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# Extreme hypoxemic tolerance and blood oxygen depletion in diving elephant seals

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**Meir JU, Champagne CD, Costa DP, Williams CL, Ponganis PJ.** Extreme hypoxemic tolerance and blood oxygen depletion in diving elephant seals. *Am J Physiol Regul Integr Comp Physiol* 297: R927–R939, 2009. First published July 29, 2009; doi:10.1152/ajpregu.00247.2009.—Species that maintain aerobic metabolism when the oxygen ($O_2$) supply is limited represent ideal models to examine the mechanisms underlying tolerance to hypoxia. The repetitive, long dives of northern elephant seals (*Mirounga angustirostris*) have remained a physiological enigma as $O_2$ stores appear inadequate to maintain aerobic metabolism. We evaluated hypoxemic tolerance and blood $O_2$ depletion by *1*) measuring arterial and venous $O_2$ partial pressure ($P_{O_2}$) during dives with a $P_{O_2}$/temperature recorder on elephant seals, *2*) characterizing the $O_2$-hemoglobin ($O_2$-Hb) dissociation curve of this species, *3*) applying the dissociation curve to $P_{O_2}$ profiles to obtain %Hb saturation ($S_{O_2}$), and *4*) calculating blood $O_2$ store depletion during diving. Optimization of $O_2$ stores was achieved by high venous $O_2$ loading and almost complete depletion of blood $O_2$ stores during dives, with net $O_2$ content depletion values up to 91% (arterial) and 100% (venous). In routine dives (>10 min) $Pv_{O_2}$ and $Pa_{O_2}$ values reached 2–10 and 12–23 mmHg, respectively. This corresponds to $S_{O_2}$ of 1–26% and $O_2$ contents of 0.3 (venous) and 2.7 ml $O_2$/dl blood (arterial), demonstrating remarkable hypoxemic tolerance as $Pa_{O_2}$ is nearly equivalent to the arterial hypoxemic threshold of seals. The contribution of the blood $O_2$ store alone to metabolic rate was nearly equivalent to resting metabolic rate, and mean temperature remained near 37°C. These data suggest that elephant seals routinely tolerate extreme hypoxemia during dives to completely utilize the blood $O_2$ store and maximize aerobic dive duration.

$P_{O_2}$; aerobic metabolism; %Hb saturation; $O_2$-Hb dissociation curve; hypoxia

ANIMALS OPERATING IN EXTREME environments often push the limits of their physiological capacity and thus represent unique examples of physiological adaptation. Species that must maintain aerobic metabolism when oxygen ($O_2$) supply is limited represent ideal models in which to examine the physiological, cellular, and biochemical mechanisms underlying tolerance to hypoxia. In addition, investigation of adaptations to hypoxia in animals at high altitude, during hibernation, or in diving environments may provide insights to the understanding and treatment of clinical conditions, such as circulatory collapse, ischemia, and hypoxemia.

The northern elephant seal (*Mirounga angustirostris*) is a consummate pinniped diver, with routine 10- to 30-min dives to depths of 400 to 800 meters and mean surface intervals of only 2 min (51, 52). The repetitive, long duration dives of this pelagic predator remain a physiological enigma in that $O_2$ stores appear insufficient to account for these frequent, long dives (34). This diving behavior makes the elephant seal an excellent model of hypoxemic tolerance and regulation of tissue $O_2$ delivery and consumption.

The aerobic dive limit [ADL; duration beyond which blood lactate concentration increases above resting levels (43)] and the concept that most dives in the wild are aerobic has dominated the interpretation of diving physiology and diving behavior for the past 25 years. Although an ADL has not been measured in elephant seals, their diving metabolism has been considered predominantly aerobic in nature because surface intervals are only 2 min in duration (51, 52). Furthermore, > 90% of their time at sea is spent submerged during 2-mo foraging trips throughout which elephant seals gain an average of 1 kg/day body mass (51, 52). With the exception of postmolt females, most dives of elephant seals are within their theoretical ADL (34, 51). The physiological mechanisms that account for the sustained, continuous diving of this species, however, are unresolved.

Hypothermia has been suggested as a mechanism of metabolic rate reduction (10, 14). In addition, moderate bradycardias of 30–40 beats/min, regulated organ perfusion during dives (5, 33), a hydrodynamic body shape (26), and prolonged gliding (92) undoubtedly contribute to the conservation of blood and muscle $O_2$ stores during dives. Another strategy that has been proposed to conserve $O_2$ during dives is partitioning of the metabolic demands of travel, foraging, and digestion into different dive types and extended surface intervals (34, 53, 80). We postulate in the present study that extreme hypoxemic tolerance in the elephant seal allows for nearly complete blood $O_2$ depletion with every dive and that it is this tolerance that especially allows the seal to make such frequent, repetitive, long dives.

The breath-hold capacity of air-breathing animals is dependent on increased $O_2$ stores, the management and depletion of those stores, and hypoxemic tolerance. In phocid seals, such as the elephant seal, approximately two-thirds of the total body $O_2$ store is in the blood (65). The contribution of the lung to total $O_2$ stores is small (<5% of total) due to a small diving lung volume following exhalation prior to submersion and also due to lung collapse at depth (25, 46). High myoglobin concentrations, especially in the primary locomotory muscles [>15× the human value (86)], account for ~25% of the total store. The blood $O_2$ store constitutes 65–71% of the total and is based upon a large blood volume (~3 times the human mass-specific volume) and a high Hb concentration (almost twice the human value) (65). Although the management of the blood $O_2$ store is central to the understanding of diving physiology, there have been few studies that quantify the rate and

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magnitude of blood $O_2$ store depletion during diving. Juvenile elephant seals are an excellent model for investigation of blood $O_2$ depletion as blood $O_2$ stores and dive behavior have been studied extensively in young elephant seals (52, 78, 82, 86).

Our project used a "backpack" partial-pressure of $O_2$ ($Po_2$) and temperature recorder to investigate blood $O_2$ transport, blood $O_2$ store depletion, and hypoxemic tolerance during dives of translocated juvenile northern elephant seals. We hypothesized that maintenance of metabolism during routine dives of these seals is due to extreme hypoxemic tolerance and almost complete depletion of the large blood $O_2$ store. The objectives of this project were to *1*) measure arterial and venous $Po_2$ ($Pa_{O_2}$ and $Pv_{O_2}$) continuously during dives, *2*) characterize the $O_2$-hemoglobin ($O_2$-Hb) dissociation curve, *3*) apply the $O_2$-Hb dissociation curve to the $Po_2$ profiles to obtain %Hb saturation ($So_2$) during dives, and *4*) calculate the magnitude of blood $O_2$ store depletion during diving and estimate its contribution to the metabolic rate during the actual dive.

Marine mammals typically have $P_{50}$ ($Po_2$ at which Hb is 50% saturated) values similar to those of humans and other terrestrial mammals (25–31 mmHg) (54, 56, 73, 79, 91). The complete $O_2$-Hb dissociation curves of whole blood of elephant seals at a temperature of 37°C and at various pHs have not been published previously. Documentation of the entire dissociation curve in whole blood is essential to estimate in vivo $So_2$ changes during the dive on the basis of the $Po_2$ profiles collected in this study.

We hypothesized that: *1*) end-of-dive $Pa_{O_2}$ and $Pv_{O_2}$ values would be $\leq$ 10 mmHg, far below hypoxemic limits of other mammals; *2*) the elephant seal $O_2$-Hb dissociation curve would be similar to those of other marine mammals with a $P_{50}$ near 30 mmHg and a Bohr effect ($\Delta \log P_{50}/\Delta pH$) between $-0.5$ to $-0.6$ (56, 73, 79, 91); *3*) application of the $O_2$-Hb dissociation curve to $Po_2$ profiles would demonstrate that end-of-dive $So_2$ is routinely $< 10\%$ in all dives and that the rate of desaturation will vary according to dive duration (i.e., the rate of desaturation will be slower during longer dives); and *4*) the contribution of the blood $O_2$ store alone to diving metabolic rate during routine (10–30 min) dives of juvenile elephant seals would be equivalent to or greater than the resting metabolic rate for this species.

Because the conventional differences in arterial and venous $O_2$ contents are diminished in a breath-hold diver with collapsed lungs (due to the lack of addition of $O_2$ from the respiratory system and continual decline in blood $O_2$ during a dive), we also hypothesized that $Pa_{O_2}$ and $Pv_{O_2}$ values would become indistinguishable, particularly toward the end of longer dives. Accordingly, hepatic sinus $Po_2$ values should approximate those in the artery. Indeed, Elsner's early work (23) with elephant seals reported higher $O_2$ contents in the posterior vena cava than the aorta at the end of forced submersions. This resulted from the slow metering of blood from the hepatic sinus and posterior vena cava back into the general circulation via the caval sphincter, the lack of $O_2$ uptake from the lung, and the return of desaturated blood via the anterior vena cava (23). Equalization of end-of-apnea $Pa_{O_2}$ and $Pv_{O_2}$ values has also been observed in sleep apnea studies (82).

## MATERIALS AND METHODS

This research was conducted on juvenile (1- to 3-yr-old) elephant seals [body mass = 195 kg (SD 46), $n = 13$] from the Año Nuevo colony (San Mateo County, CA) during the April molt season using the translocation method (5, 60) over three field seasons (2006, 2007, 2008). Seals were captured from their haul-out site on the Año Nuevo beach, transported by truck to the Long Marine Laboratory (University of California, Santa Cruz, CA) for instrumentation, allowed to recover overnight, and then transported by truck to the other side of Monterey Bay (Hopkins Marine Station) or by boat for offshore release. Thirteen seals were instrumented with a $Po_2$ electrode and thermistor [3 seals with electrodes in the extradural vein, 4 in the hepatic sinus, and 6 in the artery (5 brachial artery, 1 femoral artery)], and blood was drawn from 15 seals for $O_2$-Hb dissociation curve analysis. All procedures were approved by a University of California Santa Cruz Chancellor's Animal Research Committee and a National Marine Fisheries Service marine mammal permit (no. 87-1743-02).

