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REVIEW

In pursuit of Irving and Scholander: a review of oxygen store management in seals and penguins

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Summary

Since the introduction of the aerobic dive limit (ADL) 30 years ago, the concept that most dives of marine mammals and sea birds are aerobic in nature has dominated the interpretation of their diving behavior and foraging ecology. Although there have been many measurements of body oxygen stores, there have been few investigations of the actual depletion of those stores during dives. Yet, it is the pattern, rate and magnitude of depletion of O_2 stores that underlie the ADL. Therefore, in order to assess strategies of O_2 store management, we review (a) the magnitude of O_2 stores, (b) past studies of O_2 store depletion and (c) our recent investigations of O_2 store utilization during sleep apnea and dives of elephant seals (*Mirounga angustirostris*) and during dives of emperor penguins (*Aptenodytes forsteri*). We conclude with the implications of these findings for (a) the physiological responses underlying O_2 store utilization, (b) the physiological basis of the ADL and (c) the value of extreme hypoxemic tolerance and the significance of the avoidance of re-perfusion injury in these animals.

Key words: aerobic dive limit, blood flow, heart rate, hemoglobin, hypoxemia, marine mammals, metabolic rate, myoglobin, oxygen stores, penguins, re-perfusion injury, seals.

Introduction

In a landmark paper in diving physiology, Kooyman and co-workers demonstrated that most dives of Weddell seals (*Leptonychotes weddellii*) were aerobic in nature and did not result in elevated post-dive blood lactate concentration (Kooyman et al., 1980). The dive duration at which post-dive blood lactate concentration became significantly elevated was named the aerobic dive limit (ADL). Over the past 30 years, the proliferation of electronic dive recorders has led to documentation of dive profiles in many species. The interpretation of the diving behavior and foraging ecology of these marine mammals and sea birds has been primarily influenced by this concept of aerobic diving (Butler and Jones, 1997; Kooyman and Ponganis, 1998; Ponganis, 2011). In order to make such interpretations, there have been many studies of the magnitude of O_2 stores in marine mammals and sea birds. In fact, in the first 3 months of 2010, there were two such studies on phocid seals in this journal (Burns et al., 2010; Hassrick et al., 2010). In contrast to the large number of dive behavior and O_2 store studies, there have been few investigations of the pattern, rate and magnitude of O_2 store depletion in free-diving animals. Yet, it is these very parameters that ultimately determine the limits to dive performance and foraging success. In essence, it is still necessary to pursue the pioneering work of Irving and Scholander (Irving, 1934; Irving, 1939; Scholander, 1940), but in free-diving animals.

Therefore, in this review, we first examine the magnitude of O_2 stores in representative species. This includes discussion of the potential sources of error in the calculation of those stores because of the frequent and almost routine estimation of O_2 stores in the diving physiology literature. Second, we review different options in the strategy of O_2 store management through examination of past studies of O_2 store depletion in marine mammals and diving birds. Third, we focus on our recent investigations of O_2 store utilization during sleep apnea and dives of elephant seals (*Mirounga angustirostris*) and during dives of emperor penguins (*Aptenodytes forsteri*). Lastly, we conclude with the implications of these findings for (a) the physiological responses underlying O_2 store utilization, (b) the physiological basis of the ADL and (c) the value of extreme hypoxemic tolerance, and the significance and relevance of the obligate avoidance of re-perfusion injury in these animals.

O_2 stores

Oxygen stores are located in the respiratory system, blood and muscle. The magnitude and distribution of those stores vary in different species, and are dependent on the respiratory air volume during a dive, blood volume, hemoglobin (Hb) concentration, myoglobin (Mb) concentration and muscle mass (Table 1). Estimation of the O_2 stores from these parameters is dependent on a variety of assumptions.

Respiratory O_2 stores

Calculations of the respiratory O_2 store usually assume a 15% O_2 extraction and diving air volumes of about 50% and 100% total lung capacity in pinnipeds and cetaceans, respectively (Kooyman, 1989). These assumptions are based on the few data available from inflations of excised lungs, studies during free dives and simulated dives, and the fact that cetaceans appear to dive on inspiration (Kooyman et al., 1999; Ponganis et al., 2003a; Ridgway, 1986). In the past, respiratory data for penguins had only been obtained from simulated dives in pressure chambers (Kooyman et al., 1973c; Ponganis et al., 1999). These included a 15% O_2 extraction, and

Table 1. Oxygen store determinants in several representative species

Species	Diving respiratory volume ($ml\,kg^{-1}$)	Blood volume ($ml\,kg^{-1}$)	[Hb] ($g\,dl^{-1}$)	Muscle mass (% body mass)	[Mb] ($g\,100\,g^{-1}$)
Bottlenose dolphin	40[1]	71[2]	14[2]	30[3]	3.3[4]
Sperm whale	28[5]	200[6]	22[7]	34[8]	5.4[9]
California sea lion	48[10]	120[11]	18[11]	37[12]	5.4[11]
Harbor seal	30[13]	150[14]	23[14]	30[15]	5.5[16]
Weddell seal	27[17]	210[18]	26[18]	38[19]	5.4[18]
Elephant seal	27[17]	216[20]	25[20]	28[21]	6.5[22]
Adélie penguin	200[23]	87[21]	16[24]	40[3]	3.0[25]
King penguin	125[23]	83[26]	18[26]	33[27]	4.3[28]
Emperor penguin	117[29]	100[30]	18[30]	25, 12[30]	6.4, 2.1[30]

The bottlenose dolphin (*Tursiops truncatus*), California sea lion (*Zalophus californianus*), harbor seal (*Phoca vitulina*) and Adelie penguin (*Pygoscelis adeliae*) have classically been considered shallow divers. Deep divers are the sperm whale (*Physeter macrocephalus*), Weddell seal (*Leptonychotes weddellii*), elephant seal (*Mirounga angustirostris*), king penguin (*Aptenodytes patagonicus*) and emperor penguin (*A. forsteri*). Abbreviations: Hb, hemoglobin, Mb, myoglobin.

[1](Skrovan et al., 1999); [2](Ridgway and Johnston, 1966); [3]assumed; [4](Blessing, 1972); [5](Miller et al., 2004); [6](Sleet et al., 1981); [7](Ridgway, 1986); [8](Lockyer, 1976); [9](Scholander, 1940); [10](Kooyman and Sinnett, 1982); [11](Weise and Costa, 2007); [12](Ponganis et al., 1997c); [13](Kooyman et al., 1973b); [14](Burns et al., 2005); [15](Kooyman et al., 1983); [16](Lenfant et al., 1970); [17](Kooyman et al., 1973a; Kooyman et al., 1973b); [18](Ponganis et al., 1993a); [19](Fujise et al., 1985); [20](Simpson et al., 1970); [21](Bryden, 1972); [22](Thorson and Le Boeuf, 1994); [23](Sato et al., 2003); [24](Lenfant et al., 1969); [25](Weber et al., 1974); [26](Ponganis et al., 1999); [27](Cherel et al., 1993); [28](Baldwin, 1988); [29](Sato et al., 2011); and [30](Ponganis et al., 1997a) – the two values in muscle mass and [Mb] represent primary swimming muscle *vs* non-swimming muscle.

diving air volumes of 165 and $69\,ml\,kg^{-1}$ in Adélie (*Pygoscelis adeliae*) and king (*Aptenodytes patagonicus*) penguins, respectively. More recently, air volume in free-diving animals has also been estimated on the basis of buoyancy, body angles and predicted/observed swim velocities during gliding ascents (Miller et al., 2004; Sato et al., 2002; Sato et al., 2011). Use of the air volume calculated during the gliding ascent for estimation of the respiratory O_2 store is dependent on the assumption that the animal has not exhaled any air earlier during the dive prior to ascent. Notably, the air volumes estimated in free-diving penguins are greater than those determined during simulated dives; this results in a 10–20% increase in estimated total body O_2 stores (Sato et al., 2002). Air volume differences between free dives and simulated dives of penguins and other birds (Stephenson, 1995) as well as the relative paucity of respiratory air volume data in birds argue the need for further investigation. Indeed, even the allometric equation for respiratory air volume and body mass in birds is only based on five samples from different studies (Lasiewski and Calder, 1971).

Blood and muscle O_2 stores

Calculations of the blood O_2 store typically include the assumption that one-third of the blood volume is arterial and two-thirds are venous, that arterial Hb saturation declines from 95% to 20%, and that initial venous O_2 content is $5\,ml\,dl^{-1}$ less than initial arterial O_2 content and that all venous O_2 is consumed (Kooyman, 1989; Lenfant et al., 1970). In muscle O_2 store calculations, it is assumed that 100% saturated Mb is completely usable during the dive (Kooyman, 1989). O_2 content is calculated on the basis of $1.34\,ml\,O_2\,g^{-1}$ of either Hb or Mb at 100% saturation.

Potential sources of variation or error in the estimation of O_2 stores include (a) frequent assumptions as to the size of the respiratory air volume (diving air volume *vs* total lung capacity) and the percentage extractable O_2, (b) the accuracy of blood volume measurements and Hb determinations in animals such as seals with large blood volumes, large spleens and, consequently, fluctuating hematocrit (packed red cell volume, Hct) and Hb concentration (Hurford et al., 1996; Persson et al., 1973; Ponganis et al., 1993a), (c) the percentage distribution of blood between the arterial and venous systems in animals with large blood volumes, (d) the frequent lack of accurate muscle mass measurements and (e) the frequent assumption that Mb content is the same in all muscles. As an example, because of splenic expansion and a decrease in Hct during anesthesia of seals, the size of the calculated blood O_2 store of the Weddell seal would be underestimated by 50% if the Hct of an anesthetized seal was used in the calculation of the blood volume and its O_2 store (Ponganis et al., 1993a).

The calculation of the blood O_2 store is also dependent on the initial and final Hb saturation. The initial 95% arterial Hb saturation is reasonable given that there may be a pulmonary shunt and/or an altered ventilation–perfusion ratio (mismatch of ventilation to perfusion) in the lung, and, at least in seals, there may be some carboxyhemoglobin (carbon monoxy–Hb) present. Pugh reported a sixfold elevation in blood carbon monoxide gas content in Weddell seals, which was attributed to a high Hb content and the metabolic breakdown of Hb to bilirubin (Pugh, 1959). In regard to initial venous Hb saturation, Scholander observed that venous blood samples often appeared arterialized at the start of forced submersions (Scholander, 1940). If this occurs prior to diving, the venous portion of the blood O_2 store may be much larger (i.e. $5\,ml\,O_2\,dl^{-1}$ venous blood greater) than frequently assumed. The end-of-dive limits on arterial and venous Hb saturations are supported by forced submersion studies, in which arterial and venous P_{O_2} values were 10 and 2 mmHg, respectively, at the electroencephalographic threshold for hypoxemic brain damage (Elsner et al., 1970; Kerem and Elsner, 1973).

Magnitude and distribution of O_2 stores in selected species

The magnitude of total body O_2 stores and the distribution of O_2 in the respiratory, blood and muscle compartment are listed for several representative species of cetaceans, pinnipeds and penguins in Table 2 and Fig. 1. The calculations are based on the parameters in Table 1 and the general assumptions described above. For comparison, with use of human data (Schmidt-Nielsen, 1997) and the same assumptions, human total body O_2 stores are estimated at $24\,ml\,kg^{-1}$, with 42% in the lungs, 44% in the blood and 14% in muscle. Specific values in Table 2 vary from past estimations (Kooyman and Ponganis, 1998) because of the use of more recent data now available in the literature (Table 1). In addition, two calculations of O_2 stores are provided for both emperor penguins and elephant seals in order to demonstrate how our most recent data change both the magnitude and distribution of the total body O_2

Table 2. Magnitude and distribution of total body O_2 stores in representative species, based on the parameters in Table 1 and the standard assumptions/calculations in the text

Species	Total body O_2 store (ml O_2 kg^{-1})	% Respiratory	% Blood	% Muscle
Bottlenose dolphin	29	21	33	46
Sperm whale	74	6	61	33
California sea lion	55	13	39	48
Harbor seal	62	7	57	35
Weddell seal	89	4	65	31
Elephant seal	85	4	67	29
Elephant seal*	88	4	68	28
Adélie penguin	63	48	26	26
King penguin	55	34	32	34
Emperor penguin	63	31	30	39
Emperor penguin*	68	33	31	36

*Values calculated with greater blood and lung O_2 extraction as described in text.

store in each of these species. Despite such changes in specific estimates, the general trends in both the magnitude and distribution of O_2 stores in shallow and deep divers remain the same. Deep-diving marine mammals such as the sperm whale and Weddell seal have larger mass-specific O_2 stores than their shallow-diving counterparts (Snyder, 1983). In contrast, the magnitude of the total body O_2 store does not vary that greatly between shallow and deep-diving penguins (Table 2), although these values are somewhat higher than in other diving birds (Butler, 2000; Butler, 2004; Green et al., 2005). In the thick-billed murre (*Uria lomvia*), the total O_2 store is 45 ml O_2 kg^{-1}, while in the mallard duck (*Anas platyrhynchos*), it is only 29 ml O_2 kg^{-1} (Croll et al., 1992; Keijer and Butler, 1982).

The distribution of the total O_2 store also varies between deep and shallow divers. In animals that dive deeper and longer, the contribution of the respiratory O_2 store is less, while the proportion of O_2 in the blood and muscle compartments is greater. This pattern even occurs to some degree in penguins, which have much larger mass-specific respiratory O_2 stores in comparison to cetaceans and pinnipeds (Table 2, Fig. 1). Less reliance on the respiratory O_2 store in deep divers decreases the need for gas exchange at depth. Smaller diving air volumes in deep divers also represent a smaller nitrogen reservoir and, in mammals, promote alveolar collapse and pulmonary shunting (Fahlman et al., 2009; Kooyman, 1989; Kooyman et al., 1999). These factors should minimize the risk of excess nitrogen absorption during deep dives.

