002). Audiogram of a harbor porpoise (*Phocoena phocoena*) measured with narrow-band frequency-modulated signals. *J. Acoust. Soc. Am.* **112**, 334-344.
- Kastelein, R. A., Hagedoorn, M., Au, W. W. L. and Haan, D. (2003). Audiogram of a striped dolphin. *J. Acoust. Soc. Am.* **113**, 1130-1137. 135.
- Ketten, D. R. (1997). Structure and function in whale ears. *Bioacoustics* **8**, 103-135.
- Leatherwood, S., Perrin, W. F., Kirby, V. L., Hubbs, C. L. and Dahlein, M. (1980). Distribution and movement of Risso's dolphin *Grampus griseus* in the Eastern North Pacific. *Fish. Bull.* **77**, 951-963.
- Madsen, P. T., Kerr, I. and Payne, R. (2004). Echolocation clicks of two free-ranging, oceanic delphinids with different food preferences: false killer whales *Pseudorca crassidens* and Risso's dolphins *Grampus griseus*. *J. Acoust. Soc. Am.* **207**, 1811-1823.
- Mohl, B., Au, W. W. L., Pawloski, J. L. and Nachtigall, P. E. (1999). Dolphin hearing: Relative sensitivity as a function of point of application of a contact sound source in the jaw and head region. *J. Acoust. Soc. Am.* **105**, 3421-3424.
- Nachtigall, P. E., Au, W. W. L., Pawloski, J. and Moore, P. W. B. (1995). Risso's dolphin (*Grampus griseus*) hearing thresholds in Kaneohe Bay, Hawaii. In *Sensory Systems of Aquatic Mammals* (ed. R. A. Kastelein, J. A. Thomas and P. E. Nachtigall), pp. 49-53. Woerden, The Netherlands: DeSpil.
- Nachtigall, P. E., Lemonds, D. W. and Roitblat, H. L. (2000). Psychoacoustic studies of dolphin and whale hearing. In *Hearing by Whales and Dolphins* (ed. W. Au, A. N. Popper and R. R. Fay), pp. 330-363. New York: Springer.
- National Research Council (2005). *Marine Mammal Populations and Ocean Noise*. Washington, DC: The National Academies Press.
- Norris, K. S. (1968). The evolution of acoustic mechanisms in odontocete cetaceans. In *Evolution and Environment* (ed. E. T. Drake), pp. 297-324. New Haven, Connecticut: Yale University.
- Philips, J. D., Nachtigall, P. E., Au, W. W. L., Pawloski, J. and Roitblat, H. L. (2003). Echolocation in the Risso's dolphin, *Grampus griseus*. *J. Acoust. Soc. Am.* **113**, 605-617.
- Popov, V. V. and Klishin, V. O. (1998). EEG study of hearing in the common dolphin *Delphinus delphis*. *Aquat. Mamm.* **24**, 13-21.
- Rice, D. W. (1998). *Marine Mammals of the World: Systematics and Distribution. Special Publication 4 of the Society of Marine Mammalogy*. Lawrence, KS: Allen Press, Inc.
- Ridgway, S. H. and Carder, D. A. (1997). Hearing deficits measured in some *Tursiops truncatus*, and the discovery of a deaf/mute dolphin. *J. Acoust. Soc. Am.* **101**, 590-594.
- Thomas, J. A., Chun, N., Au, W. and Pugh, K. (1988). Underwater audiogram of a false killer whale (*Pseudorca crassidens*). *J. Acoust. Soc. Am.* **84**, 936-940.
- Supin, A., Ya., Popov, V. V. and Mass, A. M. (2001). *The Sensory Physiology of Aquatic Mammals*. Boston: Kluwer.
- Szymanski, M. D., Bain, D. E., Kiehl, K., Pennington, S., Wong, S. and Henry, K. R. (1999). Killer Whale (*Orcinus orca*) hearing: auditory brainstem response and behavioral audiograms. *J. Acoust. Soc. Am.* **106**, 1134-1141.
- Yuen, M. M. L., Nachtigall, P. E., Supin, A. Y. and Breese, M. (2005). Behavioral and auditory evoked potential audiograms of a false killer whale (*Pseudorca crassidens*). *J. Acoust. Soc. Am.* **118**, 2688-2695.