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Beaked and Baleen Whale Hearing: Modeling Responses to Underwater Noise

by

Darlene R. Ketten and David C. Mountain

June 2009

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13. ABSTRACT (maximum 200 words) Ultimately this research is to provide proof of concept for using modeling techniques to provide reliable hearing estimates for species thought to be most liable to impacts from oceanic sound sources. To address this, preliminary data were acquired that may later be applied toward calculating a model audiogram of large whale hearing that demonstrates the ability to accurately estimate hearing ranges and peak sensitivities using current anatomical and biomedical engineering techniques. The model proposed is to be created from neuroanatomical data combined with direct measures of middle and inner ear stiffness. For now, preliminary data have been collected, with anatomical measures obtained for the heads and inner ears of two beaked and two minke whales, as well as for 10 ears from these and additional animals. A partial frequency range map for the minke has been completed via CT and histologic data. Specimens have been used to redesign a piezo electric system to accommodate baleen ears, and point stiffness measures have been accomplished for the middle ears of some of the test minke specimens. These data have been compared with earlier results from multiple odontocete and land mammal ears.				
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Final Report

Beaked and Baleen Whale Hearing: Modeling Responses to Underwater Noise Project 49002200

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STATEMENT OF WORK

Estimates of the large whale audiograms will be accomplished by completing three major aims:

- I. Development of a comprehensive morphometric database for beaked and baleen whale middle and inner ears;
- II. Completion of the models through direct stiffness measures of the middle and inner ears; Generation of test waveforms and impact testing;
- III. Data dissemination and publication of model whale audiograms and impact assessments.

PROJECT SUMMARY

The primary goal of this research was to provide a proof of concept for employing modeling techniques to provide reliable hearing estimates for species thought to be most liable to impacts from common, active sound sources deployed in the oceans. These data are necessary for determining ranges of interest for playback experiments, for species specific risk assessments for hearing impacts and for effective electrode and sound source placements for proposed auditory brainstem response (ABR) measures of live stranded larger whales.

To address this question, the immediate goal was to begin the procedures and acquire the preliminary data that may ultimately be applied toward calculating a model audiogram of large whale hearing that demonstrates the ability to accurately estimate hearing ranges and peak sensitivities using current anatomical and biomedical engineering techniques. The model proposed was to be created from neuroanatomical data combined with direct measures of middle and inner ear stiffness. The validity of this model will, pending funding continuation, be tested by comparing model predictions for land and marine mammal species with published audiograms obtained previously from conventional behavioral and electrophysiological methods.

At this stage, preliminary data in all categories have been collected. Anatomical measures have been obtained for both entire heads and for inner ears of two beaked whales and two minke whales as well as for 10 ears from these and additional animals. The anatomical team (Ketten/Woods Hole Oceanographic Institution) has completed a partial frequency range map for the minke via CT and histologic data. Specimens provided to the Boston University team for comparison have been employed to redesign a piezo electric system to accommodate baleen ears, and point stiffness measures have been accomplished for the middle ears of some of these test minke specimens. These data were then compared with earlier results from multiple odontocete and land mammal ears.

DOD RELEVANCE

At present, scientific research, oil and gas exploration, and U.S. Naval operations are hampered by intense public oversight and even injunction because of a lack of knowledge about the

potential impacts of sound on the marine environment. These concerns are particularly acute for marine mammal impacts, and much of the recent public and legislative concerns have focused explicitly on the effects of sonar and seismic sources on two groups of whales: beaked whales, because of their predominance in strandings associated with military exercises using mid-frequency sonars; and baleen whales, because of their expected sensitivity to lower frequency sounds from seismic exploration sources and low frequency active sonars.

Normally, detailed information on hearing in mammals is obtained by acute experiments or by behavioural tests. But, while data on baleen whales, the animals most likely to be affected by lower frequency sources, are needed urgently, these data are also the most difficult to obtain, as these animals are the least approachable animals by conventional methods. They are not kept in captivity and seldom strand live. Further, they are unlikely to be tested successfully by current auditory brainstem response (ABR) techniques, which require the auditory centers to be relatively large compared to the rest of the brain and body and sufficiently close to the surface for signals to be strong enough to be detected by surface electrodes. ABR methods work well on smaller odontocetes, but may not be viable for baleen whales which have a brain to body mass ratio two magnitudes smaller than humans or toothed whales (0.01% vs. 1-2%, respectively). Therefore, we must employ alternative methods for obtaining reliable underwater hearing and impact estimates for very large whales.

Auditory system modeling is a well-established and increasingly sophisticated area of auditory system research. Pending additional funding, we will employ the preliminary data obtained under this project to model the acoustic properties and hearing capacities, from the inner ear through whole head reception paths, of representative species from these groups of whales in order to better understand how sounds are processed and perceived by each species and thereby to provide critical information for assessing potential impacts from any sound source.

This work addresses important gaps in our database on marine mammal hearing and will improve our understanding of physiological effects of sound. It builds on and extends previous work on hearing models for two control species (bottlenose dolphins and harbour porpoise) that were constructed under prior funding for Topic M (Marine Mammals) in FY04 NOPP programs.

These preliminary data demonstrate the feasibility and value of stiffness and mass measurements as major improvements in model accuracy and detail (Mountain *et al.* 2003, Miller *et al.* 2006). In addition, considerable progress was made in the understanding of the role of head anatomy and cochlear duct topology for underwater sound reception and low frequency hearing characteristics (Ketten 2004, Koopman *et al.* 2006, Chadwick *et al.* 2006).

