

4 Oxygen storage and transport

Oxygen transport and increased O₂ storage within the body are fundamental components of the breath-hold capacities of marine mammals and birds. The body O₂ stores are located in the respiratory system, blood, and muscle. The magnitude and distribution of these O₂ stores vary among species, and are primarily dependent on diving lung volume, blood volume, hemoglobin (Hb) concentration, muscle mass, and myoglobin (Mb) concentration. As will be reviewed, Hb and Mb are the primary O₂-binding proteins in the body.

The precise functions of other O₂-binding proteins, such as neuroglobin and cytoglobin, have not been defined yet (Burmester and Hankeln, 2009, Burmester *et al.*, 2000, Weber and Fago, 2004). Potential roles include oxygen transport and storage, and scavenging of reactive oxygen species and reactive nitrogen species (Burmester and Hankeln, 2009, Reeder, 2010, Weber and Fago, 2004). The neuroglobin amino acid sequences of seals and whales differed by only two to three amino acids from those of terrestrial controls, suggesting that differences in neuroglobin function (i.e., O₂ affinity) between terrestrial and marine mammals were unlikely (Schneuer *et al.*, 2012). The potential role of neuroglobin in the hypoxemic tolerance of marine mammals is discussed in Chapter 11.

The functional size of an O₂ store is also affected by how much O₂ can be extracted from those stores, i.e., the initial and final O₂ concentrations during a dive. This is exemplified in a classic forced submersion study of pekin ducks (*Anas platyrhynchos*) taken to their maximum breath-hold capacity (Hudson and Jones, 1986). At that end point, although blood O₂ was nearly depleted, 25% of the original respiratory O₂ store still remained. As will be reviewed later in the chapter, that large percentage of respiratory O₂ was unusable because of the O₂-binding properties of duck hemoglobin. At that O₂ fraction in the lung, the resulting P_{O₂} was low enough that duck Hb was essentially devoid of O₂, resulting in "imminent cardiovascular collapse." Thus, all the O₂ could not be extracted from the lung. The final O₂ concentration and actual size of the respiratory O₂ store in the duck were dependent ultimately on the biochemical properties and O₂ affinity of Hb.

This chapter on O₂ storage and transport is divided into four sections. First, the structure and function of Hb and Mb in diving mammals and birds will be reviewed. Then, the classic assumptions used in the calculation of body O₂ stores will be examined. Third, the many measurements of the determinants of O₂ stores in different species will be compiled (i.e., diving air volume, blood volume, Hb concentration,

muscle mass, Mb concentration). In addition, because of the high hematocrits and Hb concentrations in the blood of many divers, blood rheology and coagulation will be reviewed in this section. Lastly, the magnitudes and distribution of O₂ stores in different species will be reviewed in relation to their known diving behaviors.

4.1 Hemoglobin structure and function

Hemoglobin (Hb) in diving birds and mammals is similar in structure to that of other mammals and birds in that it is composed of four polypeptide chains, each with an iron-containing heme group that can bind an O₂ molecule. When all four heme sites are bound with O₂, Hb is fully saturated. At 100% saturation, there are 1.34 ml O₂ per gram of Hb. The concentration of Hb in blood is the primary determinant of blood O₂ content because the solubility of O₂ in blood at body temperature is quite low (0.00124 mM mm Hg⁻¹ or 0.003 ml O₂ dl⁻¹ mm Hg⁻¹) (Powell, 2000, West, 1972).

There are two forms of Hb which cannot bind to O₂. Methemoglobin, which contains iron in the ferric state (Fe⁺⁺⁺) as opposed to the usual ferrous state (Fe⁺⁺), is unable to bind O₂, but is usually found in only minimal concentrations (Reeder, 2010). Carboxyhemoglobin is also incapable of binding O₂, but again is usually only present in very low concentrations in most animals. It is formed when carbon monoxide, which has 240 times greater affinity for Hb than O₂, is present in the blood (West, 1972).

4.2 O₂-hemoglobin dissociation curves

The O₂-Hb dissociation curve (Fig. 4.1) describes the reversible binding of O₂ to Hb as a function of Hb saturation and the partial pressure of O₂ (P_{O₂}). Its sigmoidal shape is due to allosteric interactions and cooperativity between the four polypeptide subunits of the Hb molecule (Powell, 2000, West, 1972). P₅₀, the P_{O₂} at which Hb is 50% saturated, is used as an index of Hb's affinity for O₂, and its reference value is usually taken at normal body temperature and blood pH (pH 7.4 in mammals and 7.5 in birds). The O₂-Hb dissociation curve (and the P₅₀) can be shifted to the left or right by changes in three primary factors that regulate the O₂ affinity of Hb.

The three important factors that affect Hb's affinity for O₂ are pH, temperature, and the concentration of organic phosphates inside the red blood cell (Powell, 2000, West, 1972). Acidosis (decreased pH) and elevated temperatures both decrease the affinity of Hb, shifting the curve to the right and increasing the P₅₀. The effect of pH on Hb affinity for O₂ is known as the Bohr effect. Both acidosis and elevated temperature occur in exercising muscle and should facilitate unloading of O₂ from Hb. Alkalosis (increased pH) and lower temperature both increase O₂ affinity, shifting the curve to the left and decreasing the P₅₀. These factors in the lung (an increased pH due to low CO₂ and a normal as opposed to higher temperature in muscle) are considered to facilitate the loading of Hb with O₂. In mammals, 2,3-diphosphoglycerate (2,3-DPG) binds to Hb

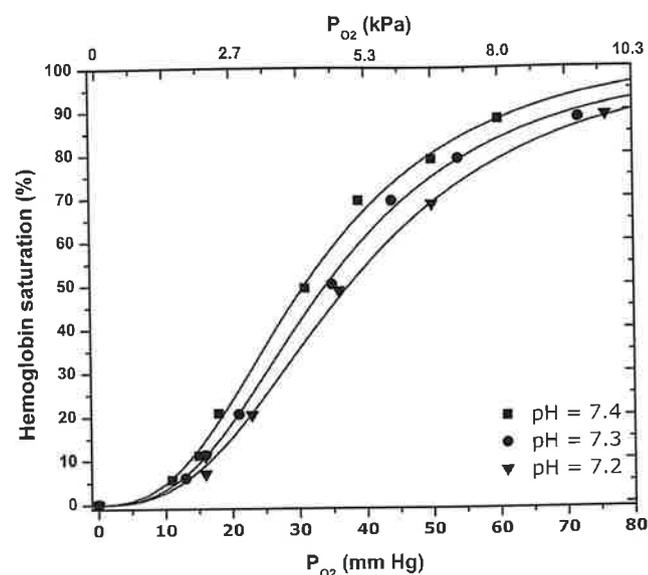


Figure 4.1 O_2 -hemoglobin dissociation curves of the northern elephant seal, *Mirounga angustirostris*, at pHs, 7.4, 7.3, and 7.2 shifted to the right with acidosis, demonstrating an increase in the P_{50} value and a decrease in O_2 affinity. The mean P_{50} of elephant seal hemoglobin at pH 7.4 was 30.5 mm Hg (4.06 kPa). Adapted from Meir et al. 2009.

and also affects its O_2 affinity. In high-altitude-adapted animals, for instance, 2,3-DPG is elevated and decreases O_2 affinity, shifting the curve to the right, increasing the P_{50} and improving O_2 unloading to the tissues (West, 1972). In bird red blood cells, myoinositol 1,3,4,5,6-pentophosphate (IPP) binds to Hb and can decrease its affinity for O_2 , shifting its curve to the right and increasing the P_{50} (Powell, 2000). Although high-altitude-adapted birds such as the bar-headed goose (*Anser indicus*) have Hbs with greater O_2 affinity (shift to the left, lower P_{50}) than low-altitude birds, IPP concentrations are similar (Petschow et al., 1977). Instead, changes in the amino acid sequence of the Hb polypeptide chains alter the binding site and affinity for IPP (Powell, 2000, Weber, 2007, Weber and Fago, 2004).

In light of reports of elevated carbon monoxide and carboxyhemoglobin in deep-diving seals (Pugh, 1959, Tift et al., 2014), it should also be noted that although carboxyHb cannot transport O_2 , these compounds can increase the O_2 affinity of the remaining Hb, and shift the dissociation curve to the left (Hlastala et al., 1976, Roughton and Darling, 1944). Roughton and Darling suggested that the left shift could be especially beneficial in increasing O_2 at low P_{O_2} values and that this could account for the 1898 observation by J. S. Haldane and L. Smith of improved hypoxic tolerance of mice on exposure to low carbon monoxide concentrations. Low concentrations of carbon monoxide have also been found to enhance oxygen delivery in more recent experimental models of brain blood flow (Koehler et al., 1984). In addition, low concentrations of carbon monoxide may also contribute to prevention of reperfusion injury in divers, as well as at least partially contribute to elevated tissue mitochondrial

volume densities in pinnipeds through effects of carbon monoxide on mitochondrial biogenesis (Lancel et al., 2009, Piantadosi, 2008, Piantadosi et al., 2008, Rhodes et al., 2009). See Chapters 7, 9, 11 and 13 for further review.

