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RESEARCH ARTICLE

Insights from venous oxygen profiles: oxygen utilization and management in diving California sea lions

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SUMMARY

The management and depletion of O_2 stores underlie the aerobic dive capacities of marine mammals. The California sea lion (*Zalophus californianus*) presumably optimizes O_2 store management during all dives, but approaches its physiological limits during deep dives to greater than 300 m depth. Blood O_2 comprises the largest component of total body O_2 stores in adult sea lions. Therefore, we investigated venous blood O_2 depletion during dives of California sea lions during maternal foraging trips to sea by: (1) recording venous partial pressure of O_2 (P_{O_2}) profiles during dives, (2) characterizing the O_2–hemoglobin (Hb) dissociation curve of sea lion Hb and (3) converting the P_{O_2} profiles into percent Hb saturation (S_{O_2}) profiles using the dissociation curve. The O_2–Hb dissociation curve was typical of other pinnipeds (P_{50}=28±2 mmHg at pH 7.4). In 43% of dives, initial venous S_{O2} values were greater than 78% (estimated resting venous S_{O_2}), indicative of arterialization of venous blood. Blood O_2 was far from depleted during routine shallow dives, with minimum venous S_{O_2} values routinely greater than 50%. However, in deep dives greater than 4 min in duration, venous S_{O_2} reached minimum values below 5% prior to the end of the dive, but then increased during the last 30–60 s of ascent. These deep dive profiles were consistent with transient venous blood O_2 depletion followed by partial restoration of venous O_2 through pulmonary gas exchange and peripheral blood flow during ascent. These differences in venous O_2 profiles between shallow and deep dives of sea lions reflect distinct strategies of O_2 store management and suggest that underlying cardiovascular responses will also differ.

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Key words: blood oxygen depletion, dive, hemoglobin saturation, oxygen–hemoglobin dissociation curve, P_{O_2}, P_{50}.

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INTRODUCTION

The pattern and magnitude of depletion of elevated body O_2 stores underlie the dive capacity, feeding behavior and foraging ecology of marine mammals. The California sea lion [*Zalophus californianus* (Lesson 1828)] is an excellent model species for investigation of O_2 store depletion in an otariid (sea lions and fur seals) because both its O_2 stores and dive behavior have been extensively studied (Feldkamp et al., 1989; Kuhn, 2006; Weise and Costa, 2007). Forty-three percent of the 52 ml O_2 kg^{-1} total body O_2 store in adult females is found in the blood (Weise and Costa, 2007). Consequently, investigation of blood O_2 depletion is especially crucial if we are to understand the physiological limits of their routine 3–5 min dives and the physiological strategies used in longer dives up to 14 min in duration (Feldkamp et al., 1989; Kuhn, 2006).

Venous blood O_2 depletion has been examined in two freely diving species, the emperor penguin (*Aptenodytes forsteri*) and the northern elephant seal (*Mirounga angustirostris*), both considered to be extreme breath-hold divers (Ponganis et al., 2007; Meir et al., 2009; Meir and Ponganis, 2009). The ability of the penguin and elephant seal to perform such long dives was attributed to extreme hypoxemic tolerance and near-complete depletion of the blood O_2 stores (Ponganis et al., 2007; Meir et al., 2009; Meir and Ponganis, 2009). During dives that exceeded 6 min in emperor penguins and 15 min in elephant seals, venous partial pressure of O_2 (P_{O_2}) often reached values less than 10 mmHg [1.3 kPa, near 0% hemoglobin (Hb) saturation (S_{O_2})] in both species (Meir et al., 2009; Meir and Ponganis, 2009). Although both emperor penguins and elephant seals deplete venous blood O_2 to low values, the depletion patterns exhibited by emperor penguins were more variable (Ponganis et al., 2007; Meir and Ponganis, 2009). For example, at the 5.6 min aerobic dive limit (ADL; the dive duration associated with the onset of post-dive blood lactate accumulation) of emperor penguins (Ponganis et al., 1997b), end-of-dive S_{O_2} ranged from ~5 to 85% saturation (Meir and Ponganis, 2009). In contrast, for dive durations of 15 min (estimated ADL for juvenile elephant seals based on the ADL from the similar sized juvenile Weddell seal) (Kooyman et al., 1983), end-of-dive S_{O_2} ranged from 0 to 30% (Meir et al., 2009). Such differences are possibly attributed to differences in: (1) the relative size of the respiratory O_2 store (4% in the elephant seal *versus* 33% in the emperor penguin) (Kooyman and Ponganis, 1998; Sato et al., 2011), (2) heart rate regulation (degree and pattern of diving bradycardia), (3) pulmonary blood flow/gas exchange early in the dive and (4) peripheral organ perfusion, severity of peripheral vasoconstriction and degree of arterio-venous (a-v) shunting.

This study took advantage of the recurrent 1–10 day maternal foraging trips of California sea lions to measure venous blood O_2 depletion by utilizing a backpack P_{O_2} recorder to document venous P_{O_2} profiles during the routine dives of these animals at sea. The goals of this research were to: (1) obtain venous P_{O_2} profiles from freely diving animals, (2) characterize the sea lion O_2–Hb

dissociation curve and apply it to convert P_{O_2} to S_{O_2} and (3) calculate the rate and magnitude of venous blood O_2 store depletion during both shallow and deep dives at sea.

We hypothesized that: (1) the O_2–Hb dissociation curve, the P_{50} and the Bohr shift would be similar to those of other marine mammals; (2) venous blood O_2 depletion would be incomplete even in long-duration dives of sea lions because of the contribution of respiratory O_2 to blood and the probable isolation of muscle from the circulation due to slower heart rates during such dives (Ponganis et al., 1997b); (3) minimum venous P_{O_2}, S_{O_2} and the magnitude of O_2 depletion would be negatively related to dive duration; and (4) depletion patterns would be highly variable in short dives (<3 min) as seen in emperor penguins due to the contribution of lung O_2 stores [16% of total body O_2 stores (Weise and Costa, 2007)], especially at shallow depths.

MATERIALS AND METHODS

O_2–Hb dissociation curve determination

O_2–Hb dissociation curves were determined on fresh whole blood using the mixing technique at 37°C (Scheid and Meyer, 1978). Blood (30–60 ml) was collected in heparinized blood tubes from stranded adult sea lions rehabilitated at Sea World San Diego (N=7) and from healthy neutered male adult sea lions maintained by the US Navy Marine Mammal Program in San Diego, CA (N=4), during routine health procedures. Samples were placed on ice during transport. Analyses began immediately upon arrival to the laboratory (~30–60 min post collection) and were completed within 8 h of collection to prevent depletion of labile organic phosphates. Blood was tonometered (tonometer 237; Instrumentation Laboratory, Bedford, MA, USA) to create 0% saturated and 100% saturated blood at the desired pH (7.2, 7.3, 7.4 and 7.5) using an appropriate mix of N_2, O_2 and CO_2. Desaturated and 100% saturated blood was mixed to obtain S_{O_2} at various points along the curve (10, 20, 50, 70, 90 and 95% S_{O_2}) and the P_{O_2} of the mix was then determined using an i-STAT blood gas analyzer (Abbott Point of Care, Princeton, NJ, USA). The use of the i-STAT also allowed verification of pH. The $\log[S_{O_2}/(100–S_{O_2})]$ *versus* $\log(P_{O_2})$ relationship was plotted, and linear regression analysis was performed to determine the equation of the O_2–Hb dissociation curve at pH 7.2, 7.3, 7.4 and 7.5. Because of the limited quantities of blood, curves were not determined at each pH for all sea lions, but when possible additional P_{50} values were obtained at each pH. The CO_2 Bohr coefficient was determined from the linear regression of $\log(P_{50})$ on pH (averaged from all P_{50} values at pH 7.2, 7.3 and 7.4) (Meir et al., 2009). The equipment and procedure used in this study were validated by determining the P_{50} in species with previously published O_2–Hb binding data (rat, sheep and California sea lion).

