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14. ABSTRACT Recent reports suggest a link between mass stranding of beaked whales and the use of naval mid-frequency sonar. These reports have increased the concern that anthropogenic sound, such as that created by military sonar or during seismic exploration, may harm marine animals, particularly whales and dolphins. Primary issues have centered around direct auditory damage, resonance of gas containing spaces, and increased risk of decompression sickness (DCS) due to alteration in diving behavior or acoustic enhancement of bubble formation and growth. While a recent survey of causes of mortality in Massachusetts suggested that the vast majority of marine mammal strandings are not associated with sonar exposure (Bogomolni et al., 2008), there appears to be a relationship between the use of military sonar and mass strandings of cetaceans (Jepson et al., 2003).					
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This has fueled an intense non-governmental organizational (NGO) scrutiny of the complex relationship between ocean noise, bubble injury and marine mammal strandings (<http://www.awionline.org/oceans/Noise/IONC/index.htm>). There has also been significant scientific interest in the topic, with a workshop held in Baltimore, MD USA in April 2004 (Cox et al., 2006). The workshop concluded that 'gas-bubble disease, induced in supersaturated tissue by a behavioral response to acoustic exposure, is a plausible mechanism for the morbidity and mortality seen in cetaceans associated with sonar exposure.' The need was stated for further investigation of the onset of lung collapse and its impact on nitrogen (N₂) gas kinetics.

Imaging the Lung Under Pressure

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LONG-TERM GOALS

This study was undertaken to improve our understanding of the mechanical properties of the respiratory system for a range of diving mammals to improve our ability to predict gas exchange during diving. Our long term aim is to determine the alveolar collapse depth and the pressure where gas exchange cease in marine mammals. We hypothesized that differences in the anatomy of the respiratory system result in significant species differences in collapse depth. Understanding how pressure affects gas exchange is vital to understand how diving mammals manage inert and metabolic gases during breath-hold diving.

OBJECTIVES

Our main objective was to determine the elasticity (compliance) of the lower (alveoli) and upper (trachea and bronchi) respiratory system in marine mammals (seal and dolphin). A number of measurements will be used to estimate volumes of the respiratory system in deceased marine mammals at varying pressures (depths). We hypothesized that the anatomical differences in the respiratory system in different species would result in volume differences at equivalent conditions. Differences in the elastic properties would result in variation of the alveolar collapse depth for animals diving with the same lung volume. The results will be used to re-parameterize a previous mathematical model that predicts the alveolar collapse depth.

APPROACH

This project was separated into two aims: A) computed tomography and associated analyses of respiratory volumes; B) mathematical modeling to estimate respiratory volumes and the level of gas exchange.

A) Ultra-high resolution spiral CT scans were used to determine volumes of the respiratory system at varying pressures in different species of marine mammals. We used recently deceased, stranded or bycaught, animals. The specimen was placed inside the pressure vessel and the lungs inflated with varying volumes of air. The volume of air injected was meant to simulate an animal with a diving lung volume of 100% or 50%, of total lung capacity (TLC). The TLC was determined before the animal was placed in the chamber as the air volume required to raise the trans-pulmonary pressure to 30 cmH₂O.

The specimens were scanned at varying pressures in the chamber to allow us to determine how varying lung volumes affected the compression of the lower respiratory tract. Specialized 3D imaging software was used to estimate the air volumes in the various parts of the respiratory system at each pressure. We attempted to measure the trans-thoracic pressures using a high resolution pressure sensor. However, this system did not provide reliable results and for future experiments we plan to use of a differential pressure transducer to allow airway pressures to be measured.

As an alternative, we analyzed the properties of the various respiratory compartments (upper and lower airways) from excised tissues (Fig. 1 and 2) to determine if there are species differences in the structural properties of the respiratory system. These data will be used to revise a previously published mathematical model to predict air volumes in the upper and lower airways during diving as described in aim B.

B) The physical properties of the upper and lower airways will be used to re-parameterize a previously published mathematical model and allow testing of the hypothesis that differences in the physical characteristics of the respiratory system result in large differences in blood and tissue N_2 levels in deep versus shallow diving marine mammals.

WORK COMPLETED

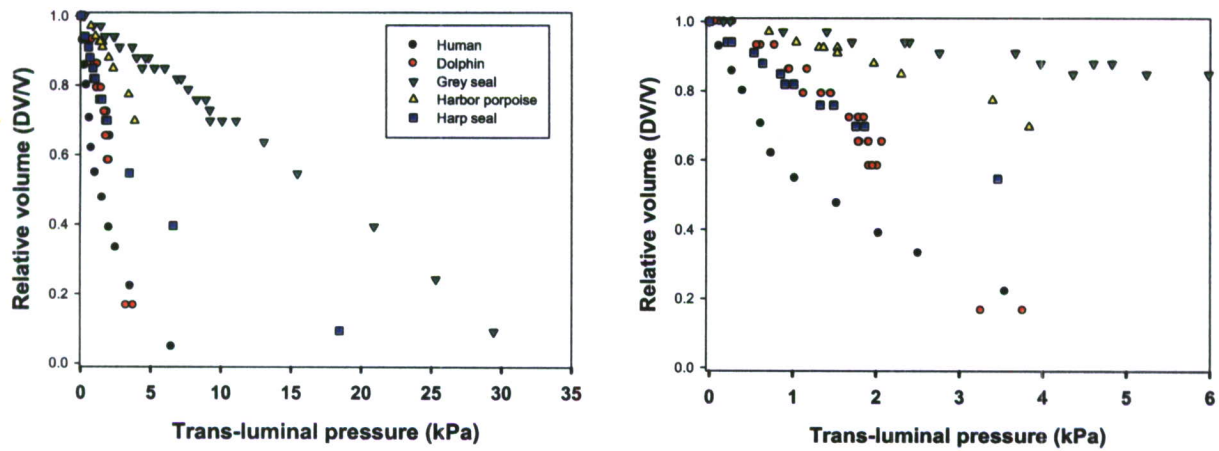
In the first year of this study, we completed the design, fabrication and testing of the pressure chamber. We also performed initial experiments to acquire images of seals and porpoises at different pressures and with different initial diving lung volumes.