PROJECT DETAILS

We proposed to develop a full research program for biophysically based models of the acoustic power flow (from the middle ear into the inner ear and ultimately to the sensory receptor cells) determined from anatomical and mechanical measurements in whales to estimate the audiogram for nine large whale species. In this funded effort, we began collection of the primary data for target species and demonstrated the adaptability of both CT and stiffness measurement technologies to these larger ears.

The proposed effort involves two integrated teams:

An **Anatomical Analysis Team (Aim I)** (WHOI) led by Darlene Ketten will characterize head, middle, and inner ear structures of the candidate species.

A **Physiological Modeling Team (Aim II)** (BU) led by David Mountain will implement auditory response models using the anatomical data and develop the species-specific model audiogram.

AIM I (WHOI): Anatomical Substrates of Hearing

This aim consists of developing a biomedical scan and histology derived database of baleen and beaked whale ear anatomy for model development.

AIM I: Accomplishments (see also Publications)

The Anatomical Analysis Team (AIM I/WHOI) had as its goal to characterize head, middle, and inner ear structures of the candidate species.

To date, five intact minke whale (*Balaenoptera acutorostrata*) and two beaked whale (*Ziphius cavirostris*) heads have been CT scanned. In addition, ten ears from seven animals and five brains with the auditory centers intact have been scanned and preserved for further analyses. Two intact ears and surrounding associated peribullar and fatty tissues have also been examined with MRI. These head and ear scans provided the first multi-individual, comprehensive, matrix-based, species-specific databases of head and ear anatomy and tissue density maps of a baleen species head with undisturbed internal anatomy. In addition, five middle and inner ears have been prepared for analysis by the BU Team, and two ears have been processed through histology.

From these tissues and techniques, mandibular fat bodies have been identified proximal to and in communication with the middle ear. These tissues are consistent in shape and volume across individuals and are similar in consistency and color to fats that are known to be an essential component of toothed whale auditory systems. Samples of these fats have been sent to Dr. Heather Koopman (University of North Carolina, Wilmington) for biochemical analyses.

The histologic sections have also yielded the first data on longitudinal variations in basilar membrane dimensions for this species. The data show the inner ear to be consistent with a 9-10 octave hearing range that is primarily adapted for mid to lower frequencies and with cochlear ratios consistent with better propagation of lower frequencies than is common in odontocete ears.

Specifics of each of these study areas are listed below.

Task 1: Middle Ear Anatomy

Three-dimensional reconstructions from the transaxial section data are used to measure cavity and ossicular dimensions at consistent orientations and to determine *in situ* angles of the ossicular chain.

Milestones

1.1: Complete CT scan of survey of intact ear complexes and gross description and measurements of ears

Accomplished:

Primary measurements were completed for five heads and ears.

- Minke whale ears were scanned at 0.1 mm increments, segmented for soft and bony tissue elements, and reconstructed as fat, bone, and fluid labyrinth elements in order to demonstrate the turn distribution, radii ratios, and axial heights.
- In addition, five intact minke heads were scanned at 1 mm increments, for a total database of ten ears, of which four also had soft tissue associations. A significant finding in the whole head scans is the presence adjacent to the middle ear cavity of fatty tissues, which may function in the same way as specialized fats in the odontocetes (i.e., as an outer ear analogue) (Figures 1A and 1B).
- Three-dimensional reconstructions of the middle ear ossicles were completed for three of these specimens and the data on position and angles were transferred to the BU team.

1.2: Identify practice specimens and distribute to BU

Accomplished:

Three of the available ears have been distributed to BU for the purpose of providing specimens both to assist with refinement of the support and stiffness measurement as well as to obtain preliminary data on middle and inner ear stiffness measures. (See BU team report for details of measurements.)

Problems Encountered:

Two persons in addition to Dr. Ketten are in training to perform the ear extractions and preparations. To date they have not been successful at maintaining ear capsule integrity. Therefore, Dr. Ketten will take responsibility for future extractions.

1.3: Complete measures of middle ear morphometry from CT

Volume estimates for these middle ears based on the first Amira software tested were unsatisfactory. Errors in that program are corrected in the new version of Amira software (version 5), which will be used in any future calculations of volumes. Tests of this software using calibrated fluid volumes show an error of less than 2%. Dr. Ketten also worked with one of the BU students to assist him with developing methods for work on middle ear ossicular density.

