Behavioral and Auditory Evoked Potential (AEP) Hearing Measurements in Odontocete Cetaceans

by

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Dedication

This dissertation is dedicated to my family for their continued love, support, and guidance. Thank you for encouraging me to ask questions and for teaching me to love the ocean.
Acknowledgments

I would like to thank the numerous institutions, organizations, and volunteers who helped make this project a reality. I would especially like to thank my advisor, David Mann, for his never-ending enthusiasm and interest in my project; I am exceptionally grateful for your guidance along this journey. His wealth of knowledge and ideas made all of this possible. I would also like to thank my committee members, Jose Torres, Gordon Bauer, Butch Rommel, and Randy Wells, for their helpful advice, support, and encouragement, and for the valuable time they each invested in my research to improve it by leaps and bounds. Finally, I would like to thank the past and present members of the Mann Lab who taught me a lot, enriched my graduate experience, and made life a little more interesting along the way.

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Behavioral and Auditory Evoked Potential (AEP) Hearing Measurements in
Odontocete Cetaceans

Mandy Lee Hill Cook

ABSTRACT

Bottlenose dolphins (Tursiops truncatus) and other odontocete cetaceans rely on sound
for communication, navigation, and foraging. Therefore, hearing is one of their primary
sensory modalities. Both natural and anthropogenic noise in the marine environment
could mask the ability of free-ranging dolphins to detect sounds, and chronic noise
exposure could cause permanent hearing losses. In addition, several mass strandings of
odontocete cetaceans, especially beaked whales, have been correlated with military
exercises involving mid-frequency sonar, highlighting unknowns regarding hearing
sensitivity in these animals.

Auditory evoked potential (AEP) methods are attractive over traditional
behavioral methods for measuring the hearing of marine mammals because they allow
rapid assessments of hearing sensitivity and can be used on untrained animals. The goals
of this study were to 1.) investigate the differences among underwater AEP, in-air AEP,
and underwater behavioral hearing measurements using two captive bottlenose dolphins,
2.) investigate the hearing abilities of a population of free-ranging bottlenose dolphins in Sarasota Bay, Florida, using AEP techniques, and 3.) report the hearing abilities of a stranded juvenile beaked whale (*Mesoplodon europaeus*) measured using AEP techniques.

For the two captive dolphins, there was generally good agreement among the hearing thresholds determined by the three test methods at frequencies above 20 kHz. At 10 and 20 kHz, in-air AEP audiograms were substantially higher (about 15 dB) than underwater behavioral and underwater AEP audiograms.

For the free-ranging dolphins of Sarasota Bay, Florida, there was considerable individual variation, up to 80 dB between individuals, in hearing abilities. There was no relationship between age, gender, or PCB load and hearing sensitivities. Hearing measured in a 52-year-old captive-born bottlenose dolphin showed similar hearing thresholds to the Sarasota dolphins up to 80 kHz, but exhibited a 50 dB drop in sensitivity at 120 kHz.

Finally, the beaked whale was most sensitive to high frequency signals between 40 and 80 kHz, but produced smaller evoked potentials to 5 kHz, the lowest frequency tested. The beaked whale hearing range and sensitivity were similar to other odontocetes that have been measured.
Chapter One:

Hearing Thresholds in Captive and Free-Ranging Cetaceans: An Introduction

Bottlenose dolphins (*Tursiops truncatus*) have an impressive ability to both produce and perceive a wide variety of sounds. These sounds include echolocation clicks used for feeding and other functions, and whistles and burst-pulse sounds used for communication (Caldwell et al. 1990; Au 1993; Thomson and Richardson 1995). Presumably, dolphins are capable of hearing all of the sounds they are capable of producing; therefore, their hearing should be sensitive over a wide range of frequencies. Additionally, because dolphins rely on sound for communication, navigation, and foraging, their sense of hearing is one of their most important senses (Au 1993; Janik and Slater 1998). This chapter presents a chronological review of the sound production and hearing abilities of odontocetes to provide a framework for the hearing studies presented in the following chapters. In particular, behavioral and auditory evoked potential techniques were used to evaluate the hearing capabilities of cetaceans.

The sound production and reception abilities of bottlenose dolphins have been studied by several prominent researchers. In 1947 the first curator of Marineland, Florida, Arthur McBride, presented evidence that Atlantic bottlenose dolphins may detect objects underwater by means of echolocation. During the dolphin capture operations that took place at night in the turbid waters of Florida’s inland waterways, he noted that
dolphins could avoid fine mesh nets and detect openings in the nets beyond visual range (McBride 1956). Kellogg and Kohler (1952) were the first to hypothesize the production of echolocation by dolphins. They performed a crude sound avoidance experiment and found that dolphins could hear frequencies up to 50 kHz. A year later Kellogg and colleagues reported that dolphins could hear up to 80 kHz (Kellogg 1953; Kellogg et al. 1953). In 1953, Forrest Wood described spotted (Stenella plagiodon) and bottlenose dolphins producing rasping and grating sounds to “echo-investigate” a transducer (Wood 1953).

Schevill and Lawrence (1953) reported Tursiops hearing frequencies as high as 120 kHz. In 1956, Schevill and Lawrence first described captive dolphins producing echolocation to find small, silent bits of food that were placed into the water. These dolphins were producing sounds inaudible to a person listening from the bank of the pond, but they could be detected using sensitive underwater listening equipment. To eliminate vision as a possible cue for fish detection, Schevill and Lawrence frequently worked on dark nights, and the pond that contained the dolphin was extremely turbid. They extended a net from a boat perpendicular to the bank, and sat at opposite ends of the boat, each holding a fish at arm’s length. They would randomly take turns placing the fish below the water surface as the dolphin swam by and, in about 75% of the tests, the dolphin chose the correct side of the net from which to obtain a fish (Schevill and Lawrence 1956).

In 1958 Kellogg published the results from a series of experiments that provided strong evidence of echolocation in dolphins. He trained the animals to swim through an obstacle course and to perform fish food discrimination tasks. These tasks required the
dolphins to select the preferred fish or to select the fish not behind a clear glass pane (Kellogg 1958, 1961).

Echolocation was unequivocally demonstrated by Kenneth Norris and colleagues in the early 1960s; they used rubber suction cups to cover the eyes of dolphins trained to perform echolocation tasks (Norris et al. 1961). The dolphin was able to successfully retrieve fish tossed into the water as they drifted downward. Many odontocetes have since been shown to produce echolocation. Most experiments testing this ability have been conducted with animals wearing eye cups and trained in object retrieval, object discrimination, or obstacle course tasks. Au and many others have studied various aspects of dolphin echolocation production since the mid-1970s (see Au 1993 for a general review).

Kellogg and colleagues first described the whistles of bottlenose dolphins (Kellogg et al. 1953), although they were mentioned by both Kullenberg in 1947 and Essapian in 1953. In the 1960s several researchers, including Dreher (1961), Lilly (1962), Dreher and Evans (1964), Schevill (1964), and Evans (1967), described the shapes and early repetitive elements of dolphin whistles. Lilly and Miller first hypothesized that dolphin whistles had specific functions (Lilly and Miller 1961a, 1961b). They assigned discrete whistle contours to specific behavioral situations, and went on to propose the dolphin “distress call” (Lilly and Miller 1961a; Lilly 1963).

David and Melba Caldwell first reported the presence of individualized whistles in captive bottlenose dolphins (Caldwell and Caldwell 1965). Over a three week period, they recorded the vocalizations of five newly-captured animals. These recordings showed that each animal from this group tended to produce an individually distinctive
whistle that remained relatively unchanged regardless of context. The Caldwells called these individualized whistles “signature” whistles (Caldwell and Caldwell 1968), and hypothesized that these whistles functioned in individual recognition. They went on to show that dolphins could correctly classify different signature whistles in as little as a 0.5 second exposure to them (Caldwell et al. 1969). Their seminal research on signature whistles during the 1960s and 1970s was summarized by Caldwell et al. (1990).

Although signature whistles were disputed by McCowan and Reiss (1995, 2001), research by others produced an overwhelming amount of evidence supporting the signature whistle hypothesis, and demonstrated that free-ranging bottlenose dolphins produce signature whistles in a variety of activity contexts (e.g., Sayigh 1992; Sayigh et al. 1990, 1995, 1999; Watwood 2003; Watwood et al. 2004, 2005; Cook et al. 2004; Janik et al. 2006). For example, free-ranging bottlenose dolphins respond significantly more often to signature whistles produced by related or familiar animals than by unrelated or unfamiliar animals (Sayigh 1992; Sayigh et al. 1999; Janik et al. 2006).

Burst-pulse sounds produced by bottlenose dolphins have recently been categorized as social and foraging sounds. Conner and Smolker (1996) reported the use of ‘pop’ calls by male dolphins during consortship. Janik (2000) reported the production of food-related bray calls, and Nowacek (2005) reported the production of pop calls and suggested that perhaps they are used to startle fish.

The hypothesis that dolphins use their lower jaws in the reception of sound, especially high frequency sounds, is generally accepted. Norris (1964, 1968) originally proposed that the mandibular foramen and the fats associated with it function as acoustic wave guides; electrophysiological (Bullock et al. 1968; McCormick et al. 1970, 1980)
and behavioral (Brill et al. 1988, 2001) studies with bottlenose dolphins support this theory. Jawphones (contact hydrophones attached by suction cups) take advantage of this sound conduction pathway and have been used by several researchers to deliver acoustic stimuli to the mandibles of bottlenose dolphins (e.g., Moore and Pawloski 1993; Brill et al. 2001; Cook et al. 2006; Finneran and Houser 2006; Houser and Finneran 2006).

Behavioral hearing measurements of bottlenose dolphins began with Kellogg and Kohler’s study in 1952, which was quickly followed by reports from both Schevill and Lawrence (1953) and Kellogg (1953; see above). C. Scott Johnson performed the most detailed behavioral hearing measurement experiments in a bottlenose dolphin published to date (Johnson 1966, 1967). He trained an 8-9-year-old male bottlenose dolphin to respond to 3-second pure-tone acoustic stimuli between 75 Hz and 150 kHz. The test procedure used a go/no-go response paradigm, and false alarms were followed by 90-second time-outs. This methodology probably caused the animal to respond very conservatively to the sound presentations and thus could have potentially elevated the results of the audiogram (Nachtigall et al. 2000). The lowest hearing thresholds occurred near 50 kHz at a level around 45 dB re 1 \( \mu \text{Pa} \), but sounds were detected by the dolphin throughout the range of 75 Hz to 150 kHz. Since Johnson’s seminal work on bottlenose dolphin audiograms, Thompson and Herman (1975), Ljungblad et al. (1982), Ridgway and Carder (1993, 1997), Au et al. (2002), Finneran et al. (2002a, 2002b, 2002c), Houser et al. (2004), Finneran and Houser (2006), Houser and Finneran (2006), and Cook et al. (in prep.) have reported additional behavioral audiograms for this cetacean species. In the last 40 years, behavioral hearing thresholds have been reported for a wide variety of cetaceans, representing 13 different species (Table 1-1).
Auditory evoked potential (AEP) techniques, described in detail in the following chapters, can be used as an alternative to traditional behavioral techniques to measure hearing in cetaceans. Research projects using these procedures were first attempted in the 1960s. Although Bullock et al. (1968) and Bullock and Ridgway (1972) reported cetacean evoked potentials recorded in response to auditory stimuli, both of these studies were done invasively (electrodes were placed near or within the inferior colliculus or the lateral lemniscus), and many of the animals were sacrificed or succumbed to the experimental procedures soon after the completion of testing (Bullock et al. 1968; Bullock and Ridgway 1972). Popov et al. (1986) reported the evoked potentials of a harbor porpoise (*Phocoena phocoena*), but these techniques were also invasive.

Ridgway (1980) reported less-invasive auditory evoked potentials recorded in bottlenose dolphins, and Popov and Supin (1990 a, b) also reported similar experimental results (see Supin et al. 2001 for a general review). The AEP hearing abilities of 18 different species of cetaceans have been measured to date (Table 1-2). Most of these studies used subdermal or surface electrodes to record the auditory evoked potentials generated in response to acoustic stimuli.

Dolphin and colleagues have also conducted several studies that examine how the use of different test signals changes the evoked potential response (e.g., Dolphin and Mountain 1992, 1993; Dolphin 1995, 1997, 2000). For example, the magnitude of the evoked potential response increases with both increased stimulus intensity and modulation depth (Dolphin and Mountain 1992). More recently, auditory evoked potential measurements and behavioral hearing measurements have been collected on the same animals to accurately compare the threshold differences generated by each
technique (e.g., Szymanski et al. 1999; Houser et al. 2004; Yuen et al. 2005; Finneran and Houser 2006; Houser and Finneran 2006; Cook et al. 2006, in prep.).

The major weaknesses of using behavioral techniques to study the hearing abilities of cetaceans include the large amounts of time required to train and test the animals (months to years) and the limited availability of animal subjects to test (e.g., generally smaller odontocetes maintained in captivity). In contrast, auditory evoked potential (AEP) techniques allow for the rapid measurement (minutes) of an individual’s hearing abilities with little or no training necessary. Thus, AEP techniques save large amounts of time, which potentially allow for larger sample sizes. Furthermore, AEP techniques allow animals to be tested in the field, in air or in the water, and with non-mobile animals, which means stranded and larger cetaceans can be examined (e.g., Popov and Klishin 1998; Ridgway and Carder 2001; André et al. 2003; Nachtigall et al. 2005; Cook et al. 2006).

