COMPARATIVE MORPHOLOGY OF THE ODONTOCETE
MELON: FUNCTIONAL AND EVOLUTIONARY
INTERPRETATIONS

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ABSTRACT OF THE THESIS

Comparative Morphology of the Odontocete Melon:
Functional and Evolutionary Interpretations

by

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Master of Science in Biology
San Diego State University, 2005

Odontocetes are a lineage of marine mammals that descended from terrestrial artiodactyls and have acquired myriad adaptations for life in an aquatic environment. Among the traits supporting an aquatic existence is an acute sense and use of sound in the ocean. Toothed whales have developed the remarkable ability to echolocate. This study explored the anatomy and evolution of the melon, a structure presumably involved in the propagation of echolocation sounds. This is the first study to examine morphological changes in the melon across odontocete families through comparative anatomy and in an evolutionary context.

X-ray computed tomography (CT) was employed to quantify the size, shape, and geometric relationship of the melon to other forehead structures. The use of CT proved to be a valuable tool to quantify melon characteristics. In addition, CT accurately captured structure in postmortem specimens. Chapter 2 compares CT scans of a live bottlenose dolphin (Tursiops truncatus) to a recently dead specimen and to frozen and thawed specimens; little disparity in internal structure and density of forehead was found across these treatment groups.

Considerable differences in the melon across odontocete lineages were found. The internal structure of the melon has a distinct pattern of density change; a higher density shell grades into a lower density core. This pattern of concentric low density layers is especially apparent in Kogia breviceps. The results of the melon morphology, combined with tissue composition data, suggest a specific function of the melon. Based on melon descriptions, predictions on sound propagation pathways are presented. For example, the melon in Ziphius cavirostris appears to be more homogeneous and without organized density layers; however, the lower density spermaceti organ and thick connective tissue theca might function to focus and propagate sound in these animals.

Inferences on the origin of the melon are presented. The results of the detailed descriptions of the soft anatomy (Chapter 3) provided the foundation for comparisons. This study presented preliminary conclusions on the evolution of the forehead structures associated with sound production/propagation; however, the morphological changes in the melon did not statistically correlate with the underlying bony structures. The presence of similar morphological structures in basal odontocetes compared to later diverging lineages suggests the melon evolved early in odontocete evolution and is a synapomorphy for odontocetes. Certain melon characters were inferred in two extinct lineages of odontocetes (Kentriodon and Eurhinodelphis) based on the results of character mapping.
Documenting morphological intricacies of odontocete echolocation proved critical to understanding the functional significance and evolutionary history of the system. The results are also important for understanding possible key innovations that lead to diversification into unexploited habitats. The sophisticated nose in odontocetes evolved in a relatively short period of time (~35mya). The superior propagation of sound in water compared to land potentially drove the radiation of odontocetes and allowed them to inhabit the light deficient oceans. Further modifications in the soft anatomy present in extant species suggest that the animals continued to adapt to the different acoustic environments of the ocean.
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CHAPTER 1

INTRODUCTION

The entirety of an organic being forms a coordinated whole, a unique and closed system, in which the parts mutually correspond and work together in the same specific action through a reciprocal relationship. None of these parts can change without the others changing as well. Consequently, each of them, taken separately, points to and reveals all the others.

– Cuvier

Odontocetes (toothed whales) are a lineage of marine mammals that descended from terrestrial artiodactyls and have acquired myriad adaptations for life in an aquatic environment (Gingerich, 2005). Among the traits supporting an aquatic existence is an acute sense and use of sound in the ocean. Toothed whales have developed the remarkable ability to echolocate. This study describes the morphology of the odontocete melon; a structure presumably involved in the propagation of echolocation sounds. The results provide further insight into the function and evolution of echolocation in odontocetes.

Echolocation is the process of emitting sounds and using the information contained in the returning echoes to sense the surrounding environment. This active system allows echolocating animals to forage, avoid obstacles, and orient in the absence of light. Echolocation was first documented in experiments with insect eating bats (Griffin and Galambos, 1941). The first evidence for dolphin echolocation came from observations of dolphins in and around nets during capture operations (McBride, 1956). Experiments with captive odontocetes have characterized echolocation as a brief and directional sound (echolocation clicks) emitted from the forehead to identify or track objects and make fine discriminations.
Acoustic tags placed on wild odontocetes have recorded high frequency clicks and echoes from prey during diving (Johnson et al., 2004).

The evolution of echolocation in odontocetes is likely a key innovation associated with their diversification into previously unexploited niches, namely light deficient habitats (Hunter, 1998). The fossil record supports this hypothesis; there is a radiation of odontocetes early in their history, Eocene/Oligocene boundary (~34-35 Ma), compared to their sister taxa, mysticetes (Fordyce, 2002). Furthermore, a suite of morphological modifications associated with echolocation are also present early in the evolution of odontocetes.

Morphological modifications for underwater sound reception and production provide important clues to the evolution of this system. The fossil record of cetaceans extends back over 53 Ma years (Gingerich, 2005). The earliest cetaceans were small amphibious species that lived in fresh and brackish waters in the warm subtropical Tethys seaway, between Eurasia, India, and Africa. The two extant groups within Cetacea, mysticetes and odontocetes, diverged in the late Eocene (35 Ma) (Gingerich, 2005).

The excellent preservation of the bony ear complex in fossil cetaceans enables an understanding of transitions in sound reception. Modifications in the ear that influenced sound reception mechanisms occurred early in cetacean evolution. An intermediate morphological stage for hearing capabilities on land and in the water is found in pakicetids. This morphology was lost in remingtonocetids and protocetids and replaced by a sound reception mechanism similar to that in modern toothed whales (Nummela et al., 2004). Further modification in the middle ear enabled mysticetes and odontocetes to increase their frequency range of hearing (Fleischer, 1978). The adaptations to the cochlea for high frequency
hearing capabilities occurred in Squalodontoidae during the Oligocene, potentially indicative of echolocation (Fleischer, 1976).

Morphology associated with sound production/propagation will most likely provide important transitions associated with echolocation. The structures associated with sound production/propagation are mainly soft tissue structures and, therefore difficult to trace in the fossil record.

The anatomy of sound production/propagation is a well investigated topic in living odontocetes, despite the limited amount of information in the fossil record. It is generally accepted that click generation begins by action of the palatopharyngeal muscle complex, as it forces the larynx up into the inferior bony nares and pressurizes the air in the bony nasal passages. The pressurized air passes through lips formed by a narrow slit in the spiracular cavity, causing vibrations in adjacent ellipsoid fat bodies. These vibrations are reflected forward by the skull and air sacs functioning as acoustic mirrors. The sound vibrations propagate through the melon anteriorly and emerge into the environment as a click. The melon (homologous to the junk in a sperm whale [Cranford, 1999]) is a fat and connective tissue structure that apparently functions to focus sound and decrease acoustic attenuation at the tissue-water boundary (Cranford and Amundin, 2004).

Investigations of the sound production anatomy combined with physiological experiments have identified five functional components in the odontocete forehead (Mead, 1975; Heyning, 1989; Cranford, 1992; Au, 1993; Cranford, 1996; Cranford et al., 1997; Cranford et al., 2000): 1) nervous system (command center), 2) respiratory system (power supply), 3) nasophonation system (signal generators), 4) nasal musculature (signal modifiers), and 5) the melon and associated structures (signal propagators).
Marino (2004) investigated morphological changes associated with the first component, specifically the evolution of brain size in cetaceans. The results show that the odontocete brain increased significantly in two critical phases: at the origin of odontocetes near the Eocene-Oligocene boundary (~35 Ma) and the origin of Delphinoidea (Delphinidae, Monodontidae, and Phocoenidae) 15 Ma. One hypothesis for the first increase in odontocete brain size is related to processing of echolocation sounds (Marino, 2004).

Modifications to the second anatomical component (respiratory system), specifically the bony nasal passages, are well documented in both fossil and extant cetaceans. The posterior movement of the nasal passages is associated with the telescoping of the cetacean skull; specific to odontocetes is the vertical position of the bony narial openings and contribution of the palatine bone to the bony nasal passages (Miller, 1923).

The third component (signal generator) is composed of soft tissues imbedded in the forehead. Comparative anatomical studies using X-ray computed tomography of the signal generators (bursae complexes) showed the variation across odontocetes, reported precise geometric locations, and identified two basic anatomic configurations: 1) unilateral configuration or “one sound source” found in sperm whales (Physeteridae, Kogiidae, and Ziphiidae); and 2) bilateral configuration or “two sound sources” in all other odontocetes, with a propensity toward directional asymmetry (right larger than the left) (Cranford, 1992; Cranford, 1996).

Nasal musculature (the fourth component) associated with the nasal region has been well described in extant odontocetes (Mead, 1975; Heyning, 1989); The morphology is difficult to determine in extinct lineages.
Modeling efforts simulated signal propagation (the fifth component) in the head of a dolphin and concluded that the skull, air sacs, and melon all play roles in sound propagation (Aroyan et al., 1992). Debate exists about the exact contribution of these auxiliary structures, but the morphology of skull, air sacs, and melon might help tease apart the functions.

Modifications to the cetacean skull are well documented. The presence of a spiracular plate in odontocetes is diagnostic of the presence of air sacs; therefore, echolocation capabilities are probable in even the earliest cetaceans (Barnes, 2000). Variation in the air sacs identified both interspecific and intraspecific differences (Schenkkan, 1973).

The melon gives odontocetes the distinct bulbous forehead; however, because of the fatty composition of the melon, dissections lose geometric and structural information, necessary to model sound propagation accurately. Previous studies investigating tissue properties of the melon are also valuable for understanding function. Chemical analysis of the melon identifies unique lipid compositions, potentially important for signal propagation (Varanasi and Malins, 1972; Litchfield et al., 1973; Litchfield and Greenberg, 1974; Litchfield et al., 1975; Koopman et al., 2003). Acoustic properties, namely sound velocity, characterize the melon tissues as an efficient medium to propagate sound waves in water without losing acoustic energy at the tissue air boundary (Norris and Harvey, 1974; Litchfield et al., 1975; Litchfield et al., 1979; Goold and Clarke, 2000; Clarke, 2003).

Acoustic experiments placing hydrophones at different locations on the surface of the forehead of an echolocating porpoise identified differences in acoustic signal dependent on location (Au et al., 2004). The results suggest that the tissues of the forehead will influence sound propagation. These studies provided functional descriptions of the melon; however function in relationship to structure has yet to be investigated. To combine structure with
function, this study quantitatively defines the melon in odontocetes to determine geometric information.

In addition to functional conclusions, structural and geometric information of the melon is important for reconstructing evolutionary history. Heyning and Mead (1990) suggest that the melon is an exaptation for echolocation and the melon is present in mysticetes, contrary to previous hypotheses that the melon is a synapomorphy for odontocetes. Defining the melon and reconstructing morphological transitions will help determine its origin and provide further insight into the evolution of echolocation.

**Benefits of Computed Tomography Imaging**

X-ray Computed Tomography (CT) was employed to quantify the size and shape of the melon, and characterize the geometric relationship of the melon to other forehead structures. The benefits of CT are well demonstrated in the medical field. As medical technology became more readily available financially and logistically, CT penetrated the worlds of paleontology, comparative morphology, and physiology. The use of CT is attractive to these fields because of the resulting high resolution images maintained in an X, Y, and Z coordinate system. With enhanced abilities to explore morphology, paleontologists can peer into delicate and rare specimens without damage; morphologists can interpret complex biological forms without losing information on the relationship to other components; and physiologists can examine *in vivo* anatomy and physiology. The use of CT in this study proved to be a valuable tool to quantify anatomical structures and to describe anatomy using postmortem material.

To produce a CT scan, an x-ray source emits a series of beams that penetrate the specimen. The attenuation of the beam is dependent on the density of electrons within the
specimen and it is picked up by a series of detectors across from the source. CT scans, like any digital image, are composed of a collection of picture elements or pixels. The pixel size or the resolution is determined by the size of the field of view. For example, if the fixed number of pixels covers a large area, then spatial resolution is limited. To construct a 3D image of the specimen, the series of scans of a given thickness are compiled.

The resulting image of the entire specimen is composed of voxels (3D picture elements) determined by the thickness of each slice, field of view, and the number of pixel elements. Each voxel has an average electron density that is adjusted to the Hounsfield scale (a calibrated measure of electron density) making the densities directly comparable. Not only is an image produced, but voxels represent a constellation of points in an X, Y, and Z coordinate system.

As a function of the X, Y, and Z coordinate system, CT scans provide an automatic scaling factor. This allows animals, big and small, to be put in the same size reference allowing ease of comparative analyses and identification of homologous structures. The maintained geometric and spatial relationships allow novel visualizations. For example, isolated soft tissue structures can be viewed with or without other soft tissue to understand its relationship to the bones.

Another benefit to CT scanning is that the images can be archived and retrieved for later analysis, as with any remote imaging technique. This non-invasive technique addresses the concern of access to and destruction of rare specimens.
OBJECTIVES

This study uses X-ray Computed Tomography as a method to describe and compare the anatomy of the odontocete melon across a spectrum of taxa. The first chapter investigates the validity of using CT to study postmortem morphology and extract tissue properties. The morphology of the odontocete melon is compared among CT scans of live and dead bottlenose dolphins. The results establish protocols for incorporating CT into morphological studies.

In the second chapter, the morphology of the melon is described and compared across odontocete families. The results offer the first quantitative descriptions of the melon in the context of the forehead anatomy. The comparative aspect of the study broadens the understanding of the complex echolocation system in odontocetes. The third chapter traces morphological characters associated with sound production/propagation onto several phylogenies to infer the transitions and origins of echolocation structures.
CHAPTER 2

DEAD TISSUE, DEAD ISSUE? EVALUATION OF MORPHOLOGY AND TISSUE PROPERTIES FROM COMPUTED TOMOGRAPHY (CT) SCANS OF LIVE, RECENTLY DEAD, AND POSTMORTEM THAWED HEADS OF THE BOTTLENOSE DOLPHIN

(TURSIOPS TRUNCATUS)

INTRODUCTION

Questions about how organisms function and interact with their environment have a long history of investigation in medical, functional morphology, physiology, paleontology, and more recently conservation research. Accurately capturing geometric information and tissue properties to model functioning systems are the primary objectives in these studies, and subsequently catalyzed the development of new techniques and technologies. For example, the application and benefits of X-ray Computed Tomography (CT) in clinical medicine are well established. It is a remote imaging tool used to examine anatomy represented by different tissue densities. As the use of medical imaging technology became ubiquitous and easily accessible, it reinvigorated many fields of study that have benefited from new methods of capturing structural and physiological information. The present study compares postmortem tissue to live tissue in bottlenose dolphins in an effort to understand postmortem effects as shown by CT scans.
X-ray CT, and most remote imaging techniques, allows morphologists to study complex forms without losing the geometric relationships between components (Cranford, 1996; Marcucci et al., 2001; Marino et al., 2003; Summers et al., 2004). X-ray CT has also allowed paleontologists to examine internal structures in delicate or rare specimens without the need to destroy the material in the process (Jungers and Minns, 1979; Conroy et al., 1990; Rogers, 1998; Rowe et al., 2001; Spoor et al., 2002; Kearney et al., 2005). Sound propagation models in biological systems can be built upon CT data extracted from tissue morphology (Aroyan et al., 1992; Terheyden et al., 2000; Aroyan, 2001). New research horizons using digital imaging techniques are still being discovered.

Although capturing CT data from live animals is feasible in human studies, new challenges arise when attempting to CT live whales. Cooperation of the animal and limited access to specimens is of concern. Only one study has overcome these challenges (Houser et al., 2004). Many investigators have employed CT on recently dead, frozen, and thawed specimens (Cranford, 1996; Maisano et al., 2002; Carpenter et al., 2004). Before the present study, there were no data to help sort out whether CT scans on postmortem specimens yield an accurate representation of live tissue.

The acoustic anatomy of odontocetes is a system in which this study is interested in looking for postmortem changes using CT. The soft tissue structures associated with the acoustic system in toothed whales are not easily described by traditional dissection alone because of the tendency for soft structures to deform as the systems are taken apart. For example, the melon (homologous to the junk in a sperm whale [Cranford, 1999]) is a fat and connective tissue organ that apparently functions to focus sound and match the impedance differences between tissue and water (Norris 1969; Cranford and Amundin, 2004). Computed
tomography provides a means to characterize the geometry and density topography within this unique organ, and has the potential to allow us to predict sound propagation pathways within the head. Because of the limited number of specimens and the challenges of studying live animals, researchers often rely on stranded animals to investigate the anatomy in toothed whales. Stranded specimens are usually transported to laboratories and sometimes frozen until scans, experiments, or observations can be made.

The main objectives of this study are to 1) evaluate the use of computed tomography for comparing structural information gathered from live and dead bottlenose dolphins, 2) record the postmortem changes in tissue properties, such as density and sound speed, in blubber, melon, muscle, and connective tissue, and 3) make general recommendations for using CT to study postmortem specimens.

**MATERIALS AND METHODS**

The heads of four bottlenose dolphins (*Tursiops truncatus*) were subjected to CT. The specimens were divided into three treatment groups: living (LIVE), recently dead (FRESH), and frozen then thawed (THAWED). The specimens are all adult bottlenose dolphins of similar size (Table 1). The heads of the two THAWED specimens (THAWED A and THAWED B) died of natural causes and were acquired from the Navy Marine Mammal Program. The heads were separated from the body with a ban saw and frozen at 4°C to retard tissue decomposition. In preparation for CT scanning, the heads were thawed in a water bath at room temperature to promote rapid and even thawing of the specimens. The FRESH specimen stranded and then died in San Diego County. The head was removed from the body and CT scanned four hours after death. A trained dolphin (LIVE)
Table 1. Specimen Life History Information

<table>
<thead>
<tr>
<th>Group</th>
<th>Name</th>
<th>Date of Death</th>
<th>Location</th>
<th>Age</th>
<th>Sex</th>
<th>Body Length (cm)</th>
<th>Weight (kg)</th>
</tr>
</thead>
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<tr>
<td>THAWED</td>
<td>Tutr790</td>
<td>3/11/1985</td>
<td>San Diego, CA</td>
<td>Adult</td>
<td>Male</td>
<td>238.76</td>
<td>136</td>
</tr>
<tr>
<td>THAWED</td>
<td>Tutr567</td>
<td>5/26/1987</td>
<td>San Diego, CA</td>
<td>Adult</td>
<td>Male</td>
<td>238</td>
<td>153</td>
</tr>
<tr>
<td>FRESH</td>
<td>Tutr2082</td>
<td>4/24/2004</td>
<td>San Diego, CA</td>
<td>Adult</td>
<td>Female</td>
<td>278</td>
<td>162</td>
</tr>
<tr>
<td>LIVE</td>
<td>Tutr21030</td>
<td>Alive</td>
<td>San Diego, CA</td>
<td>Adult</td>
<td>Female</td>
<td>222</td>
<td>122</td>
</tr>
</tbody>
</table>
Data Acquisition

X-ray CT data were collected as a series of digital images, thin cross-sections of the specimen known as serial tomograms or scans. Each scan was comprised of a matrix of values (CT numbers) expressing linear attenuation of X-rays of the material at a specific geometric location within the specimen. The information was then stored in a format specific to each scanner.

The specimens THAWED A and THAWED B were subjected to X-ray CT at the University of California, San Francisco in 1987 after being frozen for one year. Serial tomograms were taken continuously from the tip of the rostrum to the occipital condyles without intervening spaces for THAWED A and THAWED B. The slice thickness varied depending on the interest of a particular region (Appendix A). From the tip of the rostrum to the anterior border of the nasal passages the slice thickness was 5 mm. In the adjacent series of scans, through the region of the nasal air sacs and the sound generation complexes, the X-ray beam narrowed to a thickness of 1.5 mm to record the greatest detail (Cranford, 1996). The scan thickness increased to 3 mm over the posterior portion of the head.

The FRESH specimen was scanned on a GE Light Speed Plus scanner at Thorton Hospital, University of California, San Diego. Axial scans were collected continuously every 2.5 mm, and the entire head was scanned.

Houser and colleagues (2004) conducted a CT scan on the LIVE dolphin for a separate study and generously provided access to a few of their scans. Details of their scanning protocol can be found in Houser et al. (2004), but, in brief, they used a scan.
thickness of 2.5 mm, and successive slices were not continuous so that only selected regions of the LIVE animal were scanned. Protocols in their study included consideration of safe limits for exposure to ionizing radiation and did not allow continuous scanning of the entire head. There is no such concern in study of postmortem specimens. The CT scanner parameters for all specimens are compiled and detailed in Appendix A.

Image formats from the CT scanners included both DICOM and GE9800. The DICOM images from the LIVE and FRESH specimens contain information on two fields (rescale slope and rescale intercept) that adjusted the values contained in the image to Hounsfield (H) units. The DICOM standard always rescales back to the actual H values, making the volumes directly comparable (Philcock, personal communication).

