

## RESEARCH ARTICLE

# Audiogram of a stranded Blainville's beaked whale (*Mesoplodon densirostris*) measured using auditory evoked potentials

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### SUMMARY

Quantifying and understanding the impact of anthropogenic sound on marine mammals has been the focus of many researchers both in laboratory settings as well as in the field. This study presents the audiogram of a sub-adult Blainville's beaked whale that stranded in Hawaii. The hearing measurements were conducted using the non-invasive auditory brainstem response technique. A total of 11 sinusoidally amplitude modulated tones were tested ranging from 5.6 to 160 kHz. The audiogram data indicated that the region of best hearing was found between 40 and 50 kHz with thresholds below 50 dB. This frequency range partially overlaps with the frequency modulated upsweep that Blainville's beaked whales have been reported to use during echolocation. These results match the frequency range obtained from the hearing measurements of a Gervais' beaked whale previously tested using contact acoustic stimulation and emphasize the importance of obtaining rapid hearing measurements on live stranded animals to improve the understanding of poorly known species.

Key words: Blainville's beaked whale, hearing, marine mammal, acoustics, auditory evoked potential.

### INTRODUCTION

Beaked whales are deep-diving and cryptic animals belonging to the Ziphiidae family, which contains an unusually large number of species (21) for odontocetes. Most beaked whale species are characterized by a "pronounced beak, relatively small dorsal fin set far back on the body, small flippers that fit into depressions on the sides," a reduction in the number of erupted teeth and the presence of converging throat grooves [p. 19 in Jefferson et al. (Jefferson et al., 2008)]. These animals are rarely encountered in the wild and very little is known about their ecology, behavior and life history. Blainville's beaked whales *Mesoplodon densirostris* (Blainville 1817) have the widest distribution within the *Mesoplodon* genus and inhabit both temperate and tropical areas. They are usually found in waters with depths of approximately 1000 m and a steep bathymetry, where they are assumed to forage (Baird et al., 2006) on squid and deep-water fish (Mead, 1989; Heyning and Mead, 1996; Santos et al., 2001; Santos et al., 2002). Individuals are usually brownish to dark grey dorsally and lighter ventrally; they can reach up to 4.7 m in length and weigh over 1000 kg (Jefferson et al., 2008). Most animals exhibit oval white scarring, presumably from cookiecutter shark bites.

Photo identification and satellite tagging have provided some information about habitat use and site fidelity in Blainville's beaked whales. In the main Hawaiian Islands, this species may exhibit strong site fidelity and the population appears to be island associated (Schorr et al., 2009; McSweeney et al., 2007). The use of acoustic recording suction cup tags has provided valuable information about the foraging behavior of these deep-diving cetaceans. They do not initiate echolocating at depths shallower than 200 m (Johnson et al.,

2004). Their foraging dives have a mean duration of 47 min and occur at a mean depth of 840 m. These long dives are usually followed by series of shallow dives with no apparent foraging attempts. These shallow dives are hypothesized to be 'recompression dives' to help the animals recover from the long foraging bouts (Baird et al., 2006; Tyack et al., 2006).

The properties of the far-field echolocation clicks were obtained when a conspecific fortuitously echolocated on an acoustically tagged animal (Johnson et al., 2006). Blainville's beaked whale echolocation signals have been compared with those produced by a variety of bat species. They produce two distinct echolocation signals (Madsen et al., 2005; Johnson et al., 2006). During the search phase, they emit long clicks, approximately 271  $\mu$ s in duration, with a frequency-modulated upsweep component. Most of the acoustic energy is found between 26 and 51 kHz (Johnson et al., 2006). The second type of echolocation signal is a terminal buzz (Griffin, 1958), where the inter-click interval decreases towards the end of a click train as the animal presumably closes in on its prey. Similar to other odontocete clicks, the *M. densirostris* terminal buzz clicks have no frequency-modulated component, a broader bandwidth and a shorter duration (105  $\mu$ s).

