Dive, food, and exercise effects on blood microparticles in Steller sea lions (*Eumetopias jubatus*): exploring a biomarker for decompression sickness.

Fahlman, Andreas.[1*]; Moore, Michael[2]; Trites, Andrew W.[3]; Rosen, David A. S.[3]; Haulena, Martin[4]; Waller, Nigel[4]; Neale, Troy[4]; Yang, Ming[5]; Thom, Stephen R.[5]

*(1) Texas A&M University - Corpus Christi, 6300 Ocean Drive, Unit 5892, Corpus Christi, Texas, 78412, USA *Corresponding author Andreas.Fahlman@tamucc.edu*

(2) Woods Hole Oceanographic Institution, Mailstop 50, Woods Hole, Massachusetts, 02543, USA

(3) Marine Mammal Research Unit, Institute for the Oceans and Fisheries, University of British Columbia, 2202 Main Mall, AERL Room 247, Vancouver, British Columbia, V6T 1Z4, Canada

(4) Vancouver Aquarium, P.O. Box 3232, Vancouver, British Columbia, V6B 3X8, Canada

(5) Dept. of Emergency Medicine, University of Maryland, 4-013 Bressler Research Building, 655 W. Baltimore St., Baltimore, MD 21201, USA

Abstract

Recent studies of stranded marine mammals indicate that exposure to underwater military sonar may induce pathophysiological responses consistent with decompression sickness (DCS). However, DCS has been difficult to diagnose in marine mammals. We investigated whether blood microparticles (MPs, measured as number/µl plasma), which increase in response to decompression stress in terrestrial mammals, are a suitable biomarker for DCS in marine mammals. We obtained blood samples from trained Steller sea lions (*Eumetopias jubatus,* 4 adult females) wearing time-depth recorders that dove to predetermined depths (either 5 or 50 m). We hypothesized that MPs would be positively related to decompression stress (depth and duration underwater). We also tested the effect of feeding and exercise in isolation on MPs using the same blood sampling protocol. We found that feeding and exercise had no effect on blood MP levels, but that diving caused MPs to increase. However, blood MP levels did not correlate with diving depth, relative time underwater, and presumably decompression stress—possibly indicating acclimation following repeated exposure to depth.

Key Words: Sea Lion; Decompression, Stress, Apnea, Diving, Bubbles

Introduction

The purpose of this study was to improve understanding of physiological responses to diving in a breath-hold diving marine mammal—with a particular focus on the pathophysiology of decompression sickness (DCS). DCS is a systemic pathophysiological process that occurs naturally after tissues become supersaturated with nitrogen under high atmospheric pressure. In humans, DCS can also occur when nitrogen or some alternative gas is used to dilute O_2 in breathing mixtures during activities such as deep-sea diving, high altitude aviation and space exploration. Most studies of DCS are performed with terrestrial mammals. We therefore hypothesized that a comparative physiological approach studying *bona fide* diving mammals would add valuable insight into the physiology of decompression. Such an approach may yield insights into the potential physiological traits that allow breath-hold diving animals to perform prolonged apneas to great depths without apparent pressure-related problems.

There are data indicating that diving mammals may sustain DCS, at least when they are subjected to atypical stresses (11, 16, 17). Recent necropsy reports have suggested a link between mass stranding of beaked whales and the use of naval mid-frequency sonar (10). The whales experienced symptoms that were similar to those caused by inert gas bubbles in human divers (4). These reports have increased the concern that anthropogenic sound, such as that created by military sonar or during seismic exploration, may harm marine animals. Specifically, it has been suggested that alteration in physiology or diving behavior may increase the risk of DCS (3, 15). For instance, blood bubble formation has been noted in some turtles that were trapped underwater and hauled rapidly to the surface. Those that received recompression treatment recovered and were released, thereby confirming a clinical diagnosis of DCS in a

diving vertebrate (12). Bubble formation is believed to be a crucial event in the etiology of DCS, but the role bubbles play in the disease process remains unclear (22). As more is learned about DCS, it has become apparent that some of the symptoms are similar to those of other disease states (18, 35, 36).

There are a few well-defined risk factors that increase the probability of DCS, such as increasing dive duration, dive depth, ascent/decompression rate, body mass, and breathing gas (8, 19, 20, 37). Recent studies have shown that microparticles (MPs) are elevated with decompression stress (31, 32). Microparticles are cellular fragments between 0.3 and 1 um in size that are shed from various cells. MPs derived from platelets are known to activate leukocytes and cause aggregation, and can stimulate pro-inflammatory cytokines. MPs derived from decompression stress have been shown to specifically activate neutrophils and cause vascular damage (32). Microparticles correlate with depth of diving in mice (32), but correlate poorly with depth in humans (31, 33). Studies have also evaluated the effect of exercise on bubble and MP production in human scuba divers. Exercise can increase the number of circulating MPs slightly, whereas diving has a much greater effect and is also impacted by the gas used during the dive (31).