Several research questions were addressed during the course of this dissertation. Chapter Two discusses the relationship among in-air AEP audiograms, underwater AEP audiograms, and underwater behavioral audiograms. In this study, two captive male bottlenose dolphins at The Living Seas, Epcot®, Walt Disney World® Resort, Calvin and Ranier, participated in the in-air and underwater AEP measurements, and Ranier participated in the underwater behavioral measurements. In addition, the acoustic stimuli used in each of the three experiments were the same. Therefore, the confounding issues of both subject and stimulus variability were removed from this study, and the three different methodologies could be directly compared. This chapter also addresses how
well in-air AEP audiograms model or predict traditional underwater behavioral audiograms.

Chapter Three investigates the hearing abilities of free-ranging bottlenose dolphins in Sarasota Bay, Florida, using in-air AEP techniques. This is the first study to examine the hearing abilities of a population of wild odontocetes. The effects of age and gender on an individual’s hearing abilities are discussed in this chapter. In addition, predicted underwater AEP and behavioral audiograms are calculated using the AEP-behavioral audiogram transfer function presented in Chapter Two. Finally, this chapter emphasizes the need for larger sample sizes when making population-level assessments or management decisions.

Chapter Four explores the hearing abilities of a live-stranded juvenile beaked whale (*Mesoplodon europaeus*). This study highlights the importance of stranded cetaceans, especially those that cannot be maintained in captivity, for addressing key scientific questions. In addition, these are the first hearing data collected for any member of the family Ziphiidae. Because several strandings of beaked whales have also been linked to the use of Naval sonar, the results of this study are discussed in terms of hearing sensitivity to sonar-like sounds.

Chapter Five provides a brief summary of each chapter and the concluding remarks to this dissertation.

Each chapter has been formatted for the Journal of Comparative Physiology A: Neuroethology, Sensory, Neural, and Behavioral Physiology. Chapter Four was published there earlier this year.
References Cited


Caldwell MC, Caldwell DK (1968) Vocalization of naïve captive dolphins in small groups. Science 159:1121-1123

Caldwell MC, Caldwell DK, Hall NR (1969) An experimental demonstration of the ability of an Atlantic bottlenosed dolphin to discriminate between whistles of other individuals of the same species. LA County Museum of Natural History Foundation TR 6, 37 pp.


Cook MLH, Bauer GB, Fellner W, Mann DA (in prep.) Ground-truthing in-air auditory evoked potential (AEP) hearing measurements with traditional behavioral audiograms in bottlenose dolphins (*Tursiops truncatus*).


Dolphin WF, Mountain DC (1993) The envelope following response (EFR) in the
Mongolian gerbil to sinusoidally amplitude-modulated signals in the presence of
simultaneously gated pure tones. J Acoust Soc Am 94:3215-3226

33:1799-1800

Dreher JJ, Evans WE (1964) Cetacean communication. In: Tavolga WN (ed) Marine Bio-
Acoustics. Pergamon Press, New York, pp 373-393


behavioral hearing thresholds in four bottlenose dolphins (Tursiops truncatus). J
Acoust Soc Am 119:3181-3192

Finneran JJ, Carder DA, Ridgway SH (2002a) Low-frequency acoustic pressure, velocity,
and intensity thresholds in a bottlenose dolphin (Tursiops truncatus) and white
whale (Delphinapterus leucas). J Acoust Soc Am 111:447-456

Finneran JJ, Schlundt CE, Carder DA, Ridgway SH (2002b) Auditory filter shapes for the
bottlenose dolphin (Tursiops truncatus) and the white whale (Delphinapterus
leucas) derived with notched noise. J Acoust Soc Am 112:322-328

Finneran JJ, Schlundt CE, Dear R, Carder DA, Ridgway SH (2002c) Temporary shift in
masked hearing thresholds in odontocetes after exposure to single underwater
impulses from a seismic watergun. J Acoust Soc Am 111:2929-2940


Lilly JC, Miller AM (1961a) Sounds emitted by the bottlenose dolphin. Science 133:1689-1693

Lilly JC, Miller AM (1961b) Vocal exchanges between dolphins. Science 134:1873-1876


Thompson RKR, Herman LM (1975) Underwater frequency discrimination in the bottlenosed dolphin (1-140 kHz) and the human (1-8 kHz). J Acoust Soc Am 57:943-948


Table 1-1  Behavioral hearing measurements for odontocete cetaceans.  N indicates the sample size for the study.

<table>
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<tr>
<th>AUTHOR</th>
<th>YEAR</th>
<th>SPECIES</th>
<th>n</th>
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<td>2005</td>
<td><em>Delphinapterus leucas</em></td>
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<td>2-130 kHz</td>
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<td>Finneran &amp; Houser</td>
<td>2006</td>
<td><em>Tursiops truncatus</em></td>
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<td>Houser &amp; Finneran</td>
<td>2006</td>
<td><em>Tursiops truncatus</em></td>
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<tr>
<td>Cook et al.</td>
<td>in prep.</td>
<td><em>Tursiops truncatus</em></td>
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Table 1-2  Auditory evoked potential (AEP) hearing measurements for odontocete cetaceans. N indicates the sample size for the study.

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<td>Bullock et al.</td>
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<td>Bullock &amp; Ridgway</td>
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<td>Ridgway</td>
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<td>Ridgway et al.</td>
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<td>Popov et al.</td>
<td>1986</td>
<td><em>Phocoena phocoena</em></td>
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<td>Popov &amp; Supin</td>
<td>1987</td>
<td><em>Delphinapterus leucas</em></td>
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<td>Popov &amp; Supin</td>
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<td>Popov &amp; Supin</td>
<td>1990b</td>
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<td>1990c</td>
<td><em>Inia geoffrensis</em></td>
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<tr>
<td>Supin et al.</td>
<td>1993</td>
<td><em>Tursiops truncatus</em></td>
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<td>Dolphin</td>
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<td>2, 2, 1</td>
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<td>1995</td>
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<td>Popov &amp; Klishin</td>
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<td><em>Delphinus delphis</em></td>
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<td>Szymanski et al.</td>
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<td><em>Orcinus orca</em></td>
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<td>Ridgway &amp; Carder</td>
<td>2001</td>
<td><em>Eschrichtius robustus,</em> Kogia breviceps,* Physeter macrocephalus*</td>
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<td>André et al.</td>
<td>2003</td>
<td><em>Stenella coeruleoalba</em></td>
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<td>Supin et al.</td>
<td>2003</td>
<td><em>Pseudorca crassidens</em></td>
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<td>Houser et al.</td>
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<td>Beedholm &amp; Miller</td>
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<td><em>Phocoena phocoena</em></td>
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<td>80, 100, 125, &amp; 160 kHz</td>
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<td>Nachtigall et al.</td>
<td>2005</td>
<td><em>Grampus griseus</em></td>
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<td>Popov et al.</td>
<td>2005</td>
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<td>Yuen et al.</td>
<td>2005</td>
<td><em>Pseudorca crassidens</em></td>
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<tr>
<td>Cook et al.</td>
<td>2006</td>
<td><em>Mesoplodon europaeus</em></td>
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<td>Finneran &amp; Houser</td>
<td>2006</td>
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<td>Cook et al.</td>
<td>in prep.</td>
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Chapter Two:

Ground-Truthing In-Air Auditory Evoked Potential (AEP) Hearing Measurements with Traditional Behavioral Audiograms in Bottlenose Dolphins (Tursiops truncatus)

Abstract

Auditory evoked potential (AEP) methods are more attractive than traditional behavioral methods for measuring the hearing of marine mammals because they allow for rapid assessments of hearing sensitivity and can be used on untrained animals. However, few studies have compared these two measurement types using the same individual. This study investigated the differences between underwater AEP and in-air AEP measurements using two captive bottlenose dolphins (Tursiops truncatus). Underwater behavioral hearing measurements were also made with one of the dolphins using the same stimuli used for the AEP measurements. Frequencies tested ranged from 5 to 80 kHz. There was generally good agreement among the hearing thresholds determined by these three methods at frequencies above 20 kHz. At 10 and 20 kHz, in-air AEP audiograms were substantially higher (about 15 dB) than underwater behavioral and underwater AEP audiograms, suggesting multiple sound pathways to the dolphins’ ears at lower frequencies.
Introduction

Bottlenose dolphins (*Tursiops truncatus*) have an impressive ability to both produce and perceive a wide variety of sounds over a large frequency range. Because dolphins rely on sound for communication, navigation, and foraging, their sense of hearing is one of their most important senses (Au 1993; Janik and Slater 1998). The vast majority of the information known about the hearing capabilities of dolphins and other odontocetes (toothed whales) has been obtained using traditional behavioral and psychometric techniques. Behavioral audiograms have been reported for twelve odontocete species: bottlenose dolphin *Tursiops spp.* (Johnson 1966, 1967; Ljungblad et al. 1982), common dolphin *Delphinus delphis* (Belkovich and Solntseva 1970), Pacific white-sided dolphin *Lagenorhynchus obliquidens* (Tremmel et al. 1998), striped dolphin *Stenella coeruleoalba* (Kastelein et al. 2003), Risso’s dolphin *Grampus griseus* (Nachtigall et al. 1995), Amazon river dolphin *Inia geoffrensis* (Jacobs and Hall 1972), Chinese river dolphin *Lipotes vexillifer* (Wang et al. 1992), beluga whale *Delphinapterus leucas* (White et al. 1978; Awbrey et al. 1988; Finneran et al. 2005), false killer whale *Pseudorca crassidens* (Thomas et al. 1988; Yuen et al. 2005), tucuxi *Sotalia fluvialitis guianensis* (Sauerland and Dehnhardt 1998), harbor porpoise *Phocoena phocoena* (Andersen 1970; Kastelein et al. 2002), and killer whale *Orcinus orca* (Hall and Johnson 1972; Szymanski et al. 1999). Because most behavioral studies require repeated measurements using highly-trained subjects, sample sizes are generally small (one to two animals) and data can take up to several years to collect.
As an alternative to traditional behavioral techniques, electrophysiological techniques can also be used to measure hearing abilities. The auditory evoked potential (AEP) response is a non-invasive electrophysiological technique commonly used to measure hearing thresholds and other aspects of hearing (e.g., masking and sound localization) in humans, birds, fish, and other animals, including cetaceans (e.g., Corwin et al. 1982; Supin and Popov 1995; Kenyon et al. 1998; Szymanski et al. 1999; Mann et al. 2001; Lucas et al. 2002). In general, when the auditory pathway is presented with an acoustic stimulus that is above threshold levels, large numbers of neurons within the acoustic pathway are excited. If the neuronal discharges are time-locked to the acoustic stimulus, the electrical signals produced by the simultaneous firings of multiple neurons produce a synchronous discharge that can be detected by an electrode placed on the head.

AEP hearing measurement techniques are advantageous over behavioral hearing measurement techniques for several reasons: they are relatively non-invasive, they require little to no animal training, and they can be done in short time periods. Therefore very rapid estimations of an individual’s hearing threshold can be obtained. Finally, AEP techniques can be used to measure the hearing sensitivities of animals, particularly cetaceans, for which behavioral audiograms cannot be determined (e.g., Ridgway and Carder 2001; Cook et al. 2006).

One notable problem with AEP hearing measurements is that they are measures of neural activity rather than sensation and perception. Thus, they need to be validated and calibrated against a direct measure of hearing, i.e., the behavioral audiogram. Although evoked potential and behavioral techniques have been used to assess hearing in many odontocetes (Dolphin 2000; Nachtigall et al. 2000; Supin et al. 2001), they have only
rarely been measured using the same animal (Szymanski et al. 1999; Yuen et al. 2005; Cook et al. 2006; Finneran and Houser 2006; Houser and Finneran 2006). Therefore, any comparison between the two techniques is confounded by potential subject differences.

Another potential source of variability comes from the type of sound stimulus used in each study condition. In general, pure tones (generally > 1 s) are used for behavioral hearing measurements (Nachtigall et al. 2000), while short clicks, tone pips, or tone bursts (all generally < 1 s) are used for AEP measurements (Dolphin 2000). For example, Szymanski et al. (1999) collected behavioral hearing data from killer whales using 2 s tones, and AEP data using 0.5-1 ms cosine-gated tone bursts. Yuen et al. (2005) used 3 s pure tones to measure the behavioral audiogram of a false killer whale and 20 ms sinusoidally amplitude-modulated (SAM) tone bursts to measure AEP thresholds. Cook et al. (2006), Finneran and Houser (2006), and Houser and Finneran (2006) used 500 ms tones to measure behavioral hearing sensitivities in bottlenose dolphins. However, Cook et al. (2006) used 14 ms SAM tone bursts to measure AEP hearing thresholds, Finneran and Houser (2006) used 12-15 ms SAM tone bursts to measure AEP thresholds, and Houser and Finneran (2006) used 23 ms SAM tone bursts to measure the majority of their AEP thresholds (Table 2-1). As a result, any comparisons between the two techniques are also complicated by the use of different acoustic stimuli and their potential to affect hearing sensitivity.

