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Hearing and Echolocation in Stranded and Captive Odontocete Cetaceans

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Hearing and Echolocation in Stranded and Captive Odontocete Cetaceans

by

Danielle Rene Greenhow

A dissertation submitted in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy
College of Marine Science
University of South Florida

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dolphin, Pantropical spotted dolphin, auditory evoked potential (AEP), beam pattern

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DEDICATION

This dissertation is dedicated to my family and friends, especially those that left this Earth too soon. To my grandfather who always pushed me to do my best, I know you’re proud of me. To my aunt who loved me as one of her own, I will always remember how much you love me. To my uncle who always challenged me, thank you for building my determination. To my friend Michael, although you left far too soon your smile and positive spirit will always lift me up. To all of those who inspire me, either by my side or from above, this could not have been accomplished without your love and support.
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ABSTRACT

Odontocetes use echolocation to detect, track, and discriminate their prey, as well as negotiate their environment. Their hearing abilities match the frequency of greatest sensitivity to the higher frequencies used for foraging and navigation. Hearing and echolocation together provide odontocetes with a highly developed biosonar system. This dissertation examines the hearing ability of several odontocete species to understand what signals they can perceive during echolocation. The variability in hearing ranges between species is examined in the context of phylogenetic and ecological differences among taxa. An autonomous hydrophone array is also developed that could be used in an expanded form in field deployments to study echolocation signals in a wider range of species.

Methods for measuring hearing sensitivity include both psychophysical and electrophysiological procedures. Behavioral methods require a large time commitment, for both training and data collection, and can only be performed on captive dolphins. Auditory evoked potential (AEP) methods are non-invasive, rapid measurements of the brain’s response to sound stimuli and allow for audiograms to be collected on stranded, high risk dolphins. By determining the hearing abilities of odontocetes either in captivity or during stranding, data can be collected about inter- and intraspecies variability, and the occurrence of hearing impairment. It can also be used as another diagnostic tool to determine the releasability of a stranded animal.
A juvenile male short-finned pilot whale (*Globicephala macrorhynchus*) that stranded in Curacao had severe hearing impairment at all frequencies tested. Four female short-finned pilot whales tested had the best sensitivity at 40 kHz. The juveniles had greater high frequency sensitivity than the adult pilot whales. Cutoff frequencies were between 80 and 120 kHz.

Hearing sensitivity was determined for the two mother/calf pairs of Risso’s dolphins (*Grampus griseus*) before and after antibiotic treatment in order to measure any potential effects of antibiotic treatment. Greatest sensitivity occurred at 40 kHz and cutoff frequencies were around 120 kHz for all dolphins tested. Changes in hearing sensitivity after antibiotic dosage were 12 dB or less in all cases except one. The adult female Betty showed a threshold shift at 120 kHz of 54 dB from May to June, which partially demonstrates the presence of an ototoxic effect at one frequency. Dosages of antibiotics during drug treatment detailed in this study should be considered safe dosages of antibiotics for Risso’s dolphins.

AEP and behavioral methods were used to collect audiograms for three *Stenella* spp. dolphins. The frequency of best hearing for the Atlantic spotted dolphin and the spinner dolphin was 40 kHz, and their upper cutoff frequencies were above 120 kHz. The pantropical spotted dolphin had the greatest sensitivity at 10 kHz, and had severe high frequency hearing loss with a cutoff frequency between 14 and 20 kHz.

Comparisons of high frequency hearing sensitivities among the species tested show two distinct groups. Short-finned pilot whales and Risso’s dolphins have a cutoff frequency below 120 kHz, whereas *Stenella* spp. dolphins have cutoff frequencies above
120 kHz. Expanding the comparison to include other species, killer whales, pygmy killer whales, false killer whales, and long-finned pilot whales also have cutoff frequencies below 120 kHz. Common bottlenose dolphins, white-beaked dolphins, Indo-Pacific humpback dolphins, rough-toothed dolphins, and common dolphins have cutoff frequencies above 120 kHz. Genetic evidence exists for two subfamilies within Delphinidae (Vilstrup et al., 2011) and those species with cutoff frequencies below 120 kHz belong to the subfamily Globicephalinae and those species with cutoff frequencies above 120 kHz belong to the subfamily Delphininae.

An autonomous, field-deployable hydrophone array was developed to measure free-swimming echolocation. The array contained 25 hydrophones, two cameras, and a synchronization unit on a PVC frame. The distinct click train was used to time-align all 25 channels, and the light was used to synchronize the video and acoustic recordings. Echolocation beam patterns were calculated and preliminary evidence shows a free-swimming dolphin utilizes head movement, beam steering and beam focusing.

Among all areas of cetacean biology more research is necessary to gain a clearer picture of how odontocetes have adapted to function in their acoustic environment. The array system developed can be used to study how dolphins use echolocation in the wild, the impacts of anthropogenic sound on echolocation production, and the potential consequences of high frequency hearing loss.
CHAPTER ONE: HEARING AND ECHOLOCATION IN ODONTOCETE CETACEANS: AN INTRODUCTION

Odontocetes have evolved the ability to use echolocation to detect and track their prey. Their hearing abilities have also coevolved to shift the frequency of greatest sensitivity to the higher frequencies used for foraging and navigation. Hearing and echolocation together provide odontocetes with a highly developed biosonar system. This dissertation examines the hearing ability of several odontocete species to understand what signals they can perceive during echolocation. The variability in hearing ranges between species is examined in the context of phylogenetic and ecological differences among taxa. An autonomous hydrophone array is also developed that could be used in an expanded form in field deployments to study echolocation signals in a wider range of species.

HEARING IN ODONTOCETES

Sound reception pathways funnel sound through acoustic fats to transfer the sound to the auditory system. The sound is transferred to the tympanic plate and tympanic bone which vibrate and transfer sound to the middle ear complex of the malleus, incus and stapes (Nummela et al., 2007; Hemila et al., 1999; Nummela et al.,
Sound pressure on the oval window creates movement in the fluid inside the cochlea towards the round window (Ketten, 1992). This motion causes the basilar membrane to vibrate. The basilar membrane is constructed such that the narrow base that allows for high frequency detection rapidly widens towards the cochlear apex (Ketten, 2000). Within the cochlea, the organ of Corti is found on the basilar membrane and contains hair cells with hairs attached to the tectorial membrane. Sound is detected when the basilar membrane vibrates and causes the hairs to bend with respect to the membrane (Ketten and Wartzok, 1990).

Anatomical differences within the cochlea exist between odontocetes such that phocoenids and river dolphins (non-whistle producing odontocetes) produce narrow high frequency clicks, possess a Type II cochlea (echolocation peak spectra below 80 kHz, greater than two turns in cochlea) and their range of best hearing is higher (Ketten, 2000). However, the rest of the odontocetes produce broadband high frequency clicks, possess a Type I cochlea (echolocation peak spectra above 100 kHz, fewer than two turns in the cochlea), and have a wide range of good hearing where their best hearing is slightly lower than Type II animals (Ketten, 2000).

Behavioral methods were first developed to measure hearing in marine mammals in a captive setting (Hall and Johnson, 1972; Jacobs and Hall, 1972; Belkovich and Solntseva, 1970; Johnson, 1967; Johnson, 1966). However, these methods require a significant amount of time and training effort. Auditory evoked potential (AEP) methods use an involuntary brain wave response to determine hearing thresholds. AEP measurements have been used in human infants to determine hearing ability at a young age (Finitzo et al., 1998). In both captive and wild dolphins, results are comparable to
behavioral thresholds (Mann et al., 2011; Cook et al., 2006; Houser and Finneran, 2006a; Houser and Finneran, 2006b; Yuen et al., 2005; Szymanski et al., 1998; Dolphin et al., 1995). Hearing measurements can be acquired during strandings with this quick, non-invasive technique.

AEP methods can utilize several types of sound stimuli to measure different parameters of the auditory response. Amplitude modulated (AM) tones can be used to measure hearing thresholds across a range of frequencies by stimulating the auditory system with a combination of a carrier tone and a modulating signal. The envelope following response occurs whereby the auditory system is responding to the carrier frequency but is firing at the frequency of the modulating signal (Picton et al., 1987; Stapells et al., 1984; Hall, 1979; Campbell et al., 1977). The modulation rate transfer function (MRTF) compares the rate of modulation to the AEP response. By testing a range of modulation frequencies, the MRTF can be used to determine the modulation frequency to use for the carrier tone stimulus testing that will obtain the largest response (Vermeister, 1979). Testing six to eight carrier frequencies at decreasing sound stimulus levels until the response is no longer detected to determine a threshold level at each frequency can take approximately 45 minutes.

Although this is much quicker than the several months to collect a behavioral audiogram, an even more rapid single snapshot of hearing ability can be captured with the use of a click stimulus. The click stimulus is broadband, containing energy across several frequencies, and the click evoked potential shows the response of each of the auditory centers to the sound stimulus. This single stimulus can be tested in just a few
minutes and captures the potential for high frequency hearing impairment. These hearing measurements can be utilized to determine if a stranded cetacean should be released.

Audiograms have been collected on multiple species and a few species like the common bottlenose dolphin (*Tursiops truncatus*) are thoroughly represented in hearing data (Table 1-1). Bottlenose dolphins have been the representative species for many hearing studies, although it is well documented that variability exists not only within the species (Finneran *et al.*, 2008; Finneran & Houser, 2007; Finneran & Schlundt, 2007; Finneran & Houser, 2006; Houser & Finneran, 2006b; Houser *et al.*, 2004; Au *et al.*, 2002; Finneran *et al.*, 2002a; Finneran *et al.*, 2002b; Finneran *et al.*, 2002c; Ridgway & Carder, 1997; Dolphin, 1995; Supin & Popov, 1995), but also across species (Linnenschmidt *et al.*, 2013; Finneran *et al.*, 2005a; Beedholm & Miller, 2005; Kastelein *et al.*, 2002; Finneran *et al.*, 2002a; Finneran *et al.*, 2002b; Finneran *et al.*, 2002c; Szymanski *et al.*, 1999; Szymanski *et al.*, 1998; Dolphin, 1995; Popov & Supin, 1990a; Hall & Johnson, 1972). However, only a single audiogram has been collected for some odontocetes to represent the entire species (Table 1-1), and the species variability still needs to be characterized.

Odontocete hearing ranges from 0.5-160 kHz, with large amounts of inter- and intra-species variability. Common bottlenose dolphins have good hearing from 0.75-140 kHz, with a typical cutoff frequency around 120 kHz (Finneran *et al.*, 2008; Finneran & Houser, 2007; Finneran & Schlundt, 2007; Finneran & Houser, 2006; Houser & Finneran, 2006a; Houser *et al.*, 2004; Au *et al.*, 2002; Finneran *et al.*, 2002a; Finneran *et al.*, 2002b; Finneran *et al.*, 2002c; Ridgway & Carder, 1997; Dolphin, 1995; Supin &
Popov, 1995; Johnson, 1967; Johnson, 1966). However, Ljunblad et al. (1982) and Houser et al. (2008) tested bottlenose dolphins from the Pacific Ocean (then considered *T. gilli*) that possessed a higher cutoff frequency around 140 kHz. Harbor porpoise (*Phocoena phocoena*) audiograms display a broad range of hearing, with excellent high frequency hearing up to 160 kHz (Beedholm & Miller, 2005; Kastelein et al., 2002; Popov et al., 1986; Anderson, 1970). Montie et al. (2011) reported thresholds below 100 dB re 1 μPa up to 100 kHz for two male pygmy killer whales (*Feresa attenuata*) and an infant Risso’s dolphin (*Grampus griseus*) studied in Nachtigall et al. (2005) had thresholds below 100 dB re 1 μPa up to 128 kHz. However, Nachtigall et al. (1995) measured the hearing of an older adult Risso’s dolphin behaviorally and found thresholds at 100 and 110 kHz to be above 120 dB re 1 μPa.

Audiograms in the published literature include the four species listed above, as well as 19 more species in 17 genera. The common bottlenose dolphin and the beluga whale (*Delphinapterus leucas*, Finneran et al., 2005a; Finneran et al., 2002a; Finneran et al., 2002b; Finneran et al., 2002c; Dolphin, 1995; Popov & Supin, 1990a; Awbrey et al., 1988; Popov & Supin, 1987; White et al., 1978) have over 10 individual audiograms published and are well represented. The harbor porpoise has four published audiograms (Beedholm & Miller, 2005; Kastelein et al., 2002; Popov et al., 1986; Anderson, 1970), as does the false killer whale (*Pseudorca crassidens*, Yuen et al., 2005; Supin et al., 2003; Dolphin, 1995; Thomas et al., 1988). The killer whale (*Orcinus orca*, Szymanski et al., 1999; Szymanski et al., 1998; Hall and Johnson, 1972), short-beaked common dolphin (*Delphinus delphis*, Popov and Klishin, 1998; Ridgway et al., 1981; Belkovitch &
Solntseva, 1970), and Amazon River dolphin (\textit{Inia geoffrensis}, Popov & Supin, 1990a; Popov & Supin, 1990c; Jacobs and Hall, 1972) have three audiograms each in the published literature.


Characterizing variability among taxa is somewhat difficult considering some phylogenetic relationships are still under debate. However, a recent study has shown that genetic evidence links the killer whale (as a sister taxon) to the subfamily
Globicephalinae which includes the Risso’s dolphin, false killer whale, short and long-finned pilot whales, pygmy killer whale, Irrawaddy dolphin (*Orcaella brevirostris*), Australian snubfin dolphin (*Orcaella heinsohni*), and the melon-headed whale (*Peponocephala electra*) (Vilstrup *et al.*, 2011). Hearing has been studied in most of these species, with the exception of the Irrawaddy dolphin, Australian snubfin dolphin and the melon-headed whale.

The rough-toothed dolphin genetic data analysis placed the species as a sister taxon within the subfamily Delphininae, which includes the members of the *Stenella*, *Tursiops*, and *Delphis* genera, and the Indo-Pacific humpback dolphin (Vilstrup *et al.*, 2011), not within the Globicephalinae subfamily. Hearing audiograms are present in the published literature for the rough-toothed dolphin, as well as two *Stenella* species, the common bottlenose dolphin, and the Indo-Pacific humpback dolphin, but the long-beaked common dolphin (*Delphinus capensis*) and the Indo-Pacific bottlenose dolphin (*Tursiops aduncus*) are not represented. Vilstrup *et al.* (2011) reported that genetic evidence suggests that the white-beaked dolphin is not a member of either the Globicephalinae or the Delphininae subfamilies, but is likely a sister taxa to the Delphinidae family. Hearing variability within and amongst these subfamilies will be discussed in Chapter Two, Three and Four, and summarized in Chapter Six.

Inherent variability also exists within the methodologies used to measure hearing. Threshold differences between different testing methods (AEP versus behavioral) and testing environments (in-air AEP versus in-water AEP) can be as large as 26 dB and standard deviations are around 13 dB (Houser and Finneran, 2006a; Houser and Finneran,
Repeated recordings from the same individual during a single testing period have shown an 8-10 dB variability in thresholds in other studies (Finneran et al., 2008; Finneran and Houser, 2007).

With this amount of variability, it is difficult to determine what a “normal” audiogram will show. It is unknown if the differences between individuals are representative of the variability of the entire species. Furthermore, the strength of comparisons of range of best sensitivity or upper hearing limit across major groups is uncertain without data on more individuals.

HEARING IMPAIRMENT

Hearing impairment can be caused by congenital hearing loss, presbycusis (age-related hearing loss), impulsive or sustained noise exposure, or ototoxic antibiotic treatment. The relative contributions of each of these within the hearing impaired dolphin population are sometimes difficult to measure. There are several cases of suspected congenital hearing loss in marine mammals including two rough-toothed dolphins and a pilot whale (Mann et al., 2011), and a striped dolphin (André et al., 2003) that were likely born deaf. Studies that analyzed hearing loss within age groups have shown that presbycusis trends, showing increasing hearing loss with increasing age, are similar to those found in humans (Houser and Finneran, 2006b; Ridgway and Carder, 1997). Cook (2006) found two possible cases of presbycusis: a captive 52 yr old female and a wild, older adult female, but did not find age-related hearing impairment in any of the other bottlenose dolphins (ranging from 2-40 yrs old) tested in Sarasota Bay, Florida.
Measurements of noise exposure-related hearing impairment are produced through experiments on permanent or temporary threshold shift (PTS or TTS) (Southall et al., 2007; Finneran et al., 2005b; Finneran et al., 2002c; Schlundt et al., 2000; Finneran et al., 2000). Although ototoxicity is commonly listed as a potential cause for hearing loss in marine mammals (Mann et al., 2011; Finneran et al., 2005a), studies have not been conducted to measure hearing before and after antibiotic treatment.

Examining the possible impact of hearing impairment or deafness on foraging success is very challenging, and only a few studies have been able to shed light on this topic (Wright, 2011; Ridgway and Carder, 1997). Ridgway and Carder (1997) reported the presence of a deaf/mute dolphin amongst eight bottlenose dolphins that were trained to respond to a tone stimulus between 40 and 120 kHz. The deaf dolphin responded to the stimulus only in the presence of other dolphins and it was suggested that through learning to use other senses or behavioral cues from other dolphins this deaf/mute dolphin could potentially survive in the wild (Ridgway and Carder, 1997). Another dolphin with severe hearing impairment was found to have significantly reduced reaction times and significantly lower success rates at capturing prey items during a study conducted at a captive facility (Wright, 2001).

Several studies have shown that severe hearing impairment in humans leads to a reduction in quality of life (Dalton et al., 2003; Davis and Hind, 1999; Hétu et al., 1993). Because odontocetes live in an acoustic environment, it is assumed hearing impairment would limit their ability to successfully forage, navigate and maybe even communicate. However, it is unknown what level of hearing impairment would equate to the inability to capture prey or navigate. A false killer whale showed a decrease in discrimination ability
after developing high frequency hearing loss, and a reduction in peak frequency, center
frequency and source level of clicks used during the discrimination task (Kloepper et al.,
2010a; Kloepper et al., 2010b).

Hearing loss in this animal could not be quantitatively determined because the
first audiogram was collected during a masking task, but with masking present the false
killer whale could hear at 100 kHz (Thomas et al., 1990). In 2004, the upper cutoff
frequency, or limit of high frequency hearing, without masking present was around 45
kHz (Yuen et al., 2005). The task to discriminate cylinder wall thickness does not
directly translate to surviving in the wild but Kloepper et al. (2010b) compared it to
discriminating prey types and range. This comparison would follow that high frequency
hearing loss would result in limited successful foraging, but this has not been directly
studied. Possible adaptations in echolocation use may allow for hearing impaired
dolphins to overcome any limitation.

ECHOCOLOCATION

Echolocation abilities have been widely studied in both captive and wild
odontocete cetaceans. Echolocation clicks are high frequency (50-120 kHz), broadband,
directional (10 degree 3-dB beamwidth, with off-axis distortion), and short impulse
sounds produced at high source levels (up to 230 dB re 1 µPa SPL) used for foraging and
navigation (Au, 1993). Sound generation begins when the larynx is pulled forward and
dorsally causing the nasal cavity to be pressurized (Au, 1993; Amundin and Andersen,
1983). Pressurized air is then pushed past the phonic lips causing them to vibrate and
eventually achieve relaxed oscillation. It is believed that pulse production occurs either when the lips make contact or when the change in vibrational acceleration is greatest (Cranford et al., 1996). The pulse emitted reflects off internal services of the melon and air sacs causing it be focused into a beam before leaving the head. The density structure of the melon consists of an oily fat layer on the exterior with a denser core, causing sound traveling through the less dense layer to travel faster, sound in the denser core to travel slower and a focused beam to be formed. The high frequency clicks are used to obtain high-resolution information about their surroundings by actively ensonifying an area to listen for echoes.

Properties of the echolocation click emitted and the aquatic environment through which it has to travel determine the type of information a dolphin can obtain through echolocation. Water depth can affect the amount of click energy that reaches a target (i.e., prey item) and the amount of detail gained from a target prey species is dependent on the frequency of the clicks emitted. High frequency clicks have shorter wavelengths and are more susceptible to transmission loss of energy (Urick, 1983). Lower frequency clicks have longer wavelengths and can travel further through the water column (Urick, 1983). Higher resolution and detail can be obtained from a target with higher frequency clicks, even though high frequency clicks attenuate faster than lower frequency clicks.

