DIRECTIONAL HEARING AND A HEAD-RELATED TRANSFER FUNCTION (HRTF) OF A BOTTLENOSE DOLPHIN (*Tursiops truncatus*)

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ABSTRACT

Odontocete cetaceans have some of the most sensitive hearing in the animal kingdom. Despite over a half-century of research on these animals, there are still many aspects of their functional anatomy and physiology that have not been described or even explored. Directional hearing allows an individual to locate an object in space and is important in foraging, predator avoidance and social cohesion. Despite notable publications on the directional hearing in bottlenose dolphins, there is still an incomplete picture as to the three dimensional hearing capabilities of these animals. This study uses auditory evoked potential (AEP) techniques to explore the directional hearing capabilities of an Atlantic bottlenose dolphin (*Tursiops truncatus*). Audiograms obtained for different design regimes were compared. Then, using an underwater in hoop with biteplate setup was used to obtain the directional hearing thresholds in multiple angles in the horizontal plane and depths. In addition to the threshold values, the first head-related transfer function (HRTF) on any marine mammal species was collected. These studies promise to shed greater light on the plasticity of directional hearing and localization capabilities.

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List of Abbreviations and Symbols

AEP - Auditory Evoked Potentials
CNS - Central Nervous System
dB - Decibels
EFR - Envelope Following Response
HRTF - Head-Related Transfer Function
Hz - Hertz
kHz - Kilohertz
msec - Millisecond
µPa - Micropascal
µV - Microvolt

Chapter 1
Introduction

Marine mammals inhabit many types of oceanic, as well as some riverine, habitats (Reeves et al., 2002). This unique order of mammals relies upon the marine environment as their primary ecosystem. Being a mammal and living in water brings about some major physiological considerations - the most obvious one being their dependence on breathing air while living in water. Other physiological constraints include being homeothermic and inhabiting an environment which is cooler than their body temperature, maintenance of hypotonic tissues compared to the surrounding ocean, and diving, which can entail having to deal with great changes in pressure, temperature and salinity, as well as the need to breath hold for extended periods of time.

Water, with elasticity much greater than air, is an exceptional medium for the transmission of sound. The faster speed of sound in water is due to a higher bulk modulus and in spite of its higher density. In physical terms, the sound speed increases as the stiffness increases and decreases as density increases. In the case of salt water the increase in sound speed is due to the higher bulk modulus (stiffness), which overcomes the decrease in speed due to the higher density, and the net effect is to increase the sound speed (Bradbury & Vehrencamp, 2011). Temperature, salinity and depth (pressure) also play roles in the actual complex and often changeable sound profile of the specific body of water (Wartzok & Ketten, 1998).

There are three parameters of sound that can be physically measured (as opposed to qualitative parameters such as pitch and timbre): frequency, phase and amplitude. The frequency of a sound is, at its most basic, how frequently a sound wave passes an arbitrary point and is in Hertz (per second). The frequency of a sound is inversely proportional to the wavelength of the sound, which means that higher frequencies have shorter wavelengths. The speed of sound in the particular medium greatly affects the wavelength of that sound wave – the faster the sound speed of the medium, the longer the wavelength of that particular frequency. Phase is a property that describes angle of the wave as it arrives at the receiver and its units are in degrees or radians. The amplitude of

a sound is a measure of the power of that sound wave and is analogous to the loudness of the sound and its unit is the decibel (dB) with reference to 1 μPa (for water).
Marine mammals, odontocete cetaceans especially, have exploited the acoustic environment of the ocean and use hearing as their primary sensory system (Kellogg & Kohler 1952). Quite a few studies have focused on the anatomy of the hearing apparatus (Mead, 1975, Ketten, 2000). However, despite the correlation between structure and function, we do not have empirical physiological or even anatomical data as to the exact mechanism of the sound pathway(s).

Human subjects are a lot more accessible to researchers than are marine organisms. The breadth achieved in human hearing studies has been greatly increased by research on those individuals with central nervous system (CNS) or sensory issues that have required surgery. This surgical contact has allowed access to deep hearing structures and pathways (Hall, 1992), giving auditory researchers opportunities to see how the affected regions function. With the ratification of the Marine Mammal Protection Act in 1972, invasive studies on live cetaceans have been prohibited. Since that time, sensory studies of marine mammals have mainly been undertaken using behavioral conditioning or, more recently, using passive electrophysiological recordings.

Electrophysiology: Auditory Evoked Potentials

The Gold Standard in audiometry has always been the practice of obtaining behavioral audiograms. A behavioral audiogram is one in which the subject sits quietly as sounds of known frequency and amplitude are played and is then asked to respond behaviorally in some way when the sound is audible (Schusterman, 1980). The behavioral audiogram has been especially problematic for cetacean researchers in that it requires the training of the subject which is time consuming and expensive. In addition, the state of arousal of the test subject plays a crucial role in the level of the threshold; the more focused the individual, the lower the threshold. Plus, access to a great many species of cetaceans is limited to those in captivity or during a stranding event and that animal is deemed stable enough to be cared for in a rehabilitation facility, which is completely unsuited for lengthy training regimes. Therefore, due to the limited number of individuals and species in captivity, as well as the time required to train the individuals for the task,

behavioral audiograms have been obtained for only a small percentage of marine mammal species (Taylor et al. 2007,Nachtigall et. al. 2000). What was and is needed is a much faster method that does not rely on training the test subject: auditory evoked potential (AEP) techniques fit those requirements.

Auditory evoked potentials (AEPs) are fluctuations in the normal electroencephalograms (EEG) when neurons in the brain synchronously respond to an acoustic stimulus. AEP techniques were developed for human infants as a quick and painless alternative to behavioral audiometry (See review in Hall 1992). Evoked potential audiometry in cetaceans has been practiced for quite some time with good success (Supin, 2001) and the use of AEP in research facilities has allowed relatively rapid collection of otherwise complex, to the point of cumbersome, hearing data (Nachtigall et al., 2007). In addition to research on captive animals, AEP techniques have given researchers the ability to measure hearing from wild cetaceans (Nachtigall et al. 2008), and animals in stranding facilities (Nachtigall et al. 2005). To date, only a handful of evoked potential audiograms for marine mammal species have been done and of the cetacean only those belonging to the Family Odontoceti have been obtained. The number of these studies is quite small compared with the number of species of odontocetes, however it is still a much greater number than have been done behaviorally. However, because the behavioral audiogram is still the standard, the relationship between behavioral audiograms and those obtained with AEP techniques are being increasingly quantified, and are comparable at mid-frequencies, and are similar at the low and high ends of an audiogram (Yuen et al., 2005; Finneran & Houser, 2006; Houser & Finneran, 2006).

This study used the AEP technique of the envelope following response (EFR). A sinusoidally amplitude modulated (SAM) tone with a modulation frequency of 1 kHz was used to carry sounds of differing frequencies. This approach allowed us to measure a rhythmic brainstem response that "followed" or mirrors the envelope of the sound that was played to the test subject. The electrophysiological response measured during an EFR study is most likely the result of firing of multiple brainstem structures (Supin et al. 2001) anywhere from the eighth nerve itself, up to the lateral lemniscus (Hall, 1992).

Anatomy and Physiology of Hearing

The anatomy and physiology of humans is much better understood than those of any marine mammal species. In order to understand the possible mechanisms underlying directional hearing, the basics of the anatomy and physiology underlying fundamental hearing mechanisms must be explored. Human auditory anatomy may be used as a springboard for discussing the anatomy and possible physiology of dolphin hearing. The human head is a very complex structure. Within this structure, all four tissue types are represented and are organized to form structures for feeding, breathing and dealing with sensory information (afferent pathways), and the resulting actions to deal with that sensory information (efferent pathways). Hearing is the capture, transduction and integration of sound waves into neural signals. Sound waves from the environment must get from the sound source to the head and once at the head into the organ of hearing – the cochlea (see Figure 1.1).

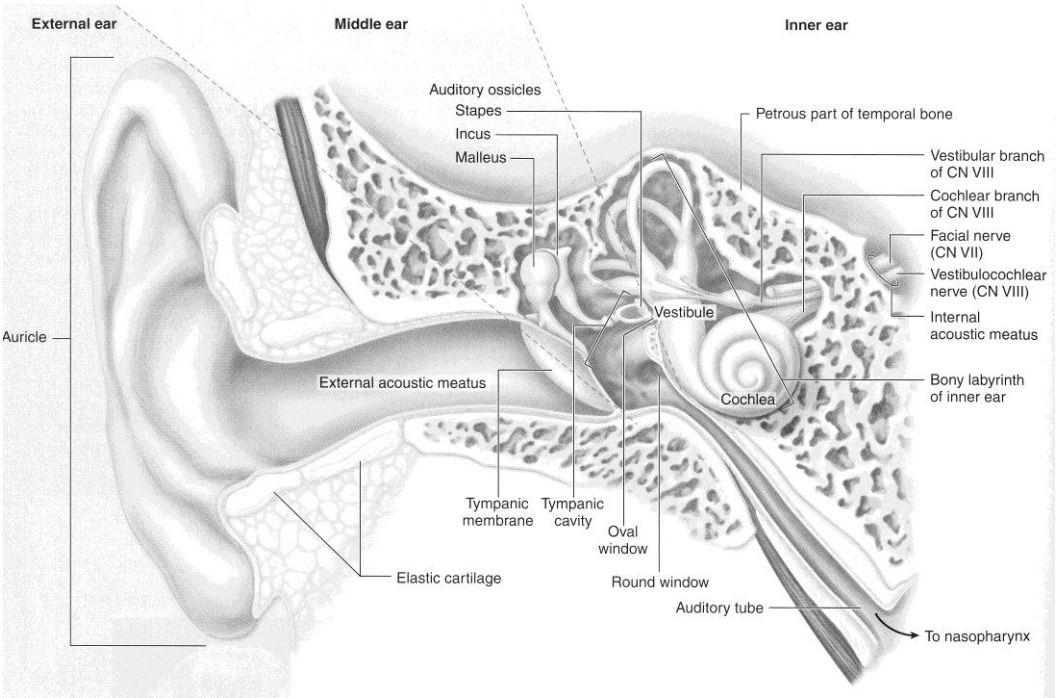

Figure 1.1
The human ear showing all three divisions: outer, middle and inner. (McKinley and O'Loughlin (2006) Figure 19.20)

The Outer Ear

The function of the outer ear is to funnel sound to the tympanic membrane and into the middle ear. During this process, the sound will be modified due to the sound wave interaction with the tissues of the head. The outer ear consists of the auricle or pinna, the external acoustic, or auditory, meatus (ear canal) and the tympanic membrane. The shape of the pinna allows sounds coming from the front of the head to reach the auditory meatus faster than sounds originating from behind the head. This differential time of arrival gives the brain cues as to the location of the sound in space. In addition, the multiple groves and bumps of the pinna seem to act like filters or amplifiers of particular sound characteristics such as the 10-15 dB increase for frequencies from 1.5-7 kHz observed in human subjects (Yost, 2006). The sound wave moves past the pinna down the external auditory meatus and impinges on the tympanic membrane, or eardrum. This structure is popularly depicted as a flat, uniformly thick, drum-like membrane. However, the actual tympanic membrane is funnel-shaped and has varying thickness and directionality of the connective tissue causing complex oscillations that somehow encode the frequency and amplitude of the sound as it is at that location.

The Middle Ear (Tympanum)

The transduction of sound waves from the external environment into the fluid environment of the inner ear is the major function of the middle ear. The middle ear is bounded on the proximal (medial) side by the oval and round windows and on the distal (lateral) side by the tympanic membrane. Sound waves impinge on the tympanic membrane and move the inner ear ossicles relative to each other.

The three inner ear ossicles are the smallest bones in the body (McKinley and O'Loughlin, 2006) and consist of the malleus, incus and stapes, all of which are held in place in the middle ear cavity by the axial ligaments. These bones allow the airborne sound to be transferred into the fluid-filled cochlea of the inner ear. The malleus is continuous (via connective tissue) with the connective tissue of the tympanic membrane and articulates with the incus, which connects to the stapes - which itself is continuous with the oval window through connective tissue. The motion of the stapes is transferred via the oval window (or vestibular window) which is a membrane-covered opening which

leads from the middle ear to the vestibule of the inner ear. The round window allows release of pressure at the opposite end of the inner ear. The ossicles do not move in a simple linear motion with one another but have a complex motion that may act as a filtering mechanism (Yost, 2006) for the sound before it moves the oval window.
The contraction of small muscles can also limit the movement of the inner ear ossicles. The tensor tympani is attached to the malleus and, when contracted, limits the oscillations of the tympanic membrane which decreases the amount of movement of the ossicular chain. The stapedius (stapedial) muscle is attached to the stapes and, when contracted, limits the stapes motion against the oval window which decreases the motion of the fluids in the inner ear. Both muscles act to decrease the propagation of sound to the inner ear. Pressure in this air-filled chamber is equalized through the opening of the eustachian tube which is continuous with the nasopharynx. If this passage is swollen or occluded in any way, it can affect hearing through the increased or decreased pressure in the middle ear relative to the outer ear.

The Inner Ear

The actual structure which transduces sound waves into neuronal signals – the Organ of Corti – is contained within the inner ear. The inner ear is comprised of a tube that decreases in size and is coiled like a snail shell – a structure called the cochlea. The cochlea itself is wrapped around spongy bone called the modiolus which is surrounded by the petrous portion of the temporal bone. The sound waves reach the inner ear through the stapes action against the oval window and the pressure wave created by this motion traverses the cochlea and is dispersed out the round window.

The cochlea is divided into three compartments: the scala vestibuli, the scala tympani and the scala media. The scala vestibuli and scala tympani are continuous with one another through the helicotrema – a small opening at the apex of the cochlea. The scala media, as its name implies, is located between these two scalae with its vestibular membrane abutting the scala vestibuli and its basilar membrane located on the scala tympani side. The scala media is attached to the outer petrous bone through the spiral ligament on the lateral side and the osseous spiral lamina on the medial side. On the

inside of the spiral ligament there are layers of cells called the stria vascularis whose functions include synthesizing the potassium rich endolymph which baths this scala.

On the basilar membrane lies the organ of Corti which is made up of multiple cells types one of which being the cells of neural transduction – the hair cells. There are 4 rows of hair cells – 3 outer rows and one inner row. Hair cells are modified epithelial cells and were named after their sterocilia that protrudes from the apical membrane, which, in the case of the 3 rows of outer hair cells, are embedded in the tectorial membrane. The scala media is bathed in endolymph which is similar to the ionic concentration of intercellular fluid in that it has a high level of potassium ions and a low concentration of sodium ions. The hair cells themselves have a resting membrane potential of -70mV. In contrast both the scala vestibuli and scala tympani are filled with perilymph which is similar in composition to interstitial fluid – high in Na+ and low in K+. The potential difference, of these two distinct fluids (with reference to the perilymph) is +80mV.

The stapes of the middle ear moves against the oval window, which creates a pressure wave that impinges on the perilymph fluid of the scala vestibuli which then moves through to the scala tympani and disperses by oscillating the round window. As a sound wave moves into the scala vestibuli from the oval window it compresses the fluid and makes it move in relation to the vestibular and tectorial membranes which then move the steriocilia of the hair cells (both vertical and shear forces). As the steriocilia move in one direction mechanically gated K+ channels open and allow K+ to flow into the hair cell which triggers the opening of Ca^{2+} channels on the hair cells. This causes them to release neurotransmitter (dopamine) towards the dendrites of the afferent neurons. These afferent neurons then fire action potentials. If the steriocilia are moved in the opposite direction, the K+ channels close which stops the influx of K+ which, in turn, stops the influx of Ca^{2+}. This action stops the release of neurotransmitter. Louder sounds cause the hair cells to open more K+ channels thereby causing the release of a lot more neurotransmitter which will result in a higher frequency of action potentials fired by the afferent neurons (Stanfield, 2010).

The basis for the neural coding of frequency (pitch) seems to be the mechanical properties of the basilar membrane itself: it is narrow and relatively stiff at the base and

becomes wider and more flaccid towards the apex (helicotrema). High frequency sounds are much more likely to make the narrow stiff base move and lower frequency sounds are much more likely to make the broad, flaccid apex shift. Efferent control of the hair cells seems to be done mainly through the modulation of the outer hair cells. However the stria vascularis and the multiple types of supporting cells within the organ of Corti most likely play important roles in modulation of the cochlear sensitivity as well (Yost, 2006).

The Central Nervous System (CNS)

Hearing, like the other senses, is a complex undertaking. Because of the inherent complexity of the CNS structures involved and the multiple neuronal types used within these structures, the processing of sound by the CNS is still not well understood. Overall, the CNS's job is to integrate sound information with those sensations from the other sensors, such as touch and sight, and then act to organize and carry out the most optimal visceral or behavioral output.

The afferent sensory neuron has an area in space that it responds to called its receptive field. It transmits this information from the environment (of a particular sense organ) to the CNS. Particular sensory modalities are encoded on what is called a labeled line. This means that the specific sensory stimuli are relayed up a specific pathway into a location in the CNS that deals with that specific modality. So how does the brain know how intense a stimulus is? A simplified model demonstrates that through the labeled line the CNS detects the frequency of action potentials (frequency coding) and the number of receptors that are activated (population coding). How does the brain know exactly where the stimulus is? It depends on the acuity of the sensory system, which is based on how large the overlaps between receptive fields and the presence of lateral inhibition (when one afferent is triggered it inhibits those directly around it which increases the contrast of the signals in the nervous system) (Stanfield, 2010).

A "simple" pathway for the incoming sound as well as the outgoing response and modulation of the pathway is not possible. The pathways for binaural hearing, like those of the other special senses, are highly complex with constant branching as well as cross-over connections to the ipsilateral side which seems to contradict, at least partially, the labeled line theory (Yost, 2006). However, as the overall purpose of this study is not an

anatomical exploration of the auditory central nervous system the discussed pathways will be brief and simplified.

Cranial nerve VIII, the vestibulocochlear nerve, a mixed nerve made up of both afferent and efferent neurons for the inner ear as well as the vestibular organs, dives through the internal auditory meatus. Action potentials, initiated by the inner hair cells of the cochlea, travel along the auditory portion of cranial nerve VIII to the cochlear nucleus. The afferent neurons (collaterals or interneurons) travel to three distinct regions within this nucleus: the anteroventral cochlear nucleus (AVCN), the posteroventral cochlear nucleus (PVCN) and the dorsal cochlear nucleus (DCN) all of which seem to have frequency mapping of the entire basilar membrane represented within each of these nucleii (Yost, 2006). However, due to the many neuronal types present in this nucleus as well as the multiple interconnections within it, basic frequency mapping seems a gross oversimplification. In fact, given just the variable neural anatomy found in this structure it is quite possibly involved in complex spectral processing of auditory stimuli. From the cochlear nucleus, information travels to the superior olivary complex in the pons. This complex is the first site that has a lot of innervation from both right and left cochlea and would, therefore, be a good guess as to where differential arrival times for both ears begin to be processed (Yost, 2006).

The auditory information from the olivary complexes travel up through the lateral lemniscus to the inferior colliculus in the midbrain. The inferior colliculi seem to have the ability to analyze both spectral as well as binaural cues and have also been implicated in the processing of information from the ears about vertical changes in the sound source which may be related to sound shaping through head related transfer functions (Yost, 2006). From the inferior colliculus, the information travels to the medial geniculate nucleus of the thalamus and then, for final processing, travels on up to the primary auditory cortex located in the Sylvian fissure of the temporal lobe.

This seeming linear path is a great oversimplification as there seems to be many locations where the acoustic information from one cochlea crosses ipsilaterally as well as multiple horizontal connections between the right and left olivary complexes, lateral lemnisci and inferior colliculi. To add to the appreciation of the complexity of this

system, there are always the connections between the auditory cortex and the cerebellum, as well as the visual cortex that are very poorly understood.

The basic descending pathways for efferent control of hearing seem to travel down from the auditory cortex back through the nuclei that the incoming afferent information ascended through. How many synapses occur between the incoming afferent information and the outgoing efferent control is unclear but one could surmise that anywhere these two directions of communications run side by side there is a great possibility for neuronal facilitation or inhibition.

Hearing in Odontocete Cetaceans

The Middle and Inner Ear

The middle and inner ears are not connected to the skull of odontocetes, instead they are housed in dense boney structures of the Tympanic bulla and Periotic bulla (see Figure 1.2) (Cranford et al., 2010).

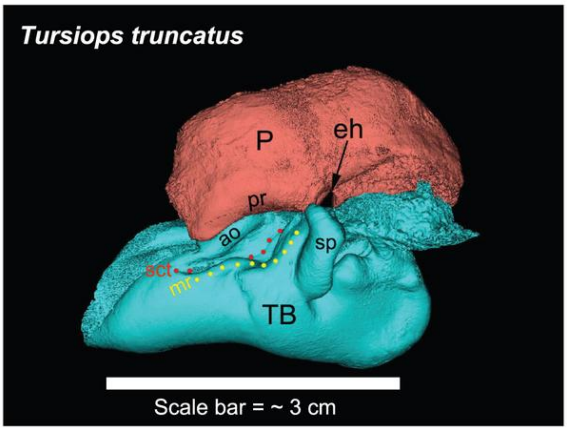

Figure 1.2
The tympano-periotic complex (TPC) of a bottlenose dolphin. "P = periotic bone; TB = tympanic bulla; eh = epitympanic hiatus; pr = parabullary ridge; ao = accessory ossicle; sp = sigmoid process; mr = mallear ridge (yellow dots); sct = sulcus for the chorda tympani (red dots)." From Cranford et al., 2010 Figure 6.

This tympano-periotic complex (TPC) is suspended by ligaments lateral to the temporal bone in the peribullar cavity (Ketten, 2000, Supin et al., 2001) and is lined with specialized, vascular, spongy epithelial tissue – the peribullar plexus. This plexus, with the fats, ligaments and air sinus's that surround the TPC, sometime referred to as

albuminous foam (Branstetter & Mercado, 2006) seem to serve as acoustic isolators (Ketten, 2000) because of the density differences between this "foam" and the dense bone of the TPC.

The tympanic bulla is lined with a thick, highly vascular sheet called the corpus cavernosum which is thought to act, by vasodilation, in preventing barotrauma to the middle ear. The eustachian tubes of odontocetes are notably rigid and tough when compared with humans and are thought to be another adaptation to a life of diving and the associated pressure changes.

Also within the Tympanic bulla are housed the three middle ear ossicles which have a recognizable mammalian shape but are modified (McCormick et al., 1970) in terms of increased stiffness and are significantly more dense for odontocete cetaceans (Ketten, 2000). The malleus is not in direct contact with the tympanic conus but is attached to the wall of the tympanic bulla through a thin process of bone called the processus gracilis. The stapes articulates directly with the oval window, as it is in humans, which transfers the sound into deflections in the fluid in the inner ear (see Figure 1.3) (Ketten, 2000).

The periotic bulla contains the inner ear structures. All cetacean cochlea examined thus far exhibit the mammalian three scalae system: the scala vestibuli and scala tympani surrounding the scala media. Yet odontocete cochlear structures demonstrate hypertrophy, characteristic basilar membrane structures (very broad and loose in mysticetes and short and rigid in odontocetes) and very dense ganglion cell distributions (Wartzok & Ketten, 1998). The ratio of afferent neurons to inner hair cells is notably high and is thought to be required to gather greater detail for processing by the CNS on ultrasonic signals, such as those used for echolocation.

