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UNIVERSITY OF CALIFORNIA, SAN DIEGO

Blood oxygen transport and depletion: the key of consummate divers

A dissertation submitted in partial satisfaction of the

requirements for the degree Doctor of Philosophy

in

Marine Biology

by

Jessica Ulrika Meir

Committee in charge:

Paul J. Ponganis, Chair
Lisa Ballance
Gerald Kooyman
Frank Powell
Terrie Williams

2009

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The dissertation of Jessica Ulrika Meir is approved, and it is acceptable in the quality and form for publication on microfilm and electronically:

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Chair

University of California, San Diego

2009

DEDICATION

This thesis is dedicated to

Gerald “Jerry” Kooyman, consummate inspiration

EPIGRAPH

"For a large number of problems there will be some animal of choice, or a few such animals, on which it can be most conveniently studied."

- August Krogh -

13th International Physiological Congress
Boston (August, 1929)

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ACKNOWLEDGEMENTS

The magnitude of work underlying a doctoral dissertation, particularly that involving field work in isolated and remote locations and large, and protected charismatic mega fauna, can certainly not be accomplished alone. I have received an abundance of assistance and support along the way, without which this work would never have come to fruition. Most importantly, I would like to acknowledge my advisor, Paul Ponganis. Despite the fact that Paul has an entirely separate career in addition to his rich research, he always makes himself available whenever necessary, for advice, questions, guidance, and revisions. Whether it involves after hours or weekend meetings, or redlines and revisions scrawled between cases in the OR, Paul's dedication, availability to his students, and the insight he provides is almost too impressive to comprehend. With the knowledge and perspective I have gained along the way, it has become more and more apparent with each passing year that attending SIO to work with Paul, the unquestionable leader pushing the boundaries of the field of "hard core" diving physiology, was a most fortunate decision.

The members of the Ponganis lab have been truly instrumental along the way. Without a doubt, Torre Knower Stockard played the most influential role in my first few years at SIO, imparting upon me everything I needed to know to function in the Ponganis lab, academically, logistically, and even personally. Without Torre's guidance, I certainly would have been lost! Then Cassondra Williams came along, finally another graduate student to share (and commiserate with!) in all levels of our hectic schedules of field season preparation, planning, and finally the fun part, a

companion for the field work! It has been wonderful having Cass as a lab-mate and colleague, and particularly, as a friend. As apparent from the dedication of this dissertation, Jerry Kooyman, a true naturalist like none I have ever known, has provided me with unparalleled academic and scientific inspiration and mentorship. Perhaps even more noteworthy, getting to know Jerry personally through long hallway and office chats about life, ranging from career paths and relationships to flight planning, and our shared adventures have left an indelible impression on me.

In addition to those already mentioned, the remaining Penguin Ranch team members (Kathi Ponganis, Katsu Sato, Robert "Red" Howard, Judy St. Leger, Tania Zenteno-Savin, Ed Stockard, Cory Champagne, Matt Tulis, Kozue Shiomi, and Brendan Tribble), McMurdo Station personnel, and UCSC faculty, students, and personnel (in particular the Costa lab, Cory Champagne, Samantha Simmons, Patrick Robinson, Jason Hassrick, and Birgitte McDonald) have provided invaluable logistical and field support for all the phases of my dissertation work. These people have not only enabled this field work to be completed, but also provided companionship and entertainment along the way.

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always striving to do and participate in everything that my older siblings were doing, and this has profoundly affected my decisions and achievements in life. As my family members are scattered around the nation far from San Diego, I was fortunate enough to be "adopted" by the Jorgensen family, who has done an outstanding job in filling this void over the past six years.

The various schools, jobs, and activities I've participated in have also endowed me with an incredibly generous helping of wonderful friends scattered around the world over the years. Certainly, there are far too many to name, but they know who they are. Particularly, I'd like to thank my best friends from childhood and college, Erin Umphrey and Jaclyn Mason, and also Jake Maule, who I always know I can depend on when in need of a friend, advice, or someone to lean on. I've made some wonderful friends here at SIO as well, and our ladies lunches, weekly gym/running/cycling activities, and Google Chat sessions have served as valuable brainstorming sessions, necessary distractions, and crucial for survival. In addition to his always entertaining companionship, Jeremy Goldbogen has provided me with countless, and very insightful, suggestions, advice, and manuscript reviews of my work.

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MATERIAL PUBLISHED/SUBMITTED FOR PUBLICATION IN THE DISSERTATION

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The dissertation author was the primary investigator and author of this paper.

Chapter 2, in full, has been accepted for publication as:

Meir, J. U., Champagne, C.D., Williams, C. L., Costa, D.P. and Ponganis, P. J. Extreme hypoxemic tolerance and oxygen depletion in diving elephant seals. (2009). *American Journal of Physiology - Regulatory, Integrative & Comparative Physiology*

The dissertation author was the primary investigator and author of this paper.

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The dissertation author was the primary investigator and author of this paper.

Chapter 4, in full, is currently being prepared for publication as:

Meir, J.U. and Ponganis, P.J. Body temperature profiles of diving elephant seals. In preparation for *Physiological and Biochemical Zoology*

The dissertation author was the primary investigator and author of this paper.

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Education

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Meir, J. U., Champagne, C.D., Williams, C. L., Costa, D.P. and Ponganis, P. J. In press. Extreme hypoxemic tolerance and oxygen depletion in diving elephant seals. *American Journal of Physiology - Regulatory, Integrative & Comparative Physiology*

Meir, J.U. and Ponganis, P.J. In press. High affinity hemoglobin and blood oxygen saturation in diving emperor penguins. *Journal of Experimental Biology*

Ponganis, P.J., Stockard, T.K., **Meir, J.U.**, Williams, C.L., Ponganis, K.V., and Howard, R. 2009. O_2 store management in diving emperor penguins. *Journal of Experimental Biology*, 212, 217-224.

Meir, J.U., Stockard, T.K., Williams, C.L., Ponganis, K.V., and Ponganis, P.J. 2008. Heart rate regulation and extreme bradycardia in diving emperor penguins. *Journal of Experimental Biology*, 211, 1169-1179.

Ponganis, P.J., Stockard, T.K., **Meir, J.U.**, Williams, C.L., Ponganis, K.V., van Dam, R.P., and Howard, R. 2007. Returning on empty: extreme blood O_2 depletion underlies dive capacity of emperor penguins. *Journal of Experimental Biology* 210, 4279-4285.

Stockard, T.K., Heil, J., **Meir, J.U.**, Sato, K., Ponganis, K.V., and Ponganis, P.J. 2005. Air sac P_{O2} and oxygen depletion during dives of emperor penguins. *Journal of Experimental Biology* 208, 2973-2980.

Awards

National Science Foundation (NSF) International Research Program Fellowship (IRFP) Postdoctoral award (~$180,000, 2-year research grant), to being Fall 2009

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Halliday Student Field Research Award ($10,000 research scholarship), Scripps Institution of Oceanography, 2007

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Diving physiology
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Marine mammals and penguins
Hypoxemic tolerance (hypoxia)

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AAAS/Science Program for Excellence in Science, honorary membership to AAAS for 2007

Sigma Xi, The Scientific Research Society

ABSTRACT OF THE DISSERTATION

Blood oxygen transport and depletion: the key of consummate divers

by

Jessica Ulrika Meir

Doctor of Philosophy in Marine Biology

University of California, San Diego, 2009

Paul J. Ponganis, Chair

To investigate the physiological enigma of the impressive dives of emperor penguins and elephant seals, blood oxygen (O_2) transport and depletion in these species were addressed with a three tiered approach. First, the transport of O_2 was examined by assessing heart rate (the principal determinant of blood O_2 depletion) during dives of emperor penguins. Secondly, O_2 transport was investigated at the biochemical level by characterizing the O_2-hemoglobin (Hb) dissociation curves for these species. Finally, the actual depletion of O_2 in the blood was documented by measuring blood O_2 partial pressure (P_{O2}) and temperature continuously during dives with a backpack recorder on translocated, juvenile elephant seals. Application of the O_2-Hb dissociation curve to P_{O2} dive data also allowed for calculation of % Hb-saturation during dives.

These studies revealed physiological responses and biochemical adaptations that contribute to the remarkable dive capacity of these species, including: 1) In

contrast to any other diving bird, but similar to that of seals, emperor penguin heart rate while diving is significantly lower than resting rate, with values as low as 6 beats per minute in longer dives. This suggests parsimonious O_2 utilization and allows extended dive time. 2) The hemoglobin of the emperor penguin has a significantly higher affinity for O_2 as compared to other birds. It is in the range of seals and other marine mammals, allowing for more complete utilization of the respiratory O_2 store and increased blood O_2 content when P_{O2} is low. 3) The elephant seal possesses exceptional tolerance to low P_{O2} in the blood, with arterial P_{O2} of 12-23 mmHg and venous P_{O2} of 2-10 mmHg at the end of routine dives. These P_{O2} values correspond to hemoglobin saturations as low as 1-26% and O_2 contents of 0.3 (venous) and 2.7 ml O_2 dl^{-1} blood (arterial). Temperature data collected during elephant seal studies revealed that core body temperature is preserved during diving, inconsistent with previous assertions of hypometabolism and a cold induced Q_{10} effect during diving. Such results support the hypothesis that these species routinely "push the envelope" of the usual physiological limits of homeotherms to achieve such extraordinary dives.

GENERAL INTRODUCTION

GENERAL INTRODUCTION

Oxygen (O_2) is the universal and essential element of most forms of life. Extreme environments, particularly those with limited O_2 availability, are especially challenging to most life forms, yet still host diverse and abundant life. Animals operating in these extreme environments often push the envelope of their physiological capacity, and thus represent unique models of physiological adaptation. Species which must maintain aerobic metabolism when O_2 supply is limited represent ideal models in which to examine the physiological, cellular, and biochemical mechanisms underlying tolerance to hypoxia and O_2 depletion. 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.

Increased O_2 stores have long been considered essential to the remarkable breath-hold capacity of diving animals. Many previous studies have addressed aspects of O_2 storage, revealing the well documented physiological adaptations of diving animals such as increased hemoglobin and myoglobin concentrations, an increased blood volume, and differences in the distribution of O_2 stores. This approach has been applied to many species in a variety of environments and has been well reviewed (Butler and Jones, 1997; Kooyman and Ponganis, 1998). Few studies, however, have addressed hypoxemic tolerance and O_2 depletion while diving. In determining the dive capacity of an animal that depends on breath-hold diving to forage, the lowest tolerable level of O_2 and the management of O_2 stores is equally as important as the

magnitude of O_2 stores. In order to understand the full aptitude of the consummate diving animals, attention must be given to hypoxemic tolerance and O_2 management strategies.

The Model Organism

Key to the success of any experimental work is the choice of the appropriate model organism or subject of study. Weighty enough to stand alone in the epigraph of this dissertation, one of the most influential physiologists throughout history, August Krogh, stated (Krogh, 1929), "For a large number of problems there will be some animal of choice, or a few such animals, on which it can be most conveniently studied." This concept has become known as "The Krogh Principle", and has provided guidance for countless studies paramount to the foundations of physiology. In seeking the most suitable organism to address questions regarding hypoxia and O_2 depletion in the diving environment, it seemed appropriate to first elucidate the physiological extremes inherent in this system. The emperor penguin (*Aptenodytes forsteri*) and the elephant seal (*Mirounga angustirostris*), undeniably the consummate avian and pinniped divers, are ideally suited for this task.

The Objectives

This dissertation focuses on fundamental characteristics of blood O_2 transport and depletion, using a three-tiered approach. First, the transport of O_2 was examined by assessing heart rate (the principal determinant of blood O_2 depletion) during dives

of emperor penguins (Chapter 1). Secondly, O_2 transport was investigated at the biochemical level by characterizing the O_2-hemoglobin (Hb) dissociation curves for these species (Chapters 2 and 3). Finally, the actual depletion of O_2 in the blood was documented by measuring blood O_2 partial pressure (P_{O2}) and temperature continuously during dives with a backpack recorder on translocated, juvenile elephant seals (Chapter 2). Application of the O_2-Hb dissociation curve to P_{O2} dive data also allowed for calculation of % Hb-saturation during dives of both species (Chapters 2 and 3). Continuous temperature measurements collected during elephant seal studies also provided a means of assessing potential relationships of a cold induced Q_{10} effect and hypometabolism while diving, and allowed detailed evaluation of temperature changes associated with dive profiles (Chapter 4).

The Experimental Setting

To conduct these studies of diving physiology in the wild, two existing, and exceptionally clever, experimental designs were utilized. Following in the footsteps of pioneer emperor penguin and seal physiologist and naturalist, Jerry Kooyman, the isolated dive hole protocol (Kooyman, 1965; Kooyman, 1968) was selected for the first objective of the study (Fig. 0-1). Basically, this consists of selecting a crack free area (with at least a 1 km radius) on the McMurdo Sound sea ice, drilling a set of experimental dive holes, and constructing a corral as a perimeter around these holes. Once this corral has been filled with emperor penguins to create the “Penguin Ranch”, it provides ease of recorder deployment and recovery and permits free-diving of the

emperor penguins. For the studies involving northern elephant seals, the translocation method (Andrews et al., 1997; Oliver et al., 1998) was used with juvenile seals from the Año Nuevo colony (San Mateo County, California) during the April molt season (Fig. 0-2). 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) 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. Subsequently, the seals make their way back to their original haul out location, diving across the bay as they transit.

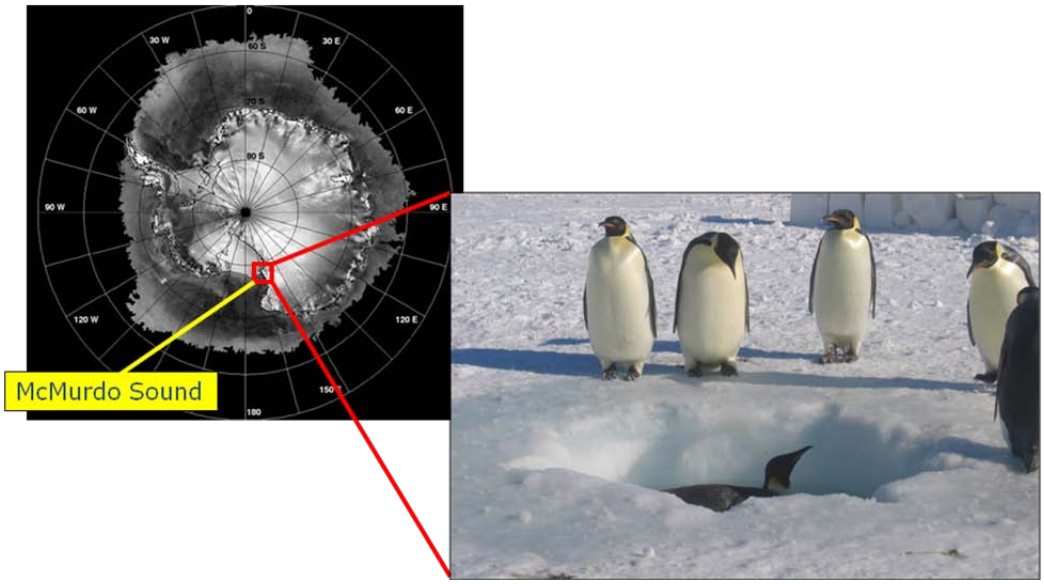

Figure 0-1. The isolated dive hole protocol (Kooyman, 1965; Kooyman, 1968). Two experimental dive holes are drilled in a crack-free area of the McMurdo Sound sea ice and enclosed in a corral, creating the Penguin Ranch.

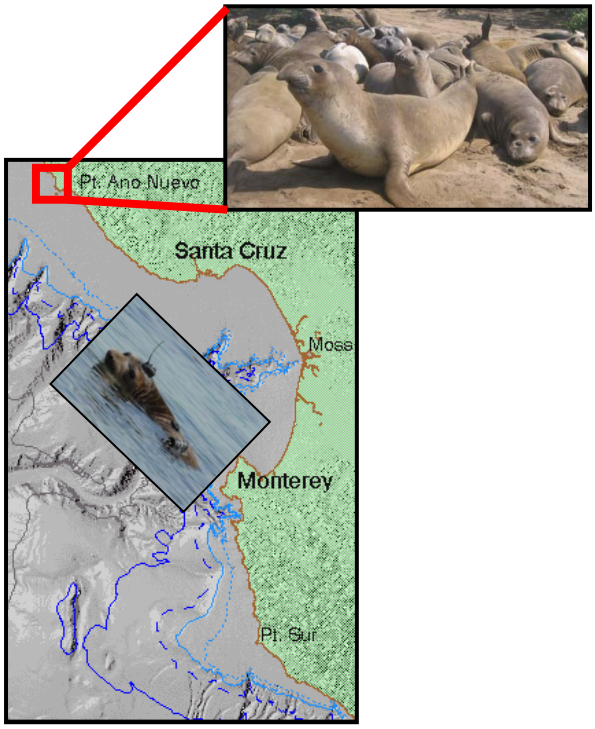

Figure 0-2. The translocation method (Andrews et al., 1997; Oliver et al., 1998). Following the translocation method, juvenile elephant seals were captured at their haul-out site at the Año Nuevo colony (San Mateo County, California), transported by truck to the Long Marine Lab (UCSC), and instrumented. After overnight recovery, seals were transported by truck to the other side of Monterey Bay (Hopkins Marine Station) or by boat for off-shore release.

CHAPTER 1: Heart rate regulation and exteme bradycardia in diving emperor penguins

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Heart rate regulation and extreme bradycardia in diving emperor penguins

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SUMMARY

To investigate the diving heart rate (f_H) response of the emperor penguin (*Aptenodytes forsteri*), the consummate avian diver, birds diving at an isolated dive hole in McMurdo Sound, Antarctica were outfitted with digital electrocardiogram recorders, two-axis accelerometers and time depth recorders (TDRs). In contrast to any other freely diving bird, a true bradycardia (f_H significantly $<f_H$ at rest) occurred during diving [dive f_H (total beats/duration)=57±2 beats min^{-1}, f_H at rest=73±2 beats min^{-1} (mean ± s.e.m.)]. For dives less than the aerobic dive limit (ADL; duration beyond which [blood lactate] increases above resting levels), dive f_H=85±3 beats min^{-1}, whereas f_H in dives greater than the ADL was significantly lower (41±1 beats min^{-1}). In dives greater than the ADL, f_H reached extremely low values: f_H during the last 5 mins of an 18 min dive was 6 beats min^{-1}. Dive f_H and minimum instantaneous f_H during dives declined significantly with increasing dive duration. Dive f_H was independent of swim stroke frequency. This suggests that progressive bradycardia and peripheral vasoconstriction (including isolation of muscle) are primary determinants of blood oxygen depletion in diving emperor penguins. Maximum instantaneous surface interval f_H in this study is the highest ever recorded for emperor penguins (256 beats min^{-1}), equivalent to f_H at $\dot{V}_{O_2}$ max., presumably facilitating oxygen loading and post-dive metabolism. The classic Scholander–Irving dive response in these emperor penguins contrasts with the absence of true bradycardia in diving ducks, cormorants, and other penguin species.

Key words: diving, electrocardiogram (ECG), emperor penguin, heart rate, respiratory sinus arrhythmia, stroke frequency.

INTRODUCTION

The emperor penguin, *Aptenodytes forsteri* Gray, is the consummate avian diver, reaching depths greater than 500 m (Kooyman and Kooyman, 1995) with durations longer than 23 min (Ponganis et al., 2007). This exceptional diving capacity has stimulated much interest in the physiology which underlies such behavior in this species (Butler and Jones, 1997; Kooyman and Ponganis, 1998; Nagy et al., 2001; Ponganis et al., 2003; Stockard et al., 2005). The duration of any given dive for an air-breathing animal is dependent on available oxygen stores, the rate of utilization of these stores, and the lowest tolerable level of oxygen (O_2). Based upon Scholander's and Irving's classic findings during forced submersion (Irving et al., 1941; Scholander, 1940; Scholander et al., 1942), it is hypothesized that a reduction in heart rate (f_H) and peripheral perfusion are the principal determinants of the depletion rate of the respiratory and blood O_2 stores. This is because the metabolic rate of the kidneys, splanchnic organs and heart, which account for about half of O_2 consumption at rest (Butler and Jones, 1997; Schmidt-Nielsen, 1983), are either perfusion dependent or directly related to f_H (Davis and Kanatous, 1999). Adjustments in f_H can influence dive duration by providing both optimal loading of O_2 stores before the dive and a reduction and redistribution of blood flow to conserve O_2 during the dive (Butler and Jones, 1997).

In comparison to pre-dive tachycardic levels, most diving animals undergo a decrease in f_H upon submersion, with the magnitude of response varying between species and with dive duration (Butler and Jones, 1997; Irving et al., 1941; Scholander, 1940). Heart rate has also been used as a measure of field metabolic rate in several studies, based on the premise that O_2 consumption is directly proportional to f_H (Bevan et al., 1994; Boyd et al., 1999; Butler, 1993; Froget et al., 2004; Green et al., 2003). A relationship between mean f_H during the dive and dive duration has been documented in several diving species, including elephant seals, grey seals and cormorants (Andrews et al., 1997; Enstipp et al., 2001; Thompson and Fedak, 1993).

The objective of this experiment was to evaluate the cardiac responses of emperor penguins on a beat-to-beat basis using a digital electrocardiogram (ECG) recorder, specifically to assess diving f_H in relationship to: (1) f_H at the surface and at rest, (2) dive duration (3) the aerobic dive limit [ADL; duration beyond which blood lactate concentration increases above resting levels (Kooyman, 1989); also termed the diving lactate threshold (DLT) (Butler and Jones, 1997)] and (4) stroke frequency. An additional goal was to investigate variations in f_H associated with respiration while the birds were at rest and at the surface, and to consider how these variations might parallel the diving f_H response. The isolated dive hole is a useful model for this study, since field metabolic rate, the ADL and respiratory and blood O_2 store depletion rates have been determined for this species under these conditions (Nagy et al., 2001; Ponganis et al., 1997; Stockard et al., 2005; Ponganis et al., 2007).

We hypothesized that: (1) dive f_H would correlate negatively with dive duration, (2) a true bradycardia (f_H significantly $<f_H$ at rest) would occur during diving, and (3) dive f_H would be independent of swim stroke frequency. Such findings would be consistent with the classic Scholander and Irving dive response (Irving et al., 1941; Scholander, 1940; Scholander et al., 1942), with isolation of muscle and dependence of its metabolism on its large myoglobin-bound O_2 store. In addition, since f_H profiles at rest revealed a possible respiratory sinus arrhythmia (RSA), the naturally occurring respiratory-induced variation of f_H, we investigated the coupling of

this potential RSA pattern and respirations with use of a chest impedance meter. Respiratory-induced variations in f_H were also compared to dive f_H values to assess any similarity in the physiological response and possible cardiorespiratory control mechanisms between these two conditions.