Background noise also affects the ability of a dolphin to perceive the echo information. In shallow coastal waters, overall background noise will be higher. Sources of noise include wind, waves (including tides), weather, vessel noise (shipping and recreational), seismic activity, and biological sound sources (Urick, 1983). Besides some biological activity, these sound sources are low frequency compared to odontocete
echolocation. However, other dolphin echolocation and the presence of snapping shrimp (family Alpheidae) provide a large amount of background noise, masking the corresponding echo from a dolphin’s echolocation click. In open water, shipping vessel noise is less concentrated and recreational vessels are limited. Also, snapping shrimp are not present. Therefore overall background noise is lower, as long as oil exploration or sonar is not present. These two sources of anthropogenic noise are loud and exist within the frequency range of sensitive odontocete hearing (Finneran et al., 2005b).

Parameters such as click source level, click rates, and click frequency can be modified by an echolocating dolphin in order to optimize the information potentially received from a target. While closing in on a target, dolphins have been shown to decrease their click source level to compensate for the increasing echo from a closer target (Linnenschmidt et al., 2012; Atem et al., 2009; Au and Bird, 2003). Click rates have been shown to accommodate for two-way travel time and some processing time (Jensen et al., 2009; Au, 1993), but can be higher or lower dependent on target distance and species (Simard et al., 2010; Ivanov, 2004; Akamatsu et al., 1998; Turl and Penner, 1989). Changes in click rate are seen in beaked whales during foraging dives (Johnson et al., 2006; Madsen et al., 2005). Both Johnson et al. (2006) and Madsen et al. (2005) observed an increase in click rate during the terminal phase of prey capture, reflected by emission of a terminal buzz.

Studies have looked at click parameters in the wild to try to determine how a dolphin might use their echolocation in different habitats (Simard et al., 2010; Soldevilla et al., 2010). Simard et al. (2010) found that click rates decrease with increasing mean water depth, indicating that target range is depth dependent. Soldevilla et al. (2010)
described the use of two click types with different spectral qualities by Pacific white-sided dolphins. Analysis using passive acoustics showed a trend that reflected population-specific use of each click type and diurnal patterns reflecting differences in prey types (Soldevilla et al., 2010).

It is unknown exactly how dolphin click parameters vary during pursuit and capture of different prey types and the variability that exists across species that tend to forage on different prey types. A species such as the sperm whale (*Physeter macrocephalus*) that forages at great depths and on large patches of squid (order Teuthida), as opposed to a single prey item, would be expected to use lower frequency clicks that would travel farther and just estimate approximate location of a prey item. However, a species such as the bottlenose dolphin that is foraging in coastal waters on fish species would be expected to use high frequency clicks that are not required to travel long distances in the shallower waters, but provide the dolphin with high resolution information on either a single prey item or a small school of fish. When this species is found in pelagic waters, it may utilize lower frequencies when cooperatively foraging in groups on larger fish bait balls, as well as higher frequencies when individually attempting to capture a fish within the assemblage.

Hearing abilities would seem to limit the usage of different frequencies of echolocation considering an emitted click with frequencies that cannot be perceived by the foraging dolphin will not provide any information or lead to foraging success. Measuring hearing abilities and echolocation in free-swimming dolphins concurrently will provide glimpses into how these two sensory systems can or may function in wild odontocete species. The impact of hearing ability limitations (either naturally or as a
result of hearing impairment) can be assessed once the diversity of echolocation and hearing abilities among odontocetes is quantified.

FORAGING ECOLOGY

Foraging ecology within the delphinid odontocetes has some limited variability in prey type with a few specialist species. Killer whales have two ecotypes that are divided based on their foraging ecology and behavior. Resident killer whales feed on fish, like the salmon (*Oncorhynchus* sp.), but the transient killer whales feed exclusively on other marine mammals (Ford *et al.*., 2010; Saulitis *et al.*., 2000; Hoelzel, 1991; Bigg *et al.*., 1987). Sperm whales have been shown to feed on squid extensively, however in some areas this is part of a shift in prey types between squid and fish species (Miller *et al.*., 2004; Whitehead *et al.*., 2003; Whitehead, 2003; Jaquet *et al.*., 2000; Santos *et al.*., 1999). The variability in prey types occurs with several delphinids, dependent on prey abundance and energy expenditure for prey capture. Most pelagic species feed cooperatively on fish schools utilizing group tactics to feed on an assemblage of prey (Gazda *et al.*., 2005; Fertl and Wursig, 1995; Bel’kovich *et al.*., 1978; Leatherwood, 1977) or utilize the migration of the deep scattering layer to feed at night (Benoit-Bird *et al.*., 2004; Norris and Dohl, 1980).

Most of the diversity in foraging ecology among odontocetes, especially delphinids, occurs in the methods of prey pursuit and capture. Foraging strategies for bottlenose dolphins vary on all levels, from individual to group to population. Causes of variation can include habitat usage (either differing by bottom type and/or water depth),
prey distribution and learning from social groupings. At the population level foraging strategy diversity is usually controlled by habitat available and prey distribution coupled together.

For example, the *Tursiops* within Sarasota Bay typically feed along seagrass beds and seawalls (Barros and Wells, 1998). In the southeastern United States mud flats and the muddy waters of the Colorado River Delta in Mexico, dolphins rush the shallow water to create a tidal wave that washes fish out of the water and onto the muddy banks, then temporarily beaching themselves to feed on the fish trapped there (Silbert and Fertl, 1995; Rigley, 1983; Hoese, 1971). Dolphins that live in slightly deeper water with sandy bottoms like those in the more open Shark Bay or in the Bahamas will tend to bottom feed on fish buried in the sand (Mann and Sargeant, 2003; Smolker *et al*., 1997; Rossbach and Herzing, 1997). Rossbach and Herzing (1997) described their foraging behavior of echolocating in a scanning manner moving horizontal to the sea floor until a prey item is detected, and then turning vertically (rostrum pointed towards the sand) while echolocating on one spot until digging in the sand to retrieve the prey item.

Inter-population variability is best seen in Shark Bay, Australia. Groups that inhabit the very shallow waters along the beaches will intentionally beach themselves in order to depredate and be fed by humans on the beach (Sargeant *et al*., 2005). It has been shown that dolphins exhibiting this behavior return to the beach to be fed repeatedly, and the group of dolphins commonly associate which would establish the propensity for social learning such that one dolphin “shows/teaches” another that food can be acquired this way (Sargeant *et al*., 2005). Other dolphins within this population inhabit deeper waters and can be seen herding fish or bottom feeding with sponges on their rostrum.
(Smolker et al., 1997). The versatility of foraging strategies seen in the wild may require perceiving different acoustic signals, or may provide a method of adaptation for an odontocete who cannot perceive their own acoustic signals due to hearing impairment. It is likely that the ability of an impaired dolphin to forage successfully will be determined by a number of factors, including the extent and magnitude of the hearing impairment, the foraging ecology of the species, and their echolocation capabilities.

AIMS OF THIS STUDY

This study examines the variability of hearing sensitivity among several odontocete species, and provides a comparison in a phylogenetic framework to the breadth of studies already conducted on other cetaceans. Because studies relying on strandings are necessarily opportunistic one cannot choose which species to study. Still, hearing was measured from three genera that were unrepresented or underrepresented in previous studies. These results are detailed in Chapter Two on short-finned pilot whales, Chapter Three on Risso's dolphins and Chapter Four on hearing sensitivity among three species in the genus *Stenella*. Chapter Three also investigates the ototoxic effects of antibiotic drug treatment of two mother/calf pairs of Risso’s dolphins where before-treatment and after-treatment hearing measurements were performed. Most studies of echolocation signals have involved captive dolphins detecting or discriminating static targets. This greatly limits the species that can be studied. Chapter Five describes the development and implementation of an autonomous, field-deployable hydrophone array system to measure free-swimming echolocation beam patterns. This system would enable
field studies of echolocation in a wider range of species to explore the relationship between hearing sensitivity and echolocation use.
Table 1-1. Summary of hearing studies on odontocete cetacean species. Scientific name, common name and citations for all studies on hearing in odontocete cetaceans are listed. Previously recognized scientific names used in literature listed are given in parenthesis. Asterisks (*) indicate behavioral hearing methods were used and pound signs (#) indicate electrophysiological hearing methods were used.

<table>
<thead>
<tr>
<th>Scientific Name</th>
<th>Common Name</th>
<th>Literature</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Feresa attenuata</em></td>
<td>Pygmy killer whale</td>
<td>Montie <em>et al.</em>, 2010#; Mann <em>et al.</em>, 2011#</td>
</tr>
<tr>
<td><em>Globicephala macrorhynchus</em></td>
<td>Short-finned pilot whale</td>
<td>Schlundt <em>et al.</em>, 2011#; Mann <em>et al.</em>, 2011#</td>
</tr>
<tr>
<td><em>Globicephala melas</em></td>
<td>Long-finned pilot whale</td>
<td>Pacini <em>et al.</em>, 2010#</td>
</tr>
<tr>
<td><em>Grampus griseus</em></td>
<td>Risso’s dolphin</td>
<td>Nachtigall <em>et al.</em>, 1995#; Nachtigall <em>et al.</em>, 2005#; Mann <em>et al.</em>, 2011#</td>
</tr>
<tr>
<td><em>Inia geoffrensis</em></td>
<td>Amazon River dolphin</td>
<td>Jacobs &amp; Hall, 1972#; Popov &amp; Supin, 1990a#; Popov &amp; Supin, 1990c#</td>
</tr>
<tr>
<td><em>Kogia breviceps</em></td>
<td>Pygmy sperm whale</td>
<td>Ridgway &amp; Carder, 2001#</td>
</tr>
<tr>
<td><em>Lagenorhynchus albirostris</em></td>
<td>White-beaked dolphin</td>
<td>Nachtigall <em>et al.</em>, 2008#</td>
</tr>
<tr>
<td><em>Lagenorhynchus obliquidens</em></td>
<td>Pacific white-sided dolphin</td>
<td>Tremel <em>et al.</em>, 1998*</td>
</tr>
<tr>
<td><em>Lipotes vexillifer</em></td>
<td>Baiji/Yangtze river dolphin</td>
<td>Wang <em>et al.</em>, 1992*</td>
</tr>
<tr>
<td><em>Mesoplodon densirostris</em></td>
<td>Blainville’s beaked whale</td>
<td>Pacini <em>et al.</em>, 2011#</td>
</tr>
<tr>
<td><em>Mesoplodon europaeus</em></td>
<td>Gervais’ beaked whale</td>
<td>Cook <em>et al.</em>, 2006#; Finneran <em>et al.</em>, 2009#; Mann <em>et al.</em>, 2011#</td>
</tr>
<tr>
<td><em>Neophocaena asiaeorientalis</em></td>
<td>Narrow-ridged finless porpoise</td>
<td>Popov <em>et al.</em>, 2005#</td>
</tr>
</tbody>
</table>
Table 1-1 (cont.)

<table>
<thead>
<tr>
<th><strong>Phocoena phocoena</strong></th>
<th>Harbor porpoise</th>
<th>Anderson, 1970*; Popov et al., 1986#; Kastelein et al., 2002*; Beedholm &amp; Miller, 2005#; Linnenschmidt et al., 2013#</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Physeter macrocephalus</strong></td>
<td>Sperm whale</td>
<td>Carder &amp; Ridgway, 1990#; Ridgway &amp; Carder, 2001#</td>
</tr>
<tr>
<td><strong>Pseudorca crassidens</strong></td>
<td>False killer whale</td>
<td>Thomas et al., 1988*; Dolphin, 1995#; Supin et al., 2003#; Yuen et al., 2005#</td>
</tr>
<tr>
<td><strong>Sotalia guianensis (S. f. guianensis)</strong></td>
<td>Guiana dolphin</td>
<td>Popov &amp; Supin, 1990a#; Sauerland &amp; Dehnhardt, 1998#</td>
</tr>
<tr>
<td><strong>Sousa chinensis</strong></td>
<td>Indo-Pacific humpback dolphin</td>
<td>Li et al., 2012#</td>
</tr>
<tr>
<td><strong>Stenella attenuata</strong></td>
<td>Pantropical spotted dolphin</td>
<td>Bullock et al., 1968#</td>
</tr>
<tr>
<td><strong>Stenella coeruleoalba</strong></td>
<td>Striped dolphin</td>
<td>Bullock et al., 1968#; Kastelein et al., 2003*; Andre et al., 2003#</td>
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<tr>
<td><strong>Stenella frontalis</strong> (S. plagiodon)</td>
<td>Atlantic spotted dolphin</td>
<td>Kellogg &amp; Kohler, 1952*; Mann et al., 2011#</td>
</tr>
<tr>
<td><strong>Stenella longirostris</strong></td>
<td>Spinner dolphin</td>
<td>Mann et al., 2011#</td>
</tr>
<tr>
<td><strong>Steno bredanensis</strong></td>
<td>Rough-toothed dolphin</td>
<td>Bullock et al., 1968#; Mann et al., 2011#</td>
</tr>
</tbody>
</table>
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study of their identification, genealogy and natural history in British Columbia 


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CHAPTER TWO: HEARING THRESHOLD MEASUREMENTS OF FIVE STRANDED SHORT-FINNED PILOT WHALES (GLOBICEPHALA MACRORHYNCHUS)\textsuperscript{1}

ABSTRACT

Approximately 26 short-finned pilot whales, Globicephala macrorhynchus, stranded in Cudjoe Key, Florida on May 5, 2011. Four animals, two adult and two juvenile females, were transported to a rehabilitation facility in Tarpon Basin, Florida. Auditory evoked potentials were recorded in response to amplitude modulated tone pips modulated at 1000 Hz. Hearing thresholds were determined at 10, 20, 40, 80 and 120 kHz for all four animals. Short-finned pilot whales had peak sensitivity at lower frequencies than other odontocetes such as bottlenose dolphins. Greatest sensitivity was around 40 kHz for all whales, while thresholds for the two adult females were 25-61 dB higher at 80 kHz than the juveniles. Click evoked potentials were similar between the four whales and comparable to other echolocating odontocetes. Click-evoked potential data from another short-finned pilot whale that had stranded in Curacao showed no response. These findings add to the limited database of pilot whale (short- and long-finned) hearing studies, of which there are only two others (Schlundt \textit{et al.}, 2011 & Pacini \textit{et al.}, 2010).

\textsuperscript{1} Portions of these results have been previously published (Greenhow \textit{et al.}, 2011) and are utilized with the permission of the publisher. The abstract appeared in Greenhow \textit{et al.}, 2011 and may be found at http://link.aip.org/link/?JAS/130/2560. Copyright (2011) Acoustical Society of America.
INTRODUCTION

Short-finned pilot whales (*Globicephala macrorhynchus*) inhabit offshore waters and feed on migrating squid and other deep-dwelling species (Olsen, 2009). They are commonly found in mass strandings, due to the behavior of a pod following a ‘pilot’ or single lead animal even when it strands. Strandings in North Carolina from 1992-2005 included 47 pilot whales, of which 7 were individual strandings and the remaining 40 whales stranded in three stranding events (Hohn *et al*., 2006). In Florida, four mass strandings of pilot whales (*Globicephala* sp.) are on record between 1978 and 1987, and in Hawaii between 1936 and 1988, 10 short-finned pilot whale stranding events occurred with only 4 of those being single stranding events (Reynolds and Odell, 1991). In some cases animals that have stranded show no signs of being compromised, and can be tagged and released into deeper waters (Wells *et al*., 2012; Wiley *et al*., 2001), while other cetaceans either die, are euthanized, or undergo rehabilitation because of their illnesses.

Some members of the subfamily Globicephalinae (Vilstrup *et al*., 2011) which includes pilot whales have been shown to have high frequency hearing (Montie *et al*., 2011; Nachtigall *et al*., 2005), with an infant Risso’s dolphin (*Grampus griseus*) hearing up to 150 kHz (Nachtigall *et al*., 2005). Montie *et al*., (2010) reported thresholds below 100 dB re 1 µPa up to 100 kHz for two male pygmy killer whales (*Feresa attenuata*) and an infant Risso’s dolphin studied in Nachtigall *et al*., (2005) had thresholds below 100 dB re 1 µPa up to 128 kHz. However, Nachtigall *et al*., (1995) measured the hearing of an older adult Risso’s dolphin behaviorally and found thresholds at 100 and 110 kHz to be above 120 dB re 1 µPa. While the first two studies indicate sensitive high frequency hearing above 120 kHz (Montie *et al*., 2011; Nachtigall *et al*., 2005), the adult Risso’s
dolphin audiogram indicates that 120 kHz is at the upper limit of the hearing range for that species (Nachtigall et al., 1995). Chapter three of this dissertation discusses results of hearing tests on four Risso’s dolphins that all have an upper cutoff frequency between 80-120 kHz (Fig. 3-3). The variability of hearing thresholds and the evidence of less sensitive high frequency hearing in this species are discussed further in that chapter.

Although methodological differences could contribute to some discrepancies in threshold measurements, these are very different audiograms for individuals of the same species. Schlundt et al. (2011), who tested a stranded juvenile male and a captive adult female short-finned pilot whale, found the female to have an upper cutoff frequency around 80 kHz and the male to be hearing impaired above 10 kHz. It was suggested that the elevated thresholds at higher frequencies of the adult may be caused by the onset of presbycusis (age-related hearing loss), given that the female was estimated to be 30-32 yr old. The only other study on pilot whale hearing (G.melas, Pacini et al., 2010) reported a juvenile male long-finned pilot whale with thresholds above 100 dB re 1 µPa at 80 and 100 kHz. Pacini et al. (2010) stated that the audiogram did not show hearing loss, with the caveat that there was a potential for ototoxic drug effect. Since baseline data does not exist for this genus, it is unknown if the narrower hearing range is characteristic for pilot whales or if these audiograms are representative of high frequency hearing loss.

Hearing impairment may limit the efficiency of tracking and capturing prey. Behavioral methods used in captivity to measure hearing in marine mammals are not feasible to use with stranded or hospitalized animals. These methods require a significant amount of time and training effort in order to condition a dolphin to respond to hearing a
tone by physical or acoustic behavior. Behavioral hearing tests also take several months in order to achieve a full audiogram because each frequency must be tested multiple times and testing is limited by the motivation of the animal. Auditory evoked potential (AEP) methods, which use brain wave activity to determine hearing thresholds, have been used in human infants to measure hearing ability (Finitzo et al., 1998). These methods have also been used in both captive and wild settings to measure odontocete hearing, and results are comparable to behavioral thresholds on captive animals (Mann et al., 2011; Cook et al., 2006; Houser and Finneran, 2006a; Houser and Finneran, 2006b; Yuen et al., 2005; Szymanski et al., 1998; Dolphin et al., 1995). AEP methods are rapid and less invasive, requiring only minimal handling for short periods of time, which make them well-suited for stranded or rehabilitated animals. Audiogram results can provide insight for resource managers to determine if an animal should be deemed releasable.

Strandings of either individuals or large groups (mass strandings) occur frequently in some areas, for various reasons (Sundaram et al., 2006; Walker et al., 2005). In most cases it is difficult to determine the exact cause of stranding, but it has been shown that hearing loss or impairment could be a potential cause (Mann et al., 2011). In strandings, animals that are not healthy enough to be pushed back out to sea, but stable enough to be rehabilitated are transferred to a nearby rehabilitation facility. Rehabilitation facilities provide a setting for accessing pelagic animals that would otherwise be difficult to access for hearing measurements.

In this study the hearing of five short-finned pilot whales from two separate stranding events was measured using AEP methods. In Curacao a single juvenile male
was tested in July of 2009. Data on the Curacao juvenile was previously reported in Mann et al., (2011) and Schlundt et al. (2011), but further data are presented here. Four female pilot whales were tested during rehabilitation in May of 2011 in the Florida Keys. This study adds to the limited data on pilot whale hearing, which suggested that they may have a narrower hearing range than delphinid cetaceans (Schlundt et al., 2011 and Pacini et al., 2010).

METHODS

A. Subjects

Curacao

A juvenile short-finned pilot whale stranded on July 14, 2009 in Willemstad, Curacao in the Netherlands Antilles. The single animal was moved down the coast about 10 miles into a sea pen just off the seawall of the public beach in Jan Thiel Bay and rehabilitated with help from Curacao Sea Aquarium. The male pilot whale was 2.5 m in length and estimated to be approximately 2 years old. He was treated with a daily intramuscular injection of 12 mg/kg of amikacin once a day for 10 days after stranding. An auditory evoked potential hearing test was performed on August 17, 2011.

