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RESEARCH ARTICLE

Anterior vena caval oxygen profiles in a deep-diving California sea lion: arteriovenous shunts, a central venous oxygen store and oxygenation during lung collapse

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ABSTRACT

Deep-diving California sea lions (*Zalophus californianus*) can maintain arterial hemoglobin saturation (S_{O_2}) above 90% despite lung collapse (lack of gas exchange) and extremely low posterior vena caval S_{O_2} in the middle of the dive. We investigated anterior vena caval P_{O_2} and S_{O_2} during dives of an adult female sea lion to investigate two hypotheses: (1) posterior vena caval S_{O_2} is not representative of the entire venous oxygen store and (2) a well-oxygenated (arterialized) central venous oxygen reservoir might account for maintenance of arterial S_{O_2} during lung collapse. During deep dives, initial anterior vena caval S_{O_2} was elevated at 83.6±8.4% (n=102), presumably owing to arteriovenous shunting. It remained high until the bottom phase of the dive and then decreased during ascent, whereas previously determined posterior vena caval S_{O_2} declined during descent and then often increased during ascent. These divergent patterns confirmed that posterior vena caval S_{O_2} was not representative of the entire venous oxygen store. Prior to and early during descent of deep dives, the high S_{O_2} values of both the anterior and posterior venae cavae may enhance arterialization of a central venous oxygen store. However, anterior vena caval S_{O_2} values at depths beyond lung collapse reached levels as low as 40%, making it unlikely that even a completely arterialized central venous oxygen store could account for maintenance of high arterial S_{O_2}. These findings suggest that maintenance of high arterial S_{O_2} during deep dives is due to persistence of some gas exchange at depths beyond presumed lung collapse.

KEY WORDS: Blood oxygen, Dive, Hemoglobin saturation, Otariid, Vena cava

INTRODUCTION

Blood oxygen profiles in deep-diving California sea lions [*Zalophus californianus* (Lesson 1828)] have revealed that arterial hemoglobin (Hb) saturation (S_{O_2}) was maintained above 90% during the deepest portions of dives beyond 200 m in depth (McDonald and Ponganis, 2012). These high S_{O_2} values occurred despite (1) the presumed lack of gas exchange at the depths beyond when 'lung collapse' (compression of alveolar air into the tracheobronchial tree) occurs, and (2) low posterior vena caval S_{O_2} values that were sometimes near 0% at the bottom of these deeper dives (McDonald and Ponganis, 2012, 2013). The maintenance of high arterial S_{O_2} in combination with the large arteriovenous (a–v) oxygen content difference were remarkable because, in the absence of pulmonary gas exchange at depth, arterial S_{O_2} should decrease and reflect mixed venous (pulmonary artery) S_{O_2}. These findings suggested that either (1) some gas exchange still occurred beyond the estimated depth of lung collapse, or (2) posterior vena caval S_{O_2} was not representative of the entire venous oxygen store, and a highly oxygenated central venous reservoir might contribute to maintenance of high arterial S_{O_2} values during lung collapse. We suspected the latter possibility was more likely for two reasons. First, pulmonary shunting was found to increase with depth in sea lions and complete lung collapse and lack of gas exchange has been predicted to occur by 160 m depth (Kooyman and Sinnett, 1982). Second, arterial partial pressure of oxygen (P_{O_2}) profiles during deep dives were consistent with significantly decreased gas exchange at such depths (McDonald and Ponganis, 2012).

Previously, we had hypothesized that arterialization of venous blood (venous S_{O_2} approaching arterial S_{O_2}) prior to deep dives and/or during early descent created a well-oxygenated central venous oxygen store (McDonald and Ponganis, 2013; Tift et al., 2017). Such arterialization of the venous blood may occur through a–v shunting, most likely through the fore- and hind-flippers as has been suggested for phocid seals (Blix et al., 2010). To avoid confusion about shunting, readers should remember that a–v shunts are distinct from pulmonary shunts. Arteriovenous shunts occur in the peripheral vasculature and, typically, increase venous oxygen content. Pulmonary shunts occur within the lungs and decrease arterial oxygen content.

The extreme bradycardia that occurs during the bottom phases of deep dives, when the lungs are collapsed, should slowly mete out this well-oxygenated blood. The low heart rate would also preserve oxygenation of the central venous blood owing to the slow return of desaturated venous blood from the periphery during the bradycardia (McDonald and Ponganis, 2014). Theoretically, arterialization of venous blood could be achieved through (1) a–v shunting prior to or early in dives, and/or (2) some splenic contraction and expulsion of oxygenated blood into the central vena cava due to further activation of the sympathetic nervous system to maintain blood pressure during the extreme bradycardia of the bottom phases of deep dives (McDonald and Ponganis, 2013; Ponganis, 2015; Ponganis et al., 1992). Lastly, arterialization of venous blood early or prior to the dive would require minimization of muscle blood flow and blood oxygen extraction by muscle; otherwise, venous saturation would decrease.

Our hypothesis of a well-oxygenated central venous oxygen store in deep-diving sea lions is analogous to the hepatic sinus oxygen reservoir proposed by Elsner et al. (1964) in northern elephant seals (*Mirounga angustirostris*). Otariids (sea lions and fur seals) do not

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have the capacious hepatic sinus/vena cava and elaborate posterior vena caval sphincter of phocid seals (Harrison and Tomlinson, 1956; King, 1977; King, 1983). However, the longest durations of lung collapse during the deep dives of sea lions are only 3 to 3.5 min (McDonald and Ponganis, 2012); therefore, even a relatively small oxygenated venous blood oxygen reservoir in the hepatic sinus may be sufficient to maintain high arterial S_{O_2} during the lung collapse phase of their dives. Furthermore, the rate of depletion of this proposed central venous oxygen store could be slowed by a high anterior vena caval S_{O_2} during lung collapse.

Ideally, to address whether an arterialized central venous oxygen store exists in sea lions and test our hypothesis that posterior vena caval oxygenation does not reflect the total venous oxygen store during a deep dive, we would have to measure pulmonary artery (mixed venous) P_{O_2} or S_{O_2} in diving sea lions. Although such catheterizations are feasible (Ponganis et al., 1991), the P_{O_2} electrodes we currently use are not long enough to reach the pulmonary artery, and other bio-logging devices and sensors are not available for such measurements in a free-diving sea lion. Therefore, as a surrogate measurement, we chose to investigate anterior vena caval P_{O_2} and S_{O_2} during dives with use of our available P_{O_2} electrodes/recorders.

The anatomical arrangement of veins draining into the anterior vena cava (King, 1977) (Fig. 1), and the relative blood flows and S_{O_2} values in those veins, should determine the anterior vena caval S_{O_2} profile. The S_{O_2} in the anterior vena cava would be dependent on possible a–v shunting, the concomitant arterial S_{O_2}, the Hb concentration of the individual animal and the influx of venous blood from peripheral tissues (dependent on the level of bradycardia and peripheral perfusion). Notably, unlike venous inflow from other tissues, venous drainage from the brain via the external jugular vein into the anterior vena cava should continue during the bradycardia of diving because cerebral blood flow is maintained during the dive response (Blix et al., 1983; Zapol et al., 1979).

We expected that anterior vena caval S_{O_2} profiles during the dive would differ from posterior vena caval profiles, supporting our hypothesis that the posterior vena caval S_{O_2} profile was not indicative of the entire venous oxygen store. We also hypothesized that the anterior vena caval S_{O_2} profiles would (1) provide evidence for arterialization of venous blood prior to deep dives, and (2) be high enough to allow maintenance of arterial S_{O_2} when it returned to the heart and mixed with well-oxygenated blood from the hepatic sinus.

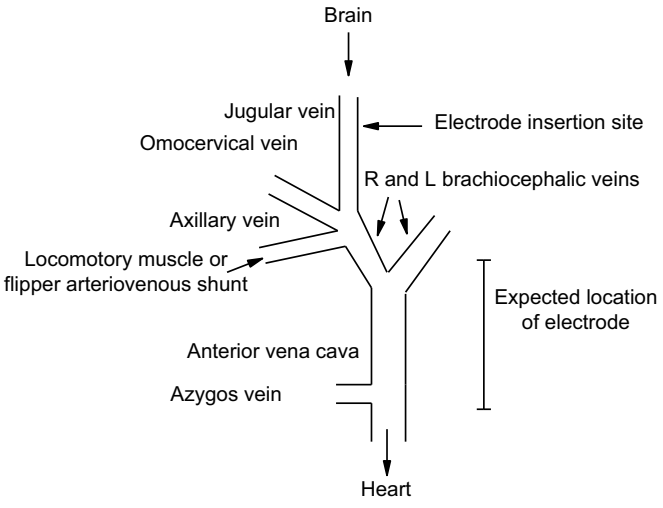

Fig. 1. The anterior vena cava and major veins in the otariid neck and upper chest (King, 1977). Venous drainage of the brain is via the external jugular (jugular) vein. Inflow of venous blood from locomotory muscles and possible arteriovenous shunts in the foreflipper primarily occurs in the axillary vein. Location of the catheterization site and approximate location of the oxygen electrode in this study are indicated in the figure.

MATERIALS AND METHODS

Instrumentation

This study was conducted on San Nicolas Island, California, in August 2013. A single adult female California sea lion was captured with a hoop net and anesthetized using a portable vaporizer-breathing circuit set up with an initial mixture of 5% isoflurane and 100% oxygen. Once anesthetized, a custom-built P_{O_2} datalogger (UUB-2PT; UFI, Morro Bay, CA, USA) with custom housing (Meer Instruments, Palomar Mountain, CA, USA), time-depth recorder with three-axis accelerometer (TDR10x; Wildlife Computers, Redmond, WA, USA) and VHF radio transmitter (mm160B; Advanced Telemetry Systems, Isanti, MN, USA) was affixed to the dorsal, midline pelage of the animal using fast-setting epoxy (Loctite, Henkel Corp., Westlake, OH, USA). Using an ultrasound machine (SonoSite Inc., Bothell, WA, USA), the external jugular vein was identified and percutaneously catheterized with a peel-away catheter (5 Fr, Cook Medical, Bloomington, IN, USA). A 30 cm P_{O_2} electrode (Licox C1.1, Integra Life Sciences, Plainsboro, NJ, USA) was then inserted into the peel-away catheter and threaded down into the anterior vena cava. The P_{O_2} electrode was connected to the P_{O_2} datalogger via a waterproof cable and connector (Impulse Enterprise, San Diego, CA, USA). Depth and P_{O_2} were recorded at 1 Hz, and three axes of acceleration were recorded at 16 Hz. Calibration and specifics on the P_{O_2} electrode have been explained in more detail in a previous publication (Stockard et al., 2005). In particular, it should be remembered that ambient pressure does not have a direct effect on P_{O_2} in the blood. Of course, ambient pressure does have an indirect effect on blood P_{O_2} through pulmonary gas exchange and the well-known effects of ambient pressure on the partial pressures of gases in the air of the lungs. Catheter placement has also been explained in more detail in previous publications (McDonald and Ponganis, 2012, 2013; Ponganis et al., 1997, 1991; Tift et al., 2017).

After instrumentation, the animal was placed in a large canine kennel to be weighed (±0.2 kg, MSI-7200 Dyna-link; Measurement Systems International, Seattle, WA, USA) and recover from anesthesia (~60 min). Once fully alert, the animal was released back onto the same beach where she was originally captured. After 7 days and two trips to sea, the animal was recaptured with hoop nets, instruments were removed under manual restraint and then the animal was released (10 min procedure). All procedures were approved by the University of California, San Diego, Animal Subjects Committee (no. S11303) and National Marine Fisheries Services (no. 14676).

Data processing and statistics

Data were analyzed as described in our previous publication (Tift et al., 2017). Briefly, dives were identified and summary data from each dive were produced using a custom-written MATLAB program (IKNOS; Y. Tremblay). This dive analysis program implements a zero-offset correction at the surface and identifies dives using a specified minimum depth (10 m) and duration (20 s). The following dive phases were identified in the data: descent (surface to 80% maximum depth), bottom (depths within 80% maximum depth) and ascent (80% maximum depth to surface). Most dives from California sea lions are shallower than 100 m (Feldkamp et al., 1989), yet many adult female California sea lions from San Nicolas

Island are known to be deep divers (Kuhn and Costa, 2014; McHuron et al., 2016) and exhibit a bimodal pattern in their maximum depth of dives, with very few dives occurring between 100 and 150 m (Fig. S1). Therefore, to compare shallow and deep dives, dives to maximum depths >100 m were classified as deep, and dives to maximum depths ≤100 m were classified as shallow.