### *$Po_2$ Electrode Deployments*

Seals at the Año Nuevo colony were immobilized with an intramuscular injection of a mixture of tiletamine HCl and zolazepam HCl (Telazol) at an approximate dose of 1 mg/kg. At the laboratory, immobilization techniques included either ketamine (3 mg/kg im or 0.5–1 mg/kg iv) or telazol (0.4–0.8 mg/kg im) followed by the administration of isoflurane and 100% $O_2$ (61, 66, 67, 69, 82) for all hepatic sinus and arterial deployments. An extradural vein catheter (14 g) was inserted postinduction for blood sample collection and was removed prior to transport.

A $Po_2$ electrode (model Licox C1.1 Revoxode; Integra Life Sciences, Plainsboro, NJ) and thermistor (model 554; Yellow Springs Instruments, Yellow Springs, OH) were inserted percutaneously through a peel-away catheter (16–20 g) into one of the following sites (1 site per animal): *1*) extradural vein, *2*) hepatic sinus-inferior vena cava, or *3*) aorta (via the brachial or femoral artery). Catheterization techniques, the $Po_2$ electrode, and thermistor have been described previously (61–63, 69, 71, 81, 82). The electrode and thermistor were connected to a custom-built microprocessor (UFI, Morro Bay, CA) in a backpack unit (5 × 16 cm, 570 g), and mounted on the middorsal region of the seal using 5-min epoxy (Loctite). $Po_2$ and temperature were sampled once every 5 or 15 s, depending on the specific recorder used for that deployment. A Mark 9 time-depth recorder (TDR; Wildlife Computers, Redmond, WA; sensitive to 0.5 m, 30 g, 6.7 × 1.7 × 1.7 cm) was attached to document the dive profile, with a 1-Hz sampling rate. The $Po_2$/temperature recorder and TDR were synchronized to the same PC clock, which was automatically synchronized to an Internet time server. A SPOT 4 satellite transmitter (4 × 3.5 cm footprint, 160 g, monitored via ARGOS; Wildlife Computers) was secured to the animal's head to track the seal during the transit back to the colony and a VHF radio transmitter (148–150 MHz, 5 × 2 cm, 34 g; Wildlife Computers) was attached to the animal's back to relocate it for recorder recovery (5, 16). Returning seals were immobilized as during capture, and all instruments removed at the beach haul-out location.

### *$Po_2$ Electrode Calibration and Verification*

As stated above, the $Po_2$ electrode and thermistor, the associated calibration procedures, and verification testing have been described previously (70, 71, 81). In addition to these protocols, additional testing was conducted to verify maintenance of electrode function (response time and potential drift) over the range of data collection in the deployments of this study. $Po_2$ electrodes were placed in saline-filled test tubes with various concentrations of bubbling $N_2$ and $O_2$ (maintained at 37°C in a water bath) and deployed for a 9-day period (equivalent to the longest recorded data in this study. Note, this is not equivalent to the number of days the animal spent at sea due to battery failure/electrode breakage.) Recalibration postdeployment was also conducted in some cases.

### *$O_2$-Hb Dissociation Curve Characterization*

$O_2$-Hb dissociation curves on fresh whole blood were determined with the mixing technique of tonometered blood (77). Blood samples

were obtained from the seals immediately after immobilization, placed on ice, and processed immediately upon return to the laboratory. All dissociation curve analyses were completed within 6 h after blood collection to prevent depletion of labile organic phosphates, such as 2,3-DPG, which would influence the dissociation curve. The mixing technique consists of the volumetric mixing of 0% $O_2$-saturated blood and 100% $O_2$-saturated blood to achieve desired $So_2$ at various points (i.e., 90, 70, 50, 40, 20, 10, 5% $So_2$) along the curve with subsequent measurement of the $Po_2$ of the resulting mixture using an i-STAT blood gas analyzer (Abbott Point of Care, Princeton, NJ) (7, 38, 59, 73). Use of an i-STAT and Tucker chamber (87) also allowed verification of pH, partial pressure of carbon dioxide ($Pco_2$), and blood $O_2$ content. The $CO_2$ Bohr effect was determined by changing the $CO_2$ concentration of the gas in the tonometer to adjust pH. Dissociation curves were determined at pH values of 7.4, 7.3, and 7.2. The $\log[So_2/(100 - So_2)]$ vs. $\log(Po_2)$ was plotted, and linear regression analysis performed to generate the equation for the $O_2$-Hb dissociation curve at each pH (all saturation points, all seals combined) (58). The Bohr coefficient was derived from linear regression of the log $P_{50}$ on pH (each point averaged from all data of all seals for pH = 7.4, 7.3, and 7.2) (58, 91). In addition, the fixed acid Bohr effect was determined by titrating with HCl to pH 7.3 and 7.2, while $Pco_2$ was maintained at the level required for the standard pH = 7.4 curve in that seal (91). The effect of lactic acid on the dissociation curve was also evaluated in whole blood by determining additional dissociation curve points after adding 5 and 10 mM concentrations of lactic acid to the blood (58).

To validate the specific equipment and methods used in this study, dissociation curves were also determined with blood from pinnipeds with previously published $O_2$-Hb binding data [*Leptonychotes weddellii, Phoca vitulina, Zalophus californianus* (54, 55)]. Mixing technique tests were also conducted to verify that no hemolysis occurred during mixing.

### *$So_2$ Calculations*

Percent Hb saturation ($So_2$) values were obtained by applying $Po_2$ data to the linear regression equation generated by the dissociation curve, $\log[So_2/(100-So_2)]$ vs. $\log(Po_2)$ plot, and solving for $So_2$ (at the appropriate pH, see below).

### *Blood $O_2$ Store Depletion Calculations*

Blood samples taken for the $O_2$-Hb dissociation curve were also used for Hb analyses [cyanomethemoglobin technique (63)] to obtain Hb concentration. Use of such blood samples for this purpose is appropriate because Hb concentration is high in blood samples obtained from seals immediately after sedation (13) (P. J. Ponganis and D. P. Costa, unpublished observations); the large decrease in Hb content observed during inhalational anesthesia (63) does not occur.

Oxygen content for initial (based on the maximum $Po_2$ value during initial descent of dive) and end-of-dive time points (minimum $Po_2$ during the dive) for each dive were calculated from the corresponding $So_2$ and $Po_2$ values, using a Hb concentration of 25 g/dl [this study, agrees well with previous findings (45, 78)] with the formula: $O_2$ content (ml $O_2$/dl blood) = (1.34 ml $O_2$/g Hb) × [Hb](g/dl) × $So_2$ + (0.003 × $Po_2$). Maximum $So_2$ was estimated with the pH 7.4 dissociation curve, and the minimum $So_2$ value was estimated with the pH dissociation curve at 7.4 or 7.3, depending on the duration of the dive (pH = 7.4 for dives < 15 min, pH = 7.3 for dives > 15 min). The effect of pH is critical in estimation of end-of-dive $So_2$. For example, from data in Weddell seals (73), a $Po_2$ of 10 mmHg corresponds to 8% $So_2$ at pH 7.4, but only 2% at pH 7.0. However, since pH values were not < 7.3, $Pco_2$ values not > 55 mmHg, and lactate levels not elevated during even long dives of Weddell seals (29, 72), it is expected that this pH effect will be small and that the $So_2$ profile obtained with the pH 7.4 dissociation curve will reflect the general pattern of change in $So_2$. This minimum change in pH is also supported by data from forced submersions of Weddell seals in which pH decreased to only 7.28 after 30-min submersions (22). Nonetheless, for greater accuracy, the pH 7.3 dissociation curve was used to estimate the end-of-dive $So_2$ value for blood $O_2$ store depletion calculations in dives > 15 min. This duration was selected based on pH data from diving Weddell seals and sleep apnea of elephant seals, demonstrating little to no change in pH for the first 15 min of diving in both short and long dives or apneas (72, 82).

The $\%O_2$ depletion in either the arterial or venous system (dependent on the site of insertion) for each dive of each seal was calculated as: $\%O_2$ content depletion = [(maximum $O_2$ content − minimum $O_2$ content)/maximum $O_2$ content] × 100. The rate of $O_2$ content depletion, (maximum $O_2$ content − minimum $O_2$ content)/dive duration, was also calculated for the arterial system, hepatic sinus, and extradural vein. This provides an overall depletion rate, not an instantaneous measure of $O_2$ consumption. Data from seals with $Pa_{O_2}$ electrodes provided an estimate of the net depletion of $O_2$ in the arterial system; those with venous probes provided estimates of net venous $O_2$ depletion.

### *Data Analysis and Statistics*

The time required for $Po_2$ to return to a venous $So_2$ ($Sv_{O_2}$) of 75% ($Po_2$ = 46 mmHg) or arterial $So_2$ ($Sa_{O_2}$) of 90% ($Po_2$ = 70 mmHg) upon completion of the dive was determined. These values were chosen as representative of $Sv_{O_2}$ and $Sa_{O_2}$ using a conservative 90% $So_2$ as typical for that of the arterial system and employing an arterial-venous $O_2$ content difference of 5 ml/dl to determine the venous value. This is because there is no typical "resting" value for these animals since $Po_2$ is constantly oscillating during continuous diving and during sleep apnea, while on land, and because surface intervals are not extended rest intervals in this species. As outlined above, the dissociation curve, calculated using linear regression, was applied to $Po_2$ values to yield $So_2$ values, and calculations of $O_2$ depletion rate and percentage of $O_2$ depletion were made. Differences between results at the three electrode insertion sites were determined with ANOVA. Correlation between dive duration and the variables of minimum $Po_2$, minimum $So_2$, maximum $So_2$, $\%O_2$ content depleted, and depletion rate was addressed with Spearman rank order correlation tests. Statistical significance was assumed at $P < 0.05$, and the significance level is quoted in the text. Values are expressed as means (SD). $Po_2$ values are expressed in mmHg (as measured). Figures include the corresponding values in kPa, assuming 1 mmHg = 0.133 kPa.

## RESULTS

### *Behavior*

Seals in this study often dived with transit patterns (travel dives) consistent with the translocation method (5, 60); however, some seals did exhibit prolonged bouts of diving consistent with foraging and drift dives of free-ranging juveniles (53). Most seals returned to Año Nuevo within 1–3.5 days (Table 1). Mean dive duration and depth data are given in Table 1.

