

Blood Oxygen Depletion in Diving California Sea Lions: How Close to the Limit?

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LONG-TERM GOALS

The management and depletion of O₂ stores underlie the dive capacities of marine mammals and are fundamental to the concept of an aerobic dive limit (ADL, dive duration associated with the onset of post-dive blood accumulation). The ADL, which is often calculated (cADL) on the basis of total body O₂ stores and an estimated diving metabolic rate, has become an essential concept in the interpretation of diving behavior and foraging ecology (Kooyman and Ponganis 1998); however, the actual rate and magnitude of O₂ store depletion during dives has not been determined in any otariid. This project will document the rate and magnitude of blood O₂ store depletion during diving in California sea lions, and use this information to develop an experimental approach to assess the aerobic dive limit (ADL) and O₂ store depletion in this and other otariid species.

OBJECTIVES

The specific objectives of this project are: 1) Document venous and arterial P_{O2} profiles in lactating California sea lions (*Zalophus californianus*) while diving during foraging trips to sea, 2) Characterize the O₂-hemoglobin (Hb) dissociation curve of sea lion Hb, 3) Use the dissociation curve to convert the P_{O2} profiles into % Hb saturation profiles that will then be used to calculate rate and magnitude of blood oxygen depletion, and 4) Refine vascular access techniques which could be used for future investigations (i.e., blood N₂ absorption during dives; blood gas (pH, P_{CO2}, P_{O2}) and blood lactate responses during dives; blood indices for stress responses to captivity, training, and/or sound exposure; application of such techniques to other species including dolphins).

APPROACH

Objective 1: We will utilize backpack P_{O2} data loggers to document venous and arterial P_{O2} profiles in lactating California sea lions. Sea lions will be captured on San Nicholas Island, Channel Islands during two summer field seasons (August 2010 and 2011). In each year, a minimum of five sea lions will be captured, anesthetized for recorder deployment, returned to the colony, and recaptured after a foraging trip to sea. In 2010 while anesthetized the inferior vena cava was catheterized percutaneously via the caudal gluteal vein with a peel-away introducer, through which a P_{O2} electrode (Licox C1.1

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Revoxode; Integra Life Sciences, Plainsboro, NJ, USA) and thermistor (model 554; Yellow Springs Instruments, Yellow Springs, OH, USA) were inserted. In 2011 while anesthetized an artery will be catheterized and only a P_{O_2} electrode inserted. The custom P_{O_2} recorder (5 x 16 cm, 570 g, UFI, Morro Bay CA) will be attached with 5-min epoxy (Loctite) glue to the fur of midline, caudal back. Additionally, sea lions will be instrumented with a Time Depth Recorder (Mk9, Wildlife Computers, Redmond, WA, USSA) to record diving behavior associated with the profiles and radio tags so we can relocate and recapture them. (Key personnel: P. Ponganis, B. McDonald, Navy Anesthesia resident, and 2 graduate students)

Objective 2: The O_2 – Hb dissociation curve will be determined at three pH values, 7.4, 7.3 and 7.2 from blood samples obtained from sea lions at Sea World's Rehabilitation Program using the mixing technique. The mixing technique (Scheid and Meyer 1978) consists of the volumetric mixing of 0%-saturated blood and 100%-saturated blood to achieve desired S_{O_2} at various points (*i.e.*, 90, 70, 50, 40, 20, 10, 5% S_{O_2}) along the curve with subsequent measurement of the P_{O_2} of the resulting mixture using an I-STAT blood gas analyzer. The $\log(S_{O_2}/(100-S_{O_2}))$ vs. $\log(P_{O_2})$ will be plotted and linear regression analysis performed in order to generate the equation for the O_2 -Hb dissociation curve at each pH (all saturation points, all sea lions combined) (Nicol 1991). (Key personnel: B. McDonald and P. Ponganis)