In the second year we continued the hyperbaric studies and completed experiments to determine the air volumes in the lower airways at different pressures. Because our experimental design required an endotracheal tube to be inserted, we were not able to accurately determine the air volumes for the normal anatomy of the upper airways. We hope to continue this work in future studies and change our approach to avoid intubations. This, together with accurately measuring airway pressures, will allow us to determine the response properties of the upper and lower airways in intact specimens.

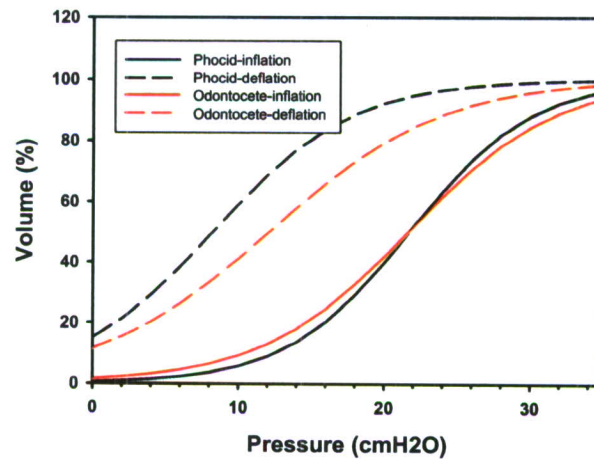
We determined the expansion/contraction states of the upper (Fig. 1) and lower (Fig. 2) airways from excised tissues from a range of marine mammals. The data showed that there are significant differences in the structural properties of the upper airways in different species (Fig. 1). Assuming an animal with lower airways with similar elastic properties and varying the compliance of the upper airways would result in a significant change in the alveolar collapse depth.

During inflation, there were no differences in the pressure-volume relationship between species or between odontocetes and phocids (Fig. 2). However, during deflation the phocid lung appears to be more compliant than that of odontocete (Fig. 2). Our previous theoretical work suggests that the alveolar collapse depth correlates with the compliance of the lower airway (alveoli) [see Fig. 3 in,1]. Thus, our results suggest that the elastic properties of the phocid lung may help facilitate collapse. However, work on live animals is required to confirm and refine these results as differences in chest wall compliance may affect the results.

RESULTS



[Figure 1 shows the relationship between inside and outside pressure (trans-luminal pressure and the relative volume in the trachea of a range of mammals. The figure shows how internal pressure increases as the trachea is compressed. The figure also shows the large range in compliances between different species, with the grey seal being the least compliant. With a total lung capacity (TLC) of 11 l, and a trachea and bronchi of 1 l, alveolar collapse would occur at 108 m for a grey seal, 150 m for a harp seal and 250 m for a human.,2]



[Figure 2 describes the inflation and deflation curves for phocids and odontocetes. The inflation volume data was expressed as a percentage of the estimated TLC in the prediction equation published by Kooyman and Sinnott [2]. The deflation data was expressed as a percentage of the maximum inflation volume before each deflation.]

IMPACT/APPLICATIONS

This work is intended to enhance our understanding of how the lungs compress during breath-hold diving in marine mammals. The results will provide information that will enable more realistic predictions of how alveolar volume varies during breath-hold diving. The study provides a proof-of-concept for a new generation of hyperbaric chambers that allow imaging under pressure.

Results from the completed study will help to improve our understanding about the physiology of marine mammals and improve modeling efforts that are aimed at estimating inert gas levels in breath-hold divers. The results can be used to determine how changes in dive behavior, from playback studies that measures avoidance patterns in deep diving whales, affect blood and tissue P_{N_2} levels. Thus, our results will enhance the fundamental understanding, interpretation and avoidance of the effect of anthropogenic sound, and enable knowledgeable decisions about sonar deployment, related training exercises and responses to NGO concerns. This should be of value to the US Navy Marine Mammal Program.

RELATED PROJECTS

Dr. Michael Moore is the PI on a related project where the hyperbaric CT chamber was being developed. The chamber was used for the experiments conducted in this study (Award Number: N000140811220). D.R. Ketten is the PI on ONR project 13123100 for assistance in scanning related research and datasets that provided the majority of support for the imaging of specimens, 3D reconstructions of specimens, and volume analysis software that will be used as well as access to previous scans on marine mammal species that may be used in this effort.

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