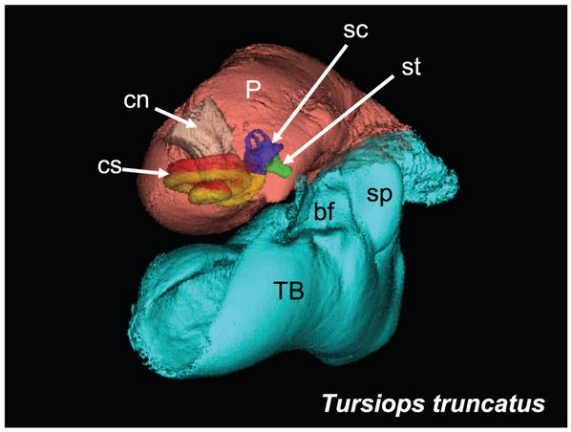

Figure 1.3
The TPC of a bottlenose dolphin cutaway and made transparent to visualize the location of the middle ear ossicles, the cochlea and cochlear nerve. "The accessory ossicle of the tympanic bone (TB) has been removed to more clearly demonstrate the relative position of the cochlear spiral (cs), the semicircular canals (sc), and the cochlear nerve (cn) or eighth nerve, all contained within the periotic (P) bone. The *scala vestibuli* (yellow) and *scala tympani* (red), components of the cochlear spiral, are shown in relationship to the semicircular canals (blue), the stapes (green), the (TB) tympanic bone (cyan) and the (P) periotic (salmon)." From Cranford et al., 2010 Figure 12.

The Outer ear

Of the three outer ear elements, the pinna, the external auditory canal and the tympanic membrane, odontocetes appear to have lost two (Ketten, 2000) – the pinna and external canal. In no odontocete species is there anything like an external pinna and in most the external canal is actually present but usually blocked by cellular or waxy debris (Supin et al., 2001, Ketten, 2000). In most cases this canal does not associate with the tympanic membrane at all (Ketten, 2000). However, the critical filtering function of the pinna and external auditory canal has been demonstrated in humans (Yost, 2006) and would, therefore, be a function that would tend to be conserved. Perhaps the function of these structures has been adapted from internal structures and conserves this critical filtering function.

The tympanic membrane in odontocetes is much more cone-shaped than humans and is termed the tympanic conus (Supin et al., 2001). There is no direct connection between the tympanic conus and the malleus of the middle ear. It would make sense that terrestrial mammals would need an external canal to funnel the sound into the middle ear so that the sound waves could be amplified and then transduced into the fluid filled

medium of the inner ear. However, odontocetes have no need for the impedance matching of air to water since they are immersed in a watery medium from which the sound could enter their watery tissues without the impedance mismatch that terrestrial mammals encounter (Supin et al., 2001).

With the lack of traditional external ears, the pathway(s) of sound from the environment into the middle and inner ears of cetaceans has been described as "bewildering" (Branstetter & Mercado, 2006). Because the head is such a complex structure (Dierauf et. al, 2001) made of many specialized tissues it would seem reasonable to conclude that as sound waves move through it – even through the primary hearing pathways, whatever they may be – the structure of those sound waves can be altered (see Figure 1.4). The calculated alteration of sound waves by the head is commonly referred to as a Head Related Transfer Function or HRTF (Yost, 2006). Dolphin heads have a unique sinus system interspersed with a series of specialized fatty-channels (Cranford et al., 2008) which are the focus of intense, ongoing, anatomical research. The fatty channels of the panbone area, of the lower jaw, were first implicated as the probable sound transmission pathway by Kenneth S. Norris in 1968. The Norris jaw-hearing hypothesis has been empirically supported by Norris (1975), Brill et al. (1988) and Mohl et al. (1999). Bullock et al. (1968), recording evoked potentials from the inferior colliculus, found that jaw-related channels seemed to be the sound path for tones above 20 kHz but found for stimuli below 20 kHz the sound path seemed to be located around the external auditory meatus. Koopman et al (2006) explored the differential distribution of types of fats and fatty acids within the mandibular fat bodies. They took samples from heads of six specimens belonging to four odontocete families; *Delphinidae*, *Phocoenidae*, *Ziphiidae* and *Kogiidae*. They found that there was a lot more structure to the distribution of fats than previously thought and describe an internal channel within the mandibular fat bodies that is made up of branched and/or short chained lipids.

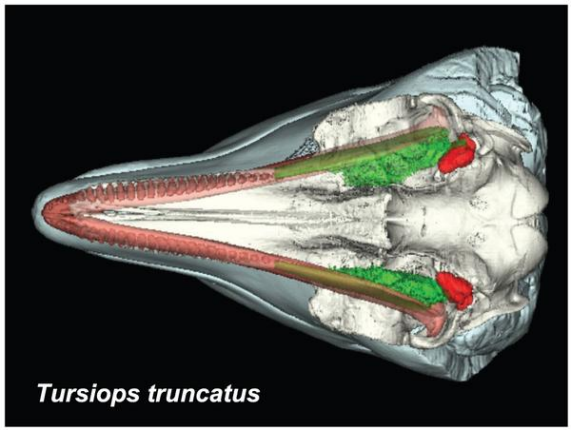

Figure 1.4
The ventral view of the head of a bottlenose dolphin. "Skin = cyan, skull = ivory, teeth and mandibles = salmon, mandibular teeth = salmon, mandibular fat bodies = green, TPC's = red." (From Cranford et al., 2010 Figure 2)

In addition to the debate about soft tissue conduction, transmission through bone conduction as a primary pathway has been considered. The work by McCormick et al. (1970) on anesthetized *Tursiops* and *Lagenorhynchus* concluded that there was no way the external auditory meatus could be the sound pathway into the middle ear and hypothesized that hearing was through bone conduction. It would make sense from a human standpoint that bone conduction be possible since it has been observed and recorded in humans (think of the woofers in the car next to you) and Ketten (2000) still makes a convincing argument for at least some kind of bone conduction in marine mammals despite the unusual density and isolation from the skull of the TPC. Renaud & Popper (1975) suggested that the external auditory canal, although occluded, might be functional at least for certain lower frequencies. Popov and Supin (1990) worked on quantifying the position of the sound receiver using ABR techniques. The transducer was moved in the horizontal position and the resulting differences in latencies were used to calculate the location of reception. Their conclusion was that the results could only be explained if the sound were directly coming into the middle ear via a sound channel around the general location of the external auditory meatus. Supin (2001) comments on this work "these results do not exclude a possibility of additional ways of specific sound transmission, especially for the frontal or near-frontal directions where the auditory sensitivity is maximal."

Finally, there is the more recent evidence from Cranford et al. (2008, 2010) suggest another 'twist' to the jaw hearing hypothesis of Norris. They suggest that the pathway of least resistance is through under the lower jaw, into and through the fatty tissue located there and right to the TPC – the gular region. They point out that the inside of the mandible has no bone and would, therefore, cause no reflections at a bone-water interface as could be experienced by even the thin bone at the panbone region.
The only conclusion to be made from the data available is that there would seem to be multiple sound pathways into the head, which are constrained by anatomical necessities as well as physical characteristics of the sound itself and have as yet to be worked out for any cetacean species.

Directional Hearing

Sound itself does not contain any spatial information. The way spatial information is extracted from a sound is entirely a result of neural processing of the sound wave information that is received by one or more sensors. Extrapolating directional properties from a sound source is a complex undertaking which can utilize the time (dependent on frequency), phase and amplitude levels of the sound, the complex interaction of the sound waves with the structure of the head and ears, a event known as a head-related transfer function (HRTF), as well as interactions with other sensory systems such as vision. The conclusion being that sound localization is a neural integration process rather than a physical process.

In order to gain spatial information from a sound, the receiver must have a way to compare the sound to some reference, either the body tissues themselves or comparison between multiple receptors. If there was no movement relative to a reference then presumably the receptors would not be activated any more than the non-excitable tissue and would not, therefore, be able to differentiate the sound information from routine body movements. Since there are no truly sound free environments in which life exists on our planet, animals have found ways to sense differential movement in response to sound waves (Bradbury & Vehrencamp, 2011).

In humans, spatial information of a sound in the horizontal plane (azimuth) is localized through comparison of time and intensity between the two ears. At mid-

frequencies, both time and intensity are used but at high frequencies localization is made primarily through interaural time differences (ITD) and at low frequencies through interaural amplitude (level) differences (IAD) between the two ears, an observation known as duplex theory of localization (Yost, 2006). Localization of the distance (range) to the source seems to be tied to the loudest sound to arrive at the ears as compared to the echoes of later arriving sound waves from reflections, a phenomenon called the "Precedence Effect." Whereas localization for humans in the vertical plane (elevation) seems to be based on spectral changes in the sound as it travels across the head, neck and torso (Yost, 2006).

Spectral changes are not easy to quantify as they have to do with the tissue reflective or resonant properties and will, therefore, be unique to the individual although most likely to be comparable within a species. A Head Related Transfer Function, or HRTF, describes how the head and torso structures of a subject can change sound characteristics as the sound moves through those structures on its way to the organ of hearing (Yost, 2006). Historically, human HRTFs have measured the differences between the sound characteristics in the free field versus the sound characteristics at the tympanic membrane (Yost, 2006). However, this type of approach cannot be used in odontocetes not only due to legal and ethical issues, but also due to the fact that there is no definitive data indicating that sound travels mainly through their highly modified external auditory meatus, in a pathway similar to that seen in humans. In fact there is building body of evidence to the contrary (Brill, 1988; Cranford, 2008). Therefore, instead of playing a sound and measuring the sound as it is at the animal's tympanic membrane, this study will be measuring the output of the subject's brain in response to a sound of known parameters. Because the output from the subject is from the brainstem, it is not sound parameters that are measured but neural output which greatly differs from traditional HRTF studies. However, it has been shown that in response to sound, neural output can follow the envelope and amplitude characteristics of that sound (Supin et al. 2001) and has the ability, therefore, to reflect changes that could have been made to that sound as it traveled around and through the head to the ears.

Directional Hearing and Sound Localization in Odontocetes

Directional hearing measurements have been performed on a limited number of marine mammal species (Popov and Supin, 2009, Kastelein et al., 2005, Supin and Popov, 1993). But overall there is little localization information due to the challenges necessitated in the setup of such a study. In 1975 Renaud and Popper behaviorally measured the minimal audible angle (MAA) in both the horizontal and vertical planes, of a bottlenose dolphin in order to quantify the angular discrimination capabilities of this animal. In their stimuli they used both pure tones from 6-100 kHz in 10 kHz increments (from 10 kHz on) as well as broadband clicks (center frequency 64 kHz). For pure tones, they saw significantly different results for certain frequencies in the horizontal plane but none in the vertical plane proved significantly different. Clicks were only measured straight ahead at the 0 point for both horizontal and vertical planes and they demonstrated that clicks had a much smaller MAA than did tones for either plane. Their conclusions were that there seemed to be two sound pathways – a low frequency pathway through the external auditory meatus and the jaw hearing pathway for higher frequencies, and that these and the other observed differences would serve as spectral cues for spatial localization.

In 1984 Au and Moore used the same bottlenose dolphin to measure receiving beam patterns in both the vertical and horizontal planes. Their stimuli were three different pure tones: 30, 60 and 120 kHz. The trials consisted of those with calibrated noise and those with noise plus the stimulus, this setup allowed them to measure the threshold of hearing relative to the level of noise. The vertical plane results showed that the thresholds were highly frequency dependent and became narrower and more directional as the frequency increased. They also saw the thresholds increase with increasing angle. The horizontal data showed the same trend of increasing directionality with increasing frequency and asymmetries between the left and right sides, although dismissed these as being "within the margin of experimental error." Their concluding beam pattern calculations showed their best hearing forward (around 0˚) at a slightly upwards angle of between 5˚-10˚ and that this supported the jaw hearing hypothesis. Popov et al. (2003) used monaural AEP techniques on a bottlenose dolphin that measured the animal's single eared response to short sound bursts with center frequencies between

8 and 128 kHz in quarter octave steps (for a total of 17 frequencies) as that sound source was moved in the horizontal plane around the dolphin's head. The ipsilateral side had lower threshold levels than when the sound source was on the contralateral side which is physically understandable. It is interesting to note that the area of best sensitivity for each frequency was not at the 0 point (straight ahead) but always offset towards the ipsilateral side. The audiograms that they obtained for each frequency showed that sound source position does affect the thresholds of the frequencies which could provide information on the spatial position of a sound, especially for a broadband signal as that used in echolocation.

The study by Popov et al (2008) measured the amplitude of the ABR as a function of the amplitude of the sound played through a contact hydrophone. These measurements were done with the contact hydrophone at three different locations: the tip of the lower jaw, the mid-mandibular area and the site of the external auditory meatus. Their data suggest the presence of two different sound channels: one for low frequencies located at the site of the external auditory meatus and one for mid- to high frequencies located around the mid-mandible, pan bone area. The authors conclude that directional hearing would be frequency dependent. The work by Mohl et al. 1999 as well as the recent and, as of now, unpublished work by Pacini et al. using contact hydrophones have yielded interesting evidence of multiple sound channels. However, Supin et al. (2001) cautions "that stimulation performed by a sound source contacting to the head surface may yield results substantially differ[ent] from those in a free acoustic field in natural conditions." Mooney et al. 2008 (with evidence from Cranford et. al. 2008, pg. 115) suggests that "adaptations in how sounds are received may be species or even sex related" The discussion concludes that "how variation in head morphology affects hearing differences across a wide range of species, or even individuals within a species, requires greater attention."

Noise

When conducting hearing tests within a natural environment, like that found in Kaneohe Bay, it is important to consider other outside sound sources that may interfere with the subject's ability to sense or perceive the test sound. For dolphin's it would not

be feasible to try to construct an anechoic chamber for testing such as is done for most human hearing tests. A quiet tank environment, where other sound sources could be minimized would be ideal but difficult to obtain. Therefore, it is left to studying hearing in a noisy, open bay, natural environment.

The most encompassing definition of noise can be had from Au and Hasting's *Principles of Marine Bioacoustics* (2008) where they describe noise as "any sound other than the desired sounds." Experimentally, sounds are specific signal types that will be played from specific spatial locations. Each experimental setup was calibrated so that the characteristics of the test signal were known at the location of the test subject, but noise could not be controlled at any of these locations. Noise sources included ocean noise from waves and water flow, environmental noise from wind and rain, biological noise from fish, snapping shrimp and the other dolphins in the pen complex, and noise from passing watercraft. The parameters of each of these noise sources were dependent on the source itself. There is a definite temporal variability to noise such as the larger amount of boat noise on the weekends and there can also be a spatial aspect to noise; the same noise coming from a reef as would not necessarily be identical to the noise in the channels between reefs. There were times that sessions were suspended or data collection was cancelled due to loud boats passing or wind or rain that interfered with the equipment. However, the nature of a natural environment precluded the possibility of quiet so, again, the best approach was to monitor and quantify the noise around the experimental setup.

There were various noise sources around or within the experimental enclosure. According to Urick (1983) shallow water noise is mostly due to wave action spurred by wind and seems to be the major physical factor in determining the noise levels at frequencies above 100 Hz. As the wind speed increases, the wave noise will increase. Wave noise is very frequency dependent; there are a lot of low frequency components and relatively fewer high frequency components so this type of noise will decrease with increasing frequency bands (Urick, 1984). Another source of noise can be the flow of water around the measuring hydrophone and in towed setups or in bays with strong currents this is an issue (Au & Hastings, 2008), but was not an issue in the slow moving waters of the pen setup. Rain creating noise must be a consideration, especially in Hawaii where rain can be a daily occurrence. The noise caused by rain is mostly white

noise (a noise with a flat power spectrum across the bandwidth measured – Yost, 2006), and increases with increasing rain intensity but its effects decrease with increasing depth (Au & Hastings, 2008).

In addition to physical noise sources, biological sources make significant contributions to the noise field of Kaneohe Bay. Fishes generate sounds through stridulatory mechanisms (rubbing two surfaces together, such as teeth – Bradbury & Vehrencamp, 2011) or movement of the swim bladder through movements of muscles or bones. The sounds made by most species of fish have not been quantified (see review in Au & Hastings, 2008) but can be assumed to be mostly comprised of low frequencies (as the frequencies that are able to be produced are usually correlated with body size). Snapping shrimp (crusteaceans of the family *Alpheidae*, genus *Synalpheus* – Au & Hastings, 2008) are a very obvious part of the sound spectrum in Kaneohe Bay. Qualitatively, shrimp snaps sound like a loud frying sound, and are made through cavitation as they close their large claw. The sound spectrum of their snaps show energy from 0-200 kHz which make them one of the most broadband sounds ever recorded (Au & Hastings, 2008) and their levels can exceed 125 dB re 1 µPa at the lower frequencies. These tiny shrimp live on the materials of the dolphin pens as well as the surrounding reefs. In addition to fish and shrimp noise, there are marine mammals in the pen complex – three *Tursiops* and one *Pseudorca*. These species are known to make sounds in many different ways, from tonal whistles, broadband clicks and burst pulses (Au & Hastings, 2008) to pectoral, head and body slapping on the surface of the water. This means that the acoustic environment of the pens can, at any given time, be rich with sounds made by the inhabitants of the experimental pens themselves. Finally, the experimental pen complex sits next to an open channel that sees quite a bit of watercraft traffic, which has quite a bit of temporal variability. This boat noise is dependent on the type of craft, number and types of engines and propellers, and the flow noise caused by the motion of the vessel in the water.

Noise can make it difficult to hear discrete signals. It is said to mask the ability of the auditory system to distinguish all, or certain characteristics, of a signal. In order to understand how noise can interfere with the perception of sound, the ear has been described as a group of bandpass filters that are differentially stimulated by specific

frequencies – these have been describes as critical bands (see discussion in Yost, 2006). If the noise is more broadband than the critical bandwidth then the energy in the noise will mask the signal. If the noise has an equal or higher amplitude than the critical band then it may obscure the signal altogether. However, if the noise is of a bandwidth that is within the critical band, the signal may be sensed. Therefore, the question really is – how many and of what size are the critical bands of a bottlenose dolphin? Understanding the critical bandwidths of a species can aid in the understanding of how different types of noise can mask specific signal types. The first type of measurement is called the critical ratio and is based on the idea that a signal will be masked by a narrow band of noise around it:

$I_t = N_o \times \Delta f$ (from Au & Hastings, 2008, pg. 347)

I_t – intensity of the tone at threshold (in μPa^2)

N_o – Noise spectral density in $\mu Pa^2/Hz$

Δf – Bandwidth of the auditory filter at the tone frequency

To express the critical ratio in dB one solves this as

$CR = 10 \text{ Log } (\Delta f)$

Au and Hastings (2008) compiled the critical ratio work for *Tursiops truncatus* and found that it was comparable to humans only at the higher frequencies. For both species the trends indicate that the critical ratio increased with increasing sound frequency (termed a constant-Q filter), therefore, it should be harder to mask higher frequencies than it would be to mask lower frequencies. However, Lemonds et al.'s work (2011) on critical ratios explored a more broad frequency range as well as used a comparative approach utilizing others work. Their finding suggested that between 40-100 kHz the filter's bandwidths were constant and at frequencies below 30 kHz the filter's bandwidth increases with increasing frequency (constant-Q). They surmised that this constant-Q filtering at lower frequencies is a mammalian vestige and the constant bandwidths at higher frequencies could be acquired through selection for good frequency discrimination. The high frequency constant bandwidth filters were further explored (Lemonds et al., 2012) and it was found that the bandwidths were not as narrow as one would assume would be observed on an animal that must interpret echoes from complex

targets. They postulated that perhaps a dolphin makes up for the lower frequency resolution with higher temporal resolution.

Another, more direct technique for measurement is simply called the critical band technique and involves changing the bandwidth of noise and determining the masked threshold for each bandwidth. Au and Hastings (2008) also reviewed the data available for these measurements and found that, unlike what is seen for humans, the critical band results do not correlate well with the critical ratio results. They have no conclusion as to why this might be but hypothesize that perhaps nonlinearities were introduced through setup parameters or perhaps the dolphin's auditory system has a greater ability to pick out signals from noise than has previously be assumed.

Regardless of the critical band or critical ratio data, the acoustic environment in and around the experimental pens, being as broadband as it can be, has the ability to mask a wide frequency range, including the signal types that were used. Renaud and Popper conducted their 1975 directional hearing study in an open pen in Kaneohe Bay with a distance of 18 m from the sound projector and the dolphin. They reasoned that because they were using supra-threshold stimuli that the noise would not affect the comparisons in the study. Au and Moore's 1984 directional hearing study was in an open pen in Kaneohe Bay with a distance of 3.5 m between the test subject and the sound transducer. In this study they used calibrated noise sources to discourage the animal from steering its hearing away from the axis they were testing. This use of masked thresholds attempted to control for some of the spatial and temporal variations of the noise field. However, as Zaitseva et al. (2002) points out, the use of masking noise does not get rid of natural noise sources that can reach levels above those of the testing noise.

Schlundt, Carder and Ridgway's 2004 hearing experiments were performed in an open pen in San Diego Bay, a bay known for heavy shipping traffic, snapping shrimp and other marine mammal noises. The authors "monitor(ed)" the noise and made a point that the thresholds obtained were not absolute thresholds but masked thresholds. The relative level of masking did not matter considering that the study was a comparison of the thresholds as the sound transducer was moved relative to the study subjects. This study did not include the use of artificial masking noise and, like Schlundt et al. (2004) is not meant as a measurement of absolute hearing but of masked hearing. Each set of

experiments were done within pen three of the experimental pen complex of the Marine Mammal Research Program. The hearing studies were comparative and assume an isotropic noise field within the small enclosure. Regardless of the isotropic assumption, the power spectrum levels of the noise for each location used in the studies were quantified (in dB re 1 $\mu Pa^2/Hz$) and reported.

Experimental Design

The test Subject was Boris, a 25-year-old male bottlenose dolphin (*Tursiops truncatus*) that is 2.6 meters long and weighs 215.5 kg (see Figure 1.5).

Figure 1.5
Boris at the Marine Mammal Research Program experimental pen complex in 2011

He was housed in the floating pen facility moored off of the Hawaii Institute of Marine Biology at Coconut Island in Kaneohe Bay, Oahu, Hawaii, and has taken part in many research experiments (e.g. Mooney et al. 2009a,b, Nachtigall et al. 2003, 2004). The test area was a 6 x 9 m floating pen that has been used for multiple acoustic experiments (Yuen et al. 2005, Mooney et al. 2009a,b). The study subject had been trained to wear AEP suction cup electrodes and to station at multiple experimental setups within the testing pen. The equipment and researcher was stationed in a test shack (shack 1) adjacent to the pen. The trainer was positioned on the wooden decking between the test pen and shack 1, which facilitated communication between the researcher, trainer(s) and test subject (see Figure 1.6).

Figure 1.6
Test trial setup in pen 3 showing Boris on station, with trainer C. Quintos on deck with the author in the equipment housing.

The equipment setup was the same system described in Taylor et al. 2007 with component and software upgrades as they had become available (see Figure 1.7).

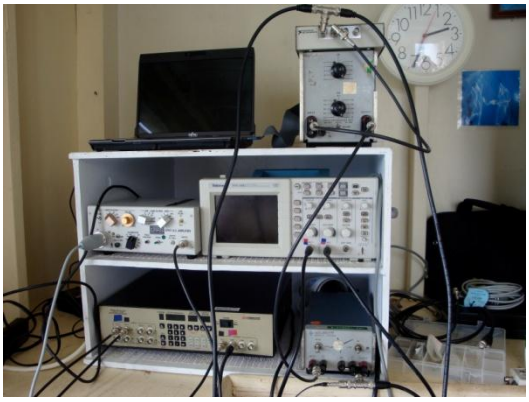

Figure 1.7
The AEP electronics as they were situated for experimentation.