The question of potential causal relationships between bubbles, MPs, platelet-neutrophil interactions, and neutrophil activation remains obscure (30). Our study aimed to examine the relationship between decompression stress (depth and time underwater) and MP levels in Steller sea lions in a controlled diving experiment, in the context of diving, feeding, and exercise. We specifically chose this species as they generally dive to between 30-40 m (24), they inhale before diving (Fahlman et al, unpublished observation), and have been trained to safely perform

repeated breath-holds without experiencing symptoms of gas bubble disease during dives up to 50 m (6). In addition, a theoretical gas dynamic model (5) predicted that for 12 repeated dives to 50 m there would be a significant increase in blood and tissue N_2 levels. Thus, we hypothesized that repeated breath-hold foraging dives would increase blood MP levels, and that the levels would increase with diving depth and time underwater. To control for the potentially confounding effect of feeding and exercise while diving, we also measured blood MP levels following exercise and feeding in separate experiments.

It is important to recognize that while we introduce the study in the context of the potential for decompression sickness, the diving experiments described here represent decompression stress as opposed to overt decompression sickness. Increase and decrease in pressure is a biomechanical stressor, in that whenever a diving mammal ascends, the gas in the lung expands, gas solubility decreases, and the animal has to manage changes consequent to that stressor. Thus our use of 'decompression stress' is considered and accurate. Diving mammals usually dive without clinical impact, but they nonetheless manage decompression stress during every dive. The question of interest to us was how do they do it? Addressing this question furthers knowledge about the physiological and behavioral mechanisms of diving that interests basic science and may have conservation impacts.

Methods

Animals

All experiments were conducted under permits from the Animal Care Committees of the University of British Columbia and the Vancouver Aquarium. Four female Steller sea lions

111 (*Eumetopias jubatus*) trained to dive to fixed depths participated in 3 dive experiments (Table 1).
112 Animals were weighed the day before a dive experiment, and had not been diving for at least 4
113 days prior to the first dive experiment. Each experiment was then separated by at least 2 days.
114
115 *Dive experiments*
116 The general experimental arrangement was as described previously (6, 9), with sea lions trained
117 to dive on command from within a respiratory dome to a specific depth. The depth was defined
118 by the length of a feeding tube delivering food to the end of the tube to reinforce the behavior.
119 Dive experiments were repeated 3 times each at depths of 5 m and 50 m. All dive experiments
120 were conducted in June.
121
122 For each experiment, pre-treatment blood samples were taken the morning of the dive before the
123 animal had been fed. Samples were drawn directly into 5 ml Cyto-Chex BCT vacutainer tubes
124 (Streck, Omaha, NE). To minimize shearing of blood components, care was exercised to obtain
125 a clean venepuncture and good sample flow. The sea lion was then transported to the dive site
126 and allowed to dive for a pre-determined duration of approximately 30 min.
127
128 Blood samples were again taken 3 and 24 hours after the sea lion had surfaced after the last dive.
129 Collecting blood samples immediately following the dive was deemed unsafe for both animals
130 and personnel. A 3 hr delay was therefore deemed acceptable before collecting the first blood
131 sample. The 24 hr post-dive sample was based on data in terrestrial mammals and allowed us to
132 trace the time-course for changes in blood MP levels (32). Food was offered during the dive and

after each blood sampling to reinforce behaviors. The same sampling protocol was repeated with only surface swimming and with feeding without access to the water.

Dive Dose

As the probability of DCS increases with both depth and dive duration, we computed a simple index, called dive dose, to estimate the relative time underwater for each experiment. Dive dose was estimated by integrating the dive depth (m) over time (sec) to calculate the index for each dive, which represents the total "depth exposure" for each animal while underwater.

Metabolic rate (rate of O_2 consumption)

The rate of O_2 consumption ($\dot{V}O_2$, L O_2 min^{-1}) before and during a dive was used as an index of the metabolic rate. The metabolic rate before and after the dive bout was assessed by measuring the gas concentrations in the metabolic dome (6). We separated metabolic rate into surface metabolic rate before diving (pre-dive), at which time the sea lion had received minimal amount of food and was post-prandial. As both the dive and surface interval durations were determined by the sea lion, and as reliable estimates for the metabolic rate of individual dives within a dive bout cannot be made (6, 7, 9), we computed the diving metabolic rate for the entire diving bout. Specifically, we divided the total volume of O_2 taken up from the beginning of the first dive until the end of the post-dive recovery period by the duration of that same period. Thus, we only computed one pre-dive and one diving metabolic rate for each experiment for each sea lion.