Animal handling and instrumentation

The field study was conducted at San Nicolas Island, CA (22°14′12.3″N, 119°32′54.3″W), during August 2010 and 2011. Adult female California sea lions were captured using customized hoop nets and only lactating females (determined by manual expression of milk from the teat) were selected for instrumentation to increase the likelihood of recovering instruments. Females were anesthetized with isoflurane gas with O_2 using a portable field vaporizer anesthesia circuit (Gales and Mattlin, 1998). After mask induction and intubation on 5% isoflurane-O_2, anesthesia was maintained with 1–2% isoflurane.

Eleven females were instrumented with custom-built P_{O_2} data loggers, time depth recorders (TDRs) and radio transmitters. With ultrasound guidance over the caudal back, a P_{O_2} electrode (model Licox C1.1 Revoxode; Integra Life Sciences, Plainsboro, NJ, USA) and thermistor (model 554; Yellow Springs Instruments, Yellow Springs, OH, USA) were placed percutaneously through a Peel-Away catheter (5 Fr; Cook Medical, Bloomington, IN, USA) into the vena cava *via* the caudal gluteal vein. Catheterization, P_{O_2} electrode and thermistor procedures have been described previously (Ponganis et al., 1991; Ponganis et al., 1997a; Stockard et al., 2005; Ponganis et al., 2007; Meir et al., 2009). The P_{O_2} electrode and thermistor were connected to a custom-built microprocessor (3991 BioLog or UUB BioLog; UFI, Morro Bay, CA, USA) in a waterproof housing (5×17 cm, 570 g or 3.2×11.4 cm, 200 g; Meer Instruments, Palomar Mountain, CA, USA) that was mounted midline above the hips with 5 min epoxy (Loctite; Henkel Corp., Westlake, OH, USA). The logger recorded P_{O_2} and temperature every 1 s (two deployments), 5 s (eight deployments) or 15 s (one deployment) depending on the specific data logger used. An Mk9 TDR (Wildlife Computers, Redmond, WA, USA; 6.7×1.7×1.7 cm, 30 g, 1 s sampling interval, ±0.5 m resolution) and a radio transmitter (mm160B, 2.0×5.6 cm, 25 g; ATS, Isanti, MN, USA) were attached in front of the P_{O_2} logger. The time on the TDR and P_{O_2} logger were synchronized to the same internet-synced computer clock.

After instrumentation, females were weighed (±0.2 kg, MSI-7200 Dyna-link; Measurement Systems International, Seattle, WA, USA) and placed in a kennel to recover from anesthesia (25–60 min). Once recovered, females were released. After one to four trips to sea, females were recaptured for instrument recovery. Instruments were removed while the sea lion was manually restrained (entire capture ~10 min). All procedures were approved under a University of California, San Diego Animals Subjects Committee permit (no. S11303) and a National Marine Fisheries Service marine mammal permit (no. 14676).

S_{O_2} and blood O_2 store depletion calculations

We obtained S_{O_2} values by applying the linear regression equation determined by the dissociation curve analysis to the P_{O_2} data collected by the data loggers and solving for S_{O_2}. Initial, maximum, minimum and end-of-dive S_{O_2} were estimated using the equation at pH 7.4 for most dives, but for dives greater than 3 min in duration, the minimum and end-of-dive S_{O_2} were estimated using the equation at pH 7.3. Although pH can shift the dissociation curve, previous work suggests that there is minimal change in pH even in forced dives of 30 min in Weddell seals (7.4 to 7.28) (Elsner et al., 1970). Therefore, we assumed little change in pH in the routine dives (<3 min) of sea lions. To be conservative, in dives longer than the calculated ADL (cADL; estimated ADL calculated from total body O_2 stores and metabolic rate) (Weise and Costa, 2007), the O_2 content at the end of dives >3 min was determined assuming a pH of 7.3.

Initial, maximum, minimum and end-of-dive O_2 content (ml O_2 dl^{-1} blood) were calculated from the corresponding P_{O_2} and S_{O_2} values from the equation:

$$O_2 \text{ content} = O_2 \text{ binding capacity of Hb} \times \text{Hb concentration} \times \left(S_{O_2} + (0.003 \times P_{O_2})\right), \quad (1)$$

using the O_2 binding capacity of Hb=1.34 ml O_2 g^{-1} Hb and Hb concentration=18 g dl^{-1} (Weise and Costa, 2007). The rate of O_2 depletion (ml O_2 dl^{-1} min^{-1}) was calculated using the equation:

$$O_2 \text{ depletion rate} = \frac{\text{Maximum } O_2 \text{ content} - \text{Minimum } O_2 \text{ content}}{\text{Time}}, \quad (2)$$

where Time is the duration between the maximum and minimum O_2 content measurements (min). Percent of the total venous O_2 content depleted during a dive was calculated from the equation:

$$\%\ O_2\ \text{content depleted} = \frac{\text{Maximum } O_2\ \text{content} - \text{Minimum } O_2\ \text{content}}{\text{Maximum } O_2\ \text{content}}. \quad (3)$$

Data processing and statistics

Prior to deployment, P_{O_2} electrodes and thermistors were calibrated in the laboratory as previously described (Stockard et al., 2005; Ponganis et al., 2007). Thermistor connections frequently broke during the deployment so we were unable to correct electrode output for P_{O_2} data using *in vivo* temperatures (Stockard et al., 2005). However, continuous venous temperature profiles were successfully collected from three sea lions while at sea for a cumulative 165 h, including 2803 dives, surface intervals and subsurface swimming. Average temperature was 36.8±0.6°C while at sea (range 33.4–39.0°C, 93% of values were within 1°C of 37°C); therefore, we assumed that body temperature was 37°C when calculating P_{O_2} and S_{O_2}. Although not ideal, this assumption would result in very small effects on P_{O_2}. For example, at a P_{O_2} of 60 mmHg (8.0 kPa), a ±1°C *in vivo* temperature difference from 37°C would result in only a 2 mmHg (0.3 kPa) difference (1% S_{O_2}). At low P_{O_2} values, the effect would be even smaller; e.g. the difference would only be 0.2 mmHg (0.1% S_{O_2}) at a P_{O_2} of 5 mmHg (0.7 kPa).

TDR data were analyzed in MATLAB (The MathWorks, Natick, MA, USA) using a custom-written dive analysis program (IKNOS; Y. Tremblay), which calculates a zero offset correction at the surface and identifies dives on the basis of a minimum depth and duration. The minimum depth for defining a dive was set at 5 m and the minimum duration was 20 s.

P_{O_2} and TDR logger clock drifts were documented pre-deployment and, when possible, post-deployment, to allow data synchronization. A custom-written MATLAB code was used to obtain initial (P_{O_2} value before dive starts), maximum, minimum and end-of-dive P_{O_2} (and S_{O_2}) and time to minimum P_{O_2} for every dive greater than 1 min in duration. Data were visually inspected to confirm processed results.

The relationship between dive duration and initial P_{O_2}, maximum P_{O_2}, minimum P_{O_2} and percent O_2 depleted during the dive were investigated using linear mixed effects models (Cran R 2.12.2, package nlme). Dives less than 2 min in duration were excluded from analysis due to the high variability of values in those dives. Dive duration was the fixed effect in all models, and to account for the lack of independence caused by repeatedly sampling the same individual over time, individual (sea lion ID) was included as a random effect. P_{O_2} data were log transformed and the proportion of O_2 depleted during a dive was arcsine transformed before analysis to meet model assumptions. Covariance and random effect structures of the full models were evaluated using Akaike's information criterion (AIC) and examination of residual plots (Zuur et al., 2009). AICs from all tested models are presented with the best model highlighted in bold.

