Markers of Decompression Stress of Mass Stranded/Live Caught and Released vs. Single Stranded Marine Mammals

Michael Moore
Biology Department
Woods Hole Oceanographic Institution
Woods Hole, MA 02543
phone: (508) 289-3228 fax: (508) 457-2089 email: mmoore@whoi.edu

Stephen Thom
Dept of Emergency Medicine
University of Maryland
4-013 Bressler Research Building
655 W. Baltimore St.
Baltimore, MD 21201
phone: (410) 706-8294 email: SThom@smail.umaryland.edu

Andreas Fahlman
Department of Life Sciences
Texas A&M– Corpus Christi
6300 Ocean Dr Unit 5892
Corpus Christi, TX 78412
phone: (361) 825 3489 fax: (361) 825 2025 email: andreas.fahlman@tamucc.edu

Award Number: N000141210388

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 that they 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 also 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: Data were obtained from trained Steller sea lions (4 adult females) wearing time-depth recorders. Sea lions dove to predetermined depths (either 5m or 50m) with blood samples collected pre-dive (0 hrs) and post-dive (3 hrs and 24 hrs). We hypothesized MPs would be positively related to decompression stress. As a proxy for decompression stress, dive depth (dive dose) was integrated for each bout. Microparticle data were transformed to a relative change from the pre-dive value (control) and positive values indicate an increase ([MPpost-dive - MPcontrol)/MPcontrol x 100). We analyzed the relationship between a dependent variable and three different experimental variables (time after dive, depth and dive dose) using linear-mixed effects models (lme, R: A Language and Environment for Statistical Computing, R Foundation for Statistical Computing, version 3.1.0, 2014). Individual animal was treated as a random effect, which accounted for the correlation between repeated measurements on the same individual [6]. In this study P-values ≤ 0.05 were considered as significant and $P \leq 0.1$ were considered a trend. Data are presented as the mean ± standard deviation (SD), unless otherwise stated.

There was a significant difference in pre-dive blood MP levels between experimental trials (AIC_{null} = 192.6, AIC_{time} = 151.5, $P < 0.01$). There was a trend toward a 26% decrease in MP levels from the first (2604 +- 352 MPs μl^{-1}) to the second experiment (1935 +- 163 MPs μl^{-1}, $P < 0.1$) and then a 57% increase from the first to the last experiment (4082 +- 788 MPs μl^{-1}, $P < 0.05$).

Diving induced a significant increase in MP levels following a dive (AIC_{null} = 485.7, AIC_{time} = 446.0, $P < 0.01$). Three hours following a dive bout the blood levels had increased by 170% and after 24 hours by 536% (Fig. 1). There was a trend for an increase with dive depth (AIC_{time} = 478, $P < 0.1$), but this was mainly because of the much higher MP-levels at 3 hours following the first 50m dive experiment (Fig.1). Feeding without diving increased the blood MP levels by 24% 3 hours after feeding (AIC_{null} = 126.0, AIC_{fed} = 110.4, $P < 0.01$), and the levels were back to control levels after 24 hours.

The effect of exercise on MP levels were tested twice and both times the pre-exercise MP levels (control) were much more variable and higher than any blood samples collected either before or after

diving (Range MP levels diving: 1763 - 24830 MPs μl^{-1}, Max levels before exercise: 18405 - 778750 MPs ul^{-1}). The reason for this is unclear but could suggest changes in blood drawing techniques where sheering of the blood could affect MP levels. However, there was a significant change in relative MP levels ($AIC_{null} = 357.3$, $AIC_{time} = 339.8$) but this change occurred between the 3hr and 24hr post-exercise blood samples (81% increase, $P < 0.05$). The MP levels at either 3hr or 24hr were not different from the pre-exercise levels ($P > 0.1$, Fig. 1).

Data analyses from the Steller sea lions experimental dive bouts support our hypothesis that MPs concentrations increase as a result of increased decompression stress. However the lack of an increase of MP count with increasing depth suggests a significant limitation in this tool as a marker for degree of decompression stress or could indicate that repeated diving results in acclimatization as has been shown in hyperbaric experiments (Montcalm-Smith, E.A., Mccarron, R.M., Porter, W.R., Lillo, R.S., Thomas, J.T., and Auker, C.R. (2010). Acclimation to decompression sickness in rats. *J Appl Physiol* 108**,** 596-603. doi: 10.1152/japplphysiol.00596.2009.).