This study addresses these differences by conducting both AEP and behavioral hearing tests using the same individual and the same acoustic stimuli: 1.) AEP hearing measurements in air with sounds presented through a jawphone; 2.) AEP hearing measurements underwater using a free-field speaker; and 3.) Underwater behavioral
hearing measurements using a free-field speaker. By comparing differences among all three experiments, it then becomes possible to derive an appropriate calibration for the in-air and underwater AEP audiograms. Thus, behavioral audiogram estimates can be calculated for animals whose hearing can only be measured using AEP techniques, including live-stranded cetaceans, free-ranging cetaceans, and other untrained animals.

Materials and Methods

Subjects

In-air and underwater AEP measurements were collected from Ranier and Calvin, two male bottlenose dolphins (Tursiops truncatus). They are currently housed at The Living Seas, Epcot®, Walt Disney World® Resort in a 5.7-million-gallon circular exhibit housing many marine species, in Lake Buena Vista, Florida. Behavioral data were collected from only Ranier due to time and research limitations. Ranier is an approximately 25-year-old male, 2.6 m in length and 190 kg in weight. Calvin is an 11-year-old male (b. 1994), 2.5 m in length and 185 kg in weight. All research was approved by the IACUC of the Walt Disney World® Animal Programs.
AEP Methods

The AEP technique involves repeatedly playing a test sound stimulus while simultaneously recording the neural evoked potential from surface electrodes. The evoked potentials from each sound presentation are continuously added together to reduce background electrical noise in the recordings and reveal the underlying auditory response (Glasscock et al. 1987; Ferraro and Durrant 1994).

A Tucker-Davis Technologies (TDT) AEP Workstation with SigGen and BioSig software and laptop computer were used to control all stimulus presentations and data acquisition. This Workstation has been previously used in field situations, and to record AEPs from cetaceans, including bottlenose dolphins and a beaked whale (Cook et al. 2006). The TDT Workstation was capable of sampling at 192 kHz, which meant it could test frequencies up to 80 kHz.

Each sound trial lasted approximately one minute and consisted of playing amplitude modulated (AM) tones at specific frequencies and levels that were programmed using BioSig software. These AM tones consisted of 14 ms tone bursts presented 21 times per second and 100% modulated at 600 Hz (Figure 2-1), a modulation rate which has been found to yield strong AEP responses in bottlenose dolphins (Supin and Popov 1995).

Using AM tones in AEP procedures results in an Envelope Following Response (EFR) in which the auditory system of the subject produces neural responses that are phase-locked with the envelope of the stimulus (Dolphin 1996, 1997; Supin and Popov 1995). The advantages of using EFR are that 1.) it results in an AEP at the frequency of
AM (Dolphin and Mountain 1992), making it easily distinguished from background electrical noise in the signal, and 2.) it has a narrow frequency spectrum, allowing for good frequency resolution in the audiogram (Dolphin 2000).

Bottlenose dolphins use their lower jaws to receive sounds (Norris 1964, 1968). Jawphones (contact hydrophones attached to the dolphin using suction cups) take advantage of this pathway, and have been used by several researchers to present acoustic stimuli to bottlenose dolphins via their lower jaws (Bullock et al. 1968; McCormick et al. 1970, 1980; Brill et al. 2001; Cook et al. 2006; Finneran and Houser 2006; Houser and Finneran 2006). A jawphone composed of an ITC-1042 transducer embedded in a suction cup (constructed from VI-SIL V-1062, Rhodia, Inc.) and powered by a Hafler P1000 amplifier was used to deliver the acoustic stimuli for the in-air AEP measurements. The jawphone suction cup is composed of a silicone-based RTV material which has an acoustic impedance similar to water (Brill et al. 2001). The jawphone was placed on the lower left jaw of each animal, corresponding to position #38 in Møhl et al. (1999), which showed the greatest AEP response in their study.

AEP signals were collected using suction cup electrodes made from standard 8 mm silver-silver chloride electrodes (Med-Associates, Inc.) embedded in either vinyl (V-F65, Anver, Inc.) or RTV silicone (VI-SIL V-1062, Rhodia, Inc.) suction cups. Each dolphin’s skin was prepared by wiping the areas of suction cup attachment with a dry gauze pad in order to remove debris. Redux® electrolyte paste (Parker Laboratories, Inc.), commonly used in human and veterinary applications, was used on the electrodes to establish a good electrical connection between the electrode and the dolphin’s skin. A recording electrode was placed dorsally at the vertex of the skull, approximately six
centimeters behind the blowhole, and a reference electrode was placed just anterior to the
dorsal fin. A ground electrode was placed between the reference and recording
electrodes with approximately 20 centimeters separating adjacent electrodes. All suction
cups were removed as soon as tests were complete.

The protected electrode leads were attached to a differential amplifier (TDT DB4-
HS4) housed in a water-resistant case. The amplifier output was connected via a fiber
optic cable to the TDT Workstation for data acquisition with the BioSig software. BioSig
controlled both stimulus presentation and data acquisition. Electrical artifacts induced by
dolphins's breathing and locomotory muscle (or skeletal muscle) movement of the
electrodes were removed by artifact rejection in BioSig (excluding all sweeps with
evoked potentials greater than a set threshold).

Sounds in these experiments were played at levels less than or equal to 160 dB re
1 µPa, which is approximately the same sound pressure level (SPL) as whistles produced
by bottlenose dolphins (mean source level: 158 dB re 1µPa; Janik 2000). Furthermore, it
is much lower than sound levels that have been found to cause temporary threshold shifts
in dolphins (180-200 dB re 1 µPa; Schlundt et al. 2000). These sounds were attenuated
in 6 or 10 dB steps and controlled by the computer using a programmable attenuator
(TDT PA5). The following frequencies were measured: 5, 10, 20, 30, 40, 60, and 80
kHz. Higher frequencies could not be measured due to the sampling rate limitations of
the equipment.

Up to 5000 averages were run for each test trial, although a few underwater AEP
measurements contained up to 16,000 averages. Once an AEP response was observed,
averaging at that test level was ended, and the next level was tested, thus minimizing the
amount of time required to collect data. An AEP response was determined to be present if the evoked signal, measured from 5 to 18 ms or from 20 to 33 ms (Calvin’s in-air AEP measurements only), was greater than the background noise, estimated from 0 to 4 ms in the same sweep (Figure 2-2). This is the same threshold determination technique used by Cook et al. (2006).

*Experiment 1: In-Air AEPs with a Jawphone*

Ranier’s in-air AEP data were collected on July 20, 2004 from 0906 hrs to 0931 hrs. Calvin’s in-air AEP data were collected on April 19, 2005 from 0905 hrs to 0938 hrs and on May 30, 2006 from 0910 hrs to 0944 hrs. Each dolphin was isolated in a medical pool and the water level was dropped or the false-bottom floor was raised. The dolphin was then placed onto a closed-cell foam mat and kept wet using wet towels and water sprayers. Animal trainers were stationed laterally around the dolphin to help support it. Once the animal was correctly stationed and not moving, the suction cup electrodes and jawphone were attached and AEP testing began (Figure 2-3). As soon as testing was complete, all suction cups were removed, the water level was raised or the false-bottom floor was lowered, and the dolphin was fed. It should be noted that both dolphins were trained to voluntarily participate in this experiment. Also, because of changes in AEP procedures as part of other experiments, Calvin’s in-air AEP data were collected in response to 15 ms tones on April 19 and May 30, but only 13 ms of the signals were analyzed to maintain consistency.
Underwater Experimental Setup

The experimental conditions were similar between the underwater AEP measurements and the underwater behavioral audiogram measurements. Both experiments were conducted from a 0.4 m × 0.5 m floating dock within the 5.7-million-gallon circular environment. An underwater PVC stationing apparatus was securely attached to the floating dock. Each dolphin was trained to station in the apparatus one meter below the water surface by placing its rostrum into a plastic chinstrap. Animal trainers helped the dolphin maintain its position within the water column by gently holding its dorsal fin during testing. This also helped to prevent the animal from moving its flukes to maintain position, which reduced data contamination for the underwater AEP measurements. An ITC-1042 transducer, identical to the one used as the jawphone, was placed one meter underwater and attached to the PVC apparatus approximately 15 cm in front of the dolphin’s rostrum (Figure 2-4).

Experiment 2: Underwater AEPs with a Free-Field Speaker

The AEP methods, stimulus control, data collection, suction cup electrodes, and sound frequencies and levels used in the underwater AEP measurements were all identical to those used for the in-air AEP measurements with a few notable exceptions. Each dolphin was trained to wear the recording and reference suction cup electrodes while the ground electrode was placed freely in the water instead of on the animal. In addition the sounds
were presented from a free-field speaker instead of an attached jawphone. Finally the animals were trained to station one meter underwater for data collection.

The dolphin was called over to the floating platform, and the area between its blowhole and dorsal fin was wiped dry with a gauze pad. The recording and reference suction cup electrodes were subsequently attached to the animal in the same locations used for the in-air AEP measurements. A hand signal was then used to send the dolphin to the underwater PVC stationing apparatus. Once the dolphin was stationed correctly, underwater AEP measurements began (Figure 2-4). If the dolphin vocalized or moved excessively during testing, the trial was ended and the dolphin was signaled to return to the surface. Otherwise, the dolphin remained stationed for up to two minutes of data collection, after which time he was recalled to the surface and rewarded. Two-minute trials were conducted in 15-20 minute sessions, up to four times a day. Behavioral training for both Ranier and Calvin began on October 23, 2003. Ranier’s underwater AEP measurements were collected on May 11, 13, 19, and 28, 2004. Calvin’s underwater AEP measurements were collected on September 1, 2, 8, and 9, 2004.

Experiment 3: Underwater Behavioral Audiogram with a Free-Field Speaker

Ranier’s behavioral audiogram was measured using a modified go/no-go procedure (Schusterman 1980), in which he was trained to vocalize in the presence of a tone and remain silent in the absence of a tone. A hand signal was used to send Ranier to the underwater PVC stationing apparatus, which was the same apparatus used for the
underwater AEP measurements. Once Ranier was stationed correctly, an underwater light illuminated, indicating the start of a trial. The time between the light illumination and sound presentation (excluding catch trials) varied from two to three seconds. Ranier was trained to whistle within six seconds of detecting a sound and to remain silent for 10 seconds if no sound was detected. He was recalled to the surface at the end of each trial.

A research assistant used a Sonatech (Model 8234-1) hydrophone and headset to monitor for the dolphin’s response (whistle or no whistle) during each trial. This information was electronically relayed to the TDT Workstation, which recorded whether Ranier’s response was correct or incorrect and then automatically determined the next trial. The TDT Workstation flashed a green LED for each of Ranier’s correct responses, and a red LED for each of Ranier’s incorrect responses. This alerted the trainer whether or not to reward Ranier’s response. Each correct response was rewarded, and each incorrect response was neither rewarded nor punished. The trainer and the assistant were both naïve as to whether a tone was present or absent during each trial, except at 5 and 10 kHz, which the assistant could hear through the headphones at only the loudest sound presentations. Continuous acoustic recordings were also collected during each session using Avisoft SASLab Pro v 4.38 (Avisoft Bioacoustics, Berlin).

The sound stimuli used for the behavioral audiogram measurements were the same as those used for the AEP measurements. Thus, each trial lasted approximately one minute and consisted of playing 600 Hz AM tone bursts 14 ms in duration and repeated 21 times per second. A modified staircase method was used. For each tone frequency, testing was started at a sound intensity level that was easily detectable, based on previously published reports for bottlenose dolphins (Johnson 1966, 1967) and on
preliminary analyses of Ranier’s in-air AEP data. Initial step sizes were 6 dB until the first error, after which 3 dB step sizes were used. Catch trials (no sound presented) varied between 25 and 50% of the total trials per session. Session with 25% catch trials allowed more sound trials to be run, but these sessions were alternated with 50% catch trial sessions in order to avoid biasing Ranier’s response pattern toward whistling. Table 2-2 shows that Ranier’s responses were not biased between the 25% and 50% catch trial sessions because similar response patterns were seen between the two types of sessions.

Run lengths for sound or catch trials varied pseudo-randomly (Gellermann 1933) with a maximum of three of one trial type in a row. A session consisted of 30 trials; this was designed to elicit approximately eight sound intensity reversals per session. A threshold was defined as two consecutive sessions with mean amplitude levels of reversals differing by no more than 3 dB. Because of logistical constraints, only Ranier participated in this experiment. Training for the underwater behavioral audiogram began on September 23, 2004. Underwater behavioral audiogram data were collected over the course of 69 sessions between December 29, 2004 and June 6, 2005.

Jawphone and Free-Field Speaker Calibrations

The jawphone was calibrated for the in-air AEP measurements by placing a Reson calibrated hydrophone (Reson TC4013; -212 dBV re 1 µPa) 10 cm from the end of the suction cup, and calibrating it in the test tank at approximately one meter water depth. For the underwater AEP and behavioral hearing measurements, the free-field speaker was
calibrated by placing a calibrated hydrophone (Reson TC4013 or HTI 96-min; -164 dBV re 1 μPa) in the center of the chinstrap either before or after each test session without the dolphins present. Background tank noise was also measured with the HTI hydrophone, which provided better sensitivity than the Reson hydrophone for measuring the low background noise levels.