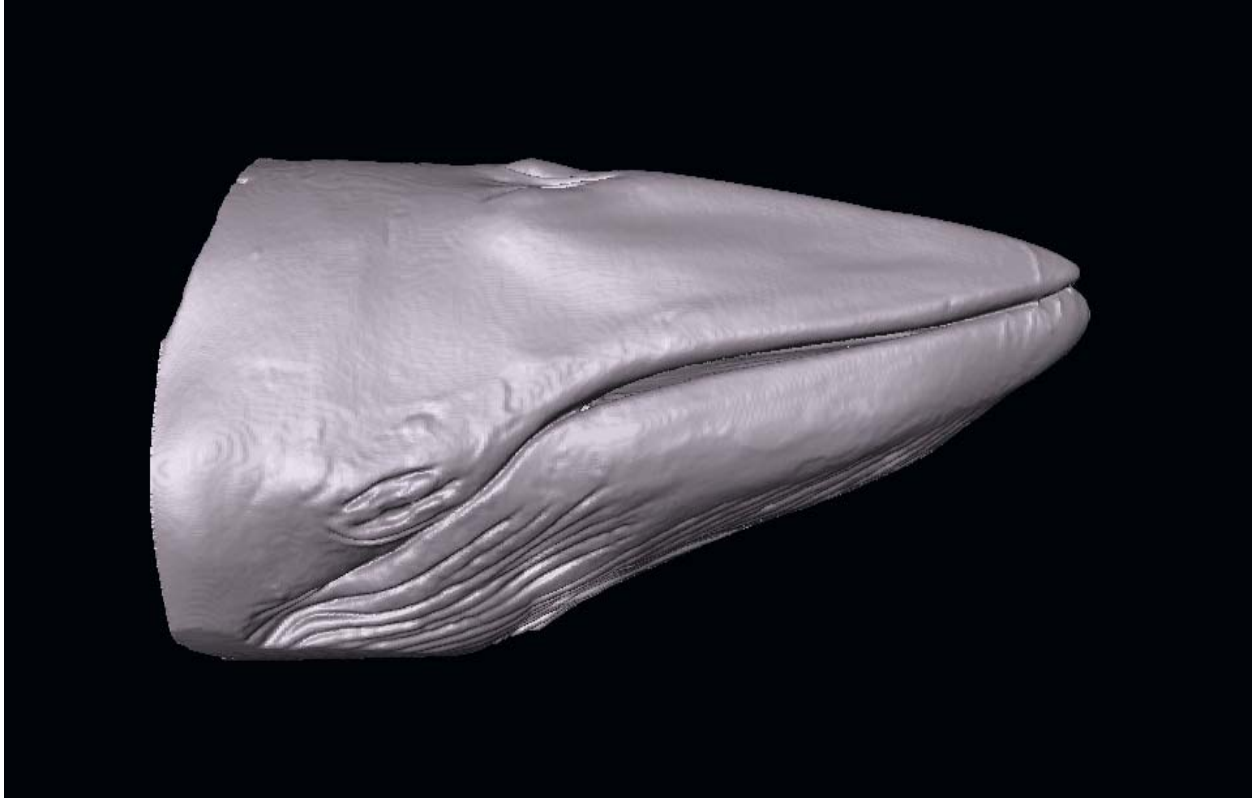


Figure 1A. 3D surface rendering from 3 mm CT scans of the head of a juvenile minke whale (*Balaenoptera acutorostrata*) using AMIRA[®] visualization software.



Figure 1B. 3D surface rendering from 3 mm CT scans of the skull of a juvenile minke whale (*Balaenoptera acutorostrata*) using AMIRA[®] visualization software. Highlighted in yellow are jaw fats.

Task 2: Cochlear Anatomy and Morphometry

The objective of this task was to obtain quantitative, topographic maps of cochlear cytoarchitecture for each ear. Measurements, reconstructions, and descriptions of minke whale inner ear anatomy were based on 3D reconstructions of registered light microscope sections (Table 1; Figure 2).

Micro-CT images of ears from other mammals that are required for controls of the model data were transferred from BU to WHOI for analysis and image reconstruction. Comparison of image resolution was made between standard and micro-CT images of the same species to determine the most useful scanning technique in assessing auditory function. A new technique was also devised for employing CT data as a guide for locating the basilar membrane within the uncut petrotic bone.

Milestones

2.1 Complete micro-CT and micro-MRI of inner ears

Accomplished:

Micro-CT has been accomplished on two control species' ears. Micro-MRI is completed on one specimen. The images obtained to date for minke whales are

unsatisfactory due to ring artifact, which results from detector failures in the machine employed. A second machine has been identified for use at the Armed Forces Institute of Pathology (AFIP) in Washington, DC, and collaborative access has been arranged for completing this milestone.

2.2 Complete additional section staining and digitizations

Two minke ears have been processed through decalcification, embedding in celloidin, sectioning, staining, and mounting.

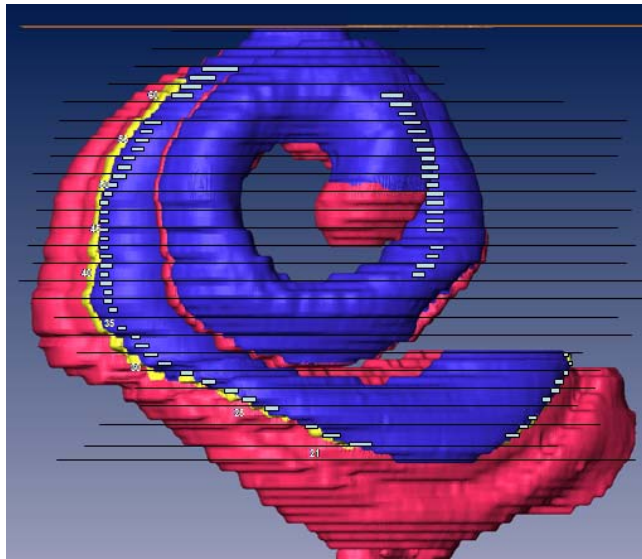
2.3 Complete basilar membrane and neural measurements from histology

Measurements from one ear are completed. A second ear has been processed through histology and is ready for analysis.

Minke Whale Frequency Estimates from Morphometry

% Length from Base	Width (mm)	Thickness (mm)	T:W	Predicted Frequency (kHz)
0	-	-	-	-
6	0.130	0.0110	0.084615	25.90
10	0.170	0.0083	0.048824	11.99
15	-	-	-	-
20	-	0.0077	-	-
25	-	0.0068	-	-
30	0.200	0.0064	0.032000	10.25
35	0.270	0.0065	0.024074	6.67
40	0.330	0.0052	0.015758	3.82
45	-	-	-	-
50	-	0.0050	-	-
55	0.460	0.0050	0.010870	0.16
60	0.510	0.0050	0.009804	0.12

Table 1. Basilar membrane Thickness to Width ratios (T:W) and predicted frequencies for minke whale.



