3 Respiratory gas exchange

Rapid replenishment of depleted oxygen stores and removal of accumulated carbon dioxide are essential during the surface intervals of breath-hold divers. Respiratory anatomy, lung volumes, and respiratory mechanics underlie pulmonary gas exchange and are the primary topics of this chapter. Marine mammals and seabirds will be reviewed separately because of the differences between the structure and function of mammalian and avian respiratory systems (Maina, 2006, Maina *et al.*, 2010).

In mammals, airflow in the lung is bidirectional, the lung is compliant, and gas exchange occurs in the alveoli, the sac-like terminations of the distal airways. In contrast, the avian respiratory system includes not only the lungs but also the air sacs. In birds, the lung is relatively rigid, gas exchange occurs in the air capillary (a tubular structure), and airflow is unidirectional and crosscurrent to the flow of blood (Powell, 2000, Scheid, 1979). Although avian lung volumes are about one-quarter less than those of mammals on a mass-specific basis, combined air sac and lung volumes are 3–5 times the lung volumes of similarly sized mammals (Maina, 2006). In addition, on average, the avian respiratory surface areas are about 15% greater than in similarly sized mammals, the blood–gas barrier in the lung about 62% thinner, and the pulmonary capillary blood volume 22% greater (Maina, 2006). The anatomical differences in the respiratory systems of birds and mammals thus potentially affect divers in regard to the size of respiratory oxygen stores, air volume/buoyancy, the magnitude of gas exchange at depth, and the pressure tolerance of a compliant versus rigid lung.

In this chapter, respiratory anatomy, respiratory mechanics, and ventilation will be reviewed first for marine mammals and then for seabirds. A recent review of cetacean lung morphometry and mechanics is also highly recommended to readers for an excellent and thorough overview of cetacean lung mechanics and pulmonary function (Piscitelli *et al.*, 2013). The effects of pressure on the respiratory system and gas exchange in these animals are examined in Chapter 12.

3.1 Marine mammal respiratory anatomy and function

3.1.1 Airway and lung anatomy in marine mammals

Although histological investigations of airway and lung structure had begun in the 1920s–1930s (for a review, see Piscitelli *et al.* 2013), modern research on respiratory

anatomy and respiratory mechanics in diving mammals began with Scholander's investigations of whales on board whaling ships. There, he measured and dissected lungs from whales with specimens probably similar to those illustrated in Fig. 3.1. His observations of cartilaginous distal airways in whales led him to hypothesize that more rigid airways would allow (1) movement of air into those airways during compression of the lungs at depth; (2) collapse of alveoli; (3) cessation of gas exchange at depth; and (4) the avoidance of excess nitrogen absorption during dives (Scholander, 1940) (see Chapter 12).

