Physiological Monitoring in Diving Mammals

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LONG-TERM GOALS

The objective with this study is to develop and calibrate an invasive data logger to measure muscle O_2 saturation in large, freely diving whales. We intend to use this data logger to measure muscle O_2 saturation and determine how blood flow to muscle is altered during diving. These data will be important to determine if muscle blood flow is reduced during diving, and important to estimate how the dive response affects muscle N_2 levels and the risk of decompression sickness (DCS).

OBJECTIVES

Recent necropsy reports suggested a link between mass stranding of beaked whales and the use of naval mid-frequency sonar. The whales experienced symptoms that were similar to those caused by inert gas bubbles in human divers. 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 certain species of deep diving whales. Primary issues have centered on direct auditory damage, resonance of gas containing spaces, and increased risk of DCS due to alteration in dive physiology or behavior, or acoustic enhancement of bubble formation and growth.

The stranding events have 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). During a workshop held in Baltimore, MD USA in April 2004 [1] it was 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.’

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One approach to study this problem has been through theoretical calculations of the plausible tissue and blood N_2 levels, and recent work suggests that beaked whales commonly experience end-dive N_2 levels that would cause a significant proportion of DCS cases in terrestrial mammals [2]. Model sensitivity analysis further suggested that level of the dive response (cardiac output and the blood flow distribution) strongly influence the N_2 levels in blood and tissue, and thereby DCS risk [2-5]. It is assumed that all breath-hold diving marine mammals experience a dive response while submerged, with a reduction in cardiac output and a re-distribution of blood to the core. But the precise knowledge of how deep diving whales distribute blood flow has never been measured. Improving such knowledge would significantly enhance our ability to predict end-dive blood and tissue N_2 levels, and determine if deep diving whales are at risk of DCS.

APPROACH

This project is separated into two aims: Aim 1) Development of a new generation of tags/data logger for marine mammals that will contain a sensor to be implanted into the muscle. The logger will collect physiological data from muscle tissue in freely diving marine mammals. The sensor will be tested and calibrated in terrestrial mammals at Massachussetts General Hospital, Boston.; Aim 2) The data logger will be tested in freely diving marine mammals in the field, and muscle O_2 saturation data will be collected.

Aim 1) A near infrared spectrophotometer connected to a data logging device will be developed and used to measure myoglobin/hemoglobin O_2 saturation in freely deep diving whales (e.g. beaked whales, sperm whales). The unit will be developed based upon the succesful construction of an oximeter used in Weddel seals [6].

A delivery device will be fabricated that will allow the optical probe to be implanted into the muscle. The sensor and photodetector will be inserted through the skin and blubber into the muscle. The flexible cable will allow the muscle to move freely, resulting in minimal discomfort. Initial experiments on terrestrial mammals and stranded or by-caught (post-mortem) marine mammals will assess the impact of the implantation to minimize the potential for inflammation and hematoma [7]. Aim 2) The data logger will be tested on a variety of diving marine mammals over 2 field seasons. We aim to perform trans-location experiments in Northern elephant seals (*Mirounga angustirostris*) with collaborators at University of California Santa Cruz (UCSC). This allows us to perform controlled field experiments and to determine if the data logger is able to collect the physiological data and to assure minimal tissue trauma.

We will implant the oximeter into the muscle of freely, deep diving whales (beaked and/or sperm whales) during 2 field seasons.

WORK COMPLETED

Aim 1:

LED/photosensor development: Our collaborators at MGH have begun development of the oximeter sensor. In the first year, testing began using a fiber optic approach. Deoxygenated blood at various dilutions was used to test that the response was linear over the physiological range.