In recent years, much of the interest in beaked whales has been generated by their association with navy sonar exercises and subsequent strandings. Several mass strandings of beaked whales have occurred following the broadcasting of low- and mid-frequency military sonars (Frantzis, 1998) (reviewed by Cox et al., 2006). In 1996, the mass stranding of 13 Cuvier's beaked whales (*Ziphius cavirostris*) was found to coincide with NATO activities using low-frequency active sonar (LFAS) (Frantzis, 1998). In 2000, 17

cetaceans including a single Blainville's beaked whale stranded in the Bahamas during a naval exercise and the interim report indicated that the use of the mid-frequency active sonar (MFAS) was the "most plausible cause" of this mass stranding (US Department of Commerce and US Department of the Navy, 2001). In 2002 during the Neo-Tapon international naval exercise, another stranding involving 14 beaked whales – including three Blainville's beaked whales – occurred and was also linked to the use of MFAS. Some common trends arise from these strandings, including bathymetry profile, sound levels used and the strong links both temporally and geographically to naval active sonars (Cox et al., 2006). Although the underlying causes of these strandings remain unknown, some hypotheses have been presented such as the formation of gas bubbles from direct ensonification or complications due to alterations in the diving behavior (Houser et al., 2001; Jepson et al., 2003; Fernandez et al., 2005; Rommel et al., 2006; Hooker et al., 2009). Unfortunately, because beaked whales are poorly understood, the underlying causes of their mass stranding remain hypothetical and call for more in-depth research on their life history. Regardless of the causes behind these strandings, the animal's ear remains the primary acoustic detector and must be better understood in order to assess what factors are likely to affect beaked whales' behavior and physiology during loud sound exposure.

It is important to understand the effects of anthropogenic sounds on marine mammals (Nowacek et al., 2007; Tyack, 2008), and tremendous efforts have been invested into understanding and quantifying the human contribution to ocean noise, designing integrative models to predict ocean noise and conducting research on marine species that are likely to be at risk (National Research Council, 2003; National Research Council, 2005). Studies looking at the effects of acute and chronic sound exposure both in the short term (Miller et al., 2000; Romano et al., 2004; Talpalar and Grossman, 2005; Di Iorio and Clark, 2010) and in the long term (Tyack, 2008) have shown that anthropogenic sound is likely to affect marine mammal populations. Southall and colleagues noted that carefully controlled studies of hearing sensitivity, particularly for high-priority species such as beaked whales, were a "critical information need" (Southall et al., 2007). As part of this effort, techniques for examining the basic hearing of marine mammals have been developed over the past 20 years and to this date, the audiogram of only one other beaked whale species, the Gervais' beaked whale *Mesoplodon europeus*, has been obtained (Cook et al., 2006; Finneran et al., 2009). The use of the envelope following response (EFR) auditory evoked potential (AEP) technique provides a unique platform to obtain audiograms rapidly with untrained animals by measuring the electrical impulses generated by the brain that occur synchronously in response to a sound stimulus (Supin et al., 2001; Nachtigall et al., 2007). The AEP technique – also sometimes referred to as the auditory steady state response – yields results comparable to more traditional behavioral audiograms (Yuen et al., 2005; Finneran and Houser, 2006), allowing hearing measurements to be obtained from species found in non-laboratory settings, such as oceanarium animals (Szymanski et al., 1999) as well as temporarily caught (Nachtigall et al., 2008) and long-term rehabilitated odontocetes (Pacini et al., 2010).

A single Blainville's beaked whale stranded in Kihei, Maui, on 16 August 2010 and was transported to the University of Hawaii at Hilo Hawaii Cetacean Rehabilitation Facility (HCRF). Hearing measurements were collected for frequencies between 5.6 and 160 kHz within the first 2 days of the animal's rehabilitation. The results provide the first basic hearing measurements for Blainville's beaked whales.

## MATERIALS AND METHODS

### Subject

The male sub-adult *Mesoplodon densirostris* was found stranded on the morning of 16 August 2010 near Kihei on the island of Maui. The animal was observed milling in very shallow waters for several hours. On physical examination, the animal appeared weak and dehydrated. Initial diagnostics indicated severe immune compromise and renal insufficiency. The animal was given mineral and electrolyte injections and transported *via* a Coast Guard flight to the HCRF. The whale was 3.5 m long and weighed approximately 800 kg. Once at the rehabilitation center, it was tube-fed every hour with a mixture of water, electrolyte solution, medications and ground squid. The hearing measurements were selected as a 'non-invasive' ancillary diagnostic test to aid in the determination of the animal's medical problem and prognosis for rehabilitation.

Hearing measurements were collected periodically while the animal was undergoing medical treatments from 16 to 18 August 2010. The animal was lightly restrained during the hearing measurements while its respiratory activity was continuously monitored. Overall behavior indicated that there was no aversive reaction to the presentation of sound or the hearing measurements.

Intensive rehabilitation efforts continued with the animal. After several days in the hospital facility, the whale developed severe gastrointestinal hemorrhage and displayed signs consistent with respiratory disease. It died on 29 August 2010.