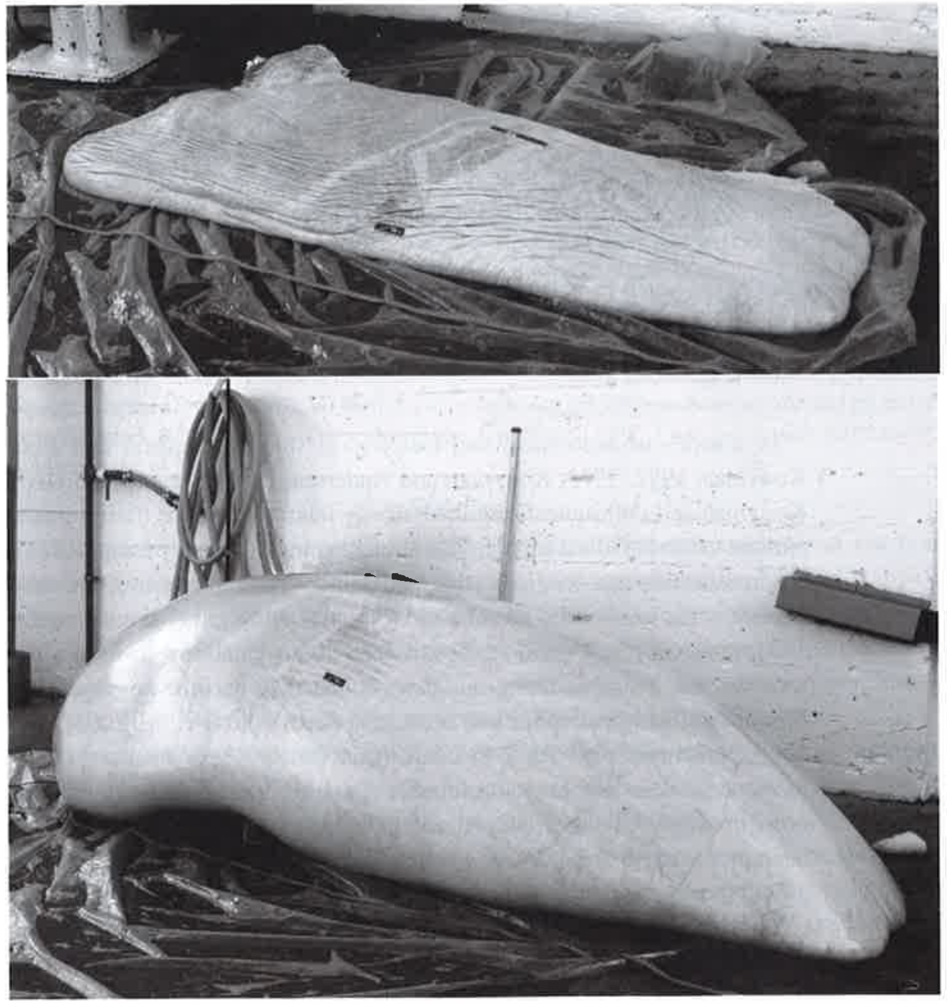

Figure 3.1 Deflated and inflated lung of a fin whale, *Balaenoptera physalus*. Such observations of lungs on a whaling vessel probably led Scholander to the hypothesis of lung collapse at depth as a mechanism for the prevention of decompression sickness in deep-diving marine mammals. Photographs with permission and courtesy from W. Vogl and M. Piscitelli.

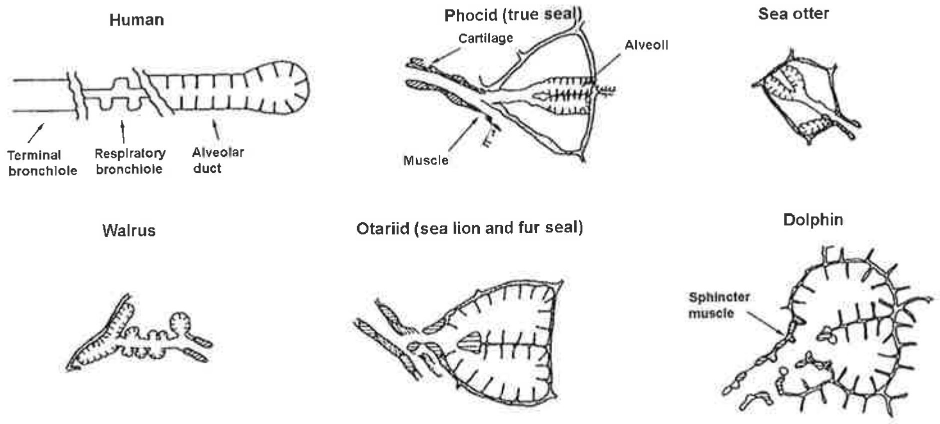

Figure 3.2 Comparison of terminal airway and alveolar diagrams of humans and marine mammals. **(a)** In humans, the terminal bronchiole extends to the respiratory bronchiole and then into the alveolar duct. **(b)** In phocid seals, the distal end of the terminal bronchiole lacks cartilage but is lined by thick smooth muscle that extends into the respiratory bronchiole. **(c)** In sea otters, some distal airways are supported by cartilage all the way to the alveolar sacs, while others lack such support over the final 1 mm. **(d)** Walrus distal airways are similar to those of the otter. **(e)** In sea lions and fur seals, airways are reinforced with cartilage all the way to the alveoli. **(f)** In dolphins, all airways have cartilaginous support. Terminal segments are marked by a series of muscular sphincters. Adapted from Kooyman, 1973.

In a series of anatomical and histological studies (Denison and Kooyman, 1973, Kooyman, 1972, 1973, Kooyman and Andersen, 1968, Tarasoff and Kooyman, 1973), Kooyman and colleagues examined airway reinforcement of diving mammals in comparison to terrestrial mammals. This strengthening of the air passages was most prominent in cetaceans and sea lions with cartilaginous reinforcement of the airways from the trachea to the level of the alveolar sac (Fig. 3.2). Respiratory bronchioles were absent.

More recent histological studies of deep-diving cetaceans have also revealed extensive vascular plexuses along the airways that may become engorged during dives to further reinforce the trachea and bronchi at depth (Cozzi *et al.*, 2005, Davenport *et al.*, 2013, Ninomiya *et al.*, 2005, Piscitelli *et al.*, 2013). These plexuses, composed primarily of large veins but also arterioles, are well developed and extend into the terminal bronchi in deep-diving whales (Piscitelli *et al.* 2013). Other possible functions that have been proposed for these vascular structures have included warming of air, dampening of airway pressures, oxygen storage, and prevention of barotrauma.

In Kooyman's examinations of phocid seals, tracheal reinforcement was minor and ranged from flexible cartilaginous rings to ventral bars of cartilage in some species. Although cartilage was absent in the distal airways of phocids, the presence of oblique muscle fibers in the bronchial walls was thought to reinforce these segments (Fig. 3.2). In walruses and sea otters, distal airways were reinforced with a mix of cartilage or muscle elements. Conceptually, more rigid airways should allow alveoli to empty and collapse more fully (no gas trapping) during compression.

