# Blood Nitrogen Uptake & Distribution during Diving in Bottlenose Dolphins


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### Background

This short summary note was prepared by Dr Pongalis for the 'Sound & Marine Life' Joint Industry Programme (JIP) who contributed part-funding for a series of experiments on dolphins trained to dive to specific depths. The experiments were conducted at the Space & Naval Warfare Systems Centre (SPAWAR) in San Diego, California, for ultrasound investigation of intravascular bubble formation in dolphins.

For the full results, please refer to the following publication: *Houser, D.S., Dankiewicz-Talmadge, L.A., Stockard, T.K., and Ponganis, P.J. (2010), "Investigation of the potential for vascular bubble formation in a repetitively deep diving dolphin", The Journal of Experimental Biology. Vol 213: 52-63, DOI:10.1242/jeb.028365*

### Project Design & Findings

This project was designed to investigate how bottlenose dolphins (*Tursiops truncatus*) avoid decompression sickness by examination of heart rate, lung volume, and blood nitrogen levels during and after diving. Recent findings in beaked whales stranded after naval exercises have led to the hypothesis that tissue nitrogen supersaturation, bubble formation, and a subsequent decompression sickness-like syndrome are the cause of beaked whale strandings associated with the use of naval sonar. In order to evaluate this hypothesis, a better understanding of cetacean diving physiology and, in particular, nitrogen uptake and distribution was required.

The project utilized a trained US Navy dolphin. Specific goals were to 1) document heart rate responses during diving activity, 2) evaluate a chest impedance meter as an index of lung volume during dives, and 3) measure blood $N_2$ levels during and after dives. These goals were relevant for several reasons. First, heart rate regulates blood flow patterns, which are a primary determinant of $N_2$ absorption and its distribution throughout the body. Second, changes in lung volume during a dive are indicative of lung compression and eventual "collapse," a point at which gas exchange and $N_2$ transfer to the blood stop. Third, blood $N_2$ analyses yield a) the maximum arterial $N_2$ pressure during a dive, which is indicative of lung collapse, and b) the venous $N_2$ wash out profile after a dive. A summary of findings is presented below. Preliminary data have been prepared for a research conference in Nyborg, Denmark, and a manuscript is in preparation for publication.

Application of a backpack electrocardiogram recorder and time depth recorder revealed that heart rate declined from pre- and post-dive surface rates of 100-150 beats per min (bpm) to 20-40 bpm

during dives (Figure 1). Such low heart rates during dives imply greatly reduced blood flow to the skin, flukes, and muscle. Consequently, blood flow and blood $N_2$ distribution during dives is restricted to central organs. This blood flow pattern has important implications for modeling of $N_2$ kinetics during dives as well as for interpretation of analyses of blood samples from different sites in the body.

A backpack, custom-designed, chest impedance recorder was also utilized to measure chest impedance with the use of suction cup electrodes. Despite reprogramming of the recorder, consistent changes in the impedance signal during dives were not obtainable. Therefore, this device or, or at least this version of such a recorder, is not useful in providing an index of lung compression during dives.

The measurement of blood $N_2$ levels during dives involved development of a catheterization technique for dolphins and connection of a backpack blood sampler to the catheter. In association with Navy veterinarians, a technique was developed to catheterize the peduncle artery of the dolphin. This involved a percutaneous technique with local anesthesia. Although this was a remarkable achievement, this was performed on animals that were temporarily removed from the water for medical exams. There is currently no attachment technique available to maintain the catheter in place in a swimming dolphin. The Navy staff did not think this was feasible. Therefore, no blood samples were obtained during dives.

