HEARING SENSITIVITIES AND SOUND PATHWAYS IN ODONTOCETES

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A mes parents, Danièle et Guy,

pour leur soutien et leur amour
inconditionnels.

A ma Tatie, tu me manques.

'Art is I, Science is We.'
Claude Bernard

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ABSTRACT

Sound is of primordial importance for marine mammals and the impact of anthropogenic noise on their life history is still largely unknown. Understanding how odontocetes or toothed whales have evolved a highly specialized hearing system has also been the focus of intensive research both in the laboratory as well as in the field. The use of auditory evoked potentials to study hearing in whales and dolphins has allowed scientists to obtain rapidly hearing measurements in species that were not easily accessible. This dissertation presents an overview of a travel system used to rapidly and non-invasively measure the hearing of odontocetes. In addition, this work presents the basic hearing measurements or audiograms of two new species of odontocetes; the long-finned pilot whale (*Globicephala melas*) and the Blainville's beaked whale (*Mesoplodon densirostris*). Hearing pathway differences in two species of odontocetes, the atlantic bottlenose dolphin (*Tursiops truncatus*) and the false killer whale (*Pseudorca crassidens*) were also collected and potential interspecies variations were discussed. The last chapter presents the results of an experiment investigating echolocation disruption in the false killer whale using an acoustic pinger to mitigate by catch in Hawaiian fisheries. This dissertation combines non only data on the basic hearing of new species and a new perspective of the complex hearing mechanisms and hearing pathways variations across odontocetes, but it also provides baseline data to address important conservation issues such as the effects of noise on marine mammals as well as the feasibility of using acoustic deterrents to mitigate interactions with fisheries. Understanding how different species of odontocetes evolve, use and react to sound is important to build successful conservation strategies that will both protect animal species as well as accommodate human activities when it is possible.

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ABR: Auditory Brainstem Response

AEP Auditory evoked potentials

EFR Envelope following response

SAM: sinusoidally amplitude modulated

HCRF: Hawaii Cetacean Rehabilitation Facility

FFT: Fast Fourier Transform

r.m.s : root-mean-square

FM: frequency modulated

LFAS : low frequency active sonar

MFAS: Mid frequency active sonar

CHAPTER 1: INTRODUCTION

MARINE ENVIRONMENT AND MAMMALS

Living in the ocean presents important physiological constraints for any organism. Maintaining homeostasis in an environment with constantly fluctuating salinity, density, pressure and temperature involves a considerable energy investment as well as morphological and physiological challenges. For warm-blooded air breathing mammals, the constraints are even greater and the few groups that have successfully evolved in the ocean have considerably modified their entire life histories. Marine mammals include animals that spend only part of their time in the water such as polar bears, walruses and other pinnipeds, to species that have completely abandoned the terrestrial life like cetaceans such as whales and dolphins. The approximately 73 species of Odontocetes or toothed-whales all belong to the order Cetacea. Toothed whales range from small animals such as the highly endangered Vaquita (*Phocoena sinus*) to the 20 meter-long sperm whale (Physeter *macrocephalus*). Odontocetes share common features such as teeth (the number of teeth can vary from over one hundred in the bottlenose dolphin (*Tursiops truncatus)* to a single tooth in the narwhal (*Monodon monoceros*), the ability to echolocate and feed on relatively large prey as opposed to baleen whales that filter smaller individual food items. However, many differences exist within this group; toothed whales vary greatly in geographical range (river dolphin vs. tropical or arctic species) feeding techniques, social interactions, and morphology.

Like all mammals, odontocetes interact with their environment using a variety of sensory modalities. The water environment imposes new challenges for any animal to navigate and communicate. The density of salt water is about 770 to 890 times the density of air at sea level (Denny, 1993), sound travels five times faster in water than in air and light dissipates almost completely in the first 200 m of the water column. In this environment, scent and vision are often limited and depend greatly on oceanographic conditions.

It appears that odontocetes have lost their olfactory sense; the nasal system seems to have been overtaken to accommodate for echolocation and the olfactory peduncle is rarely found (Oelschläger *et* Oelschläger, 2002). In addition, the olfactory lobe in the odontocete's brain, although present during the fetal development, is greatly reduced in adult cetaceans and the vomeronasal organ or Jacobson's organ (found in other mammals, its function is to detect specific chemical compounds contained within scents) and nerve are absent. Odontocetes do have active taste buds and may be sensitive to certain chemicals produced by the perianal or prostate gland (Kuznetsov, 1974, 1978) and they are capable of perceiving sour and bitter tastes (Nachtigall & Hall, 1984).

The dolphin's eyes have adapted to be able to resist temperature and pressure changes. The cornea is thicker, the lens is almost spherical and the rete mirabile is highly vascularized to protect the eye from underwater cooling (Mass *et al.*, 2002). Cetacean eyes are positioned laterally, thus providing a good panoramic vision (120-130°). In addition, odontocetes have adapted to be able to see in air as well as in water. When the animal has its head out of the water, the eyes move forward compensating for possible myopia. Underwater visual acuity is estimated to be around 8.2 arc min and aerial visual acuity of 12.5 arc min (Madsen and Herman, 1980). So far, it has not been demonstrated that odontocetes can perceive colors but appear to be most sensitive in the blue range. Because most of the sensory systems used by terrestrial mammals have limited use in the ocean, marine mammals should rely extensively on sound to obtain information from their environment because acoustic energy propagates much better in the aquatic environment than any other type of energy (electromagnetic, light or thermal) (Au, 1993).

EVOLUTION, ECHOLOCATION & HEARING

Echolocation

All odontocete species so far investigated use echolocation to navigate, find prey and avoid predators and obstacles. Echolocation has also evolved independently in one order of terrestrial mammals *Chiroptera* and over 800 species of bats are known to echolocate (1100 species of bats total). Marine mammal echolocation – or the assessment of the environment by emitting sounds and listening to echoes as the sound waves reflect off different objects in the environment – was first evidenced by McBride (1956) who recorded observations of dolphin behaviors and suggested the use of echolocation in cetaceans. Empirical data were later obtained by *Norris et al.* (1961). In their pioneering study, they put suction cups on the eyes of a bottlenose dolphin, thus eliminating vision. The animal produced pulses, and was able to swim between pipes and retrieve fish.

Extensive research has been conducted on the echolocation abilities of odontocetes such as detection range and discrimination abilities. Au and Snyder (1980) measured the maximum echolocation detection range of *Tursiops truncatus* using a 7.62 cm stainless steel water-filled target. Tests were conducted in Kane'ohe Bay (Hawai'i, USA) and results showed that the performance of the animal dropped below chance (50%) when the target was set 113 m away from the animals. Target diameter discrimination was tested in *Tursiops truncatus* and *Inia geoffrensis* and results show that both species were able to differentiate between cylinders with a 1 dB target strength difference (Evans, 1973). Target size discrimination was tested using cylinders of various lengths and results show that 70% of the time the subject (*Tursiops truncatus*) was able to discriminate a 25 mm-long target from a 30 mm-long one (Ayrapet'yants *et al.*, 1969). Using a go/no-go paradigm, Au and Pawloski (1992) tested the ability of a *Tursiops truncatus* to discriminate wall thickness differences of various cylinders. Results indicated that the subject detected thickness differences from 0.23 to 0.27 mm. Echolocation signals vary greatly across species, with broadband clicks for most delphinids, highly tonal and long clicks for harbor porpoise (*Phocoena phocoena)*

(Kamminga *et al.*, 1996), relatively low frequency for the sperm whale (*Physeter macrocephalus)* (Goold and Jones., 1995) and frequency modulated clicks for Cuvier's beaked whales (*Ziphius cavirostris)* (Zimmer *et al.*, 2005). Most recent studies showed that species such as the Risso's dolphin (*Grampus griseus)* and the Pacific white-sided dolphin (*Lagenorhynchus obliquidens*) possess clicks withdifferent spectral components and these differences can be potentially used to identify these species (Soldevilla *et al.*, 2008).These variations may be a response to various sound production mechanisms or simply an adaptation to the ecological niches these different species occupy.

Marine mammals and sound

As mentioned previously, marine mammals and more specifically odontocetes rely extensively on acoustic cues. Beyond echolocation, dolphins and whales use sounds to communicate with conspecifics as well as to localize prey and avoid predators. Whales and dolphins are known to produce a wide variety of sounds ranging from echolocation clicks to tonal whistles and burst pulses. Mysticete sounds usually lie within a low frequency range (4 Hz to 15 kHz) while odontocetes produce sounds over a wider and higher frequency range (5 kHz to over 135 kHz) (Au et al., 2000).

For many years, sound production in cetaceans was not fully understood as researchers argued over which organs were actually involved in sound production. On one hand, it was believed that sounds were produced by the larynx and these conclusions were based primarily on anatomical observations (Purves, 1967; Purves and Pillerai, 1986). On the other hand, the nasal sac complex was also believed to be a potential sound production mechanism. Wood (1968) was the first to describe the 'monkey-lips' as the site where sounds – including clicks and whistles – could be produced and Cranford et al. (1997) provided video evidence suggesting that the nasal sac system was the site of sound production.

Beyond sounds produced by conspecifics, cetaceans can also rely on acoustic signals generated by potential prey or predators; this technique is often referred to as the 'passive listening hypothesis.' Transient killer whales in British Columbia are known to forage on other marine mammals such as pinnipeds or small delphinids (Barret-Lennard et al., 1996). They have been observed to be acoustically silent, preferring a passive acoustic approach where they localize prey by listening and orienting towards the sound they make. Similarly, dolphins have been observed to orient towards fish sounds during playback experiments (Gannon et al., 2005).

Anthropogenic impacts on marine mammals

Understanding and quantifying the complex impacts of anthropogenic pressure on the oceans and more particularly on marine mammals is still mostly unknown. As top predators, marine mammals are of primary importance in the food web (Estes et al., 1998). The range of interactions between human activities varies greatly from toxic built up in beluga whales' blubber, to fishing by-catch and large whale ship strikes. One of the major concerns stakeholders are facing is the impact of anthropogenic noise on marine life. While audiograms provide basic information on hearing sensitivity, additional experiments looking at how specific sounds are likely to affect animals provide both behavioral and physiological information about the animals' potential response. Changes in behavior have been observed in cetaceans: from a total lack of behavioral response to an avoidance of the sound source (Finley et al., 1990) or to a change in emitted sounds. A Lombard response where the animals change the characteristics of their vocal repertoire (such as raising the loudness of a call) to compensate for the masking effect of a loud environment has been observed in Beluga whales (*Delphinapterus leucas*) (scheifele et al., 2005). Because man-made signals can potentially have a deterring effect on cetaceans, they have been used to prevent dolphin and whale by-catch and depredation. Pingers or acoustic deterrents have been showed to be successful in certain fisheries (Barlow and Cameron, 2006). In Hawaii, false killer whales have been reported to depredate on long-line fisheries which has not only economic impacts on the fisheries but also negative effects on the false killer whale population as individuals get injured (dorsal disfigurement) or by-caught (Baird and Gorgone, 2005).

Hearing measurements

Because most odontocetes produce clicks containing high frequencies during echolocation, one might expect these species to hear such frequencies. There are many ways to investigate hearing, but perhaps the most fundamental is the audiogram in which hearing is examined as a series of thresholds of minimum audible intensity as a function of the frequency. The results are usually plotted in a graph in which the x-axis indicates frequency in kHz, and the vertical axis sound intensity in dB. (Wartzok and Ketten, 1999). Hearing was first tested with a Atlantic bottlenose dolphin *Tursiops truncatus*, using operant conditioning with a go/no go paradigm, where the animal was trained to respond to a sound and to remain still if it could not perceive the sound or if no sound was played (Johnson, 1967). Continuous tone stimuli were presented to the animal. The data showed that this subject was capable of hearing much higher frequencies than humans with an upper limit of approximately 150 kHz.

The use of auditory evoked potentials to obtain audiograms made the data collection process much faster and allowed to test more species. Early work by Bullock *et al.* (1968) collected auditory evoked potentials directly from the inferior colliculi and the peripheral auditory nuclei from29 anesthetized dolphins (4 different species, mainly *Stenella*). Sound stimuli were either in air, in water or directly applied on the skin of the subject. They obtained audiograms similar to previous studies with an upper frequency limit of 100 to 120 kHz. In addition, they concluded that the auditory system of cetaceans was highly adapted for echolocation since the animals had a high frequency upper cut-off, good frequency and intensity discrimination, and an excellent temporal recognition to be resolve outgoing and returning clicks. They also tested the potential pathways to the inner ear and found the lower jaw to be the most sensitive to sound stimulation. While this provided pioneering information about the hearing abilities of these cetaceans; the experiment cannot be replicated because of the use of invasive techniques.

Improvements in methods have allowed investigators to obtain hearing information using a non-invasive technique called Auditory Brainstem Response (ABR) where the electrodes record evoked potentials directly from the surface of the animal's skin. Two to three electrodes embedded in soft latex suction cups are positioned on the animal: one records the brainstem activity and is usually positioned by the blowhole of the subject, while the other cups serve as ground and reference. This procedure allows the collection of hearing information without anesthesia or surgical procedures.

Since the initial hearing measurements on *Tursiops truncatus*, audiograms of other species have been obtained. Approximately 14 species have been examined so far, including the harbor porpoise *Phocoena phocoena* (Klishin *et al.,* 2000), the common dolphin *Delphinus delphis* (Popov *et al.,* 1998), the beluga whale *Delphinapterus leucas* (Klishin et al., 2000), the false killer whale *Pseudorca crassidens* (Thomas *et al*., 1988), the killer whale *Orcinus orca* (Szymanski *et al.*, 1999) and the Risso's dolphin *Grampus griseus* (Nachtigall *et al.*, 2005). No major differences were noticed across species, the shape of the audiogram resembling previous data obtained with other mammalian species with differences only in the frequency range. It is important to notice that the hearing of mysticetes or baleen whales has not been measured so far.

ADAPTATIONS

Skull and brain

If we follow the definition of skull proposed by Pabst et al. (1999), to include not only the cranium but also the derivatives of the first two visceral arches (lower jaw and hyoid apparatus), we can see that the skull is the foundation for most sensory systems in odontocetes. Although the skull does not necessarily reflect the head contour, it is important to understand the major adaptations odontocetes have undergone through evolution. First, most odontocetes have fused neck vertebrae, since there is little need to support the head against gravity in an aquatic environment. This limits rotational head movements that allow the animal to potentially turn its head towards a sound source. Belugas *Delphinapterus leucas*, narwhals *Monodon monoceros* and river dolphins *Platanistoidea* have unfused neck vertebrae and can potentially turn their head toward a sound source. Telescoping (Miller, 1923) is probably one of the most important adaptations that odontocetes have undergone. It is defined as the elongation of rostral elements and a dorso-rostral movement of caudal elements. The premaxillary and maxillary bones have been elongated while their dorsal elements have been pulled back. The bony nares have been moved to the dorsal apex of the skull and are essentially vertical to facilitate air intake at the surface.

Secondly, tooth shape has changed from heterodonty to a homodont condition, where all the teeth have the same shape and the same function. For instance, the bottlenose dolphin teeth's primary purpose is to grasp and pierce prey such as fish or cephalopods. Although it has been hypothesized that dolphin teeth had a role in receiving sound (Goodson and Klinowska, 1990; Graf *et al.*, 2008), there is no empirical data to support it and toothless animals – both in the wild and in captivity – have been observed echolocating and foraging.

In all odontocetes, an important skull asymmetry (MacLeod *et al.*, 2007) has been noted but its function is not fully understood.

Popov *et al.* (2007) noted that the brain morphology of cetaceans differs from terrestrial mammals. An essential difference is the hypertrophy of the auditory neural centers, which are several times greater than the volumes of other mammals. The structural organization of the cortex is also different in cetaceans, with a much larger cortical surface to brain ratio. It appears that the suprasylvian gyrus is the cortical projection for auditory information, which differs from terrestrial mammals where the auditory projection area is in the temporal lobe. Oelschläger and Kemp (1998) showed that in the sperm whale brain "the relative size of the teleencephalic hemisphere is the largest within the mammalian, whereas the ratio of the brain mass to the total body mass is one of the smallest." In addition, the development of the brain involves the reduced growth of the olfactory system and limbic system along with the important growth of the components of the auditory system.

Overall, it appears that dolphins and whales have undergone major brain and skull changes to adapt for the water environment and these changes greatly influence their ability to echolocate and hear high frequency sounds.

The Ear

Along with the skull, the odontocete ear structure has undergone many changes to adapt to the aquatic environment. One way that sound can be transmitted to the inner ear is through bone conduction, where sound can travel faster since the bone density is greater than the density of tissues or water. Bone conduction is defined as the conduction of sound to the inner ear through the bones of the skull, causing fluid motion within the inner ear. There are two major ways bone conduction can occur. First, by moving the entire cochlear capsule, which is referred to as the inertial lag in fluid, or by distorting one part of the capsule causing fluid to adjust to variations within the confinement of the capsule wall (McCormick *et al.*, 1980)

One of the most important changes the ear has undergone is the separation of the entire tympano-periotic complex or bulla from the rest of the skull; the only connection that remains is primarily through cartilages. This separation isolates the cochlea from the rest of the skull thus limitating spurious bone conduction of sound. In addition, this complex is well isolated and surrounded by spongy tissues filled with air spaces (Thewissen, 2002). As part of their adaptation to the marine environment, odontocetes presumably lost their pinna or external ear. The pinna plays a major role in localization in terrestrial mammal hearing, as it channels sound and 'acts as a funnel and reflector' (Batteau, 1967). Terrestrial mammals have to cope with a loss of energy as sound – produced in air – has to travel to a liquid environment found in the cochlea of the inner ear. An approximate 30 dB loss occurs when sound travels from air to liquid because of differences of impedance. To overcome this physical constraint, all terrestrial mammals possess a mechanism that amplifies the incoming sound by approximately 30dB before entering the inner ear. This amplification occurs at the middle ear, with the 3 bones, malleus, incus and stapes acting as an impedance matching lever system. Cetaceans still possess these bones even though they do not have to overcome the impedance matching problem since the density of the cochlear fluid is approximately equal to the density of seawater. Because the opening of the ear canal and the ear canal itself leading to the middle ear are very narrow, there is little possibility that this passage is used to receive acoustic signals (Ketten, 2000).

More importantly, odontocetes do not have the same sort of tympanic membrane commonly observed in terrestrial mammals. Ketten (2000) noted that because the odontocetes ossicles are more massive and have multiple stiffening elements, the tooth whale middle ear would be adapted for high frequency hearing.

The inner ear is composed of multiple cavities. The most important one for hearing is the cochlea – a snail shaped, liquid filled cavity made of three ducts. The scala vestibule and the scala tympani are filled with perilymph. The cochlear duct is located between the two scalae and contains endolymph. On the surface of the cochlear duct also called basilar membrane, cilia oscillate as sound reaches the inner ear. The length, width and thickness of the basilar membrane can provide information on the hearing capabilities of odontocetes. The organ of Corti contains the neurons that transmit sound information to the brain and is part of the eighth cranial nerve. Cetaceans have more outer hair cells than humans and significantly more ganglion cells (Wever *et al.*, 1971). Wever and colleagues believed that the high ratio of ganglion cells to hair cells (5:1 in dolphin compared to 2:1 in humans) "suggest unusual capabilities in the utilization of auditory information" such as tonal differentiation and echolocation. Indeed, in addition to frequency sensitivities, Bullock *et al.* (1968) saw three distinct categories of responses in the inferior colliculus that Ketten (2000) referred to as 'a division of signal properties among populations of neurons:' specialized in short latency signal, in the acoustic signal duration or in the change in signal rise time. While all mammals share these cochlear features, there are differences on how the sound actually reaches the inner ear. In addition there are important variations in proportions in the internal ear across cetaceans (Ketten, 1992).

Similar to the brain and skull, the cetacean ear has evolved to accommodate echolocation mechanisms and the water environment and although the hearing mechanisms are not fully understood, it appears that odontocetes have highly specialized adaptations to be able to localize and identify prey and predators using sound cues.

Acoustic fats

In odontocetes, the lower jaw is filled with endogenous fats that play a major role in sound reception (Norris and Harvey, 1974); these specialized lipids – also found in the melon – are synthesized only by odontocetes (Litchfield *et al.*, 1975; Malins and Varasani, 1975). These 'acoustic fats' have a high lipid content and cannot be used as an ener gy source by the animal even in the case of high starvation (Koopman *et al.*, 2003). In addition, wax esters which are found in jaw fats and rarely in the blubber, have very different physical properties that the rest of the usual lipids and are not found in any other mammalian species. This implies that the synthesis of these wax esters has become a physiological adaptation in odontocetes. These fats have a very complex structure which is assumed to channel sound to the ear. Interestingly, Koopman *et al.* (2006) showed that the spatial localization of wax esters and fatty alcohols remains constant across individuals (with higher concentrations close to the connection to the inner ear) creating a complex molecular arrangement forming a channel inside the mandibular. Because the impedance of these fats is very similar to the impedance of water (Varanasi and Malins., 1972), they represent a very favorable pathway for the sound to travel. In addition, it seems that the short carbon chained lipids found at the surface of the mandibular fats could channel sound inward toward the center of the mandible (Koopman *et al.*, 2006).

The composition of the mandibular fats varies greatly across species with low concentrations of *i*-5:0 and *i*-15:0 for pygmy sperm whales (*Kogia breviceps*) and Gervais beaked whales (*Mesoplodon europaeus*). In addition, it appears that younger animals have slightly different jaw fat composition than adults, suggesting that their hearing and echolocation abilities might differ as well. Koopman (2007) showed that odontocetes not only differ in the composition of the lipids in their mandibular and melon, but also in their blubber. Indeed, only beaked whales and sperm whales had high levels of wax esters in their blubber, which could be a potential adaptation to deep diving.