Florida Keys

Two of the females were estimated to be adults at the time of stranding (MMC-Gm-0611 and MMC-Gm-1011) based on body size and development. MMC-Gm-0711 was classified as a dependent calf and estimated to be between 1-3 years old. MMC-Gm-0811 was a juvenile/sub-adult and estimated to be 3-7 years old at the time of stranding. Only one adult (MMC-Gm-1011) was treated with a known ototoxic antibiotic: 20mg/kg of florfenicol via intramuscular injection once every 48 hrs for 7 days.

B. Auditory Evoked Potentials (AEPs)

During testing, each animal was temporarily restrained by volunteers at the water surface, with the jaw submerged and the blowhole above water. Sound stimuli were delivered to the animal through a jawphone consisting of an ITC-1042 piezoceramic transducer embedded in a RTV silicone suction cup placed on the left, lower jaw fat pad. Evoked potentials were measured using three gold cup electrodes (Rochester Electrode, Tampa, Florida) also embedded in silicone suction cups: a recording electrode placed 2 cm behind the blowhole, a reference electrode placed off the midline 10 cm posterior to the recording electrode, and a ground electrode placed in the water.

Modulation rate transfer functions were determined by delivering the sound stimulus at a carrier frequency of 40 kHz at 162 dB re 1µPa and altering the amplitude modulation rate. Modulation rates from 200-2000 Hz at 100 Hz steps were tested. Peak amplitude at each modulation rate was determined as mentioned above for AEP responses.

AEPs were recorded in response to amplitude modulated (AM) tone pips modulated at 1000 Hz and carrier frequencies tested ranged from 5-120 kHz. Click
evoked potentials were also recorded in response to a 0.1 ms click with a peak frequency of 62 kHz. A Tucker-Davis Technologies (TDT) RX6 real-time processor was used at a 260 kHz sample rate to generate all signals. Thresholds were determined at each frequency tested where there was a peak in the fast Fourier transform (FFT) of the recorded signal present at least 6.42 dB above the noise floor ($\alpha = 0.01$; Dobie and Wilson, 1996). The signal was a result of approximately 500 averages and the noise floor was determined from the 20 ms window prior to the stimulus beginning. Post-recording sound level calibrations were performed underwater with the jawphone and a Reson TC4041 hydrophone (-212 dBV re 1 $\mu$Pa with VP1000 pre-amplifier with 32 dB gain) mounted 10 cm apart and 30 cm underwater at the location of the hearing test. Background noise was measured with an HTI hydrophone (HTI 96-min; -164 dBV re 1 $\mu$Pa) and presented as spectrum level (dB re 1 $\mu$Pa$^2$ Hz$^{-1}$) in the audiogram.

RESULTS

A. Curacao

Auditory evoked responses were not detected from the juvenile male up to the highest presentation levels at all frequencies tested (Table 2-1). When compared to a typical common bottlenose dolphin ($Tursiops truncatus$) click evoked potential, the recorded brain response was undetectable (Fig.2-1). The pilot whale did not display any evident behavioral response during testing and post-testing recordings show that it produced tonal sounds around 7 kHz.
B. Florida Keys

The four female pilot whales were all held side by side under a canopy in a sea pen within Tarpon Basin Lagoon during the testing process. Each animal was tested in sequence and none of the four pilot whales showed any signs of agitation during any testing session.

Modulation rate transfer functions (MRTFs) were recorded in response to a 40 kHz carrier tone presented at 162 dB re 1µPa to determine the evoked response at each modulation rate. The modulation rate transfer function reflects the ability of the auditory system to follow individual pulses within the stimulus and response amplitudes are higher for rates at which the stimulus is distinguished as individual pulses (Mooney et al., 2011; Supin and Popov 1995; Vermeister 1979). The strongest peaks were present at a modulation rate of 500 Hz for both the juvenile and one adult pilot whale, whereas the strongest peaks for the calf and other adult female were at 700 Hz (Fig. 2-2). The MRTF falls off after approximately 1600 Hz (Fig. 2-2), and this reflects the high temporal resolution found in most odontocetes (Mooney et al., 2011). A modulation rate of 1000 Hz was chosen for the stimulus during threshold determination because secondary peaks occurred for all four females at this rate, and the noise floor is lower at 1000 Hz than at 500 Hz because background electrical and acoustic noise is typically lower frequency.

Pilot whale audiograms were a U-shape and similar to those found in other marine mammals. All four females had the greatest sensitivity between 20-40 kHz (Fig. 2-3). The adult female MMC-Gm-0611 and the juvenile MMC-Gm-0811 had the greatest sensitivity at 40 kHz and a cutoff frequency around 80-120 kHz. MMC-Gm-0711 had a
relatively flat sensitivity at all the lower frequencies tested (within 3 dB). The calf also had a lower threshold at 80 kHz (36-60 dB re 1µPa lower) than the other three females, and a higher upper frequency limit at 100-120 kHz (Fig. 2-3). The other adult female (MMC-Gm-1011) had a cutoff at 80 kHz and no response was detected at 120 kHz with a stimulus level up to 177 dB re 1µPa. Testing at 100 kHz was not conducted for the adults and juvenile pilot whale because handling time was limited. Click evoked potentials were recorded for all four pilot whales. There was only a slight difference among their click thresholds with the juvenile responding down to a presentation level of 100 dB<sub>peak</sub> re 1µPa and the other three whales responding down to 94 dB<sub>peak</sub> re 1µPa.

DISCUSSION

The four females in this study all had similar hearing thresholds across the frequency range tested, and thresholds below 80 kHz were similar to the bottlenose dolphin (Popov <i>et al.</i>, 2007; Houser and Finneran, 2006a). All individuals showed a decrease in sensitivity at 80 or 100 kHz (Fig. 2-3). The less sensitive thresholds at higher frequencies could reflect a species difference (as compared to the bottlenose dolphin) or the limitation of the AEP detection methods. For example, behavioral audiogram thresholds of the same individuals may yield more sensitive thresholds at all frequencies.

The upper limit of best hearing is comparable to that of the killer whale (<i>Orcinus orca</i>, Szymanski <i>et al.</i>, 1999; Szymanski <i>et al.</i>, 1998), as well as an adult Risso’s dolphin (Nachtigall <i>et al.</i>, 1995) and those found in the other pilot whale studies (Schlundt <i>et al.</i>, 2011 and Pacini <i>et al.</i>, 2010) mentioned above. Szymanski <i>et al.</i> (1998, 1999) reported a
cutoff frequency of 100 kHz for two killer whales. Finneran et al. (2009) found a cutoff frequency of 80-90 kHz for an adult Gervais’ beaked whale (*Mesoplodon europaeus*), but Cook et al. (2006) reported increasing sensitivity up to 80 kHz, the highest frequency tested. The cutoff frequency for bottlenose dolphins with normal hearing is typically 120-140 kHz (Houser and Finneran, 2006a; Houser and Finneran, 2006b).

The frequency range of greatest sensitivity for the Florida Keys pilot whales was from 20-40 kHz, with individual differences in maximum sensitive frequency. The area of best hearing overlaps with the killer whale (Szymanski et al., 1998), Gervais’ beaked whale (Finneran et al., 2009), and other pilot whales (Schlundt et al., 2011; Pacini et al., 2010). Both the adult and infant Risso’s dolphins had a wider range of best hearing, extending below 20 kHz and above 40 kHz (Nachtigall et al., 2005; Nachtigall et al., 1995). The two younger female pilot whales have lower thresholds than the two adults at the highest frequencies tested. This may indicate slight hearing impairment in the adults tested or it could simply reflect the variability within this species. It is also possible that the size of the animal would result in the electrodes being closer to the brain or the transducer being closer to the ear, leading to a stronger response or a louder stimulus during testing.

In Curacao the male juvenile pilot whale was found to have severe hearing loss across all frequencies tested. Schlundt et al. (2011) also presents an audiogram for this same individual with an elevated threshold at 10 kHz and no response at any other frequencies tested. The absence of a measurable threshold at 10 kHz in this study could be the result of higher background noise in the testing setup or the difference in testing.
methods (i.e., sound delivery method, number of signals averaged, etc.). Schlundt et al. (2011) used a transducer in the direct field and averaged over 4,000 sweeps per sound stimulus level. This study used a jawphone transducer which would increase the sound stimulus level received at the ear compared to the use of a direct field transducer, and result in a more sensitive threshold determination. However, this study used approximately 500 averages as compared to the 4,000 in Schlundt et al. (2011), which could result in a higher determined threshold.

Causes of hearing loss in marine mammals could vary from severe sound exposure or ototoxic antibiotics to congenital hearing impairment or disease. The possibility of exposure to high frequency sonar or underwater explosions is unknown for the life history of this animal. However, the pilot whale would have had to been exposed to intense sound over a prolonged period of time in order to achieve the received levels reported to cause permanent hearing loss (Southall et al., 2007), especially across all frequencies tested. The pilot whale was estimated to be 2 yrs old at the time of stranding and would not have experienced presbycusis, or hearing loss that occurs with age. The possibility exists that this pilot whale was born with severe hearing impairment. The estimated age of the animal falls within the timeframe when female pilot whales will wean their young from nursing and teach them to hunt for prey (Kaysua and Marsh, 1984). Detecting and capturing prey relies heavily on the auditory system, being able to hear the echoes off of fish and squid in the water column in order to feed (Johnson et al., 2004). The juvenile male pilot whale was malnourished at the time of stranding. If this juvenile was unable to acquire food on his own due to his hearing loss, after leaving his
mother he would have become malnourished and eventually this could have led him to strand.

Diseases and antibiotics are also known to cause hearing loss in mammals (Finneran et al., 2005; Bernard et al., 1979; Brummet et al., 1978; Black et al., 1976). After stranding, the pilot whale was given the aminoglycoside antibiotic amikacin for 10 days. This family of antibiotics has been thought to cause hearing loss in marine mammals in other studies (Finneran et al., 2005) but the evidence is inconclusive to date. The dosages in that study were also much higher and administered over a longer period of time than is the case here. It is known that aminoglycosides are ototoxic and kill hair cells causing hearing impairment in humans and rodents (Bernard et al., 1979; Brummet et al., 1978; Black et al., 1976). However, the hearing loss occurs in the high frequency range of the hearing ability of humans and guinea pigs (Cavia porcellus) (Tange et al., 1998; Aran et al., 1995), and was not as broadband as was seen here in the dolphin in Curacao.

The ages of the adult female pilot whales were not estimated so it is unknown if they were old enough to have experienced presbycusis. One of the adult females (MMC-Gm-1011) was treated with florfenicol (20mg/kg via intramuscular injection once daily every 48 hrs for 4 doses). Florfenicol is not an aminoglycoside, but is known to be ototoxic in other mammals (Tange et al., 1998). As with the male pilot whale, the life histories of these four females are unknown, and it is not possible to say whether they have been exposed to sound intense enough to cause permanent hearing loss. Comparing the previous studies on pilot whales (Schlundt et al., 2011 and Pacini et al., 2010) with
the audiograms reported here, it seems likely that the narrower hearing range found is characteristic of pilot whales. The seven pilot whales tested in total are of various ages, representing both juvenile (4 individuals) and adult (3 individuals) age classes, and represent both stranded and captive (Gm1 in Schlundt et al., 2011 was wild-caught) animals. However, it is still possible that all animals tested have experienced high frequency hearing loss due to one or multiple causes, and only future studies on pilot whale hearing will be able to rule out this possibility.

To understand the possible impact hearing impairment can have on foraging success, the dolphin’s foraging ecology must be understood. The Risso’s dolphin and both species of pilot whales typically feed on squid and deep-water fishes (Kruse et al., 1999; Aguilar Soto et al., 2008; Baird et al., 2002). In deeper water while echolocating on larger prey patches, the use of lower frequency echolocation would be advantageous because higher frequencies would be attenuated at depth. A high energy, low frequency click would travel a greater distance while maintaining enough energy to create a strong echo from the prey patch. Low resolution detail about possible prey items would provide adequate information to the foraging dolphin or whale.

Killer whales feed on multiple prey types: fish, cephalopods, and marine mammals (Saulitis et al., 2000; Bigg et al., 1987). The “transient” ecotype feeds on other marine mammals and rarely uses echolocation or social calls to avoid detection by their potential prey (Deecke et al. 2005; Barrett-Lennard et al. 1996; Guinet, 1992; Morton, 1990). Vocalizations of marine mammals fall in the range of good hearing in the reported audiograms for the species (Schusterman et al., 2001; Szymanski et al., 1999; Szymanski
et al., 1998). The killer whale predator therefore wouldn’t need sensitive high frequency hearing to forage on this prey type.

The “resident” ecotype feeds on fish and mainly Chinook salmon (Onchorhynchus tshawytscha; Ford and Ellis, 2006; Ford et al., 1998) or blue fin tuna (Thunnus sp., Guinet et al., 2007). While high frequency echolocation would yield high resolution information for a single prey item, it may not be necessary for the killer whale to detect these particular species. Compared to other species of salmon prey available, the Chinook salmon is typically the largest and has the largest echo when ensonified by an echolocation click (Au et al., 2010), and is found at greater average depths than the other species of salmon (Beacham, 1986). Near the Strait of Gibraltar where killer whales feed on blue fin tuna, it has been shown that prey pursuit occurs in the upper water column and at very fast swimming speeds, where visual tracking can occur easily until the prey is exhausted (Guinet et al., 2007). Foraging strategies that involve visual pursuit, or targeting highly echoic prey items or patches may allow these species to successfully forage with hearing impairment or without sensitive hearing above 120 kHz.

CONCLUSIONS

The hearing of four stranded female short-finned pilot whales and the severe hearing impairment of a stranded juvenile male are reported here. Hearing thresholds indicate the most sensitive hearing at 40 kHz and a cutoff frequency between 80 and 120 kHz for all four females (Fig. 2-3). Previously published studies have reported similar hearing sensitivities in a female short-finned pilot whale (Schlundt et al., 2011) and a
male long-finned pilot whale (Pacini et al., 2010). The cutoff frequencies for animals tested in this genus show an upper limit of hearing below 120 kHz, which is lower than those reported for *Tursiops* sp. and a few other delphinid species (Houser and Finneran, 2006a; Houser and Finneran, 2006b; *Stenella* spp. reported in Chapter Four). However, there are at least two species of delphinids that have reported audiograms with cutoff frequencies below 120 kHz as well (killer whale: Szymanski et al., 1999, Szymanski et al., 1998; Risso’s dolphin: Nachtigall et al., 1995). Foraging strategies in these species adapt to the lack of sensitive high frequency hearing above 120 kHz.

Of the five animals reported herein, three survived rehabilitation and were transported to captive facilities and only two survive to date. The male juvenile that stranded in Curacao died after being transferred to SeaWorld San Diego. The need for hearing tests of stranded animals as part of the release assessment has become a growing priority. Odontocetes that are released without knowing the condition of their hearing ability can present an avoidable increase in the chance of a second stranding. Making audiogram measurements a standard practice during strandings and rehabilitation care increases the knowledge of these inaccessible species. This study adds to the number of published audiograms for this species and illustrates a pattern of less sensitive high frequency hearing in several species of pelagic odontocetes which may be related to differences in foraging ecology.
Table 2-1. Highest levels tested at each carrier frequency during pilot whale AEP testing in Willemstad, Curacao. Highest levels are reported in $\text{dB}_{\text{rms}}$ re 1 µPa for tone stimuli and $\text{dB}_{\text{peak}}$ re 1 µPa for the click stimulus.

<table>
<thead>
<tr>
<th>Frequency</th>
<th>Highest Level Tested</th>
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<tbody>
<tr>
<td>5</td>
<td>130</td>
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<tr>
<td>10</td>
<td>136.7</td>
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<tr>
<td>20</td>
<td>150</td>
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<td>40</td>
<td>143</td>
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<tr>
<td>80</td>
<td>149.9</td>
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<tr>
<td>120</td>
<td>163.4</td>
</tr>
<tr>
<td>Click</td>
<td>181.3</td>
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</tbody>
</table>
Figure 2-1. Comparison of click evoked potential from a dolphin with normal hearing and pilot whale with no response. Bottlenose dolphin (*Tursiops truncatus*; unpublished data) response with normal hearing has three distinct peaks around 8.8 ms. Recorded brain response during testing of a juvenile male pilot whale in Curacao is shown in response to a click stimulus at 180 dB re 1 μPa (indicated with an arrow). The trace does not contain any distinct peaks and although background brain activity is occurring, there is no response to the stimulus being presented.
Figure 2-2. Modulation rate transfer functions for four pilot whales (*Globicephala macrorhynchus*). A 40 kHz tone stimulus was delivered at 162 dB re 1µPa at varying modulation rates from 200-2000 Hz in 100 Hz steps.
Figure 2-3. Audiograms for four short-finned pilot whales tested in the Florida Keys. Results of adult female (Gm1) audiogram from Schlundt et al., 2011 are shown as dashed line. Background noise at the test site is plotted as spectrum level (dB re 1µPa²/Hz). Comparison between the four females tested in this study and the only other full audiogram reported for the species (Gm1 in Schlundt et al., 2011) shows strong similarities at frequencies above 20 kHz.
REFERENCES


CHAPTER THREE: HEARING THRESHOLDS IN STRANDED MOTHER AND CALF RISSO'S DOLPHINS (GRampus GRISEUS) PRE- AND POST-ANTIBIOTIC DOSAGE

ABSTRACT
A mass stranding of two Risso’s dolphins, Grampus griseus, mother/calf pairs occurred on May 5, 2007 at Bonita Beach, Florida. The dolphins were transported to the Dolphin and Whale Hospital at Mote Marine Laboratory in Sarasota, Florida for rehabilitation. Hearing thresholds were determined using auditory evoked potentials measured in response to amplitude modulated (AM) tone pips modulated at 1000 Hz at carrier frequencies of 40, 80 and 120 kHz. In order to measure any potential effect of ototoxic drugs on hearing thresholds, three audiograms were collected over four months from the day of stranding prior to drug treatment to the time of release. Greatest sensitivity for all animals was at 40 kHz and audiograms were similar to previous findings for adult Risso’s dolphins. Changes in thresholds at all frequencies between consecutive hearing tests were 12 dB or less, except for one animal at 120 kHz between May and June, which increased 54 dB. Small changes in hearing sensitivity that occurred are within the range of measurement variability. There was a large threshold shift at 120 kHz for one dolphin that is partially attributable to changes in electrical background noise between tests and
partially attributable to an effect of antibiotic treatment. The results of this study show that the dosages of antibiotics given to these Risso’s dolphins during rehabilitation did not cause dramatic, widespread hearing impairment like that found in other studies with humans and rodents (Guthrie 2008; Roehm et al., 2007; Dai et al., 2006; Aran et al., 1995; Bernard et al., 1980; Bernard et al., 1979).

INTRODUCTION

It has been repeatedly shown that hearing in several terrestrial mammals including humans (Homo sapiens), mice (Mus sp.) and guinea pigs (Cavia porcellus) is affected by ototoxic drugs (Guthrie 2008; Roehm et al., 2007; Dai et al., 2006; Aran et al., 1995; Bernard et al., 1980; Bernard et al., 1979; Brummet et al., 1978; Black et al., 1976). Known ototoxic drugs include antibiotics, chemotherapy agents, some nonsteroidal anti-inflammatory drugs (NSAIDs) and loop diuretics (Brummet et al., 1980). The class of drugs known as aminoglycosides is used to treat bacterial infections and has been shown to maintain activity against antibiotic resilient infections, so they are widely used despite knowledge of their ototoxicity (Black et al., 1976). Amikacin and gentamicin, common aminoglycosides, are taken up by cochlear and vestibular hair cells and can be seen as early as the second injection of treatment in guinea pigs (Aran et al., 1995). When damage to the hair cells occurs, hearing impairment is permanent because hair cells do not regenerate in mammals (Michaels and Hellquist, 2001). The ototoxic effects of aminoglycoside treatment become present first in the loss of high frequency sensitivity and then spread to include low frequency hearing loss, due to the preferential uptake in
the hair cells at the base of the membrane (associated with high frequency hearing) compared to the hair cells at the apex (associated with low frequency hearing) (Black et al., 1976; Aran et al., 1995).