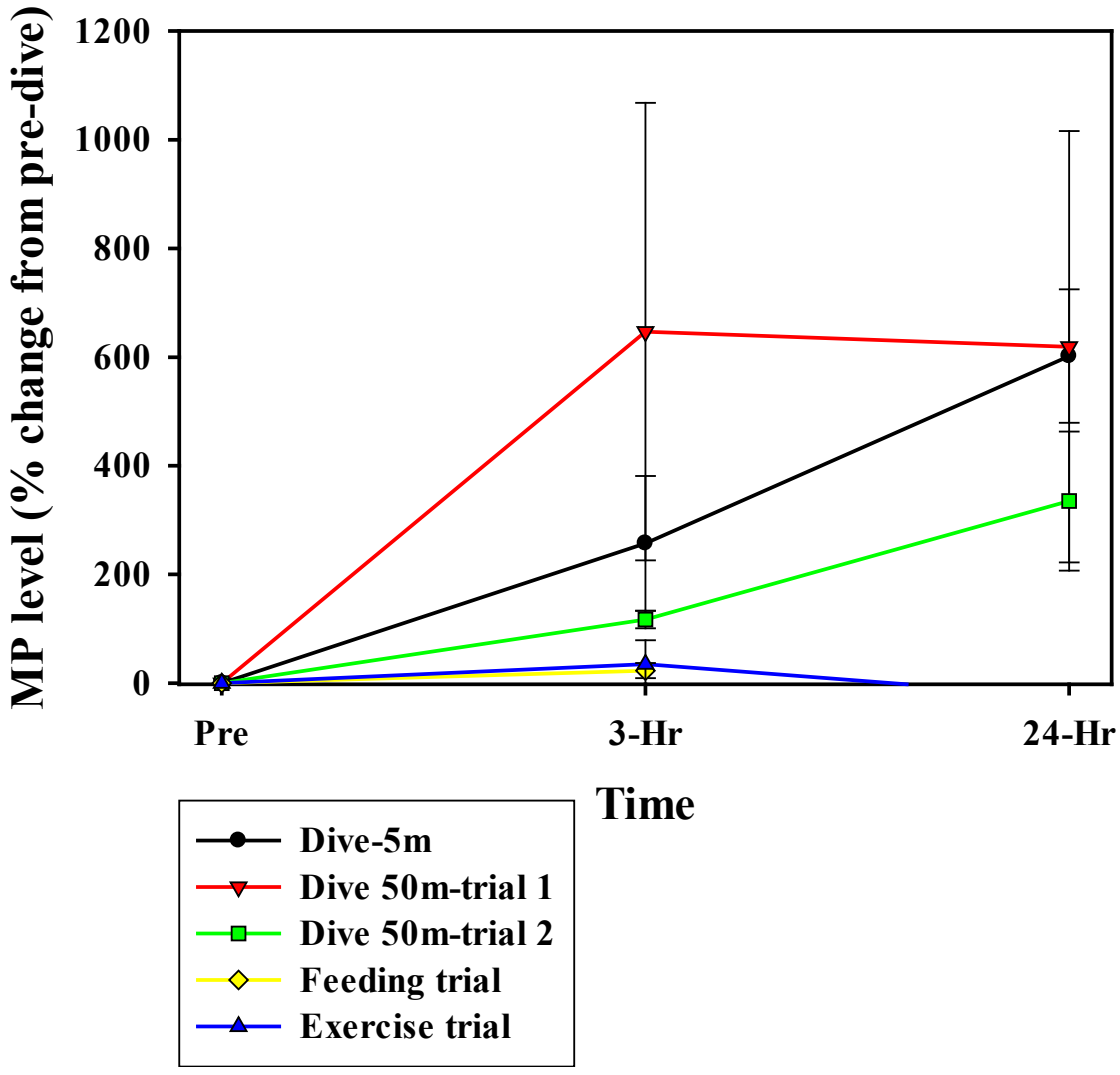

Figure 1. Average MPs for 5m and 50m dive bouts, measured at Pre-Dive (0 hrs), Post-Dive (3 hrs) and Post-Dive (24 hrs) Feeding and excercise control studies show that neither elicit a rise in MP's.