4.2.1 O_2 -hemoglobin dissociation curves: marine mammals

With this as background, it is now possible to review Hb function in diving mammals and birds. The concentration of Hb in blood is its most notable aspect in diving mammals. Many marine mammals have been found to have exceptionally high Hb concentrations in comparison to terrestrial mammals. However, the O_2 affinity of Hb in these animals was not that different from many of their terrestrial counterparts (Lenfant, 1969, Lenfant et al., 1970, Meir et al., 2009, Qvist et al., 1981). The P_{50} values were in the range of 26–30 mm Hg. The one exception was the manatee (*Trichechus manatus*) with a P_{50} of 16 mm Hg (Farmer et al., 1979, White et al., 1976). The magnitude of the Bohr effect ($\Delta \log P_{50} / \Delta \log pH$) in marine mammals was also similar to that found in terrestrial mammals (Lenfant, 1969, Lenfant et al., 1970, Meir et al., 2009, Qvist et al., 1981). Hill's coefficient (n), an index of cooperativity between the Hb subunits, was again within the normal range (Lenfant et al., 1970).

Investigations of fetal blood in Weddell seals (*Leptonychotes weddellii*) revealed that fetal blood had greater O_2 affinity and a lower Bohr effect than maternal blood, which is consistent with its lower concentration of 2,3-DPG (Qvist et al., 1981). The difference in P_{50} between maternal and fetal blood was 5–6 mm Hg (Lenfant et al., 1969a, Qvist et al., 1981). This difference in P_{50} was attributed to the lower 2,3-DPG concentrations in the fetus. Although amino acid sequencing was not performed, there did not appear to be any significant differences in the molecular structures or binding properties of isolated Hb between the fetus and the adult seal. Thus, there was no evidence in seals for a distinct fetal versus adult Hb.

4.2.2 O_2 -hemoglobin dissociation curves: seabirds

Examination of Hb in diving birds has revealed that their Hb concentrations are in the upper range of avian values (see Table 4.3). However, the most remarkable finding is the increased O_2 affinity of penguin Hbs in comparison to that of flighted birds (Fig. 4.2). This shift in O_2 affinity is probably due to specific amino acid substitutions in the polypeptide chains of Hb (Tamburrini et al., 1994, 1999). The P_{50} values of Adélie, gentoo, chinstrap, and little blue penguins (*Pygoscelis adeliae*, *P. papua*, *P. Antarctica*, *Eudyptula minor*) are 30–35 mm Hg, and that of the emperor penguin (*Aptenodytes forsteri*) is 28 mm Hg (Lenfant et al., 1969b, Meir and Ponganis, 2009, Milsom et al., 1973). In comparison, the P_{50} values of most flighted birds, including ducks and murre, are in the range of 40–55 mm Hg (Black and Tenney, 1980, Johansen et al., 1987, Lenfant et al., 1969b, Milsom et al., 1973, Petschow et al., 1977). In addition to penguins, a left-shifted dissociation curve, lower P_{50} , and higher affinity of Hb for O_2 occur in high-altitude birds (Black and Tenney, 1980, Petschow et al., 1977).

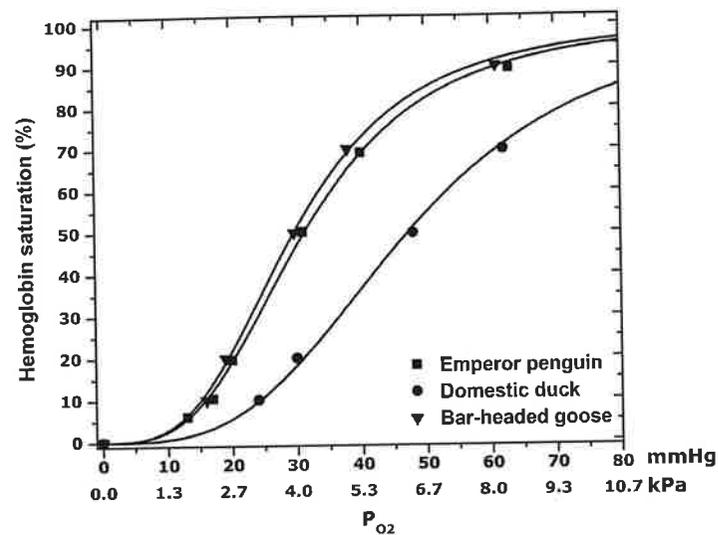


Figure 4.2 O_2 -hemoglobin dissociation curves of the emperor penguin (*Aptenodytes forsteri*) and bar-headed goose (*Anser indicus*) demonstrated a marked shift to the left, decreased P_{50} , and increased oxygen affinity in comparison to that of the domestic duck (*Anas platyrhynchos*). There was about a 14 mm Hg (1.87 kPa) difference between the P_{50} of the duck and those of the penguin and high-altitude goose. Adapted from Meir and Ponganis, 2009, and Black and Tenney, 1980.

As regard other aspects of Hb function, Hill coefficients and Bohr effects in penguins are similar to those of other birds (Lenfant *et al.*, 1969b, Meir and Ponganis, 2009).

The higher O_2 affinity of penguin Hb has several important implications in relation to the depletion of O_2 stores. In contrast to the pekin duck at the brink of "imminent cardiovascular collapse," but with 25% of its respiratory O_2 store unused, the emperor penguin can fully utilize its respiratory O_2 store (Hudson and Jones, 1986, Stockard *et al.*, 2005). One adaptation which facilitates depletion of air-sac O_2 in penguins is the higher O_2 affinity of Hb (Meir and Ponganis, 2009, Milsom *et al.*, 1973). At a P_{O_2} of 20 mm Hg, for example, the Hb of the pekin duck is devoid of O_2 , whereas that of the emperor penguin is still 27% saturated. Thus, a higher affinity Hb allows the emperor penguin to more fully deplete its O_2 store as well as to have a higher blood O_2 content at low P_{O_2} .

It should also be noted that it is probably necessary to take into account the P_{50} of Hb during calculation of the respiratory O_2 store of a given avian species. In penguins, the entire respiratory store is available, whereas in flighted birds only 75% is available (Croll *et al.*, 1992a, Stephenson *et al.*, 1989b). However, P_{50} values for most flighted, diving birds are unavailable.

4.3 Myoglobin structure and function

Myoglobin consists of a single polypeptide chain and heme group. Consequently, one Mb molecule binds one molecule of oxygen (Antonini, 1965, Wittenberg and Wittenberg, 1989). Because the molecular weight of Mb (17,000–18,000 daltons) is approximately

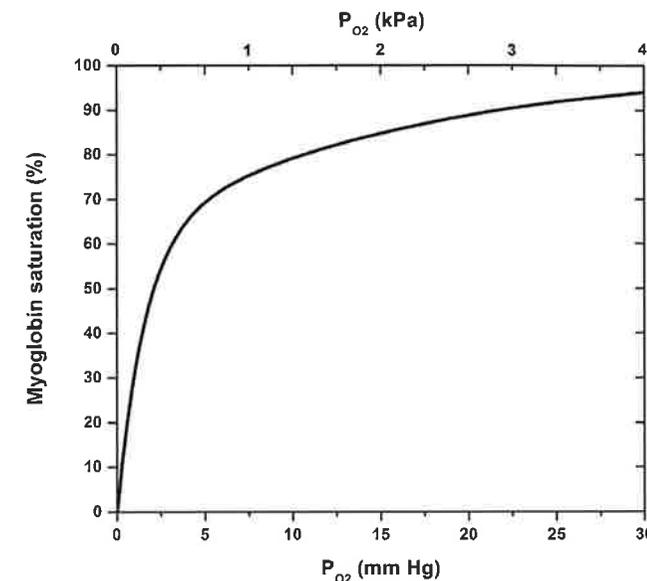


Figure 4.3 O_2 -myoglobin dissociation curve of the horse (*Equus ferus caballus*) demonstrated a sigmoidal shape with P_{50} near 2.4 mm Hg (0.32 kPa). The P_{50} of myoglobin was about ten times less than that of hemoglobin, demonstrating the much greater affinity of myoglobin for O_2 . Adapted from Schenkman *et al.*, 1997.

one-fourth that of Hb, the volume of O_2 bound per gram of Mb at 100% saturation is usually considered by most authors to be the same as Hb, i.e., 1.34 ml O_2 g Mb⁻¹. In actuality, the value for either Hb or Mb may vary dependent on the exact molecular weight of Hb or Mb in a given species. As an example, values of 1.20–1.24 ml O_2 g Mb⁻¹ have been used in some estimations of O_2 stores in birds (Croll *et al.*, 1992a, Stephenson *et al.*, 1989b).

The P_{50} of Mb at 37 to 40 °C is <4 mm Hg (0.53 kPa) in both terrestrial and most diving mammals and penguins (Antonini, 1965, Helbo and Fago, 2012, Nichols and Weber, 1989, Schenkman *et al.*, 1997, Suzuki and Imai, 1998, Weber *et al.*, 1974, Wright and Davis, 2015). In melon-headed and minke whales (*Peponocephala electra*, *Balaenoptera acutorostrata*), the P_{50} values are slightly higher at 4.9 and 4.6 mm Hg, respectively (Helbo and Fago, 2012, Wright and Davis, 2015). In general, the affinity of Mb for O_2 is at least ten-fold greater than that of Hb in most species. Once bound to Mb, O_2 in muscle is lost from the blood O_2 store and no longer available for other tissues during a dive.