### *Temperature*

Continuous temperature measurements from the thermistor were obtained from 13 seals (3 extradural vein, 4 hepatic sinus, 6 arterial) and provided correction of $Po_2$ electrode output. Average temperature during dives remained near 37°C [mean temperature of all dives of all seals pooled = 37.4°C (SD 1.3); $n$ = 3,213 dives from 13 seals]. This justifies the use of $O_2$-Hb dissociation curves obtained at 37°C for the $So_2$ calculations.

Table 1. *Individual dive, $Po_2$, $So_2$, and depletion rate data for all seals*

| Seal, No. of dives | Length of Deployment, days at sea | $Po_2$ Electrode Location | Dive Duration, min | Dive Depth, m | Max $Po_2$, mmHg | Min $Po_2$, mmHg | Max $So_2$, % | Min $So_2$, % | %$O_2$ Content Depleted | Depletion Rate, ml $O_2 \cdot dl^{-1} \cdot min^{-1}$ |
|---|---|---|---|---|---|---|---|---|---|---|
| Chick, $n$ = 267 | 3.5 | Extradural vein | 8.6 (SD 6.0) | 50.1 (SD 48.8) | 41 (SD 11) | 13 (SD 7) | 65.3 (SD 15.8) | 13.1 (SD 14.3) | 77.7 (SD 24.2) | 2.5 (SD 1.1) |
| Range | | | 1.1–43.7 | 5–380 | 16–70 | 2–46 | 16.3–90.0 | 0.1–75.2 | 4.3–99.9 | 0.6–6.8 |
| Starburst, $n$ = 106 | 3 | Extradural vein | 14.9 (SD 5.6) | 127.1 (SD 80.4) | 56 (SD 12) | 9 (SD 5) | 80.8 (SD 10.8) | 4.3 (SD 9.3) | 94.6 (SD 10.9) | 1.9 (SD 0.6) |
| Range | | | 1.6–29.9 | 9–432 | 25–75 | 3–48 | 37.2–91.6 | 0.1–77.6 | 9.8–99.9 | 0.9–4.1 |
| Patty, $n$ = 81 | 1 | Extradural vein | 9.3 (SD 4.7) | 70.3 (SD 80.3) | 41 (SD 8) | 10 (SD 3) | 66.6 (SD 12.1) | 5.8 (SD 4.6) | 90.0 (SD 11.1) | 2.5 (SD 0.8) |
| Range | | | 1–28.9 | 11–416 | 22–60 | 3–22 | 30.8–85.8 | 0.2–30.8 | 14.0–99.7 | 0.8–4.6 |
| Bodil, $n$ = 192 | 21 | Hepatic sinus | 14.2 (SD 6.5) | 214.4 (SD 157.8) | 35 (SD 7) | 19 (SD 4) | 57.4 (SD 11.4) | 20.8 (SD 9.5) | 60.6 (SD 22.9) | 0.9 (SD 0.3) |
| Range | | | 1.1–29.3 | 5.0–699.0 | 24.0–63.0 | 9–30 | 35.8–87.3 | 3.1–49.9 | 6.0–95.4 | 0.2–2.3 |
| Roberta, $n$ = 480 | 15 | Hepatic sinus | 12.2 (SD 5.7) | 156.9 (SD 135.0) | 58 (SD 19) | 12 (SD 8) | 80.5 (SD 11.0) | 11.4 (SD 13.8) | 85.6 (SD 17.4) | 2.4 (SD 1.5) |
| Range | | | 1.0–27.5 | 6.0–466.0 | 22.0–124.0 | 0–38 | 30.8–97.6 | 0–64.8 | 17.0–100 | 0.7–21.9 |
| Larry, $n$ = 218 | 9 | Hepatic sinus | 12.2 (SD 3.2) | 83.7 (SD 65.2) | 50 (SD 7) | 20 (SD 4) | 77.9 (SD 6.3) | 24.2 (SD 10.7) | 69.0 (SD 13.4) | 1.5 (SD 0.4) |
| Range | | | 5.4–22.2 | 23–504 | 29–66 | 9–29 | 47.7–88.6 | 3.1–47.7 | 44.2–96.1 | 0.9–3.5 |
| Per, $n$ = 160 | 1.2 | Hepatic sinus | 8.9 (SD 6.5) | 71.2 (SD 121.0) | 46 (SD 7.6) | 25 (SD 1) | 73.8 (SD 8.5) | 36.5 (SD 17.9) | 50.3 (SD 23.6) | 1.8 (SD 1.1) |
| Range | | | 1.0–26.3 | 6–508 | 30–68 | 5–40 | 49.9–89.3 | 0.7–67.8 | 15.1–98.6 | 0.7–10.3 |
| Jerry, $n$ = 132 | 1.4 | Arterial | 10.5 (SD 6.4) | 107.1 (SD 124.8) | 84 (SD 23) | 29 (SD 9) | 91.9 (SD 4.7) | 43.4 (SD 19.8) | 51.9 (SD 23.2) | 1.7 (SD 0.4) |
| Range | | | 1–30.5 | 5–474 | 49–140 | 15–67 | 78.1–98.2 | 10.6–89.0 | 4.9–89.2 | 1.0–3.1 |
| Sammy, $n$ = 70 | 1 | Arterial | 10.5 (SD 2.9) | 130.5 (SD 106.6) | 82 (SD 18) | 22 (SD 5) | 92.0 (SD 4) | 29.0 (SD 12) | 68.2 (SD 13.9) | 2.0 (SD 0.3) |
| Range | | | 3.2–23.2 | 7–423 | 45–140 | 12–43 | 74.1–98.2 | 8.4–71.8 | 20.0–91.0 | 1.3–2.9 |
| Knut, $n$ = 242 | 2 | Arterial | 10.0 (SD 5) | 84.7 (SD 88.5) | 93 (SD 23) | 29 (SD 9) | 93.7 (SD 3.6) | 44.1 (SD 17.9) | 52.4 (SD 20.2) | 1.9 (SD 0.6) |
| Range | | | 1.0–24.4 | 5–403 | 51–171 | 17–65 | 79.9–98.9 | 14.1–88.2 | 5.1–85.6 | 1.1–4.4 |
| Jonesie, $n$ = 217 | 3.5 | Arterial | 6.0 (SD 4) | 41.2 (SD 66.4) | 87 (SD 12) | 54 (SD 11) | 93.6 (SD 2.2) | 79.2 (SD 13.5) | 15.1 (SD 15.0) | 0.7 (SD 0.3) |
| Range | | | 1–22.0 | 5–348 | 66–116 | 23–76 | 88.6–97.1 | 26.4–91.8 | 0.7–71.9 | 0.2–1.5 |
| Grand mean | | | 10.7 (SD 5.9) | 106.7 (SD 117.8) | 60 (SD 25) | 22 (SD 15) | 79.1 (SD 15) | 28.0 (SD 25.5) | 65.3 (SD 29.0) | 1.8 (SD 1.1) |
| Grand range | | | 1–43.7 | 5–699 | 22–171 | 0–76 | 16.3–98.9 | 0–91.8 | 0.7–100 | 0.2–21.9 |

The mean (SD) and range are given for each seal. Minimum (Min) $So_2$ was determined at pH = 7.4 for dives <15 min, and pH = 7.3 for dives <15 min. Max, maximum.

### *$O_2$-Hb Dissociation Curve*

Complete $O_2$-Hb dissociation curves were determined with blood from 10 seals, with $P_{50}$ values obtained in an additional five seals. The $P_{50}$ was 30.5 mmHg at a pH of 7.4 (SD 1.2; $n$ = 15 seals) (Fig. 1). The $CO_2$ Bohr effect (slope of log $P_{50}$ vs. pH) was $-0.56$ ($y = -0.56x + 5.63$, $r^2 = 0.99$, $P < 0.0001$). The fixed acid Bohr effect (addition of HCl or lactic acid) was not significantly different from the $CO_2$ Bohr effect.

The resulting regression equations from the plots of $\log[So_2/(100 - So_2)]$ vs. $\log(Po_2)$, all saturation points, all seals combined, were: pH (7.4): $\log[So_2/(100 - So_2)] = 2.60211 \times \log(Po_2) - 3.84507$ ($n$ = 63, $r^2 = 0.99$, $P < 0.0001$); pH (7.3): $\log[So_2/(100 - So_2)] = 2.59256 \times \log(Po_2) - 3.97523$ ($n$ = 33, $r^2 = 0.98$, $P < 0.0001$); and pH (7.2): $\log[So_2/(100 - So_2)] = 2.622 \times \log(Po_2) - 4.11264$ ($n$ = 24, $r^2$ =0.98, $P < 0.0001$).

Samples from other species (*L. weddellii, P. vitulina, Z. californianus*) run for dissociation curve method validation agreed with previously published values (54, 55). Mixing technique verification tests confirmed that no hemolysis occurred while mixing blood samples in the syringes, based on clear plasma color in hematocrit tubes after centrifugation. The mean Hb concentration in this study [24.9 g/dl (SD 3.4), $n$ = 24] was equivalent to that of previous studies (45, 78). Oxygen content analyses performed with 100% $O_2$ saturated blood (Tucker Chamber) agreed within 1–2% of the maximal $O_2$ content calculated by the equation in this study, $O_2$ content (ml $O_2$/dl blood) = (1.34 ml $O_2$/g Hb) × [Hb](g/dl) × $So_2$ + (0.003 × $Po_2$), and within 4.5% of previously measured maximal $O_2$ content for this species (23).

### *$Po_2$ Profiles, $So_2$, and $O_2$ Depletion*

$Po_2$ profiles were obtained for 2,165 dives from 11 seals (extradural vein = 3 seals, 454 dives; hepatic sinus = 4 seals, 1,050 dives; arterial = 4 seals, 661 dives). The variability in the number of dives between seals is explained by the differences in their time spent at sea and, in some cases, breakage of electrodes or battery failure partway through the deployment.




Fig. 1. Oxygen-hemoglobin ($O_2$-Hb) dissociation curves from one seal at pH 7.4, 7.3, and 7.2. $Po_2$, partial pressure of oxygen.

