TEMPORAL EFFECTS OF SOUND ON THE ODONTOCETE AUDITORY SYSTEM: AN ELECTROPHYSIOLOGICAL ANALYSIS

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“You do not really understand something unless you can explain it to your grandmother.”

- Albert Einstein

“Maybe it’s something cool I don’t even know about.”

- Will Farrell as Frank Ricard in Old School
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~ TAM
ABSTRACT

Sound is likely the primary sensory modality for odontocete cetaceans (toothed whales and dolphins). As is typical of mammals, acoustic temporal patterns are important for odontocetes to detect, identify, and classify sound sources. Overexposure to certain sounds may also affect odontocete hearing capabilities. This dissertation explores the auditory capabilities of odontocetes in respect to broadband acoustic signals of various temporal characteristics. The specific goals of the work included: (i) investigate the temporal resolution of several species of odontocetes, (ii) examine the noise exposure intensities and durations required to induce temporary hearing threshold shifts, and (iii) calculate a model of predicting threshold shift occurrence and evaluate its fit to the equal energy hypothesis of noise exposure.

The results demonstrate that dolphin auditory temporal resolution is quite rapid relative to terrestrial mammals and likely capable of following echolocation clicks and echoes at very close ranges, when inter-click-intervals are very short. The odontocete temporal processing capability is similar in bandwidth across multiple species thus indicates that this trait is conserved and has likely been selected for by the need to process underwater sound and for echolocation. How odontocetes receive sound seems to differ between species and is likely dependent on the morphology of the head and acoustic fats used to gather incoming sound. Predicting effects of noise exposure and hearing temporary threshold shifts onset did not follow an equal energy model. Rather short, intense signals required significantly more energy to induce shifts than longer duration noise. Sonar signals consequently require very intense sound levels to induce threshold shifts.
It can be concluded that sounds of varying temporal characteristics are processed in a variety of ways. While there are similarities between odontocete species auditory systems, there are also divergent characteristics. The model of predicting threshold shift presented here was compared to both terrestrial mammal and marine mammal data to ensure robustness of data. Such auditory generalizations should be similarly carefully weighed when applied, especially regarding the many species for which little is known.
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<tr>
<td>ABR</td>
<td>Auditory brainstem response</td>
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<tr>
<td>AEP</td>
<td>Auditory evoked potential</td>
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<td>CT</td>
<td>Computerized tomography</td>
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<td>DAQ</td>
<td>Data acquisition</td>
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<td>EEG</td>
<td>Electroencephalogram</td>
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<tr>
<td>EFR</td>
<td>Envelope following response</td>
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<tr>
<td>FFT</td>
<td>Fast Fourier transform</td>
</tr>
<tr>
<td>ICI</td>
<td>Inter click interval</td>
</tr>
<tr>
<td>MRI</td>
<td>Magnetic resonance imaging</td>
</tr>
<tr>
<td>MRTF</td>
<td>Modulation rate transfer function</td>
</tr>
<tr>
<td>RFR</td>
<td>Rate following response</td>
</tr>
<tr>
<td>SAM</td>
<td>Sinusoidal amplitude modulated</td>
</tr>
<tr>
<td>SEL</td>
<td>Sound energy level (energy flux density)</td>
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<td>SPL</td>
<td>Sound pressure level</td>
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CHAPTER I

GENERAL INTRODUCTION

A Brief Introduction to Mammalian Hearing

Hearing is perhaps most consistently and broadly described as "the response of an animal to sound vibrations by means of a special organ for which such vibrations are the most effective stimulus," (Wever 1976; Popper et al. 1992). By such definition, the mammalian auditory system is quite distinct. This is because many mammalian species demonstrate good relative sensitivity to "sound vibrations" and sensitivity across a uniquely broad range of sound frequencies. Broadband and sensitive ears have come about through a variety of evolutionary adaptive traits including efficient sound gathering, middle ear amplification, and cochlear and basilar membrane morphology and sensitivity (see a review in Webster et al. 1992).

The mammalian ear anatomy is typically categorized in three parts; the outer, middle and inner ear. The outer ear (usually the pinna and ear canal) captures and funnels sound to the middle ear. In the middle ear, the sound is converted from the air sound pressure wave to a mechanical wave. This is done by the interaction of the ear drum membrane and the three bones in the middle ear, the malleus, incus and stapes (von Bekesy 1960; Zwislocki 1962; Zwislocki 1963; Tonndorf and Khanna 1976). Sound pressure waves strike the large area of the tympanum (eardrum) and are concentrated into the smaller area of the stapes. The three bones then act as levers to amplify the vibrations of the sound wave. The force of the vibrating stapes is
nearly 15 times larger than that of the tympanum. This provides terrestrial mammals with the ability to hear very low amplitude sounds. The levering of the stapes transduces pressure to the oval window of the cochlea, the interface between the middle ear and the fluid filled inner ear. The fluid moves the hairs of the nerve cells, which opens ion channels in the nerve cell, depolarizing the cell membrane. The neural impulses conduct electrical impulses along the auditory nerve towards the brain (see a review in Yost 1994).

How sounds are processed offers a further level of complexity. Background noise may be filtered at the peripheral hearing level by adaptations such as sensitivities to particular frequencies or temporal characteristics (Plassmann and Brändle 1992) or in the central auditory system, e.g., binaural summation or suppression responses of certain signals (Merezenich and Schreiner 1992). Of particular interest is the temporal processing of sound. Most ambient sounds fluctuate over time in both amplitude and spectral components. Acoustic temporal patterns are important for detection, identification, and classification of sound sources as well as overall effects on the auditory system. For example, humans, rats, bats and squirrel monkeys use time-of-arrival differences for low frequency sound source localization (Heffner and Heffner 1992; Brown 1994). In macaques, the detection and discrimination of time amplitude modulated signals plays an important role in within group communication (Moody 1994). Exposures to relatively short duration, intense sounds may induce muscular reflexes that reduce sound amplitude and reduce the detrimental effects on the inner ear and prevent masking of important signals (Borg 1972). Finally, noise exposure to intense sounds may permanently affect
hearing thresholds, the extent of effect being directly related to exposure duration (Ward 1997). Thus, the temporal characteristics of acoustic stimuli directly relate to how an animal processes and copes with those stimuli. Concurrently, mammalian auditory systems must be sufficiently adapted to an array of naturally fluctuating sounds.

**Odontocete Hearing**

Odontocetes, or toothed whales and dolphins (Order: Cetacea, Suborder: Odontoceti), are mammals well adapted for hearing underwater. The auditory system is likely their primary sensory modality for gaining information about their environment. Since the first cetacean audiogram in 1966 (Johnson) we have learned quite a bit about how odontocetes receive sound, what sounds are utilized and how those sounds are processed (e.g., Au et al. 2000). As odontocetes have adapted for underwater hearing, some characteristics of their auditory system differ from that of terrestrial mammals. But many structures are homologous including the need to process temporally varying sounds (Au 1993; Ketten 2000).

Any investigation of underwater hearing requires several introductory concepts. First, sound travels well underwater, whereas light attenuates relatively rapidly (Urick 1983). Light is often not a viable cue to marine animals, especially at depth, at night, or in murky waters. As water is dense medium (relative to air), sound is conducted efficiently and attenuates at a slow rate. Thus, sound is consistently available for communication, foraging, and other behaviors (Bradbury and Vehrencamp 1998). Second, sound travels rapidly underwater, nearly 1500 m/s and
almost five times faster than it does in air (Urick 1983). In order to localize and utilize many acoustic stimuli, it is important to process sounds relatively rapidly.

Third, as a result of aquatic life and these constraints, odontocetes have evolved several unique adaptations that allow them to hear well under water. These adaptations must compensate for water as the medium in which sound is perceived and they are often linked to the temporal characteristics of the surrounding acoustic environment.

For example, as water is a relatively dense medium, odontocetes have not retained external pinna which would cause significant drag. Thus, the lower jaw and associated fat bodies are required to help gather and localize sound in water (Norris 1968; Möhl et al. 1999). Sound localization depends on stimuli time-of-arrival, phase and intensity differences which vary temporally (Heffner and Heffner 1992; Au 1993; Brown 1994). Odontocetes may use their jaw morphology to help temporally identify and spatially localize sounds. Dolphins also have very broadband hearing, up to 150-180 kHz (Johnson 1966; Nachtigall et al. 2008). This bandwidth probably influences temporal resolution capabilities. This is because at higher frequencies auditory filters encompass a broad range of frequencies. These broader filters provide increased temporal resolution (Fay 1992). Another adaptation which utilizes water’s sound speed is the ability to echolocate. Consequently odontocetes process clicks and subsequent echoes rapidly, as both signals are on the order of a few tens to a few hundreds of μs in duration and often only a few ms apart (Au 1993; Madsen et al. 2004). They also have the ability to produce, withstand and detect very high amplitude signals (upwards of 220 dB re: 1 uPa p-p) and very low amplitude signals
(as low as 35 dB re: 1 μPa in the killer whale; *Orcinus orca*) (Au et al. 1974; Szymanski et al. 1999). Detection is possible (without forward masking) when these signals are very close in time (Supin et al. 2004). Thus inherent in the odontocete auditory system, very much like what is found in terrestrial mammals, the time patterns of signal fluctuations play important roles in the characteristics of their auditory system. These temporal structures, be they in timing of echolocation pulses, the varying amplitude of signals, or other patterns, are important for signal detection, identification, and classification of sounds.

Over the past 40 years the study of odontocete hearing and acoustic behavior, which includes investigation of temporal pattern influences, has yielded a substantial amount of knowledge. Their broad range of hearing and echolocation capabilities sets them (as well as many bat species) apart from other mammalian taxa. Considerable interest in odontocete underwater hearing has resulted in a broad range of topics including the audiograms of 12 species (Nachtigall et al. 2000; Kastelein 2003; Popov et al. 2005; Nachtigall et al. 2008), auditory filter bandwidths (Popov et al. 2006), sound localization (Branstetter and Mercado 2006), electrophysiological studies (Ridgway et al. 1981; Supin and Popov 1995), and anatomical investigations (Ketten 1992). Additionally many other auditory characteristics associated with thresholds, reception and perception of sound have been investigated (see Au et al. 2000).

However, challenges exist in the study of marine mammal hearing, which include: access to experimental subjects, time and money for training and maintaining animals, inability to conduct invasive studies and a focus on relatively few species.
Comparisons to terrestrial mammal hearing do describe some hearing attributes such as inner ear functionality (Ketten 1992; Yost 1994). However, despite a good deal of attention to hearing in both marine and terrestrial mammals, there is much we do not yet understand in regards to odontocete audition. For example, we have very few or no auditory measurements from most species of cetaceans. Consequently, there are few data on many individual species. This includes lack of data on substantial groups such as the beaked whales and river dolphins, both of which are niche-specialized. The relative paucity of information undermines our understanding of the diversity of odontocete hearing. Much of our understanding comes from limited measurements such as audiograms. These audiograms provided an indication of general auditory capability but little attention has been paid to higher level processing of sound.

Further, there is recent concern regarding the effects of anthropogenic produced noise on marine mammals (Dalton 2003; National Academy of Sciences, 2005; Southall et al. 2008) but few data to determine and predict those effects. Much work is required to further understand basic properties of marine mammal hearing and to mitigate potential anthropogenic effects.

There are several parallel lines of investigation that would greatly enhance our knowledge of marine mammal hearing. The first is the examination of the role of temporal patterns of acoustic signals in marine mammal audition, be it in how sounds are localized or processed, or the exposure to intense fatiguing sounds. The investigation of sounds with respect to their temporal attributes will likely help explain how odontocetes utilize and cope with various sound sources. Second, expanding the number of species investigated is important. Many odontocete species
differ in prey selection, habitat preference and morphology. These and other factors may influence particular adaptations, including auditory, which predominate in individual species. The examination of auditory characteristics across species would broaden our knowledge of the diversity and conservation of marine mammal auditory traits. Third, comparisons among odontocetes and terrestrial mammals may lead to a greater understanding of marine mammal hearing, and hearing in general. Both what is conserved and what has diverged among species may show how auditory features function and evolve. In several component studies, this dissertation aims to achieve these goals.

**Hearing Diversity and Auditory Processing**

While there are over 80 species of cetaceans, there are only 12 published audiograms (for a review see Nachtigall et al. 2000; Kastelein 2003; Popov et al. 2005; Nachtigall et al. 2008) which provide us the basic hearing sensitivities across a spectrum of frequencies. While more detailed studies focus on “model” species such as the bottlenose dolphin (*Tursiops truncatus*), the false killer whale (*Pseudorca crassidens*), and the harbor porpoise (*Phocoena phocoena*) (e.g., Au 1993; Kastelein et al. 2002; Supin et al. 2008) we know relatively little about the hearing of other species. To meet various data requirements and models, auditory and behavioral predictions are often drawn between species, but these comparisons are often based on limited information regarding auditory capabilities. While it may not be viable to examine the auditory system of every species of cetacean, it is important to understand the variation of hearing. Understanding hearing variation will provide
There are consistent attributes of odontocete hearing. The species studied to date hear a large range of frequencies from low (100 Hz) to high frequency sounds (150 kHz) (Johnson 1966; Nachtigall et al. 2000). They have the ability to produce and detect high and low amplitude signals (Au et al. 1974; Szymanski et al. 1999). The odontocete cochlea is adapted for ultrasonic perception with exceptionally narrow basilar membranes, a rigid outer spiral lamina, and high thickness-to-width ratios (Ketten 1992). These contributions tend to stiffen the basilar membrane and expand resonance to high frequencies relative to most terrestrial animals.

However, there are quite a few aspects of odontocete hearing we know little about, including possible inter-specific differences in how sound is received and auditory temporal resolution. Both play a role in the temporal processing of sounds. They may also be ideal methods of examining odontocete hearing variation as they may illustrate the diversity of hearing adaptations as well as some of what is conserved across species.

The odontocete equivalent to the terrestrial mammal “outer” ear is the head and jaw fats which collect and conduct sound to the middle and inner ear. However, head and jaw morphology and lipid compositions vary considerably among species indicating that different species (and even individuals within a species) may receive sound differently (Ketten 1997; Koopman et al. 2006; Cranford et al. 2008). This “receiver” variation may affect hearing directionality which is important for determining the direction and source of a sound. This ability is central to locating
conspecifics and localizing prey. The way that an animal receives sound may also influence how it is affected by anthropogenically produced sound. The *shaded receiver model* of odontocete hearing relates how sound is channeled through anatomical features of the head to how sound is perceived and processed (Møhl et al. 1999). This model describes how a receiver weights sound according to the direction of arrival and location where sound strikes the receiver (e.g., the odontocete head). Anatomical structures of the head affect (i.e., shade) time, amplitude and spectral components which can be used in the perception of sound. Currently one model (based on the bottlenose dolphin) is proposed for all odontocetes. Thus, investigating auditory receiver variation allows for comparison of odontocete (i) hearing diversity, (ii) directionality, and (iii) anthropogenic influences. All three features are linked to the temporal attributes of the acoustic signal as well as sound reception.

Rapid auditory temporal processing is important to marine mammals because their auditory system must be able to respond to the rapid conduction of sound underwater, which travels nearly five times faster than sound in air (Urick 1983). The odontocete auditory system must follow the rapid clicks and echoes occurring during echolocation (Au 1993). As a consequence of the complexities of an underwater environment and the adaptation of echolocation, odontocetes are often considered to have the fastest auditory processing abilities of any animal (Supin et al. 2001; Thomas et al. 2003). As all odontocetes tested echolocate and process sounds in water one might expect that rapid temporal resolution may be well conserved. As in terrestrial mammals, comparative data are limited. However, in land mammals data
suggest that temporal processing speeds are similar and probably determined by the same neural mechanisms (Fay 1994). The same may hold true for odontocetes.

The examination of temporal resolution and the manner in which the odontocete head may receive sound might provide interesting perspectives on what is conserved and what has diverged in odontocete hearing. Both attributes play key roles relative to odontocete utilization of time varying acoustic stimuli. Comparative examinations also broaden the database from the much studied bottlenose dolphin to other species, to better understand how auditory correlations may apply. The fact that there are few data on hearing diversity stresses the need for caution regarding application of auditory characteristics to species for which we know relatively little, such as mysticetes and beaked whales. Further, understanding the variation of hearing between odontocete species allows evaluation of the robustness of drawing comparisons among species.

The Physiological Effects of Noise

Excessive noise exposure can have behavioral or physiological consequences including temporary or permanent changes in hearing sensitivity shown across classes of vertebrates including fish, reptiles, birds, and mammals (Ward et al. 1958; Saunders and Dooling 1974; Popper and Clarke 1976; Mulroy 1986). Moderate levels of hearing threshold elevation are innocuous and fully recoverable and have been termed temporary threshold shift (TTS). Characterizing and understanding how TTS is induced allows prediction of stimulus characteristics which might cause permanent hearing damage, or permanent threshold shift (PTS) (Yost 1994).
occurrence and extent of threshold shift depend on three primary variables; the frequency, intensity and duration of the fatiguing noise. For example, shifts tend to be greatest at frequency regions of best sensitivity and intermittent exposures induce less shift than continuous noise exposure (because recovery occurs during intermittent stimulus bursts) (Bohne and Clark 1990). Further, there are numerous other variables that may affect threshold shifts such as variation between and within individuals or expectation of noise exposure (Bench 1971; Ward 1997). The interactions of these multiple variables makes subsequent prediction of threshold shifts complicated.

For obvious reasons, there is much incentive for determining the levels of noise that induce TTS or PTS in humans. As a result, the subject is well explored in terrestrial mammals and the primary variables (intensity, duration and frequency) that induce TTS are relatively well understood (see Ward 1997). Thus, models have been developed to predict situations that would induce TTS (Kryter et al. 1966; Kryter 1994).

Although there are several methods for predicting the effects of noise exposure on animals, the equal energy hypothesis has been the most widely accepted theory. Its appeal is its simplicity (Ward 1991). This hypothesis states that the effect associated with any sound exposure (be it continuous, intermittent, impulsive, etc...) can be predicted by a single energy level. In other words, an exposure of the same energy level will induce the same amount of TTS, regardless of its frequency, intensity or temporal attributes. This trade-off relationship between intensity and time which keeps the energy level constant is also called the 3-dB rule because
halving the exposure time and increasing the intensity by 3 dB (doubling the intensity) keeps the amount of shift constant.

Unfortunately, although the hypothesis is widely used by agencies such as the Environmental Protection Agency and National Institute for Occupational Safety and Health (EPA 1974; NIOSH 1994), peer reviewed literature often fails to support this rule because it often does not accurately predict TTS (Ward et al. 1958; Buck et al. 1984; Hamernik and Qui 2001). Thus, if, how, and when the equal energy hypothesis applies is still up for much debate.

**Noise Exposure and Marine Mammal Hearing**

Serious concern regarding the effects of noise on marine mammals has arisen over the last decade (Richardson et al. 1995; National Academy of Sciences, 2003; Wartzok et al. 2004). Much of this concern stems from recent marine mammal strandings that have been linked to naval sonar exercises indicating some physiological damage as a result of the noise (Evans et al. 2001). Increasing noise levels are a result of a variety of anthropogenic sources including mechanized shipping, naval sonar, scientific exploration, oil drilling, and construction. These noise sources may vary greatly in acoustic characteristics. For example, sonar signals are typically short duration, intense sounds (sonar) while shipping noise may be longer duration, lower amplitude signals. As usage of the oceans increases, marine noise levels are also expected to increase and the effects on marine mammals are expected to increase as well (National Academy of Sciences, 2005).
A primary concern of excessive noise exposure on marine mammals is the physiological effects of noise-induced TTS. While TTS has long been demonstrated in other taxa it was not until 2000 that Schlundt et al. found that cetaceans are also susceptible to threshold shifts. The work was fairly broad, examining shifts at multiple frequencies using several animals but as many pioneering papers do, the work raised many questions regarding TTS in odontocetes. To date, TTS research in marine mammals has focused on preliminary information such as identifying the acoustic signals that may induce shifts such as broadband noise (Nachtigall et al. 2003), tones (Finneran et al. 2005), or airguns (Finneran et al. 2002). While these studies were groundbreaking, challenges in examining TTS were revealed, reflecting the complexity of the issue. Shifts were not often reliably induced (Schlundt et al. 2000). Behavioral methodologies were slow to determine thresholds thus recovery occurred and the amount of shift was not well quantified (Nachtigall et al. 2003). Little attention was paid to recovery rates or frequency of shift occurrence (Finneran et al. 2005). Fatiguing stimuli frequency and sound pressure levels were not always well controlled (Finneran et al. 2002; Nachtigall et al. 2003). Recent experiments have improved measurements by inducing greater and more reliable shifts and using faster physiological methods of determining thresholds (Nachtigall et al. 2004; Finneran et al. 2007). However, almost no comparisons have been made to terrestrial mammals, where the wealth of TTS information has been derived. And, to draw order and find trends in odontocete TTS occurrence, comparisons can only currently be made across multiple experiments with divergent methodologies (Finneran et al. 2005).
Finally, there is a clear need to be able to predict situations that might induce TTS. For example, if exposure intensity and duration were known, would it be possible to predict TTS occurrence? Or could TTS onset be modeled by some relationship between sound intensity and duration? As noise levels in the ocean increase it is important to understand what levels will affect marine mammals and how much noise is too much.

Relating exposures of various intensities and durations has been attempted by piecing together several studies of divergent methodologies (Finneran et al. 2005). In such a manner the equal energy hypothesis, or the equal energy line, was applied to predict TTS in odontocetes. Unfortunately, because the studies varied considerably in methodologies, applying a line across the data was an inadequate way to test the hypothesis. Further, the application of the equal energy hypothesis to accurately predict TTS in terrestrial mammals is debatable. Thus, to employ the idea in marine mammals is questionable. The use of the equal energy hypothesis as a model to predict TTS is an indication of the need for empirical data on the effects of noise duration and intensity in marine mammals. In fact, in nearly ten published papers on odontocete TTS, few concrete rules to predict TTS have been established.

Thus, there is a need for a comprehensive examination of TTS in odontocete cetaceans, emphasizing the relationship of noise intensity and duration. To apply a focused approach to the equal energy concept would not only test the hypothesis in marine mammals but provide a method of plotting the actual relationship of the impact of short, intense sounds versus longer, lower amplitude sounds. If the equal energy model is not sufficient to predict TTS, then a new predictive model should be
developed. The experiments should reliably and repeatably induce shifts, determine the frequencies at which shifts are induced relative to the fatiguing noise, and examine recovery rates after noise exposure. These data could be compared to those obtained from terrestrial mammals. This would provide a means of predicting what sound exposure levels induce shifts in marine mammals and allow a comparison of marine mammal TTS and shifts of terrestrial mammals. Further, exposures of various durations and intensities would examine the temporal relationship of noise exposure. A broad experiment is particularly important given the complex sound environment of the ocean, where noise levels are increasing within the habitat of many cetaceans and many of the effects are unknown. A better understanding of TTS would allow us to predict and mitigate the situations that induce threshold shifts.

Auditory Evoked Potentials

The majority of studies on the auditory capabilities of marine mammals have used psychophysical or behavioral response paradigms similar to those employed to collect the first audiogram of a marine mammal (Johnson 1966; Nachtigall et al. 2000). However, these methods have a number of limitations including the significant time needed to train and maintain the subjects, as well as the actual time required to conduct the experiments.

An alternative to the traditional psychophysical methods is the use of auditory evoked potentials (AEPs). The AEP technique is a noninvasive and rapid method to measure hearing characteristics. Auditory evoked potential recording measures the small voltages generated by neurons in the auditory system (typically the brainstem in
mammals) in response to acoustic stimuli (Hall 2007). It requires no training of the subject and is used to assess hearing in a variety of taxa including humans and other mammals (Hecox and Galambos 1974; Dolphin and Mountain 1992), birds (Brittan-Powell et al. 2002), teleost fish (Ladich and Yan 1998), cartilaginous fish (Casper et al. 2003), and invertebrates (Lovell et al. 2005).