Fore-flipper stroke rate was calculated using a custom-written algorithm in MATLAB. The low-frequency static acceleration was filtered out using a 0.2 Hz high-pass Butterworth filter. The resulting dynamic acceleration was analyzed using power spectral density analysis to identify the dominant frequency of a flipper stroke for each axis (~0.8–1.2 strokes s^{-1}). Flipper strokes were identified using a MATLAB peak detection algorithm similar to those in other studies (Jeanniard-du-Dot et al., 2016; Sato et al., 2011). A single flipper stroke was identified when there was a prominent acceleration peak (≥0.45 m s^{-2}) in either the forward surge direction or the heave surge direction. Stroke rate was calculated both as an average stroke rate along a moving window of 10 s throughout the diving record, and as an instantaneous stroke rate (strokes s^{-1}) based on the time between two individual flipper strokes.

The P_{O_2} electrode was calibrated in the laboratory before deployment (McDonald and Ponganis, 2012, 2013). The percent hemoglobin saturation was calculated using an equation from a previously measured hemoglobin-dissociation curve in this species (McDonald and Ponganis, 2013). The minimum calculated aerobic dive limit for this species is 3.4 min (Weise and Costa, 2007), and increased P_{CO_2} accumulated during a breath hold is known to contribute to a decrease in pH as dive duration increases (Kooyman et al., 1980; Qvist et al., 1986). Therefore, P_{O_2} values collected prior to 3 min into the dive were converted to S_{O_2} using the equation for a pH of 7.4 $\{\log[S_{O_2}/(100- S_{O_2})]=2.473\times\log(P_{O_2})-3.632\}$ (r^2=0.97); all P_{O_2} values collected beyond 3 min into dives were converted to S_{O_2} using the equation for a pH of 7.3 $\{\log[S_{O_2}/(100-S_{O_2})] =2.363\times\log(P_{O_2})-3.576\}$ (r^2=0.96). The core body temperature of California sea lions is thought to remain relatively constant at 37°C while diving (McDonald and Ponganis, 2013). Therefore, the P_{O_2} electrode was only calibrated at this temperature, and effects of temperature variation on electrode output and S_{O_2} values were not considered. The time on the TDR/accelerometer instrument and P_{O_2} logger were synchronized to the same internet-synced computer clock. Mean change in S_{O_2} (ΔS_{O_2}) was calculated for the same 10 s moving window as flipper stroke rates to facilitate comparison between the two parameters. ΔS_{O_2} (initial S_{O_2}–final S_{O_2}) was also calculated for the duration of the three dive phases (descent, bottom and ascent) and for the total dive duration of every dive.

Depth of lung collapse was estimated for all dives >200 m using the linear regression provided by McDonald and Ponganis (2012): depth of lung collapse (m)=0.62×maximum depth (m)+35.66. Values and patterns of anterior vena caval S_{O_2} were compared with the estimated depths of lung collapse by extracting S_{O_2} values during depths beyond the estimated depth of lung collapse. Mean values are reported ±s.e.m., unless otherwise noted.

In evaluation of possible a–v shunting and also the maintenance of aortic S_{O_2} during lung collapse by the mixing of highly saturated anterior vena caval blood and hepatic sinus blood in the heart, we used a shunt formula based on the principle that the total amount of oxygen leaving the system must equal the sum of the amounts of oxygen in shunted blood and non-shunted blood (West, 1972). In the case of an intrapulmonary shunt, the output is total blood flow ($\dot{Q}_T$)×arterial oxygen content (Ca_{O_2}), and the input is (1) shunt flow ($\dot{Q}_S$)×venous oxygen content (Cv_{O_2}), plus (2) non-shunt flow ($\dot{Q}_T$–$\dot{Q}_S$)×pulmonary capillary oxygen content (Cc_{O_2}):

$$\dot{Q}_T \times Ca_{O_2} = (\dot{Q}_S \times Cv_{O_2}) + [(\dot{Q}_T - \dot{Q}_S) \times Cc_{O_2}]. \quad (1)$$

In these cases, all O_2 contents can be calculated as the product of the respective fractional percent hemoglobin saturation (fractional S_{O_2})×hemoglobin content×1.34 ml O_2 g^{-1} hemoglobin. Dissolved O_2 is often ignored because it is such a small fraction of the total amount of oxygen in the blood. By dividing all terms by (hemoglobin content×1.34 ml O_2 g^{-1} hemoglobin), we arrive at the basic equation, where fractional S_{O_2} is the fraction of hemoglobin that is saturated in the aorta (a), shunt (s) and the pulmonary capillary (c):

$$\dot{Q}_T \times \text{fractional } S_{O_2a} = (\dot{Q}_S \times \text{fractional } S_{O_2s}) + [(\dot{Q}_T - \dot{Q}_S) \times \text{fractional } S_{O_2c}]. \quad (2)$$

This is the basic formula that we modified for determination of the necessary saturation and flow needed in an axillary a–v shunt to achieve the observed anterior vena caval (AVC) saturation in the presence of the assumed flow and saturation from the jugular vein:

$$\dot{Q}_{AVC} \times \text{fractional } S_{O_2AVC} = (\dot{Q}_{jugular} \times \text{fractional } S_{O_2jugular}) + [(\dot{Q}_{AVC} - \dot{Q}_{jugular}) \times \text{fractional } S_{O_2a-v\ shunt}]. \quad (3)$$

In this equation, $\dot{Q}_{AVC}$–$\dot{Q}_{jugular}$ equals $\dot{Q}_{a-v\ shunt}$, i.e. a–v shunt flow. In this model, it was assumed that other venous inflow into the anterior vena cava was minimal.

The same type of formula was also used for calculation of the flows needed in the anterior vena cava and posterior vena cava (from the hepatic sinus) during lung collapse in order to achieve the observed maintenance of high arterial saturations. Calculations were based on the known cardiac output, the observed saturation in the anterior vena cava and the assumed saturation (95%) of blood coming from the hepatic sinus into the proximal posterior vena cava (and then into the right atrium to mix with the blood from the anterior vena cava).

RESULTS

Dive characteristics

A total of 198 dives with simultaneous and continuous venous P_{O_2}, depth and acceleration data were collected from one adult, female, 77.8 kg California sea lion. The mean depth of dives was 149±116 m, with a maximum depth of 399 m (Fig. S1). The mean dive duration was 194±124 s, with a maximum dive duration of 443 s. During the 7 days that the deployment lasted, she performed two trips to sea. The first trip was approximately 5 h, and the second trip was just over 24 h of continuous diving.

Shallow dives (≤100 m)

At the end of shallow dives, the mean P_{O_2} and S_{O_2} were typically higher than values at the start of the dive (Table 1). This pattern was reflected by positive ΔS_{O_2} values for the entire dive (i.e. overall increase in blood oxygen throughout the dive, see Figs 2, 3 and 4B). Most of the increase in S_{O_2} occurred early in the dive, during the descent phase of shallow dives (Fig. 5; Fig. S2). Despite these consistent patterns, the overall range in anterior vena caval S_{O_2} was highly variable among shallow dives (Figs 3 and 4B). There was no clear relationship between flipper stroke rate and ΔS_{O_2} across dive phases, or for the entire dive (Figs 4B, 5 and 6). There were often brief periods of gliding during the last portion of the ascent, when the animal was approaching the surface (Fig. S2). Rest periods at the

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Table 1. Partial pressure of oxygen (P_{O_2}) and hemoglobin saturation (S_{O_2}) in the anterior vena cava at the start and end of deep (>100 m) and shallow (<100 d) dives of a California sea lion

	Start P_{O_2}	Start S_{O_2}	End P_{O_2}	End S_{O_2}
Deep				
mmHg	59.8±10.4	83.6±8.4	21.4±5.2	30.9±11.8
kPa	7.97±1.39		2.85±0.69	
Shallow				
mmHg	40.0±7.8	66.2±10.4	45.3±8.1	72.7±9.1
kPa	5.33±1.04		6.04±1.08	

During rest periods at the surface, S_{O_2} was near 76–79% (see Figs S4 and 5). Data are expressed as means±s.e.m. *N*=102 and 96 for deep and shallow dives, respectively.

surface and shallow swim periods revealed that surface resting S_{O_2} was near 75% (Fig. S3A).

Deep dives (>100 m)

At the start of deep dives, anterior vena caval P_{O_2} and S_{O_2} were typically much higher than those at the start of shallow dives; similarly, anterior vena caval P_{O_2} and S_{O_2} at the end of deep dives were lower than those at the end of shallow dives (Table 1, Fig. 4A). On deeper dives, S_{O_2} did not decline initially; instead, high S_{O_2} was maintained until the animal reached the bottom portion of the dive, when values began to decrease and then continued to drop until the animal surfaced (Fig. 4A; Figs S2 and S4). Hence, the largest reductions in S_{O_2} of the anterior vena cava occurred during ascent. There were consistent negative relationships between ΔS_{O_2} for the entire dive duration with both maximum dive depth and duration (i.e. longer and deeper dives had more oxygen extracted from blood) (Fig. 3).

In 74 deep dives, ranging from 205 to 399 m maximum depth, mean anterior vena caval S_{O_2} at the estimated depth of lung collapse was 82.9±3.0% (Fig. S4). At the estimated depth of lung re-expansion and resumption of gas exchange, mean anterior vena caval S_{O_2} was 63.8±8.4%. The maximum and minimum S_{O_2} at the start of the lung collapse period were 88.4 and 76.4%; the corresponding values at the end of the lung collapse period were 78.9 and 40.1%, respectively.

Unlike shallow dives, the rate of decline in blood oxygen during deep dives (>100 m) mirrored flipper stroke rate throughout deep dives (Fig. 6). This relationship was due primarily to the rapid drop in S_{O_2} that occurred during the bottom and ascent phases of dives, when animals were frequently stroking (Figs 4A, 5 and 6; Fig. S2).

DISCUSSION

The diving behavior of the sea lion in this study, including dive depths and flipper stroke rate profiles (Fig. 4; Fig. S2), was similar to that seen previously in other free-diving adult female California sea lions (McDonald and Ponganis, 2012, 2013, 2014; McHuron et al., 2016; Tift et al., 2017). For example, the animal from this study consistently exhibited prolonged glides during the descent of deep dives (>100 m maximum depth), as well as short duration glides during the last 25–30 m of ascent to the surface (Fig. 4A, Fig. S2). Therefore, despite the limitation in sample size, we believe the anterior vena caval blood oxygen profiles from this study are representative of the range of dives performed by adult female California sea lions during foraging trips to sea.

Several logistical and technical factors contributed to the limited sample size in this study: (1) frequent migration of the P_{O_2} electrode out of the blood vessel during dive activity, (2) internal damage to the wires and solder joints within the underwater connector cable secondary to presumed torque on the cable, and (3) failure of either the P_{O_2} logger or TDR/accelerometer while the animal was out at sea. Out of a total of 12 deployments of anterior vena caval P_{O_2} electrodes from 2013 to 2016, the animal in this study was the only animal where all instruments and the probe functioned properly. We think that the technical problems of P_{O_2} electrode migration and cable dysfunction were due to the high degree of flexibility and maneuverability of the sea lion neck and head while swimming (Fish et al., 2003).