The shape of the O_2 -Mb dissociation curve is hyperbolic (Fig. 4.3) (Antonini, 1965, Schenkman *et al.*, 1997). Mb's affinity for O_2 is increased by lower temperatures (its P_{50} decreases). Although it was originally reported that the P_{50} of Mb did not shift with changes in pH, a small shift to the right (decreased affinity for O_2) has been detected with acidosis. The P_{50} of horse Mb at 37 °C changes from 2.39 mm Hg at pH 7.0 to

2.46 mm Hg at pH 6.5 (Schenkman *et al.*, 1997). However, unlike Hb, the affinity of Mb is not affected by changes in any known co-factors (Antonini, 1965).

In addition to its role as an O₂ store, Mb has potential roles in (a) facilitation of diffusion of O₂ to mitochondria in muscle; (b) nitric oxide production via nitrite reductase activity; and (c) the scavenging of reactive O₂ species. Readers are referred to reviews and Chapter 13 for further discussion (Flogel *et al.*, 2010, Garry *et al.*, 2003, Gros *et al.*, 2010, Helbo and Fago, 2012, Helbo *et al.*, 2013, Lin *et al.*, 2007, Ordway and Garry, 2004, Weber, 2007, Weber and Fago, 2004, Wittenberg, 1970, Wittenberg and Wittenberg, 1989, 2003).

4.4 Calculation of O₂ stores

The calculation of O₂ stores is based on a variety of anatomical and physiological parameters, many of which have not been measured in a given species. Consequently, assumptions are common, and revisions are necessary as new data become available. The earliest estimations of O₂ storage in seals and penguins were probably those of Irving and Scholander in the 1930s (Irving, 1934, 1939, Irving *et al.*, 1935b, Scholander, 1940). Primary assumptions and potential sources of error include: (1) the diving air volume (air volume in the respiratory system at the start of a dive); (2) blood volume and Hb concentration in animals with large blood volumes; (3) muscle mass; (4) the frequent assumption that Mb concentration is the same in all muscles; and (5) the net extraction of O₂ from either the respiratory system or blood during a dive. These limitations will be demonstrated in the paragraphs below as the calculation of O₂ stores is reviewed.

4.4.1 Respiratory O₂ stores

The respiratory O₂ store is determined by the diving air volume and the net extraction of O₂ from that air volume during a dive. Chapter 3 reviews diving air volumes and provides a list of measured values in marine mammals (see Table 3.1). In general, cetaceans are considered to dive at full lung capacity, and pinnipeds at 50% total lung volume (Gentry and Kooyman, 1986, Kooyman, 1989, Ponganis, 2011). However, more recent evidence suggests that sea lions inspire deeper and have larger diving air volumes for deeper dives (McDonald and Ponganis, 2012). Manatees are considered to dive on inspiration with full lung volumes, and sea otters with 60% total lung capacity (Ponganis *et al.*, 2003a).

Most ducks have been considered to dive on exhalation, and diving air volumes used in O₂ store calculations have been end-expiratory values (Keijer and Butler, 1982, Stephenson, 1995, Stephenson *et al.*, 1989b). As reviewed in Chapter 3, penguins dive on inspiration and, in free dives, diving air volume (calculated at the end of a dive) appears to increase with depth (Sato *et al.*, 2002, 2011, Wilson *et al.*, 2003). The diving air volume at the start of a penguin's dive is still unknown. In other diving birds, it is usually assumed that the allometrically predicted air-sac/lung volume represents the diving air volume (Croll *et al.*, 1992a).

The net extraction of oxygen from the diving air volume is based on the difference between the initial O₂ fraction and the lowest possible end-of-dive value. In diving mammals, this difference has been assumed to be 15% (Kooyman, 1989). This value is reasonable, especially in seals which dive on exhalation and probably have start-of-dive O₂ fractions below 20%. In addition, this assumption is consistent with expiratory and end-tidal O₂ fractions that were in the range of 2–4% after dives and forced submersions (Kooyman *et al.*, 1973a, Ponganis *et al.*, 1993a, Ridgway *et al.*, 1969, Scholander, 1940).

In ducks, respiratory O₂ fractions have been determined in the air sacs. Given that the O₂ fraction is known to vary between anterior and posterior air sacs, O₂ fractions in representative air sacs have been determined, and then averaged, based on the relative volumes of the anterior and posterior air sacs. Such calculations in ducks have provided a mean O₂ fraction of 17.6% prior to the start of diving (Keijer and Butler, 1982, Stephenson *et al.*, 1989b). In forcibly submerged ducks at maximum breath-hold duration, the mean end-of-submersion O₂ fraction was 4.1% (Hudson and Jones, 1986). Thus, the maximum decrease in the O₂ fraction is 13.5%, which represents about 75% of the available O₂ in the respiratory system. No data exist for diving seabirds, so this 13.5% change in the O₂ fraction has been used in the calculation of their respiratory O₂ stores (Croll *et al.*, 1992a).

In penguins, a change of 15% in air-sac O₂ fraction has been used in O₂ store calculations, based upon final air-sac O₂ fractions of 2–4% during simulated dives in a pressure chamber (Kooyman, 1989, Kooyman and Ponganis, 1990, Kooyman *et al.*, 1973b). Air-sac O₂ fractions have now also been determined in diving emperor penguins, and the maximum change between initial and end-of-dive O₂ fractions was 19%, with end-of-dive O₂ fractions near 0 after some dives (Ponganis *et al.*, 2010a, Stockard *et al.*, 2005). The near-complete depletion of respiratory O₂ in emperor penguins is at least partially attributable to the previously discussed increased O₂ affinity of penguin versus duck Hb. Although other penguin species also have Hbs with increased O₂ affinity, it is not known whether they tolerate as low a P_{O₂} and whether they are capable of such complete O₂ extraction from the respiratory store.

In summary, the respiratory O₂ store can be calculated as 13.5%, 15%, and 19% of the diving air volume in flighted diving birds, marine mammals, and penguins, respectively. Apart from emperor and possibly king penguins, a 15% value may also be more appropriate for the other penguin species as the limits of their hypoxemic tolerance are not known. Most estimates of respiratory O₂ stores in marine mammals and all diving birds prior to 2010 utilized either the 13.5% or 15% values. Due to the rarity of its measurement, diving air volume is probably the greater source of potential error in this calculation. For many species, this air volume still remains a dilemma just as it was for the Nobel Prize-winning physiologist August Krogh in his 1934 estimation of the lung O₂ store of the blue whale (Krogh, 1934).

4.4.2 Blood O₂ stores

Blood O₂ stores have been calculated on the basis of blood volume, Hb concentration, the net desaturation of Hb during a dive, and the assumption that one-third of the blood

volume is arterial and two-thirds venous (Kooyman, 1989, Lenfant *et al.*, 1970). Although such a calculation appears straightforward, there is potential for error in the measurement of the individual parameters

4.4.2.1 Blood O₂ stores: blood volume and Hb measurement techniques

The determination of blood volume is classically conducted by measurement of (1) red cell volume (usually by dilution of radio-labeled red cells, i.e., chromium 51); and (2) plasma volume (usually by dilution of a marker or dye such as radio-labeled albumin or Evan's blue dye). Due to the difficulty of using radio-labeled compounds in remote field locations, the majority of blood volume determinations in diving mammals and birds have been conducted with use of a formula, in which blood volume is calculated by dividing the plasma volume by the term (1-hematocrit). Plasma volume is usually measured by the Evan's blue dye technique (El-Sayed *et al.*, 1995), and the hematocrit (the packed cell volume) is determined by centrifugation.

In animals with large blood volumes and large spleens, the accurate determination of the red cell volume is potentially difficult due to prolonged mixing times for dilution of labeled red blood cells (Persson *et al.*, 1973) and to fluctuations in hematocrit secondary to the level of splenic contraction (Cross *et al.*, 1988, Turner and Hodgetts, 1959). In addition, splenic dilatation and sequestration of red blood cells during anesthesia of animals with large spleens will also contribute to prolonged mixing times, as well as to lower hematocrits. In the Weddell seal (*Leptonychotes weddellii*), for example, the difference between the hematocrit under anesthesia and during diving led to a 50% reduction in the estimated blood volume (Ponganis *et al.*, 1993a). Lastly, use of the dye dilution-hematocrit technique is dependent on the assumption that the measured hematocrit is representative of a uniform hematocrit throughout the entire circulation.

Blood volume is usually assumed to be one-third arterial and two-thirds venous in blood O₂ store calculations, (Lenfant *et al.*, 1970). This assumption is reasonable and probably not a major source of error in blood O₂ store calculations. There are few data available on the distribution of blood volume between the arterial and venous systems. Estimates for the venous fraction of blood volume in mammals have been as high as 70–80% (Alexander, 1963, Wiedeman, 1963). In animals such as seals, with large spleens and/or large central venous capacitance, the fractions of blood volume considered arterial and venous may vary dependent on the physiological state of the animal.

Hemoglobin is typically determined with commercially available spectrophotometric techniques, with the most common procedure measuring a cyanomethemoglobin derivative. Again, for purposes of O₂ store calculations, the highest circulating Hb concentration should be used, but this may be difficult to obtain as it is rarely possible to obtain blood samples during or immediately after dives (when the spleen is presumably fully contracted). Many researchers will try to obtain samples immediately after capture because presumed activation of the sympathetic nervous system should constrict the spleen and elevate both hematocrit and Hb concentration during the capture.