Cochlear Measures: $n=2$

Turns: 2.25

Basal diameter: 12.5, 12.3

Axial height: 7.25, 7.5

Length: 44.36, 44.76 mm

Basilar membrane: $n=1$

60% total length

Basal width: 0.13 mm

Thickness: 0.005 mm

Apical width: 0.51 mm (1.2 mm)

Thickness: 0.003 (0.002)

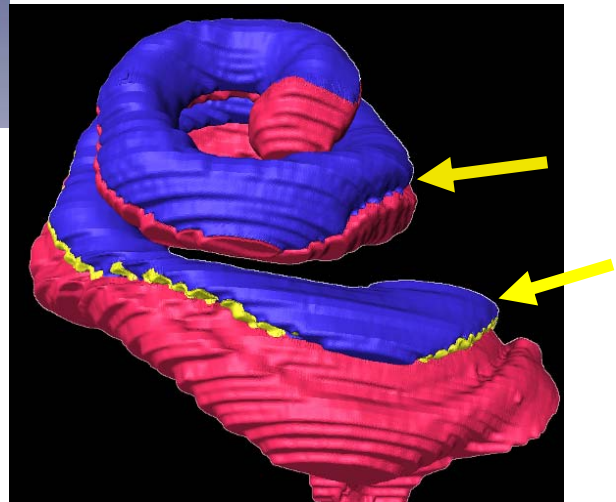


Figure 2. Three-dimensional reconstruction of the scalae and basilar membrane from celloidin histology of a minke whale temporal bone.

AIM II (BU)

Task 3: Middle Ear Stiffness

Our middle ear stiffness measurement system (Miller *et al.* 2006) has been redesigned for use on minke ears. During our preliminary experiments we discovered that it was very difficult to remove the ear from the head without separating the tympanic bone from the periotic. This separation causes a disruption of the joint between the incus and the stapes. The stapes remains in place in the oval window of the cochlea, so we were able to make stiffness measurements. But the stiffness values were radically lower than what we have seen in other species. To accurately measure these low stiffness values, we had to develop a new technique for establishing when the force probe was in contact with the stapes. The approach was to measure the distortion in the force waveform when the probe tip was displaced sinusoidally. When the tip is not in contact with the stapes, or is well engaged with the stapes, there is little distortion in the force waveform. When the static position is advanced to the point where the probe tip is barely in contact with the stapes, the force waveform becomes quite distorted. This is because for positive displacement the probe is in contact with a stiff structure (stapes), but for negative displacement the probe loses contact and registers essentially zero force. Through detailed

calibration experiments, we have established that the new method for establishing when the probe contacts the stapes is superior to the visual observation method used in the past.

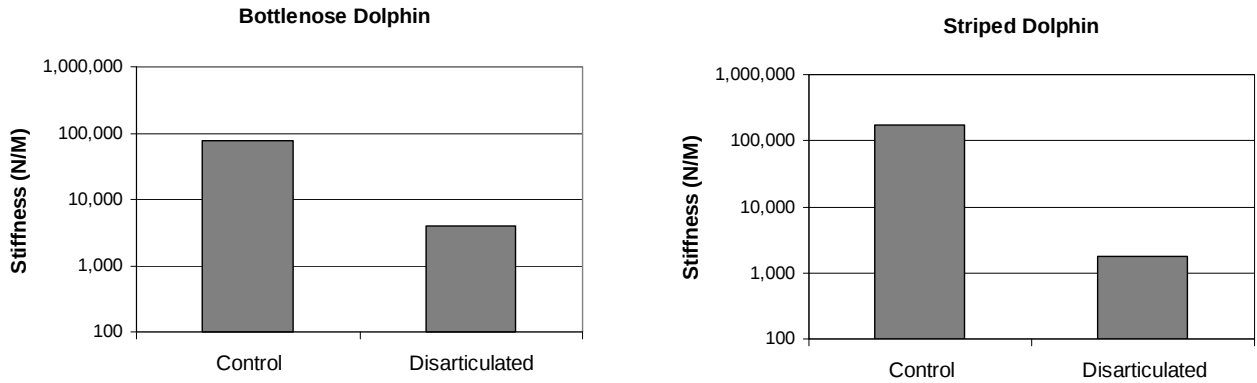


Figure 3. Effect of disrupting ossicular chain on middle-ear stiffness

We have hypothesized that cetacean middle ear stiffness is dominated by the bony connection of the malleus to the tympanic bone. If this is the case, then the low stiffness values observed in the minke ears with the ossicular chain disrupted would be expected, since we would be only measuring the stiffness of the annular ligament that holds the stapes in place and not the total stiffness of the intact middle ear. Since minke ears are in short supply, we decided to test this hypothesis in two species where ears with intact ossicular chains are easy to obtain, bottlenose dolphin and striped dolphin. In both species, we found that disrupting the middle ear caused the stiffness, measured at the stapes, to drop by over an order of magnitude (Figure 3). This result makes it clear that we need to make sure that the middle ear is intact for any stapes stiffness measurements to be meaningful for modeling purposes.