The AEP technique has been established for several decades in marine mammals but methods varied, electrophysiological tools adapted for marine mammals were not widely available, the experiments were often invasive and the method was not widely used (Bullock et al. 1968; Ridgway et al. 1981; Popov and Supin 1990). Within the last decade an emphasis on relatively simple, non-invasive AEP techniques has come about providing insights into the auditory systems of odontocetes (Dolphin et al. 1995; Supin and Popov 1995; Supin et al. 2001; Nachtigall et al. 2007).

Early dolphin AEP measurements used tone pips as stimuli and revealed dolphin AEP responses involving a series of 5-7 neurophysiological “wave” responses (Supin et al. 2001). Currently, the most efficient and reliable method to obtain AEP hearing thresholds is to use the envelope following response (EFR). The series of waves is visible at the onset of an EFR, but if a stimulus is played at a rapid enough rate, most of the waves blend together in a sinusoidal fashion (Supin and Popov 1995; Nachtigall et al. 2005). In this method, the stimulus is a sinusoidally amplitude modulated tone, and thus the animal’s EFR is a consequent sinusoidal ‘following’ of the envelope of the carrier tone. The results of this method compare favorably to those of the behavioral psychometric audiograms (Yuen et al. 2005;
Houser and Finneran 2006) and the technique is used in both laboratory and field situations (Cook and Mann 2004; Nachtigall et al. 2007; Nachtigall et al. 2008). Auditory evoked potentials are now applied beyond basic hearing threshold measurements to assessments of auditory physiology such as temporal processing, auditory filter bandwidths and mechanisms of hearing gain control (Supin et al. 2004; Mooney et al. 2006; Popov et al. 2006).

Despite the ease with which AEP methodology is presented in the literature, techniques and equipment can be challenging. Evoked potentials are recorded on the level of micro- and nano-volts and background electrical noise must be effectively and continuously reduced. Software and equipment are often not commercially available and must be custom constructed. The tools that are commercially available are typically not for dolphin AEP recordings or sea water environments and must be modified. These complex equipment packages must be simple enough to use every day and in a variety of experimental conditions. The first goal of this set of experiments was to meet these equipment and data reliability requirements in order to allow the collection of publication quality experimental data.

Specific Aims

The capabilities of marine mammal auditory systems to cope with stimuli of varying temporal attributes are not well understood. Many species have yet to be investigated and even those that have been examined provide limited data. Yet the animals must utilize or filter these sounds as they echolocate, receive external acoustic stimuli, and cope with external noise. Odontocete auditory systems must be
able to respond to and follow stimuli, recover from loud sounds, and potentially avoid or mitigate the deleterious effects of loud sound. The goal of this dissertation research was to better define odontocete auditory temporal resolution and their response to broadband stimuli of various intensities and temporal characteristics. The results may then be compared with measurements from other species to identify common traits. Broadband stimuli were used because they reflect typical sounds encountered in the wild (e.g., echolocation clicks, communication signals, much biotic and anthropogenic noise). In the light this discussion, the specific aims for this work were:

1) Develop permanent AEP tools and procedures for use in measuring marine mammal hearing.

The tools and techniques acquired here were applied to subsequent experiments.

2) Measure the auditory temporal resolution of several odontocetes using AEPs.

   o Compare these auditory capabilities among species.

The objective here was to determine the comparative auditory temporal resolutions of several species of odontocetes and in addition, to determine comparative received sensitivity of different regions of the head sensitivity of at least one additional species. These results were discussed in light of comparisons of the auditory systems between species and the applicability of such comparisons.
3) Conduct a comprehensive TIS experiment to:

- Determine the relationship of sound exposure intensity and duration of a noise stimulus required to induce TIS in an odontocete.
- Quantify the relationship between recovery duration and magnitude of TTS.
- Examine whether the equal energy hypothesis is a reasonable predictor of TTS in odontocetes.

The primary goal of this study was to provide a comprehensive experiment of TTS and noise, and in doing so, to relate noise duration and intensity, and map the animal's recovery. The final goal was to provide an algorithm which models the levels and durations of noise required to induce TTS and to predict TTS onset if sound intensity and duration are known.

4) Determine if man-made sonar signals induce TTS in an odontocete.

- Compare TTS produced by sonar signals to that produced by broadband noise

The aim here was to examine the sound levels which induce TTS using a mid-frequency sonar signal recorded in the ambient environment. The results were applied to the predictive algorithm established in the previous study in order to evaluate the algorithm and predict the situations that sonar might induce TTS in an odontocete.

Organization of Dissertation
Each chapter of this dissertation (with the exception of Chapters 1 and 7) was written for publication in peer-reviewed journals. The various journal formats have been modified into one consistent style; however there may be some material overlap between chapters. The journal that the chapter has been, or will be, submitted to is indicated at the bottom of the first page of each chapter. The final chapter, Summary and Conclusions, links the various manuscripts to the consistent theme of the odontocete auditory system's capability to process, follow, and recover from broadband stimuli of various intensities and temporal characteristics, and to the development and applicability of a model to predict the effects of such stimuli.

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CHAPTER 2

TEMPORAL RESOLUTION OF THE RISSO'S DOLPHIN, *GRampus GRiseus*,
AUDITORY SYSTEM

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Abstract

Toothed whales and dolphins (Odontocetes) are known to echolocate, producing short, broadband clicks and receiving the corresponding echoes, at extremely rapid rates. Auditory evoked potentials (AEP) and broadband click stimuli were used to determine the modulation rate transfer function (MRTF) of a neonate Risso’s dolphin, *Grampus griseus*, thus estimating the dolphin’s temporal resolution, and quantifying its physiological delay to sound stimuli. The Risso’s dolphin followed sound stimuli up to $1000 \text{ Hz}$ with a second peak response at $500 \text{ Hz}$. A weighted MRTF reflected that the animal followed a broad range of rates from $100-1000 \text{ Hz}$, but beyond $1250 \text{ Hz}$ the animal’s hearing response was simply an onset/offset response. Similar to other mammals, the dolphin’s AEP response to a single stimulus was a series of waves. The delay of the first wave, $P_1$, was $2.76 \text{ ms}$ and the duration of the multi-peaked response was $4.13 \text{ ms}$. The MRTF was similar in shape to other marine mammals except that the response delay was among the fastest measured. Results predicted that the Risso’s dolphin should have the ability to follow clicks and echoes while foraging at close range.
Introduction

Odontocetes, or toothed whales and dolphins, are known for their exceptional auditory sensory systems. In adapting to an aquatic life, odontocetes have evolved unique capabilities including broadband hearing, high frequency sensitivities, rapid neurophysiological responses, and short temporal resolutions. It is presumed that these capabilities have developed to survive in an underwater world where sound travels five times faster than in air, and where light is quickly attenuated and often limited at depth or at night. All of the odontocetes tested to date have demonstrated the ability to echolocate.

Toothed whales have demonstrated the ability to make at least two types of sounds, echolocation clicks and burst pulses. Marine mammal echolocation clicks typically consist of short, broadband pulses of sound, as short as 40 µs (Au 1993) and temporally spaced between 5 and 500 ms (Penner 1988; Madsen et al. 2004a). Echoes from targets such as fish prey will return to the animal in a similarly rapid fashion. Field recordings have demonstrated that odontocetes, including sperm whales, beaked whales, and delphinids, vary the echolocation inter click interval (ICI) and decrease the ICI when approaching prey (Johnson et al. 2004; Lammers et al. 2004; Zimmer et al. 2005). Just before prey capture, clicks are produced at their most rapid rates with minimum ICI of 5-20 ms, which is referred to as the terminal buzz. It has yet to be asked whether the auditory system of odontocetes may be able to individually follow these outgoing clicks and received echoes of this terminal buzz without the masking of either sound. To do so, the auditory neurophysiological responses and temporal resolution of toothed whales must be fast and highly derived.
Burst pulses are similar to echolocation clicks in the individual pulse, but are typically spaced with extremely short ICIs of approximately 1-10 ms (Lammers et al. 2004). These are often produced in a social context (Herzing 1996). Because of the rapid click rate of burst pulses, it is also uncertain whether the producing animal follows the click, the echo or both.

Although there have been a number of studies of the auditory system of cetaceans, there are only 10 published audiograms (Nachtigall et al. 2000) out of 83 species (Rice 1998). Further, the temporal resolution of the odontocete family has only been investigated in 6 species (Popov and Supin 1990; Dolphin et al. 1995; Szymanski et al. 1998). In order to better comprehend the physiological abilities of odontocetes, it is necessary to measure the hearing capabilities and temporal resolution of those uninvestigated species. One method to measure the temporal resolution of the auditory system is to estimate the modulation rate transfer function (MRTF) using auditory evoked potentials (AEPs). The AEP technique is a noninvasive and rapid method to measure the hearing range and temporal resolution of animals. It is a method that requires no training of the subject and is used also to assess hearing responses in human infants (Hecox and Galambos 1974).

In humans, the AEP response to a single click or tone pip is actually the summation of neurological responses from multiple sources (Kuwada et al. 1986). For this reason, AEP responses consist of several waves, PI, NII, PII, NIII, PIII and NIV and are often termed an auditory brainstem response (ABR). These waves are all visible at the onset of an envelope following response (EFR), but if a stimulus is
played at a rapid enough rate, most of the waves blend together in a sinusoidal fashion. In dolphins, a similar blending seems to occur (Supin and Popov 1995).

Evoked potentials are used to measure hearing capabilities by modulating the amplitude of a tone at a specific rate. As the subject's auditory system responds to the tone, it may follow the envelope of the loud-soft modulation of the tone by a corresponding EFR. Determining the amplitudes of the EFR at various modulation rates provides the MRTF. A typical MRTF is low pass in shape, and the corner frequency of that MRTF can be inferred as the temporal resolution of the subject (Supin et al. 2001).

Auditory evoked potentials have been used to estimate MRTFs and thus temporal resolution in several species of mammals. Human maximum MRTF responses were measured at 10-50 Hz (Kuwada et al. 1986; Rees et al. 1986). Gerbils may be the fastest measured auditory system of terrestrial mammals, with responses measured at 100-150 Hz (Dolphin and Mountain 1993). The highly specialized auditory system of odontocetes has a much faster temporal resolution with the killer whale at 800 Hz (Szymanski et al. 1998), the false killer whale at 1000 Hz (Dolphin et al. 1995), and the bottlenose dolphin at 1200 Hz (Supin and Popov 1995). These rates are 20 – 40 times that of previously measured land mammals.

Recent work has explored the acoustic capabilities of the Risso’s dolphin, *Grampus griseus*, a pelagic species of squid-eating odontocetes whose offshore habitat often limits the species’ sightings and interactions with humans. This includes the first audiogram of this species using behavioral psychoacoustic methodology (Nachtigall et al. 1996), as well as a recent redefining of the species’ audiogram using
AEP techniques (Nachtigall et al. 2005). Further investigations have demonstrated the Risso’s dolphin’s echolocation capabilities and signal characteristics (Philips et al. 2003; Madsen et al. 2004b). The goal of this study was to measure the temporal resolution from the maximum rate following response (RFR) rate of a Risso’s dolphin using AEP measurements. The MRTF of the Risso’s dolphin as well as the physiological time lag of the animal’s auditory response was measured.

**Methods**

**Subject and facility**

The study animal was an infant male Risso’s dolphin *G. griseus* that stranded off the southwest coast of Portugal in May 2004 and thus its exact age was unknown. However, due to its small size and the presence of fetal fold markings on the animal’s body, it was determined to be a neonate. For assessment and care, the animal was brought to a rehabilitation facility at ZooMarine in Guia, Albufeira, Portugal. During the experiment, the animal measured 147 cm long, weighed 47 kg, ate well and gained weight. The study was conducted for four consecutive days in late May, two weeks after the stranding. Two weeks following the experiment the animal died of pneumonia and possibly a secondary viral infection, both of which were unrelated to this work.

The animal was housed in an open-air, covered, concrete rehabilitation pool, 3 m deep and 5 m diameter. The artificial sea water depth was kept at a constant level of 1.1 m and 19°C (Figure 2.1). Pumps and filters recycling the water in the tank were turned off 15 min before the beginning and during the experiment to reduce
bubbles in the water and background noise. A desk adjacent to the tank served as the observation and data collection center, where the equipment was housed and where the experiment operators were seated.

Figure 2.1. Experimental setup shown in a cross-section of the research tank. The subject was stationed by a researcher 1 m from the transducer so that melon and lower jaw were below the surface of the water. Water depth was 1.1 m and diameter of tank was 5 m. The animal was always stationed in the same position, the blowhole directly under hanging weight. A rope was strung across the tank, from which the transducer and stationing marker were hung. Sides of the tank were lined with sound baffling cushions (not pictured).

*Experimental design and stimuli presentation*

During the experiment the animal was moved toward the middle of the tank and held in position by the experimenter. The animal became relaxed in that resting
position, decreasing its breathing rate and relaxing its muscles. During a sound presentation, the dolphin was stationed 0.5 m from the center of the tank, on axis of the diameter of the tank. The transducer used to produce the stimuli was positioned 0.5 m from the center of the pool (which was 1 m from the subject), at a depth of 30 cm, and also on axis to cross-section the tank.

The stimulus was a broadband click from 1-40 kHz designed as a rectangle function that was 50 μs in duration. The rate at which the click was played varied from 100 to 2000 Hz (Table 2.1). This broadband nature of the stimulus ensured that the animal’s presumed hearing range and the click bandwidth would overlap. Clicks were digitally generated with a computer that contained a custom LabVIEW data acquisition program that was created with a National Instruments PCI-MIO-16E-1 DAQ card. The DAQ card converted the signal from digital to analog, using an update rate of 200 kHz. The signal was then played through a custom built signal shaping box that could attenuate the click bursts in 1 dB steps. The outgoing stimulus from the signal shaping box was sent to the projecting hydrophone and was monitored using a Techtronix TDS 1002 oscilloscope. The signal was played through an ITC-1032 transducer with a resonance frequency of 38 kHz. The amplitudes of the stimuli were calibrated before data collection by placing a calibrated hydrophone, a Biomon 8261, near the dolphin’s head while the dolphin was in the correct position. The received peak-to-peak level (V) of the click stimuli was measured with the calibrated hydrophone. This Vp-p was converted to peak-equivalent rms voltage (peRMS) by subtracting 9 dB. The peRMS was taken as the RMS voltage and used
to calculate the SPL by referring to the hydrophone sensitivity. These values were taken as the received level of the click.

**AEP recording and measurement**

The animal’s ABR was recorded using two standard 10 mm gold EEG electrode sensors placed on the surface of the subject’s skin, attached by two latex suction cups. Passive conductivity of the animal’s AEPs from the skin surface to the electrode was enhanced by standard human EEG gel. One suction cup was embedded with the recording electrode, and was placed 3-4 cm behind the dolphin’s blowhole and off to the right, i.e. over the animal’s brain. The second suction cup contained the reference electrode, and was placed on the back of the animal near its dorsal fin. The animal rested at the surface with most of its head and lower jaw underwater to receive sound input through the major tissue routes to the ears (Norris 1968; Mohl et al. 1999; Ketten 2000) but with the suction cups in the air.

The received signal was then amplified 10,000x using an Iso-Dam Isolated Biological Amplifier (WPI). The Iso-Dam as well as a Krohn-Hite Filter Model 3103, with a bandpass of 100 to 3000 Hz, filtered the responses for anti-aliasing protection. The amplified and filtered responses were transferred to an analog input of the same DAQ card in the same desktop computer. The received signal was digitized at a rate of 16 kHz. In order to extract the recorded AEP from noise, the entire trial was extended to about 1 min by averaging 1000 samples of the stimuli that were presented at a rate of 20/s.
Table 2.1. Fourier transform and peak-to-peak averages of Risso's dolphin AEP when stimuli were played at varying rates (from 100-2000 Hz). Note the decline in the AEP between click rates of 1000 and 1250 Hz.

<table>
<thead>
<tr>
<th>Click rate (Hz)</th>
<th>Fourier transformed AEP (µV)</th>
<th>Weighted pk-pk AEP (µV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>0.02</td>
<td>2.35</td>
</tr>
<tr>
<td>200</td>
<td>0.14</td>
<td>2.77</td>
</tr>
<tr>
<td>300</td>
<td>0.69</td>
<td>3.01</td>
</tr>
<tr>
<td>400</td>
<td>2.80</td>
<td>4.60</td>
</tr>
<tr>
<td>500</td>
<td>3.99</td>
<td>5.37</td>
</tr>
<tr>
<td>600</td>
<td>3.35</td>
<td>4.27</td>
</tr>
<tr>
<td>700</td>
<td>1.76</td>
<td>1.87</td>
</tr>
<tr>
<td>800</td>
<td>1.21</td>
<td>1.37</td>
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<tr>
<td>900</td>
<td>2.05</td>
<td>2.13</td>
</tr>
<tr>
<td>1000</td>
<td>2.37</td>
<td>2.35</td>
</tr>
<tr>
<td>1250</td>
<td>0.15</td>
<td>0.54</td>
</tr>
<tr>
<td>1500</td>
<td>0.12</td>
<td>0.48</td>
</tr>
<tr>
<td>2000</td>
<td>0.06</td>
<td>0.37</td>
</tr>
</tbody>
</table>

The optimal stimulus amplitude that would result in a suitable EFR from the animal was initially unknown for this species. Therefore, the initial sound was low in amplitude and was slowly increased until a clear record was established (Figure 2.2).
The first stimulus was played at 77 dB re: 1 μPa sound pressure level (SPL), the second at 91 dB, the third at 96 dB and the fourth and final stimulus level was 101 dB, the level at which all of the following experimental stimuli were presented. An MRTF had not previously been determined for the Risso's dolphin, and therefore the rate at which to first present the clicks was based on prior published data of other odontocete species such as the bottlenose dolphin and false killer whale. These species follow a stimulus presentation rate of 1000 Hz relatively well (Dolphin et al. 1995; Supin and Popov 1995), and hence the initial stimulus was presented at that rate.
Figure 2.2. Click stimulus (bottom trace) and rate following responses recorded using four different stimulus amplitudes (101, 96, 91, and 77 dB re: 1 µPa). All AEPs are relative to 1 µV. Each stimulus was played for 20 ms, and AEPs were recorded during a 30 ms window, beginning at the onset of stimulus presentation.

The modulation, or click presentation, rate was then varied, presenting clicks to the animal at rates from 100 to 2000 Hz at the above SPL (Table 2.1). During the data collection, the AEP of the animal was monitored and a 30 ms window starting at the onset of the stimulus presentation was recorded by the same custom LabVIEW program mentioned above. Each window was successively averaged during collection and simultaneously viewed as the data were being collected in order to ensure good data signals, and then saved for offline data analysis.

Data analysis

To quantitatively determine the EFR magnitude, the 20-ms portion of the record that contained the response cycles were fast Fourier transformed (FFT). This provided frequency response spectra and allowed the weight of the modulation-rate fundamental to be determined. The amplitude of the FFT peak was calculated as the AEP response amplitude of the animal to the click stimulus at the corresponding modulation rate. A higher peak reflected a greater response to the corresponding click presentation rate.

Although Fourier transforming the EFR provides a rapid assessment of the animal's response at a certain frequency, it does not weigh the number of stimulus
presentations per unit time. Within a 20 ms stimulus, a 100 Hz click would be presented only twice, whereas a 1000 Hz click would be presented 20 times. Thus within the recording, the subject may only have two AEP responses at 100 Hz, but 20 responses at 1000 Hz. The Fourier transform does not account for this bias, but rather will reflect the greater energy at 1000 Hz. To adjust for this a weighted response amplitude was derived using a custom MATLAB program that would average the peak-to-peak amplitude of the physiological responses to the click at each presentation rate. The response amplitudes determined by both methods were then plotted against frequency of click presentation to determine the MRTF and estimate the temporal resolution of the Risso’s dolphin. Further analysis was conducted using EXCEL and MINITAB software.

The time lag of the subject’s auditory responses was measured using the multiple waves of an auditory brainstem response (ABR), a type of AEP involving a series of 5-7 “waves” evoked by clicks or short tone bursts of acoustic stimuli. It was possible to capture the entire ABR of a single click by using the initial response to a low rate click. In order to measure response delay, the time at which a response reached its maximum or minimum value was conservatively determined to be the onset latency of the response peak. In order to determine response duration, the point where the response wave started and stopped had to be defined as well. Within the 30 ms measured window, the onset of the signal was then defined as the point at which the first peak (PI) of the wave was 10% larger than the average noise level, and the offset was defined as the point when the declining slope of the last null wave (NIV) was 10% greater than the average noise level. The duration of a single AEP response
was measured as the time separation from waves PI to NIV. Delay mean values were measured from the first click in each of the thirteen stimulus presentations and adjusted for the system delay, 40 μs from the start of A/D conversion to activation of the loudspeaker, and transmission delay for 1 m (670 μs, c=1490 m/s).

Results

The plotted functions of the subject's response to stimuli of increasing SPL are shown in Figure 2.2. Stimulus intensity was increased from 77, 91, 96 and finally to 101 dB re: 1μPa, where the AEP was clear, level, and distinct from the background noise. The response here was a sinusoidal AEP, as is typical for higher frequency modulation rates.

![Fourier spectra](image)

Figure 2.3. Fourier spectra using a click repeated at 1.0 kHz varying the SPL. Sound intensity of the click stimuli series for each respective graph is labeled on the graph.
A SPL of 101 dB re:1 μPa was determined as the amplitude of stimulus presentation level for all modulation rates when quantifying the MRTF.

A portion of each AEP was fast Fourier transformed and viewed in the frequency spectrum (Figure 2.3). The corresponding peak at the modulation frequency was taken as the amplitude of the response. As SPL increased, the peak at 1000 Hz increased, thereby reflecting the same trend as the AEP. Again, at 101 dB, there was a very clear peak, distinct from the background noise.

Figure 2.4. Modulation rate transfer function based on peak values of Fourier transforms of the Risso’s dolphin’s AEP. Peaks typically occurred at the modulation frequency of the click presentation.

The Risso’s dolphin MRTF using the FFT data was low-pass in shape, similar to that of other odontocete MRTFs (Figure 2.4; Table 2.1). This animal showed obvious following of click rates up to 1000-1200 Hz. There was a lower frequency
peak at 500 Hz and a higher frequency peak at 1000 Hz. The MRTF was relatively broadband, spanning from 200-1200 Hz, with a clear notch at 800 Hz. Beyond 1000 Hz there was a steep, high-frequency cut-off. The function decline was more gradual at the lower frequencies, declining after 300-400 Hz. Using the weighted pk-pk results (Table 2.1), the responses to lower frequency click rates were of higher amplitude (Figure 2.5). Above the lower frequencies, the Risso’s MRTF was still the same general shape, regardless of methodology in data analysis.

![Graph showing the weighted modulation rate transfer function.](image)

Figure 2.5. Weighted modulation rate transfer function based on the average of the peak-to-peak values of the Risso’s dolphin’s AEP at each respective response.

When lower rate stimuli were used, the frequency spectrum of the EFR revealed harmonics present in the EFR (Figure 2.6). These harmonics were multiple peaks of the fundamental frequency and separated by amounts equal to the fundamental frequency. For example, when using a 400 Hz click rate, as in Figure 2.5, there was a clear peak at the fundamental frequency of 400 Hz, as well as peaks
at the harmonic frequencies of 800 and 1200 Hz. The harmonics resulted from an
AEP that deviates in shape from a normal sine wave (Figure 2.7).

Figure 2.6. Fourier spectra of records at click modulation frequencies of 0.2, 0.4, 1.0,
and 2.0 kHz. Response amplitudes are on the y-axis and in µV (note the different
scales). At 0.2 and 0.4 kHz the peaks at higher frequencies are harmonics in the AEP
found at lower stimuli modulation rates. An arrow points to the fundamental
response at 0.2 kHz.