4.4.2.2 Blood O₂ stores: initial and final Hb saturation assumptions

In oxygen store calculations, it is typically assumed that arterial blood is 95% saturated initially, and that it can decline to about 20% saturation. The latter figure is based on the hypoxemic tolerance studies of Elsner and co-workers (Elsner *et al.*, 1970b, Kerem and Elsner, 1973). The 95% value is reasonable, given that there may be some degree of pulmonary shunting or ventilation-perfusion mismatch (Kooyman and Sinnett, 1982). In addition, at least in seals, some carboxy-Hb may be present due to a six-fold elevation in carbon monoxide content in blood of seals as compared to humans (Pugh, 1959). In adult elephant seals (*Mirounga angustirostris*), carboxy-Hb can be as high as 10% (Tift *et al.*, 2014). This elevation in carbon monoxide has been attributed to the high concentrations of hemoglobin in seals and the carbon monoxide generated during the metabolic breakdown of hemoglobin to bilirubin. Although this appears counterproductive in terms of oxygen storage, it may be that low levels of carbon monoxide or carboxy-Hb contribute to regulation of vascular tone and prevention of re-perfusion injury (Herrera *et al.*, 2008, Kajimura *et al.*, 2010, Motterlini and Otterbein, 2010, Nakaoa *et al.*, 2005).

The initial venous Hb saturation level during a dive is typically considered to be the Hb saturation equivalent to 5 ml O₂ dl⁻¹ less than the initial arterial O₂ content. It has been assumed that venous blood oxygen can be completely depleted based on forced submersion findings in both ducks and seals (Hudson and Jones, 1986, Kerem and Elsner, 1973). It should be noted that the initial venous Hb saturation (at 5 ml O₂ dl⁻¹ less than the initial arterial O₂ content) is dependent on the Hb concentration. At 15 g dl⁻¹ Hb, the initial venous Hb saturation would be 74%, while at an Hb concentration of 26 g dl⁻¹, the corresponding value would be 85%. Thus, for a typical arterio-venous O₂ content difference of 5 ml O₂ dl⁻¹, the expected venous Hb saturation as well as P_{O₂} will vary dependent on the Hb content of the blood. This is important because, as will be seen in Chapter 5, venous Hb saturations and P_{O₂} can be elevated above the expected resting values. This can occur both prior to and during dives of emperor penguins and sea lions, and during dives of elephant seals (McDonald and Ponganis, 2013, Ponganis *et al.*, 2011). Such an increase in venous oxygen content is called arterialization of venous blood. If this occurs prior to diving, as in emperor penguins, the venous blood O₂ store is increased by 5 ml O₂ dl⁻¹ (Ponganis *et al.*, 2010a).

4.4.2.3 Blood O₂ stores: blood volumes, Hb concentrations

Hemoglobin concentrations and blood volumes of marine mammals may be increased as much as 50–70% above the typical human Hb concentration and blood volume of 15 g dl⁻¹ and 70 ml kg⁻¹, respectively (Tables 4.1, 4.2). The greatest increases in both Hb and blood volume are in the longest-duration divers and in highly active species (Ponganis, 2011, Ridgway and Johnston, 1966). This is exemplified in the phocid seals, in which blood volumes may be 2–3 times that of the standard human value. In contrast, Hb concentrations and blood volumes of avian divers are only at the upper range of bird values (Table 4.3). The highest reported Hb concentrations and blood volumes of avian divers are not in the penguins, but in small, flighted diving birds such as Cassin's auklet (*Ptychoramphus aleuticus*) and the ancient murrelet (*Synthliboramphus antiquus*)

Table 4.1 Hemoglobin (Hb) concentrations and blood volumes (BV) in cetaceans and manatees.

Species	Hb (g dl ⁻¹)	BV (ml kg ⁻¹)	Reference
Bottlenose dolphin <i>Tursiops truncatus</i>	14	71	A
Pacific white-sided dolphin <i>Lagenorhynchus obliquidens</i>	17	108	A
Dall porpoise <i>Phocoenoides dalli</i>	20	143	A
Commerson's dolphin <i>Cephalorhynchus commersoni</i>	18		B
Harbor porpoise <i>Phocoena phocoena</i>	19		C
Beluga whale <i>Delphinapterus leucas</i>	21	128	D
Sperm whale <i>Physeter macrocephalus</i>	22	200	E, F
Killer whale <i>Orcinus orca</i>	18	90	G, H, I, J
Pilot whale <i>Globicephala scammoni</i>	16		G, K
Gray whale <i>Eschrichtius robustus</i>	13	81	G
Bowhead whale <i>Balaena mysticetus</i>	20		L
Sei whale <i>Balaenoptera borealis</i>	16		M
Manatee <i>Trichechus manatus</i>	15	80	N

References: A: Ridgway and Johnston, 1966; B: Hedrick and Duffield, 1991; C: Reed *et al.*, 2000; D: Ridgway *et al.*, 1984; E: Ridgway, 1986; F: Sleet *et al.*, 1981; G: Gilmartin *et al.*, 1974; H: Dhindsa *et al.*, 1974; I: Lenfant *et al.*, 1968; J: Ridgway, 1972; K: Medway and Moldovan, 1966; L: Castellini *et al.*, 2006; M: Lenfant, 1969; N: Blessing, 1972b, Gallivan and Best, 1980, White *et al.*, 1976.

(Elliott *et al.*, 2010). The latter values are similar to those of the kittiwake (*Rissa tridactyla*), a non-diver (Table 4.3).

As discussed above, increased blood O₂ storage is partly achieved through elevated Hb concentrations. High blood Hb concentrations can be achieved through increased Hb content within the red blood cell as well as by an increased hematocrit (Hct, packed red blood cell volume). Both of these factors can affect blood flow properties (rheology) and the efficient transport of O₂. In this last section on the blood O₂ store, consideration will be given primarily to blood flow, blood viscosity, their effects on arterial impedance (cardiac workload), and the potential for thrombosis (clot formation). Readers are referred to an excellent review of blood rheology in marine mammals for further details (Castellini *et al.*, 2010).

Table 4.2 Hemoglobin (Hb) concentrations and blood volumes (BV) in pinnipeds and sea otters.

Species	Hb (g dl ⁻¹)	BV (ml kg ⁻¹)	Reference
California sea lion <i>Zalophus californianus</i>	18	120	A
Steller sea lion <i>Eumetopias jubata</i>	17	120	B, C
Northern fur seal <i>Callorhinus ursinus</i>	17	109	B
Antarctic fur seal <i>Arctocephalus gazella</i>	18	132	D
Australian sea lion <i>Neophoca cinerea</i>	19	178	D, E
New Zealand sea lion <i>Phocarctos hookeri</i>		150	F
Galapagos sea lion <i>Zalophus wollebaeki</i>	23	186	G
Walrus <i>Odobenus rosmarus</i>	16	106	B
Harbor seal <i>Phoca vitulina</i>	21	132	B, H
Leopard seal <i>Hydrurga leptonyx</i>	15	131	I
Gray seal <i>Halichoerus grypus</i>	20	213	J
Ribbon seal <i>Histiophoca fasciata</i>	24	132	B
Harp seal <i>Phoca groenlandica</i>	23	168	K
Hooded seal <i>Cystophora cristata</i>	23	106	K
Ringed seal <i>Phoca hispida</i>	25	158	L
Baikal seal <i>Phoca sibirica</i>	27	177	M
Weddell seal <i>Leptonychotes weddellii</i>	26	210	N
Northern elephant seal <i>Mirounga angustirostris</i>	25	216	O
Sea otter <i>Ehnydra lutris</i>	19	174	P

References: A: Weise and Costa, 2007; B: Lenfant *et al.*, 1970; C: Mellish *et al.*, 2007, Richmond *et al.*, 2006; D: Costa *et al.*, 2001; E: Fowler *et al.*, 2007; F: Costa *et al.*, 1998; G: Villegas-Amtmann and Costa, 2010; H: Burns *et al.*, 2005; I: Kuhn *et al.*, 2006; J: Noren *et al.*, 2005; K: Burns *et al.*, 2007; L: Lydersen *et al.*, 1992, St. Aubin *et al.*, 1978; M: Petrov and Shoshenko, 1987, Ponganis *et al.*, 1997b; N: Ponganis *et al.*, 1993a; O: Simpson *et al.*, 1970, Thorson and Le Boeuf, 1994; P: Thometz, 2014, Thometz *et al.* 2015.

Table 4.3 Hemoglobin (Hb) concentration and blood volume (BV) in aquatic and marine birds.

Species	Hb (g dl ⁻¹)	BV (ml kg ⁻¹)	Reference
Tufted duck <i>Aythya fuligula</i>	15–18	107–141	A
Mallard duck (pekin duck) <i>Anas platyrhynchos</i>	17	91–113	A, B
Black-legged kittiwake <i>Rissa tridactyla</i>	16	123	C
Coot <i>Fulica americana</i>	17	95	B
Red-throated loon <i>Gavia stellata</i>	21	132	B
White pelican <i>Pelecanus onocrotalus</i>	14		D
Rhinoceros auklet <i>Cerorhinca monocerata</i>	17	127	E
Cassin's auklet <i>Ptychoramphus aleuticus</i>	21		C
Ancient murrelet <i>Synthliboramphus antiquus</i>	20		C
Thick-billed murre <i>Uria lomvia</i>	18	123	F
South Georgia diving petrel <i>Pelecanoides georgicus</i>	20		G
Cormorant <i>Leucocarbo fuscescens</i>	15		H
Japanese cormorant <i>Phalacrocorax capillatus</i>		139	E
Little penguin <i>Eudyptula minor</i>	18		I
Rockhopper penguin <i>Eudyptes crestatus</i>	16		K
Magellanic penguin <i>Spheniscus magellanicus</i>	14		K
Humboldt penguin <i>Spheniscus humboldti</i>	15		L
Adélie penguin <i>Pygoscelis adeliae</i>	16	93	M
Gentoo penguin <i>Pygoscelis papua</i>	16		M
Chinstrap penguin <i>Pygoscelis antarctica</i>	20		M
King penguin <i>Aptenodytes patagonicus</i>	18	83	N

Table 4.3 (cont.)