**Results**

Calvin

Table 2-3 and Figure 2-5 present the in-air and underwater AEP hearing thresholds for Calvin. In-air AEP thresholds closely matched underwater AEP thresholds, except at 10 and 20 kHz, where the underwater thresholds were much lower than the thresholds measured in air.

Ranier

Table 2-4 and Figure 2-6 present the in-air AEP, underwater AEP, and behavioral hearing thresholds for Ranier. Underwater AEP thresholds could only be determined for four of the seven frequencies tested, and in-air AEP thresholds could only be determined for six of the seven frequencies tested. Underwater AEP thresholds were lower than in-air AEP
thresholds at 10 and 20 kHz and more closely resembled the behavioral audiogram at these frequencies. Nonetheless, there was generally good agreement among the different measurements, especially at 40, 60, and 80 kHz.

**Discussion**

While behavioral psychoacoustic methods provide the most direct measures of hearing (Nachtigall et al. 2000), the time and training required with these techniques limit their broad application. Alternatively, AEP methods can be used to rapidly assess hearing abilities and can be used on minimally or untrained animals. Studies comparing behavioral and AEP hearing measurements on the same animal have only recently been conducted (Szymanski et al. 1999; Yuen et al. 2005; Cook et al. 2006; Finneran and Houser 2006; Houser and Finneran 2006), and only two of these studies measured AEPs in air (Cook et al. 2006; Finneran and Houser 2006). Szymanski et al. (1999) found that AEP thresholds were, on average, 12 dB less sensitive than behavioral thresholds across the entire frequency range tested. Yuen et al. (2005) obtained similar results, with behavioral thresholds always lower than AEP thresholds. Behavioral hearing thresholds were also lower than AEP thresholds for two of the three animals measured by Cook et al. (2006). Behavioral and AEP hearing thresholds were in close agreement for the animals evaluated by Finneran and Houser (2006) and Houser and Finneran (2006), and measured differences between the two methods were generally the result of more sensitive behavioral measurements.
The current study measured in-air AEP, underwater AEP, and behavioral audiograms in the same individual using the same acoustic stimuli; this combination allowed for differences among methodologies to be directly compared. These results show that behavioral, underwater AEP, and in-air AEP hearing measurements produce similar thresholds, especially at higher frequencies. At 10, 20, and 60 kHz both Calvin and Ranier had higher in-air AEP thresholds than underwater AEP thresholds, and at 40 kHz they both had higher underwater AEP thresholds than in-air AEP thresholds (Tables 2-2 and 2-3). At both 30 and 80 kHz, Calvin’s underwater AEP threshold measurements were higher than his in-air AEP measurements. Ranier’s underwater AEP hearing thresholds were not determined at either of these frequencies because there were no AEP signals larger than the AEP noise floor at 80 kHz and because the 30 kHz data were contaminated by low-frequency electrical noise near the rate of amplitude modulation. Additionally, Ranier’s in-air and underwater AEP hearing thresholds were not measured at 5 kHz due to time limitations.

Ranier’s underwater behavioral hearing thresholds were lower than both his underwater AEP and in-air AEP hearing thresholds at 10, 20, 30 (in-air AEP only), and 40 kHz, while at 60 kHz his behavioral hearing threshold was higher than either AEP threshold. At 80 kHz, Ranier’s underwater behavioral and in-air AEP thresholds were very similar, differing by only 0.6 dB re 1 µPa. These results show that in-air AEP measurements collected using a jawphone accurately represent underwater behavioral measurements at higher frequencies, and exhibit both the general shape and high-frequency cutoff of behavioral audiograms. In addition, these results are consistent with previous studies, which also found similar thresholds between behavioral and in-air AEP
measurements at higher frequencies (Cook et al. 2006; Finneran and Houser 2006). Somewhat surprisingly, these measurements also demonstrate Ranier’s substantial hearing losses at 60 and 80 kHz which had previously gone undetected.

At lower frequencies, behavioral measurements resulted in the lowest measured hearing thresholds, followed by underwater AEP measurements and finally in-air AEP measurements, with the highest measured hearing thresholds. These results support the idea that bottlenose dolphins transmit lower frequency sounds to their ears using multiple sound pathways, not solely via the acoustic window area of their lower jaws (Popov et al. 2006). However, it is also possible that this is an acoustic phenomenon related to the size of the jawphone suction cup, which itself can act as an acoustic waveguide. At 20 kHz, the acoustic wavelength is approximately 7.5 cm, while the jawphone diameter is 5.0 cm. At higher frequencies, the acoustic wavelengths are shorter than the jawphone diameter.

The results of this study allow behavioral audiogram thresholds to be estimated for dolphins whose hearing can only be measured using in-air AEP techniques, including live-stranded, free-ranging, and other untrained cetaceans. The transfer function of the in-air AEP audiogram to the underwater behavioral audiogram (the numerical difference between the two hearing threshold measurements at each frequency) accounts for all differences between the two test procedures, including differences in calibration procedures. One of the challenges of in-air AEP audiograms is measuring the sound level at the dolphin ear. In this study, a free-field calibration of the jawphone measured at 10 cm was used to estimate the jawphone sound levels. However, the jawphone is not used in a free-field situation when it is attached to a dolphin in air. The calibration performed underwater is relatively straightforward, since the sound level can be
measured at the same location as the dolphin in the stationing apparatus. The transfer function thus accounts for errors in the estimation of the delivered sound level in air, as well as for differences between the AEP and behavioral methods.

When comparing among studies that measure both behavioral and AEP hearing thresholds in the same individuals, other factors must also be considered. Behavioral test paradigms and step sizes, number of AEP sweeps averaged per trial, signal lengths, background noise levels, and methods of threshold determination all factor into the final threshold value assigned to each test frequency. For example, Szymanski et al. (1999), Yuen et al. (2005), and the current study all used variations of the go/no-go test paradigm, while Cook et al. (2006), Finneran and Houser (2006), and Houser and Finneran (2006) used both go/no-go and Method of Free Response test paradigms. Behavioral step sizes in Szymanski et al. (1999) were 6-8 dB, and in both Yuen et al. (2005) and Finneran and Houser (2006) they were 2 dB. In the current study they were 3 dB. The number of AEP sweeps averaged per trial also varied considerably among studies. Szymanski et al. (1999) averaged 350 sweeps, Yuen et al. (2005) averaged 1000 sweeps, Cook et al. (2006) averaged up to 2000 sweeps, Finneran and Houser (2006) averaged between 500 and 1000 sweeps, and Houser and Finneran (2006) averaged 500 sweeps. The current study averaged up to 16,000 sweeps, due in part to the difficulty of obtaining robust AEP signals underwater.

Background noise can also affect the final hearing threshold calculations. Finneran and Houser (2006) measured behavioral hearing thresholds for one of their subjects, BLU, in an above ground pool and in San Diego Bay. Because of higher background noise levels, BLU’s behavioral hearing thresholds in San Diego Bay were
substantially elevated compared to her hearing thresholds in the pool, especially below 40 kHz (Finneran and Houser 2006).

Perhaps the most important factor in comparing among hearing thresholds in cetaceans is the method used to determine the threshold. For example, Szymanski et al. (1999) defined the behavioral threshold as “two detections at one intensity level, and two failures to detect the tone level below”; thresholds reported were the average of three determinations. Yuen et al. (2005) defined the behavioral threshold as a minimum of five reversals, and where the threshold values of two consecutive sessions varied by no more than 3 dB. Finneran and Houser (2006) defined their behavioral thresholds as 6-10 consecutive reversals averaged between 2-3 independent sessions. The current study defined a threshold as two consecutive sessions where the threshold value varied by no more than 3 dB, and was the result of at least eight reversals.

AEP threshold determination is equally variable. Szymanski et al. (1999) defined AEP thresholds as a 350 nV PIII-NIV level (peak-to-peak). Yuen et al. (2005) and Houser and Finneran (2006) calculated a linear regression of the AEP data and extrapolated to 0 V; this was defined as the AEP hearing threshold. Cook et al. (2006) defined thresholds as the quietest SPL for which an AEP was detected above the noise floor, and Finneran and Houser (2006) used magnitude-squared coherence to determine the AEP hearing thresholds in their study. Thus, differences in the way thresholds are determined could affect the final value reported at each test frequency. Until systematic calculations are used to determine these values, it will remain difficult to compare results from different studies.
Nonetheless, the results of this study and previous studies (Szymanski et al. 1999; Yuen et al. 2005; Cook et al. 2006; Finneran and Houser 2006; Houser and Finneran 2006) show that each of the three methods used to measure cetacean hearing (in-air AEPs, underwater AEPs, and underwater behavioral measurements) can reliably determine both the general shape and high-frequency cutoff of an individual’s audiogram. Furthermore, these results demonstrate that AEP hearing measurements are acceptable alternatives to traditional behavioral measurements. In situations where behavioral hearing measurements cannot be made, i.e., temporarily-captured and stranded cetaceans, AEP hearing measurements will provide valuable information regarding the auditory capabilities of these animals.
References Cited


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Table 2-1  Auditory evoked potential (AEP) and behavioral hearing studies for which the same test subjects were used.

<table>
<thead>
<tr>
<th>AUTHOR</th>
<th>YEAR</th>
<th>SPECIES (n)</th>
<th>TEST SIGNAL</th>
<th>TEST TYPE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Szymanski et al.</td>
<td>1999</td>
<td><em>Orcinus Orca</em> (2)</td>
<td>0.5 ms or 1 ms cosine-gated tone bursts</td>
<td>AEP</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2 s tones</td>
<td>Behavioral</td>
</tr>
<tr>
<td>Yuen et al.</td>
<td>2005</td>
<td><em>Pseudorca crassidens</em> (1)</td>
<td>20 ms SAM tone bursts</td>
<td>AEP</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>3 s pure tones</td>
<td>Behavioral</td>
</tr>
<tr>
<td>Cook et al.</td>
<td>2006</td>
<td><em>Tursiops truncatus</em> (3)</td>
<td>14 ms SAM tone bursts</td>
<td>AEP</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>500 ms tones</td>
<td>Behavioral</td>
</tr>
<tr>
<td>Finneran &amp;</td>
<td>2006</td>
<td><em>Tursiops truncatus</em> (4)</td>
<td>12-15 ms SAM tone bursts and/or continuous SAM tones</td>
<td>AEP</td>
</tr>
<tr>
<td>Houser</td>
<td></td>
<td></td>
<td>500 ms tones</td>
<td>Behavioral</td>
</tr>
<tr>
<td>Houser &amp;</td>
<td>2006</td>
<td><em>Tursiops truncatus</em> (3)</td>
<td>23, 32, or 62 ms SAM tone bursts</td>
<td>AEP</td>
</tr>
<tr>
<td>Finneran</td>
<td></td>
<td></td>
<td>500 ms tones</td>
<td>Behavioral</td>
</tr>
<tr>
<td>Present Study</td>
<td>2006</td>
<td><em>Tursiops truncatus</em> (2)</td>
<td>14 ms SAM tone bursts</td>
<td>AEP</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>14 ms SAM tone bursts</td>
<td>Behavioral</td>
</tr>
</tbody>
</table>
Table 2-2  Average number of hits, misses, false alarms, and correct rejections for the 25% and 50% catch trial sessions. Hits and misses are for sound trials, and false alarms and correct rejections are for catch trials. These numbers show that Ranier’s responses were not biased during the 25% catch trial sessions because similar patterns were seen during the 50% catch trial sessions.

<table>
<thead>
<tr>
<th>Frequency (kHz)</th>
<th>25% CATCH TRIALS</th>
<th>50% CATCH TRIALS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Avg. # Hits</td>
<td>Avg. # Misses</td>
</tr>
<tr>
<td>10</td>
<td>13.3</td>
<td>3.7</td>
</tr>
<tr>
<td>30</td>
<td>13.0</td>
<td>4.0</td>
</tr>
<tr>
<td>40</td>
<td>10.0</td>
<td>2.5</td>
</tr>
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</table>
Table 2-3  Calvin’s underwater AEP and in-air AEP hearing thresholds.

<table>
<thead>
<tr>
<th>FREQUENCY (kHz)</th>
<th>UNDERWATER AEP THRESHOLD (dB re 1 µPa)</th>
<th>IN-AIR AEP THRESHOLD (dB re 1 µPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>112.4</td>
<td>107.9</td>
</tr>
<tr>
<td>10</td>
<td>90.0</td>
<td>108.9</td>
</tr>
<tr>
<td>20</td>
<td>85.2</td>
<td>116.7</td>
</tr>
<tr>
<td>30</td>
<td>90.2</td>
<td>85.3</td>
</tr>
<tr>
<td>40</td>
<td>72.7</td>
<td>68.0</td>
</tr>
<tr>
<td>60</td>
<td>76.0</td>
<td>81.3</td>
</tr>
<tr>
<td>80</td>
<td>74.0</td>
<td>71.3</td>
</tr>
</tbody>
</table>
Table 2-4 Ranier’s underwater behavioral, underwater AEP, and in-air AEP hearing thresholds.