An ABR from a single click was taken out of the series of clicks in the
animal’s rate following response (RFR) (Figure 2.7b). The compaction of these
multiple ABRs in the RFR resulted in the deviation from a “normal” sine wave
response as in an EFR Figure 2.7. Thus, harmonic components as seen in Figure 2.6
were a result.
Figure 2.7. (a) Rate following response of 400 Hz click stimuli. (b) Series of AEP waves (PI-NIV) from a single click stimulus. b is a close-up section of the AEP highlighted in a, and shows the physiological delay as well as the series of response waves.

The Risso’s response was also measured at click stimulus rates of 1250, 1500, and 2000 Hz. At these rates that were greater than 1000 Hz, the Risso’s dolphin did not follow the sound as an EFR but rather only as an onset and offset response to the click stimuli (Figure 2.8).
Figure 2.8. Onset and offset response to click stimuli at a rate of 2000 Hz. Peaks at 5-6 ms and 25-26 ms are likely the onset and offset responses to the click stimuli.

The peaks of these various waves were used to measure the onset response time of the Risso’s dolphin’s initial response to an auditory stimulus. Mean response time for the Risso’s dolphin was 2.76 ms for PI, 3.29 ms for NII, 3.78 ms for PII, 4.18 ms for NIII 4.56 ms for PIII, and 5.17 ms for NIV (Table 2.2). The total duration of the multi-peaked response was also measured at 4.13 ms.

Table 2.2. Measured response delay to click stimuli. Delay mean value measured from the first click in each of the thirteen stimulus presentations, adjusted for a 710 μs system and travel time delay. Units are ms. S.D. is the standard deviation from the mean of the delay of each wave.
<table>
<thead>
<tr>
<th>Wave</th>
<th>Response delay (ms)</th>
<th>S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>PI</td>
<td>2.76</td>
<td>0.157</td>
</tr>
<tr>
<td>NII</td>
<td>3.29</td>
<td>0.099</td>
</tr>
<tr>
<td>PII</td>
<td>3.78</td>
<td>0.050</td>
</tr>
<tr>
<td>NIII</td>
<td>4.18</td>
<td>0.101</td>
</tr>
<tr>
<td>PIII</td>
<td>4.56</td>
<td>0.101</td>
</tr>
<tr>
<td>NIV</td>
<td>5.17</td>
<td>0.094</td>
</tr>
</tbody>
</table>

Discussion

The MRTF of the Risso’s dolphin presented here revealed a similar shape and temporal range to that of other odontocete cetaceans. The animal’s capability to follow stimuli presented at a rate over 1000 Hz indicated that the animal has a very high temporal resolution, beyond that of most mammals and similar to other echolocating odontocetes.

The particular shape of the MRTF revealed several peaks and notches, including the largest response peak at 500 Hz and a second, corner peak at 1000 Hz. The corner frequency of 1000 Hz is important for two reasons: (1) it is the modulation frequency used for sinusoidal amplitude modulated (SAM) waves in AEP audiograms for the species, and (2) it is the predicted temporal resolution of the subject. For this Risso’s dolphin, the AEP audiogram was conducted soon after the MRTF, and the corner frequency of 1000 Hz was used as the SAM rate in the presented tones (Nachtigall et al. 2005).
This high temporal resolution presumably evolved as an adaptation to the physical consequence that sound travels about five times faster in water than in air, and as part of the animal's echolocation ability. The echolocation click of an odontocete is very short in duration, and has been measured from a Risso's dolphin to be from 30 - 50 μs, with the inter-click interval (ICI) at a consistent 20 ms (Madsen et al. 2004b). However, in other odontocetes such as bottlenose dolphins or beaked whales, the ICIs will often vary, ranging from 5 - 500 ms (Penner 1988; Madsen et al. 2004a). Presumed burst pulses of Hawaiian spinner dolphins (*Stenella longirostris*) have an ICI as low as 1.5 - 2 ms (Lammers et al. 2004). Although variations in Risso’s dolphins ICIs have not been published, many odontocetes vary the inter click interval with target range and it is reasonable to at least compare these other odontocete results with what a Risso’s dolphin is likely to be capable of using. This Risso’s dolphin MRTF reflected that the animal’s auditory neurophysiology could follow sounds at a rate of at least 1000 Hz, or on the order of one per millisecond. Based on the MRTF, the animal measured here should be easily able to follow echolocation click trains including burst pulse trains.

The AEP of the Risso’s dolphin was made up of several peaks. This was visible at the onset of the SAM stimuli. As in other terrestrial mammals, the various peaks and valleys of the response most likely stemmed from different sources (Kuwada et al. 1986). In addition, the auditory physiological response delay of the subject was measured by the latency of these peaks. The response delay of this animal was extremely rapid, between 2.76 and 5.17 ms, for the various response waves of PI-NIV (Table 2.2). This time delay takes into account the 710 μs system
and travel time delay. These results compared favorably to those of a bottlenose dolphin *Tursiops truncatus*, for which delays were measured between 2.0 and 4.5 ms (Supin and Popov 1995). Considering our conservative method of determining a peak’s onset, the response of the Risso’s dolphin was quite fast.

However, the response delay of the Risso’s dolphin was shorter by several ms than what was previously measured for a killer whale, *Orcinus orca* (Szymanski et al. 1998). It has been suggested that the longer delay was a result of the longer neural pathways of the larger killer whale’s auditory system. This was likely to be a factor in determining response lag times. However, a comparison of the time lag of the infant Risso’s dolphin in this study (weighing 76 kg), to that of adult bottlenose dolphin (upwards of 170 kg), showed that the size of the animal does not necessarily indicate the speed of the auditory response. This was especially true if marine and terrestrial mammals are compared. Cat AEP latencies have been measured on the order of 10-50 ms in their delay, considerably longer than that of odontocetes measured (Farley and Starr 1983). Human AEP latency at the N1 wave is approximately 60-90 ms or more than an order of magnitude greater than the Risso’s dolphin measured here (Yost 1994).

Although size may be of less importance, the evolution of the auditory system played a key role in evoked potential response latency. Cetaceans have evolved in an underwater environment where sound travels five times faster than in air, and evolution favored adaptation for short echolocation clicks, short echo delays, and rapid neural processing.
The duration of a single AEP response, measured from the onset of the initial wave to the offset of the last wave in a single ABR, corresponded well with the echolocation click patterns of odontocetes. This response duration of the Risso's dolphin was measured to be approximately 4.13 ms. With a minimum echolocation click rate of 5 ms (Madsen et al. 2004a), the animal may click and follow the response, as the stimulus (the click) is occurring at a rate of 200 Hz, well within this animal's measured MRTF. However, echolocating animals are usually receiving an echo as a result of the click returning from the target. Field recordings have shown that foraging odontocetes in their final approach phase click up until they are 1 m from the target (Madsen et al. 2004a). Therefore, the click and echo additively traveled a distance of 2 m, which would take 1.34 ms considering the two-way travel time of sound in water and assuming a speed of 1490 m·s⁻¹. At this range the echolocating animal would hear a click and then the echo 1.34 ms, or 746 Hz, apart. Sounds presented at a rate of 746 Hz are well within the 1000 Hz, Risso's dolphin MRTF predicted temporal resolution. Judging from the MRTF, the Risso's dolphin should also be able to follow the clicks of these final foraging phase clicks if they are produced at a rate consistent with the published Hawaiian spinner dolphin burst pulses (Lammers et al. 2004). However, the animal may not have the ability to follow both the click and the returning echo within a burst pulse because here the clicks are being sent out faster than 1:1 processing allows.

The recovery time of the animal was of certain interest as well. This duration of the Risso's dolphin's single AEP response (Figure 2.7) was measured at 4.13 ms. However, the AEP consisted of a series of waves, each lasting 1-1.5 ms. Thus it
seemed that the animal’s auditory system may be able to recover from a single, short duration pulse of sound in 1-1.5 ms, or a series of pulses stimulating the auditory systems up to 1000 Hz rate, corresponding very well with the animal’s maximum MRTF values. This result is in accordance with the fact that AEP responses are measurements of compound neural activity (Kuwada et al. 2002). As stimulus presentation rates approach 1000 Hz, our measurement of the multi-wave response blends to reflect only the most prominent waves. However, this is likely an accurate estimation of the animal’s maximum auditory temporal processing because EFRs fall off at rates beyond 1000 Hz. Sounds modulating at a rate beyond 1000 Hz rate may only have an onset and offset response (Figure 2.8), i.e. a response to the sound stimulus turning on, and the sound stimulus turning off, and the click stimulus may not be followed. This indicated that if the animal were to produce sounds, such as echolocation clicks, at a rate less than 1000 Hz, it could easily follow the clicks.

If the animal were to click at a rate higher than 1000 Hz, which may occur in burst pulses, the animal may not follow individual clicks but rather the series of clicks as a single event. Sounds produced at a temporal rate greater than 1000 Hz would most likely reflect a response more like Figure 2.8 rather than Figure 2.2. Furthermore, the individual echoes from these types of events, where clicks are produced in an extremely rapid manner, may be physically masked by the initial click series event. Because burst pulse and terminal buzz clicks have not been measured at a rate of more than 1 per 1.5 ms it seems that in these extremely rapid sound producing cases, the clicks themselves should be followed reasonably, but the echoes
returning may overlap and thus be masked. Thus, at least in some cases, the animal may not need to detect the individual echoes of the click produced.

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References


CHAPTER 3

COMPARATIVE AUDITORY TEMPORAL RESOLUTION OF THE WHITE-BEAKED DOLPHIN, LAGENORHYPHUS ALBIROSTRIS

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Abstract

Adequate temporal resolution is required across taxa to properly utilize amplitude modulated acoustic signals. Among mammals, odontocete marine mammals are considered to have relatively rapid temporal resolution. However, multiple methods used to estimate auditory temporal resolution have left comparisons among odontocetes and other mammals somewhat vague. Here we present the estimated auditory temporal resolution of a white-beaked dolphin, *Lagenorhynchus albirostris* using auditory evoked potentials measured from simulated broadband click stimuli. Results were then compared to that of studies which used similar methodology to investigate the auditory temporal resolution of other odontocetes, marine mammals, and terrestrial mammals. The white-beaked dolphin evoked potentials followed rhythmic clicks up to a rate of approximately 1125-1250 Hz, after which the modulation rate transfer function (MRTF) cut-off steeply. The MRTF was similar in shape and bandwidth to that of other odontocetes. The estimated maximal temporal resolution of the white-beaked dolphins and other odontocetes was approximately twice that of pinnipeds and manatees, and more than ten-times faster than humans and gerbils. The exceptionally rapid temporal resolution abilities of odontocetes are likely due primarily to echolocation capabilities which require rapid processing of acoustic cues.
Introduction

Proper temporal processing of sound can be crucial for acoustic signal recognition and analysis across taxa. In certain crickets, amplitude modulated (AM) signals play a role in predator recognition (Fullard et al. 2005). Mate and competitors may be recognized by temporal cues in frogs (Rose et al. 1985). Song recognition is enhanced by proper acoustic temporal patterns in song birds and fish (Dooling and Searcy 1981; Myrberg 1986; Myrberg 1997). In some mammals such as macaques, detection and discrimination of AM signals plays an important role in within group communication (Moody 1994). In nearly all cases of deciphering temporally modulated signals, the prerequisite is that the animals must have sufficient ability to process signals up to a certain fluctuation rate.

Marine mammals provide an important case for auditory temporal processing studies because their auditory system must compensate for sound speed underwater, which is nearly five as fast as sound in air (Urick 1983). In addition to processing sound in water, odontocetes, or toothed whales and dolphins, have developed the ability to echolocate and thus have a need to process rapid clicks and subsequent rapid echoes, both of which are on the order of a few tens to a few hundreds of μs in duration and often only a few ms apart (Au 1993; Madsen et al. 2004). Because of the complexities of an underwater environment and echolocation abilities, odontocetes are often considered to have the fastest auditory processing abilities of any animal (Supin et al. 2001; Thomas et al. 2003).

One species of odontocete whose temporal resolution capabilities seem particularly intriguing is the white-beaked dolphin (Lagenorhynchus albirostris)
because it has been suggested that the white-beaked dolphin hears extremely high
frequencies, up to 250-300 kHz. (Mitson 1990; Rasmussen and Miller 2002). Recent
work demonstrates that although white-beaked dolphins do not hear quite as high as
predicted, they do hear high frequencies (up to 180 kHz) well (Nachigall et al. 2008).
High frequency hearing and corresponding peripheral auditory filter bandwidth is
associated with increased temporal resolution (Fay 1992; Supin et al. 2001). As a
general rule, the wider the filter band, as is typical at higher frequencies, the greater
the auditory temporal resolution. Dolphins and porpoises typically hear up to 150
kHz (e.g., Johnson 1967; Nachtigall et al. 2005) and it is suggested this high
frequency hearing sensitivity is related to the concurrent rapid auditory temporal
resolution (Supin et al. 2001; Mulsow and Reichmuth 2007). The same would
hypothetically hold true for white-beaked dolphins.

However, comparisons of temporal resolution across taxa can be confusing
because methods often vary, confined by the limits of experimental conditions and
thus, the scope of such evaluations must inherently be limited. For example, human
temporal resolution speeds may be referenced from 30-500 Hz based on whether the
response was determined behaviorally (Szymaszek et al. 2006), recorded from
cortical potentials (Kuwada et al. 1986), or measured from brainstem evoked
potentials (Purcell et al. 2004). In bottlenose dolphins, temporal resolution estimates
have varied from 1000 to 4000 Hz (1 – 0.264 ms) based on variation in stimulus type
and physiological versus behavioral estimations (Au et al. 1988; Dolphin et al. 1995;
Supin and Popov 1995).
One experimental method that demonstrates relative temporal resolution estimates across taxa allowing for robust comparisons is the use of sinusoidally amplitude modulated (SAM) tone stimuli and gauging responses with the auditory brainstem response (ABR). The stimuli may be presented in either click or SAM tone trains at varying presentation rates. At relatively low presentation rates, ABR responses correspond with each stimulus modulation, forming an evoked potential rate following response (RFR). The maximal rate that the subject follows forms the estimated temporal resolution of the subject (Supin et al. 2001). This method has been used in a variety of taxa including odontocetes (Dolphin et al. 1995; Supin and Popov 1995; Szymanski et al. 1998; Cook et al. 2006; Finneran et al. 2006; Mooney et al. 2006), manatees (Mann et al. 2005), pinnipeds (Mulsow and Reichmuth 2007), humans (Purcell et al. 2004), and gerbils (Dolphin and Mountain 1992). The methodological similarities allow for comparisons spanning echolocating marine mammals (dolphins), non-echolocating marine mammals (manatees and pinnipeds), humans and rodents.

The purpose of this study was two fold: 1) estimate the auditory temporal resolution of the white-beaked dolphin using SAM tones and the RFR and 2) compare the determined auditory temporal resolution with other marine and terrestrial mammals. This research was part of a larger study to measure the hearing range and sensitivity of the white-beaked dolphin.

Methods

Subject and experimental set-up
The study animal was a wild white-beaked dolphin (*Lagenorynchus albirostris*) caught-and-released within Faxaflói Bay off the coast of Keflavík, Iceland. During the months of July and August, groups of white-beaked dolphins were located in the calm bay waters and gradually approached by our 18-m modified fishing vessel, the *Hafborg*. The animals voluntarily bow-rode the vessel and twice during the research period, when a dolphin surfaced in front of the boat, it was hoop-netted, maneuvered into a dolphin-stretcher, and lifted via a hydraulic winch on board the vessel (Nachtigall et al. 2008). The dolphin was placed into a 1 x 1 x 3.7 m specially constructed plastic tank reinforced with a welded steel frame that was lined with 3 cm thick open cell mattress foam and filled with sea water. In this custom tank, the subject’s temporal resolution was measured.

This subject was an adult male, 223 kg in weight, 224 cm in length, with a girth of 139 cm. Upon capture, the animal was placed into the tank and the vessel was brought to the nearby harbor of Gardur for the hearing measurements. Conducting the experiment within this small harbor reduced water motion within the tank. The tank, lined with foam, was designed be acoustically isolated, limiting reflections that could occur in a small tank, thus the subject’s hearing thresholds could be measured in the free- and far-field and near ideal field conditions (Figure 3.1).
Figure 3.1. Experimental set-up picturing dolphin and acoustic tank. 1, projecting transducer; 2, active electrode (passive is on dorsal fin but hidden from view); 3, stretcher suspended from aluminum poles, note open flap around head and lower jaw; 4, acoustic tank lined with baffling open cell foam.

Sound stimuli were projected from an ITC-1032 transducer (resonance frequency = 38 kHz). The transducer was suspended from an overhead bar that stretched across the tank and secured at a position that was 80 cm from the animal’s rostrum and 115 cm to the approximate location of the animal’s ear, but near the foam tank wall. The transducer hung 30 cm below the water’s surface and in line with the subject’s head and lower jaw. The animal was positioned in the stretcher hanging
from two mobile steel suspension bars over the box. A large flap in the front of the stretcher, near the animal’s head, was unzipped in order to permit ‘free’ sound transmission to the animal’s head and lower jaw.

\[
\begin{array}{c}
\text{Relative amplitude} \\
0 & 0.1 & 0.2 & 0.3 & 0.4 & 0.5 \\
\end{array}
\]

\[
\begin{array}{c}
\text{Time (ms)} \ \\
0 & 5 & 10 & 15 & 20 \\
\end{array}
\]

Figure 3.2. (A) Waveform of single click stimuli, (B) Waveform of click train at 1000 Hz presentation rate.

**Acoustic measurements and stimuli**

Several days before the experiment began the tank was calibrated. The projecting transducer was placed in position and a calibrated reference hydrophone, a Reson 4034, was placed 1 m from the projector and at 30 cm depth. This position was determined to be the approximate location of the subject’s head and there was
little measurable variation in received levels within a few centimeters of the original hydrophone position. Stimuli were short, broadband pulses, 100 µs in duration with a peak frequency of 40 kHz but with a spectrum that ranged from 1-40 kHz and consisted of approximately 3 full cycles (Figure 3.2). Each of these pulses was transmitted in the tank and the received peak-to-peak voltage ($V_{p-p}$) was measured on the oscilloscope. Because of the brevity of the clicks, $V_{p-p}$ was used to calculate sound pressure levels (SPLs) and these sound levels were kept constant at 128 dB re: 1 µPa. Due to the extremely short nature of the clicks, reflections were highly unlikely and not observed. However, as a precautionary measure the received signals were simultaneously recorded to determine the spectrum and ensure that no competing signals or reflections existed. Noise level measurements were also calibrated and recorded to determine the spectrum level of the background noise (Figure 3.3).
Figure 3.3. Ambient tank-noise sampled at 1 MHz and analysis was made using a 1024 point FFT using a 5-point moving average.

The acoustic stimuli were digitally generated using a custom LabView program. The signal was then converted from digital to an analog signal with a National Instruments PCMCIA-6062E DAQ card (Austin, TX, USA) implemented into a laptop computer, using an update rate of 256 kHz. From the DAQ card, the stimuli were sent to a custom built signal shaping box which allowed for the stimulus level to be increased or decreased in 1 dB steps and from this box the signal was sent directly to the ITC transducer. An EZ OS-310M battery-powered digital oscilloscope was used to monitor the outgoing stimuli from the signal shaping box to the projecting transducer. Stimuli consisted of a series of pulses of varying modulation rates but the total pulse-series was always 19 ms long followed by 30 ms of silence. This presentation sequence prevented adaptation by the animal’s auditory responses. A total of 1000 pulse-series were presented for each modulation rate and the pulse modulation rate was varied from 125 to 3000 Hz, providing measurements at 14 different rates.

*AEP measurements*

Hearing measurements were collected using auditory evoked potential (AEP) responses to the pulsed stimuli. For each stimulus of an appropriate SPL and rate, there was a corresponding AEP response. Thus as the pulsed stimulus presentation was modulated from low to high rates a RFR could be measured and maximum
following rates could be used to estimate the animal’s AEP temporal resolution.

Responses were collected using two gold, passive electrodes embedded in latex suction cups which were attached to the animal. The electrodes were standard 10-mm EEG electrodes, the same type used for human EEG collection. The suction cups were easily placed on the animal at the beginning of each session with standard conductive gel. The active electrode was attached about 3-4 cm behind the blowhole, slightly off to the right and over the brain, while the reference electrode was attached on the dorsal fin. We chose the dorsal fin because there are few muscles and noise producing nerves in that location. The system was grounded to the water in the subject’s tank. The animal rested in the stretcher at the water’s surface with most of its head underwater to receive sound input through the major tissue routes to the ears (Mohl et al. 1999; Ketten 2000) while the suction cups, with the embedded electrodes, remained in the air to maximize signal strength.

The measured responses from the electrodes were amplified $\times 10,000$ using an Iso-Dam Biological Amplifier (WPI). Both the Iso-Dam and a Krohn-Hite Filter Model 3103 filtered the responses for anti-aliasing protection and noise reduction, using a bandpass of 300 to 3000 Hz. The amplified and filtered responses were transferred to an analog input using the same DAQ card in the same laptop computer and then digitized at 16 kHz using the same custom LabView program used for stimulus generation. Evoked potential records were recorded in 26 ms segments, beginning at the onset of the sound stimulus presentation. In order to extract the recorded AEP from noise 1000 samples were averaged per trial and each trial lasted less than 1 min.
Data analysis

To estimate the subject's response at each modulation rate, a 16-ms window of each average evoked response was fast Fourier transformed (FFT) for each modulation rate. The 256-point FFT provided response frequency spectra of the data where a peak reflected energy received, or the animal's physiological following response, at the respective modulation rate. Thus peaks were typically found in the FFT spectra at the rate at which the clicks were presented and larger peaks indicated a better "following" of that rate. The FFT peak value at each modulation rate was plotted relative to the modulation frequency to estimate the modulation rate transfer function (MRTF). This MRFT was then taken as an estimate of the subject's auditory temporal resolution. The RFR magnitudes were estimated by taking the square-root of the summed fundamental FFT peak plus its harmonics. Due to the difficulties of working with wild cetacean species, AEP data were based upon one individual. In order to ensure that our data were not strongly influenced by an individual difference, we compared the MRTF collected to that of other odontocetes. Analysis was conducted using EXCEL, MatLAB and MINITAB software.

Results

The observed AEP waveform of the white-beaked dolphin is typical of odontocetes and other mammals demonstrating several negative and positive peaks reflecting a series of neurological responses to acoustic stimuli (Figure 3.4). An onset delay was found for each AEP record, showing a period of time, usually 3-6 ms, from
the onset of the initial sound stimulus until the response was observed. When stimulus modulation rates were such that the subject’s auditory system could follow individual clicks, concurrent delays were also found between stimulus and AEP responses. Amplitudes of the AEP responses varied based on whether the response was to the first acoustic stimulus, later stimuli in a click train, or often the rate at which the click train was presented. Typically, the onset response (the first of several waves) was the largest, on the order of 1-2 μV. Later responses to later acoustic stimuli were usually less than 1 μV and on the order of 0.5 to 0.25 μV. Peak-to-peak amplitudes decreased exponentially as stimulus presentation rates increased ($r^2 = 0.93; p < 0.001; y = -0.97 \times \log(X) + 3.39; n=13$).
Figure 3.4. (A) Rate following responses in μV generated using 40 kHz pulses at four different modulation rates; 250, 625, 1000 and 2000 Hz, using a SPL of 128 dB re: 1 μPa. (B) 10 ms close-up of a selected white-beaked AEP waveform highlighted in (A).

The animal’s auditory system generally followed individual click stimuli at lower presentation rates. For example at 250 Hz, or 1 click every 4 ms, clicks were
presented at 0, 4, 8, 12 and 16 ms. The subject’s waveform response shown in Figure 3.4 reflects the animal following the individual stimuli (the last response was diminished). As presentation rates were gradually increased, the individual waveforms to each click stimulus began to blend together and become more sinusoidal in the ‘following’ of the individual click stimuli, exhibiting the typical envelope following response (EFR) shown in other odontocete auditory systems. This EFR could be seen in the AEP responses up until a rate of 1250 Hz. At higher rates, the animal’s AEP waveforms did not reflect following of individual clicks but rather, simply an onset response to the click train as a whole (as if it were one continuous stimulus).