Transfer of ototoxic drugs from mother to infant through milk has also been studied in humans (Motta et al., 2005; Mathew, 2004; Costedoat-Chalumeau et al., 2002; Celiloglu et al., 1994). Celiloglu et al. (1994) showed that gentamicin is transferred through breast milk from mother to infant and is detectable in the infant 1 hour after feeding. It was also noted that clearance of the drug would be slower in the infant and small doses delivered in the milk could accumulate before excretion (Celiloglu et al., 1994). It is hypothesized that the accumulation of ototoxic antibiotics in a nursing infant increases the risk of ototoxic effect if both the mother and calf are being treated for infections.

There is not much known about drug ototoxicity in marine mammals, even though aminoglycoside drugs are used for treatment during rehabilitation. Several studies list antibiotic treatment as a potential cause of high frequency hearing loss in marine mammals tested (Mann et al., 2011; Montie et al., 2011; Houser and Finneran et al., 2006a; Finneran et al., 2005). The studies that have been done to examine pharmacokinetics of drug treatment in marine mammals have shown that bioavailability, elimination half-life, and clearance per bioavailability rates are drastically different from a terrestrial mammal of the same size (KuKanich et al., 2004; Linnehan et al., 1999). Each study used a different method of administration and individuals were different ages and different species, but both used an allometric equation to determine dosages based on
body weight in kilograms. KuKanich et al. (2004) delivered amikacin via intramuscular injection to a killer whale (*Orcinus orca*) and a beluga (*Delphinapterus leucas*) but hearing measurements were never taken to determine if ototoxicity occurred. Linnehan et al. (1999) measured the bioavailability of orally administered enrofloxacin in eight common bottlenose dolphins (*Tursiops truncatus*) and showed that bioavailability parameters indicate slower absorption in marine mammals, as compared to terrestrial mammals of similar size. Maternal transfer of aminoglycosides in marine mammals has never been measured.

Hearing data on the pelagic, cephalopod-eating Risso’s dolphin (*Grampus griseus*) is limited. An infant Risso’s dolphin was shown to hear up to 150 kHz (Nachtigall et al., 2005), whereas Nachtigall et al. (1995) had reported a cutoff frequency between 80-100 kHz for an adult Risso’s dolphin. The stranding of four Risso’s dolphins on May 5, 2007 at Bonita Beach, Florida provided the opportunity to add to the hearing information available for the species, as well as an opportunity to examine the potential for antibiotic ototoxicity in two mothers along with their two nursing calves.

**METHODS**

**A. Subjects**

On May 5, 2007 two mother/calf pairs of Risso’s dolphins stranded at Bonita Beach, Lee County, Florida. That evening they were transported to the Dolphin and Whale Hospital at Mote Marine Lab in Sarasota, Florida for rehabilitation. One adult
female, named Betty (MML0706A), was 282 cm in total length, weighted 230 kg, and had an axillary girth of 143.2 cm. Her calf, a male named BamBam (MML0706AA), was 125 cm and weighed 31.5 kg with an axillary girth of 75 cm at the time of stranding. The other mother/calf pair consisted of two females, Wilma (MML0706B) and her calf Pebbles (MML0706BB). Wilma’s stranding weight was 174.0 kg, at a length of 256 cm and axillary girth of 134 cm. Her calf Pebbles was in critical condition at the time of stranding and her measurements were not taken. Both mothers were lactating at stranding and during rehabilitation, and both calves were estimated to be not more than a week old.

Antibiotics and other drugs were administered under veterinary care and for the purpose of treating illnesses in each of the dolphins on a case by case basis. Therefore variability exists amongst overall treatment dosages and schedules of drug treatment. Pebbles did not receive drug treatment. Both adult females (Betty and Wilma) received five known ototoxic antibiotics: amikacin, gentamicin, vancomycin, clarithromycin, and itraconazole (Table 3-1). BamBam received 21 mg/kg of amikacin once every 48 hours from May 20- June 1, 2007 and from July 14- July 30, 2007.

B. Auditory Evoked Potentials (AEPs)

Auditory evoked potentials were collected at the Dolphin and Whale Hospital at Mote Marine Laboratory while the animals were temporarily restrained at the water surface by volunteers. Testing was performed on May 5, 2007 prior to antibiotics being administered, then again on June 22, 2007 and September 17, 2007 after antibiotic treatment began. The sound stimulus was delivered to the lower, left jaw fat pad via a directly coupled ITC-1042 piezoceramic transducer embedded in a RTV silicone suction
cup (jawphone). Gold cup electrodes (Rochester Electrode, Tampa, Florida) also embedded in silicone suction cups were placed on the dorsal surface in three locations (see Fig. 3-1). The recording electrode was placed 2 cm behind the blowhole. The reference electrode was placed off the midline approximately 10 cm posterior to the recording electrode and the ground electrode was placed in the water.

All signals were generated with a Tucker-Davis Technologies (TDT) RX6 real-time processor at a 260 kHz sample rate. Calibrations were made with the jawphone and a Reson TC4013 hydrophone (-212 dBV re 1 µPa with VP1000 pre-amplifier with 32 dB gain) mounted 10 cm apart underwater at the location of the hearing test after recording audiograms, without the animal present. Modulation rate transfer functions (MRTF) were measured with a carrier frequency of 40 kHz at 162 dB re 1 µPa and modulation rates ranged from 200-2000 Hz with 100 Hz steps. Amplitude modulated (AM) tone pips were modulated at 1000 Hz and carrier frequencies of 40, 80 and 120 kHz were tested. Additional frequencies were not tested to minimize the duration of the hearing tests. Click evoked potentials were recorded in response to a 0.1 ms click with a peak frequency of 62 kHz.

The AEP noise floor was calculated from the 15 ms window prior to stimulus onset. Up to approximately 1000 sweeps were averaged at each attenuation level and thresholds were determined at each carrier frequency where a peak in the FFT of the recorded signal was at least 6.42 dB above the noise floor ($\alpha = 0.01$; Dobie and Wilson, 1996). Peak amplitude at each modulation rate was determined as mentioned above for AEP responses. Spectrum level (dB re $1 \mu Pa^2 Hz^{-1}$) background noise is presented in the
composite audiogram (see Fig. 3-3) and was measured with a Reson TC4013 hydrophone (-212 dBV re 1 µPa with VP1000 pre-amplifier with 32 dB gain).

RESULTS

During testing each animal was restrained by volunteers in the rehabilitation tank at the Dolphin and Whale Hospital at Mote Marine Laboratory. Each Risso’s dolphin was held at the water surface so that the blowhole was above water and the lower jaw was completely submerged.

Modulation rate transfer functions (MRTFs) of the three Risso’s dolphins that were measured in response to a 40 kHz carrier frequency at 131 dB re 1 µPa had a strong peak at 1000 Hz (Fig. 3-2). The modulation rate transfer function reflects the ability of the auditory system to follow individual pulses within the stimulus and response amplitudes are higher for rates at which the stimulus is distinguished as individual pulses (Mooney et al., 2011; Supin and Popov 1995; Vermeister 1979). The MRTF falls off after approximately 1600 Hz (Fig. 3-2), and this reflects the high temporal resolution found in most odontocetes (Mooney et al., 2011). Carrier frequency tone pips were modulated at 1000 Hz during threshold evoked potential recordings because other peaks in the MRTF were at frequencies where the noise floor was higher or did not occur at the same frequency for all three animals.

Pre-dosage audiograms were measured on May 5, 2007 immediately after the Risso’s dolphins were transported to the rehabilitation facility and before any antibiotics
were administered. Wilma’s calf Pebbles died less than 24 hrs after stranding and due to her critical condition at the time of stranding only 80 kHz was tested. Her threshold was within the range of thresholds for the other three dolphins tested in this study (Fig. 3-3). The lowest thresholds for all three animals were found at 40 kHz with a decrease in sensitivity at 80 and 100 kHz (Fig. 3-3). The adult Risso’s dolphin Betty had better sensitivity at 120 kHz compared to the other animals (Fig. 3-3).

As part of their treatment and rehabilitation, all three Risso’s dolphins received several ototoxic antibiotics including amikacin and gentamicin (Table 3-1). Post-dosage audiograms were collected on June 22, 2007. Lowest sensitivity was still at 40 kHz and changes at 40 and 80 kHz were 12 dB or less for all Risso’s dolphins. However, the adult female Betty showed a threshold increase of 54 dB at 120 kHz from May to June. The final audiogram was collected on September 17, 2007. Wilma died on June 29, 2007 so only Betty and BamBam were tested. Betty showed a threshold decrease of 12 dB from June to September (Fig. 3-3). Threshold level changes between June and September testing were less than 12 dB for all frequencies for both Risso’s dolphins (Fig. 3-3). Figure 4 shows the input-output functions for the three Risso’s dolphins tested pre- and post-dosage of antibiotics. The general positive relationship between increasing sound pressure level and increasing output AEP amplitude is seen at all frequencies but it is nonlinear (Fig. 3-4). Analysis of the 15 ms noise window in the raw AEP recordings used to determine thresholds based on signal to noise ratios shows that the AEP background noise (not acoustical noise) during testing at 120 kHz more than doubled from May to June, and then decreased by half in September.
Click evoked potentials were recorded in June and September. Betty responded to clicks down to a presentation level of 88 dB\text{\textsubscript{peak}} re 1 µPa in June and 96 dB\text{\textsubscript{peak}} re 1 µPa in September. BamBam had click thresholds of 100 and 102 dB\text{\textsubscript{peak}} re 1 µPa in June and September, respectively. Click responses were recorded in June for Wilma down to a presentation level of 82 dB\text{\textsubscript{peak}} re 1 µPa.

**DISCUSSION**

At 40 kHz, thresholds for Betty in May and September (59 dB re 1 µPa, Fig. 3-3) and BamBam’s thresholds for all three tests (47-59 dB re 1 µPa, Fig. 3-3) were lower than the threshold published for the infant Risso’s dolphin (63.9 dB re 1 µPa, Nachtigall et al., 2005). The two infants in this study had similar sensitivity at 80 kHz to the infant Risso’s dolphin in Nachtigall et al. (2005), whereas the two adult Risso’s dolphins reported here had higher thresholds at 80 kHz like the adult in Nachtigall et al. (1995). Cutoff thresholds, defined as the frequency where thresholds reach 120 dB re 1 µPa, were around 120 kHz for all Risso’s dolphins tested.

Audiogram threshold shifts may have been affected by inherent variability of the testing method or differences in the testing environment (i.e., background noise, electrode placement) between the three hearing tests. Threshold differences between different testing methods (i.e., AEP versus behavioral, or in-air AEP versus in-water AEP) can be as large as 26 dB and standard deviations are around 13 dB (Houser and Finneran, 2006a; Houser and Finneran, 2006b). Repeated recordings from the same individual during a
single testing period have shown an 8-10 dB variability in thresholds in other studies (Finneran et al., 2008; Finneran and Houser, 2007).

Because the thresholds in this study were determined based on the signal to noise ratio (SNR), an increase in measured background noise at a particular carrier frequency would raise the lowest signal level that could be detected. Analysis of the input-output functions (Fig. 3-4), as well as analysis of the raw AEP recordings, was used to determine if there were changes in electrical background noise from one testing period to another. Also if the location of the recording electrode changed between testing enough that it resulted in a weaker or stronger AEP response being recorded, this would affect the threshold determined during analysis. In order to determine if changes in electrode placement occurred during this study, electrode placement during each test would need to be specifically documented and this was not done due to time constraints. Montie et al. (2011) showed that the AEP wave amplitudes in pygmy killer whales (Feresa attenuata) change based on electrode placement due to the distance from the anatomical source of the wave.

The use of a jawphone directly coupled to the lower jaw removes the possibility of the dolphin moving with respect to the sound source and changing the received level and phase-locking of the sound stimulus with data acquisition during averaging. The ITC-1042 transducer is omnidirectional however, and the silicone does not prevent the stimulus from traveling via alternate water-borne pathways to the dolphin. Reflections at the air-water interface could occur that would change the received level of the stimulus at the ear. Repeated testing would have been required in order to determine if these
differences were the cause of any variability in thresholds, but in a rehabilitation setting
time with each animal is limited to minimize time spent handling the animal.

Comparisons of pre- and post-dosage thresholds did not show an ototoxic effect
on all three Risso’s dolphins. Increases in thresholds after antibiotic treatment did occur
at 40 kHz for BamBam and at all frequencies for Betty. Thresholds for BamBam and
Betty at 40 kHz increased by 6 dB, and a 12 dB increase in threshold occurred at 120 kHz
for BamBam. The result was an overall decrease in Betty’s sensitivity at 120 kHz of 42
dB from May to September. A gradual increase was evident in the female Risso’s
dolphin’s thresholds at 80 kHz from 72 dB re 1 µPa in May to 84 dB re 1 µPa in
September. However, only the increase in Betty’s threshold at 120 kHz is larger than the
typical AEP variability seen in other studies (Finneran et al., 2008; Finneran and Houser,
2007; Houser and Finneran, 2006a; Houser and Finneran, 2006b).

The gradual increase in threshold at 80 kHz matches the relationship found in
guinea pigs given aminoglycosides where the threshold continues to shift with an
increase in duration of treatment (Bernard et al., 1979). However, the input-output
function for Betty at 80 kHz in May was relatively flat across increasing stimulus
presentation levels (Fig. 3-4), and for many of the sound levels at 80 kHz, the AEP level
was similar over all of the tests. Because the threshold is defined as the lowest level
detected, it can be affected by variation in background electrical noise in the AEP
recordings. Analysis of the AEP background noise (electrical noise) during testing at 120
kHz more than doubled from May to June, and then decreased by half in September. An
increase of 10 dB in the AEP background noise in May would have limited the ability to
detect a response at lower stimulus levels. The background electrical noise is a function of the noise present, quality of electrode connection, and the number of AEP sweeps averaged. These can be difficult to control in these testing situations, and it is important to measure the electrical noise before drawing strong conclusions about hearing loss.

The presence of an ototoxic effect on hearing thresholds would be displayed in one of two ways: a temporary threshold shift (TTS) with the potential for hearing sensitivity to be recovered or a permanent threshold shift (PTS) in which the decrease in sensitivity is irreversible. Aminoglycosides can affect hearing by killing hair cells in the cochlea and inner ear or by limiting neurotransmission when it binds with calcium ions (Bernard et al., 1979). Hair cell death would cause PTS because hair cells are not regenerated in mammals (Michaels and Hellquist, 2001). Binding with calcium and affecting the action potential would cause TTS, and not PTS (Bernard et al., 1979), because calcium ions are resupplied via active diffusion. The time needed for recovery from aminoglycoside-induced TTS would be dependent on relative concentrations of calcium ions and aminoglycosides. The transition from TTS to PTS effects could occur over time or with increasing dosages, and both hair cell death and transduction limitation could cumulatively cause the resulting loss in sensitivity.

In this study antibiotics were administered after the June testing as well as after the initial testing in May. If the ototoxic antibiotics caused hair cell death a recovery of hearing sensitivity would not be expected. However, if TTS occurred a recovery of sensitivity might be seen with time. It is possible that the threshold shift from May to June is the result of the cumulative effect of five ototoxic antibiotics (gentamicin,
amikacin, clarithromycin, vancomycin and itraconazole) and the recovery occurred because only amikacin was given to Betty between the June and September tests. The improving health of the Risso’s dolphin could also have had a role in the recovery of sensitivity, if the kidneys filtered the antibiotics out of the blood stream more efficiently between the June and September testing as compared to the period between the May and June tests. As mentioned in KuKanich et al. (2004), healthy marine mammals may have higher clearance rates and therefore the antibiotic concentration in the dolphin’s system will be lower and the risk of ototoxicity will be reduced. Additionally, if the health of the nursing infant improved such that nursing frequency or amount of fluid transfer increased this would result in an increase in lactation loss of antibiotic to the infant.

Both Betty and Wilma were adult females with nursing calves who received antibiotic treatment during the testing process therefore the risk of ototoxic hearing loss should be similar. Although the ages of the two adult Risso’s dolphins are unknown, given that they are sexually mature adults still capable of producing milk it can be estimated that they were between 8-10 and 30 years of age (Baird, 2002). Age was the strongest factor in an increase in predisposition of ototoxicity from aminoglycoside therapy reported in Gatell et al. (1987). If the age difference was large enough then it could explain the threshold shift in Betty’s audiogram and the lack of possible ototoxic effect in Wilma’s audiogram. However, given their length at stranding and the estimates on length at sexual maturity, 265-277 cm in the Northwest Pacific (Amano and Miyazaki, 2004; Kaysua, 1985; Kaysua and Izumizawa, 1981), both females were in their early years of sexual maturity. There are no apparent differences in drug therapies between the
two adult females between the May and June hearing tests. Both Risso’s dolphins received all five ototoxic antibiotics at one point or another but dosages progressively decreased for Wilma (Table 3-1).

**CONCLUSIONS**

Hearing thresholds were the most sensitive at 40 kHz for three Risso’s dolphins and cutoff frequencies were between 80 and 120 kHz (Fig. 3-3). Audiograms for these dolphins are comparable to those reported for an adult Risso’s dolphin (Nachtigall *et al.*, 1995) and other members of the subfamily Globicephalinae (Vilstrup *et al.*, 2011): killer whales (Szymanski *et al.*, 1999; Szymanski *et al.*, 1998), false killer whales (*Pseudorca crassidens*; Yuen *et al.*, 2005; Supin *et al.*, 2003), short-finned pilot whales (*Globicephala macrorhynchus*; Schlundt *et al.*, 2011; Chapter 2), long-finned pilot whales (*G. melas*, Pacini *et al.*, 2010), and pygmy killer whale (Montie *et al.*, 2011).

Although this study does not report large shifts in AEP thresholds, antibiotic treatment in marine mammals has the potential to cause ototoxicity. A shift at a single frequency for a single Risso’s dolphin in this study may be caused by antibiotic drug treatment. Hearing measurements prior to and after antibiotic treatment for two Risso’s dolphins in this study did not show an ototoxic effect. Therefore, dosages for these individuals can be considered safe levels for treatment.

Previous studies that show ototoxicity from aminoglycosides in humans and rodents have shown a wide range of doses that cause hearing impairment (Aran *et al.*, 1995).
1995; Bernard et al., 1980; Bernard et al., 1979), but a comparison of dosages and metabolism of the animals tested between those studies and this one need to be made. Bernard et al. (1980) administered gentamicin or tobramycin at dosages of 5 or 7.5 mg/kg (depending on age) daily to babies for 7-10 days. It has been shown that neonate kittens (Felis sp.) are more susceptible to gentamicin than adult cats, and dosages in neonates are administered at very low levels (Bernard et al., 1979). Aran et al. (1995) treated guinea pigs for 6 days with either 60 or 450 mg/kg of amikacin. Bernard et al. (1979) treated guinea pigs with either tobramycin or netilmicin for either 14 or 28 days at four dosage levels: 25, 50, 75, and 100 mg/kg.

Dosages in both guinea pig studies are much higher than those for the human neonates and the Risso’s dolphins in this study. Dosages of known ototoxic antibiotics given to the Risso’s dolphins ranged from 1.5-21 mg/kg, and were delivered on schedules ranging from once every 48 hrs to three times daily (Table 3-1). Even if dosages administered to the Risso’s dolphins are converted to daily rates, dosages ranged from 4.5-20.8 mg/kg, and are much lower than those in Bernard et al. (1979) and Aran et al. (1995).

Drug treatment of the Risso’s dolphins in this study lasted 125 days, with ototoxic antibiotics being administered a total maximum of 36 days (Betty, three treatment periods of amikacin) and a maximum of 20 continuous days (Wilma, itraconazole). However, signs of ototoxicity were present just a few days after treatment in other studies (Aran et al., 1995; Bernard et al., 1980; Bernard et al., 1979). Dosages were not large enough to cause the same signs of ototoxicity even though drugs were administered over a longer
period of time. These dosages of antibiotics can be used to treat illnesses in the future with Risso’s dolphins. Further studies that examine hearing before and after antibiotic treatment will be able to define a suitable range of drug dosages without risk of hearing impairment to marine mammals.
Table 3-1. All drugs administered for the Risso’s dolphins tested are listed with dosage, method of administration, and dates administered. Antibiotics were administered under veterinarian care for the purpose of treating illnesses, not to directly measure individual differences in drugs administered or dosages. Ototoxic drugs are marked with an asterisk (*) and a key for abbreviations of dosages and methods of administration is given.