A custom built LabView Program generated a signal which is routed through a National Instruments DAQCard-6062E then routed through a SCB DAQCard box. The signals were 20 msec sinusoidally amplitude modulated (SAM) tones with a modulation frequency of 1 kHz and carrier frequencies that varied depending on the experiment. For the final two experiments a 20 msec train of broadband clicks, with a center frequency of 30 kHz, was used in addition to the SAM tones. The signal was then attenuated through a Hewlett Packard 350D attenuator and amplified using a Hewlett Packard 465A amplifier to increase the impedance matching of the system. The signal was then

projected through an ITC-1032 underwater transducer and visualized using a Tektronix TDS1002 Oscilloscope.

When the stimulus began the custom built LabView Program was triggered to acquire the brainwave information. The brainwaves were collected using three custom-built suction cup electrodes (Grass E5GH gold disc electrodes – see Taylor et al. 2007): an active, placed approximately 6 cm behind the blowhole, a reference, placed on the dorsal aspect just in front of the dorsal fin, and a ground, placed on the dorsal fin itself. A Grass CP511 A.C. Amplifier (10,000X) received each of these electrode inputs and also bandpass filtered the signal between 100 Hz and 3 kHz. For additional resolution the signal was again bandpass filtered at 300 Hz and 3 kHz by a Krohn-Hite Model 3362 Filter. The output was visualized using the same Tektronix TDS1002 Oscilloscope and routed into the NI 6062E DAQCard via the SCB DAQCard box and into the custom LabView Program.

In order to increase the signal-to-noise ratio, 1000 repetitions of the stimulus were played and the brainwaves acquired for each are averaged. The LabView program allowed the researcher to see the real-time averaging of the brainwaves as well as a real time Fast Fourier Transform of the incoming brainwaves.

Each frequency for each setup was calibrated using a Reson TC-4032 receiving hydrophone which has a flat response (±5 dB) up to 100 kHz.

Comparative Audiogram Study

The purpose of this study was two-fold: first to assess if audiograms done using different experimental setups were comparible and second, to obtain an audiogram using the hoop/biteplate setup as a baseline for further studies using this setup. There have been no studies comparing audiograms obtained for different setups, which was quite surprising considering how many different setups have been used to obtain hearing data for odontocete cetaceans.

The first setup stationed the test subject by the deck of pen 3, next to the trainer's location on the decking between the pen and shack 1. For the trial, the animal was stationed parallel to the deck with the blowhole and dorsal aspect above the surface of the water and the ears, lower jaw and ventral surface below the surface of the water. The

sound projector was 2m directly in front and 1m below the surface of the water which resulted in the sound source not being at the same depth as the animal's ears. The second and third setups had the test subject stationing 1m underwater and 2m from the transducer. For these trials the subject was sent from the resting at the surface to station underwater in a hoop (setup 2) or at the same location of the hoop but with a PVC biteplate attached (setup 3, see Figure 1.8). For each condition, at the conclusions of the trial(s) the test subject was recalled to the trainer and given a fish reward.

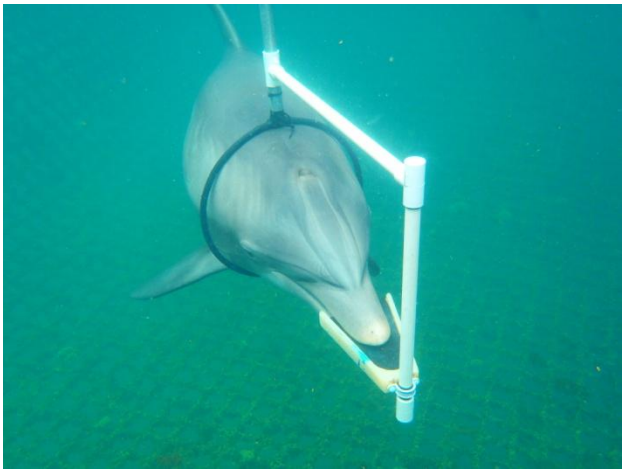

Figure 1.8
Boris stationed on the hoop/biteplate setup during a training session in 2011

The threshold of hearing (see Taylor et al., 2007) was obtained for ten frequencies: 5.6, 8, 11.2, 17, 22.5, 32, 38, 40, 43 and 48 kHz. Results were analyzed and compared between the three treatments.

Directional Hearing and HRTF Study

The test subject began each trial stationed at a Styrofoam pad at the surface beside the trainer. At a signal from the trainer, the animal would dive and position himself in the hoop/biteplate that was 1m under the surface of the water. The steel hoop was wrapped in electrical tape for comfort. The biteplate was plastic padded on both sides with closed cell neoprene. The biteplate was attached to the stem of the hoop with ¾ inch PVC piping fastened to the pole of the steel hoop with nylon screws. The ends of the PVC were open so that the apparatus would fill with water when submerged. When stationed in the hoop during a trial, the subject would grasp and hold the biteplate. His

position was monitored with an underwater camera connected to a monitor visualized by the trainer and researcher. Once the test subject was in the correct position, the trial(s) began. At the end of the trial(s) he was recalled using a bridge whistle and returned to the stationing position for a fish reward.

For each trial, the sound projector was placed in one of 21 different positions. The transducer was moved in a horizontal arc from 0° straight ahead to 90° to right and left (see Figure 1.9) in 30° increments at a distance of 1.5 m from the hoop and was moved from 1m depth to 0.5 m deeper or shallower in the vertical plane. Therefore, each position had a different depth (0.5 m, 1 m, 1.5 m) and angle (-90, -60, -30, 0, 30, 60, 90; negative angles being left and positive angles at the right).

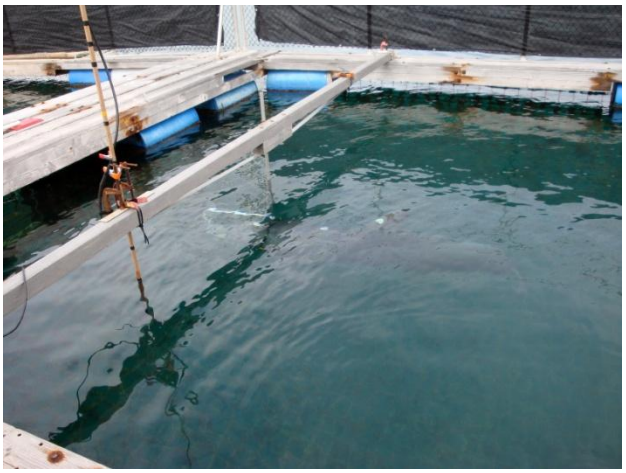

Figure 1.9
HRTF/Directional Hearing Setup showing the test subject in the hoop/biteplate (suction cup electrodes visible as white dots) with the sound transducer at the -90˚ location (1.5m to the test subjects left side).

For each position, each of four signal types was used: SAM tones of 5.6, 22.5 or 38 kHz or the broadband click with center frequency of 30 kHz. So, for every one of the 21 positions, at least 9 trials (one session) for each of the signal types was performed and the AEP threshold for hearing was calculated (see Taylor et al., 2007). During each session, for each position and signal type, the response to three specific amplitudes (120 dB, 115 dB and 110 dB re 1 µPa) was measured. These responses were analyzed, plotted and compared to obtain the HRTF. The threshold data was analyzed, plotted and compared for the study of directional hearing.

Chapter 2

Experimental Setup Comparison: Electrophysiological Audiograms Obtained for a Bottlenose Dolphin Under Three Design Regimes

Sound is an excellent medium for communication in water (Au and Hastings, 2008). Marine mammals, especially odontocetes, rely on hearing as their primary sensory modality (Ketten, 2000), and use sound to socialize, navigate and forage. Because of their reliance on sound, noise in our oceans and the concerns over its effect on marine mammal physiology and ecology is ongoing (NRC, 2003).

An audiogram is the most widely used means of assessing the frequency range of hearing. Behavioral audiometry continues to be the gold standard but relies on the cooperation, and training, of the test subject. The facilities and training required for marine mammal audiograms has precluded the testing of all but the smallest odontocetes and of them, very few of the species have been tested. Auditory evoked potential (AEP) audiograms are a comparable alternative to behavioral audiograms (Yuen et al., 2005, Houser and Finneran, 2006, Schlundt et al., 2007). Plus, they do not rely on training the test subject or the keeping of that subject for a lengthy period of time at a facility. AEP methods have also been shown to be very promising in their use on stranded animals (Nachtigall et al. 2005, Mann et al. 2010, André et al. 2007)).

In order to be able to understand the actual hearing capability of a species numerous studies spanning age and gender (from the level of the audiogram to applied hearing topics such as hearing during echolocation) must be obtained and evaluated for normal variation within and between populations. It is unrealistic to think that one group of scientists would be able to collect data in that volume and as a result data collection is done by multiple groups, working in various locations, to measure audiograms from species as opportunities arise. This seeming "shotgun" approach to the gathering of data is very robust under the scientific method because it allows many populations and species to be covered in a relatively short period of time. Studies on any marine mammal species are expensive and time consuming yet over the past 50 years considerable work has been accomplished. In addition, having multiple acoustic data-gathering groups allows depth

to the analysis by allowing for multiple interpretations. However, problems arise when study designs introduce reasonable doubt in the comparability of the data. Which raises the question of if we can really compare results across or between species or do experimental design differences render those comparisons inaccurate?

Multiple studies have addressed the variability of behavioral versus electrophysiological audiograms (Yuen et al., 2005, Houser and Finneran, 2006 Schlundt et al., 2007, Szymanski et al., 1999). Finneran and Houser (2006) also looked at the variation that can be present between in-air AEP audiograms and underwater behavioral audiograms. However, differences in study setup may confound comparisons between audiogram results. For example, some experiments have tested odontocete hearing with the subject at the surface with very light contact (Nachtigall et al. 2005, Mooney et al. 2008), some subjects were held at the surface in a stretcher (Nachtigall et al. 2008, André et al. 2007), some test subjects were stationed in a hoop underwater (Li et al. 2011, Mooney et al. 2009a,b and Nachtigall and Supin, 2008), while others were stationed underwater using a biteplate (Finneran and Schlundt 2007, Au and Moore 1983), and even some hearing studies were performed with free swimming animals (Kellogg 1953).

This study begins to address if meaningful comparisons are possible between hearing experiments that utilize different physical setups. Auditory evoked potential techniques were used to obtain audiograms for the same test subject, an adult male bottlenose dolphin (*Tursiops truncatus*), in three different experimental setups: the subject stationed at the surface, stationed in a hoop underwater, and underwater stationed in a hoop with a biteplate. It was hypothesized that there would be measureable differences in the audiograms obtained through the different setups. In addition, this study served to explore the audiogram of this test subject when stationed on the hoop with biteplate in order to ascertain if this type of setup was comparable enough to the other techniques in order to utilize for the directional hearing and Head-related transfer studies.

Materials and Methods

The test Subject was Boris a 25-year-old male bottlenose dolphin (*Tursiops trucatus*) that was 2.6 meters long and weighed 215.5 kg. He was housed in the floating

pen facility moored off of the Hawai'i Institute of Marine Biology at Coconut Island in Kāne'ohe Bay, O'ahu, Hawai'i, and has taken part in many research experiments (Mooney et al. 2009a,b, Nachtigall et al. 2003, 2004). The test area was a 6 x 9 m floating pen that has been used for multiple acoustic experiments (Yuen et al. 2005, Mooney et al. The test subject had been trained to wear AEP suction cup electrodes and to station at multiple experimental setups within the testing pen 2009a,b). The equipment and researcher were stationed in a test shack adjacent to the pen. The trainer was positioned on the wooden decking between the test pen and the equipment housing, which facilitated communication between the researcher, trainer(s) and test subject.

Surface

The test subject was stationed at a Styrofoam pad perpendicular to the experimental shack. The trainer would signal the start of the trial and the subject would position himself parallel to the deck (see Figure 2.1) at a position that is approximately 2 m from his auditory meatus to the cable of the transducer. The transducer itself was suspended at a depth of 1m from the surface of the water which was lower than the test subject's body. Once the animal was in position the stimuli were presented. At the end of the trial(s) the test subject was recalled using a bridge whistle and returned to the stationing position for a fish reward.

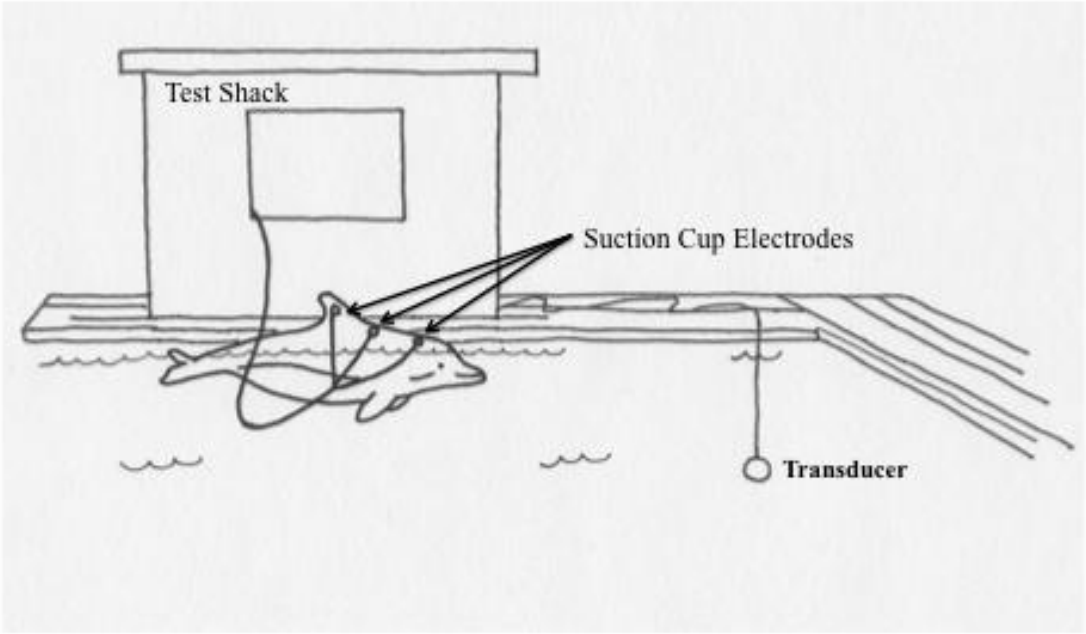

Figure 2.1
Surface setup. The test subject was stationed parralel to the deck by the test shack with the transducer at the distance of 2 m and depth of 1m (from the water's surface). The positions of the three suction cup electrodes are shown with arrows: the active electrode approximately 6 cm caudal from the blowhole, the reference electrode is just rostral to the dorsal fin and the ground electrode is on the dorsal fin itself. In this setup, all three suction cup electrodes were out of the water.

Underwater in a Hoop

The test subject was stationed at the same Styrofoam pad. At a signal from the trainer, the animal dove and positioned himself in a steel hoop wrapped with electrical tape that was 1m below the surface of the water and 2m from the signal transducer. Situated midway between the hoop and the transducer, an acoustic baffle was positioned to block sound reflecting from the surface (see Figure 2.2). The test subject's position underwater was monitored using an underwater camera which was visualized with a monitor. Once the animal was in the correct position, the trial(s) began. At the end of the trial(s) the test subject was recalled using a bridge whistle and returned to the stationing position for a fish reward.

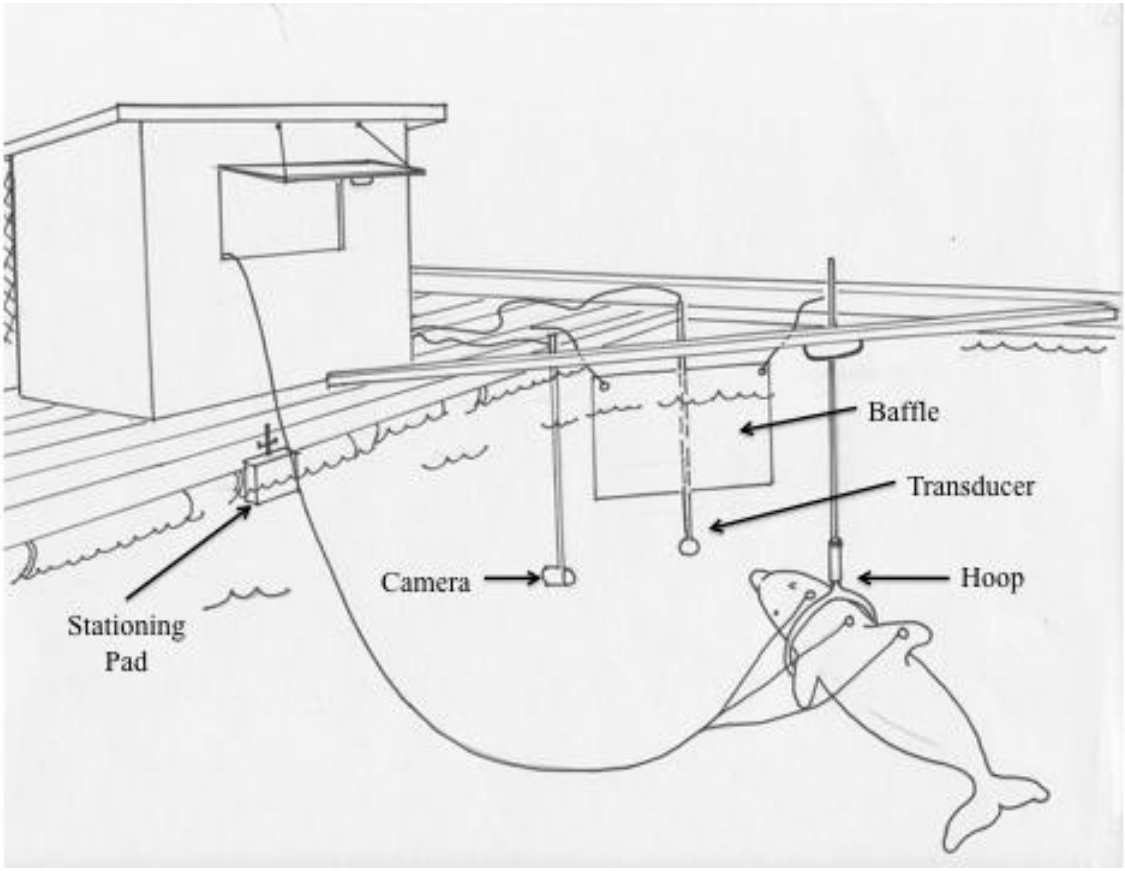

Figure 2.2
Underwater Hoop Setup. The test subject was 1m underwater and 2 meters away (in a straight line) in relation to the transducer. An acoustic baffle was fastened approximately 1m from the transducer to reduce the incidence of surface reflection of the stimuli. The position of the animal was monitored using an underwater camera to ensure proper positioning during each trial. At the end of the trial(s), the test subject was recalled and would position himself at the surface with his rostrum on the stationing pad to receive a fish reward.

Underwater in a Hoop with a Biteplate

The setup for this audiogram was identical to the hoop setup with the addition of a biteplate attached to the hoop (see Figure 2.3). The hoop was steel wrapped in electrical tape for comfort. The biteplate was plastic and padded on both sides with closed cell neoprene. The biteplate was attached to the stem of the hoop with ¾ inch PVC piping using nylon screws. The ends of the PVC were open so that the apparatus would fill with water when submerged. When stationed in the hoop, the test subject would grasp and

hold the biteplate. His position was monitored with an underwater camera connected to a monitor that could be visualized by the trainer and researcher.

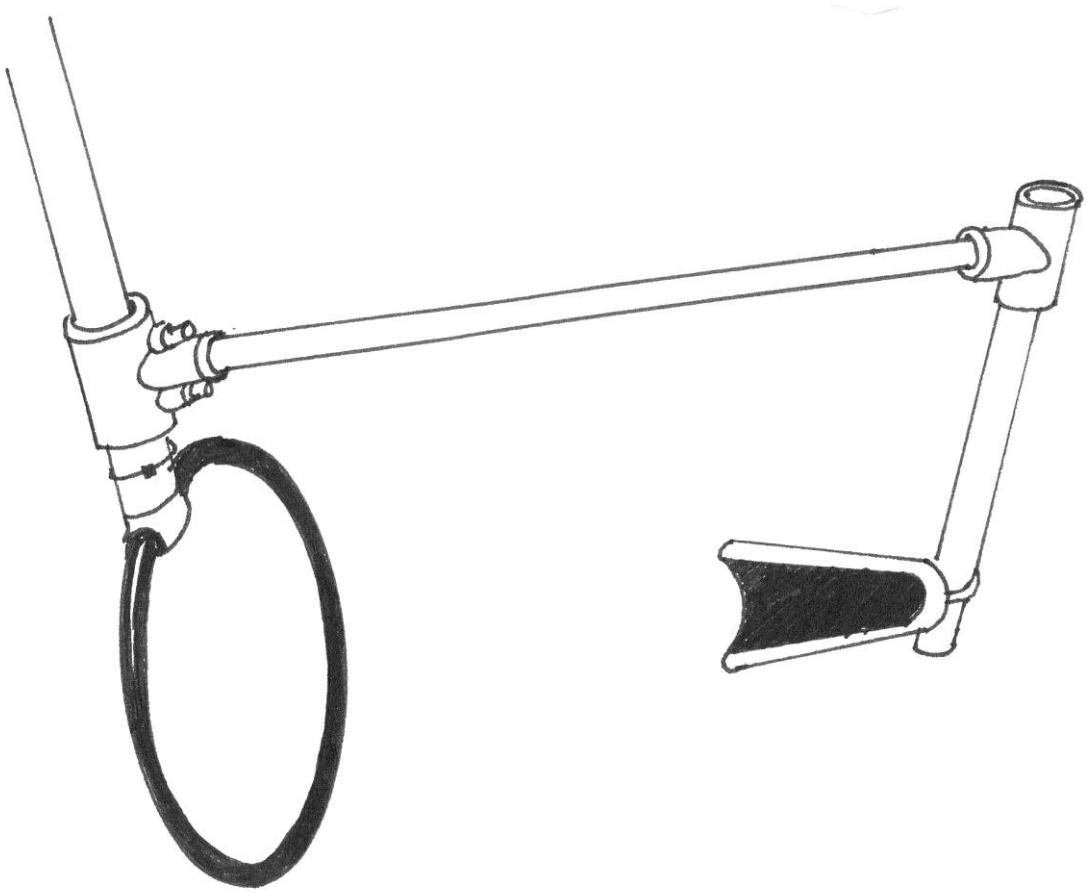

Figure 2.3
Detail on the hoop outfitted with a biteplate. A steel pole and hoop with a plastic biteplate cushioned with closed cell neoprene to protect the subject's teeth. The biteplate was held in position and mounted onto the steel pole with nylon screws and ¾ inch PVC that is open on all ends to allow it to be flooded with seawater. The pole was held with a wooden beam that was suspended over the test pen.

The equipment setup was the same system described in Taylor et al. 2007 with component and software upgrades as they had become available. A custom-built LabView Program generated a signal that was routed through a National Instruments DAQCard-6062E then routed out a SCB DAQCard box. The signal consisted of a 20 msec sinusoidally amplitude modulated (SAM) tone with a modulation frequency of 1

kHz and carrier frequencies of 5.6, 8, 11.2, 17, 22.5, 32, 38, 40, 43 and 48 kHz. The signal was then attenuated through a Hewlett Packard 350D attenuator and amplified using a Hewlett Packard 465A amplifier in order to increase the impedance matching of the system. The signal was then projected through an ITC-1032 underwater transducer and visualized using a Tektronix TDS1002 Oscilloscope.