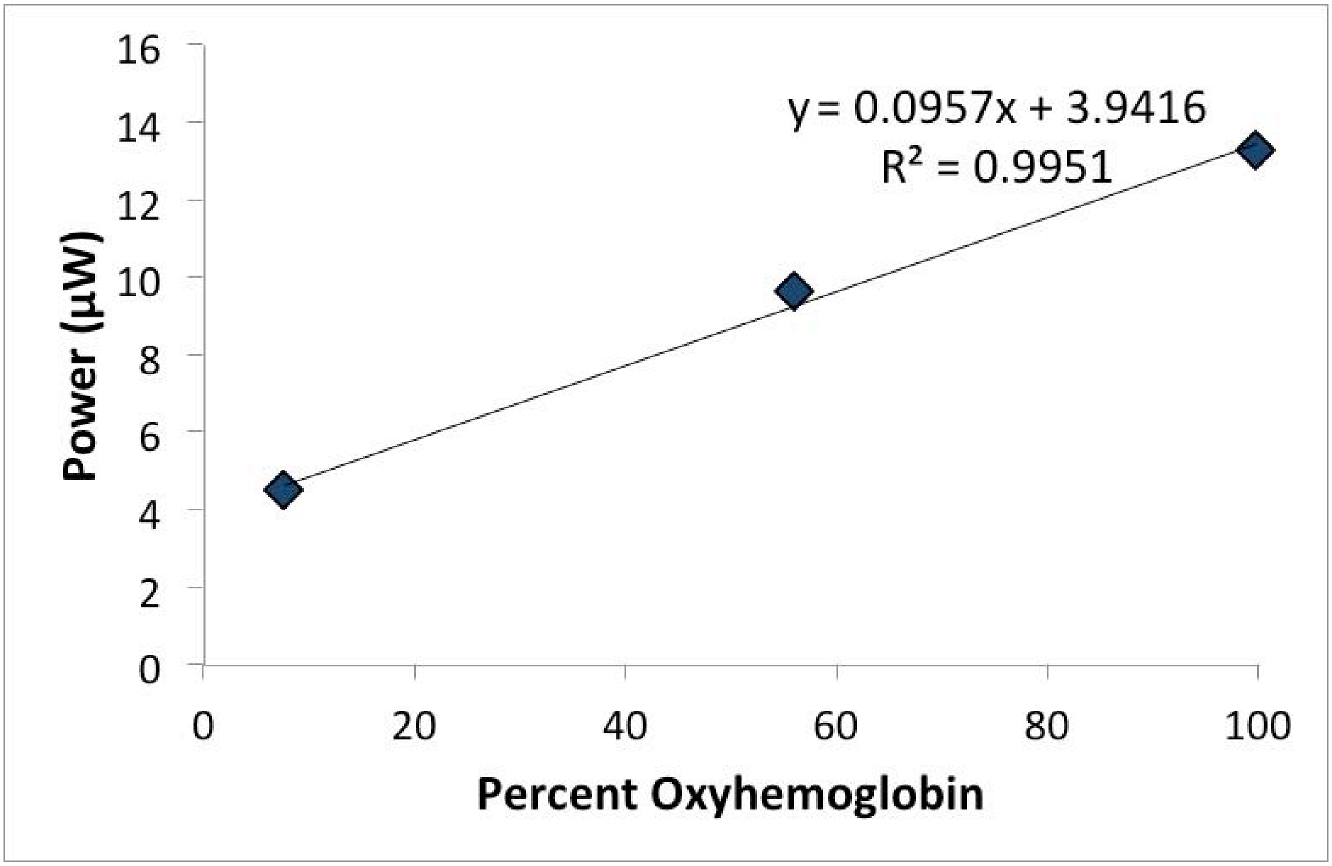

Figure 1. Correlation curve of transmitted optical power against oxyhemoglobin content.

As the approach to implant the sensing unit changed during development (see below), we are currently testing three different LEDs. One required test is to determine the optimum beam angle and distance between the two LEDs of different wavelength. In addition, we will verify linear response of sensors to changes in oxygen content of blood to determine minimum light source electric and optical power requirements to reliably measure changes in oxygenation of blood. This will allow us to determine the size of the smallest light source that we could possibly use. The difference among sensors are mainly the size and operational powers, 1) large (30 mW, [6]) 2) medium (10 mW, [8]), and 3) small (5 mW). Sensors 1 and 2 have been demonstrated to work in seals and penguins when carefully placed by surgery. Sensor 3 is commercially available designed for medical use. These sensors were used and designed to operate under specific conditions, which differ markedly from ours. This testing is in progress and should be completed later this fall.