Exercise and feeding experiments

155 Two separate experiments were conducted to evaluate the potential confounding effect of
156 exercise or feeding/digestion on blood MP levels. For the exercise experiments, the sea lions
157 performed a 30 min surface swim by following a boat driving at a speed (approximately 2 m ·
158 sec^{-1}) similar to the estimated ascent and descent rates and underwater swim speed during the
159 dive experiments. Each sea lion performed two exercise experiments. For the feeding
160 experiment, the animals received a meal of the same size as during the dive experiments over a
161 30 min period. For both experiments, a blood sample was taken before and after (3 hr and 24 hr)
162 the experiments. Thus, we tried to replicate the time course used during the experiment to
163 separately assess the effect of feeding, digestion, and exercise on blood MP levels.
164
165 *Blood MP levels*
166 All blood samples were taken by venepuncture using a butterfly needle and a vacutainer. Blood
167 MP levels were measured using flow cytometry as previously described (31, 32). Microparticle
168 concentration data were transformed to a relative change from the pre-dive value (control) such
169 that positive values indicate an increase ($[MP_{post\text{-}dive} - MP_{control})/MP_{control} \times 100$). We analyzed the
170 relationship between a dependent variable and three different experimental variables (time after
171 dive, depth, and the product of time and depth, i.e., dive dose) using linear-mixed effects models
172 (lme, R: A Language and Environment for Statistical Computing, R Foundation for Statistical
173 Computing, version 3.1.0, 2014). Individual animal was treated as a random effect, which
174 accounted for the correlation between repeated measurements on the same individual (21). We
175 used the Akaike information criterion (AIC) to select nested models. In this study P-values $\leq$
176 0.05 were considered as significant and $P \leq 0.1$ were considered a trend. Data are presented as
177 the mean ± standard deviation (SD), unless otherwise stated.

178

179 **Results**

180 *Animals*

181 Four female Steller sea lions participated in 3 dive experiments and the average weight and ages
182 are summarized in Table 1. Animals were weighed the day before a dive experiment and each
183 experiment was separated by at least 2 days.

184

185 *Dive behavior, metabolic rates and dive dose*

186 The dive metabolic rates were significantly higher for both dives to 5 m (3.22 ± 0.73 L O_2 min^{-1},
187 paired t-test, $P < 0.05$, $t = 4.42$, df=3) and 50 m (3.03 ± 0.63 L O_2 min^{-1}, $P < 0.05$, $t = 2.81$) as
188 compared with the pre-dive metabolic rates (5 m: 2.15 ± 0.50 L O_2 min^{-1}; 50 m: 2.35 ± 0.41 L O_2
189 min^{-1}). There were no differences in metabolic rate for dives to 5 m as compared with 50 m (t_{10} =
190 0.47, $P > 0.6$, Table 1). The average dive duration and inter-dive surface interval for dives to 5 m
191 (dive duration: 50 ± 11 sec; surface interval: 11 ± 3 sec) were significantly lower than dives to 50
192 m (dive duration: 105 ± 21 sec, $t_{10} = 4.49$, $P < 0.01$; surface interval: 35 ± 7 sec, $t_{10} = 6.86$, $P <$
193 0.01, Table 1).

194

195 The average dive dose (m · sec) was significantly lower for the 5 m (T1, 7868 ± 264 m · sec) as
196 compared with either the first (T2, 50848 ± 7206 m · sec) or second (T3, 44729 ± 6107 m · sec)
197 dive experiment to 50 m ($P < 0.01$, paired t-test). In addition, dive dose was significantly higher
198 during the first dive experiment to 50 m as compared with the second ($P < 0.05$, paired t-test, T2
199 vs T3)

200

201 *Blood MP levels*

202 There was a significant difference in pre-dive blood MP levels between experimental trials
203 (AIC_{null} = 192.6, AIC_{time} = 151.5, $P < 0.01$). There was a trend toward a 26% decrease in MP
204 levels from the first (2604 ± 352 MPs ul^{-1}, 5 m trial, Fig. 1) to the second experiment (1935 ±
205 163 MPs ul^{-1}, $P < 0.1$, 50m Trial 1) and then a significant 57% increase from the first to the last
206 experiment (4082 ± 788 MPs ul^{-1}, $P < 0.05$, 50m Trial 2).
207
208 There was a significant increase in MP levels following a dive (AIC_{null} = 485.7, AIC_{time} = 446.0,
209 $P < 0.01$). Blood MP levels had increased by 170% 3 hours following a dive bout and by 536%
210 after 24 hours (Fig. 1). There was a trend for an increase with dive depth (AIC_{time} = 478, $P <$
211 0.1), but this was mainly because of the much higher MP levels at 3 hours following the first 50
212 m dive experiment (Fig.1). Feeding without diving increased the blood MP levels by 24% 3
213 hours after feeding (AIC_{null} = 126.0, AIC_{fed} = 110.4, $P < 0.01$), and the levels were back to
214 control levels after 24 hours.
215
216 The effect of exercise on MP levels were tested twice and both times the pre-exercise MP levels
217 (control) were much more variable and higher than any blood samples collected either before or
218 after diving (range MP levels after diving: 1763 - 24830 MPs ul^{-1}, Max levels before exercise:
219 18405 - 778750 MPs ul^{-1}). The reason for this is unclear and warrants further investigation.
220 While there was a significant change in relative MP levels (AIC_{null} = 357.3, AIC_{time} = 339.8), this
221 change occurred between the 3-hr and 24-hr post-exercise blood samples (81% increase, $P <$
222 0.05) and neither the MP levels at 3 hr or 24 hr were different from the pre-exercise levels ($P >$

223 0.1, Fig. 1). Similarly, the MP levels increased following feeding (Pre-feeding: 2570 ± 108 MPs
224 ul^{-1}, Post-feeding: 3175 ± 414 MPs ul^{-1}; $P < 0.05$, t_6=2.83, Fig. 1).
225