Anterior vena caval oxygen profiles

Similar to prior findings in the posterior vena cava of sea lions (McDonald and Ponganis, 2013; Tift et al., 2017), anterior vena caval P_{O_2} and S_{O_2} profiles were highly variable during short duration, shallow dives (<100 m; Fig. 1). Start-of-dive S_{O_2} values during shallow dives ranged from 40 to 80% and indicated that the animal

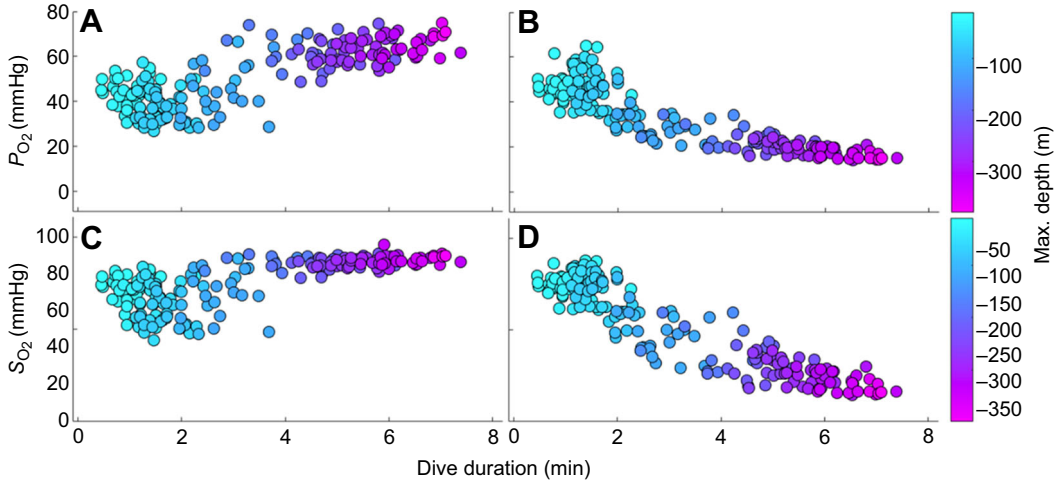

Fig. 2. Anterior vena caval partial pressure of oxygen (P_{O_2}; mmHg) and hemoglobin saturation (S_{O_2}; %) values relative to total dive duration. Values are shown at the start (A,C) and (B,D) end of every dive, and are color coded for the maximum depth (m) of the dive. Deep dives are characterized by higher initial P_{O_2} and S_{O_2} values and lower final values. For shallow dives, initial P_{O_2} and S_{O_2} are highly variable; these values can actually increase by the end of the dive. Note 1 mmHg=0.133 kPa.

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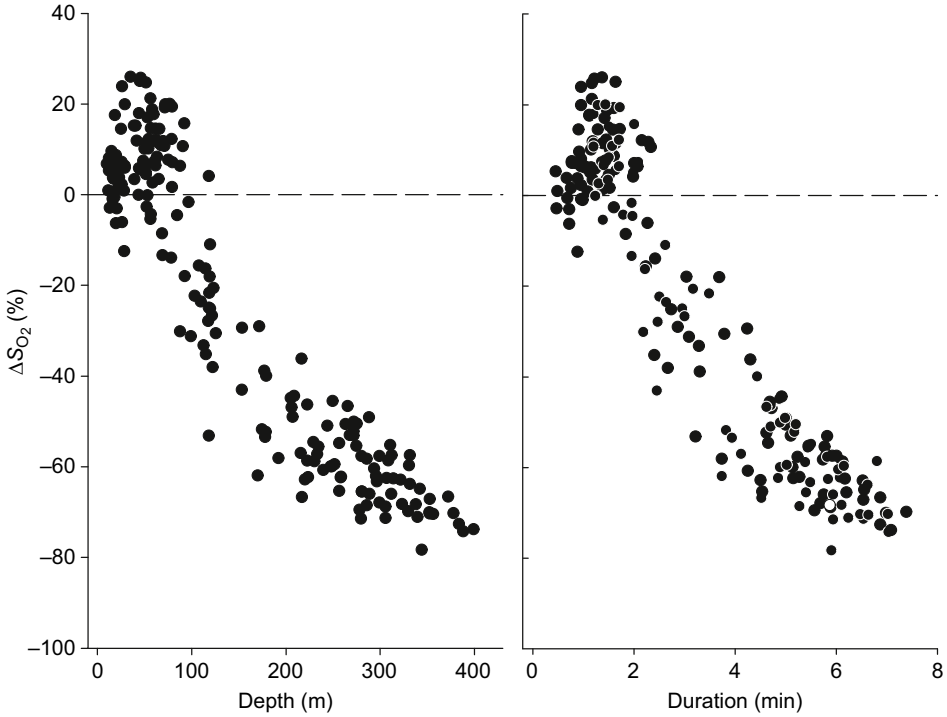

Fig. 3. Change in anterior vena caval hemoglobin saturation (ΔS_{O_2}; %) for the entire dive versus maximum dive depth and duration. ΔS_{O_2} for the entire dive duration is calculated (final S_{O_2}–initial S_{O_2}) for each dive. Positive ΔS_{O_2} values denote an overall increase in S_{O_2} for that dive, while a negative value denotes a decrease in S_{O_2} for that dive. There is a net increase in S_{O_2} for many dives <2 min in duration and <100 m in depth. In deeper, longer dives, there is a net depletion of oxygen that approaches an 80% ΔS_{O_2} in the deepest, longest dives.

did not always fully replete the venous oxygen store prior to the next shallow dive, whereas start-of-dive S_{O_2} values on deep dives were consistently higher (Table 1, Fig. 2). Such incomplete restoration of the oxygen store prior to shallow dives has been suggested based on metabolic rate studies of Steller sea lions (*Eumetopias jubatus*) (Fahlman et al., 2008). Notably, however, anterior vena caval P_{O_2} and S_{O_2} frequently increased during shallow dives (Figs 2, 3 and 4B, Table 1). This increase in venous oxygenation during shallow dives has also been observed in the posterior vena cava, although less frequently (Tift et al., 2017). A net increase in anterior vena caval S_{O_2} suggests minimal muscle blood flow and possible a–v shunting during many of these shallow dives. In other words, the blood oxygen content contributed by the a–v shunt must be greater than the tissue oxygen extraction from blood in other vessels returning into the anterior vena cava. The large range in anterior vena caval S_{O_2} during shallow dives (Fig. 4B) and even during surface intervals suggests that blood oxygen delivery to muscle and potential use of a–v shunts for thermoregulation are highly variable.

For deep, long-duration dives, initial P_{O_2} and S_{O_2} increased with dive duration while end-of-dive values consistently decreased (Figs 2 and 3). Surface interval P_{O_2} and S_{O_2} values between deep dives were typically greater than those for shallow dives, and for extended rest periods at the surface that occasionally occurred (see Fig. S3B). Based on the higher surface interval heart rates before deeper dives (McDonald and Ponganis, 2014), we think that elevated cardiac outputs and a–v shunting prior to the dive contribute to these elevations in anterior vena caval S_{O_2}. Presumably, myoglobin is already re-saturated, and muscle blood flow/blood oxygen extraction by muscle are not exceptionally high before the dive. These patterns suggest an element of planning prior to deep dives in this species.

The S_{O_2} profiles in the anterior vena cava during deep dives differed markedly from those previously recorded in the posterior vena cava (McDonald and Ponganis, 2013; Tift et al., 2017); S_{O_2} remained elevated in the anterior vena cava during most of descent (Figs 4 and 5; Fig. S2) whereas it typically declined dramatically in the posterior vena cava. During the latter portions of deep dives, anterior vena caval S_{O_2} always decreased in contrast to those in the posterior vena cava, which often increased during ascent. These findings support our hypothesis that posterior vena caval oxygen profiles are not representative of the entire venous system.

Relationship of anterior vena caval blood oxygen use to flipper stroke rate in shallow dives

Anterior vena caval S_{O_2} may be affected by flipper stroke effort because blood flow from the primary locomotory muscles of sea lions returns to the heart via the anterior vena cava (Fig. 7). However, the relationship appears to be weak between anterior vena caval blood oxygen profiles and flipper stroke rate patterns during shallow dives (Figs 4B and 5). The net increases in S_{O_2} during many shallow dives suggest that there was (1) minimal muscle blood flow (insignificant blood oxygen extraction by muscle), (2) a–v shunting or (3) a high ratio of a–v shunting to locomotory muscle blood flow. Such variability in peripheral blood flow patterns has also been suggested in diving emperor penguins (*Aptenodytes forsteri*), where increases in venous P_{O_2} and S_{O_2} were also often seen in their short duration dives (Ponganis et al., 2009; Williams et al., 2011).

Relationship of anterior vena caval blood oxygen use to flipper stroke rate in deep dives

During the initial active flipper stroking of early descent in deep dives, anterior vena caval S_{O_2} remained high (Figs 4–6; Figs S2 and S4). Maintenance of these high venous S_{O_2} values suggests that muscle blood flow and blood oxygen extraction by active muscle are minimal during the initial phase of the dive. In contrast, when the prolonged glide to the bottom of the dive ended and foreflipper stroking began, anterior vena caval S_{O_2} began to decline (Figs 4–6; Figs S2 and S4). These declines in anterior vena caval S_{O_2} were consistent with increased inflow of more desaturated blood from peripheral tissues (including muscle) that may be associated with the increased heart rate observed during the latter phases of the dive, especially during ascent (McDonald and Ponganis, 2014). As

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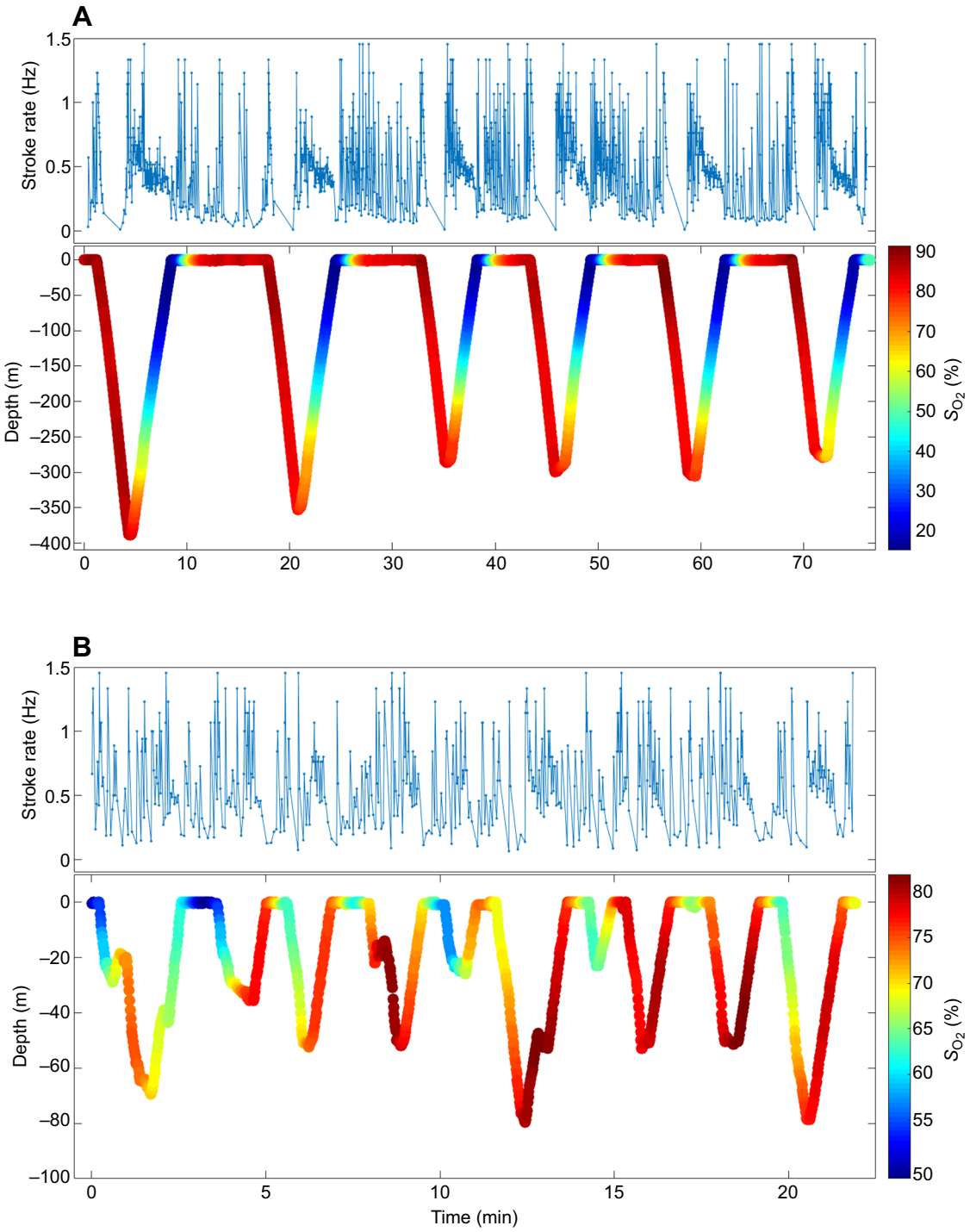

Fig. 4. Flipper stroke rate and depth profiles color-coded with anterior vena caval hemoglobin saturation (S_{O_2}) for deep (>100 m) and shallow (≤100 m) dives. (A) Deep dives exhibit a repetitive S_{O_2} profile with maintenance of high saturations throughout descent, progressive decreases in S_{O_2} during ascent, recovery of S_{O_2} during the early surface period and an increase in S_{O_2} prior to the start of the next dive. Stroke rates during deep dives are notable for a prolonged glide during descent followed by a variable stroke pattern during the bottom and ascent phases. (B) During shallow dives, there are wide variations in both flipper stroke rates and range of S_{O_2} values; the patterns are not repetitive as during deep dives.

discussed above, such variations in peripheral blood flow patterns have also been suggested for emperor penguins. An increase in muscle blood flow and blood oxygen extraction by muscle during the latter phases of deep dives would support previous suggestions that changes in heart rate may be coupled to exercise in diving mammals (Davis and Williams, 2012; Hindle et al., 2010; Signore and Jones, 1996).