Fast Fourier transforms of the EFRs provided similar indications of following responses. For example, the dolphin’s system followed the 1000 Hz click rate relatively well and therefore there was a strong peak in the FFT at 1000 Hz (Figure 3.5). When lower rate stimuli were used, the frequency spectrum revealed harmonics of the fundamental click rate. This is clear when using the 250 Hz click rate and peaks are evident at 500, 750, 1000, and 1250 Hz as well. At presentation rates of 1500 Hz and above, the dolphin did not follow individual clicks well and this was reflected by a lack of dominant peaks in the frequency spectrum.
Figure 3.5. Fourier spectra of the rate following responses at four different modulation rates; 250, 625, 1000 and 2000 Hz. At modulation rates of 250 and 625 Hz peaks can be seen at multiples of the fundamental modulation rate due to harmonics of the fundamental. Peaks at the fundamental frequency are indicated with a black arrow. Note the different y-axis scales for the response amplitude.

The dolphin's MRTF was low-pass in shape with peaks at 500-600 and 1000-1125 Hz (Figure 3.6). The MRTF was relatively broadband (1250-1500 Hz), with a rather steep high frequency cut-off after 1125-1250 Hz, reflecting high auditory temporal resolution.
Figure 3.6. (A) Comparative MRTFs of the white-beaked dolphin (solid line, diamonds) determined in this experiment, the Risso’s dolphin (dotted line, squares; Mooney et al. 2005) and the killer whale (dashed line, triangles; Szymanski et al. 1998) on a relative amplitude scale. (B) Modulation rate transfer functions of the
white-beaked dolphin (black line, diamonds) and an average of 7 odontocete species measured to date (grey line). (C) Modulation rate transfer functions of the white-beaked dolphin and the 7 odontocetes presented in B with SDs.

Discussion

White-beaked evoked potentials were very clear and distinct from the background noise despite the unique field situation for the data collection. Overall, the field methodology and consequent AEPs were found to be very similar to laboratory conditions of odontocete evoked potential recording (Nachtigall et al. 2007). The dolphin’s auditory temporal resolution was high (up to at least 1125-1250 Hz). The general characteristics of individual ABR waveforms and MRTF demonstrated results consistent with other species of odontocetes tested with similar methodologies.

The ABR waveform of the white-beaked dolphin, although basically similar, was somewhat distinct from that of other odontocetes in the series of waves generated by the click stimulus (Figure 3.4b). Odontocete ABR waveforms seem to differ slightly in the number, relative amplitude and overall pattern of negative and positive peaks (Supin et al. 2001). An ABR waveform consists of several waves. These waves are a summation of neurological responses from the general region of the “brainstem” in response to acoustic stimuli. It is logical that this pattern of waves may vary dependent upon subtle morphological of physiological differences among species. Unfortunately, reasons for this variation have yet to be thoroughly
investigated in marine mammals. Therefore, differences at this point are simply noted as species variation.

The MRTF was low pass in shape, indicating the following of individual clicks up until approximately 1250 Hz (Figure 3.6). At higher frequency modulation rates, the animal did not follow clicks as individual stimuli but rather as continuous stimuli, 19 ms in duration. This is supported by the sharp cut-off in the MRTF at 1500 Hz and the lack of “following” waveforms after the initial onset response. However, this RFR is predicted to be sufficient for the following of individual echolocation clicks and echoes (Mooney et al. 2006).

The maximum rates of white-beaked temporal processing estimated by the MRTF, near 1250 Hz, was relatively fast, but not exceptionally high, for odontocetes, as might be predicted by the extremely high frequency components of their echolocation clicks. That is, auditory filter bandwidths typically increase as the frequency of hearing increases, the higher the frequency, the wider the filter bandwidth (Yost 1994; Supin et al. 2001). White-beaked dolphin echolocation clicks contain significant energy at very high frequencies (200-300 kHz) (Rasmussen and Miller 2002) and it had been suggested, based on apparent reactions to very high frequency sounds, that white-beaked dolphins heard these very high frequencies (Mitson 1990). However, this does not seem apparent by auditory temporal resolution experiments here and is also not supported by the white-beaked dolphin audiogram (Nachtigall et al. 2008).

The white-beaked MRTF shape was very similar to other odontocetes including that of the Risso’s dolphin and killer whale (Szymanski et al. 1998;
Mooney et al. 2006), although the white-beaked MRTF is a bit higher in estimated processing frequency (Figure 3.6a). The data in all three of these studies were collected using essentially the same method.

For comparison of temporal processing within odontocetes, the white-beaked MRTF was plotted relative to a mean odontocete MRTF (Figure 3.6b and c). The mean odontocete MRTF was plotted using an average of 7 odontocete MRTFs collected using SAM tones or clicks (Supin and Popov 1995; Szymanski et al. 1998; Klishin et al. 2000; Cook et al. 2006; Mooney et al. 2006; this paper; *Pseudorca crassidens*, unpublished). All results compared were collected using similar AEP and SAM techniques. For comparison, all MRTF amplitudes were set to a relative linear scale of 0-1, where 1 was the maximum response provided in the original research. For points where the mean odontocete SDs appear large the reason is often due to relatively few points collected at that modulation rate (not all studies tested the same modulation rates). Based on the relative conserved shape of the MRTF between odontocetes, it appears that either temporal processing capabilities are comparable and conserved among odontocetes and/or the methods tend to reflect similar modulation rates. Both might well be true. At least in odontocetes, other physiological methods of measuring temporal resolution reflect similar results to SAM and AEP techniques (Dolphin et al. 1995). And SAM/AEP methods to determine temporal resolution might be constrained by the frequency spectrum of the ABR waveform (Supin and Popov 1995). However, the temporal resolution estimates calculated using the SAM/AEP technique correspond well to the processing speeds necessary to follow individual echolocation clicks and concurrent echoes.
(Mooney et al. 2006). The method also provides the important modulation rate for AEP audiograms.

Figure 3.7. Comparison of the mean odontocete MRTF with that of the mean pinniped MRTF (dotted line, asterisk's; Mulsow and Reichmuth, 2007), the manatee MRTF (solid line, circles; Mann et al. 2005), the gerbil (dotted line, triangles; Dolphin and Mountain 1992) and human (solid line, squares; Purcell et al. 2004) on a relative amplitude scale.

Relative to other non-echolocating marine mammals (pinnipeds and manatees), the mean odontocete MRTF is broader in bandwidth indicating greater temporal resolution (Figure 3.7) (Mann et al. 2005; Mulsow and Reichmuth 2007).
When temporal resolution bandwidth was estimated as the rate at which the response amplitude was 10% of the maximum response (Popov and Supin 1998), odontocetes, pinnipeds and manatees demonstrated bandwidths of 1200, 625 and 200 Hz, respectively. This indicates that odontocetes process AM sounds twice as fast as pinnipeds and nearly an order of magnitude faster than manatees. Some caveats should be considered as it is acknowledged that manatees have an unusual spike in their MRTF at 600 Hz (Mann et al. 2005). This response, although below the 10% cut-off, may reflect some temporal processing at surprisingly high rates. The MRTF bandwidth for pinnipeds may be higher if the response amplitude is plotted on a log scale, indicating a 10% (20 dB) drop at 750 Hz. However, if this adjustment was made for manatees and odontocetes as well, their estimated maximal temporal resolution would also increase. Thus it is safe to say that at least odontocete temporal resolution appears substantially faster than other marine mammals tested.

To put these high temporal resolution estimates in perspective with terrestrial mammals, the mean odontocete MRTF was compared to that of the gerbil and the human, with all three experiments using similar methodologies (Figure 3.7). Here, there is more than an order of magnitude difference in maximum temporal response, with the 10% drop for the gerbils and humans being 48 and 42 Hz, respectively (Dolphin and Mountain 1992; Purcell et al. 2004). The manatees and pinnipeds were certainly much higher than these values as well.

Even when correcting for higher pinniped MRTF values or keeping the 600 Hz point for manatees, it seems that odontocetes have very high relative temporal resolution likely as a function of three non-mutually exclusive reasons: (a) their wide
auditory filters at high frequencies (Mulsow and Reichmuth 2007), (b) adaptation to a fully aquatic environment (Supin and Popov 1995; Mann et al. 2005), and/or (c) echolocation abilities requiring discrimination of rapid clicks and echoes (Mooney et al. 2006). However, adaptation to an aquatic environment cannot be the sole reason for high odontocete MRTF values because the manatee is also exclusively marine with a much reduced temporal resolution. Obviously, the odontocete family took a quite different evolutionary path and likely the evolution of echolocation has played a significant role in temporal processing abilities. Similar fast temporal processing is seen in echolocating bats (Surlykke and Bojesen 1996; Wiegrebe and Schmidt 1996).

The high frequency hearing is a reasonable hypothesis for greater temporal resolution but it does not hold for all cases. The gerbil hears well for high frequencies, more than 40 kHz higher than a human at a 60 dB (re: 20 μN/m²) threshold, but a gerbil’s temporal resolution is similar to humans (Ryan 1976; Dolphin and Mountain 1992; Purcell et al. 2004). Thus, at least echolocating marine mammals have greater temporal resolution than non-echolocators and terrestrial mammals. Non-echolocating marine mammals (e.g. pinnipeds and manatees) are a more complex story because they apparently have greater temporal resolution than some but not all terrestrial mammals (Mulsow and Reichmuth 2007). Thus, it seems that echolocation in odontocetes is the major driving factor related to very high temporal resolution capabilities.

Two groups not presented here but which would make interesting MRTF candidates are the porpoises and mysticetes. Porpoises are echolocators but typically use a narrow-band pulse (Au et al. 1999) and have narrow critical bands overlapping
the frequency of their pulse (Popov et al. 2006). This suggests high frequency resolution but reduced temporal resolution abilities for odontocetes. Mysticetes are intriguing because they are non-echolocating cetaceans and we know very little about their hearing capabilities, so their temporal resolution is a mystery. There is obviously much to be gained by increasing our hearing and temporal resolution examinations to a broader spectrum of species and using similar techniques provides greater power to comparable data.

Acknowledgements

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References


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CHAPTER 4

HEARING PATHWAYS AND DIRECTIONAL SENSITIVITY OF THE BELUGA WHALE, *DELPHINAPTERUS LEUCAS*

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Abstract

Odontocetes are believed to receive sounds primarily through the pan bone region of the lower jaw although much variation in jaw morphology exists among species. In order to further examine this jaw hearing hypothesis we tested the head receiving sensitivity and directional hearing of a beluga whale, *Delphinapterus leucas*. Hearing thresholds were measured using auditory evoked potentials (AEPs). The subject proved to have highly directional hearing for far-field click stimuli similar to that of bottlenose dolphins and more directional than the harbor porpoise. For near-field jawphone stimulation, the beluga’s lowest thresholds were found when click stimuli were presented at the rostrum tip (76 dB re: 1 μPa) although thresholds from the pan bone region stimulation were only 2-3 dB higher. Stimulation at and behind the external auditory meatus were elevated by nearly 20 dB. Stimuli presented at the surface of the melon did not generate detectable AEP responses, although sound levels of up to 142 dB were employed. Latencies of responses were generally shortest for meatal stimulation and increased with distance. Results support a shaded receiver model for odontocete hearing but how received sounds are filtered and shaded may depend on species. We also suggest that odontocete hearing thresholds are not necessarily lowest through the pan bone region. Rather, hearing pathway variations appear to exist among odontocete species and are at least partially dependent on head morphology.
Introduction

Directional auditory sensitivity and sound source localization are crucial aspects of hearing used across taxa to identify the position of predators, prey, and conspecifics. Odontocetes (toothed whales and dolphins) have sensitive underwater hearing and use sound to orient in the marine environment, including localizing sound sources in three dimensional space. Determining the direction and source of a sound can be vital in locating other individuals and localizing targets during echolocation.

The directionality of odontocete hearing has been investigated using a variety of experimental methods, including measuring receiving beam patterns (Au and Moore 1984) and minimum audible angles (Renaud and Popper 1975), examining variation based on frequency (Supin and Popov 1993), localizing sensitivities (Mohl et al. 1999) and computational modeling (Branstetter and Mercado 2006). These studies have revealed a sophisticated hearing system that uses fine scale binaural time difference cues, spectral filtering and amplitude shading to ascertain source positions within an aqueous environment where sound travels rapidly.

Most studies on odontocete hearing and directionality have focused primarily on one species, the bottlenose dolphin, *Tursiops truncatus*. We know much less about the hearing directionality of other species of odontocetes. While auditory structures appear relatively conserved among odontocetes, subtle differences may affect hearing directionality and sound localization. When other odontocete species' auditory capabilities are investigated we often find unique results. For example, the harbor porpoise, *Phocoena phocoena*, has a relatively wide receiving beam, wider than that of the bottlenose dolphin (Kastelein et al. 2005). Kastelein et al., suggested
that although this may provide the porpoise a slightly lower signal-to-noise ratio, a broad receiver allows for predator detection and environmental cues from many angles. They also proposed the difference between the porpoise and dolphin hearing directionality was based on (head and body) morphology. More recent investigations in another odontocete, the Cuvier’s beaked whale (*Ziphius cavirostris*), have revealed fine scale anatomical differences in their auditory system, speculating adaptations in how sounds are received may be species or even sex related (Cranford et al. 2008a; Cranford et al. 2008b).

Acoustic directionality and localization is a function of several available cues including differences in sound amplitude, time of arrival, phase, and frequency components differing between the two receivers, the ears. While hearing anatomy is largely similar between odontocetes, there are apparent slight differences. These differences may affect how sound is filtered, shaded or processed, causing some variation in hearing directionality among odontocete species. To better understand this we must investigate how different species receive sounds.

The beluga whale, *Delphinapterus leucas*, is an ideal subject species to investigate the variation of odontocete hearing directionality for several reasons. First, prior studies have established baseline auditory information for the beluga including the audiogram, masked hearing thresholds, temporary threshold shift phenomena and auditory filter shapes (Aubrey et al. 1988; Klishin et al. 2000; Finneran et al. 2002).

Second, belugas are unlike other odontocetes in that they do not have fused neck vertebrae (Reynolds and Rommel 1999) providing the ability to easily turn their
head toward a sound source. In terrestrial mammals, turning toward a sound source is an important localization behavior as it allows the use of the pinnae to ‘filter’ high frequencies and thus use spectral cues to determine sound directionality (Butler 1975; Butler 1986). Although cetaceans have lost their external pinnae, they likely use the morphology of the head to shadow and filter frequencies and help localize sounds (Ketten 1997; Ketten 2000). Previous research has shown that bottlenose dolphins and belugas have directional sensitivity (Au and Moore 1984; Klishin et al. 2000). However, these studies did not measure sensitivities beyond 105° from the animals’ azimuth midline axes, despite the fact that odontocetes probably use hearing in all directions. The only study to conduct such methodology used a harbor porpoise as a subject, finding it unexpectedly broadly directional for localization purposes (Kastelein et al. 2005). Based on their ability to turn their head (Reynolds and Rommel 1999) and preliminary directionality studies (Klishin et al. 2000), it appears that a more detailed study of beluga hearing might reveal relatively narrow directional hearing.

Finally, belugas do not have a protruding rostrum and lower jaw, as found in dolphins. The best supported hypothesis of an odontocete sound receiver is the use of the lower jaw (Kobler et al. 1992). Sound is thought to enter the head through fat bodies of the lower jaw which have an impedance close to that of sea water (Varansi and Malins 1972; Koopman et al. 2006). The dolphin lower jaw ends in a thin bony plate termed the pan bone which sound passes through at the proximal end where the bone is relatively thin. Internal mandibular fat bodies then likely conduct the sound to the bony ear complex although it is not yet established how sound is actually
transmitted into the auditory bulla (Kobler et al. 1992; Ketten 2000). This lower jaw hearing hypothesis has been supported by several studies demonstrating that thresholds are lowest when a localized sound source is placed near the pan bone region of the bottlenose dolphin lower jaw (Bullock et al. 1968; McCormick et al. 1970; Möhl et al. 1999). While it is likely that odontocetes generally receive sound in this manner, there are obvious differences in head morphology across species. This may tailor niche-related subtle differences in how sound is received, for example the point of maximal jaw sensitivity. Examining differences in sound reception in odontocetes other than the bottlenose dolphin remains unexplored.

In this study the auditory evoked potential (AEP) method was utilized to address questions of beluga hearing sensitivity and directionality. The AEP technique provides a means to investigate the hearing of odontocetes both rapidly and passively (Nachtigall et al. 2005; Mooney et al. 2006; Nachtigall et al. 2007). Measurements can be made with minimal or no animal training and therefore allow more questions to be addressed. A preliminary audiogram was established to determine the subject’s baseline hearing. Thresholds were then measured up to 180° relative to the animal’s anterior-posterior azimuth midline axis to evaluate directionality of hearing. Finally, regions of best sensitivity were examined across the head of the whale.

Methods

Subject and timeline

The subject of this study was Yulka, a nine-year-old adult female beluga whale (*Delphinapterus leucas*) housed at l’Oceanogràfic marine park, Valencia,
Spain. The animal had been at the facility for three years, was 3.73 m in length and weighed approximately 600 kg. Yulka’s facilities included four separate connecting pools, two of which were public display areas, and a total water volume of 3582 m$^3$ with 800 m$^2$ of water surface. The pools were filled with cooled, filtered saltwater pumped in from the nearby Mediterranean Sea. Data sessions were conducted for 6 continuous days from April 29-May 4 2007 in three experimental situations: a) a baseline audiogram, b) thresholds of broadband clicks at three azimuth angles, 0°, 90°, and 180° and c) click thresholds at 5 jawphone source positions on the animal’s head. The first two experiments generally overlapped in procedure and thus the methods are explained together with any differences highlighted. The jawphone source position experiment, which examined relative sensitivity across the whale’s head, differed slightly in methods so it is explained separately. Within the results and discussion, the three experiments are presented in separate sections. These experiments required the cooperation of a well-trained subject and consequently this investigation was limited to one experimental animal. In order to ensure that our data were not strongly influenced by an individual difference, we compared the baseline audiogram collected to that of other beluga whales and odontocete cetaceans to demonstrate the subject heard normally.

**Experimental set-up**

Measurements were made in the rear of the large exhibition pool which was the largest beluga pool in the park that had a volume of 2,699 m$^3$ and was 5 m in depth (Figure 4.1a). Although asymmetrical in shape, the tank was approximately 25
m in diameter. Two columns in the pool supported the dome shaped roof of the facilities. All wall and column surfaces were irregular and were created to imitate the look of an ice environment but were made of concrete covered by white and blue epoxy. The size of the facilities and irregular wall shape made for a relatively free-field environment with limited interfering acoustic reflections from the sides of the tank. The tank water returned to the filtration system by four skimmers on the sides of the tank. These skimmers produced a constant low frequency noise that had peak values (107 dB re: 1 μPa RMS) in the range of 450 – 650 Hz but dropped to the measurable noise floor by 10 kHz. To record the pools total background noise, ten 1-s noise files were recorded using a custom LabView program and National Instruments PCMCIA-6062E DAQ card (Austin, TX, USA) implemented into a laptop computer. The ambient sound was collected using a Reson 4040 hydrophone (Slangerup, Denmark) connected to a Krohn-Hite filter (Brockton, MA, USA) which was connected to an NI SCI-68 break-out box and the DAQ card. Noise files were sampled at 450 kHz. The filter amplified the incoming records by 20 dB and provided a low pass filter at 200 kHz to prevent aliasing, although the resonance cut-off of the hydrophone was approximately 100 kHz. Background levels were then referenced using an 8 kHz tone calibrated at 119 dB re: 1 μPa RMS. The ten noise files were compared to ensure no extraneous signals were present, however only one file was plotted (Figure 4.1b). Ambient noise proved to be low, below the sensitivity of the acoustic recording equipment (68 dB re: 1 μPa^2 Hz^-1) at frequencies greater than 10 kHz. Below 10 kHz a low level of background noise was apparent although generally not of concern because frequencies of interest were 8 kHz and higher.
Low-noise situations such as these are valuable situations for conducting absolute hearing threshold measurements (Au et al. 2002).

Figure 4.1. (a) Experimental set-up at the rear of a large exhibition pool. 1, rope strung above the pool; 2, transducer at 0° azimuth plane, hung from the rope and directly in front of beluga; 3, trainers station; 4, beluga whale in hoop; 5, nearest skimmer; 6 and 7, directionality experiment’s transducer positions at 90° and 180°, respectively, in the azimuth plane relative to the subject’s anterior-posterior midline axis. (b) Tank background noise plotted recorded using a Reson 4040 hydrophone.
and custom built LabView program that implements a 6062E NI DAQ card in a laptop computer. Noise was sampled at 450 kHz and analysis was made with a 1024 point FFT using a 10-point moving average and plotted in dB re: 1 μPa²Hz⁻¹.

During the experiment the animal was stationed at the water's surface, 1.5 m parallel to the pool wall located to her left. The nearest walls on other sides were 4.5 m behind the animal 30 m in front and 15 m to the right, with a pillar half the distance to the wall. The animal was trained to hold a constant position by stationing in a hoop and touching its melon to a foam target placed in front of the hoop. Because belugas have an extremely flexible neck, the target and hoop were used to ensure that the subject kept her head and body still and facing the transducer. The trainer sat on a wooden platform above and to the left of the whale to closely observe the animal and assume that she maintained a consistent position. During audiogram measurements the projecting transducer was hung from a line that was placed across the pool so that the transducer was 2.15 m in front of the beluga and at a depth of 30 cm. For the directionality experiment, the transducer was suspended similarly at 0°, but for 90° and 180° the transducer was suspended from a pole and at distances of 2.15 and 3.8 m, respectively.

Acoustic signals and calibration

All signals were calibrated prior to the experiment. The projecting transducer was hung 2.15 m in front of the animal's hoop position, or in the case of the directionality experiment, signals were projected from the 0°, 90° and 180° respective
azimuth positions (Figure 4.1a). A receiving hydrophone was positioned at 30 cm depth in front of the hoop at the estimated position of the animal's ears. Two transducers were required to project the underwater stimuli: an ITC-1032 (Santa Barbara, CA, USA) was used to project lower frequency tones from 8-32 kHz and a Reson 2130 for higher frequency tones (from 50-128 kHz) and clicks. The receiving hydrophone was a Reson 4040 positioned 1.5 m from the tank wall. The projecting transducers were either 1.5 m (when directly in front of or behind the animal) or 3.65 m from the tank wall (when 90° to the animal's right). The acoustic signals were sinusoidally amplitude modulated (SAM) tones and a 100 µs click, centered at 80 kHz (-3 dB from 91-68 kHz). The calibrated signals were the same stimuli as those presented to the whale during the hearing tests. The synthesized click was specifically designed to optimize a region of the subject's best sensitivity and reflect the prominent energy found in the animal's echolocation click (Castellote and Fossa 2006). Received sound levels were calibrated at 11 frequencies which were later used to test the animal's basic hearing: 8, 11.2, 15, 23, 32, 50, 70, 80, 90, 100, and 128 kHz. Each of the SAM tone sine waves was transmitted in the tank and the received peak-to-peak voltages ($V_{pp}$) were measured with the calibrated hydrophone. This $V_{pp}$ was converted to peak-equivalent root-mean-square voltage (peRMS) by subtracting 15 dB. The peRMS was taken as the RMS voltage and used to calculate the sound pressure level (SPL) for that frequency (dB re: 1 µPa). Sound pressure levels of the clicks were measured using $V_{pp}$ as is standard to measure odontocete click intensities due to the inherent brevity of the signals (Hamernik et al. 1993). The sound levels of the clicks and SAM tones were related by integrating the $V_{pp}$ over the duration of the
respective signals to provide the energy flux density of the signals (dB re: 1 μPa²·s⁻¹) (Au et al. 2002). Signals projected from the 90° and 180° azimuth positions for directionality experiment were calibrated in the same manner but from their respective transducer positions.