RESULTS

O_2–Hb dissociation curve

Complete O_2–Hb dissociation curves were determined at pH 7.2 (N=3 sea lions), 7.3 (N=6 sea lions), 7.4 (N=7 sea lions) and 7.5 (N=1 sea lion), with additional P_{50} values determined at each pH (Fig. 1). The P_{50} at pH 7.4 was 28±2 mmHg (3.7±0.3 kPa; N=11 sea lions) and the Bohr effect was −0.57 (y=–0.57x+5.68, r^2=1.0, P=0.038). The regression equations from the $\log[S_{O_2}/(100–S_{O_2})]$ *versus* $\log(P_{O_2})$ plots including all saturation points for all sea lions combined were:

pH 7.2: $\log[S_{O_2}/(100–S_{O_2})] = 2.287 \times \log(P_{O_2}) – 3.515$ (N=26, r^2=0.98, P<0.001);

pH 7.3: $\log[S_{O_2}/(100–S_{O_2})] = 2.363 \times \log(P_{O_2}) – 3.576$ (N=48, r^2=0.96, P<0.001);

pH 7.4: $\log[S_{O_2}/(100–S_{O_2})] = 2.473 \times \log(P_{O_2}) – 3.632$ (N=44, r^2=0.97, P<0.001);

pH 7.5: $\log[S_{O_2}/(100–S_{O_2})] = 2.339 \times \log(P_{O_2}) – 3.174$ (N=6, r^2=0.99, P<0.001).

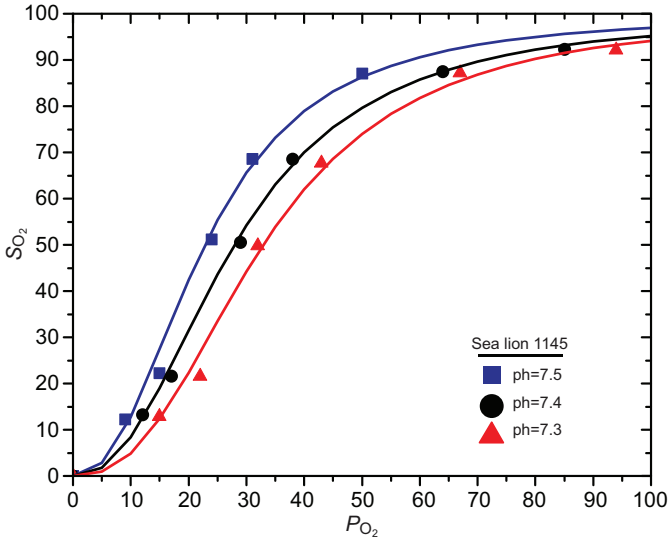

Fig. 1. O_2–Hb dissociation curves from one sea lion at pH 7.3, 7.4 and 7.5.

P_{O_2} and S_{O_2} profiles and blood O_2 depletion

P_{O_2} data were successfully obtained from eight females with trip durations ranging from 0.8 to 8.9 days (data loggers from one sea lion were not recovered and two deployments failed due to electrode failure before diving commenced). All but the two females with the shortest trip durations exhibited diving behavior typical of foraging trips (one of which did not perform any dives greater than 2 min in duration or 20 m in depth) (Feldkamp et al., 1989; Kuhn, 2006); however, an abnormal short trip following capture is not unusual in sea lions. The range in the number of profiles analyzed for each sea lion was secondary to trip duration, diving behavior and time until electrode failure (Table 1). We obtained 2596 venous P_{O_2} diving profiles from dives greater than 1 min in duration. Median dive duration was 1.7 min (range 1.0–8.1 min) and median depth was 29 m (range 5–377 m). Dive duration and depth data for each female exhibiting dives greater than 2 min in duration are given in Table 1 (seven females, 926 dives).

Blood O_2 depletion patterns differed between shallow dives and deep dives. In short-duration, shallow dives (<3 min, N=2252), P_{O_2} (and S_{O_2}) depletion patterns were highly variable, and venous blood O_2 was not usually depleted [minimum P_{O_2} ≥30 mmHg (4 kPa; S_{O_2} ≥50%) in 60.7% of dives; Fig. 2, Fig. 3A,C]. P_{O_2} (and S_{O_2}) typically increased during the initial part of the dive before decreasing until the end of the dive. In dives greater than 3 min in duration, there were two typical P_{O_2} profile shapes. The first profile type was similar in shape to the short, shallow dive profiles, with an initial increase before decreasing throughout most of the dive, but with lower minimum values than observed in short, shallow dives. However,

Table 1. Individual dive, P_{O_2} and S_{O_2} data for all dives greater than 2 min in duration (926 dives)

Sea lion	No. dives	Mass (kg)	Sampling interval (s)	Dive duration (min)	Dive depth (m)	Initial P_{O_2} (mmHg)	Initial S_{O_2} (%)	Max. P_{O_2} (mmHg)	Max. S_{O_2} (%)	Min. P_{O_2} (mmHg)	Min. S_{O_2} (%)	Depletion rate (ml O_2 dl^{-1} min^{-1})	% O_2 content depleted
Artemis	124	87.2	1	3.5±1.4	111±110	34±6	57.3±11.1	38±6	63.5±10.1	22±7	30.5±15.1	3.42±1.42	50.9±23.9
				3.0	51	34	59.1	39	66.5	23	33.4	3.07	45.3
				(2.0–8.1)	(16–377)	(15–49)	(15.0–77.5)	(19–56)	(24.8–82.9)	(4–33)	(0.9–56.4)	(0.94–8.94)	(18.5–98.6)
Athena	223	87.6	5	3.9±1.5	149±106	75±9	90.8±2.0	108±20	95.7±1.7	27±22	35.7±36.5	5.17±2.56	62.4±38.5
				4.2	189	74	90.9	106	96.0	13	10.1	5.85	89.7
				(2.0–7.4)	(10–332)	(51–136)	(79.5–97.8)	(74–173)	(90.7–98.8)	(5–72)	(1.4–90.2)	(0.80–15.17)	(2.6–98.5)
Persephone	301	75.4	5	2.9±0.7	88±47	52±8	78.8±6.9	66±13	86.7±6.0	29±13	42.6±23.4	5.05±2.23	51.2±25.5
				2.8	92	51	79.9	65	89.8	24	35.9	5.22	56.6
				(2.0–6.0)	(19–273)	(25–82)	(40.3–92.7)	(29–127)	(49.1–97.4)	(8–63)	(3.9–86.6)	(0.59–16.24)	(2.7–95.4)
Aura	59	65.0	1	2.9±1.0	54±59	33±8	55.8±15.1	37±9	61.8±15.3	23±7	33.6±15.3	3.57±1.84	46.5±19.4
				2.6	29	34	59.4	40	67.7	23	33.1	3.07	41.8
				(2.0–6.3)	(16–309)	(16–53)	(19.2–81.3)	(18–55)	(23.9–82.3)	(7–33)	(2.6–57.4)	(1.62–13.70)	(21.8–94.1)
Atalanta	162	91.0	5	2.6±0.5	49±36	42±5	70.2±7.0	47±6	75.7±5.7	34±6	57.3±10.7	2.52±1.27	24.3±12.9
				2.5	33	42	71.3	47	76.5	35	59.7	2.30	20.6
				(2.0–5.0)	(16–178)	(28–57)	(46.1–83.9)	(30–59)	(52.2–84.9)	(21–46)	(29.6–75.1)	(0.68–8.79)	(5.3–61.5)
Aphrodite	49	72.0	5	2.2±0.2	33±21	47±7	74.7±7.1	55±7	81.4±5.4	28±7	45.9±14.3	5.40±1.84	43.8±16.4
				2.1	26	47	76.1	56	83.1	28	47.0	4.87	40.9
				(2.0–2.7)	(8–108)	(31–67)	(53.2–85.4)	(36–71)	(62.2–89.8)	(13–39)	(11.7–66.8)	(2.46–10.79)	(18.0–85.0)
Amphitrite	8	83.0	15	2.4±0.3	30±30	32±5	55.2±8.0	35±3	59.4±75.4	22±1	31.6±3.0	4.03±0.70	46.6±5.4
				2.3	19	32	54.2	34	58.9	22	32.8	3.86	45.0
				(2.1–3.1)	(17–104)	(28–43)	(47.0–71.9)	(31–42)	(53.2–70.7)	(19–23)	(25.3–35.2)	(3.18–5.40)	(40.1–56.8)
Grand mean ± s.d.				3.1±1.2	93±83	52±17	75.4±14.0	67±29	81.7±13.6	28±14	41.4±25.4	4.34±2.29	48.4±29.4
Grand median				2.7	63	49	77.6	58	84.3	26	39.3	3.99	45.1
(range)				(2.0–8.1)	(8–377)	(15–136)	(15.0–97.8)	(18–173)	(23.9–98.8)	(4–72)	(0.9–90.2)	(0.59–16.24)	(2.6–98.6)