In order to measure blood $N_2$ levels after a dive, the dolphin was trained to present its fluke for blood sampling with a syringe. This allowed collection of serial samples after dives to depths of 50, 70, and 100 meters. Van Slyke analyses of these blood samples revealed that blood $N_2$ levels after these dives were essentially indistinguishable from those from the dolphin at rest (Figure 2). The lack of significantly elevated $N_2$ levels after a dive did not provide any support for the supersaturation hypothesis as a mechanism of $N_2$ bubble formation in cetaceans after exposure to naval sonar. However, it is important to note that, due to the vascular anatomy of the fluke and the observed heart rate responses, those blood samples were essentially "arterialized." Because of the efficient transfer of blood $N_2$ into the lung at the surface, and because of the lack of blood flow to the flukes during the dive (due to low heart rates), one would predict that blood from the fluke should not have elevated $N_2$ levels after a dive regardless of the blood $N_2$ level during the dive. In order to obtain a blood $N_2$ washout profile at the surface, it is necessary to obtain blood samples from deep, central veins. This is because these veins drain the central organs to which blood flow is restricted during dives. Although Navy veterinarians have developed a technique for such central venous catheterization (the brachiocephalic vein), there is again no means to maintain that catheter in that position in the swimming animal. Consequently, it is currently not possible to obtain a $N_2$ wash out curve from central venous samples in the dolphin.

**Summary**

In summary, the funds from this contract contributed to 1) documentation of heart rate responses in the diving dolphin, 2) evaluation of a chest impedance meter, 3) development of an arterial catheterization technique for dolphins, and 4) measurement of post-dive blood $N_2$ levels in blood samples from the fluke, and documentation that $N_2$ is not elevated in those "arterialized" samples. The data obtained neither refute nor support the supersaturation hypothesis of bubble formation in cetaceans exposed to naval sonar. In order to further evaluate that hypothesis, it is necessary to develop techniques that will safely maintain catheters in free-swimming dolphins. This would allow examination of blood $N_2$ absorption and distribution in the arterial and central venous systems.

This contract was greatly appreciated. Only a small portion of the contract funds were utilized because of the limited availability of the dolphin and the end of this project within a few months after the date at which contract funds were accessible.

**Figure 1**. Heart rate response of a bottlenose dolphin trained to make a series of 10 dives to 100-meters depth. The dolphin descended, stationed at a target at 100 meters, and then returned to the surface on command. Dives were approximately 3.5 min in duration.

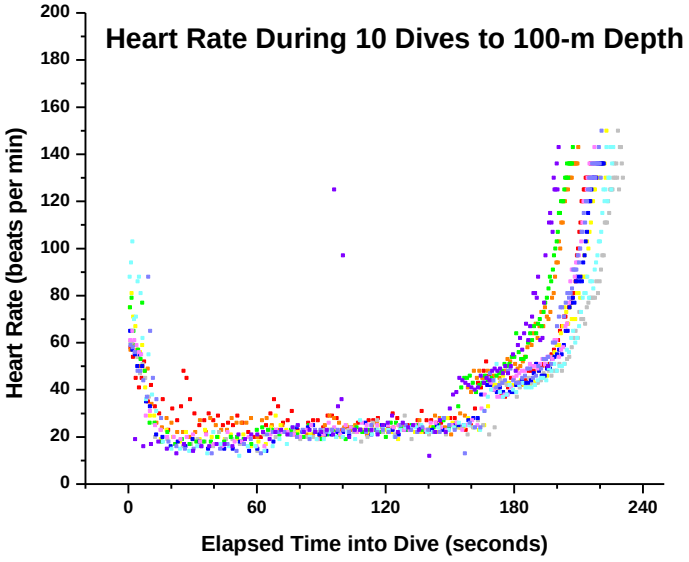


**Figure 2.** Post-dive blood nitrogen levels in a bottlenose dolphin after a series of 10 dives to either 50-, 70-, or 100-meters depth. The partial pressure of nitrogen ($P_{N2}$ in atmospheres absolute (ATA)) was not elevated in comparison to a dolphin at rest. Dive depths were equivalent to 6, 8, and 11 ATA. Blood samples were obtained from the fluke.