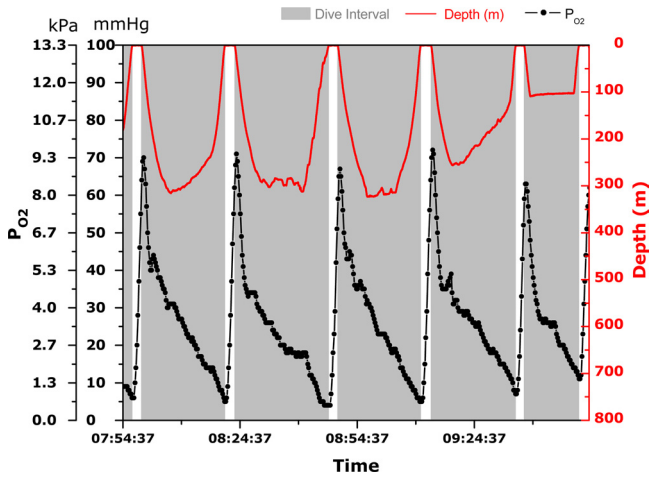


Fig. 2. Extradural vein $P_{O_2}$ and depth profiles of 2 h of diving from one seal (Starburst).

The shape of the $P_{O_2}$ profile was generally consistent among all seals in all three blood compartments. $P_{O_2}$ continued to increase (from the predive surface interval) at the start of the dive and then exhibited a progressive decline throughout the remainder of the dive (Figs. 2 and 3). The decrease of $P_{O_2}$ generally became more rapid coincident with the start of ascent, often most pronounced in the final 15–45 s of the dive (Fig. 2, 3). The decline in $P_{O_2}$ also continued briefly for ~5–25 s after the dive. After this brief postdive decline, $P_{O_2}$ increased quickly during the surface interval.

Minimum $P_{O_2}$, maximum $P_{O_2}$, and blood $O_2$ depletion rates during diving were significantly different between the three $P_{O_2}$ electrode sites (extradural vein, hepatic sinus, and arterial) (one-way ANOVA of seals grouped according to electrode site: $P < 0.001$ for all; $F$ values = 932.38, 1,298.76, 308.81, respectively) (Table 2). Minimum $P_{O_2}$ and minimum $S_{O_2}$ had a significant negative correlation with dive duration for all seals, while maximum $S_{O_2}$ had a significant positive correlation with dive duration for all but one seal (Spearman rank order correlation, Table 3). The $\%O_2$ content depleted (%total venous or arterial $O_2$ content depleted during the dive) had a significant positive correlation to dive duration in all seals, while the blood $O_2$ depletion rate had a significant negative correlation to dive duration in all but one seal (Spearman rank order correlation, Table 3).

The minimum $Pv_{O_2}$ in the extradural vein was ≤ 10 mmHg in 51% of dives (reaching as low as 2 mmHg), and ≤ 5 mmHg in 2% of dives (Table 2). In the hepatic sinus, minimum $Pv_{O_2}$ was ≤ 10 mmHg in 21% of dives and ≤ 5 mmHg in 9% of dives. Minimum $Pa_{O_2}$ was ≤ 30 mmHg in 46% of dives and ≤ 20 mmHg in 10% of dives. When only considering dives ≥ 10 min (routine dives for this species), minimum $Pa_{O_2}$ was ≤ 20 mmHg in 29% of dives.

Maximum $P_{O_2}$ (the peak value obtained during the dive), minimum $S_{O_2}$, maximum $S_{O_2}$, $\%O_2$ content depleted, and $O_2$ depletion rates are displayed in Tables 1 and 2. The depth and elapsed time (from dive start) at which maximum $P_{O_2}$ occurred was variable [extradural vein: mean = 35.8 m (SD 24.6) and 68 s (SD 31), range = 0.5–113.5 m and 1–326 s; hepatic sinus: mean = 68.5 m (SD 51.3) and 108 s (SD 66), range = 1–336.5 m and 3–757 s; arterial: mean = 20.0 m (SD 15.4), and 31 s (SD 13), range = 1–82.5 m and 1–96 s].

The average surface interval was 91 s (SD 48; $n$ = 2,093). Although the $P_{O_2}$ increased rapidly during the surface interval (Figs. 2 and 3), the time required for $P_{O_2}$ to return to an $Sv_{O_2}$ of 75% ($Pv_{O_2}$ = 46 mmHg) or 90% $Sa_{O_2}$ ($Pa_{O_2}$ = 70 mmHg) was variable. Furthermore, $P_{O_2}$ did not always reach this level during the surface interval or even during the next dive. For dives that did reach these $Sa_{O_2}$ and $Sv_{O_2}$ criteria, either during the surface interval or within the following dive (46% of dives for the extradural vein, 59% of dives for the hepatic sinus, and 86% of dives for the arterial side), the mean time to return to these $Sv_{O_2}$ and $Sa_{O_2}$ levels was 123 s (SD 23; range = 8–289 s) in the extradural vein, 149 s (SD 72; range = 1–695 s) in the hepatic sinus, and 82 s (SD 39; range = 1–356 s) for the artery.

### *$P_{O_2}$ Electrode Calibration and Verification*

Response of the $P_{O_2}$ electrode to a change in $O_2$ concentration is immediate. The near complete response time, as specified by the manufacturer and verified in this study and in Stockard et al. (81), is 60 s. Postdeployment verification tests of the probes revealed no significant changes in response time or between the original and postdeployment calibrations and no baseline drift.

## DISCUSSION

### *$P_{O_2}$ Profiles*

*$P_{O_2}$ and $S_{O_2}$ extremes.* The minimum $Pa_{O_2}$ values (12–23 mmHg) documented for elephant seals in this study correspond to routine $Sa_{O_2}$ of 8–26%, the lowest values ever measured in a freely diving seal (Table 1, Figs. 4 and 5). The extreme $P_{O_2}$ values in this study are less than that previously measured in free-diving Weddell seals (*L. weddellii*) (lowest $Pa_{O_2}$ = 18.2 mmHg, corresponding to 28% $Sa_{O_2}$) (72) and during sleep apnea of elephant seals (82). These exceptionally low values demonstrate remarkable hypoxemic tolerance for the elephant seal compared with most mammals. For example, shallow

Table 2. *Dive, $P_{O_2}$, $S_{O_2}$, and depletion rate data for seals grouped by $P_{O_2}$ electrode location (extradural vein, hepatic sinus, arterial)*

| $P_{O_2}$ Electrode Location | No. Seals/ Dives | Duration, min | Dive Depth, m | Max $P_{O_2}$, mmHg | Min $P_{O_2}$, mmHg | Max $S_{O_2}$, % | Min $S_{O_2}$, % | $\%O_2$ Content Depleted | Depletion Rate, ml $O_2 \cdot dl^{-1} \cdot min^{-1}$ |
|---|---|---|---|---|---|---|---|---|---|
| Extradural vein | 3/454 | 10.2 (SD 6.3) | 71.7 (SD 70.9) | 44 (SD 12) | 12 (SD 7) | 69.0 (SD 15.8) | 9.7 (SD 12.6) | 83.8 (SD 21.2) | 2.4 (SD 0.9) |
| Range | | 1–43.7 | 5–300 | 16–75 | 2–48 | 16.3–91.6 | 0.1–77.6 | 4.3–99.9 | 0.3–6.8 |
| Hepatic sinus | 4/1,050 | 12.0 (SD 5.8) | 139.0 (SD 135.9) | 50 (SD 16) | 17 (SD 8) | 74.6 (SD 13.3) | 19.6 (SD 16.0) | 72.2 (SD 23.1) | 1.8 (SD 1.3) |
| Range | | 1.0–29.3 | 5–699 | 22–124 | 0–40 | 30.8–97.6 | 0–67.8 | 6.0–100 | 0.2–21.9 |
| Arterial | 4/661 | 8.8 (SD 5.4) | 79.5 (SD 97.4) | 88 (SD 20) | 36 (SD 16) | 93.0 (SD 4) | 53.9 (SD 24.5) | 41.7 (SD 26.8) | 1.5 (SD 0.7) |
| Range | | 1–30.5 | 5–474 | 45–171 | 12–76 | 74.1–98.9 | 8.4–91.8 | 0.7–91.0 | 0.2–4.4 |

The mean (SD) and range are given for each location.

Table 3. *Spearman rank order correlation test data*

| Seal | No. Dives | Min $P_{O_2}$/Duration | | Min $S_{O_2}$/Duration | | Max $S_{O_2}$/Duration | | %$O_2$ Depleted/Duration | | Depletion Rate/Duration | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Spearman *R* | *P* | Spearman *R* | *P* | Spearman *R* | *P* | Spearman *R* | *P* | Spearman *R* | *P* |
| Chick | 267 | −0.809 | <0.001 | −0.825 | <0.001 | 0.616 | <0.001 | 0.894 | <0.001 | −0.679 | <0.001 |
| Starburst | 106 | −0.298 | 0.002 | −0.490 | <0.001 | 0.674 | <0.001 | 0.602 | <0.001 | −0.933 | <0.001 |
| Patty | 81 | −0.720 | <0.001 | −0.743 | <0.001 | 0.737 | <0.001 | 0.826 | <0.001 | −0.844 | <0.001 |
| Bodil | 192 | −0.793 | <0.001 | −0.868 | <0.001 | 0.584 | <0.001 | 0.881 | <0.001 | −0.076 | 0.297 |
| Larry | 218 | −0.392 | <0.001 | −0.456 | <0.001 | 0.464 | <0.001 | 0.537 | <0.001 | −0.485 | <0.001 |
| Per | 160 | −0.715 | <0.001 | −0.716 | <0.001 | 0.120 | 0.130 | 0.779 | <0.001 | −0.719 | <0.001 |
| Roberta | 480 | −0.869 | <0.001 | −0.881 | <0.001 | 0.447 | <0.001 | 0.903 | <0.001 | −0.929 | <0.001 |
| Jerry | 132 | −0.937 | <0.001 | −0.938 | <0.001 | 0.826 | <0.001 | 0.951 | <0.001 | −0.596 | <0.001 |
| Sammy | 70 | −0.707 | <0.001 | −0.721 | <0.001 | 0.726 | <0.001 | 0.772 | <0.001 | −0.375 | <0.001 |
| Knut | 242 | −0.898 | <0.001 | −0.912 | <0.001 | 0.825 | <0.001 | 0.922 | <0.001 | −0.685 | <0.001 |
| Jonesie | 217 | −0.838 | <0.001 | −0.838 | <0.001 | 0.576 | <0.001 | 0.883 | <0.001 | 0.255 | <0.001 |