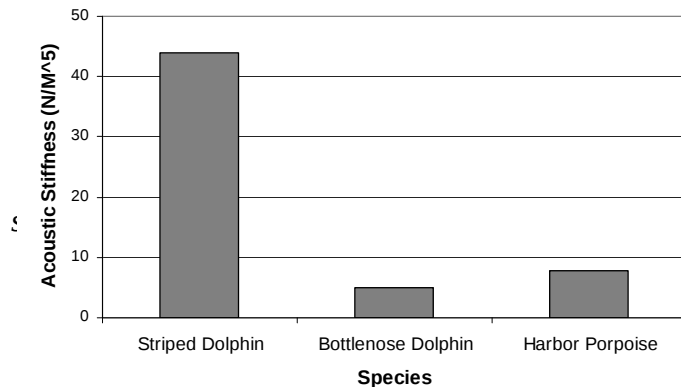


Figure 4. Middle-ear stiffness

We were also able to use data from these experiments to test our hypothesis that high middle ear stiffness is a predictor of poor low-frequency hearing. The striped dolphin has poorer low-frequency hearing than the bottlenose dolphin and harbor porpoise, but has similar sensitivity in

the high frequencies. We found that, as predicted, the striped dolphin middle-ear stiffness was significantly higher than the bottlenose dolphin and the harbor porpoise stiffness values (Figure 4). These results were reported last year at the meeting in Nyborg, Denmark.

We have shown previously that high middle ear stiffness is highly correlated with poor low-frequency hearing. To date we have not succeeded in extracting a minke ear from the head without disrupting the middle ear. So we have put considerable effort into modeling the middle ear while we try to refine our ear removal techniques. A full finite-element middle-ear model for the bottlenose dolphin has been implemented and tested. Micro-CT scans of the dolphin ear were used to create 3D reconstructions of the middle ear ossicles (Figure 5). The minke whale ear is too large to fit into our micro-CT scanner. So the minke middle ear anatomical model was created using conventional CT scans. The resulting anatomical models are then imported into the COMSOL finite-element method (FEM) modeling package using software that we developed. Since CT imaging is not well suited for imaging soft tissues, several ears were dissected and the dimensions of ligaments and muscles were measured manually. These measurements were then used to add these components to the boney components of the middle ear. A mesh of tetrahedral elements is then created (Figure 6) and material properties are assigned to the different components of the model.

The FEM model was then used to predict ossicular displacements for different types of forces applied to the middle ear. Figure 7 illustrates the results from a simulation designed to mimic our middle-ear stiffness measurements. A pressure load is applied to the stapes foot plate and the resulting displacement of the stapes, as well as other structures, calculated. The most striking aspect of the predicted movement is a rotational displacement around the axis that runs through the malleus and incus from the anterior process of the malleus to the short process of the incus. This is very much like the middle ear motion in very high frequency terrestrial mammals, such as bats and mice. Given these results and the locations of the middle-ear muscles, we predict that the cetacean middle ear functions very much like the middle ears of terrestrial mammals.

By dividing the total force acting on the stapes by the displacement of the stapes footplate, we can calculate a stiffness which can be compared to the measured values. The dolphin model is still in need of refinement, since the predicted stiffness is significantly higher than the measured stiffness. Based on the ossicular motions predicted by the model, it appears that the boney process which connects the malleus to the tympanic bone acts as a torsional spring. If we simulate the disarticulated ear, we find that the stiffness measured at the stapes decreases dramatically, which is in qualitative agreement with our experiments. In order to develop a better understanding for why the model overestimates the middle ear stiffness, we have begun a series of experiments both where stiffness is measured at the malleus in intact dolphin ears as well as where stiffness is measured at the stapes after various manipulations of the ossicular chain.

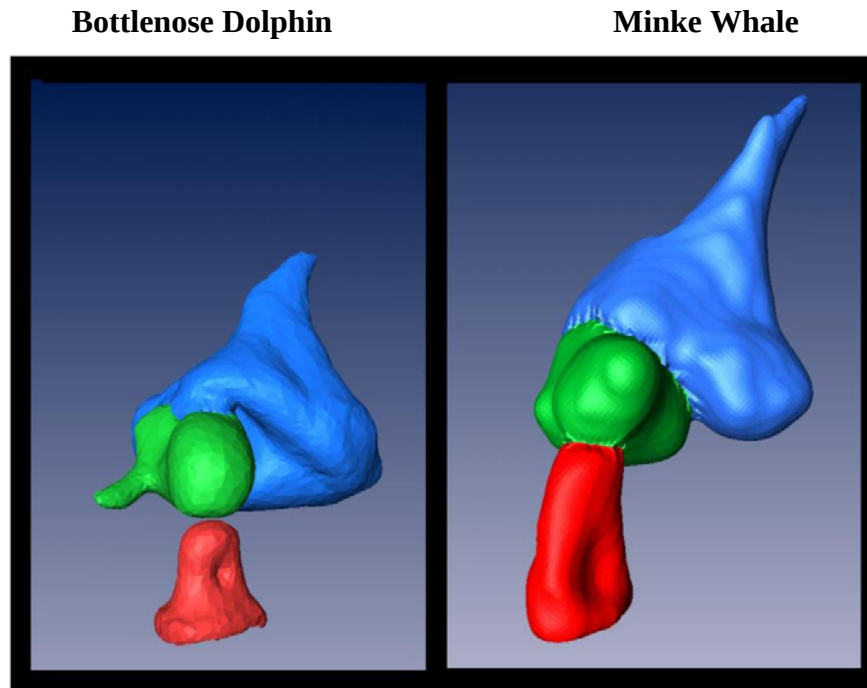


Figure 5. 3D middle ear reconstructions for bottlenose dolphin and minke whale. Note that the boney process of the minke malleus (blue) is longer and thinner than that in the dolphin, which suggests that the minke middle ear is much more compliant than the dolphin middle ear.