When the stimulus began the custom built LabView Program was triggered to acquire the brainwave information. The brainwaves were collected using three custom-built suction cup electrodes (Grass E5GH gold disc electrodes – see Taylor et al. 2007): an active, placed approximately 6 cm behind the blowhole, a reference, placed on the dorsal aspect just in front of the dorsal fin, and a ground, placed on the dorsal fin itself (see placement in Figures 2.1 and 2.2). A Grass CP511 A.C. Amplifier (10,000X) received each of these electrode inputs and bandpass filtered the signal between 100 Hz and 3 kHz. For additional resolution the signal was again bandpass filtered between 300 Hz and 3 kHz by a Krohn-Hite Model 3362 Filter. The output was visualized using the same Tektronix TDS1002 Oscilloscope and routed into the NI 6062E DAQCard via the SCB DAQCard box and into the custom LabView Program. Each frequency for each setup was calibrated using a Reson TC-4032 receiving hydrophone which has a flat response up to 100 kHz (±5 dB).

In order to increase the signal-to-noise ratio, 1000 repetitions of the stimulus were played and the brainwaves acquired for each were averaged. The LabView program allowed the researcher to see the real-time averaging of the waveforms of the brainwaves as well as the frequency domain of the incoming signal using a real time Fast Fourier Transform of the incoming brainwaves.

Threshold analysis was almost identical to that outlined in Taylor et al. 2007, Nachtigall et al. 2007 and Supin and Popov 2007. After averaging, 16 msec (excluding the first 6 and last 4 msec of the record) of the brainwave signal was 256 point Fast Fourier transformed using a custom built MatLab program and Microsoft Office Excel (2007). The magnitude of the FFT response at the modulation frequency of 1 kHz was calculated and these response magnitudes were then plotted with respect to the amplitude (in dB re 1 μPa) of the outgoing signal (at a range from 125 dB to 75 dB). A linear regression line was calculated (using a minimum of 5 points) and the level at which the

regression line crossed the zero output amplitude level was extrapolated as the threshold point.

Results

The resulting thresholds for each frequency are listed in Table 2.1. Each point was then plotted as a function of frequency, i.e. a standard audiogram (figure 2.4) with frequency on the x-axis and amplitude (in dB re 1 µPa) on the y-axis.

Table 2.1
The threshold values (in dB re: 1 µPa) for each frequency of each treatment.

Frequency (kHz)	**Surface**	**Hoop**	**Hoop w/Biteplate**
5.6	87	90	91
8	81	87	88
11.2	89	86	85
17	85	81	89
22.5	92	84	90
32	84	88	90
38	85	82	78
40	94	88	79
43	91	93	87
48	101	93	105

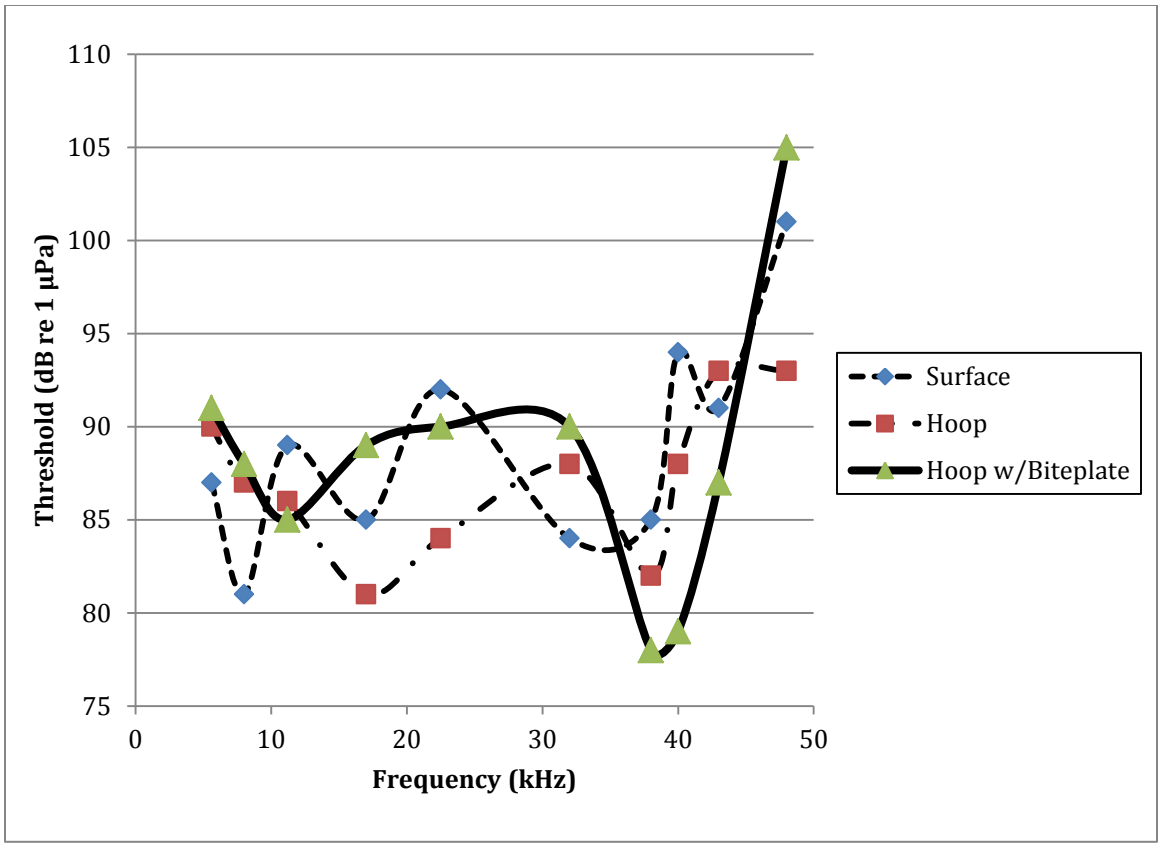

Figure 2.4
The three audiograms for each of the three treatments with the frequency (kHz) on the x-axis and the calculated threshold values (in dB re 1 µPa) on the y-axis. The audiogram from the surface treatment is in the dashed line with blue diamonds indicating the threshold levels, the audiogram underwater with the test subject stationed in the hoop is in the dotted line with the threshold points in pink boxes and the audiogram underwater with the animal stationed in the hoop with the biteplate is in the solid black line with the threshold levels in green triangles.

Discussion

It is clear that these audiograms are not the classical U-shaped curves; however, odontocete audiograms do not usually have 10 threshold points below 50 kHz and that may partially explain the non-traditional curves. It is also clear from these audiograms, as well as from prior experience, that this animal had high frequency hearing loss. The test subject's hearing, therefore, did not warrant exploration above 50 kHz.

It is not possible to perform meaningful statistical analysis between threshold levels for each frequency of each treatment because means or standard deviations are impossible for single numbers. There are not enough audiograms available for any

cetacean species to be able to robustly report the average intra-subject variability, therefore, the human literature standards could be a starting point for comparison. The acceptable level of intra-subject variability for clinical diagnosis is ±10 dB (SPL re: 20 µPa) for lower frequencies (Schmuziger et al. 2004, Frank 2001) and ±15 dB (SPL) for greater than 16 kHz (Valente, 1992). A shift of more than ±20 dB SPL between testing sessions is recorded as being clinically significant. These criteria are based on hundreds of thresholds acquired through behavioral procedures. However, it has been shown that evoked potential responses are similar to those found through behavioral methodology (Hall 1992, Gorga et al. 2006). If these clinical criteria are applied to the range of differences between the three setups, all but the thresholds at 40 and 48 kHz are well within the ±10 dB range. 40 and 48 kHz are towards the high frequency cutoff for this animal so the high frequency variability of ±15 dB might be used to explain their higher range of variability. Comparisons to hearing levels of other species should be used with caution. There is an incredible amount of inter-subject variability of around ±20 dB just within the human population. In addition, it is important to note that all of the human audiograms are done in air with reference to 20 µPa as compared to underwater with a reference to 1 µPa which does not change the range of differences but does not allow for comparison of the threshold levels unless the correction factor of +26 dB is added to the in-air audiograms (Au and Hastings 2008).

Despite the clinical comparisons, the audiograms in figure 2.4 show some interesting variation between setups. It is clear that the audiogram done at the surface demonstrates a lot more variation between points compared to the underwater setups which consist of mostly smooth curves. Surface reflection can cause great variability in sound pressure levels (Au and Hastings, 2008). During sound field calibration, sound pressure levels did not demonstrate any notable fluctuations in the sound field at any of the frequencies or locations measured, including the surface stationing position. However, the reflective nature of the air/water interface and it's unpredictability due to wind, waves or rain can make even daily calibration of the setup simply an observation of the sound pressure level at the moment of calibration. Though, if there were transient surface reflection issues, the averaging process during AEP measurement would minimize the physiological effects. Nonetheless, unless it had been possible to calibrate during

measurements, there was no way to absolutely quantify surface reflections effects on the threshold numbers for the surface position. Surface reflection would not have been a significant issue in the two underwater setups because of the depth that the animal was stationed and the presence of a baffle, midway between the transducer and the animal, which served to block surface reflections. Therefore, in terms of surface reflection issues, an underwater setup would seem to provide a less variable way to measure audiograms and were utilized in future directional hearing studies.

Consideration must also be paid to the open sea pen in which the measurements were made. All of these thresholds were collected in Kāne'ohe Bay, O'ahu, Hawai'i and are, therefore, masked thresholds. It is interesting to note the attention paid to ambient noise levels in human clinical settings. Noise and its masking effect have led to standards of maximum noise allowable during clinical measurements (Valente et al. 1992). However, these experiments were not diagnostic in nature nor was there an anechoic chamber available for use. A bay environment more closely mimics the species natural environment considering *Tursiops* are found in coastal to pelagic waters. Therefore, in order to assess the levels of noise at the setup locations, the power spectrum density (dB re 1 $\mu Pa^2/Hz$) of 20 seconds of noise between 1-79.9 kHz was sampled at a 200 kHz sampling rate and is shown in figure 2.5.

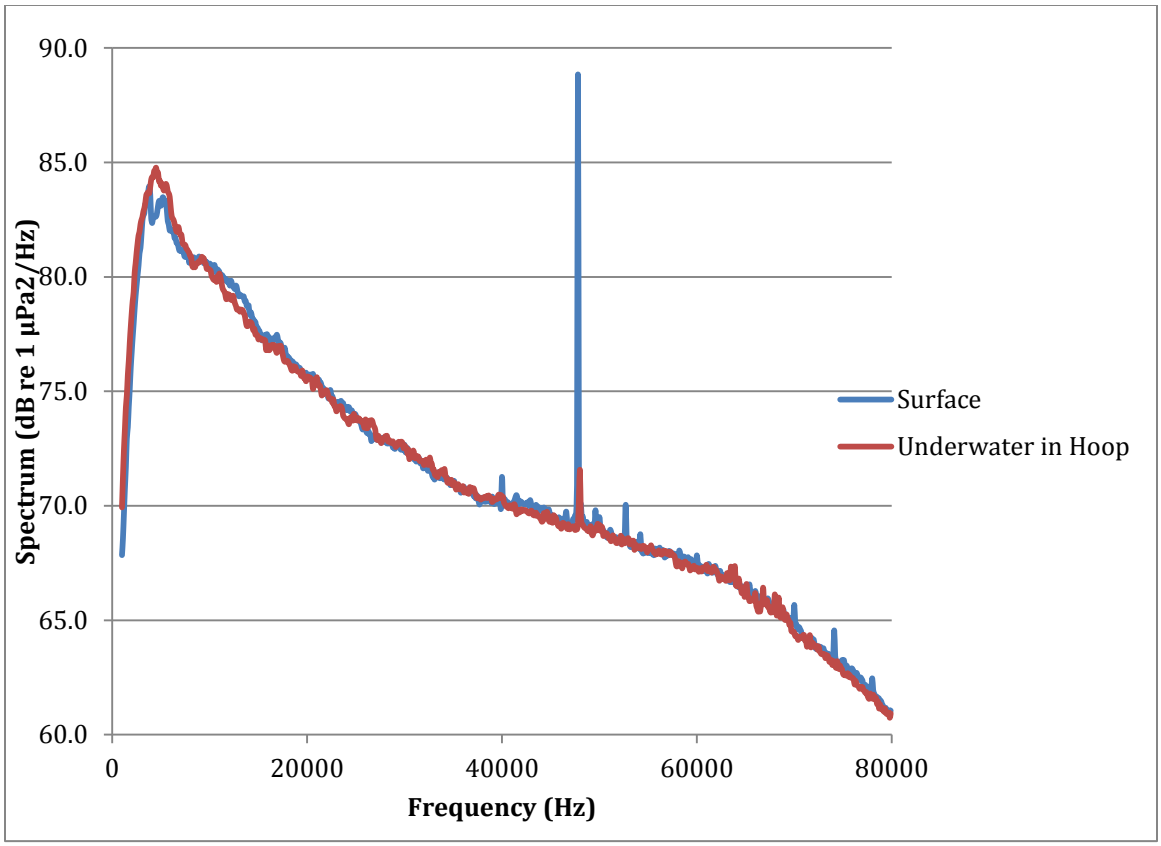

Figure 2.5
Ambient noise data for the surface position and the position underwater in the hoop.

The purpose of this study was not one of profiling the noise in the bay, but it is apparent to those that have worked outdoors that some days are noisier than others (such as weekends and holidays) and certain times of day are noisier than others. Could the time of day or day of the week have affected the threshold levels enough to reduce or accentuate the differences between treatments? The possibility cannot be ruled out.

For all of the suggested reasons, these results need to be interpreted with caution. However, taking these factors into account, what if these results are an accurate portrayal of the variation that can be caused by three different setups? It certainly would indicate that meaningful comparisons across the literature are possible. This conclusion is not to say that other differences in setup should not be taken with caution, for example, this study did not look at differences that could arise by keeping an animal in a stretcher or if that animal is free swimming, or underwater on a biteplate without the hoop? Studies addressing intra-subject as well as inter-subject variability must be obtained as well as

further studies comparing setups are clearly needed to quantify any significant differences that may arise through methodological dissimilarities.

Chapter 3

Directional Hearing of an Atlantic Bottlenose Dolphin (*Tursiops truncatus*)

We, as humans, fundamentally experience how to locate the source of a sound. We are able to pinpoint the location of a sound source but do not have to consciously think about how we actually computationally located that sound. Human hearing has been well studied – intensity, time, phase, spectral cues and even echoic differences allow us to locate an object in three-dimensional space (Yost, 2006). We tend to assume that the way humans perform this localization task is the same for other non-human animals, but is this a robust deduction?

Animals have quite a few different strategies for sound localization. Despite secondary differences, the foundations of basic hearing mechanisms are physically constrained. In order to sense pressure waves or particle motion (which is thought to be the ancestral form of hearing), one body structure must be responsive to this movement in comparison to another part which is not. This differential motion allows the sensor to relay the characteristics of this motion to a processing center that is able to compare the relative motion (Bradbury and Vehrencamp, 2011). If there are two or more such structures the relative motion of each can be determined when compared to the surrounding tissue as well as to each other. If there is a physically measureable difference between the sensors, such as frequency, intensity or phase, these differences could be used to determine the location of the source.

Human ears work through the capture of sound pressure by two membranes, one on either side of the head. These tympanic membranes, or ear drums, oscillate due to the sound pressure impinging upon them. This motion is then transferred into the organ of hearing via coupling to the middle ear ossicles. This type of ear is considered the classical mammalian ear. These ears are a type of pressure sensor. Pressure is a scalar quantity, which, unlike a vectoral quantity, has no directional information (Au and Hastings, 2008). Pressure detector ears rely on comparisons of the pressure difference (performed by an integrator, which, for mammals, is the central nervous system) in order

to gain directional information about the source of the sound (Christensen-Dalsgaard, 2005).

Some insects use pressure detector ears (Robert, 2005), yet, other insects, as well as birds, reptiles and some amphibians use another strategy: that of a pressure difference receiver. These types of tympana are sensitive to vibrations from both sides of the membrane and typically come in pairs (with certain insects have 3 or more) (Robert, 2005). The pressure difference measured between the two membranes is directional in nature and greatly depends on the frequency of the sound (Christensen-Dalsgaard, 2005). With this type of detector, if the receiver knows the sound's frequency spectrum, it can be used to locate the source of that sound.

Crustaceans, certain insects (using flagellar ears), and some fishes use another strategy for localization information; that of receptors that are sensitive to particle motion. Particle motion is a vector quantity and receptors that react to particle motion can capture the energy in a way to preserve the directional information. Typically, these receptors have distinct orientations that allow reactions to specific directions of particle motion. Some of the particular orientations of the hair cell sensors in certain fish have been studied and mapped (Au and Hastings, 2008) while other species of fish do not seem to have a particular orientation (Fay, 2005). Differential motion of the specific directional receptor populations can encode the location of the source of the motion. It is interesting to note the similarity of particle motion detection ears to crustacean statocysts and insect scolopidia, both of which can detect particle motion and can be used for body orientation (Bradbury and Vehrencamp, 2011).

Odontocete cetaceans have shown evidence of very sensitive sound localization capabilities (Renaud and Popper, 1975). These animals have recognizably mammalian ears which are comprised of two pressure detector ears, each with a single tympanic membrane, located on either side of the head. However, the tympanic membranes of these animals are cone shaped and not rigidly attached to the middle ear ossicles (Ketten, 2000). In fact, besides agreement that they have pressure detector ears (Au and Hastings, 2008), there is no consensus on how the sound pressure actually gets into the middle and inner ears of any type of cetacean. Nevertheless, their central nervous system is very adept at following sound parameters (Supin et al., 2001) and would be capable of

pressure comparisons between the two ears that would be necessary for sound localization.

In 1975, Renaud and Popper measured the minimal audible angles (MAAs) for an Atlantic bottlenose dolphin. Their results from pure tone trials showed that these dolphins had very small MAAs for both horizontal and vertical planes and exceptionally small angles in both planes in response to broadband clicks. They concluded that this study animal had very fine directional capabilities. Au and Moore (1984) used the same animal to behaviorally quantify the receiving beam pattern and found that directional sensitivity to a sound was frequency dependent (the beam narrowing with increasing frequency) and was distinct in the horizontal and vertical planes. Schlundt et al. (2004) explored hearing thresholds as a sound source was varied in depth. They used 3 pure tone signals at 2 kHz, 8 kHz and 12 kHz. For two test subjects, both Atlantic bottlenose dolphins, each signal type was played either directly in front of the animal or below the animal. They found that at 2 kHz the subject had lower threshold levels when the source was oriented below the animal's head, at 8 kHz the thresholds at both places were equivalent and at 12 kHz lower thresholds were found when the sound source was directly in front of the animal. These findings indicate that there may be different pathways for sound which are dependent on frequency. The electrophysiological findings of Popov et al. (2008) reinforce the idea of multiple sound pathways with an emphasis on a channel for low frequencies and a channel for higher frequencies. On a comparative note, in 2009, Popov and Supin compared the directional hearing sensitivities of a bottlenose dolphin and a beluga whale. The directional sensitivity of a bottlenose dolphin was measurably higher than that of a beluga (Popov and Supin, 2009). They hypothesized that the comparatively poor responses of the beluga could have been a result of the beluga not being allowed to move its neck in order to better localize the source (Mooney et al., 2008) which that species is known to do in the wild. Therefore, marine mammals have been shown to have acute localization capabilities that hint at being frequency dependent but are also subject to the animal's own movement or capability of head movement.

This study explored the directional hearing capabilities of a mature, male bottlenose dolphin using auditory evoked potential (AEP) techniques. This study is part

of a larger study in collaboration with Ted Cranford and colleagues. Cranford et al. aim to mathematically model the sound reception patterns of a bottlenose dolphin. Their modeling will utilize CAT scans of dead heads and measurements of tissue parameters. This mathematical model will then be tested against the empirical data obtained in this study to see how well the modeling compared to reality. The mathematically modeling will not be addressed in these study results. In addition, there have been no previous studies that have measured the receiving beam patterns of a bottlenose dolphin in 3-dimmentional space. The only study that is at all similar is Au and Moore's study in 1984 in which they behaviorally measured hearing in arcs along the frontal and midsagittal planes. They calculated the receiving beam pattern to be forward and a little above the animal's midline. However, that study used white noise in order to prevent beam steering by the animal. Without hindrance of beam steering, this study aims to quantify the two-dimensional hearing patterns is response to 3 tonal and one click stimuli. Since no data exist on the 2-D hearing patterns of any odontocete, the null hypothesis of a uniform hearing field is assumed.

Materials and Methods

The test subject was Boris, a 25-year-old male bottlenose dolphin (*Tursiops trucatus*) measuring 2.6 meters long and weighing 215.5 kg. He was housed in the floating pen facility moored off of the Hawai'i Institute of Marine Biology at Coconut Island in Kāne'ohe Bay, O'ahu, Hawai'i. The test area was a 6 x 9 m floating pen (pen 3) that was surrounded by wooden decking held at the surface by air-filled drums. Built on the decking is an equipment housing that was the site of the AEP equipment and the researcher. For each data collection session, a trainer was positioned on the wooden decking between the test pen and the equipment housing, which facilitated communication between the researcher, trainer(s) and test subject.

The subject began a trial stationed at a Styrofoam pad at the surface beside the trainer. At a signal from the trainer, the animal would dive and position himself in the hoop/biteplate setup that was located 1 m under the surface of the water. The hoop was steel wrapped in electrical tape for comfort. The biteplate was plastic padded on both sides with closed cell neoprene. The biteplate was attached to the stem of the hoop with

¾ inch PVC piping fastened to the pole of the steel hoop with nylon screws. The ends of the PVC were open so that the apparatus would fill with water when submerged.

When stationed in the hoop during a trial, the subject would grasp and hold the biteplate. His position was monitored with an underwater camera connected to a monitor visualized by the trainer and researcher. Once the animal was in correct position, the trial(s) began. At the end of the trial(s) he was recalled using a bridge whistle and returned to the stationing position for a fish reward.

For each trial, the sound projector was placed in one of 21 different positions. The transducer was moved in a horizontal arc from 0° straight ahead to 90° to right and left in 30° increments at a distance of 1.5 m from the hoop/biteplate and was moved from 1m depth to 0.5 m deeper or shallower in the vertical plane (See Figures 3.1 and 3.2). Therefore, each position had a different depth (0.5 m, 1 m, 1.5 m) and angle (-90˚, -60˚, -30˚, 0˚, 30˚, 60˚, 90˚; negative angles being left and positive angles at the right).