The GE9800 images were collected before the DICOM standard existed. Consequently, serial images from THAWED A and THAWED B needed to be adjusted to match the DICOM values. The GE9800 scanner added 1024 to all CT numbers in order to end up with positive integers. In order to rescale the images back to the Hounsfield units, 1024 was subtracted from all of the CT values in the GE9800 images.

The H values are a calibrated measure of electron density, which is equivalent to the change in density of 1 cubic cm of water 1 degree centigrade at standard temperature and pressure (STP). A previous study (Mull, 1984) established a calibration curve of physical density vs. CT number within the normal constraints of variation and drift. Therefore, it is possible to express the linear attenuation values in terms of physical density.

The compiled scans of each specimen are composed of calibrated H units. A three-dimensional map of the specimen is created based on the linear attenuation value within each
volumetric element or “voxel.” Voxel dimensions (pixel height, length, and width) are set by the scanning protocol prior to the actual scanning process (Appendix A).

The CT data were mapped onto a gray or color scale, thus allowing visualization of differences in tissue density by gray scale intensity or color differences. It should be noted that the decisions about display properties can have a significant effect on what can be seen or “interpreted” from CT images. For example, it is possible to change or adjust the image mapping scheme (color or gray scale) in order to emphasize particular features and diminish others (Fig. 1). The flexibility inherent in these image manipulations are a primary tool for morphologists.

Figure 1. Enhanced visualization of the CT images displayed on *Tursiops truncatus* (Tutr790). (A) All H displayed (-1024-2466) with gray scale map. (B) Adjusted scale of H displayed (-184-157) with color map. Notice the enhanced depiction of bone (white) in (A) and the highlighted distinction of boundaries in soft tissue in (B).
Image Processing

Data acquired for all specimens was processed using Analyze 5.0 /6.0, created by the Biomedical Imaging Resource at Mayo Clinic (Robb and Barillot, 1989; Robb et al., 1989; Robb, 1999). The individual scans were compiled and converted to AVW formats (a native Analyze format). The AVW format is a volumetric file. To visualize and analyze a volume of uniform elements, the voxels in each specimen were interpolated to 1.5 mm³.

Structural Comparisons

The morphological features of the forehead region were compared to evaluate the postmortem effects on structure. Hounsfield (H) values at the same location within the forehead of each specimen were compared using an image processing technique known as line profiling. Line profiles are created by choosing a line through a set of pixels in a 2-D image or “slice.” The H value is plotted against the location of each pixel along the chosen line (Fig. 2).

In order to make the line profiles comparable, the anatomic location for the line profiles were standardized. Consequently, a transverse slice through the posterior border of the antorbital notch in each specimen was selected (Fig. 3). Within this transverse slice, line profiles were traced across the width of each specimen at three locations: designated as UPPER, MIDDLE, and LOWER, as shown in Figure 3.

The resulting line profiles were plotted by group: UPPER, MIDDLE, and LOWER. Because the specimens differed slightly in size, the profiles were aligned at the mid-sagittal plane of each specimen. The resulting graph allows visual comparison of internal melon structure based on changes in H values across the same anatomic location. A linear regression was calculated for the UPPER, MIDDLE, and LOWER profiles between the
Figure 2. **Line profile image processing technique.** The graph on the right maps the changes in Hounsfield units (y-axis) along the line drawn through the image on the left with a distance plotted in mm on the x-axis. Position A is the beginning of the line profile at which the H is around 1000, then at position B and C the line crosses bone, which has a much higher density than the soft tissue as depicted in the peaks on the graph.
Figure 3. Location and transverse scans at the antorbital notch in all *Tursiops* specimens. (A) Location and orientation of transverse scan. (B) Thawed specimen (Tutr790). Labeled structures: me = melon; ma = mandibles; lano = left ant-orbital notch; raon = right ant-orbital notch. (C) Thawed specimen (Tutr567). (D) Fresh specimen (Tutr2084). The lines show the location of the three profiles analyzed. (E) Live specimen (Tutr20831).
specimens (LIVE, FRESH, and THAWED). A standard statistical package was used (SPSS, 2000).

The structure of the melon was also analyzed. In the same line profiles described above, the part of the profile containing the melon was compared in this analysis. The melon was defined by a change in slope of 6 2/3 along the gradient of the low density valley (Figs. 4-6 B). Within each segmented profile, the total distance across the melon and the change in H units on the left and right sides of the melon were calculated. Because specimens varied in size, the percent of the melon in relation to the head width was calculated. A linear regression was performed to compare the H values for just the melon to quantify the difference between the THAWED and FRESH and the LIVE specimens.

**Tissue Properties**

To compare the H values (density) in specific structures in the forehead, and determine postmortem changes in tissue properties, each specimen was processed individually using the same procedure. First, I established the best settings to highlight the tissue structures of interest using standard image processing techniques. After a range of H values for the various tissues was determined and they were used to isolate or segment organs and tissue structures.

The maximum, minimum, and average H values were recorded for the melon, cranial bones, muscle, blubber, and dense connective tissue. For the melon and cranium the mean H value from each slice containing these structures were computed and compared among the four specimens grouped by tissue type. The analysis included a subset of scans representing the same anatomic region for each specimen. In the analysis of the data, the two THAWED specimens were grouped and the variation within this group was calculated. Although the
Figure 4. Density change across the LOWER profile in all *Tursiops* specimens. The y-axis represents the change in H plotted over a specified distance across the section of the animal (mm) on the x-axis. (a) Drop in density at the blubber layer surrounding the heads, (b) connective tissue invades the sides of the melon, and (c) the melon. Right side of melon correlation $r^2 = 0.83$. Left side correlation $r^2 = 0.89$. 

Adjusted $r^2 = 0.85$
Figure 5. Density change across the MIDDLE profile in all *Tursiops* specimens. The y-axis represents the change in H plotted over a specified distance across the section of the animal (mm) on the x-axis. (a) blubber layer, (b) connective tissue layer, and (c) the melon. Right side of melon correlation $r^2 = 0.86$. Left side correlation $r^2 = 0.94$. 

Adjusted $r^2 = 0.93$
Figure 6. Density change across the UPPER profile in all *Tursiops* specimens. The y-axis represents the change in H plotted over a specified distance across the section of the animal (mm) on the x-axis. The connective tissue layer is absent and the blubber (a) grades directly into the melon. (b) Right side of melon correlation $r^2 = 0.92$. Left side correlation $r^2 = 0.96$. 
statistical power is low (1 degree of freedom), the mean H values of the three groups (THAWED, FRESH, and LIVE) for the melon, bones, muscle, blubber, and connective tissue were compared using an ANOVA.

The change in sound speed over time in different tissue types in the FRESH specimen was also measured. The difference in sound speed as a function of hours after death was measured and evaluated for postmortem effects. After the FRESH specimen was scanned, the head was cut longitudinally with a ban saw into left and right halves. The left half was frozen at -14°C and the right half was prepared immediately for sound speed measurements. Cubes of tissue approximately 2-3 cm³ were excised from the mid-sagittal surface exposed from the longitudinal cut (Fig. 7).

The speed of sound through each tissue sample was measured using a Krautkramer Branson USD10 Ultrasonic Digital Flaw Detector and two Krautkramer Branson longitudinal acoustic transducers (Alpha series, 10 MHz, 0.25 mm) attached to Mitutoyo digital calipers (model no. CD-8”CS). The Krautkramer velocimeter produced 10 MHz broadband pulses that were directed into the face of one side of each tissue cube and received at the other end yielding a transmission time. The calipers were used to determine the sample thickness, and sound speed was calculated by dividing the thickness by the transmission time. Prior to measuring the samples, the velocimeter was calibrated using room-temperature (22.5°C) distilled water, assuming a sound speed of 1490 m/s (Chen and Millero, 1977).

Sound speed was calculated for each tissue sample by averaging three trials along each axis (anterior-posterior, lateral and dorsal-ventral), assuming no significant difference
The first set of sound speed measurements were collected 7.5 hours after death. The measurements were repeated at 27, 100, 149, and 172 hours from death. In between sound speed measurements the samples were stored in zip lock bags at room temperature.

The frozen half of the head was removed from the freezer after 17 days and thawed at room temperature for 12 hours prior to sampling. Cubes of tissue, at approximately the same locations and of the same size as the fresh samples, were removed. The samples were measured using the same techniques described for the first half of the head. It was assumed
that time stopped during the freezing stage and resumed when the specimen was thawed. The samples were measured at 21, 46, 69, 93, and 120 hours from thawing.

The seven tissue samples were grouped into three tissue groups: melon (posterior melon core, middle melon core, anterior melon core, and anterior melon shell) connective tissue (connective tissue and blubber), and muscle (hyoid muscle). All data were statistically analyzed for normality of distribution and equal variance. The sound speed results for both the fresh and frozen tissue types were tested separately for significant differences as a result of time lapsed from death using an ANOVA and Bonferroni post hoc test. To identify significant differences between fresh and thawed samples, a two tailed $t$-test was used ($p = 0.05$) if the distribution for each tissue type was normal. The Wilcoxon test was used for the same purpose if the distribution was not normal.

**RESULTS**

In this section, the results of structural comparison are reported first. Then, both density and sound speed results are presented.

**Structural Comparisons**

The slumping of the dorsal and ventral regions of the superficial soft tissues in the postmortem specimens is most likely a result of disarticulating the head from the body (Fig. 3), allowing tissue masses to distort. Nevertheless, the structure of the melon and surrounding tissues were similar in the three line profiles analyzed: LOWER, MIDDLE, and UPPER (Figs. 4-6).

Comparisons between the LOWER profiles in each specimen revealed a similar drop in density at the blubber layer surrounding the heads, represented by the “valley” on the right
and left sides of each profile (denoted by “a” in Fig. 4). Deep to the skin and blubber layer, connective tissue invaded the sides of the melon, represented by the sharp peaks of higher density (denoted by “b” in Fig. 4). The low density valley in the center of all profiles is the melon (denoted by “c” in Fig. 4). In the regression analysis, comparing the FRESH and THAWED tissue to the LIVE, the FRESH and THAWED H values were highly correlated with the LIVE ($r^2 = 0.85$). The morphology within the melon, however, was conserved across all specimens; the right side has a steeper slope or change in H values than the left (Table 2).

**Table 2. Profile Lengths and Slopes**

<table>
<thead>
<tr>
<th>Profile</th>
<th>THAWED A</th>
<th>THAWED B</th>
<th>FRESH</th>
<th>LIVE</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Upper</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Melon width (mm)</td>
<td>124.5</td>
<td>108.5</td>
<td>115.5</td>
<td>97.5</td>
</tr>
<tr>
<td>% of profile</td>
<td>97</td>
<td>86</td>
<td>96</td>
<td>93</td>
</tr>
<tr>
<td>Left slope</td>
<td>-1.3</td>
<td>-1.3</td>
<td>-1.1</td>
<td>-1.6</td>
</tr>
<tr>
<td>Right slope</td>
<td>1.9</td>
<td>2.1</td>
<td>1</td>
<td>1.7</td>
</tr>
<tr>
<td><strong>Middle</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Melon width (mm)</td>
<td>111</td>
<td>111</td>
<td>106.5</td>
<td>127.5</td>
</tr>
<tr>
<td>% of profile</td>
<td>76</td>
<td>73</td>
<td>73</td>
<td>93</td>
</tr>
<tr>
<td>Left slope</td>
<td>-1.6</td>
<td>-1.6</td>
<td>-1.2</td>
<td>-1.7</td>
</tr>
<tr>
<td>Right slope</td>
<td>1.8</td>
<td>1.9</td>
<td>2.1</td>
<td>1.5</td>
</tr>
<tr>
<td><strong>Lower</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Melon width (mm)</td>
<td>108</td>
<td>108</td>
<td>102</td>
<td>117</td>
</tr>
<tr>
<td>% of profile</td>
<td>63</td>
<td>58</td>
<td>58</td>
<td>70</td>
</tr>
<tr>
<td>Left slope</td>
<td>-1.3</td>
<td>-1</td>
<td>-1.2</td>
<td>-1.1</td>
</tr>
<tr>
<td>Right slope</td>
<td>1.5</td>
<td>1.6</td>
<td>1.9</td>
<td>2.25</td>
</tr>
</tbody>
</table>

In each specimen, the MIDDLE line profile shows a decrease in H value on the lateral sides compared to the LOWER profiles, represented by the shallower gradient at the blubber layer (denoted by “a” in Fig. 5) and retreat of the peaks at the connective tissue layer (denoted by “b” in Fig. 5). In the valley representing the melon (denoted by “c” in Fig. 5) the
H values are less than the results of the LOWER profiles by approximately 10 H, showing a lower density core present in the center of the melon. The low density core creates steeper slopes on the right and left sides compared to the UPPER profiles (Fig. 5 and Table 2). The results from the LIVE specimen differed slightly from this pattern in that the right slope was only slightly steeper than the left. The overall similarities of H values in the FRESH and THAWED verse LIVE are reflected in the results of the regressions analysis ($r^2 = 0.92$). The internal structure of the melon was also very similar across all specimens ($r^2 = 0.94$ left side and $r^2 = 0.86$ right side).

A similar structure of the four heads can be seen in the UPPER line profiles ($r^2 = 0.71$). The connective tissue layer present in the MIDDLE and LOWER profiles of each forehead is absent in the UPPER line profile; instead, the blubber grades directly into the melon (denoted by “a” in Fig. 6). The lower density core of the melon is also obvious (denoted by “b” in Fig. 6). The asymmetry between the right and left side of the melon is less apparent in the UPPER line profiles compared to the MIDDLE and LOWER (Fig. 6 and Table 2).

**Tissue Property Analysis**

The H values (density) within the melon of all the specimens had a total range of 108 H units (–32 to –140). The comparison of the mean H value of the melon between the THAWED heads showed a difference of 8 H units. The difference between the pooled THAWED mean and the FRESH is 6 H units; the difference from the THAWED specimens and the LIVE is 9 H units (Tables 3 and 4). The results of the ANOVA show that there is no significant difference between the mean H values for the melon for THAWED, FRESH, and LIVE specimens ($p = 0.565$) (Fig. 8).
Figure 8. Box plot of Hounsfield values for melon tissue in all *Tursiops* specimens. The data in the bars are the mean H values from representative scans at the same anatomical landmarks (between the supra orbital process and a spot along the rostrum for the melon). The center vertical line marks the median of each group. The box edges are at the 24th and 75th percentiles and the whiskers are at the 95th and 5th percentiles. The dots represent the outliers.
The H values for a sub sample of the cranial bones (excluding the ears) ranged from 111 to 1,701 (1440 H units), a larger range than the melon but similar to known human bone density (Robb, 2001). The difference between the THAWED specimens was 19 H units. The FRESH scan differed from the THAWED mean by 142 H units. The LIVE scan differed by 41 H units from the mean of the THAWED scans (Tables 3 and 4). The results of the ANOVA show that there is no significant difference between the overall means ($p = 0.115$) (Fig. 9).

### Table 3. Summary of Hounsfield Units for *Tursiops*

<table>
<thead>
<tr>
<th>Structure</th>
<th>CT Value</th>
<th>THAWED A</th>
<th>THAWED B</th>
<th>FRESH</th>
<th>LIVE</th>
</tr>
</thead>
<tbody>
<tr>
<td>All</td>
<td>Max</td>
<td>2472</td>
<td>1834*</td>
<td>2873</td>
<td>1710*</td>
</tr>
<tr>
<td></td>
<td>Min</td>
<td>-1024</td>
<td>-1024</td>
<td>-1039</td>
<td>-1023</td>
</tr>
<tr>
<td>Bones</td>
<td>Max</td>
<td>1551.7</td>
<td>1542.3</td>
<td>1701.6</td>
<td>1504</td>
</tr>
<tr>
<td></td>
<td>Min</td>
<td>152</td>
<td>147</td>
<td>111.3</td>
<td>220.1</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>566.6 ± 62.1</td>
<td>547.3 ± 69.2</td>
<td>698.8 ± 78.4</td>
<td>607.8 ± 115.5</td>
</tr>
<tr>
<td>Melon</td>
<td>Max</td>
<td>-32.6</td>
<td>-60.2</td>
<td>-63.9</td>
<td>-51.1</td>
</tr>
<tr>
<td></td>
<td>Min</td>
<td>-127</td>
<td>-127.6</td>
<td>-123.8</td>
<td>-140.2</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>-81.3 ± 4.5</td>
<td>-89.151 ± 3.1</td>
<td>-91.4 ± 3.8</td>
<td>-94.8 ± 6</td>
</tr>
</tbody>
</table>

*Lower maximum value in THAWED B and LIVE because ears not included in the CT scan.

### Table 4. Difference in Mean H for Tissues in *Tursiops*

<table>
<thead>
<tr>
<th>Tissue</th>
<th>THAWED A vs. THAWED B</th>
<th>THAWED AB vs. FRESH</th>
<th>THAWED AB vs. LIVE</th>
<th>FRESH vs. LIVE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bones</td>
<td>19.2</td>
<td>131.5</td>
<td>41.3</td>
<td>90</td>
</tr>
<tr>
<td>Melon</td>
<td>8</td>
<td>10.4</td>
<td>13.8</td>
<td>3.4</td>
</tr>
<tr>
<td>Muscle</td>
<td>7</td>
<td>5.5</td>
<td>24</td>
<td>19</td>
</tr>
<tr>
<td>Blubber</td>
<td>14</td>
<td>1</td>
<td>6</td>
<td>4.4</td>
</tr>
<tr>
<td>Mandibular fat</td>
<td>1</td>
<td>4.5</td>
<td>25.5</td>
<td>30</td>
</tr>
</tbody>
</table>
Figure 9. Box plot of Hounsfield values for bone in all *Tursiops* specimens. The data in the bars are the mean H values from representative scans at the same anatomical landmarks (between the supra orbital process and a spot along the rostrum for the melon). The center vertical line marks the median of the each group. The box edges are at the 24th and 75th percentiles and the whiskers are at the 95th and 5th percentiles. The dots represent the outliers.
The H units for the sub-samples of the tongue (muscle) were similar in the postmortem specimens (FRESH and THAWED) but different from the LIVE dolphin. The difference in the mean H units for muscle in the THAWED specimens was 7 H units. The FRESH differed from the THAWED by 5.5 H units and the LIVE differed significantly by 24 H units (Tables 3 and 4).

The H for the sub-samples of the blubber differed in the two THAWED specimens, but THAWED A was consistent with the FRESH and the LIVE. The THAWED B had the most apparent external deformation (Fig. 3) possibly explaining the difference in the blubber value. The range of H values from the sub-samples of blubber in the THAWED A, FRESH, and LIVE was 6 H units (-43 to –49) (Tables 3 and 4).

The H values for the sub-samples of the mandibular fat were similar in the postmortem specimens but different from the LIVE dolphin. The difference in the mean H for mandibular fat in the THAWED specimens was 1 H unit. The FRESH differed from the both THAWED by 4.5 H, however the LIVE differed by 25.5 H units (Tables 3 and 4).

No significant difference was found in the sound speed for either the fresh or the thawed tissue sample groups in the time frame monitored; however, after 24 hours all tissue types showed a change in sound speed, and then remained constant until 120 hours, followed by either a drop or rise in sound speed (Fig. 10). The FRESH and THAWED melon samples increased in sound speed and then remained relatively constant until approximately 120 hours from death (Fig. 10 A and C). The blubber samples showed the most variation between the FRESH and thawed samples (Fig. 10 B and D). The muscle dropped in sound speed after 80 hours (Fig. 10 B and D). The connective tissue remained fairly constant in both the FRESH and THAWED until 100 hours after death (Fig. 10 B and D).
Figure 10. The change in sound speeds as a function of time from death for each treatment group. (A) Fresh melon samples. (B) Fresh connective tissue, blubber, and muscle samples. (C) Thawed melon samples. (D) Thawed connective tissue, blubber, and muscle samples. Zero represents time of death. For the thawed samples, we assumed that time stopped while the specimen was frozen and started again after the thawing process.
Because no significant difference ($p = 0.001$) was found in the sound speeds in the time frame analyzed, all the samples in the comparison between FRESH and LIVE tissues were included. The results of the sound speed analysis showed no apparent differences between the FRESH and THAWED groups for all tissue types ($p > 0.05$: Melon 0.729; Connective tissue 0.158; Muscle 0.239) (Fig. 11).

**DISCUSSION**

This study presents the first evaluation of using postmortem CT scans to monitor structural and physiological changes in acoustically relevant tissues and organs in *Tursiops truncatus*. Three conditions of the animals were compared: THAWED, FRESH, and LIVE. In this section, general recommendations for using postmortem material are presented. Structural, density, and sound speed comparisons suggested that postmortem material is a good representation of live tissues and organs. Based on these results, implications for examining viscoelastic properties are discussed. In addition, the use of postmortem material as input to simulate sound transmission in the dolphin head, and the potential to predict interactions of sound and live tissues are debated.