### Tank and background noise measurements

The animal was housed in the covered oval rehabilitation pool of the HCRF (9.8×7.3×1.5 m length×width×depth). Water pumps and filters were turned off during hearing measurements to limit masking background noise (Fig. 1A). The background noise was measured using a Reson TC-4040 hydrophone (−206 dB re. 1 V mPa<sup>−1</sup>; Slangrup, Denmark) and recorded as 1 min files with a Microtrack II 2 channel digital recorder (M-Audio, Irwindale, CA, USA) with a 96 kHz sampling rate. To compensate for alienated signals, one recording channel had no additional gain whereas the second channel had a 15 dB gain. Ten 1 s files were extracted using Adobe Audition 3.0, analyzed, fast Fourier transformed (FFT) using a 1024 point FFT and averaged with a customized MATLAB algorithm (Fig. 1B).

### Acoustic stimulus

The AEP measurement system used during the hearing measurements was similar to the equipment presented by Taylor and colleagues (Taylor et al., 2007) and used in the Mooney et al. (Mooney et al., 2008), Nachtigall et al. (Nachtigall et al., 2008) and Pacini et al. (Pacini et al., 2010) studies. During the hearing measurements, the animal was held at the surface in the middle of the rehabilitation pool and a projecting transducer was positioned 1 m away from the animal's head at a 30 cm depth marked by a colored tape placed on the transducer cord.

The acoustic stimuli consisted of sinusoidally amplitude modulated (SAM) tone bursts that were digitally generated using a customized Labview program and a National Instruments PCMIA-6062 E DAQ card (Austin, TX, USA) implemented into a laptop (Fig. 2). The tone bursts were 20 ms in duration and followed by 30 ms of silence, yielding a 20 s<sup>−1</sup> presentation rate. The tones were modulated at a 1000 Hz rate based on the modulation rate transfer function obtained prior to the audiogram measurements and previous results obtained with beaked whales (Finneran et al., 2009). For frequencies lower than 50 kHz, a 256 kHz update rate was used; this was increased to 512 kHz for frequencies between 50 and 100 kHz and 800 kHz for frequencies above 100 kHz. Peak-to-peak voltages were measured

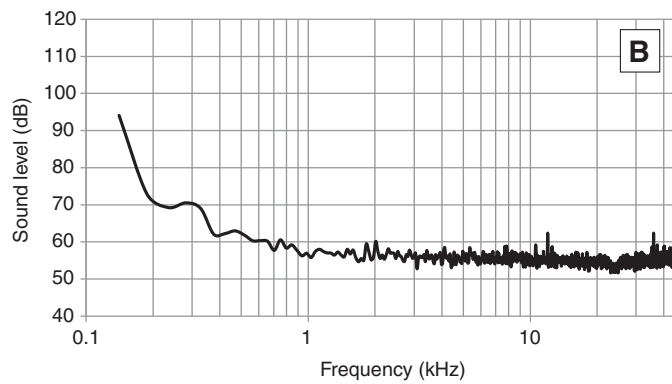
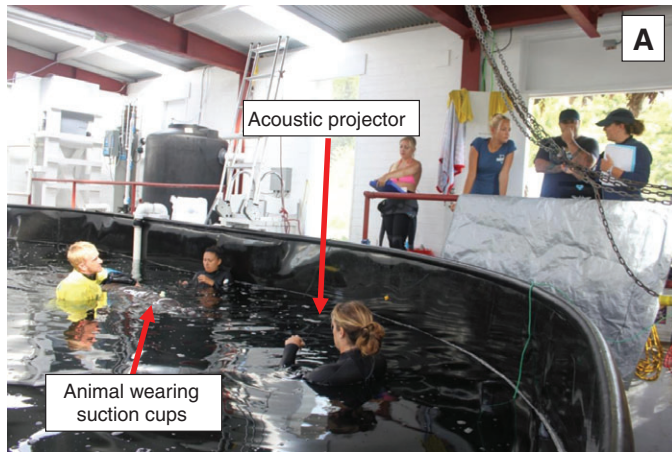


Fig. 1. (A) Rehabilitation pool where the hearing measurements on the Blainville's beaked whale (*Mesoplodon densirostris*) were conducted. The water pumps and filters are visible in the back and were turned off during the auditory tests. The projector was positioned 1 m away from the animal's head while the whale was lightly restrained. (B) Tank background noise was calculated using a 1024 point fast Fourier transform (FFT) and collected with a Reson TC-4040 hydrophone with a 96 kHz sampling rate. Sound levels are expressed in dB re.  $1 \mu\text{Pa}^2 \text{Hz}^{-1}$ .