<table>
<thead>
<tr>
<th>FREQUENCY (kHz)</th>
<th>UNDERWATER BEHAVIORAL THRESHOLD (dB re 1 µPa)</th>
<th>UNDERWATER AEP THRESHOLD (dB re 1 µPa)</th>
<th>IN-AIR AEP THRESHOLD (dB re 1 µPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>103.8</td>
<td>not tested</td>
<td>not tested</td>
</tr>
<tr>
<td>10</td>
<td>95.5</td>
<td>107.2</td>
<td>122.2</td>
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<tr>
<td>20</td>
<td>81.2</td>
<td>86.8</td>
<td>101.1</td>
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<tr>
<td>30</td>
<td>79.2</td>
<td>not determined</td>
<td>93.4</td>
</tr>
<tr>
<td>40</td>
<td>84.4</td>
<td>95.0</td>
<td>86.3</td>
</tr>
<tr>
<td>60</td>
<td>132.9</td>
<td>119.3</td>
<td>122.1</td>
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<tr>
<td>80</td>
<td>136.2</td>
<td>not determined</td>
<td>136.8</td>
</tr>
</tbody>
</table>
Figure 2-1 Amplitude-modulated (AM) tone that was presented to the dolphins via a jawphone or a free-field speaker. This example is of a 40 kHz tone modulated at 600 Hz generated using TDT SigGen software.
Figure 2-2 An example of evoked potential data collected from Calvin in response to an 80 kHz AM tone at ten sound levels. Time, in milliseconds, is on the x-axis, and sound pressure level (SPL), in dB re 1 µPa, is on the y-axis.
Figure 2-3  In-air AEP hearing tests on Ranier.
Figure 2-4  Underwater AEP hearing tests on Calvin.
Figure 2-5 In-air and underwater AEP hearing thresholds for Calvin. Spectrum level (dB re 1 µPa^2/Hz) background noise from the underwater tests is also plotted.
Figure 2-6 In-air AEP, underwater AEP, and behavioral hearing thresholds for Ranier. Spectrum level (dB re 1 µPa²/Hz) background noise from the underwater tests is also plotted.
Chapter Three:

Auditory Evoked Potential (AEP) Hearing Thresholds of Free-Ranging Bottlenose Dolphins (*Tursiops truncatus*)

Abstract

Bottlenose dolphins (*Tursiops truncatus*) rely on sound for communication, navigation, and foraging. Therefore hearing is one of their primary sensory modalities. Both natural and anthropogenic noise in the marine environment could mask the ability of free-ranging dolphins to detect sounds, and chronic noise exposure could cause permanent hearing losses. The goal of this study was to investigate the hearing abilities of a population of free-ranging bottlenose dolphins in Sarasota Bay, Florida. The hearing abilities of 62 bottlenose dolphins (32 males and 30 females), ranging in age from 2 to 36 years, were measured in the field using non-invasive auditory evoked potential (AEP) techniques during brief capture-release sessions for health assessment. Evoked potentials in response to amplitude-modulated (AM) tones ranging from 5-120 kHz elicited a robust envelope following response (EFR), and allowed an entire audiogram to be obtained in approximately 40 minutes. There was considerable individual variation, up to 80 dB between individuals, in hearing abilities. With the possible exception of dolphin F195, which did not produce a detectable evoked potential in response to a 120 dB signal at 40
kHz, none of the Sarasota dolphins demonstrated substantial hearing losses. There was no relationship between age, gender, or PCB load and hearing sensitivities. It is possible that the oldest animals in this population (> 36 years old) do exhibit hearing losses, but they were not tested in this study. Hearing measured in a 52-year-old captive-born bottlenose dolphin showed similar hearing thresholds to the Sarasota dolphins up to 80 kHz, but exhibited a 50 dB drop in sensitivity at 120 kHz. It is also possible that individuals experiencing hearing losses do not survive long in the wild as a result of compromised echolocation abilities.
Introduction

Bottlenose dolphins (*Tursiops truncatus*) have an impressive ability to both produce and perceive a wide variety of sounds including echolocation clicks, whistles, and burst-pulse sounds (Au 1993; Caldwell et al. 1990; Thomson and Richardson 1995). Because dolphins rely on these sounds for communication, navigation, and foraging, their sense of hearing is one of their most important senses (Au 1993; Janik and Slater 1998). Anthropogenic noises, including boat engine noise, ultrasonic noise from depth sounders and fish finders, marine construction, and industrial noise, could be impacting dolphins and other cetaceans by impairing their hearing or otherwise interfering with their detection of biologically relevant sounds. The effect of noise on marine mammals is currently a hotly-debated topic in scientific and environmental communities. Much of this debate is contentious due to a lack of data on the actual impacts of noise on these animals, especially on their hearing abilities.

Behavioral audiograms have been reported for several of the at least 70 odontocete (toothed whale) species (Reeves et al. 2002) including the bottlenose dolphin (Johnson 1966, 1967; Jacobs 1972; Thompson and Herman 1975). However, most behavioral paradigms require repeated measurements using highly trained animals; therefore, sample size is generally limited to one or two individuals. In addition, the subjects must be maintained in captivity for long periods, which limits the number of species available for study.

As an attractive alternative to traditional behavioral techniques, auditory evoked potential (AEP) techniques can also be used to measure hearing abilities in odontocetes.
(e.g., Ridgway et al. 1981; Supin et al. 1993; Supin and Popov 1995; Szymanski et al. 1999; Yuen et al. 2005; Finneran and Houser 2006; Houser and Finneran 2006; Cook et al. in prep.). In general, when the auditory pathway is presented with an acoustic stimulus that is above threshold levels, large numbers of neurons within the acoustic pathway are excited. The simultaneous firing of multiple neurons produces an electrical signal that can be detected by an electrode placed on the head.

AEP hearing measurements are advantageous over behavioral measurements because they are non-invasive, require little to no training on the part of the animal, and can be completed in short time segments. Thus, they allow researchers to perform very rapid estimates of an individual’s audiogram. Finally, they can be used to test hearing thresholds of animals for which behavioral audiograms cannot be determined (Ridgway and Carder 2001), including live-stranded (Popov and Klishin 1998; André et al. 2003; Nachtigall et al. 2005; Cook et al. 2006) and free-ranging odontocetes.

Data from both behavioral and AEP techniques have resulted in fewer than a dozen published audiograms for bottlenose dolphins. Thus, little is known about intra-specific variability in their hearing capacities. Only four studies published to date have even begun to examine this variability (Ridgway and Carder 1993, 1997; Finneran and Houser 2006; Houser and Finneran 2006). Ridgway and Carder (1993, 1997) found that high-frequency hearing loss is common in elderly captive bottlenose dolphins. Out of the eight individuals they tested, three males over the age of 25 (25, 29, and 35) and one female, age 33, showed hearing losses at higher frequencies. The remaining four animals, two older females, age 32 and 36, one younger male, age 9, and one younger female, age 13, showed no noticeable hearing losses. These studies suggest that hearing
loss in bottlenose dolphins may not be just a factor of increasing age, but may also depend to some extent on the gender of the individual.

The National Research Council (NRC) noted that “[audiometric] measurements from a single animal should be viewed as only a temporary substitute for average hearing capabilities across members of wild populations” (NRC 2000), yet no study to date has investigated the hearing thresholds of free-ranging dolphins. In Sarasota Bay, Florida, bottlenose dolphin capture-release projects have been carried out since 1970, with health assessment of the wild community being the primary goal of the research since the late 1980s (Wells and Scott 1990; Wells et al. 2004). These studies have collected detailed information about these animals including age, gender, and genetic relatedness to other animals in the community. In addition, individuals are sampled for levels of environmental contaminants including several PCB congeners (Wells et al. 2005). Thus, this community provides a rare opportunity to assess hearing in a natural population for which age, gender, contaminant loads, and relatedness of individuals could be correlated to differences in hearing sensitivity.

This study reports the auditory temporal resolution and evoked potential hearing measurements for 62 free-ranging bottlenose dolphins (Tursiops truncatus) determined using AEP techniques. Two different hearing tests were performed. The modulation rate transfer function (MRTF), which measures the strength of the AEP using different modulation rates, was determined for a subset of seven dolphins. The second test, the envelope following response (EFR) procedure, was used to estimate AEP hearing thresholds for all 62 animals. Because PCBs have been linked to hearing losses in mammals including humans (Murata et al. 1999; Grandjean et al. 2001), hearing
thresholds were also compared to PCB concentrations for several male dolphins. PCBs tend to bio-accumulate in male dolphins, but in female dolphins concentrations tend to decline with reproductive activity (Wells et al. 2005). This suggests that PCBs and other lipid-soluble contaminants are transferred to the fetus and/or calf, either through direct transfer across the placenta or through lactation; thus, the congener concentrations found in many of the female Sarasota dolphins may not accurately represent exposure levels (Wells et al. 2005). Finally, the hearing of a 52-year-old captive-born dolphin, the oldest bottlenose dolphin in captivity, was measured to compare with the measurements of the free-ranging dolphins.

Materials and Methods

Subjects

Sarasota Bay, FL, Bottlenose Dolphin Community

AEP hearing measurements were collected on individual bottlenose dolphins within the Sarasota Bay bottlenose dolphin community (Wells 2003). Bottlenose dolphins were encircled in a net (500 m × 5 m, 15-20 cm stretch mesh) in shallow water (< 2 m). Most individuals were then brought onboard a veterinary examination boat to be evaluated. A full assessment typically required up to one hour, after which the individual was returned to the water and released. In-air AEP data were simultaneously collected during this
onboard examination. The AEP procedures did not significantly increase the amount of time that dolphins were on the examination boat nor did they adversely impact other ongoing projects.

AEP tests were conducted during six health assessment sessions conducted between June 2003 and June 2006. Each dolphin brought onboard the examination boat for a complete veterinary work-up had its hearing tested (n=32 males and n=30 females). These animals ranged in age from 2 to 36 years (Table 3-1). During the study period, five animals were sampled twice and one animal was sampled three times, for a total of 69 AEP tests.

During the same health assessment sessions, blubber samples were taken from individuals to determine the concentrations of 63 different PCB congeners.

_Dolphin Conservation Center at Marineland_

AEP hearing measurements were collected on Nellie, a 52-year-old female bottlenose dolphin, born and raised at Marineland of Florida, on August 17, 2005 from 0931 hrs to 0955 hrs and from 1612 hrs to 1627 hrs. Because of her advanced age, she remained in the water during testing, with her lower jaw below the water and her melon and dorsal surface above the water. The jawphone was attached to her lower left jaw during AEP testing, similar to the procedure used by Cook et al. (2006) to measure the hearing of a stranded beaked whale (_Mesoplodon europaeus_). Animal trainers gently restrained
Nellie at the surface of the water during testing to keep her from making large movements.

AEP Methods

The AEP technique involves repeatedly playing a test sound stimulus while simultaneously recording the synchronized neural evoked potential from surface electrodes. Because the evoked potential from a single sound stimulus is small and is less than the electrical noise in the recordings, the neural potentials in response to each tone presentation are summed to increase the signal above the noise and reveal the underlying evoked potential (Glasscock et al. 1987; Ferraro and Durrant 1994); this process is called “signal averaging”.

All stimulus presentation and data acquisition were controlled from a Tucker-Davis Technologies (TDT) AEP Workstation with SigGen and BioSig software. The TDT Workstation was controlled with a laptop computer, and was powered using a marine battery and inverter on the veterinary examination boat. This Workstation has been used previously to record AEPs from other odontocetes in field situations (Cook et al. 2004, 2005, 2006, in prep.). This Workstation used programmed test frequencies and test levels that were controlled using BioSig software.

Two different AEP hearing tests were performed. The first test was a measurement of the MRTF, which determines how well the auditory system is able to follow the temporal envelope of an acoustic stimulus (Dolphin et al. 1995). For this test,
a 40 kHz stimulus carrier (132 dB re 1 µPa) was 100% amplitude modulated with amplitude modulation (AM) rates ranging from 200 Hz to 2000 Hz, in 100 Hz steps. The second AEP hearing test employed the EFR technique (Supin and Popov 1995; Dolphin 1996, 1997, 2000) and was used to determine the hearing thresholds of each animal. For this test, a 600 Hz AM rate was chosen 1.) because it yields a robust EFR response in bottlenose dolphins (Supin and Popov 1995; Cook et al. in prep.) and 2.) because signals modulated at 600 Hz have a relatively narrow frequency spectrum, which allows for good frequency resolution in the audiogram, especially at lower carrier frequencies.

Each trial lasted approximately one minute and consisted of playing AM tones at specific frequencies and levels. These AM tones consisted of 14 ms tone bursts modulated at 600 Hz; beginning in February 2005 the signal length was increased to 15 ms to allow for nine complete cycles of the 600 Hz modulation rate. This sound was presented 21 times per second, with simultaneous averaging of the evoked potential sweeps. Sounds in these experiments were presented at levels less than or equal to 160 dB re 1 µPa. These sound stimuli are quieter than sounds the animals are normally exposed to on a daily basis, and are much lower than sound levels that have been found to cause temporary threshold shifts in dolphins (180-200 dB re 1 µPa; Schlundt et al. 2000).

The frequencies tested were divided into two groups that spanned the dolphin hearing range from 5 kHz-120 kHz. Thus, if an animal’s time on the examination boat was less than expected, there were still data that spanned the hearing range. The following frequencies were initially measured: 10, 20, 40, and 80 kHz. Once these frequencies had been tested and if time was still available, the following frequencies were
then tested: 5, 30, and 60 kHz. In February 2005 the TDT AEP Workstation was upgraded from the RP2.1 to the RX6, so that 120 kHz could also be tested.