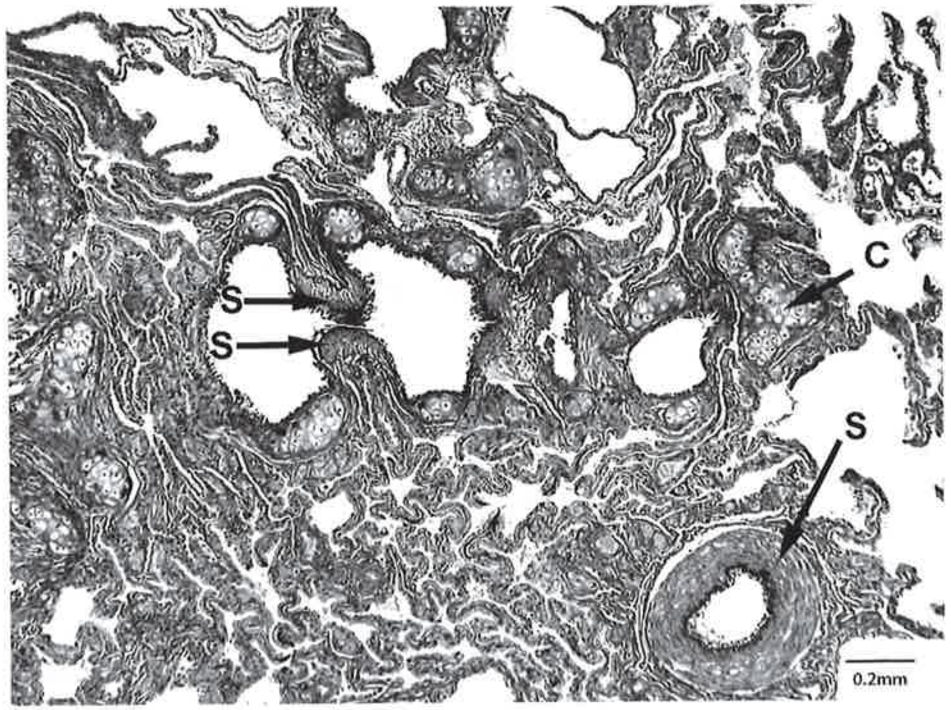

Figure 3.3 Lung micrograph of alveoli and distal bronchioles of the beluga whale, *Delphinapterus leucas*. Sagital sections and cross-sections of bronchiolar sphincter muscles are marked by arrows and the letter, S. One cross-section of cartilage is indicated by C. Photograph with permission and courtesy of M. Piscitelli.

The extensive cartilaginous reinforcement of the airways in cetaceans and sea lions should also allow higher flow rates and faster gas exchange during the brief surfacings of these animals. In cetaceans, it has been found that (1) the trachea is relatively short, (2) there is little change in the proximal vs. distal cross-sectional areas of the primary bronchi, (3) secondary bronchi are more numerous, and (4) cross-sectional areas of quaternary bronchi are high, 50–80% of that of the primary bronchi (Drabek and Kooyman, 1986).

Biomechanical compression studies have revealed that tracheas from bottlenose dolphins are relatively rigid, supporting the concept of alveolar collapse and the forcing of air into the trachea at depth (Bagnoli *et al.*, 2011). The trachea from a deep-diving pygmy sperm whale (*Kogia breviceps*) was found to be much more compressible, suggesting that eventual compression of the tracheobronchial tree further affords passage of respiratory air to the larynx and into nasal passages/sacs, where the air is necessary for formation of echolocation clicks (Davenport *et al.*, 2013).

A unique feature in the lungs of most dolphins and some odontocete whales is the presence of bronchiolar myoelastic sphincters (Belanger, 1940, Crespo and Lauria De Cidre, 2005, Goudappel and Slijper, 1958, Kooyman, 1973, Kooyman and Andersen, 1968, Kooyman and Sinnett, 1979, Ninomiya *et al.*, 2005, Piscitelli *et al.*, 2013, Wislocki, 1942). The function of these sphincters (Fig. 3.3) is unknown, although they have been postulated to (1) regulate gas distribution during diving, (2) play a role in alveolar re-expansion during ascent from depth, or (3) contribute to the rapid flow rates during exhalation.

Of historical interest, alveolar collapse as a mechanism for the prevention of caisson disease (decompression sickness) in whales was actually proposed by Damant in 1934 (Damant, 1934) in response to an article by Krogh (Krogh, 1934). In his article on whale physiology, Krogh had concluded it was unclear how whales avoided caisson disease, but speculated that the retia mirabilia had some role. Damant suggested that increased ambient pressure would compress the alveoli and block gas exchange and nitrogen uptake at depth.