The mean ± s.d. (first row), median (second row) and range (third row) are given for each sea lion. Minimum (Min.) S_{O_2} was determined at pH 7.4 for dives <3 min and at pH 7.3 for dives ≥3 min. Data are only presented for the seven sea lions that performed dives greater than 2 min in duration.

in most of the deepest and longest dives, P_{O_2} also increased initially, but then rapidly declined to less than 10 mmHg (1.3 kPa; S_{O_2} <5%) halfway through the dive, remained low for a few minutes, and finally increased during the last minute of the dive as the sea lion was ascending (Fig. 3B,D). In most of the profiles from dives greater than 3 min in duration, there was an increase in P_{O_2} (and S_{O_2}) prior to the end of the dive (88.1% of dives; Fig. 2, Fig. 4A). In general, P_{O_2} (and S_{O_2}) continued, or quickly started, to increase after surfacing (Fig. 3); yet, because many post dive intervals were short and/or sea lions were sub-surface swimming, P_{O_2} did not always recover to resting values (~78% S_{O_2} assuming a 5 ml O_2 dl^{-1} a-v difference at rest). In dives greater than 5 min, which typically were followed by a longer post-dive interval (median=4.3 min, range=1.8–126.7 min) with little evidence for sub-surface swimming during the post dive interval, median time to near-resting values (45 mmHg, 6 kPa, 75% S_{O_2}) was 28 s (range=0–67 s).

Initial, maximum and minimum P_{O_2} and S_{O_2}, percentage blood O_2 depletion and O_2 depletion rate are given for each sea lion exhibiting dives greater than 2 min in duration in Table 1. P_{O_2} (and S_{O_2}) at the start of the dive was variable (Table 1). In 43.5% of all dives, initial venous P_{O_2} was greater than 49 mmHg (6.5 kPa, S_{O_2} >78%). This was seen in seven of the eight females and most commonly in sea lions that performed the deepest dives. There was a positive, but weak, relationship between initial and maximum P_{O_2} and dive duration, with high intra-class correlation indicating that each sea lion had a different relationship (Table 2, supplementary material Fig. S1).

In dives greater than 2 min in duration, there was a negative relationship between dive duration and minimum venous P_{O_2} (Table 2, Fig. 2A). In dives greater than 4 min in duration, minimum venous P_{O_2} routinely reached values below 15 mmHg (2.0 kPa, 15% S_{O_2}; 79.3% of dives; Fig. 2A,C) and was occasionally as low as 5 mmHg (0.7 kPa, 1% S_{O_2}; Fig. 2A,C, Fig. 3A,C). This resulted in near-complete blood O_2 depletion (Fig. 5A,B). Percentage venous O_2 depletion during a dive was positively related to dive duration in dives greater than 2 min (Table 2), with over 90% of venous blood O_2 being depleted in 69.6% of dives greater than 4 min in duration. Blood O_2 depletion rates were much more variable (Fig. 5C), with individual sea lions exhibiting different relationships between dive duration and depletion rate patterns. In general, depletion rates were highly variable in dives less than 3 min in duration, but much more consistent in longer duration dives (Fig. 5C).

DISCUSSION

O_2–Hb dissociation curve

As expected, the O_2–Hb dissociation curve for the California sea lion (Fig. 1) was similar to that of other pinnipeds and terrestrial mammals (Horvath et al., 1968; Lenfant, 1969; Meir et al., 2009). The mean P_{50} of 28 mmHg (3.7 kPa) at pH 7.4 was slightly lower than the previously reported P_{50} of 28.5 and 29.5 from two sea lions (Horvath et al., 1968; Lenfant, 1969). The sea lion P_{50} falls within the range of most diving mammals (Lenfant, 1969; Meir et al., 2009). The relatively large Bohr effect was also similar to that of other pinnipeds and terrestrial mammals of similar size, and may facilitate offloading of O_2 at the tissues (Riggs, 1960; Lenfant, 1969; Lenfant et al., 1970; Snyder, 1983; Willford et al., 1990; Meir et al., 2009).

P_{O_2} and S_{O_2} profiles

In the typical short shallow dives of California sea lions (<3 min), there was great variation in minimum and final venous P_{O_2} and S_{O_2} (10–90% S_{O_2} range; Fig. 2), and the rate and magnitude of O_2 depletion (Fig. 3A,C, Fig. 5). This variability may be secondary to differences in: (1) surface duration and the magnitude of venous

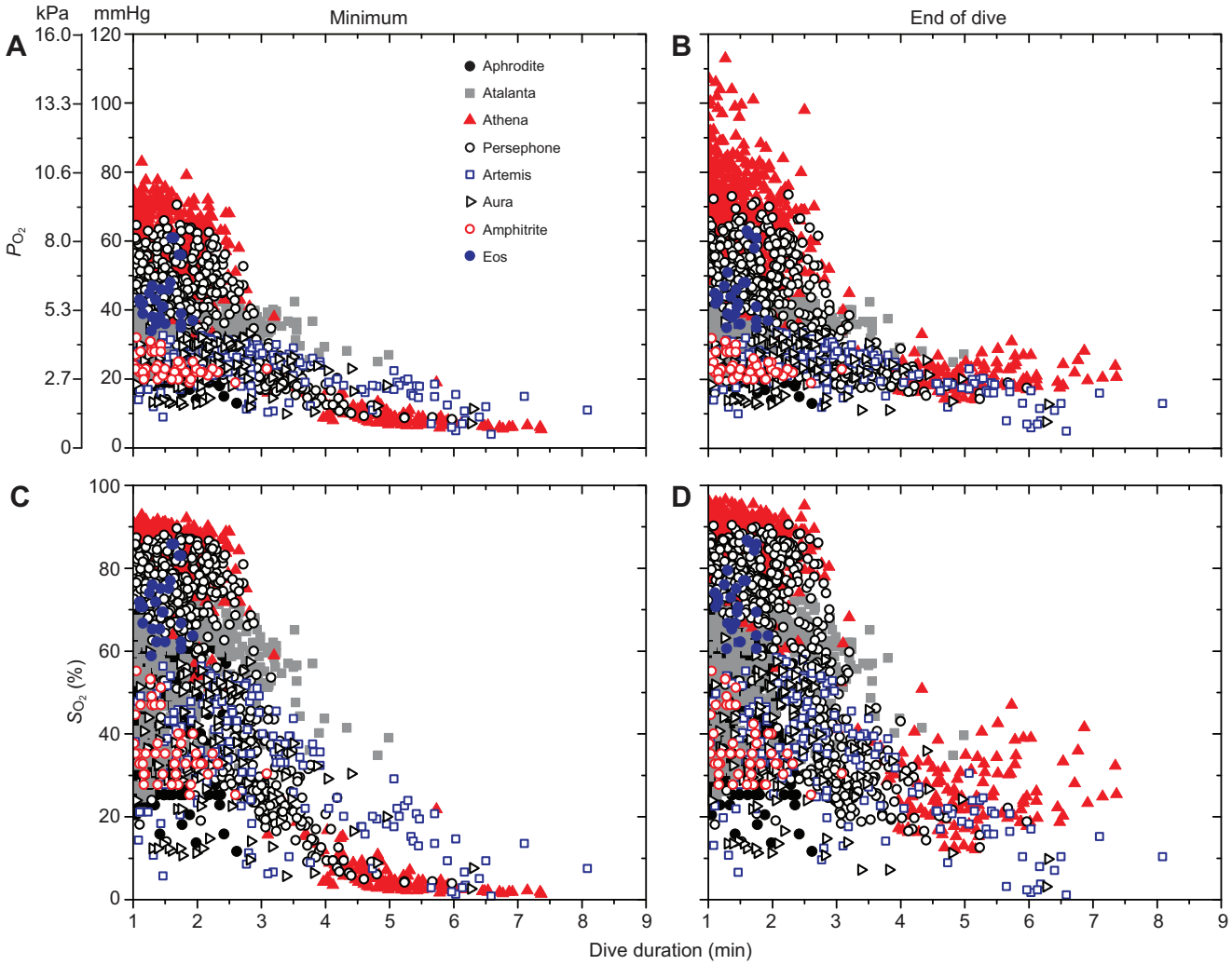

Fig. 2. (A) Minimum venous partial pressure of O_2 (P_{O_2}), (B) end-of-dive (final) venous P_{O_2}, (C) minimum venous Hb saturation (S_{O_2}) and (D) end-of-dive (final) venous S_{O_2} *versus* dive duration.