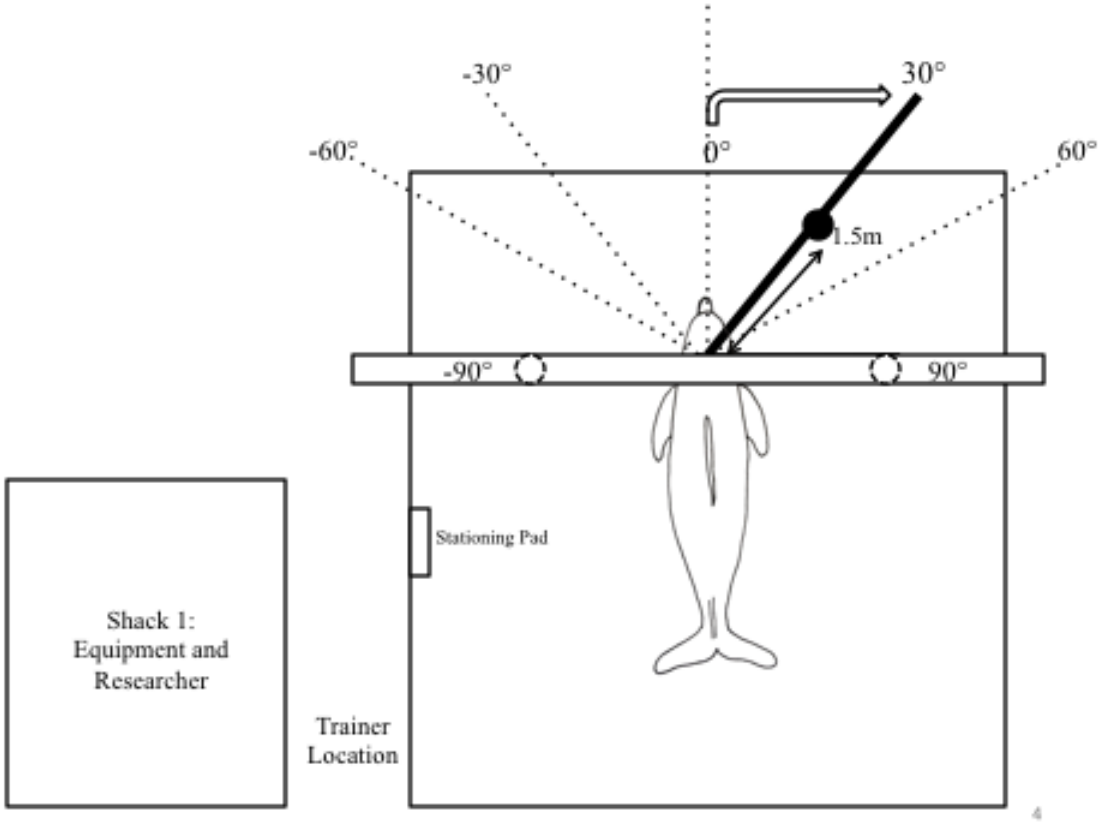

Figure 3.1
The equipment setup in pen three showing the horizontal, angular placements of the sound transducer

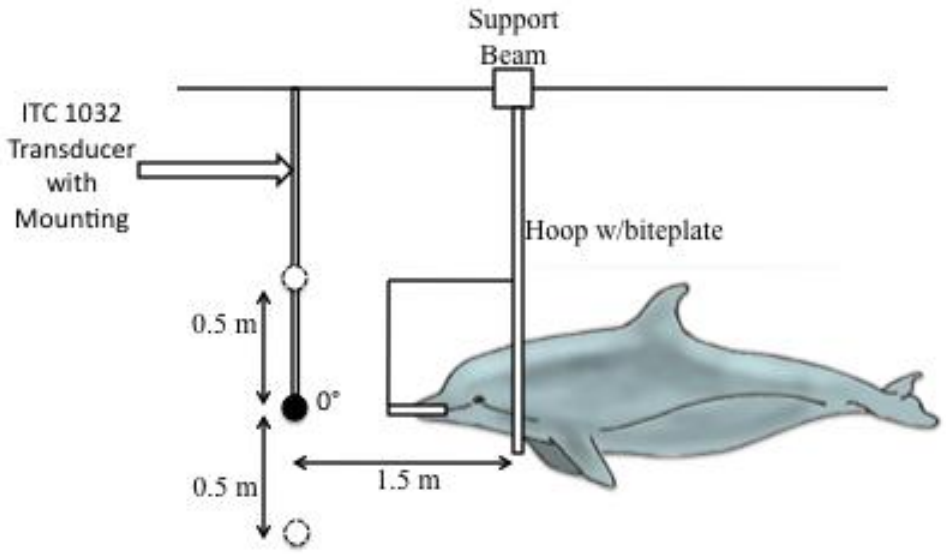

Figure 3.2
The equipment setup in pen three showing the vertical (depth) placements of the sound transducer

The equipment setup utilized the same system described in Taylor et al. 2007 with component and software upgrades as they had become available. The signals used consisted of 3 different, 20 msec sinusoidally amplitude modulated (SAM), tones, at 5.6 kHz, 22.5 kHz, and 38 kHz with a modulation frequency of 1 kHz and a broadband click with a center frequency of 30 kHz and a repetition rate of 1 kHz. A schematic of the setup of the sound generation and brainwave acquisition equipment is shown in figure 3.3.

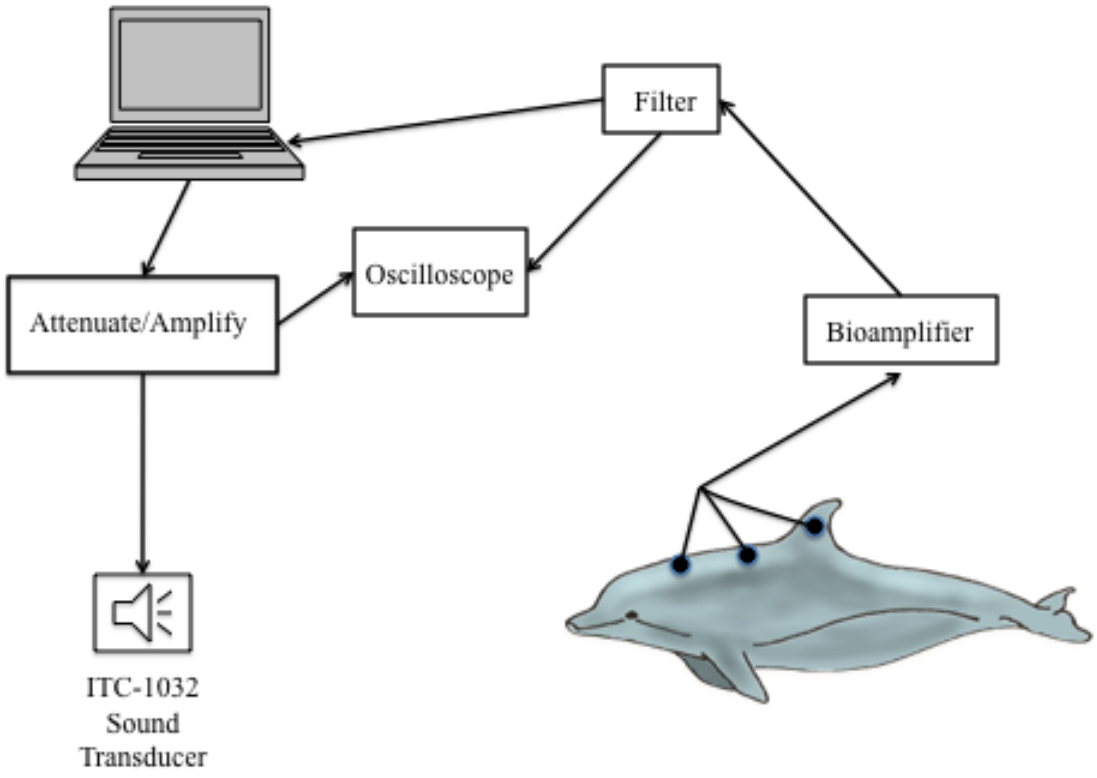

Figure 3.3
A schematic diagram demonstrating the equipment setup for the generation of sound and the reception of the brainwave information.

A custom-built LabView Program generated each signal that was attenuated then amplified (for impedance matching) and routed to an ITC-1032 underwater sound projector. The outgoing stimuli were viewed in real time on an oscilloscope. The brainwaves of the subject were captured with custom made suction cup electrodes, with the active electrode approximately 6 cm behind the blowhole (Supin et al., 2001) and the reference placed on the dorsal aspect in front of the dorsal fin and the grounding electrode placed on the dorsal fin itself (see placement in figure 3.3). The brainwaves were routed to a bioamplifier and amplified 10,000X and bandpass filtered. The brainwaves were then routed through another filter for additional reduction of noise. The information was then routed to the computer into the custom LabView Program. This program was triggered by the production of the outgoing stimulus to measure and average 1000 of the incoming brainwaves, which allowed for a much higher signal-to-

noise ratio. Sound calibration was done for each signal type and position using a Reson TC-4032 receiving hydrophone, which had a flat response (±5 dB) from 1-100 kHz.

For each threshold obtained, a minimum of 9 trials were run. Depending on the response seen for the previous trial, the next trial had a louder or softer stimulus (which could be controlled in increments of 1 dB). 16 msec (eliminating the first 6 msec and last 4 msec of each record) of each waveform was analyzed using a 256 point Fast Fourier transformed performed by means of a custom built MatLab Program. The amplitude of the FFT response (in microvolts) at 1 kHz (the modulation frequency of the stimuli) for each outgoing signal amplitude was quantified. These responses were plotted as amplitude (in dBs) versus FFT microvolt response, and a linear regression line was drawn through the linear portion of the response (Supin et al., 2001, Taylor et. al., 2007, Popov et al., 2008). The threshold value was defined as where the regression line intersected the axis.

Since all of the experiments were carried out in an open bay pen the noise field and any differences in the noise between sound source locations were explored. The power spectrum density (dB re 1 $\mu Pa^2/Hz$) was measured for the ambient noise for a frequency band between 1-79.9 kHz at multiple locations within the experimental enclosure. An omnidirectional TC4032 (Reson) receiving hydrophone captured the noise that was then band pass filtered (LP: 80 kHz, HP: 100 Hz) by a Krohn-Hite Model 3362 Filter. The output was visualized using a Tektronix TDS1002 Oscilloscope and routed into the NI 6062E DAQCard in a PC and into a custom LabView Program. This program allowed the capture 20 seconds of noise in a chosen frequency band and sampling rate (200 kHz). The noise was sampled between 1 and 79.9 kHz to completely encompass the hearing range as well as frequencies above those of the test subject. A custom MatLab program was used to analyze the raw LabView data, which allowed a view of the waveform of the noise as well as its spectrum.

Results

The threshold results were computed and are shown in levels of dB re: 1 μPa for each signal type used (Tables 3.1 – 3.4). It should be noted that for two signal types, 5.6 kHz and 22.5 kHz, multiple measurements were taken at the location of 0° 0m depth in

order to explore the normal intra-subject variation present. The 5.6 kHz repetitions had n=6 with a mean threshold of 95.2 dB, a standard deviation of ±2.2 dB and a range of 6 dB. The 22.5 repetitions had an n=7 with a mean threshold of 80.4 dB, a standard deviation of ±2.4 dB and a range of 6 dB. The threshold results for each signal type have ranges of difference in thresholds from 19 dB (38 kHz) to 38 dB (38 kHz). These ranges are well outside the observed 6 dB range observed for both 5.6 kHz and 22.5 kHz variability trials. Therefore, it can be concluded that the threshold variation seen could be a result of directional hearing sensitivities rather than dismissed as simple intra-subject variability.

In order to graphically highlight the differences in the 2-D areas measured, the results are shown as contour plots using the Minitab 16 3-D contour maps plotting function employing the Interpolation Method by means of the distance method using a power of 2 (Figures 3.4 - 3.7). The plots are laid out with the horizontal (azimuth) values on the x-axis. The negative angles are to the subject's left and the positive angular values are to the subject's right. The depth, relative to experimental subject's location, correspond to the numbers on the y-axis; 0 is at 1 m depth, 1.5m straight ahead, 0.5 is ½ m above and -0.5 m is ½ m below the transducer location at 0. The test subject's location in space corresponds to the 0°, 0m location, in the middle of each plot. The darker regions indicate the location(s) with the lowest threshold values which correspond to the locations of the most sensitive hearing.

Table 3.1
Threshold levels in dB re: 1 µPa in response to a 5.6 kHz SAM tone. The negative angles were to the left of the subject and the positive angles were to the right. Depth is in meters relative to the animal: +0.5m was at 0.5m depth, 0m was a 1m depth (straight ahead of the test subject) and -0.5m was at a depth of 1.5m

5.6 kHz	**-90°**	**-60°**	**-30°**	**0°**	**+30°**	**+60°**	**+90°**
+0.5m	86	92	90	110	94	85	100
0m	89	94	90	98	94	85	92
-0.5m	91	94	97	103	93	97	91

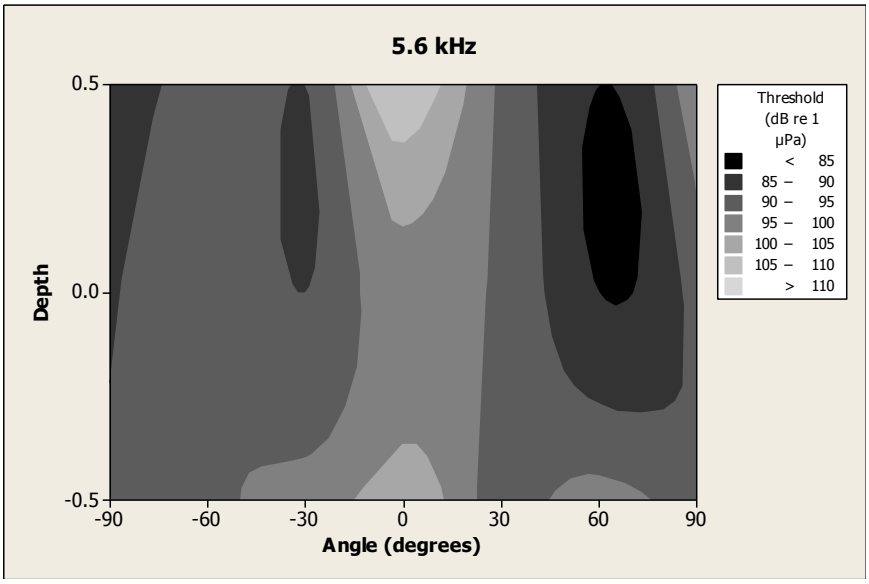

Figure 3.4
The contour plot representing the threshold values for 5.6 kHz: the darker colors represent lower hearing thresholds. The horizontal and vertical axes correspond to the azimuth and depth of the sound source.

Table 3.2
Threshold levels in dB re: 1 µPa in response to a 22.5 kHz SAM tone. The negative angles were to the left of the subject and the positive angles were to the right. Depth is in meters relative to the animal: +0.5m was at 0.5m depth, 0m was a 1m depth (straight ahead of the test subject) and -0.5m was at a depth of 1.5m

22.5 kHz	**-90°**	**-60°**	**-30°**	**0°**	**+30°**	**+60°**	**+90°**
+0.5m	88	88	94	84	92	93	98
0m	86	77	82	79	82	93	98
-0.5m	79	89	87	81	83	81	78

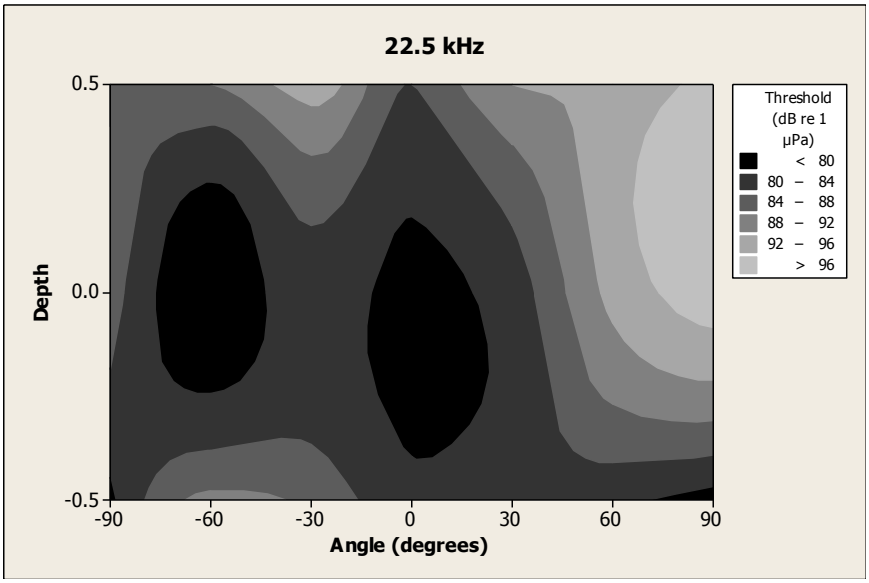

Figure 3.5
The contour plot representing the threshold values for 22.5 kHz: the darker colors represent lower hearing thresholds. The horizontal and vertical axes correspond to the azimuth and depth of the sound source.

Table 3.3
Threshold levels in dB re: 1 µPa in response to a 38 kHz SAM tone. The negative angles were to the left of the subject and the positive angles were to the right. Depth is in meters relative to the animal: +0.5m was at 0.5m depth, 0m was a 1m depth (straight ahead of the test subject) and -0.5m was at a depth of 1.5m

38 kHz	**-90°**	**-60°**	**-30°**	**0°**	**+30°**	**+60°**	**+90°**
+0.5m	90	92	94	89	73	92	105
0m	89	97	82	83	80	92	109
-0.5m	85	91	84	88	88	79	71

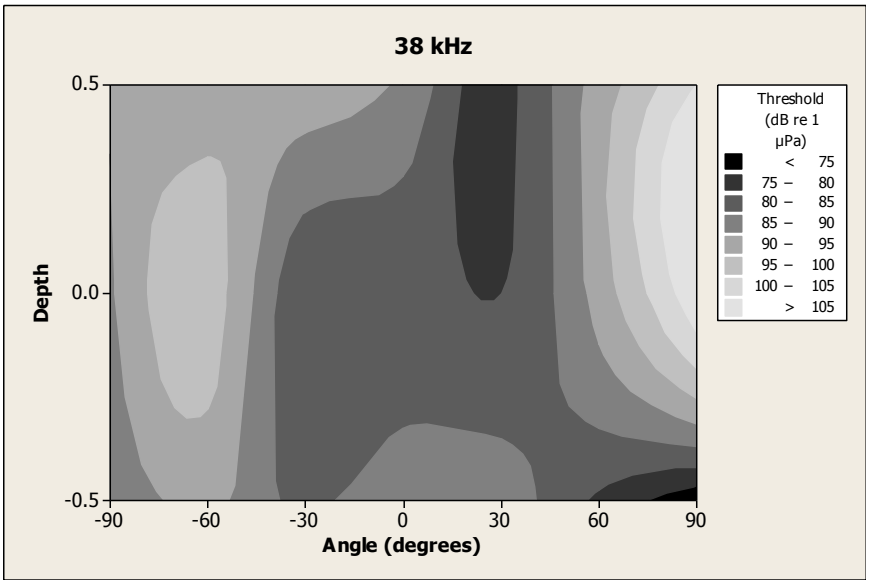

Figure 3.6
The contour plot representing the threshold values for 38 kHz: the darker colors represent lower thresholds of hearing. The horizontal and vertical axes correspond to the azimuth and depth of the sound source.

Table 3.4
Threshold levels in dB re: 1 µPa in response to a broadband click. The negative angles were to the left of the subject and the positive angles were to the right. Depth is in meters relative to the animal: +0.5m was at 0.5m depth, 0m was a 1m depth (straight ahead of the test subject) and -0.5m was at a depth of 1.5m

Clicks	**-90°**	**-60°**	**-30°**	**0°**	**+30°**	**+60°**	**+90°**
+0.5m	99	98	101	92	95	95	100
0m	92	90	93	88	92	97	87
-0.5m	88	88	87	75	87	91	87

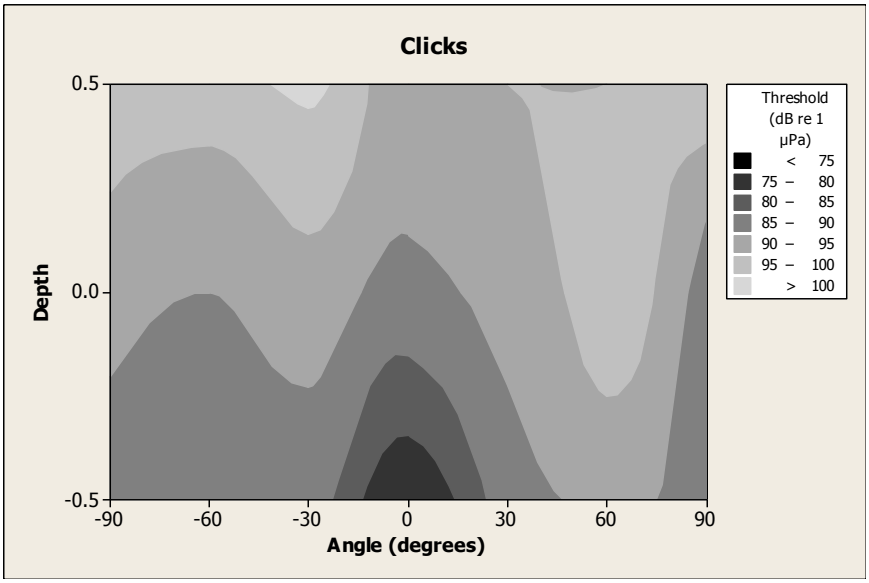

Figure 3.7
The contour plot representing the threshold values for a broadband click with center frequency of 30 kHz: the darker color represents lower hearing thresholds. The horizontal and vertical axes correspond to the azimuth and depth of the sound source.

Each of the three SAM tone signals appears to have two locations of highly sensitive hearing. These two sensitive locations for 5.6 kHz are above and to either side of the midline (Figure 3.4). The two sensitive locations for 22.5 kHz have one location towards the midline central with the other to the left of the midline (Figure 3.5). For 38 kHz the two locations of greatest sensitivity are very diverse, one above and to the right

of the midline and the other below and to the right of the midline (Figure 3.6). In contrast, clicks have a single distinct location of the most sensitive hearing; that at 0˚ and at a 0.5m depth (relative to the test subject) (Figure 3.7). No two response panels are identical. In fact, each contour is quite distinct. In general it seems that tonal sounds are heard better laterally and click stimuli are heard better medially.

Figure 3.8 shows the background noise level at the location of the test subject. Due to equipment constraints, it was not possible to measure noise during the directional hearing experiments. Therefore, the noise field measurements were done either before or after the experimental trials. To mitigate the possible effects of noise on threshold levels, testing was either suspended or canceled for boats, hard rain or high winds.

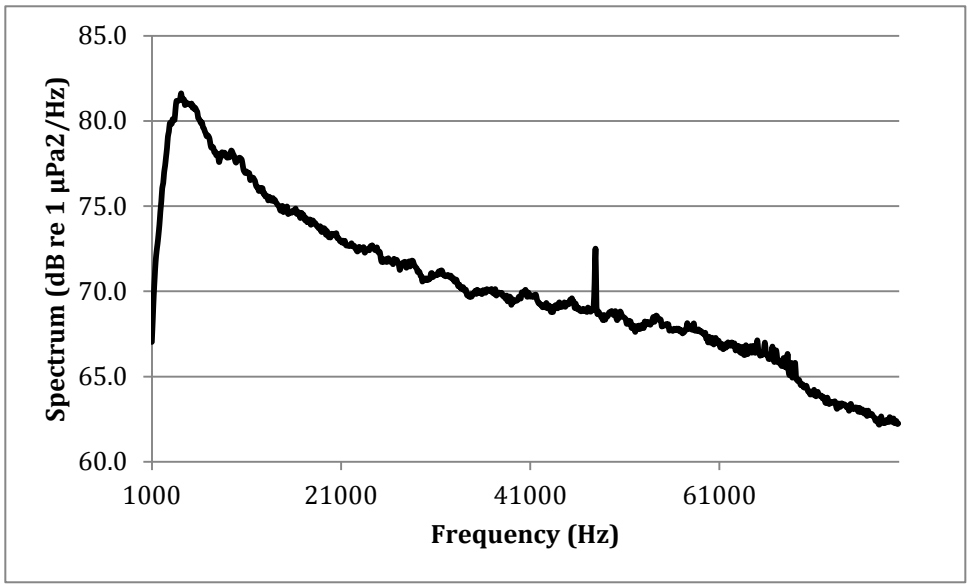

Figure 3.8
Power spectrum levels in dB re 1 µPa2/Hz for background noise measured with a 200 kHz sampling rate for 1-79.9 kHz. This measurement was taken at the location where the test subject would be stationed, underwater in a hoop, for hearing measurements.

