LONG-TERM GOALS

Understanding the behavior of gas within marine mammals as pressure varies with depth is critical for models of gas management in diving mammals. Interpretation of such data supports a mechanistic understanding of the effects, or absence of effects, of acoustic stressors on diving marine mammals.

OBJECTIVES

1. How does lung collapse progress in diving mammals with increasing depth?
2. Where residual air remains, what is the air to blood transfusion distance and rate?
3. How and why do gas bubbles develop in by-caught seals and porpoises drowned at various depths?
4. What are the gas compositions of bubbles detected in marine mammals?
5. Can ultrasound detect bubbles in intact marine mammals?
APPROACH

Specific Aims Part A (CT etc.)

1. Measurement of cadaver lung capacity and collapse in laboratory: 2D computerized tomography (CT) scans at the surface and in pressures equivalent to up to 200m depth, in cadavers in a composite, water filled, pressure vessel. 3D reconstructions of airways and adjacent tissues.

2. Histological analysis of air to blood vessel distances in sinuses, and trachea and wherever else residual gas is shown to remain in scans from Aim 1.

3. Gas bubble analysis for necropsy samples from by-caught and other stranding cases.

4. Sequential CT scan survey images of by-caught cadavers with bubbles to understand the dynamics of post mortem bubbling and to compare bubble formation incidence vs lipid content of different tissues.

Specific Aims Part B (Ultrasound)

5. Development of a bubble detection protocol with a portable ultrasound unit using by-catch known to be bubbled on CT, prior to necropsy. This protocol has substantially enhanced beach evaluation of the presence or absence of bubbles in mortality events.

RESULTS

Objective 1

Hyperbaric Computer Tomography: The hyperbaric chamber has been designed, built (Figure 1) and evolved to a point that it may be routinely used with CT spiral scans of seal and dolphin cadavers. Specimens of these animals have been intubated and given measured volumes of air, which are held in situ by a tracheal valve, while the animal is scanned at different pressures. This has enabled a novel imaging of the behavior of the chest organs in these animals at different pressures and volumes of retained gas. A paper is in preparation (Moore et al. In prep-b). Figure 2 shows CT slices of a mid-thoracic station, and 3D renderings, of inflated porpoise lungs at (top to bottom) 0, 50 and 100m depth equivalents. Note lung and chest wall compression. The area of each lung at each pressure in each 6mm slice was then measured using manual assignment of regions of interest (ROI) that included all lung tissue, but excluded the trachea and bronchi. The average CT attenuation was then measured for each slice. This allowed a calculation of the lung volumes. To estimate the amount of gas in each slice at each pressure we then normalized the attenuation on a scale from -1000 (gas) to 0 (tissue) using a published method (Patroniti et al. 2004). We then calculated the residual lung tissue volume at each pressure as the difference between lung and air volume at each pressure. We then plotted the resultant lung, gas and tissue volumes at half and full inflation for each of four species.

The resulting plots in Figure 3 show: the changing total lung volume with pressure; the compression of air to a very small volume by around 10 Atmospheres Absolute (ATA), depending on the species and level of inflation; and the residual lung tissue at each pressure. This is the first time there has been an actual visualization of diving mammal lung collapse. These plots show that the behavior of the lungs under pressure are remarkably similar between species, but by no means identical in the two seal and two odontocete species examined. They also show that the method used in the hyperbaric chamber,
and the CT scan analysis is repeatable. These numbers should of course be treated with circumspection in that it is unclear why the residual lung tissue volume decreases as the pressure increases. It must imply that the air volume estimate is not absolute. But they do suggest, at least in these dead animals, that lung collapse occurs at 8 to 10 ATA in the grey and harp seals examined, and 10 to 12 ATA in the common dolphin and harbor porpoise examined.

**Objective 2**

**Histological analysis of air to blood transfusion:** To revisit the possibility that there could be some degree of gas exchange once pulmonary alveoli are collapsed we examine the mean minimum air to erythrocyte distance in the trachea (shown after each species in microns) of a harp (193) and a harbor (72) seal, and a white sided dolphin (66) and a long finned pilot whale (224). All of these values are orders of magnitude larger than the alveolar air to erythrocyte distance of a few microns. Thus it would seem to be highly unlikely that there is significant diffusion of gas once the alveoli are collapsed. There should however be a careful analysis of the same parameter in the upper air sacs. This was not done in this project.