Shading indicates the only 2 instances in which there was no significant correlation between the 2 variables.

water blackout occurs in free-diving humans near 25 mmHg (17), and a $Pa_{O_2}$ of 19.1 mmHg, the lowest reported human arterial value, was recently measured in a climber breathing ambient air near the summit of Mt. Everest (28). This $Pa_{O_2}$ of 19.1 mmHg corresponds to an $Sa_{O_2}$ of 34.4% in the climber (28), due to hyperventilation and respiratory alkalosis in humans at this altitude. In diving elephant seals, however, a $Pa_{O_2}$ of 19.1 mmHg corresponds to an $Sa_{O_2}$ of only 18%, and $Pa_{O_2}$ as low as 12 mmHg (8% $Sa_{O_2}$ at pH = 7.4, 6% $Sa_{O_2}$ at pH = 7.3) was measured in this study. Indeed, the minimum $Pa_{O_2}$ of 12 mmHg from this study is nearly equivalent to the "critical $Pa_{O_2}$" (10 mmHg) of harbor seals and Weddell seals in forced submersion studies, as defined by EEG criteria marking the threshold of cerebral dysfunction (22, 39). A value of 8% $Sa_{O_2}$ corresponds to arterial $O_2$ content of only 2.7 ml $O_2$/dl blood in the diving elephant seal, while the limit of human tolerance on Mt. Everest corresponds to 9 ml $O_2$/dl blood. Thus, not only are elephant seals highly tolerant of a low driving force for $O_2$ in the blood ($P_{O_2}$), they are also much more tolerant of low $O_2$ content in the blood.

Minimum $Pv_{O_2}$ values in this study (2–10 mmHg) correspond to routine $Sv_{O_2}$ values of 0–4%, equivalent to (and even less than in some cases) that reached during extreme forced submersions. Again, these venous values are as low as the critical cerebral $Pv_{O_2}$ for seals, demonstrated by EEG criteria (39). In comparison, the $Pv_{O_2}$ of blood draining mammalian muscle working at maximal $O_2$ consumption is as high as 20 mmHg (76, 83), and minimum $Pv_{O_2}$ values of elephant seals in this study are even lower than the well-documented hypoxemic extremes of horses performing strenuous exercise (6, 57). These arterial and venous results support our hypothesis that elephant seals routinely tolerate extreme hypoxemia during dives to completely utilize the blood $O_2$ store and maximize aerobic dive duration.

### *Implications for Gas Exchange*

The increase in $Pa_{O_2}$ during the initial phase of descent (Fig. 3*C*) is consistent with the maintenance of pulmonary gas exchange, and the transfer of $O_2$ from the lungs to the blood at depths that are shallower than that at which lung collapse or cessation of gas exchange is predicted to occur (8, 24, 25, 46, 48). Maximum $Pa_{O_2}$ values in this study were slightly lower than that of a previous study on Weddell seals [max $Pa_{O_2}$ = 171 mmHg in this study and 232 mmHg for the Weddell seal (72)]. In part, this difference in absolute values may be due to the response time of the $P_{O_2}$ electrode. Although lower $Pa_{O_2}$ values might indicate a shallower depth of "lung collapse" in the present study, the absolute value of $Pa_{O_2}$ will be a function not only of ambient pressure, but also of the $O_2$ fraction in the lung, ventilation perfusion matching, and the degree of the pulmonary shunt (24, 25, 46, 47). All things considered for these four variables, the ambient pressure (depth) for the peak $Pa_{O_2}$ value is probably the best and most reliable estimate of the depth of lung collapse during dives in the wild.

The depth at which the maximum $Pa_{O_2}$ occurred was highly variable. If the depth at which peak $Pa_{O_2}$ occurs is indicative of the minimum depth at which there is cessation of gas exchange, then gas exchange does not always cease at the 30- to 50-m depth suggested by prior studies (25, 46). These findings are more likely consistent with a wider range of depths at which lung collapse could occur (24, 48). This may be secondary to differences in diving lung volume. Considerable variability in diving lung volume has been demonstrated in simulated dives of harbor seals and sea lions (48). One of the seals in the present study (Jonesie) had generally higher values of minimum $Sa_{O_2}$ and consistently high maximum $Sa_{O_2}$ (and consequently a lower percentage of arterial $O_2$ content depletion during dives) than other seals, even for dives of similar durations and depths [mean minimum $P_{O_2}$ = 54 mmHg (SD 11); mean maximum $P_{O_2}$ = 87 mmHg (SD 12), Table 1]. It is possible that this seal dived with a larger lung volume than the other seals. A larger lung volume should lead to increased $P_{O_2}$ in the blood, especially during shallow dives in which the lungs have not undergone collapse.

### *Implications for Blood Flow*

*Arteriovenous shunts and the spleen.* $Pv_{O_2}$ increased during the early phase of the dive (Figs. 2 and 3). This is similar to previous findings in diving emperor penguins (*Aptenodytes forsteri*) (70). The magnitude of the increase in $Pv_{O_2}$ was variable, but at times reflected arterial values. This was especially evident in some dives of one seal (Roberta, Table 1), where $Pv_{O_2}$ reached 124 mmHg in the hepatic sinus. Such high $Pv_{O_2}$ values are not consistent with blood extraction by tissues and are, indeed, indicative of arterialized blood and potential use of an arteriovenous shunt. Shunting could potentially occur in the well-described arteriovenous anastamoses in the skin (11) or perhaps through splenic blood vessels, although the spleen itself is considered to be contracted during diving (37, 85). The spleen of the phocid seal has been proposed to act as a "scuba tank" and significantly

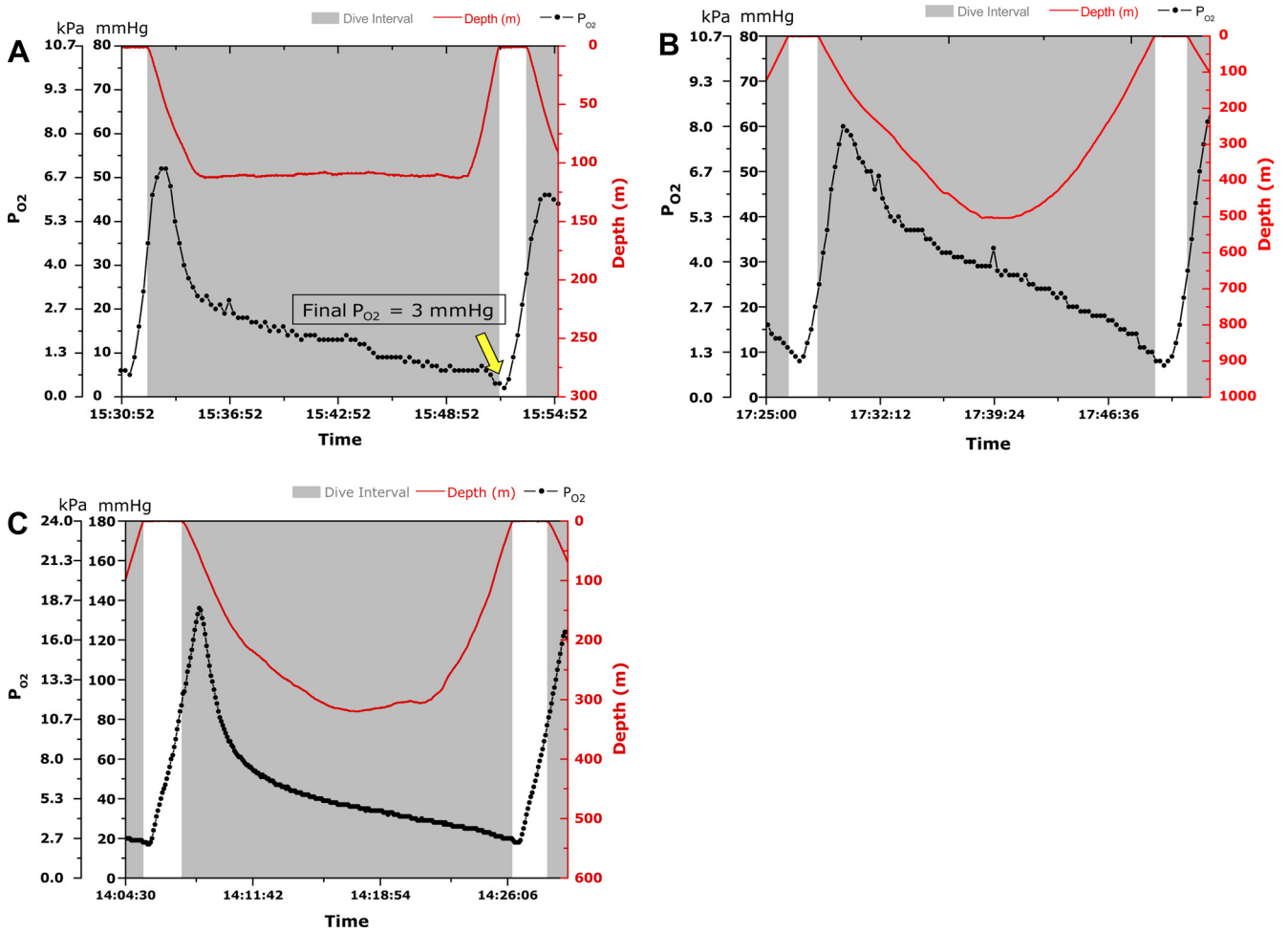


Fig. 3. $P_{O_2}$ and depth profile of individual seal's dives of ~20 min in duration from Chick ($P_{O_2}$ electrode in the extradural vein; *A*), Larry ($P_{O_2}$ electrode in the hepatic sinus; *B*), and Jerry ($P_{O_2}$ electrode in the brachial artery; *C*). Note that *y*-axis scales are different between the venous and arterial graphs to best display the data in each graph.

increase Hb concentration (and $O_2$ content) during dives, but the magnitude of such an effect in elephant seals is probably minimal due to the insufficient amount of time for splenic expansion during the very short surface intervals of these seals (37, 72, 85). In addition, this scuba tank effect would only be seen in the first dive of a bout when the spleen first contracts.