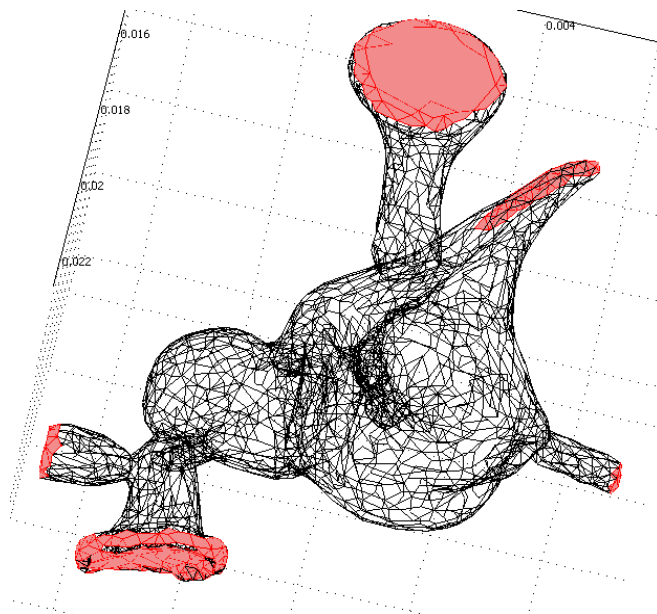


Figure 6. *Tursiops* middle ear finite-element model mesh

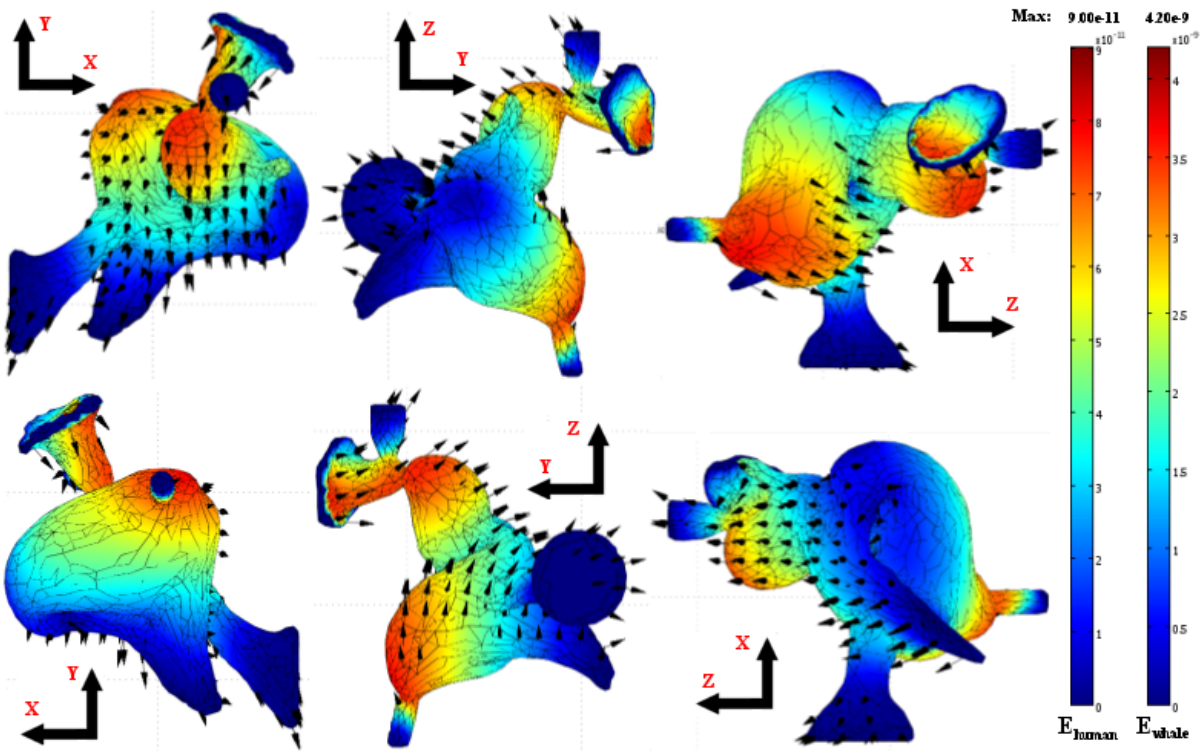


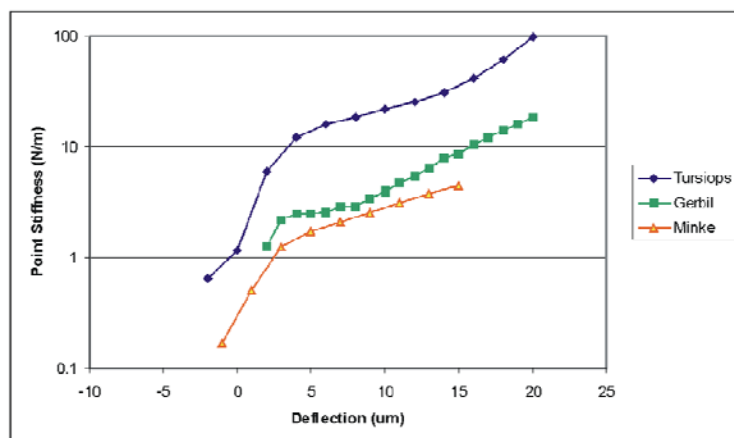
Figure 7. Predicted displacements from the dolphin FEM model for a force applied to the stapes.