Arterialization of venous blood: mechanisms and implications

Arteriovenous shunts and venous anatomy

As discussed in a prior paper (McDonald and Ponganis, 2013), a normal a–v oxygen content difference of 5 ml O_2 dl^{-1} in a sea lion with a Hb content of 18 g dl^{-1}, and an arterial S_{O_2} of 95 to 100% should result in a venous S_{O_2} of 74 to 79%. This coincides with the

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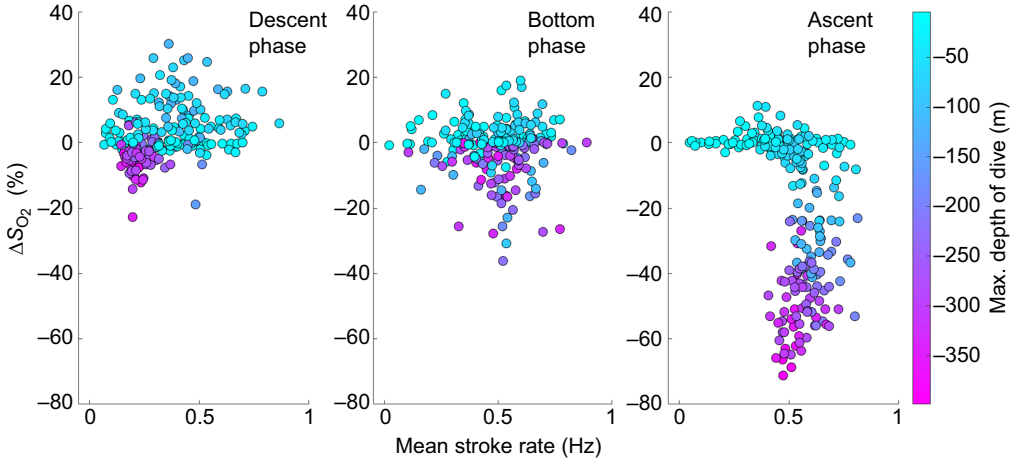

Fig. 5. Mean flipper stroke rate and mean ΔS_{O_2} for the three different dive phases. Each point represents the mean value for a given phase (descent, bottom, ascent) during one dive. Points are color coded based on the maximum depth of that individual dive. ΔS_{O_2} for each dive phase is calculated (final S_{O_2}–initial S_{O_2}) for each dive phase of every dive. Positive ΔS_{O_2} values denote an overall increase in S_{O_2} for that dive phase, while a negative value denotes a decrease in S_{O_2} for that dive phase.

S_{O_2} range observed in the anterior vena cava of the sea lion in this study during rest activity at the surface (Fig. S3). However, just as was found in the posterior vena cava (McDonald and Ponganis, 2013), S_{O_2} in the anterior vena cava was usually elevated above this level, sometimes to >90%, prior to deep dives (>100 m maximum depth) (Fig. 4; Fig. S3B). Exceptionally high P_{O_2} and S_{O_2} values were also frequently observed during shallow dives and during the early segments of deep dives (Figs 2 and 4; Figs S3B and S5). These high venous S_{O_2} values suggest that sea lions utilize some form of a–v shunting prior to and even during dives, most likely through the fore- and hind-flippers as has been suggested for phocid seals (Blix et al., 2010), and as has been reviewed in prior papers (McDonald and Ponganis, 2013; Tift et al., 2017). These high S_{O_2} values in both the anterior and posterior vena cava prior to deep dives support our hypothesis of a highly oxygenated central venous oxygen store in the sea lion.

The contribution of an a–v shunt to the elevation in anterior vena caval S_{O_2} is based on venous anatomy (Figs 1 and 7A). The anterior vena cava (the site where venous P_{O_2} was measured) is formed by the union of the two brachiocephalic veins, which each receive primary input from the external jugular (jugular), omocervical and axillary veins (King, 1977). Cerebral venous drainage into the jugular vein should result in an a–v oxygen content difference of 5–6 ml O_2 dl^{-1} of blood (Kerem and Elsner, 1973; Macmillan and Andrews, 2000; Zauner and Muizelaar, 1997), corresponding to a jugular S_{O2} of 74-79% in the sea lion (McDonald and Ponganis, 2013). Given that the sea lion brain is approximately 300 g, and cerebral blood flow is approximately 55 ml 100 g^{-1} brain tissue min^{-1}, jugular venous flow from the brain would be approximately 165 ml min^{-1} (Montie et al., 2009, 2010; Zauner and Muizelaar, 1997). With this jugular inflow into the anterior vena cava, the a–v shunt flow required to raise anterior vena caval S_{O_2} to a given level can be calculated with Eqn 3 (West, 1972) (see Materials and methods), where fractional S_{O_2AVC} is 0.92, corresponding to the observed 92% S_{O_2} in the anterior vena cava; $\dot{Q}_{jugular}$ is the estimated 165 ml min^{-1} inflow of jugular blood, and fractional $S_{O_2jugular}$ is 0.74, corresponding to 74% S_{O_2} estimated in the jugular vein; $\dot{Q}_{AVC}$–$\dot{Q}_{jugular}$ is a–v shunt flow, and fractional $S_{O_2a–v\ shunt}$ is 0.95, reflecting the observed arterial S_{O_2} value of 95%.

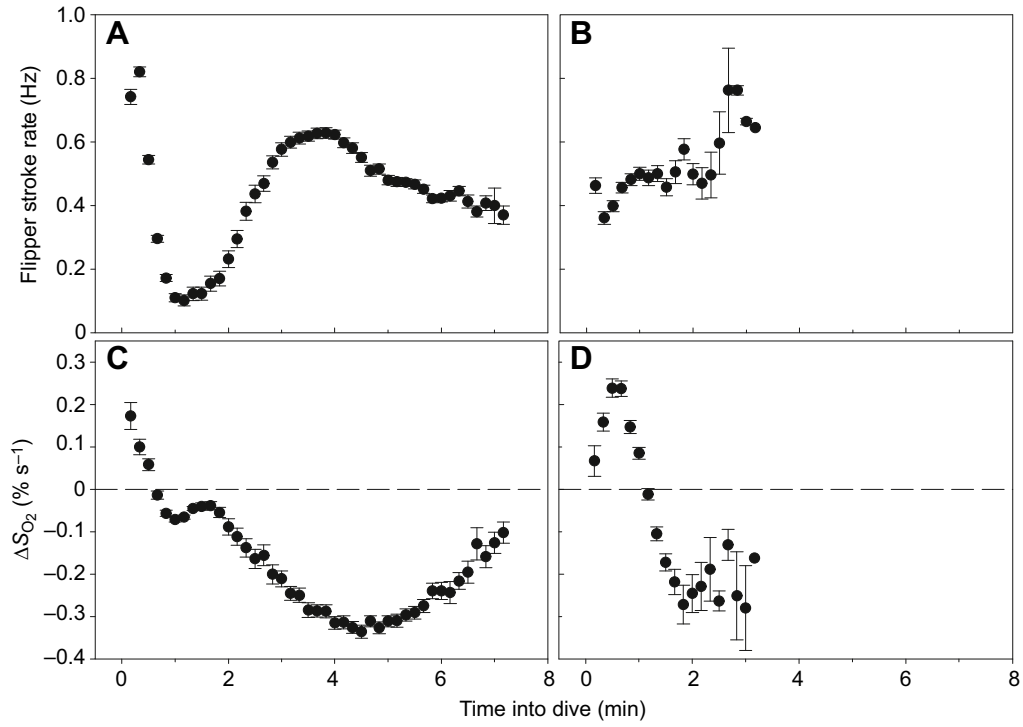

Fig. 6. Flipper stroke rate and change in anterior vena caval S_{O_2} throughout deep and shallow dives. S_{O_2} Mean (±s.e.m.) values of flipper stroke rate (A,B) and ΔS_{O_2} s^{-1} (C,D) every 10 s into dives. A and C represent deep dives (>100 m), while B and D represent shallow dives (≤100 m). Each symbol for values of ΔS_{O_2} is represented as the rate of change in S_{O_2} (Δ% s^{-1}) for each 10 s period throughout dives. Positive ΔS_{O_2} values denote an overall increase in S_{O_2} for that 10 s period in the dive, while a negative value denotes a decrease in S_{O_2} during that 10 s period of the dive. During deep dives (A,C), S_{O_2} increases in early descent despite flipper stroking; in contrast, during the bottom and ascent phases of the dive, S_{O_2} decreases at variable rates during flipper stroking. During shallow dives (B,D), varying increases in S_{O_2} at low flipper stroke rates occur during the first minute; in the latter parts of these dives, although S_{O_2} tends to decrease, both ΔS_{O_2} s^{-1} and flipper stroke rate are highly variable.

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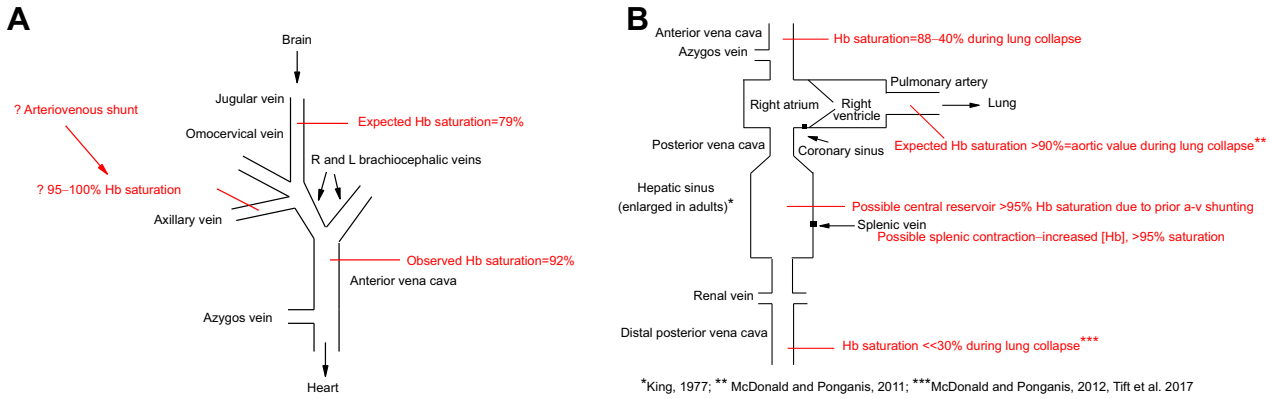

Fig. 7. Venous anatomy and hemoglobin (Hb) saturations (S_{O_2}) in a California sea lion. (A) The anterior vena cava (the site where venous P_{O_2} was measured in this study) is formed by the union of the two brachiocephalic veins, which each receive primary input from the external jugular (jugular), omocervical and axillary veins (King, 1977). Cerebral venous drainage into the jugular vein should result in an arteriovenous oxygen content difference of 5–6 ml O_2 $dl^{-1}$of blood (Kerem and Elsner, 1973; Macmillan and Andrews, 2000; Zauner and Muizelaar, 1997), corresponding to a S_{O_2} of approximately 79% in the sea lion (McDonald and Ponganis, 2013). The S_{O_2} values as high as 92% recorded in the anterior vena cava prior to deep dives and during descent imply some degree of arteriovenous shunting, probably via the foreflipper and axillary vein as has been proposed in phocid seals (Blix et al., 2010). (B) The hepatic sinus of the sea lion forms a highly oxygenated venous reservoir during deep dives due to pre-dive arteriovenous (a–v) shunting. Although pre-dive a–v shunts may increase the size of the blood oxygen store, the hepatic sinus, even with highly saturated blood, is not large enough to contribute enough blood to venous return to the heart to maintain arterial S_{O_2} above 90% during 'lung collapse' (see calculations in Discussion).

Use of these values in the equation results in a $\dot{Q}_{AVC}$ of 1155 ml min^{-1}. The calculated $\dot{Q}_{AVC}$–$\dot{Q}_{jugular}$ is 990 ml min^{-1} under these conditions.