The structural comparison revealed a strong similarity among the specimens analyzed and suggests that postmortem CT scans are a reasonable proxy to describe and quantify living animal tissues. Although some minor external deformities can be introduced from the positioning of specimens or their disarticulated parts during freezing, there is a strong correlation between the line profiles (all $r^2 > 0.71$). This suggests that the geometry of the melon and its spatial relationships with other forehead structures remains intact, even in dead specimens. X-ray CT has been used extensively to capture intricate *in situ* morphologies without sacrificing structural integrity (Stern and Webb, 1993; Estes et al., 1998; Schroeder,
Figure 11. The change in sound speeds for three tissue types (muscle [triangle], blubber [square], melon [circle]). The filled in shapes represent measurements taken hours from death. The open shapes represent measurements taken after the specimen was frozen and thawed. Zero represents time of death. For the thawed samples, we assumed that time stopped while the specimen was frozen and started again after the thawing process. (Melon $p = 0.729$; Connective tissue $p = 0.158$; Muscle $p = 0.239$.)
2001), and the results of this study expand the potential to capture structure to non-human animals and postmortem specimens.

The treatment of the specimen prior to scanning will influence interpretative power as seen in the THAWED B specimen. The handling time before freezing of the specimen prior to scanning appears to be a primary factor. Future research is needed to identify the margin in which reliable results are produced.

Hounsfield (H) values derived from CT for melon and bone were highly conserved among the three treatment groups: LIVE, FRESH, and THAWED. The maximum difference in H values for all soft tissues was 19 H units between the LIVE and FRESH and THAWED tissue. The difference in one Hounsfield unit is equivalent to raising one cubic centimeter of water one degree Celsius, a sensitive meter. The relatively small differences found in tissues in the various treatments suggest that postmortem changes in density are minimal. Some of the differences recorded might be a result of temperature differences during the scanning process. The LIVE was presumably scanned at body temperature and the FRESH and THAWED at room temperature; therefore potentially accounting for a 6 H unit difference (Kreel and Bydder, 1979). The remaining margin of difference between the LIVE and FRESH, suggests minor alterations caused by the freezing and thawing process. Because water comprises 75% of most soft tissues, and the physical properties of the tissue are consequently dominated by its presence (Duck, 1990), thawed postmortem specimens must be kept from dehydration if reliable information is to be extracted from CT. As a general recommendation, specimens should be placed in sealed plastic bags to reduce the effects of dehydration if they are to be used for postmortem studies. The melon in odontocetes is
particularly sensitive because it contains specialized acoustic fats important for sound propagation.

Although the freezing and thawing process may influence other mechanical properties of tissues, the sound speed appears to remain constant. The results suggest that sound speed in melon, connective, blubber, and muscle tissue remain constant until about 120 hours from death. The non-significant effect on sound speed from the freezing and thawing process found in this study is consistent with the results of other studies. For example, ultrasonic sound speeds through frozen and thawed tissues were compared to fresh tissues, and no significant difference was reported for the human breast (Foster et al., 1984), myocardial tissues (Dent et al., 2000), and the mammalian tissues (Van der Steen et al., 1991). A slight change in all tissue types was found after 25 hours, and this should be investigated further with a larger sample size. The observed changes might be a result of the handling process and/or subsequent loss of fluid from the tissues. For example, the large variance in the blubber results of this study and Fitzgerald (1975) may be a result of the loss of fat molecules from the collagen fiber matrix. There is also the potential that significant changes happened in the first 6 hours after death before the first data points were collected for this study (Kremkau et al., 1981).

The physical properties of mammalian viscoelastic tissues are important for understanding functioning biological tissues; however, the tissue is not easily quantified in vivo. As a result, researchers have been forced to study the physical properties of tissues after death, fixation or freezing and thawing. Several physical properties of non-contractile soft tissues have been investigated as a function of time after death. Kremkau et al. (1981) found a decrease in ultrasonic sound speed in the human brain within the first 24 hours of death, but
then found sound speed to be constant thereafter. For the human lens and vitreous, Jansson and Kock (1962) found no change in ultrasonic sound speed 70 hours after death. There was also no change found for the tensile properties of the rabbit ligaments from 2 to 96 hours after death (Viidik et al., 1965; Viidik and Lewin, 1966). Investigation into the viscoelastic properties (i.e., shear compliance, shear modulus, and mechanical loss agent) of canine intervertebral discs, whale blubber, beef fat, and human bone, however, show a dramatic change within the first 12 hours of death (Fitzgerald, 1975; Fitzgerald and Fitzgerald, 1995).

Longitudinal wave velocity is determined by the bulk modulus, rigidity modulus, and density of the tissue (Duck, 1990). Although this study did not measure the elasticity of tissues, the combined consistency of density (Figs. 8 and 9) and sound speed (Fig. 10) indicate that the bulk modulus for these tissues were conserved through each treatment. Additional evidence to support this claim is that the mechanical properties of various connective tissues that were frozen and thawed were not found to be significantly different from fresh tissue (van Brocklin and Ellis, 1965; Woo et al., 1986; Quirinia and Viidik, 1991). In contrast, Krag and Andreassen (2003) found a 20% decrease in the tensile elastic modulus of the porcine eye lens. In a study that compared the elastic shear modulus and dynamic viscosity of fresh dog vocal fold tissues to thawed tissues that were frozen at different rates, showed no difference for tissues that were quick frozen but significant changes for slowly frozen tissues (Chan and Titze, 2003). Flash freezing although not investigated in this study might produce the most reliable results when working with frozen tissues.

Although live tissue offers the most conclusive results to any physiological question, the results of this study support the use of postmortem CT data to extract some tissue properties to derive physical information about the tissues. For example, the results have
implications for modeling the acoustic function of these tissues. The potential of extracting data from CT images of postmortem animals to estimate tissue properties for finite element models is promising. Not only can models simulate propagation of biologically produced sounds, but also the interactions with sounds produced in their environment. For example, the marine realm is being increasingly inundated with anthropogenic sources of sound and some adverse reactions are documented; however, distinguishing between behavior and physical impact of sound is currently unknown.

Implementing CT to extract and simulate acoustic properties of tissues raises a series of questions concerning the reliability and limitation of the technique, as with any innovative method. This study exposes the possibilities of using CT data for various research questions, and also addresses the limitations of using this application with postmortem material.
INTRODUCTION

Odontocetes (toothed whales) are a lineage of marine mammals that descended from terrestrial artiodactyls and have acquired myriad adaptations for life in an aquatic environment (Gingerich, 2005). Among these traits is an acute sense of hearing and use of sound in the ocean. Toothed whales, in particular, have developed the remarkable ability to echolocate. This study describes the morphology of the odontocete melon, a structure presumably involved in the propagation of echolocation sounds. The goal of this paper is to provide a quantitative definition of the melon in a broad spectrum of odontocetes. The results provide further insight into sound propagation pathways in the forehead of odontocetes and a foundation for evolutionary questions.

Echolocation is the process of emitting sounds and using the information contained in the returning echoes to sense the surrounding environment. This active system allows echolocating animals to forage, to avoid obstacles, and to orient in the absence of light. Echolocation was first documented in experiments with insect eating bats (Griffin and Galambos, 1941). The first evidence for dolphin echolocation came from observations of dolphins in and around nets during capture operations (McBride, 1956). Experiments with
captive odontocetes have characterized echolocation sounds as a brief and directional sound (echolocation clicks) emitted from the forehead to identify or track objects and make fine discriminations (Norris et al., 1961; Au, 1993). Acoustic tags placed on wild odontocetes have recorded high frequency clicks and echoes from prey during diving (Johnson et al., 2004).

The sound production anatomy combined with physiological experiments have identified functional components in the odontocete forehead (Mead, 1975; Heyning, 1989; Cranford, 1992; Au, 1993; Cranford, 1996; Cranford et al., 1997; Cranford et al., 2000): 1) nervous system (command center), 2) respiratory system (power supply), 3) nasophonation system (signal generators), 4) nasal musculature (signal modifiers), and 5) the melon and associated structures (signal propagators).

It is generally accepted that click generation begins by action of the palatopharyngeal muscle complex, as it forces the larynx up into the inferior bony nares and pressurizes the air in the bony nasal passages. The pressurized air passes through lips formed by a narrow slit in the spiracular cavity, causing vibrations in adjacent ellipsoid fat bodies (bursae complexes). These vibrations are reflected forward by the skull and air sacs functioning as acoustic mirrors. The sound vibrations propagate through the melon anteriorly and emerge into the environment as a click. The melon (homologous to the junk in sperm whales [Cranford, 1999]) is a fat and connective tissue structure that apparently functions to focus sound and decrease acoustic attenuation at the tissue-water boundary (Cranford and Amundin, 2004).

Prior to this study, there were no precise anatomic descriptions of the melon. Boundaries, density, size, shape, internal organization, and geometric relationships to other
structures all influence sound propagation pathways. Establishing a quantitative definition for the melon based on these characteristics is the focus of this study.

Previous studies have described the acoustic properties of the melon to understand function. Chemical analysis of the melon shows unique lipid composition, potentially important for acoustic function (Varanasi and Malins, 1972; Litchfield et al., 1973; Litchfield and Greenberg, 1974; Litchfield et al., 1975; Koopman et al., 2003). Sound velocity measurements in the melon suggest that it plays a role in dictating beam formation (Norris and Harvey, 1974; Litchfield et al., 1979; Goold and Clarke, 2000; Clarke, 2003).

Acoustic experiments placing hydrophones at different locations on the surface of the forehead of an echolocating porpoise identified differences in beam structure and intensity dependent on location (Au et al., 2004). The results suggest that the tissues of the forehead will influence sound propagation. This study provided a foundation to test functional hypotheses based on morphology.

The melon contributes to the distinct bulbous forehead of odontocetes; however, because of the fatty composition of the melon, dissections lose the geometric and structural information important for understanding melon morphology. In this study, X-ray Computed Technology (CT) is employed to define the melon in representative species of odontocete families. The definition of the melon is based on 1) density, 2) size, 3) shape, 4) internal structure or “topography” based on density differences, and 5) geometric relationship to other forehead structures. Functional hypotheses of the sound propagation pathway are discussed based on the melon descriptions. Comparisons of melon morphology across odontocete families are also presented. The comparative aspect of the study broadens our understanding of the complex echolocation system in odontocetes.
MATERIALS AND METHODS

A total of 12 postmortem odontocete specimens (nine species) were subject to X-ray CT and subsequent morphometric analysis (Table 5). Five of the ten extant families of odontocetes are represented (Rice, 1998): Kogiidae (1 species), Ziphiidae (3 species), Pontoporiidae (1 species), Delphinidae (6 species), and Phocoenidae (1 species). Physeteridae, Platanistidae, Iniidae, Lipotidae, Monodontidae are not included. For comparative purposes, 20 additional specimens (13 species) were CT scanned and processed (Appendix B), and 6 specimens were dissected (Appendix C).

Data Collection

The CT data from the postmortem heads were acquired from three sources (Appendix A): 1) a library of previously collected scans (Cranford, 1996), 2) a medical hospital CT scanner (Fig. 12), 3) an industrial CT scanner. In preparation for the hospital scanner, the heads of the postmortem specimens were separated from the bodies and frozen soon after death to retard tissue decomposition. Before scanning the heads were thawed in a water bath to ensure homogeneity in tissue temperature. The heads were then placed in registration frame, which put all the specimens in the same orientation and provided a calibration measure.

An industrial CT scanner was used for the larger specimens. The procedure followed prior to scanning differed from the hospital scanner in that the heads scanned in the industrial scanner remained frozen and were incased in cardboard and foam. Poles of known density were attached to the cardboard frame, which allowed the scans to be calibrated and standardized orientation (Fig. 13).
### Table 5. Specimen Life History Information

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<th>Code</th>
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<th>Weight (lbs)</th>
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<td>4400*</td>
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<td>8/16/04</td>
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*Estimated weights from Carwardine (1995).*
Figure 12. Hospital CT scanner with porpoise head in registration frame. The head is thawed and wrapped in plastic bags. The registration frame is attached to the specimen at the anterior end in the nose core, and at the posterior end in the magnum foramen. The rods (labeled 1, 2, 3, and 4) are composed of different known densities.

Figure 13. Industrial CT scanner with Fin whale head in registration frame. The frozen head is centered in the cardboard tube with the attached density rods. The tube will is then filled with foam for insulation. The entire structure is wrapped in plastic.
**Image Processing**

Data acquired for all specimens were processed using Analyze 5.0/6.0, created by the Biomedical Imaging Resource at Mayo Clinic (Robb and Barillot, 1989; Robb et al., 1989; Robb, 1999). The individual scans were compiled and converted to AVW formats (a native Analyze format); the program takes the continuous serial 2D images and forms a 3D volume of each specimen.

Each 2D scan is comprised of a matrix of values (CT numbers) expressing linear attenuation of X-rays by the material (tissue) at a specific geometric location. The CT numbers are calibrated to the Hounsfield scale (–1000 to 1000) and compared as Hounsfield (H) units (Robb, 1999). The Hounsfield unit is a calibrated measure of electron density and the difference between the units is equivalent to the change in density of 1 cubic centimeter of water 1 degree centigrade at standard temperature and pressure (STP) (Robb, 1999).

Serial matrices of calibrated H units sum to a three dimensional map of the specimen. The map is composed of “voxels” that have an associated H value based on the amount of X-ray absorbed in that area. Voxel dimensions (pixel height, length, and width) are set by the scanning protocol prior to the actual scanning process (Appendix A). To create an accurate 3D reconstruction of the specimen, the voxels were made cubic by interpolating the each voxel to 1.5 mm³.

This study dealt with DICOM, GE9800, and ARACOR image formats. The DICOM images contain information on two fields (rescale slope and rescale intercept) that adjusted the values contained in the image (CT numbers) to Hounsfield (H) units. The DICOM standard always rescales back to the actual H values, making the volumes directly comparable (Philcock, personal communication).
The GE9800 images were collected before the DICOM standard existed. Consequently, GE9800 images needed to be adjusted to match the DICOM values. The GE9800 scanner added 1024 to all CT numbers in order to end up with positive integers. In order to rescale the images back to the Hounsfield units, 1024 was subtracted from all of the CT values in the GE9800 images.

ARACOR image format is collected from the industrial CT scanners. Because the specimens remained frozen during scanning, the H values were not compared as a results of the difference between thawed and frozen tissues density.

The rescaled H values are a direct representation of electron density of the tissue types and are mapped onto a gray or color scale, thus allowing visualization of differences in tissue density by intensity and/or color (see an example in Fig. 1). The interpretive process of distinguishing and defining structural boundaries from within remote images is known as segmentation and can be aided with image processing techniques.

Some boundaries of tissues in the whale heads have large differences in the densities compared to the surrounding tissue, and they segment easily using thresholding. The thresholding technique automatically segments structures based on a designated range of H values. Image display settings can also be adjusted to place more emphasis across fewer H units, thereby highlighting the tissue boundaries. This technique of image enhancement is especially useful in distinguishing soft tissue. A color map can also enhance boundaries using color.

To quantify the boundaries of structures based on differences in H values, line profiling is a useful technique. Line profiles are created by choosing a line through a set of pixels in a 2D image or “slice.” The H value is plotted against the location of each pixel
along the chosen line (Fig. 2). Actual H values can be compared to identify subtle changes within the image.

**Morphometric Analysis**

The ease of manipulating CT images to achieve a desired geometric viewpoint or perspective permits numerous measurement options. Measurements in this study were chosen for how they might pertain to the acoustic function(s) of the melon. All measurements, both soft and bony tissues, were made directly from the CT remote images. The accuracy of the measurements depends somewhat on the ability to interpret the images. Protocols were established for making interpretive decisions and extracting the measurements (Appendix D). Measurements were repeated in at least three trials to evaluate the validity and accuracy.

**MELON DEFINITION**

The boundaries of the melon are gradual changes in density as opposed to the abrupt boundaries of bone. To determine a quantitative definition of the melon, a number of image processing techniques were needed. First, CT scans of 31 odontocete specimens were processed and examined (Table 5 and Appendix B). In addition, five dissections were performed (Appendix C). The sampling represented a broad taxonomic range and various developmental stages.

Second, image display settings were adjusted to place more emphasis across fewer low density H units; thereby highlighting the distinction between the melon and forehead structures. The melon boundaries were then defined along the gradient by isolating a set of H values using visual cues. These values were used to segment the melon from the entire volume.
To quantify the boundaries of the melon within this gradient, both line profiling and thresholding techniques were employed. Hounsfield (H) values at the same location within the forehead of each specimen were collected. A homologous transverse slice through the posterior border of the antorbital notch in each specimen was selected (for example, Fig. 16 A, p. 53). Within the transverse slice, a line was drawn across the specimen halfway between the plane of the antorbital notch and the dorsal surface of the head (represented by the green line in Fig. 16 A, p. 53).

To quantify melon boundaries, the H values along the green lines are plotted over the corresponding point in the head (for example, Fig. 16 B, p. 53). The actual boundaries of the melon were determined based on a 10 H value or greater change at adjacent points in the profile. The distance between the points was conserved across all specimens (1.5 mm), and the melon was defined based on a slope of approximately $6^{2/3}$ (rise = 10/run = 1.5). This boundary was then applied to proceeding slices in the image by isolating the same boundaries. As the melon approaches the surface of the forehead, the boundary was selected by eye because of the similar density of the melon and skin.

The head, bones (skull, mandibles, ears, and hyoids), and air spaces (nasal passage, nasal air sacs, and pterygoids) were all determined using the threshold imaging processing technique. Segmentation of the bursae complexes was similar to the melon.

**Density**

The maximum, minimum, and average H values were tabulated for the melon in each specimen. The H values provided a measure of the density range within the melon tissue for each specimen.
**SIZE**

The size of the melon was estimated from volumetric measurements. Volume was calculated by counting the number of voxels or 3D picture elements contained within the melon and then multiplying this voxel count by the volume of a single voxel (1.5 mm$^3$). For comparative purposes, the volume of the melon was scaled to the skull volume.

**SHAPE**

In this study, the Sphere Fit Factor (SFF) was used to describe the shape of the melon. It was calculated as follows:

$$SFF= \frac{(\text{Melon Surface Area})^3}{9 \times (\text{Melon Volume})^2 \times 4 \times \pi}$$

This essentially measures how well the object is represented by a sphere, or how close the shape of the object is to a sphere (SFF = 1).

To evaluate the hypothesis that the shape of the forehead is a proxy for the shape of the melon, as proposed by Carrier et al. (2002), I combined the results of the shape analysis (SFF) with a measure of the curvature of the forehead (Cranford, 1996).

**INTERNAL STRUCTURE**

To describe the internal structure of the melon, line profiles at similar anatomical locations in each specimen were collected. The transverse profiles used to define the melon (described in *melon definition*) were compared to characterize the change in density across the melon. In order to get a sense of the change in topology from the posterior to anterior end of the melon, three separate scans were selected in the sagittal view (Fig. 14; for example, see Fig. 17 B, p. 54). The locations of the three scans are as follows: 1) at the centroid of the
proposed right bursae complex, 2) at the midline of the condylobasal plane, and 3) at the centroid of the left bursae complex. In each of the sagittal scans, the associated profiles displayed the change in density, H units, from the Y plane of the bursae complexes straight to the surface of the forehead (Fig. 14).

**MELON RELATIONSHIP TO FOREHEAD STRUCTURES**

The geometric relationships of the melon to other forehead structures were based on the coordinate information contained in the scans. This coordinate information allows for linear distances between structures of interest to be calculated. As a point of reference, the
centroid or center of mass of the melon, head, and bursae were computed on the basis of the classic definition of centroid: the mean value of each of the component coordinates for all of the voxels in the object. In other words, the mean value of all the X coordinates for voxels within the object provides the centroid X coordinate, the mean value of all Y coordinates provides the Y centroid coordinate, and the mean value of all Z coordinates provides the Z centroid coordinate (Fig. 14). The result is an X, Y, and Z coordinate position in the image that represents the center of mass of the object. For additional positional information, the coordinates for the center of the blowhole, tip of the rostrum, and occipital condyles were collected from the CT scan.

**Relationship of Melon to Surface of Forehead**

The region where the melon is closest to the surface of the head was explored. The linear width of this region was reported. The angle from the condylobasal plane to this location was also determined (Fig. 15).

**Results**

Descriptions of the melon are presented according to each odontocete family. The basal lineages are presented first, followed by later diverging groups of odontocetes. The melon is defined in each group in terms of density, size, shape, internal structure, and its relationship to other structures. When applicable, intraspecific variation and development of the melon are addressed. In the second section of the results, quantitative comparisons across families are made.
Figure 15. Measurements associated with the connection of the melon to the surface of the forehead. (A) Lateral view of the angle from the tip of the rostrum to the connection of the melon to the surface of the forehead, represented by the black lines. (B) Dorsal view of the width of the connection of the melon to the surface of the forehead.

**Kogiidae**

Kogiidae includes the pygmy sperm whale (*Kogia breviceps*), and dwarf sperm whale (*Kogia simus*). The closely related Physeteridae includes one species, the sperm whale (*Physeter macrocephalus*). This study examined two juvenile pygmy sperm whales.