Species	Hb (g dl ⁻¹)	BV (ml kg ⁻¹)	Reference
Emperor penguin <i>Aptenodytes forsteri</i>	18	100	O

References: A: Keijer and Butler, 1982; B: Bond and Gilbert, 1958; C: Elliott *et al.*, 2010; D: Puerta *et al.*, 1991; E: Yamamoto *et al.*, 2011; E: Yamamoto *et al.*, 2011 F: Croll *et al.*, 1992a; G: Kooyman, 1989; H: Melrose and Nicol, 1992; I: Mill and Baldwin, 1983; J: Hawkey *et al.*, 1989; K: Villouta *et al.*, 1997; L: Lenfant *et al.*, 1969b; M: Milsom *et al.*, 1973; N: Ponganis *et al.*, 1999a; O: Ponganis *et al.*, 1997a. The kittiwake is a non-diver, the mallard duck and coot are dabblers, and the pelican is a plunge diver.

4.4.2.4 Blood O₂ stores: rheology

As highlighted in the Castellini *et al.* review (2010), blood viscosity can be affected by temperature, shear rate, Hct level, and red blood cell characteristics (shape, size, deformability, aggregability, membrane composition, and cytoplasmic viscosity). A variety of adaptations in marine mammals and diving birds appear to optimize their blood rheology. For instance, although decreased temperature increases viscosity, the increase in blood viscosity in bowhead whales (*Balaena mysticetus*) was less than in human blood in samples exposed to low temperatures that would be found in the flukes of the whale (Elsner *et al.*, 2004b). Similarly, blood viscosities of little blue (*Eudyptula minor*) and Adélie penguins were less than that of the chicken at all temperatures tested (Clarke and Nicol, 1993).

Shear rate is essentially analogous to blood flow rate, and viscosity decreases curvilinearly as shear rate increases (Castellini *et al.*, 2010). Thus, viscosity will be greater under low-flow conditions (lower heart rates) and lower during periods of high flow. Furthermore, as Hct increases, viscosity will also increase, with greater increases in viscosity at lower shear rates (Castellini *et al.*, 2010). These principles were confirmed in several species of marine mammals and seabirds in early studies (Guard and Murrish, 1975, Hedrick and Duffield, 1991, Hedrick *et al.*, 1986, Wickham *et al.*, 1989, 1990a, 1990b). Notably, blood viscosity at low shear rates and high Hct was lower in seals than in pigs (Wickham *et al.*, 1989), leading to the suggestion that relatively lower viscosities in seals under these conditions lowered cardiac workload during a dive, especially when heart rate began to increase near or at the end of a dive (see Chapter 5 for cardiac responses during dives). This suggestion is based on the fact that blood viscosity contributes to systemic vascular resistance (Klabunde, 2011, Murray *et al.*, 1969), and hence to the workload of the heart (see Chapter 5). However, to make matters more complex, later studies revealed that blood viscosities at various shear rates, including low shear rates, varied in different seal species (Elsner and Meiselman, 1995, Meiselman *et al.*, 1992). For example, the ringed seal had low viscosities, especially at low shear rates, while the Weddell seal had high viscosities. This led to the suggestion that such high viscosities in some species would lead to splenic storage of red blood cells during non-diving periods (i.e., at a time with no need for a large circulating blood O₂ store) (Elsner and Meiselman, 1995). (See Chapter 5 for Hct variation and splenic contraction in diving seals.)

Differences in blood viscosities may also be affected by many other factors, including red cell size, shape, and deformability, as well as the concentration of Hb within the cell, which affects the cell's cytoplasmic viscosity (and, in turn, its deformability) (Castellini *et al.*, 2010). Differences in these variables, as well as differences in plasma proteins, may affect viscosity (Wickham *et al.*, 1989, 1990b); increased red cell aggregation in Weddell seals is probably associated with the increased fibrinogen concentrations in their blood (Meiselman *et al.*, 1992).

Increased viscosity, high Hct, periods of low blood flow, and elevated fibrinogen levels in Weddell seals all raise the question of increased risk of thrombosis in diving animals. In human patients, endothelial injury from hypoxemia and ischemia (decreased organ perfusion) is associated with changes in coagulation status and the potential for thromboembolic events (Weidman *et al.*, 2014). There have been relatively few studies of coagulation in diving mammals and birds. In northern elephant seals (*Mirounga angustirostris*), fibrinogen, antithrombin III, and platelets were reported as similar to other domestic species; the activated clotting time (ACT), prothrombin time (PT), and activated partial thromboplastin time (PTT) were relatively short in comparison to other species (Gulland *et al.*, 1996).

Factor XII of the coagulation cascade is absent in bottlenose dolphins (*Tursiops truncatus*), killer whales (*Orcinus orca*), and sei whales (*Balaenoptera borealis*); both their PT and activated PTT are prolonged (Robinson *et al.*, 1969, Saito *et al.*, 1976, Tibbs *et al.*, 2005). It has been suggested that lack of Factor XII and absence of an active extrinsic coagulation pathway may serve to decrease risk of thrombosis in stagnant, pooled blood during the dive response (Saito *et al.*, 1976, Tibbs *et al.*, 2005). In contrast, increased fibrinolytic activity does not appear to play a protective role against thrombus formation in stagnant blood in seals. Clot lysis time increased during forced submersions of gray seals (*Halichoerus grypus*), which was consistent with reduced rather than increased fibrinolytic activity (Lohman *et al.*, 1998).

In addition to potential effects of coagulation factors, decreased temperatures and decompression are known to activate platelets (Field and Tablin, 2012, Field *et al.*, 2001, Patterson *et al.*, 1993). Decreased responses of platelets to various agonists have been documented in killer whale and elephant seal platelets (Field and Tablin, 2012, Field *et al.*, 2001, Patterson *et al.*, 1993). Although elephant seal platelets are activated by exposure to 4 °C temperature, decompression-induced activation does not occur (Field and Tablin, 2012). Differences in the lipid compositions of platelet membranes of the marine mammals versus terrestrial mammals were suggested as possible mechanisms underlying the decreased sensitivities of their platelets to various agonists (Field and Tablin, 2012, Patterson *et al.*, 1998).

4.4.3 Muscle O₂ stores

Muscle O₂ stores are estimated on the basis of muscle mass, Mb concentration, and 1.34 ml O₂ g⁻¹ Mb. Although this calculation appears straightforward, there are potential sources of error.

4.4.3.1 Muscle O₂ stores: muscle mass and Mb distribution

Muscle mass for a given species is not always known, and an assumption of 30% of body mass has often been used. Although a 30% value appears reasonable for many animal species (see Tables 4.4, 4.5), the balaenopterid whales are an exception. Muscle mass in these large, lunge-feeding whales comprises 45–62% of body mass. Muscle mass is 25–29% of body mass in flighted avian divers, but 33–38% in penguins. Most measurements of muscle mass have been made by anatomical dissection. Muscle mass can also be determined through 3D reconstructions of CT or magnetic resonance scans (Ponganis *et al.*, 1997a).

Most authors have also assumed a uniform distribution of Mb concentration throughout all muscles of the body. This is not necessarily true. In emperor penguins, for example, there is a three-fold difference in Mb concentration between the leg muscles and the primary locomotory muscles, the pectoralis and supracoracoideus muscles (Ponganis *et al.*, 1997a). In Baikal seals (*Phoca sibirica*), not only are there differences in Mb contents of individual muscles, but there are also differences between superficial and deep portions of muscles as well as changes in Mb concentration with the season of the year (Neshumova *et al.*, 1983, Neshumova and Cherepanova, 1984, Petrov and Shoshenko, 1987). Mb concentrations in the epaxial muscles of dolphins and seals also vary in the cranio-caudal direction, with the highest concentrations in the caudal regions (Polasek and Davis, 2001, Polasek *et al.*, 2006). However, at least in the Baikal seal, the overall average Mb concentration for all muscles was only about 10% less than the highest value measured deep in the primary locomotory muscle (Neshumova and Cherepanova, 1984). This is at least partially due to the fact that the primary locomotory muscles are the most massive muscles in the body. Therefore, in most cases, reasonable estimates of muscle O₂ stores are possible with measurement of Mb concentration in a single muscle.

4.4.3.2 Myoglobin analyses

Most authors in the field of diving physiology have determined Mb concentrations with a spectrophotometric method (Reynafarje, 1963). In this technique, which involves measurement and subtraction of tissue absorbances at two wavelengths, the effect of Hb on the measurements is eliminated due to the assumed identical extinction coefficients of Hb at those wavelengths. However, these assumptions may not always be correct. The Hb extinction coefficients at those wavelengths can vary in different species, and, in addition, the Mb extinction coefficients used in the calculation are also not constant among species (Masuda *et al.*, 2008).