MATERIALS AND METHODS

Non-breeding emperor penguins (~15 birds/season, N=9 for this study, 25.1±0.6 kg) were captured near the McMurdo Sound ice edge or at Terra Nova Bay in October of 2001, 2004, and 2005 and were maintained for 2 months at an isolated dive hole enclosed within a corral at the Penguin Ranch (Kooyman et al., 1992) on the McMurdo Sound sea ice (77°41′, 165°59′). The birds dived and foraged under the ice daily, as evidenced by guano deposition and weight gain. Birds at the isolated dive hole typically exit the water after a dive and stand on the ice until initiating the next dive (Kooyman et al., 1992; Ponganis et al., 1997). All procedures were approved under a UCSD Animal Subjects Committee protocol and US Antarctic Treaty Permit. All animals were returned to the ice edge and released upon completion of the study.

Heart rate profiles

Investigation of respiratory sinus arrhythmia

In four emperor penguins under general isoflurane anesthesia (Ponganis et al., 2001), three subcutaneous electrodes were inserted dorsally (one just left of midline at the level of the axilla, the second just right of midline at hip level, and the reference electrode approximately 10 cm to the left of midline and 5 cm above the left hip). After recovery overnight, the electrodes were connected *via* a cable to an ECG/Impedance system (UFI, Morro Bay, CA, USA), and a BIOPAC A-D converter (BIOPAC Systems, Goleta, CA, USA) interfaced with a laptop personal computer (PC). Impedance and ECG signals were recorded at 62.5 and 250 Hz, respectively. Penguins were standing calmly in a penguin transport box (45×45×120 cm) located outside (ambient Antarctic temperature, −10 to −20°C) during the experiment, with the necessary wires and cords running back into the experimental hut where the hardware was located. Changes in the baseline impedance signal result from chest-volume-associated differences in skin impedance between inspiration and expiration and allow for the calculation of respiration rate. ECG and impedance signals were recorded simultaneously to accommodate analysis of corresponding changes between the two channels. The inspiratory–expiratory changes in the chest impedance signal were visually verified by an observer peering into the box for a portion of the recording cycle.

Heart rate at rest, during surface intervals, and during diving

Two-lead subcutaneous electrodes were inserted along the dorsal midline of nine emperor penguins (conducted separately from above protocol), roughly at the level of the axilla and just above the pelvis while birds were under general isoflurane anesthesia (Ponganis et al., 2001). The electrodes were connected to a custom-built digital ECG recorder (UFI) in an underwater housing (283 g, 16×6×2.5 cm), secured to the feathers of the mid-back with 5 min epoxy glue (Devcon; Danvers, MA, USA), a Velcro™ patch, and plastic cable ties. ECG was recorded at a sampling rate of 50 Hz. An Mk9 time depth recorder (Wildlife Computers, Redmond, WA, USA; sensitive to 0.5 m, 30 g, 6.7×1.7×1.7 cm) was also attached to record the time/depth dive profile at 1 Hz. Transducer calibration from the TDR was verified in a pressure chamber at the Scripps Institution of Oceanography. After overnight recovery, the birds were provided access to the dive hole for 1–2 days. At the end of the data collection period, the electrodes and recorders were removed under general anesthesia.

Stroke frequency and heart rate

Five of the nine birds with the ECG recorder described above were simultaneously outfitted with a two axis acceleration data logger (M190-D2GT, Little Leonardo Ltd, Tokyo, Japan; 1.5 cm diameter, 6 cm length, 16 g). The accelerometer measures depth and acceleration in the y (vertical) and x (horizontal) axes. Depth was sampled at 1 Hz (±1 m accuracy and 0.05 m resolution) and acceleration at 16 Hz *via* an accelerometer sensor (model ADXL202E; Analog Device, Inc., Norwood, MA, USA). The measuring range of the accelerometer is ±29.4 m s^{-2} with a resolution of 0.0196 m s^{-2}. The accelerometer was oriented so that the y-axis (head-to-tail) or 'surge' acceleration was in line with the spine (longitudinal axis of the animal), placed approximately midline between the apexes of the wings and secured with plastic cable ties and waterproof Tesa™ tape. In this configuration, high frequency fluctuations in the y-axis acceleration signal correspond to 'surge', or the acceleration caused by the bird's stroking wing movements (Sato et al., 2004; Watanuki et al., 2003). The unit was calibrated in seawater against the earth's gravitational acceleration at the end of each experimental run.

Data analysis and statistics

All recorders were synchronized to the same PC clock, which was automatically synchronized to an Internet time server. Data were graphed and analyzed using Excel, Origin, SPSS, Acknowledge, and R software. Using a customized peak detection program, R-wave peaks from the digital ECG records were marked and R–R intervals calculated. All peaks were visually verified in order to ensure marking accuracy. In birds with the accelerometer, the same peak detection program was used to mark the high frequency fluctuation of surge acceleration (strokes), with stroke frequency calculated from the intervals between peaks. Instantaneous f_H was plotted with dive depth from the TDR to construct dive profiles, including stroke frequency for dives with the accelerometer. These instantaneous values are also reported throughout the text. For RSA data, f_H was plotted with chest impedance signal. Heart rates for different periods (at rest, dives and surface intervals) were calculated by dividing the total number of beats during the period by the period duration. This period consisted of 1 h for birds at rest, over the entire dive for dive f_H, and over 1 min immediately prior to and immediately following a dive for the surface interval f_H (pre- and post-dive). For RSA analysis, surface interval f_H values were also analyzed until the RSA pattern emerged after the dive (up to 5.2 min).

Heart rates from the different periods and within periods were log transformed and compared by independent samples t-tests. Log-transformed dive f_H and minimum f_H were also used for linear regression to assess the relationship of these variables to dive duration and stroke frequency. Dives were also divided into two categories of duration, those that were shorter or longer in duration than the previously measured ADL (5.6 min) (Ponganis et al., 1997). This value represents the transition point of a two-phase regression analysis of post-dive lactate accumulation and dive duration in that study. Although the ADL is probably a range of values, which shifts in accordance with factors such as activity and variable oxygen consumption during the dive (Ponganis et al., 1997), the value of 5.6 min was used for this analysis to provide a fixed point by which to categorize dives of different durations.

Table 1. Individual and pooled heart rate data for emperor penguin dives at the isolated dive hole

Bird	Number of dives	f_H (beats min^{-1}) At rest	Pre-dive	Dive	Post-dive	Duration (min:s)	Max. depth (m)
19	20	84	204±6	73±6	201±6	5.23±0.85	25.4±3.0
22	12	72	193±9	56±8	189±8	7.28±1.40	54.3±11.6
43	17	72	161±6	53±5	162±5	6.75±0.63	86.4±13.3
32	17	78	182±6	57±5	191±3	7.85±1.03	39.9±2.9
39	13	68	158±7	46±5	161±7	7.90±1.10	39.6±3.9
31	10	76	143±12	45±6	156±9	8.03±1.60	48.9±6.0
35	16	73	179±7	59±9	191±4	7.15±1.18	41.0±4.0
36	6	71	112±5	36±8	143±16	8.70±1.60	45.2±6.3
49	14	63	194±3	65±7	169±5	8.83±0.90	132.9±19.7
Grand mean* (range)		73±2 (63–84)	174±3 (96–256)	57±2 (23–134)	177±3 (97–256)	7.32±0.37 (1.05–18.15)	56.8±4.3 (5–225)

f_H, heart rate; values are mean ± s.e.m., *pooled data (N=9 birds, 125 dives).
Resting data are calculated as the total number of beats divided by the 1 h duration (resting on sea ice), and thus do not represent a mean. Pre- and post-dive data are calculated from the 1 min immediately prior to and immediately post-dive.

Individual variation among birds was addressed with ANOVA and Kruskal–Wallis tests. Testing for inter-individual effects specific to the f_H *vs* dive duration relationship was addressed with a resampling approach (R custom script). In this script, the number of dives specific to an individual bird was randomly removed from the pooled data and the regression equation (linear fit of log-transformed f_H *vs* dive duration) parameters from the resulting distribution recorded, with 1000 iterations. A penguin-specific regression equation was generated from another distribution with the specific dives of the individual bird removed from the pooled data. The parameters of the penguin-specific run were then compared to the distribution of parameters from the 1000 random iterations. If the penguin-specific parameter was not within the 2.5% tails of the random distribution, it was considered to fit within the distribution of pooled data. Otherwise, the data from this penguin were considered to significantly deviate from the pooled data. This procedure was repeated for each individual bird. Unless stated otherwise, statistical significance was assumed at P<0.05 and the significance level is quoted in the text. Values are expressed as means ± s.e.m.

RESULTS

ECG data were analyzed for nine birds over the three field seasons, resulting in f_H records for 125 dives. Accelerometer records for stroke frequency analysis were obtained from five of these birds, for a total of 62 of the 125 dives. Diving behavior in this study was typical of that at the isolated dive hole, with durations ranging from 1.1–18.2 min and a mean of 7.3±0.4 min. Sixty-five percent of dives in the study were greater in duration than the previously measured ADL. Depth ranged from 5–225 m, with a mean of 56.8±4.3 m. Birds instrumented with ECG recorders/accelerometers dived to similar depths and durations as those without these recorders. Heart rate in each bird at rest, measured over a 1-h period at night when access to the dive-holes was blocked, ranged from 63-84 beats min^{-1} with a mean of 73±2 beats min^{-1} (Table 1).

Heart rate profiles: electrocardiogram signal and heart rate patterns

ECG records demonstrated high signal integrity in all dives and surface intervals included in the analysis (Fig. 1). A characteristic pattern of f_H during the dives was evident, with high f_H before and after the dive (pre- and post-dive tachycardia), and a significant reduction in f_H during the dives. The dive f_H response consisted of an immediate decline upon submersion followed by a readjustment in which f_H increased briefly and then declined once again, with a further progressive decline in longer dives (Fig. 2). In 93% of the dives, f_H reached the level of f_H at rest within 1 min after submersion. In 71% of the dives, this level was reached within the first 20 s of the dive. An increase in f_H during ascent (prior to surfacing) occurred in most dives. Surface interval f_H peaked immediately pre- and post-dive, with a maximum instantaneous f_H of 256 beats min^{-1}.

In dives longer than the ADL there were typically extended periods of very low heart rates in the latter portions of the dive, whereas in short dives there was often a gradual increase in heart rate after a nadir about halfway through the dive (Fig. 2). Consistently low levels of f_H were found only in dives longer than the ADL, with the exception of one dive. In dives longer than the ADL, f_H remained below 30 beats min^{-1} for over 1 min in 59% of dives, below 20 beats min^{-1} for over 1 min in 35% of dives, and below 10 beats min^{-1} for over 1 min in 15% of dives. The mean of minimum instantaneous f_H values in dives shorter than the ADL was 44.8±2.7 beats min^{-1} (N=44), compared with 14.3± 0.9 beats min^{-1} in dives longer than the ADL (N=81). The minimum instantaneous f_H was less than 20 beats min^{-1} in 72% of dives longer than the ADL, and less than 10 beats min^{-1} in 42% of those dives. Minimum f_H was less than or equal to 5 beats min^{-1} in 10% of dives longer than the ADL, with all of these dives being over 10.5 min in duration (Fig. 3). In the longest dive included in this study (18.2 min), the minimum f_H was 3 beats min^{-1}, with a rate of 6 beats min^{-1} for over 5 min (Fig. 2C). Instantaneous heart rate did not reach such extremely low values in short dives (Fig. 2A, Fig. 3A).

Heart rate profiles: comparisons of heart rate at rest, during dives and during surface intervals

In order to assess f_H over as wide a range of dive durations as possible, data from all birds were pooled. This was reasonable because the number of dives for each bird was relatively small, and because these data were meant as descriptive for the group, particularly in relation to a wide range of dive durations. According to ANOVA and Kruskal–Wallis tests, dive f_H and dive depth (max. depth recorded during dive) were significantly different between individual birds, while dive duration was not (Kruskal–Wallis: for f_H, depth, duration respectively, χ^2=24.265, 35.556, 10.527; P=0.002, 0.000, 0.230). The significant difference for dive f_H, however, appears only for bird 36. The resampling approach and comparison of regression equations generated from the f_H *vs* dive duration relationship also revealed differences among individuals,

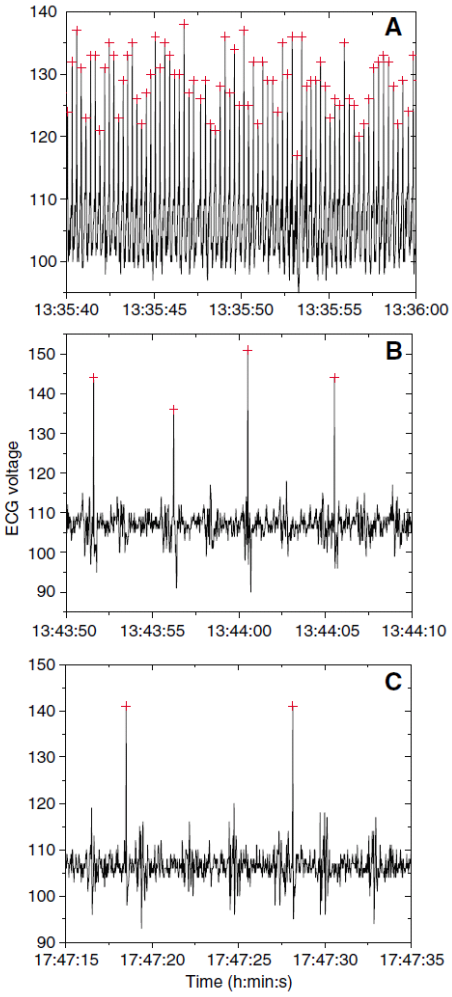

Fig. 1. Examples of ECG records of emperor penguin 35 over 20 s intervals, (A) at the surface and (B,C) during diving. From this ECG record, heart rate (f_H) was calculated as 214 beats min^{-1} (A) 12 beats min^{-1} (B) and 6 beats min^{-1} (C). Plus signs (red) represent detected peaks. The artifacts present in B and C correspond to wing strokes (as revealed by simultaneous review of stroke frequency and ECG profiles in this individual).

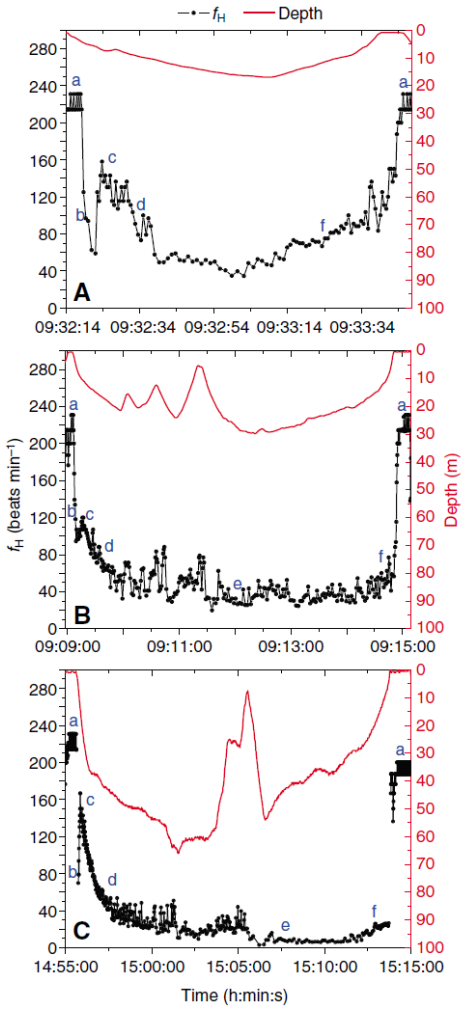

Fig. 2. Instantaneous heart rate (f_H) and dive depth profiles from (A) a typical short (~1.5 min) dive of emperor penguin 35, (B) a dive just longer than the ADL of emperor penguin 32, and (C) the longest dive in this study (18.2 min, emperor penguin 22). The f_H data gap at the end of the dive in C resulted from a brief period of unusable ECG signal. f_H in short dives does not approach extremely low values as in long dives. In C, f_H is 6 beats min^{-1} for over 5 min, reaching a minimum of 3 beats min^{-1}. Prominent features typical of dives are labeled as follows: a, surface interval tachycardia (pre- and post-dive); b, initial f_H decline, immediately upon submersion; c, readjustment of f_H (transient increase); d, secondary decline in f_H; e, progressive bradycardia in long dives; f, increase in f_H during ascent (prior to surfacing).

but when applying a Bonferonni correction due to multiple sampling (P<0.006), this difference was once again present for only one individual. Notably, if this one statistically deviating individual were removed, the results presented above did not change appreciably. Since inclusion of an outlier can only serve to weaken any resulting relationship, this analysis further reinforces the robustness of conclusions of the pooled data. Taking this into account, all nine individuals were included in the pooled dataset in all analyses in the study. Mean data from each of the f_H periods is shown for each bird in Table 1 (N=9, 125 dives).

The mean of dive f_H for pooled data (determined as the total number of beats divided by the dive duration) was 33% of the pre-dive f_H, and 78% of the mean f_H at rest (Table 2). This is similar to

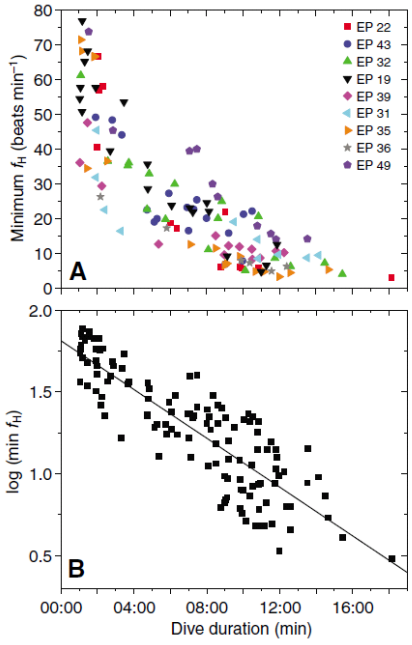

Fig. 3. (A) Minimum instantaneous heart rate (f_H) during the dive *vs* dive duration. Individual birds (EP) are denoted by colors and symbols (see legend), N=9 birds, 125 dives. (B) Log transformation of minimum f_H *vs* dive duration with regression equation for pooled dives: y=–107.27233x+1.81157, r^2=0.738, P<0.0001.

Table 2. Heart rate data

(A) Mean heart rate data divided between dives less than the aerobic dive limit and greater than the aerobic dive limit (5.6 min)

Period	f_H (beats min^{-1})	% of pre-dive f_H	% of f_H at rest
All dives	57±2	33	78
Dives<ADL	85±3	49	116
Dives>ADL	41±1	24	56

f_H, heart rate (mean ± s.e.m.); ADL, aerobic dive limit.

(B) Statistical analysis (*t*-test) of log-transformed data (analyzed with SPSS and R)

Dataset	Significant?	P	t
All *vs* rest	Yes	0.021	–2.337
<ADL *vs* rest	No	0.173	1.384
>ADL*vs* rest	Yes	0.000	–6.607
<ADL *vs* pre-dive	Yes	0.000	–17.837
>ADL *vs* pre-dive	Yes	0.000	–42.122
<ADL *vs* >ADL	Yes	0.000	14.380

ADL, aerobic dive limit.

that reported in a previous study of emperor penguins, in which diving f_H was about 85% of the resting value (Kooyman et al., 1992). Although dive f_H was significantly lower than both pre-dive and at rest values for pooled data, for individual birds, the mean dive f_H was not significantly lower than rates at rest when considering dives of all duration collectively. To address f_H differences in relation to the previously measured ADL, dives from all birds were pooled and divided into those shorter than the ADL (35.2% of the dives in the study) and those longer than the ADL (64.8% of the dives in the study). For pooled data, f_H in dives longer than the ADL was significantly less than: (1) f_H in dives shorter than the ADL, (2) f_H at rest, and (3) f_H during surface intervals (Table 2). This was also true for each individual bird, with the exception of bird 49, in which f_H in dives longer than the ADL was not significantly lower than f_H at rest, but like other birds, was significantly lower when compared to f_H in dives shorter than the ADL.

Heart rate data of all birds were log transformed for regression analysis (Figs 3, 4, 7). Log transformation resulted in homoscedasticity and a linear fit for the f_H and dive duration relationship (Fig. 4B). Minimum instantaneous f_H recorded during the dive also decreased with dive duration, with a shape similar to that of the dive f_H *vs* dive duration relationship and a similarly negative linear relationship between the log of the minimum f_H *vs* dive duration (Fig. 3). There were no significant relationships between dive duration and max. depth of the dive, or between f_H and max. depth.

A distinct respiratory sinus arrhythmia (RSA) was documented in emperor penguins at rest (Fig. 5). The change in the baseline chest impedance signal during respiration was visually verified as corresponding to actual breaths. The inspiration tachycardia is associated with the upstroke in the impedance signal. These patterns were present in all four of the penguins studied. Mean f_H at rest, peak instantaneous f_H (corresponding to inspiration), minimum instantaneous f_H (corresponding to expiration), the sinus arrhythmia index (SAI, difference between inspiratory and expiratory f_H), and the inferred respiration rate for a 10 min period are given for each bird in Table 3. The grand mean of minimum instantaneous f_H in the RSA (all minima during the 10 min period for all four birds) was 45.0±0.9 beats min^{-1} (N=181). During surface intervals, the RSA pattern was not evident until 107±10 s (N=43) after the dive.