*Muscle blood flow.* Increases in $Pv_{O_2}$ during the initial period of descent are also consistent with a lack of blood flow to muscle. The initial descent consists of a period of continuous stroking for 30–200 s when the elephant seal must overcome its positive buoyancy (92). This range of time overlaps completely with the time in which $P_{O_2}$ continues to increase toward the maximum $P_{O_2}$ during the dive [mean time to maximum $P_{O_2}$ = 31 s (SD 13) in the arterial system, 68 s (SD 31) in the extradural vein, and 108 s (SD 66) in the hepatic sinus]. If active locomotory muscles were being perfused during this period of high activity, $Pv_{O_2}$ would be expected to decline due to $O_2$ extraction, not to increase as we observed here (Figs. 2 and 3). One might consider the subsequent decline in $Pv_{O_2}$ during the later part of descent as evidence for a resumption of muscle blood flow and muscle $O_2$ extraction, especially in the extradural vein since it drains the paraspinal muscles. During this phase of the dive, however, near-infrared spectroscopy studies in Weddell seals provided no evidence of routine muscle $O_2$ resaturation, which would be expected if muscle blood flow resumed (30). In summary, these $Pv_{O_2}$ and $Sv_{O_2}$ data do not provide evidence for maintenance of muscle blood flow during the descent phase of the dive, and therefore have major implications for modeling convective $O_2$ transport in seals (18, 19).

*Extradural vein flow.* Minimum $P_{O_2}$ in the extradural vein reached values between 2 and 10 mmHg routinely in all seals (Table 2). These final $P_{O_2}$ values were generally lower than those in the hepatic sinus, perhaps reflecting flow patterns and return of more desaturated blood within the extradural vein. This could include blood flow from the intracranial drainage and paraspinal muscles (31, 39). If there is an increase in muscle blood flow and delivery of $O_2$ to muscle at the end of the dive coincident with the ascent tachycardia in these animals (5), the $Pv_{O_2}$ and $Sv_{O_2}$ of the extradural vein blood that has drained locomotory muscle would reflect this increased $O_2$ extraction. Because minimum $Pv_{O_2}$, maximum $Pv_{O_2}$, and blood $O_2$ depletion rates were significantly different between the extradural vein and hepatic sinus, it does not seem justified to conclude that the extradural vein can be considered representative of either the entire venous system or the hepatic sinus. With the drainage of blood from the brain and other tissues into

this vein, the extradural vein may be indicative of how low venous $O_2$ decreases in the peripheral veins, but not in the central hepatic sinus venous reservoir during diving.

*Ascent tachycardia and tissue perfusion.* The rate of $Pv_{O_2}$ (and often $Pa_{O_2}$) and $Sv_{O_2}$ decline generally became more rapid coincident with the start of ascent (Figs. 2, 3, and 5), often becoming even steeper in the final 15–45 s of the dive. The declines in $Pv_{O_2}$ are concurrent with the increase in heart rate which occurs during ascent in this species (5). This ascent or "anticipatory" tachycardia is characterized by a gradual increase in heart rate upon ascent; the rate of increase becomes most pronounced in the last 15 s of the dive. It has been hypothesized to increase blood flow and $O_2$ delivery to depleted tissues, thereby lowering the $P_{O_2}$ in the blood and providing a larger gradient to maximize $O_2$ uptake at the surface (84). Such a function for the ascent tachycardia would be consistent with the $Pv_{O_2}$ profile. This decline in $Pv_{O_2}$ during the ascent is also congruent with the continuous stroking that occurs during the initial ascent in dives of elephant seals (92). Thus, the steeper decline of $Pv_{O_2}$ during ascent supports the concept of blood flow to locomotory muscle and muscle $O_2$ uptake during this period of intense stroking. Blood flow to muscle during this period also agrees with previous studies of Weddell seals that demonstrated the temperature of the primary locomotory muscle does not increase during diving, that myoglobin is not completely depleted of $O_2$ in the muscle during diving, and that plasma lactate concentration is slightly increased near the end of extended dives (29, 30, 64).

Alternatively, because gas exchange has presumably resumed at shallower depths, the steeper decrease in $Pa_{O_2}$, and subsequently $Pv_{O_2}$ in the final 15–45 s of the dive might also reflect the decline in alveolar $P_{O_2}$ due to the large decreases in ambient pressure during the final phase of ascent. In addition, if alveolar $P_{O_2}$ during final ascent were less than $Pv_{O_2}$, $O_2$ would diffuse from the lung capillary into the alveolus and result in a more rapid decline in $Pa_{O_2}$ (35, 50).

The continued decline in $P_{O_2}$ after the end of the dive is likely explained by a combination of: *1*) the response time of the $P_{O_2}$ electrode (81), *2*) the sampling rate of the $P_{O_2}$ electrode (once every 5- or 15-s, depending on the recorder), *3*) the lag time in circulation from the heart through the arterial system and into the veins, *4*) a delay in inhalation due to presumed maximal expiration that immediately follows the dive, and *5*) increased blood $O_2$ extraction by previously ischemic tissues. In a study of $Sa_{O_2}$ during apnea in humans, this continual decline occurred until ~30 s after breathing resumed (1), compared with ~5–25 s after the dives of the elephant seals in this study. A similar decline in $Pv_{O_2}$ that continued into the postdive period was also observed in diving emperor penguins (70).

### *Implications for Blood $O_2$ Store Management*

*Hypoxemic tolerance and the hepatic sinus-blood $O_2$ reservoir.* There was considerable overlap between minimum hepatic sinus $Sv_{O_2}$ values and minimum $Sa_{O_2}$ values in this study (Fig. 4), with the exception of one of the seals with a hepatic sinus electrode (Roberta). Arterial and venous blood $O_2$ equilibration was expected due to the diminishing differences in arterial and venous $O_2$ contents in a breath-hold diver with collapsed lungs and is similar to results from previous forced submersion and sleep apnea studies (23, 82).

Despite the existing overlap of arterial and hepatic sinus $P_{O_2}$ values, minimum $Pa_{O_2}$ and $Sa_{O_2}$ values at the end of the dive were slightly higher than those in the hepatic sinus (Fig. 4, Table 2). The mean of minimum $Pa_{O_2}$ was also significantly different from the $Pv_{O_2}$ in the hepatic sinus. These slightly elevated values on the arterial side could reflect resumption of lung gas exchange and $O_2$ uptake from the lungs during the final phase of the seal's ascent. Increased perfusion of the lung secondary to the ascent tachycardia could enhance the potential effect of the resumption of gas exchange in maintaining higher $Pa_{O_2}$ at the end of the dive. As discussed previously, this hypothesis assumes that some lung $O_2$ was preserved during the dive due to the cessation of gas exchange at depth. This could serve to increase the arterial $O_2$ content at the end of the dive, preventing the $P_{O_2}$ from dropping too low and mitigating the risk of shallow water blackout (17).

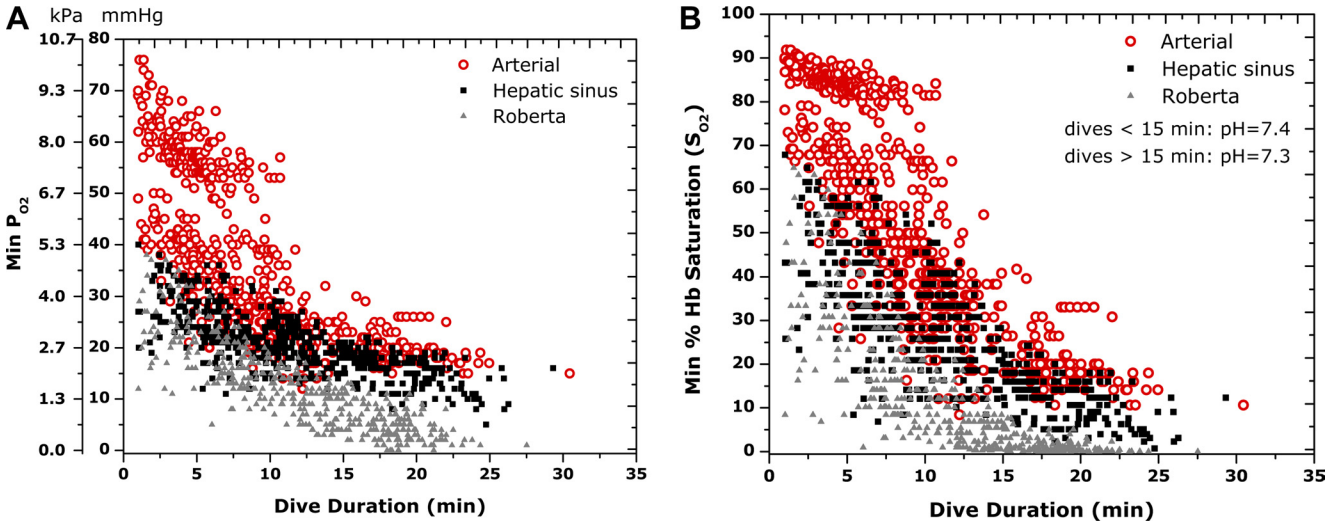


Fig. 4. Minimum $P_{O_2}$ during the dive vs. dive duration in seals with electrodes in the hepatic sinus and in the artery (*A*) and calculated $S_{O_2}$ (*B*) for these dives. Note the overlap of hepatic sinus and arterial minimum $S_{O_2}$ values with the exception of lower values in one seal with an electrode in the hepatic sinus (Roberta). Minimum $S_{O_2}$ was determined at pH = 7.4 for dives < 15 min, and pH = 7.3 for dives > 15 min. Minimum $S_{O_2}$ had a significant negative correlation with dive duration in all seals (Spearman rank order correlation, see Table 3).