Discussion

In general these results are in agreement with previous evidence. Brill et al. (2001) found that the left side of their dolphin had more sensitive hearing than the right and hypothesized asymmetrical hearing structures that would allow for greater localization of sound. The contour plots of each signal type demonstrate asymmetry, with the exception of the single, centralized pathway for the clicks. Asymmetries in hearing pathways are assumed to arise through selection for increased localization

capability (Bradbury and Vehrencamp, 2011). Obvious asymmetries have been documented in other species such as in the anatomical position of the barn owl's ears (Christensen-Dalsgaard, 2005), the funnel-like shaping of the feathers around various species of bird's ears as well as the asymmetrical pinna of bats (Bradbury and Vehrencamp, 2011). These asymmetries are thought to have been selected for as a method for especially increasing vertical localization abilities (Yost, 2006).

In 1984, Au and Moore published the receiving beam pattern of a bottlenose dolphin. They measured the masked thresholds of a bottlenose dolphin in both a vertical and horizontal arc to pure tones of 30 kHz, 60 kHz and 120 kHz. The study limited any kind of beam steering by the animals by using two sound sources, one for the signal and one that played white noise. They found the beam to be very frequency dependent in its shape but with the best overall hearing to be at 0˚ and slightly above the midline. The frequencies used in this study were much lower than Au and Moore's due to the limits of the subject's hearing. In addition, Au and Moore did not explore responses to broadband stimuli as was explored in this study. It is difficult to see how the contour data compares with the Au and Moore study hence the horizontal data is plotted at 1m depth (directly in front of the test subject) (Figure 3.9) as well as plotted from 0˚ at all three depths (Figure 3.10) in order to provide a more straightforward comparison. Please note that the angular discrimination used in this experiment was different from that used in Au and Moore (1984).

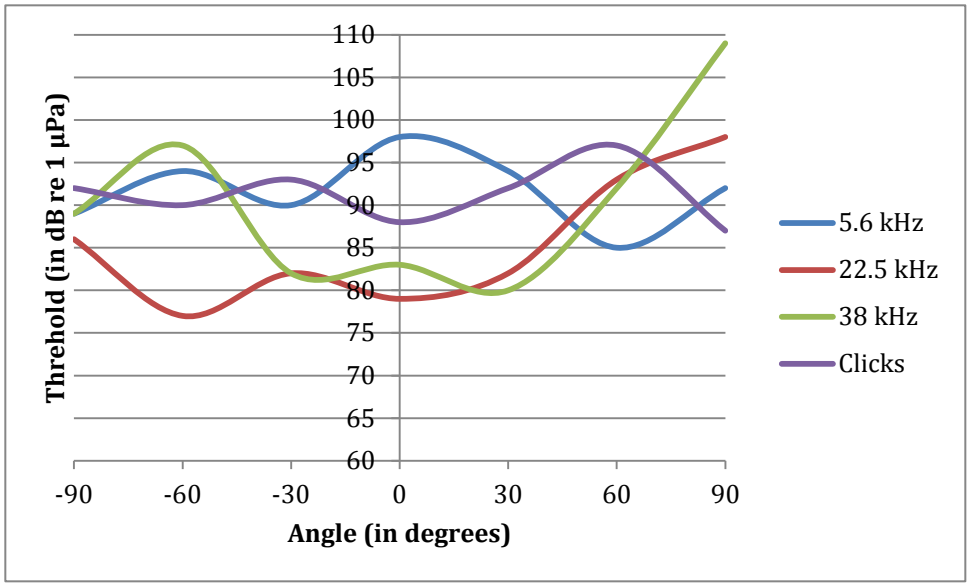

Figure 3.9
Threshold levels for each of the four signal types at 1m depth with each of the seven angles shown; the negative angles being to the left of the subject and the positive angles to the subject's right.

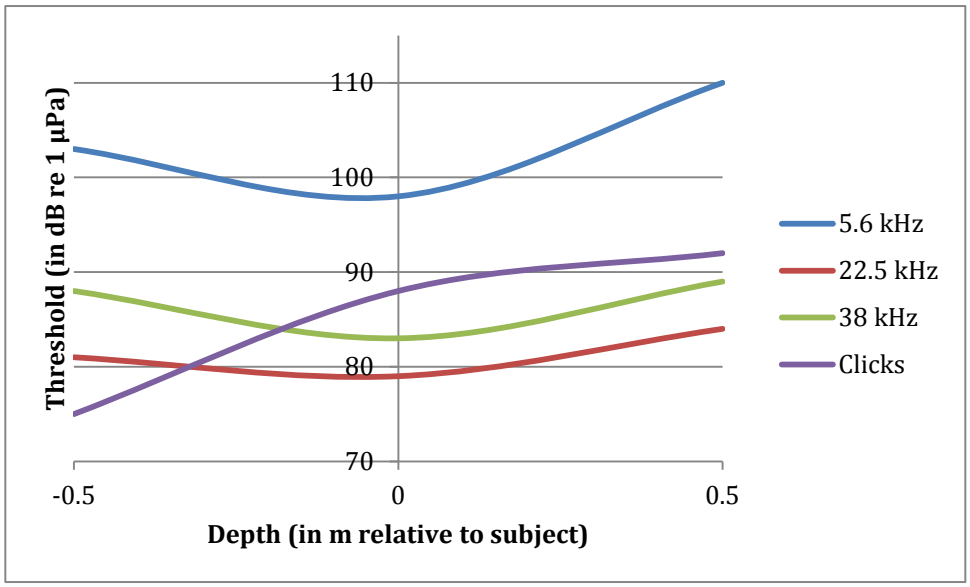

Figure 3.10
Threshold levels for each of the four signal types at 0° angle and all three depths: 0.5 m, 0 m (directly in front of the experimental subject) and -0.5m

The horizontal plane reveals a lot of variation due to signal type with 5.6 kHz and Clicks probably the most alike. However, in none of these signal types is the best sensitivity observed at the midline. In fact, there is a marked left-right asymmetry for

22.5 kHz and 38 kHz. When looking at the effect of depth, there are no noticeable dissimilarities between any of the SAM tones. However, the clicks did show noticeably lower thresholds at depth. Perhaps the difference lies in the way Au and Moore situated the animal with the pan-bone facing the midline rather than facing the rostrum at the midline as was done in this study. Another explanation for the differences observed could be the different signal types used.

The null hypothesis of a uniform receiving field was disproven for all signal types used. What is the functional significance of asymmetrical, signal dependent directional hearing? Collecting the adequate stimulus from multiple pathways would allow exploitation of the greatest amount of energy and, therefore information, from the stimulus source. Multi-pathway inputs are common in sensory systems. Humans have one primary sound pathway into their ears: around the pinna, through the external auditory meatus to the tympanic membrane, through the middle ear (via ossicular chain motion) and into the inner ear. In addition to this pathway, sound, especially of lower frequencies, can often travel through bone conduction directly into the inner ear. Also, sound can move through soft tissue pathways into the inner ear, bypassing the external and sometimes middle ears (such as seen when we hear our own voices, heartbeat etc).

An analogous, multi-pathway, situation is also observed in other sensory modalities such as vision. The most sensitive color vision is at the fovea of the eye which is the location on the retina with the highest level of cones. The eyes can be positioned so that the light coming from the object of interest is focused on that fovea. However, in low light situations and for moving objects, the most sensitive locations for detection are in regions surrounding the fovea. These locations have the richest abundance of rods, which are more sensitive to photons than are cones. During low light situations, orienting the surrounding rod-rich locations towards the object, rather than the rod-poor fovea, would allow for more sensitive reception. An even more striking, multipath example is found in the visual system of bottlenose dolphins and other odontocetes. In the completely constricted pupil, there remain two "pin-hole" openings rather than one, as is seen in humans (Supin et al., 2001). These two openings correspond to 2 highly sensitive receptive field locations on the retina (as well as the underlying ganglion cell structure). This remarkable construct, coupled with adaptations that also allow for low-light,

underwater vision, allows these animals to have sensitive vision both in and outside of the water (Supin et al., 2001).

It would seem an obvious conclusion that dolphins would exhibit multiple pathways for sound in order to exploit as much information from the sound source as possible just like is evident in their visual system. Anatomical evidence such as the specialized acoustical fatty acids that are found around the head and jaw of odontocetes (see Koopman et al., 2006) would lend evidence for the presence of multiple pathways that sound could be channeled through. Ulinsky (1984) discusses receptive fields of sensory systems and suggests that larger receptive fields or a greater amount of receptive fields allow a larger array of information to be acquired. In addition, one would expect the sound pathways through their heads to be much more complex due to their watery surround having comparable impedance to that of their tissue (Au and Hastings, 2008). Because of the similarity between the impedance of water and tissue, sound is able to move from the water to the soft tissues with little reflection. In 1999 Møhl et al. proposed a shaded receiver model for odontocete hearing that demonstrated multiple sites on a dolphin's head that had a very high acoustic sensitivity. The limitation in comparison to that study was the stimuli that they used. Their sound source was a suction cup hydrophone that delivered one specific sound type directly to the dolphins head, rather than the free field sound source used in the current study. Sound sources applied directly to a dolphins head, although helpful for exploring sound channels specific to that type of stimulus, does not really reflect any sort of natural acoustical situation that dolphins experience in the wild. Au and Moore's (1984) setup as well as this experimental setup much more closely mimic a natural acoustical free field environment. In both this experiment and that of Au and Moore, variation in the receiving patterns for different frequencies were observed and resemble that of a shaded receiver. However, the shaded receiver model does not seem wholly adequate to explain the rich variation in directional hearing that has been observed.

Sound is of primary importance for odontocetes and may even be their primary sensory system (Kellogg & Kohler 1952). Therefore the pathways of hearing and subsequent localizations of specific signal types would be of selective advantage to this group and would drive that selection towards a sophisticated receiving system. Evolution

has left odontocetes with a highly derived head that allows for efficient breathing at the surface, swallowing underwater, biosonar production and sound reception. It would make sense that each piece of the head could be exploited for multiple functions and would therefore, allow for multiple sound pathways depending on the anatomical differences over the entire head. The evidence suggests that there are, not only multiple pathways for sound to be channeled through, but that these pathways, or beams, are frequency, or signal-type, dependent. The static nature of the shaded receiver model does not seem adequate to describe the frequency-dependent variation in receiving beam patterns that have been observed. A muti-beam receiver would be capable of simultaneous localization and classification of signal types which would allow for the efficient recognition and targeting of prey and constructive interaction with conspecifics, both of which would lead to greater fitness and overall survivorship.

Chapter 4

A Head-Related Transfer Function of a Bottlenose Dolphin (*Tursiops truncatus*)

The ability to localize sound has been suggested to be a major force within the evolution of hearing (Fay and Popper, 2005). However, sound, in itself, does not have positional information – only frequency, amplitude and phase, none of which are directional in nature. In order to localize a sound to a particular point of space, the three dimensional factors, azimuth, elevation and range, must be distinguishable.

In order to localize a sound in the horizontal axis (azimuth), the duplex theory of sound applies (Yost, 2006). If the sound has a frequency high enough that it's wavelength is smaller than the head of the receiver, the sound is said to cast a shadow on the ipsilateral side of the head. Because of this sound shadowing, the subject can tell the sound's location through the time of arrival difference between the two (or more) ears – a quantity known as the interaural time difference or ITD. However, if the sound is of a low enough frequency to have a wavelength as large as or larger than the head, the head will not cast a discernible sound shadow and the ITD will not be sufficiently different between the ears to be able to code for a location. Therefore, another cue – the amplitude, or level, of the sound can be exploited. The sound level difference between the ears (known as the interaural level difference (ILD)) can be used as a cue because a sound originating at one side of the head should be sensed as louder on that side than on the opposite side. So the ITD and the ILD together can be used to discriminate a sound's location in the horizontal plane – a theory known as the Duplex Theory of Sound Localization.

The cues that the central nervous system (CNS) could use to detect the distance, or range, to a sound source is of ongoing debate. However, it is believed that the Precedence Effect is used (Yost, 2006). The Precedence Effect observes that the first sound arriving at the ears has taken the shortest linear path and will be the loudest. This direct sound pathway would also have the most minimal echoes off of the surroundings, and therefore contain the most direct information about the sound source. In contrast, the

farther away the sound source, the softer a sound will be and the more echoes it would contain due to the sound's interaction with environmental factors. However, this hypothesis relies on the subject having prior knowledge of the sound and its characteristics or the ability to learn this information so it would be suspect in a young or inexperienced receiver.

The location of a sound in the vertical plane (elevation) can cause ITD and/or ILD differences that can be used for information as to the elevation of the sound source. However, sounds originating along the midsagital plane of the receiver would have the same ITD and ILDs along the entire plane – an area known as the cone of confusion. If the receiver has asymmetrically placed ears, such as are seen in owl species, then this cone of confusion does not exist as there can be differences in the ITDs and the ILDs around that midsaggital plane (Brown and May, 2005). But for those with symmetrical ears, this cone of confusion could be a problem.

Mathematically, it has been difficult to quantify the cues that can be used to locate the elevation of a sound, however, it seems reasonable that spectral cue differences between the sounds arriving at each ear would dependent on the location of the sound source itself. For example, if a sound came from above the subject, it would have to pass over the top of the head, face and pinna before entering the external ear canal. In contrast, a sound originating from below the subject would have to pass over the lower half of the body, including the torso and neck before entering the ear canal. As a sound travels around, and in some cases through, an anatomical structure the properties of that structure can alter the properties of the sound.

A linear filter is one that can alter the sound amplitude but leaves the frequency spectrum unaltered. A nonlinear filter is one where the frequency spectrum of the sound changes, with addition or subtraction of sinusoids caused by the sound's interaction with anatomical structures. There is data to suggest that multiple components of the human hearing system acts as a nonlinear filter and that these nonlinearities are properties that may be essential for sound processing by the central nervous system (Yost, 2006). If the sound is filtered by any anatomical structure in a measurable way, that alteration can be anticipated by the central nervous system and used as a localization cue.

A Head-Related Transfer Function (HRTF) is the spectral change that a sound undergoes as a result of passing over the head, neck, torso and outer ears of a subject (Yost, 2006). It is thought that the HRTF allows a subject to localize a sound with great precision, especially in the vertical plane, because of the discrete linearities and nonlinearities introduced by the head structures which can be used by the CNS as specific directional cues. There is relatively little data on the directional hearing capabilities of cetaceans (see an excellent review in Branstetter and Mercado, 2006). However it would seem reasonable that as mammals, the land ancestors of modern cetaceans would have had similar basic anatomy to land mammals today and that through evolution, natural selection would favor those individuals that were better able to localize sound in water and therefore more successful at foraging and predator avoidance. The pinna of land mammals is highly functional and thought essential for good directional hearing because it allows for predictable filtering of the incoming sound (Yost, 2006). Since odontocete cetaceans have no readily observable outer ear structures and yet demonstrate exceptional directional hearing (Renaud and Popper, 1975, Au and Moore, 1984, Popov and Supin, 2009) it could be deduced that the spectral cue function of the pinna could have been taken over by refinement of other internal head structures.

Measurements to quantify human HRTFs have been and are still being done (See the ARI HRTF Database, 2012). These measurements consist of a sound played via a calibrated sound source at a fixed distance from the head. The sound is then measured at a point inside the external auditory meatus by a small, calibrated microphone and the differences between the two sounds are quantified. This is a difficult process for many reasons some of which are correcting for the presence of the microphone in the ear and its effect on the sound field in the EAM as well as the differences that arise between different individual test subjects. The equivalent outer ear structure of a bottlenose dolphin is not known and because of this, as well as for legal and ethical reasons, no microphone was placed inside the head of the test subject. Instead auditory evoked potential (AEP) procedures were utilized in order to measure the brainwave output in response to a calibrated sound played in the free field from various locations. Considering the anatomical complexity of a dolphin's head, it was expected that differences in brainwave output would arise between locations.

Materials and Methods

The test Subject was Boris, a 25-year-old male bottlenose dolphin (*Tursiops trucatus*) that is 2.6 meters long and weighs 215.5 kg and is housed in the floating pen facility moored off of the Hawaii Institute of Marine Biology at Coconut Island in Kāne'ohe Bay, O'ahu, Hawaii, and has taken part in many research experiments (Mooney et al. 2009a,b, Nachtigall et al. 2003, 2004). The test area was a 6 x 9 m floating pen that has been used for multiple acoustic experiments (Yuen et al. 2005, Mooney et al. 2009a,b). The test subject had been trained to wear AEP suction cup electrodes and to station underwater in a hoop outfitted with a biteplate. The equipment and researcher was stationed in a test shack adjacent to the pen. The trainer was positioned on the wooden decking between the test pen and the equipment housing, which facilitated communication between the researcher, trainers and test subject.

The test subject began a trial stationed at a Styrofoam pad at the surface beside the trainer. At a signal from the trainer, the test subject would dive and position himself in the hoop/biteplate that was 1 meter under the surface of the water. The hoop was steel wrapped in electrical tape for comfort. The biteplate was plastic padded on both sides with closed cell neoprene. The biteplate was attached to the stem of the hoop with ¾ inch PVC piping and fastened to the pole of the steel hoop with nylon screws. The ends of the PVC were open so that the apparatus would fill with water when submerged. When stationed in the hoop during a trial, the animal would grasp and hold the biteplate. His position was monitored with an underwater camera connected to a monitor visualized by the trainer and researcher. Once the subject was in the correct position, the trial(s) began. At the end of the trial(s) he was recalled using a bridge whistle and returned to the stationing position for a fish reward.

For each trial, the sound projector was placed in one of 21 different positions. The transducer was moved in a horizontal arc from 0º straight ahead to 90º to right and left in 30º degree increments at a distance of 1.5 m from the hoop/biteplate. The sound transducer was also moved from a 1m depth to 0.5 m deeper or shallower in the vertical plane (See Figures 4.1 and 4.2). Therefore, each position had a different depth (0.5 m, 1

m, 1.5 m) and angle (-90˚, -60˚, -30˚, 0˚, 30˚, 60˚, 90˚; negative angles being left and positive angles at the right).

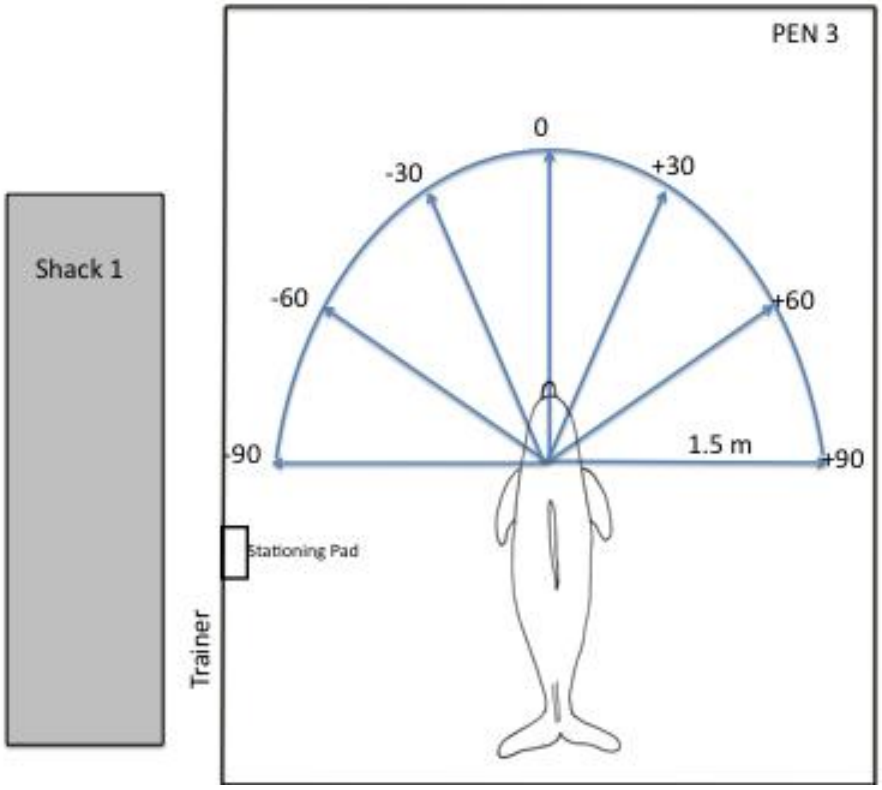

Figure 4.1
A graphical representation of the horizontal angles used for the locations of the sound transducer in relation to the subject's position.

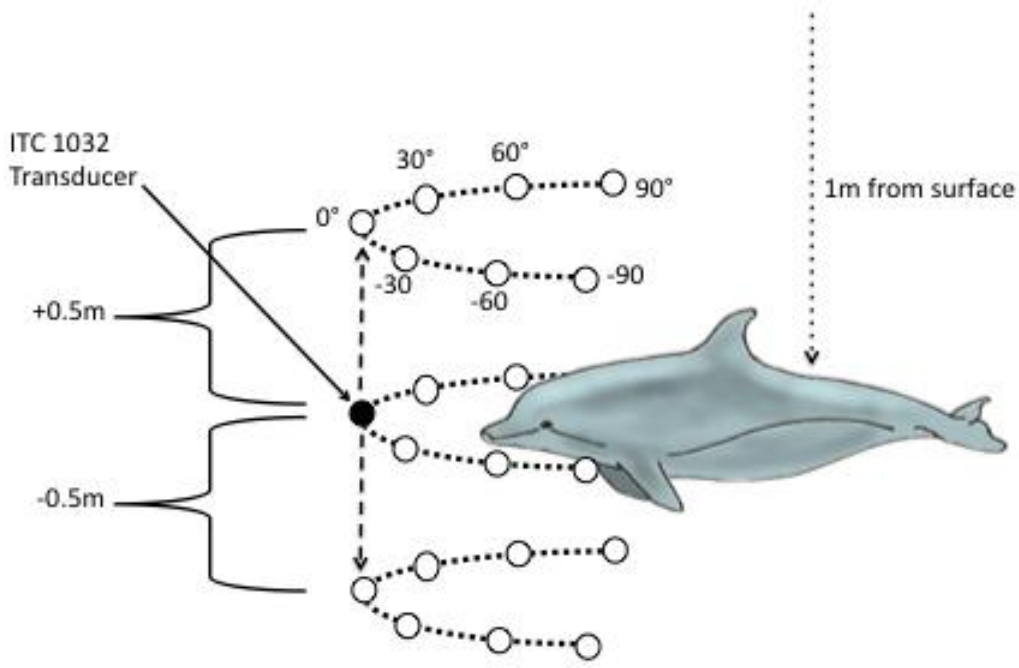

Figure 4.2
A representation of the horizontal angles and depth of the sound transducer's positions in relation to the test subject which resulted in 21 positions that each of the four signal types were presented from.

The equipment setup is the same system described in Taylor et al. 2007 with component and software upgrades as they had become available. The signals used consisted of three 20 msec sinusoidally amplitude modulated (SAM) tones, at 5.6 kHz, 22.5 kHz, and 38 kHz, each with a modulation frequency of 1 kHz, and a broadband click with a center frequency of 30 kHz. A custom built LabView Program generated each signal which was routed through a National Instruments DAQCard-6062E then routed through a SCB DAQCard box. The signal was then attenuated through a Hewlett Packard 350D attenuator and amplified using a Hewlett Packard 465A amplifier to increase the impedance matching of the system. The signal was then projected through an ITC-1032 underwater transducer and visualized using a Tektronix TDS1002 Oscilloscope.