Prior investigations of O_2 store depletion

Forced submersions – seals

To date, the rate, pattern and magnitude of O_2 store depletion have been most comprehensively studied in the forced submersion experiments of Scholander, Irving and colleagues on young harbor seals (*Phoca vitulina*), gray seals (*Halichoerus grypus*) and hooded seals (*Cystophora cristata*) (Irving et al., 1941; Scholander, 1940; Scholander et al., 1942). The O_2 content of 'alveolar air' declined to 3% (~21 mmHg or 2.8 kPa) (Scholander, 1940). Under conditions of severe bradycardia (slow heart rate, i.e. less than resting) and peripheral vasoconstriction in seals, the isolated muscle O_2 store was almost completely depleted in about 10 min, while the blood (and respiratory) O_2 store lasted about 20–25 min (Fig. 2A). In comparison, arterial Hb saturations can decrease to as low as 50–80% in less than 2 min during breath holds and dives of humans, and in less than 1 min during human sleep apnea (Dempsey et al., 2010; Lindholm and Lundgren, 2009; Qvist et al., 1993; Yumino and Bradley, 2008). These slow rates of blood O_2 depletion during the bradycardia and vasoconstriction of forced submersions conserve O_2 for the brain and heart because of the now well-demonstrated redistribution of blood flow away from O_2-consuming peripheral tissues (Blix et al., 1983; Elsner et al., 1966; Irving, 1939; Scholander, 1940; Zapol et al., 1979).

The muscle O_2 depletion rate during forced submersions, 10 ml O_2 kg^{-1} muscle min^{-1}, was considered elevated at about 2.5 times the resting rate as a result of struggling (Scholander et al., 1942). Under these conditions, muscle lactate began to accumulate at a Mb saturation of 10–20% (Fig. 2B). Again, 70 years since their publication, these remain the only studies in marine mammals in which muscle lactate and O_2 content have been measured simultaneously.

Further studies by Elsner and colleagues established that blood O_2 in seals could be depleted to arterial and venous P_{O_2} values as low as 10 and 2 mmHg, respectively (1.3 and 0.03 kPa) (Elsner et al., 1970; Kerem and Elsner, 1973). In forced submersions of Weddell seals conducted by Zapol, Hochachka and colleagues, arterial P_{O_2} averaged 32 mmHg (4.5 kPa) at 8–12 min of submersion

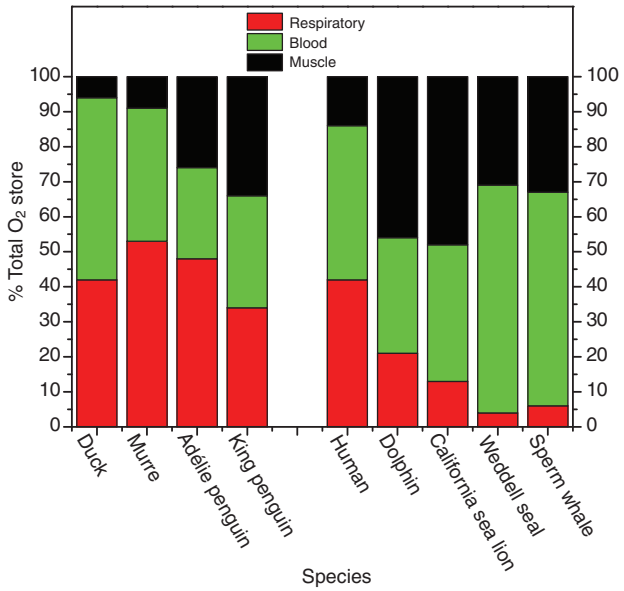

Fig. 1. Distribution of O_2 stores in various species, including the duck (Keijer and Butler, 1982), murre (Croll et al., 1992) and human. Marine mammal, penguin and human calculations based on Table 1 or as described in text. Duck and murre calculations as in references.

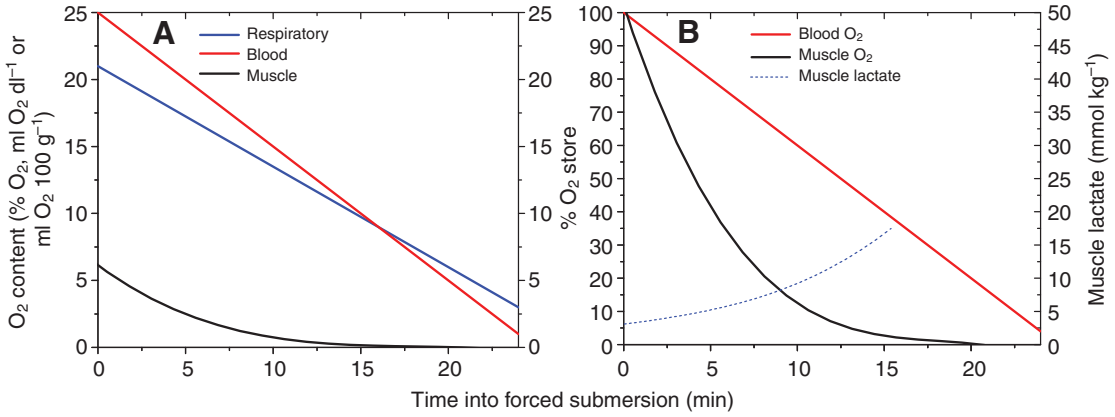

Fig. 2. (A) General depletion patterns of respiratory, blood (arterial) and muscle O_2 stores during forced submersion of seals. Adapted from Scholander (Scholander, 1940) and Scholander et al. (Scholander et al., 1942). The slowest depletion profile of muscle O_2 from that work is illustrated. (B) Muscle lactate accumulation in relation to O_2 store depletion during forced submersion. Adapted from Scholander et al. (Scholander et al., 1942).

(Zapol et al., 1979); arterial and mixed venous P_{O_2} reached 20 mmHg (<3 kPa) during a 46 min forced submersion (Hochachka et al., 1977). In addition, as demonstrated by values for hepatic sinus O_2 content that were greater than arterial values near the end of forced submersions, blood O_2 uptake from the lungs did not occur during the later portions of forced submersions of elephant seals (Elsner et al., 1964).

Comparison of the blood O_2 depletion data in the forcibly submerged harbor seal (*P. vitulina*) with those during asphyxia in the anesthetized, paralyzed dog revealed that the arterial blood O_2 content decreased at a rate 2–4 times faster in the dog (Kerem and Elsner, 1973). Declines in arterial and venous O_2 content of seals during forced submersions were about 1–2 ml O_2 dl^{-1} min^{-1} (Elsner, 1969; Elsner et al., 1964; Irving et al., 1941; Kerem and Elsner, 1973; Scholander, 1940). Arterial P_{O_2} at the asphyxial end point (hypoxemic EEG threshold) was 14 mmHg (about 2 kPa) in the dog *vs* 10 mmHg (1.3 kPa) in the seal. However, that asphyxial end point was reached at 4.25 min in the dog and 18.5 min in the seal. Arterial blood O_2 content at the asphyxial end points were similar in the two species.

Free dives – seals

Investigations of diving physiology and O_2 store depletion in free-diving seals have been primarily and most extensively conducted with the isolated dive hole technique developed by Kooyman on the sea ice of McMurdo Sound (Kooyman, 1968; Kooyman, 1985; Kooyman, 2006; Kooyman and Kooyman, 2009). In a study conducted by Zapol and colleagues, arterial blood O_2 depletion rates in free-diving Weddell seals were about 0.8 ml O_2 dl^{-1} min^{-1} (Qvist et al., 1986), in the same range as described above during forced submersions of much smaller seals. In comparison, arterial O_2 content declined at 6 ml O_2 dl^{-1} min^{-1} in Korean diving ama (Qvist et al., 1993).

The lowest arterial P_{O_2} reported near the end of a dive of a Weddell seal was 18 mmHg (2.4 kPa), corresponding to 28% Hb saturation (Qvist et al., 1986). End-tidal P_{O_2} values were in a similar range (Kooyman et al., 1973a; Ponganis et al., 1993a). After dives of 26 and 34 min duration, end-tidal P_{O_2} values were 13–14 mmHg (<2 kPa). These arterial and end-tidal values, which were much greater than those at the end of extreme forced submersions, also raised the question as to how far the blood O_2 store was depleted during routine diving. This question was also raised by findings during spontaneous sleep apneas of Weddell seals, in which, arterial P_{O_2} declined at variable rates to only about 25 mmHg (about 3.5 kPa) (Kooyman et al., 1980).

In contrast to the situation during forced submersions of seals, muscle Mb desaturation was incomplete in free-diving Weddell seals (Guyton et al., 1995). Combined with the relatively mild bradycardias of free-diving Weddell seals (Hill et al., 1987), this finding suggested that muscle blood flow persisted to some degree during free dives and that the blood O_2 store was not isolated from muscle. This contrasted with the severe bradycardia and peripheral vasoconstriction during forced submersions. Muscle O_2 depletion rates in free-diving Weddell seals averaged about 7 ml O_2 kg^{-1} muscle min^{-1} (5.1% change in Mb saturation min^{-1}) during dives of less than 17 min (near the ADL), and 3.4 ml O_2 kg^{-1} muscle min^{-1} (2.5% change in Mb saturation min^{-1}) in longer dives (Guyton et al., 1995). These values were lower than those during Scholander's forced submersion experiments, but greater than the 2 ml O_2 kg^{-1} muscle min^{-1} of tourniqueted human muscle at rest (Blei et al., 1993; Tran et al., 1999). The low Mb desaturation rates in swimming Weddell seals could be consistent with blood O_2 supplementation of muscle metabolism and/or a lower muscle metabolic rate due to hydrodynamics, prolonged gliding and a low cost of swimming (Williams et al., 2000; Williams et al., 2004). In support of the concept of such blood O_2 supplementation of muscle, it is notable that maintenance of some muscle blood flow during restrained submersions of trained *vs* naïve seals was accompanied by a slower rate of Mb desaturation in the trained seals (Jobsis et al., 2001).

Trained dives – cetaceans

Limited data are also available for cetaceans during trained dives and stationary breath holds. This research, pioneered by Ridgway, and more recently extended by Williams and colleagues, has largely been conducted by or in association with the US Navy Marine Mammal Program. After dives of a trained bottlenose dolphin (*Tursiops truncatus*), the O_2 concentration of exhaled air was as low as 3% or 22 mmHg (3 kPa) (Ridgway et al., 1969). During stationary breath holds of dolphins, venous P_{O_2} in blood vessels of the tail fluke reached values as low as 18–20 mmHg (about 2–3 kPa) (Williams et al., 1999). In beluga whales (*Delphinapterus leucas*), fluke venous P_{O_2} values were near 20 mmHg (about 3 kPa) after stationary breath holds between 10 and 17 min duration (Shaffer et al., 1997).

Forced submersions – penguins and ducks

In birds, O_2 store depletion has been determined in both penguins and ducks. In his 1940 monograph, Scholander examined blood and muscle O_2 depletion in macaroni (*Eudyptes chrysolophus*) and gentoo (*Pygoscelis papua*) penguins (Scholander, 1940). Arterial blood O_2 content declined quickly from 21 to 3 ml O_2 dl^{-1} within 5 min, yielding an overall blood O_2 depletion rate of 3.6 ml O_2 dl^{-1} min^{-1}. We are unaware of comparable data from an avian non-diver. Muscle O_2 content declined from 40 to 0 ml O_2 kg^{-1} muscle by 5 min, yielding a minimum muscle O_2 depletion rate of 8 ml O_2 kg^{-1} muscle min^{-1}. This muscle O_2 depletion rate during forced submersion of the penguin is in the same range as resting muscle O_2 consumption (11 O_2 kg^{-1} muscle min^{-1}) reported in the pekin duck (*A. platyrhynchos*) (Grubb, 1981).

During forced submersion studies of pekin ducks by Jones and colleagues, air sac P_{O_2} was near 30 mmHg (4 kPa, 25% of the initial value), and arterial and venous P_{O_2} values were 30 and 23 mmHg, respectively (4 and 3 kPa), at the point of 'imminent cardiovascular collapse' (Hudson and Jones, 1986). Because of the P_{50} (P_{O_2} at 50% Hb saturation) of duck Hb and the Bohr effect, blood O_2 content was zero at those blood P_{O_2} values. In comparison to Scholander's penguins, both blood and muscle O_2 depletion rates were lower in the smaller pekin duck. Arterial and venous O_2 content depletion rates during forced submersions in ducks were 2.2 and 1.4 ml O_2 dl^{-1} min^{-1}, respectively (Stephenson and Jones, 1992). The muscle O_2 store was estimated to be depleted within 45 s; this would result in a minimum O_2 depletion rate of about 5 ml O_2 kg^{-1} muscle min^{-1} (Stephenson and Jones, 1992). As muscle O_2 depletion was not directly measured in this study, the actual depletion rate may have been faster, i.e. near the previously cited 11 ml O_2 kg^{-1} muscle min^{-1} resting value (Grubb, 1981).

Simulated dives – penguins

During simulated dives of 5 min duration in Adélie and gentoo (*P. papua*) penguins in a pressure chamber, air sac O_2 concentration decreased at a rate of 2.2% min^{-1} to minimum values near 2% (about 15 mmHg or 2 kPa) (Kooyman et al., 1973c). Arterial P_{O_2} declined from 80 mmHg (10.2 kPa) to 20–30 mmHg (2.6–4.3 kPa). These end-of-dive values were similar to those above in pekin ducks at the point of 'imminent cardiovascular collapse'. However, the extreme limit to hypoxemic tolerance in penguins was not determined. Blood O_2 content and muscle O_2 depletion were not examined in this study.

Recent investigations of O_2 store depletion

Because of the scarcity of data on the rate and magnitude of O_2 store depletion during dives, we have attempted to examine O_2 store depletion patterns in three situations. Serial blood gas sampling and ^{1}H nuclear magnetic resonance (NMR) spectroscopy have allowed analyses of both blood and muscle O_2 store depletion during spontaneous sleep apneas (breath holds) of the northern elephant seal (*M. angustirostris*) (Ponganis et al., 2002; Ponganis et al., 2008; Stockard et al., 2007). The refinement of catheterization techniques for the elephant seal in the sleep apnea studies and the development of a backpack P_{O_2} recorder have also allowed investigation of blood O_2 depletion in translocated, free-diving elephant seals (Meir et al., 2009). Lastly, application of the P_{O_2} recorder and a backpack near-infrared Mb saturation recorder have allowed examination of O_2 store depletion in the air sacs, blood and muscle of emperor penguins diving at an isolated dive hole in the sea ice of Antarctica (Meir and Ponganis, 2009; Ponganis et al., 2009; Ponganis et al., 2007; Stockard et al., 2005; Williams et al., 2011).