Hearing pathways

Norris (1964) introduced the idea that odontocetes could potentially hear through their fat-filled lower jaw. The odontocete mandible is hollow and has no medial bony wall – also called lamina – and the fats extend up to the hearing tympano-periotic complex. In addition, the mandible seems to have evolved to represent a compromise between a strong mechanism for prey capture and handling and a thin posterior lateral wall or 'vibrating plate' for hearing (Nummela *et al.*, 2004).

Bullock *et al.* (1968) showed that this area was most sensitive to sound stimuli that were applied directly on the skin of the animal. Brain responses were recorded at the inferior colliculi for *Stenella coeruleoalba*.

In 1988, Brill *et al.* used a go/no-go paradigm (Schusterman, 1980) where the animal (*Tursiops truncatus*) was trained to report the presence of a metal target. The subject was wearing eye cups to eliminate visual cues and trials were performed with only eye cups or with eye cups and a neoprene hood covering its lower jaw but not its external auditory meati. The performance of the animal was reported to drop significantly when the animal was wearing the neoprene hood covering the lower jaw. These results indicated that the subject was not able to assess the presence of the target when its panbone was acoustically isolated adding experimental support to Norris' hypothesis of hearing through the lower jaw.

Møhl *et al.* (1999) used auditory brainstem response (ABR) to further explore Norris' acoustic window hypothesis. Sound stimuli were presented using a jawphone, which is a hydrophone embedded in a suction cup that can play sounds directly on the surface of the skin of the animal. Results showed that the *Tursiops truncatus* subject was most sensitive when sounds were presented "just forward of the panbone, about 25cm behind the tip of the lower jaw." The authors also measured the delay of the ABR response as a function of distance from a reference point. The area of lowest delay was situated behind the eyes. Møhl *et al.* concluded that the head of *Tursiops truncatus* acts as a shaded receiver to sound stimuli because the areas of minimum delay and best hearing did not coincide. Therefore the entire head probably acts as an acoustic receiver with several areas of better hearing sensitivity both for sound intensity and time of arrival.

Popov *et al.*'s work (2003) showed that the shape of the audiogram (from 8 to 128 kHz) varies with the angle at which the sound stimulus is presented in the far field indicating that while an audiogram at midline – or right in front of the animal – is a good approximation of hearing sensitivity, some frequencies are better perceived at different angles. For instance, lower frequencies were heard better at 90°. Since a same acoustic signal is perceived differently depending on the angle, the authors concluded that these spectral changes can provide additional localization cues for the animal. While this work did not directly investigate hearing pathways, these findings remain important to this issue because they provide information on how the frequency of the acoustic signal has an effect on the angles of best hearing, thus potentially on the regions of sound reception.

Popov *et al.* (2007) used ABR latencies to calculate the position of the sound-receiving point. Their results showed that when frequency varied, the reception loci changed as well. For low frequencies between 16-22.5 kHz, the area of best hearing corresponded to the meatus and for higher frequencies (from 32 to 128 kHz) the area was calculated to be the proximal part of the lower jaw. They concluded that Norris' hypothesis was only partially correct and that *Tursiops truncatus* exhibits at least two frequency-dependent acoustic windows. These results were validated by another publication by Popov *et al.*(2008) where the authors investigated hearing channels but using a contact hydrophone along the lower jaw and the meatus. The frequency stimulus varied from 8 to 128 kHz. Their results indicated that the meatus was more sensitive to lower frequencies while the higher frequencies were perceived best 20 cm away from the rostrum, which corresponds to Norris' original 'acoustic window'. Since all odontocetes have acoustic fats, it has been assumed that they have the same function for all echolocating species. Although Koopman *et al.* (2006) showed that the lipid composition and arrangement could vary between cetacean species; limited research has investigated hearing pathways in other species.

Mooney *et al.* (2008) tested 5 points on the head of a beluga whale (*Delphinapterus leucas*) using a contact hydrophone while measuring hearing with evoked potentials. Both the panbone and the tip of the lower jaw were found to be almost equally sensitive to click-like stimuli. While the panbone region is commonly accepted to be the primary pathway for sound reception, the tip of the upper jaw was always believed to be well isolated. It appears that – at least for *Delphinapterus leucas* – the tip of the upper jaw could be the principal site for sound reception, indicating that areas of best hearing might well vary probably between species of odontocetes.

Finally, Norris and Harvey suspected that sound could also travel through the mouth and throat especially when the animal was close to its target (1974). While this hypothesis was only partially validated by the work of Møhl *et al*. (199) advancements in medical imaging have allowed researchers to build models of new hearing mechanisms and pathways in the head of cetaceans which may add new support to this notion.

NEW QUESTIONS AND NEW TECHNOLOGIES

3D imaging has helped to understand how sound could potentially reach the inner ear. While most of these models still remain unverified with live animals, they provide a visualization and an approximation of internal mechanisms in a non-invasive way. They also help to understand how the different head structures interact with each other. Physical properties such as elasticity and sound velocity of tissue allow the construction of a 3D model of the head and the test of different scenarios for sound production and reception. Aroyan (2001) used three dimensional acoustic propagation and the physical properties of the head tissues in *Delphinus delphis* to obtain a 3D model of both echolocation emission and reception. His model showed that the 'acoustic window' situated on the lower jaw acted as a 'focal lens', improving the hearing directivity of received sounds. In addition, the lower jaw fats were found to transmit sounds effectively to the bullae that each contains the tympano-periotic complex. To this date, no experiment has been conducted on *Delphinus delphis* to test these findings.

Cranford *et al.* (2008) recently showed that a new hearing pathway – through the tissues of the throat – was hypothesized through finite element modeling of the head of a Cuvier’s beaked whale (*Ziphius cavirostris*). The authors noted that Møhl *et al.* (1999) found that the throat or ventral midline was essentially as sensitive as the panbone. Cranford also mentioned that this pathway could have evolved with the absence of the lamina. Jaw hearing might be more recent evolutionary speaking since odontocetes ancestors, archaeocetes did not have a lamina but their mandible wall was thicker than actual odontocetes. While these findings usually represent only models and have important limitations, they emphasize that new directions of research about hearing and sound paths of odontocetes can be explored through non-invasive techniques. Most importantly, for these results to be valuable, they should always be validated by empirical data obtained with live animals.

SPECIES LIFE HISTORY

This dissertation encompasses work collected with four different species of the sub-order Odontoceti (Order *CETACEA*) from two different families Delphinidae and Ziphiidae. *Pseudorca crassidens* and *Glocicephala melas* are in the same sub-family Globicephalinae (LeDuc et al., 1999) and *Tursiops truncatus* in the Delphininae sub-family. The Blainville’s beaked whale is In the Hyperoondontinae sub-family and the Ziphiidae family.

All odontocetes share some common life history features. They are all long lived and usually give birth to only one offspring. The gestation period is approximately about one year for most odontocetes and neonates are relatively large compared to other mammalian offspring (Chivers, 2002). The offspring are able to swim almost immediately right after birth. Sexual dimorphism varies between species but in odontocetes, males tend to be larger. As opposed to most mysticetes that have seasonal breeding periods, mating season in odontocetes is more variable and happens throughout the year for some species (Perrin and Reilly, 1984).

The following paragraphs present a brief overview of the life histories of two of the species studied: the Atlantic bottlenose dolphin *Tursiops truncatus* and the false killer whale *Pseudorca crassidens*. The life histories of the long-finned pilot whale *Globicephala melas* and the Blainville's beaked whale *Mesoplodon densirostris* will be presented in more detail in their respective chapters.

Atlantic bottlenose dolphin *Tursiops truncatus* (Montagu, 1821)

Bottlenose dolphins are probably the best known species of odontocetes. Ancient Greeks described them as messengers of Poseidon and helpers of humankind and dolphins were associated with River gods in the Hindu mythology. This species is probably the most familiar cetacean as it has been successfully kept in captivity for many decades and is presented in the media. Bottlenose dolphins are found worldwide, and tend to be more frequently observed in coastal and continental shelf waters and usually remain within 45° North and South of the equator. (Jefferson et al., 2008) Individuals are usually 1.9 to 3.8 meters in length and can weigh up to 600 kg. Males tend to be slightly larger than females. Body coloration ranges from light gray to almost black depending on the populations and the belly is usually lighter gray. The beak is short and distinctly separated from the melon by a crease. The "dorsal fin is tall and falcate and set near the middle of the back. The flippers are recurved and pointed at the tips." (pp 217).

This species behavior and life history are amongst the best known for any cetacean as most of the research both in the field and in captivity has been conducted with *Tursiops truncatus*. Bottlenose dolphins are opportunistic feeders and have been reported foraging on fish, cephalopods and crustaceans depending on the habitat they occupy. This species is highly social and groups of up to 20 individuals although larger herds of up to several hundred individuals can be observed in the open ocean. Bottlenose dolphins are often observed travelling with other cetacean species and hybridization cases have been reported.

Figure 1. An adult Altlantic bottlenose dolphin (*Tursiops truncatus*) and her calf.

This species is so widely spread that it has been difficult to assess the range and size of the different populations and therefore assess the vulnerability of this species to anthropogenic threats. *Tursiops truncatus* has been hunted in the Black sea, Japan and Taiwan and is often observed depredating or as by-caught in many fisheries such as long-line, gill nets and trawlers. This species is currently listed as *least concern* on the IUCN status database.

False killer whale *Pseudorca crassidens* (Owen, 1846)

Pseudorca crassidens is part of the often called ‘black fish’ along with pygmy killer whale, pilot whale and melon headed whales. Individuals are usually 5 to 6 meters in length and can weigh up to 2,000 kg. The false killer whale has a “long, slender, cigar-shaped body, a rounded overhanging melon and no discernable beak… The flippers have rounded tips and a characteristic hump on the leading edge.” (Jefferson et al., 2008, pp

171) Overall the animals are dark grey to black with a lighter ventral coloration. *Pseudorca crassidens* are found in tropical and subtropical regions, primarily in deep waters. They have been observed feeding on schooling fish and cephalopods as well as larger pelagic fish such as Mahi-Mahi (*Coryphaena hippurus*). Reports of *Pseudorca crassidens* feeding on other marine mammals have also been recorded but do not seem to represent the majority of their diet.

False killer whales are highly social animals and have often been observed sharing food. Groups vary in size from 10 to 60 individuals and have shown some site fidelity around the islands of Hawaii. Because of the increasing concern of the detrimental interactions between this species and the Hawaiian longline fishery, a take reduction team has been implemented under the Marine Mammal Protection Act (1972) to find solutions to both protect the island associated false killer whale population as well as to maintain the fishery.

False killer whales are known to be amongst the species that mass strand and are currently listed as *data deficient* on IUCN status species list.

Figure 2. A female false killer whale (*Pseudorca crassidens*)

RESEARCH QUESTIONS AND STUDY OBJECTIVES

It has been assumed that during echolocation, all odontocetes rely on the fat-filled panbone regions of their lower jaw to hear returning echoes. However, given the important morphological variations in size and shape of the head, melon, rostrum and teeth, there is a possibility that not all odontocete species hear the same way. Recent work – through modeling and empirical studies – has emphasized that variations between species may exist. In addition, multiple loci for hearing reception may also exist and depend primarily on the frequency range of the acoustic signal.

The main goal of this project is to look at hearing ranges in different species of odontocetes in order to obtain a better understanding of the processes by which the entire head acts as an acoustic receiver. This project investigates the potential hearing pathways in different odontocetes species using a non-invasive physiological test called auditory brainstem response (ABR).

Additionally as part of better understanding hearing processes in echolocating whales and dolphins, a study was conducted to investigate the echolocation ability of a false killer whale in the presence of an echolocation disrupter.

This projects aimed to answer three major questions:

1. **What are the hearing ranges of different species of odontocetes?**
2. **What are the regions of high sensitivities on the head of *Tursiops truncatus* and *Pseudorca crassidens* to echolocation click stimulation?**
3. **Can a deterring acoustic device affect the echolocation ability of *Pseudorca crassidens* ?**

To answer these questions, the project included multiple sub-objectives:

- Developing and improving auditory evoked potential tools and procedures in order to test the hearing of marine mammals.
- Collecting audiograms of multiple species.

- Developing a jawphone or a piezo-electric projector embedded in a suction cup.
- Determining the regions of high sensitivities for *Tursiops truncatus*.
- Determining the regions of high sensitivities for *Pseudorca crassidens*

Audiograms of different species of odontocetes

Frequency sensitivity was evaluated by calculating the minimum audible intensity as a function of the sound frequency. The first part of this project aimed to obtain audiograms by collecting hearing measurements of two new species, the long-finned pilot whale *Globicephala melas* and the Blainville's beaked whale *Mesoplodon densirostris*. The relevance of these audiograms was evaluated in view of the increasing anthropogenic impact on marine life.

Hearing sensitivities regions in *Tursiops truncatus* and *Pseudorca crassidens*

The hearing sensitivities regions were investigated for two species: *Tursiops truncatus* and *Pseudorca crassidens*. These experiments were based on previous work done by Møhl *et al.* (1999) and although one experiment has already been conducted with the Atlantic bottlenose dolphin, it was replicated for comparison purposes. Both experimental subjects have been housed in the same environment for over 20 years and complete medical records were available. The audiograms of both subjects showed a profound broadband high frequency hearing loss. A contact hydrophone was built specifically for this experiment and a computer-generated broadband click was used as the acoustic stimulus.

Echolocation disruption in the false killer whale

The final chapter investigated the effects of an echolocation disruption system in *Pseudorca crassidens.* The animal's performance during a detection task was evaluated and compared between baseline and treatment conditions. The application of such device to mitigate false killer whale bycatch in the Hawaiian long line fisheries was also evaluated.

CHAPTER 2: PORTABLE AUDITORY EVOKED POTENTIAL SYSTEM TO ASSESS ODONTOCETE HEARING

NB: This chapter is currently in press as

Pacini, AP, Nachtigall PE and Kloepper, LN (2011) Portable auditory evoked potential system to assess odontocete hearing. In: The Effects of Noise on Aquatic Life. Popper, A.N. and Hawkins, A. (Eds) Springer: New York, in press

INTRODUCTION

The hearing of marine mammals has been extensively studied in the last decades and focused primarily on species available in captivity such as the bottlenose dolphin *Tursiops truncatus*. Recent work has shown that mass stranding events could be related to anthropogenic sound exposure such as naval sonar activities, seismic surveys, or oil drilling exploration. Although little is known about the hearing abilities of most odontocete species, it is primordial to be able to obtain a rapid hearing assessment of stranded animals. A portable system was designed to be easily transported and used during stranding events, at rehabilitation facilities, and in laboratory settings. This overview provides a description of this system as well as an overview of the data collected so far.

MATERIALS AND METHODS

The system was first presented by Taylor et al. (2007), and additional modifications have been implanted to provide more flexibility and portability to the system.

Background Noise Measurements

For any new facility or stranding, background noise measurements were collected with a RESON TC-4032 (−170 dB re 1 V/mPa; Slangerup, Denmark) connected to a Microtrak II two-channel digital recorder (M-Audio, Irwindale, CA). Noise files were recorded at a 96-kHz sampling rate with one channel with no gain and the second channel with variable gain. Files of 1-s duration were then extracted, averaged, and analyzed with Adobe Audition 3.0 and a custom Matlab program.

Acoustic Stimulus Presentation

At the start of each data session, the animal was stationed at the surface and three electrodes encased in soft latex rubber suction cups were positioned on the animal’s back. The acoustic stimuli were presented through a hydrophone positioned 1 or 2 m in front of the animal’s ears at a depth of 50 cm. Different hydrophones were used depending on the range of frequencies tested: an ITC 1032 (Santa Barbara, CA) for frequencies between 4 and 50 kHz and a RESON 4013 for frequencies above 50 kHz. Because of the limited time usually available during stranding events, hydrophones were normally calibrated after the data collection.

Sound stimuli were sinusoidally amplitude-modulated (SAM) tone bursts. The tones were usually modulated at 1,000 Hz based on modulation rate transfer functions obtained in the past or before the hearing measurements. For frequencies below 50 kHz, the update rate was 256 kHz and was raised to 512 or 800 kHz for frequencies above 50 kHz. The tones were digitally synthesized with a custom Labview program and a National Instrument PCMIA-6062 E DAQ card (Austin, TX) implemented in a laptop. The tone bursts were normally 19 ms in duration followed by 30 ms of silence so that the acoustic stimuli were presented at a rate of 20 ms^{-1}. Output voltages were measured peak to peak with a Tecktronix TPS 2014 oscilloscope (Beaverton, OR) and were then converted to equivalent root mean square voltages (peRMS) to calculate the sound pressure level (SPL) for each individual frequency. SPL was varied in 1 to 10 dB steps with a Hewlett-Packard P-350D (Palo Alto, CA) attenuator.

Auditory Brainstem Response Recording (ABR)

Three gold human EEG electrodes embedded in latex suction cups were used to collect the animal’s neurological responses to the acoustic stimulus. The first electrode was positioned 5 cm posterior to the blowhole, the second electrode on the back, and the third ground electrode on the dorsal fin of the subject. The three suction cups were connected to a Grass CP-511 bioamplifier (West Warwick, RI), and the signal was amplified 10,000 times and filtered from 300 to 3,000 Hz. Additional filtering was performed by a Krohn-Hite 3384 bypass filter (Brockton, MA) with similar settings. The response signal was then digitized at a 16-kHz rate with the same PCMIA computer card that generated the acoustic stimulus. A complete record consisted of collecting and averaging 1,000 responses, which were 26 ms long and triggered with the acoustic stimulus.

Figure 3: Travel system

Data Analysis

During a stranding event, a complete audiogram can be collected in less than an hour and can include up to 10 frequencies ranging from 4 to 128 kHz. For each frequency, an average of seven stimulus-level trials was necessary to obtain a threshold. SPL was decreased until no response was visible for at least two trials. Previous work has shown that SAM tone bursts generate an envelope following response (EFR) (Nachtigall et al. 2007; Popov and Supin 2007). For each frequency and SPL, a 256-point fast Fourier transform (FFT) was performed on a 16 ms window of the EFR. Each FFT provided a frequency spectrum, and the peak response at 1 000 Hz was used to estimate the response of the subject to the acoustic stimulus. For a given frequency, the peak responses at the repetition rate were then plotted as a function of the stimulus SPL.A linear regression was used to calculate the hypothetical zero value that was used as the approximation of the threshold for that frequency. An audiogram consisted of compiling all the thresholds for each frequency and the corresponding SPL threshold (Fig. 4).

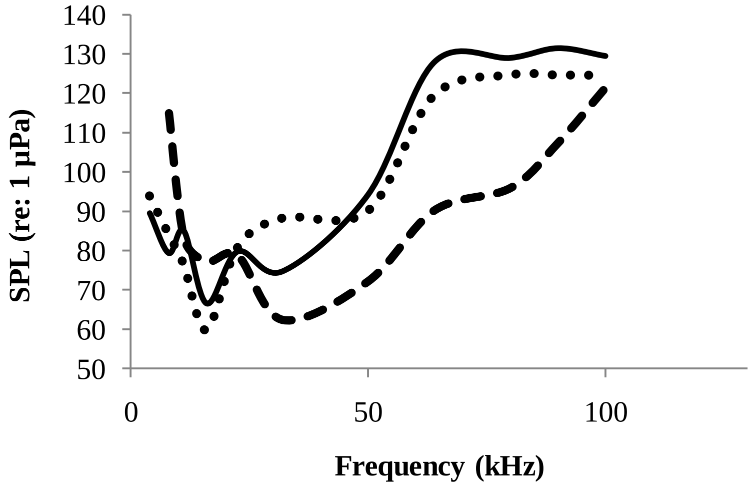


Figure 4: Audiograms of three captive adults *Tursiops truncatus* collected in Moorea, French Polynesia.

CONCLUSION

Absolute thresholds cannot be obtained using the auditory evoked potential (AEP) because of the inherent biological electrical noise. However, previous studies have indicated that behavioral audiograms are very similar to hearing measurements obtained with the AEP technique (Yuen et al. 2005). Therefore, this method seems to be ideal to measure hearing in stranded animals either during their rehabilitation or soon after the stranding event if portable pools are available. In addition to the basic system, additional measurements can be collected. The use of a jawphone to produce sound in localized areas directly on the head of the subject has been used to examine sound paths with the described system (Mooney et al. 2008). Modulation rate transfer function can easily be collected by varying the modulation rate of the acoustic stimulus

(Mooney et al. 2009). This system has been used in many situations including in the laboratory, in collaboration with marine mammal parks, in long-term rehabilitation facilities, in portable pools after stranding events, and during capture and release of wild animals. Continuous improvements will provide a reliable and adaptable platform to use on new species and perhaps on mysticetes.

CHAPTER 3: AUDIOGRAM OF A FORMELY STRANDED LONG-FINNED PILOT WHALE (Globicephala melas) MEASURED USING AUDITORY EVOKED POTENTIALS

NB: This chapter has been published in The Journal of Experimental biology as

Pacini, A. F., Nachtigall, P. E., Kloepper, L. N., Linnenschmidt, M., Sogorb, A. and Matias, S. (2010). Audiogram of a formely stranded long-finned pilot whale (*Globicephala melas*) measured using auditory evoked potentials*. J. Exp. Biol* **213**, 3138-3143.

ABSTRACT

Long finned pilot whales are highly social odontocetes found in the Northern Atlantic Ocean. This species is particularly known for its interaction with fisheries as well as its mass strandings. Even though it has been successfully kept in captivity, limited information is available on the sensory abilities of this species while recent remote tagging work has provided more information about their behavior in the wild. This study investigates the hearing abilities of a rehabilitated two year old male long-finned pilot whale. A complete audiogram was collected using auditory evoked potentials techniques that included measurements of nine frequencies from 4 to 100 kHz presented as sinusoidally amplitude modulated tones. The results indicated that the region of best hearing was between 11.2 and 50 kHz and the subject had relatively poor high frequency hearing compared to other odontocete species. This study emphasizes the importance of collecting basic hearing measurements on new species, understanding diagnostic life-histories as well as continuously increasing the sample size of audiometry measurements within and between odontocete species as animals become available.