Stroke frequency and heart rate

Emperor penguins diving at the isolated dive hole stroked continuously throughout the dive, with no prolonged periods of gliding. The highest stroke frequencies (~1.5–2.2 Hz) occurred during the initial descent (Fig. 6). Excluding descents, stroke frequency during the dive was very consistent, with typical values around 0.45–0.70 Hz, depending on the individual bird. This steady stroking frequency was usually achieved after the first 20–30 s of the dive. Dive stroke frequency (the calculated mean of all values during the dive) in relatively short dives was greater than that of long dives because of the high stroke frequencies associated with the initial descent and a relatively short post-descent period, during which stroke frequency was low and stable. Longer dives had inherently longer post-descent periods (Fig. 6). Because of this bias, dives in which the initial descent period constituted 20% or more of dive duration (generally dives shorter than 2.5 min, 15 out of the 62 dives analyzed) were not considered when assessing the relationship between dive stroke frequency and dive duration or to dive f_H. This was further justified by the fact that stroke frequency patterns showed no differences in short and long dives (Fig. 6). With the exclusion of these dives, there is no significant correlation between stroke frequency and the log of f_H during the dive (y=0.508x+1.27, r^2=0.082, P=0.051; Fig. 7A). Stroke frequency had only a weak negative relationship with dive duration

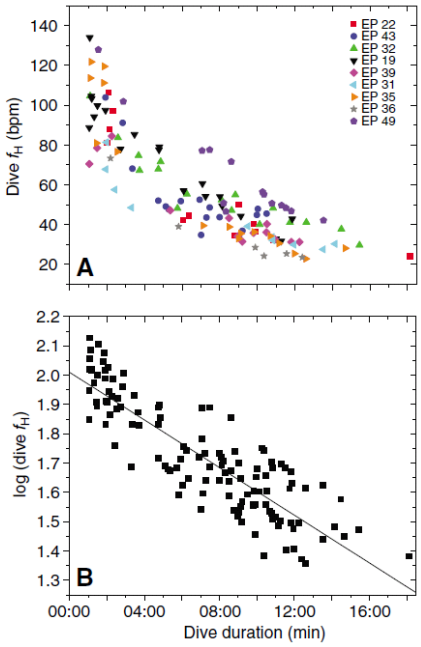

Fig. 4. (A) Dive heart rate (f_H) (total number of beats/dive duration) *vs* dive duration for emperor penguins diving at the isolated dive hole. Individual birds (EP) are denoted by colors and symbols (see legend), N=9 birds, 125 dives. (B) Log transformation of dive f_H *vs* dive duration with regression equation for pooled dives: y=–58.5361x+2.00996, r^2=0.755, P<0.0001.

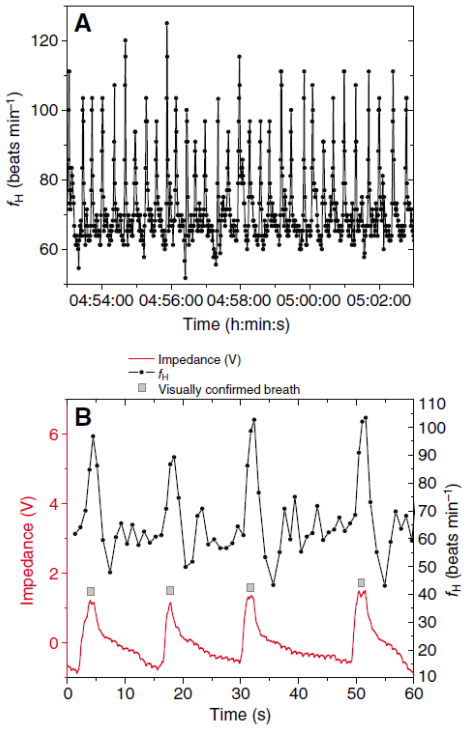

Fig. 5. (A) Heart rate (f_H) over a 10 min period in emperor penguin 22 resting on the sea ice. Note the regular, momentary tachycardias. (B) Heart rate (black) and impedance signal (red) from emperor penguin 32 during 1 min at rest. The maxima in the impedance signal were visually verified as corresponding to inspiration (grey squares). The tachycardia associated with each visually confirmed inspiration is associated with the upstroke in the impedance signal. Respiratory rate in this case is counted as 4 breaths min^{-1}.

(y=–17.70x+0.72, r^2=0.267, P=0.0002; Fig. 7B). The lack of coupling of stroke frequency and f_H is most apparent when considering individual dive profiles (Fig. 6). Simultaneous profiles of depth, stroke frequency, and instantaneous f_H during a dive revealed that changes in stroke frequency are not consistently associated with changes in f_H.

DISCUSSION

Heart rate profiles: at rest

Heart rates during rest periods in this study were equivalent to those of both previous studies and to allometric predictions (Calder, 1968; Grubb, 1983; Kooyman et al., 1992). Respiratory sinus arrhythmias (RSAs) have been documented in a variety of birds and diving species (Andrews et al., 1997; Bartholomew, 1954; Butler and Taylor, 1983; Casson and Ronald, 1975; Castellini et al., 1994a; Castellini et al., 1994b; Enstipp et al., 1999; Irving, 1939) and are more pronounced in some species than others [for example, in dogs (Hamlin et al., 1966)]. Previous work on elephant seals has demonstrated that RSAs can be used to estimate respiratory frequency in diving animals, even while at sea (Andrews et al., 2000). Apparent RSA has recently been used to determine probable breathing frequency in resting king penguins (Halsey et al., 2008). The complete ECG record afforded by the digital recorders in this study reveal an RSA pattern in emperor penguins and validate the inference of respiration rate from the regular, momentary tachycardias present in f_H data of birds at rest (Fig. 5). Analysis of these tachycardias in ECG records of emperor penguins at rest on the sea ice in McMurdo

Table 3. Mean heart rate at rest, during inspiration (peak instantaneous heart rate) and expiration (minimum instantaneous heart rate), sinus arrythmia index and the inferred number of breaths min^{-1} for each bird

	f_H (beats min^{-1})				
	Mean at rest	Peak (inspiration)	Minimum (expiration)	SAI	No. breaths min^{-1} (10 min period)
Bird 31	68.6	93.3±1.7	56.3±0.8	37.0±1.6	4.4
Bird 32	67.2	98.6±1.8	48.9±1.3	49.7±2.0	4.3
Bird 33	42.9	80.0±3.6	31.7±0.6	48.4±3.4	3.5
Bird 34	50.3	83.5±2.3	37.8±1.0	45.7±2.2	4.3

f_H, heart rate; SAI, sinus arrythmia index (difference between inspiratory and expiratory heart rates). The f_H value at rest is calculated as the total number of beats divided by the duration, and thus does not represent a mean.

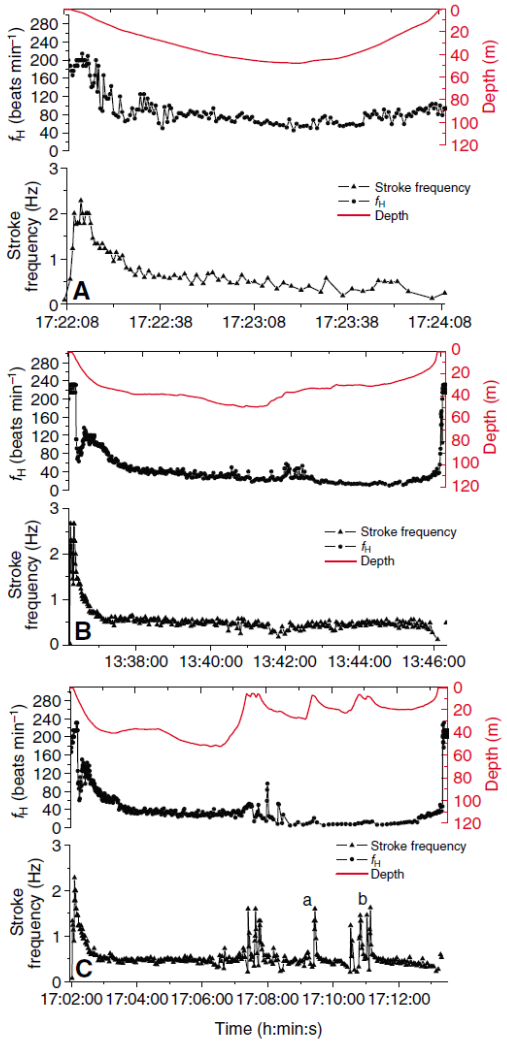

Fig. 6. Heart rate (f_H), stroke frequency, and dive profiles of: (A) a relatively short (<2 min) dive of emperor penguin 31; (B) a ~10 min dive of emperor penguin 35; (C) a ~12 min, variable depth dive of emperor penguin 35. In B f_H drops by a factor of 2 by the latter half of the dive, while stroke frequency remains constant. An increase in f_H is also apparent prior to surfacing, again with no corresponding increase in stroke frequency. In C stroke frequency is always highest during descents (labeled a and b), but f_H does not increase during descents. f_H remained near 5 beats min^{-1} for much of the second half of the dive compared to around 35 beats min^{-1} in the first half. Stroke frequency, however, remains near 0.45 Hz, increasing only during descents and while near the surface.

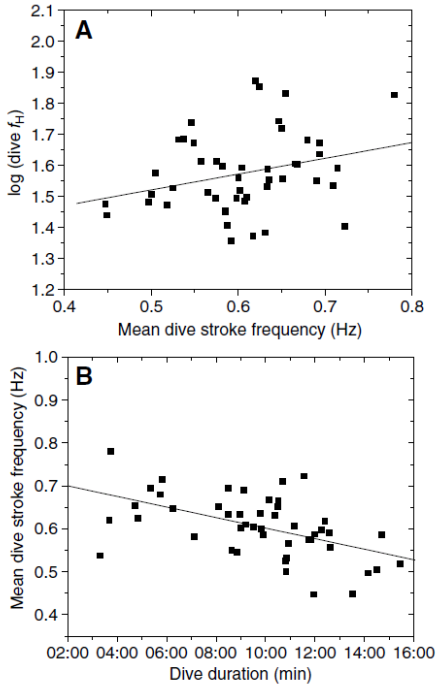

Fig. 7. Log transformation of dive heart rate (f_H) *vs* mean dive stroke frequency (A) and mean dive stroke frequency *vs* dive duration (B), N=5 birds, 47 dives. As seen in the linear fits in A the log of dive f_H does not have a significant relationship to stroke frequency (y=0.508x+1.27, r^2=0.082, P=0.051), and in B, dive stroke frequency has a significant, but weak negative relationship to dive duration (y=–17.70x+0.72, r^2=0.267, P=0.0002).

Sound reveals a mean respiration rate of 4.1±0.3 breaths min^{-1} (mean ± s.e.m., N=9; 1 h period/bird). Respiration rates of the birds standing calmly in the carrier box for this portion of the study (Table 3) were equivalent. These data are comparable to observed emperor penguin respiratory rates from a previous study (Kooyman et al., 1971) and are significantly lower than predicted respiratory rates based on allometry (Calder, 1968) (t-test, P=0.000).

The grand mean of minimum instantaneous f_H in the RSA (all minima during the 10 min period for all four birds; mean= 45.0±0.9 beats min^{-1}, N=181) is strikingly similar to the minimum f_H in dives shorter than the ADL (mean=44.8±2.7 beats min^{-1}, N=44). That is, f_H during dives shorter than the ADL falls to a level only equivalent to that reached during routine expiration at rest, as opposed to a much more extreme drop in dives longer than the ADL (mean=14.3±0.9 beats min^{-1}, N=81). This finding is similar to the f_H response during sleep apnea of elephant seals, in which the apneic f_H was comparable to the minimum value during normal respiratory sinus arrhythmia (Castellini et al., 1994a; Castellini et al., 1994b). Similar to what was conjectured for elephant seals, perhaps the reduction in f_H during dives shorter than the ADL in emperor penguins is controlled by a similar level or mechanism of cardiorespiratory control to that governing the respiratory sinus arrhythmia, with a further reduction in f_H (and true bradycardia as defined in this work) occurring only in dives longer than the ADL.

Heart rate profiles: diving

Heart rates of emperor penguins in dives shorter than their ADL (<5.6 min) in this study are in agreement with f_H of other diving

birds. For example, cormorants and several penguin species show a significant decrease in diving f_H as compared to pre-dive values, with f_H maintained throughout the dive at a level similar to that at rest (Butler and Woakes, 1984; Enstipp et al., 2001; Froget et al., 2004; Millard et al., 1973). Mean f_H in the emperor penguin in pooled dives, and, more notably, in dives longer than the ADL, is significantly lower than both surface interval f_H and f_H at rest (Table 2), consistent with findings from the previous study of f_H in diving emperor penguins (Kooyman et al., 1992). This demonstrates a true bradycardia (f_H significantly <f_H at rest) for the diving emperor penguin at the isolated dive hole. Such a response has never been documented in any other diving bird, even in dives greater in length than their calculated aerobic dive limit (cADL; though this cannot be considered a direct comparison to the measured ADL as reported here) (Butler and Woakes, 1984; Enstipp et al., 2001; Froget et al., 2004; Millard et al., 1973).

An immediate decline in f_H upon submersion was characteristic of all dives in the study. The intensity of bradycardia during the dive increased with dive duration. This applies to individual dives (lower f_H as the dive progresses) and to the dive f_H and dive duration relationship (Figs 2, 4). Because f_H is indicative of the rate of blood O_2 transport and delivery to the tissues, the bradycardia documented in this study is probably associated with a reduction in blood flow to muscle and other organs. Although it occurs immediately upon submersion, the initial decline in f_H in the emperor penguin is gradual, as described in Scholander's forced submersion studies with penguins (Scholander, 1940). This initial decline occurs more gradually than that seen in some diving seals (Andrews et al., 1997; Thompson and Fedak, 1993). Emperor penguins have 19% of their O_2 stores in the respiratory system (Kooyman and Ponganis, 1998) and dive upon inspiration (Kooyman et al., 1971). Thus, the gradual decrease in f_H in the beginning of dives may reflect the continued uptake from respiratory O_2 stores during this period. Deep diving seals, however, with <5% of O_2 stores in the lungs, would not benefit from maintenance of f_H (i.e. continued O_2 extraction) in this respect, consistent with the immediate drop to low f_H in these species.

Heart rate decreased to extremely low values in long dives, in some cases remaining below 5–6 beats min^{-1} for several minutes (Fig. 2C, Fig. 6C). Consistently low levels of f_H were frequent in dives longer than the ADL. These data demonstrate that such extreme responses are a common component of emperor penguin diving physiology. The minimum instantaneous f_H during the dive decreased significantly with increasing dive duration (Fig. 3). This distribution emphasizes that extremely low values are only present in longer dives, with the degree of bradycardia reflecting dive duration. Such extremely low values of f_H have been demonstrated in free diving seals (Andrews et al., 1997; Thompson and Fedak, 1993), but in birds, have only been documented in forced submersion or exclusion studies (Butler, 1982; Butler and Jones, 1968; Folkow et al., 1967; Jones, 1969; Jones et al., 1988; Jones and Holeton, 1972; Ponganis and Kooyman, 2000; Stephenson et al., 1986). Low f_H, present in later portions of long dives, may reflect a reinforcement of the initial bradycardia by low O_2 partial pressure (P_{O_2}), through a possible chemoreceptor response (Butler and Stephenson, 1988; Enstipp et al., 2001). It is also interesting to note that some extremely low f_Hs correspond to sudden reversals (descent immediately following an ascent) in the depth profile (Fig. 2C, Fig. 6C), as has been documented in other diving species (Andrews et al., 1997). These reversals in the depth profile in emperor penguins diving at the isolated dive hole have been associated with the ascents and descents of foraging events (Ponganis et al., 2000). In addition, in some cases, such reversals might indicate an additional descent after a return toward a hole in which exit was blocked by other birds or an occasional Weddell seal.

Assuming stroke volume is constant (Butler and Jones, 1997), the ratio of dive f_H to f_H at rest is equal to that of the respective cardiac outputs. Considering the mean of dive f_H for all dives longer than the ADL, this indicates a mean 44% reduction in cardiac output during these dives. For the longest dive in the study (18.2 min), mean cardiac output would be decreased by 77%. During the periods of very low f_H toward the end of longer dives (Fig. 2C, Fig. 6B,C), cardiac output may be reduced by as much as 93% for five or more minutes. This is consistent with a low blood O_2 depletion rate while diving, and demonstrates an extreme diving response, as originally proposed by Scholander and Irving in their early work with forced submersion studies (Irving et al., 1941; Scholander, 1940; Scholander et al., 1942).

An increase in f_H during ascent (prior to surfacing) was a common feature in most dives. Such a response has been observed in many diving species, including other penguins, and has been termed an 'ascent' or 'anticipatory tachycardia' (Andrews et al., 1997; Froget et al., 2004; Green et al., 2003; Millard et al., 1973; Thompson and Fedak, 1993). It has been hypothesized that this response may serve to replenish the O_2 supply to depleted tissues, thereby lowering the P_{O2} in blood, and providing a larger gradient to maximize O_2 uptake at the surface, and perhaps to minimize recovery time (Butler and Jones, 1997; Millard et al., 1973; Thompson and Fedak, 1993). If the number of beats in this period of increased f_H during ascent are counted and multiplied by the stroke volume of the emperor penguin (Kooyman et al., 1992), an estimate of the amount of blood circulating in that time period can be obtained. Such a calculation provides the percentage of blood available that could contribute to the supply of depleted tissues at the end of the dive. For example, considering the longest dive in this study (Fig. 2C) and the known blood volume of an emperor penguin (Kooyman and Ponganis, 1998), 92% of the blood volume would be circulated during this ascent period. For the 10 min and 11 min dives in Fig. 6B,C, these values are 47% and 43%, respectively. For shorter dives of 1.5 min and 5.8 min (Fig. 2A,B), 96% and 69% of the blood volume is circulated during this ascent period. These values imply that over a wide range of dive durations, a significant portion of the blood volume is circulated during the period of increased heart rate during ascent. The distribution of this increased blood flow to tissue (brain, heart, central organs and/or muscle) is unknown but would be dependent on sympathetic responses in individual birds during the ascent.

Heart rate profiles: surface intervals

Heart rate during surface intervals was measured over 1 min immediately prior to and following a dive because total surface interval data (i.e. the entire time period between dives) were not considered relevant for the present study. Time spent on the surface post-dive at the isolated dive hole is not necessarily an indicator of diving recovery, as these emperor penguins are subject to other influences and distractions such as interactions with other birds in the corral or activities and personnel at the Penguin Ranch campsite.

Pre- and post-dive periods of tachycardia presumably serve to load O_2 stores pre-dive, and to eliminate carbon dioxide and replenish O_2 stores post-dive. Tachycardia and high respiration rates facilitate rapid gas exchange during the surface interval and allow for a quick recovery from dives (Fedak et al., 1988; Kooyman et al., 1971; Le Boeuf et al., 2000). The maximum instantaneous surface interval f_H in this study is the highest ever recorded for emperor penguins (256 beats min^{-1}), and is in the range of f_H

recorded during maximum O_2 consumption ($\dot{V}_{O_2max}$) of emperor penguins swimming in a flume (Kooyman and Ponganis, 1994). The similarity of the peak values obtained in these two studies, and the consistent range of the f_H values immediately prior to and following dives among individual birds suggest that emperor penguins are operating at a maximum of oxygen uptake during these periods of the surface interval.

While f_H is at its highest immediately pre- and post-dive, there is little variation in f_H and a distinct RSA pattern is not evident. As the f_H decreased after the initial extreme tachycardia during the post-dive surface period, the RSA pattern did emerge in many cases. Similar RSA patterns have been demonstrated in sleep apnea studies of elephant seals. Although the RSA is present in seals throughout the post-apneic period, it is much more pronounced as the tachycardia decreases during recovery (Castellini et al., 1994a). A decrease in the sinus arrhythmia index (the difference between inspiratory and expiratory f_H) at high f_H has also been observed in other mammals (Mazza et al., 1980). The time until the RSA pattern emerged after dives in this study ranged from 11–311 s with a mean of 107±10 s (N=43). By this time, when respiration can once again be inferred by the RSA, it is not significantly elevated above the resting range. Previous data on emperor penguins document vigorous hyperventilation after dives greater than 1 min in duration, with rates of up to 25 breaths min^{-1} (mean ~15 breaths min^{-1}) during the first minute after surfacing (Kooyman et al., 1971). The time required for post-dive respiratory rate to return to resting levels (~3 min), as inferred by post-dive RSA patterns, is consistent with that observed in the previous study (Kooyman et al., 1971).

Stroke frequency and heart rate

Consistent with a previous study (van Dam et al., 2002), emperor penguins diving at the isolated dive hole stroked continuously throughout the dive, with a stroke-glide pattern but no prolonged periods of gliding. The highest stroke frequencies (1.5–2.2 Hz) occurred during initial descent, as the penguin overcame its positive buoyancy at shallow depth (Sato et al., 2002; van Dam et al., 2002). High stroke frequencies also occurred occasionally later in the dive, during descents from shallow depths.

The classical exercise response includes increases in f_H and muscle blood flow in response to increasing work effort, in both terrestrial and marine animals (Fedak et al., 1988; Williams et al., 1991). Consequently, if working skeletal muscle were perfused during diving, f_H during the dive would be expected to increase with stroke frequency, reflecting the increase in work effort. As discussed in the results, it is clear that there is no significant correlation between mean stroke frequency and dive f_H (Fig. 7A). This is also apparent when considering individual dive profiles. Review of simultaneous depth, stroke frequency, and instantaneous f_H profiles reveals that high stroke frequencies are always associated with descents but not usually associated with corresponding changes in f_H (Fig. 6). As discussed previously, f_H profiles during the dive are characterized by an immediate decrease in f_H upon submersion, followed by a readjustment period where f_H briefly increases before gradually decreasing. Despite this immediate and quite drastic reduction in f_H, stroke frequency is at its maximum during this initial descent period. According to classical exercise physiology, if f_H were significantly influenced by stroke frequency, high f_H would be expected during the descent when stroke frequency is at its maximum, however, the reverse holds true. Heart rates are conversely at their most significant rate of decrease in this period, reaching the level of f_H at rest within the first 20 s in 71% of dives, whereas maximum stroke frequency is sustained. Excluding descents, dives are characterized by constant stroke frequencies for the entire duration, yet f_H is variable and toward the end of longer dives, is often 2–4 times lower than during the first half of the dive (Fig. 6). An increase in f_H during ascent was also present in most dives, while stroke frequency remains constant in this period.

Circulatory adjustments are expected in order to maintain blood pressure because of reduced cardiac output during diving (reductions in f_H, and unchanged or slightly decreased stroke volume) (Butler and Jones, 1997; Irving, 1938). A substantial redistribution of blood flow during forced submersion and diving studies has been documented in many birds and mammals, often with more pronounced changes as the dive approaches or exceeds the ADL (Butler and Jones, 1997; Davis et al., 1983; Irving, 1938; Kooyman, 1989; Millard et al., 1973; Murdaugh et al., 1966). The 'true bradycardia' and the lack of coupling of f_H and stroke frequency in dives of emperor penguins at the isolated dive hole are consistent with decreased or absent muscle blood flow during dives. This assumption is supported by prior studies. In emperor penguins, the temperature of the pectoral muscle, one of the two primary underwater locomotory muscles of penguins, consistently increased during diving and decreased during the surface interval (Ponganis et al., 2003). The magnitude and pattern of the temperature fluctuations were consistent with what would be predicted if there were little to no muscle blood flow during dives, and if the metabolic rate of the muscle were calculated on the basis of complete depletion of myoglobin-bound O_2 stores within the ADL (Ponganis et al., 2003). Recent data also demonstrate that blood lactate levels in emperor penguins do not increase significantly during the dive, even in dives longer than the ADL (P. J. Ponganis, unpublished data). Blood lactate does not increase until the post-dive surface interval period (Ponganis et al., 1997). The true bradycardia, the lack of a f_H–stroke frequency relationship, pectoral muscle temperature increases and lactate washout into the blood during the post-dive period all support the concept of peripheral vasoconstriction, isolation of muscle from the blood O_2 store and reliance of muscle metabolism on a myoglobin-bound O_2 store.