3.1.2 Respiratory mechanics in marine mammals

The changes in respiratory mechanics suggested by the anatomical and histological findings in the above studies were investigated in the 1960s–1970s. Almost all of this research, which focused on flow rates and emptying of the lung, was conducted at or in association with Scholander's Physiological Research Lab at Scripps Institution of Oceanography.

In studies on excised lungs, it was found that dog lungs contained 27% of total lung capacity (TLC) at their relaxation volume (pleural pressure = 0), whereas sea lion lungs had a smaller relative relaxation volume (18% of TLC) (Denison *et al.*, 1971). Furthermore, when pleural pressure on the dog lungs was increased to +5 cm H_2O, only another 1% of TLC was expulsed before emptying stopped. In contrast, the sea lion lungs continued to empty with application of pleural pressures as high as +30 cm H_2O (the limit in the study). At that point, the mean gas volume of the lungs was 6% of TLC. A relaxation volume <17% of TLC in harbor porpoise lungs (*Phocoena phocoena*) (Kooyman and Sinnett, 1979), and low volumes in fin whales (*Balaenoptera physalus*) and sei whales (*B. borealis*) (Leith *et al.*, 1972) have also been reported. More recently, a mean 7% minimum air volume of excised lungs has been reported in a study of three phocid seal species and five odontocete species (Fahlman *et al.*, 2011). All these studies support the concept that reinforcement of the distal airways in diving mammals allows for the movement of air from the alveoli into the bronchi during lung compression, thus promoting collapse of the alveoli and cessation of gas exchange at depth.

Maximum expiratory flow rates, as high as 162 l s^{-1} in bottlenose dolphins, and 202 l s^{-1} in young gray whales (*Eschrichtius robustus*) (Kooyman and Cornell, 1981, Kooyman *et al.*, 1975), are necessary in cetaceans since exhalation and inhalation occur in less than 1 sec (Kooyman and Cornell, 1981, Kooyman *et al.*, 1975, Olsen *et al.*, 1969a, 1969b). Such flows allow for a tidal volume as high as 88% of TLC in the pilot whale (*Globicephala melena*) (Olsen *et al.*, 1969a, 1969b). In comparison, the average human tidal volume is less than 10% of TLC (Camporesi and Bosco, 2003). Such a high volume turnover is probably similar in other whales. In terms of vital capacity (VC, the volume of air from maximum inspiration to maximum expiration), maximum expiratory flow rates in cetaceans and sea lions are in the range of 5–8 VC s^{-1} (Kerem *et al.*, 1975, Kooyman and Cornell, 1981, Kooyman and Sinnett, 1979). Such exhalation is not active as elastic recoil of the lung has been found to be the sole driving force during expiration of the pilot whale (Olsen *et al.*, 1969a, 1969b). These high flow rates minimize the time for exhalation/inhalation and thus allow animals to porpoise through the water. With such rapid breaths, the animals can spend most of their travel time below the surface, where drag is less (Williams *et al.*, 1992c). In contrast, maximum human expiratory flow rates are about 2 VC s^{-1} (Camporesi and Bosco, 2003).

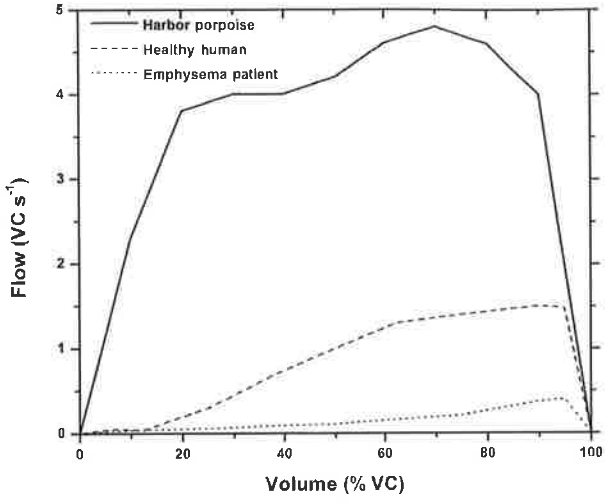

Figure 3.4 Flow volume loops (expiratory flow rate vs. lung volume) demonstrate the magnitude of high flow rates in the harbor porpoise (*Phocoena phocoena*) in comparison to a healthy human and to a patient with emphysema (with even lower flow rates). Importantly, cartilaginous reinforcement of the entire tracheo-bronchiolar tree in the porpoise allows for maintenance of maximum flow rates even at lower lung volumes (Kooyman and Sinnett, 1979). This contrasts markedly with the human emphysema patient and even with the normal, healthy human (Hyatt *et al.*, 1958). Maintenance of such high flow rates during a breath allows rapid respirations with tidal volumes equal to total lung volume in cetaceans. (VC = vital capacity, the volume of air exhaled from maximum inspiration to maximum exhalation). Adapted from above publications.