INTRODUCTION

Pilot whales have a tendency to follow conspecific "pilot" leaders which may explain their common name and also make them particularly vulnerable to drive fisheries and mass stranding events (Kritzler, 1952; Fehring et al., 1976; Ellis, 1982). Long-finned pilot whales (*Globicephala melas)* inhabit the deep waters of the North Atlantic and feed on squid and other prey normally found down to 600 m (Baird et al., 2002; Aguilar Soto et al., 2008). They do not usually dive as deeply as other pelagic odontocete cetaceans such as beaked whales or sperm whales (Heide-Jorgensen et al., 2002). Long-finned pilot whales are very similar to the short finned pilot whale (*Globicephala macrorhynchus*) and differ primarily by the habitats they occupy, the long-finned pilot whale being found primarily in subpolar oceanic regions while the short-finned pilot whale is found in tropical and subtropical regions. Both species are classified as Data Deficient on the IUCN Redlist and have been included in the 2005 Atlantic pelagic longline take reduction team initiated under the Marine Mammal Protection Act. Early reports (Sergeant, 1962) on pilot whales indicated that they were excellent animals for taxonomic studies because they (Santa Barbara, CA)are" common, widely distributed and prone to strand in herds"(p 412). Both male and female long-finned pilot whales can reach over 6 m in length, appear black with a lighter color blaze in the chest area, and have a distinct and exaggerated bulbous melon (Olsen, 2009) that primarily grows post-partum (Sergeant, 1962). An infant pilot whale, prior to growing the distinctively large melon, may resemble a false killer whale. While the acoustic signals of the pilot whale have long been recorded and analyzed (Scheville, 1964; Busnel and Dziedzic, 1966), and the whales are assumed to echolocate (Evans, 1973), hearing studies have not been attempted despite the fact that they have long been kept successfully in captivity (Kritzler, 1952; Brown and Norris, 1956; Brown, 1960; Bowers and Henderson, 1972). Hearing among members of the subfamily *Globicephalinae* such as false killer whales, pilot whales, melon-headed whales, pygmy killer whales and Risso's dolphins have not been extensively studied. Two studies on the hearing of single adult false killer whales demonstrated high frequency hearing up to 100 kHz (Thomas et al., 1988) with a fully adult animal along with the development of presbycusis with increased age and a lowering of the upper frequency thresholds to near 30 kHz in an older animal (Yuen et al., 2005). A study of an infant Risso's dolphin indicated high frequency hearing up to 150 kHz (Nachtigall et al., 2005) with an apparent similar loss of hearing in an older animal with upper frequency limits just below 100 kHz (Nachtigall et al., 1995). The hearing of the pygmy killer whale, melon headed whale and pilot whale has not yet been measured.

Most young odontocetes hear frequencies over 100 kHz (Nachtigall et al., 2000; Houser and Finneran, 2006) and may lose their high frequency hearing with age (Ridgway and Carder, 1997). While behavioral experiments remain as the ideal method of evaluating hearing and measuring audiograms, more rapid measurements can be obtained using the envelope following response (EFR) auditory evoked potential (AEP) procedure (Supin et al., 2001), which measure the brain waves in response to patterned sounds (Nachtigall et al., 2007). Behavioral experiments require captive trained animals and up to a year to complete, while AEP experiments can be conducted rapidly. AEP measures can even be gathered effectively with catch-and-release experiments, during temporary capture and measurement on board a boat, (Nachtigall et al., 2008) and only require 90 minutes to complete an audiogram. Fortunately, the AEP experiments provide data comparable to that of the behavioral experiments (Yuen et al., 2005; Houser and Finneran, 2006) and can be used with animals that are not fully trained or adapted to laboratory and captive environments.

Estimation of a species or population hearing parameters is best done using a large sample size in a quiet laboratory environment. Unfortunately these experimental conditions are rarely available when dealing with cetaceans. While the quiet laboratory environment with many test subjects is the ideal baseline setting for determining species hearing thresholds, most audiograms of marine mammals, particularly odontocetes, have been estimated from single animals measured in environments of opportunity. Recently most of those hearing measures have been conducted rapidly using evoked auditory potentials (Nachtigall et al., 2007). Species or population hearing estimates and variability must be estimated from individual animal data gathered separately with factors such as age, health and medical record taken into account. The current study involved a two year old male long-finned pilot whale (*Globicephala melas*) that was rescued from a stranding near the beach of Nazaré, North of Lisbon, Portugal on August 27, 2006. The animal was very young, not yet weaned, and in poor health. The whale was first treated at the Sociedade Portuguesa de Vida Selvagem (SPVS) facility in Quiaios – Figueira da Foz; then transferred to the Lisbon Zoo in November 2006 where veterinary treatment continued. Through an extraordinary effort from the zoo staff, the animal's health stabilized and the animal began eating solid squid and fish after about a year. The subject was trained to remain still at the surface while frequencies between 4 and 100 kHz were presented and the audiogram was measured using the AEP envelope following response (EFR) procedures.

MATERIALS AND METHODS

Subject

During its rehabilitation, the whale was trained to station, gate, target control, slide-out and bow; the medical training involved blood and gastric sampling, chuffing for blowhole samples and ultrasound measurements. During the time of the study, the animal also participated in the Zoo's dolphin public presentations three times per day performing bows and a beaching at the end of each show. The subject was further trained to remain still at the surface and accept soft latex suction cup attachments in order to examine its hearing using AEP (Fig 5). Hearing tests were conducted from April 27 to May 3, 2009 in the holding pool of the main show area of the Lisbon Zoo (Fig 6a) at quiet times between, before and after the daily dolphin presentations.

Figure 5: Experimental set-up with the animal wearing electrodes embedded in latex suction cups. The subject remained at the surface one meter away from the hydrophone placed 30cm below the surface.

Tank and background noise levels

The back pool of the Lisbon Zoo dolphinarium measured 10 x 36.5 m (Fig. 6a) and 6 m deep filled with artificial sea water. Water pumps were located over 100 m from the dolphinarium providing a quiet environment with limited ambient noise. It was assumed that the background noise measurements would be very similar between pools. Background noise measurements taken prior to the hearing measurements revealed a quiet back ground noise environment. A RESON TC-4032 hydrophone (-170 dB re 1 V/mPa) (Slangerup, Denmark) was used to measure the ambient noise of the experimental pool. One minute files were recorded on a Microtrack II 2 channel digital recorder (M-Audio, Irwindale, CA, USA) with a sampling rate of 96 kHz. One channel had a 15dB gain while the other channel had no gain to compensate for alienated signals.

Files were then transferred to a laptop and ten files of one second each were extracted using Adobe Audition 3.0, analyzed and averaged using a custom Matlab algorithm.

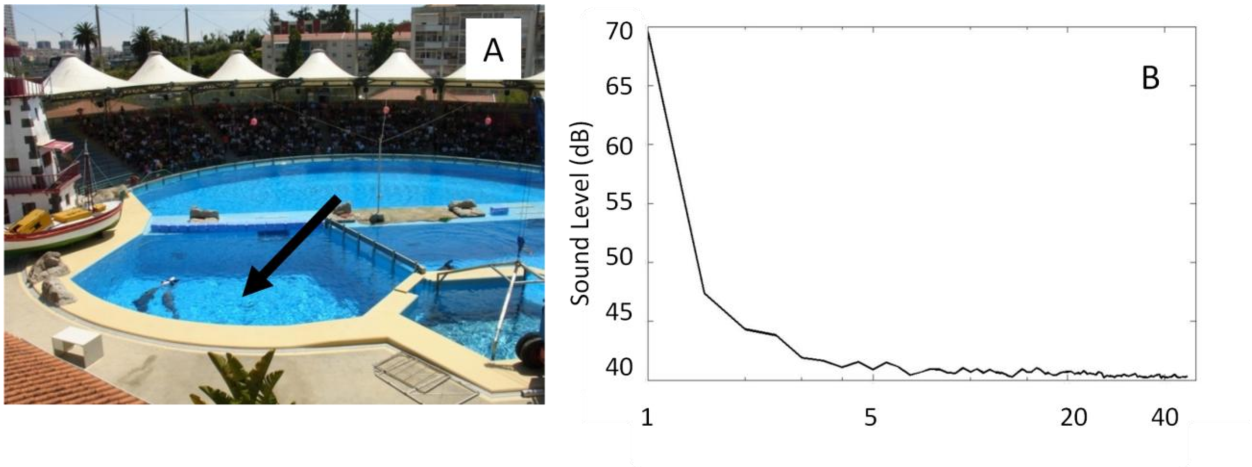


Figure 6: A. Experimental pool in the back of the main show pool at the Lisbon Zoo. B. Tank background noise calculated with a 1024 point FFT and collected with a Reson 4032 with a 96 kHz sampling rate. Sound levels are expressed in dB 1 µPa^2 Hz^{-1}.

Acoustic stimulus generation and presentation

Both acoustic stimulation and electrophysiological measurements were collected using the same system described by Taylor et al. (2007) which has been used both with captive animals (Mooney et al., 2008) and during capture-release experiments (Nachtigall et al., 2007). At the beginning of each session, the animal was positioned at the water surface parallel to the side of the pool and approximately 50 cm away from the tank wall. Three latex suction cups were positioned on the back of the animal and the acoustic stimulation was presented in front of the subject 1m away from its auditory meati (Fig 5). Two hydrophones were used to present the acoustic stimulation: an ITC-1032 (Santa Barbara, CA, USA) for frequencies between 4 and 50 kHz and a Reson TC-4013 (Slangerup, Denmark) for frequencies above 50 kHz. Because of the curvature of the experimental pool, the hydrophones were positioned approximately 1 m away from the tank wall in order to be in front of the animal at a 30 cm depth. Each hydrophone was calibrated prior to the hearing measurements.

For the audiogram measurements, sound stimuli were sinusoidally amplitude modulated (SAM) tone bursts. The tones were digitally synthesized using a custom Labview program using a National Instruments PCMIA-6062 E DAQ card (Austin, TX, USA) implemented in a laptop. The tone bursts were 19 ms in duration followed by 30 ms of silence so that the acoustic stimuli were presented at a rate of 20 ms^{-1}. The tones were modulated at a rate of 1000 Hz based on the modulation rate transfer function obtained with other species and the update rate was 256 kHz for frequencies below 50 kHz and 512 kHz for carrier frequencies above 50 kHz. Output voltages were measured as Vp-p using a Tektronix TPS 2014 oscilloscope (Beaverton, OR, USA). The measured voltages were then converted to peak-equivalent root mean square voltage (peRMS) by substracting 15 dB. This peRMS voltage was then used to calculate the Sound Pressure Level (SPL) for each individual frequency (Mooney *et al.*, 2008). Sound pressure levels were then varied using a Hewlett-Packard (Palo Alto, CA, USA) P-350D attenuator which could attenuate in 1 and 10 dB steps.

Electrophysiology

The animal's response to the acoustic stimulus was recorded using 3 Grass 10 mm gold EEG electrodes (West Warwick, RI, USA) embedded in latex suction cups. The active electrode was positioned three to five centimeters posterior to the blowhole of the subject, the second electrode on the back and the third one acted as a ground and was positioned on the dorsal fin of the subject (Fig 5). The three sensors were connected to a Grass CP-511 bio-amplifier (West Warwick, RI, USA) where the signal was amplified 10,000 times and filtered from 300 to 3000 Hz. The response was filtered again with a Krohn-Hite 3384 by-pass filter (Brockton, MA, USA) with the same settings. Using the same card that generated the acoustic stimulus, the brain response was then digitized at 16 kHz and transferred to the same laptop computer. A record consisted in collecting and averaging 1000 responses that were 26 ms each in duration and triggered with the acoustic stimulus played to the animal.

Audiogram threshold determination & data analysis

A total of 16 sessions were required to complete the experiment, each session lasting 30-45 minutes. A session usually consisted of collecting thresholds for two frequencies and each threshold was measured at least once. A threshold was evaluated using data obtained from an average of seven trials or records for each of the nine stimulus frequencies. The sound pressure level for the first trial was selected according to previous odontocete audiograms (Thomas et al., 1988; Nachtigall et al., 2005; Nachtigall et al., 2008) and was usually 10-20 dB above the published thresholds. The SPL was then varied in 5-10 dB steps until no evoked potential was observed for at least 2 trials (Fig. 6). Previous work has shown that SAM tones bursts generate envelope following response (EFR). For each frequency and SPL, a 256 point Fast Fourier Transform (FFT) was performed on a 16 ms window of the corresponding EFR. Each FFT provided a frequency spectrum and the peak response at 1000 Hz (Fig. 8a) was used to estimate the response of the subject to the acoustic stimulation. For each frequency, the peak responses at 1000 Hz were plotted against the corresponding SPL. A linear regression addressing the data points was extended to calculate the hypothetical zero value which would be used to predict the threshold (Fig. 8b). An absolute threshold could not be obtained with AEP because of the inherent biological electrical noise; nevertheless comparisons between behavioral and ABR techniques have shown that both techniques yield similar results (Yuen et al., 2005).

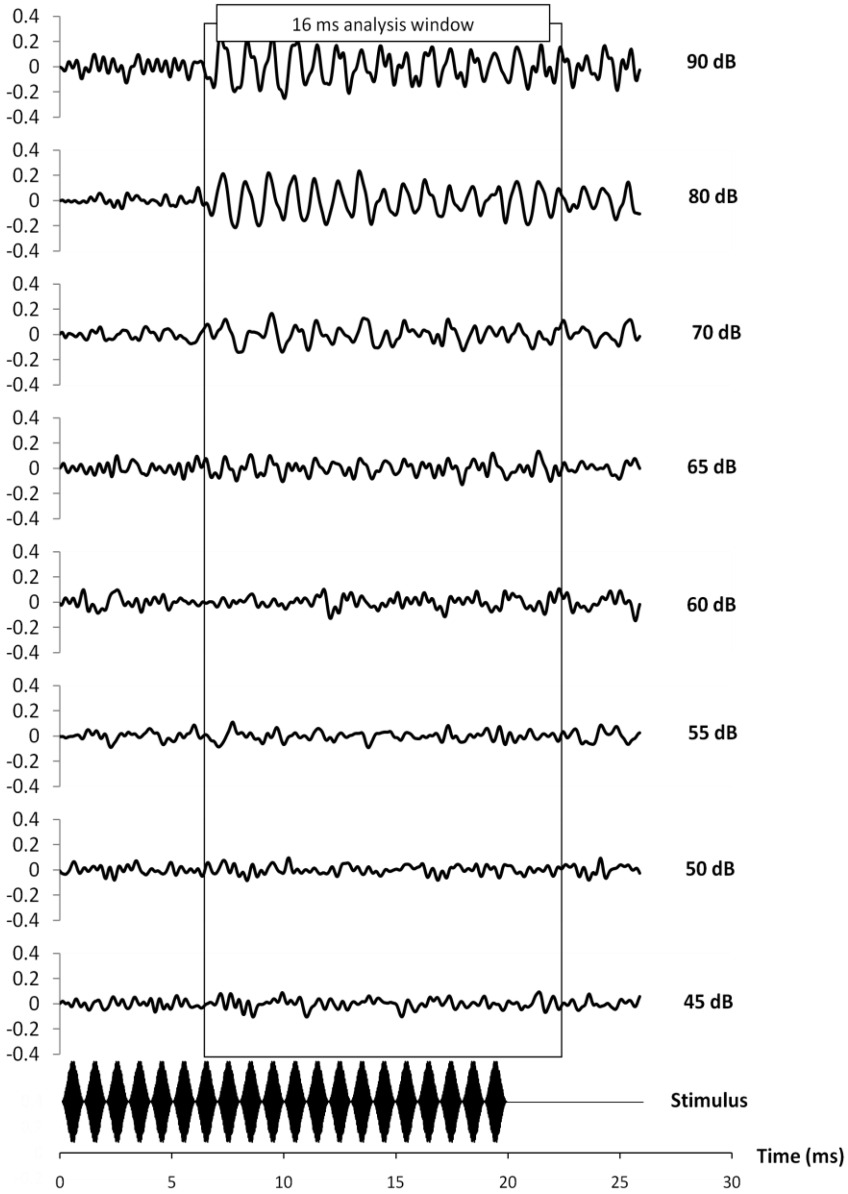


Figure 7: Sinusoidal envelope of stimulus and envelope following response to threshold of 32 kHz tone from 90 to 45 dB re. 1µPa.

RESULTS

The deep waters of the experimental pool of the Lisbon Zoo dolphinarium provided a quiet environment for the hearing measurements. Most of the ambient noise above 2000 Hz was below 40 dB (Fig. 6b) and below the sensitivity of the recording equipment and represented an excellent environment for absolute hearing measurements (Au et al., 2002).

The EFR obtained from the animal followed the typical response obtained with other species (Nachtigall et al., 2005; Popov et al., 2005; Mooney et al., 2008) with a delay of 4-6 ms which corresponded to the latency of the neurophysiological response. When the sound stimulus SPL was well above the threshold level, a completely formed response was recorded and as the SPL decreased, the response disappeared in the ambient biological noise. Fig. 7 shows the EFR response to a SAM tone with a 32 kHz center frequency. At 90 dB, the EFR was complete and closely followed the envelope of the acoustic stimulus; however the EFR started becoming less visible at approximately 65 dB and was completely indiscernible from the ambient noise at 60 dB. The FFT of the signal indicated similar results where the amplitude of the EFR peak at 1000 Hz increased with SPL (Fig. 8a). The linear regression indicated that the threshold for that specific frequency was similar to both the EFR and FFT and was calculated to be at 57 dB (Fig. 8b).

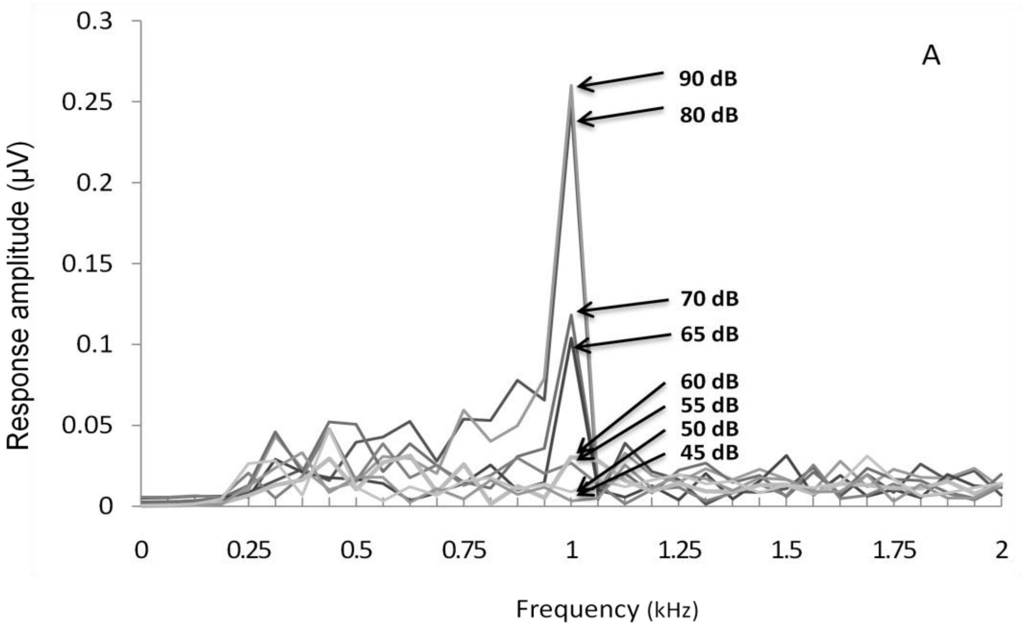


Figure 8A: Fast Fourier Transform of the envelope following response amplitudes of a long-finned pilot whale. Response (µV rms) is to a sinusoidal amplitude modulated tone with a 32 kHz carrier frequency and a 1 kHz modulation rate.

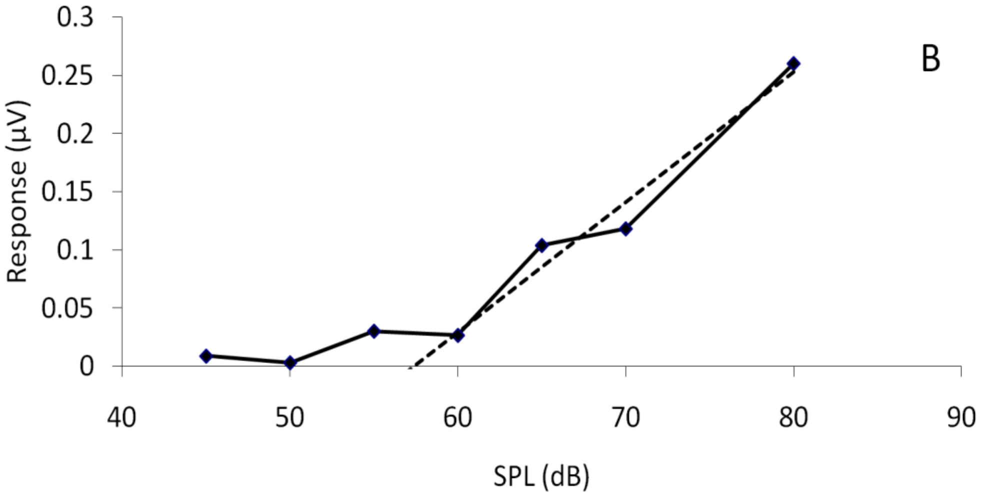


Figure 8B: Plot of the EFR response amplitude versus SPL for the 32 kHz SAM tones. (solid line, diamonds) and the linear regression (broken line) calculated The threshold for 32kHz corresponded to the intersection of the regression line with the hypothetical zero response value. The linear regression was based on 4 points from 60 to 80 dB. The response was calculated here to be at 57 dB re 1 µPa.