Review of f_H profiles also reveals that during the initial descent of a dive, f_H immediately decreases, then transiently increases, and gradually decreases again (Figs 2, 6). This pattern has also been seen in diving king and macaroni penguins (Froget et al., 2004; Green et al., 2003). It might be argued that this initial readjustment in f_H could imply a redistribution of blood back to muscle after intense stroking effort associated with the descent. This suggestion was offered to explain the comparable f_H readjustment pattern in deep dives of king penguins and a delayed increase of pectoral muscle temperature observed in these birds (Froget et al., 2004). The hypothesis posed for king penguins was based on the fact that the readjustment was seen only in deep (long) dives in that study (not in shallow, short dives), and was thereby suggested to reflect an increase in work effort possibly due to increased buoyancy from a higher inspired air volume before deep, long dives (Froget et al., 2004; Sato et al., 2002). Contrary to these king penguin results, however, emperor penguin dives at the isolated dive hole demonstrated this initial f_H readjustment in all dives. Emperor and king penguins exhibit differences in diving behavior, diving capacity, and ecological constraints, and thus physiological disparities may simply be due to the fact that the two species rely on different diving strategies. In order to make a more appropriate comparison between these two species, however, it would be necessary to obtain f_H profiles of emperor penguins during deep dives while foraging at sea. Regardless of the distribution of blood flow during the transient increase in f_H during descent, increased cardiac output should

facilitate respiratory gas exchange and the transfer of O_2 from the respiratory store to the blood O_2 store during this time period.

The value of digital ECG

Although digital ECG records result in more laborious data processing than their R-wave detector (f_H data logger) counterparts, they reveal the full extent of the cardiac response, allowing validation of extremely high f_H values associated with surface intervals and extremely low f_H during the latter parts of long dives. Application of digital ECG could resolve studies employing f_H data loggers, which have resulted in disparate f_H data for the same species (Green et al., 2005; Green et al., 2003). The detailed beat-to-beat analysis afforded by digital ECG also reveals physiological patterns such as the RSA.

Differences in diving f_H between this and the previous study on f_H in emperor penguins (Kooyman et al., 1992) can most likely be attributed to the difference in technology, as the previous study used an R-wave recorder which averaged f_H over 15 s intervals. Most of the dives in that study were much shorter than those in this one, which also contributes to the higher dive f_H found by Kooyman et al. (Kooyman et al., 1992). However, even in a 10 min dive in the previous study, f_H was reported to decline to a minimum of only about 30 beats min^{-1}, as compared to instantaneous minimum f_H values of 5–10 beats min^{-1} for dives of similar duration in this study. Although some of the dive f_H values for particular durations were consistent between the two studies, Kooyman et al. (Kooyman et al., 1992) did not find a correlation between f_H and dive duration, as compared to the present study (Fig. 4). These differences in findings may be secondary to differences in sample size and the range of dive durations, to a loss of variation in f_H due to averaging by the R-wave detector, or to possible miscounting of T-waves or other waveforms in the ECG profile by the R-wave detector in the earlier study. Digital ECG avoids the latter two potential problems by providing the entire record of the ECG signal. It also eliminates imposing an upper limit for f_H, as was necessary with the R-wave recorder to avoid the incorrect counting of T waves as R waves (Kooyman et al., 1992). In order to avoid such double counting in the previous study, it was necessary to impose an upper limit of 120 beats min^{-1}, which was significantly below surface interval f_H revealed by both the post-dive analog ECG in that study and the digital ECG in this study.

In summary, we have validated the hypotheses posed in this study, and have determined that: (1) in contrast to any other free diving bird, a true bradycardia and extremely low f_H occur routinely during dives of emperor penguins at the isolated dive hole, (2) peak surface interval f_Hs (the highest surface interval f_Hs ever recorded for this species) are likely consistent with maximum oxygen uptake, (3) respiratory rate can be inferred from the RSA in this species, and (4) f_H and stroke frequency are not coupled in diving emperor penguins.

LIST OF SYMBOLS AND ABBREVIATIONS

ADL	aerobic dive limit
DLT	diving lactate threshold
ECG	electrocardiogram
f_H	heart rate
P_{O_2}	partial pressure of oxygen
RSA	respiratory sinus arrhythmia
SAI	sinus arrhythmia index
TDR	time depth recorder
V_{O_2max}	maximum rate of oxygen consumption

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CHAPTER 2: Extreme hypoxemic tolerance and blood oxygen depletion in diving elephant seals

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: R000–R000, 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 (Po_2) during dives with a Po_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 Po_2 profiles to obtain %Hb saturation (So_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 So_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.

Po_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 S_{O_2} at various points (i.e., 90, 70, 50, 40, 20, 10, 5% S_{O_2}) along the curve with subsequent measurement of the P_{O_2} of the resulting mixture using an i-STAT blood gas analyzer (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 (P_{CO_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 $\log[S_{O_2}/(100 - S_{O_2})]$ vs. $\log(P_{O_2})$ 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 P_{CO_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.

S_{O_2} Calculations

Percent Hb saturation (S_{O_2}) values were obtained by applying P_{O_2} data to the linear regression equation generated by the dissociation curve, $\log[S_{O_2}/(100-S_{O_2})]$ vs. $\log(P_{O_2})$ plot, and solving for S_{O_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 P_{O_2} value during initial descent of dive) and end-of-dive time points (minimum P_{O_2} during the dive) for each dive were calculated from the corresponding S_{O_2} and P_{O_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) × S_{O_2} + (0.003 × P_{O_2}). Maximum S_{O_2} was estimated with the pH 7.4 dissociation curve, and the minimum S_{O_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 S_{O_2}. For example, from data in Weddell seals (73), a P_{O_2} of 10 mmHg corresponds to 8% S_{O_2} at pH 7.4, but only 2% at pH 7.0. However, since pH values were not < 7.3, P_{CO_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 S_{O_2} profile obtained with the pH 7.4 dissociation curve will reflect the general pattern of change in S_{O_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 S_{O_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 P_{O_2} to return to a venous S_{O_2} (Sv_{O_2}) of 75% (P_{O_2} = 46 mmHg) or arterial S_{O_2} (Sa_{O_2}) of 90% (P_{O_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% S_{O_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 P_{O_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 P_{O_2} values to yield S_{O_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 P_{O_2}, minimum S_{O_2}, maximum S_{O_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). P_{O_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 P_{O_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 S_{O_2} calculations.

Table 1. *Individual dive, P_{O_2}, S_{O_2}, and depletion rate data for all seals*

Seal, No. of dives	Length of Deployment, days at sea	P_{O_2} Electrode Location	Dive 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 $O_2 \cdot dl^{-1} \cdot min^{-1}$
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(SD19.8)	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) S_{O_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 $\log[S_{O_2}/(100 - S_{O_2})]$ vs. $\log(P_{O_2})$, all saturation points, all seals combined, were: pH (7.4): $\log[S_{O_2}/(100 - S_{O_2})] = 2.60211 \times \log(P_{O_2}) - 3.84507$ (n = 63, $r^2 = 0.99$, $P < 0.0001$); pH (7.3): $\log[S_{O_2}/(100 - S_{O_2})] = 2.59256 \times \log(P_{O_2}) - 3.97523$ (n = 33, $r^2 = 0.98$, $P < 0.0001$); and pH (7.2): $\log[S_{O_2}/(100 - S_{O_2})] = 2.622 \times \log(P_{O_2}) - 4.11264$ (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) × S_{O_2} + (0.003 × P_{O_2}), and within 4.5% of previously measured maximal O_2 content for this species (23).

P_{O_2} Profiles, S_{O_2}, and O_2 Depletion

P_{O_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.

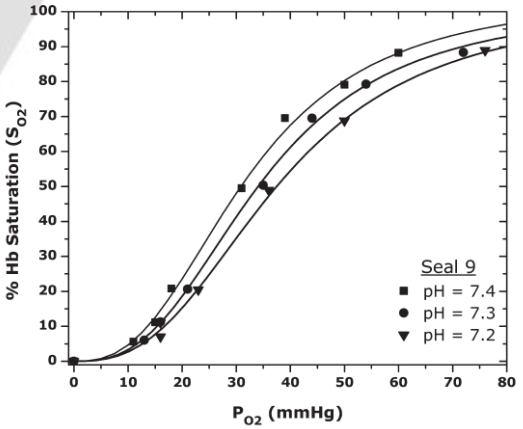

Fig. 1. Oxygen-hemoglobin (O_2-Hb) dissociation curves from one seal at pH 7.4, 7.3, and 7.2. P_{O_2}, partial pressure of oxygen.

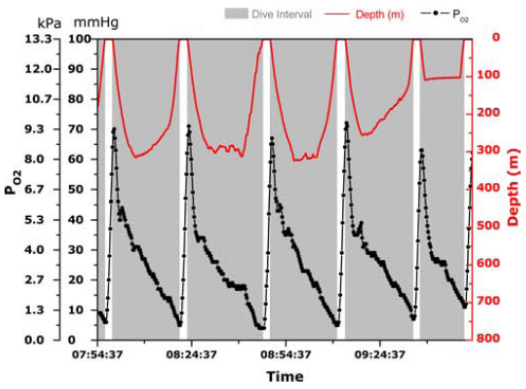

Fig. 2. Extradural vein Po_2 and depth profiles of 2 h of diving from one seal (Starburst).

The shape of the Po_2 profile was generally consistent among all seals in all three blood compartments. Po_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 Po_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 Po_2 also continued briefly for ~5–25 s after the dive. After this brief postdive decline, Po_2 increased quickly during the surface interval.

Minimum Po_2, maximum Po_2, and blood O_2 depletion rates during diving were significantly different between the three Po_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 Po_2 and minimum So_2 had a significant negative correlation with dive duration for all seals, while maximum So_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 Po_2 (the peak value obtained during the dive), minimum So_2, maximum So_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 Po_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 Po_2 increased rapidly during the surface interval (Figs. 2 and 3), the time required for Po_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, Po_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.

Po_2 Electrode Calibration and Verification

Response of the Po_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

Po_2 Profiles

Po_2 and So_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 Po_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, Po_2, So_2, and depletion rate data for seals grouped by Po_2 electrode location (extradural vein, hepatic sinus, arterial)*

Po_2 Electrode Location	No. Seals/ Dives	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 $O_2 \cdot dl^{-1} \cdot min^{-1}$
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 (Po_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 Po_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 Po_2 = 54 mmHg (SD 11); mean maximum Po_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 Po_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

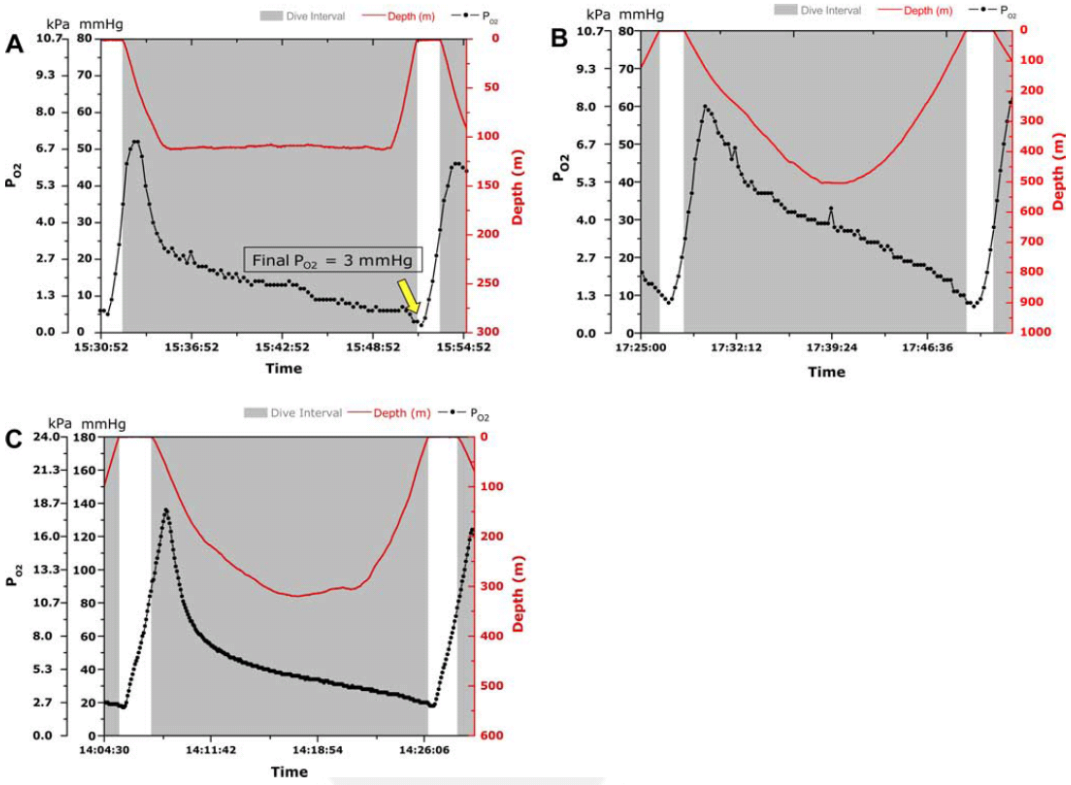

Fig. 3. P_{O_2} and depth profile of individual seal's dives of ~20 min in duration from Chick (P_{O_2} electrode in the extradural vein; *A*), Larry (P_{O_2} electrode in the hepatic sinus; *B*), and Jerry (P_{O_2} electrode in the brachial artery; *C*). Note that *y*-axis scales are different between 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 Po_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 Po_2 due to the large decreases in ambient pressure during the final phase of ascent. In addition, if alveolar Po_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 Po_2 after the end of the dive is likely explained by a combination of: *1*) the response time of the Po_2 electrode (81), *2*) the sampling rate of the Po_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 Po_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 Po_2 from dropping too low and mitigating the risk of shallow water blackout (17).

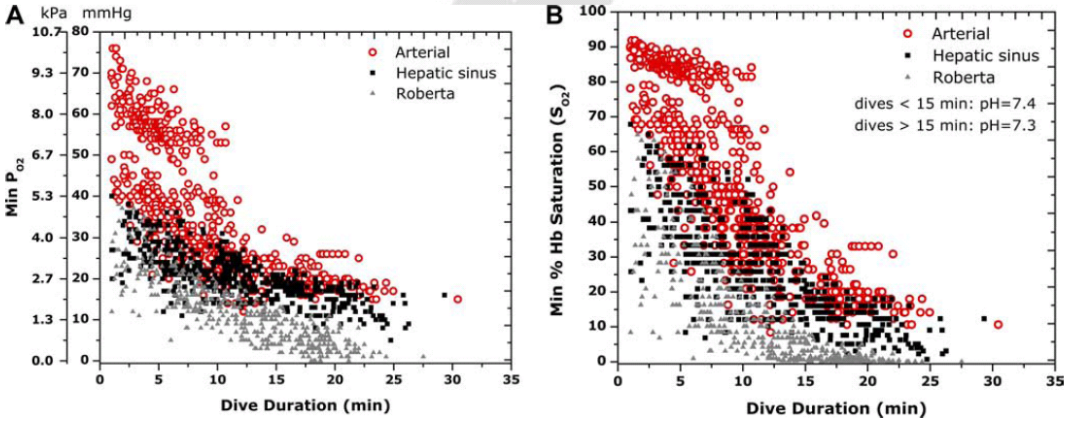

Fig. 4. Minimum Po_2 during the dive vs. dive duration in seals with electrodes in the hepatic sinus and in the artery (*A*) and calculated So_2 (*B*) for these dives. Note the overlap of hepatic sinus and arterial minimum So_2 values with the exception of lower values in one seal with an electrode in the hepatic sinus (Roberta). Minimum So_2 was determined at pH = 7.4 for dives < 15 min, and pH = 7.3 for dives > 15 min. Minimum So_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 Po_2/So_2 increased rapidly during this period (Figs. 2, 3, and 5). These surface interval Po_2/So_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 Po_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 Po_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 Po_2/So_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 Po_2 of the first postdive exhalation, which presumably approximates the end-of-dive Pa_{O_2}, declined significantly with dive duration, reaching a Po_2 of 14 mmHg after a 32-min dive (44, 63). Although end-tidal Po_2 increased greatly during the second breath, the time required for end-tidal Po_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 Po_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 Po_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 Po_2 findings contrast with the surface interval Pa_{O_2} profiles in the present study and with the surface interval blood sample Po_2 data from Weddell seals in prior studies (49, 72).

There are likely several reasons why end-tidal Po_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 Po_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 Po_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 Po_2 difference in seals during the surface interval. This hypothesis would account for the differences between prior end-tidal Po_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 Pco_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 Pco_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 So_2 from Po_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, Pco_2, and Po_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 So_2 and intracellular pH, the tonometry protocol should cause similar intracellular changes in 2,3-DPG concentration.

So_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 Po_2 value at 100% So_2. To ensure complete saturation in the tonometry of the 100% O_2-saturated blood for O_2-Hb dissociation curve determination, a high Po_2 (>200 mmHg) was used for that sample. Thus, the actual inflection point in Po_2 at which saturation first

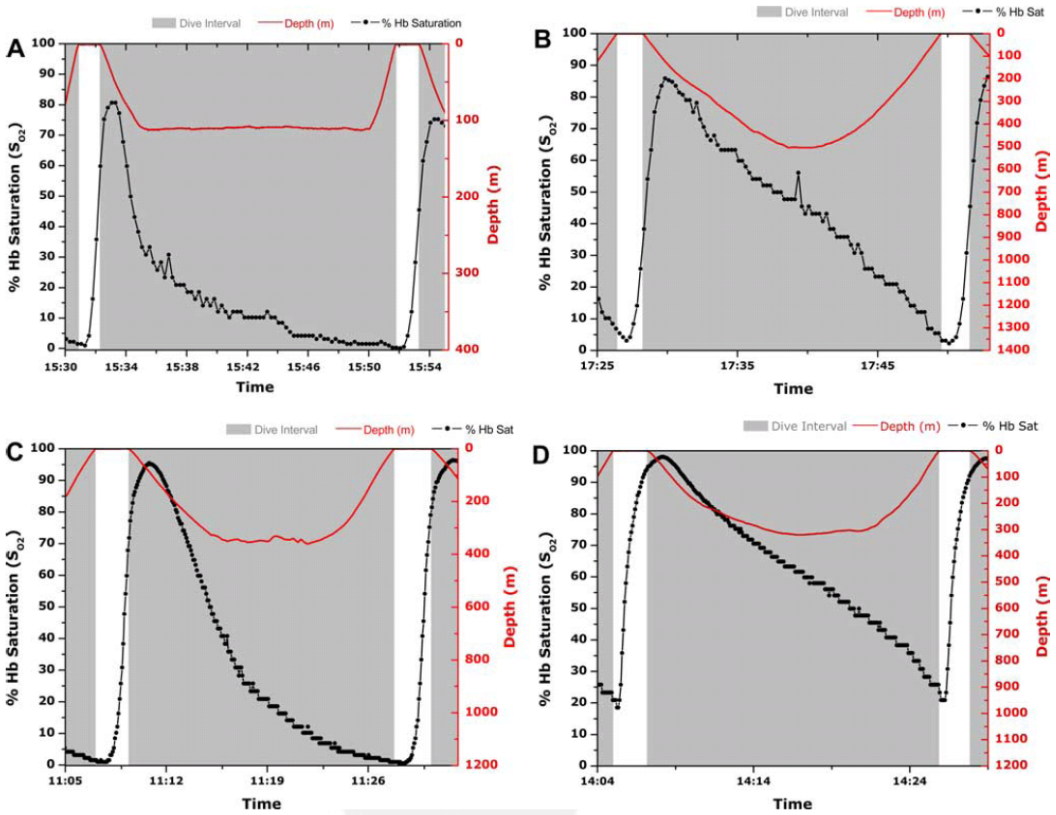

Fig. 5. Decline in So_2 during individual dives for Chick (extradural vein; *A*), Larry (hepatic sinus; *B*), Roberta (the seal whose maximum Pv_{O_2} was higher and minimum Po_2 lower in the hepatic sinus than other seals; *C*), and Jerry (arterial; *D*). The So_2 was determined at pH = 7.4 throughout the entire dive to maintain consistency and to provide a conservative estimate of continuous So_2.

reaches 100% was not determined. The lack of this data point in the regression equations results in a slightly lower calculated So_2 for Po_2 values that approach the inflection point of 100% So_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 Po_2 and So_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 Po_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 Po_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 Po_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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CHAPTER 3: High affinity hemoglobin and blood oxygen saturation in diving emperor penguins

ABSTRACT

The emperor penguin (*Aptenodytes forsteri*) thrives in the Antarctic underwater environment, diving to depths greater than 500 meters and for durations longer than 23 minutes. To examine mechanisms underlying the exceptional diving ability of this species and further describe blood oxygen (O_2) transport and depletion while diving, we characterized the O_2-hemoglobin (Hb) dissociation curve of the emperor penguin in whole blood. This allowed us to 1) investigate the biochemical adaptation of Hb in this species, and 2) address blood O_2 depletion during diving by applying the dissociation curve to previously collected partial pressure of O_2 (P_{O2}) profiles to estimate *in vivo* Hb saturation (S_{O2}) changes during dives. This investigation revealed enhanced Hb-O_2 affinity (P_{50}=28 mmHg, pH=7.5) in the emperor penguin, similar to high-altitude birds and other penguin species. This allows for increased O_2 at low blood P_{O2} levels during diving and more complete depletion of the respiratory O_2 store. S_{O2} profiles during diving demonstrated that arterial S_{O2} levels are maintained near 100% throughout much of the dive, not decreasing significantly until the final ascent phase. End-of-dive venous S_{O2} values were widely distributed and optimization of the venous blood O_2 store resulted from arterialization and near complete depletion of venous blood O_2 during longer dives. The estimated contribution of the blood O_2 store to diving metabolic rate was low and highly variable. This pattern is due, in part, to the influx of O_2 from the lungs into the blood during diving, and variable rates of tissue O_2 uptake.