using a Tektronix TPS 2014 oscilloscope (Beaverton, OR, USA) and then converted to peak equivalent root mean square (r.m.s.) voltages by subtracting 15 dB. Sound pressure levels (SPLs) were varied in 1 to 10 dB steps using a Hewlett-Packard P-350D attenuator (Palo Alto, CA, USA). These r.m.s. voltages were then used to calculate the SPL for each frequency. Two hydrophones were used to present the acoustic stimulus: an ITC-1032 (Santa Barbara, CA, USA) for the low frequencies between 5.6 and 40 kHz and a Reson TC-4013 for frequencies above 50 kHz. Due to time constraints and equipment limitations, a total of 11 frequencies were tested from 5.6 to 160 kHz and all sound stimuli were calibrated post data collection.

#### Electrophysiology measurements

Three Grass (West Warwick, RI, USA) 10 mm gold EEG electrodes embedded in latex suction cups were positioned on the animal. The active electrode was positioned over the brain 10 cm behind the blow hole and 3–4 cm to the right side of the animal's head, the reference electrode was positioned on the back of the subject and the ground electrode was positioned laterally on the animal's dorsal fin (Fig. 3). The electrophysiological signal was amplified 10,000 times and filtered from 300 to 3000 Hz using a Grass CP-511 bio-amplifier (West Warwick, RI, USA). Additional by-pass filtering was obtained

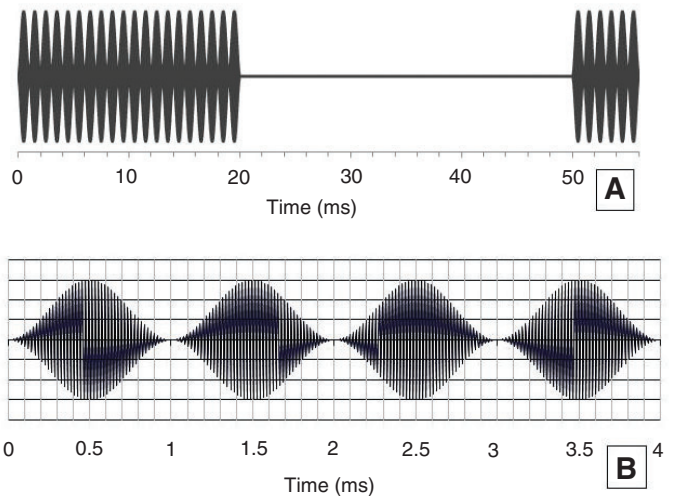


Fig. 2. (A) Sinusoidally amplitude modulated (SAM) tone stimulus used during the audiogram acquisition (150 kHz carrier frequency). (B) A 4 ms close-up of the same signal.

with a Krohn-Hite 3384 filter (Brockton, MA, USA). The same laptop computer and card were used to present the acoustic stimulus and to digitize the electrophysiological response using a 16 kHz sampling rate.

A full record – or trial – took approximately 90 s and consisted of collecting and averaging 1000 responses, each 26 ms long and triggered by the acoustic stimulus (Mooney et al., 2008; Pacini et al., 2010).

#### Data analysis

The complete audiogram was obtained over the course of 48 h. The data collection effort was divided into sessions of 20 min to avoid interfering with other medical and diagnostic tests, feeding and resting periods.

Each threshold was calculated using at least seven trials or records for each frequency. The level of the first sound for each frequency was chosen based on previous audiograms and was 15–20 dB above the published thresholds for other odontocetes (Johnson, 1967; Nachtigall et al., 2008; Finneran et al., 2009; Pacini et al., 2010). The SPL was then varied in 5–10 dB steps until the evoked potential response was low enough to not be discernable from the ambient biological noise for at least two trials. SAM tone bursts are known to generate a rhythmic response known as an EFR (Fig. 4) (Supin et al., 2001; Nachtigall et al., 2007). At each SPL, a 16 ms window of the EFR was analyzed using 256 point FFT. The peak response at 1000 Hz on the obtained frequency spectrum was used to estimate the animal's response to the acoustic stimulus (Fig. 5). For each frequency, the peak responses at 1000 Hz were then plotted against the stimulus SPL and a linear regression addressing the data points was used to evaluate the hypothetical zero value used to predict the threshold (Fig. 6). The auditory brainstem response technique does not yield absolute thresholds because of the inherent biological noise, but previous work has shown that the results are comparable to behavioral audiograms (Yuen et al., 2005).