226 **Discussion**

227 This experimental study of Steller sea lions indicates that, as is true of terrestrial mammals, MPs
228 increase in response to diving. There is variability in baseline MPs numbers at the outset of
229 diving studies, similar to observations with mice and humans, which indicates that stressors other
230 than diving impact MPs levels. There was not a consistent increase in MPs with depth of a dive
231 or dive dose (relative time and depth underwater). This finding is consistent with studies of
232 human divers, but differs from results with inbred mice. More broadly, this raises the question
233 whether measurement of MPs in sea lions can reflect decompression stress. We do not have a
234 clear understanding of the reason for this; thus further experimental study is warranted to further
235 define what variables affect blood MP levels.
236

237 It has been over 100 years since some of the first studies on human issues related to
238 decompression following exposure to pressure were published (2). Since then, several studies
239 have attempted to understand the etiology of this disease and to find ways to prevent, or at least
240 significantly reduce, the risk of decompression sickness in human divers (e.g. 25, 37, 38). They
241 have shown that the primary reason for development of clinical signs is due to an increasing inert
242 gas burden (8, 37), with gas being released from solution as pressure and solubility is reduced
243 during the decompression phase. Bubbles form in tissues or within the vasculature where they
244 can block flow or initiate an inflammatory response (18, 35, 36).

Several physiological changes occur following a decompression event. For example, a reduction in platelet count or complement activation appear to correlate with the levels of decompression stress (26, 35). Circulating MPs also correlate with the magnitude of the decompression in uniform 2-hour dives in mice (32), although more recent work with human scuba divers found no correlation between MP elevations and depth of diving (29). It is notable in the human studies that duration of a dive and relationship between eating and diving were not addressed. Circulating blood MP levels may still be a useful tool to diagnose DCS, however, because human divers with DCS have significantly higher levels of MPs with specific surface proteins than asymptomatic, control divers (29).

We hypothesized that blood MP levels may be a good indicator of the level of decompression stress in breath-hold diving mammals given that MP levels indicate decompression stress in divers breathing pressurized air. We therefore allowed Steller sea lions to dive repeatedly to two different depths, 5 m and 50 m, assuming that dives to 50 m would cause a greater decompression stress. However, we found high variability in the circulating blood MP levels of the animals diving to 50 m (T2 vs T3, Fig. 1). The risk of DCS is not only affected by dive depth alone, but dive duration and ascent rate also alters the risk (37), and it has been shown that only a 5% change in the inert gas burden can result in a 50% change in the probability of DCS (8).

We used the integrated depth as a simple estimate of decompression stress (dive dose). The dive dose was significantly greater for deeper dives, but the dive dose was also significantly greater

for the first dive (T2) to 50 m as compared to the second (T3). This may explain the lower circulating MP levels during T3 as compared with T2, but not the shallower dive. The responses during breath-hold diving versus scuba diving may be different. For example, the breath-hold dives included animals foraging, exercising, and with increasing levels of hypoxia. This may cause a greater variability in the response as compared with experimental dives on scuba diving humans or animals in a pressure chamber that are continuously breathing.

Mild oxidative stress and hypoxia increase MP levels, and it is also possible that higher activity levels during shallow dives elevate blood MP levels (1, 34). During breath-hold diving, there is a rapid and short period of hyperoxia when the lungs compress and the pulmonary gas pressure increases and gas diffusion continues. Blood and tissue PO_2 continuously decrease throughout most of the dive, with levels < 10 mmHg commonly reported in diving California sea lions (23). Thus, while dive duration and end-dive PO_2 correlate, there was considerable variability between dives and individuals (23). As sea lions are generally quite active while diving, this variability could reflect variation in underwater activity.

The dive pattern for the Steller sea lions in our study was similar to those previously reported for the same animals (7, 9, 13, 14, 27, 28), where deeper dives were longer and associated with longer surface durations to replenish the O_2 stores. While there were no significant differences in the metabolic rate over an entire dive bout (Table 1), the diving metabolic rate correlated with underwater activity, and the activity was generally greater for shallower dives (7, 9). Thus, shallow dives are shorter, but of higher intensity—while the deep long dives are prolonged

289 events of lower intensity. Consequently, the sea lions may have become more hypoxic during the
290 high intensity shallower dives, which could have independently increased MP levels.

291

292 There is anecdotal evidence suggesting that repeated hyperbaric exposures help to reduce DCS
293 incidence, and a controlled study showed that rats acclimated over as little as 4 days showed
294 reduced DCS incidence compared with control animals (25). In our study, the sea lions had not
295 been diving actively for at least 4 days before the first dive experiment. The active diving to 5 m
296 (T1, Table 1) appeared to increase blood MP levels for at least 24 hrs, which then returned to
297 pre-dive levels prior to the 2nd experiment (Fig. 1). The blood MP levels again increased during
298 the first dive experiment to 50 m, at levels that exceeded the 5 m dives (Fig. 1). However, the
299 pre-dive MP levels did not return to control levels between the first and second dive to 50 m (T2
300 vs T3, table 1), suggesting that the 48 hr recovery time between experiments may not have been
301 sufficient. Thus, while the pre-dive MP levels showed no systematic pattern before diving the
302 animals may have become acclimated and less responsive to decompression stress, similar to that
303 observed in rats (25).