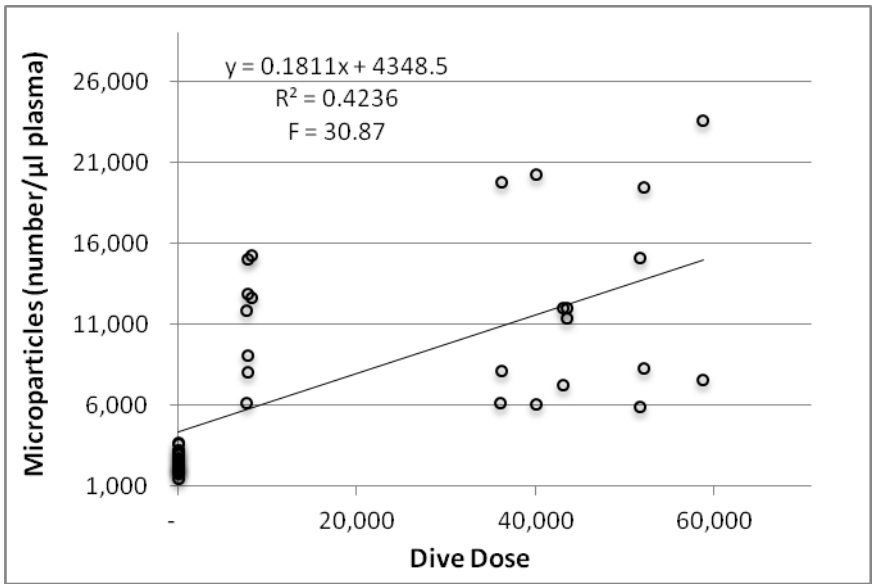

Figure 2. Regression analysis shows positive correlation between MPs and dive dose (estimated by integrating dive depth measurements from time-depth recorder)

Aim 2: Samples from a total of 9 stranded animals from 3 species were analyzed, serially when practical, with approximately one hour between sampling. A dissapointingly small sample size was obtained. No significant trends were observed comparing species, time since stranded or mass versus single stranded.

		Serial 1		**Serial 2**	
	N	**MP < 1um**	**MP 1-3 um**	**MP < 1um**	**MP 1-3 um**
Delphinus delphis	7	3098 +/- 2092	18 +/- 16	1725 +/- 1917	15 +/- 10
Lagenorhynchus acutus	2	8871 & 15625		13669	
Phocoena phocoena	1	7553			

Aim 3: A total of 123 blood samples from 58 wild-caught dolphins in Sarasota, Florida, were analyzed for MPs. The samples were obtained Pre-, Mid-, and Post-procedure. Whilst earlier analysis of year 1 and 2 data in this study there appeared to be an increase in MP count with removal of the animal from the water, analysis of all MP counts now available for this study showed no signficant impact of removal from, or return to the water (Table 1). In 2014 an additional MP size fraction (1 – 3 µm was analyzed.

Table 1 - Microparticle counts (/µl plasma) in Tursiops truncatus during temporary restraint on deck for health assessment

	At Capture in Water	On Boat	Before Release in Water
<1 µm			
Mean	5461	5607	5891
St Dev	5296	2360	4816
N	58	25	40
1 to 3 µm			
Mean	699	531	628
St Dev	703	704	1167
N	19	13	14

Plans: we have recived a no cost extension and hope to collect samples from deep diving *Tursiops truncatus* off Bermuda, and to augment the stranding sample size in the coming year.

IMPACT/APPLICATIONS

These results are equivocal given the lack of a pressure related increase in the sea lion experiment. If confirmed as a biomarker of decompression sickness, MPs might serve as a diagnostic tool for stranding events. 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

1. Fernandez, A., et al., *Gas and fat embolic syndrome involving a mass stranding of beaked whales (Family Ziphiidae) exposed to anthropogenic sonar signals.* Vet Pathol, 2005. **42**: p. 446-457.
2. Kayar, S.R., E.C. Parker, and A.L. Harabin, *Metabolism and thermoregulation in guinea pigs in hyperbaric hydrogen: Effects of pressure.* Journal of Thermal Biology, 1997. **22**(1): p. 31-41.
3. Ward, C., et al., *Complement activation involvement in decompression sickness of rabbits.* Undersea Biomed Res., 1990. **17**: p. 51-66.
4. Thom, S., et al., *Microparticles initiate decompression induced neutrophil activation and subsequent vascular injuries.* Journal of Applied physiology, 2011. **110**: p. 340-351.
5. Vince, R., et al., *Release of VCAM-1 associated endothelial microparticles following simulated SCUBA dives.* European Journal of Applied Physiology, 2009. **105**(4): p. 507-513.
6. Littell, R.C., P.R. Henry, and C.B. Ammerman, *Statistical analysis of repeated measures data using SAS procedures.* Journal of Animal Science 1998. **76**: p. 1216-1231.