

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

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20 **Abstract**

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

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

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**42 Introduction**

43 The purpose of this study was to improve understanding of physiological responses to diving in a  
44 breath-hold diving marine mammal—with a particular focus on the pathophysiology of  
45 decompression sickness (DCS). DCS is a systemic pathophysiological process that occurs  
46 naturally after tissues become supersaturated with nitrogen under high atmospheric pressure. In  
47 humans, DCS can also occur when nitrogen or some alternative gas is used to dilute O<sub>2</sub> in  
48 breathing mixtures during activities such as deep-sea diving, high altitude aviation and space  
49 exploration. Most studies of DCS are performed with terrestrial mammals. We therefore  
50 hypothesized that a comparative physiological approach studying *bona fide* diving mammals  
51 would add valuable insight into the physiology of decompression. Such an approach may yield  
52 insights into the potential physiological traits that allow breath-hold diving animals to perform  
53 prolonged apneas to great depths without apparent pressure-related problems.

54

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

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

69

70 There are a few well-defined risk factors that increase the probability of DCS, such as increasing  
71 dive duration, dive depth, ascent/decompression rate, body mass, and breathing gas (8, 19, 20,  
72 37). Recent studies have shown that microparticles (MPs) are elevated with decompression stress  
73 (31, 32). Microparticles are cellular fragments between 0.3 and 1  $\mu\text{m}$  in size that are shed from  
74 various cells. MPs derived from platelets are known to activate leukocytes and cause  
75 aggregation, and can stimulate pro-inflammatory cytokines. MPs derived from decompression  
76 stress have been shown to specifically activate neutrophils and cause vascular damage (32).  
77 Microparticles correlate with depth of diving in mice (32), but correlate poorly with depth in  
78 humans (31, 33). Studies have also evaluated the effect of exercise on bubble and MP production  
79 in human scuba divers. Exercise can increase the number of circulating MPs slightly, whereas  
80 diving has a much greater effect and is also impacted by the gas used during the dive (31).

81

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

88 repeated breath-holds without experiencing symptoms of gas bubble disease during dives up to  
89 50 m (6). In addition, a theoretical gas dynamic model (5) predicted that for 12 repeated dives to  
90 50 m there would be a significant increase in blood and tissue N<sub>2</sub> levels. Thus, we hypothesized  
91 that repeated breath-hold foraging dives would increase blood MP levels, and that the levels  
92 would increase with diving depth and time underwater. To control for the potentially  
93 confounding effect of feeding and exercise while diving, we also measured blood MP levels  
94 following exercise and feeding in separate experiments.

95

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

106

## 107 **Methods**

### 108 *Animals*

109 All experiments were conducted under permits from the Animal Care Committees of the  
110 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

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

135

#### 136 *Dive Dose*

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

141

#### 142 *Metabolic rate (rate of O<sub>2</sub> consumption)*

143 The rate of O<sub>2</sub> consumption ( $\dot{V}O_2$ , L O<sub>2</sub> min<sup>-1</sup>) before and during a dive was used as an index of  
144 the metabolic rate. The metabolic rate before and after the dive bout was assessed by measuring  
145 the gas concentrations in the metabolic dome (6). We separated metabolic rate into surface  
146 metabolic rate before diving (pre-dive), at which time the sea lion had received minimal amount  
147 of food and was post-prandial. As both the dive and surface interval durations were determined  
148 by the sea lion, and as reliable estimates for the metabolic rate of individual dives within a dive  
149 bout cannot be made (6, 7, 9), we computed the diving metabolic rate for the entire diving bout.  
150 Specifically, we divided the total volume of O<sub>2</sub> taken up from the beginning of the first dive until  
151 the end of the post-dive recovery period by the duration of that same period. Thus, we only  
152 computed one pre-dive and one diving metabolic rate for each experiment for each sea lion.

153

#### 154 *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  $2 \text{ m} \cdot$   
158  $\text{sec}^{-1}$ ) 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 ( $(\text{MP}_{\text{post-dive}} - \text{MP}_{\text{control}})/\text{MP}_{\text{control}} \times 100$ ). 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  $\pm$  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 ( $3.22 \pm 0.73 \text{ L O}_2 \text{ min}^{-1}$ ,  
187 paired t-test,  $P < 0.05$ ,  $t = 4.42$ ,  $df=3$ ) and 50 m ( $3.03 \pm 0.63 \text{ L O}_2 \text{ min}^{-1}$ ,  $P < 0.05$ ,  $t = 2.81$ ) as  
188 compared with the pre-dive metabolic rates (5 m:  $2.15 \pm 0.50 \text{ L O}_2 \text{ min}^{-1}$ ; 50 m:  $2.35 \pm 0.41 \text{ L O}_2$   
189  $\text{min}^{-1}$ ). 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:  $50 \pm 11 \text{ sec}$ ; surface interval:  $11 \pm 3 \text{ sec}$ ) were significantly lower than dives to 50  
192 m (dive duration:  $105 \pm 21 \text{ sec}$ ,  $t_{10} = 4.49$ ,  $P < 0.01$ ; surface interval:  $35 \pm 7 \text{ sec}$ ,  $t_{10} = 6.86$ ,  $P <$   
193  $0.01$ , Table 1).