The small differences observed between $Pa_{O_2}$ and hepatic sinus $Pv_{O_2}$, however, may simply be secondary to variation among different dives by different seals. If $Pa_{O_2}$ and hepatic sinus $Pv_{O_2}$ do indeed equalize during dives, then the hepatic sinus $Pv_{O_2}$ profiles of Roberta (appropriately named after Robert Elsner) reflect arterial values, and demonstrate extreme in vivo cerebral hypoxemic tolerance in the elephant seal. Many of the hepatic sinus $Pv_{O_2}$ values in this seal often approached 0, and were far less than 10 mmHg, the threshold for electroencephalographic (EEG) changes indicative of hypoxemic brain damage (39).

*Surface interval.* As expected from hyperventilation and tachycardia during the surface interval (2, 5), the $P_{O_2}/S_{O_2}$ increased rapidly during this period (Figs. 2, 3, and 5). These surface interval $P_{O_2}/S_{O_2}$ data are similar to $Pa_{O_2}$ values obtained by blood samples during the recovery period in previous studies of Weddell seals (49, 72). The time required for $P_{O_2}$ to return to representative arterial and venous values (90% $Sa_{O_2}$ and 75% $Sv_{O_2}$) was variable and did not always reach this level during the surface interval or even during the next dive. The continued increase in $P_{O_2}$ during the initial descent emphasizes the importance of moderate heart rates and continued gas exchange during this early portion of the dive (5, 69). These resaturation times may reflect the time necessary to recover from the frequently low $P_{O_2}/S_{O_2}$ values at the end of the dive and, especially in the venous system, may be secondary to the circulatory lag time and the reloading of $O_2$ in tissues as previously discussed.

The mean time for $Pa_{O_2}$ to reach 70 mmHg (90% saturation) in these elephant seals was 82 s. In Weddell seals, end-tidal $P_{O_2}$ of the first postdive exhalation, which presumably approximates the end-of-dive $Pa_{O_2}$, declined significantly with dive duration, reaching a $P_{O_2}$ of 14 mmHg after a 32-min dive (44, 63). Although end-tidal $P_{O_2}$ increased greatly during the second breath, the time required for end-tidal $P_{O_2}$ to reach 60 mmHg after long dives (>20 min) of Weddell seals (63) ranged from 0.8 to 2.5 min, similar to the recovery time for $Pa_{O_2}$ in the present study. In comparison, the lowest mean end-tidal $P_{O_2}$ values in grey seals and harbor porpoises were relatively high, at ~70 mmHg after mean dive durations of < 4 min and 1 min, respectively (9, 74). In these previous studies, Reed et al. (74) found that end-tidal $P_{O_2}$ returned to baseline within the mean 0.8-min surface interval, and Boutilier et al. (9) hypothesized that reoxygenation of arterial blood occurs rapidly, within the first three or four breaths postdive. These end-tidal $P_{O_2}$ findings contrast with the surface interval $Pa_{O_2}$ profiles in the present study and with the surface interval blood sample $P_{O_2}$ data from Weddell seals in prior studies (49, 72).

There are likely several reasons why end-tidal $P_{O_2}$ values during the surface interval may not accurately reflect $Pa_{O_2}$. During the surface interval, ventilation, heart rate, and cardiac output are at their peak in elephant seals (2, 5, 44, 69). In addition, mixed $Pv_{O_2}$ is probably extremely low as reflected by our extradural vein and hepatic sinus $P_{O_2}$ data. The surface interval is a period of maximum oxygen uptake for the seal, analogous in many ways to exercise at maximum $O_2$ consumption for an athlete (89). Exercise-induced hypoxemia with a widening of the alveolar-arterial $P_{O_2}$ difference to as much as 30 mmHg frequently occurs under such conditions (89). This relative hypoxemia is considered secondary to *1*) diffusion limitation due to increased cardiac output and decreased pulmonary transit time, *2*) ventilation-perfusion inequality, and *3*) intra- and extrapulmonary shunts (36, 75, 89). With low mixed $Pv_{O_2}$, any shunt of venous blood to the arterial side will lower the $Pa_{O_2}$ and contribute further to arterial hypoxemia (89). Although shunting constituted only 0.5% of cardiac output in exercising athletes (88), the shunt in harbor seals prior to submersion was 8% (48). Therefore, we hypothesize that all of these factors (diffusion limitation, ventilation-perfusion inequality, shunting, extremely low mixed $Pv_{O_2}$) contribute to widening of the alveolar-arterial $P_{O_2}$ difference in seals during the surface interval. This hypothesis would account for the differences between prior end-tidal $P_{O_2}$ profiles and the $Pa_{O_2}$ profiles during the surface interval in this study. In addition, after repeated deep dives, Weddell seals often cough up copious foamy secretions (presumably surfactant) during surface intervals (P. J. Ponganis, unpublished observation). Some impairment of gas exchange may occur prior to clearance of these secretions from the airway in these deep divers.

### *$O_2$-Hb Dissociation Curve*

The $O_2$-Hb dissociation curve for the elephant seal (Fig. 1) is similar to that of other pinnipeds and equivalent to the $P_{50}$ quoted for this species from previously unpublished observations listed in a book chapter (54–56). These data are also in agreement with the theory that long-duration divers like many pinnipeds may benefit from Hb with relatively lower $O_2$ affinity (slightly lower than terrestrial animals and shorter-duration divers) (54, 79). Since these species generally dive upon expiration and the lungs do not comprise a significant portion of total $O_2$ stores, lower affinity Hb could promote diffusion of $O_2$ into the tissues to enhance tolerance to apnea. As expected, the Bohr effect was similar to that of other pinnipeds (56, 73, 79, 91).

The protocols used in this study cover the range of $P_{CO_2}$ and pH values found in previous studies of diving Weddell seals (72). Since the blood pH changes in free-diving Weddell seals are most often secondary to elevations in $P_{CO_2}$ (a respiratory acidosis) and because no difference was seen in the Bohr effect between fixed acid and $CO_2$ for the elephant seals in this study, the protocol used should be sufficient for calculations of $S_{O_2}$ from $P_{O_2}$. Most importantly, based on these arguments, the whole blood tonometry protocols for these $O_2$-Hb dissociation curve studies should mimic the external blood environment (i.e., the range of pH, $P_{CO_2}$, and $P_{O_2}$ values) of red blood cells during diving. Since the concentration of 2,3-DPG, a modulator of Hb affinity, is affected by changes in $S_{O_2}$ and intracellular pH, the tonometry protocol should cause similar intracellular changes in 2,3-DPG concentration.

### *$S_{O_2}$ Profiles*

*Implications to $O_2$ store utilization.* The maximum $Sv_{O_2}$ was highly variable both between different seals and between dives of individual seals, indicating differences in $O_2$ loading during the surface intervals preceding dives (Tables 1 and 2; Fig. 5). It should also be noted that maximum saturation values in this study were likely slightly underestimated, due to the lack of determination of a true, critical $P_{O_2}$ value at 100% $S_{O_2}$. To ensure complete saturation in the tonometry of the 100% $O_2$-saturated blood for $O_2$-Hb dissociation curve determination, a high $P_{O_2}$ (>200 mmHg) was used for that sample. Thus, the actual inflection point in $P_{O_2}$ at which saturation first

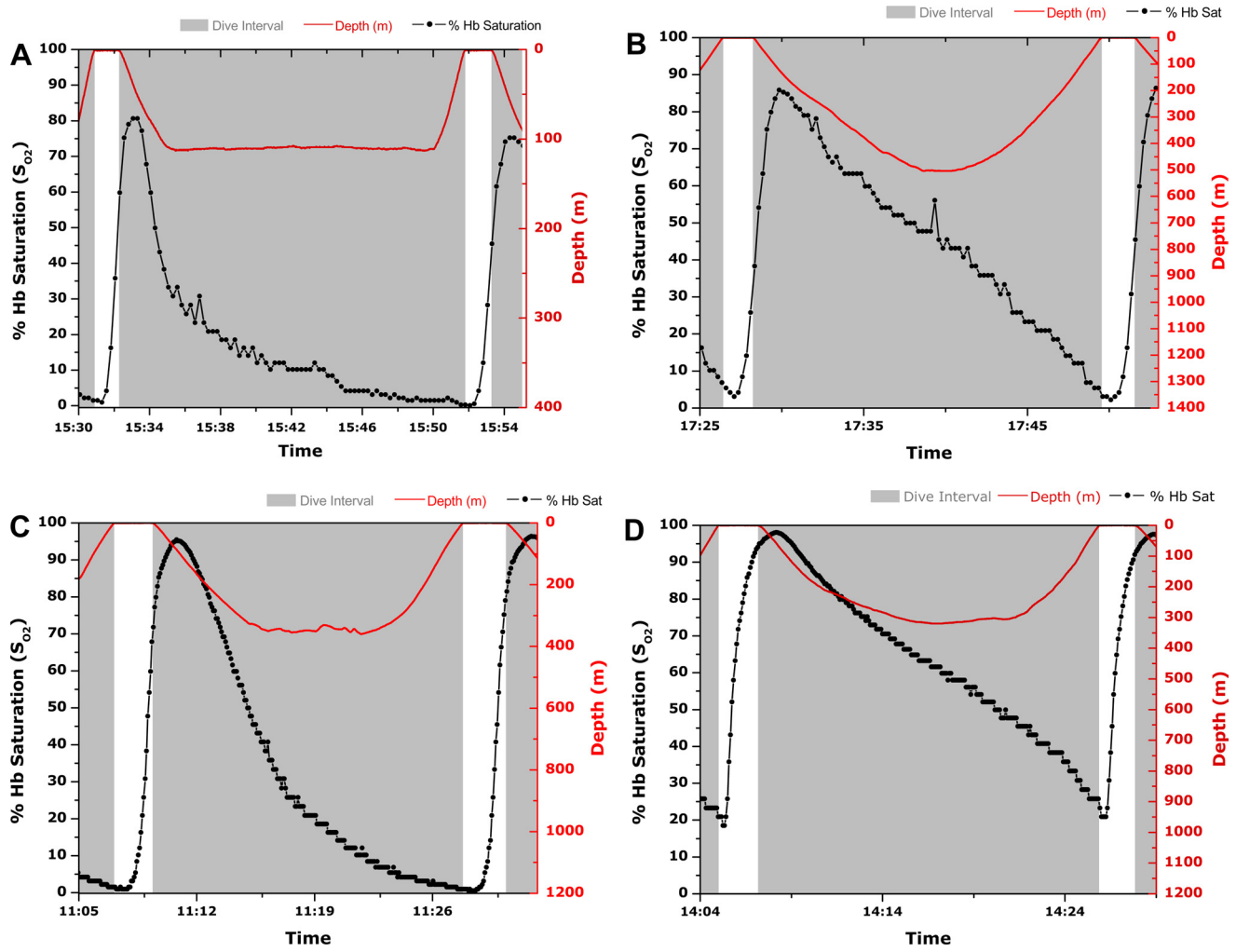


Fig. 5. Decline in $S_{O_2}$ during individual dives for Chick (extradural vein; *A*), Larry (hepatic sinus; *B*), Roberta (the seal whose maximum $Pv_{O_2}$ was higher and minimum $P_{O_2}$ lower in the hepatic sinus than other seals; *C*), and Jerry (arterial; *D*). The $S_{O_2}$ was determined at pH = 7.4 throughout the entire dive to maintain consistency and to provide a conservative estimate of continuous $S_{O_2}$.