The complete audiogram (Fig. 9) had the common U-shape found in mammals and was overall similar to other odontocete audiograms (Johnson et al., 1967; Thomas et al., 1988; Szymanski et al., 1999; Kastelein et al., 2002; Yuen et al., 2005) with a steep slope in the high frequency region and a more leveled slope in the lower frequencies. The region of best hearing was found to be between 11.2 and 50 kHz with thresholds below 70 dB (Table 1). The best hearing was found at 40 kHz with a 53.1 dB threshold. Overall threshold measurements had low values mainly because of the low ambient noise of the pool where the measurements were conducted. The slope of the thresholds became very steep above 50 kHz and the poorest sensitivity was measured at both ends of the frequency spectrum with 77 dB at 4 kHz and 124 dB at 100 kHz.

Table 1. Auditory evoked potential thresholds for each frequency tested.

Frequency (kHz)	**AEP Threshold dB re: 1μPa**
4	76.7
11.2	69.8
22.5	63.9
32	57.4
40	53.1
50	70.2
64	75.7
80	102.6
100	124.4

DISCUSSION

The hearing measurements obtained with this *Globicephala melas* indicate that the animal had overall hearing abilities similar to other odontocete species. The U-shaped audiogram with a region of best hearing up to 50 kHz and threshold values in the 50 dB range emphasize the quality of threshold measurements in a quiet environment. The high frequency hearing region was found to be relatively poor compared to the hearing capabilities of other toothed whales that can hear up to 150 kHz (Johnson, 1967; Nachtigall et al., 2005; Popov et al., 2005). Although these results represent the first audiogram for this species, one should interpret these data carefully. For most odontocetes, only one or two audiograms are available per species (Nachtigall et al., 2000). Extensive work with *Tursiops truncatus* has shown that intraspecific variations in hearing measurements exist and are often related to the age of the subjects. Popov et al. (2007) measured the hearing capabilities of 14 *Tursiops truncatus* and showed that thresholds between individuals varied with frequency. In addition, the authors showed that one animal exhibited significant high frequency hearing loss above 50 kHz. Houser et al. (2008) noted these types of variations while measuring hearing sensitivity in captive *Tursiops truncatus gilli* individuals. While it is important to obtain baseline hearing information, it is also primordial when possible to quantify variability within and between species. The Risso's dolphin audiogram was first obtained by Nachtigall et al. (1995) and was collected with a relatively old individual, using standard behavioral techniques; the results indicated good hearing up to 80 kHz. Nachtigall et al. (2005) collected hearing measurements with a neonate *Grampus griseus* which had excellent hearing up to 150 kHz. In addition, reports of deaf odontocetes have been recorded and emphasize that hearing abilities can vary greatly between individuals (Ridgway and Carder, 1997; André et al., 2003). Research conducted on groups of genetically homogeneous mice and guinea pigs has shown that the response to acoustic injury varied greatly between individuals (Maison and Liberman, 2000; Yoshida and Liberman, 2000). Thus, even with a controlled noise environment, a genetically homogeneous subject pool, interanimal auditory differences persist. These results reiterate how important it is to remain cautious while extrapolating population or species hearing ranges from measurements of a single individual.

Many factors are known to cause high frequency hearing loss. Presbycusis or the loss of hearing with aging is usually characterized with a broadband loss of hearing across the entire frequency range, although this loss might be greater in higher frequencies

(Demeester et al., 2009). Given the young age of the subject of this experiment, it seems very unlikely that presbycusis was a factor in the interpretation of this audiogram. Environmental noise has also been shown to be involved in the loss of high frequency hearing. Given the low background noise measured at the facility, it seems again unlikely that it could have caused the relatively poor high frequency hearing of the experimental subject. It should be noted however, that the causes of the stranding were unknown and that background noise measurements of the facility where the animal was first rehabilitated were not collected. The observed audiogram can potentially be due to an event that caused high frequency hearing loss that occurred prior to the animal's stranding.

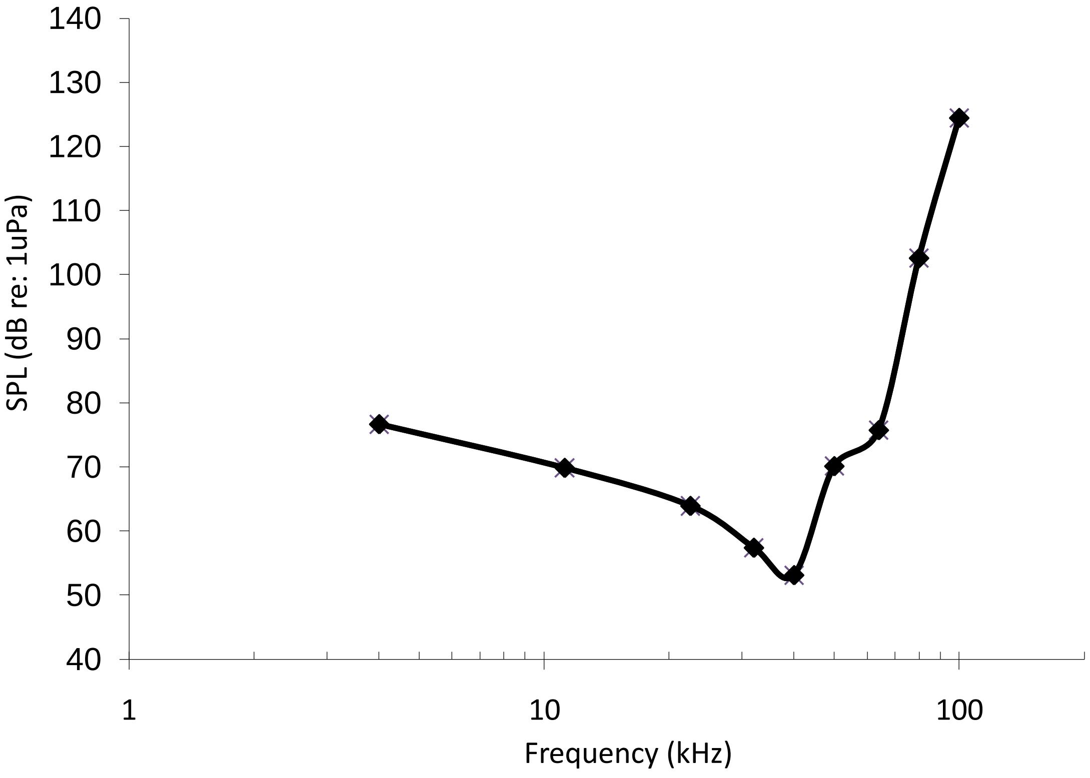


Figure 9: Audiogram of the pilot whale stationing at the surface using auditory evoked potential techniques.

Two more parsimonious explanations can be proposed to account for the relatively poor high frequency hearing. First, the species *Globicephalas melas* might not have good high frequency hearing compared to other odontocete species. In fact, the audiogram is not dissimilar to the hearing sensitivities recorded from *Pseudorca crassidens* (Thomas *et al.*, 1988). Thus, the present results might reflect adequately this species' audiogram. Pilot whales can be up to 6 m long and it has been shown that larger mammals tend to produce lower frequencies (Heffner et Heffner, 1983; Wang et al., 1995). Whistle and click frequency spectra have been collected with free ranging *Globicephala melas* and have shown that this species produced sounds usually lower in frequency than other smaller odontocetes (Steiner, 1981; Weilgart et al., 1990). No outgoing echolocation clicks produced by the subject were collected during the experiment and the Zoo staff noted that during its rehabilitation the subject was overall very acoustically silent compared to the four bottlenose dolphins housed in the same facility. Thus, the hypothesis that the hearing measurements of this subject are representative of the species cannot be completely excluded.

The second hypothesis is supported by the medical records of the animal which indicated that during its initial rehabilitation, the pilot whale was administered ototoxic drugs including aminoglycoside antibiotics gentamycin and netilmicin which are known to frequently cause hearing impairment in humans (Bernard et al., 1978, Brummett et al., 1978). In addition, during the first stages of its rehabilitation, the pilot whale subject was administered several other potentially ototoxic medicines thus increasing the probability that the different drugs had a combined effect on the hearing of the subject (Harpur, 1982). High frequency hearing loss is known to be one of the primary effects of ototoxic medicines well before the appearance of other symptoms such as tinnitus - also known as ear buzzing - and other broadband hearing loss (Tange et al., 1985; Fausti et al., 1992). Previous work by Finneran et al. (2005) compared the hearing sensitivities of two captive beluga whales and showed that one individual had high frequency hearing loss above 37 kHz. Both animals were born in captivity and had similar life histories; however the animal with high frequency hearing loss was administered aminoglycoside antibiotic amikacin which was concluded to be the likely cause of the observed differences in hearing ranges.

In addition to hearing loss, ototoxic medicines are known to cause tinnitus, distorted hearing, a feeling a fullness in the ears as well as dizziness or vertigo, which can be measured in human patients, but are difficult to quantify in animal subjects. The implications of such side effects of ototoxic medicine must be taken into account especially in the case of temporary rehabilitation. A loss of high frequency hearing might result in a decrease of echolocation performance as well as a lowering of foraging abilities.

These results show limited high frequency hearing but do not demonstrate high frequency hearing loss because no measurements were collected prior to administering the drugs. This study provides basic information regarding the hearing capabilities of the long finned pilot whale *Globicephala melas*, but the extrapolation to all long-finned pilot whales and their close relatives the short-finned pilot whales must be tempered by the possibility of potential effects of ototoxic medicine on hearing abilities.

CHAPTER 4: AUDIOGRAM OF A STRANDED BLAINVILLE'S BEAKED WHALE (*Mesoplodon densirostris*) MEASURED USING AUDITORY EVOKED POTENTIALS

This chapter was published in the Journal of Experimental Biology as

Pacini, A.F., Nachtigall, P.E. , Quintos, C.T. , Schofield, T.D. , Look, D.A. , Levine, G. A. DVM and Turner J.P.(2011) Audiogram of a stranded Blainville's Beaked Whales (Mesoplodon densirostris) measured using auditory evoked potentials. *J. Exp. Biol.***214**(4): 2409-2415

ABSTRACT

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 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.

INTRODUCTION

Beaked whales are deep diving and cryptic animals belonging to the Ziphiidae family containing 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," the reduction in the number of erupted teeth and the presence of converging throat grooves (Jefferson, Webber and Pitman, 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 temperate and tropical areas. They are usually found in waters with depths around 1000 m with a steep bathymetry where they are assumed to forage (Baird et al., 2006) on squid and deep water fish (Mead, 1989; Santos et al., 2001).

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, Webber & Pitman, 2008). Most animals exhibit oval white scarring presumably from cookie cutter 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 200m (Johnson et al., 2004). Their foraging dives have a mean duration of 47 min and are at an average 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, 2008; Tyack et al., 2006).

The properties of the far field echolocation clicks were obtained when a conspecific fortuitously echolocated on an acoustically-tagged animal. Blainville's beaked whale echolocation signals have been compared to those produced by a variety of bat species. They produce two distinct echolocation signals (Johnson et al., 2006; Madsen et al., 2005). During the search phase, they emit a long click, approximately 271 µs 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 *Mesoplodon densirostris* terminal buzz clicks have no frequency modulated component, a broader bandwidth and a shorter duration (105 µs).

Much of the interest in beaked whales in recent years 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 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 3 Blainville's beaked whales occurred and was also linked to the use of mid-frequency active sonar (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). While 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 behavior and physiology. Regardless of the cause 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.

It is important to understand the effects of anthropogenic sounds on marine mammals (Tyack, 2008; Nowacek, 2007), and tremendous efforts have been invested into understanding and quantifying the human contribution on ocean noise, designing integrative models to predict ocean noise and into conducting research on marine species that are likely to be at risk (National Research Council, 2003; 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) as well as in the long term (Tyack, 2008) have shown that anthropogenic sound is likely to affect marine mammal populations. Southall et al. (2007) noted that carefully controlled studies of hearing sensitivity, particularly for high-priority species such as beaked whales, were a “critical information need”. 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 europeaus* has been obtained (Cook et al., 2006; Finneran et al., 2009). The use of 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 (ASSR) - yields results comparable to more traditional behavioral audiograms (Yuen et al., 2005; Finneran & Houser, 2006) allowing hearing measurements of species found in non-laboratory settings to be obtained such as oceanarium animals (Szymanski et al., 1999) as well as temporarily caught odontocetes (Nachtigall et al., 2008) and long-term rehabilitated odontocetes (Pacini et al., 2010).

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

MATERIALS AND METHODS

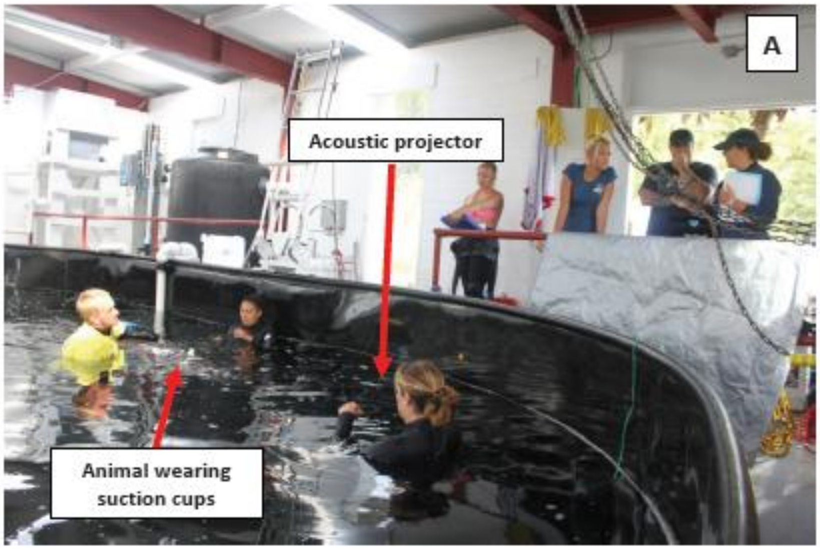
Subject

The male sub-adult *Mesoplodon densirostris* was found stranded on the morning of August 16th,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 University of Hawaii Hilo Cetacean Rehabilitation Facility (HCRF). The whale was 3.5 meters long and weighed approximately 800 kg. Once at the rehabilitation center, he 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 it was undergoing medical treatments from August 16th until August 18th. The animal was lightly restrained during the hearing measurements while monitoring its respiratory rate. Overall behavior indicated that there was no aversive reaction to the presentation of sound or the hearing measurements.

Intensive care of 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 August 29th, 2010.

Tank and Background noise measurements

The animal was housed in the covered oval rehabilitation pool of the Hilo rehabilitation center. The oval pool was 9.8 m long, 7.3 m wide and 1.5 m deep. Water pumps and filters were turned off during hearing measurements to limit masking background noise (Figure 10.A). The background noise was measured using a Reson TC-4040 hydrophone (- 206 dB dr 1V/mPa; Slangerup, Denmark) and recorded as one minute files with a Microtrack II 2 channel digital recorder (M-Audio, Irwindale, CA, USA) with a 96 kHz sampling rate. Alienated signals were compensated by having a channel with no gain and the other channel with a 15 dB gain. Ten one second files were extracted using Adobe Audition 3.0, analyzed, fast Fourier transformed using a 1024 point FFT and averaged with a customized Matlab algorithm (Figure 10.B)



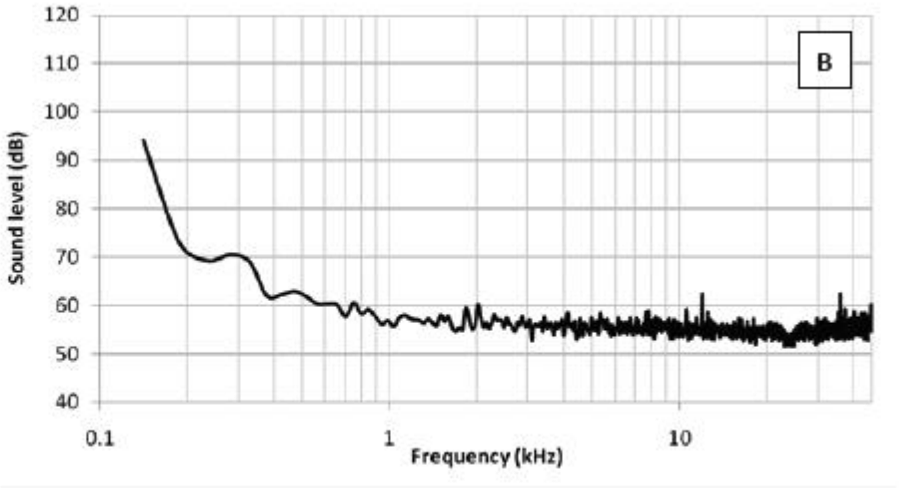


Figure 10: (A) Rehabilitation pool where the hearing measurements were conducted. The water pumps and filters are visible in the back and were turned off during the auditory tests. The projector was positioned one meter away from the animal's head while the whale was lightly restrained (B) Tank background 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 1 $\mu Pa^2 Hz^{-1}$.

Acoustic stimulus

The AEP measurement system used during the hearing measurements was similar to the equipment presented by Taylor et al (2007) and used in the Mooney et al. (2008), Nachtigall et al. (2008) and 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 Instrument PCMIA-6062 E DAQ card (Austin, TX, USA) implemented in a laptop (Figure 11). The tone bursts were 20 ms in duration and followed by 30 ms of silence yielding a 20 s^{-1} 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 and increased to 512 kHz for frequencies between 50 kHz and 100 kHz and 800 kHz for frequencies above 100 kHz. Peak-to-peak voltages (Vp-p) were measured using a Tektronix TPS 2014 oscilloscope (Beaverton, OR, USA) and then converted to peak equivalent root mean square (r.m.s.) voltages by substracting 15 dB. 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 sound pressure level (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 constraint 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.

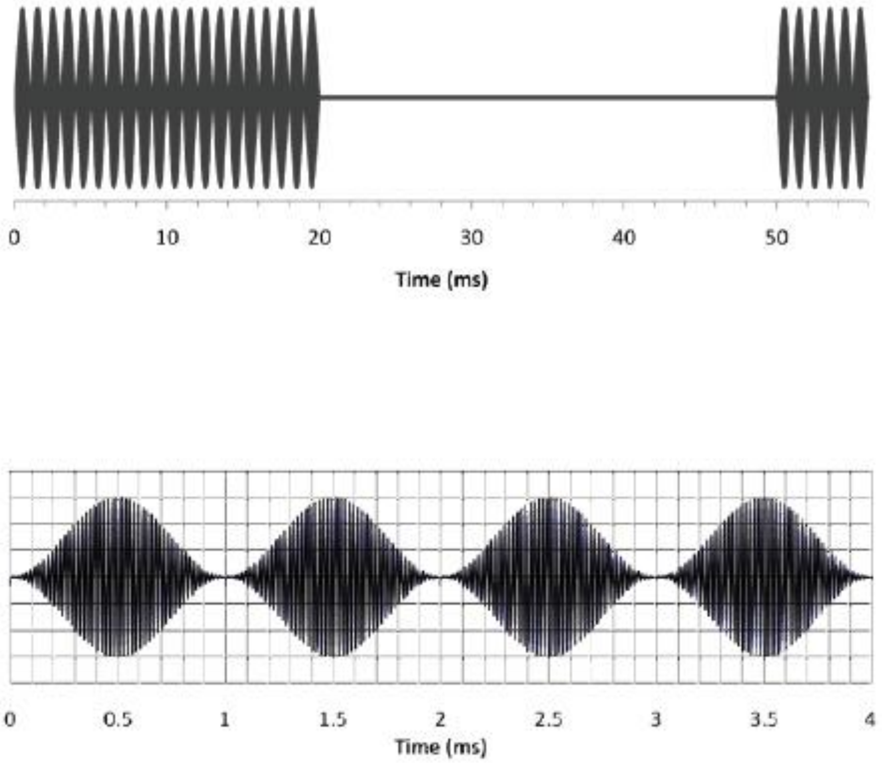


Figure 11: (A) Sinusoidal amplitude modulated stimulus using during the audiogram acquisition (Frequency 150 kHz) (B) 4 ms seconds close-up of the same signal.

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 off to the right side of the animal's head, the reference on the back of the subject while the ground electrode was positioned laterally on the animal's dorsal fin (Figure 12). 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 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 sec and consisted of collecting and averaging 1000 responses, each 26 ms long and triggered with the acoustic stimulus (Mooney et al, 2008;Pacini et al., 2010).

Figure 12 : Experimental set up, the active suction cup is visible behind the blow hole of the beaked whale. The animal was lightly restrained during hearing measurements.

Data analysis

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

Each threshold was calculated using at least 7 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 (Pacini et al., 2010; Finneran et al., 2009; Nachtigall et al., 2008, Johnson, 1967) The SPL was then varied in 5-10 dB steps until the evoked potential response was low enough so as 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 (Figure 13) (Supin et al., 2001; Nachtigall et al., 2007). At each SPL, a 16 ms window of the EFR was analyzed using 256 point fast Fourier Transform (FFT). The peak response at 1000 Hz on the obtained frequency spectrum was used to estimate the animal's response to the acoustic stimulus (Figure 14). 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 (Figure 15). The auditory brainstem response (ABR) technique does not yield absolute thresholds due to the inherent biological noise, but previous work has shown that the results are comparable to behavioral audiograms. (Yuen et al., 2005)

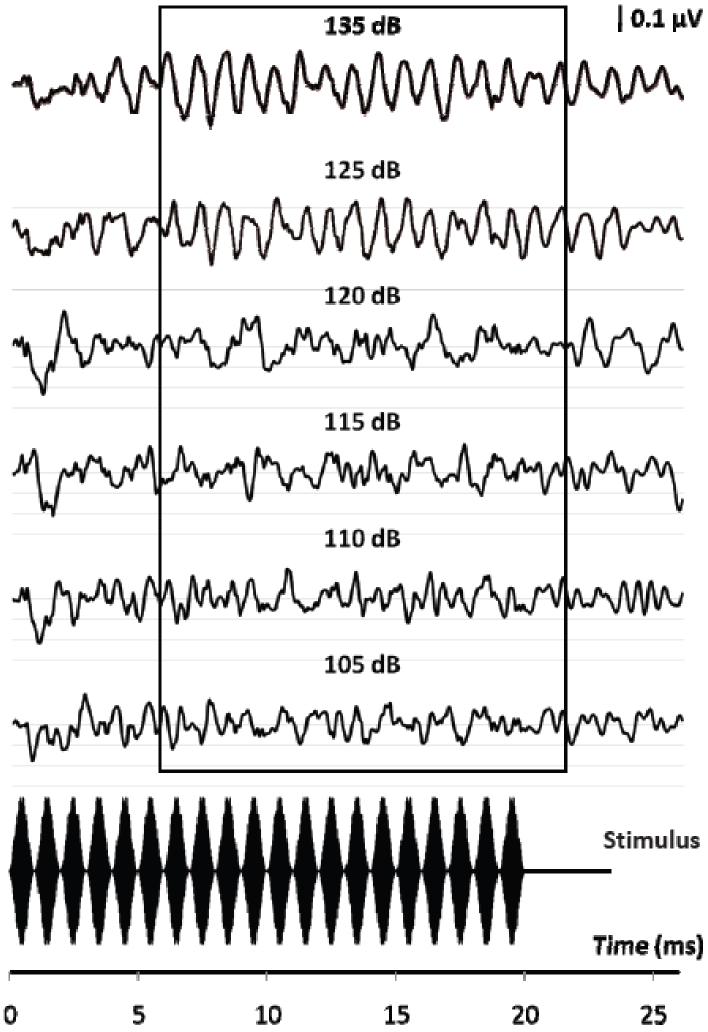


Figure 13: Sinusoidal envelope following response (EFR) to threshold of 150 kHz SAM tone from 105 to 135 dB re. 1 µPa. The box indicates the 16ms analysis window for the Fast Fourier transform.

