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RESEARCH ARTICLE

Penguin lungs and air sacs: implications for baroprotection, oxygen stores and buoyancy

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ABSTRACT

The anatomy and volume of the penguin respiratory system contribute significantly to pulmonary baroprotection, the body O_2 store, buoyancy and hence the overall diving physiology of penguins. Therefore, three-dimensional reconstructions from computerized tomographic (CT) scans of live penguins were utilized to measure lung volumes, air sac volumes, tracheobronchial volumes and total body volumes at different inflation pressures in three species with different dive capacities [Adélie (*Pygoscelis adeliae*), king (*Aptenodytes patagonicus*) and emperor (*A. forsteri*) penguins]. Lung volumes scaled to body mass according to published avian allometrics. Air sac volumes at 30 cm H_2O (2.94 kPa) inflation pressure, the assumed maximum volume possible prior to deep dives, were two to three times allometric air sac predictions and also two to three times previously determined end-of-dive total air volumes. Although it is unknown whether penguins inhale to such high volumes prior to dives, these values were supported by (a) body density/buoyancy calculations, (b) prior air volume measurements in free-diving ducks and (c) previous suggestions that penguins may exhale air prior to the final portions of deep dives. Based upon air capillary volumes, parabronchial volumes and tracheobronchial volumes estimated from the measured lung/airway volumes and the only available morphometry study of a penguin lung, the presumed maximum air sac volumes resulted in air sac volume to air capillary/parabronchial/tracheobronchial volume ratios that were not large enough to prevent barotrauma to the non-collapsing, rigid air capillaries during the deepest dives of all three species, and during many routine dives of king and emperor penguins. We conclude that volume reduction of airways and lung air spaces, via compression, constriction or blood engorgement, must occur to provide pulmonary baroprotection at depth. It is also possible that relative air capillary and parabronchial volumes are smaller in these deeper-diving species than in the spheniscid penguin of the morphometry study. If penguins do inhale to this maximum air sac volume prior to their deepest dives, the magnitude and distribution of the body O_2 store would change considerably. In emperor penguins, total body O_2 would increase by 75%, and the respiratory fraction would increase from 33% to 61%. We emphasize that the maximum pre-dive respiratory air volume is still unknown in penguins. However, even lesser increases in air sac volume prior to a dive would still significantly increase the O_2 store. More refined evaluations of the respiratory O_2 store and baroprotective mechanisms in penguins await further investigation of species-specific lung morphometry, start-of-dive air volumes and body buoyancy, and the possibility of air exhalation during dives.

INTRODUCTION

The anatomy and function of the respiratory system play important roles in the dive performance of penguins. The estimated oxygen store of the lungs and air sacs of various penguin species constitutes 30–45% of the total body oxygen store in comparison to 5–20% in most marine mammals (Burns et al., 2007; Ponganis et al., 2011). In addition, although the air sacs of birds are compliant and presumably collapse under ambient pressure at depth (Jones and Furilla, 1987; Powell, 2015), requirements for the avoidance of pulmonary barotrauma ('lung squeeze') in the relatively rigid avian lung may limit the routine and maximum dive depths of penguins. It is unknown how tissue disruption in the walls of the air capillaries and parabronchi is avoided at depth. Lastly, respiratory air volume also affects body density, buoyancy and the potential cost of locomotion and diving (Lovvorn and Jones, 1991; Wilson et al., 1992).

Despite the importance of the respiratory system, there have been few measurements of the actual volumes of the lungs and air sacs in penguins, and no investigations of mechanisms by which penguins avoid pulmonary barotrauma (Kooyman et al., 1999). It has only been reported that the lungs of emperor penguins, *Aptenodytes forsteri* Gray 1844, have a thickened air–blood barrier (Welsch and Aschauer, 1986).

Estimates of respiratory air volumes and oxygen stores in penguins are based on four publications – two simulated-dive studies in compression chambers (Kooyman et al., 1973; Ponganis et al., 1999) and two free-dive studies at sea (Sato et al., 2002, 2011). Air volumes of the free-diving penguins were greater than those of the penguins in the pressure chamber studies for all three species examined [Adélie, *Pygoscelis adeliae* (Hombron and Jacquinot 1841); king, *Aptenodytes patagonicus* Miller 1778; and emperor penguins]. Such differences in air volumes had also been found in free-diving versus forcibly submerged ducks (Stephenson, 1995).