A jawphone composed of an ITC-1042 transducer embedded in a suction cup (constructed from VI-SIL V-1062, Rhodia, Inc.) and powered by a Hafler P1000 amplifier was used to deliver the acoustic stimulus. The jawphone suction cup is composed of an RTV silicone-based material which has an acoustic impedance similar to water (Brill et al. 2001). The jawphone was placed on the lower left jaw of each animal corresponding to position #38 in Møhl et al. (1999), which showed the greatest AEP response in their study. The jawphone was calibrated by placing a Reson calibrated hydrophone (Reson TC4013; -212 dBV re 1 µPa) 10 cm from the end of the suction cup, and calibrating it in the field at approximately one meter water depth. Sound levels were controlled by the computer with a programmable attenuator (TDT PA5).

AEP signals were collected with vinyl (V-F65, Anver, Inc.) or RTV silicone (VI-SIL V-1062, Rhodia, Inc.) suction cups that incorporated standard 8 mm Ag-AgCl electrodes (Med-Associates, Inc.). The skin of each individual was prepared by wiping the areas of suction cup attachment with a dry gauze pad in order to remove debris. Redux® electrolyte paste (Parker Laboratories, Inc.) was used on the electrodes to establish a good electrical connection between each electrode and the dolphin’s skin. All suction cups were removed as soon as tests were complete.

Recordings were made with three suction cup electrodes attached to a differential amplifier (TDT DB4-HS4). A recording electrode was placed dorsally at the vertex of the skull, approximately six centimeters behind the blowhole. The reference electrode was located just anterior to the dorsal fin. A ground electrode was placed between the
reference and recording electrodes, with approximately 20 centimeters separating adjacent electrodes.

The output signal of the amplifier was connected via a fiber optic cable to the TDT Workstation for data acquisition with the BioSig software, which was located in the bow of the examination boat. BioSig controlled both stimulus presentation and data acquisition. Electrical artifacts induced by dolphin breathing and movement of the electrodes were removed by artifact rejection in BioSig (excluding all sweeps with evoked potentials greater than a set threshold). Information about the test subject, placement of the jawphone, date, time, amplifier gain, number of sweeps, and any additional information was stored with the AEP data in BioSig and was also recorded separately in a field notebook.

The number of sweeps analyzed ranged from 200 to 7176, with an average of 1795 (± 1424) sweeps analyzed for each trial. Once an AEP response was observed, averaging at that test level ended, and the next level was tested. Evoked potential levels in response to the AM tones were measured by performing a 1220-point Fast Fourier Transform (FFT) on the portion of the evoked potential waveform containing the evoked potential in response to the sound. Evoked potentials were included in the analysis if there was a peak in the spectrum that was greater in amplitude than an estimate of the noise level from the same sweep.

Because input-output functions, plots of evoked potential strength against sound pressure level (SPL), are non-linear, they were not used to extrapolate hearing thresholds. Rather, the lowest SPL for which an evoked potential was detected with a signal strength less than or equal to -150 dBV (31.62 nV) was determined to be the threshold SPL for
each individual at each frequency. Thus, if an evoked potential signal greater than -150 dBV was still detected at the lowest SPL presented for an individual at a given frequency it was excluded from further analyses. The -150 dBV cutoff level indicated that sufficient averaging had been performed and that extraneous electrical noise did not produce artificially high thresholds.

A multiple linear regression model (STATISTICA v. 6, StatSoft, Inc.) was calculated for each frequency to determine if hearing thresholds were affected by the age and/or gender of the individual. Correlations between hearing thresholds and PCB concentrations were also calculated for the male dolphins at each frequency.

**Results**

**MRTF**

The MRTFs of seven bottlenose dolphins were measured to determine the effect of AM rate on the evoked potential amplitude (Figure 3-1). Responses were detected at all modulation rates tested from 200 to 2000 Hz. Although there was a large amount of variability among the individuals tested, a 600 Hz modulation rate consistently gave a robust response to the 40 kHz stimulus carrier with high signal-to-noise ratios. Moderate peaks occurred at modulation rates of 1000-1200 Hz, while a trough occurred at 800 Hz.
I-O Functions and AEP Audiograms

Input-output functions were plotted for each animal at each frequency to compare evoked potential strength to the SPL of the stimulus. Although the input-output functions were non-linear, in general, higher SPLs resulted in larger evoked potentials and lower SPLs resulted in smaller evoked potentials (Figure 3-2).

Threshold AEP values were used to calculate the mean male and mean female Sarasota Bay bottlenose dolphin AEP audiograms (Figure 3-3). A multiple linear regression performed on each frequency determined that hearing thresholds were not significantly influenced by the age and/or gender of the individual being tested (p > 0.05 for gender and p > 0.05 for age at each frequency). For purposes of illustration and clarity, simple linear regressions for male and female data are plotted separately for each frequency in Figure 3-4. Note that the coefficients of determination ($r^2$) are generally low, except for when only a few data points are available, such as at 5 kHz. In the case of 5 kHz and some of the other frequencies tested, the slope of the regressions are opposite of what one would expect for age-related hearing loss.

F195

AEPs measured on one individual, F195, indicated that this female may have had substantial mid-frequency hearing losses. She showed no evoked potential response to the 40 kHz tone burst at 120 dB re 1 µPa after 1100 sweeps, while FB75, a 31-year-old
female, showed a strong response to the same stimulus after only 86 sweeps (Figure 3-5). In addition, F195 showed no evoked potential response to the 20 kHz tone burst at 153 dB re 1 µPa after 1000 sweeps, while FB75 showed a strong response to the same stimulus after only 348 sweeps. Although the exact age of F195 remains unknown, she was likely old when her hearing was tested based on the worn condition of her few remaining teeth. To definitively determine the extent of F195’s possible hearing losses, additional AEP hearing data would need to be collected.

PCBs and Hearing Thresholds

There were no strong relationships among hearing thresholds and the concentrations levels of total PCBs or of the 69 PCB congeners. The largest positive correlation in the correlation matrix over all frequencies (5, 10, 20, 30, 40, 60, 80, and 120 kHz) was 0.55 for PCB 174 at 20 kHz. This correlation did not hold for this PCB at other frequencies.

Nellie at Marineland

Animals under the age of two or over the age of 40-45 years are not generally sampled during health assessments in Sarasota Bay. As a result it was not possible to measure the hearing of the oldest individuals in this population. However, during this study period, the hearing of the oldest known bottlenose dolphin in captivity, Nellie, was measured.
Nellie’s AEP audiogram was very similar to the mean Sarasota male and female dolphin audiograms, except at 120 kHz, where she exhibited a substantial hearing loss (Figure 3-6). At the four lower frequencies tested (5, 10, 20, and 40 kHz), Nellie’s audiogram was slightly lower than the mean Sarasota audiograms, and at 80 kHz, Nellie’s hearing threshold was slightly higher than the mean Sarasota audiograms.

Discussion

The MRTF data collected from seven dolphins in this study are very similar to MRTF data collected previously on captive dolphins (Supin et al. 2001), with large peaks at 600 Hz and 1000 Hz. These data demonstrate the high temporal resolution of free-ranging bottlenose dolphins. The robust evoked potential values measured at 600 Hz justify the use of the 600 Hz AM rate for EFR data collection on bottlenose dolphins.

The results of the PCB concentrations-hearing thresholds correlation matrices suggest that the hearing thresholds of bottlenose dolphins are not negatively affected by PCB levels, at least at levels of exposure occurring in Sarasota Bay. It is thought that PCBs cause hearing loss by blocking thyroid hormones during fetal development, resulting in inner ear defects (Goldey et al. 1995). Therefore, the PCB concentrations of a young calf’s mother or its own PCB concentrations before the age of three may be more relevant to the calf’s hearing thresholds than its own PCB concentrations later in life. However, because animals under the age of two are rarely sampled during health assessments, it is difficult to measure PCB concentrations in new mothers and very young
calves. Additionally, because PCBs are lipid-soluble and are excreted in milk (Wells et al. 2005), measuring their concentrations in mothers of older calves may not accurately represent the load received by that calf as a developing fetus and newborn. Concurrent AEP and PCB data are presently available for very few calves; therefore, these analyses were not conducted. However, these are important analyses worthy of future consideration.

The two most interesting findings from the AEP audiogram data are as follows: first, there is a large amount of variability among the hearing thresholds of free-ranging bottlenose dolphins that occurs independently of the age or gender of the individual; second, none of the individuals tested had a substantial hearing loss, with the possible exception of F195.

There are two obvious explanations for the results of this study. First, it is possible that the free-ranging bottlenose dolphins of Sarasota Bay, Florida, experience no significant hearing losses during the majority of their lifetime. Alternatively, it is possible that individuals that experience significant hearing losses do not survive long in the wild because hearing is so vital for both navigation and foraging.

Most published AEP studies have been conducted on captive (e.g., Ridgway and Carder 1993, 1997; Szymanski et al. 1999; Yuen et al. 2005; Finneran and Houser 2006; Houser and Finneran 2006; Cook et al. in prep.) or stranded (Popov and Klishin 1998; André et al. 2003; Nachtigall et al. 2005; Cook et al. 2006) odontocetes, where the pressures of food-finding, predator avoidance, and navigation have largely been removed. Because of this, hearing losses reported in these animals (Ridgway and Carder 1993, 1997; Finneran and Houser 2006; Houser and Finneran 2006; Cook et al. in prep.), while
most likely detrimental to the individual, are not as life-threatening as they might be for free-ranging animals.

The results of Nellie’s AEP testing indicate that she has a significant high-frequency hearing loss. Although data from one individual must be interpreted cautiously, they do support the idea that bottlenose dolphins could experience presbycusis, increasing hearing loss with increasing age (Ridgway and Carder 1993, 1997). Nellie’s hearing at lower frequencies further supports the idea that bottlenose dolphins transmit these frequencies to their inner ears using more than just the acoustic window of their lower jaws (Popov et al. 2006; Cook et al. in prep.). Because the Sarasota animals’ hearing was measured in air using a jawphone, alternate sound pathways to the inner ear were unavailable. Therefore, the hearing thresholds determined for these animals at lower frequencies (5, 10, and 20 kHz) are likely elevated compared to analogous underwater measurements.

Cook et al. (in prep.) found that AEP hearing measurements in air using a jawphone were up to 32 dB (20.0 ± 8.0 dB) higher than AEP hearing measurements made underwater for two captive bottlenose dolphins at 10 and 20 kHz. In addition, in-air AEP measurements were approximately 20 dB (20.3 ± 6.2 dB) higher than underwater behavioral measurements for one captive dolphin at 10, 20, and 30 kHz (Cook et al. in prep.). At 40, 60, and 80 kHz, however, there was good agreement between the in-air and underwater AEP measurements (Cook et al. in prep.). Using the results of Cook et al. (in prep.), the mean in-air AEP measurements for the Sarasota animals were adjusted at 10 and 20 kHz to more accurately represent their likely AEP hearing thresholds in water. These adjusted values were determined by subtracting the mean difference
between in-air AEP and underwater AEP measurements for the two captive dolphins at each frequency (Cook et al. in prep.) from the mean in-air AEP measurements for the Sarasota animals at each corresponding frequency. Figure 3-7 shows these adjusted AEP audiograms.

The mean in-air AEP measurements for the Sarasota animals were also modified at 10, 20, and 30 kHz to model their theoretical behavioral hearing thresholds. These values were calculated by subtracting the difference between in-air AEP and underwater behavioral hearing measurements for one captive dolphin at each frequency (Cook et al. in prep.) from the mean in-air AEP measurements for the Sarasota animals at each corresponding frequency. Nellie’s underwater AEP hearing thresholds were also adjusted to model her theoretical behavioral hearing thresholds at these frequencies using the surface AEP-behavioral audiogram transfer function from Cook et al. (2006). The data point representing her hearing threshold at 120 kHz was removed because there was no correction value at this frequency. These audiograms are shown in Figure 3-8.

With the possible exception of F195, the free-ranging bottlenose dolphins of Sarasota Bay do not exhibit substantial hearing losses. The animals exhibiting hearing losses in the two studies by Ridgway and Carder (1993, 1997) were all at least 25 years old; however, none of the six 25-year-old or older animals tested in this study showed any hearing deficits. Because they were not tested, it is not possible to say whether or not the very oldest animals in the Sarasota Bay population have higher hearing thresholds.

The considerable variability in hearing thresholds among these individuals further substantiates the idea that data from individual animals do not accurately represent entire populations (NRC 2000). For example, at 80 kHz there was as much as a 47 dB hearing
threshold difference between individuals within the Sarasota dolphin population. This hearing variability can perhaps be best appreciated in terms of echolocation: assuming a spherical spreading loss model of $1/r^2$, a 47 dB hearing difference could result in minimum signal detection differences of up to 15-fold. So, a target detectable by a dolphin with good hearing at 150 m would only be detectable at 10 m by a dolphin with a 47 dB hearing deficit. The substantial differences in hearing thresholds in these dolphins could be the result of several factors working independently or in concert with each other, including genetic differences and differences in levels of instantaneous or chronic environmental noise exposure. For perspective on noise exposure, more than 41,000 boats are registered within the home range of the resident Sarasota dolphin community (Florida Fish and Wildlife Conservation Commission 2002, unpublished data), and there are occasional marine construction/demolition projects that introduce exceptionally loud noise into the environment from time to time (R. Wells, personal communication).