Objective 3: The percent hemoglobin O_2 saturation (S_{O_2}) values during dives will be obtained by applying P_{O_2} data from the P_{O_2} recorder to the linear regression equation generated by the $\log(S_{O_2}/(100-S_{O_2}))$ vs. $\log(P_{O_2})$ plot and solving for S_{O_2} at the appropriate pH. From this we can calculate the blood O_2 content at the start (maximum O_2 content) and end (minimum O_2 content) of a dive by using the corresponding % Hb saturation, a Hb concentration of 18 g dl⁻¹ (Weise and Costa 2007), and 1.34 ml O_2 g⁻¹ Hb at 100% saturation (Kooyman and Ponganis 1998). The percentage of net O_2 depletion and the rate of O_2 content depletion in either the arterial or venous system (dependent on the site of insertion) for each dive of each sea lion will be calculated. We will also estimate the net contribution of blood O_2 to metabolic rate during diving (ml O_2 kg⁻¹ min) from these data by applying the start and end-of-dive data to the total blood volume of the sea lion (Weise and Costa 2007). (Key personnel: B. McDonald and P. Ponganis)

Objective 4: As part of this project we will also refine vascular access techniques used both in the field and laboratory setting. The use of a portable ultrasound will allow visualization and easy access to the caudal gluteal vein. The ultrasound will also be essential in locating an artery for electrode placement. Before the second field season, we will preform neocropies to identify potential arterial access points. (Key personnel: P. Ponganis)

WORK COMPLETED

To date we have made progress on Objectives 1, 2 and 4. The first field season was completed in August 2010. We captured, instrumented and recovered backpack P_{O_2} recorders and Time Depth Recorders from 6 lactating California sea lions. P_{O_2} electrodes and thermistors were successfully placed in the caudal gluteal vein using sterile techniques in the field in all 6 females. Processing and analysis of the data has begun. Additionally, preparations for the O_2 -Hb dissociation analysis have begun.

RESULTS

We have obtained venous P_{O_2} profiles from 3513 dives from 5 females. 32.5% of the dives were between 1 and 3.5 minutes and 3.9% were greater than 3.5 min. Preliminary analysis of the data indicate, as predicted, that venous blood O_2 is not depleted during routine short dives (<3.5 min, Fig. 1). However, in dives over 5 minutes, venous P_{O_2} values regularly got below 10 mmHg and as low at 5 mmHg in dives over 7 min (Fig. 2). The sea lions ability to continue diving with P_{O_2} values less than 10 mmHg for over two minutes and the lack of consistant extended surface intervals following these dives, suggest that sea lions might have greater tolerance to hypoxia than suspected.

