Effect of Breath-Hold on Blood Gas Analysis in Captive Pacific White-Sided Dolphins (Lagenorhynchus obliquidens)

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ABSTRACT. The effect of a breath-hold on blood gas was evaluated in captive Pacific white-sided dolphins (Lagenorhynchus obliquidens). Serial blood collections were performed from a vessel on the ventral surface of the flukes during breath-hold. In total, 178 blood samples were taken from three dolphins for five trials in each animal. During a breath-hold, partial pressure of oxygen (P\textsubscript{O\textsubscript{2}}) decreased from 152.5 to 21.8 mmHg and partial pressure of carbon dioxide (P\textsubscript{CO\textsubscript{2}}) conversely increased from 31.8 to 83.6 mmHg. The range of pH was 7.54 to 7.25, suggesting drastic change from alkalemia to acidemia. These wide ranges of blood gas imply a considerable change of oxygen affinity caused by the Bohr effect during breath-hold, which enable effective uptake and distribution of oxygen to metabolizing tissues.

KEY WORDS: blood gas, Pacific white-sided dolphin, P\textsubscript{O\textsubscript{2}}, pH, P\textsubscript{CO\textsubscript{2}}.

The Pacific white-sided dolphin, Lagenorhynchus obliquidens, belongs to the suborder Odontoceti (toothed whale), family Delphinidae, and is characterized by a distinctive black and white coloration [16]. This dolphin has a barely noticeable beak and a falcate large dorsal fin. Adults range between 2.1 and 2.4 m in the length and weight between 85 and 150 kg in the body weight. Pacific white-sided dolphins are fast, powerful swimmers, sometimes traveling up to 55 km/hr. They engage in bow-wave riding and aerial behavior. These dolphins are frequently seen with vessels.

Blood gas parameters, including partial pressure of oxygen (P\textsubscript{O\textsubscript{2}}) and carbon dioxide (P\textsubscript{CO\textsubscript{2}}) and pH, have been recorded from central arteries and veins found in flukes, and values correlate well with those taken from the carotid artery in small dolphins [11, 13]. However, these studies were performed under general anesthesia and then data were obtained. A healthy animal cannot be referred to the results directly. On the other hand, there are some studies on P\textsubscript{O\textsubscript{2}}, P\textsubscript{CO\textsubscript{2}}, and pH, during apnea to examine diving response and swimming activity in cetaceans. According to these studies, it is concluded that a harbour porpoise is not physiologically adapted for long dives [8]. The calculated aerobic dive limit (oxygen store/metabolic rate) for an adult bottlenose dolphin is 268 sec [17], and its value for a white whale is 9–10 min [14]. White whales are also not well adapted for high-speed swimming. Cetaceans are remarkably active during prolonged periods of apnea. It is important to understand the physiological characteristics with analyzing blood gas parameters from trained animals during apnea. The goal for our project is to assess the metabolic rate of Pacific white-sided dolphins by several studies. In the present study, the effect of a breath-hold on blood gas analysis was evaluated in captive Pacific white-sided dolphins.

Animals: Three adult Pacific white-sided dolphins (two males and one female) were used for this study (Table 1). The dolphins were fed 4–5 times per day on 9–11 kg of mackerel supplemented with vitamins (Vita-Zu Mammal Tablet, MAZURI, Japan SLC, Inc.). The rectal temperature was recorded with a clinical thermometer (CTM 303, TER-UMO, Tokyo, Japan) for daily heath care. Blood samples were taken from the fluke as a monthly clinical examination.

Environment: Two males were housed in an outdoor pen (7 m × 3 m × 3.5 m deep), which was a part of a total sea water volume of 5,000 m\textsuperscript{3} (45 m × 25 m, oval-shaped, with a depth of 3.5 to 5.5 m). One female was kept in an indoor pool (5.8 m × 3.8 m × 3.3 m deep, 90 m\textsuperscript{3}). During the experimental period, the water temperatures of the outdoor pen and the indoor pool values ranged from ranged from 11.6 to 15.8°C and 11.9 to 16.1°C, respectively. In our pool,

Table 1. Animals used in this study

<table>
<thead>
<tr>
<th>ID Number</th>
<th>Arrival date</th>
<th>Sex</th>
<th>Body weight*</th>
<th>Body length*</th>
</tr>
</thead>
<tbody>
<tr>
<td>EAM.183</td>
<td>1978/02/16</td>
<td>Male</td>
<td>125</td>
<td>218</td>
</tr>
<tr>
<td>EAM.201</td>
<td>1981/03/28</td>
<td>Male</td>
<td>141</td>
<td>230</td>
</tr>
<tr>
<td>EAM.240</td>
<td>1996/03/01</td>
<td>Female</td>
<td>90</td>
<td>205</td>
</tr>
</tbody>
</table>

* Body weight (kg) and Body length (cm) measured at March 2003.
sodium hypochlorite was used for good water quality and levels of free chlorine ranged from 0.05 to 2.0 ppm.