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<thead>
<tr>
<th>Animal</th>
<th>Drug</th>
<th>Dosage</th>
<th>Route of administration</th>
<th>Dates started and ended</th>
</tr>
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<tbody>
<tr>
<td>Betty (MML0706A)</td>
<td>*Amikacin sulfate 21mg/kg q 48 hours IM</td>
<td>10 May-20 May; 9 June-22 June; 23 Aug-3 Sept</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>*Vancomycin hydrochloride capsules (vancocin HCL) 1.8 mg/kg TID PO</td>
<td>20 May-28 May</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>*Clarithromycin tablets (Biaxin) 9.75 mg/kg BID PO</td>
<td>16 May-1 June</td>
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<tr>
<td></td>
<td>*Gentamicin sulfate 2.5 mg/kg TID PO</td>
<td>7 May-20 May</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>Baytril 2.5 mg/kg BID 3.9 mg/kg BID IM PO</td>
<td>5 May-10 May 9 June-18 June</td>
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<td></td>
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<td></td>
<td>Vitamin K 0.58 mg/kg BID IM</td>
<td>11 May-14 May</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>*Itraconazole (Sporanox) 4.7 mg/kg BID PO</td>
<td>21 May-8 June</td>
<td></td>
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<td></td>
<td>Vitamin E/Sel 5 mg/kg BID IM</td>
<td>5 May</td>
<td></td>
<td></td>
</tr>
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<td>Augmentin 9.5 mg/kg BID 11 mg/kg BID PO PO</td>
<td>4 June-8 June; 25 July-7 Sept</td>
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<td></td>
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<td>Levaquin 9.8 mg/kg SID PO</td>
<td>15 May-16 May</td>
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<td>Panacur 11 mg/kg SID PO</td>
<td>14 May</td>
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<td></td>
<td>Doxycycline 1.5 mg/kg BID PO</td>
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<td>Metronidazole 7.5 mg/kg TID PO</td>
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<td>BamBam (MML0706AA)</td>
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<td>20 May-1 June; 14 July-30 July</td>
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</tr>
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<td>Ceftazidime 17 mg/kg BID IM</td>
<td>6 May-18 May</td>
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<tr>
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<td>Rocephin 20 mg/kg SID IM</td>
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<td>Cedax 6.5 mg/kg SID PO</td>
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</tr>
<tr>
<td></td>
<td>Baytril 4.9 mg/kg SID IM</td>
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<td>Augmentin 10 mg/kg SID PO</td>
<td>1 June-10 July; 26 July-7 Sept</td>
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<td></td>
<td>Vitamin K 0.58 mg/kg BID IM</td>
<td>14 July-24 July</td>
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<td>Wilma (MML0706B)</td>
<td>*Vancomycin hydrochloride capsules (vancocin HCL)</td>
<td>1.5 mg/kg TID</td>
<td>PO</td>
<td>20 May- 27 May</td>
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<tr>
<td>------------------</td>
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</tr>
<tr>
<td>*Amikacin sulfate</td>
<td>21 mg/kg q 48 hours 20.9mg/kg q 48 hours 2.5 mg/kg TID</td>
<td>IM</td>
<td>IM</td>
<td>9 May- 20 May</td>
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<td>Baytril</td>
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<td>5 mg/kg BID</td>
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<td>9 May- 20 May</td>
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<td>*Itraconazole (Sporanox)</td>
<td>5 mg/kg SID</td>
<td>PO</td>
<td>20 May- 8 June</td>
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<td>Doxycycline</td>
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<td>Diazepam</td>
<td>0.04 mg/kg BID</td>
<td>PO</td>
<td>12 June- 29 June</td>
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</tr>
</tbody>
</table>

*Ototoxic Drugs TID=Three times daily; BID=Twice daily; SID=Once daily; q 48=Once every 48 hours IM=Intramuscular injection PO=Orally
Figure 3-1. Adult female Risso’s dolphin (*Grampus griseus*) named Betty (MML 0706A) is shown during AEP measurements. AEPs were measured using electrodes on the dorsal surface: the recording (A, directly behind the blowhole) and reference (B) electrodes. The sound was delivered via jawphone (C), an ITC 1042 transducer embedded in a RTV silicone suction cup, placed on the left, lower jaw fat pad.
Figure 3-2. Modulation rate transfer functions for three Risso’s dolphins (*Grampus griseus*). A 40 kHz tone stimulus was delivered at 131 dB re 1µPa at varying modulation rates from 200-2000 Hz in 100 Hz steps. Betty (circles) was not tested below 700 Hz due to time restrictions.
Figure 3-3. Audiograms for four Risso’s dolphins (*Grampus griseus*) tested pre- (May, circles) and post-dosage (June, squares and Sept, diamonds) of ototoxic drugs is shown. Pebbles (bottom right panel) died prior to subsequent testing and Wilma (bottom left panel) died prior to testing in September. Background noise is presented as spectrum level (dB re 1 μPa² Hz⁻¹) in each panel.
Figure 3-4. Input-output functions for three Risso’s dolphins (*Grampus griseus*) tested pre- (May, solid lines and circles) and post-dosage (June, long dashes and squares; Sept, short dashes and diamonds). Note differences in y-axis scale for Betty and BamBam at 120 kHz.
REFERENCES


CHAPTER FOUR: COMPARISON OF THREE *STENELLA* SPP. AUDIOGRAMS MEASURED USING AUDITORY EVOKED POTENTIALS AND BEHAVIORAL METHODS

ABSTRACT
The oceanic dolphin genus *Stenella* is underrepresented in cetacean hearing data. In this study the hearing of three *Stenella* spp. dolphins was measured using auditory evoked potential (AEP) methods. A single male juvenile Atlantic spotted dolphin (*Stenella frontalis*) was rehabilitated in Key Largo, Florida after being sighted alone and emaciated. The Atlantic spotted dolphin’s range of best sensitivity was at 40 kHz, with good hearing up to 128 kHz. A female spinner dolphin (*Stenella longirostris*) was housed at Mote Marine Laboratory at the time of testing. Peak sensitivity was at 40 kHz and the spinner dolphin had good hearing up to 120 kHz, the highest frequency tested. A pantropical spotted dolphin (*Stenella attenuata*) was housed at Mote Marine Laboratory and hearing thresholds were determined using AEP and behavioral methods. The pantropical spotted dolphin had the greatest sensitivity at 10 kHz, with a cutoff frequency between 14 and 20 kHz. The source of the dramatic high-frequency hearing loss is not known; it could be congenital hearing loss or due to ototoxic drug treatment during rehabilitation.
INTRODUCTION

There are five species in the oceanic dolphin genus *Stenella*: the pantropical spotted dolphin (*S. attenuata*), the Atlantic spotted dolphin (*S. frontalis*), the striped dolphin (*S. coeruleoalba*), the spinner dolphin (*S. longirostris*), and the Clymene dolphin (*S. clymene*) (Rice, 1998). All the dolphins of this genus are found in pelagic, temperate and tropical waters and have distinguishing body patterns of either spots or stripes (Rice, 1998). Pantropical spotted dolphins are nocturnal feeders that are commonly associated with yellowfin tuna (*Thunnus albacores*) and as a result have been studied in the past due to their high bycatch rates by tuna purse-seine fisheries (Scott and Chivers, 2009). Atlantic spotted dolphins are found in the Atlantic Ocean from the Gulf of Mexico to Brazil, and across to Gabon (Rice, 1998). The striped dolphin is found in the Atlantic, Pacific and Indian Oceans, with an independent population in the Mediterranean Sea (Aguilar, 2000). The spinner dolphin is known for its acrobatics, and has the largest geographical variation in body coloration and morphology (Rice, 1998). The Clymene dolphin, or short-snouted spinner dolphin, is often confused with the spinner dolphin or the common dolphin (*Delphis* sp.) with which they often travel and is only found in the Atlantic Ocean (Fertl et al., 2003).

Methods for measuring hearing in marine mammals have been evolving since the 1950s. Behavioral methodologies have been used in captive settings where a dolphin responds to the presence of sound stimuli (Cook, 2006; Houser and Finneran, 2006a; Finneran and Houser, 2006; Finneran et al., 2005; Houser et al., 2004; Kastelein et al., 2003; Szymanski et al., 1999; Nachtigall et al., 1995; Kellogg, 1953; Kellogg and Kohler, 1952). Although the sound stimulus and method of delivery can vary, most
behavioral studies require an extended training period and the construction of an apparatus to ensure the consistency of stimulus reception from trial to trial. The test animal is trained to station in the apparatus during sound presentation and to respond either vocally or physically, by touching a paddle or stationing elsewhere in the tank. Achieving the baseline behavior alone can take up to several months, depending on the animal and trainer, and the audiogram collection process is lengthy as well. At most aquariums training time is spent on husbandry (medical) behaviors and behaviors for the entertainment of an audience (Luck and Jiang, 2007; Waples and Gales, 2002).

Electrophysiological methods have been adapted to quickly capture the electrical response of the brain to an acoustic stimulus. Measuring the auditory evoked potential (AEP), in captive and stranding situations, has been used to obtain audiograms for several species that wouldn’t be accessible otherwise, including the killer whale (*Orcinus orca*, Szymanski *et al*., 1999), false killer whale (*Pseudorca crassidens*, Yuen *et al*., 2005; Supin *et al*., 2003), beluga (*Delphinapterus leucas*, Popov and Supin, 1987), Blainville’s beaked whale (*Mesoplodon densirostris*, Pacini *et al*., 2011), Gervais’ beaked whale (*Mesoplodon europaeus*, Finneran *et al*., 2009; Cook *et al*., 2006), pygmy killer whale (*Feresa attenuata*, Montie *et al*., 2011), Indo-Pacific humpback dolphin (*Sousa chinensis*, Li *et al*., 2012), striped dolphin (Kastelein *et al*., 2003; André *et al*., 2003), long-finned pilot whale (*Globicephala melas*, Pacini *et al*., 2010), short-finned pilot whale (*Globicephala macrorhynchus*, Schlundt *et al*., 2011; Chapter 2), and Risso’s dolphin (*Grampus griseus*, Nachtigall *et al*., 2005; Nachtigall *et al*., 1995; Chapter 3). However, it has been shown that behavioral thresholds are more sensitive, especially at lower frequencies, and can be up to 20 dB lower (Finneran and Houser, 2006; Houser and
Finneran, 2006a; Yuen et al., 2005; Syzmanski et al., 1999). When the resources exist, it is generally accepted to be advantageous to measure hearing thresholds behaviorally.

Existing studies of hearing in Stenella dolphins are limited. Bullock et al. (1968) reported several audiograms from Japanese Stenella spp. dolphins with a range of good hearing from 40-80 kHz. Kastelein et al. (2003) measured the hearing of a single striped dolphin in the Netherlands with good hearing ranging from 29 to 123 kHz and a cutoff frequency of 160 kHz. Another study found deafness in a stranded striped dolphin with all thresholds above 115 dB re 1 µPa from 16-128 kHz (André et al., 2003). Mann et al. (2011) previously reported normal hearing in a single spinner dolphin and the stranded Atlantic spotted dolphin. The audiogram of the spinner dolphin and the Atlantic spotted dolphin measured using auditory evoked potentials is presented here along with the AEP and behavioral audiogram from a formerly stranded pantropical spotted dolphin.

METHODS

A. Subjects

Cutter (Stenella frontalis)

On February 14, 2009 a juvenile Atlantic spotted dolphin was captured in Key West, Florida after being sighted and monitored during the previous week swimming in circles alone in shallow water and malnourished. The male spotted dolphin (“Cutter”; MMC SF0209) was transported to Tarpon Basin lagoon in Key Largo, Florida for rehabilitation. At stranding Cutter was 127 lbs., 173 cm in length with 87 cm maximal
girth, and estimated to be between 2-5 years of age. Cutter did not receive any ototoxic antibiotics prior to testing. Testing occurred on March 9, 2009.

**Harley (Stenella longirostris)**

The spinner dolphin named Harley (MML 0509) stranded on April 20, 2004 on Mustang Island in Port Aransas, Texas. At the time of stranding, the dolphin was 155 cm in length and estimated to be a year old. On March 28, 2005 the spinner dolphin was transferred from Animal Rehabilitation Keep at the University of Texas Marine Science Institute to Mote Marine Laboratory, where she was housed at the time of testing on December 19, 2006. Harley was approximately three years old at the time of testing. Antibiotic drug treatment information was not available for this animal.

**Moonshine (Stenella attenuata)**

A pantropical spotted dolphin named Moonshine (MML 0326) stranded in Marathon, Florida on April 28, 2003 and was rehabilitated by the Marine Animal Rescue Society (MARS) in Miami, Florida, before being transferred to Mote Marine Laboratory on June 29, 2003. He was estimated to be 2 yrs old at the time of stranding. Moonshine was housed at Mote Marine Laboratory during testing. Auditory evoked potentials were measured on May 10, 2004 and September 7, 2012 (3 and 11 yrs old at the time of testing, respectively). Behavioral thresholds were collected from July to October 2012. During rehabilitation at MARS, the only available drug information shows that Moonshine received itraconazole for 10 days, which was discontinued on June 24, 2009. While a permanent resident at Mote Marine Laboratory, he received five ototoxic antibiotics (amikacin, gentamicin, clarythromycin, vancomycin, and itraconazole) orally.
from June 29, 2003 to June 6, 2005. The health of this dolphin does not currently allow further AEP data to be collected to complete the AEP audiogram.

B. Auditory Evoked Potentials (AEPs)

AEP testing was conducted either in air or in water for the two spotted dolphins. An ITC-1042 piezoceramic transducer embedded in a RTV silicone suction cup (jawphone) was placed on the left, lower jaw fat pad to deliver the sound stimuli. Three gold cup electrodes (Rochester Electrode, Tampa, Florida) also embedded in silicone suction cups were used to measure auditory evoked potentials. The recording electrode was placed 2 cm behind the blowhole, a reference electrode was 10 cm off the midline posterior to the recording electrode, and the ground electrode was placed in the water (in-water testing) or on the dorsal surface of the dolphin posterior to the reference electrode (in-air testing).

All signals were generated by a Tucker-Davis Technologies (TDT) RX6 real-time processor at a 260 kHz sample rate. Amplitude modulated (AM) tone pips were modulated at either 600 or 1000 Hz and carrier frequencies from 5-128 kHz were used to elicit auditory evoked potentials. The AEP stimulus was 15 ms and presented 21 times per second at 100% modulation. Sound level calibrations were performed post-recording, underwater with the jawphone and a Reson TC4041 hydrophone (-212 dBV re 1 µPa with VP1000 pre-amplifier with 32 dB gain) mounted 30 cm underwater and 10 cm apart at the location of the hearing test. Where there was a peak in the FFT of the recorded AEP signal present at least 6.42 dB above the noise floor ($\alpha = 0.01$; Dobie and Wilson, 1996), thresholds were determined as the lowest level at which a response was detected.
The recorded signal was a result of at least 500 averages and the noise floor was determined from the 20 ms window prior to the stimulus starting. Background noise was measured with an HTI hydrophone (HTI 96-min; -164 dBV re 1 μPa) and is presented as spectrum level (dB re 1 μPa$^2$ Hz$^{-1}$).

*Cutter (Stenella frontalis)*

Evoked potentials were measured for the Atlantic spotted dolphin on March 9, 2009. Cutter was temporarily restrained by volunteers at the water surface in a sea pen in Tarpon Basin Lagoon, with the jaw submerged and the blowhole above water. Carrier frequencies from 5-128 kHz were tested with a modulation rate of 1000 Hz. Click evoked potentials were recorded in response to a 0.1 ms click with a peak frequency of 62 kHz.

*Harley (Stenella longirostris)*

Evoked potentials were measured for Harley on December 19, 2006 and May 27, 2007 in the dolphin main pool at Mote Marine Laboratory. The modulation rate transfer function was measured using a 40 kHz carrier frequency and AM modulation rates from 200 Hz to 1200 Hz. AEPs were recorded in response to 10, 20, 40 and 80 kHz tones modulated at 600 Hz. In May 2007, evoked potentials were also recorded in response to 120 kHz modulated at 600 Hz. Click evoked potentials were recorded in response to a 0.1 ms click with a peak frequency of 62 kHz.

*Moonshine (Stenella attenuata)*

Evoked potentials were measured for Moonshine on May 10, 2004 in air while the dolphin was restrained on a foam mat. The modulation rate transfer function (MRTF) was measured using a 60 kHz carrier frequency and AM modulation rates from 200 Hz to
2000 Hz. AEPs were measured in response to 10, 20, 40, and 80 kHz modulated at 600 Hz. The pantropical spotted dolphin was tested again on September 7, 2012 in the dolphin main pool at Mote Marine Laboratory. Evoked potentials were recorded at carrier frequencies of 10, 20 and 40 kHz with a 1000 Hz AM rate.

C. Behavioral methods

The behavioral audiogram for Moonshine was conducted in the dolphin main pool at Mote Marine Laboratory. The animal was trained to station in an underwater apparatus attached to a floating dock, with its head region in a hoop station up to its pectoral fins and its body resting on a pad, during data collection sessions (Fig. 4-1). The stimulus was presented from a free-field transducer mounted 1.04 m from the center of the hoop station, 0.66 m from the ear. A light mounted 0.5 m to the right of the approximate location of the right eye turned on for 2 sec to indicate the beginning of the trial and after a 1 sec delay the presentation window began. The dolphin was trained on a go-no go paradigm where presence of a sound stimulus was indicated by touching a paddle on the apparatus, to the left of the animal. If there was no stimulus present (catch trial), the dolphin was trained to remain in the hoop station for the duration of the presentation window. Correct responses were indicated by using a Lubell UW30 underwater speaker to play a file recording of the trainer’s whistle. This secondary reinforcer signals that food will be delivered for the correct response.

The 1 sec pure tone stimulus windowed by a $10 \text{ ms} \cos^2$ gate was presented using a modified up/down staircase method. The sound stimulus level was increased when an incorrect response was given, and decreased when a correct response was given. Step size
was 6 dB until three correct responses for sound present trials were obtained and then testing continued with 3 dB steps. This method facilitates presentation of lower sound levels sooner so the animal does not get fatigued.

Sessions continued until 8 reversals occurred and the frequency presented was changed after the threshold criterion was met. Frequencies tested were 5, 8, 10, 12, 14, 20, 40, 60, 80, 100 and 120 kHz. A low frequency speaker (UW30) was used for frequencies below 20 kHz, except 5 and 8 kHz, and an ITC-1042 transducer was used for the higher frequencies. The high frequency transducer was used at 5 and 8 kHz because the low frequency speaker had distortion at these frequencies. Thresholds were determined as the mean of two consecutive sessions where the reversal averages were within 6 dB. Calibration of the stimulus transducer occurred before each session with a Reson TC4013 hydrophone (-212 dBV re 1 μPa with VP1000 pre-amplifier with 32 dB gain) placed at the position of the ear, 0.66 m from the transducer. Session recordings were collected with a Reson TC4013 hydrophone (-212 dBV re 1 μPa with VP1000 pre-amplifier with 32 dB gain) mounted 0.66 m from the transducer and 0.5 m to the right of the dolphin’s head region. Background noise is presented as spectrum level (dB re 1 μPa² Hz⁻¹) (see Fig. 4-2)

RESULTS

A. Cutter (S. frontalis) AEP

The Atlantic spotted dolphin had the greatest sensitivity at 40 kHz with a
threshold of 57 dB re 1 µPa, and good hearing between 40 and 80 kHz (Fig. 4-2). A typical mammalian U-shape audiogram was seen, with sensitivity decreasing at 17 dB per octave below 40 kHz, and higher thresholds at higher frequencies. Thresholds at the highest frequencies tested were below 100 dB re 1 µPa so a cutoff frequency was not reached. Click evoked potentials were recorded and a response was detected down to a presentation level of 98 dBpeak.