When the stimulus began the custom built LabView Program was triggered to acquire the brainwave information. The brainwaves were collected using three custom-

built suction cup electrodes (Grass E5GH gold disc electrodes – see Taylor et al. 2007): an active, placed approximately 6 cm behind the blowhole, a reference, placed on the dorsal aspect just in front of the dorsal fin, and a ground placed on the dorsal fin itself. A Grass CP511 A.C. Amplifier (10,000X) received each of these electrode inputs and also bandpass filtered the signal between 300 Hz and 3 kHz. For additional resolution the signal was again bandpass filtered between 300 Hz and 3 kHz by a Krohn-Hite Model 3362 Filter. The output was visualized using the same Tektronix TDS1002 Oscilloscope and routed into the NI 6062E DAQCard via the SCB DAQCard box and into the custom LabView Program. In order to increase the signal-to-noise ratio, 1000 repetitions of the stimulus was played and the brainwaves acquired for each were averaged. The LabView program allowed the researcher to see the real-time averaging of the brainwaves as well as the real time Fast Fourier Transform of the incoming brainwaves. Sound calibration was done for each signal type and position using a Reson TC-4032 receiving hydrophone which has a flat response up to 100 kHz.

The brainwave data was analyzed using a custom built MatLab Program. This program took the brainwaves and plotted them in microvolts and then analyzed them for their frequency spectrum using a Fast Fourier Transform (see Figure 4.3). The peak values at 1 kHz (the modulation rate of each signal) were calculated in μV for each of the three sound amplitudes: 120 dB, 115 dB and 110 dB (re: 1 μPa).

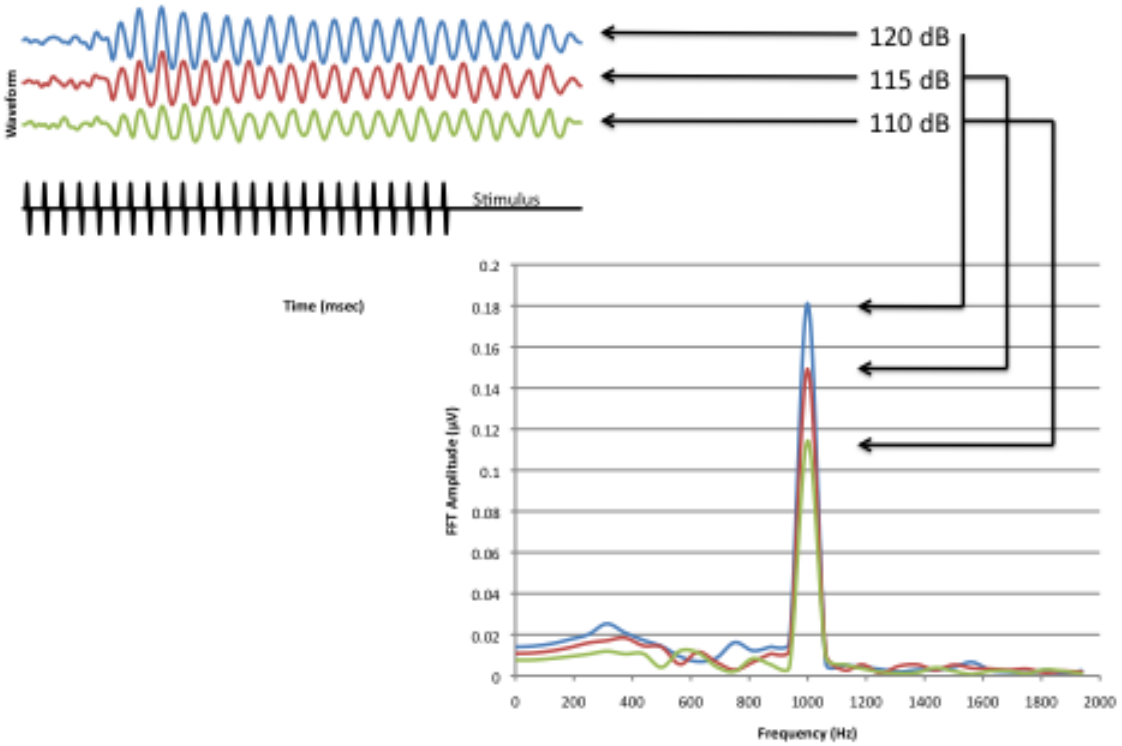

Figure 4.3
The 26 msec long AEP waveforms for 3 amplitudes: 120 dB, 115 dB and 110 dB (all re: 1 µPa) in response to a 20 msec click stimuli. Each of these waveforms was fast Fourier transformed in order to obtain the frequency spectrum. The levels (in µV) were calculated for each peak at 1 kHz (the modulation rate for each signal).

Results

The FFT peak value at 1 kHz for output levels of 120 dB, 115 dB and 110 dB are enumerated in Tables 4.2-4.5 for each of the signal types. In order to address some of the underlying intra-subject variation, two signal types, 5.6 kHz, and 22.5 kHz had multiple measurements taken at one position, that of 0° 0m depth. The means and standard deviations for each amplitude level are listed in Table 4.1.

The inter-subject range for all three amplitudes at 5.6 kHz and 22.5 kHz are smaller than the range of responses seen for the HRTF measurements (Compare tables 4.1 to tables 4.2 and 4.3). Therefore, the amplitude differences seen for these two signals can be deduced as arising from spectral changes as the sound travels through the head rather than from normal intra-subject variability. There are no intra-subject variability

data for 38 kHz or for the broadband clicks. Again, deduction would indicate that it is a likely scenario that the variation seen across the HRTF data for those two signal types would also most likely arise from anatomical spectral changes rather than from just basic intra-subject variability.

Table 4.1
Intra-subject variation for 2 signal types at 0° 0m depth, all values are in μV

	N	Mean 120	SD 120	Range 120	Mean 115	SD 115	Range 115	Mean 110	SD 110	Range 110
5.6 kHz	6	0.0276	0.0093	0.0287	0.0254	0.0048	0.0128	0.0191	0.0047	0.0121
22.5 kHz	7	0.0833	0.0130	0.0367	0.0628	0.0078	0.0219	0.0559	0.0095	0.0258

In order to visualize the differences between the 21 signal locations contour plots (Minitab 16 – Interpolation Method, distance method with a power of 2) of the brainwave values (in μV) for each signal type (5.6 kHz, 22.5 kHz, 38 kHz and clicks), for each of the three amplitudes: 120 dB, 115 dB and 110 dB (re: 1 μPa) are shown in Figures 4.4 - 4.7. The azimuth is plotted in degrees using standard HRTF notation (Cheng and Wakefield, 2001) with the angle to the left of the subject being a negative angle and the angles to the subject's right as positive values, each in increments of 30 degrees. The elevation coordinates are the depths of the signal transducer is relation to the test subject: +0.5 was ½ meter above the test subject and ½ meter under the surface of the water, 0 was straight ahead of the subject at 1 meter depth, -0.5 was ½ meter below the subject and 1.5 meters below the surface of the water. The darker colors in the contour plot figures denote higher brain response outputs at those locations.

Table 4.2
The FFT Output amplitude (in μV) for output levels of 120 dB, 115 dB and 110 dB (re: 1μPa) for 5.6 kHz

120 dB	-90°	-60°	-30°	0°	30°	60°	90°
+0.5m	0.0177	0.0213	0.0294	0.0571	0.0336	0.0310	0.0144
0m	0.0362	0.0345	0.0436	0.0385	0.0238	0.0321	0.0186
-0.5m	0.0360	0.0383	0.0507	0.0389	0.0407	0.0268	0.0285

115 dB	-90°	-60°	-30°	0°	30°	60°	90°
+0.5m	0.0136	0.0261	0.0116	0.0410	0.0282	0.0187	0.0110
0m	0.0290	0.0285	0.0288	0.0364	0.0128	0.0330	0.0245
-0.5m	0.0302	0.0354	0.0535	0.0282	0.0401	0.0224	0.0285

110 dB	-90°	-60°	-30°	0°	30°	60°	90°
+0.5m	0.0067	0.0098	0.0103	0.0197	0.0199	0.0170	0.0039
0m	0.0198	0.0185	0.0258	0.0089	0.0222	0.0208	0.0076
-0.5m	0.0173	0.0320	0.0309	0.0165	0.0342	0.0219	0.0205

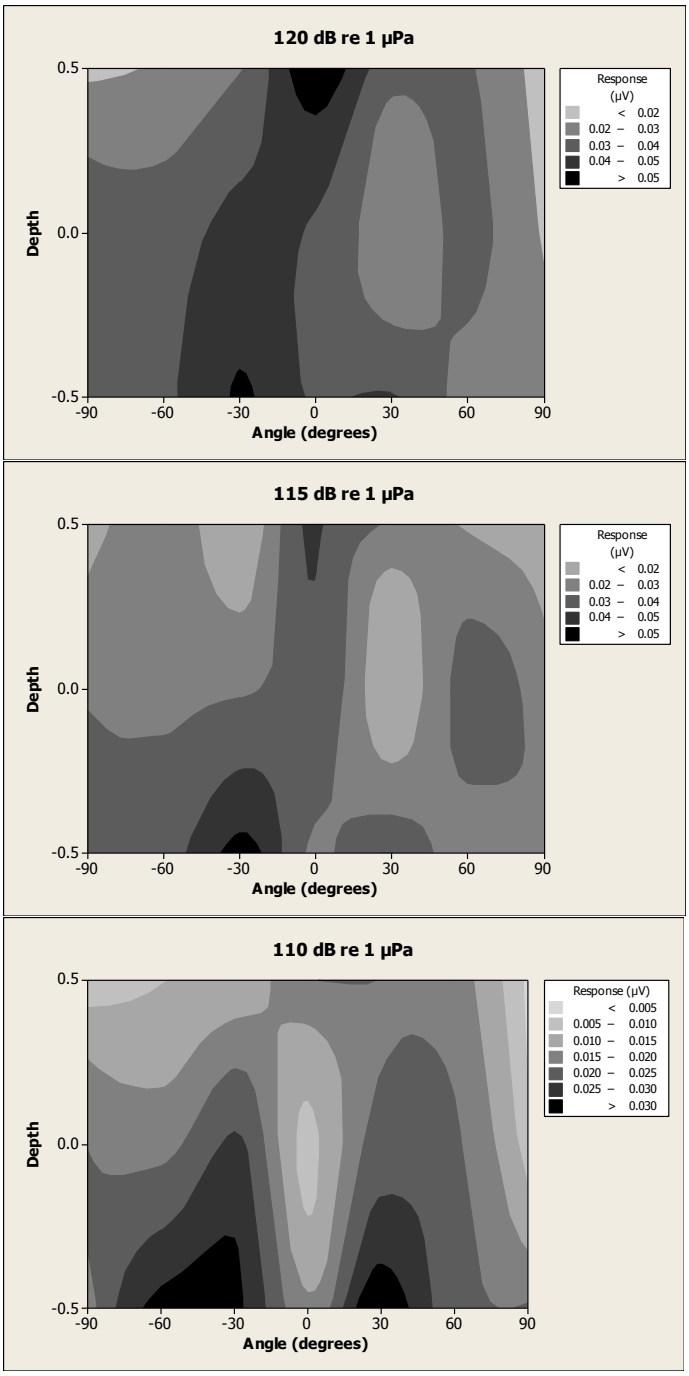

Figure 4.4
Contour plots for the Fourier transform outputs in µV for a signal of 5.6 kHz with decreasing amplitude from 120 dB to 110 dB (all re: 1 µPa) in 5 dB decrements. The subject's position was at 0m depth and 0˚

Table 4.3
The FFT Output amplitude (in μV) for output levels of 120 dB, 115 dB and 110 dB (re: 1μPa) for 22.5 kHz

120 dB	-90°	-60°	-30°	0°	30°	60°	90°
+0.5m	0.0360	0.0327	0.0349	0.0362	0.0034	0.0299	0.0358
0m	0.0425	0.0435	0.1268	0.0621	0.0594	0.0461	0.0280
-0.5m	0.0534	0.0684	0.1215	0.1676	0.0509	0.0328	0.0392

115 dB	-90°	-60°	-30°	0°	30°	60°	90°
+0.5m	0.0289	0.0211	0.0284	0.0417	0.0175	0.0185	0.0231
0m	0.0380	0.0445	0.0560	0.0529	0.0538	0.0347	0.0367
-0.5m	0.0342	0.0503	0.0802	0.0953	0.0463	0.0206	0.0284

110 dB	-90°	-60°	-30°	0°	30°	60°	90°
+0.5m	0.0202	0.0246	0.0188	0.0357	0.0175	0.0142	0.0182
0m	0.0255	0.0325	0.0475	0.0466	0.0486	0.0377	0.0285
-0.5m	0.0292	0.0428	0.0599	0.0544	0.0470	0.0363	0.0243

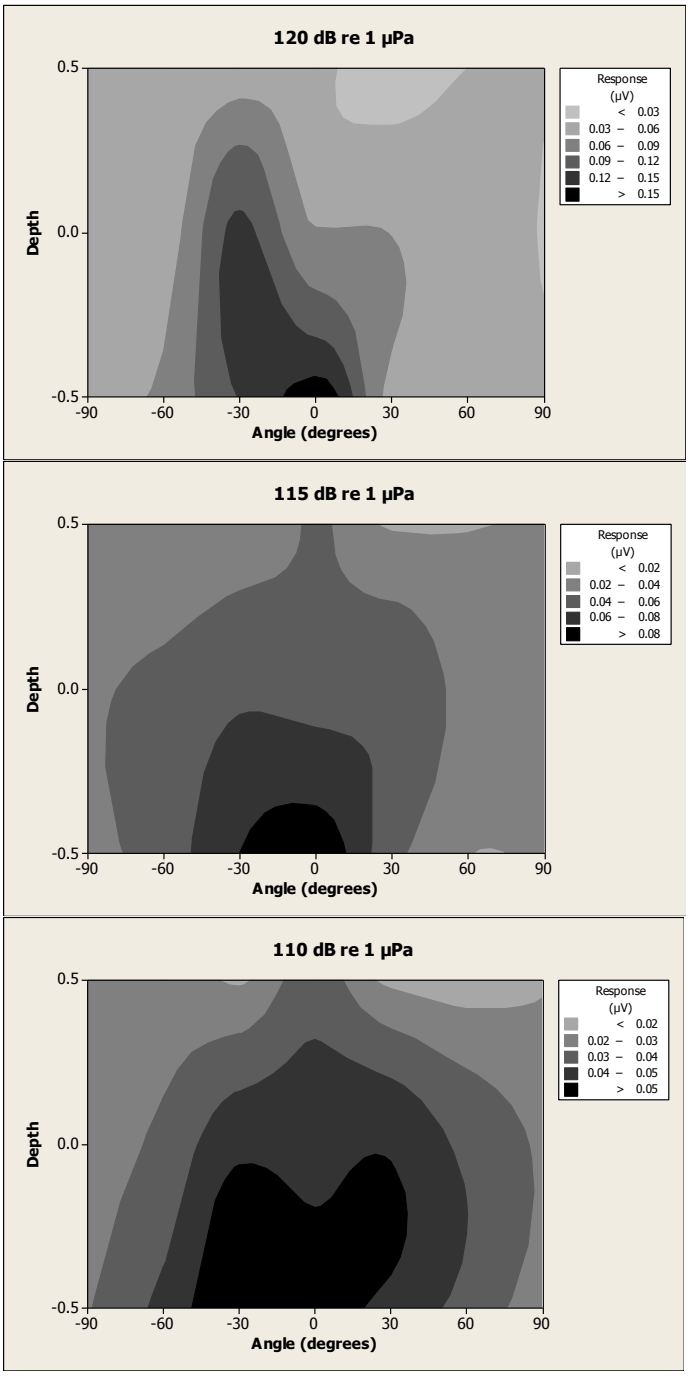

Figure 4.5
Contour plots for the Fourier transform outputs in µV for a signal of 22.5 kHz with decreasing amplitude from 120 dB to 110 dB (all re: 1 µPa) in 5 dB decrements. The subject's position was at 0m depth and 0˚

Table 4.4
The FFT Output amplitude (in μV) for output levels of 120 dB, 115 dB and 110 dB (re: 1μPa) for 38 kHz

120 dB	-90°	-60°	-30°	0°	30°	60°	90°
+0.5m	0.0423	0.0420	0.0463	0.0445	0.0303	0.0350	0.0193
0m	0.0350	0.0138	0.0722	0.0966	0.0563	0.0494	0.0148
-0.5m	0.0498	0.0575	0.0842	0.1595	0.1384	0.0695	0.0516

115 dB	-90°	-60°	-30°	0°	30°	60°	90°
+0.5m	0.0379	0.0341	0.0414	0.0375	0.0266	0.0095	0.0146
0m	0.0313	0.0165	0.0655	0.0694	0.0409	0.0372	0.0180
-0.5m	0.0435	0.0595	0.1165	0.1401	0.1157	0.0583	0.0236

110 dB	-90°	-60°	-30°	0°	30°	60°	90°
+0.5m	0.0346	0.0189	0.0282	0.0393	0.0296	0.0200	0.0087
0m	0.0229	0.0063	0.0497	0.0555	0.0329	0.0303	0.0037
-0.5m	0.0293	0.0383	0.0612	0.1062	0.0723	0.0441	0.0284

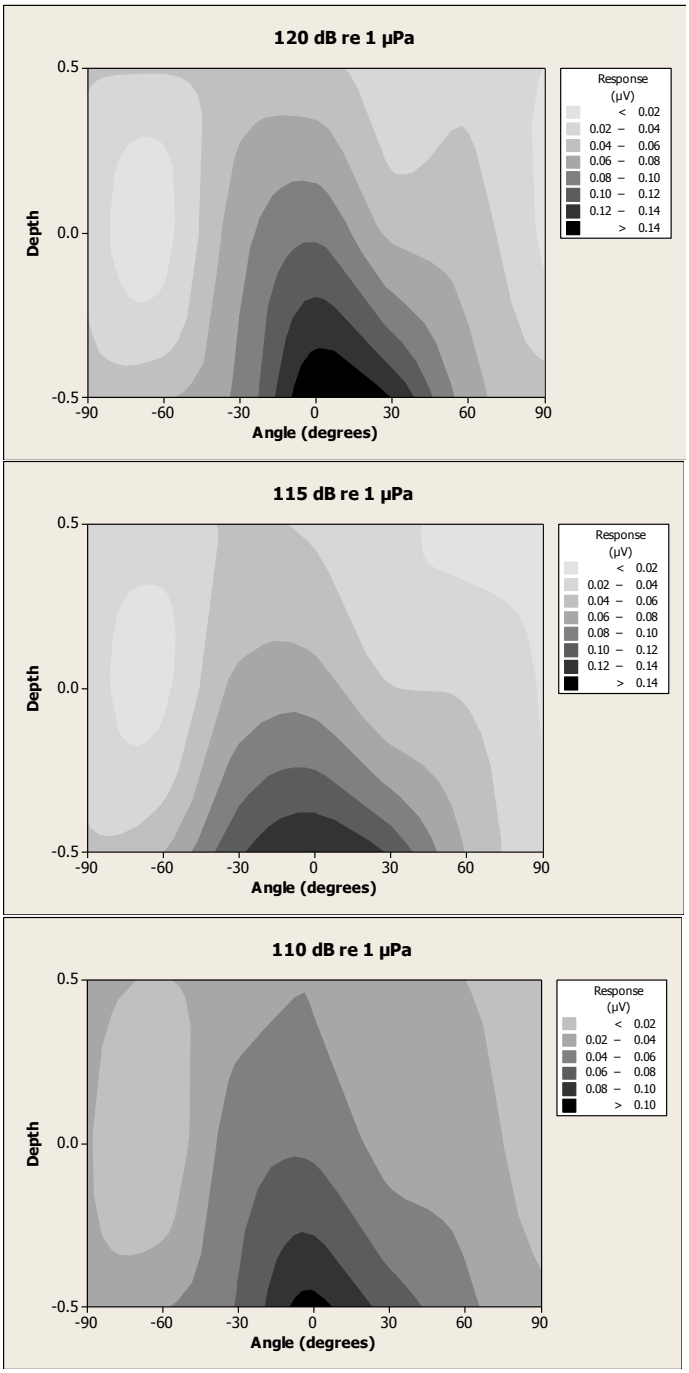

Figure 4.6
Contour plots for the Fourier transform outputs in µV for a signal of 38 kHz with decreasing amplitude from 120 dB to 110 dB (all re: 1 µPa) in 5 dB decrements. The subject's position was at 0m depth and 0˚

Table 4.5
The FFT Output amplitude (in μV) for output levels of 120 dB, 115 dB and 110 dB (re: 1μPa) for broadband clicks

120 dB	-90°	-60°	-30°	0°	30°	60°	90°
+0.5m	0.0751	0.1092	0.0865	0.1811	0.0767	0.1110	0.0292
0m	0.1241	0.0881	0.2321	0.1515	0.2077	0.1963	0.0866
-0.5m	0.1175	0.1493	0.2114	0.1829	0.1697	0.1302	0.1564

115 dB	-90°	-60°	-30°	0°	30°	60°	90°
+0.5m	0.0527	0.0694	0.0583	0.1492	0.0599	0.0719	0.0279
0m	0.1061	0.0916	0.1893	0.1267	0.1734	0.1535	0.0753
-0.5m	0.1085	0.1085	0.1839	0.1964	0.1533	0.1047	0.1498

110 dB	-90°	-60°	-30°	0°	30°	60°	90°
+0.5m	0.0252	0.0505	0.0375	0.1143	0.0454	0.0708	0.0157
0m	0.0808	0.0529	0.1292	0.1013	0.1294	0.1222	0.0682
-0.5m	0.0900	0.1039	0.1406	0.2153	0.0965	0.0912	0.1294

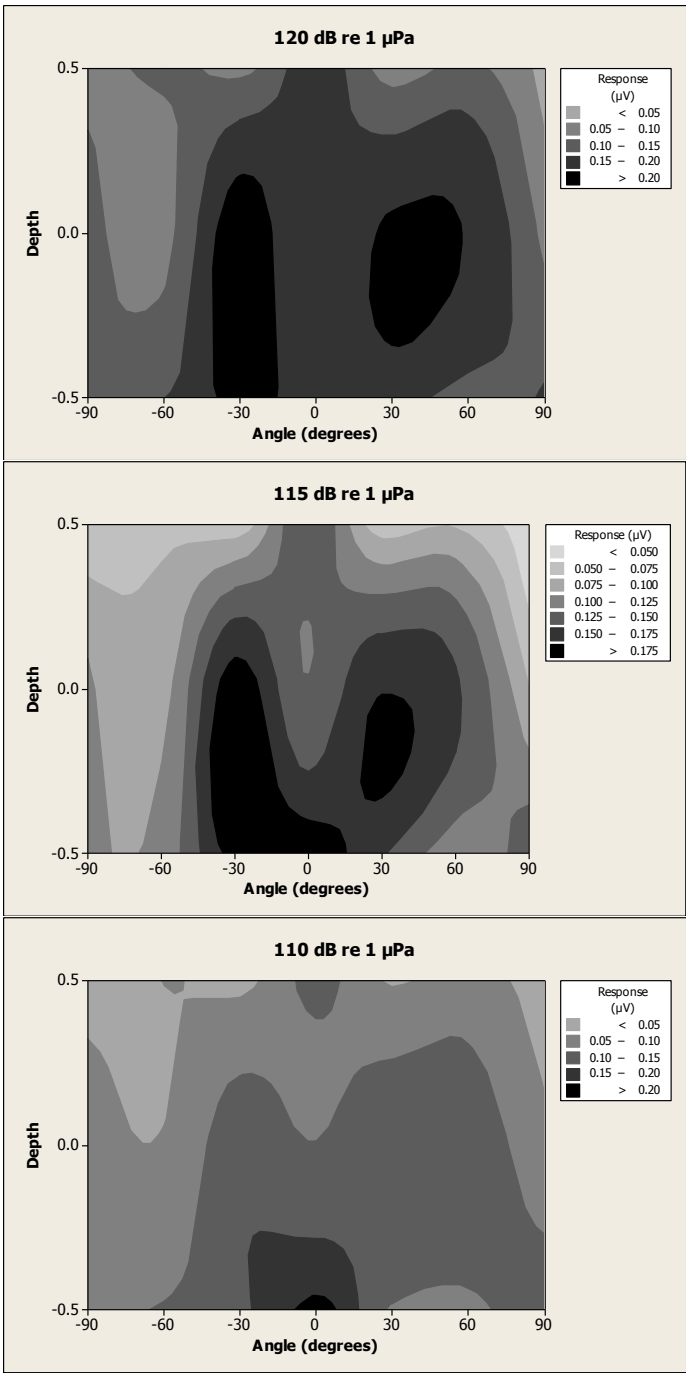

Figure 4.7
Contour plots for the Fourier transform outputs in µV for a broadband click signal with decreasing amplitude from 120 dB to 110 dB (all re: 1 µPa) in 5 dB decrements. The subject's position was at 0m depth and 0˚

Since all experiments were performed in an open bay pen, noise was a consideration. When the test subject was stationed in the hoop/biteplate setup his right ear was directed towards a coral reef environment that is approximately 25 m away from pen 3 and his left ear was facing an open channel area that sees quite a lot of small craft traffic. Therefore, the noise field around the test subject could be quite different especially at the 90˚ angles. In order to deal with possible noise field inconsistencies of a natural environment, Au and Moore (1984) chose to play white noise in relation to the sound signal in order to perform a masked hearing threshold. In their experiments either the noise level would be lessened in relation to an unchanging signal level or they would vary the signal level in relation to a consistent noise level. In addition, their transducer was at a distance of 3.5m (whereas this HRTF setup held the transducer 1.5 m from the hoop). The presence of this masking noise could have improved the odds of less spatial differences in the noise field but it could not address any temporal disturbances in the noise field. It was decided not to add another noise source into our setup, deciding that the open pen area was more similar to a natural bay environment than a uniform noise field, which made our measurements also masked measurements. This experiment used a maximum depth of 1.5 m for the transducer and 1 m for the test subject. According to Urick (1984) the noise field at these depths is usually dominated by rain and wind noise, which would factor into the hearing of any cetacean behavior that occurs close to the surface of the water such as breathing. The sound field was monitored and is shown for the position of the test subject in Figure 4.8. Testing sessions were suspended or cancelled for wind, significant rain and boat noise.