SYMBOLS/ABBREVIATIONS

ADL aerobic dive limit
Hb hemoglobin
P_{O2} partial pressure of oxygen (Pa_{O2} or Pv_{O2}: arterial or venous)
P_{CO2} partial pressure of carbon dioxide
P_{50} partial pressure of O_2 at which Hb is 50% saturated
S_{O2} % hemoglobin saturation (Sa_{O2} or Sv_{O2}: arterial or venous)

INTRODUCTION

Extreme environments, particularly those with limited oxygen (O_2) availability, are especially challenging to most life forms, yet still host diverse and abundant life. Species which flourish in such environments represent ideal models in which to examine the physiological, cellular, and biochemical mechanisms underlying tolerance to hypoxia and O_2 depletion. The emperor penguin (*Aptenodytes forsteri*), the consummate avian diver, thrives in the extreme Antarctic underwater environment, diving to depths greater than 500 meters (Kooyman and Kooyman, 1995; Wienecke et al., 2007) and for durations longer than 23 minutes (Ponganis et al., 2007). Recent measurements of air sac and blood partial pressure of O_2 (P_{O2}) in diving emperor penguins have revealed exceptional tolerance to low O_2 in this species (Ponganis et al., 2007; Stockard et al., 2005). It has been hypothesized that this underlying tolerance, well below the limits of many birds and mammals, may necessitate biochemical and molecular adaptations including a shift in the emperor penguin O_2-hemoglobin (Hb) dissociation curve, relative to other birds.

The O_2-Hb dissociation curve of whole blood of emperor penguins has not been determined. In general, the hemoglobin of birds has lower O_2 affinity than that of mammals. This may reflect a shift toward favoring O_2 unloading to the tissues, as

the avian respiratory system is inherently more efficient at O_2 uptake (Piiper and Scheid, 1975; Powell, 2000; Powell et al., 1989). Consequently, the P_{50} (P_{O2} at which Hb is 50% saturated) values of most birds (44-52 mmHg) are much higher than those of mammals (25-31 mmHg) (Christensen and Dill, 1935; Hirsowitz et al., 1977; Lenfant et al., 1970; Lutz, 1980; Wastl and Leiner, 1931). However, Adélie (*Pygoscelis adeliae*), Chinstrap *(P. antarctica)*, and Gentoo penguins *(P. papua)*, and the bar-headed goose (*Anser indicus*), a bird adapted to life at high altitudes, have P_{50} values in the mammalian range (Black and Tenney, 1980; Lenfant et al., 1969b; Milsom et al., 1973; Petschow et al., 1977), favoring O_2 uptake from the lungs when P_{O2} is low. The P_{50} for isolated emperor penguin Hb (36 mmHg) has been measured (Tamburrini et al., 1994). Determination of the P_{50} and dissociation curve in whole blood for this species remains necessary, however, as the P_{50} of isolated Hb of this species was highly sensitive to the presence of various co-factors (Tamburrini et al., 1994).

We characterized the O_2-Hb dissociation curve of the emperor penguin in whole blood in order to 1) investigate the biochemical adaptation of Hb in emperor penguins, and 2) address blood O_2 depletion during diving by applying the dissociation curve to previously collected P_{O2} profiles (Ponganis et al., 2009; Ponganis et al., 2007) to estimate *in vivo* Hb saturation (S_{O2}) changes during dives. It was hypothesized that the P_{50} of the emperor penguin would be similar to that of the bar-headed goose and other penguin species, an adaptation which could contribute to tolerance to low O_2 in the emperor penguin (Black and Tenney, 1980; Lenfant et al.,

1969b; Milsom et al., 1973; Ponganis et al., 2007). Hemoglobin with O_2 affinity in this range would also be consistent with blood P_{O2} and O_2 contents of emperor penguins at rest (Ponganis et al., 2007). The magnitude of the Bohr effect was expected to be similar to those in other birds and penguins (-0.4 to -0.6) (Lenfant et al., 1969b). A similar range of Bohr effects, which should favor O_2 unloading to the tissues, has also been observed in a variety of marine mammals (Lenfant et al., 1970; Qvist et al., 1981; Willford et al., 1990). An accurate determination of the Bohr effect in emperor penguin whole blood also allows refinement of the estimation of S_{O2} from P_{O2} profiles since P_{CO2} and pH data are available at various points in dives of this species (Ponganis et al., 2009; Ponganis et al., 2007).

The extent and rate of respiratory O_2 store depletion have been previously determined for the emperor penguin (Stockard et al., 2005). The addition of blood O_2 store depletion data provides the next component of the investigation of total O_2 consumption in this exceptional diver. It was hypothesized that S_{O2} profiles would reflect the wide range of P_{O2} values at the end of dives (Ponganis et al., 2009; Ponganis et al., 2007) and that the rate of decrease of blood O_2 would vary inversely with dive duration since heart rate in this species progressively decreases during longer dives (Meir et al., 2008). An inverse relationship to dive duration has been demonstrated in the respiratory O_2 store depletion rate of the diving emperor penguin (Stockard et al., 2005) and in blood O_2 store depletion rates of seals (Elsner et al., 1964; Stockard et al., 2007).

MATERIALS AND METHODS

As in past studies (Kooyman et al., 1992; Ponganis et al., 2001), non-breeding emperor penguins (*Aptenodytes forsteri* Gray; ~15 birds/season, 20-30 kg) were captured near the McMurdo Sound ice edge or at Terra Nova Bay in October of 2003-2005, 2007, and 2008 and were maintained for 6 weeks at an isolated dive hole enclosed within a corral at the Penguin Ranch on the McMurdo Sound sea ice (77°41', 165°59'). All procedures were approved under a UCSD Animal Subjects Committee protocol and U.S. Antarctic Treaty Permit. All birds were returned to the ice edge and released upon completion of the study.

P_{O2} Electrode Deployments

P_{O2} electrodes (Licox C1.1 Revoxode; Integra LifeSciences, Plainsboro, NJ, USA) and thermistors (model 554,Yellow Springs Instruments, Yellow Springs, OH, USA) were inserted percutaneously into the aorta or vena cava of emperor penguins under general isoflurane anesthesia as described previously (Ponganis et al., 2007; Ponganis et al., 2001; Ponganis et al., 2004; Ponganis et al., 2003). An additional arterial data set was obtained in 2008 from a bird equipped with only an arterial P_{O2} electrode as described in the previous study (Ponganis et al., 2009). The electrodes were connected to a custom-built P_{O2}/temperature recorder (UFI, Morro Bay, CA, USA) and an Mk9 time depth recorder (TDR) was also attached (Wildlife Computers, Redmond, WA, USA) as previously described (Stockard et al., 2005). After overnight recovery from anesthesia, birds were allowed to dive at the isolated dive hole. The

instrumented birds dived for 1–2 days, after which catheters, probes and recorders were removed under general anesthesia. All P_{O2} values were corrected to 38°C for construction of the P_{O2} profiles, as previously reviewed (Ponganis et al., 2007).

O_2-Hb Dissociation Curve Characterization

O_2-Hb dissociation curves on fresh whole blood were determined with the mixing technique of tonometered blood (Scheid and Meyer, 1978). Blood samples were obtained from the Penguin Ranch emperor penguins during anesthesia, placed on ice, and processed immediately. All dissociation curve analyses were completed within six hours after blood collection in order to prevent depletion of labile organic phosphates such as inositol pentaphosphate (IPP) which would influence the dissociation curve, and to avoid prolonged metabolism of these nucleated avian red blood cells. The mixing technique consisted of the volumetric mixing of 0% O_2-saturated blood and 100% O_2-saturated blood to achieve desired S_{O2} at various points (*i.e.*, 90, 70, 50, 40, 20, 10, 5% S_{O2}) along the curve with subsequent measurement of the P_{O2} of the resulting mixture using an i-STAT blood gas analyzer (37^{o}C; Abbott Point of Care, Princeton, New Jersey, USA) (Black and Tenney, 1980; Johansen et al., 1987; Nörgaard-Pedersen et al., 1972; Qvist et al., 1981). Use of the i-STAT analyzer also allowed verification of pH and P_{CO2}, and Tucker chamber analyses (Tucker, 1967) provided verification of blood O_2 content. The CO_2 Bohr effect was determined by changing the CO_2 concentration of the gas in the tonometer in order to adjust pH. Dissociation curves were determined at pH values of 7.5, 7.4, 7.3, and 7.2. The

log[S_{O2}/(100-S_{O2})] vs. log(P_{O2}) was plotted and linear regression analysis performed in order to generate the equation for the O_2-Hb dissociation curve at each pH (Nicol, 1991). As in similar studies (Nicol, 1991), all S_{O2} points from all birds were combined in order to generate a general O_2-Hb dissociation curve for this species. This is justified as, with the exception of disease, the structure of hemoglobin varies among species, not individuals of a species. The Bohr coefficient was derived from linear regression of the log P_{50} on pH (each point averaged from all data of all birds for pH=7.5, 7.4, 7.3, and 7.2) (Nicol, 1991; Willford et al., 1990). In addition, the fixed acid Bohr effect was determined by titrating with HCl to pH 7.4, 7.3 and 7.2 while P_{CO2} was maintained at the level required for the standard pH=7.5 curve in that penguin (Willford et al., 1990). The effect of lactic acid on the dissociation curve was also evaluated in whole blood by determining additional dissociation curve points after adding lactic acid (SIGMA L6661) to the blood to a final concentration of 5 and 10 mM (Nicol, 1991).

In order to validate the specific equipment and methods used in this study, dissociation curves were also determined with blood from an avian (chicken, *Gallus gallus domesticus*) and pinniped (*Leptonychotes weddelli, Phoca vitulina, Zalophus californianus*) species with previously published O_2-Hb binding data (Bartels et al., 1966; Hirsowitz et al., 1977; Lenfant, 1969; Lenfant et al., 1969a). Mixing technique tests were also conducted to verify that no hemolysis occurred during mixing.

% Hb Saturation (S_{O2}) Calculations

Percent hemoglobin O_2 saturation (S_{O2}) values were obtained by applying data from P_{O2} profiles [from past (Ponganis et al., 2009; Ponganis et al., 2007) and current studies] to the linear regression equation generated by the $\log[S_{O2}/(100-S_{O2})]$ vs. $\log(P_{O2})$ plot and solving for S_{O2} (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 hemoglobin analyses [cyanomethemoglobin technique (Ponganis et al., 1993)] in order to obtain Hb concentration. Oxygen content for initial and final (end-of-dive) time points for each dive were calculated from the corresponding S_{O2} values, using a Hb concentration of 18.3 g dl^{-1} ([this study, agrees well with previous findings (Kooyman and Ponganis, 1998; Ponganis et al., 1997a)] with the formula: [O_2 Content (ml O_2 dl^{-1} blood) = (1.34 ml O_2 g^{-1} Hb) x [Hb](g dl^{-1}) x S_{O2} + (0.003 x P_{O2})]. Initial S_{O2} (S_{O2} at the start of the dive) was estimated with the pH=7.5 dissociation curve, and the final S_{O2} value (from the final P_{O2} value, measured within the last 5 or 15 seconds of the dive depending on the specific recorder) was estimated with the pH dissociation curve at 7.4. The effect of pH is critical in estimation of final S_{O2}. For example, from data in Weddell seals (Qvist et al., 1981), a P_{O2} of 10 mmHg corresponds to 8% S_{O2} at pH 7.4, but only 2% at pH 7.0. Blood lactate concentration does not increase significantly during dives of emperor penguins, even during dives longer in duration than the previously measured aerobic dive limit [ADL; dive duration associated with the onset

of post-dive blood lactate accumulation (Kooyman et al., 1983); 5.6 min for the emperor penguin (Ponganis et al., 1997b)] for this species (Ponganis et al., 2009). The rise in blood lactate does not occur until the post-dive period in this species (Ponganis et al., 1997b; Ponganis et al., 2009). Based on lactate, P_{CO2}, and pH measurements obtained from emperor penguin blood samples drawn at various time points during diving (Ponganis et al., 2009), a pH of 7.4 was deemed most representative of conditions at the end of the dive.

The percentage of net O_2 content depletion in either the arterial or venous system (dependent on the site of insertion) for each dive of each penguin was calculated [% O_2 Content Depletion = (initial O_2 content – final O_2 content)/initial O_2 content x 100]. The rate of O_2 content depletion [(initial O_2 content – final O_2 content)/dive duration) was also calculated for the arterial and venous systems. This provides an overall depletion rate, not an instantaneous measure of O_2 consumption. Data from penguins with arterial P_{O2} 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

As outlined above, the dissociation curve, calculated using linear regression, was applied to P_{O2} values to yield S_{O2} values, and calculations of O_2 depletion rate and percentage of O_2 depletion were made. Differences between arterial and venous results were determined with one-way ANOVA. Correlation between dive duration

and the variables of final S_{O2}, pre-dive S_{O2}, % 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 $\pm$ s.d.. P_{O2} values are expressed in mmHg (as measured). Figures include the corresponding values in kPa, assuming 1 mmHg= 0.133 kPa.

RESULTS

Dive behavior of emperor penguins at the Penguin Ranch for the dives associated with the P_{O2} profiles in this study has been described (Ponganis et al., 2009; Ponganis et al., 2007). Dive duration and depth data are given in Table 1.

O_2-Hb Dissociation Curve

Complete O_2-Hb dissociation curves were determined with blood from 12 penguins, with additional P_{50} values at specific pH levels and fixed acid Bohr effects obtained in an additional 2 penguins. The P_{50} was 28 $\pm$ 1 mmHg (n=12 penguins) at pH=7.5 (Fig. 1A). The CO_2 Bohr effect (slope of log P_{50} vs. pH) was -0.45 ($y= -0.45x+4.81$, $r^2=0.98$, $P=0.008$). The fixed acid Bohr effect (addition of HCl or lactic acid) was not significantly different from that of CO_2.

The resulting regression equations from the plots of $\log[S_{O2}/(100-S_{O2})]$ vs. $\log(P_{O2})$ (all saturation points, all penguins combined) were:

pH (7.5):$\log[S_{O2}/(100-S_{O2})] = 2.92589 \times \log(P_{O2}) - 4.24338$ (n=43, $r^2=0.98$, $P<0.0001$)

pH (7.4): $\log[S_{O2}/(100-S_{O2})]=2.94767 \times \log(P_{O2}) - 4.39858$ (n=70, $r^2=0.98$, $P<0.0001$)

pH (7.3): $\log[S_{O2}/(100-S_{O2})] = 3.04945 \times \log(P_{O2}) - 4.72019$ (n=38, $r^2=0.99$, $P<0.0001$)

pH (7.2): $\log[S_{O2}/(100-S_{O2})] = 3.15958 \times \log(P_{O2}) - 4.97618$ (n=9, $r^2=0.99$, $P<0.0001$)

Samples from other species (chicken and pinnipeds) run for dissociation curve method validation agreed with previously published values (Bartels et al., 1966; Hirsowitz et al., 1977; Lenfant, 1969; Lenfant et al., 1969a). 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 hemoglobin concentration in this study (18.3 ± 1.1 g dl^{-1}, n=17) was equivalent to that of previous studies (Kooyman and Ponganis, 1998; Ponganis et al., 1997a). Oxygen content analyses performed with 100% O_2-saturated blood (Tucker Chamber) agreed within 5% of the maximal O_2 content calculated by the equation in this study [O_2 Content (ml O_2 dl^{-1} blood) = (1.34 ml O_2 g^{-1} Hb) x [Hb](g dl^{-1}) x S_{O2} + (0.003 x P_{O2})].

P_{O2} Profiles

In addition to the arterial P_{O2} data collected in previous studies (Ponganis et al., 2009; Ponganis et al., 2007), an arterial P_{O2} and dive profile was obtained for one more emperor penguin in the 2008 Antarctic field season (Fig. 2A). This increased the arterial data sample size to 6 penguins (71 dives total). The venous data were from 9 penguins (130 total dives). These profiles have been adequately described in previous studies (Ponganis et al., 2009; Ponganis et al., 2007).

S_{O2} and Blood O_2 Depletion

Sa_{O2} often remained near 100% for much of the dive, reflecting the large increase in arterial P_{O2} at the beginning of the dive and the fact that despite a subsequent decrease, P_{O2} remained relatively high until the later dive phase (Fig. 2A) (Ponganis et al., 2007). A rapid decline in Sa_{O2} generally began during the final ascent phase of the dive (Fig. 2A). Sv_{O2} profiles reflected venous P_{O2} profiles (Ponganis et al., 2007) in that they were quite variable among dives (Fig. 2B, 3), with marked fluctuations, transient increases during the dive, and a large range of final Sv_{O2} values (Fig. 4). Re-oxygenation occurred rapidly upon return to the surface, as demonstrated by the return in both Sa_{O2} and Sv_{O2} to values consistent with those at rest within approximately two minutes post-dive (Fig. 2). Pre-dive and initial Sv_{O2} values (Table 1, Figs. 2B, 3) were often higher than those corresponding to the mean venous P_{O2} of emperor penguins at rest (Ponganis et al., 2007).

Final Sv_{O2} was $\leq$ 20% in 28% of dives, $\leq$ 5% in 15% of dives, and $\leq$ 2% in 6% of dives. With the exception of only 1 dive, Sv_{O2} decreased below 20% only in dives greater than the previously measured ADL (Ponganis et al., 1997b) (Table 1, Fig. 4). Max S_{O2} (the peak S_{O2} during the dive), pre-dive S_{O2}, initial S_{O2}, final S_{O2}, ΔS_{O2} (initial S_{O2}-final S_{O2}), % O_2 content depletion, and blood O_2 store depletion rate data are given in Table 1. Max S_{O2}, initial S_{O2}, final S_{O2}, ΔS_{O2}, and % O_2 content depletion were all significantly different between the arterial and venous compartments [One-way ANOVA: $P \leq 0.001$ for all except ΔS_{O2} (P=0.035); F values = 144.40, 180.42, 59.14, 4.53, 10.93 respectively]. The blood O_2 store depletion rates

between the 2 compartments, however, were not significantly different (One-way ANOVA: P=0.94; F value = 0.00627).

Both final Sa_{O2} and Sv_{O2} demonstrated a strong and significant negative correlation to dive duration (Spearman Rank Order Correlation, Table 2) (Fig. 4). The % of O_2 content depleted during the dive showed a strong and significant positive correlation to dive duration for both compartments (Spearman Rank Order Correlation, Table 2). The pre-dive Sv_{O2} had a significant, but weak positive correlation to dive duration, while pre-dive Sa_{O2} was not significantly related to dive duration (Spearman Rank Order Correlation, Table 2). Blood O_2 store depletion rate had a significant, but weak, positive relationship to dive duration in both the arterial and venous compartments (Spearman Rank Order Correlation, Table 2).

DISCUSSION

O_2-Hb Dissociation Curve

As hypothesized due to its potential to contribute to tolerance to low O_2 in this species (Ponganis et al., 2007), the O_2-Hb dissociation curve of the emperor penguin is indeed left-shifted relative to most birds, similar to that of other penguin species and the high-flying bar-headed goose (Black and Tenney, 1980; Lenfant et al., 1969b; Milsom et al., 1973; Petschow et al., 1977). Left-shifted hemoglobin (higher Hb-O_2 affinity) is advantageous for the diving emperor penguin, as it implies that more O_2 is available at any given P_{O2}. This becomes particularly relevant for a breath hold diver that often experiences low P_{O2} values during diving. For example, at a P_{O2} of 20

mmHg, a pekin duck would be stripped of all its O_2, while the hemoglobin of the emperor penguin, with its left-shifted dissociation curve, is still 27% saturated (pH=7.5; 21.5% at pH=7.4) at this same P_{O2} (Fig. 1B). This advantage could also assist in the prevention of deleterious effects such as shallow water blackout if Pa_{O2} drops to low levels during diving.

An increased Hb-O_2 affinity also allows for more complete depletion of the respiratory O_2 store. As opposed to marine mammals whose lungs do not comprise a significant portion of total O_2 stores, the respiratory store of the diving emperor penguin remains a significant contributor to O_2 stores while diving [19% of total O_2 stores (Kooyman and Ponganis, 1998)], and this species dives upon inspiration (Kooyman et al., 1971). The increased O_2 affinity of emperor penguin Hb allows for continued extraction of this O_2 from the respiratory O_2 store for use during diving. In contrast, a pekin duck forcibly submerged to the point of "imminent cardiovascular collapse" is left with 25% of its respiratory O_2 store unused, as the blood contained no O_2 at an air sac P_{O2} near 30 mmHg (Hudson and Jones, 1986).

Presumably, the biochemical adaptation underlying the increased Hb-O_2 affinity is achieved with specific amino acid substitution(s), similar to that of other species. In two species of high-altitude birds with increased Hb-O_2 affinity (bar-headed and Andean geese), the same $\alpha_1\beta_1$ intradimer contact site is disrupted, accomplished by two different amino acid substitutions (on the α-chain in one of the species, and on the corresponding β-chain site in the other) (Perutz, 1983; Weber and Fago, 2004). This same intradimer contact site is altered in high O_2 affinity fish Hb,

and even reconstituted human Hb demonstrates significantly higher affinity for O_2 when this single site is altered (Jessen et al., 1991; Weber and Fago, 2004). Upon examination of the published amino acid sequence of emperor penguin hemoglobin (Tamburrini et al., 1994), it is revealed that this specific site is not altered in emperor or rockhopper (*Eudyptes crestatus*) penguins. However, as compared to human Hb, there are changes in six α-chain and five β-chain $\alpha_1\beta_1$ contact sites, and one α-chain $\alpha_1\beta_2$ contact site. It is possible that one of these modifications accounts for the emperor penguin's increased Hb-O_2 affinity, though other structural features might also be responsible.

We hypothesize that the small Bohr effect (-0.25) found in the previous study of isolated emperor penguin Hb may be secondary to experimental conditions and the sensitivity of the isolated Hb to co-factors (Tamburrini et al., 1994). It was hypothesized in that study that the low Bohr effect prevents "stripping" of O_2 during the long dives of emperor penguins (Tamburrini et al., 1994). However, such a low Bohr effect for Hb in whole blood was not found in this study, nor is it present in other diving penguins and marine mammals (Lenfant et al., 1970; Lenfant et al., 1969b). A small Bohr effect is a potential disadvantage in terms of unloading O_2 to the tissues in a diving animal, especially in those with relatively high Hb-O_2 affinities. Blood pH data during dives has also demonstrated that emperor penguin blood does not become very acidotic during the dive, indicating that the effects of the Bohr shift may not be particularly relevant during diving in this species (Ponganis et al., 2007).

S_{O2} Extremes

Final Sv_{O2} reached very low levels, $\leq$ 5% in 15% of dives (corresponding to O_2 contents < 1.2 ml O_2 dl^{-1} blood) and approaching 0% in some dives, particularly in those with durations longer than the previously measured ADL (Ponganis et al., 1997b) (Table 1, Figs. 2B, 3, 4). This resulted in a large % venous O_2 content depletion during the dive (Table 1). The wide range of final Sv_{O2} values for dives of similar duration (Fig. 4) and the fluctuations in the venous P_{O2} and S_{O2} profiles during dives (Fig. 3) suggest significant variability in tissue O_2 extraction and blood flow patterns during dives. As previously suggested (Ponganis et al., 2009), this may reflect differences or changes in the peripheral vascular response including regulation of blood flow to muscle and other organs as well as through arterio-venous (a-v) shunts.