**B. Harley (S. longirostris) AEP**

Evoked potentials recorded in response to a 40 kHz carrier frequency and modulation rates from 200-2000 Hz produced an MRTF with peaks at 600 and 1000 Hz (Fig. 4-3). The modulation rate transfer function reflects the ability of the auditory system to follow individual pulses within the stimulus and response amplitudes are higher for rates at which the stimulus is distinguished as individual pulses (Mooney *et al.*, 2011; Supin and Popov 1995; Vermeister 1979). The MRTF falls off after approximately 1600 Hz (Fig. 4-3), and this reflects the high temporal resolution found in most odontocetes (Mooney *et al.*, 2011). Hearing sensitivity was the lowest at 40 kHz and increased at both higher and lower frequencies (Fig. 4-2), typical of a mammalian audiogram. Click responses were detected down to a presentation level of 100 dBpeak.

**C. Moonshine (S. attenuata) AEP**

On May 10, 2004 the modulation rate transfer function was tested on both the left and right jaw with a 60 kHz carrier frequency at 110 dB re 1 µPa. No evoked potentials were observed at AM rates from 200-1200 Hz. Sound stimuli at 10 kHz were tested up to a 110 dB re 1 µPa presentation level, and stimuli at 20, 40, and 80 kHz were tested up to
100 dB re 1 µPa. No evoked potentials were detected. During recording, electrode impedance was 5 kOhm and only approximately 100 averages were collected.

On September 7, 2012 evoked potentials were measured at 10, 20 and 40 kHz modulated at 1000 Hz. At 10 kHz, the threshold for the pantropical spotted dolphin was 120 dB re 1 µPa (Fig. 2). No responses were detected at 20 kHz up to 136 dB re 1 µPa or at 40 kHz up to 105 dB re 1 µPa.

D. Moonshine (S. attenuata) Behavioral

Collection of the pantropical spotted dolphin's behavioral audiogram occurred from July to October, 2012 with 2-5 sessions per frequency (Table 4-1). Testing at 8, 12, and 14 kHz was added after initial testing revealed increased sensitivity between 5 and 20 kHz. Thresholds for the pantropical spotted dolphin showed a cutoff frequency between 14 and 20 kHz, with the greatest sensitivity (52 dB re 1 µPa) at 10 kHz (Fig. 4-2). Spectrum level background noise was measured during a catch trial where no sound stimulus was presented. Peaks in background noise are from electrical noise, and at 10 kHz the electrical noise floor of the hydrophone limited background noise measurement (Fig. 4-2). Equivalent spectral noise for the Reson TC4013 is approximately 50 dB re 1 µPa Hz^{-1/2} at 10 kHz (Teledyne-Reson Technical Note 3). False alarm responses were low (Table 4-1) with an overall false alarm rate of 0.6%.

At 5 kHz, there was only an 8 dB difference in sensitivities between the two spotted dolphins. The behavioral threshold at 10 kHz for the pantropical spotted dolphin was 68 dB more sensitive than the AEP threshold. Elevated behavioral thresholds above 12 kHz for the pantropical spotted dolphin were 46-80 dB higher than those measured for
the Atlantic spotted dolphin, but only 29-46 dB higher than those measured for the spinner dolphin. The pantropical spotted dolphin’s threshold at 12 kHz was comparable to the Atlantic spotted dolphin’s threshold at 10 kHz. The greatest differences between thresholds for the Atlantic spotted dolphin and the spinner dolphin were at 40 kHz (24 dB) and 80 kHz (34 dB).

DISCUSSION

Differences between thresholds for the Atlantic spotted dolphin and the spinner dolphin range from 12-34 dB. The range of best hearing for the Atlantic spotted dolphin and the spinner dolphin is comparable to other Stenella spp. dolphins (Kastelein et al., 2003; André et al., 2003; Bullock et al., 1986). Both the Atlantic spotted dolphin and the spinner dolphin had good high frequency hearing similar to the striped dolphin in Kastelein et al. (2003).

When compared to other pelagic dolphins not in the genus Stenella, the frequency of highest sensitivity is higher for the common dolphin (Delphinus delphis, Popov and Klishin, 1998) compared to all of the dolphins in this study. At 40 and 80 kHz, thresholds for the Atlantic spotted dolphin are comparable to the common dolphin (Popov and Klishin, 1998), whereas the thresholds for the spinner dolphin are comparable to common bottlenose dolphins (Tursiops truncatus, Houser et al., 2008; Finneran and Schlundt, 2007; Houser and Finneran, 2006a; Houser and Finneran, 2006b; Au et al., 2002; Ljungblad et al., 1982). The good high frequency hearing of the Atlantic spotted dolphin
and the spinner dolphin is similar to the common bottlenose dolphin (Houser et al., 2008; Houser and Finneran, 2006a; Houser and Finneran, 2006b).

Thresholds for the pantropical spotted dolphin at 10 kHz measured by AEP and behaviorally differ by more than 60 dB. Although other studies have shown that behavioral methods are more sensitive, differences between behavioral methods and AEP are typically only up to 20 dB (Finneran and Houser, 2006; Houser and Finneran, 2006a; Yuen et al., 2005; Syzmanski et al., 1999). A response to the sound stimulus was not detected at 40 or 80 kHz, but the highest stimulus level tested was below behavioral thresholds. Also, electrode impedances are usually 1 kOhm or below during AEP testing, but during testing on May 10, 2004 impedances were 5 kOhm. Differences in electrode impedances could be due to different electrode sizes being used or the location of electrode placement. Higher impedances indicate a weaker electrical connection between the electrode and the surface of the skin of the dolphin, and would result in a recorded response with lower amplitude. A smaller response will then be harder to detect above background noise.

Thresholds for the pantropical spotted dolphin are similar to the striped dolphin at 8 and 20 kHz, and the low sensitivity recorded behaviorally at 10 kHz is similar to the sensitivity at slightly higher frequencies (between 16 and 32 kHz) for the striped dolphin (Kastelein et al., 2003). Compared to the striped dolphin with hearing loss (André et al., 2003), the pantropical spotted dolphin has comparable hearing loss from 20-60 kHz. However, the hearing loss in the pantropical spotted dolphin is greater than that of the striped dolphin above 60 kHz (André et al., 2003).
Hearing loss is evident in the audiogram of the pantropical spotted dolphin above 12 kHz. If thresholds for the Atlantic spotted dolphin are used as a baseline, the pantropical spotted dolphin’s thresholds represent a 46-80 dB loss of sensitivity. The American Speech-Language-Hearing Association lists the following categories of hearing impairment for humans: 41 to 55 dB hearing loss as moderate, 56 to 70 dB hearing loss as moderately severe, 71 to 90 dB hearing loss as severe, and over 90 dB hearing loss as profound (Clark, 1981; ASLHA, 2013). Using these definitions and the thresholds for the Atlantic spotted dolphin, the pantropical spotted dolphin’s hearing loss progresses from moderate at 20 kHz (46 dB hearing loss) to severe at 80 kHz (80 dB hearing loss), and moderately severe at 120 kHz (62 dB hearing loss). If the thresholds of the striped dolphin (Kastelein et al., 2003) were used, the pantropical spotted dolphin’s hearing at 40 kHz would be considered severe and the difference of 104 dB at 120 kHz would represent profound hearing loss in humans. However, a comparison of thresholds between the spinner dolphin and the pantropical spotted dolphin would only represent moderate hearing loss at 40 and 80 kHz for the pantropical spotted dolphin.

Several studies have shown that severe hearing impairment in humans leads to a reduction in quality of life (Dalton et al., 2003; Davis and Hind, 1999; Hétu et al., 1993). Because odontocetes live in an acoustic environment, it is assumed hearing impairment would limit their ability to successfully forage, navigate and maybe even communicate. However, it is unknown what level of hearing impairment would equate to the inability to capture prey or navigate. A false killer whale showed a decrease in discrimination ability after developing high frequency hearing loss, and a reduction in peak frequency, center frequency and source level of clicks used during the task (Kloeper et al., 2010a;
Kloeppe et al., 2010b). Hearing loss in this animal could not be quantitatively determined because the first audiogram was collected during a masking task, but with masking present the false killer whale could hear at 100 kHz (Thomas et al., 1990). In 2004, the cutoff frequency for the same false killer whale without masking was around 45 kHz (Yuen et al., 2005). The task to discriminate cylinder wall thickness does not directly translate to surviving in the wild but Kloeppe et al. (2010b) compared it to discriminating prey types and range. This comparison would follow that high frequency hearing loss would result in limited successful foraging.

Hearing loss can be caused by noise exposure (chronic or transient), presbycusis (age-related hearing loss), congenital hearing loss, and ototoxic drug treatment. The life history and acoustic exposure of the pantropical spotted dolphin is unknown prior to his stranding. During rehabilitation and captivity, the pantropical spotted dolphin was not exposed to noise at high received levels or for prolonged periods known to cause permanent hearing loss (Southall et al., 2007). Given his young age, presbycusis is not likely the cause of his hearing impairment. Congenital hearing loss is a possible factor, but there is no way to determine this without testing hearing at birth or examining possible genetic links. There are several cases of suspected congenital hearing loss in marine mammals including two rough-toothed dolphins (Steno bredanensis) and a short-finned pilot whale (Mann et al., 2011), and a striped dolphin (André et al., 2003).

Aminoglycosidic antibiotics (amikacin, gentamicin, vancomycin, and clarythromycin), as well as itraconazole, are known to cause ototoxicity and hearing impairment in humans and rodents (Bernard et al., 1979; Brummet et al., 1978; Black et
al., 1976), and are suggested to cause hearing loss in marine mammals (Finneran et al., 2005). Preferential uptake of ototoxic antibiotics at the base of the basilar membrane (associated with high frequency hearing) as compared to the apex (associated with low frequency hearing) causes high frequency hearing loss that can spread to lower frequencies (Tange, 1998; Aran et al., 1995). Antibiotic treatment records for Moonshine indicate administration of several ototoxic antibiotics over an extended time period. Although it was originally thought that oral treatment with these drugs did not cause ototoxicity due to decreased absorption, it has been shown that over time hearing impairment can occur (Brummet, 1980; Ballantyne, 1973; Gibson, 1967; Halpern and Heller, 1961). However, without performing a hearing assessment at the time of stranding, it is impossible to determine if the administration of ototoxic antibiotics caused the hearing loss evident in Moonshine’s audiogram. Initial AEP testing was conducted almost a year after Moonshine was moved to Mote Marine Laboratory. The absence of an evoked potential response at elevated levels at 20, 40 and 80 kHz may indicate that hearing loss occurred prior to the time of testing. However, a response was not detected and electrode impedances were higher than normal.

CONCLUSIONS

Three species from the genus Stenella are reported here. Audiograms for the Atlantic spotted dolphin and the spinner dolphin have the lowest thresholds at 40 kHz, and good high frequency hearing up to the highest frequencies tested (120 or 128 kHz). Cutoff frequencies for these two dolphins were not reached. Sensitive high
frequency hearing is also found in common bottlenose dolphins (Houser et al., 2008; Houser and Finneran, 2006a; Houser and Finneran, 2006b), common dolphins (Popov and Klishin, 1998), a striped dolphin (Kastelein et al., 2003), belugas (Finneran et al., 2005; Klishin et al., 2000), and an Indo-Pacific humpback dolphin (Li et al., 2012). Popov and Klishin (1998) tested a rescued male common dolphin with frequencies up to 152 kHz and found a cutoff frequency around 150 kHz. Kastelein et al. (2003) reported the hearing of a female striped dolphin with a cutoff frequency above 160 kHz, the highest frequency tested. One of the two belugas tested in Finneran et al. (2005) and a beluga tested in Klishin et al. (2000) had a cutoff frequency above the highest frequency tested which was 130 kHz. Finally, the audiogram of a captive male Indo-Pacific humpback dolphin had a cutoff frequency around 150 kHz (Li et al., 2012). Members of the *Stenella*, *Delphinus*, and *Tursiops* genera belong to the subfamily Delphininae, along with the Indo-Pacific humpback dolphin and the rough-toothed dolphin (Vilstrup et al., 2011). It appears that members of this subfamily all possess good high frequency hearing with cutoff frequencies above 120 kHz.

Members of this subfamily feed cooperatively on fish schools utilizing group tactics to feed on an assemblage of prey (Benoit-Bird and Au, 2009; Baird et al., 2008; Gazda et al., 2005; Pitman and Stinchcomb, 2002; Van Parijs and Corkeron, 2001; Karczmarski et al., 2000; Fertl and Wursig, 1995; Bel’kovich et al., 1978) or utilize the migration of the deep scattering layer to feed at night (Pusineri et al., 2007; Benoit-Bird, 2004; Norris and Dohl, 1980). While some dolphins in the Delphininae subfamily are almost exclusively found in deeper waters (i.e., rough-toothed dolphins, Baird et al., 2008) and some only in shallow coastal waters (i.e., Indo-Pacific humpback dolphin,
Karczmarski et al., 2000), the *Stenella* sp., *Delphinus* sp., and *Tursiops* sp. dolphins can be found inshore or nearshore, as well as in pelagic waters (Benoit-Bird and Au, 2009; Pusineri et al., 2007; Gazda et al., 2005; Rice, 1998; Fertl and Wursig, 1995; Norris and Dohl, 1980).

This diversity of foraging strategies would require a range of echolocation frequencies in order to obtain both large scale information on prey patches, as well as high resolution information on individual prey items. Foraging in shallow waters can occur using adaptive foraging strategies that likely minimize use of echolocation like those found in the southeastern United States or Mexico (Silbert and Fertl, 1995; Rigley, 1983; Hoese, 1971), or those found in Australia or the Bahamas (Mann and Sargeant, 2003; Smolker et al., 1997; Rossbach and Herzing, 1997). Van Parijs and Corkeron (2001) recorded broad band clicks during foraging activities in Indo-Pacific humpback dolphins, but the recordings were limited to below 22 kHz because of the sampling equipment. Although rough-toothed dolphins inhabit deep waters, they are thought to forage on near-surface fishes (Baird et al., 2008; Pitman and Stinchcomb, 2002), and therefore may be able to gain high resolution information with high frequency clicks, without losing the energy of the outgoing click through transmission loss.

Severe hearing impairment that is present across almost the entire range of hearing would reduce the ability of a dolphin to perceive echoes within that frequency range. Thresholds measured with AEP and behavioral methods for the male pantropical spotted dolphin indicate severe high frequency impairment from 12-120 kHz (Fig. 4-2). Although this is an audiogram of a previously unreported species, hearing abilities of this species would not be expected to be drastically different from the other members of the
genus given that their sensory morphology (Nummela et al., 2007; Sassu and Cozzi, 2007; Zook et al., 1988; Oelschlager, 1986; Ketten et al., 1983) and autecology (Benoit-Bird, 2004; Schotten et al., 2004; Psarakos et al., 2003; Baird et al., 2001; Herzing, 1997) are very similar. Also, while Stenella sp. dolphins are social and vocal animals (Schotten et al., 2004; Au and Herzing, 2003; Lammers et al., 2003; Psarakos et al., 2003) this animal is relatively quiet and only produces low frequency sounds (H. Harley and A. Cardwell, personal communication). Two cases of a deaf/mute dolphin have been reported in the literature (Andre et al., 2003; Ridgway and Carder, 1997). Andre et al. (2003) reported a deaf/mute female striped dolphin and Ridgway and Carder (1997) reported a deaf/mute female common bottlenose dolphin.

The pantropical spotted dolphin was the most sensitive at 10 kHz, with a threshold of 52 dB re 1 µPa that was just above the noise floor (Fig. 4-2). Determining a threshold above the background noise is often difficult at lower sound stimulus levels because the background noise creates a masking effect. Masking occurs when the background noise interferes with the detection or perception of the stimulus signal (Reichmuth, 2012; Trickey et al., 2010; Branstetter and Finneran, 2008). The presence of masking in the auditory system of dolphins is determined by the bank of overlapping, continuous band-pass filters, and the location of the frequency band of noise relative to that of the signal (Fletcher, 1940).

Studies have shown that masking occurs when the noise is present in the 1/3 octave band around a tonal signal (Southall et al., 2003; Southall et al., 2000; Au and Moore, 1990). The minimal detectable difference between noise and a pure tone around
10 kHz in a bottlenose dolphin is 25-30 dB (Johnson, 1968; Johnson, 1986). The difference between the behavioral threshold of the pantropical spotted dolphin and the noise spectrum level was 29 dB at 8 kHz and 49 dB at 12 kHz. This indicates that the determination of the threshold at 8 kHz may be noise-limited. The difference between the behavioral threshold and the noise spectrum level at 10 kHz was 5 dB. Masking release, or a decrease in hearing threshold, occurs when the noise bandwidth increases beyond a critical bandwidth (Trickey et al., 2010). It is likely that Moonshine was experiencing masking release during the behavioral audiogram testing of the 10 kHz tone stimulus because his hearing indicates that he wouldn’t hear most of the noise in the band where masking would occur. Masking release would explain the low behavioral threshold at this frequency, as well as a portion of the large difference between the behavioral and AEP thresholds.
Table 4-1. Summary of sessions for behavioral audiogram of Moonshine (*Stenella attenuata*). Number of sessions and total number of trials do not include the training period or sessions with equipment malfunctions. For frequencies with more than two sessions, thresholds were determined from the last two sessions. Total number of false alarm responses is reported for each frequency, rather than per session, because the false alarm rate was low.

<table>
<thead>
<tr>
<th>Frequency</th>
<th>No. of sessions</th>
<th>Total no. of trials</th>
<th>False alarm rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>3</td>
<td>187</td>
<td>0.000</td>
</tr>
<tr>
<td>8</td>
<td>4</td>
<td>331</td>
<td>0.003</td>
</tr>
<tr>
<td>10</td>
<td>4</td>
<td>240</td>
<td>0.017</td>
</tr>
<tr>
<td>12</td>
<td>3</td>
<td>186</td>
<td>0.005</td>
</tr>
<tr>
<td>14</td>
<td>3</td>
<td>158</td>
<td>0.019</td>
</tr>
<tr>
<td>20</td>
<td>2</td>
<td>107</td>
<td>0.000</td>
</tr>
<tr>
<td>40</td>
<td>2</td>
<td>111</td>
<td>0.009</td>
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<tr>
<td>60</td>
<td>4</td>
<td>246</td>
<td>0.000</td>
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<td>3</td>
<td>171</td>
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<tr>
<td>100</td>
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</tr>
<tr>
<td>120</td>
<td>5</td>
<td>252</td>
<td>0.008</td>
</tr>
</tbody>
</table>
Figure 4-1. Underwater stationing apparatus for behavioral audiogram mounted below a floating dock. During the sound presentation window (1 s), Moonshine’s head region was placed in the hoop station up to his pectoral fins. The recording hydrophone was 0.5 m off to the right and 0.66 m from the location of the ear. During calibration, a Reson TC4013 hydrophone was placed in line with the transducer and hoop station, 0.66 m from the transducer.
Figure 4-2. Audiograms for three *Stenella* spp. dolphins. Thresholds for Moonshine (*S. attenuata*) determined by behavioral audiogram (dashed line) are an average of 2 consecutive trials, with an average of eight reversals within 6 dB re 1 µPa. AEP thresholds for Moonshine (circles) are shown, and open circles indicate the highest level tested without a response detected. Thresholds for Cutter (solid line; *S. frontalis*) and Harley (dotted line; *S. longirostris*) were determined by AEP.
Figure 4-3. Modulation rate transfer function (MRTF) for a spinner dolphin (*Stenella longirostris*). Evoked potentials were recorded in response to a 40 kHz carrier frequency and modulation rates from 200 Hz to 1200 Hz were tested in 100 Hz steps.
REFERENCES


CHAPTER FIVE: METHODS FOR DETERMINING FREE-SWIMMING POSITONING AND ECHOLOCATION BEAM PATTERNS IN CAPTIVE BOTTLENOSE DOLPHINS

ABSTRACT

Echolocation beam patterns have been studied in captive settings with a stationed dolphin echolocating on a target at a set distance. However, in the wild dolphins are free-swimming, allowing them to orient their head, and echolocating on a number of targets at various distances, possibly requiring them to change the parameters of their outgoing echolocation to obtain information from their environment. Commercial hydrophones are expensive and available data acquisition systems are limited by either the number of channels or the single channel sample rate. Development of an autonomous, field-deployable hydrophone array was necessary to record free-swimming dolphin echolocation at a high sample rate with multiple channels. Echolocation beams were recorded using a 25-element autonomous, self-contained hydrophone array during free-

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2 Portions of these results have been previously published (Greenhow et al., 2013) and are utilized with the permission of the publisher. Portions appeared in Greenhow D, Harley H, Fellner W, Cardwell A, and Mann D. “Methods for determining free-swimming positioning and echolocation beam patterns.” Proceed. of Meetings on Acoustics 19, 010004-8 (2013) and may be found at http://dx.doi.org/10.1121/1.4799416. Copyright (2013) Acoustical Society of America. This article may be downloaded for personal use only. Any other use requires prior permission of the author and the Acoustical Society of America.
swimming echoic match tasks in order to examine echolocation beam patterns in the context of target discrimination, as a proxy for foraging in the wild. The hydrophones were sampled at 400 kHz and analyzed in real-time using an embedded computer system with a dsPIC microcontroller. Through several serial buffers, the maximum and minimum values and their time indices were recorded for each 0.32 ms window, to reduce the amount of data that needed to be stored. Data was continuously written from the final buffer to an on-board Secure Digital flash memory card. Two GoPro HERO2 cameras mounted to the 1.4 m by 1.4 m PVC array recorded the position of the dolphin during approach and investigation of the sample object presented in front of the array. To synchronize the acoustic recorders and video cameras, a synchronization unit was mounted on the array and simultaneously emitted light pulses (LED) and acoustic clicks. Post-recording analysis of timeseries data using custom-designed MATLAB graphical user interfaces shows preliminary evidence of three strategies utilized during object inspection: head movement with concurrent beam movement, dynamic beamwidth changes, and beam steering without head movement. Analysis of video recordings with MaxTRAQ software is still needed to determine quantitative position of the dolphin during echolocation, as well as calculations of beamwidth and the degree of beam steering.