Implantation and delivery system:

Initially, we intended to implant a fiber optic cable into the muscle. The fiber optic cable would be connected to an external logger, which would house the LED and photosensors. The cable would be inserted using a needle delivery device developed by our colleagues at Paxarms. During the development we discovered that there is remarkably little structure linking the blubber to the sub-dermal sheath in cetaceans. This allows the blubber to move laterally over muscle. Therefore, a cable crossing the blubber/muscle interface would experience sheer forces of varying degree. To assess the potential stress to the cable, Paxarms built a testing platform, consisting of a blubber sample glued to a sheet of foam (Fig. 2). The data showed that there would be considerable movement of the cable, which would interfere with the readings and potentially cause tissue trauma. One possibility would be to anchor the probe within the muscle. As the properties of muscle and foam might be quite different, the muscle being more viscous and elastic, the viscosity of muscle may make the tissue sticky and thereby anchoring the cable. However, before considering this approach tests were made on blubber/muscle samples from stranded (post-mortem) animals to determine the effect in real tissue. The data confirmed the blubber foam results and also indicated that anchoring the cable would cause trauma to the tissue.

Figure 2 shows the testing platform that was used to determine the mechanical forces acting on the implanted cable. The top left panel shows the testing platform, consisting of a blubber sample glued to a foam pad (the muscle). The cable was inserted through the blubber and foam (top right). Maximum lateral movement showed that the cable moved in and out of the sample a maximum 4 cm.

We therefore modified our approach and we will implant a small probe that will hold the LED and photodetector (Fig. 3). The probe will be implanted into the muscle using a custom-built rifle with a laser scope (Fig. 4) that will allow us to adjust the power of the delivery to account for different species and distances. The sensor probe will be connected to the external data logger (Fig. 5) with flexible wires that will allow lateral movement of the muscle/blubber interface without pulling the probe or causing sheer stress to the wire. Figure 5 shows the prototype logger housing that will be attached externally and released using a locking mechanism. The data logger will house the board that drives the LEDs and sensors on the probe and will log the data. The board has been tested, and Figure 6 shows the output when the sensors are placed on the finger of a human.