Final Sa_{O2} reached only as low as 47% (Table 1, Fig. 4) (corresponding to an O_2 content of 11.5 ml O_2 dl^{-1} blood) in dives as long as 12 min. Such high values contrast with the lower values found in elephant seals (*Mirounga angustirostris*) (Meir et al., 2009) and, again, are considered secondary to the size of the respiratory O_2 store in emperor penguins and to the maintenance and efficiency of respiratory gas exchange during these dives. Such high Sa_{O2} may well minimize the risk of shallow water black out in these birds (Fig. 2A). It should be noted that many of these final Sa_{O2} values are greater than would have been predicted from final air sac P_{O2} values for dives of similar duration (Ponganis et al., 2009; Ponganis et al., 2007). It is unclear why final Pa_{O2} values are higher than those recorded in the air sacs, especially

since air sac P_{O2} prior to dives and during dives is greater than Pa_{O2} (Ponganis et al., 2009). Sample size, type of dive, circulatory lag time, and the location of the sampled air sac may contribute to these differences. Regardless of the explanation, it is apparent that emperor penguins can perform dives as long as 12 min in which final arterial P_{O2} and S_{O2} are relatively well preserved.

It should also be noted that there is considerable overlap between final arterial and many final venous S_{O2} values of the dives in this study (Fig. 4). Such overlap may be secondary to the previously suggested intra-dive a-v shunting (Ponganis et al., 2009) and the close equilibration of air sac, arterial, and venous P_{O2} in the latter parts of such dives. Equilibration of arterial and venous P_{O2} and S_{O2} values toward the end of long breath holds has been reported in seals (Elsner et al., 1964; Stockard et al., 2007). As the sample size for arterial records was smaller than that of venous records, and because there were no dives longer than 12 min for arterial data (compared to > 23 min for venous data), we believe that final Sa_{O2} values in longer dives may well be lower than those documented in this study.

Both Sa_{O2} and Sv_{O2} returned to values equivalent to those at rest within about two minutes post-dive (Fig. 2), consistent with P_{O2} profiles of this species (Ponganis et al., 2009; Ponganis et al., 2007). In the previous study, no relationship was shown between surface interval duration and the time to return to the P_{O2} value at rest (Ponganis et al., 2009). Thus, re-oxygenation occurred very rapidly upon return to the surface, and does not represent a limitation to the onset of the subsequent dive.

S_{O2} Profiles and Implications to O_2 Store Utilization

As previously discussed (Ponganis et al., 2009), the initial transient increase in Pa_{O2} during dives reflected the compression hyperoxia observed during diving in the air sacs of emperor penguins (Stockard et al., 2005) and demonstrated the maintenance of pulmonary gas exchange and transfer of O_2 from the respiratory to the blood O_2 store. Even as Pa_{O2} declined from the early peak value, its value was relatively high for much of the dive (Fig. 2A). As a consequence, Sa_{O2} remained near 100% for much of the dive (Fig. 2A), preserving a high O_2 content in the arterial system for critical organs like the brain. Sa_{O2} generally did not decrease significantly until the final ascent phase of the dive, consistent with the decline in ambient pressure and decrease in both air sac and arterial P_{O2} during ascent (Ponganis et al., 2007; Stockard et al., 2005) (Fig. 2A). The net transfer of O_2 from the lungs to the blood also resulted in some final Pa_{O2} values greater than the Pa_{O2} of birds at rest and some final Pv_{O2} values not only greater than that at rest, but even greater than Pv_{O2} at the start of the dive (Ponganis et al., 2007). The corresponding S_{O2} values resulted in negative values for ΔS_{O2}, % O_2 content depleted, and blood O_2 depletion rates (*i.e.*, an overall increase in S_{O2} during the dive) in such dives (Table 1, Fig. 5). These findings are consistent with the significant role of the left-shifted dissociation curve and with the contribution of respiratory O_2 to total O_2 stores in this species.

In contrast, although there was also a transient increase in Pa_{O2} and consequently Sa_{O2} in diving elephant seals at the beginning of dives, max Pa_{O2} in the emperor penguin was nearly twice that in the elephant seal [mean max $Pa_{O2} = 88 \pm 20$

mmHg for the elephant seal (Meir et al., 2009) and 157 $\pm$ 52 mmHg in the emperor penguin]. Like the emperor penguin, Sa_{O2} peaked near 100% in the elephant seal, but this high level of Sa_{O2} was not maintained and instead decreased rapidly after the peak (Meir et al., 2009). These findings reflect the difference in the magnitude of the respiratory O_2 store between these two species. Unlike emperor penguins, elephant seals dive upon expiration and the lungs do not comprise a significant portion of total O_2 stores (Falke et al., 1985; Kooyman and Ponganis, 1998).

It should be noted that maximum S_{O2} values in this study were likely slightly underestimated, due to the lack of determination of a true, critical P_{O2} value at 100% S_{O2}. In order to ensure complete saturation in the tonometry of the 100% O_2-saturated blood for O_2-Hb dissociation curve determination, a high P_{O2} (>200 mmHg) was used for that sample. Thus, the actual inflection point in P_{O2} at which S_{O2} first reaches 100% was not determined (Fig. 1A). The lack of this data point in the regression equations results in a slightly lower calculated S_{O2} for P_{O2} values that approach the inflection point of 100% S_{O2}. In turn, this implies that net depletion values and O_2 depletion rates are slightly underestimated.

Arterio-Venous Shunts

Pre-dive and initial Sv_{O2} values were often higher than those corresponding to Pv_{O2} of emperor penguins at rest (Ponganis et al., 2007), reaching as high as 95% (Table 1, Figs. 2B, 3). Although maximum Sv_{O2} during a dive was much more variable than Sa_{O2} (Table 1), the mean of maximum Sv_{O2} was also quite high at 81%,

and, in some dives, max Sv_{O2} values were almost 100% (Table 1, Figs. 2B, 3). These "arterialized" venous values imply some degree of arterio-venous (a-v) shunting or at least a lack of tissue O_2 uptake from the blood. In addition to previous findings in these species, including increases in Pv_{O2} during the descent period, the lack of blood lactate accumulation during the dive, muscle temperature profiles, dramatic bradycardias and a lack of association between heart rate and stroke frequency during diving, these results further support the concept that muscle is isolated from the circulation during dives of emperor penguins (Meir et al., 2008; Ponganis et al., 2009; Ponganis et al., 2007; Ponganis et al., 2003).

Such high values during surface intervals may be secondary to an increased gas exchange rate, enhanced reloading of O_2 stores and improved ventilation perfusion matching due to a more pronounced hyperventilation and tachycardia before such dives. In addition to the maximization of the blood O_2 store by the arterialization of its venous blood, these birds often made full use of this component of their O_2 store by decreasing Sv_{O2} to near 0 % in longer dives, as discussed above (Table 1, Figs. 2B, 3). For example, in the longest recorded dive to date for an emperor penguin (23.1 min), pre-dive S_{O2} was 91-95%, initial S_{O2} was 91%, and final S_{O2} decreased to as low as 1%, demonstrating a near complete utilization of the venous blood O_2 store in this remarkable dive (Fig. 2B).

Based on pre-dive S_{O2} values, the magnitude of a peripheral a-v shunt prior to the dive can be estimated with a shunt equation. This can be calculated using the following equation, assuming a 5 ml O_2 dl^{-1} a-v O_2 difference at rest (Ponganis et al.,

2007): (venous O_2 content = [arterial O_2 content x (% a-v shunt)] – [(arterial O_2 content – 5 ml O_2 dl^{-1}) x (1- (% a-v shunt))]. For example, the maximum pre-dive Sv_{O2} in this study was 95.8% (Table 1). Using O_2 contents calculated for this level of Sv_{O2} (23.5 ml O_2 dl^{-1}) and the maximal pre-dive Sa_{O2} (24.4 ml O_2 dl^{-1}) an arterio-venous shunt of 82% would be necessary to reach this venous S_{O2}. For the pre-dive Sv_{O2} of 91.3% (22.4 ml O_2 dl^{-1}) prior to the 23.1 min dive (Fig. 2B), the magnitude of the a-v shunt would be 60%. These calculations should be considered slight overestimates since the Sv_{O2} values are vena caval and not true mixed venous samples (myocardial O_2 extraction would decrease the mixed venous Sv_{O2} further).

Intrapulmonary Shunts

As discussed in previous studies, the mean Pa_{O2} of emperor penguins at rest (68 $\pm$ 7 mmHg) is less than 2/3 of the mean value in the air sac (Ponganis et al., 2009; Ponganis et al., 2007), likely due mainly to ventilation-perfusion mismatch (Powell, 2000; Powell et al., 1989). Using the O_2-Hb dissociation curve and the classic pulmonary shunt equation [% shunt = (Capillary O_2 content – Arterial O_2 content)/(Capillary O_2 content – Venous O_2 content)], calculations can be made to estimate the size of the pulmonary shunt in the emperor penguin both at rest and in the pre-dive period. Calculating S_{O2} from the previously measured mean Pa_{O2} and Pv_{O2} values at rest (Ponganis et al., 2007) and assuming capillary S_{O2}=100%, based on air sac P_{O2} measurements of 120 mmHg from previous studies (Stockard et al., 2005), and a [Hb] = 18.3 g dl^{-1} (this study) to calculate O_2 content, the extent of the

intrapulmonary shunt in a resting emperor penguin is 28%. Again, this value may be slightly overestimated since mixed venous values were not used in the calculation. Nonetheless, shunting of pulmonary blood flow is usually very small in birds, less than 1-2.7% of cardiac output in anesthetized artificially ventilated geese and ducks [representing the true intrapulmonary shunt; (Burger et al., 1979; Powell and Wagner, 1982)], or 6.3-8% in ducks [representing intrapulmonary plus extrapulmonary shunts; (Bickler et al., 1986)]. The high % shunt value in emperor penguins may also be due in part to an overestimation of the capillary O_2 content because capillary P_{O2} and saturation may be decreased secondary to the thickened parabronchial capillary blood-to-air barrier reported in emperor penguins (Welsch and Aschauer, 1986). Using pre-dive Sa_{O2} and Sv_{O2} values (mean values for pre-dive Sv_{O2} and Sa_{O2} in this study, Table 1) instead of values at rest, however, the intrapulmonary shunt is reduced to 14.3%. This suggests that the hyperventilation and tachycardia characteristic of the pre-dive period in this species (Kooyman et al., 1971; Kooyman et al., 1992; Meir et al., 2008) improves ventilation-perfusion matching prior to the dive.

Blood O_2 Store Contribution to Metabolic Rate

Since the value to predict blood volume of an emperor penguin of a given mass is known (100 ml kg^{-1}) (Kooyman and Ponganis, 1998; Ponganis et al., 1997a), a calculation of the contribution of the blood O_2 store to overall metabolic rate was made: Blood O_2 store contribution = [((final O_2 content – initial O_2 content)/dive duration) x Blood Volume], with the assumption that one-third of blood volume is

arterial, and two-thirds venous in distribution (Kooyman, 1989). Using the mean values for all arterial and all venous dives combined, the blood O_2 store contribution alone to metabolic rate from the arterial compartment is 0.3 $\pm$ 0.2 ml O_2 kg^{-1} min^{-1} (range = -0.1 - 0.7) and from the venous compartment is 0.6 $\pm$ 0.9 ml O_2 kg^{-1} min^{-1} (range = -4.1 – 3.1) (venous). These values are only approximately 5% (arterial) and 10% (venous) of the resting metabolic rate measured for this species (Kooyman and Ponganis, 1994; LeMaho et al., 1976; Pinshow et al., 1977). If dives in which S_{O2} increased during the dive are excluded from the analysis, the blood O_2 store contribution alone to metabolic rate from the arterial compartment is 0.4 $\pm$ 0.2 ml O_2 kg^{-1} min^{-1} (range=0.0 - 0.7) and from the venous compartment is 0.9 $\pm$ 0.6 ml O_2 kg^{-1} min^{-1} (range=0.1 – 3.1). In comparison, respiratory O_2 store depletion rates were 2.3 and 5.3 times that in the venous and arterial blood O_2 store compartments, respectively [mean = 2.1$\pm$ 0.8 ml O_2 kg^{-1} min^{-1} (Stockard et al., 2005)].

As previously discussed, since S_{O2} was sometimes higher at the end of the dive than at the initial time point, negative values resulted for Δ S_{O2}, % O_2 content depletion, and depletion rates (Table 1, Fig. 5). For example, the venous O_2 store contribution to metabolic rate was -1.8 ml O_2 kg^{-1} min^{-1} for a 1.6 min dive. Assuming a gain in the venous blood O_2 store of 1.8 ml O_2 kg^{-1} min^{-1} in this 1.6 min dive, this indicates a minimum loss of 2.9 ml O_2 kg^{-1} from the respiratory system during the dive. If the respiratory system is initially 20% O_2 at 69 ml kg^{-1} (Ponganis et al., 1999), it contains approximately 14 ml O_2 kg^{-1} at the beginning of the dive. For the 1.6 min dive, a minimum of 21% (2.9/14) of the respiratory O_2 was transferred into the blood.

This represents a minimum value, as it does not include respiratory O_2 that entered the blood and replaced any O_2 taken up by tissue from the blood. However, such calculations are useful to illustrate the maintenance of gas exchange during the dive and the potential magnitude of the transfer of respiratory O_2 into the blood.

These calculations illustrate the complexity of estimating the contribution of the net blood O_2 store to metabolic rate as the blood O_2 depletion rate in a penguin (or any diver with a large respiratory O_2 store) is a function of both the transfer of O_2 into the blood and the uptake of O_2 from blood by the tissues. Simultaneous air sac and blood P_{O2} data would allow calculation of the net contribution of these O_2 stores to diving metabolic rate, but such measurements are not currently feasible. However, because the separately determined O_2 contributions from the lungs and blood are both low, these data are consistent with 1) a significant contribution from the exceptionally large muscle O_2 store to diving metabolic rate in emperor penguins (Kooyman and Ponganis, 1998; Ponganis et al., 1997a), and 2) the low field metabolic rate and the true diving bradycardia (heart rate while diving significantly lower than that of heart rate at rest) exhibited by emperor penguins at the isolated dive hole (Meir et al., 2008; Nagy et al., 2001).

Summary

This investigation has revealed enhanced O_2 affinity of emperor penguin hemoglobin, similar to that of high-altitude geese and other penguin species. These species are distantly related (Sphenisciformes and Anseriformes) (Hackett et al., 2008)

and have evolved in different environments under different constraints. The conditions of hypoxia that high-altitude birds and penguin species encounter are also different, ranging from that of extended hypoxia during sustained flight for the bar-headed goose, to transient, though perhaps frequent hypoxia experienced by diving penguins. Despite these differences, both bar-headed geese and emperor penguins have evolved hemoglobin with remarkably similar O_2 affinity. As neither of the two amino acid substitutions responsible for the increased Hb affinity in high-altitude birds is present in emperor penguin hemoglobin, the mechanisms underlying this shared trait are also different. Perhaps this level of O_2 affinity is closer to the physiological limit of maximizing O_2 uptake during hypoxia. As this is also the range of O_2 affinity optimized by mammalian hemoglobins, including those of diving mammals, it demonstrates the compromise between O_2 uptake from the respiratory system and O_2 unloading to the tissues intrinsic to the O_2-Hb dissociation curve.

S_{O2} profiles during diving, based on the O_2-Hb dissociation curve, demonstrated 1) the maintenance of Sa_{O2} levels near 100% throughout much of the dive, 2) a wide range of final Sv_{O2} values and optimization of the venous blood O_2 store resulting from arterialization and near complete depletion of venous blood O_2 during longer dives, and 3) that estimated contribution of the blood O_2 store to diving metabolic rate was low and highly variable. This pattern is due, in part, to the influx of O_2 from the lungs into the blood during diving, and variable rates of tissue O_2 uptake.

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Table 3-1. Dive, S_{O2}, % O_2 content depletion, and depletion rate data for the emperor penguins in this study, grouped as arterial or venous. Mean ± s.d. and range are given. Pre-dive, initial and maximum S_{O2} were determined at pH=7.5, final S_{O2} at pH=7.4.

P_{O2} Electrode Location	**Duration** (min)	**Maximum Depth** (m)	**Pre-dive S_{O2}** (%)	**Initial S_{O2}** (%)	**Maximum S_{O2}** (%)	**Final S_{O2}** (%)	**Δ S_{O2}** (initial S_{O2}-final S_{O2})	**% O_2 Content Depletion**	**Depletion Rate** (ml O_2 dl^{-1} min^{-1})
Venous (n=9 penguins, 130 dives)	6.1 ± 4.6 (0.8-23.1)	38.6 ± 24.7 (4-155)	74.1 ± 13.7 (29.7-95.8)	75.6 ± 13.0 (38.4-95.3)	80.9 ± 12.7 (49.5-98.8)	46.6 ± 30.2 (0-97.0)	29.0 ± 30.3 (-27.8-92.3)	37.8 ± 40.0 (-58.0-100)	0.9 ± 1.4 (-6.2-4.7)
Arterial (n=6 penguins, 71 dives)	5.1 ± 2.1 (1.1-11.9)	29.1 ± 17.3 (11-91.5)	96.2 ± 2.3 (85.7-99.3)	96.5 ± 2.3 (85.7-99.2)	99.1 ± 0.8 (93.5-100)	75.6 ± 13.4 (47.4-98.9)	20.9 ± 14.1 (-5.0-50.7)	21.6 ± 14.5 (-5.5-51.7)	0.9 ± 0.6 (-0.4-2.0)

Table 3-2. Spearman Rank Order Correlation results
(* Correlation is significant at the 0.05 level).

		Final S_{O2}/Duration		Pre-dive S_{O2}/Duration		% O_2 Content Depletion/Duration		Depletion Rate/Duration	
Location	***N***	**Spearman *R***	***P***	**Spearman *R***	***P***	**Spearman *R***	***P***	**Spearman *R***	***P***
Venous	130	-0.722*	<0.001	0.216*	0.007	0.804*	<0.001	0.272*	0.001
Arterial	71	-0.677*	<0.001	0.174	0.074	0.667*	<0.001	0.210*	0.040

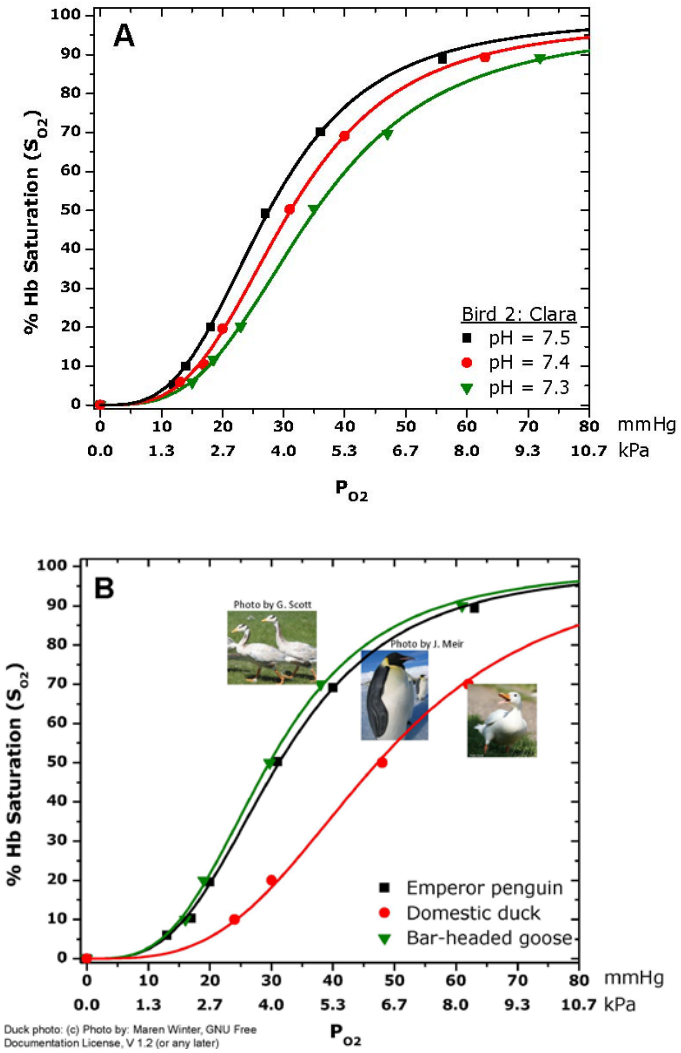

Figure 3-1. An example of oxygen-hemoglobin dissociation (O_2-Hb) curves from a) one penguin at pH 7.5, 7.4, and 7.3, and b) the emperor penguin, the bar-headed goose (*Anser indicus*) (Black and Tenney, 1980) and the domestic duck (*Anas platyrhynchos*, forma domestica), (Hudson and Jones, 1986) at pH=7.4. Note that like the bar-headed goose, the O_2-Hb dissociation curve of the emperor penguin is significantly left-shifted as compared to the domestic duck (and most birds).

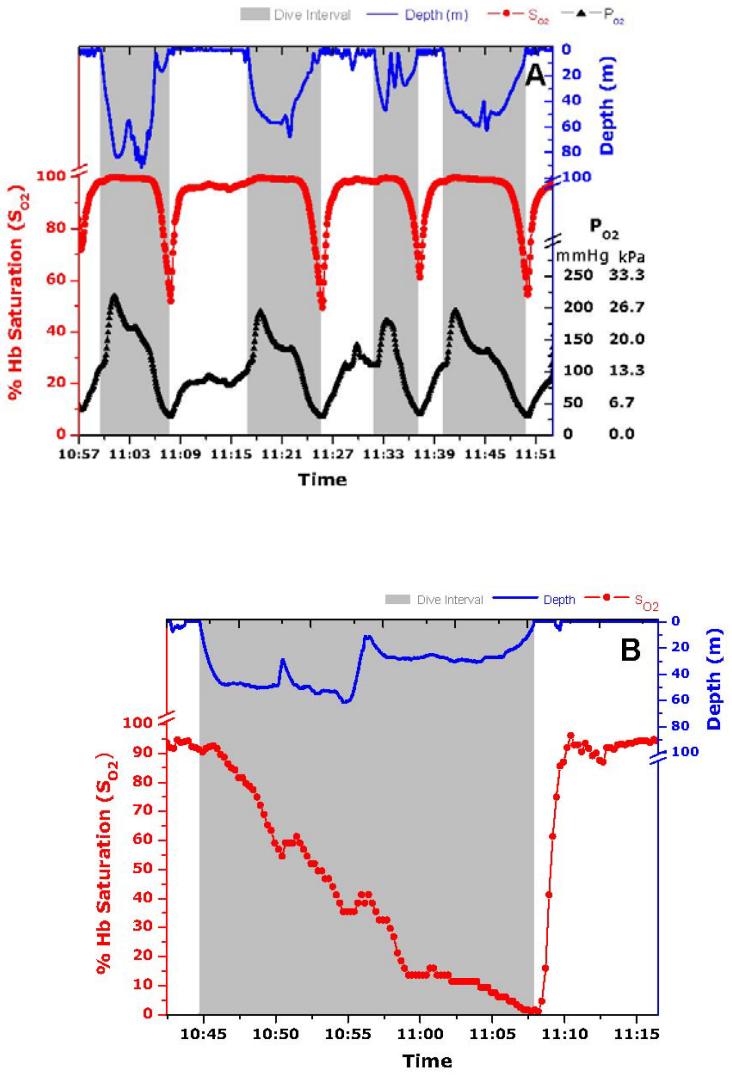

Figure 3-2. The S_{O2} profile during a) one hour of diving of Bird 1 (2008) (arterial S_{O2}) with Pa_{O2} superimposed. Note that Pa_{O2}/Sa_{O2} are at low levels at the start of the timeline as a prior dive occurred just before this series, and b) the current record dive (23.1 min) of an emperor penguin [Bird 19 (Ponganis et al., 2007), venous S_{O2}]. Note the arterialization of the venous blood O_2 store in this dive, as Sv_{O2} before the dive is as high as 95% and the initial Sv_{O2} of the dive is 91%. Sv_{O2} decreased to 1% by the end of this long dive. S_{O2} was determined at pH=7.5 throughout the entire dive to maintain consistency and to provide a conservative estimate of continuous S_{O2}.