The waveform of the received signals was viewed with a Tektronix TPS 2014 oscilloscope (Beaverton, OR, USA), to confirm that there were no competing reflections produced from other signals or reflections in the tank. In this environment there were no constructing or destructing interferences observed with the transmitted signal. Had these sorts of interferences been present they would have been apparent in SAM reflections on the oscilloscope screen; they would also have been extremely unlikely because of the transient properties of the short SAM tone-bursts.

For the hearing tests, acoustic stimuli were digitally created using a custom LabView program and DAQ card installed in a laptop computer. Both stimulus types, clicks and SAM tones, were repeated or modulated at a rate of 1000 Hz, the previously determined effective rate for beluga whales (Klishin et al. 2000). Stimuli were presented in 19-ms stimulus trains and alternating with 30 ms of silence and presented at a rate of 20 s⁻¹. Signal trains were played 1000 times, thus each trial lasted approximately 50 s. Lower frequency stimuli (8-32 kHz) were synthesized using an update rate of 256 kHz while those of 50 kHz and above, as well as the clicks, had an update rate of 512 kHz. The signals were sent from the computer to an HP-Attenuator 350D that could attenuate in 1-dB steps. The oscilloscope was used to monitor the outgoing stimuli from the attenuator to the projecting transducer.
**AEP Measurements**

Auditory evoked potentials were collected using passive, gold EEG electrodes imbedded in custom-built latex suction cups. The electrodes were standard 10-mm EEG electrodes, the same type used for human EEG collection. The suction cups were placed on the animal at the beginning of each session with standard conductive gel. The active electrode was attached about 3-4 cm behind the blowhole, slightly off to the right and over the brain. The reference electrode was attached posterior to the active electrode, on the animal’s back, and near the third ground electrode. Placement of the active electrode proved quite challenging as the mobile head and skin surface of the beluga allowed the animal to easily dislodge the suction cup. Thus the first research session was dedicated to determining the best region for cup placement in regard to a position where it would not be displaced and yet still received the best AEP signal. The optimal position proved to be several cm behind the blowhole but just anterior to creases from the beluga’s neck. For the audiogram and subsequent directionality experiment the animal rested at the surface with its blowhole and the electrodes remaining out of the water, while most of its head was underwater. This configuration maximized data collection efficiency and AEP signal strength.

The electrodes were connected to a Grass CP511 bio-amplifier and filter (West Warwick, RI, USA), set to amplify the AEPs by 10,000x and filter the responses between 300 and 3000 Hz. The responses were then run through a Krohn-Hite 3384 filter with the same filter settings to further protect against aliasing. The amplified and filtered responses were transferred to an analog input of the same DAQ.
card in the same computer and digitized at 16 kHz. In order to extract the AEPs from noise, 1000 response records were collected and averaged for each trial (one frequency and SPL). Each AEP record was 26 ms in duration and began simultaneously with stimuli presentation.

Threshold measurements and AEP analysis

The procedure was identical for all threshold measurements and each threshold was measured once. Before each session, a carrier frequency or click was selected as the stimulus and the initial SPL of the stimulus was determined for the first trial. For the following trials stimulus intensity was determined on the AEP responses in the prior trials but generally SPL’s were decreased in 5-10 dB steps between trials until no response was visible for 2-3 trials. Each threshold measurement took approximately 5-10 min and 2-4 thresholds were collected each session. Usually 2 sessions were conducted each day. Frequencies and start intensities for the audiogram were determined by referencing a previous beluga AEP audiogram paper (Klishin et al. 2000). Click start intensities were determined by the subject’s measured audiogram thresholds at the click center frequency (80 kHz). Stimulus intensity levels began 20-30 dB above the estimated threshold values. An average of 7 intensity levels were presented for each of the 19 different thresholds measured.

In odontocetes, a clear and defined SAM tone or click train produces an AEP response that ‘follows’ the envelope of that stimulus. This response has been termed the envelope following response (EFR) (Supin et al. 2001). In this experiment a 16-
ms portion of the EFR was fast Fourier transformed (FFT) for each frequency and intensity level (Figure 4.2a). This window contained a whole number of response cycles. The 256-point FFT provided a response frequency spectrum of the data where a peak reflected the energy received or the animal’s physiological response to the 1000 Hz modulation rate. A larger EFR response was reflected as a higher FFT peak value. The peak FFT amplitude at the modulation or repetition rate was used to estimate the magnitude of the response evoked by the SAM stimulus.

![Figure 4.2](image)

Figure 4.2 (a) Fourier transform of the envelope following responses measured using 11.2 kHz SAM tones as the carrier frequency, a 1000 Hz modulation rate and stimulus intensities from 90 to 58 dB re: 1 μPa. Sound pressure levels of stimuli are labeled in dB indicating their corresponding AEP response spectra. (b) Plot of the peak value of each Fourier spectra at the 1000 Hz modulation frequency (solid line-
diamonds) for each SPL presented and best fit regression (dotted line-open circles) used to determine the threshold at 11.2 kHz.

For each of the frequencies or projecting transducer placements, the FFT peak at each stimulus intensity level was plotted as response intensity as a function of the SPL of the stimulus (Figure 4.2b). A linear regression addressing the data points obtained was hypothetically extended to zero, the theoretical point where there would be no response to the stimulus. This zero point had to be extrapolated because of the low level of biological electrical noise always present in the records that would mask the actual zero point. However, by estimating the zero response level it was possible to predict the threshold for each frequency and transducer placement presented to the animal. Analysis was conducted using Excel, Matlab, and Minitab software.

*Jawphone presented stimuli*

To measure head relative sensitivity to click stimuli we used a custom built jawphone transducer. The piezo-ceramic transducer element was imbedded in a latex suction cup that was easily and gently attached to the beluga’s skin. The transducer’s frequency response was from 40-100 kHz. The jawphone was calibrated in the free- and far-field at standard 1 m distance from a receiving hydrophone. Click SPLs were determined by \(V_{pp}\) in the manner previously described. In this way thresholds from jawphone measurements could be compared to far-field thresholds while recognizing the differences between free-field and contact measurements (Cook et al. 2006; Finneran and Houser 2006).
The subject’s head and the jawphone were kept out of the water to ensure that the surface of the jawphone was the only sound pathway to the animal. Therefore, the animal was stationed in varying positions, from positions identical to the previous measurements (for melon placement), to lying on its side for pan bone, meatus and behind meatus placements, and then to holding vertical in the water column with its head out of the water for the lower rostral jawphone placement. Electrode placements could generally be kept constant except when the animal was vertical. When the animal was vertical, the reference and ground electrodes were moved anteriorly to keep all electrodes out of the water. Moving the ground and reference electrodes to comparable locations had no effect on threshold determination, based on initial measurements establishing the best electrode placements. Similar results have been established in prior experiments which demonstrate that if the recording electrode is kept constant, moving the reference and ground have minimal effects on AEP magnitude or latency (Beattie et al. 1986; Finneran and Houser 2006; Houser and Finneran 2006).

In order to determine the regions of ‘best’ response, two primary variables were analyzed. The first was the relative threshold of response at the varying positions. The second variable was latency of the peak response. These were measured by establishing the time (ms) between stimulus onset and the point of maximal change in neuronal firing. This was determined by measuring the time to the rising front of the most prominent peak (IV). Generally this was measured for the first 2-3 AEP responses, i.e., the responses to the maximal SPL presented and 1-2 attenuation levels below because peak IV was unambiguous at these levels.
Results

The baseline audiogram in the free field revealed that the subject had quite sensitive hearing with thresholds below 60 dB re: 1 μPa between 32 and 80 kHz and below 70 dB at 11.2 and 90 kHz (Figure 4.3; Table 4.1). As is typical of odontocete hearing thresholds they increased gradually at lower frequencies (< 32 kHz) and more steeply for higher frequencies to the least sensitive threshold measured of 102.8 dB at 128 kHz. The whale also had a distinct notch in the audiogram at 50 kHz.

Table 4.1. Auditory evoked potential (AEP) thresholds of a beluga whale in dB (re: 1 μPa).

<table>
<thead>
<tr>
<th>Frequency (kHz)</th>
<th>Threshold (dB)</th>
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<tbody>
<tr>
<td>8</td>
<td>90.2</td>
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<tr>
<td>11.2</td>
<td>68.5</td>
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<td>15</td>
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<td>75.0</td>
</tr>
<tr>
<td>128</td>
<td>102.4</td>
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Figure 4.3. AEP audiogram of the beluga whale subject stationed at the surface.

Thresholds in dB (re: 1 μPa) were measured from 8 to 128 kHz using SAM tones.

*Directional sensitivity*

Thresholds were also measured using far-field broadband clicks with the source placed in several positions. Directly along the anterior azimuth midline, with the source directly in front of the animal, click thresholds were measured at 85 dB (Figure 4.4). The threshold at 90° relative to the midline and the animal's ears was 105 dB, dropping off 20 dB from along the animal's anterior/posterior axis. At 180°, or directly behind the animal, the threshold was an additional 9 dB higher or 114 dB.
Figure 4.4 (a) Response thresholds to click stimuli presented at azimuth angles of 0°, 90° and 180° where 0° and 180° are along the anterior-posterior midline and 90° is at a right angle to the beluga's external auditory meatus. Thresholds are presented in dB (re: 1 µPa) and click levels were measured in $V_{pp}$ and stimuli were presented in the far-field. (b) Sketch of the directivity experiment set-up. Indicated are the distances (both for the calibration and the experiment) from the transducer to the whale’s ears.

Received sensitivities from jawphone stimuli

The beluga's sensitivity and AEP latency were measured at various locations on the animal’s head (Figure 4.5a) using a contact jawphone for stimulus presentation. The same broadband clicks as used in the directionality experiment
were also presented – but they were presented via the jawphone. The region of maximum sensitivity (76 dB) was found to be at the tip of the lower jaw (2) of the animal (Figure 4.5b). The pan bone area (3) was found to have a slightly higher but similar threshold (78 dB). Sensitivities dropped off considerably at the position of the external auditory meatus (4) and 12 cm behind the meatus (5), with thresholds of 92 and 100 dB respectively. Interestingly, no response at all could be detected when the jawphone was placed on the whale’s melon (6), despite SPLs of 142 dB presented to the animal. This lack of response from the melon presentation was further demonstrated when the subject briefly lowered her melon and thus the jawphone into the water during the trial at 130 dB (Figure 4.6). A response was immediately detected by a peak developing in the FFT at the modulation frequency of 1 kHz. The trial was then quickly stopped. When the 130 dB trial was repeated, ensuring that the jawphone remained out of the water, no response was detected and the FFT reflected a minimum at the modulation frequency.
Figure 4.5. (a) Diagram of beluga’s head for AEP recording with points of stimulation indicated. 1, location of active AEP electrode; 2, rostrum tip; 3, pan bone; 4, external auditory meatus; 5, behind meatus; 6, melon. (b) Response thresholds to click stimuli based on 5 different jawphone placements. Thresholds are presented in dB re: 1 μPa using p-p SPLs measured at 1 m.
Figure 4.6. Fourier transform of beluga EFR when click stimuli were presented from the melon. Bold line with peak at 1 kHz reflects when jawphone was dipped into the water and indicates subject's auditory system heard and was following the clicks. The finer line was when the jawphone remained out of the water for the entire record and indicates the animal did not detect the click stimuli. Both situations are indicated on the graph. Stimuli for both records were presented at 130 dB.

In order to determine the latency of the AEP response the precise waveform characteristics had to be identified. The 19 individual near-sinusoidal EFR waves produced from higher intensity stimuli were examined. By counting backwards from the last wave (found at approximately 25 ms after stimulus and recording onset) it was possible to determine the four initial AEP waves that were a response to the onset stimulus (Figure 4.7a). These four initial waves and the subsequent EFR were similar to those of a previously measured beluga whale AEPs (Klishin et al. 2000). After the
initial waves were identified, their latencies were measured from stimulus onset. In this manner, AEP latencies were determined for the four locations in which the jawphone generated AEP responses. The latency of only the three largest waves (II, III and IV) were measured and only at relatively higher stimulus intensities in order to avoid ambiguous measurements of responses that were close to background noise levels. Because they showed the largest response (up to 1 \mu V), the patterns of wave IV were considered the most faithful measure of AEP latency, although all waves measured showed the same general trend. Minimum latencies for all three waves were found when the jawphone was placed at the external auditory meatus or 12 cm behind the meatus \textit{(Figure 4.7b)}. Latencies increased at pan bone and rostrum tip placements respectively. For wave IV, response latency was shortest from the meatus at 6.5 ms. Waves II and III had minimum latencies of 4.1875 and 5.375 ms respectively, measured from 12 cm behind the meatus. Maximum AEP response latencies were all measured during rostrum tip stimulation and measured 4.875, 5.1825 and 6.75 ms for waves II, III and IV respectively. The difference between maximum and minimum latency was quantified for each wave. The greatest difference in latency duration was 0.6875 ms, for Wave II, measured as the difference between rostrum tip stimulation and 12 cm behind the meatus. Wave IV had considerably less variation in latency differences (0.25 ms) between stimulation points, found between the meatus and rostrum tip.
Figure 4.7. (a) Four initial AEP (I-IV) waves and succeeding EFR to click stimuli presented at the pan bone region using a SPL of 105 dB re: 1 μPa. Waves II, III and IV were used. (b) Latency of response (ms) to various jawphone locations using the three response waves of greatest amplitude, II (bottom-dashed line), III (middle-dotted line) and IV (top-solid line).

Discussion

Audiogram

The free-field AEP audiogram revealed beluga whale thresholds of greater sensitivity than previously published. Although hearing thresholds of the beluga have

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been reported before, previous studies found differing results. Either beluga thresholds were shown to be less sensitive ($n = 1$; Klishin et al. 2000), the study’s limited focus to lower frequencies ($n = 3$; Aubrey et al. 1988), or the research was not published in peer-reviewed journals ($n = 2$; White et al. 1978). However, these differences in thresholds were minor and likely reflect a combination of individual (i.e. Klishin et al.,) and methodological differences (e.g. a focus on lower frequencies). Further, of the two published audiograms that encompass low and high frequencies (Klishin et al. 2000; this study), both are AEP audiograms. This seems to highlight the relative emphasis on AEPs for current marine mammal hearing work. If the audiogram here is compared to the audiogram by White et al., the thresholds actually track each other very closely. Both studies found overall low thresholds, near 45 dB for some frequencies and a steep high-frequency cutoff near 100-128 kHz. The two studies also revealed two highly sensitive regions ($< 60$ dB), a lower frequency region centering near 32 kHz and a higher frequency region from 70-80 kHz. Between these frequency bands, both studies found a clear notch at approximately 50 kHz. Unfortunately, while the data of White et al. is the first report of beluga thresholds and reflects the audiogram presented here, their data were only published as a technical report thus caution is required when considering the results. Additionally, whether this notch is found in all belugas cannot be certain, but to be observed in three of the four animals for which there are complete audiograms was intriguing.

*Directional sensitivity*
Klishin et al. (2000) measured the thresholds of a beluga as a function of sound source azimuth using tone pips and clicks from 0-105° difference from the animal’s anterior-posterior azimuth midline. Thresholds were found to be elevated by approximately 20 dB at 90° regardless of stimulus. Their results were quite similar to our study which used broadband clicks and found a 20 dB increase in thresholds from 0° to 90°. Continuing to an azimuth of 180°, or along the anterior-posterior midline but behind the animal, the threshold dropped an additional 9 dB. This near 30 dB drop in sensitivity from anterior to posterior along the animal’s midline is quite dramatic and reflects a highly directional hearing system.

This is in contrast to the harbor porpoise, the only other odontocete in which 180° sensitivity has been measured (Kastelein et al. 2005). These porpoise thresholds were measured using frequency modulated tones (16, 64, and 100 kHz) in a 360° horizontal plane around the porpoise. The animal appeared to have greater relative sensitivity at higher angles (less directional) with sensitivities dropping off by no more that 15 dB from 0° to 180° regardless of the frequency tested. This suggests that the mechanisms for producing directional hearing may not work as well for smaller odontocetes (e.g. porpoises or perhaps calves) with shorter distances between receivers (i.e. the ears or jaw fats). A larger distance between receivers may provide a more directive beam, especially at higher frequencies where phase, amplitude and spectral differences, potential cues for sound source localization, are likely greater. The beluga’s body size may also have shadowed the clicks somewhat indicating body and orientation play important roles in directionality. Similar shadowing by the head and body has been demonstrated in terrestrial mammals and plays a greater role at
higher frequencies which are more easily shaded (Brown 1994). The same is likely true for marine mammals and supports why odontocetes have greater directional hearing at higher frequencies (Au and Moore 1984; Klishin et al. 2000; Kastelein et al. 2005). Thus a better examination of beluga hearing directionality across frequencies and angles would test this hypothesis.

In order to map the bottlenose dolphin receiving beam pattern in front of the animal, Au and Moore (1984) used 2-s tones of 30, 60, or 120 kHz up to 90° in the horizontal plane, although not all frequencies were tested at all angles. At 90° and 30 kHz, thresholds dropped only 10 dB. Higher frequencies appeared to be narrower in receiving beam thresholds however, they were not measured beyond 50° thus it is difficult to draw conclusions. Higher relative thresholds at 90° for the beluga may indicate that it is more directional in its hearing than the dolphin. Unfortunately, with limited data these comparisons are purely speculative.

Based on the results of this study, the beluga seems to have more directional hearing than the harbor porpoise and potentially similar directionality for the bottlenose dolphin. It is possible that the unfused vertebrae, and thus the highly movable head, of the beluga have allowed for adaptations of highly directional hearing. The ability to move and rotate the head has resulted in good directional hearing in terrestrial mammals because sound localization is enhanced by turning toward the source (Brown 1994). The size and/or shape of the beluga body and head are considerably larger than that of the harbor porpoise. This may serve to shadow off-axis high frequency signals and result in greater directionality. Further, head morphology including acoustic fat location, material composition likely play an
important role in sound wave guiding, like that of the terrestrial mammal pinna (Brown 1994; Müller 2004). Narrow receiving beams, i.e. a directional receiver, will also enhance signal-to-noise ratios (S/N). During echolocation, when echoes are returning primarily from directly ahead of the animal, lower noise would potentially allow easier echo detection, especially in high clutter environments. Finally, hearing directionality may aid the belugas in detecting and localizing the acoustic signals of conspecifics. Belugas are highly social animals with a complex repertoire of social sounds which range in temporal and frequency components (Castellote and Fossa 2006). Acute directional hearing for higher frequency and broadband signals (Branstetter and Mercado 2006) would likely aid in using acoustics to maintain fine scale cohesion and coordination by enhanced localization capabilities. Broad directional sensitivities at lower frequencies would allow for detection of conspecifics at longer ranges when signals may be attenuated. Again, frequency and hearing directionality should be investigated further.

**Receiving pathways from jawphone stimuli**

The lowest thresholds were measured when the jawphone was placed at the tip of the lower jaw (rostrum tip) and pan bone region. This is both in contrast and agreement to what was previously found with the bottlenose dolphin (Møhl et al. 1999). Møhl et al., used a jawphone to project clicks to a bottlenose dolphin. The jawphone was moved around the animal’s head and lower jaw, measuring AEP responses to various transducer placements. The authors found that the dolphin’s rostrum tip was not very sensitive while the pan bone region was highly sensitive.
The magnitude of lower rostrum tip sensitivity measured here was unexpected and may indicate there are acoustic fat channels which begin at the beluga rostrum tip that effectively guide sound to the ears. A similar pathway has been recently proposed in the Couvier’s beaked whale (Cranford et al. 2008a). In the dolphin, these channels do not start as far anterior on the rostrum but good sensitivity is found in several locations along the outer part of the jaw (Bullock et al. 1968; McCormick et al. 1970; Möhl et al. 1999). Out data also reflects good reception of sound in the pan bone region, supporting Norris’s jaw hearing hypothesis in the beluga (Kobler et al. 1992). While we cannot fully eliminate the possibility of bone conduction from the rostrum tip, we find this idea unlikely as it has not been supported in other studies (Möhl et al. 1999; Ketten 2000) and simple impedance matching from water to acoustic fats transfers sound waves better (Varansi and Malins 1972) and would excite a greater AEP response than from water-to-bone. The stimulation point we chose on the rostrum tip was also centered on the lower jaw. If sound is effectively conducted from this region, it may stimulate both ears (as opposed to primarily ipsilateral stimulation from the pan bone region)(McCormick et al. 1970) and excite a greater relative AEP response. The relative sensitivity from this region may also play a role in directional hearing and sound localization. For example, sound from a source directly in front would be primarily received on the rostrum tip (and perhaps both pan bone areas) and then conducted to both ears well. But greater shadowing, and thus amplitude differences, may occur if sound is primarily received from the side and other locations. It should also be noted that thresholds were only measured with broadband clicks. By using tones, thresholds and relative sensitivities to jawphone
placement may change (Popov et al. 2008). If so, the spectral properties of a perceived sound would depend on the direction of the sound source and demonstrate how spectral properties may influence sound reception and localization.

Our lack of detected responses from melon stimulation was unexpected and indicates that the beluga melon is not a good acoustic receiver, at least for this individual and this set of circumstances. It appears that the beluga bulla and ears are likely well insulated from the melon, perhaps to reduce the hearing of, and masking by, self-generated echolocation clicks as has been shown by Supin et al (2006). As evoked potential responses recorded after from melon stimulation were measured in the bottlenose dolphin (Bullock et al. 1968; Mohl et al. 1999), this may indicate that there is some subtle variation in the melon morphology of odontocetes and the bottlenose dolphin may not be as effectively insulated from its own clicks. Or slight differences in transducer placement on the melon might greatly affect how sound is propagated through this tissue. Thus differences in responses between the beluga and dolphin are not species variations but experiment methodological differences and reinforcing the beamforming effect of the odontocete melon (Au et al. 2006).

The latencies of AEP responses were found to generally increase with distance from the external auditory meatus. Precise beluga head morphology has not been sufficiently described, but if tympanic bulla and middle ear locations are in locations similar to delphinids, they are roughly internal from the meatus. However, latencies of waves II and III and the mean latency of all waves were found to be fastest from 12 cm behind the meatus. This may also simply be data scatter as wave IV, the most prominent wave, reflects a clear trend of increasing latency from meatus
to rostrum tip. It may also indicate that the tympanic bulla is slightly posterior from
the meatus and oriented more toward the posterior, or that a sound pathway from
behind the meatus enables more rapid sound conduction. A third explanation is that
the peribullary sinuses act as reflective boundaries which help channel sound,
presumably from in front of the animal, toward the ears (Cranford et al. 2008b).
These sinuses may change volume depending on the state of the animal and therefore
affect acoustic delays from sound which enters behind the ear.

In order to further examine the shaded receiver model, latencies of AEP
responses from the four stimulus locations were compared to the estimated sound
velocity profile for the odontocete lower jaw acoustic fats (138 cm·ms⁻¹) (Blomberg
and Jensen 1976; Kobler et al. 1992). These jaw delays were, on average, 0.05 ms
slower than those predicted for a straight line path from sound source to the location
of shortest latency. However, rostrum tip stimulation reflected the smallest deviation
with a mean delay of only 0.01 ms difference from a straight line path. The
consistently higher values of measured delays vs. straight line predictions lend further
support to the shaded receiver model. Delay differences for each stimulus location
from a predicted straight line path support the theory of various sound channels,
based on acoustic fat composition. Thus, we agree with previous work which
suggests that odontocete localization and directionality depend on multiple factors in
addition to simple shaded receiver model data (Branstetter and Mercado 2006;
Koopman et al. 2006; Cranford et al. 2008a).