Blood and muscle O_2 store depletion – sleep apnea – elephant seals

The spontaneous breath holds that occur during sleep in seals represent a convenient model to investigate O_2 store depletion during an apneic period. Close access to the sleeping seal allows collection of blood samples as well as the opportunity to conduct ^{1}H NMR spectroscopy to investigate Mb desaturation. During these apneic periods, heart rate is near 50 beats min^{-1}, cardiac output is maintained at resting levels and muscle blood flow declines but persists at an average value near 50% the eupneic (during a breath) level (Ponganis et al., 2006). Hence, the muscle O_2 store is not completely isolated from the circulation during these breath holds. The lack of change in blood lactate concentration during and after the apneas is also consistent with adequate organ perfusion and O_2 delivery during the breath hold (Castellini et al., 1986).

Serial blood gas analyses during sleep apneas in young elephant seals revealed that arterial and venous P_{O_2} values quickly equilibrated within the first minute, indicative of minimum gas exchange in the lung after the first minute (Stockard et al., 2007). The minimal role of the lung as an O_2 store is consistent with the exhalation of air observed at the start of the apnea, the low lung volumes determined in simulated dives (Kooyman et al., 1973b), prior observations of the equilibration of arterial and venous O_2 contents during forced submersions (Elsner et al., 1964), and Elsner's proposal that the large hepatic sinus–vena cava of the seal acts as a venous blood O_2 reservoir (Elsner et al., 1964).

Fig. 3 demonstrates the decline in arterial and venous P_{O_2} and O_2 content during sleep apneas as long as 10.9 min (Stockard et al., 2007). It is apparent that arterial P_{O_2} and the corresponding Hb saturation during most of the breath hold (i.e. beyond 1 min) is in a

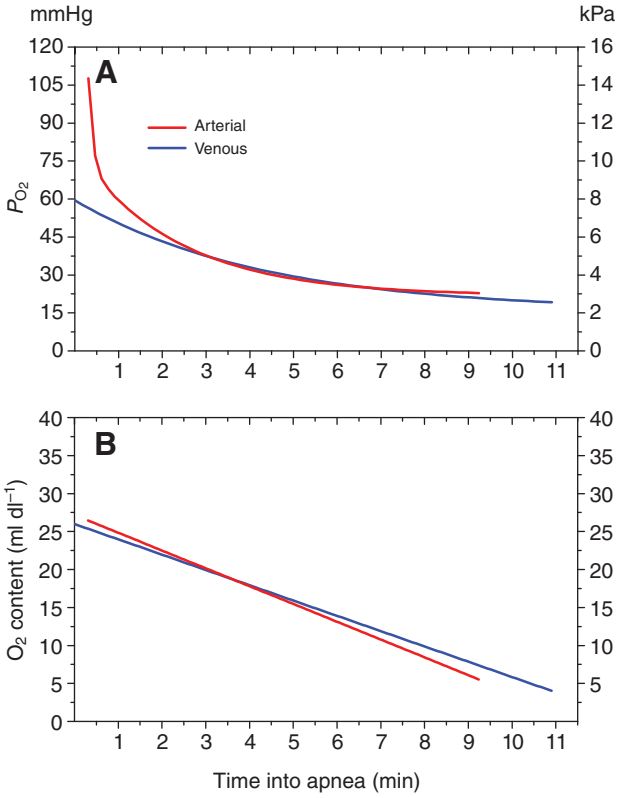

Fig. 3. Changes in arterial and venous P_{O_2} (A) and O_2 content (B) during sleep apnea of elephant seals. Adapted from Stockard et al. (Stockard et al., 2007).

range that would be considered critical [<60 mmHg (7.8 kPa) and 90% saturation] and, indeed, severe [<50 mmHg (6.5 kPa) and 80% saturation] in most human patients (Mason et al., 2005; Nunn, 1977). The lowest arterial P_{O_2} values during sleep apnea are less than the mean value (25 mmHg, 3.3 kPa) of humans breathing ambient air at 8400 m near the summit of Mount Everest (Grocott et al., 2009).

Blood O_2 content declined by about 2 ml O_2 dl^{-1} min^{-1} during sleep apnea. This was about twice the rate previously observed during forced submersions and was consistent with the maintenance of cardiac output during the breath hold. For a typical 7 min apnea in these young seals, about 56% of the blood O_2 store was consumed (Stockard et al., 2007). That portion alone of the blood O_2 store was sufficient to provide enough O_2 for a metabolic rate of 4.2 ml O_2 kg^{-1} min^{-1} during the apnea. This estimation of the blood O_2 store was based on the previously reviewed O_2 store assumptions and on a blood volume of 196 ml kg^{-1} and Hb concentration of 23.5 g dl^{-1} in these young seals (Stockard et al., 2007).

The development of ^{1}H NMR spectroscopy techniques to analyze Mb saturation by Jue and associates allowed investigation of Mb desaturation in elephant seals sleeping inside a magnetic resonance imaging scanner (Ponganis et al., 2008). Mb desaturated rapidly with the onset of apnea (Fig. 4B), and settled at a steady-state level near 80% saturation by 4 min into the apneic period. In these young seals, that initial desaturation rate corresponded to a muscle O_2 depletion rate of 1–2.3 ml O_2 kg^{-1} muscle min^{-1} during the first 4 min of the apnea. The Mb saturation remained at the 80–85% level until the end of apnea, after which it quickly re-saturated to >95% saturation. Low muscle blood flow and blood-to-muscle O_2 transfer during the apnea allowed maintenance of the steady-state 80% saturation throughout the apnea. Assuming all muscle in the body desaturated to that level, the O_2 from muscle, in addition to that provided by the blood, would yield a metabolic rate of 4.7 ml O_2 kg^{-1} min^{-1} during a 7 min apnea in these young elephant seals. This O_2 store depletion rate of 4.7 ml O_2 kg^{-1} min^{-1} is 26% greater than the metabolic rate predicted at rest for a mammal of this size (Kleiber, 1975), and is consistent with the maintenance of heart rate and cardiac output during the breath-hold period.

In summary, it is the blood O_2 store that is primarily utilized during sleep apnea in elephant seals. The pattern of O_2 store utilization contrasts strikingly with that during forced submersion (Fig. 4A). Blood O_2 depletion rates are greater and muscle O_2 depletion rates slower during sleep apnea than during forced submersion (Fig. 4). Higher heart rates and tissue perfusion result in greater blood O_2 extraction. The lower rate of muscle O_2 depletion is secondary to both maintenance of some blood O_2 delivery and a lower muscle metabolic rate during sleep than during the stress of a forced submersion. Under these conditions, Mb is able to function not only as an O_2 store but also as the primary intracellular O_2 transporter because of its translational diffusion coefficient and high concentration in seal muscle (Gros et al., 2010; Ponganis et al., 2008).

For typical 7 min apneas of these young elephant seals, the blood O_2 store is far from depleted; more than 40% of the initial blood O_2 still remains at the end of the breath hold. In addition, the muscle O_2 store is only 20% depleted during sleep apnea. Thus, although heart rate, cardiac output and metabolic rate are maintained near resting levels, O_2 store depletion does not limit the breath-hold duration during sleep apneas.

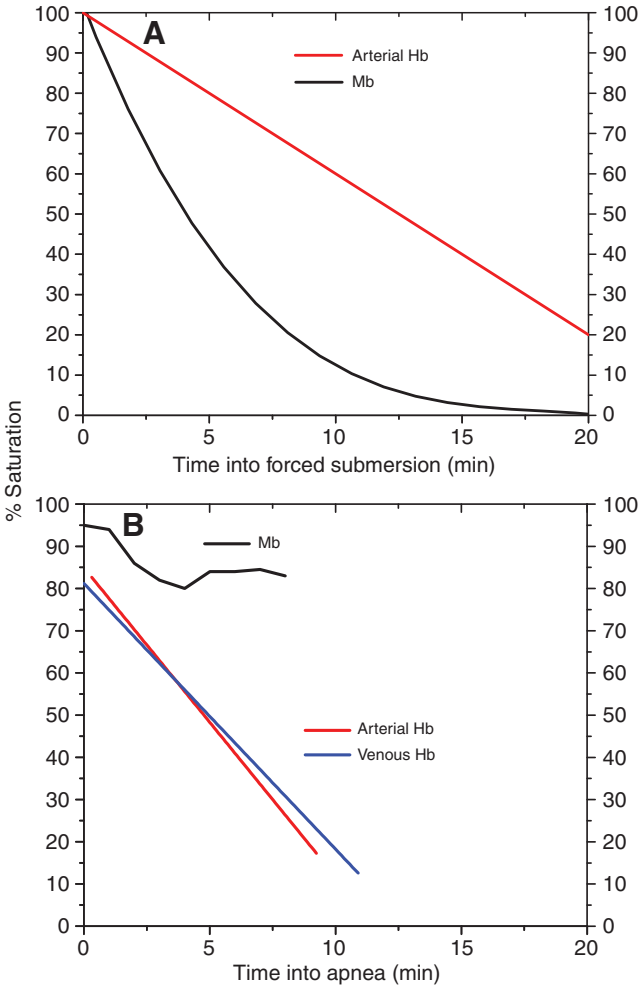

Fig. 4. Comparison of hemoglobin (Hb) and myoglobin (Mb) desaturation patterns between forced submersion (A) and sleep apnea (B) in seals. Higher heart rates, maintenance of tissue perfusion (including muscle) and a lower muscle metabolic rate during sleep apnea lead to a more rapid decline in Hb saturation and preservation of Mb saturation. Adapted from Scholander et al. (Scholander et al., 1942), Stockard et al. (Stockard et al., 2007) and Ponganis et al. (Ponganis et al., 2008).

Blood O_2 store depletion – free dives – elephant seals

Blood O_2 store depletion during diving was evaluated with the use of indwelling P_{O_2} electrodes in translocated, juvenile elephant seals, and conversion of the P_{O_2} profiles to Hb saturation profiles with use of the elephant seal O_2–Hb dissociation curve (Meir et al., 2009). This translocation model was developed by Oliver and LeBoeuf (Oliver et al., 1998). Mean dive heart rates of such translocated seals ranged from 31 to 48 beats min^{-1} (Andrews et al., 1997). The typical 2–3 day return trips from release sites to the rookery provided routine dives of 10–20 min duration and 100–200 m depth, and occasional dives as long as 44 min and as deep as 700 m (Meir et al., 2009). During these trips, dives were spontaneous, voluntary and, in contrast to Weddell seals diving under sea ice at an isolated dive hole (Kooyman, 1968), unrestricted in access to the surface. Blood P_{O_2} was recorded at three sites – the aorta, the hepatic sinus and the extradural vein (Fig. 5).

Arterial P_{O_2} values were as low as 12–23 mmHg at the end of dives, corresponding to routine Hb saturations of 8–26%, again demonstrating exceptional hypoxemic tolerance. The lowest arterial P_{O_2} measured in this study (12 mmHg) is nearly as low as the 'critical P_{O_2}' of harbor seals and Weddell seals (10 mmHg)

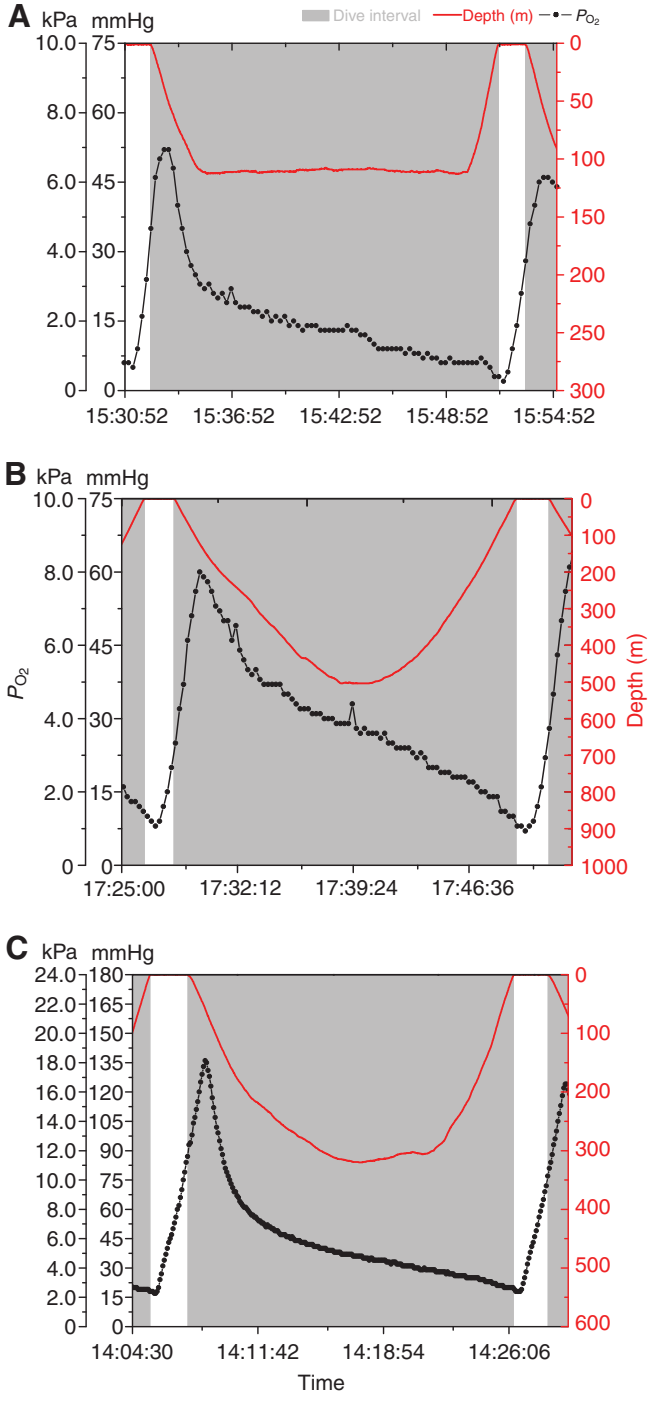

Fig. 5. Typical P_{O_2} profiles in extradural vein (A), hepatic sinus (B) and arterial (C) blood during free dives of young elephant seals. Adapted from Meir et al. (Meir et al., 2009).

(Elsner et al., 1970; Kerem and Elsner, 1973). Routine venous P_{O_2} values and Hb saturations were as low as 2–10 mmHg (0–4%), equivalent to or even lower than those measured in forced submersion studies, and even lower than the well-documented hypoxemic extremes of horses performing strenuous exercise (Bayly et al., 1989; Manohar et al., 2001). These low end-of-dive P_{O_2} values result in near-complete depletion of blood O_2 stores during routine dives, with net O_2 content depletion values up to 91% and 100% in the arterial and venous stores, respectively.