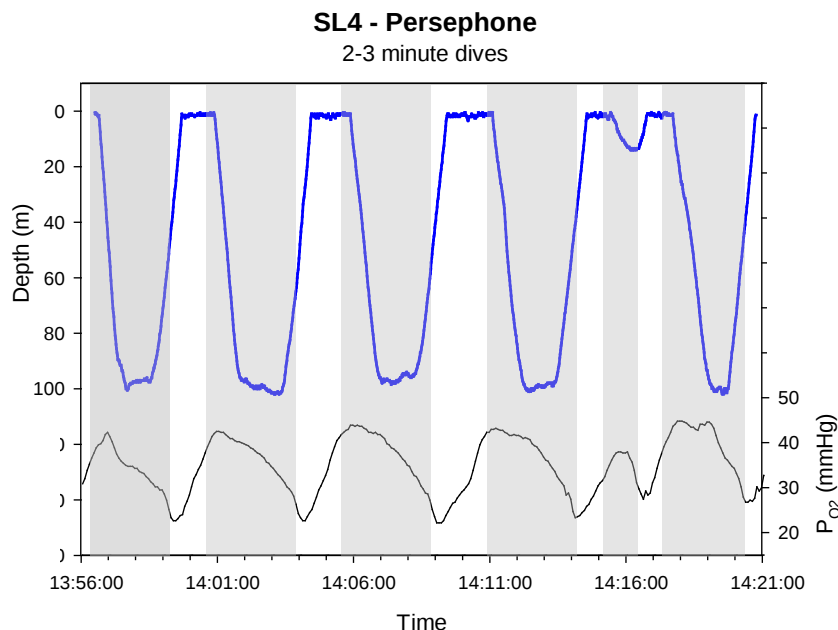
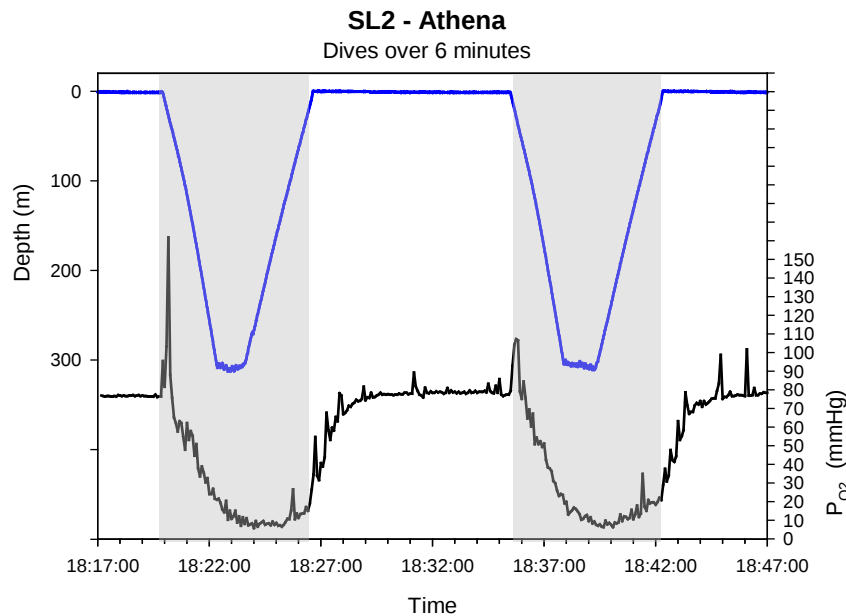


Figure 1. Venous P_{O_2} profiles from routine 2-3 minute dives. During these routine short duration dives venous P_{O_2} did not go below 20 mmHg.



***Figure 2. Venous P_{O_2} profiles from 2 dives over 6 minutes.
 P_{O_2} values reached 6 mmHg during both dives.***

IMPACT/APPLICATIONS

This project directly assesses blood O_2 depletion in diving California sea lions and develops catheterization techniques (including ultrasound guidance) for these animals. These findings thus extend beyond just the foraging ecology of sea lions, and are relevant to many important topics in diving physiology including: a) the role of the diving bradycardia in gas exchange, reduction of organ blood flow, and management of O_2 stores (Davis et al. 1983; Scholander 1940), b) the significance of myoglobin, hydrodynamics, and locomotory swim patterns in depletion of the muscle O_2 store and the ADL (Noren et al. 2001; Williams et al. 2000), c) time partitioning of energetic demands into different dive types or surface intervals in order to conserve O_2 stores during diving (Sparling et al. 2007; Williams et al. 2004), d) the role of neuroglobin and cytoglobin in hypoxemic tolerance (Williams et al. 2008), e) the role of free radicals and oxidative stress in diving mammals (Elsner et al. 1998) and f) the basic assumptions of many recent computer models of the uptake and distribution of N_2 during diving (Fahlman et al. 2009; Houser et al. 2001).

Refinement of vascular access techniques in this project also provides the groundwork to apply this technology to other otariids, and possibly other marine mammals, including dolphins. Development of such approaches is especially pertinent to the question of nitrogen uptake and its role in beaked whale strandings after exposure to naval sonar (Cox 2006). And, in fact, given that access to cetaceans for physiological investigations is so difficult, the sea lion, with a lung O_2 store as significant as that in cetaceans (Ponganis et al. 2003), may be an appropriate surrogate model for further investigation of blood N_2 uptake during diving.

RELATED PROJECTS

None.

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