194

195 The average dive dose ( $\text{m} \cdot \text{sec}$ ) was significantly lower for the 5 m (T1,  $7868 \pm 264 \text{ m} \cdot \text{sec}$ ) as  
196 compared with either the first (T2,  $50848 \pm 7206 \text{ m} \cdot \text{sec}$ ) or second (T3,  $44729 \pm 6107 \text{ m} \cdot \text{sec}$ )  
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 ( $AIC_{\text{null}} = 192.6$ ,  $AIC_{\text{time}} = 151.5$ ,  $P < 0.01$ ). There was a trend toward a 26% decrease in MP  
204 levels from the first ( $2604 \pm 352$  MPs  $\text{ul}^{-1}$ , 5 m trial, Fig. 1) to the second experiment ( $1935 \pm$   
205  $163$  MPs  $\text{ul}^{-1}$ ,  $P < 0.1$ , 50m Trial 1) and then a significant 57% increase from the first to the last  
206 experiment ( $4082 \pm 788$  MPs  $\text{ul}^{-1}$ ,  $P < 0.05$ , 50m Trial 2).

207

208 There was a significant increase in MP levels following a dive ( $AIC_{\text{null}} = 485.7$ ,  $AIC_{\text{time}} = 446.0$ ,  
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 ( $AIC_{\text{time}} = 478$ ,  $P <$   
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 ( $AIC_{\text{null}} = 126.0$ ,  $AIC_{\text{fed}} = 110.4$ ,  $P < 0.01$ ), 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  $\text{ul}^{-1}$ , Max levels before exercise:  
219 18405 - 778750 MPs  $\text{ul}^{-1}$ ). The reason for this is unclear and warrants further investigation.

220 While there was a significant change in relative MP levels ( $AIC_{\text{null}} = 357.3$ ,  $AIC_{\text{time}} = 339.8$ ), 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:  $2570 \pm 108$  MPs  
224  $\text{ul}^{-1}$ , Post-feeding:  $3175 \pm 414$  MPs  $\text{ul}^{-1}$ ;  $P < 0.05$ ,  $t_6=2.83$ , 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).

245

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

255

256 We hypothesized that blood MP levels may be a good indicator of the level of decompression  
257 stress in breath-hold diving mammals given that MP levels indicate decompression stress in  
258 divers breathing pressurized air. We therefore allowed Steller sea lions to dive repeatedly to two  
259 different depths, 5 m and 50 m, assuming that dives to 50 m would cause a greater  
260 decompression stress. However, we found high variability in the circulating blood MP levels of  
261 the animals diving to 50 m (T2 vs T3, Fig. 1). The risk of DCS is not only affected by dive depth  
262 alone, but dive duration and ascent rate also alters the risk (37), and it has been shown that only a  
263 5% change in the inert gas burden can result in a 50% change in the probability of DCS (8).

264

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

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

273

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

282

283 The dive pattern for the Steller sea lions in our study was similar to those previously reported for  
284 the same animals (7, 9, 13, 14, 27, 28), where deeper dives were longer and associated with  
285 longer surface durations to replenish the  $O_2$  stores. While there were no significant differences in  
286 the metabolic rate over an entire dive bout (Table 1), the diving metabolic rate correlated with  
287 underwater activity, and the activity was generally greater for shallower dives (7, 9). Thus,  
288 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 2<sup>nd</sup> 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

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

339

#### 340 **Authors' contributions**

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

349

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352 animal care staff and research technicians at Vancouver Aquarium and the UBC Open Water  
353 Research Station who made this study possible. All research protocols were approved by the  
354 UBC Animal Care Committee, and the animal care committee of Woods Hole Oceanographic  
355 Institution. None of the authors have competing interests.

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 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 1<sup>st</sup> 50m dive, T3-2<sup>nd</sup> 50 m dive). The pre-dive metabolic rate was the measured oxygen  
 367 consumption rate ( $\dot{V}O_2$ , L O<sub>2</sub> min<sup>-1</sup>) during the last 2-3 min in the respirometry dome before  
 368 diving. Dive metabolic rate was the cumulative volume of O<sub>2</sub> 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).