Masuda *et al.* found that use of the Reynafarje technique overestimated rat heart cytoplasmic Mb concentrations by 0.10 mM, which was 37% greater than the 0.26 mM value determined by ¹H NMR spectroscopy. The authors attributed this overestimation to differences in extinction coefficients as well as to baseline errors in spectral subtraction, and suggested use of a baseline corrected spectra of a standard Hb and Mb solution to correct such errors. Whether these findings invalidate the many Mb determinations with the Reynafarje technique in diving animals is unclear. Marine mammals have much higher Mb concentrations (i.e., 4.5 g 100 g⁻¹ muscle is equivalent to 3.8 mM in

Table 4.4 Measured muscle mass as percentage of body mass in marine mammals.

Species	Muscle (%)	Reference
Bottlenose dolphin <i>Tursiops truncatus</i>	36	A
Sperm whale <i>Physeter macrocephalus</i>	34	B
Sei whale <i>Balaenoptera borealis</i>	58	B
Fin whale <i>Balaenoptera physalus</i>	45	B
Bryde's whale <i>Balaenoptera brydei</i>	46	B
Minke whale <i>Balaenoptera acutorostrata</i>	62	B
Pacific right whale <i>Balaena glacialis siboldi</i>	31	B
Humpback whale <i>Megaptera novaeangliae</i>	30	B
Manatee <i>Trichechus manatus</i>	40	C
California sea lion <i>Zalophus californianus</i>	37	D
Harp seal <i>Phoca groenlandica</i>	26	E
Hooded seal <i>Cystophora cristata</i>	28	E
Ringed seal <i>Phoca hispida</i>	30	F
Baikal seal <i>Phoca sibirica</i>	30	G
Weddell seal <i>Leptonychotes weddellii</i>	35	H
Southern elephant seal <i>Mirounga leonina</i>	28	I
Crabeater seal <i>Lobodon carcinophagus</i>	44	J
Sea otter <i>Enhydra lutris</i>	33	K

References: A: Goforth, 1986; B: Lockyer, 1976; C: Scholander and Irving, 1941; D: Ponganis *et al.*, 1997c; E: Burns *et al.*, 2007, 2010; F: Lydersen *et al.*, 1992; G: Neshumova and Cherepanova, 1984; H: Fujise *et al.*, 1985; I: Bryden, 1972; J: Bryden and Erickson, 1976; K: Thometz, 2014, Thometz *et al.* 2015.

In most calculations of muscle oxygen stores in species for which muscle mass has not been determined, a value of 30% of body mass is usually assumed.

Table 4.5 Muscle mass (as a percentage of body mass) in aquatic and marine birds.

Species	Muscle (%)	Reference
Tufted duck <i>Aythya fuligula</i>	25	A
Black-legged kittiwake <i>Rissa tridactyla</i>	21	B
Cassin's auklet <i>Ptychoramphus aleuticus</i>	26	B
Ancient murrelet <i>Synthliboramphus antiquus</i>	25	B
Rhinoceros auklet <i>Cerorhinca monocerata</i>	27	C
Thick-billed murre <i>Uria lomvia</i>	29	B, D
King penguin <i>Aptenodytes pagagonicus</i>	33 – pre-breeding 37 – pre-molt	E
Emperor penguin <i>Aptenodytes forsteri</i>	38	F

References: A: Keijer and Butler, 1982; B: Elliott *et al.*, 2010; C: Yamamoto *et al.*, 2011; D: Croll *et al.*, 1992a; E: Chérel *et al.*, 1993; F: Ponganis *et al.*, 1997a.

The kittiwake represents a non-diver.

cytoplasm), and Masuda *et al.* estimated that use of the Reynafarje technique would only lead to a 3.6% error in sperm whale (*Physeter macrocephalus*) myoglobin concentration. Such an error would have minimal effect on O₂ store calculations given the high concentrations found in sperm whale muscle. Clearly, however, a combined spectrophotometric and ¹H NMR study in marine mammal muscles would be valuable in the evaluation of this technique and the confirmation of past determinations of Mb concentrations in marine mammals and diving birds.

Thus, it is important to remember potential limitations of available data in the calculation of muscle O₂ stores. These include available muscle mass data, variation in Mb concentrations in different muscles, and even potential limitations in the analytic technique used to determine Mb concentration. In addition, in muscle samples that have been preserved frozen for long time periods (i.e., months or longer), it is important to verify that dehydration has not occurred. This is most easily accomplished by freeze drying fresh and stored samples and comparing water contents.

4.4.3.3 Myoglobin concentrations

In marine mammals, Mb concentrations vary almost 100-fold, from 0.1 g 100 g⁻¹ muscle in manatees to 9.5 g 100 g⁻¹ muscle in hooded seals (see Table 4.6, 4.7). It is in the deep, long-duration divers that Mb concentrations are highest. These animals

Table 4.6 Myoglobin (Mb) concentrations in cetaceans and the manatee.

Species	Mb (g 100 g ⁻¹)	Reference
Bottlenose dolphin <i>Tursiops truncatus</i>	2.7–3.2	A, G
Pacific white-sided dolphin <i>Lagenorhynchus obliquidens</i>	3.5	A
Killer whale <i>Orcinus orca</i>	3.1	B
Northern right whale dolphin <i>Lissodelphis borealis</i>	1.8	A
Indus River dolphin <i>Platanista indi</i>	2.6	C
Common dolphin <i>Delphinus delphis</i> , <i>D. capensis</i>	3.6	A
Striped dolphin <i>Stenella coeruleoabla</i>	5.8	A
Spinner dolphin <i>Stenella longirostris</i>	5.5	D
Spinner dolphin <i>Stenella attenuata</i>	2.5	E
Fraser's dolphin <i>Lagenodelphis hosei</i>	7.1	D
Harbor porpoise <i>Phocoena phocoena</i>	4.0	A, F
Beluga whale <i>Delphinapterus leucas</i>	3.4	A
Short-finned pilot whale <i>Globicephala macrorhynchus</i>	6.8	G
Pygmy sperm whale <i>Kogia breviceps</i>	4.3–5.9	A, H
Cuvier's beaked whale <i>Ziphius cavirostris</i>	4.3	A
Beaked whale (four species) <i>Mesoplodon</i> sp.	7.3	H
Narwhal <i>Monodon monoceros</i>	7.9	I
Northern bottlenose whale <i>Hyperoodon ampullatus</i>	6.3	J
Sperm whale <i>Physeter macrocephalus</i>	5.4	J
Sei whale <i>Balaenoptera borealis</i>	0.9	K
Fin whale <i>Balaenoptera physalus</i>	1.1–2.4	A, K, L
Blue whale <i>Balaenoptera musculus</i>	0.8	M
Bowhead whale <i>Balaena mysticetus</i>	3.5	A

Table 4.6 (cont.)

Species	Mb (g 100 g ⁻¹)	Reference
Manatee <i>Trichechus manatus</i>	0.1	F

References: A: Noren and Williams, 2000; B: Noren *et al.*, 2012b; C: Blessing, 1972a; D: Dolar *et al.*, 1999; E: Castellini and Somero, 1981; F: Blessing, 1972b; G: Velten *et al.*, 2013; H Kielhorn *et al.*, 2013; I: Williams *et al.*, 2011b; J: Scholander, 1940; K: Tawara, 1950; L: Hochachka and Foreman, 1993; M: Lawrie, 1953. Data in this table are from primary locomotory muscles in adult animals. Values are in g 100 g⁻¹ muscle wet weight.

include phocid seals (hooded, harp, ribbon, Weddell, and elephant seals), otariids (Galapagos (*Zalophus wollebaeki*) and California sea lions (*Z. californianus*)) and cetaceans such as the sperm whale, bottlenose whales, and the narwhal (*Monodon monoceros*). Among species with limited documentation of diving behavior, high Mb concentrations suggest deep-diving capacity in striped (*Stenella coeruleoabla*), spinner (*Stenella longirostris*), and Fraser's (*Lagenodelphis hosei*) dolphins. However, not all deep divers have the most extreme Mb concentrations. Cuvier's beaked whale (*Ziphius cavirostris*), the mammalian dive record holder, is an example; Mb content is high, but not the highest among marine mammals (Table 4.6). And, conversely, in the Baikal seal, elevated Mb concentrations may support yet undocumented, long-duration dives during the Siberian winter when the lake freezes over.

Elevated Mb concentrations are also a hallmark of the penguins, with the highest values in the deep-diving king and emperor penguins (Table 4.8). Although Mb concentrations are higher in the primary swimming muscles versus the legs of penguins, the Mb concentration is still considerably elevated in the leg muscles. In flighted diving birds, Mb concentrations are elevated only two- to three-fold, with higher concentrations in the primary underwater locomotory muscles. The Japanese cormorant, however, is an exception in regard to Mb distribution.

4.5 Magnitude and distribution of total body O₂ stores

Both the magnitude and distribution of total body O₂ stores of divers versus non-divers vary between mammals and birds (Tables 4.9–4.11). In comparison to humans (“a non-diver”), mass specific body O₂ stores are elevated 1.5- to almost five-fold in all marine mammals except the manatee. In general, diving mammals with greater diving capacities have significantly larger O₂ stores.

In contrast, total body O₂ is not exceptionally elevated in diving birds. Even in the premier avian diver, the emperor penguin, the total mass-specific O₂ store is only elevated about one-third above that of the non-diving black-legged kittiwake (*Rissa tridactyla*). The distribution of O₂ stores between mammals and birds also differs, and the most striking difference in is the respiratory compartment. In all diving mammals

Table 4.7 Myoglobin (Mb) concentrations in pinnpeds and sea otters.