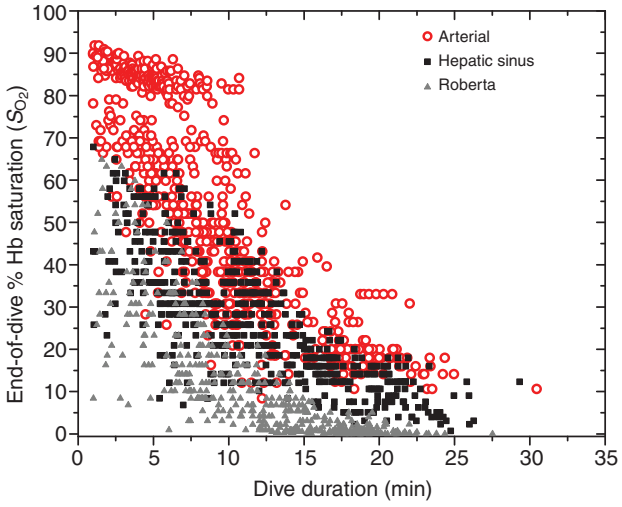

Fig. 6. End-of-dive arterial and hepatic sinus Hb saturations in young elephant seals demonstrate extensive overlap, consistent with the role of the hepatic sinus as a blood O_2 reservoir and the minor role of the lung as an O_2 store during diving in seals. One animal (Roberta) performed many foraging dives during which hepatic sinus saturations routinely approached zero, implying exceptional hypoxemic tolerance. Adapted from Meir et al. (Meir et al., 2009).

It is notable that venous P_{O_2} continued to increase during the early phase of the dive, sometimes reflecting arterial values (Fig. 5). Such 'arterialization' of venous blood (P_{O_2} values greater than those typically found in venous blood) is not consistent with blood extraction by the tissues or blood flow to muscle in this period, and is suggestive of an arterio-venous (a–v) shunt. There was also substantial overlap between end-of-dive arterial and hepatic sinus Hb saturation values (Fig. 6). This is consistent with previous forced submersion and sleep apnea studies, and is expected in a breath-hold diver with collapsed lungs, and supports the concept of the hepatic sinus–venae cavae as a significant O_2 reservoir.

The rate of decrease in venous P_{O_2} (and often arterial P_{O_2}) and venous Hb saturation often became steeper concurrent with the ascent of the dive, usually most pronounced in the final 15–45 s (see Fig. 5A,B and the declines in saturation during ascent in Fig. 7). This period is coincident with both the ascent or 'anticipatory' tachycardia (increased heart rate) (Andrews et al., 1997) and the intense stroking that occur in this species (Williams et al., 2000). These P_{O_2} data support the hypotheses that: (a) the ascent tachycardia serves to increase blood flow and O_2 delivery to depleted tissues at the end of the dive, maximizing the gradient for O_2 uptake at the surface, and (b) some blood flow to muscle occurs in this period (Thompson and Fedak, 1993). Some muscle blood flow during this period is also consistent with the lack of elevated muscle temperature during dives of seals and the slight elevations in blood lactate near the end of long dives (Guppy et al., 1986; Ponganis et al., 1993b). Increased muscle blood flow during increased exertion is also predicted by a numerical model of blood O_2 transport in which the duration of aerobic metabolism during a dive is optimized by coupling muscle blood flow to muscle O_2 demand (Davis and Kanatous, 1999).

The arterialization of venous blood early in a dive does not increase the magnitude of the total body O_2 stores as this increase in blood O_2 is due to O_2 transfer from the lung store (already included in the O_2 store calculation (Kooyman et al., 1999; Meir et al., 2009). However, if depletion of arterial O_2 to below 20% Hb

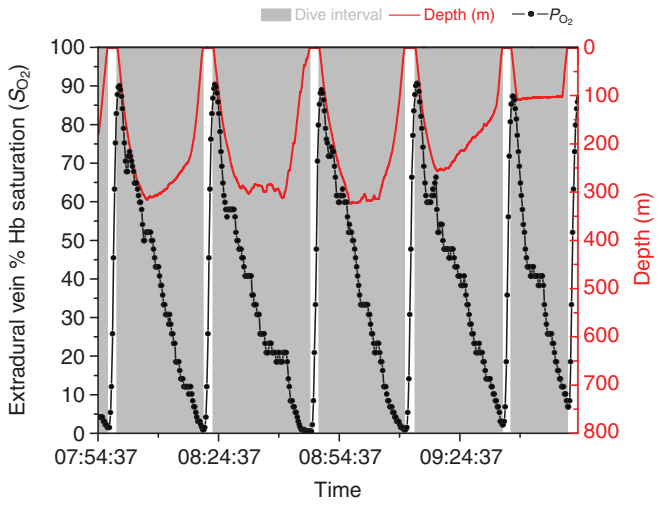

Fig. 7. Extradural vein Hb saturation profiles during dives of elephant seals. Start-of-dive Hb saturations near 85% are consistent with a standard arterio-venous (a–v) content difference of 5 ml O_2 dl^{-1} in blood with such a high O_2 capacity. Note also the declines in saturation during ascent, especially the more rapid decline near the end of the second dive; this is consistent with increased muscle perfusion and blood O_2 extraction during the 'ascent tachycardia', as proposed by Thompson and Fedak (Thompson and Fedak, 1993). Adapted from Meir et al. (Meir et al., 2009).

saturation (typical end-of-dive arterial Hb saturation value used for such calculations) occurs during dives (as documented in this study with values as low as 8% S_{a,O_2}), the available O_2 store would increase by about 3 ml O_2 kg^{-1} to 88 ml O_2 kg^{-1} (with parameters specified in Table 1).

Meir et al.'s results also indicate that at the whole animal level, juvenile elephant seals are not 'hypometabolic' during diving, and that they do not require any significant anaerobic metabolism during routine dives (Meir et al., 2009). For example, the contribution of the venous O_2 store alone to metabolic rate is >100% of the allometrically predicted basal metabolic rate, even for routine dives >10 min.

Air sac, blood and muscle O_2 store depletion – free dives – emperor penguins

Oxygen store depletion has been investigated in emperor penguins diving freely at an isolated dive hole in McMurdo Sound. This approach, again pioneered by Kooyman (Kooyman et al., 1971), has allowed examination of heart rate, swim speed, stroke frequency and temperature during diving (Kooyman et al., 1992; Meir et al., 2008; Ponganis et al., 2001; Ponganis et al., 2004; Ponganis et al., 2003b; van Dam et al., 2002). Most importantly, an ADL of 5.6 min has been documented by blood lactate determinations in birds diving at the isolated dive hole (Ponganis et al., 1997b). During these dives, the penguins travel as far as 1.2 km from the dive hole, and primarily feed on the sub-ice fish *Pagothenia borchgrevinki* (Ponganis et al., 2000; Shiomi et al., 2008). Because of the availability of this prey item, most dives are less than 100 m – relatively shallow for emperor penguins.

Air sac and blood O_2 depletion during dives have been examined with the use of a P_{O_2} electrode and custom-built recorder (Ponganis et al., 2009; Ponganis et al., 2007; Stockard et al., 2005). As in research with the elephant seal, P_{O_2} profiles have been converted to Hb saturation profiles by determination of O_2–Hb dissociation curves of emperor penguins, and application of the curve to the P_{O_2}

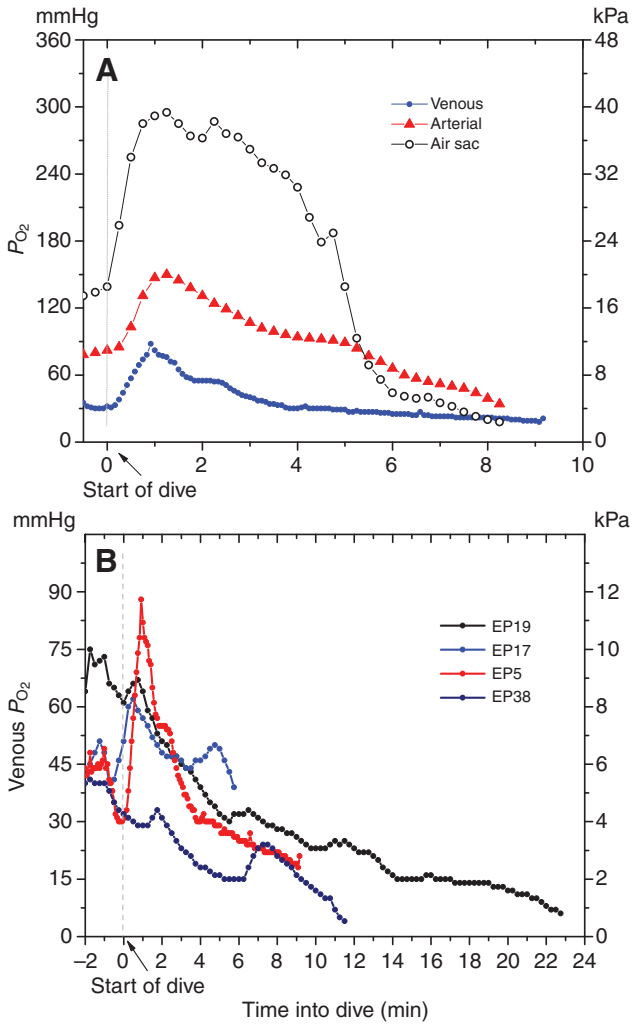

Fig. 8. (A) Air sac, arterial and venous P_{O_2} profiles of emperor penguins during different dives of 8–9 min duration demonstrate compression hyperoxia and the transfer of respiratory O_2 to blood. Adapted from Stockard et al. (Stockard et al., 2005) and Ponganis et al. (Ponganis et al., 2009). (B) Variable patterns of venous P_{O_2} profiles during dives of emperor penguins (EP) are consistent with variable peripheral vascular responses, including mixed muscle blood flow responses and intermittent use of a–v shunts. Adapted from Ponganis et al. (Ponganis et al., 2009).

data (Meir and Ponganis, 2009). Investigation of pectoral muscle O_2 depletion has been conducted with the development of a backpack near-infrared recorder and probe (Williams et al., 2011). In these studies, the birds are typically instrumented under general anesthesia in the evening and, after overnight recovery, are allowed to dive for 1–2 days at the dive hole before recapture, anesthesia and removal of the recorder.

Air sac P_{O_2} profiles in 5–75 m deep dives of up to 11 min duration revealed a compression hyperoxia followed by a decrease in P_{O_2} as the respiratory O_2 fraction declined secondary to O_2 consumption and as the ambient pressure decreased during ascent (Fig. 8) (Stockard et al., 2005). Final P_{O_2} declined exponentially and reached values as low as 0 mmHg; it was less than 20 mmHg (2.7 kPa) in 42% of dives. By comparison, the inspiratory P_{O_2} for a bar-headed goose (*Anser indicus*) at a simulated altitude of 11,580 m was 23 mmHg (3.1 kPa), and air sac P_{O_2} of an Adélie penguin at the end of a simulated dive was 15 mmHg (2.0 kPa)

(Black and Tenney, 1980; Kooyman et al., 1973c). End-tidal P_{O_2} values of a climber breathing ambient air on Mount Everest (35 mmHg, 4.7 kPa) and human shallow-water black-out thresholds (25 mmHg, 3.3 kPa) were also greater than many of the end-of-dive P_{O_2} values in the air sacs of emperor penguins (Ferretti et al., 1991; Ferrigno and Lundgren, 2003; West et al., 1983).

The low end-of-dive air sac P_{O_2} values in emperor penguins contrast with the air sac P_{O_2} values near 30 mmHg (4 kPa) of ducks at the point of imminent cardiovascular collapse (Hudson and Jones, 1986). Such hypoxic tolerance is afforded, in part, by a left shift of the O_2–Hb dissociation curve of the emperor penguin (and other penguins as well as high altitude flying birds) in comparison to that of the duck (Meir and Ponganis, 2009). At a P_{O_2} of 20 mmHg (2.7 kPa), the Hb of the duck would be devoid of O_2 while the Hb of the emperor penguin would still be 27% saturated.

Initial air sac P_{O_2} values during dives indicated that initial O_2 fractions could be as high as 19%, which is greater than the 15% value observed in simulated dives in a pressure chamber (Kooyman et al., 1973c). Complete consumption of the 19% O_2 fraction resulted in a greater respiratory O_2 store than that typically calculated with the pressure chamber results (Kooyman, 1989; Ponganis et al., 2010). The overall rate of change in the respiratory O_2 fraction during dives of up to 11 min duration ranged between 5 and 2% min^{-1}. By comparison, the respiratory O_2 fraction changed about 2% min^{-1} during simulated dives of penguins (Kooyman et al., 1973c).

Arterial P_{O_2} profiles of diving emperor penguins were characterized by a compression hyperoxia followed by a decline to values as low as 26–30 mmHg during dives of up to 12 min duration (Ponganis et al., 2009; Ponganis et al., 2007) (see Fig. 8A). The pattern was similar to that observed in the air sac, but it was unclear why these end-of-dive arterial values were generally greater than air sac values for dives of similar duration. The magnitude of air sac P_{O_2} depletion was quite variable, and it is possible that differences in physiological responses and depth profiles of individual dives may have at least partially contributed to differences between the air sac and arterial studies. Regardless of the mechanisms, it is notable that arterial Hb saturation of emperor penguins can be maintained near 100% during almost the entire dive (Fig. 9), even during dives as long as 12 min (Meir and Ponganis, 2009).

Arterial Hb saturation declined primarily during the ascent, and reached a minimum value of only 47%. These final arterial P_{O_2} values and Hb saturations were greater than those of the bar-headed goose at a simulated altitude of 11,580 m (22 mmHg, 28% saturation) and similar to those of humans breathing ambient air near the summit of Mount Everest (25 mmHg, 54% saturation) (Black and Tenney, 1980; Grocott et al., 2009). Whether lower arterial P_{O_2} values and Hb saturation occur in other dives of emperor penguins is unknown and awaits further investigation. During the ascent, the arterial Hb desaturation rate was about 25% min^{-1}, although the average value over a dive of 9 min duration was much lower, about 5–6% min^{-1} (Meir and Ponganis, 2009).

The ability to maintain arterial Hb saturation near 100% during most of the duration of a dive in emperor penguins contrasts with that in elephant seals, where arterial Hb saturation is usually between 70% and 30% during the majority of the dive (Fig. 9). This highlights the size and role of the respiratory O_2 store in penguins and emphasizes the significance of lung-to-blood O_2 transfer during dives of penguins.