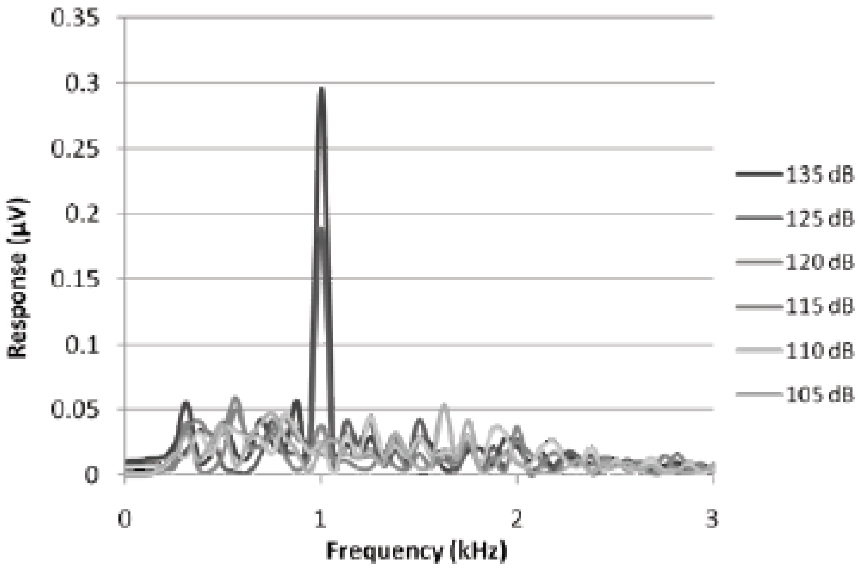


Figure 14: 256 point FFT of EFR. The response is expressed in µV r.m.s. to a 150 kHz SAM tone with a 1 kHz modulation rate.

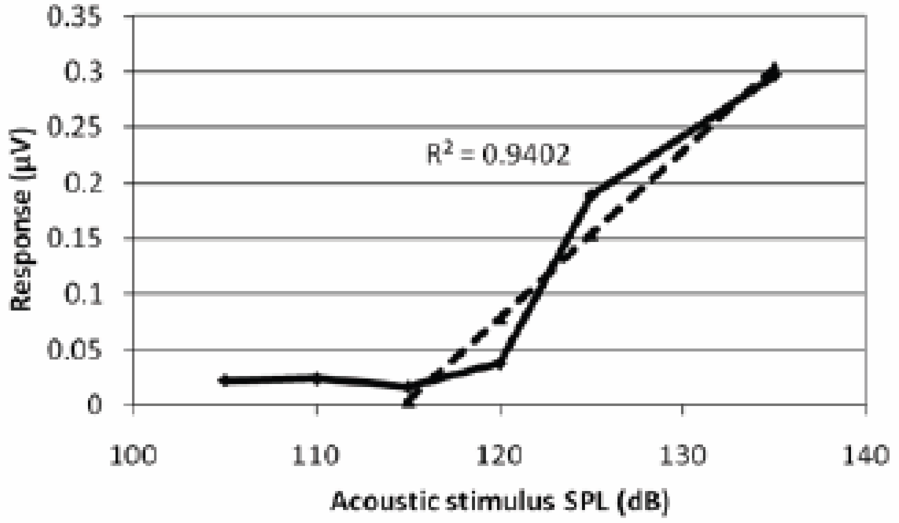


Figure 15: Plot of the EFR response amplitude at 1 kHz versus the corresponding acoustic stimulus SPL for the 150 kHz tone (solid line) and the linear regression (broken line) calculated from 115 to 135 dB. The threshold was estimated as the interestion of the regression line with the zero value.

RESULTS

The rehabilitation pool at HRCF provided a relatively quiet environment for the hearing measurements because most of the energy was below 1 kHz. Above 1 kHz, most of the ambient noise was below 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 Figure 10B.

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 with other odontocete species (Nachtigall et al., 2008; Finneran and Houser, 2006; Supin, 2001; Szymanski et al., 1999). 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. Figure 15 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 (Figure 16) had the common U-shape found in mammalian species and the hearing range was similar to typical odontocete audiograms (Johnson, 1967, Houser et al., 2008, Thomas et al., 1988) 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 broad 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 around 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 the 50 dB ranges in this relatively quiet environment (Au et al., 2002).

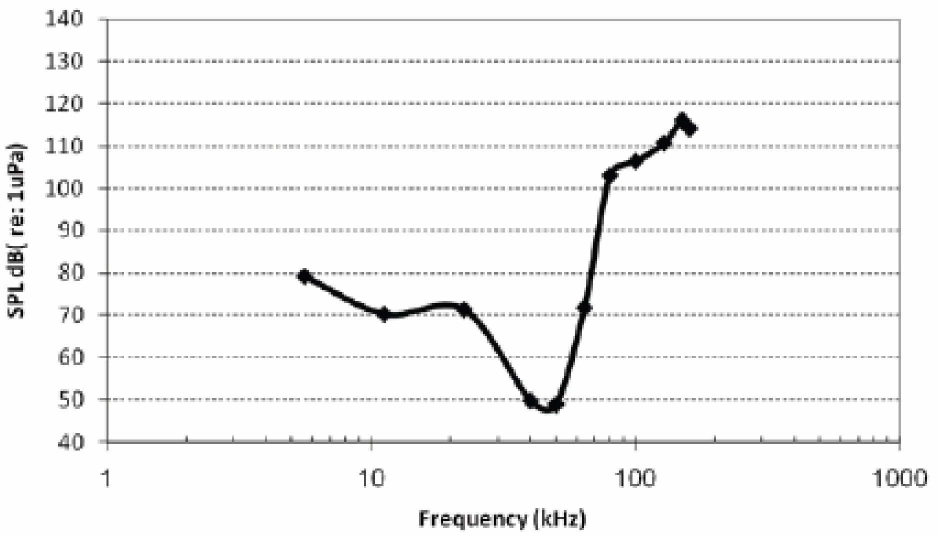


Figure 16: Audiogram of the *Mesoplodon densirostris*

Table 2: Audiogram thresholds of the *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

DISCUSSION

The audiogram of this *M. densirostris* is similar to audiograms of other odontocete species with a U-shape curve and good hearing in the human ultrasonic range. However, unlike most audiograms, the range of best hearing is relatively narrow and the upper frequency limit plateaus around 100 kHz. While such features are sometimes observed with other odontocete individuals (Houser et al., 2003; Finneran et Houser, 2006), it is difficult to assess whether or not they are representative of the species or simply specific to the individual tested. Thresholds below 50 dB indicate 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 is relatively low compared to small odontocetes that have an area of best hearing around 40-50 kHz. In young bottlenose dolphins, the best hearing usually lies around 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 kHz 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 the Gervais' beaked whale *Mesoplodon europeaus* (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 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 that particular study, 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 comparable results to far-field audiograms in bottlenose dolphins (Finneran et al., 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 to 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. europeaus* was between 40-60 kHz. Additionally, while no responses could be detected above 80 kHz for the *M. europeaus* (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. (2009) - that comparison between jawphone and free field stimulation might have represented a difficulty. Alternatively, the Gervais beaked whale simply did not hear overall 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 animal in this study was a sub-adult male whose teeth had not yet erupted. In comparison, the *M. europeaus* 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; Demeester et al., 2009; Houser et al., 2008, 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* 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 path for sound to reach the ear complex, this gular pathway where sounds enters “the head from below and between the lower jaws…continues toward the bony ear complexes through the internal mandibular fat bodies” (Cranford et al., 2008). While there was no possibility of testing hearing pathways, it should be noted that if the unlikely event that this specific sound path was not directly stimulated, the free-field audiogram would be a partial representation of the frequency hearing range of the *Mesoplodon 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 200m (Tyack et al., 2006: Johnson et al., 2004). 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 et Pollack, 1979).

The audiogram collected in this study - combined with the acoustic data obtained by 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 to 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. (2006) hypothesized that the use of FM signals during the search phase might improve the detection and discrimination of specific prey in a scattered environment and thus "maximizing the net energy return of foraging during long breath-hold dives." (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" (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).

While acoustic tagging research has provided a more comprehensive picture of beaked whales' ecology and behavior, 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 (Dalebout et al., 2002: Reyes et al., 1991). Most of the knowledge about this beaked whale 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, 2009b). MFAS uses frequencies between 1-10 kHz (D'amico and Pittenger, 2009). The Blainville's beaked whale hearing threshold at 5.6 kHz indicated that the animal 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 two weeks after the end of the biannual international Rim of the Pacific (RIMPAC) exercise. The animal died 13 days after it stranded in Maui. At the time of the writing of the manuscript, histopathology of the organ systems have not been 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 et al. (2009), collecting data during a stranding event is not ideal; due to 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* 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 critically important species. This type of research – although not as controlled as laboratory settings – 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.

CHAPTER 5: HEARING SENSITIVITIES USING A CONTACT HYDROPHONE IN THE ATLANTIC BOTTLENOSE DOLPHIN (*Tursiops truncatus*) AND THE FALSE KILLER WHALE(*Pseudorca crassidens*)

ABSTRACT

Hearing pathways have been assumed to be similar for all echolocating whales and dolphins based on morphological data obtained for several species combined with behavioral and electrophysiological work conducted primarily with *Tursiops truncatus*. The jaw hearing hypothesis, as it was originally presented by Norris, has been revisited in the view of recent findings. This study presents the hearing sensitivities of the Atlantic bottlenose dolphin *Tursiops truncatus* and the false killer whale *Pseudorca crassidens* to click like stimuli. The responses were measured using auditory evoked potential techniques. The results obtained with *Tursiops truncatus* were very similar to work obtained in the past. However a zone of good sensitivity – at the tip of the upper jaw - was found in the false killer whale that had not been reported before. These results indicate that sound pathways might vary between species.

INTRODUCTION

As part of their adaptation to the aquatic environment, odontocetes, or toothed whales, have evolved to rely extensively on acoustic cues. One of their major adaptations is the use of echolocation where an animal produces a signal and listens to its echo to localize and discriminate objects in its environment (Au, 1993). Understanding how toothed whales process acoustic information has been the focus of extensive research over for the past 60 years. While the sound production mechanisms are primarily studied using a combination of traditional psychological and acoustic measurements (Kloepper et al., 2010; Lammers et Castellote, 2009; Madsen et al., 2010), the use of medical imaging techniques has allowed researchers to non-invasively investigate the internal mechanisms the animals rely on to produce, listen and process such a wide variety of sounds (Houser et al., 2004; Montie et al., 2011; Cranford et al., 2008). The anthropogenic impact of sound has been of primarily importance in the recent years as several strandings of whales and dolphins have been temporally linked to the use of loud sounds such as military sonar and seismic survey tools (Frantzis, 1998; Cox et al., 2006; Nowacek et al., 2007). With this increasing need to understand the hearing mechanisms of marine mammals, new techniques have allowed researchers to rapidly obtain hearing measurements from stranded animals. The auditory brainstem response or ABR where the brain's response to pattern sounds is studied is often used to collect audiograms of untrained animals (Popov et al., 2007; Nachtigall et al., 2008; Finneran et al., 2009; Pacini et al., 2010; Mann et al., 2010; Mulsow et al., 2011). In the laboratory, this technique provides a unique platform to study in-depth hearing mechanisms (Nachtigall and Supin, 2008; Mooney et al., 2008).

Fraser and Purves (1960) provided morphological evidence that odontocete hearing was similar to terrestrial mammals: the sound enters the head via the ear opening or auditory meatus and follows the typical hearing pathway through the ear canal, the middle and inner ear. Their results were based on dissections and data collected from specimens previously frozen. While their anatomic work is remarkably detailed, their observations were not collected with live animals and required invasive procedures that included the disruption or the alteration of the bulla arrangement. Therefore their results were often questioned and nowadays are difficult to replicate or validate with live animals.

Norris (1968) listed 5 adaptations found in modern odontocetes that he believed were instrumental in understanding both the sound production and sound reception mechanisms. They were: 1.Telescoping (Miller, 1923) or the 'sliding of the maxillary and pre-maxillary bones over other skull bones as the nostrils moved dorsally', 2. The presence of air sinuses which could potentially provide acoustic isolation, 3. The thinning of the posterior region of the lower mandible also referred to as the panbone, 4. The modifications of the tympanic bulla and middle ear ossicles, and 5. 'The development of a cartilage-filled merosostral canal, lined dorsally on both sides by unusually dense premaxillary bones and below by the vomer to form a long subcylindrical channel, running from the median mesethmoid division of the bony nares to the tip of the rostrum."(page 298)

The 'acoustic window' hypothesis (Norris, 1968) has been supported by several experiments. The same year, Bullock and colleagues (1968) demonstrated that the lower jaw and some areas of the melon were the most sensitive to contact acoustic stimulation. Because these measurements required invasive procedures, the conclusions were not widely accepted and called for further examination. Brill et al. (1988) behaviorally tested the ability of an Atlantic bottlenose dolphin to detect a target using solely echolocation when the lower jaw was covered with a neoprene hood blocking the returning echo. Their results indicated that the performance of the subject dropped significantly when the animal was not able to hear the returning echoes. Finneran and Houser (2006) compared behavioral audiograms to hearing measurements collected using acoustic contact stimulation positioned on the panbone region of Atlantic bottlenose dolphin and showed that both methods yielded comparable results indicating that this area of the lower jaw was intricately involved in sound reception. Møhl et al. (1999) used ABR techniques to measure the differences in response as a function of sound source on the head of a bottlenose dolphin. The results indicated that the area of best sensitivity (mid region of the lower jaw) and the region of minimum latency (rear of the lower jaw) were different. They proposed a shaded receiver model where the entire head would act as an acoustic receiver and the acoustic input perceived at the ear would be the combination of both intensity and time delay information, thus allowing the animal to obtain precise information about the characteristics of the return echo from multiple input loci. This study was amongst the first to provide evidence of a potentially more complex hearing mechanism than originally thought.

Sound localization seems to be achieved by 'filtering' cues at different locations on the head of the animal. While measuring minimum audible angles, Renaud and Popper (1975) noted that for low frequencies, the bottlenose dolphin appeared to rely on the external auditory meatus (the outer ear area) but with frequencies above 20 kHz, the animal was using lateral sides of the lower jaw – the panbone – to detect and localize sounds. Surprisingly, their results also indicated acute vertical localization abilities but they did not postulate on potential sound pathways that could be involved; instead the authors argued that the subject was probably using intensity discrimination between the highly sensitive lower jaw and the well isolated melon.

The concept that multiple loci might be available to the animal to discriminate not only frequencies but also differences in time of arrival and sound direction has been explored by Popov and colleagues in a series of experiments which indicated that low frequencies tended to be detected at angles that would correspond to the meatus and high frequency sounds (above 20 kHz) via the panbone (Popov et al., 2003; Popov et al., 2007). Recent work has provided new insights on this problematic, and perhaps a new, vision on how different odontocetes might integrate acoustic information. Koopman et al. (2006) investigated the chemical composition of the mandibular fats in different species of odontocetes and showed that while the structure and overall organization of these fats remain fairly consistent across families with the shortest lipid chain located in the center of the jaw, the actual chemical composition varies with species to perhaps accommodate differences in life history and ecology. A study investigating hearing directionality and pathways in the beluga whale *Delphinapterus leucas* showed that the sensitivity to broadband clicks was similar when the sound source was presented at the tip of the lower jaw or over the panbone region (Mooney et al., 2008). In contrast, the auditory meatus yielded thresholds 16 to 24 dB (12 cm behind meatus) less sensitive. The hypothesis that perhaps the entire lower jaw area was involved in sound reception was also presented by Cranford et al. (2008). Using finite element modeling, the authors simulated sound reception in a Cuvier's beaked whale (*Ziphius cavirostris*) and found that the sound entered the lower jaw via an opening in the lower jaw that they referred to as the 'gular pathway.' These two studies, while very different in terms of methods, provide reasons to suspect that hearing pathways might not only vary with frequency but also among species. Based on these recent findings, the purpose of this study was to measure and compare the free field audiograms and hearing thresholds to broadband clicks presented with a contact hydrophone at different loci on the head of a *Tursiops truncatus* and a *Pseudorca crassidens* using auditory evoked potentials.

MATERIALS AND METHODS

Experimental subjects

The experiment was conducted with two animals housed at the Marine Mammal Research Program floating pen complex in Kane'ohe Bay, HI. The Atlantic bottlenose dolphin *Tursiops truncatus* was a 25 –year old female named BJ. She was approximately 2.5 meters in length, weighed 190 kg and had participated in several echolocation experiments (Ibsen et al., 2007; Li et al., in press). The false killer whale *Pseudorca crassidens* was an approximately 35 year old female named Kina that weighed 540 kg and was 4 meters in length. Kina has also participated in extensive hearing and echolocation research projects (Nachtigall and Supin, 2008; Yuen et al., 2005; Kloepper et al., 2010). All measurements were collected in July and August, 2009.

Acoustic presentation and calibration

The measurements were collected with the system described by Taylor et al. (2007) and used in other auditory studies both in the field and in laboratory settings (Mooney et al., 2008, Nachtigall et al., 2008, Pacini et al., 2010). The acoustic stimuli consisted of sinusoidally amplitude modulated (SAM) tone bursts or click trains. Each was 20 ms in duration followed by 30 ms of silence and was used to elicit the envelope following response (EFR). A 1000 Hz modulation rate was selected for both species based on data available on modulation rate transfer function obtained for odontocetes (Supin et al., 1995; Mooney et al., 2006; Finneran et al., 2007). Acoustic stimuli were created using a custom Labview program and digitized with a National Instrument PCMIA-6062 E DAQ card (Austin, TX, USA) implemented in a laptop computer.

The audiograms were collected as each animal was positioned at the surface one meter away from the 30 cm deep hydrophone. Because both animals are known to have suffered high frequency hearing loss (Yuen et al., 2005; Ibsen et al., 2007) only low frequencies matching known hearing rangeswere tested using an ITC 1032 hydrophone (Santa Barbara, CA, USA). Sound stimuli were presented as sinusoidally amplitude modulated tone bursts. For the bottlenose dolphin, a total of 8 frequencies ranging from 6.7 to 49 kHz were tested. For *Pseudorca crassidens*, 10 frequencies from 4 to 40 kHz were tested. The update rate varied between 128 and 512 kHz depending on the frequency of interest. The outgoing signal played to the animal was monitored using a Tektronix TPS 2014 oscilloscope (Beaverton, OR, USA). For each trial, peak to peak voltage was measured and converted to peak equivalent root mean square (r.m.s.) voltages by substracting 15 dB. A Hewlett-Packard P-350D attenuator (Palo Alto, CA, USA) was used to vary the SPL in 1 or 10 dB steps. These r.m.s. voltages were then used to calculate the sound pressure level (SPL) for each frequency. The system was calibrated prior to collecting data in the experimental pen with a RESON 4040 hydrophone positioned one meter away from the respective projectors.

To investigate the relative sensitivity on the head of each animal, a jawphone consisting of a piezo-electric element embedded in a suction cup similar to Mooney et al. (2008) was used. The jawphone was calibrated in the far field (1 meter away) prior to the experiment using the same ITC 4040 hydrophone. It was estimated that the frequency response of the transducer was linear from 30 to 110 kHz. The sound stimulus consisted of short pulses, 100 µs long with a peak frequency of 50 kHz but with a frequency spectrum from 0 to 110 kHz for both subjects. Due to the previously reported high frequency hearing loss of the subjects (Yuen et al., 2005: Ibsen et al., 2007), the center frequency of the click was selected to cover the widest frequency range possible ensuring the animals would detect the lower frequency portion of the click.

Similar to audiogram measurements, the outgoing signals were monitored using the same Tektronix TPS 2014 oscilloscope and peak to peak voltages were measured while SPL were varied with the same Hewlett-Packard P-350D attenuator in 1 to 10 dB steps. 21 points for *Tursiops truncatus* and 35 points for *Pseudorca crassidens* were selected on the head and in the mouth of each animal and for each point. The jawphone was positioned underwater to avoid trapping air bubbles between the projector and the skin of the animal. Each location was marked to ensure that the suction cup would not move between trials. After the jawphone was positioned, the portion of the area tested was kept out of the water by positioning the animal so that the head remained dry during trials. Because many points were tested, the subject's position was not consistent between loci being tested. The animals were sometimes positioned vertically with their head sticking out of the water, lateral loci were tested by positioning the animal laterally to the deck (while still in the water)and points inside the mouth required the animal to be upside down. As the jawphone relies on near field stimulation, thresholds were calculated as relative thresholds in reference to the panbone region threshold for each animal. Therefore positive thresholds indicate less sensitive loci that required louder sound stimuli to generate a response as opposed to negative thresholds that indicate areas of better sensitivity than the reference.