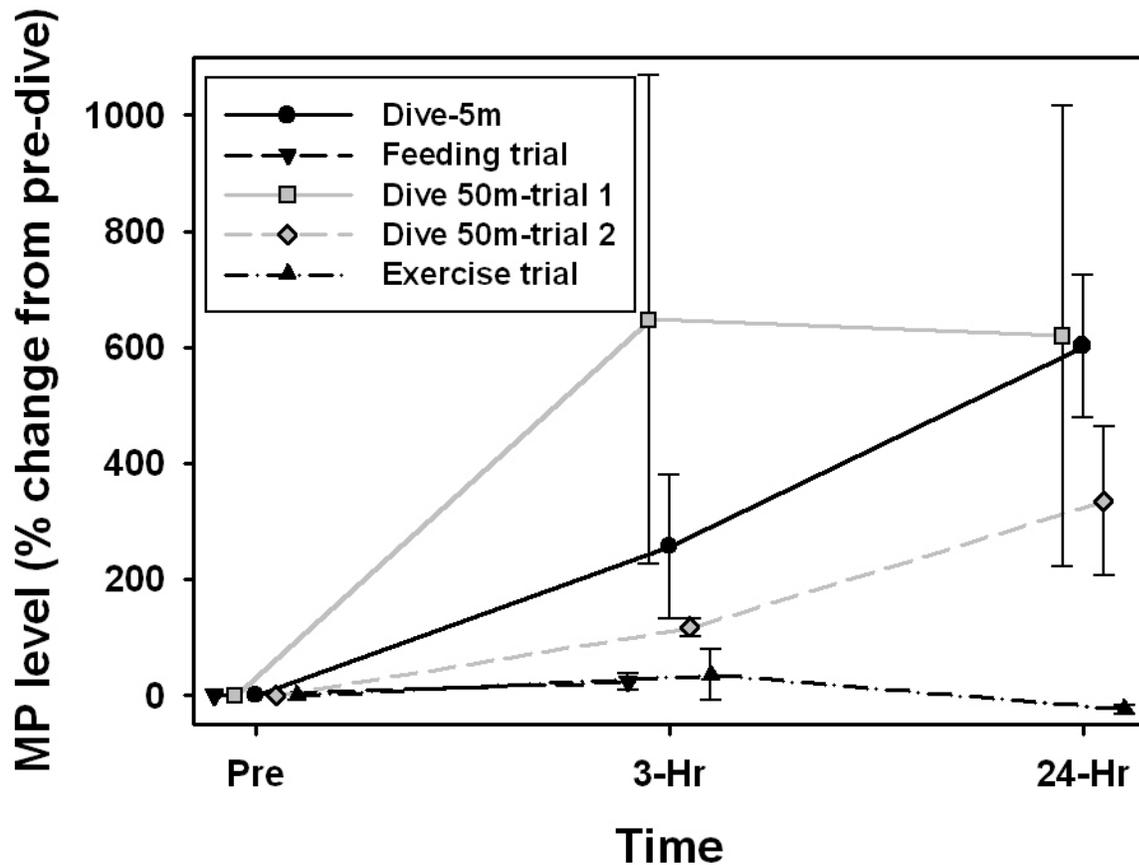
371

Ani mal	Age (yea rs)	$M_b$ (kg)	Dive duration (sec)			Surface interval (sec)			Pre-dive $\dot{V}O_2$ (L O <sub>2</sub> min <sup>-1</sup> )			Dive $\dot{V}O_2$ (L O <sub>2</sub> min <sup>-1</sup> )		
			T1	T2	T3	T1	T2	T3	T1	T2	T3	T1	T2	T3
F97H A	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
F00B O	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
F97S I	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)											
F00Y	12	204±	66±	85±1	71±2	11±	24±	35±	1.95	2.32	2.44	2.71	3.71	3.88
A		1	9	8	2	3	12	23						
			(23)											
Gran	13.5	191±	50±	111±	100±	11±	34±	35±	2.15±0	2.32±0	2.38±0	3.22±0	2.86±0	3.20±0
d	±1.7	32	11	22	21	3	9	5	.50	.39	.49	.73	.74	.55
mean														

372

373 Figure 1. Mean ( $\pm$  SD) blood microparticle levels before, and 3 hours or 24 hours following a  
 374 dive or exercise bout, or before and 3 hours following a feeding event. Dive bouts were either to  
 375 5 m or 50 m and the latter was repeated twice (see text). All data points are samples from 4 sea  
 376 lions except 2<sup>nd</sup> dive trial to 50 m at 3 and 24 hours.



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