Venous P_{O_2} and Hb saturation profiles during dives of emperor penguins were remarkable for the variability in the rate and pattern of decline throughout the dive (Fig. 8B, Fig. 10). Venous P_{O_2} could transiently rise during the early portion of some dives while it declined in others. Initial or peak P_{O_2} values were often consistent with early, even pre-dive arterialization of venous blood (i.e. >90% Hb saturation). P_{O_2} and Hb saturation declined at variable rates and, at times, with fluctuations during the later portions of dives (Fig. 8B, Fig. 10). The transient elevations in P_{O_2} and Hb saturations during dives are not consistent with muscle blood flow and blood O_2 extraction by muscle during such dives. Rather, these increases in P_{O_2} emphasize (a) the potential role of a–v shunts in arterialization of the venous O_2 store and, again, (b) the significance of net lung-to-blood O_2 transfer during dives of penguins. In contrast, muscle blood flow may occur in those dives in which venous P_{O_2} and Hb saturation decline significantly during the early portion of the dive. This plasticity in the peripheral blood flow is also suggested by the range of venous P_{O_2} values and Hb saturations for a dive of given duration. For example, near 6 min dive duration, final venous Hb saturations ranged from 3% to 75%. End-of-dive venous Hb saturations indicated that the entire venous O_2 store could be consumed; 15% of dives had a final venous Hb saturation ≤5%.

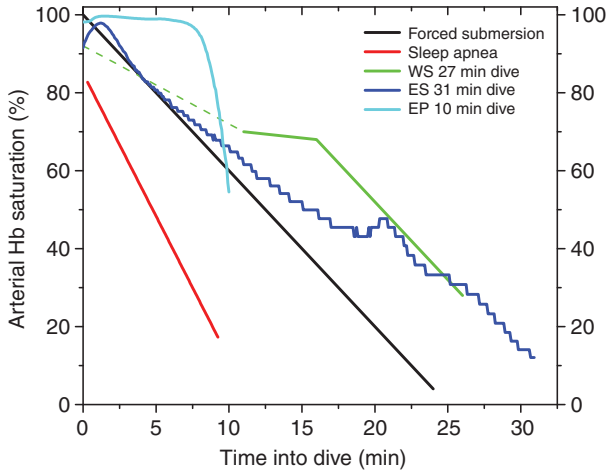

Fig. 9. Typical arterial Hb saturation profiles during forced submersions of seals, sleep apnea of seals and dives of Weddell seals (WS), elephant seals (ES) and emperor penguins (EP). The large respiratory O_2 store of the emperor penguin can allow for maintenance of 100% arterial Hb saturation during almost 80% of a 10 min dive, while seals almost routinely experience clinically significant hypoxemia with Hb saturations less than 80% during much of a dive or breath hold. During the dashed segment of the WS profile, no data were available as blood was sampled only in the last part of the dive (solid line). Adapted from Scholander et al. (Scholander et al., 1942), Qvist et al. (Qvist et al., 1986), Stockard et al. (Stockard et al., 2007), Meir et al. (Meir et al., 2009), and Meir and Ponganis (Meir and Ponganis, 2009).

Mb desaturation profiles of diving emperor penguins revealed two distinct patterns (Fig. 11): a monotonic decline (type A) and a mid-dive plateau pattern (type B) (Williams et al., 2011). In type A dives, Mb saturation generally followed a monotonic decline throughout the dive and, in dives near the ADL, the final Mb saturation was near 0%. Mean desaturation rate in type A dives was 14.4±3.8% min^{-1}. Type A dives are consistent with isolation of muscle from the circulation during dives. The desaturation rate is similar to rapid Mb desaturation observed in forced submersion studies (Scholander et al., 1942) and is higher than desaturation rates observed in Weddell seals, where muscle perfusion was suspected during dives (Guyton et al., 1995) (see Fig. 12). Mean

muscle O_2 consumption (12.4±3.3 ml O_2 kg^{-1} muscle min^{-1}) based on the Mb desaturation rate in diving emperor penguins is low, less than one-tenth the pectoralis–supracoracoideus muscle O_2 consumption calculated from emperor penguins swimming maximally in a flume (160 ml O_2 kg^{-1} muscle min^{-1}) (Kooyman and Ponganis, 1994; Ponganis et al., 1997a), demonstrating the efficiency of underwater locomotion in diving emperor penguins.

In type B dives, Mb desaturation rate during the initial descent was either rapid, as in type A dives, or moderate. Desaturation rate then slowed significantly, often leveling off completely until the ascent phase when desaturation rate again increased (Fig. 11B and Fig. 12). Given the continuous, almost constant, stroking pattern throughout these dives, the minimum change in Mb saturation implies the muscle O_2 store was supplemented by the circulation during these periods. Such intermittent muscle blood flow is consistent with the variable venous P_{O2} profiles discussed above. The increase in Mb desaturation rate during the ascent phase implies that muscle blood flow was reduced during this portion of the dive. The type B plateau period of Mb desaturation in diving emperor penguins is similar to that during sleep apnea in elephant seals, during some dives of Weddell seals and during exercise in humans (Guyton et al., 1995; Ponganis et al., 2008; Richardson et al., 1995). Saturation values in type B dives of 8–10 min duration often declined to below 10% (Fig. 11B and Fig. 12); mean desaturation rate in type B dives was 9.8±2.4% min^{-1}. Despite the potential supplementation of the Mb O_2 stores during dives, the near-complete O_2 depletion of Mb in longer type B dives (Fig. 11) is supportive of the concept that the onset of post-dive lactate accumulation is secondary to muscle O_2 depletion.

The results of these studies of O_2 store depletion in emperor penguins emphasize the significance of the relatively large respiratory O_2 store in penguins as well as the apparent plasticity in peripheral vascular responses. In contrast to seals, this allows for maintenance of the penguin's arterial Hb saturation during much of the dive (Fig. 9). We hypothesize that not only are the transient elevations in heart rate during the early segments of dives of penguins important for lung-to-blood O_2 transfer but also the increased cardiac output can be utilized either to enhance the venous O_2 store through the use of a–v shunts or to supplement the muscle O_2 store through maintenance of muscle blood flow (Figs 10 and 11).

As recently reviewed (Ponganis et al., 2010; Sato et al., 2011), the findings in these latest studies have increased the estimated total body O_2 stores in emperor penguins to 68 ml O_2 kg^{-1}, well above prior estimates of 56 ml O_2 kg^{-1}. This is primarily due to the large diving air volumes, greater respiratory O_2 extraction and the pre-dive arterialization of venous blood measured in recent studies. The respiratory system contains 33% of the total O_2, while the blood and muscle compartments hold 31% and 36%, respectively. Note that pre-dive arterialization of venous blood can occur in emperor penguins whereas in elephant seals, arterialization of venous blood appears to occur primarily during early descent (Meir et al., 2009; Meir and Ponganis, 2009). Therefore, in contrast to the elephant seal, the venous O_2 store of the emperor penguin can be optimized to arterial levels before the dive.

The average depletion rates of all the body O_2 stores of the emperor penguin contributed 6.8 ml O_2 kg^{-1} min^{-1} to whole-body metabolic rate for dives of about 10 min duration. The muscle O_2 store contributed most (53%), while the respiratory and blood compartments contributed 31% and 16%, respectively (Williams et al., 2011). This average total O_2 store depletion rate during diving demonstrates the low metabolic cost of diving in emperor penguins as it is similar to measured and predicted resting metabolic rates. It must be emphasized that O_2 depletion rates are highly variable, and that there is probably a wide range of diving metabolic rates dependent on the activity and duration of an individual dive. In addition, the actual metabolic rate during dives, especially dives longer than the ADL, may be greater because of phosphocreatine breakdown and glycolysis.

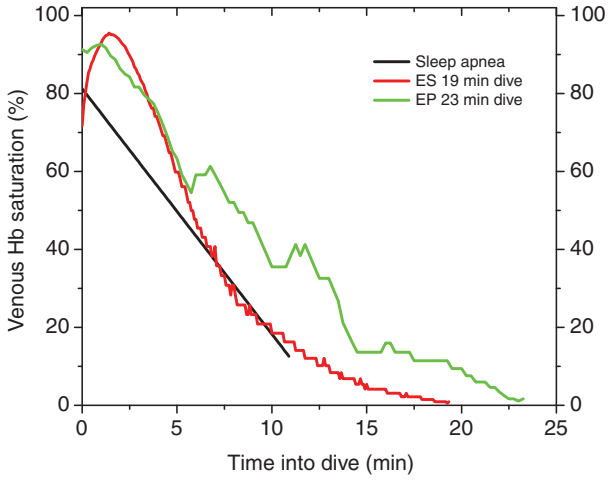

Fig. 10. Venous Hb desaturation profiles during sleep apnea and dives of elephant seals (ES), and during dives of emperor penguins (EP). Of note is the pre-dive arterialization of venous blood prior to a 23 min dive of an emperor penguin, and the arterialization of venous blood during the early descent of the seal. It should also be noted that venous Hb desaturation profiles are quite variable in emperor penguins as reflected in the different venous P_{O2} profiles in Fig. 8B. Adapted from Stockard et al. (Stockard et al., 2007), Meir et al. (Meir et al., 2009), and Meir and Ponganis (Meir and Ponganis, 2009).

Conclusions

These recent investigations of O_2 store depletion in elephant seals and emperor penguins highlight differences in O_2 store management associated with (a) the magnitude and distribution of O_2 stores, (b) the intensity of cardiovascular responses during a breath hold or dive and (c) locomotory muscle workload. The depletion patterns of these O_2 stores during dives also provide insight into the processes underlying the ADL, i.e. the duration of aerobic metabolism and the onset of post-dive blood lactate accumulation. In addition, effective utilization of the entire O_2 store demonstrates the importance of extreme hypoxemic (low arterial O_2) tolerance and avoidance of re-perfusion injury.

Magnitude and utilization of O_2 stores

Recent findings demonstrate that assumptions previously made in the calculation of O_2 stores may underestimate the true magnitude of the respiratory and blood O_2 stores (Ponganis et al., 2010). First, the volume of respiratory air during spontaneous breath holds, especially in penguins, may be greater than the commonly assumed values determined during simulated dives (Sato et al., 2002; Sato et al., 2011). Second, again in penguins, the start-of-dive respiratory O_2 fraction and the net extraction of respiratory O_2 may be greater than those obtained during simulated dives and forced submersion. Third, in emperor penguins, arterialization of the venous blood prior to the dive suggests that the venous portion of the blood O_2 store can be larger than that typically assumed in the O_2 store calculation. And lastly, especially as evidenced by our data in

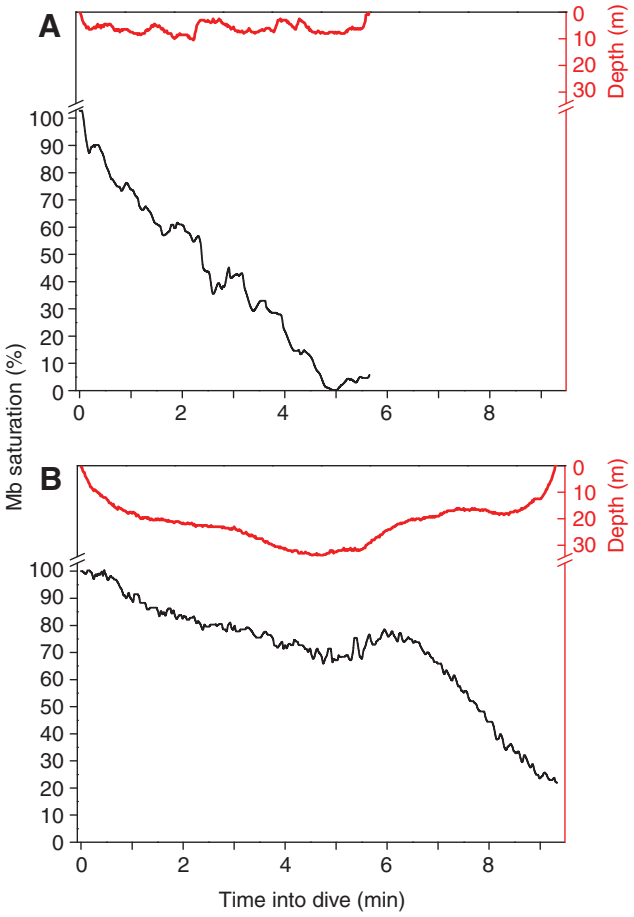

Fig. 11. Mb desaturation profiles during dives of emperor penguins are classified into two types, type A (A) and type B (B). It is postulated that muscle is completely ischemic in type A dives, but that transient muscle blood flow in the middle of the dive accounts for the type B profile. Adapted from Williams et al. (Williams et al., 2011).

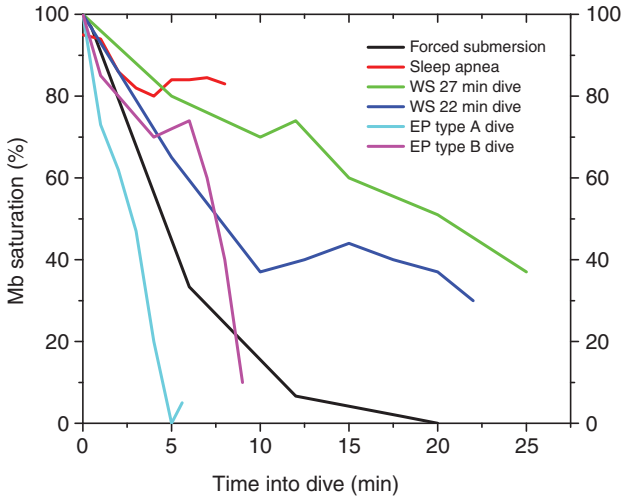

Fig. 12. Examples of Mb desaturation profiles during forced submersions of seals, sleep apnea of elephant seals, and dives of Weddell seals (WS) and emperor penguins (EP). The rate of depletion of Mb-bound O_2 is a function of Mb concentration, muscle metabolic rate and the magnitude of blood-to-muscle O_2 transfer (dependent on the degree of arterial hypoxemia and the degree of muscle ischemia during a given breath hold or dive). In comparison, tourniqueted human muscle at rest desaturates within about 4–5 min (Blei et al., 1993; Tran et al., 1999). Adapted from Scholander et al. (Scholander et al., 1942), Ponganis et al. (Ponganis et al., 2008), Guyton et al. (Guyton et al., 1995) and Williams et al. (Williams et al., 2011).

elephant seals, end-of-dive arterial saturations may well be less than the assumed 20% value, again increasing the size of the calculated O_2 store.