With the increasing portability and decreasing cost of AEP equipment, hearing threshold data from larger sample sizes of a wider variety of odontocetes should continue to become more easily obtained. In addition, AEP measurements on temporarily-captured and stranded animals will continue to provide powerful insights into the auditory capabilities of these animals.
References Cited


Cook MLH, Bauer GB, Fellner W, Mann DA (in prep.) Ground-truthing in-air auditory evoked potential (AEP) hearing measurements with traditional behavioral audiograms in bottlenose dolphins (*Tursiops truncatus*).


Thompson RKR, Herman LM (1975) Underwater frequency discrimination in the bottlenosed dolphin (1-140 kHz) and the human (1-8 kHz). J Acoust Soc Am 57:943-948


Table 3-1  Freeze-brand (FB) number, gender, age at AEP testing, and health assessment (H.A.) session for each animal tested. Animals tested during multiple sessions are listed separately for each session. F173 was tested, but no usable data were obtained; therefore, she was excluded from all subsequent analyses.

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Figure 3-1  Mean MRTF measured for seven free-ranging bottlenose dolphins (*Tursiops truncatus*). Individual MRTFs were measured from 200 to 2000 Hz using a 40 kHz carrier frequency at ~130 dB re 1 µPa. Mean (± SD) evoked potential level (nV) is plotted against AM rate (Hz).
Figure 3-2 Input-output function for FB75 for seven test frequencies. Sound pressure level (SPL), in dB re 1 µPa, is plotted on the x-axis and evoked potential (EP) level, in dBV, is plotted on the y-axis. The input-output functions are generally non-linear.
Figure 3-3  Mean (± SD) AEP audiograms measured for 32 male and 29 female free-ranging bottlenose dolphins (*Tursiops truncatus*).
**Figure 3-4** Plots of dolphin age, in years, versus SPL at hearing threshold, in dB re 1 µPa, for each frequency, separated by gender. Regression equations, $r^2$ values, and sample sizes are reported on each plot for each frequency.
Figure 3-4 (Continued)
Figure 3-4 (Continued)
Figure 3-5  F195 showed no EP response to the 40 kHz tone burst at 120 dB re 1 µPa even after 1100 sweeps (top), while FB75 showed a strong EP to the same tone burst at the same SPL after only 86 sweeps (bottom). It is likely that F195 exhibited a mid-frequency hearing loss.
Figure 3-6  Nellie’s AEP audiogram compared to the mean (± SD) AEP audiograms measured for 32 male and 29 female free-ranging bottlenose dolphins (*Tursiops truncatus*).
Figure 3-7  Nellie’s AEP audiogram compared to the predicted underwater mean (± SD) AEP audiograms measured for 32 male and 29 female free-ranging bottlenose dolphins (*Tursiops truncatus*). The audiograms of the free-ranging animals have been adjusted at 10 and 20 kHz to more accurately represent underwater AEP hearing thresholds.
Figure 3-8 Predicted behavioral audiograms based on AEP-behavioral audiogram transfer functions. The mean (± SD) male and female AEP audiograms of the free-ranging animals have been adjusted at 10, 20, and 30 kHz, and Nellie’s AEP audiogram has been adjusted at each frequency (except 120 kHz) to more accurately represent theoretical underwater behavioral hearing thresholds.
Chapter Four:

Beaked Whale Auditory Evoked Potential Hearing Measurements

Abstract

Several mass strandings of beaked whales have recently been correlated with military exercises involving mid-frequency sonar, highlighting unknowns regarding hearing sensitivity in these species. The hearing abilities of a stranded juvenile beaked whale (*Mesoplodon europaeus*) were measured with auditory evoked potentials (AEPs). The beaked whale’s modulation rate transfer function (MRTF), measured with a 40 kHz carrier, showed responses up to an 1800 Hz amplitude modulation (AM) rate. The MRTF was strongest at the 1000 Hz and 1200 Hz AM rates. The envelope following response (EFR) input-output functions were non-linear. The beaked whale was most sensitive to high frequency signals between 40-80 kHz, but produced smaller evoked potentials to 5 kHz, the lowest frequency tested. The beaked whale hearing range and sensitivity are similar to other odontocetes that have been measured.
Introduction

Beaked whales (e.g., *Ziphius cavirostris* and *Mesoplodon densirostris*) produce echolocation clicks with estimated source levels of 200-220 dB re 1 µPa peak-peak at 1 m (Johnson et al. 2004; Zimmer et al. 2005) and with the energy of the click centered on 42 kHz and -10 dB bandwidths of 22 kHz (Zimmer et al. 2005). Given that other odontocetes demonstrate similar structure in their echolocation clicks and have sensitive hearing within the range of echolocation frequencies, it seems likely that the beaked whales would also have good high-frequency hearing sensitivity. However, no direct assessment of hearing sensitivity has ever been performed on a beaked whale to verify this assumption. This lack of information is an impediment to understanding the effects that anthropogenic sound can have on marine mammals, particularly since several mass strandings of beaked whales have been linked both spatially and temporally to military exercises involving mid-frequency sonar (Balcomb and Claridge 2001; US Dept. of Commerce 2001; Frantzis 1998; Simmonds and Lopez-Jurado 1991).

Auditory evoked potential (AEP) techniques are commonly used to measure hearing thresholds and other aspects of hearing in humans, birds, fishes, and other animals, including cetaceans (e.g., Ridgway et al. 1981; Corwin et al. 1982; Szymanski et al. 1999; Lucas et al. 2002). In general, when the auditory pathway is presented with an acoustic stimulus that is above threshold levels, large numbers of neurons within the acoustic pathway are excited. If the neuronal discharges are time-locked to the acoustic stimulus, the electrical signals produced by the simultaneous firings of multiple neurons produce an evoked potential (EP) that can be detected by an electrode placed on the head.
AEP hearing measurements are advantageous over behavioral hearing measurements because they may be performed non-invasively, they require no training on the part of the animal, and they can be done in a short time frame. Therefore, they allow researchers to perform rapid estimations of an individual’s hearing thresholds. This often becomes critical when working with stranded marine mammals because of time limitations and the nature of the stranding event itself.

This study reports the auditory temporal resolution and evoked potential hearing measurements of a live-stranded juvenile male beaked whale (*Mesoplodon europaeus*) as determined using auditory evoked potential techniques. The modulation rate transfer function (MRTF), which measures the strength of the AEP using different modulation rates, was first measured for the animal. The results of MRTF testing determined the amplitude-modulation (AM) rate employed in the envelope following response (EFR) procedure used to estimate AEP hearing thresholds. To determine the similarity between these AEP EFR hearing threshold estimates and traditional behavioral hearing threshold estimates, AEP hearing measurements were conducted on captive bottlenose dolphins (*Tursiops truncatus*) for whom behavioral hearing abilities had previously been measured (Houser et al. 2004). This study is the first to report data on the auditory system of any whale in the family Ziphiidae, and provides insights regarding the use of military sonar and coincidental mass strandings of several species of whales from this family.
Materials and Methods

Subject

A single, 181 kg juvenile male beaked whale (*Mesoplodon europaeus*; HBOI-Me-0402) live-stranded ocean-side near the south edge of St. Lucie Inlet, FL, on July 20, 2004. It presented in underweight nutritional condition with decreased post-nuchal fat and a slight concavity to its epaxial muscles with visible peduncular vertebral processes and ribs, as well as a prominent scapular ridge. The animal was transported to Harbor Branch Oceanographic Institution, where it was maintained in an aboveground pool (approximately 1.5 m depth) until its death on July 22, 2004 at 1821 hrs.

AEP measurements were performed on the animal on July 22, 2004 from 1527 hrs to 1611 hrs, under the direct supervision of Dr. Greg Bossart, V.M.D., Ph.D. and in accordance with NMFS Permit No. 932-1489-06. During this time the animal was stationed at the surface of the water with passive restraint, and remained relatively motionless.

AEP Methods

Evoked potentials were measured by repeatedly playing a sound stimulus while simultaneously recording the neural evoked potential from surface electrodes. Because the evoked potential from a single sound stimulus is small and is less than the electrical
noise in the recordings, the neural potentials in response to each tone presentation are averaged together to reduce noise and reveal the underlying evoked potential (Ferraro and Durrant 1994).

Stimulus Control and Data Collection

All stimulus presentation and data acquisition were controlled from a Tucker-Davis Technologies (TDT) AEP Workstation. The TDT Workstation was run from a laptop computer. This Workstation has pre-programmed test frequencies and test levels that were run with BioSig software.

Two hearing tests were performed. The first test was a measurement of the MRTF, which determines how well the auditory system is able to follow the temporal envelope of an acoustic stimulus (Dolphin et al. 1995). For this test, a 40 kHz stimulus carrier (130 dB re 1 µPa) was 100% amplitude modulated with AM rates ranging from 200 Hz to 1800 Hz, in 200 Hz steps. The MRTF results were used to determine the AM rate that yielded the strongest AEP response. This AM rate was then used to conduct the second hearing test, hearing threshold determination, using the EFR technique.

AM tones used in an AEP procedure result in an EFR in which the auditory system of the subject produces neural responses that are phase-locked with the envelope of the stimulus (Dolphin 1996; Dolphin 1997). The advantages of such a stimulus are that it results in an AEP at the frequency of AM, which can be distinguished from background electrical noise in the electrode signal, and that it has a narrow frequency
spectrum, which allows for good frequency resolution in the audiogram. Each trial lasted approximately one minute and consisted of playing AM tones at specific frequencies and levels. These AM tones consisted of 14 ms tone bursts modulated at 1200 Hz, the AM rate that yielded the strongest AEP response. This sound was presented 21 times per second, with simultaneous averaging of the evoked potential.

Jawphone and AEP Electrodes

The hypothesis that dolphins use their lower jaws in the reception of sound is generally accepted. Norris (1964, 1968) originally proposed that the mandibular foramen and the fats associated with it function as acoustic wave guides; electrophysiological (Bullock et al. 1968; McCormick et al. 1970, 1980) and behavioral (Brill et al. 1988, 2001) studies with bottlenose dolphins support this theory. Taking advantage of this sound reception pathway, jawphones (contact hydrophones attached by suction cups) have been used by several researchers to deliver acoustic stimuli to the lower jaw of bottlenose dolphins (e.g., Moore and Pawloski 1993; Brill et al. 2001; Houser and Finneran 2005). A jawphone composed of an ITC-1042 transducer embedded in a suction cup with an acoustic impedance similar to water (constructed from VI-SIL V-1062, Rhodia, Inc.) and powered by a Hafler P1000 amplifier was used to deliver the acoustic stimulus in this study. The jawphone was placed on the lower left jaw of the animal corresponding to a position scaled to that of position #38 in Møhl et al. (1999), which showed the greatest AEP response in their study on bottlenose dolphins. The jawphone was located below
the water surface during data collection. The jawphone was calibrated in reference to the sound level 10 cm from the suction cup using a calibrated hydrophone (Reson TC4013; -212 dB re 1 V/µPa). Sound levels were controlled by the computer with a programmable attenuator (TDT PA5).

The sound field in water is complicated by constructive and destructive interference from reflections off of the water surface and bottom. Thus, it is often more difficult to deliver a consistent sound stimulus in shallow water than in air. More precise stimulus levels were presented to the animal via the jawphone than if a free-standing underwater speaker were used to deliver sounds because the distance between the ear and the jawphone did not change as it might with a free speaker.

Evoked potentials were collected with suction cup electrodes made from standard 8 mm silver-silver chloride electrodes (Med-Associates, Inc.) embedded in a RTV silicone rubber compound (VI-SIL V-1062, Rhodia, Inc.). Redux® electrolyte paste (Parker Laboratories, Inc.) was used on the electrodes to establish a good electrical connection between the electrodes and the whale’s skin. All electrodes and suction cups were removed as soon as testing was complete.

Recordings were made with two suction cup electrodes and a ground electrode attached to a differential amplifier (TDT DB4-HS4). The recording electrode was placed behind the nuchal crest approximately 2 cm lateral to the dorsal midline and approximately 15 cm behind the blowhole. The reference electrode was placed approximately 20 cm caudal to the recording electrode, and a ground electrode was placed in the water. The output of the amplifier was connected via a fiber optic cable to the TDT Workstation for data acquisition with the BioSig software. BioSig controlled
both stimulus presentation and data acquisition. Electrical artifacts induced by the whale breathing and movement of the electrodes were removed by artifact rejection in BioSig (excluding all sweeps with evoked potentials greater than 90 µV).

Sounds

The carrier frequencies tested included 5, 10, 20, 40, 60, and 80 kHz. Sound pressure levels (SPLs) were attenuated in 10 dB steps. Up to 2000 sweeps were averaged for each test trial, although most trials consisted of about 500 sweeps. Once an evoked potential was observed, averaging at that test level was ended, and the next level was tested. Evoked potential levels in response to the AM tones were measured by performing a 1220-point Fast Fourier Transform (FFT) on the evoked potential waveform from 5-20 ms (the portion containing the EP in response to the sound). EPs were included in the analysis if there was a peak in the spectrum that was greater in amplitude than an estimate of the noise level from 0-5 ms in the same sweep.