blood O_2 repletion prior to a dive, (2) dive duration and (3) metabolic rate during an individual dive. For example, observed start-of-dive venous S_{O_2} values were as low as 15%, consistent with prior suggestions that the blood O_2 store is not necessarily restored prior to each dive (Fahlman et al., 2008b). And, in regard to metabolic rate, there has also been as much as fivefold variation in diving metabolic rate during short dives of another otariid, the Steller sea lion (Fahlman et al., 2008b).

In addition to highly variable venous blood O_2 depletion patterns in short dives, venous O_2 was not necessarily depleted at the end of dives at the cADL (3 min), with end-of-dive S_{O_2} values ranging from 10 to 80%. This is similar to the high variability in S_{O_2} at the end of dives at the ADL (5.6 min) in emperor penguins (range 5–85%) (Meir and Ponganis, 2009). Given that the venous O_2 store is not depleted at most estimates of the ADL (3 min) in sea lions, we hypothesize that, like the ADL of emperor penguins, the ADL of sea lions is determined by the depletion of the muscle O_2 store (Williams et al., 2011). Lack of depletion of the venous O_2 store at the ADL is not consistent with previous theoretical models of O_2 store management in which both the blood and muscle O_2 store are simultaneously depleted in order to maximize aerobic dive duration (Davis and Kanatous, 1999).

In all dives, there was a decrease between maximum and minimum venous blood O_2 during a dive, but in 15% of the dives (all <3 min in duration) end-of-dive venous blood O_2 was equal to or greater than the start-of-dive value. This indicates that pulmonary gas exchange can continue during these shallow dives, and that, as in emperor penguins, venous blood O_2 may even increase during the breath hold, perhaps due to a-v shunting during the dive (Ponganis et al., 2007). The lung O_2 store of the sea lion constitutes 16% of the total body O_2 store (Weise and Costa, 2007), making it a significant potential O_2 source during diving, especially in shallow dives when lungs do not collapse (Kooyman and Sinnett, 1982; McDonald and Ponganis, 2012).

In the longer-duration dives of California sea lions (>3 min), there was much less variability in minimum and final venous blood O_2, and in the rate and magnitude of O_2 depletion (Figs 2, 5). In contrast to shallow dives, there was near-complete O_2 depletion in most dives greater than 4 min in duration. We had hypothesized that venous blood O_2 depletion would be incomplete even in long dives because of the contribution of respiratory O_2 to blood and the likely isolation of muscle from the circulation during such dives. Yet, sea lions regularly depleted venous O_2 to levels equivalent to those recorded for both elephant seals and emperor penguins (Ponganis et al., 2007;

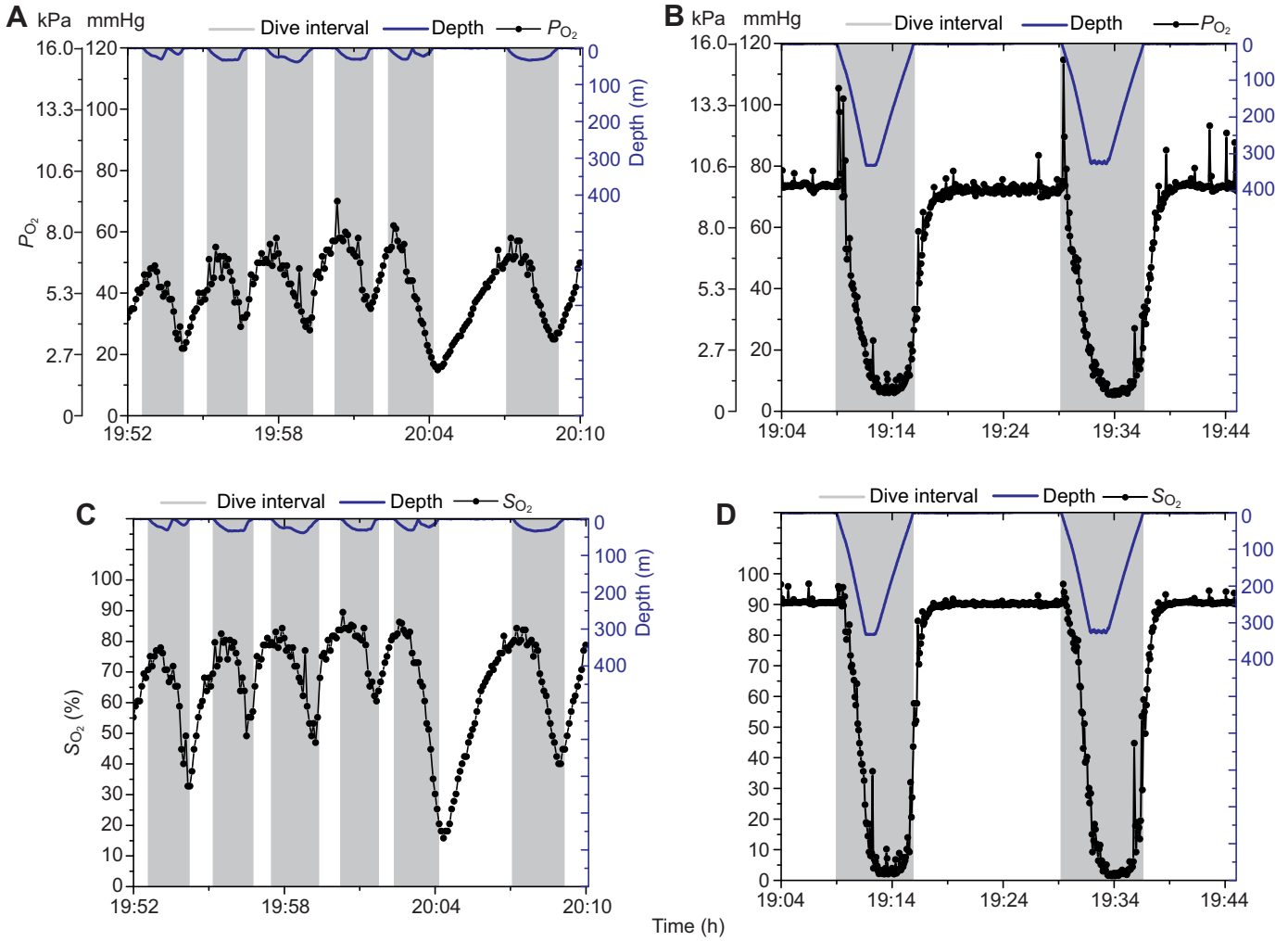

Fig. 3. Venous P_{O2} during short, shallow dives (Aphrodite; A) and deep dives (Athena; B) and the corresponding venous S_{O2} profiles during short, shallow dives (C) and deep dives (D). In both shallow and deep dives, there was an initial increase in P_{O2} and S_{O2}. The transient increase in venous P_{O2} and saturation at the start of dives is not consistent with muscle O_2 extraction, suggesting that there is no blood flow to muscle. The S_{O2} displayed was determined at pH 7.4 to be consistent throughout the dive.