Differences and similarities found between the sensitivities and latencies of
beluga and dolphin AEP responses beg the question: Why? There were certainly
some differences in experimental design between this and the Möhl et al. (1999) study, but procedures generally overlapped. Thus it seems likely there are differences in the way belugas and bottlenose dolphins receive sound, based in part on their head morphologies. How variation in head morphology affects hearing differences across a wide range of species, or even individuals within a species, requires greater attention. The directionality related differences also indicate that other auditory capabilities vary between species and extrapolating from only one species leaves limited conclusions. This underlines the importance of investigating hearing in the dozens of cetaceans not yet examined and stresses the need for caution regarding application of auditory characteristics to species for which we know relatively little, such as mysticetes and beaked whales. Because the variation in latency and sensitivity were found, this work supports the idea of a shaded receiver model in the beluga that includes variations based on hearing directionality and head morphology. Certainly, the hypothesis of a shaded receiver model for sound localization and much of odontocete directional hearing requires substantial additional data.

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CHAPTER 5

PREDICTING TEMPORARY THRESHOLD SHIFTS IN A BOTTLENOSE DOLPHIN (*Tursiops truncatus*): THE EFFECTS OF NOISE LEVEL AND DURATION

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Abstract

Noise levels in the ocean are increasing and are expected to affect marine mammals. To examine the auditory effects of noise on odontocetes, a bottlenose dolphin (*Tursiops truncatus*) was exposed to octave-band noise (4-8 kHz) of varying durations (1.875-30 min) and intensities (130-178 dB re: 1 μPa). Hearing thresholds were measured using auditory evoked potentials and temporary threshold shift (TTS) occurrence was quantified in an effort to determine the sound energy levels (SEls; dB re: 1 μPa^2·s) that induce TTS, and develop a model to predict TTS onset. If SEL was kept constant, significant shifts were induced at longer duration exposures but not for shorter exposures. Increased SELs were required to induce shifts in shorter duration exposures. The results do not support an equal-energy model to predict TTS onset, but rather a logarithmic algorithm that increases in sound energy as exposure duration decreases was a better predictor of TTS. Recovery to baseline hearing values was also logarithmic (approximately -1.8 dB/doubling of time) but functions indicated variability including greater shifts inducing faster recovery rates and longer recoveries from longer duration exposures. The data reflect the complexity of TTS in mammals that should be taken into account when predicting odontocete TTS.
Introduction

Anthropogenic noise in the ocean stems from a variety of sources including mechanized shipping, naval sonar, scientific study, oil exploration and drilling, and construction. As usage of the oceans increases, marine noise levels are also expected to rise (National Academy of Sciences, 2005). Serious concern regarding the effects of this noise on marine mammals, as major utilizers of sound in the ocean, has been emerging during the last decade (Richardson et al. 1995; National Academy of Sciences, 2003; Wartzok et al. 2004; National Academy of Sciences, 2005). Excessive noise exposure in marine mammals can induce a variety of behavioral and physiological consequences including temporary or permanent changes in hearing sensitivity. In order to mitigate these effects in wild populations it is necessary to better understand their causes.

Permanent threshold changes are indicative of hearing damage and are referred to as permanent threshold shifts (PTS). Moderate levels of hearing threshold elevation are usually innocuous and fully recoverable and have been termed temporary threshold shift (TTS). These threshold shifts have been demonstrated across vertebrates including fish, reptiles, birds, and mammals (Ward et al. 1958; Saunders and Dooling 1974; Popper and Clarke 1976; Mulroy 1986). Characterizing and understanding how TTS is induced allows an extraction and prediction of PTS levels. Further, TTS exposure conditions may be considered the reasonable limit of excessive noise exposure. There is much concern, for obvious reasons, for determining the levels of noise that induce TTS or PTS in humans. As a result, the subject is well explored in some terrestrial mammals and the variables which relate to TTS (intensity, duration and
frequency) are relatively well studied. Thus, models have been developed to predict situations that would induce TTS and PTS (Ward et al. 1959; Kryter et al. 1965; Ward et al. 1976).

But as TTS has long been demonstrated in other taxa, it was not until recently that it was shown that cetaceans are also susceptible to threshold shifts (Schlundt et al. 2000). Further research has shown a relatively robust and resilient marine mammal hearing system and has demonstrated shifts using broadband noise, tones, and seismic waterguns (Finneran et al. 2002; Nachtigall et al. 2003; Finneran et al. 2005). Yet there is much we do not know regarding TTS occurrence and noise effects in cetaceans. For example: Do short, loud sounds have the same effects as longer, quieter sounds of equivalent energy? If we know the intensity and duration of a noise exposure can we predict TTS?

Answers to these questions require the investigation of a wide range of fatiguing noise levels and durations to develop a predictive model based on empirical evidence. In pinnipeds, several studies have done just that (Kastak et al. 2005; Kastak et al. 2007). However, in odontocetes, predictive TTS models have been developed based on comparisons across studies which have quite different methodologies and fatiguing stimuli and the models developed are consequently straightforward but general (Finneran et al. 2005). Thus there is a need for a comprehensive study that encompasses a range of noise level and duration conditions in order to accurately predict the effects of noise on a representative odontocete.

The goal of this study was to examine the relationship between fatiguing noise amplitude and duration in inducing TTS in an odontocete cetacean and in doing so,
develop a model which predicts the noise levels and durations that would affect TTS and determine if a simple time-intensity trade-off (equal energy) rule could be applied to these predictions. A secondary goal was tracking the recovery from TTS to establish the recovery rates. To achieve this, the auditory evoked potential (AEP) technique was utilized which allowed for rapid and repeated auditory threshold measurements. The AEP method compares favorably with behavioral tests (Yuen et al. 2005; Houser and Finneran 2006) and has been applied previously in other marine mammal audiometric work including TTS investigations (Nachtigall et al. 2004; Finneran et al. 2007; Nachtigall et al. 2007).

Methods

Subject and experimental procedure

The subject used in this experiment was Boris, an 18-year-old male Atlantic bottlenose dolphin, *Tursiops truncatus*. Boris was born and raised in the dolphin breeding colony at the marine mammal research facility in Kaneohe Bay, Oahu, HI. The animal has had substantial cooperative experience with hearing research experiments, including AEP and TTS work (Nachtigall et al. 2003; Nachtigall et al. 2004).

All threshold testing was conducted in the floating, open-water sea pens of the Hawaii Institute of Marine Biology, moored off of Coconut Island in Kaneohe Bay. The experiment began in May 2004 by the establishment of a baseline audiogram of the subject. Controlled noise exposures for this experiment were introduced in February 2005 and were conducted through September 2006. There were a total of
57 noise exposures. Exposures were permitted once every four days; however, more typically exposures were once per week and often there would be several weeks without exposure sessions. The animal’s hearing was monitored and always returned to baseline levels prior to another exposure. Control sessions were paired with noise exposure sessions and were experimentally identical excluding the presentation of a fatiguing sound. Because noise exposure sessions were usually once per week, a greater number of control and training threshold sessions were conducted resulting in 82 control sessions (thus some controls were conducted more than once), and 201 days and nearly 300 hearing thresholds measured that were not associated with a fatiguing noise exposure.

Noise sessions consisted of three phases: (1) a baseline threshold measurement to ensure the subject’s threshold was similar to its “normal”, or average threshold, (2) the noise exposure session, and (3) follow-up thresholds measurements that were designed to determine the amount of threshold shift and track the subject’s recovery until the subject’s hearing returned to the normal threshold range. Baseline threshold measurements were conducted in the threshold measurement pen using auditory evoked potentials (Figure 5.1). Five AEP records, of 50 s each, were collected per threshold determination, thus a threshold could be estimated in 4-5 min. After the initial threshold measurements the dolphin voluntarily moved to a separate pen for the fatiguing noise exposure. Immediately after the noise exposure Boris returned to the threshold measurement pen for the follow-up threshold measurements. These measurements would begin 1-2 min after the cessation of the noise exposure.
and were conducted with their middle point at 5, 10, 20, 40, and 80 min post-noise exposure to thoroughly track the subject's hearing recovery.

Figure 5.1. Diagram of dolphin audiogram threshold testing and fatiguing noise pen experimental set-up. 1a and b – Trainer positions; 2 – Assistant position; 3 – Hoop stations for noise and threshold tests; Equipment shack which housed the AEP and noise exposure equipment is also indicated.

Evoked potential measurements

At the beginning of each hearing threshold measurement, Boris entered the hearing test pen and he immediately stationed in front of the trainer. The trainer would then gently place two standard 10-mm gold EEG electrodes, embedded in latex suction-cups on the animal. The recording electrode was located 4-10 cm behind the blow-hole, just to the left or right of the animal's midline. The reference electrode was placed on the subject's dorsal fin, which minimized electrical noise from extraneous muscle or nerve movements. Signal conduction was enhanced by the use
of standard electro-gel placed between the skin and the electrodes. The animal then returned to station. Upon the trainers cue, Boris re-stationed in a hoop 1 m below the surface and facing a sound projecting transducer 2 m away. An acoustic baffle was hung at the surface, 1 m from both the dolphin and the transducer, to prevent extraneous acoustic surface reflections from interfering in the hearing threshold area. The dolphin remained in the hoop for 1-3 AEP trials (~1-3 min), after which he was recalled to the trainer for a breath and fish rewards. Boris was then quickly resent to the hoop for the remaining AEP trials. If the hearing thresholds were part of the post-exposure threshold measurements, the dolphin was given a break, either resting at station or being taken out of the test pen, until it was time to begin the next threshold trials.

The sound stimuli were sinusoidally amplitude modulated (SAM) tone bursts digitally generated with a custom LabView program. The tones were converted from digital to analog using an update rate of 200 kHz and a National Instruments PCI-MIO-16E-1 DAQ card implemented into a desktop computer. Individual tone bursts were 20 ms in duration with a 30-ms offset time and presented 1000 times in succession. Based on prior established dolphin modulation rates (Supin and Popov 1995; Mooney et al. 2006), carrier frequencies were modulated at 1000 Hz and with a modulation depth of 100%. The analog signals were sent from the computer to a custom-built signal shaping box that could attenuate the tones in 1 dB steps. From the signal shaping box, outgoing signals were sent to the projecting transducer, an ITC-1032, and concurrently monitored on a Techtronix TDS 1002 oscilloscope. For each session, thresholds were collected at 1 of 5 frequencies: 5.6, 8, 11.2, 16 or 22.5
kHz. Signals typically started 15-20 dB above the baseline threshold for the first trial, and were reduced in 5 dB steps for the remainder of the 5 trials. If TTS was apparent and thresholds were reached before 4-5 trials, the SPLs were increased to best track the threshold.

The received AEPs were amplified and filtered using an Iso-Dam Isolated Biological Amplifier and a Krohn-Hite 3102 filter, both with a bandpass of 300-3000 Hz. They were then digitized by the DAQ card at a rate of 16 kHz. A 30-ms AEP record was collected simultaneously with each stimulus presentation and to extract the AEP from noise 1000 of these records were averaged for each AEP trial.

Noise exposure

The noise exposure pen was equipped with a hoop fixed where the subject was required to station for the exposure 2 m from the fatiguing noise transducer. When the subject entered the noise exposure pen he immediately stationed in front of the trainer. Upon a visual cue from the trainer, the dolphin swam and stationed in the hoop. Typically, several warm-up trials were initiated where the subject was sent to the hoop for 1-2 min with the noise off. When the equipment, animal and trainer were ready, the trainer would indicate to the fatiguing noise equipment operator to turn the noise on. Because the subject initially demonstrated a startle response that might have inhibited the experiment when an intense fatiguing noise was abruptly turned on, the experimental procedure required the sound be ramped up, from 130 dB re: 1 μPa to 10 dB below the deemed exposure level or a maximum value of 160 dB over the course of a 30 s trial. The animal was then recalled back to the trainer and
the sound was turned up to the planned experimental intensity. The dolphin was then sent back to the hoop to begin what was considered the timed exposure. The warm-up trials were on average 140-5 dB of noise for 30 s and well below TTS levels shown here and elsewhere (Nachtigall et al. 2004; Finneran et al. 2007), thus were not considered part of the noise exposure.

To examine the effects of noise duration and intensity on dolphin hearing, both exposure duration and intensity were varied. Hoop exposure duration was set at: 30, 15, 7.5, 5.625, 3.75 or 1.875 min. Fatiguing noise intensities ranged from 130-178 dB re: 1 μPa where, irrespective of the initial ramp-up, sound pressure levels (SPLs) were kept constant throughout the exposure. The fatiguing noise was octave-band noise from 4-8 kHz (Figure 5.2). It was generated by a custom-built white noise generator and then filtered using a custom bandpass filter. Noise was then amplified using a Hafler P3000 amplifier, monitored on the oscilloscope and played through a Massa TR-61A transducer (peak frequency 5.5 kHz). The noise level was calibrated 11 times throughout the experiment to ensure no drift occurred with the equipment. To calibrate, a Biomon 8235 hydrophone (-173 dB sensitivity and ± 1 dB from 1-40 kHz) was placed in the center of hoop when the animal was not present. The hydrophone was connected to the oscilloscope to monitor the noise levels, an RMS voltmeter, and the same DAQ card and a custom LabView program to record received levels. The noise was then measured with the voltmeter and calculated to noise spectral density (dB re: 1 μPa²·Hz⁻¹) from 4-8 kHz. These values were then converted to SPLs. Energy flux density (dB re: 1 μPa²·s), or sound exposure level (SEL), was also calculated for each exposure intensity and duration.
Figure 5.2. (a) Fatiguing noise spectral density recorded from the hoop calibration position in the noise pen. The octave-band noise is from 4-8 kHz with a center frequency of 5.6 kHz. The SPL in this case is approximately 160 dB re: 1 μPa. (b) Ambient sound at experimental pens in Kaneohe Bay, Oahu, HI measured with a Biomon 8235 and plotted as noise spectral density using a 1024-point FFT. Four noise samples were averaged to create the plot.

The dolphin's behavior was continuously monitored during the noise exposure by both the trainer and an assistant. While the trainer was responsible for interacting with the dolphin, the assistant recorded behavioral alterations including: number of respirations during surface intervals, latency of time from surface station to the hoop (delay), excessive head or body movement, or any apparent reactions to the fatiguing sound (or reactions within control trials). Because the dolphin had significant experience in TTS, AEP, and other psychoacoustic experiments, his “normal”
behavior was well known and behavioral changes were easily noted. The animal also had significant previous training required to maintain participation in research and husbandry activities and thus reactions to avoid such activities were expected to be minimized. The dependent variables of respirations, latency, and behavioral modification were run in respective one-way ANOVAs to determine if behavioral changes were observed. The assistant also informed the trainer of the duration of the surface intervals and time in the hoop, which were varied somewhat from trial-to-trial to prevent the dolphin from predicting when each trial and hoop session was over. However, the total time in the hoop for each noise exposures or control session was pre-established and maintained as the exposure time for that session (i.e. 30, 15… min).

Data analysis

A 16-ms window of each averaged AEP response to the SAM tones was fast Fourier transformed (FFT) to view the response in the frequency domain. The FFT was then gauged for a response at the 1000 Hz modulation rate, where a peak reflected energy received, or the animal’s physiological “following” of the envelope of the carrier frequency. A larger envelope following response (EFR) in the AEP waveform transformed to a higher peak value at 1000 Hz. The peak values were then plotted against the stimulus sound pressure level and a linear regression was obtained, addressing the peak values. The point where the regression line crossed the abscissa was taken as the theoretical sound level at which no AEP response would be induced and thus was considered the animal’s threshold (Supin et al. 2001).
To determine TTS, the mean and standard deviations of these thresholds were calculated for each of the five frequencies tested. A threshold shift was then determined as a threshold, after a control or actual noise exposure, where the threshold exceeded 1 SD above the mean threshold. A TTS was defined as a demonstrated "recovery" back to within ±1 SD of the mean. These shifts were deemed greater than the day-to-day variation that was found in the baseline thresholds and were distinguishable due to over 300 thresholds measured during the course of the experiment. The consequent recoveries provided confirmation of the shift. All analyses were completed with EXCEL, MatLab, and MiniTab software.

Results

Baseline hearing thresholds for the dolphin subject were relatively consistent, varying ±2-3 dB SD (mean = 2.8 dB) for all the frequencies tested with more than 40 thresholds (not associated with a noise exposure) acquired for each frequency (Figure 5.3a). Hearing was most sensitive at the higher frequencies (16 and 22.5 kHz) and followed a typical mammalian curve. Baseline thresholds and TTS were primarily explored at 11.2 kHz. The subject demonstrated hearing recovery from all threshold shifts and baseline thresholds did not significantly increase over the duration of the experiment (Figure 5.4). No TTS was measured after control conditions. The experimental matrix for all exposures ≥160 dB and occurrences of TTS are listed in Table 1.
Table 5.1. Noise exposure experimental matrix where the fatiguing sound was octave-band white noise from 4-8 kHz. Parameters listed include: audiogram test frequency (kHz); exposure duration (min); noise sound pressure level (SPL) and energy flux density (SEL); and number of exposures and number of shifts at corresponding test conditions.
<table>
<thead>
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<th>Frequency (kHz)</th>
<th>Duration (min)</th>
<th>SPL (dB re: 1 µPa)</th>
<th>SEL (dB re: 1 µPa²s)</th>
<th>(No. exposures-no. shifts)</th>
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Figure 5.3. (a) Mean hearing thresholds (± 1 SD) of Boris for 5.6-22.5 kHz in SPL (dB re: 1 μPa). The fatiguing noise band, from 4-8 kHz, is depicted at the bottom, relative to the hearing thresholds tested. (b) Mean (± 1 SD) amount of TTS (dB re: 1 μPa) at each of the five frequencies tested. The * indicates that 5.6, 8, and 11.2 kHz demonstrated mean shifts that were significantly greater than their average threshold (one-way ANOVA and Tukey’s pairwise comparison; p<0.001; F_{5,82} = 16.69).
Figure 5.4. Thresholds over the duration of the experiment for (a) 11.2 and (b) 8 kHz.
Black diamonds indicated thresholds measured after a fatiguing noise exposure.
Middle line indicates mean threshold value and top and bottom lines indicate ± 1 SD from the mean threshold.

Threshold shifts and frequency

Temporary threshold shifts were recorded in 26 of 57 noise exposure sessions and at all frequencies tested (Figure 5.3b). However, TTS at the higher, more sensitive frequencies (16 and 22.5 kHz) did not occur frequently and significant increases in the mean thresholds post-noise exposure were not recorded at those frequencies. Mean post-exposure thresholds for 5.6, 8, and 11.2 kHz demonstrated significant increases in hearing thresholds, relative to the baseline hearing threshold
(one-way ANOVA, $F_{5.82} = 16.69, p < 0.001$; subsequent Tukey's pairwise comparison). These frequencies fell immediately within the center frequency of the fatiguing noise (5.6 kHz), and one-half (8 kHz) and one-octave above the noise center frequency. The greatest mean threshold shift occurred at 8 kHz (8.3 dB) although 5.6 and 11.2 kHz both demonstrated an average TTS of 6 and 6.1 dB respectively.

Of all the thresholds measured at each particular frequency, TTS was found at 11.2 kHz after 51% of post-noise thresholds (n=29) and in 71% (n=21) of the post-exposures when the SEL was $> 185$ dB. Thresholds were measured in 7 instances post-noise exposure for each of the other frequencies tested, and TTS occurrence was found to be 43%, 71%, 29% and 14% for 5.6, 8, 16, and 22.5 kHz respectively.

![Figure 5.5. Sound exposure levels required to induce TTS as duration of exposure changes at all threshold frequencies tested. Shorter duration exposures required](image-url)
greater SEL to induce TTS. Dotted line indicates an equal energy line of 195 dB SEL. Black diamonds indicate TTS occurrence; open circles indicate no TTS.

**Threshold shifts and sound energy levels**

Fatiguing sound exposure energy could be adjusted in at least two ways, either by altering the exposure duration or by varying the intensity level of the sound. Generally, as either exposure duration or sound intensity increased, if the other variable remained constant, a greater incidence of TTS could be expected. For example, at the start of the experiment, exposure duration was held at 30 min, but noise intensities (SPL), and concomitant SELs, were gradually increased (Figure 5.5). Post-noise exposure thresholds did not demonstrate TTS until approximately 155 dB SPL or 187 dB energy flux density, when a shift was initially induced and measured at 11.2 kHz, although this shift was relatively small (3 dB). In the following several sessions, as SEL was gradually increased to 192.5 dB (160 dB SPL), the amount of TTS at 11.2 kHz also increased (Figure 5.6a).
Figure 5.6. (a) Amount of threshold shift measured at 8 (closed symbols) and 11.2 (open symbols) kHz for SELs of 192.5 dB and below. Increasing SELs and corresponding symbols are labeled at the right demonstrating at constant exposure duration but increasing SEL, TTS increased. A regression line was used to illustrate
that with decreasing noise exposure duration, TTS decreased when SEL was held constant ($r^2 = 0.85; p < 0.001; y = 0.341517 x + 2.56609; n = 13$). (b) Amount of TTS for SELs of 192.5 dB and higher. Threshold frequencies are not discriminated but SEL symbols are labeled to the right. Note at shorter durations amount of shift is clustered and higher SELs (top circle) are required to induce significant TTS.

One initial goal of the experiment was to examine the equal energy hypothesis by keeping SEL constant and varying exposure duration and sound intensity level to determine the effects on TTS. Thus, after TTS was measured at all relevant threshold frequencies (5.6-22.5 kHz) using a 30-min, 160-dB SPL noise exposure, exposure duration was halved and sound intensity was increased by 3 dB, keeping SEL constant and exposures and thresholds were measured again. However, TTS did not stay constant as exposure duration decreased. Threshold shift occurrence decreased from 80-86% for 15-30 min exposures to 71% at 7.5 min, and to zero significant TTS occurrences at 3.75 and 1.875 min. The amount of TTS also decreased with exposure duration (Figure 5.6a) and could be predicted by a significant linear relationship ($r^2 = 0.77; p < 0.001; y = 0.341517 x + 2.56609; F_{1,12} = 36.29$). Thus, for the same SELs, exposures of 30 min produced nearly 12 dB TTS whereas 1.875 min of exposure did not generate a shift.

In order to induce TTS at durations of 1.875-3.75 min, fatiguing sound energy flux density had to be increased from 192.5 to 198.5 dB SEL. Intermediate levels at 195.5 dB SEL did not induce significant amounts of TTS. At 198.5 dB SEL, significant TTS was induced at 8 and 11.2 kHz in 7 of 8 exposures, with a mean shift.
of 5.4 dB and one shift of 11 dB (Figure 5.6b). Overall, no significant relationship
was found between the amount of shift (measured at 5.6, 8, and 11.2 kHz) and SEL
($r^2 = 0.02; p = 0.42$).

Recovery from threshold shifts

Following noise exposures AEP measurements were recorded for up to 80
min afterward to track the subject’s recovery. In all noise exposure sessions, the
subject fully recovered to baseline values within the 80 min. When shifts occurred,
recovery to within ±1 SD of the baseline thresholds was typically seen by 20 min
(15/26). In only 3 instances was the subject not within 1 SD of the baseline threshold
values by 40 min after the noise exposure. These were either 15 or 30 min exposures
at a SEL of 192.5 dB. The shifts were 12, 9.3 and 9.6 dB for 11.2, 11.2 and 5.6 kHz
respectively. Recovery was rare within 10 min of the noise exposure, only occurring
on 3 occasions and TTSs were 3, 4.7 and 5.8 dB.