Task 4: Basilar Membrane Stiffness

For the minke project we needed to redesign both our ear holder and the force probe. Our previous force probe design (Olson and Mountain 1991) was not sensitive enough to measure very soft structures, and we suspected that the apical regions of the minke basilar membrane would be significantly more compliant than comparable regions for the other two species for which we have extensive data (gerbil and bottlenose dolphin). The new probe has now been extensively tested and calibrated.

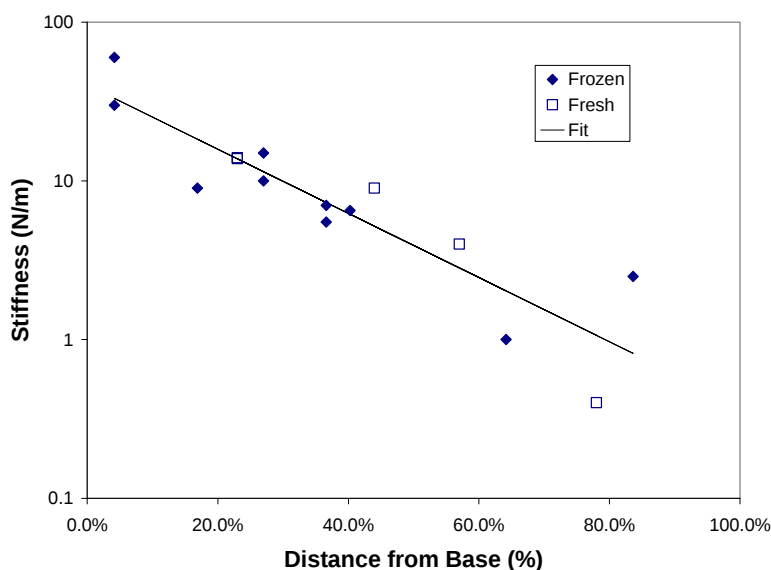
Stiffness measurements are made by bringing the probe near the basilar membrane and then advancing it in 1 μm steps until the stiffness increases rapidly and then levels off to a shallower slope. This break point is assumed to be the point where the probe has fully engaged the basilar membrane but has not deflected it to the point where the stiffness is radically increased.

Although separation of the tympanic from the periotic is a problem for the middle ear measurements, it does not affect the basilar membrane. We have obtained high quality data from one minke ear at multiple locations in the basal turn. The minke stiffness values were slightly less than what we have measured in gerbil and much less than those found in bottlenose dolphin (Figure 8). This suggests that the high-frequency limit for hearing in minke whales will be significantly less than the high-frequency limit for dolphins (~ 140 kHz) and somewhat less than the high-frequency limit in gerbil (~ 60 kHz).

**Figure 8.**

Comparison of minke whale, gerbil, and dolphin (*tursiops*) basilar membrane stiffness.

Since the majority of the cetacean ears that we get have been frozen and then thawed, we have conducted a series of control experiments using gerbil ears. In these experiments we extract the bulla from anesthetized gerbils and freeze them. The ears are then subsequently thawed and basilar membrane stiffness measured. Our preliminary results indicate that the basilar membrane stiffness is reduced to 60-70% of its normal value by the freeze-thaw process.

**Figure 9.**

Bottlenose dolphin basilar membrane stiffness plotted as a function of cochlear location. Data are from two fresh ears and three ears that were frozen and then thawed for data collection.

We recently received two bottlenose dolphin ears that were harvested from a stranded animal and that were refrigerated rather than frozen. We were able to begin basilar membrane stiffness measurements approximately 24 hours after the animal died. The stiffness data from these ears fell within the range of measurements that we reported previously for frozen ears (Figure 9). We therefore conclude that basilar membrane stiffness measurements made on ears that are frozen

shortly after death of an animal and then thawed are representative of the normal values or slightly more compliant. In other words, the measurements from frozen ears should be treated as a lower bound on the normal stiffness, but a bound that is not far from the physiological value.

Task 5: Modeling

Our first modeling subtask was to develop a scaling rule for the compliance to frequency map transformations. After reviewing the available data for species where both the compliance map and the frequency-place map were available, we decided that the most reliable data were our own gerbil data and von Békésy's (1960) human data. These data are complementary in the sense that they overlap in the mid-frequency range, but the gerbil data extend to higher frequencies than the human data, while the human data extend to lower frequencies than the gerbil data. A polynomial function was fitted to these data (Figure 10) and will be used to predict the minke frequency-place map as soon as more minke basilar-membrane stiffness data become available. Using this mapping function, we predict that the best frequency for the minke basal turn is 20-30 kHz. This value is in good agreement with estimates based on anatomical observations.

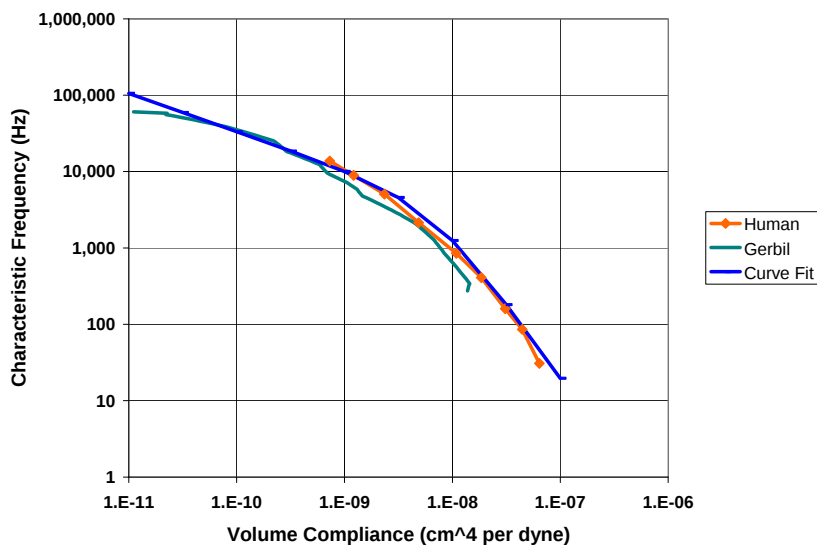


Figure 10.
Compliance-frequency map.