The difference in arterial Hb saturation profiles between emperor penguins and elephant seals emphasizes the role of the large respiratory O_2 store in emperor penguins (Fig. 9). In dives of emperor penguins, arterial Hb saturation can be maintained near 100% during much of the dive, even during dives as long as 12 min. Although lower arterial Hb saturations may occur as suggested by low end-of-dive air sac P_{O_2} values on some dives, 12 min is remarkable because more than 99% of dives of emperor penguins at sea are less than that duration (Kooyman and Kooyman, 1995; Wienecke et al., 2007). In contrast, seals, which exhale prior to diving, reach arterial Hb saturations of 60–70% within a few minutes after the start of a dive, and within a minute after initiation of a sleep apnea breath hold (Fig. 9). Elephant seals routinely experience arterial P_{O_2} values and Hb saturations that would be considered critical in humans.

We also conclude that heart rate, peripheral blood flow distribution and muscle workload are the primary determinants of the rate and pattern of O_2 store utilization. This is exemplified by sleep apnea in elephant seals, during which higher heart rate, higher cardiac output and presumed organ perfusion, and maintenance of muscle blood flow result in a faster rate of blood O_2 depletion and a slower muscle O_2 depletion rate than during forced submersions (Figs 3 and 4). In seals, sleep apnea and forced submersion can be considered to represent opposite ends of the spectrum of physiological responses and O_2 store management during a breath hold. Diving fits in between those two extremes with its exact position in the spectrum determined by the nature and circumstances of a given dive. During sleep apnea, average muscle blood flow is only one-half the eupneic level. In that regard, it is notable that heart rates during dives of elephant seals are even lower than those during sleep apnea. Therefore, we further hypothesize that muscle blood flow, at least until the start of increased heart rate during ascent (the 'ascent tachycardia'), is low to nil during dives of seals. In contrast, in emperor penguins, highly variable venous P_{O_2} profiles and two distinct patterns of muscle Mb desaturation suggest that regulation of blood flow to muscle and to peripheral a–v shunts is quite plastic both within dives and between different types of dives (Figs 10 and 11). The variability in the muscle blood flow response in emperor penguins is probably associated with the relatively larger magnitude of their respiratory O_2 store and with the transient elevations in heart rate observed in the initial portions of their dives (Meir et al., 2008). In penguins, in contrast to seals, a high heart rate early in the dive and a large respiratory O_2 store allow for increased blood O_2 uptake from the lungs, and either enhancement of the blood O_2 store through a–v shunting (arterialization of venous blood) or supplementation of aerobic muscle metabolism through muscle blood flow.

The degree of muscle perfusion and, indeed, the general tissue distribution of blood flow during a dive remain key issues in understanding the rate and pattern of O_2 store depletion. Differences in renal and hepatic clearances between short, aerobic dives and long, exploratory dives of Weddell seals presumably

reflect differences in heart rate, renal/hepatic perfusion and blood O_2 depletion rates during those types of dive (Davis et al., 1983; Guppy et al., 1986). Numerical modeling of blood flow distribution and tissue O_2 consumption during dives suggests that heart rate and muscle blood flow should be tailored to muscle workload in order to optimize the duration of aerobic metabolism (Davis and Kanatous, 1999). However, studies of California sea lions, Steller sea lions (*Eumetopias jubatus*), harbor seals, gray seals, bottlenose dolphins and emperor penguins have all reported that, in general, diving heart rate does not correlate with locomotory effort (Fedak et al., 1988; Hindle et al., 2010; Thompson and Fedak, 1993; Williams et al., 1999; Williams et al., 1991; Meir et al., 2008). Finer scale analyses of heart rate–stroke rate relationships would be useful. And, unfortunately, muscle blood flow has yet to be measured during diving. Further resolution of this topic awaits future research.

Physiological basis of the ADL

We conclude that the most plausible physiological basis of the ADL is the depletion of the primary locomotory muscle O_2 store and the net accumulation of intramuscular lactate. We suspect, as demonstrated by Scholander and colleagues' findings in Fig. 2B, that such lactate accumulation will begin when Mb saturation declines to about 10–20%. After that threshold, lactate accumulation and phosphocreatine breakdown will increase.

We also hypothesize that a low rate of muscle O_2 consumption (locomotory effort) and possible maintenance of muscle perfusion and blood-to-muscle O_2 transfer may delay the onset of lactate accumulation and prolong the ADL in individual dives (Kooyman and Ponganis, 1998; Williams et al., 2011; Williams et al., 2000). In emperor penguins, we think that vascular responses and the degree of muscle ischemia are quite plastic during different dives, consistent with our observed heart rate, venous P_{O_2} and Mb desaturation profiles. In seals, some muscle blood flow may also occur, especially during the ascent tachycardia. This is consistent with minor elevations of blood lactate concentration late in long dives (Guppy et al., 1986), a lack of elevation of muscle temperature during dives (Ponganis et al., 1993b) and steeper declines in extradural vein Hb saturation profiles during late ascent (Meir et al., 2009).

Our hypothesis that muscle O_2 depletion underlies the ADL is not consistent with the significant muscle Mb saturation remaining in Weddell seals even during dives beyond the ADL (Guyton et al., 1995) (see Fig. 12). We suspect that this difference is due to the specific muscle (latissimus dorsi) examined in the Weddell seal study. The longissimus dorsi–iliocostalis muscle complex has been considered the primary locomotory muscle for the hindlimb propulsion of phocid seals (Howell, 1930; Kanatous et al., 1999). On an anatomical basis, the latissimus dorsi muscle participates in movement of the forelimb, not the hindlimb (Howell, 1930). Therefore, we suspect that the slow muscle desaturation rate observed in the Weddell seal was due, in part, to the low metabolic rate of a muscle not primarily used in phocid propulsion. We suggest that the longissimus dorsi muscle would have a higher rate of Mb desaturation. Again, we emphasize that more studies are needed to unravel this important aspect of O_2 store utilization in diving animals.

Hypoxemic tolerance and avoidance of re-perfusion injury

To fully deplete and take advantage of their large O_2 stores, diving mammals and birds should have extreme hypoxemic tolerance. Although such tolerance had been demonstrated during forced submersions of seals, extreme respiratory and blood O_2 depletion have now been recorded during free dives of both emperor penguins and elephant seals. As already reviewed, these extremes are below inspiratory values found in bar-headed geese at simulated altitude as well as below end-tidal and blood levels in humans breathing ambient air near the summit of Mount Everest. This tolerance is best exemplified in the elephant seal. This seal functions during most of its dive under what would be considered hypoxic conditions in human patients. Indeed, human breath-hold divers experience cardiac arrhythmias and neurological complications with arterial saturations that, while low for humans, are far higher than those routinely observed in seals (Andersson et al., 2009a; Andersson et al., 2009b; Lindholm and Lundgren, 2009; Liner and Andersson, 2009). And, sleep apnea patients develop pulmonary hypertension and right ventricular failure with chronic episodic exposures to hypoxia (Dempsey et al., 2010). Again, in contrast, chronic hypoxia has no apparent sequelae in the elephant seal, even after months-long trips to sea, during which it spends 80 to 90% of its time diving (LeBoeuf et al., 1988).

The mechanisms underlying such tolerance as well as avoidance of re-perfusion injury remain to be fully determined. Higher brain capillary densities, shorter O_2 diffusion distances, mild hypothermia, intrinsic neuronal tolerance, neuroglobin and cytogoblin function, greater glycolytic and buffering capacities or other biochemical adaptations may all play significant roles (Blix et al., 2010; Castellini and Somero, 1981; Folkow et al., 2008; Fuson et al., 2003; Halasz et al., 1974; Kanatous et al., 2002; Kerem and Elsner, 1973; Kerem et al., 1973; Odden et al., 1999; Ramirez et al., 2007; Williams et al., 2008). For example, the shift in the O_2–Hb dissociation curve of penguins is an advantage in comparison to other birds (Meir and Ponganis, 2009). As regards hypothermic protective mechanisms, although temperature fluctuations and regional heterothermy occur during dives, it should be noted that core hypothermia, at least in emperor penguins and elephant seals, has not been observed (Meir and Ponganis, 2010; Ponganis et al., 2004). However, re-perfusion injury may be avoided with enhanced O_2 free radical scavenging, especially through elevated glutathione levels and increased activities of enzymes involved in glutathione recycling (Elsner et al., 1998; Vázquez-Medina et al., 2006; Vázquez-Medina et al., 2007; Zenteno-Savin et al., 2010). And perhaps, in seals, a slight elevation in carboxyhemoglobin levels may even contribute to protection against re-perfusion injury (Nakaoa et al., 2005). Investigation of these processes is required and is relevant not only to the biology of diving but also to a better understanding and potential treatment of human ischemic (decreased perfusion) events (i.e. stroke, myocardial ischemia, organ preservation for transplantation).

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References

Andersson, J. P. A., Liner, M. H. and Jonsson, H. (2009a). Asystole and increased serum myoglobin levels associated with 'packing blackout' in a competitive breath-hold diver. *Clin. Physiol. Funct. Imaging* **29**, 458-461.

Andersson, J. P. A., Liner, M. H. and Jonsson, H. (2009b). Increased serum levels of the brain damage marker S100B after apnea in trained breath-hold divers: a study including respiratory and cardiovascular observations. *J. Appl. Physiol.* **107**, 809-815.

Andrews, R. D., Jones, D. R., Williams, J. D., Thorson, P. H., Oliver, G. W., Costa, D. P. and Le Boeuf, B. J. (1997). Heart rates of northern elephant seals diving at sea and resting on the beach. *J. Exp. Biol.* **200**, 2083-2095.

Baldwin, J. (1988). Prediciting the swimming and diving behavior of penguins from muscle biochemistry. *Hydrobiologia* **165**, 255-261.

Bayly, W. M., Hodgson, D. R., Schulz, D. A., Dempsey, J. A. and Gollnick, P. D. (1989). Exercise-induced hypercapnia in the horse. *J. Appl. Physiol.* **67**, 1958-1966.