The tachycardia of 120 beats min^{-1} prior to deep dives results in a cardiac output of 14.4 l min^{-1} based on a minimum stroke volume of 1.5 ml kg^{-1} in an 80 kg sea lion (Ponganis et al., 1991). Thus, the 990 ml min^{-1} a–v shunt flow would be only 6.9% of cardiac output. A slightly larger a–v shunt flow and percentage of cardiac output could compensate for additional desaturated blood from any venous tributaries, such as the omocervical vein. Even during late descent, with a heart rate of 15 beats min^{-1}, cardiac output would be 1.6 l min^{-1}, which should be more than sufficient to account for brain, a–v shunt and coronary blood flow. Furthermore, the bradycardia and peripheral vasoconstriction during this phase of the dive would also minimize inflow of any desaturated blood from the omocervical vein and other vessels into the brachiocephalic veins.

The magnitude of the hypothesized a–v shunt may decrease during late descent because elevated carbon dioxide increases cerebral blood flow in sea lions (Dormer et al., 1977) and decreases the percentage of cardiac output available for an a–v shunt. However, in regard to S_{O_2}, the hypercarbia-induced increase in cerebral blood flow will result in more oxygen being delivered to the brain and an increased S_{O_2} in the jugular vein, because brain metabolism will remain constant (Macmillan and Andrews, 2000; Schell and Cole, 2000). This would mitigate the decrease in a–v shunting and again contribute to maintenance of elevated anterior vena cava oxygen content. In addition, hypercarbia can also depress brain metabolic rate (Xu et al., 2011), which should also elevate or at least conserve jugular S_{O_2}. These processes could help account for the maintenance of relatively high anterior vena caval S_{O_2} during this phase of the dive.

Anterior and posterior vena caval profiles during deep dives

Comparison of S_{O_2}, heart rate and stroke rate profiles collected during deep dives of different animals provide a general physiological overview of deep dives of sea lions (Figs 7B, 8 and 9). The near equivalence of aortic S_{O2} with both anterior and posterior vena caval S_{O_2} values early in deep dives (Fig. 8) supports the hypothesis of arterialization of anterior and posterior vena caval blood via a–v shunts prior to a deep dive. During the first minute of deep dives (Fig. 8; Figs S5 and S6), maintenance of high venous S_{O_2} values and low a–v O_2 content differences (<2 ml O_2 dl^{-1}) suggest continued a–v shunting and insignificant muscle blood flow at a time when stroke rate is highest during the dive. Otherwise, blood oxygen extraction by muscle should decrease venous S_{O_2}, especially in the anterior vena cava (due to flow of desaturated blood from fore-flipper locomotory muscles into the axillary vein and anterior vena cava).

As a deep dive progresses into the bottom phase of the dive, S_{O_2} profiles diverge with maintenance of S_{O_2} in the aorta and anterior vena cava and a progressive decline in S_{O_2} in the posterior vena cava (Fig. 8). By 3 min into a deep dive, the posterior vena caval a–v O_2 content difference is up to 20 ml O_2 dl^{-1}, while the anterior vena caval O_2 content difference is still only 2 ml O_2 dl^{-1} (Fig. S5). As already suggested (McDonald and Ponganis, 2013; Tift et al., 2017), we suspect that the severe bradycardia during the bottom phase of the dive results in extremely low influx of highly desaturated blood from the distal posterior vena cava into the central blood oxygen store. These low S_{O_2} values in the posterior vena cava are probably due to prolonged tissue transit time and almost complete blood oxygen extraction from the small volume of blood perfusing tissues in the posterior regions of the body. Such intense vasoconstriction in the posterior body during the severe bradycardia is, in part, secondary to the dense sympathetic nerve fiber innervation of the proximal segments of arteries supplying abdominal organs and the posterior body in pinnipeds (White et al., 1973). In contrast, in the anterior regions of the body, such innervation is sparse, the brain is still well perfused (Zapol et al., 1979) and some element of an a–v shunt may still exist. As discussed in previous paragraphs, these blood flow patterns could account for the maintenance of higher S_{O_2} values in the anterior vena cava at that time.

When heart rate increases as the dive progresses into the ascent phase, we suspect that the observed decreases in anterior vena caval S_{O_2} are due to influx of relatively desaturated blood from other tissues in the anterior region of the body (potentially even including muscle tissue). At the same time, increases in peripheral perfusion of the posterior regions of the body would actually increase S_{O_2} in the posterior vena cava because of the increase in peripheral perfusion and decrease in tissue transit time of more oxygenated

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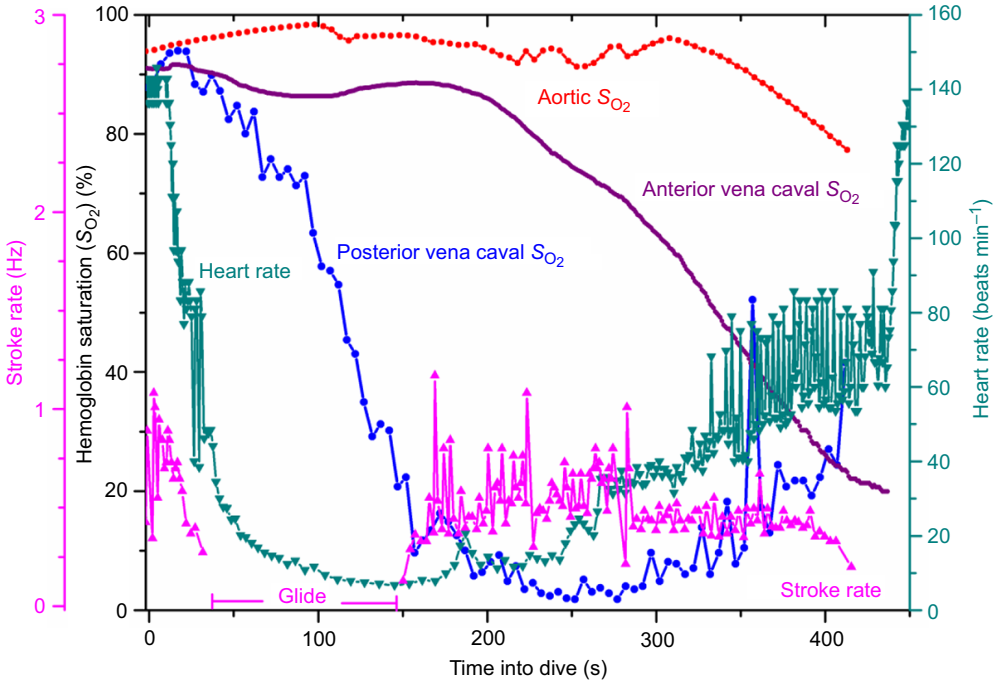

Fig. 8. Stroke rate profile, heart rate profile and hemoglobin (Hb) saturation (S_{O_2}) profiles (aorta, anterior vena cava and posterior vena cava) from deep (300 to 400 m) dives. These profiles are remarkable for prolonged gliding during descent to depth, extreme bradycardia at maximal depths, near equivalence of all S_{O_2} profiles at the start of the dive, maintenance of aortic S_{O_2} during the dive, and divergent patterns of venous S_{O_2} in the anterior and posterior venae cavae. See Discussion for review. The stroke rate and anterior vena caval S_{O_2} profiles were from a single dive of one sea lion in this study; the other profiles were from single dives of individual sea lions in prior studies (McDonald and Ponganis, 2013; McDonald and Ponganis, 2014).

blood from the anterior region of the animal that has mixed in with blood from the posterior portion of the body. This description provides a general overview and we suspect that there will be considerable variability in saturation profiles depending on circumstances and physiological responses of individual dives.

Venous anatomy and a possible central blood oxygen reservoir

Although adult sea lions have been reported to have large hepatic sinuses and even a modified posterior vena caval sling, these features are not as well developed as in phocid seals (King, 1977). We hypothesized that a–v shunting prior to a dive oxygenates venous blood, and that the hepatic sinus could serve as an important venous oxygen store in sea lions (Fig. 9). It was not likely to be as large as in phocids (Elsner et al., 1964), but it might be sufficient for the shorter dive durations and periods of lung collapse in sea lions. The volume of the hepatic sinus of the adult female California sea lion is typically 1 liter, maximally 2 liters (J. St. Leger, personal communication). Given that blood volume in an average 80 kg adult female California sea lion is 120 ml kg^{-1} (Weise and Costa, 2007), a 1–2 liter hepatic sinus would only store approximately 10 to 21% of total blood volume. Therefore, we set out to determine, with the use of shunt calculations, whether the reported size of the hepatic sinus and proposed central venous blood oxygen store was large enough to account for maintenance of arterial S_{O_2} during the period of lung collapse (see Materials and methods for review of shunt calculations).

Maintenance of arterial hemoglobin saturation during lung collapse

Despite the hypothesized central venous oxygen store in sea lions, it is still difficult to account for maintenance of arterial S_{O_2} values above 90% during the period of estimated lung collapse when gas exchange is presumed to not occur (Fig. 7B; Fig. S6). First, although we only have a limited amount of arterial and anterior vena caval data, the observed S_{O2} values in the anterior and posterior venae cavae at the start of the dive never reach levels high enough to create a central oxygen reservoir with greater than 95% saturation (Figs 7B and 8). Conceivably, splenic contraction during deep dives could elevate venous S_{O_2} and Hb content, but sea lion spleens are not especially large (Ponganis et al., 1992). Second, during periods of lung collapse in deep dives, the anterior vena caval S_{O_2} values do not remain high the entire time (Fig. S4). The S_{O_2} values can range from as high as 88% at the start of complete lung collapse to as low as 40% at the start of lung re-expansion.

With the mean anterior vena caval S_{O_2} of 83% at the start of complete lung collapse, an assumed hepatic sinus S_{O_2} of 95% (representing posterior vena caval inflow), an aortic S_{O_2} of 92% (McDonald and Ponganis, 2013) and a cardiac output of 840 ml min^{-1} at the start of complete lung collapse (based on a heart rate of 7 beats min^{-1} from Fig. 8), the shunt equation:

$$\text{Cardiac output} \times \text{fractional } S_{O_2a} = (\dot{Q}_{AVC} \times \text{fractional } S_{O_2AVC}) + [(\text{cardiac output} - \dot{Q}_{AVC}) \times \text{fractional } S_{O_2 hepaticsinus}], \quad (4)$$

results in an anterior vena caval flow of 208 ml min^{-1}, and hepatic sinus (posterior vena caval) flow of 632 ml min^{-1} into the heart (note: fractional S_{O_2a} is 0.92, corresponding to an aortic S_{O_2} of 92%, $\dot{Q}_{AVC}$ is anterior vena caval flow into the right atrium, fractional S_{O_2AVC} is 0.83, corresponding to 83% S_{O_2} in the anterior vena cava; cardiac output–$\dot{Q}_{AVC}$ is the posterior vena caval flow from the hepatic sinus into the right atrium, and fractional $S_{O_2 hepaticsinus}$ is 95%, the assumed S_{O_2} in the hepatic sinus).

Under these conditions, for maintenance of aortic S_{O_2} at 92% during 3 min of lack of gas exchange (complete lung collapse), the hepatic sinus would have to be at least 1.9 l in volume. Given that these conditions were optimal for maintenance of aortic S_{O_2} (low heart rate and high anterior vena caval S_{O_2}), it is unlikely that the hepatic sinus is large enough to contribute a sufficient volume of highly saturated blood to venous return in order to maintain high aortic S_{O_2} during 3 min of complete absence of gas exchange. Furthermore, any venous inflow from the coronary sinus, thebesian veins and bronchial veins into the heart would also decrease aortic S_{O_2}.