Species	Mb (g 100 g ⁻¹)	Reference
California sea lion <i>Zalophus californianus</i>	5.4	A
Steller sea lion <i>Eumetopias jubata</i>	2.7	B
Northern fur seal <i>Callorhinus ursinus</i>	3.5	C
Antarctic fur seal <i>Arctocephalus gazella</i>	2.4	D
Australian sea lion <i>Neophoca cinerea</i>	2.7	E
Galapagos sea lion <i>Zalophus wollebaeki</i>	5.3	F
Walrus <i>Odobenus rosmarus</i>	3.0	C
Harbor seal <i>Phoca vitulina</i>	5.5	C
Gray seal <i>Haliuchoerus grypus</i>	5.4	D
Leopard seal <i>Hydrurga leptonyx</i>	5.1	G
Harp seal <i>Phoca groenlandica</i>	8.6	H
Hooded seal <i>Cystophora cristata</i>	9.5	H
Ribbon seal <i>Histiophoca fasciata</i>	8.1	C
Ringed seal <i>Phoca hispida</i>	4.1	I
Baikal seal <i>Phoca sibirica</i>	6.0	J
Weddell seal <i>Leptonychotes weddellii</i>	5.4	K
Northern elephant seal <i>Mirounga angustirostris</i>	7.8	L
Sea otter <i>Enhydra lutris</i>	3.3	M

References: A: Weise and Costa, 2007; B: Richmond *et al.*, 2006; C: Lenfant *et al.*, 1970; D: Reed *et al.*, 1994a; E: Fowler *et al.*, 2007; F: Villegas-Amtmann and Costa, 2010; G: Kuhn *et al.*, 2006; H: Burns *et al.*, 2007, 2010; I: Lydersen *et al.*, 1992; J: Neshumova and Cherepanova, 1984; K: Ponganis *et al.*, 1993a; L: Hassrick *et al.*, 2010, Thorson and Le Boeuf, 1994; M: Thometz, 2014, Thometz *et al.*, 2015.

Data in this table are from primary locomotory muscles in adult animals. Values are in g 100 g⁻¹ muscle wet weight.

Table 4.8 Myoglobin (Mb) concentrations in pectoral and leg muscles of aquatic and marine birds.

Species	Mb Concentration (g 100 g ⁻¹ muscle)		Reference
	Pectoral	Leg	
Tufted duck <i>Aythya fuligula</i>	0.6–0.7	1.0	A
Black-headed gull <i>Larus ridibundus</i>	0.6		B
Black-legged kittiwake <i>Rissa tridactyla</i>	0.4		C
Manx shearwater <i>Puffinus puffinus</i>	0.6		B
Rhinoceros auklet <i>Cerorhinca monocerata</i>	1.8	1.2	D
Cassin's auklet <i>Ptychoramphus aleuticus</i>	1.1	0.8	C
Ancient murrelet <i>Synthliboramphus antiquus</i>	1.0	0.9	C
Atlantic puffin <i>Fratercula arctica</i>	1.3	0.8	E
Common murre <i>Uria aalge</i>	1.4	0.6	E
Thick-billed murre <i>Uria lomvia</i>	1.9		F
Japanese cormorant <i>Phalacrocorax capillatus</i>	1.6	1.1	D
Little penguin <i>Eudyptula minor</i>	2.8		G
Rockhopper penguin <i>Eudyptes crestatus</i>	3.7		B
Royal penguin <i>Eudyptes schlegeli</i>	4.3		B
Adélie penguin <i>Pygoscelis adeliae</i>	3.0		H
Gentoo penguin <i>Pygoscelis papua</i>	4.4		H
King penguin <i>Aptenodytes patagonicus</i>	4.3		B
Emperor penguin <i>Aptenodytes forsteri</i>	6.4	2.0	I

References: A: Keijer and Butler, 1982; Stephenson *et al.*, 1989b; B: Baldwin *et al.*, 1984; C: Elliott *et al.*, 2010; D: Yamamoto *et al.*, 2011; E: Davis and Guderley, 1987; F: Croll *et al.*, 1992a; G: Mill and Baldwin, 1983; H: Weber *et al.*, 1974; I: Ponganis *et al.*, 1997a.

The gull and kittiwake represent non-divers.

Table 4.9 Mass specific total body oxygen stores and percentage distribution among lung, blood, and muscle O₂ stores in humans, some cetaceans, manatees, and sea otters.

Species	Total O ₂ store (ml O ₂ kg ⁻¹)	Lung (%)	Blood (%)	Muscle (%)	Reference
Human	24	42	44	14	A
Bottlenose dolphin <i>Tursiops truncatus</i>	34	27	33	40	B
Pacific white-sided dolphin <i>Lagenorhynchus obliquidens</i>	40	23	49	28	B
Killer whale <i>Orcinus orca</i>	36	23	36	41	
Beluga whale <i>Delphinapterus lucus</i>	51	17	51	32	C
Narwhal <i>Monodon monoceros</i>	75	12	38	50	D
Sperm whale <i>Physeter macrocephalus</i>	81	5	64	30	E
Manatee <i>Trichechus manatus</i>	21	33	60	7	F, G
Sea otter <i>Enhydra lutris</i>	69	45	33	21	H

A: Ponganis *et al.*, 2011; B: Noren *et al.*, 2012b; C: Shaffer *et al.*, 1997; D: Williams *et al.*, 2011b; E: Miller *et al.*, 2004b, Sleet *et al.*, 1981; F: Gallivan *et al.*, 1986; G: Lenfant *et al.*, 1970; H: Thometz, 2014, Thometz *et al.* 2015).

Calculations and values as per cited references.

except the sea otter (*Enhydra lutris*), the lung O₂ stores comprise a smaller percentage of the total O₂ store than in humans. Diving birds, on the other hand, especially flighted diving birds, have about 50% of the total body O₂ store in the respiratory system. This is similar to that of non-divers such as the black-legged kittiwake and the dabbling mallard duck. In the deep-diving king and emperor penguins, about one-third of the total body O₂ store is still located in the airs sacs and lungs. In part, this difference in the distribution of O₂ stores between mammals and birds is due to the relative magnitudes of their respective respiratory air volumes (Lasiewski and Calder, 1971). Importantly, these differences in the magnitudes and distribution of O₂ stores between mammals and birds have implications for the cardiovascular responses and pulmonary gas exchange required during dives for the effective utilization of those O₂ stores (see Chapter 5).

Despite these differences in O₂ store distribution between diving mammals and birds, there are also similar trends among them. In penguins and all marine mammals except the manatee, muscle is a significant component of the total O₂ store in comparison to their non-diving counterparts. A large muscle O₂ store, and especially a high muscle O₂ content concentrated in the primary underwater locomotory muscles, will again have

Table 4.10 Mass specific total body oxygen stores and percentage distribution among lung, blood, and muscle O₂ stores in pinnipeds.

Species	Total O ₂ store (ml O ₂ kg ⁻¹)	Lung (%)	Blood (%)	Muscle (%)	Reference
California sea lion <i>Zalophus californianus</i>	55	13	39	48	A
Steller sea lion <i>Eumetopias jubata</i>	40	20	45	35	B
Australian sea lion <i>Neophoca cinerea</i>	56	10	70	20	C
New Zealand sea lion <i>Phocarctos hookeri</i>	48	12	63	25	D, E
Galapagos sea lion <i>Zalophus wolfebaeki</i>	74	8	67	25	F
Northern fur seal <i>Callorhinus ursinus</i>	42	24	43	33	E, G
Antarctic fur seal <i>Arctocephalus gazella</i>	44	25	55	20	H
Walrus <i>Odobenus rosmarus</i>	38	24	50	26	G, I
Harbor seal <i>Phoca vitulina</i>	62	7	57	35	G, I, J
Crabeater seal <i>Lobodon carcinophagus</i>	43	12	67	21	E
Leopard seal <i>Hydrurga leptonyx</i>	51	7	46	47	K
Gray seal <i>Halichoerus grypus</i>	61	6	68	26	L
Ribbon seal <i>Histiophoca fasciata</i>	72	8	49	43	E, G
Harp seal <i>Phoca groenlandica</i>	73	7	49	44	E
Hooded seal <i>Cystophora cristata</i>	90	7	51	42	E
Ringed seal <i>Phoca hispida</i>	52	12	44	44	E, M
Baikal seal <i>Phoca sibirica</i>	79	5	66	29	N
Weddell seal <i>Leptonychotes weddellii</i>	89	4	66	30	O
Northern elephant seal <i>Mirounga angustirostris</i>	94	3	71	26	P

A: Weise and Costa, 2007; B: Richmond *et al.*, 2006; C: Fowler *et al.*, 2007; D: Costa *et al.*, 1998; E: Burns *et al.*, 2007; F: Villegas-Amtmann and Costa, 2010; G: Lenfant *et al.*, 1970; H: Costa *et al.*, 2001; I: Ponganis, 2011; J: Burns *et al.*, 2005; K: Kuhn *et al.*, 2006; L: Noren *et al.*, 2005; M: Lydersen *et al.*, 1992; N: Neshumova and Cherepanova, 1984, Neshumova *et al.*, 1983, Petrov and Shoshenko, 1987, Ponganis *et al.*, 1997b; O: Ponganis *et al.*, 1993a; P: Bryden, 1972, Simpson *et al.*, 1970, Thorson and Le Boeuf, 1994.