The forehead morphological features of *Kogia breviceps* are similar to the sperm whale (Cranford, 1999) in that they show extreme deviations from all other odontocetes (Figs. 16-18). For example, although the melon (also known as junk in sperm whales) lies dorsal to the bones of the rostrum; unique to the sperm whales is that the melon extends beyond the anterior tip of the rostrum (Fig. 18). The melon in the pygmy sperm whale is embedded in connective tissue and blubber on the dorsal and lateral borders (Fig. 16).
Figure 16. Definition of melon boundaries in *Kogia breviceps* based on change in Hounsfield units in the CT scan (Kobr72). (A) Transverse tomographic reconstruction through the anterior portion of the supraorbital processes. The black line shows the location of the line profile displayed in part (B) of the figure (me = melon; sk = skull; ma = mandible; rsop = right supraorbital notch; lsop = left supraorbital notch). Left and dorsal are indicated by L and D, respectively. (B) Graph of line profile showing the change in density along the blackline in (A). The y-axis represents the change in H units plotted over a specified distance (mm) along the black line in (A) on the x-axis. The melon boundaries defined by a change in 10 H units at adjacent locations are indicated by the black arrows.
Figure 17. Sagittal profile and hypothetical acoustic pathway for *Kogia breviceps.*

(A) Vertical tomographic reconstruction (parasagittal section) from *Kogia breviceps* highlighting the internal structure of the melon with a color scale (bc = brain case; ma = mandibles; me = melon; mp = right nasal passage; ro = rostrum; sk = skull; so = spermaceti organ). Anterior and dorsal are indicated as A and D, respectively. (B) Line profiles through two vertical slices (mid-sagittal, right parasagittal at the centroid of the spermaceti organ) showing the change in density from the proposed sound generator to the surface of the forehead. The y-axis represents the change in HU plotted over a specified distance across the forehead (mm) on the x-axis. (C) Hypothetical acoustic pathway through the head. The red star represents the location of the sound source (bursae complex). The dotted line represents the hypothetical pathway of sound through the head.
Figure 18. 3D reconstructions of the melon, head and spermaceti organ in *Kogia breviceps*. (A) Dorsal view. (B) Right lateral view (me = melon; sk = skull; so = spermaceti organ), anterior and left are indicated by A and L, respectively. The red dotted lines represent the width of the connection of the melon to the surface of the forehead (A) and the angle from the tip of the rostrum to the melon surface connection (B).
In the pygmy sperm whale, the melon attaches directly to the spermaceti organ in the posterior region (Fig. 18). The melon was defined in the CT images by a range of H values (-123 to -23) that were distinct from the surrounding tissue mass (Table 6). The internal structure of the melon in the pygmy sperm whale has a highly organized pattern of density, from higher density in the outer melon to lower density in the center of the melon (Figs. 17 and 18). This pattern of concentric low density layers is especially apparent in the two juvenile *Kogia breviceps* examined, compared to all other species examined. The separate density layers are thin and continuous on all borders of the melon.

The anterior region of the melon follows the contour of the external morphology of the forehead. The melon extends laterally both right and left in a wing-like structure in the posterior region (Fig. 18). The melon is closest to the surface of the forehead along the anterior region of the forehead; the width of the connection is 2.4 cm. The angle from the tip of the rostrum is 115.7°.

When compared to the shape of a sphere using the sphere fit factor, the melon in the pygmy sperm whale deviates from the shape by 0.83 (SFF = 1.83), most likely due to the lateral wing-like projections.

**Ziphiidae**

Ziphiidae includes four genera and 21 species (Dalebout et al., 2002). This study examined and quantified the forehead anatomy in three specimens: two specimens of Cuvier’s beaked whale (*Ziphius cavirostris*) and one specimen of *Mesoplodon perrini*. Not only were we able to make comparisons with other odontocetes, but also report some preliminary data on developmental variation based on the difference between the adult and neonate Cuvier’s beaked whale.
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Although the differences in bony features of the skull in ziphiids are not as extreme as sperm whales, some striking differences in the soft tissues are apparent (Figs. 19-27). The melon in the ziphiids, examined in this study, is dorsal to the bony rostrum and does not extend beyond the anterior tip. In the neonates, *Z. cavirostris* and *M. perrini*, the boundaries of the melon at the anterior region are not distinct and the region is relatively homogeneous with the surrounding tissue (Figs. 22 A and 24 A). In contrast, the posterior region of the melon, in the neonates, shows not only differentiation from the surrounding tissues but also a distinct shape (Figs. 22 A, 24 A, 25, 27). Because of the possible incomplete development of the melon in the two neonate specimens, the descriptions will concentrate on the adult *Z. cavirostris* specimen.

The melon in the adult *Ziphius cavirostris* although distinct from the surrounding forehead tissue appears to be more homogeneous in density; the profiles show a shallower gradient in density change (Fig. 20). The adult *Ziphius cavirostris* specimen remained frozen during the scanning process; therefore the density values are not comparable with other specimens.

The fat body structures in the forehead of *Ziphius cavirostris* are divided into two separate regions. The lower density region is nested within a bony basin-like structure formed by the premaxillaries, maxillaries, and vomer. Based on its position in relation to the nasal passages, the lower density portion seems to be homologous to the spermaceti organ found in sperm whales, as suggested by Norris and Harvey (1972). Additional evidence to support the homologous relationship of the spermaceti organ in *Ziphius cavirostris* is the lower density tissue present in the spermaceti organ relative to the melon. This pattern is present in the pygmy sperm whale. The spermaceti organ in the adult *Ziphius cavirostris*
Figure 19. Definition of melon boundaries in *Ziphius cavirostris* (neonate) based on change in Hounsfield units in the CT scan (ZicaN). (A) Transverse tomographic reconstruction through the anterior portion of the supraorbital processes. The black line shows the location of the line profile displayed in part (B) of the figure (me = melon; sk = skull; ma = mandible; rsop = right supraorbital notch; lsop = left supraorbital notch). Left and dorsal are indicated by L and D, respectively. (B) Graph of line profile showing the change in density along the black line in (A). The y-axis represents the change in H units plotted over a specified distance (mm) along the black line in (A) on the x-axis. The melon boundaries defined by a change in 10 H units at adjacent locations are indicated by the black arrows.
Figure 20. Definition of melon boundaries in *Ziphius cavirostris* (adult) based on change in Hounsfield units in the CT scan (ZicaA). (A) Transverse tomographic reconstruction through the anterior portion of the supraorbital processes. The black line shows the location of the line profile displayed in part (B) of the figure (me = melon; sk = skull; ma = mandible; rsop = right supraorbital notch; lsop = left supraorbital notch). Left and dorsal are indicated by L and D, respectively. (B) Graph of line profile showing the change in density along the black line in (A). The y-axis represents the change in H units plotted over a specified distance (mm) along the black line in (A) on the x-axis. The melon boundaries defined by a change in 10 H units at adjacent locations are indicated by the black arrows.
Figure 21. Definition of melon boundaries in *Mesoplodon perrini* based on change in Hounsfield units in the CT scan (Mepe). (A) Transverse tomographic reconstruction through the anterior portion of the supraorbital processes. The black line shows the location of the line profile displayed in part (B) of the figure (me = melon; sk = skull; ma = mandible; rsop = right supraorbital notch; lsop = left supraorbital notch). Left and dorsal are indicated by L and D, respectively. (B) Graph of line profile showing the change in density along the black line in (A). The y-axis represents the change in H units plotted over a specified distance (mm) along the black line in (A) on the x-axis. The melon boundaries defined by a change in 10 H units at adjacent locations are indicated by the black arrows.
Figure 22. Sagittal profile and hypothetical acoustic pathway for a neonate Cuvier’s beaked whale (*Ziphius cavirostris*). (A) Vertical tomographic reconstruction (parasagittal section) from *Ziphius cavirostris* (neonate) highlighting the internal structure of the melon with a color scale (bc = brain case; ma = mandibles; me = melon; rnp = right nasal passage; ro = rostrum; sk = skull). Anterior and dorsal are indicated as A and D, respectively. (B) Line profiles through two vertical slices (mid-sagittal, right parasagittal at the centroid of the spermaceti organ) showing the change in density from the proposed sound generator to the surface of the forehead. The y-axis represents the change in HU plotted over a specified distance across the forehead (mm) on the x-axis. (C) Hypothetical acoustic pathway through the head. The red star represents the location of the sound source (bursae complex?). The dotted line represents the hypothetical pathway of sound through the head.
Figure 23. Sagittal profile and hypothetical acoustic pathway for an adult Cuvier’s beaked whale (*Ziphius cavirostris*). (A) Vertical tomographic reconstruction (parasagittal section) from *Ziphius cavirostris* (adult) highlighting the internal structure of the melon with a color scale (bc = brain case; ma = mandibles; me = melon; rnp = right nasal passage; ro = rostrum; sk = skull; so = spermaceti organ. Anterior and dorsal are indicated as A and D, respectively. (B) Line profiles through two vertical slices (mid-sagittal, right parasagittal at the centroid of the spermaceti organ) showing the change in density from the proposed sound generator to the surface of the forehead. The y-axis represents the change in HU plotted over a specified distance across the forehead (mm) on the x-axis. (C) Hypothetical acoustic pathway through the head. The red star represents the location of the sound source (bursae complex). The dotted line represents the hypothetical pathway of sound through the head.
Figure 24. Sagittal profile and hypothetical acoustic pathway for *Mesoplodon perrini*. (A) Vertical tomographic reconstruction (parasagittal section) from *Mesoplodon perrini* highlighting the internal structure of the melon with a color scale (bc = brain case; ma = mandibles; me = melon; rnp = right nasal passage; ro = rostrum; sk = skull. Anterior and dorsal are indicated as A and D, respectively. (B) Line profiles through three vertical slices (mid-sagittal, right parasagittal at the centroid of the right bursae complex?, and left parasagittal at the centroid of the left bursae complex?) showing the change in density from the proposed sound generator to the surface of the forehead. The y-axis represents the change in HU plotted over a specified distance across the forehead (mm) on the x-axis. (C) Hypothetical acoustic pathway through the head. The red star represents the location of the sound source (bursae complex?). The dotted line represents the hypothetical pathway of sound through the head.
Figure 25. 3D reconstructions of the melon and skull in *Ziphius cavirostris* (neonate). (A) Dorsal view. (B) Right lateral view (me = melon; sk = skull; pme = posterior melon extension), anterior and left are indicated by A and L, respectively. The red dotted lines represent the width of the connection of the melon to the surface of the forehead (A) and the angle from the tip of the rostrum to the melon surface connection (B).
Figure 26. 3D reconstructions of the melon, head and spermaceti organ in *Ziphius cavirostris* (adult). (A) Dorsal view. (B) Right lateral view (me = melon; sk = skull; so = spermaceti organ), anterior and left are indicated by A and L, respectively. The red dotted lines represent the width of the connection of the melon to the surface of the forehead (A) and the angle from the tip of the rostrum to the melon surface connection (B).
Figure 27. 3D reconstructions of the melon and skull in *Mesoplodon perrini*. (A) Dorsal view. (B) Right lateral view (me = melon; sk = skull; lbc? = possible left bursae complex; rbc = possible right bursae complex), anterior and left are indicated by A and L, respectively. The red dotted lines represent the width of the connection of the melon to the surface of the forehead (A) and the angle from the tip of the rostrum to the melon surface connection (B).
extends posterior to the dorsal region of the right bony nasal passage in two branches (Fig. 26). The right branch is double the width of the left. The anterior terminus of the spermaceti organ ends abruptly in an extremely dense bone, the vomer, which fills the meso-rostral canal. The melon contacts the middle section of the spermaceti organ, and extends anteriorly towards the surface of the forehead (Figs. 23 and 26). The melon is closest to the surface of the forehead along the dorsal region; the width of the connection is 19.4 cm (Fig. 26 A). The angle from the tip of the rostrum to this location is 34.8° (Fig. 26 B). The dorsal and lateral sides of the melon are surrounded by an extremely dense connective tissue structure or theca.

The shape of the melon in *Z. cavirostris* is not sphere like (SFF = 14.3), instead it has a box-like morphology; the anterior terminus ends bluntly just anterior to the premaxillary, maxilla, and vomer bone basin (Figs. 23 A and 26 B).

**Pontoporiidae**

There are currently four recognized families of river dolphins with one species represented in each (Rice, 1998). Because of the remote locations where these animals live and the low population sizes, specimens are difficult to acquire. Consequently, only one family is represented in this study Pontoporiidae, by the franciscana (*Pontoporia blainvillei*).

The melon is defined by a -200 to -30 H value range in the CT images and shows a distinct gradient to a lower density central core (Figs. 28-30, Table 6). A thick connective tissue blanket (theca) covers the dorsal and lateral sides of the melon (Fig. 28). The ventral region of the melon in *Pontoporia blainvillei* extends anterior along the rostrum and ends posterior to the crease between the forehead and the rostrum. The franciscana melon has a relatively flat anterior surface, similar to *Z. cavirostris*. The posterior morphology is similar
Figure 28. Definition of melon boundaries in *Pontoporia blainvillei* based on change in Hounsfield units in the CT scan (Pobl1663). (A) Transverse tomographic reconstruction through the anterior portion of the supraorbital processes. The black line shows the location of the line profile displayed in part (B) of the figure (me = melon; sk = skull; ma = mandible; rsop = right supraorbital notch; lsop = left supraorbital notch). Left and dorsal are indicated by L and D, respectively. (B) Graph of line profile showing the change in density along the black line in (A). The y-axis represents the change in H units plotted over a specified distance (mm) along the black line in (A) on the x-axis. The melon boundaries defined by a change in 10 H units at adjacent locations are indicated by the black arrows. The blurred image is a result of the lower resolution scan (Appendix A).
Figure 29. Sagittal profile and hypothetical acoustic pathway for *Pontoporia blainvillei*. (A) Vertical tomographic reconstruction (parasagittal section) from *Pontoporia blainvillei* highlighting the internal structure of the melon with a color scale (bc = brain case; ma = mandibles; me = melon; rnp = right nasal passage; ro = rostrum; sk = skull. Anterior and dorsal are indicated as A and D, respectively. (B) Line profiles through three vertical slices (mid-sagittal, right parasagittal at the centroid of the right bursae complex, and left parasagittal at the centroid of the left bursae complex) showing the change in density from the proposed sound generator to the surface of the forehead. The y-axis represents the change in HU plotted over a specified distance across the forehead (mm) on the x-axis. (C) Hypothetical acoustic pathway through the head. The red star represents the location of the sound source (bursae complex). The dotted line represents the hypothetical pathway of sound through the head.
Figure 30. 3D reconstructions of the melon, skull, and bursae complexes in *Pontoporia blainvillei*. (A) Dorsal view. (B) Right lateral view (me = melon; sk = skull; lbc = left bursae complex; rbc = right bursae complex), anterior and left are indicated by A and L, respectively. The red dotted lines represent the width of the connection of the melon to the surface of the forehead (A) and the angle from the tip of the rostrum to the melon surface connection (B).
to the delphinids in that the melon extends from the main body of the melon to the right bursae complex. The extension is long (34% of total melon length) in *P. blainvillei* and twisted in a corkscrew fashion (Fig. 30). The melon is closest to the surface of the forehead along the anterior region; the width of the connection is 2.0 cm (Fig. 30 A). The angle from the tip of the rostrum to this location is 22.2° (Fig. 30 B).

When compared to a perfect sphere, the melon in *Pontoporia* deviates (1.31 units) from the shape of a sphere (SFF = 2.31), most likely due to the posterior branch and the blunt anterior region.

**Delphinidae**

The family Delphinidae (true dolphins) is the most diverse and specious group of odontocetes and includes 16 genera and at least 36 species (Figs. 31-42). Within this family, four genera (representing four species) were examined in this study: *Tursiops, Delphinus, Lissodelphis*, and *Lagenorhynchus*. *Tursiops* and *Delphinus* were adults; *Lissodelphis* and *Lagenorhynchus* were juveniles. Despite the similar external head morphology found in this family, we found diversity in the shape of the melon and its relationships to other forehead structures.

Three *T. truncatus* specimens were analyzed. All specimens are adults of similar size. The melon was defined in *T. truncatus* by a range of -128 to -60; the range was similar to *D. delphis* (-131 to -28) and *L. borealis* (-127 to -44) (Table 6). The range of density was less in the juvenile *L. obliquidens* (-90 to -40). The gradient toward a lower density core was present in all delphinid specimens (Fig. 43). The melon is embedded in the connective tissues of the forehead and extends anterior along the rostrum in all delphinid species examined.
Figure 31. Definition of melon boundaries in *Tursiops truncatus* based on change in Hounsfield units in the CT scan (Tutr790). (A) Transverse tomographic reconstruction through the anterior portion of the supraorbital processes. The black line shows the location of the line profile displayed in part (B) of the figure (me = melon; sk = skull; ma = mandible; rsop = right supraorbital notch; lsop = left supraorbital notch). Left and dorsal are indicated by L and D, respectively. (B) Graph of line profile showing the change in density along the black line in (A). The y-axis represents the change in H units plotted over a specified distance (mm) along the black line in (A) on the x-axis. The melon boundaries defined by a change in 10 H units at adjacent locations are indicated by the black arrows.
Figure 32. Definition of melon boundaries in *Delphinus delphis* based on change in Hounsfield units in the CT scan (Dede17). (A) Transverse tomographic reconstruction through the anterior portion of the supraorbital processes. The black line shows the location of the line profile displayed in part (B) of the figure (me = melon; sk = skull; ma = mandible; rsop = right supraorbital notch; lsop = left supraorbital notch). Left and dorsal are indicated by L and D, respectively. (B) Graph of line profile showing the change in density along the black line in (A). The y-axis represents the change in H units plotted over a specified distance (mm) along the black line in (A) on the x-axis. The melon boundaries defined by a change in 10 H units at adjacent locations are indicated by the black arrows.
Figure 33. Definition of melon boundaries in Lissodelphis borealis based on change in Hounsfield units in the CT scan (Libo1110). (A) Transverse tomographic reconstruction through the anterior portion of the supraorbital processes. The black line shows the location of the line profile displayed in part (B) of the figure (me = melon; sk = skull; ma = mandible; rsop = right supraorbital notch; lsop = left supraorbital notch). Left and dorsal are indicated by L and D, respectively. (B) Graph of line profile showing the change in density along the black line in (A). The y-axis represents the change in H units plotted over a specified distance (mm) along the black line in (A) on the x-axis. The melon boundaries defined by a change in 10 H units at adjacent locations are indicated by the black arrows.
Figure 34. Definition of melon boundaries in *Lagenorhynchus obliquidens* based on change in Hounsfield units in the CT scan (Labo176). (A) Transverse tomographic reconstruction through the anterior portion of the supraorbital processes. The black line shows the location of the line profile displayed in part (B) of the figure (me = melon; sk = skull; ma = mandible; rsop = right supraorbital notch; lsop = left supraorbital notch). Left and dorsal are indicated by L and D, respectively. (B) Graph of line profile showing the change in density along the black line in (A). The y-axis represents the change in H units plotted over a specified distance (mm) along the black line in (A) on the x-axis. The melon boundaries defined by a change in 10 H units at adjacent locations are indicated by the black arrows.
Figure 35. Sagittal profile and hypothetical acoustic pathway for *Tursiops truncatus*. (A) Vertical tomographic reconstruction (parasagittal section) from *Tursiops truncatus* highlighting the internal structure of the melon with a color scale (bc = brain case; ma = mandibles; me = melon; mp = right nasal passage; ro = rostrum; sk = skull). Anterior and dorsal are indicated as A and D, respectively. (B) Line profiles through three vertical slices (mid-sagittal, right parasagittal at the centroid of the right bursae complex, and left para-sagittal at the centroid of the left bursae complex) showing the change in density from the proposed sound generator to the surface of the forehead. The y-axis represents the change in HU plotted over a specified distance across the forehead (mm) on the x-axis. (C) Hypothetical acoustic pathway through the head. The red star represents the location of the sound source (bursae complex). The dotted line represents the hypothetical pathway of sound through the head.
Figure 36. Sagittal profile and hypothetical acoustic pathway for Delphinus delphis. (A) Vertical tomographic reconstruction (parasagittal section) from Delphinus delphis highlighting the internal structure of the melon with a color scale (bc = brain case; ma = mandibles; me = melon; rnp = right nasal passage; ro = rostrum; sk = skull). Anterior and dorsal are indicated as A and D, respectively. (B) Line profiles through three vertical slices (mid-sagittal, right parasagittal at the centroid of the right bursae complex, and left parasagittal at the centroid of the left bursae complex) showing the change in density from the proposed sound generator to the surface of the forehead. The y-axis represents the change in HU plotted over a specified distance across the forehead (mm) on the x-axis. (C) Hypothetical acoustic pathway through the head. The red star represents the location of the sound source (bursae complex). The dotted line represents the hypothetical pathway of sound through the head.
Figure 37. **Sagittal profile and hypothetical acoustic pathway for Lissodelphis borealis.**

