LONG-TERM GOALS

The long-term goal of this study is to develop a biomarker of decompression stress in cetaceans to better understand the link between anthropogenic interactions and barometric stress. We aim to analyze blood samples from captive, wild-caught, and stranded marine mammals in order to compare concentrations of Microparticles (MPs). If confirmed as an indicator of decompression stress, the use of MPs could be applied as a diagnostic tool for stranding events. Development of an effective diagnosis tool has significant implications to the military when the cause of strandings is in question.

OBJECTIVES

Recent necropsy reports have suggested a link between mass stranding of beaked whales and the use of naval mid-frequency sonar [1]. 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. It has been suggested that alteration in physiology or diving behavior may increase the risk of decompression sickness (DCS).
Bubble formation is believed to be the crucial event in the etiology of DCS, but the role bubbles play in the disease process remains unclear. As we learn more about DCS, it has become apparent that some of the symptoms are similar to those of other disease states [2, 3]. Recent studies have shown that Microparticles (MPs) correlate with the level of decompression stress in both the mouse [4] and human [5]. MPs are particles between 0.3 to 1 µm in size that are shed from various cells. MPs derived from platelets are known to activate leukocytes and cause aggregation, can stimulate pro-inflammatory cytokines, and MPs derived from decompression stress have been shown to activate neutrophils and cause vascular damage [4]. In addition, our recent investigations have confirmed that MPs are present in stranded odontocetes and phocids and can be detected by standard assays. Thus, MPs may be suitable biomarkers to assess decompression stress. The study is aimed at verifying a relationship between decompression stress and MPs in sea lions and then transferring this knowledge to assess decompression stress of cetaceans in the field.

**APPROACH**

This project is separated into three aims:

**Aim 1:** The relationship between decompression stress and MPs was calibrated by conducting voluntary dive trials in Steller sea lions housed at the Open Water (OW) Research Laboratory in Vancouver, Canada. By analyzing blood samples before and after a dive bout to depths of 5m and 50m, we aimed to verify a correlation exists between MPs and decompression stress (number of dives, duration and depth) in marine mammals. In addition, the metabolic cost (using respirometry), and environmental variables were measured during each dive series. B-mode ultrasound was used to determine whether bubbles are present before and after the dive bout and correlate the presence of bubbles with the measured decompression stress and MPs. Dive experiments were repeated 3 times and at 2 different depths, 5 and 50 m. The first experiment started on June 12, 2012 when the animals dove to 5 m, while the dive depth of the second and third experiments was to 50 m and started on June 18 and 21, 2012. For each experiment, blood samples were taken the morning of the dive experiment before the animal had been fed or had been diving for at least 3 days. The sea lion was then transported to the dive site and allowed to dive for a pre-determined duration, approximately 30 min. Blood samples were again taken 3 and 24 hours after the sea lion had surfaced after the last dive.

**Aims 2 and 3:** We have sampled and analyzed single and mass stranded dolphins (Aim 2) and live-restrained dolphins (Aim 3) for MPs. We have also collected associated data to determine which stressors correlate with changes in MPs.

**WORK COMPLETED**

**Aim 1:** In the first year, four adult female Steller sea lions participated in experimental dive bouts to depths of either 5m or 50m at the OW Research Lab. For each experimental dive bout, we collected and analyzed blood samples from each animal pre-dive and post-dive. Statistical analyses of dive data in the current year support our hypothesis that MPs concentrations are positively related to decompression stress; however stressors such as feeding and exercise may affect MPs levels. Data were collected in the first year to isolate the effect of feeding.
In the second and third year, surface swim trials were conducted in order to estimate the effects of exercise on MPs levels. By isolating feeding and exercise impacts, we can assess the effects of diving to depth on MPs concentrations.

Based on the Steller sea lion experimental dive bout data, an abstract was prepared and submitted to the Society for Marine Mammalogy 2013 Biennial Conference on the Biology of Marine Mammals in New Zealand. Dr. Fahlman’s graduate student Lauren Gonzalez presented this work at the conference in December 2013.

**Aim 2:** Blood samples from single and mass stranded marine mammals in Cape Cod were collected and analyzed for MPs concentrations. We plan to collect additional samples from stranded animals in the final year to compare against previous data.

**Aim 3:** In the third year, blood samples from wild-caught dolphins in Sarasota were collected and analyzed for MPs concentrations for Pre-, Mid-, and Post - deck restraint for health assessment. Analysis of the accumulated data from this field-work was undertaken.

**RESULTS**

**Aim 1:** We have continued to analyze the results of the experimental study of changes in Steller sea lion Microparticle count with diving, feeding and exercise, the data from which have been reported in previous reports. A manuscript is now in late draft stage and will be submitted for publication shortly. Results suggest that MPs do change with diving, however there are a number of concerns that have to be addressed before there can be clarity as to the relationship between diving stress and MPs. The most obvious concern with the data is that there is not a clear cut increase in MPs with depth of dive. We do not have a clear understanding of the reason for this, although we are suspicious that our data may be confounded by the potential for acclimation with successive dive bouts, thus further experimental study is warranted to establish the degree to which measurement of MPs in sea lions is a valuable indicator of decompression stress.

**Aim 2:** We have collected and analyzed further samples from beached cetaceans in the Cape Cod Area. We will continue to do this over the next six months and then compile a final analysis of these results, and combine them with the results from Aim 3 to summarize our findings with regard to microparticle counts in wild cetaceans.

**Aim 3:** As mentioned in prospect in our 2014 report, we attempted to collect blood samples from deep diving *Tursiops* around the island of Bermuda in September 2015. This required major logistical planning in collaboration with Dolphin Quest Bermuda, the Bermuda Aquarium, and Chicago Zoological Society. Hoop net capture of bow riding Tursiops was attempted on three different occasions, but was unsuccessful. We believe that samples from these animals are extremely important, and will attempt this again in 2016.

**Plans:** In the final year of this project we will finalize and submit two papers from this study, as described above.
IMPACT/APPLICATIONS

Our current assessment of the sea lion experimental data is that MP levels do increase with diving, but not with feeding or exercise, but that the diving response is not depth related. We discuss possible reasons for this. The ability to effectively diagnose decompression sickness in marine mammals could aid in our understanding of anthropogenic sound on marine mammals, which would ultimately improve management practices. This could be especially significant in stranding events where the use and role of sonar is in question.

REFERENCES