**INTRODUCTION**

Echolocation clicks are high frequency (50-120 kHz), broadband, and short impulse sounds produced at high source levels (up to 230 dB re 1 µPa SPL) used for
foraging and navigation (Au, 1993). Click production is a result of pressurized air being pushed past the phonic lips and it is believed that pulse production occurs either when the lips make contact or when the change in vibrational acceleration is greatest (Cranford et al., 1996). The pulse emitted reflects off internal services of the melon and air sacs causing it be focused into a beam before leaving the head. The density structure of the melon consists of an oily fat layer on the exterior with a denser core, causing sound traveling through the less dense layer to travel faster, sound in the denser core to travel slower and a focused beam to be formed (Cranford et al., 1996). The high frequency clicks are used to obtain high-resolution information about their surroundings by actively ensonifying an area to listen for echoes.

Echolocation abilities have been widely studied in both captive and wild odontocete cetaceans to determine the acoustic parameters of the echolocation beam (Wahlberg et al., 2011; Atem et al., 2009; Johnson et al., 2006; Madsen et al., 2005; Au and Herzing, 2003; Au et al., 1999; Houser et al., 1999; Akamatsu et al., 1998; Au et al., 1995; Au, 1993). Studies on the bottlenose dolphin (Tursiops sp.) have shown that echolocation click source levels are higher in the wild than in captivity (Wahlberg et al., 2011; Au, 1993). Au et al. (1995) reported four click types used by a false killer whale (Pseudorca crassidens) during a target detection task where source levels increased with an increase in the center frequency of clicks. This relationship was also found in wild Atlantic spotted dolphins (Stenella frontalis, Au and Herzing, 2003). Houser et al. (1999) reported variability in click spectra and bandwidth associated with individual variability as well as differences due to the type of task being performed by two common bottlenose dolphins (T. truncatus).
Earlier studies in captivity showed that bottlenose dolphins emitted clicks at a rate equal to the two-way travel time between the dolphin and a target plus some internal processing time (Au, 1993). However, Au et al. (1999) showed that clicks emitted by a harbor porpoise (*Phocoena phocoena*) during a target detection task had intervals greater than the two-way travel time and greater than that of the bottlenose dolphin. Also, echolocation recordings in the wild have shown longer click intervals for the baiji (*Lipotes vexillifer*), Indo-Pacific finless porpoise (*Neophocaena phocaenoides*), and common bottlenose dolphin than those measured in captivity, most likely due to larger target distances (Akamatsu et al., 1998). However, Simard et al. (2010) found that click rates decrease with increasing mean water depth, indicating that target range is depth dependent. Atem et al. (2009) also showed an increase in source level with increasing distance between the dolphin and the ensonified target in captive harbor porpoises and free-ranging white-beaked dolphins (*Lagenorhynchus albirostris*).

Echolocation acoustic parameters as well as click intervals can change depending on the task and the environment the dolphin is in. Although previous studies have indicated a relationship with simple target distance (Atem et al., 2009; Houser et al., 1999; Akamatsu et al., 1998), a relationship between acoustic parameters of the echolocation click could also be related to prey type and habitat. Soldevilla et al. (2010a) showed patterns of increased echolocation activity and echolocation rate at night by Risso’s dolphins (*Grampus griseus*) in the Southern California Bight and determined that these patterns are consistent with nocturnal foraging on diel patterns of migrating squid. Also, it has been suggested that patterns of spatial variability in click type (two types based on frequency spectrum) usage could correlate with different prey types (Soldevilla
et al., 2010b). Both Johnson et al. (2006) and Madsen et al. (2005) observed an increase in click rate during the terminal phase of prey capture, reflected by emission of a terminal buzz. Similar patterns of echolocation click rates have been recorded in captive harbor porpoises (Verfub et al., 2009). It is unknown if patterns of click frequency or bandwidth are correlated with foraging activity, prey type or prey distribution in delphinids.

A. Echolocation beam patterns

All studies conducted to calculate beam patterns, or the distribution of acoustic parameters spatially within the echolocation beam, have used a limited number of hydrophones and those studies conducted in captivity have required that the dolphin be stationed on either a biteplate or a chin cup (Ibsen et al., 2012; Kloeppeper et al., 2012; Koblitz et al., 2012; Au et al., 1999; Au et al., 1986). This allows for an easier calculation of beam angles because the head is stationary, and also minimizes the numbers of hydrophones needed to measure beam angle. Au et al. (1986) used seven hydrophones and calculated that the outgoing beam of a common bottlenose dolphin was directed five degrees in the upward vertical. Au et al. (1999) measured the echolocation beam of a harbor porpoise and found an approximately symmetrical 3-dB beamwidth of 16 degrees. Another study of the same species found a dorso-ventrally compressed beam (13 and 11 degrees in the horizontal and vertical, respectively) with a directivity index of 24 (Koblitz et al., 2012).

Ibsen et al. (2012) used a 16 hydrophone star array and reported circular frequency contours in the beams of a stationed false killer whale and a free-swimming
common bottlenose dolphin. However, only visual confirmation was used to measure positioning of the free-swimming dolphin, and therefore orientation of the beam with respect to the head region cannot be determined (Ibsen et al., 2012). Species variability in beam symmetry, and horizontal and vertical location of the beam may aid a foraging odontocete in obtaining information about their environment and potential prey items. However, it is generally accepted that some differences are a direct result of anatomical differences in the melon (Wahlberg, 2011; Cranford et al., 2008; Soldevilla et al., 2005; Zimmer et al., 2005; Cranford et al., 1996).

In the wild, foraging dolphins actively scan their environment both physically and acoustically, by orienting their head or steering their echolocation beam. Moore et al. (2008) have shown that echolocation beams can be directed off the axis of head orientation of a bite plate stationed common bottlenose dolphin (18 degrees in the horizontal, 12 degrees in the upward vertical, and 4 degrees in the downward vertical) and the beam width can vary during an echolocation task. Measuring free-swimming echolocation beam patterns becomes an even more challenging task with this knowledge because the main axis and energy of the beam can be present in a larger possible area than if the beam was always directly in front of the animal and only 10 degrees, as previously reported (Au, 1993). A wild echolocating dolphin could potentially use multiple tactics in order to investigate their environment. It is unknown to what extent dolphins steer their echolocation beam while swimming, or if beam focusing, or narrowing of the beamwidth, is used to facilitate acquisition of information.
Steering and widening of the echolocation beam would allow the dolphin to perceive more of their environment while in motion, while steering and beam focusing would allow the dolphin to obtain higher resolution detail on a target of interest in a shorter amount of time. Furthermore, the relationship between these two parameters (beam movement and beamwidth) during free-swimming target discrimination is unknown. With so many dynamic parameters of the echolocation beam, foraging dolphins could be utilizing any number of strategies based on their foraging ecology and environment.

B. Hardware limitations

Commercial hydrophones have been used to study dolphin echolocation; however, they are often very expensive making the cost of a large array costly (e.g. a 25-hydrophone array with Reson hydrophones would cost approximately $50,000). Furthermore, data acquisition systems that allow for simultaneous sampling on a large number of channels at a high sample rate (>300 kHz/channel) are very large and expensive. Most systems that allow for sampling on more than 8 channels (i.e., IOtech Personal DAQ 3000 or National Instruments USB-6251, 16 channels) are limited to a 1MHz aggregate sampling rate and those with a higher bandwidth usually limit sampling to 2 or 3 channels. The option to synchronize multiple units is available with some software configurations, but it becomes cumbersome to synchronize the number of units needed to achieve the required single channel sample rate on 25 channels. Developing a cost-effective hydrophone that records at a high sample rate and allows for on-board real-
time analysis to minimize data storage provides a way to capture echolocation beam patterns emitted by free-swimming dolphins.

The goals of this project were to design an autonomous hydrophone array that was field deployable and could be expanded to any number of hydrophones. This array system was used to record echolocation during free-swimming approach and inspection of sample objects during a three choice alternative match-to-sample task. Free-swimming inspection of a target in a field of acoustic clutter (the array) and discrimination of that target (in a match-to-sample paradigm) is used to examine echolocation beam patterns in the context of inspecting and discriminating prey items in the wild while foraging.

HYDROPHONE ARRAY SYSTEM DESIGN AND IMPLEMENTATION

A. Acoustic and visual recording hardware

Custom-made autonomous hydrophones composed of a spherical piezoceramic and signal conditioning board were developed to minimize cost to construct a 25-element array capable of recording and analyzing acoustic data in real-time (Fig. 5-1). On-board electronics sampled at 400 kHz at 16-bit resolution and stored three values for every 128 points recorded. Through several serial buffers, the maximum and minimum values and their time indices (combined into a single 16 bit value) were recorded for each 0.32 ms window, to reduce the amount of data that needed to be stored. Data was continuously written from the final buffer with 2.5 µs resolution to a 32 GB Secure Digital flash memory card. Data reduction from the raw signal sampled at 400 kHz to the recorded
signal (equivalent sample rate of 3,125 Hz) did not retain enough detail for frequency analysis, but maintained click peak amplitude (Fig. 5-2). Each recording hydrophone can run for up to 12 hours on one 1.5 Ah lithium-ion rechargeable battery. Memory cards were removed and data were downloaded directly to a laptop. An ITC-1042 transducer operated by an Arduino Uno processor board was used to produce a distinct pulse train, was amplified by a Hafler P1000 amplifier and emitted from the center of the array, to synchronize the 25 hydrophone elements.

Cameras were used to determine spatial orientation of the head region during echolocation recordings. The two GoPro HD HERO 2 cameras recorded at 30 frames per second with a resolution of 1280 X 960. The 170-degree wide angle field of view of the camera was limited when placed in the dive housing, but still allowed for adequate view of the array as well as the dolphin’s approach and departure. The dive housing used on the camera during deployment has a flat lens to increase underwater sharpness and limit vignetting, or a decrease in brightness at the image’s edges. The camera’s field of view was calibrated using a frame with 8 fixed points in space recorded on both cameras. The calibration frame was constructed with PVC, aluminum rods and white sphere markers. Analysis using MaxTRAQ and MaxTRAQ3D software calculated the precise location of both cameras in 3D space in order to determine spatial orientation of the dolphin during session recordings.

B. Hardware calibration

On-board electronics were calibrated using a Tucker-Davis Technologies (TDT) RP2.1 real-time processor to produce a tone sweep from 10-120 kHz at 0.05 V.
Hydrophones were calibrated using an underwater apparatus and an ITC-1042 transducer placed 1 m from the hydrophone. Tone bursts from 10-120 kHz were recorded on both the custom hydrophone and a Reson TC 4013 connected to a preamplifier in order to calculate calibration values. Average sensitivity was -190 dB re 1 µPa and the response curve was flat across the range tested (Fig. 5-3).

Video recordings of the calibration frame from each camera were loaded into MaxTRAQ, and the eight fixed-point markers found in at least 10 frames, to determine the position of the two cameras in 3-D space. Using natural markers (rostrum tip, eyes, etc.) on the dolphin, three points on the head region of the animal were selected in the video recording corresponding to the time of emission of the click train of interest. The cameras were synchronized to the acoustic recordings using the LED flash that was coincident with the synchronizing click train emission.

C. Array testing

Four male common bottlenose dolphins housed at Epcot’s The Seas at Walt Disney World® Resort were used for this study. The study was conducted in one of two off-display holding pools (7.0 m x 7.6 m x 2.1 m).

In order to examine echolocation beam parameters in a pseudo-representative situation of prey discrimination during foraging in the wild, a three-choice alternative match to sample paradigm was used. After stationing and voluntary eye cup placement, the dolphin performing the task was trained to swim directly across the testing pool to inspect the sample object. The sample object was suspended from a carbon fiber pole.
with monofilament line. It was lowered to the center of the array vertically and just to the right of the center hydrophone horizontally so the path of the incoming beam would not be obstructed by the sample object. After several seconds of examination by the dolphin, the sample object was removed. This cued the animal to swim to the side wall of the pool to choose among the three match objects.

Match objects were hung from a single PVC apparatus using monofilament line with equal spacing between objects. The experimental setup is depicted in figure 5-4. The dolphin was trained to station in front of the match object and to emit a whistle. After 3 s, an assistant who was naïve to the sample’s identity reported to the trainer his choice. Object sets were chosen for their small size (smaller than the distance between hydrophones as to not impede the incoming beam being recorded) and their high echoic properties, as well as their echoic contrast with each other.

D. Data analysis and synchronization

Post-recording acoustical analysis was conducted using a custom-designed embedded MATLAB GUIs to determine the location of the distinct synchronizing pulse train closest to the click train of interest on each of the 25 hydrophones and using an offset calculation to time-align all recordings. Using the center hydrophone as the reference, beam patterns were then determined by calculating the received level in decibels on the center hydrophone and those surrounding it (Fig. 5-5). The main GUI was used to visualize the beam pattern and the corresponding video frame (Fig. 5-6).

Beam patterns were calculated for clicks recorded on the center hydrophone and results were variable. During inspection, the dolphin clicked both at a distance and when
directly in front of the array, although to a lesser extent at an intermediate distance. Received levels ranged from 148 dB to 190 dB. Recorded beams had beamwidths that extended beyond the area covered by the array.

EXAMPLE RESULTS

Echolocation beams were recorded during sample object inspection of four object sets over 20 sessions. Post-recording analysis of timeseries data using custom-designed MATLAB graphical user interfaces showed preliminary evidence of three strategies utilized during object inspection: beam steering, dynamic beamwidth changes with little to no head movement, and head movement with concurrent beam changes. Figure 5-6 shows a dolphin positioned in front of the hydrophone located in the center row, in the second column from the left. The received level at the hydrophone directly in front of the dolphin was 10 dB lower than the highest received level for this click (Fig. 5-6). The highest received level, and presumably the center of the echolocation beam, was recorded on two hydrophones in the far right column, in the center row and the row below it. Although preliminary visual analysis of the video frame from both cameras indicated the body was turned or titled to the right side of the array, head positioning did not line up with the location of the center of the beam. This could be evidence of beam steering during inspection. The object was not located in this area of the array, so it is likely the dolphin was inspecting the array itself.

All three strategies of physical and acoustical scanning used during object inspection were found after analysis of four sequential clicks within a click train emitted
by a dolphin during a match-to-sample trial while wearing eyecups. The first click of the
click train had a broad beam pattern, with the center of the beam recorded on the
hydrophone one row below the center hydrophone (Fig. 5-7 A). The video frame on the
right side of Fig. 5-7 A shows the dolphin positioned horizontally between the far left
column and the second from the left column of hydrophones, at the depth of the center
row of hydrophones, slightly tilted to the right side of the array (Fig. 5-7 A). Since the
depth of the hydrophone with the highest received level was greater than the position of
the dolphin, the echolocation beam seemed to be steered vertically downward.

The second click in the click train appeared to have two focal points, with equal
received levels on two hydrophones not located next to each other (Fig. 5-7 B). Further
analysis of the positioning of the dolphin and the sample object will be able to determine
if this is an artifact caused by the object blocking part of the beam, causing less energy to
be received on the hydrophones between to the two foci. The beam of the second click
seemed to be as wide as the beam of the first click and movement of the head towards the
bottom of the array seemed to be the only change from the first click to the second click
(Fig. 5-7).

Echolocation beam focusing was utilized between the second and third clicks in
the click train (Fig. 5-7). The center of the beam was in the same location as it was for the
first click, but received levels within 3 dB of the highest received level are recorded on
only two other hydrophones: one directly above and one directly to the right of the
hydrophone with the highest received level (Fig. 5-7 C). The third click in the click train
occurred in less than 33 ms after the second click and the frame rate of the camera was
unable to capture changes in positioning of the dolphin. Assuming minimal changes in the position of the head, this illustrates beam focusing from click to click with little to no head movement between clicks.

Finally, the fourth click in the click train illustrates head movement and dynamic beam changes between clicks (Fig. 5-7). The video frame shown on the right of Fig. 5-7 D shows the dolphin in relatively the same body position but with his rostrum pointed lower on the array. Changes in beam patterns illustrated movement of the center of the beam and widening of the beam from the third click to the fourth click in the click train (Fig. 5-7). The highest received level was recorded on the hydrophone in the second row from the bottom, in the column second from the right of the array and received levels within 3-dB spanned across the three hydrophones in the vertical center of the array (Fig. 5-7 D).

Further analysis of video recordings to determine exact positioning of the dolphin’s head region during echolocation emission is needed to calculate the dolphin’s distance from the array, angle to the array and the degree of beam steering and changes in beamwidth in between clicks.

DISCUSSION

A. Echolocation beam patterns

Preliminary evidence suggested that a free-swimming, echolocating dolphin uses physical movement, beam steering, and beam focusing during object inspection.
Although free-swimming beam patterns have not been extensively studied in delphinids, their echolocating counterpart, the bat, has been. Several studies report the dynamic beam patterns emitted by an echolocating bat in flight during prey pursuit and capture (Jakobsen and Surlykke, 2010; Chiu et al., 2010; Surlykke et al., 2009; Ghose and Moss, 2003; Hartley and Suthers, 1987). Jakobsen and Surlykke (2010) recorded the beam patterns of two species of insectivorous bats (order Chiroptera; this study: *Myotis daubentonii* and *Eptesicus serotinus*) and found that bats broaden the beam during prey pursuit. Also, bats tend to narrow their echolocation beam to “lock on” to a prey target before capture (Ghose and Moss, 2003; Hartley and Suthers, 1987). In the wild, echolocation beams are highly directional compared to beams measured in captivity, especially when bats are foraging over a water surface or vegetation (Surlykke et al., 2009). Surlykke et al. (2009) further found that higher source levels were a result of focused energy caused by higher frequencies being emitted in highly directional beams.

Furthermore, even gleaning bats that listen to their prey and capture prey from the ground use echolocation to discriminate between prey items (Schmidt et al., 2000). Bat echolocation studies have even looked into echolocation beam steering during competitive foraging (Chiu et al., 2010). Chiu et al. (2010) found that when two bats are foraging on the same prey item the trailing bat will focus its beam on the leading bat and the leading bat will focus its beam on the prey item. When the paths of the two foraging bats are oriented so that the bats are flying at each other, echolocation beams are steered away from the other bat so signal jamming does not occur (Chiu et al., 2010).
Moore et al. (2008) first reported that echolocating dolphins steer the maximum response axis of the beam to detect targets off-axis of the head orientation. Methods for steering of the beam were suggested to be through manipulation of air sacs and the melon volume or geometry, and phase shifting between the two pairs of phonic lips used for click production (Moore et al., 2008). Anatomical analysis has revealed an array of air sacs located in the melon and nasal passages that could be either inflated or deflated to change the reflective properties (Cranford et al., 1996), and therefore change the path of the internally reflected echolocation click. Solntseva and Rodionov (2007) suggested that contraction of the nasiolabial muscle would increase pressure within the melon. This contraction would also change the shape and volume of the melon, and possibly change the gradient of lipids through which a click would have to travel before leaving the melon (Moore et al., 2008). Phase shifting between the two pairs of phonic lips to steer the axis of the echolocation beam would hinge on the two pairs of phonic lips operating separately and producing two clicks that combine to form the resulting click recorded external to the head of a delphinid. This topic is still highly debated but seems well supported with recent findings as well as earlier indirect evidence (Cranford et al., 2011; Starkhammar et al., 2011).