304

305 There are other possible reasons that the relationship between blood MP levels and
306 decompression stress (time and depth) did not correlate. Using blood MP as a biomarker is a
307 relatively new concept and as this is the first study to look at how blood MP levels change in a
308 diving mammals, we undertook separate experiments to determine the effect of digestion or
309 exercise on blood MP levels. For the former, we gave the sea lions a meal of similar size to what
310 they caught during diving. For exercise, we had the sea lions perform a surface swim running at

311 a similar speed that we estimated the sea lions were swimming underwater while foraging.
312 Neither of these variables seemed to significantly change the MP levels above the control levels
313 (Fig. 1).

314

315 In summary, blood MP levels seem to be a useful biomarker to identify decompression stress.
316 However, the magnitude of decompression stress (dive dose or depth) correlated poorly with
317 blood MP levels in Steller sea lions. We suggest that hypoxia or acclimation may affect the
318 response to decompression stress during breath-hold diving, which may explain the variation in
319 biomarker levels for dives to different depths. We conclude that neither digestion nor exercise
320 affected the blood MP levels and should not have influenced our findings. Thus, blood MP levels
321 may be a useful index to identify decompression stress, but more research is needed on Steller
322 sea lions and other species of marine mammals to verify our findings and separate the potential
323 confounding effects of hypoxia and acclimation.

324

325 **Perspectives and Significance**

326 Marine mammals are diverse group of animals with over 100 species that obtain their food from
327 the ocean. Million of years of evolution have enabled them with physiological traits to master the
328 art of free diving, and avoid the problems that increasing pressure have on physiological
329 homeostasis. These animals appear to avoid the risk of DCS during natural dives, but recent
330 studies have shown that under certain circumstances departure from normal physiology or
331 behavior may cause bubbles to form. While the diving physiology of marine mammals has been
332 studied for well over 70 years, this area of their physiology has received relatively limited
333 attention. To improve understanding about the potential traits that reduce DCS risk, we
334 investigated changes in blood MP levels during a natural diving bout in Steller sea lions. The

results help improve basic understanding about their physiology, and are important to assess the potential ecological impact of these species to changes in the environment. Thus, comparative studies on the diving physiology of marine mammals may have significant relevance for conservation efforts.

Authors' contributions

AF and MM conceived of the study, designed the experiments, obtained funding, collected and analyzed the data, carried out the statistical analysis, and drafted the paper; AWT, DR participated in the design of the experiments and data collection, provided access to trained animals, helped draft the manuscript; MH participated in the design of the experiments, provided veterinary support during anesthesia, participated in data collection; NW and TN helped design experiments, participated in data collection and were in charge of animal training; MY helped with microparticle analysis; SRT helped design experiments and performed analysis of microparticle levels. All authors gave final approval for publication.

Acknowledgements:

We thank Stephen Raverty for help with shipping samples, and extend a special thanks to all the animal care staff and research technicians at Vancouver Aquarium and the UBC Open Water Research Station who made this study possible. All research protocols were approved by the UBC Animal Care Committee, and the animal care committee of Woods Hole Oceanographic Institution. None of the authors have competing interests.

Funding

358 Funding for this project was provided by the Office of Naval Research to MM (ONR Award #
359 N00014-12-10388) and SRT (ONR Award # N00014-13-10614). Additional support was
360 provided by the National Oceanic and Atmospheric Administration through the North Pacific
361 Marine Science Foundation and the North Pacific Universities Marine Mammal Research
362 Consortium.