**Objective 3**

**Formation of gas bubbles in seals and dolphins drowned at depth in nets:** As part of this study we completed analysis of a series of net drowned seal and dolphin cases. Our findings were published in the attached publication to which we refer the reader (Moore et al. 2009). In summary gas bubbles were found in 15 of 23 gillnet-drowned bycaught harp (*Pagophilus groenlandicus*), harbor (*Phoca vitulina*) and gray (*Halichoerus grypus*) seals, common (*Delphinus delphis*) and white-sided (*Lagenorhyncus acutus*) dolphins, and harbor porpoises (*Phoecaena phoecaena*) but in only 1 of 41 stranded marine mammals. Cases with minimal scavenging and bloating were chilled as practical and necropsied within 24 to 72 hours of collection. Bubbles were commonly visible grossly and histologically in bycaught cases. Affected tissues included lung, liver, heart, brain, skeletal muscle, gonad, lymph nodes, blood, intestine, pancreas, spleen, and eye. Computed tomography performed on 4 animals also identified gas bubbles in various tissues. Mean ± SD net lead line depths (m) were 92 ± 44 and ascent rates (ms⁻¹) 0.3 ± 0.2 for affected animals and 76 ± 33 and 0.2 ± 0.1, respectively, for unaffected animals. The relatively good carcass condition of these cases, comparable to 2 stranded cases that showed no gas formation on computed tomography (even after 3 days of refrigeration in one case), along with the histologic absence of bacteria and autolytic changes, indicate that peri- or postmortem phase change of supersaturated blood and tissues is most likely. This work suggests that diving mammals are routinely supersaturated and that these mammals presumably manage gas exchange and decompression anatomically and behaviorally. It provides a unique illustration of such supersaturated tissues. We suggest that greater attention be paid to the radiology and pathology of bycatch mortality as a possible model to better understand gas bubble disease in marine mammals.

**Objective 4 - Composition of gas bubbles in marine mammals.** After proposing to undertake this work it became apparent that colleagues in the Fernandez group in the Canary Islands were undertaking a very similar study, and rather than duplicate their work we felt it prudent to await their results and hopefully collaborate in the near future.

**Objective 5**

**Ultrasound detection of Gas Emboli:** Calibration of B mode ultrasound imaging protocols, using a Teravet t3000 (http://www.terason.com/products/t3000.asp) on bubbled bycatch in parallel with CT and gross necropsy has also enabled deployment of ultrasound on live stranded and wild caught dolphins (Moore et al. In prep-a).


**Validation of ultrasound as a technique for gas bubble detection:** Bycatch dolphin cadavers were evaluated in the lab using B-mode ultrasound. Ultrasound was performed prior to CT and necropsy and all cadavers demonstrated widespread ring-down artifact consistent with the presence of gas bubbles. CT confirmed the presence of gas prior to disruption of the cadaver and demonstrated the extensive distribution of bubbling that involved all abdominal organs, blubber, vasculature and eyes. Gas bubbles were confirmed during necropsy observations. Gross necropsy and histopathology results confirmed that these dolphins had drowned without evidence of another cause of death. This lab-based study confirmed that B-mode ultrasound is capable of detecting gas bubbles.

**Evaluation of live stranded cetaceans for gas bubble formation:** Twenty-one live dolphins that stranded in the vicinity of Cape Cod, MA, USA during 2009-2010 and were employed for the study. These included 8 Atlantic white-sided dolphin and 13 common dolphin. Seventeen dolphins mass-stranded (more than one animal stranding at the same time) in four unrelated events. Four dolphins stranded individually. Nine dolphins either died or were euthanized and 12 dolphins were relocated and released at a site remote from the stranding location. Of those 12 dolphins that were released, two dolphins in two separate mass-strandings were each tagged with a satellite transmitter, and demonstrated normal behavioral pattern post release. One dolphin that was part of a mass-stranding event re-stranded the next day and was euthanized. A second dolphin that stranded as an individual was found re-stranded and dead the next day.

All 21 dolphins underwent ultrasound examination on the beach prior to any other procedure, and were repeatedly examined via ultrasound during the relocation process in those determined fit for release. The time between the dolphins physically stranding on the beach and the ultrasound being performed varied from within two minutes for three dolphins that mass stranded at a location where another dolphin was already being attended to and eight hours (estimated) for a dolphin found at the high tide water level early in the morning. In all 21 dolphins gas bubbles were identified in the region of the kidney bilaterally using ultrasound (Figure 4). In 2/21 dolphins, gas was concurrently identified in the portal vasculature and of those two dolphins, one was successfully released and one died. Gas was not identified in either eye of any dolphin.

Four of nine dolphins that died or were euthanized underwent CT scanning prior to necropsy. CTs were performed in three dolphins within twelve hours of death with the dolphin being kept cool for the time between death and scanning. In one dolphin the CT scan took place within two hours of death. In all four dolphins, gas-attenuating regions were identified in the region of both kidneys as was identified via ultrasound while the dolphins were still alive. There was no CT evidence of gas accumulation in the liver or eyes of these four dolphins, correlating with the ultrasound results for those individuals. On CT examination, all four dolphins had abnormal gas accumulations in locations other than the kidneys. In those dolphins, abnormal gas was identified between the blubber layer and the muscle, in multiple linear accumulations within the musculature that were presumptively intravascular, in the coronary arteries of the heart, within the brain and in the spinal canal. It was not always possible to determine whether the gas was intravascular or intraparenchymatous (within the body of the organ) via CT. In addition to abnormal accumulations, gas accumulations considered to be normal volumes based on location were identified in the paranasal sinuses, within the upper and lower airways and within the gastrointestinal tract.