A preliminary cochlear model for the minke whale has been implemented (Figure 11), but more experimental data are needed before the modeling work can be completed. CT scans were used to create 3D reconstructions of the cochlear fluid compartments (Figure 12). The area-distance functions from these reconstructions were used to compute impedance-distance functions for the fluid impedances. Since we do not yet have a complete stiffness map for the minke basilar membrane, we scaled our bottlenose dolphin stiffness map based on our preliminary data from the basal turn of the minke cochlea.

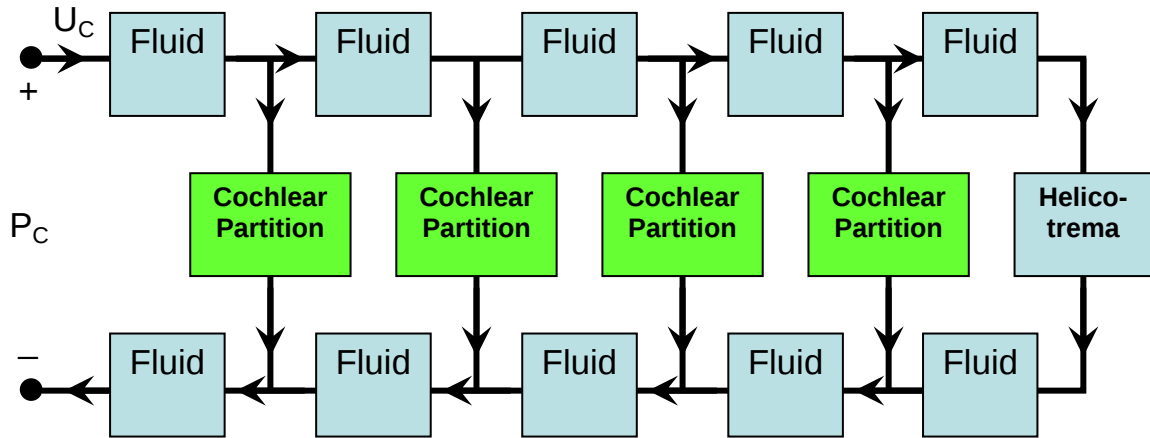


Figure 11. Cochlear finite-difference model

The final step in developing the basilar membrane model is to convert the point stiffness data into a volume (acoustic) compliance map for the cochlear partition. This conversion depends on accurate measurement of the basilar membrane dimensions using conventional histological techniques, which has been completed as part of Task 2.

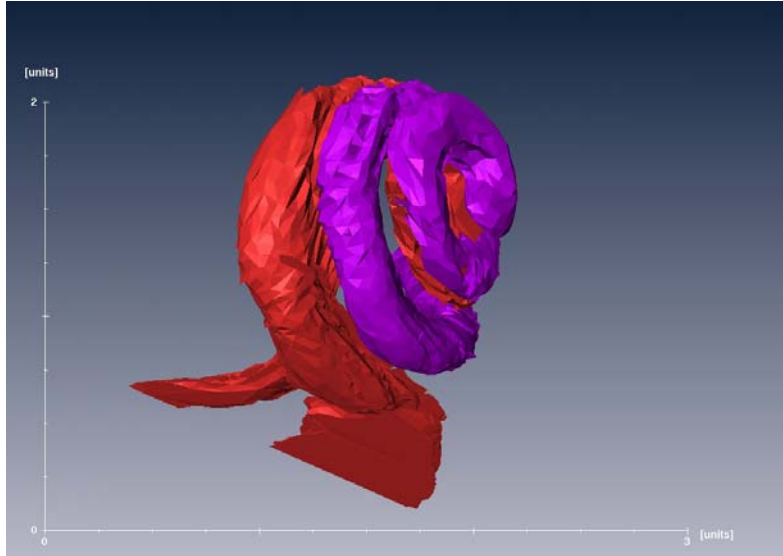


Figure 12.

Minke whale cochlear reconstruction. The scala tympani compartment is shown in red and the scala vestibuli compartment is shown in purple.

We have not yet created a full audiogram model, since the full model requires accurate estimates for middle-ear stiffness.

Summary

Our most important findings are that both the middle ear and the cochlea in cetaceans appear to function in much the same way that they do in terrestrial mammals. This means that the use of computational models derived from our extensive knowledge of terrestrial auditory physiology to predict cetacean hearing capabilities is justified.

AIM III: Reporting/ Education/Outreach

Task 6: Reporting

This task comprised peer reviewed publication and development of website incorporating images and data from this project.

Milestones

6.1 Manuscript preparation and submission

Publications related to this effort from the Ketten WHOI laboratory are listed at the end of this report.

6.2 Complete website development for WHOI scanner

The WHOI scanner website has been completed and Beta tested: www.whoi.edu/csi. Visualizations available include 2D series, 3D shaded surface reconstructions, and 3D multi-tissue reconstructions. Both still and animated data sets are represented for multiple species.

6.3 Augment BU Ear Lab site with baleen data (See BU report for details on this section.)

Publications Acknowledging Support

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