Flow volume curves of the bottlenose dolphin and of excised harbor porpoise lungs are remarkable for the maintenance of high flows at low lung volumes (Kooyman and Cornell, 1981, Kooyman and Sinnett, 1979). This contrasts with human flow volume curves, in which expiratory flow decreases as volume decreases (Fig. 3.4). This difference, which contributes to the short exhalation time, is considered secondary to the cartilaginous reinforcement of distal airways in the dolphin.

3.1.3 Lung volumes of marine mammals

Lung volumes of diving mammals are in the general range of terrestrial mammals (Kooyman, 1973, Fahlman *et al.*, 2011, Piscitelli *et al.*, 2013) (Table 3.1). Notable exceptions are the small lungs of the deep-diving whales and the large lungs of the

Table 3.1 Lung volumes in diving mammals.

Species	TLC ml kg^{-1}	Reference	DLV ml kg^{-1}	Reference
Harbor porpoise *Phocoena phocoena*	80–130^a	A		
Bottlenose dolphin *Tursiops truncatus*	50–91^{a,f}	B	40–50^d	C
Minke whale *Balaenoptera acutorostrata*	72^a	D		
Sei whale *Balaenoptera borealis*	61–126^a	E		
Fin whale *Balaenoptera physalus*	61–126^a	E		
Pilot whale *Globicephala melena*	10^b	F		
Kogiid whales *Kogia* sp.	20–49^a	G		
N. bottlenosed whale *Hyperoodon ampullatus*	28^a	H		
Sperm whale *Physeter macrocephalus*			28^d	I
Manatee *Trichechus manutus*	65^g	J		
Northern fur seal *Callorhinus ursinus*	145^a	K		
Steller sea lion *Eumetopias jubata*	110^a	K		
California sea lion *Zalophus californianus*			48^e	L
Walrus *Odonbenus rosmarus*	116^a	K		
Harbor seal *Phoca vitulina*	91^a	K	23–39^e	M
Hooded seal *Cystophora cristata*	80^a			N
Ribbon seal *Histriophoca fasciata*	86^a	K		
Weddell seal *Leptonychotes weddellii*	48^c	O	22^e – 27^c	P
Elephant seal *Mirounga angustirostris*			20^e	Q
Sea otter *Enhydra lutris*	345^a	K	207^e	R

References: A: Kooyman and Sinnett, 1979; B: Kooyman and Cornell, 1981, Ridgway *et al.*, 1969; C: Skrovan *et al.*, 1999; D: Folkow and Blix, 1992; E: Leith *et al.*, 1972; F: Olsen *et al.*, 1969b; G: Piscitelli *et al.*, 2010; H: Scholander, 1940; I: Miller *et al.*, 2004b; J: Scholander and Irving, 1941; K: Lenfant *et al.*, 1970; L: Kooyman and Sinnett, 1982; M: Kooyman and Sinnett, 1982, Kooyman *et al.*, 1973b; N: Burns *et al.*, 2007; O: Kooyman *et al.*, 1971b; P: Kooyman *et al.*, 1973a, 1973b; Q: Kooyman *et al.*, 1973b; R: Ponganis *et al.*, 2003a.

Techniques: ^{a} inflation of excised lungs, ^{b} helium dilution, ^{c} nitrogen washout, ^{d} buoyancy – swim velocity calculations, ^{e} compression during simulated dives, ^{f} tidal volume measurement, ^{g} inspiratory capacity. Abbreviations: TLC: total lung capacity, DLV: diving lung volume.

shallow-diving sea otter. Inflation of excised lungs of bottlenosed whales (*Hyperoodon ampullatus*) and pygmy and dwarf sperm whales (*Kogia breviceps*, *K. sima*) revealed lung volumes of 28 and 21 ml kg^{-1}, respectively (Piscitelli *et al.*, 2010, Scholander, 1940). Allometric analyses of lung mass in cetaceans revealed that kogiids, physeterids, ziphiids, and mysticetes all had relative lung masses similar to terrestrial mammals, while delphinids, phocoenids, and monodonts had relatively larger lungs masses (Piscitelli *et al.*, 2010, 2013). Inflation of excised lungs of the sea otter yielded a volume of 345 ml kg^{-1} (Lenfant *et al.*, 1970). The high lung volume in the otter presumably contributes to its buoyancy at the surface, where it feeds, grooms, and cares for its young. Such buoyancy in the otter also elevates more of the body out of the water while the animal is at the surface; this should reduce body heat loss due to conduction in water.