The decrease in anterior vena caval S_{O_2} and the increase in heart rate during the lung collapse period (Fig. 8) also reinforce the

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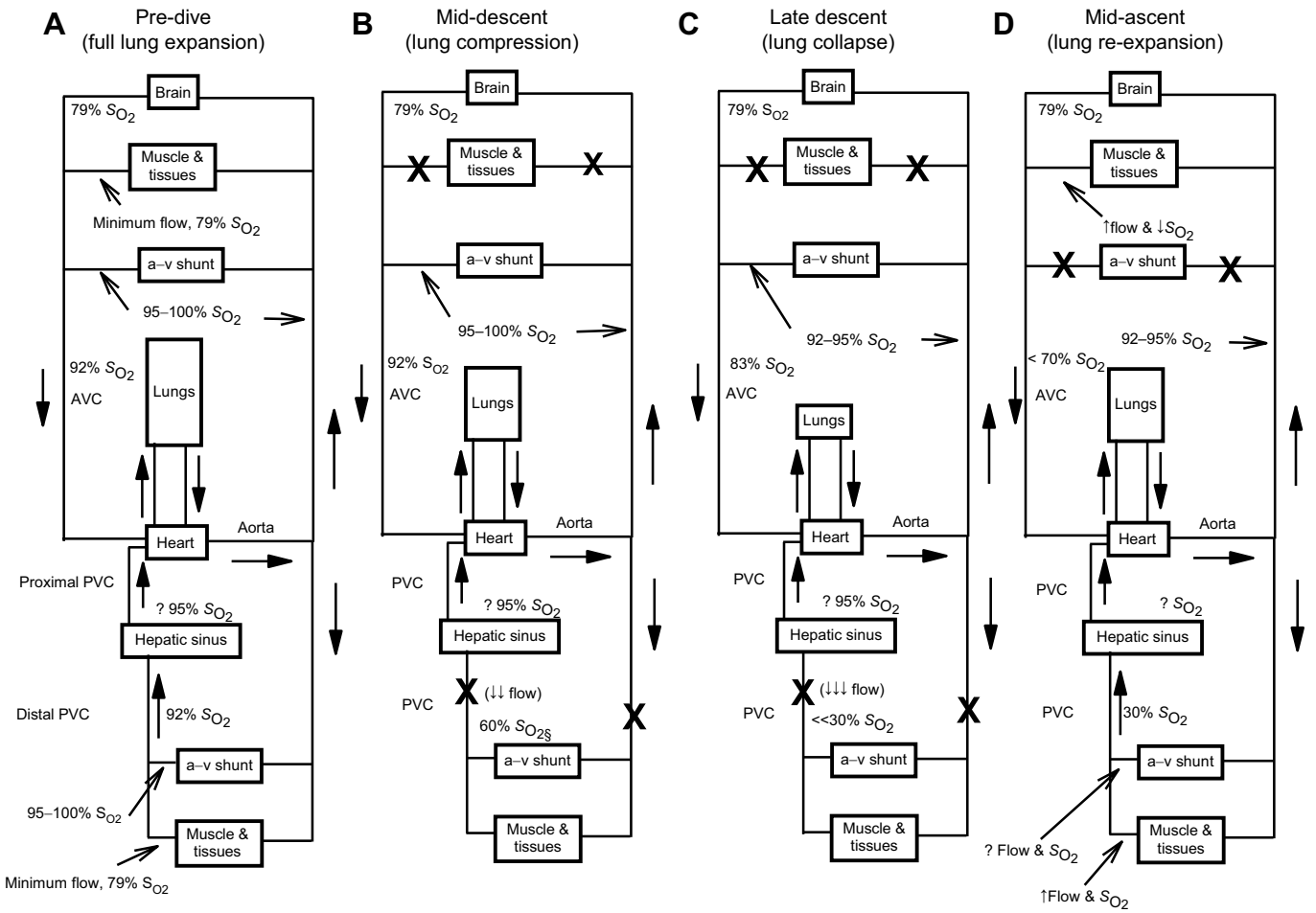

Fig. 9. Circulation patterns hypothesized to account for observed hemoglobin saturations (S_{O_2}) in a deep-diving California sea lion. (A) In the pre-dive state, with high heart rates and maximal ventilation, we postulate that high cardiac outputs contribute to a highly oxygenated central venous oxygen reservoir (in the hepatic sinus) through arteriovenous (a–v) shunting in both the hind- and fore-flippers, probably through the numerous a–v anastomoses in the flippers of sea lions (Bryden and Molyneux, 1978). The S_{O_2} values in the aorta, posterior vena cava (PVC) and anterior vena cava (AVC) were measured in this and prior studies (see Fig. 8), or for the brain circulation, the S_{O_2} was estimated as in the Discussion. Pre-dive flows in muscle that had already been re-oxygenated during the surface interval were assumed to be minimal and with typical venous S_{O_2} (Bangsbo and Hellsten, 1998; Walløe and Wesche, 1988). (B) As heart rate decreases and the lung compresses during early to mid-descent, we postulate that maintenance of brain blood flow and an a–v shunt in combination with a lack of significant muscle blood flow (indicated by X) contribute to the high saturation (92%) in the AVC. Although initial flow patterns are probably similar in the posterior body, as heart rate decreases further, distal arterial flow is severely constricted in proximal segments of arteries supplying abdominal organs and the posterior body owing to dense sympathetic innervation (White et al., 1973), thus accounting for the low S_{O_2} values of blood slowly entering the PVC. (C) During the severe bradycardia and vasoconstriction of late descent, we postulate that maintenance of brain blood flow and an anterior a–v shunt (probably via the fore-flipper) account for the high mean S_{O_2} (88%) in the AVC at the start of 'complete' lung collapse. At the same time, PVC S_{O_2} remains low. We postulate that hepatic sinus S_{O_2} remains high despite the low PVC S_{O_2} because there is little flow in the PVC during the severe bradycardia of this phase of the dive. The high AVC S_{O_2} in combination with highly saturated hepatic sinus blood was postulated to account for the maintenance of aortic S_{O_2} during lung collapse. However, as shown in Fig. 8, AVC S_{O_2} decreases and heart rate increases during the latter part of the period of lung collapse, making this hypothesis unlikely. Some gas exchange presumably still occurs during the lung collapse period in order to account for the high aortic S_{O_2}. (D) At the start of lung re-expansion, mean AVC S_{O_2} was 64%, and PVC S_{O_2} could be less than 10%. With the increase in heart rate during ascent (see Fig. 8), we postulate that the decline in AVC S_{O_2} is secondary to re-perfusion of tissues, including muscle, during the ascent as well as closure of the a–v shunt. At the same time, increased blood flow in the posterior body increases O_2 delivery and decreases tissue transit time, thus accounting for an increase in PVC S_{O_2}.

argument that the hepatic sinus is not large enough to maintain aortic S_{O_2}. At the start of lung re-expansion, mean anterior vena caval S_{O_2} was 64% and heart rate was near 33 beats min^{-1} (cardiac output=3.9 l min^{-1}). In order to maintain aortic S_{O_2} at 92% during those conditions, the above shunt calculation resulted in an anterior vena caval flow of 383 ml min^{-1} and hepatic sinus flow of 3577 ml min^{-1}. At this flow rate, the assumed 95% saturated blood in a 1-liter hepatic sinus would be emptied in approximately 15 s and be replaced with desaturated blood returning from the posterior vena cava. There is also no mechanism to account for an almost 10-fold greater return of blood to the heart from the posterior vena cava than from the anterior vena cava. In addition, even at the lower heart rates earlier in the lung collapse period, there would be some desaturated blood returning to the hepatic sinus and decreasing the assumed 95% value in the equation.

These calculations indicate that although a–v shunts may arterialize the venous blood and increase the magnitude of the total blood oxygen store, the size of the hepatic sinus in the sea lion is inadequate to maintain aortic saturation during a period of complete lung collapse and lack of gas exchange. How then does aortic S_{O_2} remain elevated above 90% during the presumed period of lung collapse? We suggest that some gas exchange may still occur

even at or below depths where lung collapse has been predicted to occur. These predictions of lung collapse have been based on the depths at which there were characteristic changes in the arterial P_{O_2} profile of deep-diving sea lions (McDonald and Ponganis, 2012). As originally pointed out in that study, although the changes in arterial P_{O_2} profile were consistent with a significant decrease in gas exchange, continuation of some gas exchange at great depths could not be ruled out. Resolution of this question requires further anatomical and physiological investigations.

Conclusions

Blood oxygen profiles in the anterior vena cava demonstrated that previously documented oxygen profiles in the posterior vena cava were not representative of the entire venous oxygen store. Similar to findings in the posterior vena cava, there was marked variation in anterior vena caval S_{O_2} profiles during shallow dives (<100 m in maximum depth). Notably, these shallow and short duration dives (1) often began before full re-saturation of the venous oxygen store, and (2) often had a net increase in venous S_{O_2} during the dive. During deep dives, although S_{O_2} values in both the anterior and posterior venae cavae were often >90% at the start of dives, the S_{O_2} profiles markedly differed between the two blood vessels as the dives progressed. Anterior vena caval S_{O_2} remained high until the bottom phase of the dive and then decreased during ascent, while posterior vena caval S_{O_2} declined markedly during descent and then often increased during ascent. These differences likely result from variations in peripheral blood flow patterns and tissue transit times of blood entering the respective venae cavae. We conclude that regulation of peripheral blood flow, tissue oxygen extraction and a–v shunting is complex and variable during dives of sea lions, and that mixed venous S_{O_2} measurements are needed to completely assess venous blood oxygen profiles and net oxygen depletion during dives.

Although our findings support the hypothesis of a highly oxygenated central venous oxygen reservoir at the start of deep dives, declines in anterior vena caval S_{O_2} and increases in heart rate during periods of lung collapse indicate that the hepatic sinus of the sea lion is not large enough to account for the maintenance of arterial S_{O_2} values greater than 90% during the period of lung collapse. Although arterial P_{O_2} profiles are indicative of periods of significantly decreased gas exchange during deep dives of sea lions (McDonald and Ponganis, 2012), our findings suggest that some gas exchange may still persist. Further investigations are needed to address this question.

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Competing interests

The authors declare no competing or financial interests.

Author contributions

Conceptualization: M.S.T., P.J.P.; Methodology: M.S.T., P.J.P.; Software: M.S.T., L.A.H., P.J.P.; Validation: M.S.T., L.A.H., P.J.P.; Formal analysis: M.S.T., L.A.H.; Investigation: M.S.T., L.A.H., P.J.P.; Resources: M.S.T., P.J.P.; Data curation: M.S.T.; Writing - original draft: M.S.T., P.J.P.; Writing - review & editing: M.S.T., L.A.H., P.J.P.; Visualization: M.S.T., P.J.P.; Supervision: M.S.T., P.J.P.; Project administration: M.S.T., P.J.P.; Funding acquisition: M.S.T., P.J.P.