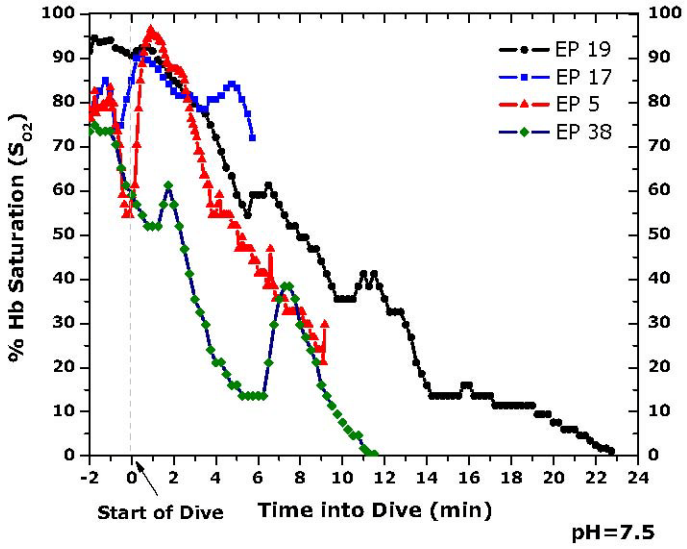

Figure 3-3. Comparisons of the Sv_{O2} profile [based on P_{O2} profiles from a prior study (Ponganis et al., 2009)] in dives of four emperor penguins. S_{O2} was determined at pH=7.5 throughout the entire dive to maintain consistency and to provide a conservative estimate of continuous S_{O2}. Note the variability of the S_{O2} profile among different dives.

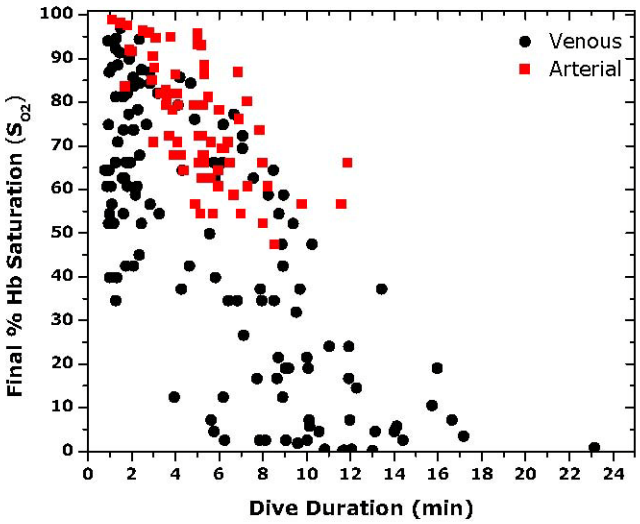

Figure 3-4. The final S_{O2} vs. dive duration from the compiled P_{O2} profiles of all dives [venous and arterial; (Ponganis et al., 2009; Ponganis et al., 2007) and current study]. Note the significant amount of overlap between arterial and venous values.

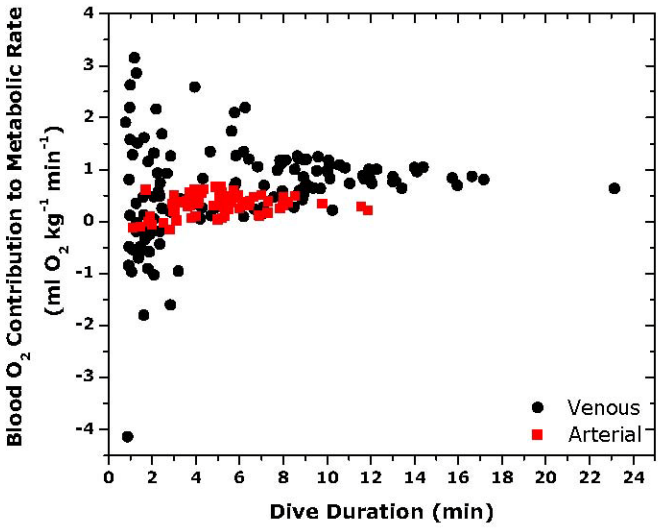

Figure 3-5. The blood O_2 contribution to metabolic rate vs. dive duration for all venous and arterial dives. Note that since S_{O2} was sometimes higher at the end of the dive than at the initial time point, negative values resulted in some cases for this variable.

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CHAPTER 4: Body temperature profiles of diving elephant seals

ABSTRACT

Hypothermia-induced reductions in metabolic rate have been proposed to suppress metabolism and prolong the duration of aerobic metabolism during dives. To determine if core hypothermia might account for the repetitive, long duration dives of the northern elephant seal, *Mirounga angustirostris*, blood temperature profiles were obtained in translocated, juvenile elephant seals equipped with a thermistor and backpack recorder. Mean temperatures of dives [extradural vein = 36.7 $\pm$ 0.5°C (n=381 dives, 3 seals), hepatic sinus = 37.2 $\pm$ 0.7°C (n=1050 dives, 4 seals) and arterial = 37.7 $\pm$ 1.5°C (n=1778 dives, 6 seals)] were significantly, though very weakly, related to dive duration. Mean venous temperatures of all dives of individual seals ranged between 36 and 38°C, while mean arterial temperatures ranged between 35 and 39°C. Transient decreases in venous and arterial temperatures to as low as 30-33°C did occur in some dives > 30 min, however only in 0.1% of dives in the study. The lack of significant core hypothermia during routine dives (10-30 min), and the weak correlation of mean temperature with dive duration do not support the hypothesis that a cold induced Q_{10} effect contributes to metabolic suppression of central tissues during dives. The wide range of arterial temperatures while diving and transient declines in temperature during long dives suggest that alterations in blood flow patterns and peripheral heat loss contribute to thermoregulation during diving.

LIST OF ABBREVIATIONS

ADL aerobic dive limit
Q_{10} ratio of given reaction rate and given reaction rate at a 10°C lower temperature
P_{O2} partial pressure of oxygen
S_{O2} % hemoglobin saturation

INTRODUCTION

The northern elephant seal, *Mirounga angustirostris*, is the consummate pinniped diver, with routine 10- to 30-minute dives to depths of 400 to 800 meters and mean surface intervals of only two minutes (Le Boeuf et al., 1988; Le Boeuf et al., 1996). Although an aerobic dive limit [ADL; dive duration associated with the onset of post-dive blood lactate accumulation (Kooyman et al., 1983)] has not been measured in these seals, such short surface intervals suggest that diving metabolism is predominantly aerobic in nature (Le Boeuf et al., 1988; Le Boeuf et al., 1996). 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 (Hindell et al., 1992). Moderate bradycardias of 30-40 beats per min (bpm) and regulated organ perfusion during dives (Andrews et al., 1997; Hindell and Lea, 1998), a hydrodynamic body shape (Fish, 1994), and prolonged gliding (Williams et al., 2000) undoubtedly contribute to the conservation of blood and muscle O_2 stores during dives. However, because estimates of O_2 stores and calculated aerobic dive limits (cADL) for this species have not accounted for continued aerobic metabolism during such diving behavior, some researchers have suggested that some form of "hypometabolism" must occur (Hindell et al., 1992; Le Boeuf et al., 1988). Partitioning of the metabolic demands of travel, foraging and digestion into different dive types and extended surface intervals has also been proposed (Crocker et al., 1997; Hindell et al., 1992; Le Boeuf et al., 1992; Sparling et al., 2007).

Regional temperature reductions have been documented in diving animals, particularly in birds, (Bevan et al., 1998; Culik et al., 1996; Handrich et al., 1997; Hill et al., 1987), raising hypotheses that metabolic rate reduction may be facilitated by a decrease in temperature and associated Q_{10} effect (Boyd and Croxall, 1996; Butler, 2004). Although regional heterothermy occurs in diving emperor penguins, core body temperature is preserved, suggesting that a cold induced Q_{10} effect on central organ metabolic rate does not occur. Regional heterothermy, however, may contribute to a reduction in metabolic rate during a dive in that metabolic energy need not be expended to maintain peripheral tissue temperature during the dive. In that case, metabolic costs would occur during the surface interval period when peripheral re-warming occurs (Ponganis et al., 2001; Ponganis et al., 2004; Ponganis et al., 2003).

In seals, brain temperature has been shown to decrease by 2.5-3°C during forced submersions protocols (Odden et al., 1999; Scholander et al., 1942). Such declines in brain temperature were hypothesized to provide a 15-20% reduction in brain O_2 consumption as well as neuroprotection against hypoxic and ischemic injury (Odden et al., 1999). In free diving Weddell seals, aortic temperature declined by 1-3°C in particularly long dives, though temperatures during and after dives of less than 20 min (the ADL in adults of this species) were higher (Hill et al., 1987; Kooyman et al., 1980). Temperature in locomotory muscle of Weddell seals, however, remains near 37°C during diving (Ponganis et al., 1993b).

Elephant seals spend >90% of time at sea submerged, foraging and gaining an average of 1 kg body mass per day during a two-month trip to sea (Le Boeuf et al.,

1988; Le Boeuf et al., 1996). Because the lifestyle and level of activity of elephant seals in the wild are not consistent with metabolic suppression during dives, we hypothesized that core hypothermia does not extend aerobic dive duration through a Q_{10} effect in elephant seals. Our project used a "backpack" partial pressure of O_2 (P_{O2}) and temperature recorder to measure arterial and venous temperature continuously during dives of translocated juvenile northern elephant seals. Despite some degree of peripheral cooling associated with diving due to decreased peripheral perfusion and conductive and convective heat loss to seawater, we hypothesized that core body temperature (as approximated by hepatic sinus and arterial temperatures) would remain near 37°C during the elephant seal's routine dives (10-30 min) and would not correlate with dive duration. Such patterns of regional heterothermy and preservation of core body temperature have been documented in emperor penguins, dolphins, and humans (Heath and Ridgway, 1999; Ponganis et al., 2003; Sessler, 2000).

MATERIALS AND METHODS

This research was conducted on juvenile (1-3 years old) elephant seals [mass = 195 kg (SD 46), n=13] from the Año Nuevo colony (San Mateo County, California) during the April molt season using the translocation method (Andrews et al., 1997; Oliver et al., 1998) 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) 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 P_{O2} electrode and thermistor. All procedures were approved by a UCSC Chancellor's Animal Research Committee and a National Marine Fisheries Service marine mammal permit (87-1743-02).

Thermistor 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 1mg kg^{-1}. At the laboratory, immobilization techniques included either ketamine (3 mg kg^{-1} IM or 0.5-1 mg kg^{-1} IV) or telazol (0.4-0.8 mg kg^{-1} IM) followed by the administration of isoflurane and 100% O_2 (Ponganis et al., 2006a; Ponganis et al., 1991; Ponganis et al., 1990; Ponganis et al., 2006b; Stockard et al., 2007) for all hepatic sinus and arterial deployments. An extradural vein catheter (14 g) was inserted post induction for blood sample collection and was removed prior to transport.

A P_{O2} electrode (Licox C1.1 Revoxode; Integra Life Sciences, Plainsboro, NJ, USA) and thermistor (model 554; Yellow Springs Instruments, Yellow Springs, OH, USA) were inserted percutaneously through a peel-away catheter (16-20 g) into one of the following sites (1 site per animal): a) extradural vein, b) hepatic sinus – inferior vena cava, or c) aorta (via the brachial or femoral artery). Catheterization techniques, the P_{O2} electrode, and thermistor have been described previously (Ponganis et al.,

2006a; Ponganis et al., 1997; Ponganis et al., 1993a; Ponganis et al., 2006b; Ponganis et al., 2007; Stockard et al., 2005; Stockard et al., 2007). The electrode and thermistor were connected to a custom built microprocessor (UFI, Morro Bay, CA, USA) in a backpack unit (5 x 16 cm, 570 g), and mounted on the mid-dorsal region of the seal using 5 min epoxy (Loctite). P_{O2} and temperature were sampled once every 5 or 15 seconds, depending on the specific recorder used for that deployment. A Mark 9 Wildlife Computers time depth recorder (TDR; Wildlife Computers, Redmond, WA, USA; sensitive to 0.5 m, 30 g, 6.7x1.7x1.7 cm) was attached in order to document the dive profile, with a 1 Hz sampling rate for both depth and ambient temperature. The P_{O2}/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 x 3.5 cm footprint, 160 g, monitored via ARGOS; Wildlife Computers, Redmond, WA, USA) 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 x 2 cm, 34 g; Wildlife Computers, Redmond, WA, USA) was attached to the animal's back to relocate it for recorder recovery (Andrews et al., 1997; Costa et al., 2003). Returning seals were immobilized as during capture, and all instruments removed at the beach haul-out location. In addition to the previously described protocols included above, thermistors were also re-calibrated post-deployment.

Data analysis and statistics

Differences between results at the three thermistor insertion sites were determined with one-way ANOVA. Correlation between dive duration and mean temperature was addressed with linear regression. Statistical significance was assumed at $P<0.05$ and the significance level is quoted in the text. Values are expressed as means (SD).

RESULTS

Behavior

Seals in this study often dived with transit patterns (travel dives) consistent with the translocation method (Andrews et al., 1997; Oliver et al., 1998); however, some seals did exhibit prolonged bouts of diving consistent with foraging and drift dives of free-ranging juveniles (Le Boeuf et al., 1996; Le Boeuf et al., 1992). 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). Mean temperature was significantly different between the 3 thermistor placement sites (One-way ANOVA, $P<0.0001$, $F=151.41$). Mean temperature in the extadural vein remained near 37°C during dives (36.7 ± 0.5°C; n=381 dives, 3 seals) (Fig. 2a). Mean temperature in the

hepatic sinus (37.2 ± 0.7°C; n=1050 dives, 4 seals) was slightly more variable among seals than that in the extradural vein, but also remained near 37°C during diving (Fig. 2b). Mean temperature in the artery (37.7 ± 1.5°C; n=1778 dives, 6 seals), however, was higher than those of the venous temperatures and was more variable between seals, with a wider distribution of temperatures (Fig. 2c, 4). Mean temperature was significantly, though very weakly, related to dive duration in all compartments (extradural: $y= -0.03x + 36.93$, $r^2=0.11$, $P<0.001$; hepatic sinus: $y= -0.06x + 37.85$, $r^2=0.23$, $P<0.001$; arterial: $y= -0.11x + 38.83$, $r^2=0.18$, $P<0.001$).

Temperature decreased dramatically and rapidly to 30-33°C during four, prolonged (≥30 min) dives in this study, with subsequent re-warming prior to the end of the dive (two dives with thermistors in the artery, and two in the extradural vein) (Fig. 3). Arterial temperatures while diving were often as high as 40-41°C (Fig. 4).

DISCUSSION

Mean Temperatures

Arterial and central venous (hepatic sinus) temperatures, which should approximate core body temperature, did not decline significantly during routine dives [10-30 min; (Le Boeuf et al., 1988; Le Boeuf et al., 1996)] (Figs. 1, 4). Mean arterial and venous temperatures during dives remained near 37°C, with only weak correlations to dive durations (Fig. 2). Moreover, mean arterial temperatures of dives of individual seals were greater than 37°C in all but one seal (Table 1). While it is certainly advantageous not to overheat during dives (Liu and Yenari, 2007; Odden et

al., 1999), these results are contrary to the hypothesis that a cold induced Q_{10} effect contributes to metabolic suppression of central body tissues and prolongs the duration of aerobic metabolism during dives.

Arterial Temperatures

Although arterial temperatures of up to 40-41°C while diving were unexpected, we believe the readings are accurate because 1) the initial increase in temperature was coincident with the start of diving in all seals with arterial thermistors and temperatures as high as 40°C were measured in all but one seal (Knut) while diving (Fig. 4a, b), 2) these profiles were documented in two different arterial locations [aorta accessed *via* the femoral artery in one seal (Sir Richard) and *via* the brachial artery in all other seals], 3) temperature decreased during each surface interval between dives (usually by 0.5-1.0°C) (Fig. 4a), with the exception of one seal, Jerry, whose arterial temperature increased by about 1°C during surface intervals), 4) temperature decreased after conclusion of the diving bout (once again, with the exception of Knut), and 5) four out of the six thermistors placed in the artery were fully functional upon removal from the animal, with no change between original and post-deployment calibrations.

Alternatively, a local inflammatory response to the arterial probe, or an infection can elevate body temperatures. However, the association of fluctuations in temperature with different activities, the lack of sustained temperature elevations, and the timeframe of these responses in this study are not consistent with these

alternatives. In addition, as lethargy is a powerful component of the behavior that accompanies infective fever (Skinner et al., 2009), it is unlikely that a seal would engage in sustained diving while febrile.

The temperature profiles of one seal (Butler) revealed three distinct phases of temperature ranges, corresponding to differences in the dive profiles (Fig. 4b). During the initial portion of his diving (the first ~15 hours), Butler's temperature remained between 36-37°C with a series of dives between 300-600 meters. This was also the period in which the dramatic temperature decreases during two sustained dives occurred. After this initial period, diving for the next 2.5 days was shallower (100-200 m) and temperature was higher (38-40°C) throughout this period. Mean dive duration during this phase was 10.2 min (SD 2.4, range=3.1-19.4 min). The final phase consisted of a transition to deeper (500-600 m) and longer [mean duration = 16.7 min (SD 3.0, range = 8.5-28.4 min)] dives, consistent with intense foraging at-sea (according to dive profile and position over a seamount revealed by the satellite transmitter). At the onset of this period of deeper diving, temperature declined from 39°C to 36°C, and remained between 36-37°C throughout the next 3.5 days of diving. Although temperature was clearly lower in this part of the dive bout than in the preceding period, it still remained close to 37°C. Such patterns are inconsistent with fever induced by infection, and again, are contradictory to metabolic suppression and a cold induced Q_{10} effect during diving. It is more probable that warming of ingested cold prey contributed to lower body temperatures during foraging dive bouts.

In addition to Butler, another seal (Jonesie) also exhibited a difference in temperature range in two separate dive bouts. The first bout consisted of 350-450 m dives with a mean duration of 11.9 min (SD 7.9, range=1.1-25.4), during which temperature remained between 36-38°C. At the end of this bout, temperature increased to 40°C. During the next dive bout, consisting of shorter [mean duration=6.0 min (SD 4.0, range=1.0-22.0)] and shallower (100-300 m) dives, temperature remained near 39-40°C. Thus, dive profiles and the associated temperature responses in this study, particularly illustrated by these two seals, indicate differences in temperature during diving based on the type of dive bout. Dive bouts consisting of short, shallower dives, likely indicative of transit, are associated with higher arterial temperatures during diving, while in bouts with longer, deeper dives, characteristic of at-sea foraging, temperature was not elevated, remaining near 37°C. These responses appear to be more related to the nature of the entire bout (or phase of bout) than to individual dives, as there was not a strong relationship between mean temperature of the dive and dive duration for individual dives (Fig. 2). It should also be noted that the differences in temperature during these bouts did not simply reflect seawater temperature stratification related to depth, as revealed by ambient temperature records provided by TDR data. Despite the fact that deeper dives did include transit through slightly colder water, the minimum ambient temperature was only approximately 2°C cooler in the deeper dives, and mean temperature during the bout was equivalent for the different bout types (shallower, shorter dives vs. longer, deeper dives).

Only one seal (Knut) had mean arterial dive temperatures between 34 and 36°C, a range which Odden et al. (1999) suggested would lead to decreased O_2 consumption and neuroprotection during forced submersions. Certainly, other seals could make routine dives without such reductions in arterial temperature. It is unclear why Knut's mean and max arterial temperatures were lower than those of other seals (Table 1). Dive durations and depths for this seal were similar to that of other seals, though the length of deployment in this seal was quite short. Detailed analysis correlating specific dive types and the associated temperature profile is underway and may elucidate the relationship between dive types and temperatures among and between seals.

Increased arterial temperatures during diving have also been documented in emperor penguins, with a similar range and profile to that documented in the elephant seal (Ponganis et al., 2004). Mean aortic temperature in diving emperor penguins was higher than that at rest in all emperor penguins studied, with temperature during the dive increasing by 0.1-3.5°C (up to a temperature of 40.1°C) above the pre-dive level (Ponganis et al., 2004). A temperature increase of this magnitude was documented only in the aorta of emperor penguins, and was not characteristic of deep venous sites (Ponganis et al., 2003), again, analogous to the elephant seal temperature profiles in this study. Aortic temperature in the emperor penguin also declined during each surface interval, consistent with the pattern observed in all but one of the elephant seals with thermistors in the artery in this study. For both of these species, a decrease in arterial temperature during the surface interval is consistent with increased heart

rate, increased blood flow and re-warming of the periphery during this period (Fig. 4a) (Andrews et al., 1997; Meir et al., 2008; Ponganis et al., 2003). Temperatures in the 39-40°C range are not uncommon in mammals, ranging from Schmidt-Nielsen's classic example of the camel during hot days in the African desert, to that of zebras, exercising horses, and even human long distance runners (Fuller et al., 2000; Irving, 1959; Mitchell et al., 2006; Schmidt-Nielsen et al., 1957; Taylor et al., 1998).