363

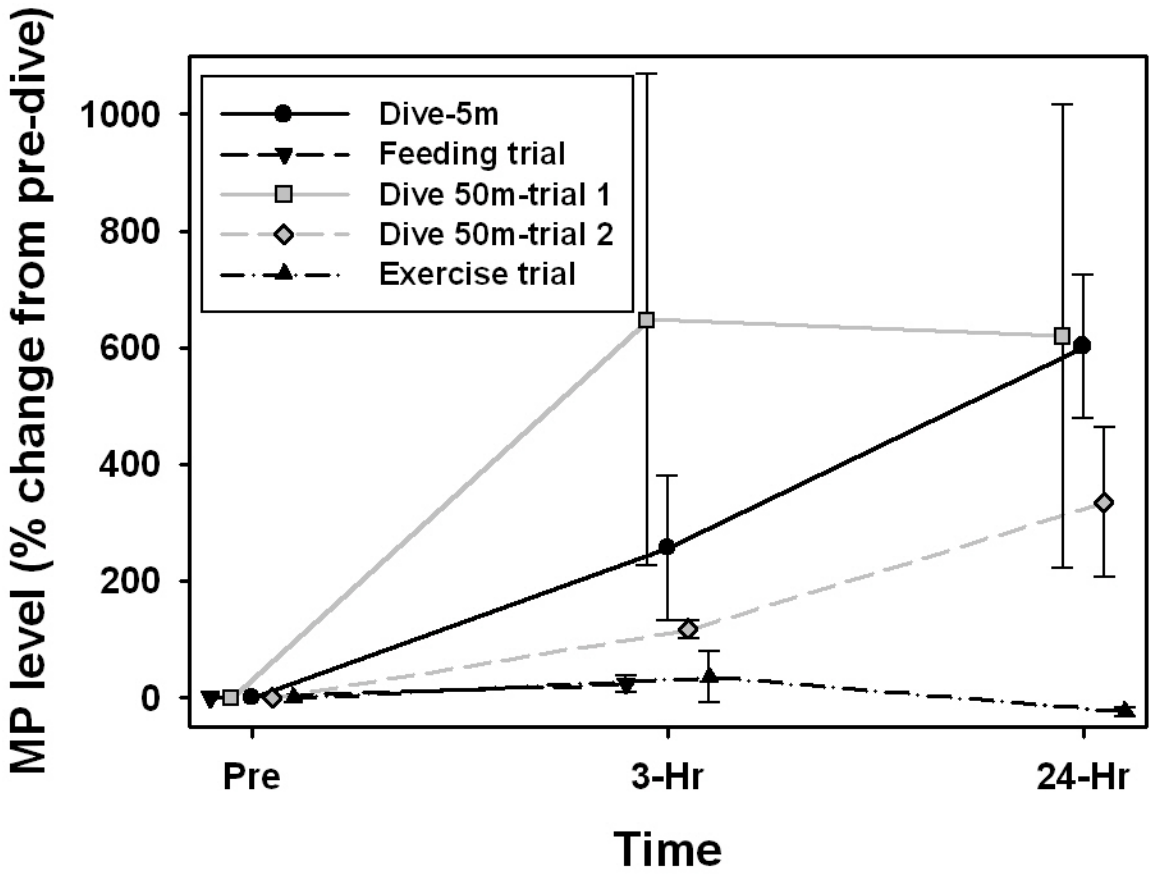
364 **Table 1.** Animal ID, age, body mass, and average dive duration (number in parenthesis is
365 number of dives), and measured pre-dive and dive metabolic rate for each trial (T1-5 m dive, T2-
366 1st 50m dive, T3-2nd 50 m dive). The pre-dive metabolic rate was the measured oxygen
367 consumption rate ($\dot{V}O_2$, L O_2 min^{-1}) during the last 2-3 min in the respirometry dome before
368 diving. Dive metabolic rate was the cumulative volume of O_2 consumed from after the first dive
369 until the end of the post-dive recovery period divided by the time from the start of the first dive
370 until the end of the post-dive recovery period (6).

371

Animal	Age (years)	M_b (kg)	Dive duration (sec)			Surface interval (sec)			Pre-dive $\dot{V}O_2$ (L O_2 min^{-1})			Dive $\dot{V}O_2$ (L O_2 min^{-1})		
			T1	T2	T3	T1	T2	T3	T1	T2	T3	T1	T2	T3
F97HA	15	171±1	47±17 (31)	135±48	120±47	11±4	44±14	42±11	2.67	2.59	2.88	3.38	2.16	2.91
F00BO	12	160±1	47±15 (29)	122±19	105±8	14±12	38±9	30±6	1.54	1.76	1.70	2.59	2.33	2.62
F97SI	15	229±2	39±12	100±21	102±13	7±3	31±12	34±5	2.42	2.59	2.48	4.18	3.22	3.37

			(38)											
F00YA	12	204±1	66±9 (23)	85±18	71±22	11±3	24±12	35±23	1.95	2.32	2.44	2.71	3.71	3.88
Grand mean	13.5±1.7	191±32	50±11	111±22	100±21	11±3	34±9	35±5	2.15±0.50	2.32±0.39	2.38±0.49	3.22±0.73	2.86±0.74	3.20±0.55

Figure 1. Mean (± SD) blood microparticle levels before, and 3 hours or 24 hours following a dive or exercise bout, or before and 3 hours following a feeding event. Dive bouts were either to 5 m or 50 m and the latter was repeated twice (see text). All data points are samples from 4 sea lions except 2nd dive trial to 50 m at 3 and 24 hours.



377

378

379

380

381 **References**

382 1. **Ayers L, Stoewhas AC, Ferry B, Latshang TD, Lo Cascio CM, Sadler R,**
383 **Stadelmann K, Tesler N, Huber R, Achermann P, Bloch KE, and Kohler M**. Circulating
384 levels of cell-derived microparticles are reduced by mild hypobaric hypoxia: data from a
385 randomised controlled trial. *Eur J Appl Physiol* 114: 1067-1073, 2014.
386 2. **Boycott AE, Damant GCC, and Haldane JS**. The prevention of decompression-air
387 illness. *J Hyg* 8: 342-443, 1908.