reaches 100% was not determined. The lack of this data point in the regression equations results in a slightly lower calculated $S_{O_2}$ for $P_{O_2}$ values that approach the inflection point of 100% $S_{O_2}$. In turn, this implies that net depletion values and $O_2$ depletion rates are slightly underestimated.

Particularly high maximum $Sv_{O_2}$ in the hepatic sinus (almost 100%) was evident in one of the seals (Roberta) (Table 1, Fig. 5*C*). This individual was also the seal with extremely low minimum $P_{O_2}$ and $S_{O_2}$ in the hepatic sinus (Table 1, Fig. 4). Not only was the blood $O_2$ store maximized in this seal by the arterialization of its venous blood, but it made full use of the large $O_2$ store by decreasing its $P_{O_2}$ to near zero. This pattern of maximal $O_2$ loading and depletion may be due to a difference in the diving behavior of this animal. This seal remained at sea for an extended amount of time (15 days), performing dives indicative of foraging as opposed to more typical behavior of a direct route of transit dives across the bay and back to the colony. A complete analysis of dive type and behavior is beyond the scope of this report, but would serve to reveal such differences in $O_2$ store loading and utilization between different dive types.

In general, the elephant seal is almost completely depleting its blood $O_2$ stores during routine dives, with net $O_2$ content depletion values as high as 91 and 100% (arterial and venous, respectively) (Table 2). This highly efficient use of available $O_2$ stores is much greater than that previously predicted by models or measured in studies of sleep apnea. For example, in a computer model of $O_2$ transport in Weddell seals, the end-of-dive limits of arterial and venous blood $O_2$ depletion were 38 and 27%, respectively (18). In a typical 7-min sleep apnea of a young elephant seal, with a final $P_{O_2}$ near 25 mmHg, only 56% of the blood $O_2$ store was utilized (82). Oxygen depletion data from this study have implications to a variety of topics in diving physiology and physiological ecology, particularly those which rely on accurate estimates of the extent of usable $O_2$ stores and will provide valuable input for recent modeling studies focused on diving animals (18, 24). As in the above case of the seal making full use of its venous $O_2$ store, the true maximum value for the blood $O_2$ store may be the entire blood volume with completely oxygenated Hb.

### *Blood $O_2$ Store Depletion*

*Blood $O_2$ depletion rates.* It is realized that the lack of simultaneous $P_{O_2}$ data from more than one site in the same seal

is a potential criticism in the calculation of blood $O_2$ store depletion rates, but this was not feasible due to the technical complexities of electrode placement and concerns regarding animal welfare. The mean $\%O_2$ content depletion for dives in this study ranged from 42% (SD 27) (arterial) to 72% (SD 23) (hepatic sinus) and 84% (SD 21) (extradural vein) (Table 2). These changes in $O_2$ content corresponded to blood $O_2$ depletion rates from 1.5 ml $O_2 \cdot dl^{-1} \cdot min^{-1}$ (SD 0.6) (arterial) to 1.8 (SD 1.3) (hepatic sinus) and 2.4 (SD 0.9) (extradural vein). One seal, Jonesie, had minimum $Sa_{O_2}$ values that were consistently higher than the other seals. Consequently, this seal had a blood $O_2$ depletion rate that was less than one-half the rate of the other seals with arterial electrodes (Table 1). The mean arterial blood $O_2$ depletion rate (1.5 ml $O_2 \cdot dl^{-1} \cdot min^{-1}$) is 35% lower (or if Jonesie's data are excluded from the arterial group, 20% lower) than that measured during sleep apnea of elephant seals (2.3 ml $O_2 \cdot dl^{-1} \cdot min^{-1}$) (82) but greater than that measured during forced submersions of this species (1.3 ml $O_2 \cdot dl^{-1} \cdot min^{-1}$) (20, 23) (Tables 1 and 2). Qvist et al. (72) documented an even lower arterial $O_2$ depletion rate (0.8 ml $O_2 \cdot dl^{-1} \cdot min^{-1}$) in diving Weddell seals. Venous $O_2$ depletion rates in this study were similar to that during sleep apnea (2 ml $O_2 \cdot dl^{-1} \cdot min^{-1}$) (82) and were about two times the rate of venous $O_2$ depletion during forced submersions (1.0 ml $O_2 \cdot dl^{-1} \cdot min^{-1}$) (20, 23). These data are consistent with the facts that: *1*) mean heart rate during sleep apnea on land is not significantly different from that during diving on the continental shelf for this species (5), *2*) cardiac output during sleep apnea is maintained at resting levels (69), *3*) cardiac output and heart rate in submerged swimming harbor seals are essentially equivalent to those at rest (67), and *4*) a more dramatic bradycardia (and reduced cardiac output) is associated with forced submersion (20).

### *Blood $O_2$ Contribution to Metabolic Rate During the Breath Hold*

Since the blood volume of juvenile elephant seals is known [216 ml/kg (SD 28)] (78, 86), a calculation of the contribution of the blood $O_2$ store to total metabolic rate while diving can be made: blood $O_2$ store contribution (ml $O_2 \cdot kg^{-1} \cdot min^{-1}$) = [(maximum $O_2$ content − minimum $O_2$ content)/dive duration] × blood volume, with the assumption that one-third of blood volume is arterial and two-thirds venous in distribution (42). Using the example of Roberta, the seal that demonstrated optimum loading and utilization of the blood $O_2$ store during diving, the mean value of the contribution of her venous blood $O_2$ store alone to the $O_2$ needs of her dives was 3.4 ml $O_2 \cdot kg^{-1} \cdot min^{-1}$ (mean of calculated blood $O_2$ store contribution for all dives of this seal. This represents the venous $O_2$ store contribution only as this seal had an electrode in the hepatic sinus). This value is equivalent to: *1*) 76% of the resting metabolic rate of a juvenile elephant seal, quietly breathing at the water's surface (also 76% of the diving metabolic rate for an adult Weddell seal) (63, 90); *2*) the metabolic rate of a juvenile elephant seal diving in a metabolic chamber (90); and *3*) 117% of the allometrically predicted basal metabolic rate for an elephant seal of this mass (41).

In examining individual dives of this seal, the contribution of the venous blood $O_2$ store to metabolic rate is *1*) 3.6 ml $O_2 \cdot kg^{-1} \cdot min^{-1}$ in a 7-min dive, *2*) 3.0 ml $O_2 \cdot kg^{-1} \cdot min^{-1}$ in a 12.2-min dive, and *3*) 1.7 ml $O_2 \cdot kg^{-1} \cdot min^{-1}$ during an extended dive of 27.5 min. Even for dives equivalent to the mean dive duration for this seal (12.2 min), the contribution of the venous blood $O_2$ store toward total metabolic rate is > 100% of the allometrically predicted basal metabolic rate. Since the venous blood $O_2$ store contribution alone to metabolic rate (even without considering the arterial $O_2$ store or the significant muscle $O_2$ store for this animal) represents such a large percentage of resting metabolic rate and because mean temperature during dives remained near 37°C, these data suggest that at the level of the whole animal, juvenile elephant seals are not "hypometabolic" while diving and that they do not require any significant anaerobic metabolism during routine dives. Comparatively, during a typical 7-min breath hold during sleep apnea of an elephant seal, blood $O_2$ depletion (arterial and venous) contributed 4.2 ml $O_2 \cdot kg^{-1} \cdot min^{-1}$ to total body metabolic rate during apnea (82). The range of blood $O_2$ depletion values in this study reinforce the fact that cardiovascular adjustments and, consequently, $O_2$ depletion during routine dives are intermediate between those of sleep apnea and forced submersion.

### *Perspectives and Significance*

These blood $O_2$ data are the first ever measured in a truly free-diving seal with unrestrained access to the surface. The results of this study indicate that elephant seals possess exceptional hypoxemic tolerance. In dives > 10 min (routine dives for an elephant seal), $Pv_{O_2}$ declined to as low as 2–10 mmHg, and $Pa_{O_2}$ decreased to between 12 and 23 mmHg in all seals. These values correspond to $So_2$ values of 1–26% and $O_2$ contents as low as 0.3 ml $O_2$/dl blood (venous) and 2.7 ml $O_2$/dl blood (arterial). The underlying mechanisms of this extreme hypoxemic tolerance remain to be revealed, but may include intrinsic neuronal tolerance as demonstrated in the hooded seal (*Cystophora cristata*) (27), increased brain capillarization (39), increased glycogen content in the brain and/or heart (32, 40), alterations in reactive $O_2$ species production or scavenging systems (21), increased tissue buffering capacity (15), or potential roles of the neurally located neuroglobin or more widespread cytoglobin $O_2$-binding proteins (12). The elucidation of these mechanisms may also provide applications relevant to enhancing the survival of humans experiencing circulatory collapse, ischemic injury, and other trauma.

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