Electrophysiology

Three Grass (West Warwick, RI, USA) 10 mm gold EEG electrodes embedded in latex suction cups were positioned on the animal. The active cup was positioned 2-4 cm behind the blow hole and the two remaining cups acted as reference were positioned on the back and on the dorsal fin of the animal.

The electrophysiological response was amplified 10,000 times and filtered from 300 to 3,000 Hz with a Grass CP-511 bio-amplifier (West Warwick, RI, USA). A Krohn-Hite 3384 filter (Brockton, MA, USA) was also used to provide additional filtering at the same frequencies. The electrophysiological signal was digitized at a 16 kHz rate using the same acquisition card and laptop. A full record - or trial - took approximately 90 sec and consisted of collecting and averaging 1000 responses, each 26 to 30 ms long and triggered with the acoustic stimulus (Mooney et al., 2008;Pacini et al., 2010).

The complete data set for both animals was collected over the course of two months and one or two sessions were conducted a day. Each session consisted on testing either

2 frequencies for the audiogram or 2 points for the jawphone experiment. Each frequency or jawphone locus consisted of collecting 7 to 9 trials or records. Individual thresholds were calculated based on at least 7 records. For the audiograms, the first level presented to the dolphins was chosen based on previous audiograms obtained with the animals. The SPL was then decreased in 5-10 dB steps until the evoked potential was not discernable from the biological noise for at least two trials. Because of the inherent biological noise generated by the animal, thresholds can only be approximated. Past studies have shown that ABR results are comparable to more conservative behavioral techniques (Yuen et al., 2005; Houser et al., 2006). Both SAM tone bursts and click trains are known to generate a rhythmic envelope following response (EFR) (Popov and Supin, 1998, 2007) which can be used to approximate hearing thresholds. A 16 ms analysis window of the signal was used to obtain a 256 point Fast Fourier Transform (FFT) of the signal. The amplitude of the peak at 1000 Hz – corresponding to the modulation rate - was measured and plotted against the SPL. A linear regression was then fitted to the data and used to calculate the hypothetical zero value which is used to approximate the threshold for a given frequency or locus.

RESULTS

Audiogram

SAM tones are known to elicit rhythmic evoked potential responses. The results for both animals followed the typical EFR with a 4-6 ms delay typical of odontocete response. When the SPL was above the threshold level, a complete EFR was visible and decreased in amplitude as the SPL was lowered. Around threshold, the response was not discernable from the biological noise generated by the animal’s body. Both animals displayed a typical U-shaped audiogram with a steep slope in the high frequency range and a more leveled slope in the lower frequency region (Figure 17). The two audiograms also indicated that the animals had significant high frequency hearing loss with no visible response beyond 40 kHz (128 dB) and 49 kHz (128.3 dB) for *Tursiops truncatus* and *Pseudorca crassidens* respectively. For the bottlenose dolphin*,* the range of best hearing was found between 16 and 40 kHz with thresholds below 95 dB. The false killer whale heard best between 11.2 and 27 kHz with thresholds below 91 dB. The experiments were conducted in Kaneohe Bay, HI, USA which is known to be a very noise environment (Lammers et al., 2008) mainly due to snapping shrimp.

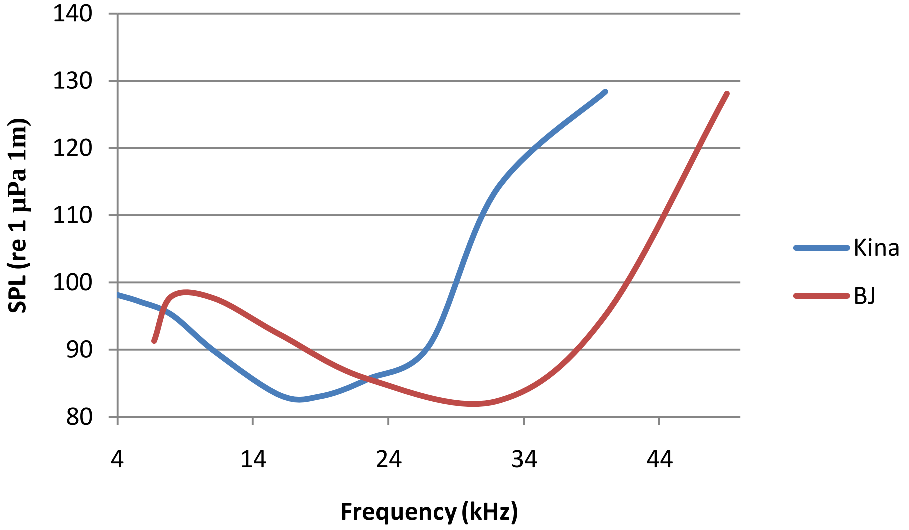


Figure 17: Audiograms of both subjects, in red the *Tursiops truncatus* and in blue the *Pseudorca crassidens*

Jawphone

Similar to SAM tone bursts, click trains generated a rhythmic response that can be used to evaluate and compare thresholds at different locations on the head of each animal. While keeping the stimulus constant between loci (Figure 18), the different thresholds for the sound were estimated using techniques similar to audiogram calculations. For *Tursiops truncatus*, all the results are presented in Table 1. Both sides of the panbone were most sensitive to broadband clicks and the left panbone threshold was arbitrarily assigned a 0 dB value. In comparison, the auditory meati were 15.1 and 16.5 dB less sensitive.

Table 3: Summary of hearing thresholds obtained with jawphone stimulation for *Tursiops truncatus*

points on head	Threshold (Relative SPL dB re 1 µPa)
Melon middle	29.6
Melon base	15.6
Rostrum tip upper jaw	24.6
Rostrum tip lower jaw	17
Right meatus	15.1
Left meatus	16.5
Right panbone	0.3
Left panbone	0
Right melon	19.5
Left melon	18.1
Gular pathway 8cm	23.5
Gular pathway 16cm	15.9
Gular pathway 20cm	13.5
Gular pathway 24cm	6.3
Inside mouth middle	19.5
Inside mouth left side	13.4
Inside mouth right side	26.1
Inside panbone back right side	17.5
Inside panbone back left side	24.7
Inside panbone tooth right side	13
Inside panbone tooth left side	20.2

The melon region was overall fairly isolated with thresholds ranging from 15.6 at the base of the melon to 29.6 dB 4 cm dorsally of the melon/rostrum junction. The upper and lower tips of the rostrum were respectively 24.6 and 17 dB less sensitive than the panbone region.

An increased sensitivity along the gular region was noted with thresholds decreasing from 23.5 dB 8 cm away from the tip of the rostrum to 6.3 dB 24 cm away, which approximately corresponding to the ventral region of the panbones. Inside the mouth of the animals, points at the tip of the lower jaw were between 13.4 to 26.1 dB less sensitive than the panbone. Points were selected on each side of the back of the mouth in the panbone area, close to the teeth the thresholds were 13 to 20.2 dB above the panbone thresholds. Thresholds were also collected on the ventral region of the panbone and thresholds ranged from 17.5 to 24.7 dB. Overall, results on the two sides of the head of *Tursiops truncatus* were comparable with little variation (between 0.3 and 1.4 dB between sides), as opposed to a marked asymmetry in the thresholds obtained inside the mouth of the subject (7.2 to 12.7 dB).

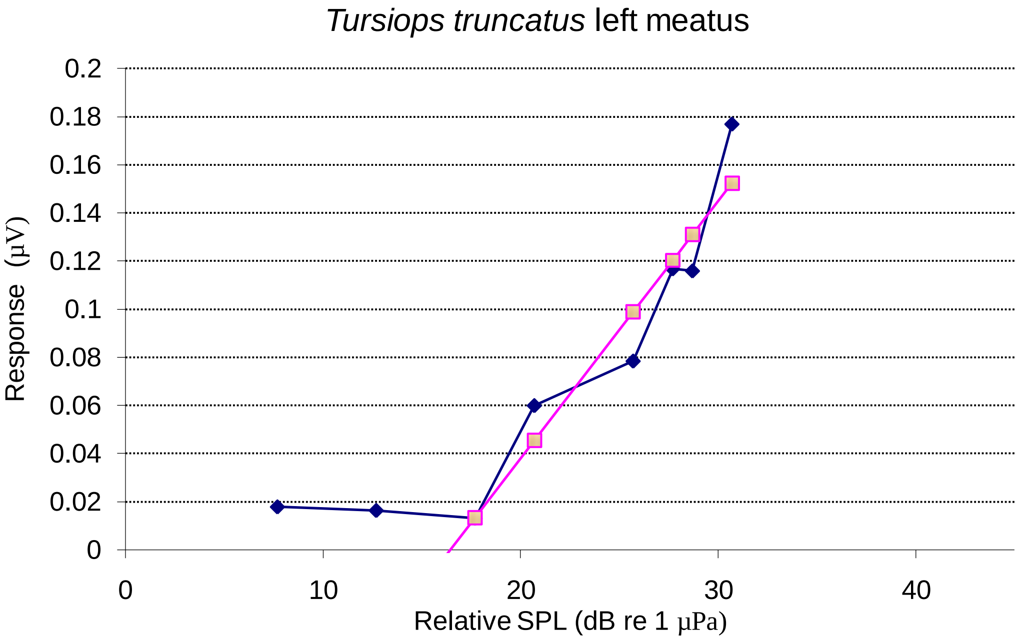


Figure 18: Plot of the EFR response amplitude of the *Tursiops truncatus* at 1 kHz versus the corresponding acoustic stimulus relative SPL for the click like stimulus (blue line) and the linear regression (pink).The threshold was estimated as the interestion of the regression line with the zero value, in this case at 16.5 dB re 1µPa.

For *Pseudorca crassidens*, the most sensitive areas on the panbone were again arbitrarily assigned a zero value threshold (Table 2). Each panbone was tested 3 times to control for variation (SD = 1.08 for right panbone and SD = 0.4 for the left panbone). Three additional points were tested around the panbone area and the upper jaw, they were found to be between 4.4 dB and 13 dB less sensitive than the reference points. No response could be detected on the left side at a locus behind the panbone. The auditory meati had similar thresholds 3.1 dB more sensitive than the panbone.

No increase in sensitivity was observed along the gular region of the lower jaw as thresholds varied between 1.5 and 14.2 dB above the reference. The dorsal area of the melon was very well isolated with thresholds between 7.4 and 11.6 dB, no response was observed on the dorsal region of the melon midline.

The tip of the upper jaw was found to be very sensitive to a click stimulus with a threshold on average 2.3 dB (SD = 1.94 dB) less sensitive than the panbone. Because this point was unusually sensitive measurements were conducted 10 times. The most sensitive record was 12.6 dB more sensitive than the panbone.

Inside the mouth, individual teeth on the lower jaw were tested and showed little sensitivity with +13.4 and 14.6 dB. The subject was missing an upper jaw tooth and the contact hydrophone was applied directly on the scar tissues but no response could be detected. Two points near the panbone were tested on each side and were 2.8 to 10.5 dB above the outer most sensitive region of the panbone. The inside region of the upper lips was tested and the thresholds were 7.7 to 9.5 dB above the reference. One position on the palate was tested close to the tip of the rostrum, and was 2 dB more sensitive than the panbone. Similar to *Tursiops truncatus*, the results were similar on both sides of the head of the *Pseudorca crassidens* with a mean absolute variation of 1.76 dB (SD = 1.35).

DISCUSSION

The audiograms of these two individuals showed important hearing loss in the high frequency domain. Previous work with the same animals – in particular with the false killer whale – has shown that presbycusis or hearing loss due to age was probably causing the poor high frequency hearing (Kloepper et al, 2010). In the bottlenose dolphin, audiograms were periodically collected as part of the annual diagnostics exam and presbycusis was also considered to be the most likely cause of the poor high frequency hearing. Additionally, Kane'ohe Bay is known to be a loud environment (Au et al., 2002; Lammers et al., 2008) primarily due to the high biological activity such as snapping shrimps. Noisy environments are known to exacerbate hearing loss (Mills and

Going, 1982). Loud ambient noise has also been shown to create a semi-masking environment and the relatively high thresholds obtained here reflect these masking effects (Au et al., 2002).

The jawphone results obtained with *Tursiops truncatus* were in relative agreement with previous work obtained with this species (Møhl et al., 1999: Brill et al., 2001; Finneran et al., 2006). Differences with work obtained by Møhl et al. (1999) and Brill et al. (2001) could be attributed to factors such as variations in sound presentation and acoustic stimulus (duration, bandwidth and frequency). Inherent variability in hearing abilities between individuals is also to be expected especially when high frequency hearing loss is observed (Popov et al., 2007; Houser et al., 2008). Interestingly in *Tursiops truncatus*, the panbone was the most sensitive area to broadband clicks even with a high frequency cut-off of 49 kHz. The animal was probably detecting the lower half of the energy present in the click. The asymmetry observed inside the mouth of the subject might be due to the stimulus presentation, as some water remained in the back of the mouth and could have created interference and additional sound pathways. Additionally, maintaining the jawphone at a constant position between trials created difficulty and while extensive precaution was taken to keep the jawphone in place, inter-trial variations could not be completely excluded. Testing the hearing sensitivities in the bottlenose dolphin not only provided additional data to complement previously obtained results, thus increasing the sample size for that species; but it also constituted a control for comparison with another species. Since the results obtained with the *Tursiops truncatus* were in agreement with previous studies, it could be assumed that the differences observed with *Pseudorca crassidens* were not solely due to the experimental paradigm, equipment or sound presentation or even the hearing loss.

Table 4: Summary of hearing thresholds obtained with jawphone stimulation for *Pseudorca crassidens.* Positive thresholds indicate points less sensitive than the reference (panbone) and negative value, points more sensitive

points on head	**thresholds (relative SPL dB re 1 μPa)**
gular 0	2.1
gular 8	11.2
gular 16	14.2
gular 24	1.5
front middle melon 2cm	2.3
front melon 2cm up 5 off Right	-1.5
front melon 2cm up 5 off Left	0.3
front middle melon 10cm up	11.1
front middle melon 10cm up 5cm Right	11.6
front middle melon 10cm up 5cm Left	7.4
front midlle melon 15m up 18cm from tip of mouth	no resp
right meatus 2cm	-3.1
left meatus 2cm	-3.1
right panbone 4.5cm of crease 3.5cm down	0
left panbone 4.5cm of crease 3.5cm down	0.6
right panbone 9cm of crease 3.5cm down	9.1
left panbone 9cm of crease 3.5cm down	9.6
right panbone 6cm down	5.2
left panbone 6cm down	7.2
right mouth 5cm off crease 2cm up	13
left mouth 5cm off crease 2cm up	9.2
inside mouth upper jaw middle palate	-2
left crease of mouth	2.8
right crease of mouth	3
inside mouth right lip	7.7
inside mouth left lip	9.5
inside mouth lower jaw middle	no resp
inside left tooth	13.4
inside right tooth	14.6
missing tooth	no resp
left panbone inside mouth	8.8
right panbone inside mouth	10.5
right panbone 6cm crease 2.5down	4.4
right panbone 11cm crease 5.5cm down	5.9
left panbone 6cm crease 2.5down	no resp
left panbone 11cm crease 5.5cm down	no resp

The results obtained with the false killer whale raised interesting questions about potential sound pathways in *Pseudorca crassidens*. Because of the acute high frequency hearing loss, one should assume that the results obtained in this experiment might differ with a subject with normal hearing. One indication that the animal only detected the low frequency component of the click stimulus was the good sensitivity found 2 cm behind each meatus. Popov et al. (2003) showed the potential existence of two pathways, the panbone for high frequency sounds and the meatus for lower frequencies. Because the *Pseudorca crassidens* subject had a more pronounced high frequency hearing loss, it seems possible that most of the energy encoded by the cochlea would lie within the low frequency range detected around the meatus region. Similarly if the panbone acted as a high frequency filter, a smaller response would be expected as the false killer whale would not detect the high frequency portion of the broadband click even though these sounds were transmitted through the acoustic fats. The 'gular' region presented by Cranford et al. (2008) was tested for both species. While it was evidenced that in *Tursiops truncatus* the hearing sensitivity improved along the ventral region of the lower jaw, this pattern was not visible with *Pseudorca crassidens*. As mentioned previously, many factors could potentially cause the observed variations between the two species including presbycusis and limited sampling size. More importantly, these results show that while the gular hypothesis might be an active pathway in some species, it might not apply to all odontocetes.

Pseudorca crassidens being much larger than *Tursiops truncatus*, points inside the mouth were more easily accessible. The small responses detected on the lower jaw teeth indicated that teeth could potentially channel sound. However, because the responses were small compared to the panbone or the meatus, it seems unlikely that they would be the primary path for sound detection. In addition to these results, the wearing off or total absence of teeth in Risso's dolphins, Pilot whales and sperm whales would prevent these species from echolocating and hearing high frequency sounds. Previous audiograms for two out of these three species combined with acoustic information on their echolocation abilities indicated that at least in the three species mentioned above teeth would not be the primary sound pathway (Møhl et al., 2000; Nachtigall et al., 2005; Pacini et al., 2010; Aguilar de Soto et al., 2008).

Using a jawphone as a sound source comes with many caveats, the main one being the use of near field stimulation. The near field constitutes a range where the sound levels presented to the animal cannot be evaluated with the usual units and references used in acoustic measurements. More importantly, the sound levels do not decrease linearly with distance from the sound source. As the sound stimulus was presented via a contact hydrophone, it is difficult to translate or convert the results obtained here with conventional hearing as it is an integrative and complex process that requires both time and frequency detection and discrimination.

Changing the position of the animal depending on the point tested might have also increased the variability between loci as the AEP recording cups were sometimes submerged. A more controlled environment where the animal was completely outside of the water or where every locus is repeatedly tested to quantify inter trial variations would be difficult to achieve but would also provide additional information on the variability of these measurements.

An area of unusually good hearing was found at the tip of the upper jaw of the false killer whale. While the midline was the most sensitive, the area surrounding this point and on the palate were also as sensitive as the panbone indicating that this entire zone was sensitive to acoustic energy. Such a pathway has not been reported with the bottlenose dolphin or any other species (Møhl et al, 1999; Mooney et al, 2008) but Norris (1969) noticed that the mesorostral canal filled with cartilage could potentially channel sound. Whether it was involved in outgoing clicks or on the receiving end was not specified in Norris' work. Cartilages are known to have a density close to water (Reference) and the bones surrounding this canal have been reported to be abnormally dense (Norris, 1968), thus creating an exceptionally isolated pathway for sound to travel along. It should be noted that unlike most points, the midline region likely triggered a response in both ears thus creating a larger series of evoked potentials in the brain. This response in return cannot be directly compared to the threshold obtained by stimulating the panbone or meatus individually (ispilateral response).

The Archaeocetes represent a group of primitive whales from which Odontocetes and Mysticetes have radiated. Archaeocetes lived in the Eocene (55-34 million years ago) and had already reentered the aquatic environment. Fossil records indicate that some of the Dorudontinae (Zygorhiza) had already undergone telescoping but the premaxillary bones were still in contact and no mesorostral canal was present. However by the Oligocene (34-24 million years ago), both Mysticetes and Odontocetes had an open mesorostral groove (Fordyce, 2002). Stimpert (2007) presented acoustic recording of foraging humpback whales (*Megaptera novaeangliae*) that had some of the characteristics of echolocation signals. Whether mysticetes echo-range or not is still subject to controversy. However the evolution and presence of open mesorostral canal in both living cetacean groups indicate that perhaps the opening between the premaxillae might represent a primitive sound path. In beaked whales, particularly in *Mesoplodon densirostris*, the mesorostral region ossified (MacLeod, 2002) to become the densest bone in the skull, and was hypothesized to prevent damage to the rostrum during male-male interactions (Heyning, 1984; MacLeod, 2002); as a ballast to accommodate the deep dives beaked whales undergo (De Buffrénil and Casinos, 1995) or as a sound transmission mechanism (Zioupos et al., 1997).

The intricacies of the function of this sound path are difficult to assess based on the data collected in this study. One of the major issues with the mesorostral “hearing” hypothesis is the absence of direct morphological connection between the mesorostral canal and the tympano-periotic complex. The acoustic fats located on the posterior end of the lower jaw have been showed to connect directly to the tympano-periotic complex, thus carrying sound from the environment directly to the hearing receptors (Aroyan, 2001). Bone conduction would appear to be a potential candidate to explain how sound could enter the head through the mesorostral canal. Bone conduction has been often rejected as a good sound transmission mechanism for marine mammals as the ear organs are encased in the dense bulla and surrounded by air sacs. As the bulla is separated from the skull, the transmission of sound via the jaw and skull bones appeared limited. Humans – whose high frequency limit is around 20 kHz – have been showed to detect tones up to 128 kHz through bone conduction. Frequencies above the high frequency cut-off were however perceived as the highest frequency the individuals could detect (Kunze and Kietz, 1849). A similar sort of bone conduction would have limited used for an echolocating animal, as slight changes in frequency in the returning echoes would likely go undetected. Additionally, because bone conduction involves an actual pressure wave travelling through dense bones, an inherent loss of directionality would occur and would be disadvantageous to an animal chasing a prey. On the other hand, because the mesorostral opening is located on the mid-line of the upper jaw, directionality would not necessarily be a requirement and the animal could potentially detect frequencies above its hearing range. In fact, if the mesorostral canal is the only region that would accurately transmit sound through bone conduction, it would provide the animal an additional sound input and potentially explain the good directional hearing in the vertical plane observed by Renaud and Popper, 1975. Whether or not this hearing mechanism is functional could only be tested through behavioral experimentation. Behavioral tests such as the experiment conducted by Brill et al. (1988) – where the principal hearing pathway was blocked and the detection ability of the animal was then tested – would provide additional evidence supporting the mesorostral hearing pathway. Additional information such as EFR latency and changes in response as a function of stimulus frequency should also be investigated.