(A) Vertical tomographic reconstruction (parasagittal section) from *Lissodelphis borealis* highlighting the internal structure of the melon with a color scale (bc = brain case; ma = mandibles; me = melon; rnp = right nasal passage; ro = rostrum; sk = skull). Anterior and dorsal are indicated as A and D, respectively. (B) Line profiles through three vertical slices (mid-sagittal, right parasagittal at the centroid of the right bursae complex, and left parasagittal at the centroid of the left bursae complex) showing the change in density from the proposed sound generator to the surface of the forehead. The y-axis represents the change in HU plotted over a specified distance across the forehead (mm) on the x-axis. (C) Hypothetical acoustic pathway through the head. The red star represents the location of the sound source (bursae complex). The dotted line represents the hypothetical pathway of sound through the head.
Figure 38. Sagittal profile and hypothetical acoustic pathway for *Lagenorhynchus obliquidens*. (A) Vertical tomographic reconstruction (parasagittal section) from *Lagenorhynchus obliquidens* highlighting the internal structure of the melon with a color scale (bc = brain case; ma = mandibles; me = melon; rnp = right nasal passage; ro = rostrum; sk = skull). Anterior and dorsal are indicated as A and D, respectively. (B) Line profiles through three vertical slices (mid-sagittal, right parasagittal at the centroid of the right bursae complex, and left parasagittal at the centroid of the left bursae complex) showing the change in density from the proposed sound generator to the surface of the forehead. The y-axis represents the change in HU plotted over a specified distance across the forehead (mm) on the x-axis. (C) Hypothetical acoustic pathway through the head. The red star represents the location of the sound source (bursae complex). The dotted line represents the hypothetical pathway of sound through the head.
Figure 39. 3D reconstructions of the melon, skull, and bursae complexes in *Tursiops truncatus*. (A) Dorsal view. (B) Right lateral view (me = melon; sk = skull; lbc = left bursae complex; rbc = right bursae complex), anterior and left are indicated by A and L, respectively. The red dotted lines represent the width of the connection of the melon to the surface of the forehead (A) and the angle from the tip of the rostrum to the melon surface connection (B).
Figure 40. 3D reconstructions of the melon, skull, and bursae complexes in *Delphinus delphis*. (A) Dorsal view. (B) Right lateral view (me = melon; sk = skull; lbc = left bursae complex; rbc = right bursae complex), anterior and left are indicated by A and L, respectively. The red dotted lines represent the width of the connection of the melon to the surface of the forehead (A) and the angle from the tip of the rostrum to the melon surface connection (B).
Figure 41. 3D reconstructions of the melon, skull, and bursae complexes in *Lissodelphis borealis*. (A) Dorsal view. (B) Right lateral view (me = melon; sk = skull; lbc = left bursae complex; rbc = right bursae complex), anterior and left are indicated by A and L, respectively. The red dotted lines represent the width of the connection of the melon to the surface of the forehead (A) and the angle from the tip of the rostrum to the melon surface connection (B).
Figure 42. 3D reconstructions of the melon, skull, and bursae complexes in *Lagenorhynchus obliquidens*. (A) Dorsal view. (B) Right lateral view (me = melon; sk = skull; lbc = left bursae complex; rbc = right bursae complex; lfb = left fat basin; rfb = right fat basin), anterior and left are indicated by A and L, respectively. The red dotted lines represent the width of the connection of the melon to the surface of the forehead (A) and the angle from the tip of the rostrum to the melon surface connection (B).
Figure 43. Composite graph of forehead line profiles in representatives of Delphinidae family. The profiles correspond to the black lines displayed in part (A) of Figs. 31-34. The graph of line profile is showing the change in density. The y-axis represents the change in H units plotted over a specified distance (mm).
The internal structure of the melon differs within the family (Fig. 32). The posterior region of the melon in *T. truncatus*, *D. delphis*, and *L. borealis* extends from the main body of the melon and connects to the right bursae complex (Figs. 39-41). On the left side there is an increase in density between the melon and the left bursae complex. The melon in *L. obliquidens* is interrupted by an increase in density (14.2 mm) between the melon and two fat body basins (Cranford, 1996) that connect directly to both the right and left bursae complexes (Fig. 42).

In all delphinid specimens analyzed, the dorsal surface of the middle melon lies just below the surface of the forehead (Figs. 39-41). In *Tursiops* and *Delphinus*, the melon follows the curvature of the forehead, and the width of the connection is 5.4 cm and at an angle of 33° from the tip of the rostrum. The connection of the melon to the surface of the forehead in the juvenile *Lissodelphis* is difficult to define and appears to extend to the tip of the rostrum. Examination of the internal morphology in *Lissodelphis* showed a width of 9.2 cm at the melon surface connection (Fig. 41 A) and an angle of 30.7° from the tip of the rostrum to the melon surface connection (Fig. 41 B). In *Lagenorhynchus* the distance is 2.5 cm and the angle is 34.0° (Fig. 42).

In *L. obliquidens* the ventral melon is invaded by connective tissue, creating a blocky anterior surface to the low density core of the melon (Fig. 38). This ventral invasion of connective tissue was previously described by Cranford (1996) as a connective tissue post. The blocky anterior surface of the core of the melon is also present in *L. borealis*; however there is not a distinct invasion of connective tissue in the ventral region (Fig. 37). The morphology of the anterior region of the melon core in *T. truncatus* and *D. delphis* follows
the same sloping and tapered appearance as the melon shell and the external forehead (Figs. 35 and 36).

Disparity in melon shape within Delphinidae is reflected in the comparison of the melon to a sphere. *T. truncatus* and *D. delphis* the melon deviated from a sphere by approximately 2 units (SFF = 3.4 and 3.2, respectively), and the SFF for *L. borealis* and *L. obliquidens* was 4.3 and 5.6, respectively.

### Phocoenidae

The family Phocoenidae (porpoises) is represented by three genera (six species) (Figs. 44-46). The morphology of the melon in one harbor porpoise (*Phocoena phocoena*) was examined in this study.

Although slight disparity between the right and left premaxillary bones and the bursae complexes exists (Cranford, 1996), the melon is symmetrically placed on the skull (Fig. 46 A). The melon in the porpoise is defined by a relatively small range of H units (-96 to -37) (Table 6). The smaller disparity in H values is reflected in the lesser degree of density gradient within the melon (Fig. 44).

The dorsal and lateral sides of the melon in *Phocoena phocoena* are embedded in a similar connective tissue structure and as seen in delphinids (Fig. 44). Anterior morphology of the melon surface runs just deep to the skin layer, and then tapers to a point along the rostrum (Fig. 45). Some major differences exist in the posterior region of the melon in *Phocoena phocoena*, both in morphology and relationship to other structures. Unlike the right posterior extension of the melon in *T. truncatus*, *D. delphis*, and *L. borealis*, the melon extends and tapers along the midline of the skull and ends just anterior to the bony nasal passages (Fig. 46A). In the porpoise, there is no direct contact between the melon and the
Figure 44. Definition of melon boundaries in *Phocoena phocoena* based on change in Hounsfield units in the CT scan (Phph964). (A) Transverse tomographic reconstruction through the anterior portion of the supraorbital processes. The black line shows the location of the line profile displayed in part (B) of the figure (me = melon; sk = skull; ma = mandible; rsop = right supraorbital notch; lsop = left supraorbital notch). Left and dorsal are indicated by L and D, respectively. (B) Graph of line profile showing the change in density along the black line in (A). The y-axis represents the change in H units plotted over a specified distance (mm) along the black line in (A) on the x-axis. The melon boundaries defined by a change in 10 H units at adjacent locations are indicated by the black arrows.
Figure 45. Sagittal profile and hypothetical acoustic pathway for *Phocoena phocoena*. (A) Vertical tomographic reconstruction (parasagittal section) from *Phocoena phocoena* highlighting the internal structure of the melon with a color scale (bc = brain case; ma = mandibles; me = melon; rnp = right nasal passage; ro = rostrum; sk = skull). Anterior and dorsal are indicated as A and D, respectively. (B) Line profiles through three vertical slices (mid-sagittal, right parasagittal at the centroid of the right bursae complex, and left para-sagittal at the centroid of the left bursae complex) showing the change in density from the proposed sound generator to the surface of the forehead. The y-axis represents the change in HU plotted over a specified distance across the forehead (mm) on the x-axis. (C) Hypothetical acoustic pathway through the head. The red star represents the location of the sound source (bursae complex). The dotted line represents the hypothetical pathway of sound through the head.
Figure 46. 3D reconstructions of the melon, skull, and bursae complexes in *Phocoena phocoena*. (A) Dorsal view. (B) Right lateral view (me = melon; sk = skull; lbc = left bursae complex; rbc = right bursae complex), anterior and left are indicated by A and L, respectively. The red dotted lines represent the width of the connection of the melon to the surface of the forehead (A) and the angle from the tip of the rostrum to the melon surface connection (B).
right or left bursae complexes. The complexes are both 21.5 mm from the midline of the skull, and 26 mm from the posterior tip of the melon. The connection of the melon to the surface of the forehead has a width of 3.8 cm at an angle of 44° from the tip of the rostrum (Fig. 46).

The symmetry of the melon porpoise is reflected its shape, which of all odontocetes measured most closely resembles a sphere (SFF = 1.78).

**Morphometric Comparisons**

The overall range of the H values for the melon in all specimens analyzed was 169 H units (-322 to 22) (Table 6). When compared to normal mammalian adipose tissue, toothed whale melon tissue covers a larger range of H values (Robb, 1999). The mean H value for all specimens do not differ significantly (ANOVA, $p < 0.01$) (Fig. 47); however, the degree of density gradient did seem to vary according to family (Fig. 48).

The comparison of melon size scaled to skull size revealed a relatively linear relationship, although the data was best explained by a logarithmic curve (adjusted $r^2 = 0.73$) (Fig. 49). In general, a larger skull translates to a larger melon. This trend is the opposite when the adult *Ziphius* is compared to the neonate *Ziphius*. The melon boundaries in the neonate at the anterior region are not distinguishable from the surrounding tissue. By removing the neonates *Ziphius* and *Mesoplodon* from size comparison, the logarithmic correlation between skull size to melon size is stronger (adjusted $r^2 = 0.85$).

The disparity in melon shape was measured by the SFF. Interestingly, two phylogenetically distant species, *Kogia breviceps* and *Phocoena phocoena*, have a similar SFF value, implying a similar melon shape compared to a sphere (Fig. 50). Within the delphinids, the SFF varies considerably. Although *Tursiops* and *Delphinus* share a similar shape reflected in
Figure 47. Box plot of average Hounsfield units (density) of melon in all specimens analyzed. The species on the x-axis are organized by phylogenetic relationships (to the left are the basal lineages). The boxes represent the range of average density in each scan containing the melon. The central line marks the median of each group. The boxes are at the 24th and 75th percentiles and the whiskers are at the 95th and 5th percentiles. The dots are outliers. The overlying line graph connects the mean H value (diamonds) in each specimen.
Figure 48. Composite graph of forehead line profiles in representatives of five odontocete families. The profiles correspond to the black lines displayed in part (A) of the Figs. 16, 19, 28, 31, and 44. The graph of line profile is showing the change in density. The y-axis represents the change in H units plotted over a specified distance (mm).
Figure 49. Comparison of the size of the melon to the skull for various odontocete species.
the SFF value (SFF = 3), the melon in *Lissodelphis* and *Lagenorhynchus* differ from this
shape factor (SFF = 4.3 and 5.6, respectively).

In all specimens, the melon follows the contour of the forehead in the dorsal region
where the melon connects to the surface of the forehead. The posterior and anterior regions
of the melon deviate from the shape of the external forehead. The melon is a complex
structure embedded in the tissues of the forehead, and the morphology appears to be species
specific and not reflected in the external forehead shape. For example, the species with the
most sphere-like melons (*Kogia breviceps* and *Phocoena phocoena*) had very different
external morphology; *Kogia breviceps* has a block-like forehead and *Phocoena phocoena* has
a sloping external forehead (Fig. 51).

The angle from the tip of the rostrum to the melon surface connection in the pygmy
sperm whale differed from the rest of the specimens as a result of the anterior projection of
the melon past the tip of the rostrum (Fig. 52). The angle was also dependant on the length of
the rostrum; *Phocoena* and *Lagenorhynchus* have the shortest rostra, and the next largest
angles (Fig. 52). The smallest width of melon surface connection relative to postcranial
length was measured in *Kogia breviceps, Lagenorhynchus obliquidens*, and *Pontoporia
blainvillei*. 
Figure 50. **Shape comparison of the melon in various odontocete species.** The shape of the melon was compared to a sphere using the Sphere fit factor (SFF). The species on the x-axis are organized by phylogenetic relationships (to the left are the basal lineages).
Figure 51. Shape of the melon compared to curvature of the forehead in various odontocete species. The y-axis on the left indicates the shape of the melon, represented by the circles on the graph. The y-axis on the right represents the curvature of the forehead, represented by the triangles on the graph.
Figure 52. The angle from the tip of the rostrum to the connection of the melon to the surface of the forehead for various species of odontocetes. To standardize the angles, each specimen’s condylo-basal plane is along the x-axis. Zero on the x-axis represents the tip of the rostrum, and the length of the radiating lines represents the distance from the tip of the rostrum to the surface of the forehead (mm). The y-axis indicates the angle from the tip of the rostrum to the surface of the head at the melon surface connection.
DISCUSSION

The results of this study provide the first definition and description of the melon for a broad spectrum of odontocete species; thereby, providing a means to look at melon variation across odontocete lineages, within the Delphinidae family, and development in *Ziphius cavirostris*. Based on these morphological descriptions, this study concludes with functional hypotheses on the sound propagation pathways through the forehead of odontocetes.

The comparisons of melon morphology across odontocete families reveal difference dependent on the lineages (Fig. 48). For example, the melon of the pygmy sperm whale extends anterior past the tip of the bony rostrum. *Pontoporia blainvillei* has features of the melon that are similar to both Delphinidae and Ziphiidae, possibly related to phylogenetic history.

The comparisons of melon structure within Delphinidae showed some salient comparisons. Intraspecific variation was low based on the comparisons of the three adult *Tursiops* specimens (see Chapter 2). *Tursiops* and *Delphinus* had very similar melons in terms of morphology and density. *Lissodelphis* was similar to *Tursiops* and *Delphinus* in the posterior melon region; however, the internal structures of the melon were more similar to *Lagenorhynchus*. Unlike all other delphinids examined, in *Lagenorhynchus* the connection of the main body of the melon to both bursae complexes was interrupted by an increase in density. The differences might be related difference in developmental stages, *Lagenorhynchus* and *Lissodelphis* are juveniles. The distinct boundaries present in *Lagenorhynchus*, unlike the neonate *Mesoplodon* and *Ziphius*, lead me to believe that these differences are functionally important. Furthermore, antidotal evidence shows that week old killer whales can produce echolocation clicks (A. Bowles, personal communication). Combining the
morphological difference with acoustic signals might explain some of these discrepancies. Delphinids might be partitioning the available habitat based on acoustic capabilities; therefore, it is possible that their morphology is adapted to produce different sounds.

Difference in the melon between the adult and neonate Cuvier’s beaked whales suggest that the posterior region develops first, then the anterior sections. Because sounds are produced near the posterior region of the melon, the neonate most likely has some ability to focus and propagate sound with the melon, but the lack distinct boundaries in the anterior melon might limit propagation. The thickened theca and slight projection of the vertex of the skull present in the neonate might also influence sound propagation.

The highly organized morphology present in the juvenile pygmy sperm whales is curious in light of the difference found in the beaked whales. The two juvenile pygmy sperm whales examined had the most organized arrangement of tissues in the melon. The rate of construction of the melon appears to differ across species. Further comparisons with series of age classes in different species are necessary to thoroughly understand the development of the melon.

Melon tissue properties, size, shape, internal topology, and geometric relationships to other structures are combined to describe potential sound pathways in the melon. First, I will review melon tissue properties, both from previous studies and this study.

The chemical composition of melon lipids have been shown to be acoustically active and isolated from metabolism (Varanasi and Malins, 1972; Litchfield et al., 1973; Litchfield and Greenberg, 1974; Norris and Harvey, 1974; Morris, 1986). The high concentration of the “acoustic fat” in the forehead alludes to a function related to sound propagation. The unique fat is also present in the blubber of some animals (Koopman et al., 2003) indicating that these
special lipids are an exadaption (secondary function) in sound transmission. The melon appears to refract sound wave produced in the bursae complexes towards the low density center, based on studies mapping sound velocity through the melon tissue (Norris and Harvey, 1974; Goold and Clarke, 2000; Mohl et al., 2003; Soldevilla et al., 2004).

The results of this study add to this hypothesis; the specific density range found in the melon will influence sound propagation. When compared to normal mammalian adipose tissue, the range of H values in the melon tissues includes lower densities, which potentially serve an acoustic function. Soldevilla et al. (2004) found that H units correlated with sound velocity and density. In most species, the melon topology is from a higher density outer core that grades into a lower density core (Fig. 48 and Table 6). Because sound refracts towards the lower density material, the gradation of density in the melon should refract sound beams towards the center as sound enters and passes through the melon (Norris and Harvey, 1974).

The unique tissue properties combined with melon morphology allows for predictions on the pathways of sound in the heads of odontocetes and refinement of the proposed role of the melon. In this study, the predicted the pathways of sound in the melon are based on three criteria: 1) the relationship of the melon to the sound source (bursae complex or spermaceti organ), 2) the pattern of density change in the internal melon, and 3) the shortest distance of the melon to the surface of the forehead.

The first criterion is important because a sound wave radiates from the specific source and the position is critical to understanding interactions with other tissues. The second criterion is based on the physical properties of sound propagation. The speed of sound depends on density and elasticity of the tissue. Sound bends or refracts towards lower density as it propagates (in all but very specialized conditions). Consequently, sagittal line profiles of
the change in density in the melon provide some information on sound propagation within
the melon. The third criterion identifies the location on the forehead where the melon is
closest to the surface of the head. This location represents a place where the sounds would
experience least acoustic impedance or loss of acoustic energy as the sound leaves the head
and enters the water. The area and angle from the tip of the rostrum of the melon surface
connection between will help identify the region of the most direct and high intensity sound
beam on live animals; and potentially helpful to test these hypothesis.

In *Kogia breviceps* the melon connects directly to the spermaceti organ or signal
generator (Fig. 18). The spermaceti organ is lower in density than the outer layers of the
melon (Appendix E and Table 6). The melon tissues in *Kogia breviceps* quickly grade into an
inner core that is of similar density to the spermaceti organ (Fig. 17). Based on the morphol-
ogy, the sound generated in the phonic lips is reflected anterior into the spermaceti organ by
a combination of the skull and air sacs system. When the sound enters the tissues of the
melon, it is refracted towards the low density core, and enters the environment at the anterior
face of the forehead (Figs. 17 and 18). The lateral wings off the posterior region of the melon
toward the surface of the skin in *Kogia breviceps* present a possible alternate sound pathway
(Fig. 18 A).

In the adult *Ziphius*, there appears to be specific pathway of sound through the head,
and subsequent focusing of sound, although quite different from other specimens examined.
Sound is likely produced by vibrations near the posterior branches of the spermaceti organ
and focused anterior into the low density spermaceti organ, by the amphitheater shape of the
skull in this region. The extremely dense bone basin composed of the premaxillary bones and
surrounding the spermaceti organ will keep the sound within the fat channel. The sound
channel of the spermaceti organ in *Ziphius* terminates at the dense vomer bone (densest bone in the head) filling the meso-rostral canal; any sound reaching this boundary will be reflected posteriorly, back through the spermaceti organ, as a result of the impedance mismatch of the fat and bone. This reflection of sound should be apparent in the acoustic signal, like the sperm whale’s succession of decaying reverberations (Cranford, 1999). The posterior bony border of the spermaceti organ in *Ziphius* is dense and angled slightly dorsal and would potentially reflect the returning sound beam off the bone into the melon. The melon is surrounded dorsally and laterally by dense connective tissue of the theca. Although there is a shallower gradient of densities within the melon of *Ziphius* (Fig. 48), the spermaceti organ and the theca create a narrow pathway of sound through the melon to the surface of the head in *Ziphius* (Fig. 23).

The melon in *Pontoporia blainvillei* extends just anterior to the premaxillary bosses in a corkscrew fashion and connects directly to the right bursae complex. Sound produced in the right bursae complex will refract towards the low density posterior melon extension; sounds produced on the left side will have a less direct pathway to the melon (Fig. 30 A). The pattern of density in the melon of *Pontoporia blainvillei* forms a high density shell into a low density core, potentially refracting sound toward the center of the melon. The melon is closest to the surface of the head about midway between the blowhole and the connection of the forehead with the beak (Fig. 30 B). The anterior terminus of the melon is blunt, and does not follow the sloping contour of the forehead (Fig. 29 B). The least attenuated sound beam would exit the *Pontoporia blainvillei* head near this location (Fig. 29 C).