Meir et al., 2009; Meir and Ponganis, 2009), and often much earlier in the dive (Fig. 3). In long-duration dives, elephant seals exhibited an increase in venous P_{O2} at the beginning of a dive, as seen in sea lions, but then usually exhibited a steady decline throughout the remainder of the dive. In contrast, after the initial increase, sea lions showed a rapid decline in S_{O2} during the first half of the dive, after which it remained low for a few minutes before increasing during the last 30–60 s of the dive during the ascent. The rapid decrease in venous S_{O2} during the first half of the sea lion's dive may reflect increased blood O_2 extraction by perfused, working muscle. However, much of the decline is during descent, when sea lions are likely minimizing effort with a stroke and glide locomotory pattern (Williams, 2001; Fahlman et al., 2008a). Alternatively, this rapid decline in venous P_{O2} observed in most long dives may be secondary to a severe bradycardia, low tissue perfusion and prolonged tissue transit time, resulting in near-complete extraction of blood O_2.

Implications for blood flow and gas exchange

Pre-dive arterio-venous shunting

Initial venous S_{O2} values greater than 78% (and sometimes as high as 95%) in over 40% of dives indicate that sea lions are able to enhance their blood O_2 stores by arterialization of venous blood (Fig. 3D). These elevated, pre-dive venous S_{O2} values suggest the use of a-v shunts, as has been hypothesized for emperor penguins (Ponganis et al., 2007; Meir and Ponganis, 2009). This shunting could occur in the well-described a-v anastomoses in the skin of California sea lions (Bryden and Molyneux, 1978) or potentially in undescribed a-v shunts in other locations.

Alternatively, high initial venous S_{O2} values could also be secondary to a lack of O_2 extraction in tissue (i.e. muscle) hyperperfused with well-oxygenated blood during the hyperventilation and tachycardia of the surface intervals. Artificial hyperperfusion through muscle can arterialize venous blood (Grassi et al., 1998); however, post-exercise hyperemia does not result in venous hyperoxia in healthy subjects (Bangsbo and Hellsten, 1998), and once the muscle is recovered, muscle blood flow decreases to resting rates (Walløe and Wesche, 1988; Bangsbo and Hellsten, 1998). Furthermore, hyperventilation at rest, at least in humans, is not associated with increased venous saturations (Huckabee, 1958). Therefore, the use of a-v shunts is the most plausible explanation for elevated S_{O2} values observed during surface intervals, as it is unlikely that the arterialization of pre-dive venous S_{O2} is due to

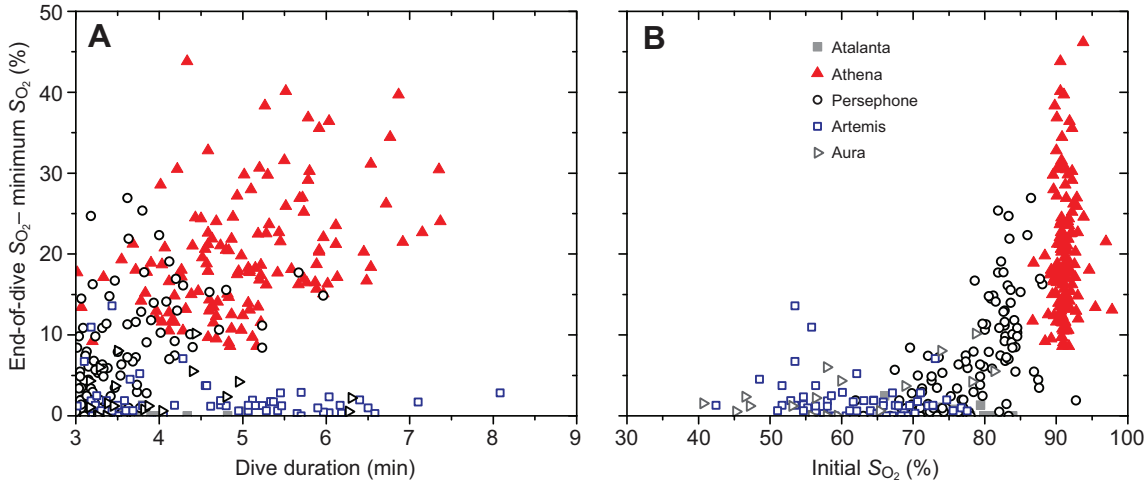

Fig. 4. The relationship between the increase in S_{O_2} at the end of a dive (end-of-dive S_{O_2} – minimum S_{O_2}) and (A) dive duration and (B) initial S_{O_2} for dives greater than 3 min in duration (N=5 sea lions). For two sea lions, the increase in S_{O_2} at the end of the dive was greater in longer dives (A). However, the relationship between initial S_{O_2} and increase in S_{O_2} at the end of the dive suggests that the sea lions that arterialize their venous blood before dives also exhibit the greatest increase in S_{O_2} at the end of a dive.

hyperperfusion of resting muscle with already fully saturated myoglobin.

The magnitude of the a-v shunt before the dive can be estimated using the shunt equation: venous O_2 content = [arterial O_2 content × % a-v shunt] + [(arterial O_2 content – 5 ml O_2 dl^{-1}) × (1 – % a-v shunt)], assuming a 5 ml O_2 dl^{-1} a-v O_2 difference at rest, using an initial arterial O_2 content of 24.07 ml O_2 dl^{-1} (McDonald and Ponganis, 2012), and assuming that the vena caval O_2 content is close to the mixed venous value. For example, before a 7.4 min dive, one of the sea lions had an initial venous S_{O_2} of 90.7% (22.1 ml O_2 dl^{-1}). In order to obtain the elevated venous S_{O_2} observed, an a-v shunt of 60.4% is required. This magnitude of shunt is similar to what was calculated in an emperor penguin before a 23 min dive (Meir and Ponganis, 2009).

Sea lions appear to maximize venous blood O_2 utilization by arterializing venous blood before dives and then depleting venous O_2 to less than 1% saturation during deep dives. An initial venous S_{O_2} of 90%, in contrast to the 78% that would be used in a classic O_2 store calculation, allows sea lions to dive with an additional 3 ml O_2 dl^{-1} in venous blood. This additional O_2 results in an approximate 16% increase in venous blood O_2. Thus, in addition to a role in thermoregulation (Bryden and Molyneux,

Table 2. Mixed effect model results examining the relationship between dive duration and initial, maximum, minimum venous blood O_2 and blood O_2 depletion

	Model variables			Fixed effect					Random effect
Model	Fixed effect	Random effect	AIC	Coefficient	Error	d.f.	t	P	ICC (%)
Duration *vs* Initial P_{O_2}	Duration		–1376.5						
	Duration	Sea lion ID (intercept)	–2384.1						
	Duration	**Sea lion ID (intercept + slope)**	**–2405.1**	0.00032	0.00011	918	3.01	0.003	89.8
		Sea lion ID (intercept)	–2383.2						
Duration *vs* Max. P_{O_2}	Duration		–916.1						
	Duration	Sea lion ID (intercept)	–2134.4						
	Duration	**Sea lion ID (intercept + slope)**	**–2138.0**	0.00030	0.00012	918	2.48	0.013	96.3
		Sea lion ID (intercept)	–2091.5						
Duration *vs* Min. P_{O_2}	Duration		–936.2						
	Duration	**Sea lion ID (intercept)**	**–1034.1**	–0.00322	0.00008	918	–41.93	<0.001	60.7
	Duration	Sea lion ID (intercept + slope)		Did not converge					
		Sea lion ID (intercept)	–501.8						
Duration *vs* % O_2 depletion	Duration		–320.8						
	Duration	Sea lion ID (intercept)	–549.1						
	Duration	**Sea lion ID (intercept + slope)**	**–687.0**	0.00354	0.00055	918	6.42	<0.001	98.4
		Sea lion ID (intercept)	234.3						

Akaike's information criterion (AIC) is reported for all models. The fixed effects and intraclass correlation coefficient (ICC) are only presented for the top model (indicated in bold). Dive duration is always the fixed effect and individual sea lion is always the random effect (with random intercept or random intercept and slope). P_{O_2} data were log transformed and percent O_2 depletion data were arcsin transformed before analysis. N=7 sea lions; dives <2 min in duration were excluded from the model.