#### RESULTS

The rehabilitation pool at HRCF provided a relatively quiet environment for the hearing measurements as most of the energy was below 1 kHz. Above 1 kHz, most of the ambient noise was below





Fig. 3. Experimental setup; the active suction cup is visible behind the blow hole of the beaked whale. The animal was lightly restrained during hearing measurements.

60 dB and below the sensitivity of the recording equipment. All hearing data were collected with the pumps and filters turned off, providing limited masking effects. The background noise is plotted in Fig. 1B.

The EFR had a delay of 4–6 ms, which corresponded to the latency of the neurophysiological response. Overall, the EFR was similar to measurements obtained in other odontocete species (Szymanski et al., 1999; Supin et al., 2001; Finneran and Houser, 2006; Nachtigall et al., 2008). With a SPL well above the threshold level, the EFR formed a complete rhythmic response, which decreased with the SPL. As the SPL approached the threshold level, the rhythmic EFR disappeared in the inherent biological noise. Fig. 4 shows the EFR to a SAM tone at 150 kHz. At 135 dB, the EFR was fully formed and closely followed the envelope of the acoustic stimulus. The EFR decreased in magnitude as the SPL of the acoustic stimulus decreased. At 115 dB, the rhythmic pattern was indiscernible from the background noise. The linear regression for that specific frequency yielded a 116.0 dB threshold.

The audiogram (Fig. 7) had the common U-shape found in mammalian species and the hearing range was similar to typical odontocete audiograms (Johnson, 1967; Thomas et al., 1988; Houser et al., 2008), with a steep slope in the high-frequency region and a more leveled slope in the low-frequency range. The area of best hearing was found between 40 and 50 kHz, forming a distinct notch in the audiogram. The best hearing was found at 50 kHz with a 48.9 dB threshold (Table 1). Past 50 kHz, the slope of the threshold curve increased rapidly and leveled off at approximately 80 kHz. The ranges of poorest hearing were found at both ends of the frequency spectrum, with thresholds of 79 dB for 5.6 kHz and 116 dB for 150 kHz. Overall, the low ambient noise of the pool provided a quiet environment, and masking effects were low, yielding threshold measurements with comparatively low values down to 50 dB (Au et al., 2002).

#### DISCUSSION

The audiogram of this *M. densirostris* was similar to audiograms of other odontocete species, with a U-shaped curve and good hearing in the human ultrasonic range. However, unlike most

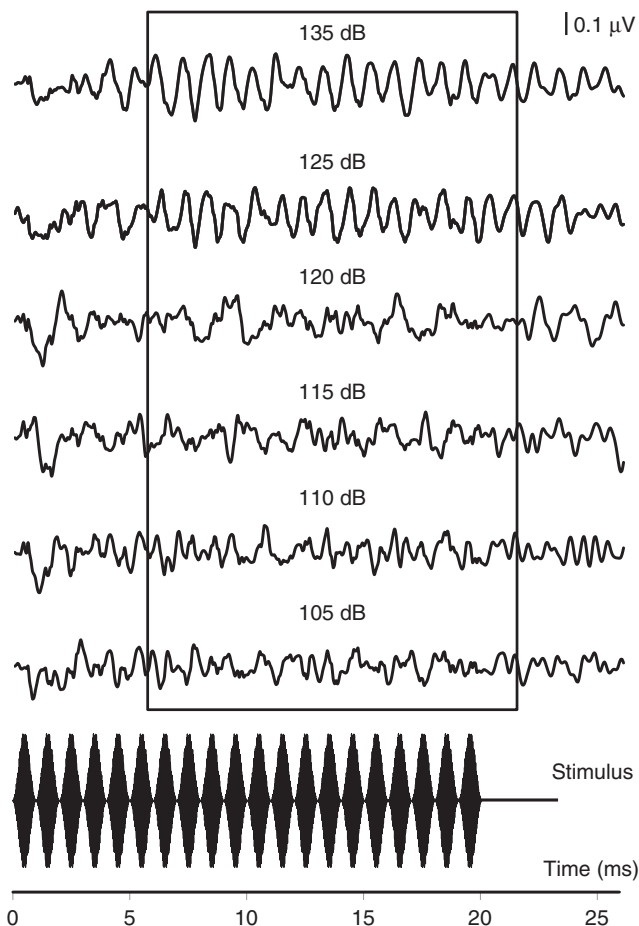


Fig. 4. Envelope following response (EFR) to a 150 kHz SAM stimulus from 105 to 135 dB re. 1  $\mu$ Pa. The box indicates the 16 ms analysis window for the FFT.