Nine of nine dolphins that died or were euthanized underwent necropsy with tissue sampling for histologic exam. In all nine dolphins, gas accumulations in the region of the kidney was confirmed. This was mostly subcapsular but some renal intravascular bubbling was also identified. Gas bubbling
at necropsy was often more extensive than had been determined from CT images due to the ability to spread out the gastrointestinal tract and its associated mesentery and evaluate the portal and mesenteric vascular system.

DISCUSSION

The major limitation of the hyperbaric CT work to date is that the act of intubating and closing off the upper airways with a hard tube and valve system hampers the compliance of the upper airways. Our analysis of data generated from the hyperbaric chamber is based upon a series of experiments whereby the trachea was intubated with a valved endotracheal tube. This allows closure of the respiratory tract and accurate measurement of volume changes of the lung vs. pressure with different degrees of inspiration. It does not however, allow the compliance of the non diffusional upper airway structures, such as the trachea, larynx, internal nares and vestibular sacs to be figured in to the overall behavior of the lung biomechanics. However upper respiratory tract compliance is central to the behavior of the lung in terms of depth of lung and alveolar collapse. In hindsight this was an unfortunate aspect of the protocol, but it did at least enable a data series of lung compression. We therefore need to refine our protocol to enable the study without intubation as in a pending proposal. Furthermore, the first iteration of this work used an inspired volume taken to depth and then scanned at a series of reducing pressures. In the future we will scan at a greater number of pressures, including the maximum capacity of the chamber (250psi, 17ATA, 176m), starting at surface pressure and increasing in steps and then decreasing again.

The findings of the ultrasound work are very intriguing and perhaps unexpected, although on reflection we should not be so surprised. If these animals are routinely supersaturated then why would they not be routinely bubbled? The next step is to move from the stranding beach and the live capture boat, to a diving mammal at sea doing what it does normally. At the April 2010 Woods Hole DCS workshop there was substantial discussion about the relative merits of screening for bubbles with routine B mode ultrasound vs. intravascular Doppler probes. The latter has a greater chance of detection, but requires catheterization which would not be practical for deep diving odontocetes.

IMPACT/APPLICATIONS

The above findings add weight to the hypothesis that diving marine mammals manage bubbles routinely and therefore have to manage the risk of decompression sickness (DCS). The work of Houser et al.(Houser et al. 2010) does not refute this hypothesis in that their experimental animals were maintained in shallow water, and thus were not fully saturated at the time of their relatively brief diving experiments. We suspect that there are physiological, behavioral and anatomical mechanisms that normally minimize the clinical impact of bubbles. The findings of Fernandez et al. (Fernandez et al. 2005) would suggest that acoustic stressors may push bubble impacts from subclinical to clinical.

TRANSITIONS

We anticipate that the ability to image substantive biological material under different pressures will have a diverse range of academic and industrial applications once the method has been published. We have demonstrated the current HCT system to a representative from Creare Inc., who is currently in Phase 1 of an SBIR related to a comparable hyperbaric system for MRU.
RELATED PROJECTS

Dr Fahlman has analyzed inflation and deflation pressure-volume loops in breath-hold excised seal and dolphin lungs to show that mass-specific lung capacity does not differ between species that normally dive in shallow water compared to those that commonly do deep dives and that that the elastic inflation properties in excised marine mammal lungs do not differ between different species or between phocids and odontocetes.

REFERENCES


PUBLICATIONS


PATENTS

None

HONORS/AWARDS/PRIZES

None
Figure 1. Hyperbaric imaging system. The chamber consists of a fiberglass pipe with end flanges and caps rated to 250PSI. A dead seal or dolphin is placed in the imaging portion (arrow), restrained with suction cups to the chamber wall, and the chamber is filled with water. A hydraulic press allows pressurization to the desired depth equivalent. The chamber rests on a carriage on a pair of rails, to accommodate the loaded weight as that exceeds the scanner table capacity. The rails are motor driven to follow the movement of the scanner table. Spiral CT scans are acquired of the subject at a series of pressures.
Figure 2. The images at left show CT sections though the same mid-thoracic station of a harbor porpoise (IFAW10-045Pp) at depth equivalents from top to bottom of 0, 50 and 100m. The darker the tissue, the more air it contains. The images at right show a 3D rendering of the same CT series at each pressure. 3D renderings were made by Eric Montie (Uni. S FL.). As the pressure increases the amount of air in the lung decreases.
Figure 3. Observed lung total, air and tissue volumes vs. pressure in the lungs of seals and dolphins. A valved endotracheal tube was inserted, and sealed after the lungs were fully inflated at surface pressure. The three curves for each case show the volume of the lung, including air, and the volume of air as observed by CT attenuation, and the volume of the remaining lung tissue.
Figure 4 - Ultrasonogram of the right kidney of a common dolphin live stranded in Wellfleet, MA. The brilliant white areas are reflections from gas bubbles. After two hours of stabilization and health assessment it was released back to deep water at Herring Cove Beach, Provincetown, MA. Satellite tag data showed it reunited with other releasees and was sighted doing well in a large group of conspecifics.