AEP Audiogram Calibration

AEP measurements were also conducted using the same methods and equipment as above on three bottlenose dolphins (Tursiops truncatus) for which behavioral audiograms had already been measured (WEN: 21 yr. old male, BLU: 39 yr. old female, and BEN: 41 yr.)
old male; Houser et al. 2004). WEN and BEN were tested in San Diego Bay, while BLU
was tested in a 6.1 m diameter, 1.5 m deep above-ground pool.

Results

MRTF

The beaked whale MRTF was measured to determine the effect of AM rate on the evoked
potential amplitude (Figure 4-1). While responses were detected at all modulation rates
tested, a 1200 Hz modulation rate gave a robust response to the 40 kHz stimulus carrier
with a high signal-to-noise ratio. Thus, this modulation rate was chosen for subsequent
EFR measurements. Strong peaks occurred at modulation rates of 600 and 1000-1200
Hz, while a trough occurred at 800 Hz. Evoked potentials were detected in response to
AM rates up to 1800 Hz, the highest AM rate tested.

EFR

An EFR was detected at each frequency tested, but was strongest at the highest
frequencies tested (40, 60, and 80 kHz). Input-output functions were plotted for each
frequency to compare evoked potential strength to the SPL of the stimulus (Figure 4-2).
The input-output functions were non-linear. In general, higher SPLs resulted in larger
evoked potentials, except at 80 kHz where mid-level sounds (110-128 dB re 1 µPa) evoked the strongest potentials. Because of the non-linearity in these data, the input-output functions were not used to extrapolate hearing thresholds (Popov and Supin 1990). Rather, only the lowest SPLs for which an evoked potential was detected at each frequency are reported here (Figure 4-3). It is also important to note that the whale showed no reaction to the presentation of the acoustic stimuli.

To establish the equivalence between these AEP EFR hearing threshold estimates and traditional behavioral hearing threshold estimates, AEP hearing measurements were conducted on three bottlenose dolphins (WEN, BLU, BEN) for whom behavioral hearing abilities had previously been measured by the U.S. Navy Marine Mammal Program (Houser and Finneran 2005; Finneran et al. 2005). The AEP thresholds tended to be higher than the behavioral thresholds, especially at lower frequencies (Figure 4-4).

Discussion

The lowest detected AEPs of this beaked whale resemble hearing thresholds of other cetaceans reported in the literature (Johnson 1966; Nachtigall et al. 2000) with decreasing hearing sensitivity at lower frequencies and increasing sensitivity at higher frequencies. These findings show that beaked whales are capable of detecting sounds between 5 and 80 kHz, and are most likely capable of detecting frequencies much higher than 80 kHz; however, higher frequencies could not be tested due to the sampling rate limitations of the equipment. The results of the MRTF procedure suggest that beaked whales have a
high temporal resolution, similar to that of other cetaceans (Supin et al. 2001). Beaked whale (*Ziphius cavirostris*) echolocation clicks have energy centered on 42 kHz, with energy up to about 80 kHz (Zimmer et al. 2005). This range appears to be lower than the high frequency limits of the beaked whale tested in this study, based on the data obtained at 80 kHz. It is important to note however, that the whale tested was a juvenile of a different genus.

Although behavioral psychoacoustic methods provide the most direct measures of hearing abilities (Nachtigall et al. 2000), the training and time involved with these techniques can limit their broad application. Alternatively, AEP techniques allow for rapid hearing assessment of untrained or minimally trained animals. However, the equivalence between hearing thresholds determined using these two testing paradigms has only recently been investigated (Szymanski et al. 1999; Houser et al. 2004; Yuen et al. 2005). Therefore, the hearing abilities of bottlenose dolphins measured behaviorally in a direct-field were compared with hearing estimates made with a jawphone in the same testing configuration that was used with the beaked whale (i.e., at the surface with the jawphone attached). The most similar situation was BLU who was tested in a pool similar to that of this beaked whale. WEN and BEN were tested in San Diego Bay, which has much higher ambient noise levels compared to the test pool (Finneran et al. 2005). The results with BLU showed that the AEP audiogram had consistently higher thresholds than the behavioral audiogram, with the greatest differences at the lowest frequencies.

The U.S. Navy’s mid-frequency tactical sonar AN/SQS-53 has center frequencies of 2.6 and 3.3 kHz and nominal source levels of 235 dB re 1 µPa at 1 m; the AN/SQS-56
has center frequencies of 6.8 to 8.2 kHz and nominal source levels of 223 dB re 1 µPa at 1 m (U.S. Dept. of Commerce 2001). Several hypotheses have been put forth concerning the potential mechanism of sonar-induced stranding including acoustic or pressure trauma, *in vivo* bubble formation, and high auditory sensitivity of beaked whales to mid-range sonar (Balcomb and Claridge 2001; U.S. Dept. of Commerce 2001; Jepson et al. 2003; Fernández et al. 2004). The lowest SPL to produce a detectable evoked potential in the beaked whale at 5 kHz was 132 dB re 1 µPa. Based on the differences between AEP thresholds and behavioral thresholds observed in captive bottlenose dolphins (Figure 4-4), it is likely that the beaked whale behavioral threshold at 5 kHz would be lower than 132 dB re 1 µPa. However, until a beaked whale can be kept alive in captivity, the behavioral data will be impossible to obtain.

The hearing sensitivity of the beaked whale at 5 kHz appears to be similar to or less than that of bottlenose dolphins measured with evoked potentials. Thus, the beaked whale AEP measurements do not support the hypothesis that these species have a particularly high auditory sensitivity at the frequencies used in mid-range sonar. The data presented here, along with accurate sound propagation models, should be useful for estimating minimum distances at which beaked whales could acoustically detect mid-frequency sonar.
References Cited


Figure 4-1  Beaked whale (*Mesoplodon europaeus*) modulation rate transfer function measured with a 40 kHz carrier tone at 130 dB re 1 μPa at various amplitude modulation rates.
Figure 4-2 Beaked whale (*Mesoplodon europaeus*) input-output functions of evoked potential level as a function of stimulus sound pressure level (SPL). Carrier tones were amplitude modulated at 1200 Hz.
Figure 4-3  Lowest sound pressure levels (SPLs) for which an evoked potential could be detected at each test frequency.
Figure 4-4  Comparison between auditory evoked potential (AEP) and behavioral hearing thresholds determined for three bottlenose dolphins (*Tursiops truncatus*): a) WEN, b) BLU, and c) BEN.
Chapter Five:

Hearing Thresholds in Captive and Free-Ranging Cetaceans: Concluding Remarks

In-air AEP, underwater AEP, and underwater behavioral audiograms have been measured in several species of cetaceans by many prominent researchers. Several of these studies have been discussed in detail throughout this dissertation.

Chapter One presented a brief overview of the sound production and hearing abilities of odontocetes in order to provide a framework for the auditory evoked potential (AEP) and behavioral hearing studies that were presented in the chapters that followed.

Chapter Two investigated the differences between underwater AEP and in-air AEP measurements in two bottlenose dolphins (Tursiops truncatus). Underwater behavioral hearing measurements were also conducted with one of the dolphins using the same stimuli used for the AEP measurements. There was generally good agreement among the hearing thresholds determined by these three methods at frequencies above 20 kHz. At 10 and 20 kHz, in-air AEP audiograms were considerably higher than underwater behavioral and underwater AEP audiograms. This suggests multiple sound pathways to the dolphins’ ears at lower frequencies and/or poor transmission of lower frequency stimuli through the jawphone. This chapter also provided an in-air AEP to underwater behavioral audiogram transfer function that could be applied to the in-air AEP data. Thus, it validated the use of in-air AEP hearing measurements for animals
whose hearing cannot be measured using traditional techniques, including live-stranded and free-ranging cetaceans.

Chapter Three presented the first hearing measurements ever collected on free-ranging bottlenose dolphins. The hearing abilities of 62 bottlenose dolphins (32 males and 30 females), ranging in age from 2 to 36 years, were measured in the field using AEP techniques during brief capture-release sessions for health assessment. Evoked potentials in response to AM tones ranging from 5-120 kHz elicited a robust envelope following response. There was considerable individual variation in hearing abilities, up to 80 dB, between individuals. With the possible exception of dolphin F195, which did not produce a detectable evoked potential in response to a 120 dB re 1 µPa signal at 40 kHz, none of the Sarasota dolphins demonstrated substantial hearing losses. There was no relationship among age, gender, or PCB load and hearing sensitivities. Because they were not tested, it is not possible to say whether or not the very oldest animals (> 36 years old) in the Sarasota Bay population have higher hearing thresholds. Hearing measured in a 52-year-old captive-born bottlenose dolphin showed similar hearing thresholds to the Sarasota dolphins up to 80 kHz, but exhibited a 50 dB drop in sensitivity at 120 kHz. It is possible that individuals experiencing hearing losses do not survive long in the wild as a result of compromised echolocation abilities.

Chapter Four provided the first hearing measurements made on any member from the Ziphiidae family, a juvenile beaked whale, *Mesoplodon europaeus*, measured with auditory evoked potentials. The beaked whale’s modulation rate transfer function measured with a 40 kHz carrier showed responses up to an 1800 Hz amplitude modulation rate. The MRTF was strongest at the 1000 Hz and 1200 Hz AM rates. The
envelope following response input-output functions were non-linear. The beaked whale was most sensitive to high frequency signals between 40-80 kHz, but produced smaller evoked potentials to 5 kHz, the lowest frequency tested. The beaked whale hearing range and sensitivity were similar to other odontocetes that have been measured. These hearing data were discussed in terms of sonar-type sounds, as several species from this family of cetaceans have been shown to strand in close spatial and temporal proximity to Naval sonar exercises (Balcomb and Claridge 2001; US Dept. of Commerce 2001; Frantzis 1998; Simmonds and Lopez-Jurado 1991).

These studies show that for odontocete cetaceans, auditory evoked potential hearing measurements capture both the shape and upper hearing cutoff of behaviorally determined audiograms. Furthermore, AEP hearing measurements can be adjusted with a transfer function to estimate the behavioral threshold. Thus, AEP audiograms are a good approximation of hearing abilities for animals whose hearing cannot be measured behaviorally. The ease and rapidity of AEP data collection compared to behavioral methods dictates their expanded, though not exclusive, use in marine mammal audiometry.

The hearing abilities of a large population of animals can be highly variable from individual to individual, regardless of age or gender. This underscores the need for larger numbers of individuals to be sampled prior to management or policy decisions. Unlike previous studies on captive dolphins (Ridgway and Carder 1993, 1997; Finneran and Houser 2006; Houser and Finneran 2006), the wild dolphins in Sarasota Bay, Florida, did not exhibit substantial hearing losses, with the possible exception of F195. Also unlike previous studies (Ridgway and Carder 1993, 1997), hearing loss in the Sarasota animals
did not increase with increasing age, and males were no more likely than females to have higher hearing thresholds.

Perhaps the most important use of auditory evoked potential hearing measurements is in the hearing assessment of stranded cetaceans. Many whales, dolphins, and porpoises cannot be maintained in captivity and are difficult to find and study in the wild. Stranded animals, therefore, can provide valuable data that may otherwise never be obtained. AEP hearing data collected from stranded animals provide key information about their basic biology, and allow more informed decisions to be made regarding their management, conservation, and protection.

Finally, AEP hearing work with stranded cetaceans will allow for the effects of aminoglycosidic antibiotics commonly used in marine mammal rehabilitation to be carefully monitored. For example, gentamicin sulfate, amikacin sulfate, and vancomycin hydrochloride capsules (vancocin HCL) are all currently used to treat stranded cetaceans in very poor health. However, it is unknown if these drugs cause hearing losses in cetaceans similar to the known hearing losses they cause in both rodents (Rybak and Whitworth 2005) and humans (García et al. 2001; Black et al. 2004). Because the foremost goal of the rehabilitation process is to successfully return the animal to the wild, it is important to know whether or not these drugs do more harm than good. AEP hearing measurements collected on stranded individuals shortly after the stranding event (prior to treatment with aminoglycosidic antibiotics), followed with repeat measurements throughout the rehabilitation process will allow for dose-effect tables to be determined for these drugs and for the ethical consequences of their administration to be considered for odontocete cetaceans.
References Cited


About the Author

Mandy Lee Hill Cook graduated summa cum laude from the University of North Carolina at Wilmington (UNCW) in May 1999 with a Bachelor of Science degree in Marine Biology and a minor in Spanish. She completed an undergraduate honors thesis entitled “Quantification of Signature Whistle Production by Free-ranging Bottlenose Dolphins (Tursiops truncatus)” during her senior year. She graduated with a Master of Science Degree in Marine Biology from UNCW in May 2002. Her thesis was titled “Signature Whistle Production, Development, and Perception in Free-ranging Bottlenose Dolphins”, and her major advisor was Dr. Laela Sayigh. While in graduate school at the University of South Florida, Mandy received numerous awards including the Von Rosenstiel, Getting, and Lake Endowed Fellowships through the College of Marine Science, and a P.E.O. Scholar Award. Mandy currently lives with her husband in Hillsboro, Oregon.