In cetaceans and manatees, tidal volumes are large, 80–90% of TLC (Reynolds III and Odell, 1991, Ridgway, 1986), and in Weddell seals post-dive volumes are 75% of vital capacity (Kooyman *et al.*, 1971b). The large post-dive tidal volumes and increased ventilatory rates of Weddell seals allow for a maximum post-dive minute ventilation that is ten times the minimum value at rest, and a post-dive O_2 uptake rate that is eight times that at rest (Kooyman *et al.*, 1971b, 1973a). Harbor seals (*Phoca vitulina*) and California sea lions (*Zalophus californianus*) swimming vigorously in a water flume and taking a breath at each surfacing are able to attain maximum O_2 uptake rates that are 8–10 times that of the animals at rest (Ponganis *et al.*, 1990, 1991). These maximum O_2 uptakes in seals and sea lions are less than those of highly aerobic animals such as dogs and horses, but are equivalent to those of less specialized species such as goats and calves (Taylor *et al.*, 1987).

Post-dive respiratory rates in northern elephant seals (*Mirounga angustirostris*) average 22 breaths min^{-1} (Andrews *et al.*, 2000). Among odontocete cetaceans, post-dive breathing rates are 5–10, 6, and 5 breaths min^{-1}, respectively, in bottlenose dolphins (*Tursiops truncatus*), Blainville's beaked whales (*Mesoplodon densirostris*), and sperm whales (*Physeter macrocephalus*) (Baird *et al.*, 2006b, Drouout *et al.*, 2004, Williams et al., 1999a). In baleen whales, post-dive respiratory rates in fin whales (*Balaenoptera physalus*) and humpback whales (*Megaptera novaeangliae*) are about 4–5 breaths min^{-1} (Goldbogen *et al.*, 2008, Lafortuna *et al.*, 2003). Blue whales (*Balaenoptera musculus*) average ten breaths during post-dive surface intervals of about 3-min duration (Goldbogen *et al.*, 2011).

Diving lung volumes, defined as the lung volume at the start of a dive, are important determinants of the size of the respiratory O_2 store during a dive. Cetaceans and otariid pinnipeds appear to dive on inspiration, while phocid pinnipeds usually dive on expiration (Kooyman, 1989, Ridgway, 1986). Consequently, the diving lung volumes of cetaceans are probably near TLC. This assumption is supported by the similarity of the calculated diving lung volume of the sperm whale, a deep-diving odontocete, to the measured TLC of another deep diver, the bottlenosed whale (Miller *et al.*, 2004b, Scholander, 1940). Determinations of diving lung volumes in pinnipeds during free dives and simulated dives have yielded values that are 40–50% of TLC (Kooyman and Sinnett, 1982, Kooyman *et al.*, 1971b, 1973b). More recently, blood oxygen profiles in deep-diving sea lions have provided evidence that lung collapse occurs at deeper depths in dives of greater maximum depth (McDonald and Ponganis, 2012). In other words, the sea lion appears to inhale deeper prior to deeper dives. Such increased lung volumes potentially increase the magnitude of the lung oxygen store, which has been most commonly calculated with values of 50% TLC in these species.

3.2 Seabird respiratory anatomy and function

3.2.1 Airway, air sac, and lung anatomy in diving birds

Although the data available for lung anatomy and function might be considered limited for marine mammals, there is even less description of respiratory anatomy and function in diving birds. Histological and detailed anatomical descriptions of upper airway structure in diving birds do not exist. In general, the avian trachea bifurcates into the primary bronchi, which extend into the lungs and also exit into the posterior air sacs. Within the lung, secondary bronchi branch off from the primary bronchi, eventually leading to the parabronchi, from which extend the air capillaries, which intertwine with pulmonary blood capillaries and are the site of gas exchange in the bird lung (Duncker, 1972, Duncker, 1974, Powell, 2000, Scheid, 1979, West *et al.*, 1977, Woodward and Maina, 2008). Secondary bronchi extend from the parabronchi back to the anterior air sacs. The air sacs of birds are poorly vascularized and not considered sites of gas exchange. There are nine air sacs: two cervical sacs, a single clavicular sac, two anterior thoracic sacs, two posterior thoracic sacs, and two abdominal sacs, all of which are connected to various secondary bronchi and some parabronchi. In general, air sac volumes are about ten times greater than lung air volumes (Duncker, 1972, Scheid *et al.*, 1974). Fig. 3.5 demonstrates the lung and air sac system of a penguin in a three-dimensional reconstruction from computerized tomographic (CT) scans.

Airflow through the various bronchi and air sacs is complex, but during inspiration and expiration air flow is unidirectional (caudal to cranial) in the parabronchi (Powell, 2000, Scheid, 1979, Scheid and Piiper, 1987). During inspiration, air flows both into the parabronchi and posterior air sacs, with air exiting the parabronchi into the anterior air sacs (Scheid, 1979). During exhalation, air flows predominantly from the posterior air sacs into the parabronchi, exiting into the anterior air sacs, and then out through secondary bronchi into the trachea (Scheid, 1979).