Møhl et al. (1999) presented a shaded receiver model that was later on supported by the double acoustic window hypothesis presented by Popov et al. (2003; 2007). Both authors presented evidence that parts of the head – if not the entire head – acted as an acoustic receiver. Odontocetes have evolved dramatic morphological adaptations to accommodate leaving in an aquatic environment and relying on acoustic cues. This study provides partial evidence that other pathways might exist, and that one of the potential functions of the mesorostral canal would be to transmit sound. As hypothesized by Møhl and his colleagues (1999), hearing in odontocetes may combine multiple loci for acoustic receptions as well as multiple mechanisms. When odontocetes first re-entered the aquatic environment, terrestrial hearing, and in particular the middle ear, became of limited use as the need to overcome the impedance difference between air and water disappeared. Bone conduction might have occurred in the early stages of this re-adaptation to an acoustically transparent environment. If it is the case, the results observed with the *Pseudorca crassidens* would just represent artifacts of this earlier adaptation to the marine environment.

This experiment – as most research conducted with marine mammals – relies on a small sample size and the extrapolation to the species level is very difficult. Inter and intra specific variations are known to exist (Popov et al., 2007; Houser et al., 2008) and are likely to also occur in sound pathways experiments. These results provided partial evidence indicating that sound pathways might vary across species and that the shaded receiver hypothesis presented by Møhl et al. (1999) might be even more complex than originally thought. While it did not directly demonstrate that *Pseudorca crassidens* relies on the mesorostral canal as a hearing pathway, it showed that sound might reach the hearing apparatus through channels and mechanisms that had not been previously reported. Understanding hearing pathways implies not only taking into account the areas on the animal’s head that are likely to channel and transmit sound to the auditory apparatus but also understanding how all this information get integrated at the tympano-periotic complex. The recent use of 3D imaging and modeling in association with empirical data collected with trained animals promises to provide insight on the complex hearing processes in odontocetes (Cranford et al., 2008, Ketten and Montie, 2008).

CHAPTER 6: FALSE KILLER WHALE (*Pseudorca crassidens*) ECHOLOCATION AND ACOUSTIC DISRUPTION: IMPLICATIONS FOR LONG-LINE BYCATCH AND DEPREDATION

NB: This chapter has been published as below in The Canadian Journal of Zoology,

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ABSTRACT

False killer whales (*Pseudorca crassidens*; Owen, 1846) depredate fish caught by the North Pacific pelagic long-line fishery resulting in loss of target species catch and the whales themselves becoming bycaught. This incidental take of false killer whales exceeds sustainable levels. In an effort to address a potential solution to reducing this depredation and bycatch we tested an acoustic device designed to deter false killer whales from approaching long-lines by reducing the whales' echolocation performance capabilities. The device produced a series of complex, broadband signals (1-250 kHz) at high intensity levels (up to 182 dB). In the experiment, a trained false killer whale was asked to detect a target in the presence or absence of the acoustic device. Baseline performance capabilities were 95% correct responses. Initially, the device reduced the whale's echolocation performance to chance levels. However, subsequent sessions demonstrated improvement in echolocation performance up to 85%. This improvement was likely a result of behaviorally adapting to the task and a decrease in the source level of the echolocation "disruptor." The results underscore the challenges in using acoustic devices to reduce depredation and bycatch, and demonstrate the need for concern regarding anthropogenic noise levels and effects on odontocete echolocation capabilities.

INTRODUCTION

It is well documented that marine mammals interact with fisheries around the world and this widespread relationship between fishers and marine mammals rarely seem to benefit both parties. For example, dolphin-tuna associations in the Eastern Tropical Pacific have been utilized to locate and identify tuna schools for harvesting which at one time resulted in substantial herding and incidental catch of dolphin species (NRC 1992). Both pinnipeds and cetaceans have learned to remove target fish from various fishing operations such as aquaculture facilities (Quick et al. 2004), gillnets (Cox et al. 2003), long-lines (Thode et al. 2007) and trawlers (Broadhurst 1998). This often results in damage to fishing gear and harvestable fish. As a consequence of these and other marine mammal-fisheries interactions, two primary challenges can be established including: (a) loss of target catch for fisherman, which may have deleterious economic and social consequences (Gilman et al. 2006), and (b) incidental catch of marine mammals (bycatch), reducing populations and resulting in potential ecological changes (Read et al. 2006). Thus, mitigation measures are often suggested to reduce both the loss of target catch and bycatch rates of marine mammals.

Situations that present a particularly important need to reduce these interactions include populations or species of marine mammals that are of limited size. In this case, further reduction in numbers can threaten a population's survival, perhaps leading to extinction (Slooten et al. 2006; Turvey et al. 2007). The population of false killer whales (*Pseudorca crassidens*; Owen, 1846) in Hawaiian waters has considerable interaction with long-line fisheries (Baird and Gorgone 2005); whales are removing target catch, such as tunas, from the long-lines (depredation) and as a result, are being occasionally caught on the long-line hooks (Gilman et al. 2006). Fisherman may lose a substantial amount of marketable fish and whales may be seriously injured or killed in this process. The rate of bycatch exceeds the "Potential Biological Removal" (PBR) set for the population by the NOAA-National Marine Fisheries Service resulting in a stock of “strategic” importance (Caretta et al. 2006) indicating that such bycatch rates could result in a decline of the population size.

Methods to reduce marine mammal bycatch depend on the fishery but may include: (1) reducing fishing seasons or regions (Murray et al. 2000), (2) establishing marine protected areas (MPAs) with fishery regulations (Dawson and Slooten 1993), (3) modifying the gear to prevent entanglement (Mooney et al. 2004; Mooney et al. 2007) or (4) deterring animals from approaching the fishing area (Quick et al. 2004). Limiting fishing capabilities through regulation or MPAs may have the negative consequences of reducing the economic intake of fisherman and some social components of fishing communities. However, developing technology which reduces bycatch but maintains target species catch has obvious benefits to both fisheries and ecosystems.

We investigated an acoustic tool proposed to deter false killer whales from approaching long-lines, thus reducing depredation and bycatch. The device, a *SaveWave Long-line Saver*, was designed to emit sounds hypothesized to reduce echolocation capabilities, one of the primary sensory modalities of odontocete marine mammals. This, in turn was proposed to discourage false killer whales from attempting to remove target catch from long-line gear. Similar acoustic devices such as pingers have reduced harbor porpoise bycatch in gillnet fisheries (Kraus et al. 1997) by presumably alerting the animal of the net’s presence. In long-line fisheries the goal of an acoustic device would likely not be a warning of gear presence but rather to disrupt the animal’s depredative behavior. The efficacy of an acoustic device or disruptive tool has not yet been addressed in reducing marine mammal bycatch in long-line fisheries. The goals of this experiment were to (a) characterize the sounds produced by the device and (b) determine the tool’s efficacy in reducing false killer whale echolocation performance, thus evaluating its potential to reduce depredation and bycatch.

MATERIALS AND METHODS

Animal subject and background

The study animal was an approximately 30-year old-female false killer whale (4.1 m and 528.4 kg), housed in the open-water sea pens off the Hawaii Institute of Marine Biology's Coconut Island, Kaneohe Bay, Oahu, Hawaii, USA. The animal has had considerable experience with echolocation research including target detection tasks similar to the experiment presented here (see Nachtigall and Supin 2008). Experiments were conducted in March and April of 2007, over 15 experimental sessions, each consisting of 50 trials. All sessions were designed around a target detection task in the presence, or lack of, an acoustic device which emitted broadband acoustic signals. The device, a *SaveWave Long-line Saver* (Delft, Netherlands), was intended to reduce false killer whale depredation and bycatch on long-line fishing sets by producing a loud, broadband acoustic signal. This signal was proposed to decrease a false killer whale's echolocation performance abilities, and consequently, deter them from approaching a long-line, attempting to depredate it, and becoming hooked. We sought to investigate the capability of this device in "jamming" a false killer whale's sonar in a series of target detection experiments.

Experimental procedure

The experiments were conducted across two experimental pens and are diagramed in Fig. 19. The animal was tested in the first pen which was supported by floats, bounded a wire net, and was 8 x 10 m in size (1). At the far end of this enclosure was an equipment shack (2) which housed the electronics for the experiment and an operator. A second "enclosure," 7 x 10 m in area (3), was used to suspend the echolocation targets. This structure was without the wire net, and the supporting floats were positioned to the side to prevent any potential acoustic reflections during the echolocation task. A trainer (4) sat along the side of the first pen to provide instructions and monitor the animal during the experiment. A hoop-opening 55 cm in diameter was fixed between the two pens which served as a hoop station (5) for the animal during the echolocation task, so that it could be positioned in pen 1, but required to echolocate into the free-field of pen 2. Near the hoop station was an underwater camera (6) which allowed the animal's behavior to be monitored from the shack. One meter in front of the hoop was an acoustical baffle (7) which could be raised or lowered and prevented the animal from echolocating into the target pen until the proper time. Just behind the baffle was an acoustically transparent plastic screen that did not preclude sound transmission but inhibited the whale from seeing the target. A Reson 4013 hydrophone (8; Slangerup, Denmark) was positioned 2 m in front of the hoop and 1.34 cm in front of the whale to detect the animal's echolocation pulses. An aluminum cylinder, 3.8 cm in diameter, 12.7 cm in length, served as the target (9) for which the animal was trained to echolocate for and was hung 8 m in front of the animal from an aluminum pipe and pulley, the pipe spanning the width of the target pen. The cylinder was hung from a thin monofilament line and could be pulled out of, or lowered into, the water. Near the hoop station was a response ball (10) mounted above the water surface serving as a target-present response indicator. Except for early training sessions and the last session, the *Long-line Saver* (11) was suspended 8 m from the whale and 1 m to the side of the target.

Each session began with the false killer whale stationing near the trainer. Upon a cue from the trainer, the animal would leave the surface station and swim to the hoop station, 1 m below the water's surface. When the animal was in position, the target would either be gently lowered into the water (for a target-present trial), or lowered and then raised (for a target-absent trial). The acoustic baffle was then lowered allowing a free and direct path between whale and target. This was also the whale's cue to echolocate. A go/no-go paradigm was employed in which the go response was associated with target present and the no-go was associated with target absent (Schusterman 1980). If the whale detected a target, it would back out of the hoop and touch the response ball with its rostrum (a go). If the whale did not detect the target, the animal remained in the hoop for approximately 5 s (a no-go), until the trainer blew a whistle recalling the animal. Only correctly indentifying target presence (a hit) or absence (correct rejection) was rewarded with fish, and both present and absent trials were equally rewarded. Incorrect responses included false alarms (responding when no target was present) or a miss (failure to respond when the target was present).

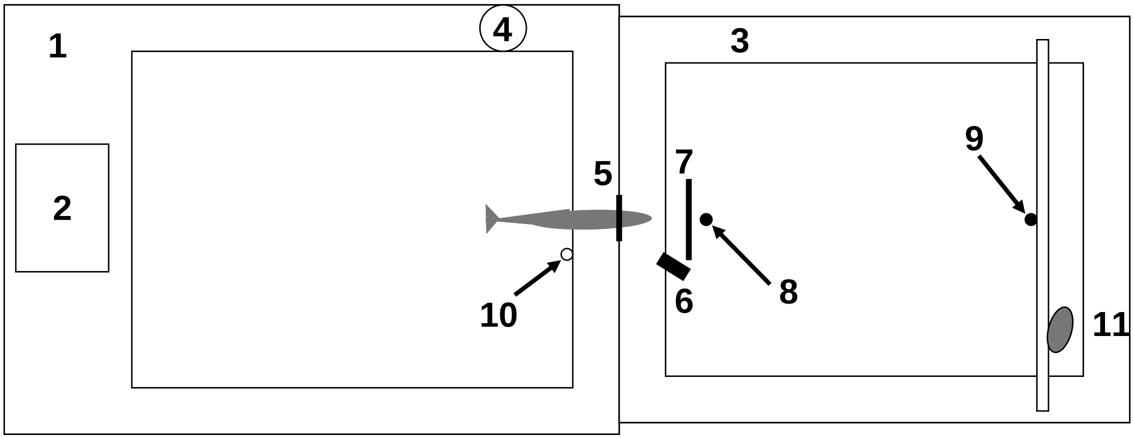

Figure 19: The experimental set-up for the target detection task. The various components are referred to in the text.

The *Long-line Saver* was designed to reduce false killer whale echolocation capabilities, thus the experiment was designed to determine if the device would reduce the echolocation performance of detecting the aluminum target. Two initial sessions were devoted to establishing the animal's baseline performance level, without the acoustic device in the water. Two subsequent sessions were run with the device in the water but turned off, with the device being placed 8 m from the whale, 1.5 m to the side of the target. A session was then run with the device on for the entire session. The sessions were then broken up so that the acoustic device was off for 10 trials, on for 20 trials, and off for 10 trials. Five "warm-up" and "cool-down" trials preceded and followed the experimental trials. These ABBA sessions were run until the animal's performance level stabilized again, four sessions later. A session was then run with the device in the water but off, ensuring that whale's detection baseline performance was consistent. Then, to determine how distance and reduced sound pressure levels (SPLs) might affect detection performance, the device was moved 30 m from the whale, but still in line with the target. All sessions were 50 trials, comprised of 5 blocks of 10 trials. Each block was randomized to target present vs. absent following a Gellermann series (Gellermann 1933).

Acoustic signals

The *Long-line Saver* was half-spherical in shape, with a diameter of 38.1 cm and weighing 24 kg (Fig. 20). The acoustic signals emitted by the *SaveWave* device were a complicated assortment varied up- and downsweeps, pure tones and harmonics. We calibrated the sound levels emitted before the experiment on three separate days with essentially the same results. The *Long-line Saver* device was hung off a dock at 1 m depth in the open seawater environment of Kaneohe Bay. Signals were received 2 m from the source using a calibrated Biomon 8235 hydrophone (sensitivity -182 ±2 dB up to 300 kHz; Santa Barbara, CA) which was connected to a Krohn-Hite 3364 filter (Brockton, MA) for anti-alias bandpass filtering (300 Hz-250 kHz). Ten 1-s files were recorded from three different directions (front, side and back) on each of the three calibration days to get a general estimate of the directionality of the device. Files were recorded using a custom LabView program working with a National Instruments DAQ card (6062E; Austin, TX) implemented into a laptop computer. Signals were sampled at a rate of 512 kHz. The *Long-line Saver* sounds were extremely broadband (from 1 to 250 kHz) but signals were not recorded above 250 kHz as this was considered well beyond the range of hearing for false killer whales and other odontocetes (Thomas et al. 1988; Yuen et al. 2005). These sounds were referenced to calibrated pure tones of 20, 40, 60, 80, 100, and 120 kHz, produced by a Wavetek function generator (Everett, WA) connected to a Reson 4040 transducer and recorded in the same manner as the *Long-line Saver* signals above. All signals were monitored as they were recorded using a Tektronix TDS 1002 oscilloscope (Richarson, TX).

The recorded signals were assessed off-line using CoolEdit (Adobe Systems, San Jose, CA) and MatLab (MathWorks, Natick, MA) programs. Portions were selected from each of the pure tones and the broadband SaveWave noise files and analyzed with a 2048-

point fast Fourier transform (FFT). The peak values of the FFTs were compared between tones and the noise files to determine the peak intensity of the *Long-line Saver*. Before the experiment, the device produced a relatively intense source level (SL = 182 dB re: 1 µPa) from the forward direction but signals dropped off to the side and back, at 174 and 164 dB, respectively. After the experiment and approximately 25 hours of use, the *Long-line Saver* device dropped off in sound pressure level to 162 dB peak intensity at the source and in the center of the front facing beam. Peak received levels at the animal were 164 dB.

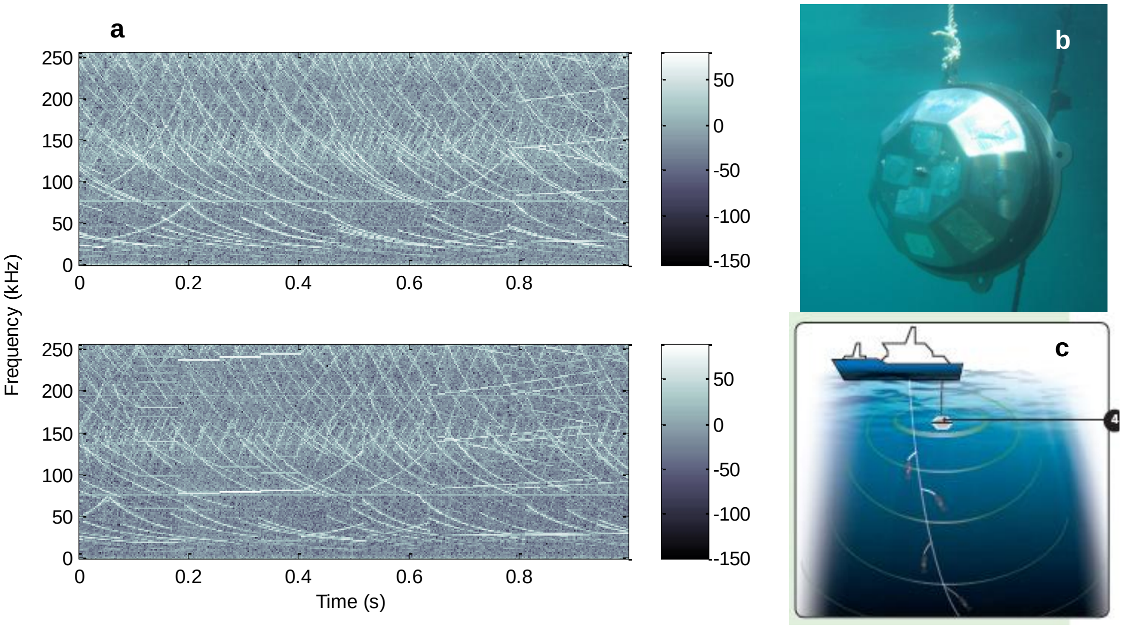

Figure 20. (a) Sample spectrograms of the various acoustic signals produced by the acoustic deterrent. (b) The *Long-line Saver* hung in sea water for calibration. (c) A diagram for the suggested use and deployment of the device.

Data collection and analysis

The primary data collected were based on the performance of the false killer whale in the echolocation task. Correct responses (hits and correct rejections) were measured against incorrect response (misses and false alarms) and compared using a two-tailed t-test. A number of complimentary parameters were collected for each trial to measure additional effects of the *Long-line Saver*. Because the *Saver* might be considered an acoustic harassment device, several of the parameters were designed to assess the acoustic device's influence on the animal's behavior. This included the delay (s) from when the trainer sent the whale to the hoop to when it actually stationed in the hoop, the number of echolocation clicks used, the latency of time (s) for the whale to respond (only in target present responses) and any overt behavioral alterations during the experiment. Within each category the variable was averaged and compared using a two-tailed t-test. The animal's behavior in the hoop and at the surface was video-recorded. MiniTab and EXCEL software were used in the behavioral data analysis. All methods and animal care abided by national and university animal care guidelines (IACUC permit # 93-005-13; National Academy of Sciences 1996).

RESULTS

Echolocation performance

The experiment was initiated by establishing the false killer whale's baseline echolocation performance ability and ensuring that the percentage of correct responses was stable. Across the first four sessions, the animal demonstrated a mean 97% correct response rate (±1.15 s.d.) to the presence and absence of the cylinder target (Fig. 21). For the first two sessions, the acoustic device was not yet suspended in the water and the animal responded 98% correct. The single errors in both sessions were misses. In the following two baseline sessions (3 and 4) the *Long-line Saver* was hung in its experimental position but the sound was not turned on and the whale's performance was a similar 96 %. The acoustic device was kept in the water for the remainder of the experiment. The errors in these sessions were made within the first 5 trials of both sessions and 3 of the 4 errors were false alarms.

During session 5, the *Long-line Saver* was turned on as the animal first entered the hoop station for the first echolocation trial of the session. The sound then remained on for the entire session (50 trials; approximately 1 hr) including all subsequent target presence/absence trials. The animal's performance dropped off to 46% for this session.

Eleven errors were false alarms and 16 errors were misses. We then returned to a session with the *Long-line Saver* off for the session's entirety. With the device off, the whale responded correctly for 96% of the trials, reaffirming its baseline performance capabilities.

We then switched to an ABBA format of the trial order within sessions to hopefully reduce frustration of the animal in the apparently difficult task of target detection when the *Long-line Saver* was emitting sound. During this portion of the experiment the animal's detection performance improved significantly when the *Long-line Saver* was on (Fig. 22), although performance was never at the level of trials without sound. In the first session, the animal correctly identified target presence/absence at 60% with the *Long-line Saver* on and 95% with it off. Of the incorrect responses when the device was on, 87.5% were false alarms. For the remaining three sessions, the animal's performance leveled off at 85% for sound-on trials and 98% for sound-off trials. Over the course of these four sessions, the animal detected target presence/absence significantly better when the *Long-line Saver* was off (mean on = 78.8%; mean off = 96.2%, two-tailed t-test, p = 0.03).

After performance stabilized, we returned to a full session with the *Long-line Saver* off to re-established baseline capabilities and the whale demonstrated 94% correct responses. In the final session we moved the device 30 m from the animal, turned the acoustic device on, and returned to the original echolocation task. The animal was 100% correct in identifying the presence or absence of the target.