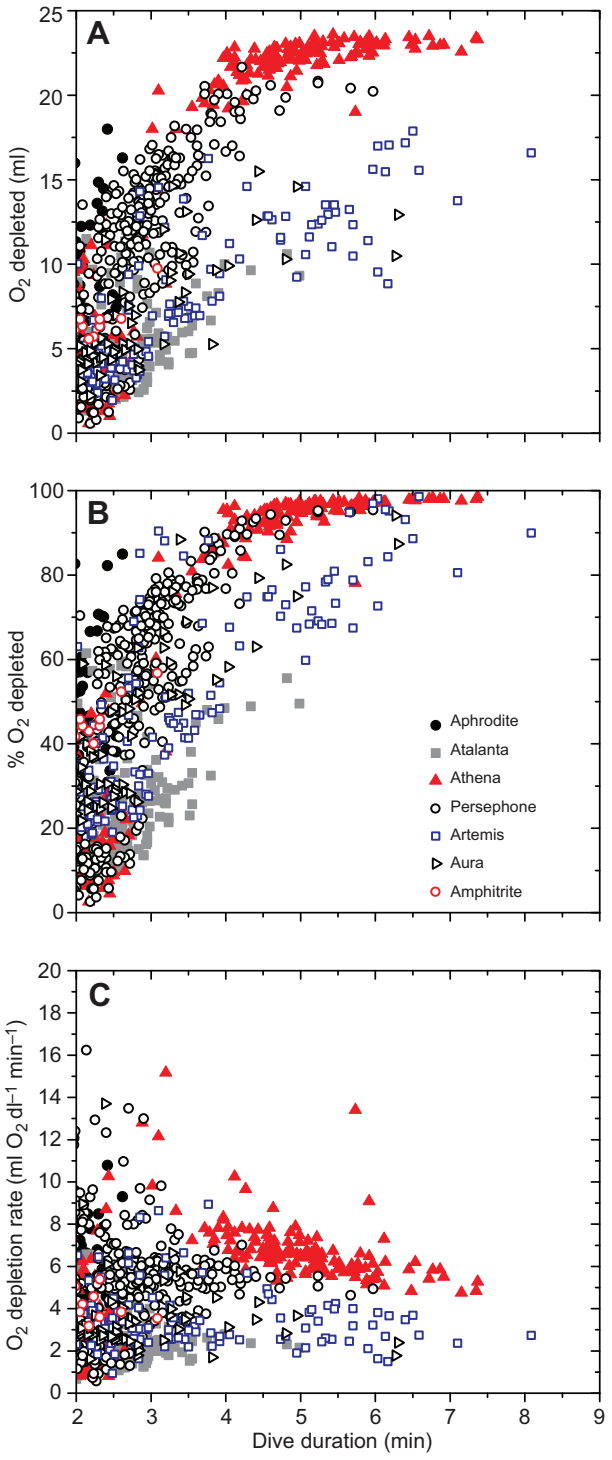

Fig. 5. (A) Venous blood O_2 depleted (ml), (B) percent of venous blood O_2 depleted and (C) venous blood O_2 depletion rate in relation to dive duration. In short dives blood O_2 depletion is variable, but in dives greater than 5 min venous blood O_2 is often almost 100% depleted. Depletion rate is also variable in short dives and there is no consistent relationship between dive duration and depletion rate, although depletion rate is consistently lower in the longest dives.

1978; Molyneux and Bryden, 1981), a-v anastomoses in the sea lion may contribute to enhanced blood O_2 storage and extension of the duration of aerobic metabolism in long-duration dives.

Muscle blood flow during dives

The increase in venous O_2 during the initial descent of a dive (Fig. 3) is inconsistent with O_2 extraction by working tissues, suggesting that diving sea lions are able to restrict blood flow to locomotory muscles. If sea lions were perfusing muscle during the initial descent, we would expect to see a decline in S_{O_2} because sea lions have a high stroke rate during the initial descent, presumably to overcome buoyancy (Fahlman et al., 2008a; Hindle et al., 2010). However, venous S_{O_2} often increased to values greater than 85% during early descent (Table 1, Fig. 3), implying that muscle is ischemic during the initial phase of the dive. This is also similar to venous S_{O_2} profiles obtained for both emperor penguins and elephant seals (Meir et al., 2009; Meir and Ponganis, 2009).

Although the increase in S_{O_2} suggests sea lions are not perfusing muscle early in the dive, the high variability in profile shape later in the dive, especially in shorter dives, may partially be explained by different patterns of muscle blood flow during diving as suggested in emperor penguins by different myoglobin saturation profiles (Williams et al., 2011). In addition, in deep dives of sea lions, resumption of some muscle blood flow may account for the rapid decline in venous S_{O_2} during the latter part of descent. If heart rate is low and muscle blood flow only partially resumes during the latter descent, the rapid decline in venous S_{O_2} may be due, at least partly, to enhanced muscle O_2 extraction to replace muscle O_2 consumed during the beginning of the dive. Increased O_2 demand in poorly perfused muscle could thus account for the rapid decline in venous S_{O_2} to extremely low levels during late descent.

Gas exchange

As already mentioned, increases in venous S_{O_2} during early descent and net increases in venous S_{O_2} during some shallow dives are consistent with maintenance of gas exchange at shallow depths. The increases in venous S_{O_2} during ascent from deep dives (Fig. 3D, Fig. 4A) are also consistent with arterial P_{O_2} profiles of deep dives, which indicate resumption of gas exchange in re-expanded lungs during the 'ascent tachycardia' (Ponganis et al., 1997a; McDonald and Ponganis, 2012). The deep dives of California sea lions are associated with lung compression, and there appears to be complete or near-complete cessation of pulmonary gas exchange (~100% pulmonary shunt or near-complete 'lung collapse') at ~200 m depth (Kooyman and Sinnett, 1982; McDonald and Ponganis, 2012). We have proposed that in addition to minimization of nitrogen absorption, lung collapse preserves a pulmonary O_2 reservoir that supplements blood O_2 during ascent in the sea lion (McDonald and Ponganis, 2012). Our finding that end-of-dive venous S_{O_2} is usually greater than the minimum S_{O_2} during deep dives reinforces this hypothesis. Increased pulmonary blood flow, secondary to the ascent tachycardia routinely observed in diving sea lions (Ponganis et al., 1997a; Hindle et al., 2010), could facilitate O_2 uptake by the blood and maintain or even improve arterial S_{O_2}. Similarly, increased blood flow secondary to the ascent tachycardia may result in blood passing through a-v shunts, thereby minimizing peripheral extraction of blood O_2 and supplementing venous blood O_2, thus accounting for the observed increases in end-of-dive venous S_{O_2}. The possible use of shunts during ascent is supported by the positive relationship between initial S_{O_2} and the magnitude of increase in S_{O_2} at the end of the dive (Fig. 4B), indicating that when sea lions appear to be using a-v shunts during the surface interval, they may also use shunts during ascent. Alternatively, the increase in venous S_{O_2} near the end of a dive may be secondary to increased tissue blood flow with a constant or decreased a-v O_2 difference during the ascent tachycardia (i.e. a relative increase in muscle blood flow despite

unchanged or decreased blood O_2 extraction by muscle). However, as already discussed in relation to pre-dive arterialization of venous blood, there is no evidence for this available in the literature.

Contribution of venous O_2 to metabolic rate

The contribution of venous blood O_2 to diving metabolic rate was highly variable, especially in short dives. We were able to calculate the contribution of venous blood O_2 to metabolic rate while diving because the Hb concentration and the mass-specific blood volume of adult female California sea lions are known (18 g dl^{-1}, 110 ml kg^{-1}) (Weise and Costa, 2007). We used the following equation: venous blood O_2 contribution to metabolic rate (ml O_2 kg^{-1} min^{-1}) = [(maximum O_2 content – minimum O_2 content) / dive duration] × (mass-specific blood volume) × (% venous blood), assuming two-thirds of the blood volume is venous.