Inspiration and expirations are accomplished through activation of specific thoracic wall and abdominal muscles (Powell, 2000). Lung volume changes only minimally during the respiratory cycle (Jones *et al.*, 1985). During flight, wing upstrokes during inspiration and downstrokes during exhalation augment airflow through expansion and compression effects on the thoracoabdominal cavity (Boggs, 1997, Butler and Bishop, 2000). Differential air-sac pressures due to wing movements in swimming penguins also suggest that wing movements may induce air-sac pressure oscillations with secondary enhanced diffusion and/or airflow from the air sacs through the lung during the breath hold in diving penguins (Boggs *et al.*, 2001).

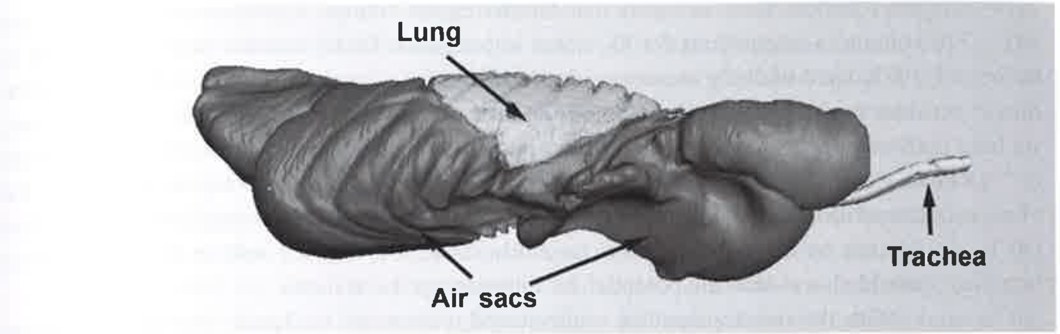

Figure 3.5 Three-dimensional computerized tomographic scan reconstruction of the air sacs and lungs of an emperor penguin (*Aptenodytes forsteri*). Adapted from Ponganis et al., 2015.

3.2.2 Lung/air-sac volumes of diving birds

Measurements of the combined gas volumes of air sacs and lung in birds are rare, and have usually been conducted by tracer gas dilution or inflation/cast injection to a given pressure. Indeed, the allometric equation relating air-sac/lung air volumes to body mass in all birds is based on samples collected in different studies from only five species (Lasiewski and Calder, 1971). This equation is often used to calculate diving air volume in seabirds because marine birds such as murres are reported to dive on inspiration (Croll *et al.*, 1992a). In the marine birds, total air-sac/lung volume should be equivalent to the diving air volume.

In aquatic birds, the most detailed examinations of air-sac and lung volumes have been in ducks. Ducks, however, usually exhale prior to diving (Butler and Woakes, 1979) Therefore, end-expiratory lung volumes have been used to estimate their diving air volumes. In the unrestrained lesser scaup (*Aythya affinis*), tidal volumes were 65–75 ml kg^{-1}, and the entire end-expiratory gas volume of the air sacs and lungs was 335 ml kg^{-1} (Stephenson, 1994). This total respiratory air volume was greater than that in restrained ducks in the same study (139 ml kg^{-1}). In this study, plumage air was 177 ml kg^{-1}; thus, in consideration of the contribution of respiratory and plumage air to body buoyancy, plumage air composed about 35% the total in an unrestrained duck. In the pekin duck (*Anas platyrhynchos*) under restrained conditions, respiratory air volume ranged from 108 to 220 ml kg^{-1} (Hudson and Jones, 1986). In unrestrained tufted ducks (*Aythya fuligula*), end-expiratory respiratory air volume was 165–232 ml kg^{-1} (Stephenson *et al.*, 1989b).

In penguins, diving air volumes (160–165 ml kg^{-1}) were first reported in gentoo and Adélie penguins (*Pygoscelis papua*, *P. adeliae*) from simulated dives in a pressure chamber (Kooyman *et al.*, 1973c). This value, determined from the volume of water pumped into the chamber from once it was filled until the chamber pressure began to rise, included both respiratory air and plumage air (the latter estimated to be about 10% of the total based on volume displacement measurements of birds washed with detergent). Gastrointestinal gas was assumed to be negligible. Because of these findings, most authors have assumed that the diving air volume represents the respiratory air volume in calculations for O_2 stores in penguins. These measurements, determined in 1973, were the only measurements available for penguin respiratory O_2 store calculations for 26 years, until diving air volumes of king penguins (*Aptenodytes patagonicus*, 69 ml kg^{-1}) were determined with the same technique and even the same pressure chamber (Ponganis *et al.*, 1999a). Because of the smaller diving air volume of the deeper-diving king penguin, it was suggested that deeper-diving penguins had less reliance on the respiratory O_2 store. The smaller diving air volume in the deep diver would also reduce the potential for nitrogen uptake at depth.