Black, C. P. and Tenney, S. M. (1980). Oxygen transport during progressive hypoxia in high-altitude and sea-level waterfowl. *Respir. Physiol.* **39**, 217-239.
Blei, M. L., Conley, K. E. and Kushmerick, M. J. (1993). Separate measures of ATP utilization and recovery in human skeletal muscle. *J. Physiol.* **465**, 203-222.
Blessing, M. H. (1972). Studies on the concentration of myoglobin in the sea-cow and porpoise. *Comp. Physiol. Biochem.* **41A**, 475-480.
Blix, A. S., Elsner, R. W. and Kjekhus, J. K. (1983). Cardiac output and its distribution through capillaries and A-V shunts in diving seals. *Acta Physiol. Scand.* **118**, 109-116.
Blix, A. S., Walloe, L., Messelt, E. B. and Folkow, L. P. (2010). Selective brain cooling and its vascular basis in diving seals. *J. Exp. Biol.* **213**, 2610-2616.
Bryden, M. M. (1972). Body size and composition of elephant seals (*Mirounga leonina*): absolute measurements and estimates from bone dimensions. *J. Zool.* **167**, 265-276.
Burns, J. M., Costa, D. P., Frost, K. and Harvey, J. T. (2005). Physiological development in juvenile harbor seals. *Physiol. Biochem. Zool.* **78**, 1057-1068.
Burns, J. M., Skomp, N., Bishop, N., Lestyk, K. and Hammill, M. (2010). Development of aerobic and anaerobic metabolism in cardiac and skeletal muscles from harp and hooded seals. *J. Exp. Biol.* **213**, 740-748.
Butler, P. J. (2000). Energetic costs of surface swimming and diving of birds. *Physiol. Biochem. Zool.* **73**, 699-705.
Butler, P. J. (2004). Metabolic regulation in diving birds and mammals. *Respir. Physiol. Neurobiol.* **141**, 297-315.
Butler, P. J. and Jones, D. R. (1997). The physiology of diving of birds and mammals. *Physiol. Rev.* **77**, 837-899.
Castellini, M. A. and Somero, G. N. (1981). Buffering capacity of vertebrate muscle: correlations with potentials for anaerobic function. *J. Comp. Physiol. B* **143**, 191-198.
Castellini, M. A., Costa, D. P. and Huntley, A. (1986). Hematocrit variation during sleep apnea in elephant seal pups. *Am. J. Physiol.* **251**, R429-R431.
Cherel, Y., Charassin, J.-B. and Handrich, Y. (1993). Comparison of body reserve buildup in prefasting chicks and adults of king penguins (*Aptenodytes patagonicus*). *Physiol. Zool.* **66**, 750-770.
Croll, D. A., Gaston, A. J., Burger, A. E. and Konnoff, D. (1992). Foraging behavior and physiological adaptation for diving in thick-billed murres. *Ecology* **73**, 344-356.
Davis, R. W. and Kanatous, S. B. (1999). Convective oxygen transport and tissue oxygen consumption in Weddell seals during aerobic dives. *J. Exp. Biol.* **202**, 1091-1113.
Davis, R. W., Castellini, M. A., Kooyman, G. L. and Maue, R. (1983). Renal GFR and hepatic blood flow during voluntary diving in Weddell seals. *Am. J. Physiol.* **245**, 743-748.
Dempsey, J. A., Veasey, S. C., Morgan, B. J. and O'Donnell, C. P. (2010). Pathophysiology of sleep apnea. *Physiol. Rev.* **90**, 47-112.
Elsner, R. (1969). Cardiovascular adjustments to diving. In *The Biology of Marine Mammals* (ed. H. T. Andersen), pp. 117-145. New York: Academic Press.
Elsner, R., Franklin, D. L., Van Citters, R. L. and Kenney, D. W. (1966). Cardiovascular defense against asphyxia. *Science* **153**, 941-949.
Elsner, R., Shurley, J. T., Hammond, D. D. and Brooks, R. E. (1970). Cerebral tolerance to hypoxemia in asphyxiated Weddell seals. *Respir. Physiol.* **9**, 287-297.
Elsner, R., Oyasaetr, S., Almaas, R. and Sauagstad, O. D. (1998). Diving seals, ischemia-reperfusion, and oxygen radicals. *Comp. Biochem. Physiol.* **119A**, 975-980.
Elsner, R. W., Scholander, P. F., Craig, A. B., Dimond, E. G., Irving, L., Pilson, M., Johansen, K. and Bradstreet, E. (1964). A venous blood oxygen reservoir in the diving elephant seal. *Physiologist* **7**, 124.
Fahlman, A., Hooker, S. K., Olszowka, A., Bostrom, B. L. and Jones, D. R. (2009). Estimating the effect of lung collapse and pulmonary shunt on gas exchange during breath-hold diving: the Scholander and Kooyman legacy. *Respir. Physiol. Neurobiol.* **165**, 28-39.
Fedak, M. A., Pullen, M. R. and Kanwisher, J. (1988). Circulatory responses of seals to periodic breathing: heart rate and breathing during exercise and diving in the laboratory and open sea. *Can. J. Zool.* **66**, 53-60.
Ferretti, G., Costa, M., Ferrigno, M., Grassi, B., Marconi, C., Lundgren, C. E. G. and Cerretelli, P. (1991). Alveolar gas composition and exchange during deep breath-hold diving and dry breath holds in elite divers. *J. Appl. Physiol.* **70**, 794-802.
Ferrigno, M. and Lundgren, C. E. (2003). Breath-hold diving. In *Physiology and Medicine of Diving* (ed. A. O. Brubakk and T. S. Neuman), pp. 153-180. Edinburgh: Saunders.
Folkow, L. P., Ramirez, J.-M., Ludvigsen, S., Ramirez, N. and Blix, A. S. (2008). Remarkable neuronal hypoxia tolerance in the deep-diving adult hooded seal (*Cystophora cristata*). *Neurosci. Lett.* **446**, 147-150.
Fujise, Y., Hidaka, H., Tatsukawa, R. and Miyazaki, N. (1985). External measurements and organ weights of five Weddell seals (*Leptonychotes weddelli*) caught near Syowa Station. *Antarct. Rec.* **85**, 96-101.
Fuson, A. L., Cowan, D. F., Kanatous, S. B., Polasek, L. K. and Davis, R. W. (2003). Adaptations to hypoxia in the heart, kidneys, and splanchnic organs of harbor seals (*Phoca vitulina*). *J. Exp. Biol.* **206**, 4139-4154.
Green, J. A., Halsey, L. G. and Butler, P. J. (2005). To what extent is the foraging behaviour of aquatic birds constrained by their physiology? *Physiol. Biochem. Zool.* **78**, 766-781.
Grocott, M. P. W., Martin, D. S., Levett, D. Z. H., McMorrow, R., Windsor, J. and Montgomery, H. E. (2009). Arterial blood gases and oxygen content in climbers on Mount Everest. *N. Engl. J. Med.* **360**, 140-149.
Gros, G., Wittenberg, B. A. and Jue, T. (2010). Myoglobin's old and new clothes: from molecular structure to function in living cells. *J. Exp. Biol.* **213**, 2713-2725.
Grubb, B. R. (1981). Blood flow and oxygen consumption in avian skeletal muscle during hypoxia. *J. Appl. Physiol.* **50**, 450-455.
Guppy, M., Hill, R. D., Liggins, G. C., Zapol, W. M. and Hochachka, P. W. (1986). Micro-computer assisted metabolic studies of voluntary diving of Weddell seals. *Am. J. Physiol.* **250**, 175-187.
Guyton, G. P., Stanek, K. S., Schneider, R. C., Hochachka, P. W., Hurford, W. E., Zapol, D. G. and Zapol, W. M. (1995). Myoglobin-saturation in free-diving Weddell seals. *J. Appl. Physiol.* **79**, 1148-1155.
Halasz, N. A., Elsner, R. and Garvie, R. S. (1974). Renal recovery from ischemia: a comparative study of harbor seal and dog kidneys. *Am. J. Physiol.* **227**, 1331-1335.
Hassrick, J. L., Crocker, D. E., Teutschel, N. M., McDonald, B. I., Robinson, P. W., Simmons, S. E. and Costa, D. P. (2010). Condition and mass impact oxygen stores and dive duration in adult female northern elephant seals. *J. Exp. Biol.* **213**, 585-592.
Hill, R. D., Schneider, R. C., Liggins, G. C., Schuette, A. H., Elliott, R. L., Guppy, M., Hochachka, P. W. and Zapol, W. M. (1987). Heart rate and body temperature during free diving of Weddell seals. *Am. J. Physiol.* **253**, R344-R351.
Hindle, A. G., Young, B. L., Rosen, D. A. S., Haulena, M. and Trites, A. W. (2010). Dive response differs between shallow- and deep-diving Steller sea lions. *J. Exp. Mar. Biol. Ecol.* **394**, 141-148.
Hochachka, P. W., Liggins, G. C., Qvist, J., Schneider, R. C., Snider, M. T., Wonders, T. R. and Zapol, W. M. (1977). Pulmonary metabolism for diving: conditioning blood for the brain. *Science* **198**, 831-834.
Howell, A. B. (1930). *Aquatic Mammals*. New York: Dover.
Hudson, D. M. and Jones, D. R. (1986). The influence of body mass on the endurance to restrained submersion in the Pekin duck. *J. Exp. Biol.* **120**, 351-367.
Hurford, W. E., Hochachka, P. W., Schneider, R. C., Guyton, G. P., Stanek, K. S., Zapol, D. G., Liggins, G. C. and Zapol, W. M. (1996). Splenic contraction, catecholamine release, and blood volume redistribution during diving in the Weddell seal. *J. Appl. Physiol.* **80**, 298-306.
Irving, L. (1934). On the ability of warm-blooded animals to survive without breathing. *Sci. Mon.* **38**, 422-428.
Irving, L. (1939). Respiration in diving mammals. *Physiol. Rev.* **19**, 112-134.
Irving, L., Scholander, P. F. and Grinnell, S. W. (1941). Significance of the heart rate to the diving ability of seals. *J. Cell. Comp. Physiol.* **18**, 283-297.
Jobsis, P. D., Ponganis, P. J. and Kooyman, G. L. (2001). Effects of training on forced submersion responses in harbor seals. *J. Exp. Biol.* **204**, 3877-3885.
Kanatous, S. B., DiMichele, L. V., Cowan, D. F. and Davis, R. W. (1999). High aerobic capacities in the skeletal muscles of pinnipeds: adaptations to diving hypoxia. *J. Appl. Physiol.* **86**, 1247-1256.
Kanatous, S. B., Davis, R. W., Watson, R., Polasek, L., Willliams, T. M. and Mathieu-Costello, O. (2002). Aerobic capacities in the skeletal muscles of Weddell seals: key to longer dive durations? *J. Exp. Biol.* **205**, 3601-3608.
Keijer, E. and Butler, P. J. (1982). Volumes of the respiratory and circulatory systems in tufted and mallard ducks. *J. Exp. Biol.* **101**, 213-220.
Kerem, D. and Elsner, R. (1973). Cerebral tolerance to asphyxial hypoxia in the harbor seal. *Respir. Physiol.* **19**, 188-200.
Kerem, D. H., Hammond, D. D. and Elsner, R. (1973). Tissue glycogen levels in the Weddell seals (*Leptonychotes weddelli*): a possible adaptation to asphyxial hypoxia. *Comp. Biochem. Physiol.* **45**, 731-736.
Kleiber, M. (1975). *The Fire of Life*. New York: Wiley and Sons.
Kooyman, G. L. (1968). An analysis of some behavioral and physiological characteristics related to diving in the Weddell seal. *Antarct. Res. Ser.* **11**, 227-261.
Kooyman, G. L. (1985). Physiology without restraint in diving mammals. *Mar. Mamm. Sci.* **1**, 166-178.
Kooyman, G. L. (1989). *Diverse Divers Physiology and Behavior*. Berlin: Springer-Verlag.
Kooyman, G. L. (2006). Mysteries of adaptation to hypoxia and pressure in marine mammals. The Kenneth S. Norris lifetime achievement award lecture. *Mari. Mamm. Sci.* **22**, 507-526.
Kooyman, G. L. and Kooyman, T. G. (1995). Diving behavior of emperor penguins nurturing chicks at Coulman Island, Antarctica. *Condor* **97**, 536-549.
Kooyman, G. L. and Ponganis, P. J. (1994). Emperor penguin oxygen consumption, heart rate and plasma lactate levels during graded swimming exercise. *J. Exp. Biol.* **195**, 199-209.
Kooyman, G. L. and Ponganis, P. J. (1998). The physiological basis of diving to depth: birds and mammals. *Annu. Rev. Physiol.* **60**, 19-32.
Kooyman, G. L. and Sinnett, E. E. (1982). Pulmonary shunts in harbor seals and sea lions during simulated dives to depth. *Physiol. Zool.* **55**, 105-111.
Kooyman, G. L., Drabek, C. M., Elsner, R. and Campbell, W. B. (1971). Diving behavior of the emperor penguin, *Aptenodytes forsteri. Auk* **88**, 775-795.
Kooyman, G. L., Kerem, D. H., Campbell, W. B. and Wright, J. J. (1973a). Pulmonary gas exchange in freely diving Weddell seals (*Leptonychotes weddelli*). *Respir. Physiol.* **17**, 283-290.
Kooyman, G. L., Schroeder, J. P., Denison, D. M., Hammond, D. D., Wright, J. J. and Bergman, W. D. (1973b). Blood N_2 tensions of seals during simulated deep dives. *Am. J. Physiol.* **223**, 1016-1020.
Kooyman, G. L., Schroeder, J. P., Greene, D. G. and Smith, V. A. (1973c). Gas exchange in penguins during simulated dives to 30 and 68 m. *Am. J. Physiol.* **225**, 1467-1471.
Kooyman, G. L., Wahrenbrock, E. A., Castellini, M. A., Davis, R. W. and Sinnett, E. E. (1980). Aerobic and anaerobic metabolism during diving in Weddell seals: evidence of preferred pathways from blood chemistry and behavior. *J. Comp. Physiol.* **138**, 335-346.
Kooyman, G. L., Castellini, M. A., Davis, R. W. and Maue, R. A. (1983). Aerobic dive limits in immature Weddell seals. *J. Comp. Physiol.* **151**, 171-174.
Kooyman, G. L., Ponganis, P. J., Castellini, M. A., Ponganis, E. P., Ponganis, K. V., Thorson, P. H., Eckert, S. A. and LeMaho, Y. (1992). Heart rates and swim speeds of emperor penguins diving under sea ice. *J. Exp. Biol.* **165**, 161-180.
Kooyman, G. L., Ponganis, P. J. and Howard, R. S. (1999). Diving animals. In *The Lung at Depth* (ed. C. Lundgren and J. Miller), pp. 587-620. New York: Marcel Dekker.
Kooyman, M. M. and Kooyman, G. L. (2009). Historical perspectives. *Aquat. Mamm.* **35**, 523-556.
Lasiewski, R. C. and Calder, W. A. (1971). A preliminary allometric analysis of respiratory variables in resting birds. *Respir. Physiol.* **11**, 152-166.