audiograms, the range of best hearing was relatively narrow and the upper frequency limit levelled off at approximately 100 kHz. Although such features have sometimes been observed in other odontocete individuals (Finneran et al., 2005; Houser et al., 2008), it is difficult to assess whether they are representative of the species or simply specific to the individual tested. Thresholds below 50 dB indicated that the environment was likely suitable for hearing measurements and that masking effects were negligible. The high-frequency cut-off of the animal's hearing was relatively low compared with small odontocetes, which have an area of best hearing at approximately 40–50 kHz. In young bottlenose dolphins, the best hearing usually lies at approximately 80 kHz (Johnson, 1967) and up to 120–140 kHz for harbor porpoises and white-beaked dolphins, with an area of best hearing between 100–140 and 45–128 kHz, respectively (Nachtigall et al., 2008; Kastelein et al., 2002). The audiogram of *M. densirostris* was similar to larger odontocete audiograms such as those of the Gervais' beaked whale *Mesoplodon europaeus* (Finneran et al., 2009) the long-finned pilot whale *Globicephala melas* (Pacini et al., 2010) and the killer whale *Orcinus orca* (Szymanski et al., 1999), indicating that size might influence not only the sound production mechanisms (Wang et al., 1995) but also the hearing range of the animals, a pattern well documented in terrestrial mammals (Heffner and Heffner, 1983).

In comparison to the hearing measurements of Gervais' beaked whales (Cook et al., 2006; Finneran et al., 2009), the audiogram

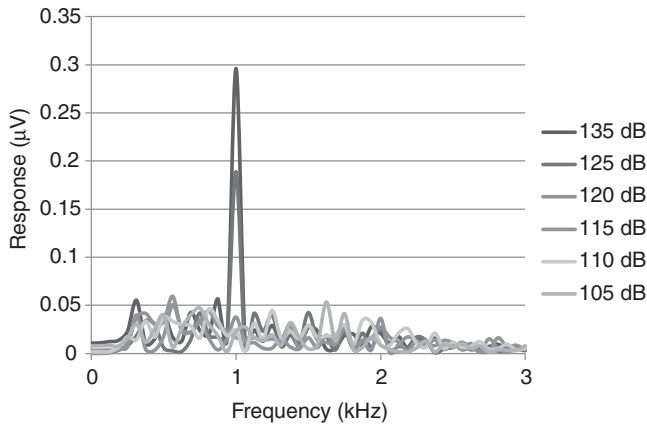


Fig. 5. The 256 point FFT of the EFR to a 150 kHz SAM tone with a 1 kHz modulation rate. The response is expressed in  $\mu\text{V}$  r.m.s.

obtained here is similar in shape but very different in threshold values. Most of the thresholds were at least 20 dB more sensitive than the Gervais' beaked whale thresholds. In these two studies, the acoustic stimulus was presented *via* a contact hydrophone positioned underwater on the panbone region of the lower jaw. Acoustic stimulation *via* jawphone stimulates only one ear whereas both ears were stimulated in the free field, which might account for the differences in threshold measurements. The jawphone technique has been shown to produce results comparable to far-field audiograms in bottlenose dolphins (Finneran and Houser, 2006) and was preferred by the investigators to limit the effects of the animal's head movements on the threshold calculations. The authors, however, noted that this underwater jawphone method had not been directly compared with free-field stimulation for beaked whale species and that the threshold values should be interpreted carefully as they were extrapolated from calibrations obtained with *Tursiops truncatus*.

Similar to the present hearing measurement of *M. densirostris*, the range of lowest thresholds or most sensitive hearing with *M. europaeus* was between 40 and 60 kHz. Additionally, although no responses could be detected above 80 kHz for *M. europaeus* (Finneran et al., 2009), the free-field sound presentation yielded thresholds in the 100 dB range for frequencies between 80 and 160 kHz for *M. densirostris*. These results may indicate – as suspected by Finneran et al. (Finneran et al., 2009) – that comparison

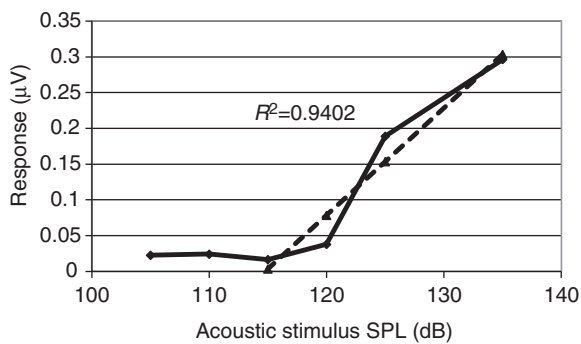


Fig. 6. Plot of the EFR amplitude at 1 kHz *versus* the corresponding acoustic stimulus sound pressure level (SPL) for the 150 kHz tone (solid line) and the linear regression (dashed line) calculated from 115 to 135 dB. The threshold was estimated as the intersection of the regression line with the zero value.