3. **Cox TM, Ragen TJ, Read AJ, Vos E, Baird RW, Balcomb K, Barlow J, Caldwell J, Cranford T, Crum L, D'Amico A, D'Spain G, Fernández A, Finneran J, Gentry R, Gerth W, Gulland F, Hildebrand J, Houser D, Hullar T, Jepson PD, Ketten DR, MacLeod CD, Miller P, Moore S, Mountain D, Palka D, Ponganis P, Rommel S, Rowles T, Taylor B, Tyack P, Wartzok D, Gisiner R, Mead J, and Benner L**. Understanding the impacts of anthropogenic sound on beaked whales. *Journal of Cetacean Research and Management* 7: 177-187, 2006.

4. **Eckenhoff RG, Olstad CS, and Carrod G**. Human dose-response relationship for decompression and endogenous bubble formation. *J Appl Physiol* 69: 914-918, 1990.

5. **Fahlman A, Hooker SK, Olszowka A, Bostrom BL, and Jones DR**. Estimating the effect of lung collapse and pulmonary shunt on gas exchange during breath-hold diving: the Scholander and Kooyman legacy *Resp Physiol Neurobiol* 165: 28-39, 2009.

6. **Fahlman A, Svärd C, Rosen DAS, Jones DR, and Trites AW**. Metabolic costs of foraging and the management of O_2 and CO_2 stores in Steller sea lions. *J Exp Biol* 211: 3573-3580, 2008.

7. **Fahlman A, Svärd C, Rosen DAS, Wilson RS, and Trites AW**. Activity as a proxy to estimate metabolic rate and to partition the metabolic cost of diving vs. breathing in pre- and post-fasted Steller sea lions. *Aquat Biol* 18: 175-184, 2013.

8. **Fahlman A, Tikuisis P, Himm JF, Weathersby PK, and Kayar SR**. On the likelihood of decompression sickness during H_2 biochemical decompression in pigs. *J Appl Physiol* 91: 2720-2729, 2001.

9. **Fahlman A, Wilson R, Svärd C, Rosen DAS, and Trites AW**. Activity and diving metabolism correlate in Steller sea lion *Eumetopias jubatus*. *Aquat Biol* 2: 75-84, 2008.

10. **Fernandez A, Edwards JF, Rodriguez F, Espinosa De Los Monteros A, Herraez P, Castro P, Jaber JR, Martin V, and Arbelo M**. Gas and fat embolic syndrome involving a mass stranding of beaked whales (Family *Ziphiidae*) exposed to anthropogenic sonar signals. *Vet Pathol* 42: 446-457, 2005.

11. **Fernandez A, Edwards JF, Rodruiquez F, Espinosa de los Monteros A, Herraez MP, Castro P, Jaber JR, Martin V, and Arbelo M**. ‘‘Gas and Fat Embolic Syndrome’’ Involving a Mass Stranding of Beaked Whales (Family *Ziphiidae*) Exposed to Anthropogenic Sonar Signals. *Vet Pathol* 42: 446-457, 2005.

12. **García-Párraga D, Crespo-Picazo JL, Bernaldo de Quirós Y, Cervera V, Martí-Bonmati L, Díaz-Delgado J, Arbelo M, Moore MJ, Jepson PD, and Fernández A**. Decompression Sickness (“the bends”) in Sea Turtles. *Dis Aquat Organ* 111: 191-205, 2014.

13. **Hastie GD, Rosen DAS, and Trites AW**. The influence of depth on a breath-hold diver: Predicting the diving metabolism of Steller sea lions (*Eumetopias jubatus*). *J Exp Mar Biol Ecol* 336: 163-170, 2006.

14. **Hastie GD, Rosen DAS, and Trites AW**. Reductions in oxygen consumption during dives and estimated submergence limitations of Steller sea lions (*Eumetopias jubatus*). *Mar Mam Sci* 23: 272-286, 2007.

15. **Hooker SK, Fahlman A, Moore MJ, Aguilar de Soto N, Bernaldo de Quiros Y, Brubakk AO, Costa DP, Costidis AM, Dennison S, Falke KJ, Fernandez A, Ferrigno M, Fitz-Clarke JR, Garner MM, Houser DS, Jepson PD, Ketten DR, Kvadsheim PH, Madsen PT, Pollock NW, Rotstein DS, Rowles TK, Simmons SE, Van Bonn W, Weathersby PK, Weise MJ, Williams TM, and Tyack PL**. Deadly diving? Physiological and behavioural