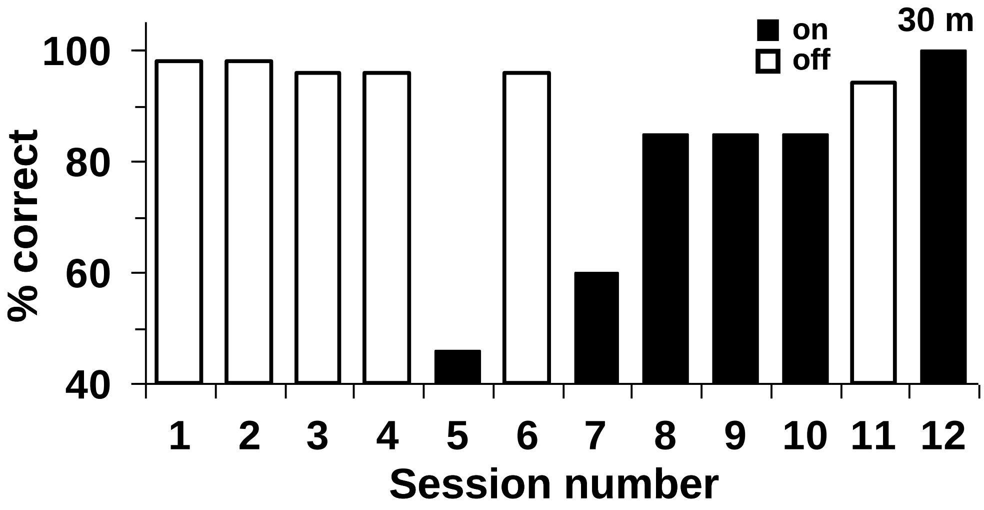

Figure 21. Target detection performance displayed in percent correct responses for sessions where the acoustic deterrent was on (black) or off (white). The *Long-line Saver* was suspended 8 m from the animal during its echolocation task except for the last session when the device was placed 30 m from the whale.

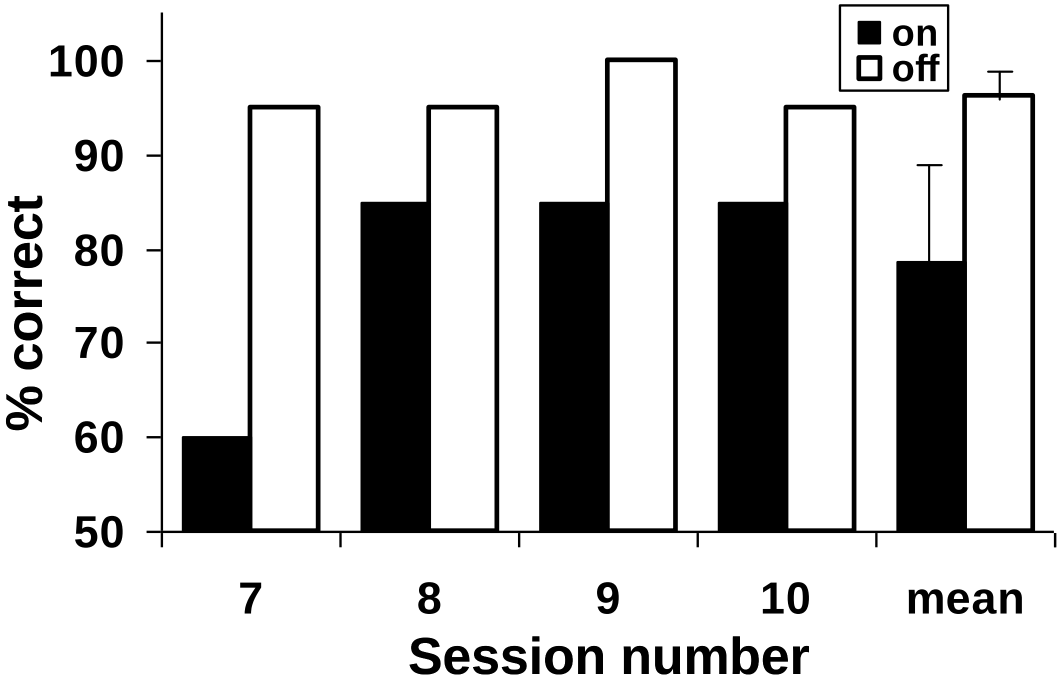

Figure 22. Target detection performance in correct responses for sessions in ABBA format, as well as the mean performance of those sessions.

Receiver Operating Characteristics

The animal’s hits (correct detections) and false alarm probabilities were plotted in receiver operating characteristic (ROC) curves (Fig. 23) to assess how the whale might make decisions of detecting the target within background noise (the acoustic disruption device). As noted, when the sound was off the animal’s hit rate was very high, near 100%, and the probability of false alarms was relatively low (≤ 20%; Fig. 23a,c). Such clear responses indicate the ease at which the whale could detect the target in the standard situation. However, when the acoustic disruption device was turned on, it was obvious that the echolocation detection task became more difficult. Hit rates initially dropped below 40% and false alarm rates increased to near 50% (Fig. 23a), indicating that the animal became less “conservative” and was apparently lowering its detection threshold or guessing. However, as sessions continued, false alarm rate dropped and hit rate increased, as the whale improved and returned to a more conservative approach. For a more detailed look at the decisions made when the acoustic device was first turned on, we analyzed the first two sessions with the device on, broken up into 10-trial blocks (Fig. 23b). Notably, the animal’s false alarm and hit rate changed substantially throughout the sessions. Nearly all hovered around the major diagonal, indicating the animal’s likelihood of a correct response was near chance thus supporting that whale was guessing. Or by changing strategies from conservative to “liberal,” the whale could have been searching for a strategy to improve her chances of correct detection. While overall, the whales’ strategy across the two sessions was 50% for both hits and false alarm, the last trial block (large square) showed improvement to 60% hit rate and 20% false alarms.

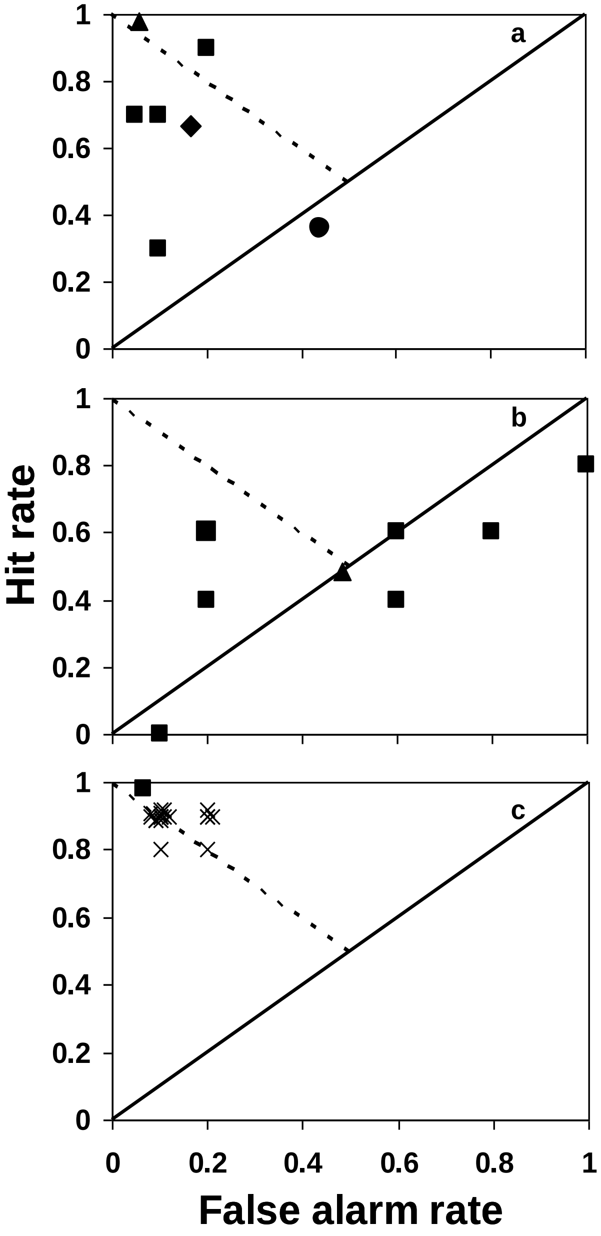

Figure 23. ROC graphs for the false killer whale performing the echolocation task. (a) All data when acoustic *Long-line Saver* was in the water. 'Circle' indicates session 5 when sound was on for the entirety. 'Squares' indicate sessions 7-9, when device was *on* for an ABBA format. 'Diamond' indicates a summary of all trials with the acoustic device was *on*. 'Triangle' indicates all sessions when device was *off*. (b) Sessions 5 and 7, when acoustic device was *on*. 'Squares' indicate 10 trial blocks. 'Large square' indicates last block and apparent improvement in correct responses. Triangle indicates summation of data. (c) Sessions 1-4, 6, 11, when acoustic device was *off*. 'X's indicate 10 trial blocks. 'Square' indicates summation of data.

Table 5. Two tailed t-tests for behavioral alterations associated with the use of the *Long-line Saver* acoustic device.

Behavioral parameter	*Long-line Saver* status	Trials (*n*)	Mean value	SD	Significant Difference
Latency to hoop (*s*)	On	36	5.175	0.522	*p* < 0.001
	Off	149	4.772	0.380	
Delay (*s*)	On	82	9.79	1.13	No difference
	Off	173	9.93	1.34	
Number of clicks	On	128	21.1	10.4	*p* < 0.001
	Off	297	12.63	6.70	

Behavior

In addition to echolocation capabilities, we monitored several behavioral parameters to address potential reactions to the acoustic device. The animal did not demonstrate any significant behavioral alterations in the presence of the acoustic stimulus. Nor did the animal increase its swimming time (delay-s) from the trainer station to the echolocation-hoop station (Table 1; Fig. 24). However, the false killer whale did take significantly longer to decide target presence when the *Long-line Saver* was turned on. Additionally, the animal used a significantly greater number of echolocation clicks when the acoustic device was on.

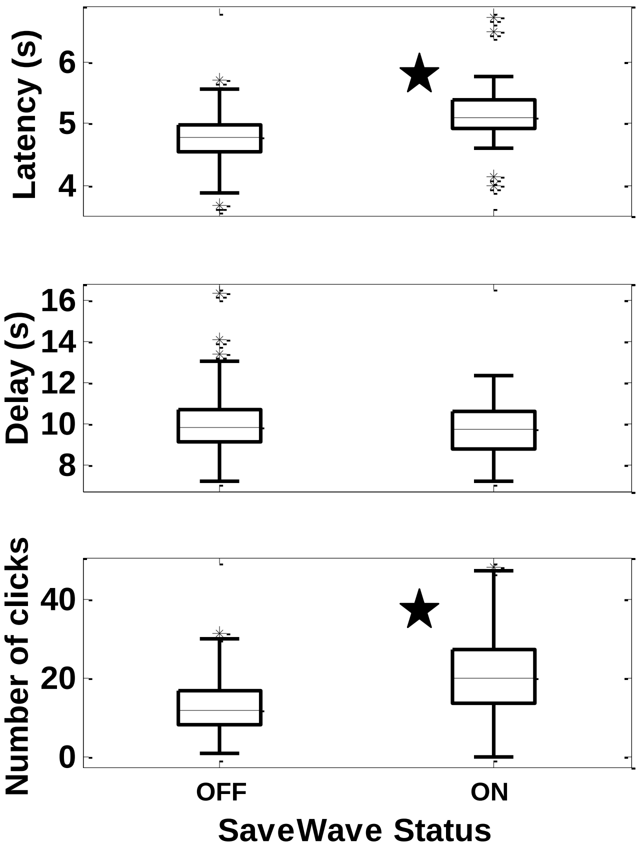

Figure 24. Behavioral responses during sessions when the *Long-line Saver* was on. Responses noted include: Latency (*s*) to the echolocation hoop station, delay (*s*) to respond to target presence, and number of echolocation clicks used per trial. Large stars indicate significant differences between groups.

DISCUSSION

The *SaveWave Long-line Saver* did work to significantly reduce the echolocation performance of an experienced and well-practiced false killer whale. The increased number of echolocation clicks used and latency of time to decide target presence or absence indicate that the device apparently made the echolocation task more difficult. However, the animal's performance in the presence of the device improved over the duration of the experiment, from 46%, or essentially guessing, to a respectable 85%. This indicates that: either the false killer whale devised a strategy to improve its target detection capabilities in noise, or that the decrease in sound pressure levels over time allowed for easier target detection, or both, contributed to the animal's improved performance.

Unfortunately the decrease in sound pressure levels over a relatively short period of time (~25 hrs of use) confounds the results of the experiment somewhat because it makes it difficult to discern whether the whale's improvement was from learning or reduction in sound. However, moving the device 30 m away from the animal (typically the device was 8 m away) obviously decreased the received sound levels at the animal and consequently, the whale was 100% correct in identifying target presence/absence during that session. Thus, the output of the device and received levels at target animals has a crucial effect on echolocation performance and the effectiveness of the *Long-line Saver*. This may be crucial in many long-line situations with gear tens of km in length (Gilman et al. 2006). If the acoustic device has a limited radius of effectiveness, then its use in deterring depredation may well also be restricted. Perhaps a better acoustic solution might be the implementation of smaller but loud acoustic devices fixed repeatedly on the long-line to cover the line effectively.

The decrease in SL also indicates how the device may have worked to reduce echolocation performance. At the start of the experiment, when the *Long-line Saver* was operating at higher SLs, the device effectively reduced echolocation performance to chance level. As SLs apparently decreased but signal types remained consistent, performance improved. Thus, it was not the complex acoustic signals that were the basis of reducing the echolocation performance but more likely, the masking of the echoes by the intense noise. Consequently, the sound pressure (or received level) and frequency spectrum of the acoustic disruptor-masker may be more important than the type of signal itself.

Interestingly, echolocation performance was reduced slightly (85%) even when the device's source levels had dropped. Bottlenose dolphins demonstrate similar decreases in echolocation performance in the presence of background noise (Au and Penner 1981). These decreases in performance occur at noise spectrum levels that are above background but not necessarily significantly higher in intensity (Au 1993). This is because masker levels are relative to the echo level, thus the lower the echo level, the lower the effective masker-noise level may be. This raises concern for animals which echolocate and forage in environments that have high levels of anthropogenically induced background noise. The animals’ echo detection may be reduced by the surrounding noise levels. This is particularly true if the animal is echolocating on targets which may not have strong echo returns (i.e., squid, monofilament nylon line, or fishing hooks), making certain items difficult to detect.

Detecting the target in noise also revealed that when the task was difficult and the target was not easy to detect, the animal seemed to vary its strategy from high false alarms and high hit rates, to low false alarms but lower hit rates. While this may simply have been guessing during a trying task, this may also indicate the animal was “searching” for a strategy that would yield greater success. Further, the incidence of less conservative, higher false alarm rates indicates that this false killer whale might have attempted a fairly liberal target detection strategy. This is quite different than what has been shown typical for bottlenose dolphins, which tend to be conservative and not allow false alarm rates to rise above a certain level (Au and Snyder 1980). Research along similar lines with other odontocetes that live in varying niches may find further evidence for differing decision strategies. Further, the study whale is well experienced in experimental investigations. Different strategies may be used by wild or younger animals inhabiting a complex, pelagic environment.

This device was intended to be suspended from the side of a boat and broadcast sound into the ocean environment in order to deter false killer whales (Fig. 20). Sound intensity levels were apparently engineered to be relatively high (up to 182 dB peak energy). The device might be considered more of an ‘acoustic harassment’ tool, rather than a deterrent (Quick et al. 2004). Received levels at our false killer whale were approximately no more than 164 dB peak energy for relatively short durations of time (4-6 s) thus well below any physiological effects (Nachtigall et al. 2004; Finneran et al. 2005; Mooney et al. 2009). Nor did the animal demonstrate any overt behavioral modifications to approaching the hoop station, where the maximum received levels were measured. However, this animal was well trained in many experimental procedures such as this echolocation task, and obvious behavioral reactions would not have been expected. More dramatic reactions to acoustic harassment devices would be expected and have been noted in wild, naïve animals (Quick et al. 2004).

The complexities and relative ineffectiveness of this well-engineered acoustic device underscores the challenges related to deterring odontocetes from depredation and becoming caught on long-lines. The simple mass of this device unfortunately causes it to be difficult to handle and operate. Further, applying a single device from a boat and expecting acoustic disruption on long-line km's away does not seem to be a realistic means of reducing echolocation performance, depredation or bycatch. However, it seems that the masking of echolocation signals reduces echolocation performance. Thus, smaller, loud, more numerous devices might cause such effects, and should be tested. As these devices would likely be costly and difficult to implement and maintain, government support would likely enhance implementation, if the devices prove successful in echolocation disruption. Mechanistic devices which shroud catch and reduce depredation should also be explored for their detection possibilities in captive research settings. It is obvious that devices to decrease bycatch and depredation must meet certain characteristics including they actually succeed, are relatively easy to disseminate and implement, are cost-effective, and they not decrease target species catch rates (McPherson 2003; Gilman et al. 2006). However, as seen here and in other studies (Cox et al. 2001; Gilman et al. 2006), cetacean species often habituate to certain devices. Such modifications and assessments are often seen in animal foraging strategies (Pyke et al. 1977; Lima and Bednekoff 1999). Solutions to these fisheries-related issues will probably need to be equally flexible. It is likely multiple tools, including acoustic and mechanistic, should be applied to deter depredation and bycatch. To achieve these goals researchers, fisherman and regulatory agencies will need to work closely in order to find suitable resolutions.

CHAPTER 7: CONCLUSION

The results presented in this dissertation represent the combination of basic research and data addressing the impacts of anthropogenic activities on marine mammals. This work provides data on the hearing abilities on two new species and additional insights on the complex hearing mechanisms of two other species. The following summarizes these findings and provides an overview of future work.

- The second chapter reviewed the design of a portable auditory evoked potential measurement system. The building of a system that could accommodate both field research as well as laboratory experiments has allowed AEP work to be obtained in various settings and research conditions (Taylor et al., 2007; Mooney et al., 2008; Nachtigall et al., 2008). The versatile combination of the different pieces of equipment has made the data collection more rapidly obtainable during stranding events while the reliability of the system was constantly evaluated in the laboratory. The creation of such platform provides researchers a useful technique to complement their limited knowledge on marine mammal hearing as well a reliable medical diagnostic tool (Finneran, 2009; Mann et al., 2010; Houser et al. 2008; Taylor et al., 2007).
- The third chapter presented some of the results obtained with this same system at a public facility with a *Globicephala melas* where the animal was desensitized to most of the experimental set-up. Working in collaboration with marine parks that house non-releasable stranded animals allows scientists to obtain crucial information on species that are not commonly found in laboratory settings. This particular work demonstrated that the hearing of the young individual might have been impaired due to the ototoxic medicines that were administered to the whale during its rehabilitation. Two additional audiograms obtained with another *Globicephala* species showed similar threshold levels which indicated that perhaps pilot whales did not have an extensive high frequency hearing range (Schlundt et al., 2011).
- The work described in the fourth chapter provided the first hearing measurements of a species of concern *Mesoplodon densirostris* in the debate over the impact of military

sonar on marine mammals. This audiogram is part of an on-going effort to better understand both the behavior and the physiology of a rare group of cetaceans (NRC, 2003; 2005). The data indicated that the range of best hearing was centered on the frequencies of this species' echolocation signals (Johnson et al., 2006). This unique adaptation has been previously observed in echolocating bats (Neuweiler, 1984) and provides new information about the ecology of this species.

- The fifth chapter investigated how sound entered and was transmitted through the head of two different species of odontocetes and showed that *Pseudorca crassidens* might possess more hearing pathways than previously thought. This new sound path - through the mesorostral canal – had been suspected to be involved in odontocetes but no empirical evidence had been presented to support this hypothesis (Norris, 1968). While the work conducted with a jawphone does not fully replicate hearing mechanisms, it provides insights on how sound might enter and get processed in the animal's head. Most importantly it provides additional support to the recent hypothesis that hearing pathways might vary across odontocetes (Mooney et al., 2008; Cranford et al., 2008) and represent empirical data that can be used for comparison with 3D modeling. Understanding the variations across odontocete species might ultimately help to understand why certain animals are more sensitive to anthropogenic noise.
- The sixth chapter investigated the echolocation disruption of a *Pseudorca crassidens* caused by an acoustic pinger. Initially, the sounds generated by the acoustic disrupter did lower the echolocation abilities of the animal but these effects decreased after several sessions. It was not clear what generated the performance improvement; a decrease in pinger sound levels combined with a behavioral adaptation of the subject is likely to have caused such an improvement over time. This study indicated that while the acoustic deterrent could potentially deter false killer whales from depredating long line fisheries, the results underscore the challenges in using acoustic devices to reduce depredation and bycatch,. Additionally, it emphasized the need for concern regarding anthropogenic noise levels and effects on odontocete echolocation capabilities.

This dissertation combines non only data on the basic hearing of two new species of odontocetes and a new perspective of the complex hearing mechanisms and hearing pathways variations across odontocetes, but it also provides baseline data to address important conservation issues such as the effects of noise on marine mammals as well as the feasibility of using acoustic deterrents to mitigate interactions with fisheries. Understanding how different species of odontocetes evolve and use and react to sound is important to build successful conservation strategies that will both protect animals at the species level as well as accommodate human activities when it is possible.

Unfortunately while providing crucial answers about the odontocete hearing mechanisms and potential variations between species, this work inevitably generates many new questions about sound pathways and the mechanisms involved in hearing. One of the major limitations of most physiological and sensory work obtained with marine mammals is the limited sample size. While the need for this type of data is primordial for conservation and management strategies, the available information is limited and renders such effort very difficult. One approach is to continuously complement these data with new information, which can be achieved by collaborating with both public facilities and stranding networks. Testing a new species is a rare opportunity where researchers can start understanding and quantifying inter- and intra-specific variations. Future research includes collecting audiograms of new species as they become available. Each audiogram has the potential to complement not only behavioral, anatomic and physiological work obtained in the field but also to provide empirical data to compare to modeling based on medical imaging.

Additional experiments exploring the mechanisms involved in hearing pathways will also be conducted using a combination of ABR methods and behavioral testing. These empirical data will then again be compared to 3D modeling as more species get tested. Beyond the excitement of understanding the complex hearing mechanisms of odontocetes, this work emphasizes that technological and medical techniques today have the potential to provide powerful tools to address important issues threatening marine mammals.

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