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Supplementary information

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References

Bangsbo, J. and Hellsten, Y. (1998). Muscle blood flow and oxygen uptake in recovery from exercise. *Acta Physiol. Scand.* **162**, 305-312.
Blix, A. S., Elsner, R. and Kjekshus, J. K. (1983). Cardiac output and its distribution through capillaries and A-V shunts in diving seals. *Acta Physiol. Scand.* **118**, 109-116.
Blix, A. S., Walloe, L., Messelt, E. B. and Folkow, L. P. (2010). Selective brain cooling and its vascular basis in diving seals. *J. Exp. Biol.* **213**, 2610-2616.
Bryden, M. M. and Molyneux, G. S. (1978). Arteriovenous anastomoses in the skin of seals II. The California sea lion (*Zalopohus californianus*) and the northern fur seal (*Callorhinus ursinus*) (Pinnipedia: Otariidae). *Anat. Rec.* **191**, 253-260.
Davis, R. W. and Williams, T. M. (2012). The marine mammal dive response is exercise modulated to maximize aerobic dive duration. *J. Comp. Physiol. A* **198**, 583-591.
Dormer, K. J., Denn, M. J. and Stone, H. L. (1977). Cerebral blood flow in the sea lion (*Zalophus californianus*) during voluntary dives. *Comp. Biochem. Physiol. A Physiol.* **58**, 11-18.
Elsner, R. W., Scholander, P. F., Craig, A. B., Dimond, E. G., Irving, L., Pilson, M., Johansen, K. and Bradstreet, E. (1964). A venous blood oxygen reservoir in the diving elephant seal. *The Physiologist* **7**, 124.
Fahlman, A., Svard, C., Rosen, D. A. S., Jones, D. R. and Trites, A. W. (2008). Metabolic costs of foraging and management of O_2 and CO_2 stores in Steller sea lions. *J. Exp. Biol.* **211**, 3573-3580.
Feldkamp, S. D., Delong, R. L. and Antonelis, G. A. (1989). Diving patterns of California sea lions, *Zalophus californianus*. *Can. J Zool.* **67**, 872-883.
Fish, F. E., Hurley, J. and Costa, D. P. (2003). Maneuverability by the sea lion *Zalophus californianus*: turning performance of an unstable body design. *J. Exp. Biol.* **206**, 667-674.
Harrison, R. J. and Tomlinson, J. D. W. (1956). Observations on the venous system in certain Pinnipedia and Cetacea. *Proc. Zool. Soc. Lond.* **126**, 205-234.
Hindle, A. G., Young, B. L., Rosen, D. A. S., Haulena, M. and Trites, A. W. (2010). Dive response differs between shallow- and deep-diving Steller sea lions. *J. Exp. Mar. Biol. Ecol.* **394**, 141-148.
Jeanniard-Du-Dot, T., Trites, A. W., Arnould, J. P. Y., Speakman, J. R. and Guinet, C. (2016). Flipper strokes can predict energy expenditure and locomotion costs in free-ranging northern and Antarctic fur seals. *Sci. Rep.* **6**, 33912.
Kerem, D. and Elsner, R. (1973). Cerebral tolerance to asphyxial hypoxia in the harbor seal. *Respir. Physiol.* **19**, 188-200.
King, J. E. (1977). Comparative anatomy of the major blood vessels of the sea lion *Neophoca* and *Phocarctos*; with comments on the differences between the otariid and phocid vascular systems. *J. Zool.* **181**, 69-94.
King, J. E. (1983). *Seals of the World*. Ithaca, NY: Cornell University Press.
Kooyman, G. L. and Sinnett, E. E. (1982). Pulmonary shunts in harbor seals and sea lions during simulated dives to depth. *Physiol. Zool.* **55**, 105-111.
Kooyman, G. L., Wahrenbrock, E. A., Castellini, M. A., Davis, R. W. and Sinnett, E. E. (1980). Aerobic and anaerobic metabolism during voluntary diving in Weddell seals: evidence of preferred pathways from blood chemistry and behavior. *J. Comp. Physiol.* **138**, 335-346.
Kuhn, C. E. and Costa, D. P. (2014). Interannual variation in the at-sea behavior of California sea lions (*Zalophus californianus*). *Mar. Mammal Sci.* **30**, 1297-1319.
Macmillan, C. S. A. and Andrews, P. J. D. (2000). Cerebrovenous oxygen saturation monitoring: practical considerations and clinical relevance. *Intensive Care Med.* **26**, 1028-1036.
Mcdonald, B. I. and Ponganis, P. J. (2012). Lung collapse in the diving sea lion: hold the nitrogen and save the oxygen. *Biol. Lett.* **8**, 1047-1049.
Mcdonald, B. I. and Ponganis, P. J. (2013). Insights from venous oxygen profiles: oxygen utilization and management in diving California sea lions. *J. Exp. Biol.* **216**, 3332-3341.
Mcdonald, B. I. and Ponganis, P. J. (2014). Deep-diving sea lions exhibit extreme bradycardia in long-duration dives. *J. Exp. Biol.* **217**, 1525-1534.
Mchuron, E. A., Robinson, P. W., Simmons, S. E., Kuhn, C. E., Fowler, M. and Costa, D. P. (2016). Foraging strategies of a generalist marine predator inhabiting a dynamic environment. *Oecologia* **182**, 995-1005.
Montie, E. W., Pussini, N., Schneider, G. E., Battey, T. W. K., Dennison, S., Barakos, J. and Gulland, F. (2009). Neuroanatomy and volumes of brain structures of a live California sea lion (*Zalophus californianus*) from magnetic resonance images. *Anat. Rec.* **292**, 1523-1547.
Montie, E. W., Wheeler, E., Pussini, N., Battey, T. W. K., Barakos, J., Dennison, S., Colegrove, K. and Gulland, F. (2010). Magnetic resonance imaging quality and volumes of brain structures from live and postmortem imaging of California sea lions with clinical signs of domoic acid toxicosis. *Dis. Aquat. Organ.* **91**, 243-256.
Ponganis, P. J. (2015). *Diving Physiology of Marine Mammals and Seabirds*. Cornwall: Cambridge University Press.

Ponganis, P. J., Kooyman, G. L. and Zornow, M. H. (1991). Cardiac output in swimming California sea lions, Zalophus californianus. *Physiol. Zool.* **64**, 1296-1306.
Ponganis, P. J., Kooyman, G. L., Sartoris, D. and Jobsis, P. F. (1992). Pinniped splenic volumes. *Am. J. Physiol.* **262**, R322-R325.
Ponganis, P. J., Kooyman, G. L., Winter, L. M. and Starke, L. N. (1997). Heart rate and plasma lactate responses during submerged swimming and trained diving in California sea lions (*Zalophus californianus*). *J. Comp. Physiol. B* **167**, 9-16.
Ponganis, P. J., Stockard, T. K., Meir, J. U., Williams, C. L., Ponganis, K. V. and Howard, R. (2009). O_2 store management in diving emperor penguins. *J. Exp. Biol.* **212**, 217-224.
Qvist, J., Hill, R. D., Schneider, R. C., Falke, K. J., Liggins, G. C., Guppy, M., Elliot, R. L., Hochachka, P. W. and Zapol, W. M. (1986). Hemoglobin concentrations and blood gas tensions of free-diving Weddell seals. *J. Appl. Physiol.* **61**, 1560-1569.
Sato, K., Shiomi, K., Marshall, G., Kooyman, G. L. and Ponganis, P. J. (2011). Stroke rates and diving air volumes of emperor penguins: implications for dive performance. *J. Exp. Biol.* **214**, 2854-2863.
Schell, R. M. and Cole, D. J. (2000). Cerebral monitoring: jugular venous oxymetry. *Anesth. Analg.* **90**, 559-566.
Signore, P. E. and Jones, D. R. (1996). Autonomic nervous control of heart rate in muskrats during exercise in air and under water. *J. Exp. Biol.* **199**, 1563-1568.
Stockard, T. K., Heil, J., Meir, J. U., Sato, K., Ponganis, K. V. and Ponganis, P. J. (2005). Air sac P_{O2} and oxygen depletion during dives of emperor penguins. *J. Exp. Biol.* **208**, 2973-2981.
Tift, M. S., Hückstädt, L. A., Mcdonald, B. I., Thorson, P. H. and Ponganis, P. J. (2017). Flipper stroke rate and venous oxygen levels in free-ranging California sea lions. *J. Exp. Biol.* **220**, 1533-1540.
Walløe, L. and Wesche, J. (1988). Time course and magnitude of blood flow changes in the human quadriceps muscles during and following rhythmic exercise. *J. Physiol.* **405**, 257-273.
Weise, M. J. and Costa, D. P. (2007). Total body oxygen stores and physiological diving capacity of California sea lions as a function of sex and age. *J. Exp. Biol.* **210**, 278-289.
West, J. B. (1972). *Respiratory Physiology – the Essentials*. Baltimore, MD: Williams & Wilkins.
White, F. N., Ikeda, M. and Elsner, R. W. (1973). Andrenergic innervation of large arteries in the seal. *Comp. Gen. Physiol.* **4**, 271-276.
Williams, C. L., Meir, J. U. and Ponganis, P. J. (2011). What triggers the aerobic dive limit? Muscle oxygen depletion during dives of emperor penguins. *J. Exp. Biol.* **214**, 1801-1812.
Xu, F., Uh, J., Brier, M. R., Hart, J., Jr, Yezhuvath, U. S., Gu, J., Yang, Y. and Lu, H. (2011). The influence of carbon dioxide on brain activity and metabolism in conscious humans. *J. Cereb. Blood Flow Metab.* **31**, 58-67.
Zapol, W. M., Liggins, G. C., Schneider, R. C., Qvist, J., Snider, M. T., Creasy, R. K. and Hochachka, P. W. (1979). Regional blood flow during simulated diving in the conscious Weddell seal. *J. Appl. Physiol.* **47**, 968-973.
Zauner, A. and Muizelaar, J. P. (1997). Brain metabolism and cerebral blood flow. In *Head Injury* (ed. P. Reilly and R. Bullock), pp. 89-99. London: Chapman & Hall.

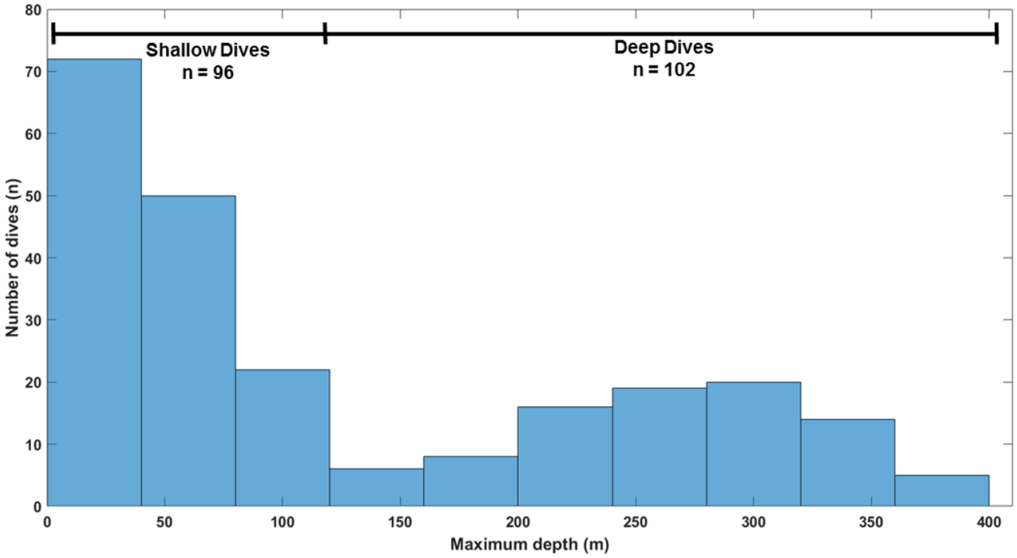

Fig. S1. Histogram of maximum dive depths from the sea lion in this study.

This bimodal pattern was also seen in the animals seen in Tift et al., 2017 from San Nicolas Island. For analysis, deep dives were characterized as maximum depths > 100m, and shallow dives were dives with maximum depths ≤ 100 m.

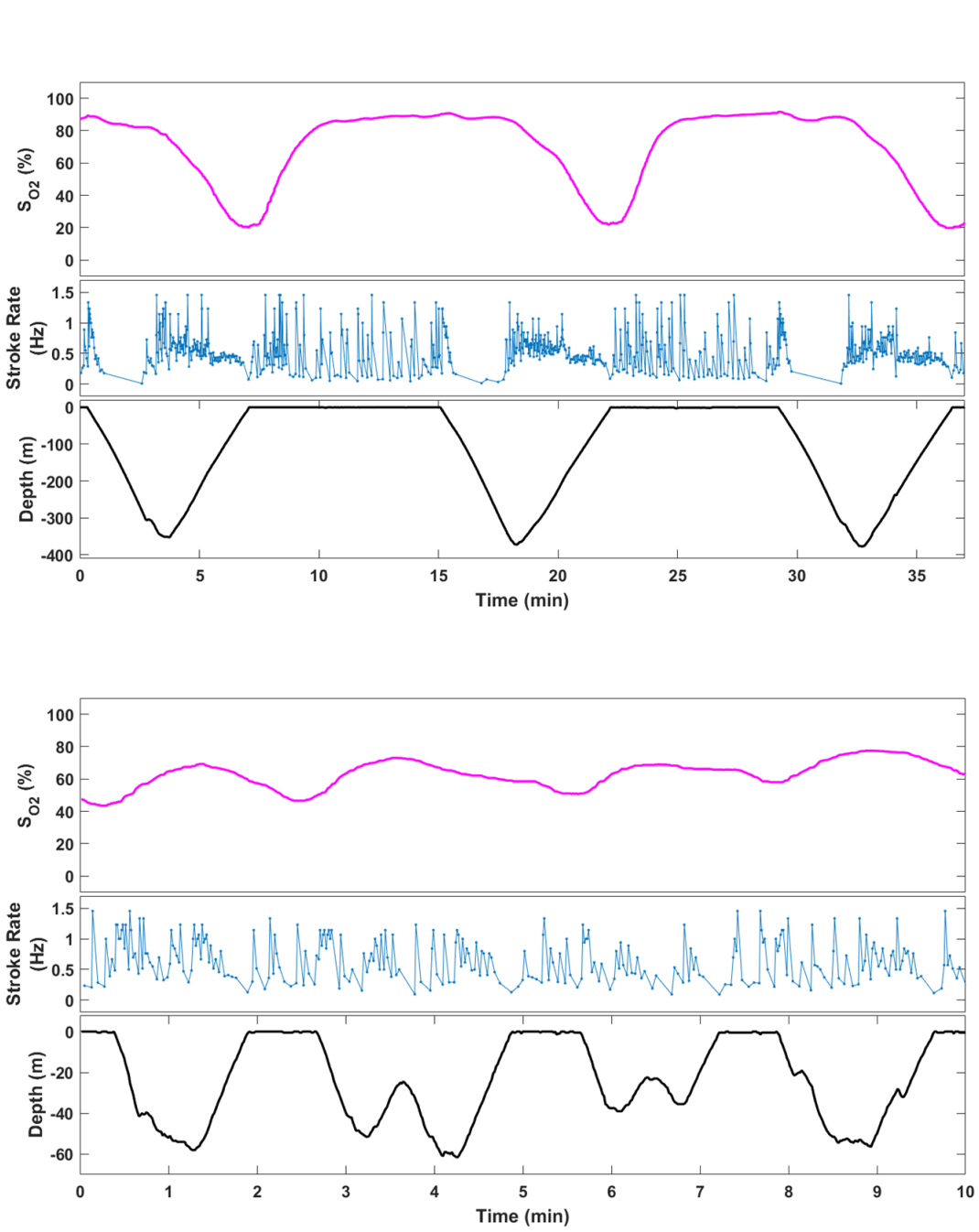

Fig. S2. Anterior vena caval hemoglobin saturation (S_{O2}), flipper stroke rate, and dive depth for a series of deep (>100m, A) and shallow (≤ 100m, B) dives from a free-ranging California sea lion. Notice the maintenance of S_{O2} through approximately half the duration of the deep dives (A) until flipper stroke rates increase near the bottom of the dive. Also

notice the inconsistent patterns in S_{O2} during shallow dives (B), with values both increasing and decreasing throughout different dives.