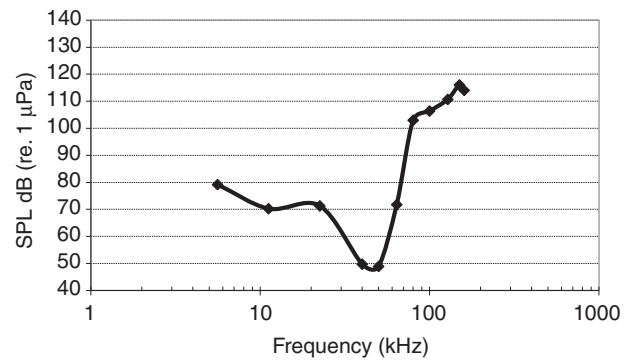


Fig. 7. Audiogram of *M. densirostris*.

between jawphone and free-field stimulation may not always be easily calculated. Alternatively, the Gervais' beaked whale may not hear as well as the Blainville's beaked whale in this study. Variability in threshold levels between individuals, even within a species, is not uncommon (Finneran and Houser, 2006).

Many factors are known to influence hearing, from variations across individuals (Houser et al., 2008; Popov et al., 2007) to environmental factors such as acoustic ambient noise (Kei et al., 2008). Whether the two complete beaked whale audiograms are representative of beaked whale hearing or just ends of the spectrum of individual variation can only be determined as more audiograms become available. The *M. densirostris* in this study was a sub-adult male whose teeth had not yet erupted. In comparison, the *M. europaeus* was a mature adult of unknown age and hearing loss could not be ruled out. Younger animals tend to hear better and presbycusis, or hearing loss due to age, has been documented in marine mammals and is likely to occur in the high-frequency range (Ridgway and Carder, 1996; Houser et al., 2008; Demeester et al., 2009; Kloepper et al., 2010). The subject in the present study was not full grown and presbycusis does not appear to be a potential cause of the observed limited high-frequency hearing. In addition, the *M. densirostris* in the present study was not administered any ototoxic medicine during its rehabilitation. Hearing pathways were not investigated with the animal due to the limited time available to collect the data. A recent study investigating sound pathways in *Ziphius cavirostris* using finite element model with CT scan data showed the existence of a potential new gular pathway for sound to reach the ear complex, where sounds enters “the head from below and between the lower jaws... (and) continues toward the bony ear complexes through the internal mandibular fat bodies” [p. 1 in Cranford et al. (Cranford et al., 2008)]. Although there was no possibility of testing hearing pathways, it should be noted that in the unlikely event that this specific sound path was not directly stimulated, the free-field audiogram

Table 1. Audiogram thresholds of *Mesoplodon densirostris*

| Frequency of sound stimulus (kHz) | Threshold (dB) |
|-----------------------------------|----------------|
| 5.6                               | 79.2           |
| 11.2                              | 70.2           |
| 22.5                              | 71.3           |
| 40                                | 49.7           |
| 50                                | 48.9           |
| 64                                | 71.7           |
| 80                                | 102.9          |
| 100                               | 106.4          |
| 128                               | 110.6          |
| 150                               | 116.0          |
| 160                               | 114.3          |

presented in this study would be a partial representation of the frequency hearing range of the *M. densirostris*.

Acoustic tagging has provided information on the echolocation behavior of Blainville's beaked whales. In general, beaked whales are deep divers and, so far, echolocation has only been detected when individuals are below a depth of 200 m (Johnson et al., 2004; Tyack et al., 2006). The clicks used during the searching phase of a foraging bout differ from most odontocete clicks. The signals are longer in duration and are characterized by a frequency-modulated (FM) upsweep with a  $-10$  dB bandwidth between 26 and 51 kHz (Johnson et al., 2006). The buzz phase clicks used in the final approach before prey capture have a broader bandwidth and are very similar to other odontocetes' clicks. FM bats appear to use a similar method of prey detection and capture (Madsen et al., 2005) and their best hearing usually lies within the range of echolocation frequencies of their signals (Neuweiler, 1984). Some species have even been shown to possess a cochlear acoustic fovea centered on the area of their echolocation clicks (Schuller and Pollack, 1979).