Arterial temperature profiles were complex, perhaps secondary to thermoregulatory processes and the role of arteries in delivering heat to the peripheral heat exchangers. Elevated temperatures during diving may be a product of a marine mammal's constraint of balancing thermoregulation with the dive response, the natures of which may be considered as in direct conflict (Whittow, 1987). The decreased heart rate and reduction of peripheral blood flow characteristic of the dive response present a challenge to an extremely well insulated, exercising marine mammal that may need to dissipate heat in order to thermoregulate. Rather than sacrifice the O_2 conserving dive response, dolphins (*Tursiops truncatus*) delay their dissipation of excess heat until the post-dive period (Noren et al., 1999; Williams et al., 1999). The greatest period of heat transfer in these animals occurred immediately following the dive, corresponding to the release of the cardiovascular system from the dive response (Williams et al., 1999). In contrast, restrained harbor seals have been shown to override the dive response during conditions of heat stress (Hammel et al., 1977). Such responses demonstrate the remarkable flexibility of marine mammals in balancing their physiological and behavioral requirements.

These increased temperatures during diving might also be associated with blood flow through the seal's large pericardial plexus or other venous plexuses, which are embedded in brown adipose tissue (BAT) (Blix et al., 1975; Harrison and Tomlinson, 1956). Blix et al. hypothesized (1979) that the venous plexuses and thermogenic BAT may function as a heat exchanger. Regardless of the specific mechanism responsible for these increased arterial temperatures, it should be noted that dive behavior of these seals was typical for this species (profile and duration, including long dives). This further supports the concept that significant hypothermia and a cold induced reduction in metabolic rate are not essential for diving in this species.

Dramatic Temperature Declines in Sustained Dives

Temperature declined dramatically during four, sustained (≥30 min) dives in this study (two dives with thermistors in the artery, and two in the extradural vein). During a 43 min dive, temperature declined to 30°C in the extradural vein, re-warming to 35°C by the end of the dive (Fig. 3a). Temperature in the artery decreased to 32.7°C during an approximately 30 min dive, with rapid re-warming to 36°C by the end of the dive (Fig. 3b). Since these periods of temperature decrease were documented not only in the extradural vein [in which blood flow is variable and slow during forced submersion (Nordgarden et al., 2000)], but also in the artery, they may represent transient changes throughout much of the circulatory system. Though these

profiles are quite remarkable, it should be stressed that this type of response was documented in only 0.1% (4 out of 3209 dives) of the dives in this study.

The temperature decrease in these sustained dives occurred very rapidly. Temperature in the extradural vein declined by a mean of 5.5°C (SD 1.5, n=2 dives), at a rate of 0.6°C min^{-1} (Fig. 3a). The magnitude and rate of decrease in the artery was lower than that in the extradural vein, with a mean decrease of 2.4°C (SD 0.9, n=2), occurring at a rate of 0.4°C min^{-1} (Fig. 3b). These rates are 2-3x that measured for brain temperature decreases during forced submersion protocols (Odden et al., 1999; Scholander et al., 1942).

In these long dives, re-warming occurred prior to the end of the dive in all cases, also occurring rapidly. In three of the four dives, temperature began to increase at the onset of the final ascent of the dive (Fig. 3b), concurrent with the increase in heart rate (ascent or "anticipatory" tachycardia) which occurs during ascent in this species (Andrews et al., 1997). The ascent tachycardia has been hypothesized to increase blood flow and O_2 delivery to depleted tissues, thereby lowering the P_{O2} in the blood and providing a larger gradient to maximize O_2 uptake at the surface (Thompson and Fedak, 1993). Such a function for the ascent tachycardia would be consistent with the temperature increase in the extradural vein in this period, as warm blood from the muscle would drain into the extradural vein. This re-warming of blood during the ascent is also congruent with the continuous stroking which occurs during the initial ascent in dives of elephant seals (Williams et al., 2000). Thus, the increase in temperature during ascent supports the concept of blood flow to locomotory muscle

during this period of intense stroking. Blood flow to muscle during this period of the dive also agrees with previous studies of Weddell seals which demonstrated that 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 (Guppy et al., 1986; Guyton et al., 1995; Ponganis et al., 1993b). As outlined in the related study involving these deployments, P_{O2} profiles of these elephant seals also support these arguments, as the rate of P_{O2} decline becomes more rapid coincident with the start of ascent, with an even steeper decline in the final 15-45 seconds of the dive (Meir et al., 2009) (Fig. 3a).

Simultaneous records of temperature and P_{O2} can also further elucidate patterns of blood flow. For example, in the long dive with the temperature decline in the extradural vein, P_{O2} remained relatively unchanged during the middle of the dive, suggesting that blood in this vessel might be stagnant (Fig. 3a). However, because blood temperature decreased during that time period, the temperature profile provided evidence for maintenance of at least some blood flow.

Potential Mechanisms of Cooling

Scholander used his original documentation of the decrease in brain temperature in forcibly submerged harbor seals as an argument for "hypometabolism", as he theorized that while diving, circulation is slowed to the periphery, resulting in diminished heat loss (Scholander et al., 1942). Thus, internal temperature during this

period should increase, not decrease as he observed. Scholander attributed the decrease in internal temperature during the forced submersion to the lowering of internal heat production. An inhibition of shivering in hypothermic seals during forced submersions could also lead to decreased heat production (Kvadsheim et al., 2005). The rapid decrease in temperature documented in this study, however, seems most consistent with peripheral vasodilation, resulting in a bolus of cool blood returning from the periphery, likely associated with a the dumping of heat through a thermal window such as the flippers, or shunting through the numerous arterio-venous anastamoses in the skin of pinnipeds (Molyneux and Bryden, 1975; Molyneux and Bryden, 1978). Other researchers have also considered increased blood flow through the skin and/or flippers as the primary mechanism of cooling to account for decreases in aortic temperature of Weddell seals prior to diving (Hill et al., 1987) and decreases in brain temperature in hooded and harp seals during forced submersions (Blix et al., 2002). In addition, yet undocumented heat exchange retes in this species may contribute to heat transfer. If an arterial-venous temperature gradient does indeed exist in an individual seal, this could serve as a mechanism of rapid heat loss. Simultaneous deployments of both arterial and venous thermistors could elucidate this issue, though this was not currently feasible due to the technical complexities of probe placement.

Attributing these large decreases in temperature to vasodilation of the periphery, however, does conflict with the fact that elephant seals are experiencing the most intense phase of their bradycardia at the same time period as the temperature

decrease (Andrews et al., 1997). Although an intense bradycardia is not consistent with increased blood flow to the periphery, brain temperature does decline during forced submersions of seals when their heart rates are only 8-10 bpm (Odden et al., 1999). As previously mentioned, temperature decreases during the dive could also be related to the ingestion of cold prey. However, many of the dives in this study had profiles consistent with foraging activity, while these dramatic temperature decreases occurred in only 4 (0.1%) of the total dives in this study.

Implications to Related Studies

Because mean temperature while diving remained near 37°C, the use of the O_2-Hb dissociation curve at this temperature was justified to convert P_{O2} data into % Hb saturation (S_{O2}) in a related study (Meir et al., 2009). Significant temperature decreases occurred in only 0.1% of dives in this study, and even in those dives, re-warming occurred prior to the end of the dive (Fig. 3a, b). Assuming standard effects of increased temperature on mammalian O_2-Hb dissociation curves, discrepancies in actual S_{O2} for dives with increased arterial temperatures would result in lower arterial S_{O2} than that calculated with the 37°C curve. Consequently, for dives with elevated temperatures, calculations of arterial S_{O2} and O_2 depletion in diving elephant seals in the related study are conservative. This implies that hypoxemic tolerance in this species may be even more impressive than previously stated (Meir et al., 2009).

Summary

The results of this study indicate that core body temperature, as approximated by continuous arterial and central venous (hepatic sinus) temperature measurements, is preserved during diving, with mean temperature remaining near 37°C during routine dives. However, during a few prolonged dives, there were significant transient declines in temperature to 30-33°C. In addition, mean arterial dive temperatures of individual seals spanned a 4.2°C range. Our understanding of thermoregulation during diving remains far from complete. As Laurence Irving stated most eloquently, "Variations in body temperature are both normal and adaptive in the sense that they bring about better economy in the animal heat machine and fit animals to exploit special situations which would be otherwise unutilized" (Irving, 1959).

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Table 4-1. Individual dive and temperature data for all seals. The mean (SD) and range are given for each seal

Seal (# of dives)	Length of Deployment (days at sea)	Thermistor Location	Dive Duration (min)	Max Depth (m)	Mean Temp	Max Temp	Min Temp
Chick (n=267)	3.5	Extradural	8.6 (SD 6.0) (1.1-43.7)	50.1 (SD 48.8) (5-380)	36.5 (SD 0.3) (34.3-37.1)	36.7 (SD 0.2) (35.8-37.3)	36.2 (SD 0.6) (30-36.9)
Starburst (n=33)	3	Extradural	12.7 (SD 5.9) (5.1-29.9)	105.8 (SD 71.7) (41-432)	36.8 (SD 0.3) (35.4-37.1)	37.0 (SD 0.1) (36.7-37.3)	36.4 (SD 0.6) (33.9-37.0)
Patty (n=81)	1	Extradural	9.3 (SD 4.7) (1-28.9)	70.3 (SD 80.3) (11-416)	37.4 (SD 0.3) (36.4-38.0)	37.5 (SD 0.3) (36.5-38.0)	37.2 (SD 0.4) (35.8-37.9)
Bodil (n=192)	21	Hepatic sinus	14.2 (SD 6.5) (1.1-29.3)	214.4 (SD 157.8) (5.0-699.0)	37.1 (SD 0.5) (36.1-38.0)	37.2 (SD 0.5) (36.2-38.0)	37.0 (SD 0.6) (35.9-37.9)
Roberta (n=480)	15	Hepatic sinus	12.2 (SD 5.7) (1.0-27.5)	156.9 (SD 135.0) (6.0-466.0)	36.7 (SD 0.4) (35.8-37.7)	36.8 (SD 0.4) (35.9-37.7)	36.6 (SD 0.5) (35.6-37.5)
Larry (n=218)	9	Hepatic sinus	12.2 (SD 3.2) (5.4-22.2)	83.7 (SD 65.2) (23-504)	37.9 (0.6) (36.4-39.0)	38.0 (SD 0.6) (36.5-39.1)	37.8 (SD 0.7) (36.2-38.9)
Per (n=160)	1.2	Hepatic sinus	8.9 (SD 6.5) (1.0-26.3)	71.2 (SD 121.0) (6-508)	37.5 (SD 0.6) (36.1-38.1)	37.5 (SD 0.6) (36.2-38.1)	37.4 (SD 0.7) (35.9-38.1)
Sir Richard (n=312)	2	Arterial (femoral)	5.8 (2.3) (1.1-11.7)	65.8 (28.4) (6-234)	39.0 (0.5) (37.3-40.2)	39.1 (0.5) (37.3-40.2)	38.9 (SD 0.5) (37.2-40.1)
Jerry (n=132)	1.4	Arterial (brachial)	10.5 (SD 6.4) (1-30.5)	107.1 (SD 124.8) (5-474)	38.9 (0.9) (37.3-40.3)	39.4 (0.9) (37.4-40.6)	38.7 (SD 0.9) (36.4-40.2)
Sammy (n=70)	1	Arterial (brachial)	10.5 (SD 2.9) (3.2-23.2)	130.5 (SD 106.6) (7-423)	39.7 (0.3) (39.3-40.6)	39.9 (0.4) (39.4-41.0)	39.1 (0.7) (36.2-40.0)
Knut (n=242)	2	Arterial (brachial)	10.0 (SD 5) (1.0-24.4)	84.7 (SD 88.5) (5-403)	35.1 (0.6) (33.8-36.5)	35.2 (0.6) (34-36.6)	34.9 (0.7) (33.1-36.4)
Jonesie (n=401)	3.5	Arterial (brachial)	7.6 (SD 6.4) (1-25.4))	75.2 (SD 113.7) (5-443)	38.1 (0.7) (36.5-39.0)	38.2 (0.7) (36.5-39.1)	37.9 (SD 0.8) (36.2-39.0)
Butler (n=621)	8	Arterial (brachial)	13.4 (4.5) (1.4-33.0)	268.2 (SD 146.0) (5-630)	37.4 (SD 1.2) (34.9-39.6)	37.5 (SD 1.2) (35.7-39.7)	37.2 (1.2) (32.7-39.5)

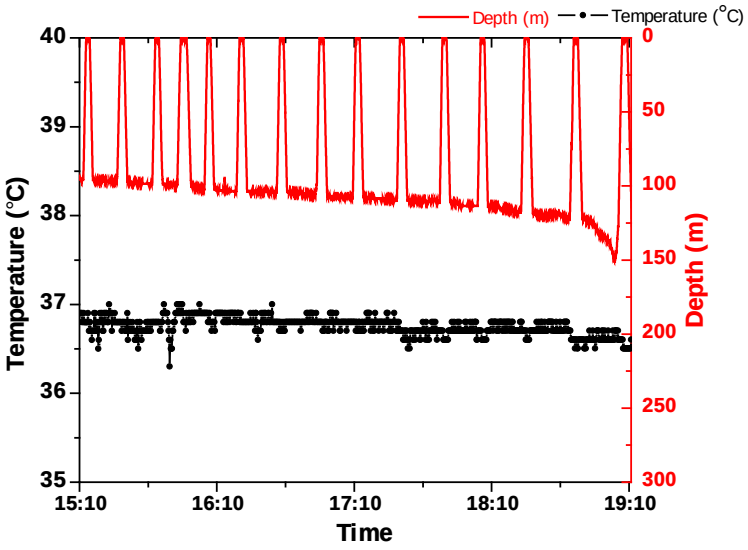
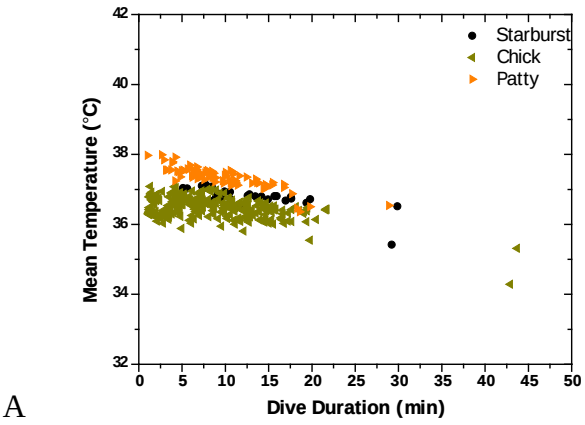
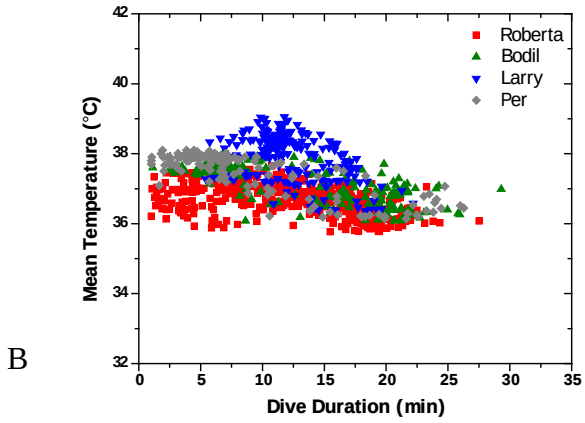


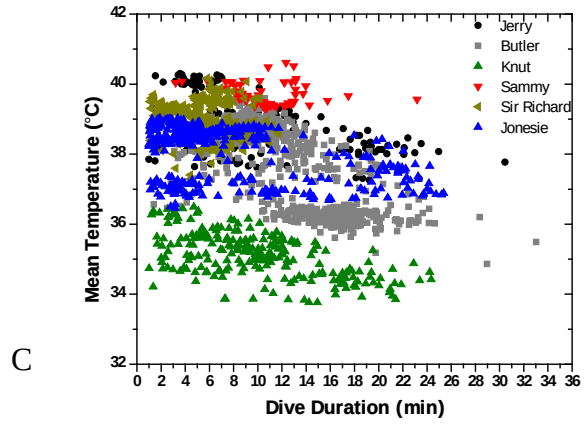
Figure 4-1. Dive and extradural vein temperature profile from four hours of diving in Starburst. Note that temperature remains near 37°C throughout the entire period.



A



B



C

Figure 4-2. Mean temperature during individual dives vs. dive duration for the a) extradural vein, b) hepatic sinus, and c) artery.

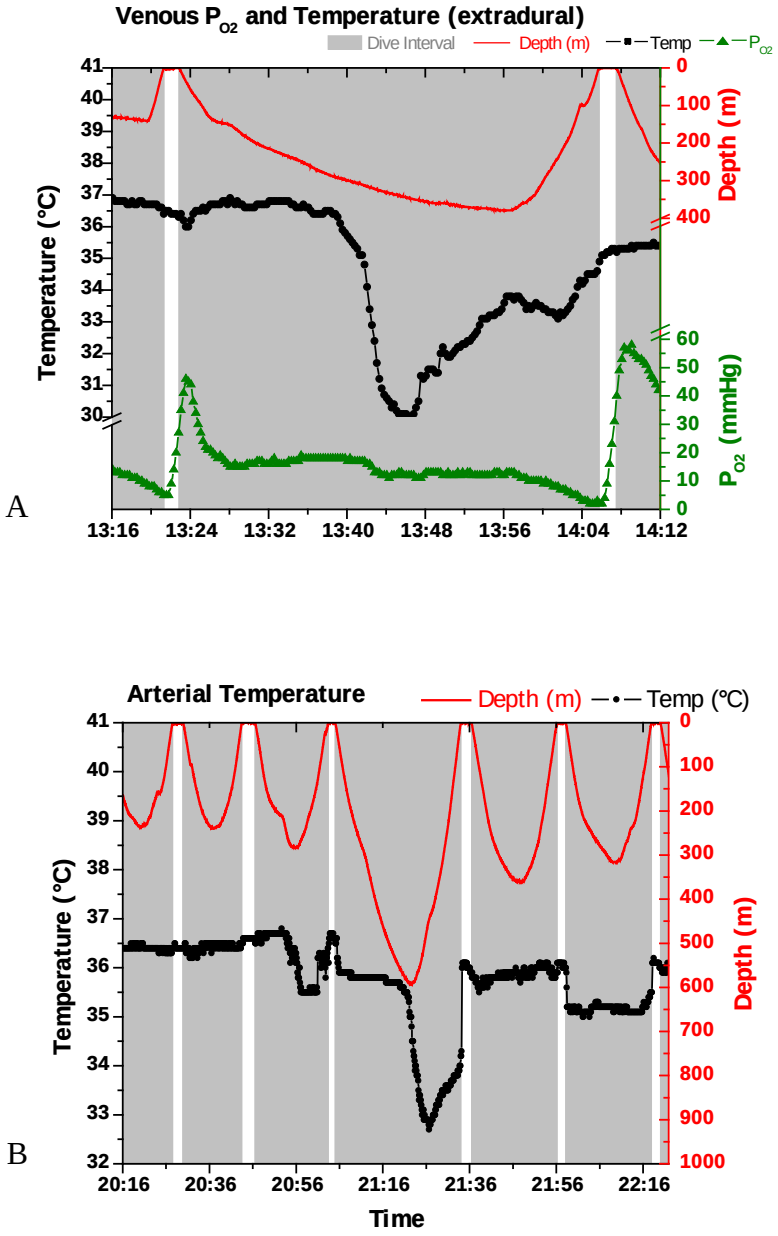


Figure 4-3. Dive profiles of a) extradural vein temperature and P_{O2} during a 43 min dive of Chick. Temperature declined to 30°C during the dive, re-warming to 35°C by the end of the dive, and b) arterial temperature over a sequence of dives of Butler. Temperature in the artery decreased to 32.7°C during an approximately 30 min dive, with rapid re-warming to 36°C.

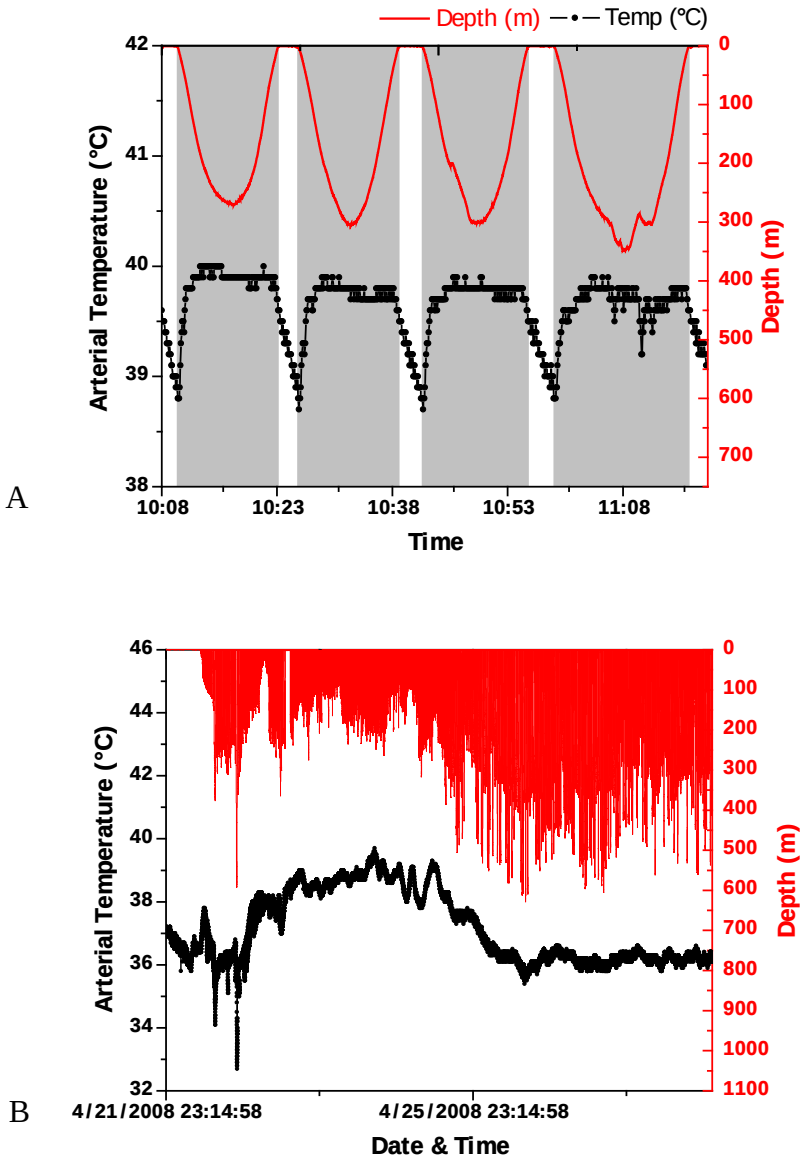


Figure 4-4. The arterial temperature and dive profile of a) 4 dives of Sammy. Note the near 40°C temperature during the dives, with decreases in temperature during each surface interval, and b) Butler's 8 day diving record. Note that temperature is higher (38-40°C) during the phase of the bout consisting of shorter, shallower dives, decreasing to 36-37°C during the final phase of deep, long dives consistent with at-sea foraging.

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