Further investigation into how dolphins use their echolocation in the wild will shed light on potential mechanics of beam steering. Examining the variability of beam patterns during free-swimming object detection, inspection and discrimination is also important as a proxy for how dolphins may use their echolocation in the wild in different foraging or navigational situations.
B. Array design and implementation

The 25-element array system developed for this study has provided many advantages as well as many challenges. This system allows for simultaneous recording on 25 channels at 400 kHz with on-board data storage. Each unit is self-contained and autonomous meaning that a large array of recorders can be used without any power or data cables. The component cost for each unit is about $300. Furthermore, the array is easily reconfigurable and any number of hydrophones can be added to the array. The only cables in this system come from the LED and acoustic pinger (ITC-1042) located in the center of the array, which will be redesigned in the future to be battery operated. The synchronizing acoustic and light pulses are needed to overcome the largest challenge presented by autonomous recorders, synchronizing all of the recorders and the cameras. Even though the setup is minimal, it takes two people 30 minutes to turn on all of the recorders and position them in the array. Synchronization challenges included producing a loud enough acoustic signal from the center of the array to be recorded on all hydrophones, and automating alignment across 25 acoustic time series in the presence of other signals.

During MTS sessions, the array system provided a large field of acoustic clutter behind the sample object during inspection by the dolphin. The air-filled housings containing the electronics for each unit are highly acoustically reflective, and the large reflection of acoustic energy could mask the echo from the target or confuse the dolphin about which object needed to be matched. Target strength recordings from individual housings were made with and without a neoprene covering over the housing to determine
if the neoprene would mask the echo from the housing. Recordings showed that the echo from the housing was not masked by the neoprene, but also that the reflection was minimal compared to that from wall or water surfaces surrounding the array. The system could be potted in acoustically transparent material to minimize reflections. Also, extra training and testing had to be implemented to ensure the presence of the array did not make the task too difficult for the dolphin.

Future work will continue with analysis of video recordings to determine the positioning of the echolocating dolphin with respect to the array. Sessions will be examined to compare echolocation during training and learning of novel object sets during MTS tasks, as well as potential echolocation changes that occur with increased experience with the array. The hydrophone array system can also be used to record wild dolphin echolocation beam patterns to shed light on the differences between captive and wild dolphin echolocation use during target inspection.
Figure 5-1. The 25-element hydrophone array mounted on the PVC frame with two GoPro cameras (left camera indicated with an arrow, right camera is out of frame), LED (A) and acoustic pinger (B). Complete array measures 1.4 m by 1.4 m, with hydrophones approximately 21.6 cm apart. Cameras are mounted on each side, and the LED and acoustic pinger (ITC-1042) are mounted in the center of the array.
Figure 5-2. Graphical representation of data reduction by on-board processing. Through several serial buffers, the maximum and minimum values and their time indices (combined into a single 16 bit value) were recorded for each 0.32 ms window, to reduce the amount of data that needed to be stored. Data reduction from the raw signal (left panel) sampled at 400 kHz to the recorded signal (right panel; equivalent sample rate of 3,125 Hz) did not retain enough detail for frequency analysis, but maintained click amplitude (indicated with an arrow).
Figure 5-3. Hydrophone response curve from 20-120 kHz. Mean response curve of 25 hydrophones calibrated at 1 m. Mean hydrophone sensitivity was -190 dB re 1V/µPa +/- 1.16 dB. Bars represent standard deviation.
Figure 5-4. Behavioral experimental setup: diagram showing the overhead view of the pool. The dolphin stations (1), then is sent across the pool wearing eye cups to examine the sample object which is suspended in front of the hydrophone array (2). After the sample object is pulled out of the water, the animal swims to the side wall to choose from the three alternative objects (3). When the dolphin has chosen the match object he stations in front of it and emits a whistle. After being bridged, he returns to the original station (1). Paths (indicated by lines and arrows) approaching either the sample object or the match objects are unconstrained and vary between dolphins.
Figure 5-5. Graphical user interface (GUI) visualizing the beam pattern of an individual click and the corresponding location in the timeseries. The plot on the left shows relative amplitude level in decibels of the click on the corresponding hydrophone both numerically and chromatically. The plot on the right shows all 25 time-aligned timeseries for several click trains and an ‘x’ on the x-axis indicates the location of the click.
Figure 5-6. Graphical user interface (GUI) visualizing the beam pattern of an individual click and the corresponding video frame. The plot on the left shows relative amplitude level in decibels of the click on the corresponding hydrophone both numerically and chromatically. For orientation purposes, both panels are oriented as if the viewer is standing in the plane of the array, facing the echolocating dolphin.
Figure 5-7. Echolocation beam patterns for a series of clicks within a click train and the corresponding video frames. Panels A-D show the beam pattern and corresponding video frame for four sequential clicks from a click train recorded during object inspection. Beam patterns are visualized numerically and chromatically with higher numbers and higher intensity colors indicating greater received levels. The time lapse from the first click (A) to the fourth click (D) is 85 ms.
REFERENCES


CHAPTER SIX: HEARING AND ECHOLOCATION IN ODONTOCETE CETACEANS: DISCUSSION

SUMMARY

In this dissertation, hearing measurements were made using auditory evoked potential (AEP) and behavioral methods on 12 odontocete cetaceans in five species. Opportunistic stranding events were used to collect audiograms for under-represented species of odontocetes, as well as measure the effect of antibiotic treatment. Variability within and among species was demonstrated, as well as the presence of hearing impairment in at least two dolphins. Free-swimming echolocation beam patterns were measured during object inspection and analysis showed preliminary evidence of three strategies utilized during object inspection: beam steering, dynamic beamwidth changes with little to no head movement, and head movement with concurrent beam changes.

Stranding events are a prime opportunity to gather information on less accessible odontocetes. With the use of rapid, non-invasive techniques, hearing data are easily collected in this setting. Audiograms are a vital tool for assessment of release and should become routine during stranding and rehabilitation. Antibiotic drug treatment can cause hearing loss, but only monitored treatment paired with auditory evaluation can determine the case-by-case risks. The results of this dissertation also show the need for hearing data
beyond a representative species or individual in order to accurately make inter- and intraspecies comparisons.

A short-finned pilot whale (*Globicephala macrorhynchus*) audiogram was only previously reported in a single study (Schlundt *et al*., 2011). The audiograms of four short-finned pilot whales, as well as the extent of deafness in another short-finned pilot whale, are discussed in Chapter Two. The range of best sensitivity of these delphinids was similar to the common bottlenose dolphin (*Tursiops truncatus*) (Fig. 6-1; Popov *et al*., 2007; Houser and Finneran, 2006; Finneran & Houser, 2007; Finneran & Schlundt, 2007) and other pilot whales (Fig. 6-1; Schlundt *et al*., 2011; *G. melas*, Pacini *et al*., 2010). The cutoff frequency around 80-100 kHz of pilot whales (Chapter Two; Schlundt *et al*., 2011; Pacini *et al*., 2010) is much lower than the cutoff frequency for common bottlenose dolphins (Fig. 6-1; Ljungblad *et al*., 1982; Au *et al*., 2002; Finneran *et al*., 2002a; Finneran *et al*., 2002b; Finneran *et al*., 2002c; Houser *et al*., 2004; Finneran & Houser, 2006; Houser & Finneran, 2006; Finneran *et al*., 2008; Finneran & Houser, 2007; Finneran & Schlundt, 2007; Houser *et al*., 2008). The pilot whale limit of high frequency hearing is comparable to the cutoff frequency of the killer whale (*Orcinus orca*, Szymanski *et al*., 1998; Szymanski *et al*., 1999) and the pygmy killer whale (*Feresa attenuata*, Montie *et al*., 2011) (Fig. 6-1).

The Risso’s dolphins (*Grampus griseus*) measured in Chapter Three had a cutoff frequency between 80 and 120 kHz. Two studies by Nachtigall and colleagues (1995 and 2005) illustrated differences between infant and adult hearing thresholds. Differences in hearing thresholds above 40 kHz of the mothers and calves reported here were consistent
with previous studies where younger Risso’s dolphins are more sensitive at higher frequencies (Nachtigall et al., 1995; Nachtigall et al., 2005). After antibiotic drug treatment, a single animal had an elevated threshold at 120 kHz. Although a portion of the threshold shift can be attributed to increases in background electrical noise, an effect of antibiotic ototoxicity is present. All other changes in hearing thresholds were within the range of typical variability from AEP hearing measurements. Dosages of antibiotics during drug treatment detailed in Chapter Three should be considered safe dosages of antibiotics for Risso’s dolphins and were much lower than dosages in studies that show ototoxicity in guinea pigs (Cavia porcellus, Aran et al., 1995; Bernard et al., 1979).

Variability among the hearing abilities within the genus Stenella was examined in Chapter Four. Severe hearing loss was evident in the pantropical spotted dolphin (S. attenuata) with a cutoff frequency between 14-20 kHz. Causes of this broad spectrum hearing impairment are unknown; however congenital hearing loss and antibiotic treatment during rehabilitation are possible causes. It is possible the pantropical spotted dolphin was experiencing masking release at 10 kHz because his hearing thresholds at nearby frequencies indicate that he would not be able to hear the background noise in the band where masking would occur. The presence of masking release along with differences inherent to the two testing methods could explain the 60 dB difference between his behavioral and AEP threshold at 10 kHz. The Atlantic spotted dolphin (S. frontalis) and the spinner dolphin (S. longirostris) had the best sensitivity at 40 kHz and a cutoff frequency was not reached. Although it is undetermined where their cutoff occurs, it is much higher than the limit of high frequency hearing for the short-finned pilot whale, and the Risso’s dolphins.
Modulation rate transfer function (MRTF) data was presented for the short-finned pilot whales, Risso’s dolphins, and the spinner dolphin tested (Fig. 2-2, Fig. 3-2, and Fig. 4-3). The MRTF reflects the ability of the auditory system to follow individual pulses within the stimulus and response amplitudes are higher for rates at which the stimulus is distinguished as individual pulses (Mooney et al., 2011; Supin and Popov 1995; Vermeister, 1979). Although there is large variability in the amplitude of the responses, and the location of peaks, all MRTFs fall off after approximately 1600 Hz (Fig. 2-2, Fig. 3-2, and Fig. 4-3). This reflects the high temporal resolution found in most odontocetes (Mooney et al., 2011).

In Chapter Five, free-swimming echolocation beams were collected with a hydrophone array system developed to be autonomous, expanded to numerous channels, and field-deployable. Acoustic data was collected with a custom-made hydrophone programmed to record at a 400 kHz sample rate, while utilizing serial buffers to reduce the amount of data stored to a manageable level. Synchronization and acoustic data analysis were conducted with the design of custom-made embedded MATLAB graphical user interfaces (GUIs). Preliminary evidence suggested that a free-swimming, echolocating dolphin uses physical movement, beam steering, and beam focusing during object inspection. Further analysis of the video recordings will determine positioning of the head region and allow for calculation of movement, the degree of beam steering, and beamwidths.

Examining echolocation beam patterns in the context of a dolphin’s hearing abilities provides insight on how these mammals use the two sensory components of their
biosonar system to survive in the wild. The array system developed as part of this
dissertation can be used to study how dolphins use echolocation in the wild, the impacts
of anthropogenic sound on echolocation production, and the potential consequences of
high frequency hearing impairment. Intra- and inter-species variability in echolocation
beam patterns can be compared to variability in hearing abilities to determine if
odontocete cetaceans are manipulating their echolocation beams to match the range of
good hearing.

**AUDITORY ANATOMY AND HEARING**

Ranges of best sensitivity among odontocete species have been correlated with
differences in peak spectra of the sound the species produces and classified as two types
(Ketten, 2000; Ketten, 1992; Ketten and Wartzok, 1990). Type I odontocetes have lower
frequency hearing than the harbor porpoises (*Phocoena phocoena*) and riverine dolphins
(families Platanistidae, Inidae, Lipotidae and Pontoporiidae) of Type II. However, all the
dolphins tested in these studies are categorized as Type I, but there is a pattern of lower
cutoff frequencies for the pilot whales and Risso’s dolphins compared to the spotted and
common bottlenose dolphins. In general, adaption for high frequency hearing occurs by
increasing stiffness of tissues in the middle and inner ear or conversely better low
frequency hearing is achieved by increasing mass (Ketten, 2000).

There are several areas in which this can and does occur within the cetacean ear.
Odontocetes are not as large as mysticetes, but are massive by most standards. The
middle ear bones of a marine mammal are proportionately high in mass, with differences
in mass of the middle ear bones among odontocetes not directly proportional to the overall mass of the animal (Hemila et al., 2010). Also, the basilar membrane of the inner ear plays an important role in frequency-dependent hearing. The base of the basilar membrane is associated with high frequency hearing and the apex is associated with low frequency hearing, and the gradient from narrow, thick and stiff to wide, thin and pliable facilitates frequency tuning (Ketten, 2000; Ketten, 1992). Also within the inner ear, the variation in length of the outer lamina is species-specific (Ketten, 2000).

Anatomical examination of these membranes is very challenging considering potential post-mortem and fixation degradation. However, it is possible that the overall and localized rigidity within the basilar membrane could explain differences in the limit of high frequency hearing abilities within odontocete cetaceans. Increasing the support by the outer lamina, or the documented length variation, among species could also contribute to an increase in higher frequency hearing. Common bottlenose dolphins, some species of spotted dolphins, and Risso’s dolphins may have basilar membranes with more rigid bases, more extensive laminar support, and therefore higher cutoff frequencies. Other studies have shown morphometric variations in the area of the mandible where sound is received (Barroso et al., 2012), and this could explain differences in dolphin cutoff frequencies.

FORAGING ECOLOGY

Comparisons of high frequency hearing sensitivities among the species tested in this dissertation show two distinct groups. Short-finned pilot whales and Risso’s dolphins
have a cutoff frequency below 120 kHz, whereas members of the genus *Stenella* have cutoff frequencies above 120 kHz (Fig. 6-1). Expanding the comparison to include previously published audiograms for other species, killer whales (e.g., Szymanski *et al*., 1999), pygmy killer whales (Montie *et al*., 2011), false killer whales (*Pseudorca crassidens*; e.g., Yuen *et al*., 2005), and long-finned pilot whales (Pacini *et al*., 2010) also have cutoff frequencies below 120 kHz (Fig. 6-1). Common bottlenose dolphins (e.g., Houser *et al*., 2008), white-beaked dolphins (*Lagenorhynchus albirostris*, Nachtingall *et al*., 2008), Indo-Pacific humpback dolphins (*Sousa chinensis*, Li *et al*., 2012), rough-toothed dolphins (*Steno bredanensis*; e.g., Mann *et al*., 2011), and common dolphins (*Delphinus delphis*; e.g., Popov and Klishin, 1998) have cutoff frequencies above 120 kHz like the *Stenella* spp. dolphins (Fig. 6-1).

Figure 6-2 shows the same data from figure 6-1, with the two groupings emphasized and a phylogenetic tree from Vilstrup *et al*. (2011) that reports two subfamilies of delphinids. The subfamily Globicephalinae includes the species reported with cutoff frequencies below 120 kHz and the subfamily Delphininae (along with the sister taxon of the white-beaked dolphin) includes the species reported with cutoff frequencies above 120 kHz (Fig. 6-2; Vilstrup *et al*., 2011). Other species not included in the Vilstrup *et al*. (2011) analysis have audiograms in the published literature. The upper cutoff frequency for the beluga (*Delphinapterus leucas*; e.g., Finneran *et al*., 2005), the Pacific white-sided dolphin (*Lagenorhynchus obliquidens*, Tremel *et al*., 1998), the baiji (*Lipotes vexillifer*, Wang *et al*., 1992), Blainville’s beaked whale (*Mesoplodon densirostris*, Pacini *et al*., 2011), Narrow-ridged finless porpoise (*Neophocaena...
The foraging ecology of delphinids discussed in Chapters 1, 2, and 4 demonstrates the diversity of prey types, foraging strategies, and foraging habitats. In deeper water while echolocating on larger prey patches, the use of lower frequency echolocation would be advantageous because higher frequencies would be attenuated at depth. A high energy,
low frequency click would travel a greater distance while maintaining enough energy to create a strong echo from the prey patch. Low resolution detail about possible prey items would provide adequate information to the foraging dolphin or whale. Foraging strategies that involve visual pursuit, or targeting highly echoic prey items or patches may allow some species to successfully forage without sensitive hearing above 120 kHz. Adaptations in foraging strategies or echolocation use could potentially allow a dolphin to overcome hearing impairment and still forage successfully.

A wild echolocating dolphin could potentially use multiple tactics in order to investigate their environment. It is unknown to what extent dolphins steer their echolocation beam while swimming, or if beam focusing is used to facilitate acquisition of information. Steering and widening of the echolocation beam would allow the dolphin to perceive more of their environment while in motion, while steering and beam focusing would allow the dolphin to obtain higher resolution detail on a target of interest in a shorter amount of time.

Soldevilla et al. (2010a) showed patterns of increased echolocation activity and echolocation rate at night by Risso’s dolphins in the Southern California Bight and determined that these patterns are consistent with nocturnal foraging on diel patterns of migrating squid. Also, it has been suggested that patterns of spatial variability in click type (two types based on frequency spectrum) usage could correlate with different prey types (Soldevilla et al., 2010b). Both Johnson et al. (2006) and Madsen et al. (2005) observed an increase in click rate during the terminal phase of prey capture, reflected by emission of a terminal buzz. Similar patterns of echolocation click rates have been
recorded in captive harbor porpoises (Verfub et al., 2009). It is unknown if patterns of click frequency or bandwidth are correlated with foraging activity, prey type or prey distribution in delphinids. Species variability in beam symmetry, and horizontal and vertical location of the beam may aid a foraging odontocete in obtaining information about their environment and potential prey items. With so many dynamic parameters of the echolocation beam, foraging dolphins could be utilizing any number of strategies based on their foraging ecology and environment.

Among all areas of cetacean biology more research is necessary to gain a clearer picture of how these marine mammals have adapted to function in their fully aquatic, acoustic environment. Hearing in the odontocete cetaceans has coevolved with echolocation, enabling these animals to gain information through sound. How they fully utilize that echolocation in different environments during navigation and foraging and to what extent their hearing abilities contribute to or limit their foraging ecology are questions still to be answered.
Figure 6-1. High frequency hearing in delphinids. Audiograms are shown for all species available in published literature and those tested in this study. Note that the frequency scale is focused on hearing above 40 kHz. An asterisk (*) indicates the one audiogram that represents behavioral thresholds. All other thresholds reported are from AEP studies.
Figure 6-2. Phylogenetic grouping of high frequency sensitivity at the subfamily level. The graph on the left is a reproduction of Fig. 6-1 with species bimodally color coded based on subfamily groupings. The phylogenetic tree on the right is modified from Figure 1 in Vilstrup et al. (2011). Color boxes indicate subfamily members represented in the same color in the graph on the left.
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ABOUT THE AUTHOR

Danielle Rene Greenhow graduated with honors from Eckerd College in May 2006 with a Bachelor of Science degree in Marine Science and a minor in Mathematics. She completed a senior thesis entitled “SEAS In-situ Spectrophotometric Phosphate Profiling in the Gulf of Mexico.” Although her passion is marine biology, her work before, during, and after her senior thesis with Dr. Lori Adornato and Dr. Bob Byrne allowed her to develop an invaluable appreciation for marine chemistry. While working on her doctoral dissertation project at the University of South Florida, Danielle has traveled to Curacao, Japan, Cancun, Montreal, Oregon and California collecting data or attending professional conferences. She received two conference travel grants, funding from the College of Marine Science assistantships, a Florida Fish Scholarship and a George Lorton fellowship in Marine Science that facilitated accomplishing her goals while being a graduate student. Completing her doctoral work on cetacean hearing and echolocation, Danielle looks forward to continuing to follow her childhood dreams of research with marine mammals, as well as motivating and encouraging younger generations to develop a passion and follow it.