**Blood collection:** The present study was performed in February and March of 2003. All samples were obtained between 13:30–14:00, and were analyzed within one hr after collection. Each dolphin was trained to maintain the ventral side up position, submerge their blowhole under the surface of the water and present its flukes voluntarily for collection of blood samples [3]. To obtain a sample, a 21 gauge butterfly needle was placed in a vessel on the ventral surface of the flukes. In each blood collection, serial samples from 1.0 to 1.5 ml were taken with a needle placed into a blood vessel. Blood was collected as much as possible while the animal held its breath. Time in sec was counted simultaneously from final breath to the end of the collection at which the animal gave up breath hold or all the prepared syringes were used.

Samples collected for blood gas measurements were drawn into 2.5 ml heparinized syringes (PREZA-PAK®II Arterial Blood Sampling Kit, TERUMO) and measured partial pressures of oxygen ($P_{O_2}$) and carbon dioxide ($P_{CO_2}$) and pH with a blood gas analyzer (Rapidlab 840®, Bayer Medical Ltd.). After removing air bubbles, capped syringes were stored in ice slurry until they were assayed.

The Institutional Animal Care and Use Committee at Tokai University suggested that these treatments for the experiment agree with Guidelines for the Care and Use of Animals for Scientific Purposes at Tokai University on Apr 1st 2008.

**Statistical analysis:** After $P_{O_2}$, $P_{CO_2}$, pH and breath-hold duration were log-transformed, relationships between breath-hold duration and each blood parameter were analyzed using with liner regression. On the result of the analysis, slope and the intercept value of $P$ were < 0.0001 in all cases.

In total, 178 blood samples were collected from three dolphins for 5 trials in each animal. Time of breath-holds ranged from 61 to 148 sec. The responses of dolphins were very similar. Until 90 sec, samples were taken from all three dolphins. However, for over 91 sec blood collections could only be done in EAM.183 without one data of EAM.201. Blood data were used from 0 to 90 sec for general statistical analysis.

**pH:** Blood pH decreased from 7.54 to 7.25 during breath-holds. A significant negative correlation between pH and breath-hold duration was observed (Fig. 1a). In the first pH for each trail, 10 out of 15 data were more than 7.40, and the other 5 data values were over 7.45. The median value from 0 to 15 sec for a breath-hold was 7.43. However, in the final pH for each trail, 11 out of 15 data were less than 7.30. For a breath-hold of over 46 sec, three medians were also less than 7.30. As breath-hold duration became longer, medians for every 15 sec decreased from 7.43 to 7.29.

**$P_{O_2}$:** $P_{O_2}$ decreased from 152.5 to 21.8 mmHg. A significant negative correlation between $P_{O_2}$ and the breath-hold duration was observed (Fig. 1b). As breath-hold duration became longer, medians for every 15 sec decreased from 118.5 to 33.6 mmHg. Median from 16 to 30 sec was 103.5 mmHg. For 61 sec, two medians were lower than 40 mmHg. For 90 sec, all data was lower than 40 mmHg (Fig.
ventral surface of the flukes. In the initial present study, we took blood samples from a vessel on the level after a ventilation would be remarkably high. On the Therefore, the present findings showed how the first oxygen the second sampling should be less influenced by air. 

This suggests the possibility that at least the first blood was an air space of 0.39 m approximately 160 mmHg. Before blood sampling, there was an air space of 0.39 m in the tube of the butterfly needle. After the second sampling, this space was filled with blood. This suggests the possibility that at least the first sample might be influenced by air. However, the data after the second sampling should be less influenced by air. Therefore, the present findings showed how the first oxygen level after a ventilation would be remarkably high. On the other hand, the median for every 15 sec increased continuously, peaking from 61 to 75 sec. However, the median from 76 to 90 sec was smaller than the value from 46 to 60 sec. Over 45 sec, all data was higher than 60 mmHg. Individual differences for PO2 were larger than the other two parameters. 