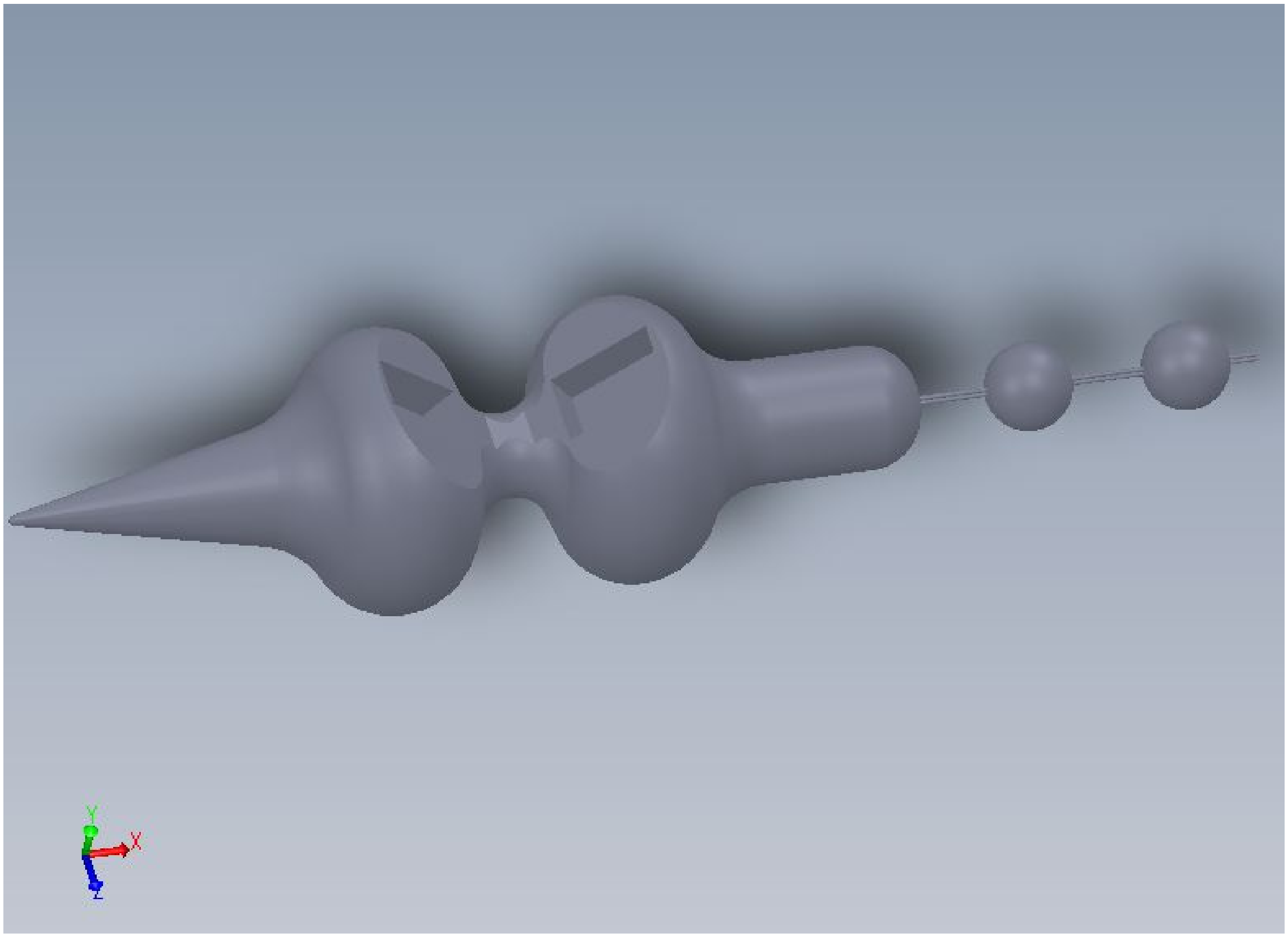

Figure 3 shows a prototype of the ballistic end that will be implanted into the muscle and hold the LED and photosensor. The probe is shaped to reduce movement in the muscle and will be connected to the external tag with flexible wires.

Figure 4 Prototype of rifle, Paxarms Remote Delivery System (PRDS) that will be used to implant the oximeter probe into the muscle. The PRDS consist of a rifle with a laser scope and a guideline.

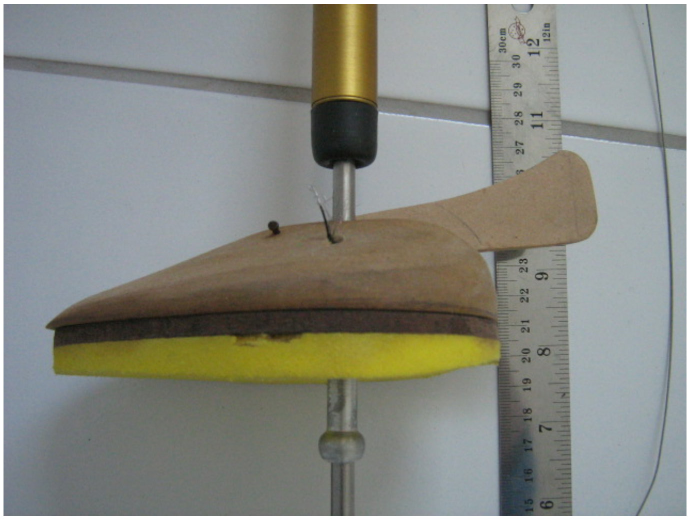

Figure 5 Mock up tag viewed from the side showing the foam pad at the bottom and the tag housing with locking wing.