Examples of recovery functions after noise exposure are plotted in Figure 5.7.
Note the difference that can be found. Although all exposures were a constant SEL at
192.5 dB, the durations and intensities varied. While the greatest shift was found at
the 30 min exposure, the 7.5 min exposure demonstrated greater TTS than the 15 min
exposure. However recovery from the 7.5 min exposure was most rapid. Neither the
3.75 nor 1.875 min exposures induced threshold shifts. The 15 min recovery function
exemplifies a “bounce” effect where between the 5 to 10 min thresholds, TTS appears
to still be increasing.
Figure 5.7. Thresholds at 11.2 kHz before and after exposures for 192.5 dB SEL for 5 exposure durations (min): 30 (triangle), 15 (X), 7.5 (open square), 3.75 (open circle), and 1.875 (diamond). Thresholds were measured 5, 10, 20, 40, and 80 min after noise ended. Dotted line indicates noise exposure. Arrows indicate mean threshold ± 1 SD.

Recovery rates followed a logarithmic function which held relatively constant across various methods of analysis (Figure 5.8). Greater shifts demonstrated initially steeper slopes of recovery and lesser shifts reflected more gradual recovery rates. This was best seen by breaking the recovery functions into separate groups (> 7 dB shifts, < 7 dB shifts, ≥15 min exposure and < 15 min exposure). The slopes of these groups varies some but were roughly similar and were calculated as -7.4, -4.4, -6.3 and -5.2*\log(\text{min})\), respectively, and all were linear in log time. The steeper slopes of
> 7 dB shifts and ≥15 min exposure reflected the greater mean shifts of those groups, 8.3 and 7.2 dB, respectively. The more mild slope of the < 7 dB shifts group was a result of the relatively lower TTS values (mean = 4.6 dB) of the grouping. On average, all recoveries could be approximated by a function of -1.8 dB/doubling of time.
Figure 5.8. Threshold shift (dB re: 1 μPa) recovery functions demonstrating linear logarithmic recoveries in log time across multiple analysis methods: (a) TTS > 7 dB
(linear time), (b) TTS > 7 dB (log time), (c) TTS < 7 dB, (d) TTS from longer
duration exposures (30-15 min), and (e) TTS from shorter duration exposures (7.5-
1.875 min). Rate of recovery in all cases was approximately -1.8 dB/doubling of
time.

**Behavioral reactions**

The subject's behavior during both fatiguing noise and control sessions was
recorded by an assistant and for analysis, behavioral comparisons were made between
the control and exposure sessions. Sessions were also divided into groupings of those
with > 10 trails, 10-6 trials, and 5-1 trials, which were rough proxies for long,
moderate and short duration exposures. No significant difference in overt behavioral
changes was found between the exposure and control sessions. However, the subject
did exhibit a significant increase in respirations during noise sessions, for the longer
duration exposures and during noise exposures overall (Figure 5.9a; one-way
ANOVA and subsequent Tukey's pairwise comparison; p < 0.001; F7,459 = 31.98).
Mean respirations (± SD) for these longer durations and all control exposures were
6.14 (± 3.21) and 5.98 (± 3.10), respectively, and 8.81 (± 2.99) and 7.31 (± 3.59) for
the equivalent noise exposures. The delay (s) from the inter-trial station to
hoop/noise exposure station was also significantly greater during noise exposure
sessions and this was across the groupings of sessions (p < 0.001, F7,459 = 25.31).
Mean values for these differences for the groups of > 10 trails, 10-6 trials, 5-1 trials
and all control trials were 6.61 (± 4.48), 6.01 (± 2.85), 5.92 (± 3.64), and 6.41 (±
4.01), respectively. For the noise sessions, delay means were 9.28 (± 5.50), 8.02 (± 3.51), 8.00 (± 3.97) and 8.77 (± 4.95) for the respective groups.

Figure 5.9. Mean no. of respirations (a) and delay from inter-trial stationing pad to hoop station (b) for various noise exposure and control trials. For comparison, we grouped the sessions by number of trials as well as summed data from all trials of all sessions. There were significantly greater respirations, indicated by the *, during
noise sessions with >10 trials and all the sessions grouped together (one-way ANOVA and Tukey’s pairwise comparison; \( p < 0.001; F_{7,455} = 31.98 \)). The hoop delay was significantly greater for all groups of noise exposure trials (\( p < 0.001, F_{7,4559} = 25.31 \)).

**Discussion**

The data presented here provide a comprehensive examination of the interaction of fatiguing noise duration and amplitude on TTS in a bottlenose dolphin. Shorter duration exposures were found to require greater amounts of energy (higher SELs) to induce similar amounts of TTS relative to longer duration exposures indicating there is not a simple equal-energy approach to accurately predict TTS in dolphins. Small behavioral changes in number of respirations and delay to the exposure station were evident in noise exposure conditions versus control. Mean TTS levels induced here were relatively small compared to those often demonstrated in terrestrial mammals, and some other marine mammals (Ward et al. 1959; Finneran et al. 2007; Kastak et al. 2007). However, the data presented here demonstrate the SELs required for TTS onset across a range of exposure durations and TTS onset can certainly be considered helpful in assessing the effects of noise on wild populations.

It should be noted the major caveat in this study is the limited sample size. Substantial constraints exist when working with large pelagic marine animals both in the wild and in laboratory conditions. Marine mammals are particularly challenging because of the political and legal considerations that must be taken into account when working with protected and endangered species. In addition, these experiments
required the cooperation of a well-trained subject. Consequently, this investigation was limited to one experimental animal. In order to ensure that our data were not strongly influenced by a one-time occurrence, the measurements were often repeated, and results and trends compared to other TTS research in marine and terrestrial mammals. However, it must be noted that in TTS studies both within and between individual variation may be substantial (Ward 1997), thus all data must be taken with a degree of caution and additional data with additional animals and cetacean species is advised.

The frequency for which the greatest TTS was observed was one-half octave above the center frequency of the noise. Other significant levels of mean TTS were found at the center frequency of the noise and 1 octave above the center frequency. These trends reflect what has been demonstrated previously with the same animal in similar noise exposure conditions (Nachtigall et al. 2004) as well as results collected with terrestrial mammals and pinnipeds (Ward et al. 1959; Ward 1962; Kastak et al. 2005), indicating that the frequency trend of odontocete noise-induced TTS appears relatively conserved in marine mammals. The experiment was designed using frequencies below the regions of best auditory sensitivity for a bottlenose dolphin but similar to that of introduced anthropogenic noise. It is likely that at higher frequencies (20-100 kHz) and regions of better sensitivity, TTS levels might have been greater (Mills 1982). Further, snapping shrimp produce high levels of background noise in Kaneohe Bay, creating an environment for masking (Figure 5.2b). In a quieter situation, we would have likely seen greater levels of TTS (Humes 1980), thus these results may be somewhat conservative in their TTS onset levels.
Based on the SEL levels that induced TTS, the data were evaluated to determine a model that would predict TTS onset. Several algorithms have been suggested to predict noise levels that induce TTS including the equal energy rule which assumes that as long as noise exposure energy levels are constant, similar threshold shifts will be induced regardless of the noise temporal pattern (Ward et al. 1959). This is often termed the "3-dB rule" as a halving of sound exposure duration and a 3-dB increase in sound intensity (or vice-versa) maintains a constant energy level and should theoretically induce similar shifts (Kryter et al. 1966). This rule has been employed for human standards (NIOSH 1998) and has recently been proposed for use in predicting TTS in odontocetes (Finneran et al. 2005). However, despite the fact that it is applied in many situations, empirical studies often do not support the equal energy hypothesis as an accurate means to predict TTS, demonstrating that the trade-off between time and energy is not necessarily linear (Buck et al. 1984; Ward 1991; Hamernik and Qui 2001).

Our data generally follow an equal energy line of 195 dB SEL however the TTS instances more often split the line rather than falling upon it (Figure 5.5). At shorter duration exposures, greater SELs were required to induce TTS relative to longer duration exposures. Further, when SEL was held constant (sound duration decreased but SPLs increased), the amount of TTS did not hold constant as would be predicted by an equal energy hypothesis (Figure 5.6a). Rather, TTS levels also decreased. At shorter duration exposures, increased SELs were required to induce significant levels of TTS (Figure 5.6b). This non-coherence to the equal energy rule was previously demonstrated in pinnipeds (Kastak et al. 2005; Kastak et al. 2007).
These data also indicate that equal energy levels do not induce similar TTS levels in odontocete cetaceans.

To better predict TTS onset, a model of increasing energy as exposure level decreases appears to fit these data more closely (Figure 5.10). Described are both a linear relationship to the threshold shift data, as well as a logarithmic relationship. The linear model does approximate the trend sufficiently well ($r^2 = 0.57; p < 0.001; y = -0.210353x + 197.138; F_{1,22} = 28.6473$). However the logarithmic estimation does a much better job of predicting the threshold shifts and would apparently make a better model for predicting TTS ($r^2 = 0.78; p < 0.001; y = -6.1771\log(x) + 200.21; F_{2,21} = 78.71$).

![Figure 5.10. The SELs required to induce TTS at higher noise levels where threshold frequencies are discriminated. Just TTS occurrence is plotted. Two methods to](image-url)

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predict TTS are also graphed: a linear estimation of TTS (dotted line) \( r^2 = 0.57; p < 0.001; y = -0.210353 x + 197.138; n = 23 \) and a logarithmic estimation (\( r^2 = 0.81; p < 0.001; y = -12.113 \log(x) + 202.25; n = 23 \)). Note that in both cases, the slope is positive indicating that for shorter time durations, greater energy is required to induce TTS.

Interestingly, although the equal energy model is often proposed to predict the occurrence of human TTS there is often contradiction to this rule and this is found across taxa, including in humans (Mills et al. 1981), guinea pigs (Buck et al. 1984), and chinchillas (Ward 1991). Threshold shift occurrence depends on many factors in addition to fatiguing noise energy including frequency, intensity, duration and time-intervals of exposure (Bohne and Clark 1990). Therefore using energy and SEL alone is an insufficient metric for predicting TTS. Although SEL combines both exposure intensity and duration these two factors do not necessarily contribute equally to TTS onset. It is vital to present both SPL and duration in reporting and predicting TTS. Further, as more information is collected regarding odontocete TTS, it becomes increasingly obvious that the subject is quite complex. The best way to predict it may be to investigate TTS using a range of variables to determine what exposures produce the same TTS, and address an equal-TTS based approach to reducing deleterious noise exposures (Kryter et al. 1966; Ward 1991).

When TTS onset was examined relative to sensation level (SL), or the difference in dB between the fatiguing noise SPL and the absolute threshold, two points were relatively apparent (Figure 5.11). First, there is a relationship between
SL and exposure duration which is not surprising because shorter duration exposures require higher SPLs to induce shifts, but the clarity of the trend may be a means of predicting TTS based on SLs and duration. Second, at longer durations, TTS onset was found at very low SLs relative to other marine mammals (Kastak et al. 2005). Although shifts are likely to decrease and perhaps reach asymptote as exposure levels approach effective quite levels, these SLs at which shifts are induced may cause some concern for potential TTS effects for very long duration sounds, such as shipping. Further, it poses the need for measures on effective quiet (the level at which no duration of exposure will induce TTS) for marine mammals.

![Figure 5.11. Sensation levels (SL; dB re: 1 μPa) at which TTS was induced for various exposure durations. TTS could be induced at much lower SLs for longer duration exposures.](image)

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When considering the behavioral data, it is important to realize that these experiments were primarily designed as hearing investigations not investigations of behavioral effects of noise. Thus some caution must be taken when interpreting behavior during these studies and extrapolating to other conditions. We assume behavioral changes associated with noise exposure indicate aversion to the fatiguing noise, but this is only an assumption. There were no overt behavioral changes that were significantly associated with the presence of the fatiguing noise. However, the dolphin was reinforced throughout his extensive research training and husbandry experience to limit any deviations from expected procedures and in the interest of the present study, such considerations were taken here. Thus, major behavioral changes would have been unexpected. However, more subtle changes such as significant increases in respiration rates and delay from the inter-trial station to the hoop station were noted (Figure 5.9). Fatiguing noise levels at the surface inter-trial station were 10 dB lower in SPL then at the hoop. Presumably, this may have been a passive method of deferring noise exposure by the dolphin. Interestingly, only the longer duration exposures reflected higher respiration rates inferring that potentially the shorter duration exposures, although higher in SPL, may have been less adverse. The only occasion on which the dolphin exhibited an obvious reaction to fatiguing noise exposure was when an amplifier electrically shorted during the exposure, creating an unplanned, unusual, and relatively loud sound. The dolphin immediately pulled out of the hoop and swam vigorously around the noise exposure pen, burst-pulsing and
jaw popping at the transducer and not heeding the trainer for several minutes. However, this was an isolated event and typically the animal stationed properly and observed the trainers’ cues.

Some previous TTS studies have often demonstrated more obvious behavioral reactions to noise exposure (Kastak et al. 1999; Schlundt et al. 2000). However, it seems more often that there are only small behavioral changes as these animals are exceptionally well trained. Thus, motivation regarding certain tasks can override presumed reactions to potentially adverse stimuli. Such overriding motivations have been found in wild individuals, for example pinnipeds raiding fish farms that are equipped with acoustic harassment devices (Quick et al. 2004). The prevalence of such behaviors both in wild and captive animals deserves further attention. Further, our documented alternations were not obvious indicating the importance of detailed observations when examining the effects of noise on marine mammals.

Hearing recovery rates generally followed a logarithmic trend and -1.8 dB recovery/doubling of time and both are similar to some previous results, particularly for shifts that are not greater than 10-15 dB (Nachtigall et al. 2004; Finneran et al. 2007). However, there was variability in the slopes and greater shifts reflected faster recovery rates. A similar trend was found by Finneran et al. (2007) which is not only a reflection of the logarithmic recovery functions but indicates robustness of both data sets. An interesting note is that the shifts that took 40 min to demonstrate recovery were all of longer duration exposures. This may support the trend that longer duration exposures will often induce greater amounts of TTS which concurrently requires a greater amount of time for recovery. Concurrently, in humans, recovery
functions depend somewhat on the exposure situation, and longer duration exposures have demonstrated longer recovery times (Mills 1982). This may also be demonstrated here. Further, these longer recoveries were recorded on shifts measured at 5.6 and 11.2 kHz, frequencies which did not produce the highest mean TTS. If these shifts were recorded at 8 kHz, greater shifts and longer recoveries might have been found. This underlies the importance of measuring multiple frequencies simultaneously, an important AEP advancement when investigating TTS (Finneran et al. 2007). Also noted here were the occasional TTS “bounces” where thresholds collected immediately after the noise exposure demonstrated slightly less shift than a later threshold (Spieth and Trittipoe 1958; Finneran et al. 2005). This occasional delayed shift was unexpected but not unusual in mammals.

Conclusions

This work demonstrates TTS onset in an odontocete across a range of exposure durations and sound levels and the results indicate that shorter duration exposures often require greater sound energy to induce TTS than longer duration exposures. Recovery functions were relatively consistent but did show some indications that different exposure situations may relate to different recovery rates. The sample size was limited but repetitive exposures and comparisons between studies increased the robustness of the data. These results are inconsistent with an equal energy model of TTS supporting the notion that, as in terrestrial mammals, predicting odontocete TTS is quite complicated. It is suggested that TTS onset is considered sufficient to conclude physiological effects of noise exposure in marine
mammals. Future investigations should continue to explore the range of variables that relate to threshold shifts to develop an equal-TIS model to better predict and mitigate situations in which anthropogenic noise may affect marine mammals.

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References


SONAR INDUCES TEMPORARY HEARING LOSS IN DOLPHINS

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Abstract

There is increasing concern that anthropogenic ocean noise is affecting marine mammals (National Academy of Sciences 2005; Southall et al. 2008). It has been suggested that several recent cetacean mass strandings have been caused by animal interactions with concurrent use of naval mid-frequency sonar (Evans et al. 2001; Jepson et al. 2003). Exposure to high intensity sonar may cause deleterious physiological damage (hearing loss or bubble formation) or behavioral modifications (such as prolonged diving or rapid surfacing) (Finneran et al. 2005; Jepson et al. 2005; Tyack et al. 2006). However, mechanisms by which sonar directly affects marine mammals and produces strandings remains to be empirically demonstrated. Using controlled experimental studies, we show that mid-frequency sonar can induce temporary hearing loss in a bottlenose dolphin, demonstrating the sound levels that sonar can cause deleterious physiological effects in bottlenose dolphins. Temporary hearing threshold shifts were induced by multiple high intensity signals (214 dB energy flux density) played in succession. Although source levels (203 dB re: 1 μPa RMS) were lower than what might typically be produced at the source by naval sonar, modeling the attenuation of these sonar signals indicates that these sound levels would likely occur at relatively close ranges to a ship (40 m). The necessity of repeated “ping” exposures indicates that an animal would need to be in the sonar beam for multiple pings in order to induce shifts. A comparison of behavioral observations during control and sonar exposure sessions indicates mild behavioral alterations during the potentially aversive sonar exposures. Our results demonstrate that mid-frequency sonar can have physiological effects on marine mammals,
specifically inducing temporary hearing loss, supporting concern for sonar use in marine mammal habitats. However concern must be balanced by evidence that effects are generated after exposure to multiple sonar pings at a location relatively close to the sound source for an extended period of time.
Introduction, Results and Discussion

Within the past decade there have been multiple instances when cetacean species have stranded immediately following naval training activities involving mid-frequency tactical sonar (Evans et al. 2001; Jepson et al. 2003). These temporally and spatially overlapping events seem to indicate that high intensity sonar induced these marine mammal strandings and subsequent research has often assumed a cause-and-effect relationship (Johnson et al. 2004; Fernandez et al. 2005; Jepson et al. 2005; Tyack et al. 2006). However, it has yet to be shown that exposure to sonar signals actually produces effects which could cause a multi-species stranding event.

Increases in noise levels have been correlated with some behavioral changes in wild populations of cetaceans (Miller et al. 2000; Foote et al. 2004). As in other mammals, noise exposures in toothed whales (odontocetes), and seals and sea lions (pinnipeds), may cause auditory physiological effects such as temporary hearing loss, or temporary threshold shifts (TTS) (Kastak and Schusterman 1996; Schlundt et al. 2000). Further effects of noise exposure, such as decompression sickness, changes in dive profile, or acoustically induced bubble-formation, have also been postulated (Jepson et al. 2003; Tyack et al. 2006), but these typically lack controlled experimental situations to examine and evaluate potential deleterious noise effects. Thus, despite much supposition, there are limited data on the actual effects, behavioral or physiological, of mid-frequency sonar on marine mammals.

We directly tested the possibility that sonar may temporarily affect odontocete auditory capabilities in a controlled noise exposure setting, using actual recorded mid-frequency sonar pings. We also compared behavior patterns between control and
noise exposure sessions to examine potential behavioral changes induced by the noise exposure. Finally, we addressed a model to predict the onset of these temporary auditory shifts for sonar and longer duration noise exposures.

Figure 6.1. Spectrogram, (a), and waveform, (b), of sonar ping used in the noise exposure and recorded off of San Juan Island, WA. Fundamental of the first signal is a slight downsweep, just above 3 kHz and 0.5 s in duration. The signal is then off for 0.5 s, and then a downsweep just below 4 kHz is played for 0.5 s. This “ping” sequence was repeated 3 times, with a 0.5 s of silence between the pings. A series of 3 pings was followed by 24 s of silence and then repeated. This repetition schedule
was repeated up to 5 times for an intermittent exposure of up to just over 2 min for exposure levels up to 214 dB re: 1 μPa²·s. Each individual ping was 203 dB re: 1 μPa. Note the harmonics of the fundamental for each signal.

The mid-frequency sonar signal (3-4 kHz) was recorded off San Juan Island, Puget Sound, WA, USA in the presence of a U.S. Navy ship operating nearby during the summer of 2005 (Figure 6.1). There are multiple uses and types of mid-frequency sonar signals (Evans et al. 2001), and we chose this signal because it was actively used in a naval training exercise, was a high-quality recording from the ambient environment, and was openly available. We played signals in 3-ping successive blocks, each block spaced 24-s apart, simulating a 'typical' mid-frequency sonar application (Evans et al. 2001). Intense sonar sounds were presented to a captive-born, well-trained Atlantic bottlenosed dolphin accustomed to noise exposure experiments (Nachtigall et al. 2003; Nachtigall et al. 2004). We were permitted to work with only one experimental subject, thus in order to ensure robustness of data and limit any individual biases, multiple hearing thresholds were obtained on the subject and data were compared to the subject's known individual variation, variation of other individuals of the same species (Nachtigall et al. 2000) and prior threshold shift work on this and other odontocetes (Schlundt et al. 2000; Finneran et al. 2002; Nachtigall et al. 2003; Nachtigall et al. 2004; Finneran et al. 2005; Finneran et al. 2007). Hearing thresholds for a 5.6 kHz carrier frequency were measured before and after noise exposure using the physiological method of auditory evoked potentials (AEPs) which allowed us to rapidly determine the amount of threshold shift as well as
track the subject’s recovery (Figure 6.2) (Nachtigall et al. 2004; Finneran et al. 2007). Sonar sound pressure levels (dB re: 1 µPa) were gradually increased over multiple exposure sessions until a threshold shift was induced. These exposure levels were then repeated. If a shift was not induced, sound intensity or exposure duration was then increased, resulting in higher sound exposure levels (SEL; dB re: 1 µPa²-s), until a significant threshold shift was reliably induced (deemed three consecutive sessions with threshold shifts).

![Auditory evoked potential (AEP) records](image)

Figure 6.2. Auditory evoked potential (AEP) records for hearing tests after a sonar exposure (left graphs) and control session (right graphs). (a) AEP waveforms to 5 sound pressure levels, 105-125 dB, in 5 dB steps. (b) Fast Fourier transforms (FFT) of the waveform records, corresponding to respective sound levels. A peak at 1 kHz indicates an AEP response to sound stimuli. Note the peak is lost near 110 dB for the
noise exposure, but still present in the equivalent control record. (c) Linear regressions of the FFT peak values. The sound level at which the regression line hits zero response is taken as the hearing threshold. The noise exposure points flatten-out at 110 dB, thus only the 4 points where responses were found (and highest regression value) were used. This resulted in a threshold 7 dB higher for the sonar exposure session.

Control sessions, in which no sound exposure occurred, showed no changes in hearing thresholds. Sessions in which the animal was exposed to sonar pings demonstrated significant elevation in thresholds, 5 and 10 min after exposure. These shifts required at least 3 blocks of sonar pings and showed significant declines in hearing capability relative to the subject’s average hearing threshold (Figure 6.3). These shifts were not reliably induced using 3 or 4 blocks of sonar pings. Only the 5 3-ping blocks (214 dB re: 1 uPa^2·s total sound exposure level) induced shifts for 3 consecutive research sessions. Recovery back to the range of normal hearing (mean threshold ± 1 s.d.; 100.3 ± 2.8 dB; n=57) typically (80 %) occurred within 20 min after sonar exposure (n=10) and always within 40 min.
Figure 6.3. (a) Mean amount of hearing threshold shift, for sonar (black bars; n = 6 shift sessions) and control exposure sessions (grey; n = 18 control sessions). Significant differences were found when comparing thresholds related to sonar exposure and mean threshold at 5.6 kHz (one-way ANOVA, $F_{5.75} = 8.82$, $P < 0.001$). A Tukey's pairwise comparison was subsequently used to determine differences were at 5 and 10 min after sonar exposure. Control exposures failed to demonstrate significant differences from the average threshold shifts (one-way ANOVA, $F_{5.103} = 1.32$, $P = 0.263$). Zero is the baseline hearing threshold and the dotted line is 1 s.d. greater than that mean threshold. (b) Behavioral reactions to the sound exposure.
Significant differences were found between sonar exposure (n = 10) and control sessions (n = 18) in mean no. of respirations ($P = 0.018$) and mean latency ($P < 0.001$) from trainer station to the sonar exposure station (s) (two-tailed t-tests for unequal variances).