Within delphinids there is variation in melon morphology, suggesting different pathways of sound through the forehead. *Tursiops* and *Delphinus* have elliptical shaped
melons, with similar shaped cores of lower density that taper to a point along the rostrum. Similar to *Pontoporia*, the melon in *Tursiops* and *Delphinus* connects directly to the right bursae complex (Figs. 39 and 40 A). Sound produced in the right bursae complex will refract toward the low density posterior melon extension. Sounds produced on the left side will have a less direct pathway to the decreasing density layers of the melon.

The melon in *Tursiops* and *Delphinus* follows the curvature of the forehead, and the least attenuated sound will most likely exit the head at an angle of 33° from the tip of the rostrum and with a width of 5.4 cm (Figs. 39 and 40). The anterior extension of the low density core along the rostrum could potentially project an additional sound beam (Figs. 35 and 36).

Although the posterior melon in *Lissodelphis borealis* is similar to *Tursiops* and *Delphinus* in that the right extension connects to the right bursae complex, the internal structure is different. Instead of concentric elliptical density layers that taper at the anterior terminus, the inner melon has a blunt anterior terminus midway along the sloping external forehead (Fig. 37). Unlike *Tursiops* and *Delphinus*, there is less of a potential for a sound pathway anterior to the midpoint on the melon. Because the low density tissues of the melon are closest to the surface at a similar location along the forehead, the angle (30.7°) is similar to *Tursiops* and *Delphinus* (Fig. 41).

The main body of the melon in *Lagenorhynchus obliquidens* does not connect to either bursae complex. The potential acoustic pathway does not have a direct low density pathway, like all other delphinid specimens analyzed (Fig. 38 and 42), potentially influencing sound propagation. The low density fat bodies that attach to the left and right bursae complexes might function to direct the sound into the melon. Assuming that sound that enters
the posterior melon of \textit{Lagenorhynchus}, sounds waves will refract to the low density core
and exit the head along the midline of the sloping forehead at an angle of 43.0° from the tip
of the rostrum (Fig. 42). The dorsal invasion of connective tissue into the melon just anterior
to where the melon is closest to the surface of the head supports this predicted pathway of
sound (Fig. 38 C).

Like, \textit{Lagenorhynchus} there is not a low density connection between the bursae and
the melon in \textit{Phocoena phocoena}. The anterior morphology of the melon is similar to
\textit{Tursiops} and \textit{Delphinus} in that the main body of the melon has concentric elliptical density
layers that taper at the anterior terminus (Figs. 45 and 46). The sound that enters the melon
will focus to the low density core, and exit the head where the melon is closest to the surface
of the forehead, 44° from the tip of the rostrum (Fig. 46). There is a potential for sound to
exit the head just anterior to the tip of the rostrum if the sound follows the low density cores;
however, the sound will be attenuated more at this location because of the connective tissue
border at the anterior melon (Fig. 45 C).

The morphological descriptions presented in this study allow us to predict sound
pathways based on the morphology likely to influence sound propagation; however, form
does not necessarily equate with function, but consider the following example.

In a recent study, a series of hydrophones were placed on the head of a submerged
echolocating harbor porpoise (\textit{Phocoena phocoena}). The objective was to determine
differences in acoustic signal based on hydrophone position (Au et al., 2004) (Fig. 53). The
results show a significantly higher sound pressure levels in the hydrophone located along the
midline of the head and at the midpoint between the blowhole and the tip of the rostrum. The
hydrophone at the anterior tip of the rostrum along the midline also recorded a high sound
Figure 53. Experimental evidence for sound propagation pathway in *Phocoena phocoena*. (A) Photograph of hydrophone arrangement on the surface of an echolocating harbor porpoise (*Phocoena phocoena*) from the Au et al. (2004) experiment. (B) Resulting graph of acoustic data from Au (2004) (distance across the specimen is on the x-axis and sound pressure level is on the y-axis). The data are overlaying a CT scan of a porpoise. The red star represents the location of the sound source, and the red dotted line represents the hypothetical sound propagation pathway.
pressure level; although still lower than hydrophone at the middle of the forehead. A drop in amplitude 3.5 cm to the right and left of the middle hydrophone was also recorded.

The predictions based on morphology in *Phocoena phocoena* presented in this study seem to coincide with some of the variation found in the acoustic signals. Based on the morphological predictions in this study, the most directional and high intensity beam will exit the head near the hydrophone along the midline of the head and halfway between the tip of the rostrum and the blowhole. In addition, the width of the connection of the melon to the surface of the head was 3.8 cm suggesting that the acoustic signal would change outside this distance, again similar to the results of the hydrophone study (Fig. 53).

Additional hypothetical sound pathways need to be evaluated in other specimens through either direct collection of acoustics on the surface of the forehead and/or modeling. Captive animals provide subjects to rigorously test the difference in the beam along the forehead, and if the predictions based on morphology hold true, the morphology might potentially be useful for predicting acoustic pathways based on morphology. This is of particular importance when recordings are unavailable or not feasible. With recent technological advances, modeling offers a promising technique for understanding the acoustic pathway through the head and the contributions of the intricate anatomical components of the forehead. For example, transmission line modeling (TLM) was used to examine wave propagation through the head of a porpoise (Goodson et al., 2004) and proved to be a practical technique. X-ray CT provides the raw data on geometric relationships and tissue properties to accurately model transmission pathways (Soldevilla et al., 2004). With X-ray CT as the input for models, the foundation for a better functional understanding of echolocation is evolving.
This study provides the first quantitative definition of the melon for a number of odontocete species. Expanding the definition to a broader spectrum of species and age classes will corroborate interspecific, intraspecific, developmental, and functional predictions presented. This study also provides the foundation to address the evolution of the melon and understand the possible contribution of natural selection and/or constraints to explain the variation found in structure of the melon.
CHAPTER 4

INSIGHTS INTO THE EVOLUTION OF ECHOLOCATION

INTRODUCTION

Odontocetes (toothed whales) emit directional high intensity sounds and use the information from the returning echoes to sense their surrounding environment, this is defined as echolocation. Echolocation in odontocetes is demonstrated in both experimental settings and field recordings (Au, 1993; Johnson et al., 2004).

The evolution of echolocation in odontocetes is likely a key innovation associated with their diversification into previously unexploited niches, namely light deficient habitats (Hunter, 1998). The fossil record supports this hypothesis; there is a radiation of odontocetes early in their history, Eocene/Oligocene boundary (~34-35 Ma), compared to their sister taxa, mysticetes (Fordyce, 2002). Furthermore, a suite of morphological modifications associated with echolocation are also present early in the evolution of odontocetes. This study investigates the origin and transitions in the sound production/propagation anatomy in cetaceans.

The anatomy associated with echolocation can be divided into sound reception and sound production/propagation components. The morphological transitions in sound reception are well preserved in the bony ear complex of fossil cetaceans. Modifications in the ear that influenced sound reception mechanisms occurred early in cetacean evolution. An intermediate morphological stage for hearing capabilities on land and in the water is found in
Morphology associated with sound production will most likely provide additional evidence for the evolution of echolocation; however, the structures associated with sound production/propagation are mainly soft tissue structures, therefore difficult to trace in the fossil record.

The anatomy of sound production/propagation is a well investigated topic in living odontocetes, despite the limited amount of information in the fossil record. It is generally accepted that click generation begins by action of the palatopharyngeal muscle complex, as it forces the larynx up into the interior bony nares and pressurizes the air in the bony nasal passages. The pressurized air passes through lips formed by a narrow slit in the spiracular cavity, causing vibrations in adjacent ellipsoid fat bodies. These vibrations are reflected forward by the skull and air sacs functioning as acoustic mirrors. The sound vibrations propagate through the melon anteriorly and emerge into the environment as a click. The melon (homologous to the junk in a sperm whale [Cranford, 1999]) is a fat and connective tissue structure that apparently functions to focus sound and decrease acoustic attenuation at the tissue-water boundary (Cranford and Amundin, 2004).

Investigations of the sound production anatomy combined with physiological experiments have identified five functional components in the odontocete forehead (Mead,
1975; Heyning, 1989; Cranford, 1992; Au, 1993; Cranford, 1996; Cranford et al., 1997; Cranford et al., 2000): 1) nervous system (command center), 2) respiratory system (power supply), 3) nasophonation system (signal generators), 4) nasal musculature (signal modifiers), and 5) the melon and associated structures (signal propagators).

Marino et al. (2004) investigated morphological changes associated with the first component, specifically the evolution of brain size in cetaceans. Their results show that the odontocete brain increased significantly in two critical phases: at the origin of odontocetes near the Eocene-Oligocene boundary (~35 Ma) and the origin of Delphinoidea (Delphinidae, Monodontidae, and Phocoenidae) 15 Ma. One hypothesis for the first increase in odontocete brain size is related to the processing of echolocation sounds (Marino, 2004).

This study investigates transitions in the respiratory system, the nasophonation system, and melon and associated structures (components 2, 3, and 5). Reconstructing the morphological transitions in these anatomical components may help resolve current uncertainties regarding the origin of the structures. For example, Heyning and Mead (1990) suggest that the melon is an exaptation for echolocation and is present in mysticetes, contrary to previous hypotheses that the melon is a synapomorphy for odontocetes. Defining the melon and reconstructing morphological transitions will help determine its origin and provide further insight into the evolution of echolocation.

In this study, both soft and bony characters are described and mapped onto recent cetacean phylogenies. The present study provides the first description and reconstruction of the changes in the morphologies associated with the production/propagation of echolocation sounds in fossil odontocetes. The results help resolve the origin of the production/
propagation of echolocation sounds, demonstrate how the morphology is modified in extant taxa, and reconstruct fossil soft tissue anatomy.

**MATERIALS AND METHODS**

To infer transformations in the evolution of the odontocete forehead anatomy, morphological characters were optimized onto three phylogenies (Messenger and McGuire, 1998; Geisler and Sanders, 2003) built from different characters sets and taxa. The first phylogeny used was the most recent and comprehensive morphological study (Geisler and Sanders, 2003). The phylogeny included both fossil and extant species and was used to reconstruct ancestral morphologies and infer fossil soft anatomy (Fig. 54). There is little agreement regarding the position of the river dolphins in Geisler and Sanders (2003) (Messenger and McGuire, 1998; Cassens et al., 2000). Therefore, a second phylogeny was used to compare the effect of the different positions of the river dolphins on ancestral reconstructions (Fig. 55). The third phylogeny used was Messenger and McGuire (1988) (Fig. 56). They presented a combined analysis of morphological and molecular characters, but only extant taxa were included. The tree was pruned according to taxa available for this study.

Characters were optimized onto the three topologies using the program Mesquite (Maddison and Maddison., 2004). Using a phylogenetic tree and a distribution of character states in the observed (terminal) taxa, Mesquite reconstructs the character states at ancestral nodes. Both parsimony and maximum likelihood resolving options were used to reconstruct ancestral states. Parsimony reconstruction methods find the ancestral states that minimize the number of steps of character change given the tree and observed character distribution. The likelihood reconstruction methods find the ancestral states that maximize the probability that
Figure 54. Pruned phylogeny from Geisler and Sanders (2003). Phylogeny is built from strict consensus of 21 most parsimonious trees for the matrix of 56 extinct (†) and extant taxa codes for 304 morphological characters.
Figure 55. Composite phylogeny based on Geisler and Sanders (2003) and Messenger and McGuire (1998). The position of the river dolphins (Pontoporiidae) is changed based on Cassens et al. (2000).
Figure 56. Strict consensus of tree obtained in the combined analysis of the morphology and modified DNA sequence data set (Messenger and McGuire, 1998). The numbers above and below the nodes represent the bootstrap values with morphological characters weighted as transitions and transversions, respectively. Solid boxes represent the sampled extant taxa and dotted boxes represent sampled extinct taxa.
the observed states would evolve under a stochastic model of evolution. The likelihood
reconstruction finds, at each node, the state assignment that maximizes the probability of
arriving at the observed states in the terminal taxa, given the model of evolution (Mk1), and
allowing the states at all other nodes to vary. The maximum likelihood method was preferred
because the equivocal character states could be reconstructed based on relative likelihood,
but when character coding was incomplete or polymorphic, parsimony was employed. All
soft tissue reconstruction used the parsimony option. Neither ACCTRAN nor DELTRAN
were employed as both require all nodes to be dichotomous.

The ingroup consists of 13 extant and 11 fossil odontocete species. The analysis
includes at least one representative from 8 of the 10 extant odontocete families (Ziphiidae,
Physateridae, Kogiidae, Pontoporiidae, Iniidae, Monodontidae, Delphinidae, and
Phocoenidae) (Rice, 1998). The river dolphin families Lipotidae and Platanistidae were
unavailable and therefore not included. Based on availability, three extinct families were
examined and included in the analysis (Squalodontidae, Eurhinodelphidae, and
Kentriodontidae) of the 10 described extinct families of odontocetes (Agorophiidae,
Squalodontidae, Eurhinodelphidae, Waipatiidae, Squalodelphinidae, Dalpiaziniidae,
Kentriodontidae, Albireonidae, and Odobenotopsidae [Barnes, 2000]). Extinct species of the
families Kogiidae, Physeteridae, Monodontidae, and Delphinidae were also included. The
outgroups included two modern mysticetes (*Eschrichtius robustus*, gray whale and
*Balaenoptera physalus*, fin whale) and one extinct basilosaurid archaeocete (*Zygorhiza
kochii*). Appendix F comprises a list of the specimens examined.

Morphological characters serve as the raw data from which phylogenetic inferences
are based. Soft tissue characters were collected from X-ray Computed Tomography (CT)
scans of extant specimens. The CT data for the postmortem heads were acquired from three sources (Appendix A, B, and F): 1) a library of previously collected scans (Cranford, 1992; Cranford, 1996), 2) a medical hospital scanner, and 3) an industrial scanner.

In preparation for the hospital scanner, the heads of the postmortem specimens were separated and frozen soon after death to retard decomposition. Before scanning, the heads were thawed in a water bath to ensure homogeneity in tissue temperature and placed in registration frame to put all the specimens in the same orientation and provide a calibration measure.

The industrial scanner was used for the larger specimens. The procedure followed prior to scanning differed from the hospital scanner in that the heads scanned in the industrial scanner remained frozen and were incased in cardboard and foam. A registration frame was also used in the industrial CT scanner, but in this case, density poles were inserted within the cardboard frame and allowed the scans to be calibrated.

Data acquired for all specimens was processed using Analyze 5.0 /6.0, created by the Biomedical Imaging Resource at Mayo Clinic (Robb and Barillot, 1989; Robb et al., 1989; Robb, 1999). The individual scans were compiled and converted to AVW formats (a native Analyze format) that takes serial 2-D images and forms a 3D volume of each specimen. The slice thickness in all scans was interpolated to 1.5 mm (Appendix A).

The digital images were compared to dissected specimens (Appendix C). Additional soft tissue characters were drawn from the literature (Cranford, 1996). Osteological characters were collected from CT scans of the modern taxa and museum specimens (modern and fossil) at the United States Natural History Museum, Smithsonian Institution, Washington, DC (USNM). All characters are described in Appendix G.
Character states for 24 characters (6 bony and 18 soft) were entered into Mesquite software program (Maddison and Maddison, 2004) as unordered and unweighted (Appendices H and I). Six characters were binary and 18 multistate. Character states not assigned because of incomplete preservation, inapplicability to the taxon, or ambiguous morphologies were entered as a “?” in the matrix. Polymorphic characters were entered as (0/1) (Appendices H and I).

To test the correlations between characters, pairwise comparisons were performed in Mesquite. When the phylogeny is insufficiently resolved, or when less confidence can be placed on ancestral state reconstructions, pairwise comparisons are sometimes used (Nunn and Barton, 2001). The method of pairwise comparisons has the advantage of avoiding assumptions about ancestral states, branch lengths, and elaborate models of evolution (Maddison, 2000). The pairwise comparison method loses information by focusing on only a subset of branches and comparisons (Felsenstein, 1985), and may have low power to detect correlations (Grafen and Ridely, 1996). To determine the number of pairwise comparisons, the characters were mapped on a pruned phylogeny (Messenger and McGuire, 1998) that was modified with the resolved sister relationship between *Lissodelphis* and *Lagenorhynchus* based on molecular data (R. McGowen, personal communication). Taxa included were only those with complete character information. Using the most pairs option in Mesquite, four pairs of independent taxa were identified under the criteria that internal nodes are not reconstructed and branches are not used more than once (Fig. 57).
Figure 57. All possible pairwise comparisons on pruned phylogeny of Messenger and McGuire (1998). The letters correspond to the pairwise contrasts determined in Mesquite used to test character correlations. The key requirement is that the internal nodes are not reconstructed and branches are not used more than once, four pairwise comparisons are extracted from this phylogeny.
RESULTS

Morphological transitions in three of the five anatomic components of echolocation (power supply, signal generations, and signal propagators) are presented. Characters 14-15, 18-20, and 22-23 describe the transitions in the power supply for the generation of echolocation sounds. The changes in echolocation signal generators (bursae complexes) are highlighted in characters 11-13, and 23. Signal propagation by the skull, melon, and theca are illustrated in characters 1-10, 16-17, 21, and 23-24. For the three echolocation components, inferences on the origin of structures, modification in extant taxa, and fossil reconstructions of soft tissue are reported.

Power Supply

The power supply for an echolocation click begins in the respiratory system: the action of the palatopharyngeal muscle complex forces the larynx up into the inferior bony nares and pressurizes the air in the bony nasal passages (Cranford et al., 1997). The modifications in the respiratory system (component 2), namely the bony structures, are well documented in both fossil and extant cetaceans. A posterior location and dorsal orientation of the nasal passages is associated with the telescoping of the odontocete skull (Miller, 1923). The narial opening moved from a typical anterior position in most mammals to a dorso posterior position, apparently associated with efficiency of surface breathing (Barnes and Mitchell, 1978).

The dorsal position of the nasal passages combined with the concave dorsal surface of the skull and the elevation of the facial bones potentially make room for the signal generators located directly above the bony nasal passages (power supply). Furthermore, the location,
position, size, and shape of the bony nasal passages potentially influence the amount of air pressure built up in the bony nasal passage.

The dorsally positioned nasal passages and concave skull most likely evolved in a common ancestor to all odontocetes using the maximum likelihood option on the Geisler and Sanders (2003) phylogeny (Fig. 58). The likelihood of dorsally positioned nasal passages being present in the common ancestor of all odontocetes is 0.962, and the likelihood of the concave skull is 0.836. Based on these characters, I concluded that the power supply for echolocation clicks originated in odontocetes.

Further modification in the bony nasal passages within odontocetes, specifically in the size and shape of the nasal passages, implies a specialized function. The right nasal passage opening is smaller and less circular in most extant odontocetes (Fig. 59 B), possibly adapted to create a higher pressure chamber on the right side. In contrast, the larger and more circular opening on the left is potentially modified for breathing (Fig. 60 B).

Alignments of soft tissue structures supporting this hypothesis are: the position of the right bursae complex dorsal to the right nasal passage, and the left nasal passages with the blowhole. In *Tursiops*, *Delphinus*, *Lagenorhynchus*, and *Lissodelphis*, the right bursae complex is slightly larger and located directly dorsal to the right nasal passage. This soft tissue character appears to correlate with the smaller and less circular opening of the right nasal passage (Fig. 59), although this correlation is not statistically significant. The position of the blowhole shows a relationship with the larger and more circular opening of the left nasal passage (Fig. 60). This is particularly evident in the pygmy sperm whale (Character 14, Fig. 60). In *Phocoena* and *Pontoporia*, there is little disparity in the circularity or size of the nasal passages. These species also have little difference in the size of the right and left bursae
Figure 58. Mirror diagram showing character transitions associated with the power supply in echolocation sound production (Characters 22 [A] and 23 [B]). Characters reconstructed on Geisler and Sanders (2003) phylogeny using maximum likelihood option. The shaded circles represent the likelihood of a specific character states in the hypothetical ancestor.
Figure 59. Mirror diagram showing the specialization of the right bony nasal passage and associated soft tissue character (Characters 11 [A] and 19 [B]). Characters traced on Messenger and McGuire (1998) phylogeny using parsimony to reconstruct ancestral morphologies. The boxes at the terminal nodes indicate the character state present in the taxa; no boxes means the state was inferred. The branches that show multiple colors are equivocal and the ancestral conditions cannot be inferred.
Figure 60. Mirror diagram showing the specialization of the left bony nasal passage and associated soft tissue character (Characters 15 and 19). Characters traced on Messenger and McGuire (1998) phylogeny using parsimony to reconstruct ancestral morphologies. The boxes at the terminal nodes indicate the character state present in the taxa; no boxes means the state was inferred. The branches that show multiple colors are equivocal and the ancestral conditions cannot be inferred.
complexes (Figs. 59 and 60). In the pygmy sperm whale and Cuvier’s beaked whale, the spermaceti organ is positioned dorsal to the right nasal passage (Figs. 59 and 60). The opening of the left bony nasal passage in the pygmy sperm whale is twice as large as the right.