Calculations and values as per cited references.

Table 4.11 Mass specific total body oxygen stores and percentage distribution among respiratory, blood, and muscle O₂ compartments in aquatic and marine birds.

Species	Body mass (g)	Total O ₂ store ml O ₂ kg ⁻¹	Respiratory (%)	Blood (%)	Muscle (%)	Reference
Black-legged kittiwake <i>Rissa tridactyla</i>	359	50	56	42	2	A
Mallard duck (pekin duck) <i>Anas platyrhynchos</i>	1080	29	43	51	6	B
Tufted duck <i>Aythya fuligula</i>	674	42	48	48	4	B
Thick-billed murre <i>Uria lomvia</i>	1029	45	47	44	6	C
Ancient murrelet <i>Synthliboramphus antiquus</i>	197	60	50	44	6	A
Cassin's auklet <i>Ptychoramphus aleuticus</i>	171	61	50	44	6	A
Rhinoceros auklet <i>Cerorhinca monocerata</i>	560	55	49	40	11	D
Adélie penguin <i>Pygoscelis adeliae</i>	3800	63	48	26	26	E, F
King penguin <i>Aptenodytes patagonicus</i>	12 000	55	34	30	39	F
Emperor penguin <i>Aptenodytes forsteri</i>	25 000	68	33	31	36	F

A: Elliott *et al.*, 2010; B: Keijer and Butler, 1982; C: Croll *et al.*, 1992a; D: Yamamoto *et al.*, 2011; E: Chappell *et al.*, 1993; F: Ponganis *et al.*, 2011.

Calculations and values as per cited references. Typical body masses are included since many readers will probably not be familiar with the body sizes of these species.

implications for the nature of cardiovascular responses required for effective utilization of O₂ stores during dives (Chapter 5).

In marine mammals, there is decreased dependence on the respiratory O₂ store in deeper divers. This is especially evident in deep-diving mammals such as the sperm whale, elephant seal, hooded seal, and Weddell seal. Less reliance on the respiratory O₂ store decreases the need for pulmonary gas exchange at depth and should decrease the risks of excess blood N₂ uptake at depth, including the risk of decompression sickness. The same argument, although to a lesser extent, could be applied to the smaller mass-specific end-of-dive air volumes in king (*Aptenodytes patagonicus*) and emperor penguins (*A. forsteri*). However, start-of-dive air volumes in penguins are not documented, and recent air-sac volume measurements suggest that air-sac volumes may be much greater than expected in penguins (Ponganis *et al.*, 2015). If penguins do inhale to such large volumes, the total body O₂ store and respiratory O₂ fraction would increase greatly. Further assessments of start-of-dive air volumes in penguins are needed.

As a percentage of the total body O₂ store in diving mammals, the blood and muscle O₂ stores range from 54% in the sea otter to 97% in the northern elephant seal (see Tables 4.9, 4.10). The relative distribution of O₂ between these two stores is affected by more than just the Hb and Mb concentrations. This is exemplified in three deep-diving phocid seals with total body O₂ stores near 90 ml O₂ kg⁻¹ and more than 93% of that O₂ in blood and muscle. Hb concentrations and blood volumes are especially elevated in the elephant seal and Weddell seals (Table 4.2), while Mb content of hooded seal muscle is extremely elevated (Table 4.7). Such differences may be significant from a physiological perspective. Provided the metabolic cost to muscle during dives is similar among the three species, the need for delivery of O₂ from blood to muscle to maintain aerobic metabolism during long dives would appear to be less in the hooded seal than in the Weddell seal and elephant seal. This, in turn, may be reflected in the nature of cardiovascular responses during the dives of these animals. Following the same argument but in the opposite direction, the very small muscle O₂ store and low Mb concentration in manatees suggest that maintenance of muscle blood flow and blood-to-muscle O₂ transfer is necessary in order to maintain aerobic muscle metabolism during their dives.

At least two features of the distribution of O₂ stores in diving birds are particularly notable. The first is the large respiratory O₂ store, especially in flighted diving birds. The large respiratory O₂ store is most likely a consequence of avian respiratory anatomy and ventilation requirements during flight. The structure of the air-sac-lung system, its volume, and a high respiratory rate all contribute to ventilation during flight (Butler, 1991, Lasiewski and Calder, 1971, Maina and King, 1987). Because of this large respiratory store and also the small muscle O₂ store, both pulmonary gas exchange and blood-to-muscle O₂ transfer might be expected to occur during diving in flighted birds in order to maintain aerobic metabolism. This may account for the minimal elevations in Mb concentrations in flighted diving birds.

Second, it should also be noted that the relatively reduced size of the respiratory O₂ store in king and emperor penguins is not due to a decrease in their respiratory air volumes. The estimated volume of the respiratory system (diving air volume) in diving king and emperor penguins is similar to that predicted by avian allometric equations (Sato *et al.*, 2002, 2011). In these birds, the relative magnitude of the respiratory O₂ stores is reduced primarily because of an increased muscle O₂ store. The latter is secondary to both a much higher Mb concentration and a larger muscle mass in the penguins as compared to flighted diving birds. It should also be noted that the size of the respiratory O₂ store may also vary with depth of dive as estimated diving air volume increases with maximum depth of dive in penguins (Sato *et al.*, 2002, 2006, 2011). An increase in air volume with depth has also been suggested in deep-diving shags (Cook *et al.*, 2008, 2010).

4.6

Advantage of size in the rate of O₂ store utilization

The maximum four- to five-fold difference in mass-specific total body O₂ stores between humans and the best-diving mammals cannot account for the differences in their respective breath-hold capacities. The same argument applies to the minimal

increases in mass-specific O₂ storage in diving birds. Scholander emphasized this in his 1940 monograph. At least two additional factors contribute to that breath-hold capacity. The first is the advantage of increased body size (Noren and Williams, 2000, Noren *et al.*, 2012b). The second is the regulation of the depletion rate of those O₂ stores through changes in heart rate and organ perfusion. The latter is the subject of the next chapter. Increased body size, as noted by Krogh in 1934, confers an advantage in divers through the well-known relationship of metabolic rate to body mass (Kleiber, 1975, Krogh, 1934). Furthermore, not only is the resting or basal mass-specific metabolic rate lower in larger animals, but the metabolic cost of locomotion is also less in larger animals (Heglund *et al.*, 1982a, 1982b, Taylor *et al.*, 1982). While the former is at least partially a function of surface area–volume relationships, the latter is considered dependent on the velocity of shortening of active muscle units and the rate at which those units are activated (both higher in smaller animals). Thus, a given-sized O₂ store will last longer in the larger animal both at rest and during locomotion. Conversely, high metabolic rates secondary to small body size may also account for the high mass specific O₂ stores of the smallest marine mammal, the sea otter.

5

Cardiovascular dive response

The physiological hallmark of diving in marine mammals and birds is a decrease in heart rate relative to the pre-dive or surface heart rate (Irving *et al.*, 1941b, Scholander, 1963). Regulation of heart rate, cardiac output, and the degree of peripheral vasoconstriction during dives is essential to the management and utilization of body O₂ stores because (a) the magnitude and distribution of cardiac output to peripheral tissues contributes to rates of tissue O₂ delivery and tissue O₂ consumption; and (b) cardiac output contributes directly to the rate of blood O₂ uptake from the lungs (Hogan *et al.*, 1993, Kviety and Granger, 1982, Lutz *et al.*, 1975, Taylor *et al.*, 1987, Valtin, 1973). Although these responses were investigated as early as 1870 by Bert (see Irving *et al.*, 1941b for review) and also examined under conditions of asphyxia (Irving, 1934, 1938, 1939, Irving *et al.*, 1935a), the slowing of heart rate to below resting levels (bradycardia) and the constriction of peripheral blood vessels (vasoconstriction) of seals and penguins during forced submersions were first thoroughly documented in Scholander's 1940 monograph.

During Scholander's forced submersion experiments, heart rates of seals were as low as 10 beats min⁻¹ (bpm). Vasoconstriction and the circulatory isolation of muscle were convincingly demonstrated by (a) the depletion of muscle O₂ with a concomitant increase in muscle lactate concentration during the submersion, and (b) the subsequent wash-out of lactate into blood during the post-submersion period (Fig. 5.1). This dive reflex (severe bradycardia in combination with peripheral vasoconstriction) isolated peripheral organs and tissues from the circulation, decreased the rate of blood O₂ depletion (Fig. 5.2), and conserved that blood O₂ for the heart and brain, thus prolonging the duration of the breath hold (Irving *et al.*, 1941b, Scholander, 1940). It was not until the 1960s that Elsner found that the reductions in heart rates of seals and sea lions during trained submersions were not as severe as during forced submersions (Elsner, 1965, Elsner *et al.*, 1964a). Since that time, diving physiologists have continued to investigate the nature, plasticity, and consequences of dive responses in multiple species under different conditions.

Chapter 5 is the longest and probably most detailed section of this book because the dive response represents the core of diving physiology. As such, it is essential that students and researchers understand the physiological mechanisms and implications of the dive response. The chapter will lay the ground work for understanding the role of heart rate and vasoconstriction in (a) the conservation and preservation of O₂ for the heart and brain; (b) the regulation of metabolic rate and the depletion of O₂ stores,