Observations of the animal’s behavior during control and noise exposure sessions, demonstrated no significant overt behavioral changes between the sonar exposure and control sessions. While this animal previously demonstrated an uneasiness with intense sound (Nachtingall et al. 2003) the lack of overt differences in the animal’s behavior when intense sound was presented likely is a result of previous exposure, habituation and good training. Interestingly, however, the number of respirations per surface interval (mean ± 1 s.d.; sonar = 6.95 ± 2.54; control = 5.38 ± 2.13) and the latency of time from surface station to noise exposure station (sonar = 8.60 ± 3.20; control = 5.42 ± 1.08) were both significantly increased during the sonar exposures (Figure 6.3b). Although the actual implications of these behavioral changes are unclear, it is apparent that there is a link between subtle behavioral responses and the noise exposure of the animal.

Finally, we compared the onset of threshold shift found in these data to that of other studies in order to devise a model to predict when sonar signals, as well as other types of noise, would affect odontocete hearing (Finneran et al. 2002; Mooney et al. 2008). Previous models suggested that temporary hearing threshold shifts in marine mammals could be predicted by an equal energy model (Finneran et al. 2002; Finneran et al. 2005) based on similar extrapolations in humans and terrestrial
mammals (Kryter 1994). This model states that shifts may be predicted based on the total energy in the exposure sound regardless of exposure temporal pattern. However, this model does not hold for odontocetes because as sound exposure duration decreases, increasing sound exposure levels are required to induce threshold shifts (Figure 6.4a and b). This is true for various types of noise (broadband noise vs. frequency modulated sonar pings) where exposure levels required to induce shifts increase linearly with log time.

To establish a new model we evaluated threshold shift onset and growth using data compiled from both sonar ping and broadband noise temporary threshold shift experiments where the fatiguing stimuli varied across a broad range of sound intensities (157-203 dB) and exposure durations (30-0.25 min) (Mooney et al. 2008). Based on these data it is possible to predict when noise exposure related hearing shifts will occur across a range of exposure durations and intensities. Thus, if sound exposure duration and intensity are known, the occurrence of temporary, physiological auditory effects can be evaluated (Figure 6.4c). Both the logarithmic model and surface plot demonstrate that with very short acoustic signals, like sonar sounds, sound energy levels must be very high, at least 210-214 dB re: 1 uPa^2·s, to induce threshold shifts. This indicates that the animal would have to be close to the source and/or exposed repeatedly in a short time period. Let us take these two points separately. First, a 53-C mid-frequency sonar is assumed to operate at a 235 dB source level (Evans et al. 2001). The sound levels used in this experiment were 203 dB, constrained by the difficulty and safety of producing higher sound levels. If one assumes a typical sound attenuation rate of 6 dB/doubling of distance (Urick 1983), a
235 dB source level sonar would drop to 203 dB approximately 40 m from the sonar source, a distance that can be considered "close" with respect to naval ships. Second, the animal would then have to remain that close for the approximate 2-2.5 min of operating the sonar to receive a total sound energy level of near 214 dB. Alternatively, the animal could initially be located closer to the sonar source and receive a more intense signal, and, within the exposure duration, not move a distance that would exceed that required to provide the sound energy levels that would induce threshold shifts.

Figure 6.4. Predicting the temporary physiological auditory effects of sonar and noise exposure based on onset of hearing threshold shifts. (a) Exposure duration (log-min) vs. sound exposure level (dB re: 1 μPa²-s) and regression line to best fit the shift onset \(F_{1,28} = 256.6, P < 0.001, y = 10.5 \log x + 204.7, r^2 = 0.902\). (b) Similar effects plotted on a linear time scale. (c) Surface plot of measured and predicted odontocete hearing threshold shifts for sonar and noise exposures under a range of exposure.
sound pressure levels (dB re: 1 μPa) and durations (min) using the equation of the line plotted in a and b. Hearing shifts were not predicted past 40 dB as those values near the range of permanent effects and beyond the scope of the experiment.

Each of these scenarios entails the subject being relatively close to the sonar source for a somewhat prolonged duration. Exceptions may be if the sonar signals are rapidly repeated (which is unlikely due to overlap of returning echoes) or oceanographic conditions in which sound levels do not attenuate regularly and thus remain intense. In that case, temporary threshold shifts might be induced at longer ranges if the animal were to remain in the high level sound field for a prolonged duration. Perhaps such a situation could occur with multiple sonar sources over an underwater canyon.

Our results demonstrate that mid-frequency sonar can induce at least temporary physiological auditory effects in odontocete cetaceans. Our data also reveal that subtle behavioral changes may also be caused by noise exposure. These behavioral conditions should be monitored in further experimental and field studies to examine the robustness of data presented here. In contrast to previous studies, we show that predicting these noise induced threshold shifts cannot be based solely on an equal energy model but rather, sound exposure levels must increase as noise duration decreases for continued shift onset. This agrees with some terrestrial mammal and pinniped noise exposure results (Ward 1991; Kastak et al. 2005). Our data indicate that short acoustic signals, such as sonar pings, must be of high intensity with repeated exposures to induce auditory physiological effects. The data presented here
are novel, thus we suggest that controlled exposure studies such as this be applied to a
greater number of cetaceans in both laboratory and field conditions to explore the
variability likely to be found at population levels. The findings also reflect the need
for caution when employing such acoustic signals in the wild as well as the careful
consideration by naval and regulatory agencies of such applicable data.

Methods

The study was conducted at the open seawater pens at the Hawaii Institute of
Marine Biology, University of Hawaii (UH) using a 20-year-old adult male bottlenose
dolphin (*Tursiops truncatus*). All procedures complied with UH Institutional Animal
Care Committee and the National Marine Fisheries Service permit # 978-1567-02.

Hearing thresholds were measured using the auditory evoked potential technique
described in previous studies (Nachtigall et al. 2004; Mooney et al. 2006). A mean
threshold at 5.6 kHz was established before the experiment, and a temporary
threshold shift was defined as a threshold (after a mock- or actual noise exposure) that
exceeded 1 s.d. above the mean threshold and consequently returned to within 1 s.d.
variation. Recovery was always tracked 40 min past the sonar exposure. The
experiment began with a baseline threshold measurement to ensure the subject’s
threshold was near its average threshold. Then the dolphin voluntarily moved to a
noise exposure pen for mock- or actual sonar exposure. The sonar exposure could be
minimized to a 1-2 min trial, immediately after which respirations were counted for
30 s. The subject was then resent to the hoop briefly (1-2 s), before returning to the
threshold testing pen for follow-up hearing tests. These began 2-2.5 min after the
cessation of the sonar signal. The sonar ping was generated digitally from a .wav file, played with a custom LabView program, and converted to an analog signal using a NI-6062E DAQ card in a laptop computer. The signal was amplified using a Crown MA-5002 amplifier and played through a Massa TR-61A transducer. Behavioral observations during sonar or control exposures were noted by the animal trainer and an assistant. The threshold shift model was created using data from this and a separate experiment using octave-band noise as the fatiguing sound (Mooney et al. 2008). Threshold shift growth was estimated by amount of shift relative to octave-band fatiguing sound energy flux density (SEL) where shift ($\geq 0$) = 3672.65 - 40.3299*(SEL) + 0.110712*(SEL)$^2$. Shifts were not estimated above 40 dB, which was considered near permanent hearing damage.

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Author Contributions

T.A.M. and P.E.N. designed the experiment. Data was collected by T.A.M. and S.V. Analysis and manuscript preparation was performed by T.A.M. and P.E.N.
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References


CHAPTER 7

SUMMARY AND CONCLUSIONS

The aim of this dissertation was to explore how the odontocete auditory system processes a diversity of broadband sounds with varying temporal characteristics. This included investigating: a) responses to click stimuli of rapid rates to determine temporal processing capabilities across several species, b) the directional reception of short, broadband clicks across the head of a beluga whale, c) the effects of intense fatiguing stimuli (noise and sonar pings) which induced temporary threshold shifts across a range of durations, d) and predicting threshold shift onset from empirically derived results. Investigations were made in a comparative light, examining results relative to other odontocetes and marine mammals, as well as terrestrial mammals.

The main findings of the experiments were:

- The auditory temporal resolution and AEP delay of the Risso's dolphin is quite rapid, even for echolocating odontocetes. Based on this temporal resolution, it appears that the auditory system of this Risso's dolphin, and likely other odontocetes, is sufficient to not only follow individual echolocation clicks and echoes but to do so at very close ranges where time between clicks and echoes is extremely short.
• The shape and bandwidths of MRTFs, are conserved among odontocetes suggesting that their auditory temporal resolution is also similar between species. Similar parallels are found in terrestrial mammals. The hearing processing speed within odontocetes likely derived from the need to process fast acoustic cues in water and concurrent echolocation.

• Receiving sensitivity in the beluga whale is quite different from that of the bottlenose dolphin indicating that how odontocetes receive sound may vary substantially between species. Although a shaded receiver model was supported, the model will differ between species likely depending on head and acoustic fat morphology. This hearing diversity emphasizes the differences found between species and caution is suggested when applying auditory models to species we know little about.

• Intense, short-duration fatiguing noise requires significantly more energy to induce TTS than longer, lower amplitude sounds. This does not support an equal energy model of temporary threshold shift but rather a model that predicts that shorter signals require greater energy to induce TTS. The complexity of TTS demonstrated in odontocetes supports investigations of how TTS is induced using a wide range of noise variables to develop an equal-TTS model to predict shift onset. Recovery from exposures was logarithmic but exposures of longer duration and greater shifts initiated slightly faster recovery rates. Finally, many trends such as recovery rate and
frequencies of shift relative to fatiguing noise were similar to terrestrial mammal data.

- The short-duration, but intense, sonar pings required very high sound energy levels (214 dB re: 1 μPa²-s) to induce TTS. Subtle behavioral reactions were also demonstrated significantly more often during noise exposures. Results indicated that the animal would likely have to be relatively close to the sonar source and within the beam for multiple ping exposures for shifts to occur. However, it was demonstrated that intense mid-frequency sonar can physiologically affect an odontocete species' auditory system, thus caution should be taken when applying such sonar in the habitat of wild populations.

Implications and Applications

In addition to the chapter summaries above, three broad points may be taken from this dissertation, relating the entirety of the chapters. The first point is that within the odontocetes that have been examined, their temporal processing capabilities are strikingly similar (Mooney et al. 2006; Chapters 2 and 3) reinforcing some clear consistencies within odontocete hearing. Odontocetes respond to sound rapidly and follow individual sounds up to very high relative rates. This is not surprising as they have similar ecological and adaptive constraints, meaning they are all facultatively aquatic and all seem to echolocate. In order to utilize sound under both constraints, high-speed sound processing is required. Other auditory adaptations, such as hearing ranges (Nachtigall et al. 2000), the shape and partitioning
of the auditory bulla and middle and inner ear morphology (Ketten 2000), are also
grossly consistent between species. Temporal resolution might also be considered
among these defining attributes of odontocete hearing.

We may also see similar consistency within terrestrial mammals across their
functional morphology and physiology. This might include the middle ear bones,
unique among mammals, allowing for the transduction of high frequency sounds
(Rosowski 1992; Yost 1994). Another example is the traveling wave of the cochlear
basilar membrane allows for detection across a broad range of frequencies (von
Bekesy 1960). And terrestrial temporal processing speeds are also relatively
consistent, cutting off at approximately 50-150 Hz (Fay 1994; Long 1994). These
“constants” allow us to draw trends used to predict certain attributes for species we
have not yet examined. High frequency hearing limits can be reasonably predicted by
the distance between a species’ ears and the basilar membrane stiffness (Heffner and
Heffner 1992; Echteler et al. 1994). The functional hearing range (in octaves) can be
estimated by the number of cochlear turns (Echteler et al. 1994). Thus, mammalian
evolutionary history has obviously played a strong role in both the development of
auditory adaptations and the consequent conservation of these apparently successful
derivations.

Why certain auditory traits are well conserved is an interesting point. The
auditory sense, including proper temporal resolution and processing, facilitates the
detection of prey, predators and mate recognition, all strong evolutionary pressures.
Across taxa we see hearing used for basic biological events. Moths detecting bats
and vice-versa (Surlykke 1984; Moss and Zagaeski 1994), and frogs recognizing
mates (Rose et al. 1985) are examples of adaptations which utilize and detect temporal modulating signals. Adequate time processing and resolution of auditory cues and echolocation signals is undoubtedly fitness-enhancing. Additionally, the mammalian ear is a leap in complexity which allows for utilization of a greater range of acoustic signals. These signals are not available to many other animals, which may further reinforce evolutionary conservation of such adaptations. The mammalian middle ear structures and cochlear-basilar membrane complex allow for increased sensitivity across a broad range of frequencies (Rosowski 1992). Although ontogeny may be costly, the improvements in predator and prey detection (by improved sensitivity), as well as other potential adaptive characteristics (e.g. intraspecific communication outside the frequency range of predators or prey) provide ample situations to increase fitness and promote the conservation of such traits. For example, sensitive hearing and detection of time-amplitude modulated signals allows for long distance group communication and identification in elephants (McComb et al. 2003). Temporal processing (and localization) of high frequency signals by dolphins and bats allows for the detection of prey, in many cases where the prey likely do not hear the predators. Thus detection, identification and classification of sounds play an important role in various fitness enhancing events and included in the perception of the sounds is processing of their temporal components. Not only is hearing a vital biological tool, mammalian and odontocete hearing systems have adapted several unique auditory traits that appear conserved across many taxa.

The second point from this dissertation is that while there are constants, there is also much diversity in odontocete auditory systems. Results of the beluga study are
a prime example of this (Chapter 4). Previously, all odontocetes were considered to primarily receive sound through the pan bone region of the lower jaw (Bullock et al. 1968; Norris 1968; Mehl et al. 1999). However, this study has shown that different species may receive sounds differently, likely based on the morphology of their head and jaw fats, which are the acoustic receivers. Prior research reflects other auditory variations including directional sensitivity (Kastelein et al. 2005), auditory filter bandwidths (Popov et al. 2006), and gradients in the acoustic fats (Koopman et al. 2006). This reinforces the importance of understanding the auditory system in the dozens of cetaceans that have been not yet been examined thoroughly and stresses the importance of considering such diversity when applying models to species for which we know relatively little, such as mysticetes and beaked whales.

Within a broader perspective we also see similar diversity within other groups of animals. For example, humans are good auditory generalists, in that we hear many frequencies (<20 kHz) relatively well (Echteler et al. 1994). Our cochlear map, which estimates the proportion of the cochlea devoted to each octave, is relatively consistent across our hearing range. This provides good hearing across a range of frequencies. While rodents are known for their good high frequency hearing, the mole rat, *Spalax ehrenbergi*, specializes in low frequencies, 0.6-1 kHz, presumably for subterranean localization. Thus its cochlear dimensions do not adhere to the typical mammalian trends of predicting hearing range, high frequency hearing limit, and temporal resolution listed above (Echteler et al. 1994). Another hearing specialist, the horseshoe bat, *Rhinolophus ferrumequinum*, breaks nearly all mammalian hearing trends. It hears very high frequencies (100 kHz), has cochlear specializations for frequency analysis
near 80 kHz (Bruns 1976), and echolocates and processes very rapid signals (Weibchenacher et al. 2002). In fact, bats in general show a broad range of auditory diversity with often species specific adaptations in discrimination of (higher) echolocation frequencies (Pollak 1992), sensitivities in (lower) social communication frequencies (Bohn et al. 2004), morphology dependent head related transfer functions (Chiu and Moss 2007), cochlear innervation (Henson Jr. 1978), and other characteristics.

Thus, while there are many commonalities, divergence that seems to be found in odontocetes is not all that uncommon. The diversity likely has two contributing factors. First, acoustics and hearing are certainly not the only driving evolutionary forces. For example, odontocete head morphology also likely has much to do with foraging tactics and prey selection. Spinner dolphins (Stenella longirostris), which typically feed on small mesopelagic fish, squids and crustaceans, have a bony, almost sharp, rostrum (Benoit-Bird and Au 2002; Benoit-Bird and Au 2003). This may affect reception of sound but the particular shape may be a result of a feature that developed to grab small prey items. In contrast, false killer whales, whose habitat often overlaps that of spinner dolphins, have a rounded, blunt head (Reynolds and Rommel 1999). Acoustic fats likely are found up to the tip of the rostrum and probably guides sound in a different manner. Their prey items include large pelagic fish such as mahi-mahi (Coryphaena hippurus), marlin (Istiophoridae), and tunas (Scombridae), for which a rounded head may be better suited at grabbing and tearing apart those fish. Sexual selection may also play a role in adapting traits that are auditory related. For example, both sperm whale echolocation clicks and head morphology have been suggested to convey male fitness
Further, humpback whale (*Megaptera novaeangliae*) song is tied to reproduction although the exact function is unknown (Darling and Berube 2001). The auditory and sound production mechanisms of a humpback whale are likely related to enhance sound reception and projection. Thus, while auditory diversity exists, the reasons behind divergent adaptations likely stem from several evolutionary pressures, auditory processes being just one.

Second, as temporal processing speeds in odontocetes are likely based on medium (water) and echolocation requirements, other auditory characteristics may also depend on niche. To return to the false killer whale and spinner dolphin examples, false killer whales would probably detect larger prey at longer ranges in relatively open pelagic environments and spinner dolphins need to identify much smaller prey in cluttered meso-pelagic patches at short ranges. The requirements for localization in the two situations are quite different, thus reception of sound and head morphology can be quite divergent. The pressures for conserved characteristics versus divergent niches can certainly lead to some traits which are constant while others are unique.

Therefore, although generalizations can be made which enhance comparative biology, there are specializations for specific niches which do not fit the predicted trends. This is true for odontocetes as well as other mammals. Models and predictions of auditory systems should then be fit with caution. In light of this discussion, it is important to address the appropriateness of the TTS-onset model proposed here.

The third point of this dissertation work is that shorter duration sounds affect the odontocete auditory system differently than longer duration sounds. Specifically, shorter duration sounds require greater energy levels to induce TTS in a bottlenose
dolphin, results which are inconsistent with an equal energy model (Chapters 5 and 6). From these data, a model to predict odontocete TTS can be derived. However these and other noise exposure data were acquired primarily from one species, the bottlenose dolphin, and relatively few experimental subjects. Thus the application of these data to populations and across species must be considered.

Interestingly, the trends in the TTS experiments (frequency of shift, recovery functions, and temporal effects) follow guidelines found in terrestrial mammal models (Bohne and Clark 1990; Ward 1991). These results are also comparable to other marine mammal TTS studies (Finneran et al. 2007; Kastak et al. 2007). Important to the TTS-onset model, research from other taxa, including humans, chinchillas, guinea pigs and pinnipeds, also refute the equal energy hypothesis yet support a line of increasing energy with decreasing exposure duration (Mills et al. 1981; Buck et al. 1984; Ward 1991; Kastak et al. 2005). The reason for the comparability of data across taxa may be that TTS and PTS are primarily physiological inner ear phenomena (Harding et al. 1992; Nordmann et al. 2000). In both situations, hearing sensitivity is altered by noise exposure detrimentally affecting the hair cells and associated cells of the inner ear. As the gross morphology of the inner ear of marine and terrestrial mammals is largely similar (Ketten 1992), it is consistent that noise exposure effects and TTS are also similar. While individual variation is nearly a constant in TTS research (Clark 1991) and all results must be taken with consideration, the compatibility of these data at least reinforces their reliability. Further, they may suggest that although odontocete “outer” ears are
adapted for aquatic life, the inner ear and the physiological effects of noise are fairly consistent in mammals.

The TTS data here seem comparable to other studies thus applying the results is well supported as long as the caveats are considered. Additionally, by addressing the relationship between noise exposure duration and intensity, the results fill a gap in predicting threshold shift onset and occurrence. If the sound level and duration are known, one can predict the occurrence of physiological effects. Further, if TTS onset can be considered the limit of a reasonable amount of noise exposure then this model also would predict noise exposure limits. There are current ongoing legal arguments on what levels and situations naval sonar use should be permitted (e.g., Lester 2008; Southall et al. 2008). This dissertation and the consequent published manuscripts will supply much needed information for sound judgments based on sound scientific evidence.

Future Research

Although the results presented here lead to interesting conclusions, they also breed future questions which remain to be addressed. Evoked potentials can be applied in many of these experiments. I have noted several of these below:

- Expand audiograms in other species. There is an obvious lack of data on hearing in cetaceans and expanding our hearing range database is the first step. This is especially true for groups of species which we know little about, including beaked whales, mysticetes, and river dolphins.
• Explore receiving sensitivities, MRTFs, and other characteristics. From the data presented here, we know that hearing range information is not enough. Species vary in how they receive and process sound and consequently our effects of noise exposure may also vary. There are very little comparative data in this area and much of it shows striking differences. This would involve investigating new species as well as applying MRI/CT scanning techniques with AEPs to examine the heads (receivers) of various marine mammals. Of particular interest are species which are morphologically divergent such as the river dolphins, beaked whales and porpoises.

• Auditory scene analysis. This is the processing of the auditory scene and sorting of information, a topic applied in bats but not yet addressed in marine mammals. A combination AEP/psychophysical experiment could provide insight into how odontocetes use their acoustic sense to derive information about their environment. How odontocetes electrophysiologically follow single or multiple targets during echolocation and how complexity of an auditory scene affects detection and discrimination are introductions into this investigation.

• Intermittent exposure TIS. Exposure duration plays a crucial role in inducing TIS. We know that many exposures are not from continuous sound and recovery may occur between exposures. This not only further refutes an equal
energy theory but complicates predicting TTS onset and effects. Fatiguing stimuli can be sonar signals, white noise or other sounds. The duration and consistency of the intermittent (sound-off) period which allows full recovery is particularly important. There are no data in this area for marine mammals.

- Effective quiet. This is defined as the maximal sound pressure level that will not cause TTS nor retard its recovery, regardless of duration. It is an important metric in predicting and mitigating the effects of noise exposure, especially because anthropogenic noise is often of low amplitude and long duration (shipping, oil drilling) but effects are unknown. This experiment could be conducted by inducing TTS from an established fatiguing noise. Recovery thresholds could then be tracked whilst the animal is exposed to varying levels of background noise, measuring the rate of recovery relative to the level of background noise.

- Loudness contours. This is a relative concept of perceiving several sounds as "equally loud" and the perceived loudness can be used to estimate the effects of noise (physiological and behavioral). Loudness curves are derived using psychoacoustic methodologies and do not exist for marine mammals. Currently, human contours are applied to cetaceans in noise exposure models, thus appropriate measurements are required.
• Startle reactions. Behavioral reactions to sound are as important as physiological effects, however, in the field, controlled exposure experiments are logistically challenging. Startle reactions are one example of behavioral investigations that examine the influences of sound exposure, especially short, loud sounds (e.g. sonar, airguns) on marine mammals.

• TTS/noise effects in other marine species. Much of this dissertation has focused on effects of noise in odontocete cetaceans. However, fish and invertebrates are also vulnerable to the impacts of loud sound. With the huge numbers of marine species, there is also a relative paucity of information available for these groups as well. In particular interest are invertebrate species including cephalopods, and crustaceans such as prawns. These animals have the ability to hear but there are no data that examining the effect of noise.

• Behavioral effects of noise in wild/other species. There is little information on controlled exposure experiments in wild marine animals, including marine mammals or fishes. Yet, this provides the in situ empirical data. Exposures to air guns, sonar, or continuous low frequency noise are all potential real-world and experimental sound sources. Tracking changes (or not) in acoustic communication, habitat use or effects on fitness would provide valuable information. Soniferous and hearing specialist fish would be of interest as
they are relatively easily tracked and monitored, and sound seems to play an important role in their ecology.

Conclusion

The initial goal of this dissertation was to explore and quantify how the odontocete auditory system processes broadband sound of varying temporal characteristics. In doing so, I identified several novel ideas regarding odontocete hearing including the apparent consistency in temporal resolution capabilities, variation in how odontocetes receive sound and the relationship between noise duration and intensity. I addressed the equal energy theory of noise exposure as well as immediately applicable mid-frequency sonar issues. Here and in other works, there has been a great deal learned about the auditory system of whales and dolphins and marine species in general, but there is obviously much more to be gained. I look forward to continuing these investigations.

References


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