With the development of sophisticated underwater backpack recorders, it became possible to estimate diving air volume in free-diving penguins (Sato *et al.*, 2002). Based on buoyancy–swim speed calculations, and recorded swim speeds, body angles, and depth profiles during the final gliding ascents to the surface by penguins, Sato and colleagues estimated the air volume that would most closely predict the observed swim speed during that period. This air volume was taken to represent the start-of-dive respiratory volume, assuming that plumage air was minimal and no air was exhaled during the dive. In both Adélie and king penguins, diving air volume increased with maximum depth of dive, from 100 to 200 ml kg^{-1}, and from 50 to 125 ml kg^{-1}, respectively, in each species. Not only were these values variable with depth, but the highest values were approximately 20–60% greater than those measured in each species in the pressure chamber dives. Larger values in free-diving animals would be consistent with Stephenson's prior findings in restrained versus unrestrained lesser scaups.

More recently, similar techniques applied to emperor penguins (*A. forsteri*) at sea revealed a mean maximal air volume for deep dives of 117 ml kg^{-1}, while the mean value for the shallowest dives was 64 ml kg^{-1} (Sato *et al.*, 2011). There is at least one caveat, however, in using this technique to estimate start-of-dive air volumes and respiratory O_2 stores. These calculations are based on measurements collected during the final ascent to the surface. If the bird exhales prior to that final ascent, these volumes will underestimate the start-of-dive air volume. The data in emperor penguins were quite variable for any depth, and the calculated volumes actually decreased for the deepest dives. Either the penguins may exhale early, or they are, indeed, beginning deep dives with smaller air volumes. It should be noted that initial stroke rates on descent increased with dive depth, consistent with greater buoyancy and larger air volumes for deeper dives (Williams *et al.*, 2012).

As discussed above, estimations of respiratory O_2 stores in most diving birds are currently derived from respiratory air volumes predicted by allometric equations (Lasiewski and Calder, 1971). In ducks, estimations can be based on the results from the inert gas dilution methods reviewed above, and, in penguins, on results from swim speed–buoyancy calculations (Sato *et al.*, 2002, 2011, Scheid *et al.*, 1974, Stephenson, 1995, Stephenson *et al.*, 1989b). It is notable, however, that the end-expiratory air volume measured in the unrestrained diving duck is about twice the allometrically predicted value, and the highest diving air volumes calculated for free-diving Adélie and emperor penguins are about 30–50% greater than predicted values. These variations in values raise questions as to the exact size of the air sacs and lungs in diving birds.

Most recently, lung and air sac volumes in Adélie, king and emperor penguins were determined from 3D reconstructions from CT scans (Ponganis *et al.*, 2015). The volumes of the lungs scaled allometrically according to published equations (Lasiewski and Calder, 1971). However, maximal air-sac volumes during positive pressure breath holds were 2.2–3 times greater than allometrically predicted values. Maximal total air volumes of the lungs and air sacs were calculated to be 311, 368, and 374 ml kg^{-1} in Adélie, king, and emperor penguins, respectively. Whether the birds can spontaneously inspire to that volume is uncertain because it is well known that the volume of the abdominal air sacs during spontaneous ventilation is much less than their maximal capacity (Scheid, 1979). Although uncertainty still exists as to the exact volume of the air sacs prior to a dive, certainly all the values estimated in free-diving penguins are less than the maximal value measured by CT scan in each species (see Chapter 12 for further review). If start-of-dive air-sac volumes are, indeed, greater than calculated end-of-dive values, the O_2 store will be greater as will initial buoyancy that must be overcome as the bird descends.

In regard to ventilation, the tracheal volumes of birds are about 4.5 times greater than those of similarly sized mammals (Hinds and Calder, 1971). As a consequence, at rest, birds have a larger tidal volume than similarly sized mammals (Bouverot, 1978). During exercise, respiratory rate increases and tidal volume may increase or remain unchanged, dependent on the species (Butler, 1991). In most species studied, both minute ventilation and oxygen uptake increase similarly during flapping flight to values as high as ten times resting rates (Butler, 1991, Butler and Bishop, 2000). Ventilation in emperor penguins swimming in a flume was sufficient to allow a maximal O_2 uptake about eight times the measured resting rate (Kooyman and Ponganis, 1994).

There are few data available for post-dive breathing rates in birds. Respiratory rate after the longest reported dive (27.6 min) of an emperor penguin decreased from 22 to 16 breaths min^{-1} over the first 5 min of the post-dive interval; the initial rate was similar to that reported after shorter-duration dives, but respiratory rate remained elevated longer after the long dive (Kooyman *et al.*, 1971a, Sato *et al.*, 2011). Respiratory rates of emperor penguins at rest have been reported as low as 3 breaths min^{-1} (Kooyman *et al.*, 1971a, Meir *et al.*, 2008).