LeBoeuf, B. J., Costa, D. P., Huntley, A. C. and Feldkamp, S. D. (1988). Continuous, deep diving in female northern elephant seals, *Mirounga angustirostris*. *Can. J. Zool.* **66,** 446-458.
Lenfant, C., Kooyman, G. L., Elsner, R. and Drabek, C. M. (1969). Respiratory function of the blood of the Adelie penguins (*Pygoscelis adleiae*). *Am. J. Physiol.* **216**, 1598-1600.
Lenfant, C., Johansen, K. and Torrance, J. D. (1970). Gas transport and oxygen storage capacity in some pinnipeds and the sea otter. *Respir. Physiol.* **9**, 277-286.
Lindholm, P. and Lundgren, C. E. G. (2009). The physiology and pathophysiology of human breath-hold diving. *J. Appl. Physiol.* **106**, 284-292.
Liner, M. H. and Andersson, J. P. A. (2009). Hypoxic syncope in a competitive breath-hold diver with elevation of the brain damage marker S100B. *Aviat. Space Environ. Med.* **80**, 1066-1068.
Lockyer, C. (1976). Body weights of some species of large whales. *J. Cons. Int. Explor. Mer* **36**, 259-273.
Manohar, M., Goetz, T. E. and Hassan, A. S. (2001). Effect of prior high-intensity exercise on exercise-induced arterial hypoxemia in thoroughbred horses. *J. Appl. Physiol.* **90**, 2371-2377.
Mason, R. J., Murray, J. F., Broaddus, V. C. and Nadel, T. A. (2005). *Murray and Nadel's Textbook of Respiratory Medicine*. Philadelphia: Elsevier Saunders.
Meir, J. U. and Ponganis, P. J. (2009). High-affinity hemoglobin and blood oxygen saturation in diving emperor penguins. *J. Exp. Biol.* **212**, 3330-3338.
Meir, J. U. and Ponganis, P. J. (2010). Blood temperature profiles of diving elephant seals. *Physiol. Biochem. Zool.* **83**, 531-540.
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. *J. Exp. Biol.* **211**, 1169-1179.
Meir, J. U., Champagne, C. D., Costa, D. P., Williams, C. L. and Ponganis, P. J. (2009). Extreme hypoxemic tolerance and blood oxygen depletion in diving elephant seals. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **297**, R927-R939.
Miller, P. J., Johnson, M. P., Tyack, P. L. and Terray, E. A. (2004). Swimming gait, passive drag and buoyancy of diving sperm whales. *J. Exp. Biol.* **207**, 1953-1967.
Nakaoa, A., Netoa, J. S., Kannob, S., Stolzc, D. B., Kimizuka, K., Liud, F., Bache, F. H., Billarb, T. R., Choid, A. M., Otterbeind, L. E. et al. (2005). Protection against ischemia/reperfusion injury in cardicac and renal transplantation with carbon monoxide, bilverdin, and both. *Am. J. Transplant.* **5**, 282-291.
Nunn, J. F. (1977). *Applied Respiratory Physiology*. London: Butterworths.
Odden, A., Folkow, L. P., Caputa, M., Hotvedt, R. and Blix, A. S. (1999). Brain cooling in seals. *Acta Physiol. Scand.* **166**, 77-78.
Oliver, G. W., Morris, P. A., Thorson, P. H. and Le Boeuf, B. J. (1998). Homing behavior of juvenile northern elephant seals. *Mar. Mamm. Sci.* **14**, 245-256.
Persson, S. G. B., Ekman, L., Lyden, G. and Tufvesson, G. (1973). Circulation effects of splenectomy in the horse I-IV. II. Effect on plasma volume and total and circulating red cell volume. *Zentrabl. Veterinaermed.* **20**, 456-468.
Ponganis, P. J. (2011). Diving mammals. In *Comprehensive Physiology* (ed. R. Terjung), pp. 447-465. Hoboken, NJ: John Wiley & Sons, Inc.
Ponganis, P. J., Kooyman, G. L. and Castellini, M. A. (1993a). Determinants of the aerobic dive limit of Weddell seals: analysis of diving metabolic rates, post-dive end tidal P_{O_2}'s, and blood and muscle oxygen stores. *Physiol. Zool.* **66**, 732-749.
Ponganis, P. J., Kooyman, G. L., Castellini, M. A., Ponganis, E. P. and Ponganis, K. V. (1993b). Muscle temperature and swim velocity profiles during diving in a Weddell seal, *Leptonychotes weddellii*. *J. Exp. Biol.* **183**, 341-348.
Ponganis, P. J., Costello, M. L., Starke, L. N., Mathieu-Costello, O. and Kooyman, G. L. (1997a). Structural and biochemical characteristics of locomotory muscles of emperor penguins, *Aptenodytes forsteri*. *Respir. Physiol.* **109**, 73-80.
Ponganis, P. J., Kooyman, G. L., Starke, L. N., Kooyman, C. A. and Kooyman, T. G. (1997b). Post-dive blood lactate concentrations in emperor penguins, *Aptenodytes forsteri*. *J. Exp. Biol.* **200**, 1623-1626.
Ponganis, P. J., Kooyman, G. L., Winter, L. M. and Starke, L. N. (1997c). Heart rate and plasma lactate responses during submerged swimming and diving in California sea lions (*Zalophus californianus*). *J. Comp. Physiol. B* **167**, 9-16.
Ponganis, P. J., Kooyman, G. L., Van Dam, R. and Le Maho, Y. (1999). Physiological responses of king penguins during simulated diving to 136 m depth. *J. Exp. Biol.* **202**, 2819-2822.
Ponganis, P. J., Van Dam, R. P., Marshall, G., Knower, T. and Levenson, D. H. (2000). Sub-ice foraging behavior of emperor penguins. *J. Exp. Biol.* **203**, 3275-3278.
Ponganis, P. J., Van Dam, R. P., Knower, T. and Levenson, D. H. (2001). Temperature regulation in emperor penguins foraging under sea ice. *Comp. Biochem. Physiol.* **129A**, 811-820.
Ponganis, P. J., Kreutzer, U., Sailasuta, N., Knower, T., Hurd, R. and Jue, T. (2002). Detection of myoglobin desaturation in *Mirounga angustirostris* during apnea. *Am. J. Physiol.* **282**, R267-R272.
Ponganis, P. J., Kooyman, G. L. and Ridgway, S. H. (2003a). Comparative diving physiology. In *Bennett and Elliott's Physiology and Medicine of Diving* (ed. A. O. Brubakk and T. S. Neuman), pp. 211-226. Edinburgh: Saunders.
Ponganis, P. J., Van Dam, R. P., Levenson, D. H., Knower, T., Ponganis, K. V. and Marshall, G. (2003b). Regional heterothermy and conservation of core temperature in emperor penguins diving under sea ice. *Comp. Biochem. Physiol.* **135A**, 477-487.
Ponganis, P. J., van Dam, R. P., Knower, T., Levenson, D. H. and Ponganis, K. V. (2004). Deep dives and aortic temperatures of emperor penguins: new directions for bio-logging at the isolated dive hole. *Mem. Natl. Inst. Polar Res. Special Issue* **58**, 155-161.
Ponganis, P. J., Stockard, T., Levenson, D. H., Berg, L. and Barnov, E. A. (2006). Cardiac output and muscle blood flow during rest-associated apneas in elephant seals. *Comp. Biochem. Physiol.* **144A**, 105-111.
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. *J. Exp. Biol.* **210**, 4279-4285.
Ponganis, P. J., Kreutzer, U., Stockard, T. K., Lin, P. C., Sailasuta, N., Tran, T.-K., Hurd, R. and Jue, T. (2008). Blood flow and metabolic regulation in seal muscle during apnea. *J. Exp. Biol.* **211**, 3323-3332.
Ponganis, P. J., Stockard, T. K., Meir, J. U., Williams, C. L., Ponganis, K. V. and Howard, R. (2009). O_2 store management in diving emperor penguins. *J. Exp. Biol.* **212**, 217-224.
Ponganis, P. J., Meir, J. U. and Williams, C. L. (2010). Oxygen store depletion and the aerobic dive limit in emperor penguins. *Aquat. Biol.* **8**, 237-245.
Pugh, L. G. C. E. (1959). Carbon monoxide content of the blood and other observations on Weddell seals. *Nature* **183**, 74-76.
Qvist, J., Hill, R. D., Schneider, R. C., Falke, K. J., Liggins, G. C., Guppy, M., Elliott, R. L., Hochachka, P. W. and Zapol, W. M. (1986). Hemoglobin concentrations and blood gas tensions of free-diving Weddell seals. *J. Appl. Physiol.* **61**, 1560-1569.
Qvist, J., Hurford, W. E., Park, Y. S., Radermacher, P., Falke, K. J., Ahn, D. W., Guyton, G. P., Stanek, K. S., Hong, S. K., Weber, R. E. et al. (1993). Arterial blood gas tensions during breath-hold diving in the Korean ama. *J. Appl. Physiol.* **75**, 285-293.
Ramirez, J.-M., Folkow, L. P. and Blix, A. S. (2007). Hypoxia tolerance in mammals and birds: from the wilderness to the clinic. *Annu. Rev. Physiol.* **69**, 113-143.
Richardson, R. S., Noyszewski, E. A., Kendrick, K. F., Leigh, J. J. and Wagner, P. D. (1995). Myoglobin O_2 dissociation during exercise. Evidence of limited O_2 transport. *J. Clin. Invest.* **96**, 1916-1926.
Ridgway, S. H. (1986). Diving by cetaceans. In *Diving in Animals and Man* (ed. A. O. Brubakk, J. W. Kanwisher and G. Sundnes), pp. 33-62. Trondheim: Royal Norwegian Society of Science and Letters.
Ridgway, S. H. and Johnston, D. G. (1966). Blood oxygen and ecology of porpoises of three genera. *Science* **151**, 456-458.
Ridgway, S. H., Scronce, B. L. and Kanwisher, J. (1969). Respiration and deep diving in the bottlenose porpoise. *Science* **166**, 1651-1654.
Sato, K., Naito, Y., Kato, A., Niizuma, Y., Watanuki, Y., Charassin, J. B., Bost, C.-A., Handrich, Y. and Le Maho, Y. (2002). Buoyancy and maximal diving depth in penguins: do they control inhaling air volume? *J. Exp. Biol.* **205**, 1189-1197.
Sato, K., Mitani, Y., Camerson, M. F., Siniff, D. B. and Naito, Y. (2003). Factors affecting stroking patterns and body angle in diving Weddell seals under natural conditions. *J. Exp. Biol.* **206**, 1461-1470.
Sato, K., Shiomi, K., Marshall, G., Kooyman, G. L. and Ponganis, P. J. (2011). Stroke rates and diving air volumes of emperor penguins: implications for dive performance. *J. Exp. Biol.* **214**, 2854-2863.
Schmidt-Nielsen, K. (1997). *Animal Physiology: Adaptation and Environment*. Cambridge: Cambridge University Press.
Scholander, P. F. (1940). Experimental investigations on the respiratory function in diving mammals and birds. *Hvalradets Skrifter* **22**, 1-131.
Scholander, P. F., Irving, L. and Grinnell, S. W. (1942). Aerobic and anaerobic changes in seal muscle during diving. *J. Biol. Chem.* **142**, 431-440.
Shaffer, S. A., Costa, D. P., Williams, T. M. and Ridgway, S. H. (1997). Diving and swimming performance of white whales, *Delphinapterus leucas*: an assessment of plasma lactate and blood gas levels and respiratory rates. *J. Exp. Biol.* **200**, 3091-3099.
Shiomi, K., Sato, K., Mitamura, H., Arai, N., Naito, Y. and Ponganis, P. J. (2008). Effect of ocean current on the dead-reckoning estimation of 3D dive paths of emperor penguins. *Aquat. Biol.* **3**, 265-270.
Simpson, J. G., Gilmartin, W. G. and Ridgway, S. H. (1970). Blood volume and other hematologic values in young elephant seals (*Mirounga angustirostris*). *Am. J. Vet. Res.* **31**, 1449-1452.
Skrovan, R. C., Williams, T. M., Berry, P. S., Moore, P. W. and Davis, R. W. (1999). The diving physiology of bottlenose dolphins (*Tursiops truncatus*). II. Biomechanics and changes in buoyancy at depth. *J. Exp. Biol.* **202**, 2749-2761.
Sleet, R. B., Sumich, J. L. and Weber, L. J. (1981). Estimates of total blood volume and total body weight of sperm whale (*Physeter catodon*). *Can. J. Zool.* **59**, 567-570.
Snyder, G. K. (1983). Respiratory adaptations in diving mammals. *Respir. Physiol.* **54**, 269-294.
Stephenson, R. (1995). Respiratory and plumage gas volumes in unrestrained diving ducks (*Aythya affinis*). *Respir. Physiol.* **100**, 129-137.
Stephenson, R. and Jones, D. R. (1992). Metabolic responses to forced dives in Pekin duck measured by indirect calorimetry and ^{31}P-MRS. *Am. J. Physiol.* **263**, R1309-R1317.
Stockard, T. K., Heil, J., Meir, J. U., Sato, K., Ponganis, K. V. and Ponganis, P. J. (2005). Air sac P_{O_2} and oxygen depletion during dives of emperor penguins. *J. Exp. Biol.* **208**, 2973-2981.
Stockard, T. K., Levenson, D. H., Berg, L., Fransioli, J. R., Baranov, E. A. and Ponganis, P. J. (2007). Blood oxygen depletion during rest-associated apneas of northern elephant seals (*Mirounga angustirostris*). *J. Exp. Biol.* **210**, 2607-2617.
Thompson, D. and Fedak, M. A. (1993). Cardiac responses of grey seals during diving at sea. *J. Exp. Biol.* **174**, 139-164.
Thorson, P. H. and Le Boeuf, B. J. (1994). Developmental aspects of diving in northern elephant seal pups. In *Elephant Seals: Population Ecology, Behavior, and Physiology* (ed. B. J. Le Boeuf and R. M. Laws), pp. 271-289. Berkeley: University of California Press.
Tran, T.-K., Sailasuta, N., Kreutzer, U., Hurd, R., Chung, Y., Mole, P., Kuno, S. and Jue, T. (1999). Comparative analysis of NMR and NIRS measurements of intracellular P_{O_2} in human skeletal muscle. *Am. J. Physiol.* **276**, R1682-R1690.
van Dam, R. P., Ponganis, P. J., Ponganis, K. V., Levenson, D. H. and Marshall, G. (2002). Stroke frequencies of emperor penguins diving under sea ice. *J. Exp. Biol.* **205**, 3769-3774.
Vázquez-Medina, J. P., Zenteno-Savín, T. and Elsner, R. (2006). Antioxidant enzymes in ringed seal tissues: potential protection against dive-associated ischemia/reperfusion. *Comp. Biochem. Physiol.* **142C**, 198-204.

Vázquez-Medina, J. P., Zenteno-Savín, T. and Elsner, R. (2007). Glutathione protection against dive-associated ischemia/reperfusion in ringed seal tissues. *J. Exp. Mar. Biol. Ecol.* **345**, 110-118.
Weber, R. E., Hemmingsen, E. A. and Johansen, K. (1974). Functional and biochemical studies of penguin myoglobin. *Comp. Biochem. Physiol.* **49B**, 197-214.
Weise, M. J. and Costa, D. P. (2007). Total body oxygen stores and physiological diving capacity of California sea lions as a function of sex and age. *J. Exp. Biol.* **210**, 278-289.
West, J. B., Hackett, P. H., Maret, K. H., Milledge, J. S., Peters, R. M., Jr, Pizzo, C. J. and Winslow, R. M. (1983). Pulmonary gas exchange on the summit of Mt. Everest. *J. Appl. Physiol.* **55**, 678-687.
Wienecke, B., Robertson, G., Kirkwood, R. and Lawton, K. (2007). Extreme dives by free-ranging emperor penguins. *Polar Biol.* **30**, 133-142.
Williams, C. L., Meir, J. U. and Ponganis, P. J. (2011). What triggers the aerobic dive limit? Muscle oxygen depletion during dives of emperor penguins. *J. Exp. Biol.* **214**, 1801-1812.
Williams, T. M., Kooyman, G. L. and Croll, D. A. (1991). The effects of submergence on heart rate and oxygen consumption of swimming seals and sea lions. *J. Comp. Physiol. B* **160**, 637-644.
Williams, T. M., Haun, J. E. and Friedl, W. A. (1999). The diving physiology of bottlenose dolphins (*Tursiops truncatus*). I. Balancing the demands of exercise for energy conservation at depth. *J. Exp. Biol.* **202**, 2739-2748.
Williams, T. M., Davis, R. W., Fuiman, L. A., Francis, J., Le Boeuf, B. J., Horning, M., Calambokidis, J. and Croll, D. A. (2000). Sink or swim: strategies for cost-efficient diving by marine mammals. *Science* **288**, 133-136.
Williams, T. M., Fuiman, L. A., Horning, M. and Davis, R. W. (2004). The cost of foraging by a marine predator, the Weddell seal *Leptonychotes weddellii*: pricing by the stroke. *J. Exp. Biol.* **207**, 973-982.
Williams, T. M., Zavanelli, M., Miller, M. A., Goldbeck, R. A., Morledge, M., Casper, D., Pabst, D. A., McClellan, W., Cantin, L. P. and Kliger, D. S. (2008). Running, swimming, and diving modifies neuroprotecting globins in the mammalian brain. *Proc. R. Soc. B* **275**, 751-758.
Yumino, D. and Bradley, T. D. (2008). Central sleep apnea and Cheyne-Stokes respiration. *Proc. Am. Thorac. Soc.* **5**, 226-236.
Zapol, W. M., Liggins, G. C., Schneider, R. C., Qvist, J., Snider, M. T., Creasy, R. K. and Hochachka, P. W. (1979). Regional blood flow during simulated diving in the conscious Weddell seal. *J. Appl. Physiol.* **47**, 968-973.
Zenteno-Savin, T., St. Leger, J. and Ponganis, P. J. (2010). Hypoxemic and ischemic tolerance in emperor penguins. *Comp. Biochem. Physiol.* **152C**, 18-23.