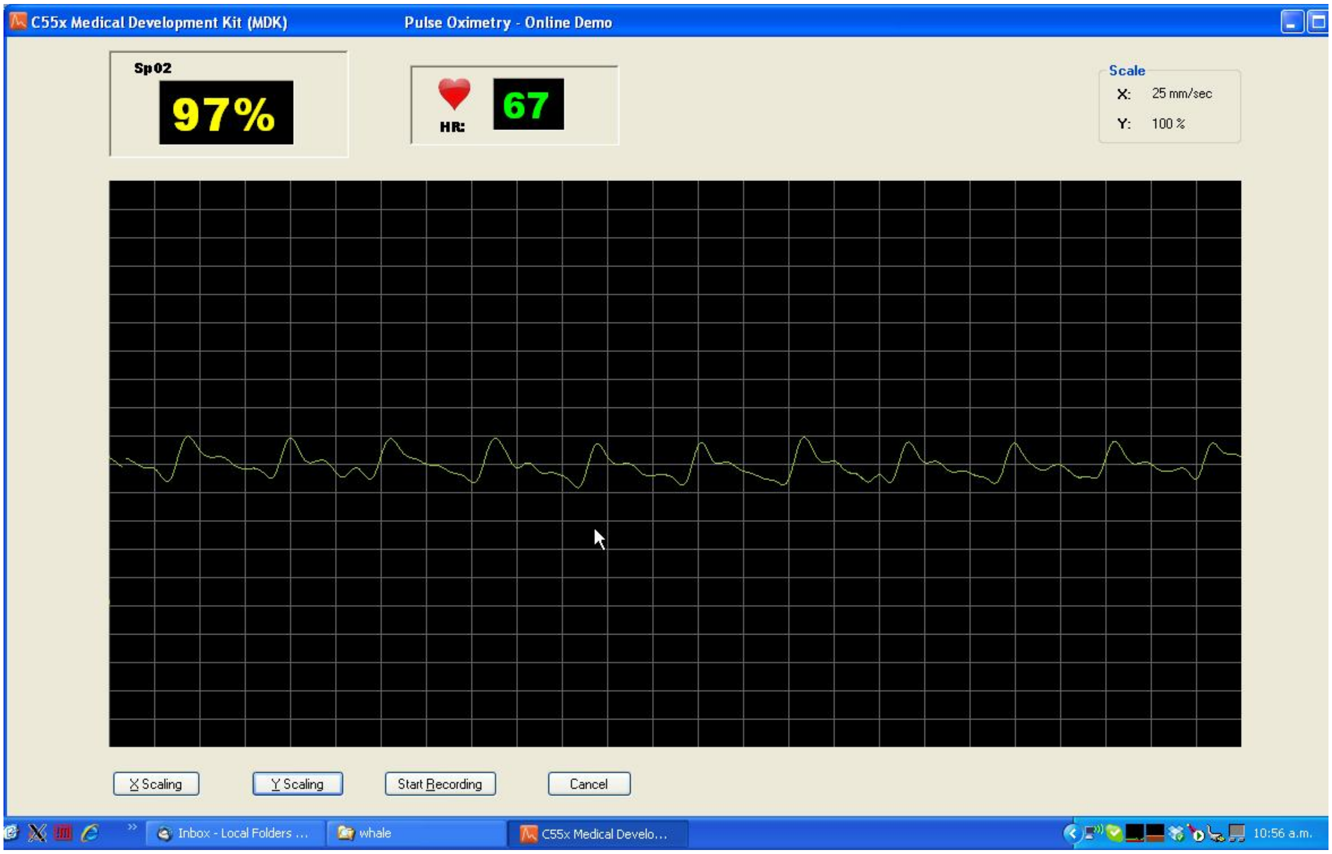

Figure 6. Computer board output from sensor and LEDs when placed on the finger of a human. Calibration experiments are on-going to define the relationship between output and oxygen saturation in live animals from 100% to 0% O2 saturation.

Aim 2: We expect to have the first prototype of the probe and logger ready for testing on post-mortem stranded whales in the fall 2011, and testing in live elephant seals should begin in the first half of 2012.

We consider the Northern elephant seal to be a suitable species to perform the first tests. This species perform long and deep dives, similar to sperm and beaked whales. It is also possible to perform controlled translocation experiments in this species where the instruments can be retrieved within a few days of release. For the field experiments on elephant seals, we are planning experiments in collaboration with researchers at UCSC in Santa Cruz.

For tag deployment on whales, we have developed collaboration with Norwegian colleagues at the Norwegian Defense Research Establishment in Horten and the Polar Institute in Tromsø, Norway. We are currently discussing the time-frame and logistics for this tagging effort and how to combine it with on-going work to minimize the logistical burden and to reduce animal impact.

RESULTS

Aim 1) All the components of the data logger (oximeter probe and data logger housing and electronics) and delivery system (custom-built rifle) are currently in the development stage and will be tested in the next few months. We have shown that we are able to detect differences in blood oxygenation using

LEDs and reflectance sensors. We have evaluated and tested the lateral movement betweel whale blubber and muscle to assess the mechanical forces acting on the optical probe.

Aim 2) We are planning the field experiments in year 2 and 3 and all the animal care protocols and permits have been approved.

IMPACT/APPLICATIONS

This work is intended to enhance our understanding of how the dive response alters muscle blood flow and metabolism in large, freely diving whales. The results will provide information that will enable more realistic predictions of how the dive response varies during breath-hold diving at different activities. The study will also provide a new generation of data loggers that are able to collect physiological data in large whales with minimal impact.

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.

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