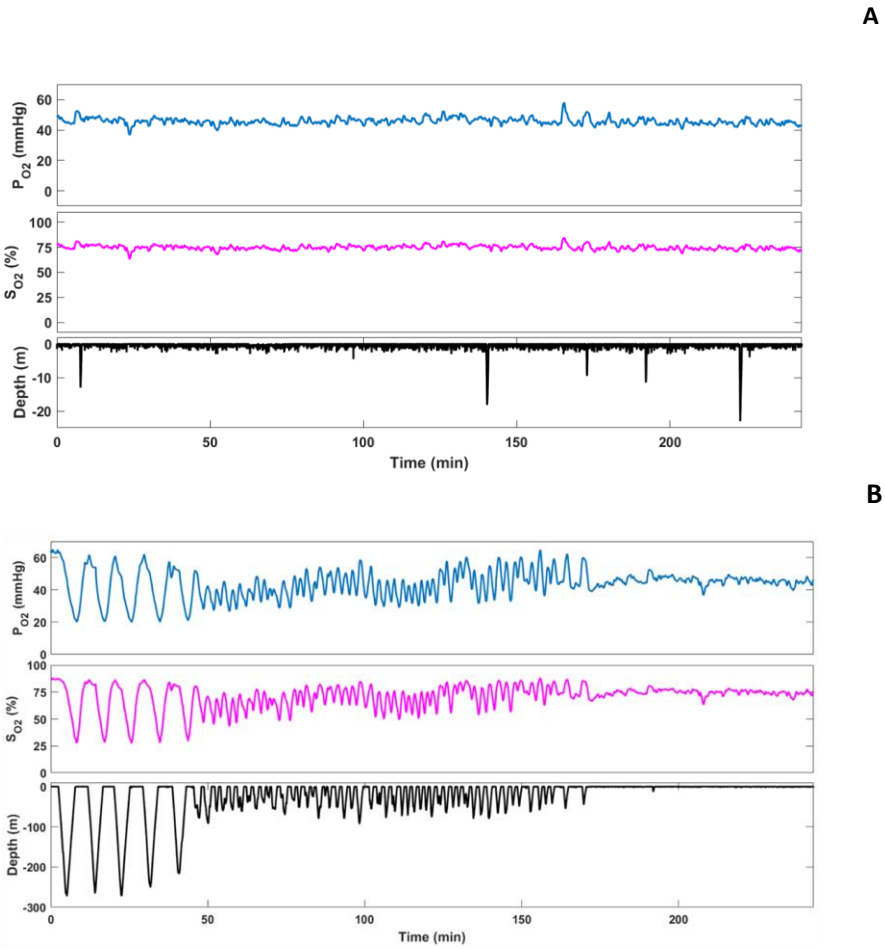

Fig. S3. Surface P_{O2} and S_{O2} profiles at rest and between/after deep dives.

A. A period of 300 min when the animal was resting at the surface and performing very shallow dives (< 20m) reveals surface resting S_{O2} and P_{O2} values. Note the surface resting S_{O2} of 75% is near the on-land resting values of 78% from McDonald and Ponganis (2013). These saturations are consistent with an arteriovenous oxygen content difference of about 5 ml O_2 $dl^{-1}$when the sea

lion is at rest. **B.** The record shows patterns in blood oxygen for deep dives, shallow dives, and a long surface period. Note the mean S_{O2} for the long surface period is ~75%, which is consistent with the estimated resting venous S_{O2} value (78%) from an animal on land from McDonald and Ponganis (2013). Note 1 mm Hg = 0.133 kPa.

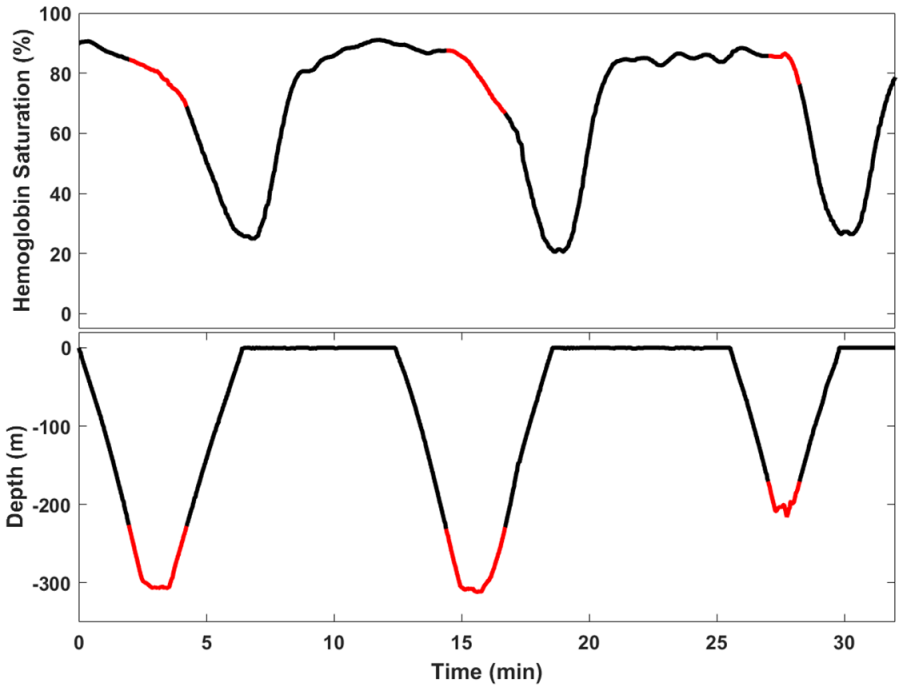

Fig. S4. Anterior vena caval hemoglobin saturation (S_{O2}) and dive depth for 3 deep dives from a free-ranging California sea lion, with periods of estimated lung collapse highlighted in red. The equation for the estimation of lung collapse was obtained from McDonald and Ponganis (2012). Desaturation of anterior vena cava blood during the lung collapse period makes it difficult to account for the maintenance of high arterial S_{O2} during this phase of the dive.

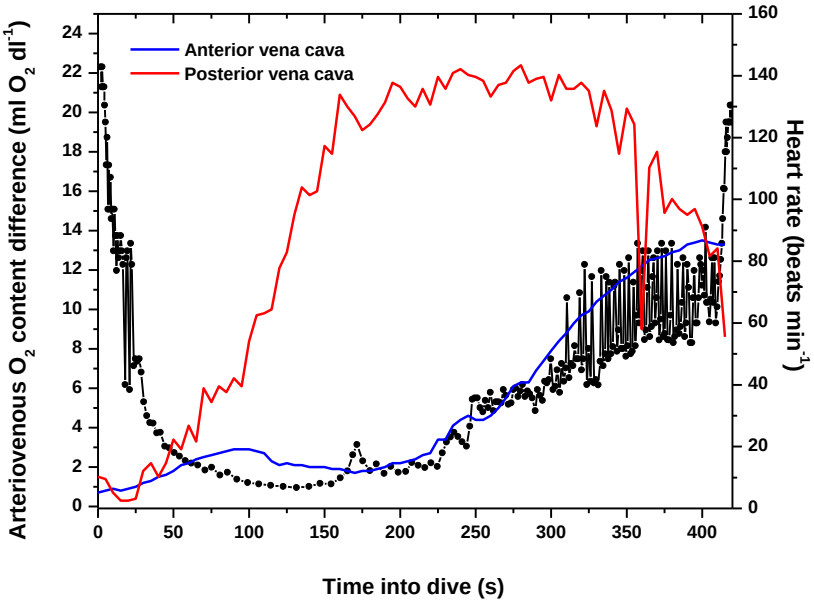

Fig. S5. Heart rate and arteriovenous (a-v) oxygen content difference profiles in the anterior and posterior venae cavae during representative deep dives. The oxygen content differences were calculated from the data in Figure 8. The extremely low a-v oxygen content differences in both venae cavae early in the dive support the hypotheses of minimal muscle blood flow and possible a-v shunting during this phase of the dive. As the dive progresses and the diving bradycardia intensifies during descent, it is postulated that decreased peripheral blood flow in the posterior body and increased tissue transit time allow for increased blood oxygen extraction by tissues, resulting in decreased posterior vena caval hemoglobin saturation (S_{O2}) and a widened a-v oxygen content difference. In contrast, in the anterior vena cava during the descent phase of the dive, it is postulated that continued cerebral venous drainage and foreflipper a-v shunting continue during the bradycardia, maintain S_{O2}, and minimize the a-v oxygen content difference. During the latter phases of the dive, it is postulated that increased heart rate and peripheral perfusion contribute to a decrease in anterior vena caval S_{O2} and an increase in the a-v oxygen content difference. Increased blood flow during ascent in the posterior

regions of the body (previously severely ischemic during descent) is hypothesized to result in an increase in posterior vena caval S_{O2} and a decrease in the posterior vena caval oxygen content difference.

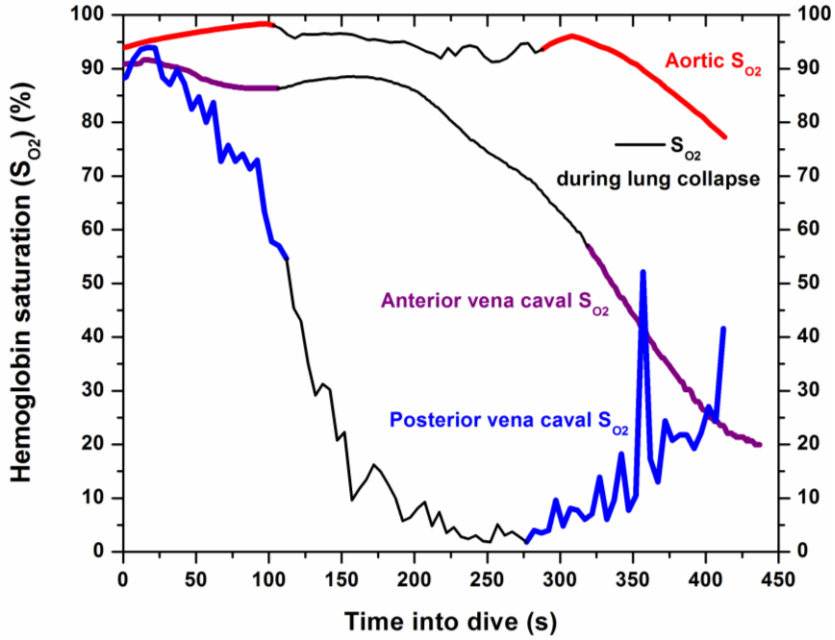

Fig. S6. Hemoglobin saturation (S_{O2}) profiles in the aorta, anterior vena cava and posterior vena cava of three sea lions performing dives to 310 m, 377 m, and 310 m, respectively. The declines in anterior vena caval and posterior vena caval S_{O2} make it difficult to account for maintenance of arterial S_{O2} values > 90% during the latter part of the lung collapse period even with a well-oxygenated central venous oxygen reservoir. Each profile is from a single dive of a given sea lion. Data sources are this study and a prior study (McDonald and Ponganis, 2013). Periods of lung collapse during each dive vary dependent on the dive profile and maximum dive depth of the individual dive.