Using Athena, the sea lion that demonstrated maximum O_2 loading and unloading, as an example, the average contribution of venous blood O_2 to diving metabolic rate for all dives was 1.9±1.0 ml O_2 kg^{-1} min^{-1}. However, the majority of these dives (87% <3 min in duration) were 1–2 min in duration with minimal changes in venous S_{O_2}, presumably due to continued pulmonary gas exchange and transfer of lung O_2 into the blood. We think that maintenance of such gas exchange masks the contribution of venous O_2 to metabolic rate in sea lions just as was proposed in emperor penguins (Meir and Ponganis, 2009). In longer dives, the contribution of venous O_2 to metabolic rate was higher. For example, the venous O_2 contribution to metabolic rate in Athena was 4.4 ml O_2 kg^{-1} min^{-1} for a 3 min dive, 3.3 ml O_2 kg^{-1} min^{-1} for a 5 min dive, and 2.4 ml O_2 kg^{-1} min^{-1} for a 7 min dive; ~43, 32 and 24%, respectively, of the metabolic rate of a sea lion resting at the surface (Hurley and Costa, 2001), and 133, 100 and 73%, respectively, of the allometrically predicted basal metabolic rate for a sea lion of this size (Kleiber, 1975). These contributions to diving metabolic rate do not include O_2 from arterial blood or from the respiratory and muscle O_2 stores. This venous O_2 contribution to metabolic rate in the sea lion for dives greater than 3 min is in the general range of the venous contribution of 3.4 ml O_2 kg^{-1} min^{-1} for an elephant seal (Meir et al., 2009), and much higher than the contribution of venous blood to metabolic rate calculated in emperor penguin (0.6 ml O_2 kg^{-1} min^{-1}) (Meir and Ponganis, 2009).

Arterio-venous O_2 content difference in deep dives

Application of the O_2–Hb dissociation curve to arterial profiles from deep dives of sea lions (McDonald and Ponganis, 2012) allows comparison of the arterial S_{O_2} profile to the venous S_{O_2} profiles of this study (Fig. 6). As, we have previously emphasized, arterial S_{O_2} is well maintained during these long, deep dives (McDonald and Ponganis, 2012). It is notable that during the middle of these dives, arterial O_2 content is still near maximal values at 23 ml O_2 dl^{-1}, while venous O_2 content is almost 0 ml O_2 dl^{-1}, yielding an a-v O_2 content difference (Δa-v O_2) of 23 ml O_2 dl^{-1}, more than four times the typical value assumed in most animals at rest. As previously emphasized for a low venous S_{O_2}, this large Δa-v O_2 is consistent with extreme hypoperfusion of tissue and complete blood O_2 extraction. Thus, these S_{O_2} profiles and the large Δa-v O_2 suggest that sea lions are extremely bradycardic during these segments of the dive.

During extreme bradycardia and tissue hypoperfusion, venous return is low and venous mixing is slow. Under such conditions, we raise the caveat that a P_{O_2} electrode in the distal posterior vena cava may not provide data representative of the entire venous blood O_2 store. Under such conditions, the position of the electrode in this study may only allow assessment of P_{O_2} in blood slowly draining peripheral tissues, but not in mixed venous blood (in the pulmonary artery). Therefore, there are limitations to our interpretations, and to our calculations of the contribution of venous O_2 to diving metabolic rate. Mixed venous blood P_{O_2} and S_{O_2} would be ideal, but were not feasible in this study because of the length of the P_{O_2} electrode and size of the animal.

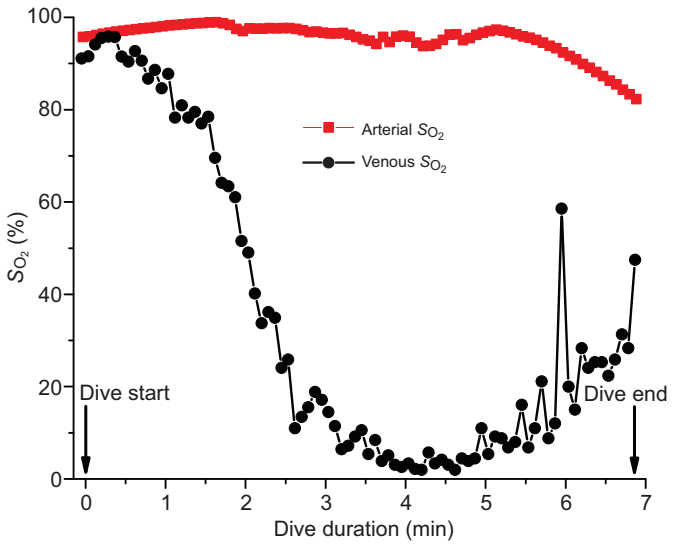

Fig. 6. Arterial and venous S_{O_2} in two 7 min dives to ~310 m. Arterial S_{O_2} is well-maintained during long, deep dives, while venous O_2 content is nearly depleted, resulting in an a-v O_2 content difference of 23 ml O_2 dl^{-1}, more than four times the typical value assumed in most animals at rest. This large a-v O_2 content difference is consistent with extreme hypoperfusion of tissue and complete blood O_2 extraction. Data are from two sea lions performing dives with similar depth profiles [arterial data are from McDonald and Ponganis (McDonald and Ponganis, 2012)]. The S_{O_2} displayed was determined at pH 7.4 throughout the entire dive to maintain consistency and to provide a conservative estimate of continuous S_{O_2}.

The arterial and venous S_{O_2} profiles of deep dives also have other implications. If our venous S_{O_2} profiles truly reflect mixed venous S_{O_2}, and if there is complete cessation of gas exchange as we have previously suggested due to lung collapse near 200 m, then it is unclear how arterial P_{O_2} and S_{O_2} can be maintained at such high levels during this middle portion of the deep dive. One would expect that arterial S_{O_2} should rapidly approach mixed venous S_{O_2} under conditions of complete lung collapse. Therefore, we conclude that either (1) mixed venous S_{O_2} is greater than the distal vena cava S_{O_2} during this segment of the dive, or (2) some degree of gas exchange still persists even at maximal depths, thus allowing for maintenance of arterial S_{O_2}. It is again notable that, under either condition, a severe bradycardia is optimal. We hope to address these questions in future studies.

Lastly, the transient rises in venous S_{O_2} shown in Fig. 6, especially those in late ascent, are again consistent with intermittent large pulses of well-oxygenated arterial blood into the venous system. These transient increases in venous S_{O_2}, as well as the near equivalence of arterial and venous S_{O_2} early in the dive (Fig. 6), again reinforce our hypothesis that the a-v shunts are utilized in the diving sea lion. Such 'spikes' in venous S_{O_2} were not uncommon (Fig. 3).

Conclusions

These are the first blood O_2 depletion data from a diving animal during natural foraging trips. We were able to take advantage of the foraging behavior of lactating sea lions to obtain data on how

they manage O_2 during natural dives. Our results suggest that sea lions are optimizing the size of the venous blood O_2 store and the magnitude of its depletion during long dives by arterializing venous blood before a dive and then depleting it to extremely low levels, resulting in a net O_2 content depletion of up to 99%. In addition, the increase in venous O_2 at the end of deep dives supports the hypothesis that lung collapse preserves an O_2 reservoir that the sea lions can use as they ascend. The high variability in O_2 depletion patterns, both between and within individuals, suggests that the O_2 management strategy is variable and can be adapted to the current O_2 demands of the dive. Oxygen store depletion during shallow dives certainly differs from that of deep dives. Future work investigating diving heart rate and stroke rate during diving will further elucidate the mechanisms underlying O_2 store management in freely diving California sea lions.

LIST OF SYMBOLS AND ABBREVIATIONS

a-v	arterio-venous
ADL	aerobic dive limit
cADL	calculated aerobic dive limit
Hb	hemoglobin
P_{O_2}	partial pressure of oxygen
S_{O_2}	percent Hb saturation
TDR	time-depth recorder
Δa-v O_2	arterio-venous oxygen difference

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AUTHOR CONTRIBUTIONS

B.I.M. and P.J.P. conceived and performed the study, B.I.M. conducted data analysis, and B.I.M. and P.J.P. wrote the manuscript.

COMPETING INTERESTS

No competing interests declared.

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