In cetaceans, there is a uniquely structured arteriovenous plexus in the flukes and flippers. Their veins are periarterial venous retia [1, 2]. Consequently, samples can typically be mixed venous/arterial blood. All blood samples were assumed to be a mixture of venous and arterial blood [1]. However, it is difficult to distinguish whether sampled blood was arterial, venous or mixture blood. An arterial blood PO2 for a healthy human is 95 mmHg [5]. In the present study, we took blood samples from a vessel on the ventral surface of the flukes. In the initial PO2 for each trail, all 15 data were higher than 95 mmHg. The data, therefore, suggests that sampled blood was possibly arterial blood.

The pH level drastically decreased from 7.54 to 7.35 at maximum range with a considerable concentration of PCO2 from 54.2 to 83.6 mmHg during prolonged apnea. These data suggest a variation from higher to lower oxygen affinity of blood provided by the Bohr effect during a breath-hold. Observed initial higher pH and suggested higher oxygen affinity of blood in small cetaceans could be important in maximizing the oxygen loading from the lungs during dives [15]. Whereas, a lower oxygen affinity at the end of a breath-hold supports an effective oxygen release from blood to metabolizing peripheral tissues when the oxygen pool has already been almost exhausted. A wide variation of pH during breath-holds with the Bohr effect would ensure effective delivery of oxygen.

In the present study, PO2 was more than 140 mmHg within first 15 sec of apnea, but exponentially decreased to less than 40 mmHg after 90 sec. The first level of PO2 was higher than those of bottlenose dolphins [17] and white whales [14]. The oxygen pressure of the atmosphere is approximately 160 mmHg. Before blood sampling, there was an air space of 0.39 m/ in the tube of the butterfly needle. After the second sampling, this space was filled with blood. This suggests the possibility that at least the first sample might be influenced by air. However, the data after the second sampling should be less influenced by air. Therefore, the present findings showed how the first oxygen level after a ventilation would be remarkably high. On the other hand, the final level of PO2 was almost same in these two species [14, 17].

The short-duration of a diving dolphin and porpoise has relative lung volumes comparable to a terrestrial mammal’s dive following inspiration and appear to use the lungs as an oxygen store [15]. Our measurements for RBC, Hb and Ht are comparable to values reported for Pacific white-sided dolphins [1, 9, 10]. Relative to similar-sized terrestrial mammals, RBC values for pigs [6] were higher than for Pacific white-sided dolphins. Conversely, Hb and Ht values for Pacific white-sided dolphins are higher than those for pigs. A large blood volume, a high Hb level and a high Ht give white whales a large blood oxygen-carrying capacity [12]. The blood oxygen stores in small cetaceans vary up to about 3-fold greater than the average for terrestrial mammals [4, 15]. Even if lung volumes were similar, however, from the levels of Hb and Ht, it suggests that the blood oxygen stores in Pacific white-sided dolphins are higher than those in pigs.

In Pacific white-sided dolphins, our data revealed that pH and PO2 decreased, and PCO2 increased after breath-holds similar to bottlenose dolphins [17] and white whales [14]. The magnitudes of variation for pH, PO2, and PCO2 in Pacific white-sided dolphins (Fig. 1) were larger than these for 2 cetaceans. Relative to human blood pH standard [5], pH for only one breath at a time changed from alkalemia (pH > 7.44) to acidaemia (pH < 7.36). In the present study, blood pH was as same level as the respiratory acidosis due to hypercapnaemia. When blood gas analysis is applied to cetaceans for clinical examination, an attention should be paid to reading pH, PO2, and PCO2 and to how long blood was sampled after a breath-hold.

Pacific white-sided dolphin is distributed in both coastal shallow and oceanic deep waters, but it is unclear how deep and long they dive. Our results indicated that their blood oxygen stores are higher than those of bottlenose dolphin, but lower than Dall’s porpoise (Phocoenoides dalli) [10]. Dall’s porpoises are known to dive to forage in the mesopelagic zone deep to about 500 m [7], but field observation indicated that Pacific white-sided dolphins breathe about once every 20 sec [16]. In the present study, the maximum breath-hold duration was 148 sec. The information from the fields and the present results from blood gas analysis imply that Pacific white-sided dolphin is not a long and deep diver. In conclusion, the present findings suggest the physiological response of Pacific white-sided dolphin is to consume as much of the oxygen stored in the lungs into the tissues, and its suitability for short-duration rather than long-duration diving. The present study may be useful also for reading blood gas data for clinical examination for Pacific white-sided dolphin.

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REFERENCES