The extinct odontocete lineages (*Kentriodon* and *Eurhinodelphis*) have dorsally positioned and symmetrical bony nasal passages. When reconstructing the soft tissue anatomy using parsimony, the size disparities between the right and left bursae complexes were equivocal. Both *Kentriodon* and *Eurhinodelphis* most likely had a left position of the blowhole, similar to modern dolphins.

**Signal Generators**

In the production of an echolocation click, the pressurized air passes through lips formed by a narrow slit in the spiracular cavity, causing vibrations in adjacent ellipsoid fat bodies (bursae complexes) (Cranford and Amundin, 2004). The signal generators are soft tissue structures and difficult to trace in fossil species. As mentioned previously, the dorsal position of the nasal passages, the concave dorsal surface of the skull, and the elevation of the facial bones potentially make room for the signal generators located dorsal the openings of the bony nasal passages. The presence of bursae complexes most likely evolved early in the evolution of odontocetes based on the slight elevation of the skull posterior to the nasal passages found in Squalodontidae, but absent in mysticetes and archaeocetes (Fig. 58).

Modifications in the number and size disparity in the signal generators (bursae complexes) are present in extant odontocetes. Comparative anatomical studies using X-ray CT of the signal generators (bursae complexes) showed the variation across odontocetes and identified two basic anatomic configurations of the signal generators: 1) unilateral configura-
tion or “one sound source” found in sperm whales (*Ziphius cavirostris*, *Kogia breviceps*, and the giant sperm whale); and 2) bilateral configuration or “two sound sources” in all other odontocetes, with a propensity toward directional asymmetry (right larger than the left) (Cranford, 1992, 1996). In *Tursiops, Delphinus, Lagenorhynchus*, and *Lissodelphis*, the right bursae complex is slightly larger than the left. *Phocoena* and *Pontoporia* have little difference in the size of the bursae complexes. A hypertrophied right anterior dorsal bursa or spermaceti organ is present in the *Ziphius cavirostris*, *Kogia breviceps*, and the giant sperm whale, suggesting that particular morphology was favored early in odontocete evolution.

Reconstructions of the number and size disparity of the bursae in the fossil lineages (*Kentriodon* and *Eurhinodelphis*) using the parsimony option in Mesquite, indicate that the left and right bursae complexes were present. The size disparities between the right and left bursae were equivocal in *Kentriodon* and *Eurhinodelphis*.

**Signal Propagation**

Signal propagation is most likely accomplished through a combination of the skull, air sacs, and melon (Aroyan et al., 1992). The components all play an integrated role in creating an amphitheater of acoustic mirrors that reflect sound anteriorly. Modifications to the cetacean skull are well documented. The presence of a spiracular plate in odontocetes is diagnostic of the presence of air sacs; therefore, echolocation capabilities are probable in even the earliest cetaceans (Barnes, 2000). The amphitheater shape of the skull accomplished by a concave dorsal surface and elevation of the facial bones posterior to the nasal passages is present in basal odontocetes and absent in mysticetes and archaeocetes (Fig. 58). The amphitheater shape of the skull suggests some degree of reflection of sound by the skull evolved in early odontocetes. Inferring the origin of the connective tissue theca is equivocal.
The skull posterior to the nasal passages in odontocete lineages is further modified, mainly in the degree of the projection of the skull anterior to the nasal passages, creating a dorsal bony reflection surface. Although *Eurhinodelphis* has a slight projection over the bony nasal passages, the most extreme projection of the skull is present in *Ziphius cavirostris*. In addition, *Ziphius cavirostris* has a thick theca along the dorsal and lateral sides of the forehead. The presence of the theca can be inferred in archaic dolphins (*Kentriodon* and *Eurhinodelphis*). Using the parsimony option, both the Geisler and Sanders (2003) phylogeny and the composite tree topology suggest that *Kentriodon* and *Eurhinodelphis* had a theca similar to dolphins.

The melon, a soft tissue structure embedded in the tissue lining the dorsal surface of the skull and composed of unique fats, is presumably involved in the propagation of echolocation sounds. Although the elevation of the vertex of the skull and the concavity of the skull do not correlate significantly to the presence of a melon, these characters offer information on the morphology underlying the melon and allow inferences on origin of the melon. The absence of a similar soft tissue structure in the same location and density in extant mysticetes, the lack of elevation of the skull, and orientation of nasal passages imply that the melon is unique to odontocetes.

Within extant odontocetes, the melon varies in composition, shape, and internal structure; factors that will most likely influence sound propagation. Previous studies identified the unique chemical composition of the melon and the variation across odontocetes (Varanasi and Malins, 1972; Litchfield et al., 1975, 1979; Koopman et al., 2003) (Fig. 61). The density composition of the soft tissue in basal odontocete species (*Ziphius cavirostris* and sperm whales) consists of two soft tissue structures associated with sound propagation:
Figure 61. Comparison of morphological reconstructions on different phylogenies for fatty acid branching in the melon (Character 10). Red stars indicate the different reconstruction. (A) Topology for Geisler and Sanders (2003) showing a resolved character state for Eurhinodelphiidae† and Kentriodontidae†. (B) Composite tree topology showing an unresolved character state for Eurhinodelphiidae† and Kentriodontidae†.
the lower density spermaceti organ, and the melon. Kogiidae and later diverging extant odontocetes have gradients of higher density tissue in the outer melon and a low density core. This pattern of concentric low density layers is especially apparent in *Kogia breviceps*.

The shape of the melon also varies across extant odontocetes, making it difficult to infer melon shapes without internal examination. For example, within the family Delphinidae the shape of the melon in *Lagenorhynchus* is different from other analyzed species within the same family; the ancestral melon shape to the family is equivocal. Modification of the melon occurred early in odontocete evolution based on the variation in composition and shape of the melon present in basal odontocetes.

Inferring the specific morphology of the melon is equivocal in basal odontocete lineages outside the crown group. Using the parsimony option, the morphology in archaic dolphins (*Kentriodon* and *Eurhinodelphis*) can be inferred. Ten characters (1-10) associated with the morphology of the melon were traced onto both the Geisler and Sanders (2003) and composite topologies. In a comparison between the two topologies of the inferred melon character states for *Kentriodon* and *Eurhinodelphis*, there were four conflicting hypotheses. For example, Geisler and Sanders (2003) inferred a blunt anterior morphology of the melon core (character 5) for both *Kentriodon* and *Eurhinodelphis*. The composite tree failed to resolve this character state. The chemical composition of the melon also varied depending on the topology (Fig. 61). The Geisler and Sanders (2003) tree suggested the presence of iso12 and no iso5 in the melons of *Kentriodon* and *Eurhinodelphis*; the carbon structure could not be reconstructed. In the composite topology, the carbon structure in the melon was all triglycerides, but the fatty acid branching is equivocal.
The informative character states of the melon that were the same on both topologies included: 1) right posterior extension from main body of melon; 2) elliptical shaped melon; 3) anterior terminus of the melon ends posterior to the tip of the rostrum; 4) two fat body complexes present; and 5) intermediate sized melon relative to head size (see Fig. 64, p. 135).

**DISCUSSION**

Comparisons of forehead soft and hard anatomy in fossil and extant odontocetes provide the first quantitative descriptions and reconstructions of changes in structures associated with the production/propagation of echolocation sounds. For the purposes of this study, the morphology associated with the ability to produce clicks is divided into three functional categories: power supply, signal generators, and signal propagators. Inferences were made on the origin, modification, and fossil morphology of the three components. Combination of these results with transitions in the sound reception pathways and ears will provide a more complete picture of the evolution of echolocation.

The results of phylogenetic inferences suggest that the three components of echolocation investigated (power supply, signal generators, and signal propagators) originated in odontocetes. The main evidence to support this hypothesis is the posterior location and dorsal orientation of the bony nasal passages combined with the concave skull and elevation of the facial bones present in basal odontocetes and absent in mysticetes and archaeocetes (Fig. 62). This morphology creates a dorsally oriented power supply, room for the signal generators and melon, and an amphitheater to reflect the sound forward.

The origin of the melon in odontocetes is further supported by the absence of a melon in mysticetes based on density and positional comparisons, contrary to a previous hypothesis.
Figure 62. Origin of characters associated with the echolocation power supply, signal generators, and signal propagators on pruned Geisler and Sanders (2003) tree.
by Heyning (1989). To completely rule out the presence of the melon in mysticetes, comparisons with adults and chemical composition analyses in the forehead region are needed.

Further morphological modifications imply specializations of the echolocation system in odontocete lineages. The most prominent alterations in the bony nasal passages, signal generators, and melon are present in basal odontocetes (Ziphiidae and Kogiidae), suggesting that specialization in the sound production/propagation originated early in odontocete evolution (Figs. 59 and 60).

A comparison of the morphology with echolocation signals highlights a possible acoustic function of these modifications. Echolocation signals sort into two broad categories (Cranford, 2000): 1) a brief broadband click with a duration of 40-70µs (*Tursiops truncatus, Inia geoffrensis*), and 2) narrowband click with a duration greater than 100µs (*Phocoena phocoena, Cephalorhynchus commersonii, Cephalorhynchus hectori, Phocoenoides dalli, and Kogia sp.*). The variation noted in click structure correlated to the anatomy (Cranford, 1996). The animals with broadband signal (*Tursiops truncatus, Inia geoffrensis*) have similar morphologies in the sound production structures: two different size sound sources (bursae complexes) (Fig. 63). Species in the narrowband category (phocoenids, ziphiids, and physeterides) have one sound source (or symmetrical bursae complexes). Animals in the narrowband category represent diverse taxa. The convergence in signal type may be a result of habitat.

Parsimony reconstructions of the anatomy associated with echolocation sound production/propagation were used to infer odontocete fossil anatomy. *Kentriodon†* and *Eurhinodelphis†* are nested within odontocetes. The extinct dolphins most likely had a
Figure 63. Mirror diagram showing echolocation signal types and morphology of signal generators (Character 14 [B]). Characters traced on Messenger and McGuire (1998) phylogeny using parsimony to reconstruct ancestral morphologies. The boxes at the terminal nodes indicate the character state present in the taxa; no boxes means the state was inferred. The branches that show multiple colors are equivocal and the ancestral conditions cannot be inferred.
slightly left position of the blowhole, right and left bursae complex, a theca similar to dolphins, right posterior melon extension, a right position of the melon compared to the midline of the skull, and the anterior terminus of the melon ends posterior to the tip of the rostrum (Fig. 64).

The analysis with the two conflicting phylogenies revealed some discrepancy in the fossil reconstructions, affecting parsimony hypotheses. Resolving odontocete relationships is needed to make accurate inferences on ancestral conditions and fossil soft anatomy.

The timing of the ability to produce and propagate echolocation clicks does not appear to correspond with the origin of fully aquatic hearing capabilities (Nummela et al., 2004) (Fig. 65); however, specializations to the inner ear are unique to odontocetes (Fleischer, 1976). The results of this study support the hypothesis that the ability to produce echolocation clicks evolved separately from underwater hearing (Fordyce and Barnes, 1994) (Fig. 65). The ability to interpret the vast amounts of acoustic information in the marine environment through specialized hearing was most likely crucial to cetacean evolution and invasion into the marine realm. Investigations on sound reception pathways might provide further information on the evolution of echolocation sound reception.

Another important component to the behavior of echolocation is the interpretation of the complex signals possibly reflected in the neural anatomy. The odontocete brain increased significantly in two critical phases: at the origin of odontocetes near the Eocene-Oligocene boundary (~50 ma) and the origin of Delphinoidea (Delphinidae, Monodontidae, and Phocoenidae) 15 Ma (Marino et al., 2004). I would argue that the first increase in encephalization (EQ) in odontocetes seems to originate around the same time as the ability to produce and propagate echolocation sounds; the second increase in EQ in Delphinoidea does not
Figure 64. Inferred soft tissue morphology in *Kentriodon pernix* (USNM 10670) pictured on the CT scan of a skull of *Tursiops truncatus*. (a) right position of the posterior extension of the melon. (b) anterior terminus of the melon posterior to the tip of the rostrum (c) presence of two fat body complexes (the size disparity is unknown).
Figure 65. Complied character transitions assumed to be associated with echolocation. Hearing characters are from Nummela et al. (2004) and Fleischer (1976). The brain characters are from Marino et al. (2004). The sound production and propagation characters are from this study.
appear to correspond with a specialization in the morphology of the echolocation system (Marino, 2004). Identifying changes in functional areas of the brain is needed to link changes in echolocation anatomy with brain changes.

This study assumes that echolocation in odontocetes is reflected in the intricate morphology associated with both the production and reception of clicks. The assumption that bone is a proxy for soft tissue changes is conjectural, especially because no statistically significant osteological correlates were identified.

Future work should be directed at describing the melon in all extant odontocete species to increase the number of pairwise comparisons for the purpose of testing character correlation. In addition to the problems with character correlations, phylogenetic relationships are contentious. In this study, characters are traced onto various topologies to address this problem. A well supported phylogeny using molecular and morphological characters and including extinct odontocetes is crucial to reconstruct the fossil and ancestral morphology accurately. A well supported tree might allow comparisons with habitats to explore morphologic transitions and echolocation signal type. The available evidence to date confirms that the ability to echolocate is unique to odontocetes and is most likely associated with their diversification, 34-35 Ma.
CHAPTER 5

CONCLUSIONS

Odontocetes have acquired myriad adaptations for life in an aquatic environment. Toothed whales developed the ability to echolocate; an active system which allows animals to forage, avoid obstacles, and orient in the absence of light. This study explored the anatomy and evolution of the melon, a structure presumably involved in the propagation of echolocation sounds.

X-ray Computed Tomography (CT) was employed to quantify the size, shape, internal topology, and geometric relationship of the melon to other forehead structures. The results of the descriptions were analyzed in both a comparative anatomical and evolutionary context. Predictions on the pathway of sound through the odontocete head were presented, and inferences were made on the origin of the melon.

The use of CT proved to be a valuable tool. Chapter 2 compared CT scans of a live bottlenose dolphin (*Tursiops truncatus*) to a recently dead specimen and to frozen and thawed specimens; little disparity in internal structure and density of forehead was found across these treatment groups. The results have implications for future anatomic studies using postmortem material. In addition, using CT data as input for finite element modeling is conceivable. Increasing the number of specimens analyzed in the different treatment groups will corroborate the accuracy of these applications.

Considerable differences in the melon across the major odontocete lineages were found. The internal structure of the melon has a distinct pattern of density change: higher
density shell grades into a lower density core. This pattern of concentric low density layers is especially apparent in *Kogia breviceps*. The density gradient present in all other families, combined with tissue composition data, suggests a specific function of the melon. In *Ziphius cavirostris*, the melon appears to be more homogeneous and without organized density layers. The lower density spermaceti organ and thick connective tissue theca might function to focus the sound; however, it is important to note that this specimen was frozen during the scanning process.

Predictions of the pathway of sound were made based on melon morphology. The results of the pathway of sound through the harbor porpoise (*Phocoena phocoena*) were similar to recorded differences in acoustic signal (Au et al., 2004). Additional work is necessary to determine if function relates to the form in other odontocete species. Hydrophones on the surface of echolocating animals can be used to test these morphological predictions. And, modeling might help determine the function of the melon in rare animals.

This study presented preliminary conclusions on the evolution of the forehead structures associated with sound production/propagation; however, the morphological changes in the melon did not statistically correlate with the underlying bony structures. Transitions thought to be associated with echolocation production/propagation are the dorsal orientation of the bony nasal passages, posterior movement of the facial bones, and concavity of the dorsal skull. These characteristics are present in basal odontocetes, but absent in their closest fossil and living relatives. In addition, no melon was found in the two juvenile baleen whales examined. The melon most likely evolved early in odontocete evolution and is a synapomorphy for odontocetes, contrary to previous hypotheses (Heyning and Mead, 1990).
Variations in the nasal passages, position of blowhole, and shape of the melon in later diverging toothed whales allude to specializations in the echolocation system. For example, the smaller right bony nasal passage aligns with the right bursae complex and the right posterior extension of the melon in most delphinids, suggesting that the right side is dedicated to propagation of echolocation clicks. The alignment of the left bony nasal passage with the blowhole complements this argument and implies that the left side is mainly dedicated to breathing.

Phylogenetic inferences of the transitions in the echolocation sound production/propagation were combined with previous studies on changes in the ears and brain to produce a more comprehensive look at the evolution of echolocation. Hearing in water evolved in archaeocetes (Nummela et al., 2004); however, specializations in the inner ear are unique to odontocetes (Fleischer, 1976). The pathway of sound to the ear needs to be examined to investigate the correlation of sound production/propagation and ability to hear high frequency sounds.

The first increase in encephalization (EQ) in odontocetes seems to occur at the same time as the ability to produce echolocation sounds; the second increase in EQ in Delphinoidea (Marino, 2004) does not appear to correspond with a specialization in the morphology of sound production and propagation. Identification of changes in functional areas of the brain is needed to link changes in echolocation anatomy with brain changes.

Documenting morphological intricacies of odontocete echolocation proved critical to understanding the functional significance and evolutionary history of the system. The results are also important in understanding possible key innovations that have led to diversification into unexploited habitats. The sophisticated nose in odontocetes evolved in a relatively short
period of time (~35mya). The superior propagation of sound in water, compared to land, potentially drove the radiation of odontocetes and allowed them to inhabit the light deficient oceans. Further modifications in the soft anatomy present in extant species suggest that the animals continued to adapt to different acoustic environments of the ocean.
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CHAPTER 1


Heyning JE. 1989. Comparative facial anatomy of beaked whales (Ziphiidae) and a systematic revision among the families of extant Odontoceti. Contrib Sci 405:1-64N.


**CHAPTER 2**


multidimensional medical image display and analysis. Comput Med Imaging Graph 13.


SPSS, I. 2000. SYSTAT.


CHAPTER 3


Heyning JE. 1989. Comparative facial anatomy of beaked whales (Ziphiidae) and a systematic revision among the families of extant Odontoceti. Contrib Sci 405:1-64N.


CHAPTER 4


Heyning JE. 1989. Comparative facial anatomy of beaked whales (Ziphiidae) and a systematic revision among the families of extant Odontoceti. Contrib Sci 405:1-64N.


**CHAPTER 5**


APPENDIX G


APPENDIX A

X-RAY COMPUTED TOMOGRAPHY PARAMETERS
<table>
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<th><strong>Kogia breviceps</strong></th>
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<th><strong>Ziphius cavirostris Neonate</strong></th>
<th><strong>Mesoplodon perrini</strong></th>
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SFOV = scanning field of view; KV = kilovolts; MA = milli-amps
APPENDIX B

ADDITIONAL CT SCANNED SPECIMENS
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APPENDIX C

DISSECTED SPECIMENS
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APPENDIX D

INSTRUCTIONS FOR EXTRACTING, LOGGING, AND EXPORTING MEASUREMENTS USING ANALYZE 5.0/6.0
I. Region of Interest (ROI)
   a. Options
      i. Generate/Sample options: select which measurements
      ii. Configure log stats to set measurements desired
   b. Sampling
      i. In main ROI window highlight “sample images”
      ii. Click in image (should automatically create sample and log window)
   c. Log
      i. Generate/ Sample options: turn log on
      ii. Right click in log window will allow you to edit the output
   d. Export to excel worksheet
      i. Right click “save log as” choose location, name
      ii. Open excel new document
      iii. Open stats file just saved (remember to highlight “all files” for the file type)
      iv. In text import window
         1. Fixed width (next)
         2. Divide data sheet to desired width (just click on arrows) (next)
         3. General (finish)
         4. If it does not look correct play with the above parameters

II. Volume Render
   a. First only render objects to be measured in View/ Objects/Attribute/ Display
   b. Options
      i. Tools Menu/ Measure: choose- point, line, angle, volume, or area,
      ii. Separate window will appear
   c. Sampling
      i. Click on image (must be on image)
   d. Log
      i. Click on log button in sample window
   e. Export to excel worksheet
      1. Fixed width (next)
      2. Divide data sheet to desired width (just click on arrows) (next)
      3. General (finish)
      4. If it does not look correct play with the above parameters
APPENDIX E

SUMMARY OF HOUNSFIELD UNITS

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APPENDIX F

SPECIMENS USED IN PHYLOGENETIC ANALYSIS
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APPENDIX G

CHARACTER DESCRIPTIONS
SOFT TISSUE CHARACTERS

MELON

**Character 1.** Melon: 0= absent, 1=present.

The melon was defined in CT images as a low-density area located anterior to the spiracular cavity and extending anteriorly, dorsal to the premaxillary bones. A quantitative definition of the melon was established based on density (see Chapter III, Methods: Melon Definition).

A melon was not found in CT scans of two neonate mysticetes, despite a previous study that proposed a small melon present in mysticetes (Heyning and Mead, 1990). The presence of the melon distinguishes odontocetes from mysticetes.