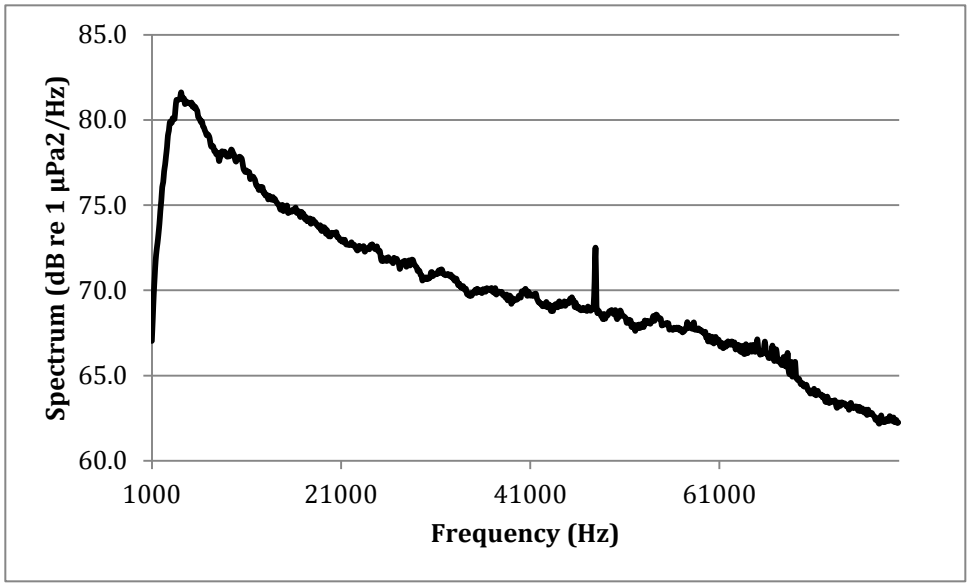

Figure 4.8
Power spectrum levels in dB re 1 µPa2/Hz for background noise measured with a 200 kHz sampling rate for 1-79.9 kHz. This measurement was taken at the location where the test subject would be stationed, underwater in a hoop, for hearing measurements.

Discussion

A head-related transfer function can provide spectral cues that can be used for sound localization. A traditional HRTF is a sound-to-sound comparison and measures how the head/neck/torso and external ear structures affect the properties of the incoming sound. In contrast, this study relied on the brain output from the brainstem/midbrain region. The data not only includes the HRTF but also the middle ear transfer function, the inner ear transfer function, and the neural transfer functions. These additional transfer functions make this study a sound-to-neural output comparison rather than the traditional sound-to-sound comparison. However, the neural output is related to the amplitude and frequency of the sound.

The brain's response to sound is a sigmoidal function dependent upon the amplitude and frequency of the sound (Supin et al., 2001). At high sound amplitudes, the neural output will reach saturation, and the firing frequency of the neurons, and the resulting voltage output, cannot increase to physiological constraints. As the sound levels decrease, the neural output will decrease in a linear manner until the noise floor is reached. The noise floor represents the level of brain activity from which auditory outputs cannot be distinguished from other neural output (or the noise floor of the

recording system). The response to frequency is dictated by the ear's tuning to that frequency or frequency band. Therefore, depending on the frequency of the stimulus, the ear could be saturated (like some of the click locations), or towards the top of the linear portion near saturation (like 22.5 and 38 kHz) and some may be towards the bottom of the linear portion near the noise floor (such as some of the 5.6 kHz locations). Regardless, the value in this transfer function is in the intra-frequency comparisons and comparisons between signal types as viewed in the contour plots.

Could these spectral dissimilarities between amplitudes and between signal types be enough to designate location? The differences between the responses for 120 dB and 110 dB for 5.6 kHz is quite striking; maximal levels moving from the midline to more lateral positions as amplitude decreases. 22.5 kHz also sees a change between 120 dB and 110 dB having a widening of sensitivity at the lower amplitude. The responses at 38 kHz are interesting in its uniformity in responses between amplitudes. The response to the broadband clicks change markedly from sensitivity at ±30º to greatest sensitivity from below at 0º. If scientific equipment, measuring far-field evoked responses, can quantify positional differences in responses, it is an almost certainty that the dolphin's central nervous system (CNS) can detect and process these differences between locations. In fact, using evoked potential methodology could be very advantageous in quantifying spectral differences. These measurements represent the central nervous system's output and if there is a measureable difference in a far-field CNS electrophysiological experiment it would seem reasonable that there was a lot more detailed information making its way into the CNS that was not quantified including the contralateral and ipsilateral contributions to the output. In fact, Popov et al. (2003) used AEP measurements to explore ipsilateral and contralateral hearing thresholds to sound from different locations in the horizontal plane, and found a measureable difference in the response between the ears. Since humans and other animals are known to exploit binaural differences for directional cues, it would be interesting to explore the HRTF from each ear in response to sounds from changing azimuth, range and elevation.

The results of this HRTF experiment indicate that spectral cues arising from the sound interacting with the head anatomy of the subject were quantifiable. Because variation was observed not only between responses to different signal types but also

between amplitudes of the same signal type, the assumption is that the dolphin's central nervous system can as well. Because this study included only one subject the numbers should be interpreted with caution. However, it is proposed that the comparison between response amplitudes as well as between response patterns to the different signal types is robust and can be used as an example of spectral shading done by the head anatomy of a bottlenose dolphin.

Chapter 5
Conclusions and Model Revision

The previous chapters have outlined studies that encompassed measurements all the way from an ecosystem level down to an organismal and even anatomical level. One cannot help but be struck by the variable and dynamic nature of these systems. Biology, because of the fundamental nature of living systems, often seems a messy and chaotic science. However, the worth of gaining empirical descriptions of the processes that allow the complex organizational attributes of life is invaluable.

Methodological Comparisons

It was very enlightening to peruse the literature and find a dearth of comparative studies that addressed fundamental methodological differences. There are a number of publications addressing the large differences between studies, such as between the thresholds results obtained through behavioral methods contrasted with those that used electrophysiological methods. But there were no studies addressing the differences that could arise through simpler methodological differences in basic threshold testing such as is routinely seen between groups of researchers.

This comparative study was a basic step in that it addressed three different orientations that have been or could be used to station an animal for hearing studies. It was very surprising that this study yielded no extreme differences between treatments. This finding, although comforting in its ease for comparisons within the literature, should be taken with cautious optimism. The study was done with the same level of rigor as the collection of any standard audiogram would naturally dictate. However, the study was done with a single, middle-aged, male bottlenose dolphin and broad sweeping conclusions are hardly warranted. The conclusions in this case should be taken with robust skepticism and urge others to do so as well. This being said, adherence to the scientific method dictates that there should be constant questioning of the validity of any findings, especially one's own.

Noise Considerations

It would take many studies over many decades in order to really quantify the noise field in Kāneʻohe Bay, Oʻahu, Hawaiʻi. There are many factors that produce and effect the distribution of noise, namely: physical, chemical and biological. Physical factors can be as obvious as wind and rain, or as subtle as the flow of water past a physical barrier such as a reef. Chemical considerations can be seen in the interaction of fresh water and seawater, dissolved organic matter, or even the types of salts within a water column. Biological factors come in the form of the plant and animal life interacting with each other as well as their physical surroundings. Put all of these factors together and the noise field is still incomplete. The variable nature of the noise field must be considered: temporal variability (from impulsive sources up to decadal fluctuations) as well as spatial variability (from near field to across ocean basins) plays large roles. There is no way for one person, or even one group of people, to address all of these considerations. It is left to individuals and groups to gather as much data and take as many readings of noise as possible. This approach will, over time, give the scientific community a much more complete picture of what could be considered the average noise for specific locations.

Noise is a particularly poignant issue for those participating in hearing research. Given that hearing levels are affected by ambient noise levels (Yost, 2006, Au and Hastings, 2008), the levels of noise present during hearing experiments must be addressed. Researchers are left with two decisions: build an artificial anechoic testing location, or gather data in a natural setting. One could argue that midway between these choices would be to use animals in captivity such as dolphins in tanks. With the pumps turned off, artificial sea tanks can be very quiet locations for testing. However, the need to understand hearing levels as they relate to an individual's natural, functional state would seem to be more biologically relevant.

Quantification of noise as it relates to spatial hearing tests is essential. Other studies of directional hearing have ignored the noise (Renaud and Popper, 1975), taken note of the noise (Schlundt et al., 2004) or tried to control for the noise by incorporating levels of white noise into their design (Au and Moore, 1984). Ignoring the noise, while convenient, does not eliminate its possible effects on the experimental outcome.

However, using white noise does not eliminate the effects that temporal variation in the ambient noise field make exert upon the hearing levels of the test subject at that point in time. Therefore, the most parsimonious route would seem to be to take note of the noise. Previous chapters have addressed the measurements performed in order to quantify the noise fields within the testing environment used for these dissertation studies. However, the intriguing nature of these findings is compelling.

Figure 5.1 are noise spectrum waveform for all 21 locations from which sound was played for the directional hearing and HRTF studies. In addition, the waveform in the black line is the noise spectrum at the location of the test subject for these same experiments. The noise within the testing location appears to be more of a pink noise (Yost, 2006) that decreases with increasing frequency.

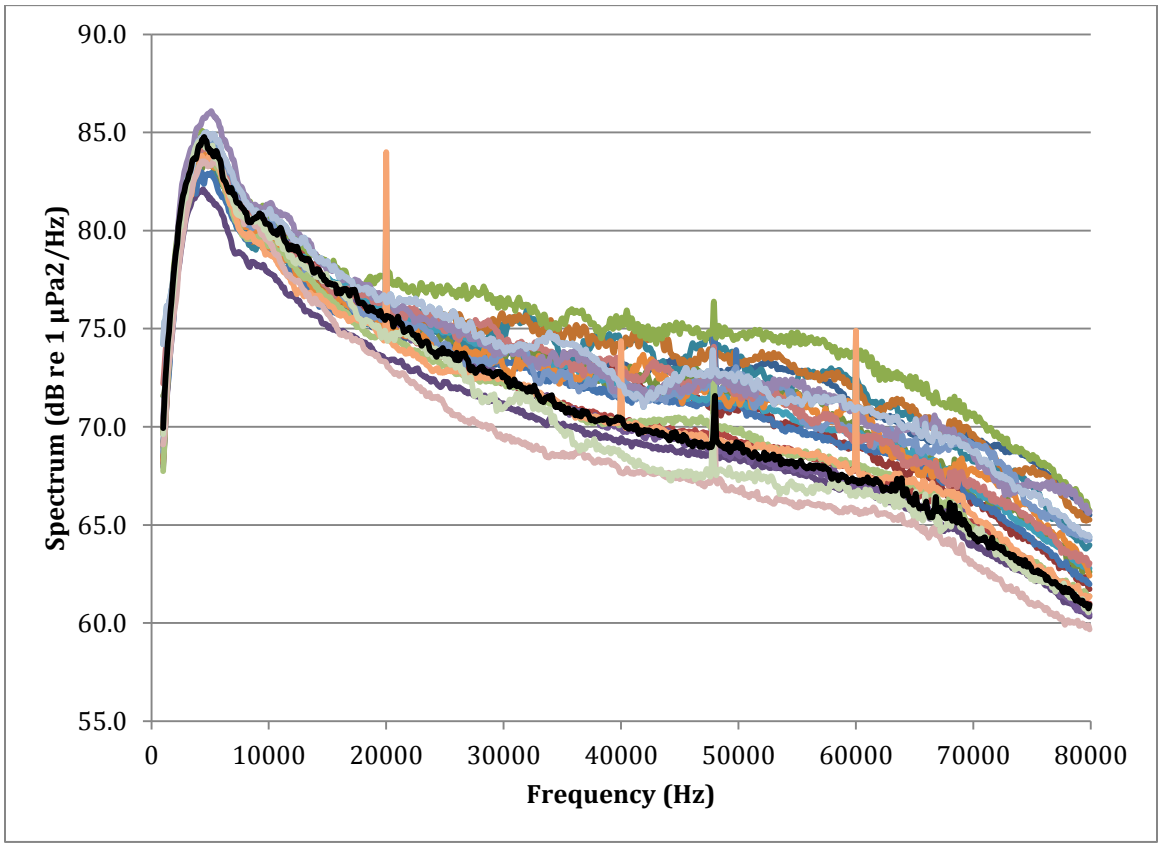

Figure 5.1
The power spectrum density (dB re 1 $\mu Pa^2/Hz$) of noise between 1-79.9 kHz for the 22 spatial locations (3 depths, 7 angles per depth – in color and one central location that corresponds to the location of the experimental subject's ears – the line in black) for the directional hearing and HRTF studies.

When the noise bands within the noise waveform were analyzed, some points (90° at 1.5m for the bands from 31-45.9 kHz and 30° at 0.5m for the bands from 31-45.9 kHz) appear to have hearing threshold values at or below the mean value of the noise measurements for those locations. The most obvious explanation is that temporal variation due to vocalizations from the inhabitants of the pens during the noise measurements increased the mean noise levels above what they were during actual threshold measurements. This explanation was very likely correct. However, does a signal have to be above the noise level in order to be heard? Physics would suggest that the energy level of the signal must be above that of the frequency band of noise that encompasses the signal, in order to be heard. The reasoning behind this explanation has to do with the physiology of hearing. The basilar membrane of the inner ear seems to be divided into bands that respond maximally to specific frequencies – areas called critical bands. How large a frequency band is contained within a critical band is dependent upon species (see Lemonds et al. 2011, 2012 and Yost, 2006) and is still under debate. Noise centered on and around a critical band would cause masking effects that increase with increasing noise levels.

The brain has the ability to pull signals out of the noise as is demonstrated by the cocktail party effect. The cocktail party effect is the curious ability to hear one's name being spoken amidst a noisy background (masking). This effect is thought to be possible because the location of the sound voice (speaker) is distinct within the background masking noise and that distinct location will allow differential spatial signals to be interpreted by the brain, which enables location of that source. That argument is a reasonable one from the point of view of the afferent pathways for sound. There could also be another explanation based on the efferent pathways for sound.

The efferent pathways of hearing, and of all other senses, are not well quantified or understood. Efferent pathways are modulatory pathways that alter the sensitivity of the receptors themselves, or the neural pathways from the receptors to the central nervous system. It is interesting to note the large amount of efferent innervation to the basilar membrane in both humans and cetaceans (Yost, 2006, Ketten, 2000, Wever et al., 1971). In fact, for both species, the number of outer hair cells is significantly greater than the number of inner hair cells, which is noteworthy considering that the outer hair cells are

mostly under the control of efferent fibers. The process of afferent pathway and efferent pathway interaction could be similar to that of noise reduction through greater bins sizes of fast Fourier transform (FFT) analysis (Au and Hastings, 2008). In the case of the basilar membrane, when the brain perceives a signal (of specific frequency(s)) or is "looking for" a signal with a certain frequency spectra, the FFT that the brain is trying to find could have an improved signal to noise ratio (S/N) through the amplification of the movement at that (those) sections of basilar membrane (through efferent modulation) and decrease motility on the rest of the basilar membrane (through efferent modulation as well as afferent processes such as lateral inhibition). If man-made sonar receivers have multiple sophisticated means of improving the S/N then it would seem parsimonious for nature to have selected for such receivers concurrent with the selection for biosonar.

There are many future directions for noise exploration in Kāneʻohe Bay. It would be useful to obtain a system that could measure noise levels at the same time as hearing tests so that the temporal and spatial nature of the noise was synchronized with the hearing or echolocation tests. It would also be fundamentally interesting to systematically explore the ability of the test subjects to perceive signals below the noise floor.

Dynamic Multi-Beam Receiver Model

The directional hearing results indicate that this bottlenose dolphins has a multi-beam receiving system. These beams are asymmetrical and signal dependent. The ecological ramifications of this distinct directional hearing could allow an individual to ensonify prey using clicks, listen to the echoes of the clicks while simultaneously monitoring its surround for tonal calls of conspecifics. This strategy would optimize foraging while maintaining social cohesion. In addition, the HRTF data reveal that the head anatomy affects the properties of the sound allowing for spectral cues that would allow for exceptional localization capabilities. These data clearing indicate that the receiving system of this animal is very complex.

The head anatomy of dolphins is very sophisticated. There are measurable asymmetries in the skulls and soft tissues of odontocetes (Mead, 1975). MacLeod et al. (2007) argued that this asymmetry was due to a respiratory constraint that separated the

trachea from the esophagus which allowed this group to swallow prey underwater without the risk of aspiration. They further argued that the level of asymmetry was related to the relative prey size captured and that the selective pressures for the asymmetry was due to that feeding constraint and not that of the constraint caused by biosonar production that had previously been argued by Cranford et al. (1996). It would seem to be prematurely exclusionary to rule out one selective pressure for another. Rarely is there only one selective pressure acting on a single anatomical structure let alone a complex, multi-tissue structure such as the head. It is proposed that there was another strong selective pressure at work on the cetacean skull – that of the need to conserve the function of the external ears. In humans, the outer ears do not just passively funnel sound towards the tympanic membrane. The complex bumps and grooves of the pinna and the shape and tissue properties of the external ear canal actually filter the sound before it ever even makes it to the tympanic membrane. Some frequencies are attenuated and some frequencies are actually amplified and in some cases the frequency components are altered (Yost, 2006). This alteration of the sound by the external ears demonstrates their functionality as non-linear filters. This nonlinearity can allow for spectral cues that the central nervous system can monitor in order to aid in the location of the incoming sound source. Considering the complex structure of a dolphin's head, it is parsimonious to conclude that the functionality of the external ears as non-linear filter which contribute spectral cues would have been preserved in order to maximize cues for localization.

The physiology of hearing is very sophisticated. Mammals have two ears. Each of these two ears has a distinct receptive field that is partially overlapping with the receptive field of the other. Therefore, each ear would account for at least one beam of the receiver. In addition, each ear can be manipulated in terms of sensitivity to amplitudes and frequencies. How can the sensitivity of the ear be changed? As previously mentioned, there is an amazing amount of efferent control of the ears. Of the four rows of hair cells, three of them are the outer hair cells which are embedded into the tectorial membrane, whereas the inner hair cells are not coupled to the tectorial membrane. Almost all innervation to the outer hair cells is efferent which can come from the auditory cortex or from lower brainstem or midbrain structures such as the inferior colliculus or olivary complexes (Yost, 2006). This "top-down" control is thought to act

on the outer hair cells to make them more or less rigid – their steriocillia more or less reactive to endolymph changes that will enhance or inhibit hyperpolarizations of these hair cells. If the membrane potential of these cells is changed, their connection to the tectorial membrane may be effected in a way to allow that membrane to oscillate more or less in response to sound. If the mechanics of the tectorial membrane were changed then the motion of the inner hair cells, which are mostly afferent pathways, could be affected. These changes could allow portions of the basilar membrane to become more or less sensitive. Since the basilar membrane is tonotopically arranged then this change in sensitivity would correspond to a change in sensitivity to certain frequencies or patterns of frequencies. Therefore, each ear can independently regulate its sensitivity to different frequencies or patterns of frequencies. This independent regulation could result in receptive beams from each ear being sensitive to different signal types and being able to change what signal types they are sensitive too if the receiver has prior knowledge of the signal spectrum. Hence, these multi-beam receptors, through their physiology, are dynamic.

The dynamic, multi-beam receiver that these studies have shown is just that – dynamic. Sensory systems are redolent of dynamic variation. For example, vision, which demonstrates multiple pathways, can also change the location of focus depending on how the lens is stretched; a situation called accommodation. A recent study by Kloepper et al. (2012) demonstrated that the outgoing echolocation beam of a false killer whale (*Pseudorca crassidens*) could be changed in order to focus on a target at varying distances from the test subject. This acoustic "squinting" (Kloepper) is thought to maximize the acoustic energy on particular prey items and minimize echoic distractions. Kloepper et al. also suggests that this dynamic focus of the sonar beam allows for "cuing the animal to 'pay attention to' targets at a certain distance along its acoustic axis" (pg. 1310).

The dynamic multi-beam receiver model demonstrates the acoustic version of accommodation. If an subject is going to focus a sonar beam on a specific location in order to more efficiently ensonify prey items, it would make sense that the receiving system should also be able to be "focused" in a way that would maximize returning echoes and minimize the echoes from the surround. Why have sonar that can change its

focal point without a dynamic receiver that is capable of accommodating that focal point? The mechanism(s) that would allow accommodation are the same efferent pathways that can allow each ear to be dynamic receivers. These modulating pathways could differentially modify not only the sensitivity of the basilar membrane (or particular parts of the basilar membrane) but also the sensitivity of the nuclei or tracts in the brain for a more long-termed potentiation.

The results obtained for these studies are compelling. When you combine these findings with those of previous studies, the amalgam is complex yet remarkably logical. A simplistic hearing system would not be adaptive for a creature that uses sonar and lives in a multifaceted and noisy environment. Therefore, the use of an accommodating, dynamic, multi-beam receiver would make sense on all levels from anatomical up to ecological.

How accurate would it be to compare these results from one individual to the species as a whole as well as in contrast with other species? As always, a study with one test subject, although common in marine mammal science, should be taken with caution. There should be follow up experiments with other individuals of the same species, as well as for both genders with multiple age groups. This goal is an expensive and time consuming one, but one that could yield valuable information on the average level of variability and capability within a population.

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