The audiogram collected in this study – combined with the acoustic data obtained by Johnson and colleagues (Johnson et al., 2006) – indicates that the area of best hearing partially overlaps with the frequency spectrum of the FM signals used by *M. densirostris* ( $-10$  dB bandwidth from 26 to 51 kHz). Other odontocetes such as the bottlenose dolphins use broadband echolocation clicks and are thought to rely on an energy detector receiver model using these short, pulsed signals (Au, 1993). Beaked whales could rely on an energy-detecting ear where FM clicks contain more energy at certain frequencies, and longer clicks would provide additional energy within that frequency range to detect and identify prey.

Beaked whale FM clicks resemble the FM signals used by bats, which are believed to rely on a matched filter receiver model where the animal innately compares the received echo with the outgoing click to obtain ranging information. Why and whether beaked whales would rely on a different technique from other odontocetes remains unknown and might be related to their unique life history. Johnson et al. hypothesized that the use of FM signals during the search phase might improve the detection and discrimination of specific prey in a scattered environment, thus “maximizing the net energy return of foraging during long breath-hold dives” [p. 5047 in Johnson et al. (Johnson et al., 2006)]. If *M. densirostris* relies on a different echolocation strategy to locate and identify their prey and use “prey-specific signatures in the returning echoes” [p. 5047 in Johnson et al. (Johnson et al., 2006)] (Madsen et al., 2005), extremely sensitive hearing in the frequency range of the FM clicks would represent a definite advantage to cross correlate the returning echo to the emitted signal. Interestingly, the audiogram range of best hearing does not overlap as well with the frequency range of the terminal buzz clicks, which indicates that the animal might not fully hear these broadband clicks ( $-10$  dB bandwidth from 25 to 80 kHz) (Johnson et al., 2006).

Although acoustic tagging research has provided a more comprehensive picture of the ecology and behavior of beaked whales, these species remain amongst the most cryptic marine mammals. Some species have been only identified only within the last 10 years and have never been observed alive (Reyes et al., 1991; Dalebout et al., 2002). Most of the knowledge about beaked whales has been obtained through strandings. In recent years, special interest has arisen after multiple unusual mass strandings have been linked to military exercises (reviewed in Cox et al., 2006; Rommel et al., 2006; Nowacek et al., 2007; D'Amico et al., 2009; Filadelfo et al., 2009a; Filadelfo et al., 2009b). MFAS uses frequencies between 1 and 10 kHz (D'Amico and Pittenger, 2009). The Blainville's beaked

whale hearing threshold at 5.6 kHz indicated that the animal in the present study was able to detect this frequency at levels as low as 79 dB in a quiet environment.

At the time of the stranding of the animal examined in this study, no naval activity was reported. The animal stranded 2 weeks after the end of the biannual international Rim of the Pacific (RIMPAC) exercise and it died 13 days after it stranded in Maui. At the time of writing, histopathology of the organ systems were not completed. Based on the gross post mortem examination, organ cultures, viral serology and PCR testing, it has been hypothesized that the whale was likely suffering from a systemic viral infection that caused weakness and anorexia, which ultimately led to dehydration and stranding. The immune-compromised whale then developed a peracute bronchopneumonia with subsequent gastrointestinal ulcerations.

As any work obtained from a stranded animal, the present audiogram should be interpreted carefully. Strandings provide a rare opportunity to obtain physiological information about poorly known species. One of the main difficulties in studying marine mammals arises from the limited sample size available to researchers. As noted by Finneran and colleagues (Finneran et al., 2009), collecting data during a stranding event is not ideal; because of the unstable health of the animal and the limited time allocated to measurements, factors such as electrode placement and head movements must be carefully monitored and accounted for during the analysis, thus increasing potential errors in the measurements obtained.

This audiogram of a *M. densirostris* individual contributes to the ongoing effort to better understand the effects of noise on marine life. More importantly, these results provide valuable information about the hearing abilities of a species implicated in strandings related to naval exercises. In addition, they provide baseline data about the acoustic abilities of a poorly known but crucially important species. This type of research – although not as controlled as that in a laboratory setting – allows the scientific and management communities to obtain crucial physiological information using non-invasive techniques and provides a diagnostic tool to rapidly measure the hearing of wild animals.

#### LIST OF ABBREVIATIONS

|      |   |
|------|---|
| AEP  | auditory evoked potential               |
| EFR  | envelope following response             |
| FFT  | fast Fourier transform                  |
| FM   | frequency modulated                     |
| HCRF | Hawaii Cetacean Rehabilitation Facility |
| LFAS | low-frequency active sonar              |
| MFAS | mid-frequency active sonar              |
| SAM  | sinusoidally amplitude modulated        |

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