**Character 2.** Density change in the melon: 0=homogeneous (0-20 H units), 1=intermediate heterogeneity (20-40 H units), 2= large heterogeneity (40-60 H units), 3=extreme heterogeneity (60+ H units) (Fig. 1).

To determine the range of density in the melon, the H values in CT scans were used. CT data is collected as a series of digital images or thin cross-sections of the specimen known as serial tomograms or scans. Each scan is comprised of a matrix of values (CT numbers) expressing linear attenuation of X-rays of the material at a specific geometric location within the specimen. The CT numbers are calibrated to the Hounsfield scale (–1000 to 1000) and renamed Hounsfield (H) units. The H unit is a calibrated measure of electron density which is equivalent to the change in density of 1 cubic cm of water 1 degree centigrade at standard temperature and pressure. When data sets are imported into image processing program, the images are rescaled to the same Hounsfield scale, making the H values directly comparable.

The minimum and maximum H value in the melon was collected transversely every 1.5 mm. The difference between the average minimum and average maximum values was calculated.

*Kogia breviceps* had the most heterogeneous melon density. *Lissodelphis borealis, Delphinus delphis*, and *Pontoporia blainvillei* showed a large degree of density difference within the melon. *Ziphius cavirostris, Phocoena phocoena* and *Lagenorhynchus obliquidens* showed intermediate tissue differentiation in the melon (Table 6). The neonate outgroups *Eschrichtius robustus* and *Balaenoptera physalus* had no tissue differentiation anterodorsal to the bony nasal passages.

**Character 3.** Posterior melon: 0=no extension, 1= right posterior extension, 2=centered posterior extension (Fig. 1).

The main body of the melon has an elliptical shape; however, the posterior melon varies. *Ziphius cavirostris* and *Kogia breviceps* have no posterior extension from the main body of the melon. All other odontocetes examined had a posterior extension from the melon. In *Tursiops truncatus, Lissodelphis borealis, Delphinus delphis*, and *Pontoporia blainvillei*, the posterior extension from the main body of the melon is to the right of the midline of the skull. In *Phocoena phocoena* the posterior extension of the melon is positioned in line with the midline of the skull.
Character 4. Anterior terminus of the melon: 0= projects anterior to the tip of the bony rostrum, 1= no projection over the tip of the rostrum (Fig. 2).

The melon in *Kogia breviceps* extends anteriorly to the tip of the bony rostrum. In all other odontocete taxa examined the melon did not extend anterior to the tip of the bony rostrum.
Fig. 2: Anterior melon morphology. Pictured here is a dorsal view of 3D reconstruction and outline of (A) *Tursiops truncatus*, and (B) *Kogia breviceps* (anterior projection). The red dotted line represents the transverse plane at the tip of the bony rostrum.

**Character 5.** Melon core: 0= tapers anteriorly, 1= no anterior taper (Fig. 3).

The lowest density core of the melon varied in the anterior region. In *Tursiops*, *Delphinus*, and *Phocoena* the melon tapers to a point along the midline of the skull. In *Kogia breviceps*, *Lissodelphis borealis*, *Lagenorhynchus obliquidens*, *Ziphius cavirostris*, and *Pontoporia blainvillei*, the anterior region does not taper along the skull, instead is has more of a blunt appearance.
Character 6. Position of the melon in relation to the midline of the skull: 0= right (>3mm), 1= along the midline (0-3mm) (Fig. 4).

The offset of the melon from the mid-sagittal plane was calculated from the comparison of the X coordinates of the centroids (center of mass) of the head and the melon. The centroid was computed on the basis of the classic definition of centroid: the mean value of each of the component coordinates for all of the voxels (volumetric picture elements) in the object. In other words, the mean value of all the X coordinates for voxels within the object provides the centroid X coordinate, the mean value of all Y coordinates provides the Y centroid coordinate, and the mean value of all Z coordinates provides the Z centroid coordinate (see Fig. 5 for descriptions of X, Y, and Z planes). The result is an X, Y, and Z coordinate position in the image that represents the center of mass of the object.

The melon in *Phocoena phocoena, Kogia breviceps, and Pontoporia blainvillei* are in line with the midline of the skull. In all other taxa examined the melon has an asymmetrical position to the right.

Character 7. Shape of the melon: 0=perfect sphere (SFF=0), 1=ellipse (SFF1-3), 2=irregular (SFF>3).

The Sphere Fit Factor (SFF) measures how well the melon is represented by a sphere, or how close the shape of the melon is to a spherical shape. The Sphere Fit Factor is calculated by:

\[ \text{SFF} = \frac{(\text{Object SA})^3}{9 \times (\text{Object V})^2 \times 4 \times \pi}. \]

*Ziphius cavirostris, Lissodelphis borealis, and Lagenorhynchus obliquidens* had the most irregularly shaped melon. *Delphinus delphis, Pontoporia blainvillei, and Phocoena phocoena* had a more elliptically shaped melon.

Character 8. Size of the melon: 0=small (1-2% of head), 1=intermediate (2-5% of head), 2= large (>5% of head).

The sizes of anatomic structures were estimated from volumetric measurements with in the CT scan. For comparative purposes, melon volumes were scaled to the total head volume. Volume in a CT scans is calculated by counting the number of voxels (3D picture elements)
contained within a given structure. The voxels are then multiplied by volume of a single voxel (1.5 mm³) to get a cubic measurement.

*Kogia breviceps, Lissodelphis borealis* and *Tursiops truncatus* have the largest melons relative to head size. *Delphinus delphis, Pontoporia blainvillei,* and *Phocoena phocoena* had intermediate sized melons. *Lagenorhynchus obliquidens* and *Ziphius cavirostris* had the smallest melons.

**Character 9:** Carbon structure of fat tissue in the melon: 0= all triglycerides (low average carbon number, straight-chain unsaturated acids, normal mammalian adipose tissue), 1= mainly waxy esters (highest levels of average carbon numbers) and some triglycerides (highest level of carbon numbers: 42.0 to 50.0), 2= similar amount of triglycerides (intermediate average carbon number 33.1 to 40.8) and waxy esters (high levels of average carbon numbers) 3= mainly triglycerides (average carbon number 25.5 to 33.3, rich in isovaleric acid (iso5:0) and long chain iso acids:) and some waxy esters (intermediate levels of average carbon numbers), 4= all triglycerides (average carbon number 25.5 to 33.3, rich in isovaleric acid (iso5:0) and long chain iso acids) with little or no waxy esters.

Information collected from previous studies analyzing the composition of the tissues in the forehead region (Koopman et al., 2003; Litchfield and Greenberg, 1974; Litchfield et al., 1973; Lithcfield and Greenberg, 1974; Varanasi and Malins, 1972).

*Plantanistids, physeterids, and Iniidae* all have mainly waxy esters present in the melon. *Ziphiidae* has similar amounts of triglycerides and waxy esters. *Delphinids* have mainly triglycerides and some waxy esters present. *Monodontidae and Phocoenidae* have only triglycerides.

**Character 10:** Fatty acid branches off carbon chain: 0= long-straight chains, 1= iso12 present, no iso5, 2= combination of iso5 and iso12, 3= mainly iso5.

Most fatty acids are long, straight chains of even numbers of carbons (Stryer, 1988), but iso5:0 is short (containing only five carbon atoms) and has a branched structure. Rather than following the typical pattern exhibited by most fatty acids, of synthesis via the sequential addition of two-carbon units in the cytoplasm (Stryer, 1988), iso5:0 is produced in mitochondria (as iso5:0-coA) as one of the intermediate steps in the catabolism of leucine (Morii and Kaneda, 1982; Tanaka et al., (1966)). There is considerable evidence that iso5:0 can be extremely toxic to mammals. Information collected from previous studies analyzing the composition of the tissues in the forehead region (Koopman et al., 2003; Litchfield et al., 1973; Litchfield and Greenberg, 1974; Varanasi and Malins, 1972).

*Plantanistids, physeterids, Inia,* and *ziphiids* have iso12 present, and no iso5. *Monodontids* have a combination of iso5 and iso12. *Delphinids and phocoenids* have mainly iso5.

**BURSAE COMPLEXES**

**Character 11.** Bursae complexes (spermaceti organ): 0=spermaceti organ (right anterior bursa), 1= two pairs of same size dorsal bursae, 2= two pairs of different size dorsal bursae (Fig. 4).

The bursae are ellipsoid fat bodies, encapsulated by a connective tissue pouch (Cranford, 1996). They are normally found near the posterodorsal terminus of the melon at the boundary of the spiracular cavity in right and left bilateral pairs. The spermaceti organ is homologous to the
right anterior bursa (Cranford, 1996; Heyning and Mead, 1990; Mead, 1975). The bursae and spermaceti organ were located in the CT images by low-density areas located between the posterior terminus of the melon and the anterior wall of the spiracular.

*Kogia breviceps* and *Ziphius cavirostris* have a spermaceti organ. *Lissodelphis borealis, Tursiops truncatus,* and *Delphinus delphis* have two pairs of different size dorsal bursae (right larger than left). *Lagenorhynchus obliquidens, Pontoporia blainvillei,* and *Phocoena phocoena* have two of the same size pairs of dorsal bursae, one dorsal to the right nasal passages and one dorsal to the left nasal passage.

Fig. 4: Bursae Complex. Pictured here is a dorsal view of 3D reconstruction and labeled outline (melon in yellow, Bursae in pink). (A) *Tursiops truncatus* (two pairs of dorsal bursae) and (B) *Kogia breviceps* (spermaceti organ).

**Character 12.** Attachment of melon body to bursae complexes: 0= attachment to right bursae complex, 1= attachment to right bursae complex, but density increases, 2= no attachment (Fig. 4 and 5).
The relationship between the posterior melon and bursae complexes differs among odontocetes. In *Ziphius cavirostris* and *Kogia breviceps* the melon attaches directly to the spermaceti organ. *Tursiops truncatus, Lissodelphis borealis, Delphinus delphis,* and *Pontoporia blainvillei* the melon extends posteriorly to the right and attaches directly to the right bursae complex; on the left side of the melon there is a gap between the main body of the melon and the left bursae complex. Although the two low density fatty basins of the *Lagenorhynchus obliquidens* melon attach to right and left bursae complexes, there is an increase in density between the main melon body and the fatty basins. In *Phocoena phocoena* and the melon does not attach to the bursae complexes; there is a gap between the melon and right and left bursae complexes.

![Image of melon attachment](image)

**Fig. 5:** Melon attachment of melon to bursae complexes. Pictures here is a dorsal view of 3D reconstruction (melon in yellow, bursae complexes in pink) and labeled outline are pictured (lbc=left bursae complex, rbc=right bursae complex). (A) *Phocoena phocoena* and (B) *Lagenorhynchus obliquidens*

**Character 13.** Disparity between right and left bursae complexes: 0=right and left same size (0-2mm³), 1=right larger than left (2-4 mm³), and 2= extreme asymmetry (>4 mm³ or no left).

The volume (mm³) of the right and left bursae complexes were calculated from the CT scans. The size of the left was compared to the right. See character 7 for volume calculation method. The results were compared to Cranford (1996).
*Kogia breviceps* and *Ziphius cavirostris* have a spermaceti organ, therefore extreme asymmetry in the bursae complexes (no left). *Tursiops truncatus, Lissodelphis borealis, Delphinus delphis*, and have a significantly larger right complex than the left. *Pontoporia blainvillei, Lagenorhynchus obliquidens, and Phocoena phocoena* showed only a slight difference between the size of the right and left bursae complexes.

**EXTERNAL FOREHEAD**

**Character 14.** Orientation of the blowhole opening: 0=no curve, 1= apex of curve anteriorly directed, 2= apex of curve directed posteriorly (Fig. 6).

The position of the blowhole was analyzed using 3D reconstructions of the head from the CT scans and examining the dorsal surface. Observed states were compared to photographs when available.

In *Kogia breviceps* the apex of the blowhole is anterior, unlike *Ziphius cavirostris, Tursiops truncatus, Lissodelphis borealis, Lagenorhynchus obliquidens, Delphinus delphis, Pontoporia blainvillei, and Phocoena phocoena* in which the blowhole curve is posteriorly directed.
Fig. 6: Orientation of the blowhole opening. Pictured here are 3D reconstructions and labeled outlines of (A) *Phocoena phocoena*, (B) *Kogia breviceps*, and (C) *Tursiops truncatus*. (bh=blowhole, np=bony nasal passage). (D) *Ziphius cavirostris*. Black dotted line represents the midline of the skull.

**Character 15.** Position of the blowhole in relation to the midline of the skull: 0= centered (0-2mm), 1=slight left position (2-4mm), 2= extreme left position (4mm+). The shift of the blowhole opening from the mid sagittal plane of the specimen was calculated from the x coordinate of the centroid (see character 6 for description) of the entire head and compared to the x coordinate of the middle of the blowhole slit to analyze the magnitude (mm) of the shift from the midline of the specimen.
In *Phocoena phocoena* and *Lagenorhynchus obliquidens* the blowhole is in a centered position. The blowhole has a left position in *Tursiops truncatus, Lissodelphis borealis, Delphinus delphis, Pontoporia blainvillei* and *Kogia breviceps* (refer to Fig. 7).

**Character 16.** Curve of the external forehead (from the crease of forehead to the blowhole): 0=flat 1=blocky, 2=bulbous, 3=sloping.

The curve of the external forehead was analyzed from the tip of the rostrum to blowhole in the mid sagittal plane (center of occipital condyles to the center of the tip of the rostrum). Some character states were collected from published photographs. The results were compared to Cranford (1996) measure of forehead radius curvature, estimated from the anterodorsal surface of the forehead (FROC).

*Kogia breviceps* has a blocky forehead (Fig. 2). *Tursiops truncatus* (0.036), *Lissodelphis borealis, Lagenorhynchus obliquidens* (0.034), and *Delphinus delphis* (0.036) all have a sloping forehead. *Phocoena phocoena* (0.095) and *Pontoporia blainvillei* (0.07) the forehead is sloping, but not as gradual as the dolphins. From photographs it is apparent that *Delphinapterus leucas* has a more malleable forehead and therefore a bulbous forehead (O'Corry-Crowe, 2000).

**ACCESSORY FOREHEAD STRUCTURES**

**Character 17.** Dense connective tissue theca: 0=absent, 1=present, 2=thickened (Fig. 7).

The theca is defined as a dense connective tissue capsule that embraces the posterior portion of the melon and merges with the nasal plug muscle ventrally (Cranford, 1996). The theca was defined in the CT images by a steep density gradient of the tissues around the posterior portion of the melon and confirmed in dissections and previous studies (Cranford, 1996).

*Tursiops truncatus, Lissodelphis borealis, Delphinus delphis, Pontoporia blainvillei* and *Phocoena phocoena* have a connective tissue structure dorsal to the posterior melon. In *Ziphius cavirostris* the theca is thicker and penetrates deeper in the region surrounding the blowhole.

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![Fig. 7: Connective tissue theca (in purple) in *Ziphius cavirostris* in relation to melon (in yellow).](image)

(A) Right lateral view. (B) Dorsal view.
**Character 18:** Orientation of the spiracular cavity from the external opening to bony nasal passages: 0= straight, 1=slight posterior curve, 2=extreme posterior curve (Fig. 8).

In *Phocoena phocoena* and *Pontoporia blainvillei* the external opening of the spiracular cavity (blowhole) is directly dorsal to the bony nasal passages. The blowhole is slightly anterior to the bony nasal passages in *Tursiops truncatus*, *Lissodelphis borealis*, and *Delphinus delphis*. *Kogia breviceps* and *Ziphius cavirostris* have an extreme posterior position of the blowhole; therefore the external opening of the nasal passage is anterior to the opening of the bony nasal passages.

![Fig. 8: Orientation of bony nasal passages (green) in relation to the blowhole (red) in (A) *Tursiops truncatus* and (B) *Ziphius cavirostris*. The dotted line represents the soft tissue spiracular cavity.](image)

**BONY CHARACTERS**

**Character 19.** Difference in size of the opening of the bony nasal passages: 0= same size (difference 0-2mm), 1=left larger than right (2-10mm), 2=left more than 2x the size of right (10mm+).

The maximum width of the nasal passages was measured. The extinct taxa *Kentryodon pernix†, Kentryodon westmorelandi†, Eurinodelphis bosi†*, and the extant *Phocoena phocoena* and *Lagenorhynchus obliquidens* show little disparity in the size of the nasal openings. In all other delphinids the left nasal passage was larger the right.

**Character 20:** Difference in the circularity of the bony nasal openings. 0=same, 1=left more circular (Fig. 9).

The circularity was measured at the anterior surface of the bony nasal passages. Circularity/(4*Pi) is the circularity value divided by 4*Pi. This value should be 1 for a perfect circle.

The measurement was calculated in the CT images; therefore the distribution is unknown for extinct taxa. In *Phocoena phocoena* and *Pontoporia blainvillei* the left and right nasal openings had the same circularity value. In all other odontocetes measured, the left bony nasal passage opening is more circular than the right.
Character 21. Difference in the width of the premaxillary bones at the anterior nasal septum: 0= same size (0-2mm), 1=right larger than left (2-10mm), 2=right more than 2x the size of left (10-20mm).

The width of left and right premaxillary bones in the plane perpendicular to the nasal septum or the anterior edge of the bone nasal passages were measured from CT reconstructions of skulls and on museum specimens using digital calipers. The results were compared to published data (Cranford, 1996).

The distribution of this character indicates that *Balaenoptera* and *Eschrichtius* share the have symmetrical bone widths as found in *Zygorhiza kochii*† and *Squalodon cavertensis*†. In *Phocoena phocoena* and *Pontoporia blainvillei* there is no difference between the width of the right and left premaxillary; however in dephinids the right premaxillary is wider than the left. In the Ziphiid specimen measured, Kogiidae, and physeterids the right more that two times the size of the left.

Character 22: Orientation of the bony nasal passage openings: 0=anterior, 1=intermediate, 2=dorsoposterior (Fig.10).

The orientation of the bony nasal passages was examined by both the plane of the nasal septum and the direction of the boney nasal passageway. The extinct archaeocete family, Basilosauridae, have anteriorly facing bony nasal septum and the passages extend posterior at a slight angle above the concylobasal plane. Mysticetes have nasal septum and nasal passages are elevated at about the same angle above the condylobasal plane of the skull. Most odontocetes have dorsally orientated nasal septum and nasal passages.
A. Basilosauridae†

B. Balaenopteridae

C. Balaenidae

D. Agorophidae†

E. Physeteridae

F. Squalodontidae†
Fig. 10: Lateral view of cetacean skulls showing the orientation of the bony nasal passages. All pictures are adopted from Fordyce (2002). The blue arrows are marking the tip of the rostrum. The red dotted arrows are showing the orientation of the bony nasal passages; the head of the arrow is in the same orientation of the nasal septum. (A) Basilosauridae (Archaeocete) is represented by the skull and mandible of *Dorodon atrox* (Eocene Egypt), (B) Balaenopteridae (Mysticete) is represented by a drawing of *Megaptera hibachi* (Pliocene, Chili), (C) Balaenidae (Mysticete) is represented by *Balaena mysticetus* (Extant, Artic), (D) Agorophidae (Odontocete) is represented by *Agorophius pygmaeus* (Oligocene, South Carolina), (E) Physeteridae (Odontocete) is represented by *Physeter macrocephalus* (Extant), (F) Squalodontidae (Odontocete) is represented by Squalodon-like squalodontid (Oligocene, New Zealand).

Character 23. Dorsal skull surface: 0=flat, 1= convex, 2=concave, 3= deep basin (Fig. 10)

The dorsal surface of the skull was described from the tip of the bony rostrum to the suture of the nasal bones. In Basilosauidae, the surface is relatively flat, similar to terrestrial ancestors. The mysticetes examined show a slight convex morphology; however the right whales have an exaggerated arch. All odontocetes have a concave morphology, although the extinct basal lineages have a less obvious concavity. *Ziphius cavirostris*, *Kogia breviceps*, and *Physeter macrocephalus* have a deep supracranial basin.

Character 24. Anterior projection of the vertex of the skull over the nasal passages: 0= no projection, 1=slight projection, 2= projection over the nasal passages.

Archaeocetes and mysticetes do not have a dorsal orientation of the bony nasal passage openings; therefore this character was coded as 0 even thought the nasal bones hang over the posterior nasal openings. All odontocetes have dorsal oriented nasal passages and an elevation of the facial bones posterior to the nasal openings; however the position of the vertex in relation to the nasal passages varies. In *Ziphius cavirostris* the vertex projects over the nasal passages; therefore the passages are not visible in the dorsal view. *Kogia breviceps* shows a slight projection over the nasal passages. In the Delphinidae, Phocoenidae, Pontoporia, Monodontidae, and Iniidae the vertex of the skull does not project anteriorly over the nasal passages.
APPENDIX H

CHARACTER DATA MATRIX FOR

CETACEAN FOSSIL AND EXTANT TAXA
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APPENDIX I

CHARACTER DATA MATRIX

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