433 management of decompression stress in diving mammals. *Proceedings of the Royal Society of*
434 *London B Biological Sciences* 279: 1041-1050, 2012.
435 16. **Jepson PD, Arbelo M, Deaville R, Patterson IAP, Castro P, Baker JR, Pocknell AM,**
436 **Rodruiquez F, Howie FE, Espinosa A, Reid RJ, Jaber JR, Martin V, Cunningham AA, and**
437 **Fernandez A**. Gas-bubble lesions in stranded cetaceans. *Nature* 425: 575-576, 2003.
438 17. **Jepson PD, Deaville R, Patterson IAP, Pocknell AM, Ross HM, Baker JR, Howie**
439 **FE, Reid RJ, Colloff A, and Cunningham AA**. Acute and chronic gas bubble lesions in
440 cetaceans stranded in the United Kingdom. *Vet Pathol* 42: 291-305, 2005.
441 18. **Kayar SR, Aukhert EO, Axley MJ, Homer LD, and Harabin AL**. Lower
442 decompression sickness risk in rats by intravenous injection of foreign protein. *Undersea*
443 *Hyperbar Med* 24: 329-335, 1997.
444 19. **Lillo RS, Himm JF, Weathersby PK, Temple DJ, Gault KA, and Dromsky DM**.
445 Using animal data to improve prediction of human decompression risk following air-saturation
446 dives. *J Appl Physiol* 93: 216-226, 2002.
447 20. **Lillo RS, and MacCallum ME**. Decompression comparison of N2 and O2 in rats.
448 *Undersea Biomed Res* 18: 317-331, 1991.
449 21. **Littell RC, Henry PR, and Ammerman CB**. Statistical analysis of repeated measures
450 data using SAS procedures. *J Anim Sci* 76: 1216-1231, 1998.
451 22. **Mahon RT, and Regis DP**. Decompression and decompression sickness. *Comp Physiol*
452 4: 1157-1175, 2014.
453 23. **McDonald BI, and Ponganis PJ**. Insights from venous oxygen profiles: oxygen
454 utilization and management in diving California sea lions. *J Exp Biol* 216: 3332-3341, 2013.
455 24. **Merrick RL, and Loughlin TR**. Foraging behavior of adult female and young-of-the-
456 year Steller sea lions in Alaskan waters. *Can J Zool* 75: 776-786, 1997.
457 25. **Montcalm-Smith EA, McCarron RM, Porter WR, Lillo RS, Thomas JT, and Auker**
458 **CR**. Acclimation to decompression sickness in rats. *J Appl Physiol* 108: 596-603, 2010.
459 26. **Pontier JM, Jimenez C, and Blatteau JE**. Blood platelet count and bubble formation
460 after a dive to 30 msw for 30 min. *Aviat Space Env Med* 79: 1096-1099, 2008.
461 27. **Suzuki I, Sato, K., Fahlman, A., Naito, Y. Miyazaki, N. Trites, A.W.** Drag, but not
462 buoyancy, affects swim speed in captive Steller sea lions. *Biol Open* 3: 379-386, 2014.
463 28. **Svärd C, Fahlman A, Rosen DAS, Joy R, and Trites AW**. Fasting affects the surface
464 and diving metabolic rates of Steller sea lions *Eumetopias jubatus*. *Aquat Biol* 8: 71-82, 2009.
465 29. **Thom SR, Bennett M, Banham ND, Chin W, Blake DF, Rosen A, Pollock NW,**
466 **Madden D, Barak OF, Marroni A, Balestra C, Germonpre P, Pieri M, Cialoni D, Le P-NJ,**
467 **Logue C, Lambert D, Hardy KR, Sward D, Yang M, Bhopale VM, and Dujic Z**. Association
468 of microparticles and neutrophil activiation with decompression sickness. . *J Appl Physiol* 2015.
469 30. **Thom SR, Milovanova TN, Bogush M, Bhopale VM, Yang M, Bushmann K, Pollock**
470 **NW, Ljubkovic M, Denoble P, and Dujic Z**. Microparticle production, neutrophil activation,
471 and intravascular bubbles following open-water SCUBA diving. *J Appl Physiol* 112: 1268-1278,
472 2012.
473 31. **Thom SR, Milovanova TN, Bogush M, Yang M, Bhopale VM, Pollock NW,**
474 **Ljubkovic M, Denoble P, Madden D, Lozo M, and Dujic Z**. Bubbles, microparticles, and
475 neutrophil activation: changes with exercise level and breathing gas during open-water SCUBA
476 diving. *J Appl Physiol* 114: 1396-1405, 2013.

32. **Thom SR, Yang M, Bhopale VM, Huang S, and Milovanova TN**. Microparticles initiate decompression-induced neutrophil activation and subsequent vascular injuries. *J Appl Physiol* 110: 340-351, 2011.
33. **Vince R, McNaughton L, Taylor L, Midgley A, Laden G, and Madden L**. Release of VCAM-1 associated endothelial microparticles following simulated SCUBA dives. *Eur J Appl Physiol* 105: 507-513, 2009.
34. **Vince RV, Chrismas B, Midgley AW, McNaughton LR, and Madden LA**. Hypoxia mediated release of endothelial microparticles and increased association of S100A12 with circulating neutrophils. *Oxid Med Cell Long* 2: 2-6, 2009.
35. **Ward CA, McCullough D, and Fraser WD**. Relation between complement activation and susceptibility to decompression sickness. *J Appl Physiol* 62: 1160-1166, 1987.
36. **Ward CA, McCullough D, Yee D, Stanga D, and Fraser WD**. Complement activation involvement in decompression sickness of rabbits. *Undersea Biomed Res* 17: 51-66, 1990.
37. **Weathersby PK, Homer LD, and Flynn ET**. On the likelihood of decompression sickness. *J Appl Physiol* 57: 815-825, 1984.
38. **Wisloff U, Richardson RS, and Brubakk AO**. NOS inhibition increases bubble formation and reduces survival in sedentary but not exercised rats. *J Physiol* 546: 577-582, 2003.