Free-diving penguins also appeared to inhale more air prior to deeper dives (Sato et al., 2002, 2011). Diving air volume in free-diving penguins increased with maximum depth of dive. The largest diving air volumes in Adélie and emperor penguins were 30–50% greater than the allometrically predicted respiratory volumes (Sato et al., 2002, 2011). Could respiratory air volumes in diving penguins actually be greater than the allometrically predicted values? In this regard, it is notable that the end-of-dive respiratory air volumes of free-diving lesser scaups (*Aythya affinis*) were not only greater than those during forced submersion (Stephenson, 1995) but also approximately twice the value predicted by allometric equations

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for a bird of that body mass (Lasiewski and Calder, 1971). And this was in a bird that exhales prior to diving.

Because of the importance of air sac and lung volumes of penguins in the potential prevention of barotrauma, in the magnitude of the respiratory O_2 store, and in buoyancy and locomotory cost, we utilized computerized tomography (CT) and three-dimensional (3D) reconstructions to measure air sac, lung and airway volumes to further define the anatomy of the respiratory system of penguins. By measuring lung volume, and using the lung morphometric data of Maina and co-workers (Maina and King, 1987; Maina and Nathaniel, 2001), we could determine the air capillary volume and parabronchial volume of the penguin lung. We hypothesized that (a) mass-specific lung volumes would be smaller in deeper divers, (b) mass-specific air sac volumes would be less in deeper divers and (c) the ratios of air sac volume to air capillary+parabronchial+tracheobronchial volume would be greater than the ratio of the ambient pressure at maximum depth of dive to ambient pressure at the surface. Thus, we postulated that the ratio of air sac volume to the air volumes of the 'rigid' lungs and airways would be large enough to allow compression of adequate air volume into the air capillaries, parabronchi and tracheobronchial tree to prevent barotrauma to those structures. Otherwise, the volume of the airway and/or those lung structures must decrease in order to prevent barotrauma at depth.

We also suspected that the air sac capacity after a maximum inspiration of a penguin would be greater than that measured over time in restrained birds or birds at rest. Therefore, we postulated that maximum air sac capacity would be large enough to support the prior reports of diving air volumes that were often greater than allometric predictions (Sato et al., 2002, 2011). Such measurements would provide better insight into the potential magnitude of the respiratory oxygen store. Lastly, we took advantage of the CT scan technology to estimate body density/buoyancy at different respiratory air volumes by calculating body density from total body volumes at different inflation pressures and the known body masses of the penguins. Such calculations would document the buoyancy the bird must overcome to descend to depth, as well as provide an evaluation of how realistic the air volumes were for a diving penguin.

RESULTS

CT scans were conducted on five Adélie, four king and six emperor penguins under managed care in captivity (Table 1). Sample size was limited to four king penguins because of animal availability. Furthermore, because of concerns over body temperature regulation, length of scan time and radiation dose, scans at all five inflation pressures (0, 10, 20, 30, 40 cm H_2O; 0, 0.98, 1.96, 2.94, 3.92 kPa) were only conducted on the smaller Adélie penguins and two king penguins. Therefore, scans at 0, 30 and 40 cm H_2O (0, 2.94 and 3.92 kPa) inflation pressure were conducted on the other two king penguins and on five emperor penguins. In the sixth emperor penguin, scans were conducted at 0, 10 and 20 cm H_2O (0, 0.98 and 1.96 kPa) inflation pressure.

3D reconstructions from the CT scans (Fig. 1) allowed calculation of lung volume, total and anterior/posterior air sac volume, tracheobronchial volume, solid tissue volume and total body volume. The anterior air sacs included the interclavicular, cervical and anterior thoracic air sacs. The posterior thoracic and abdominal
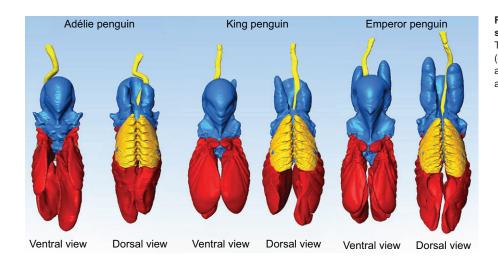
Table 1. Lung volume and air sac volume calculated from 3D reconstructions of CT scans of Adélie, king and emperor penguins
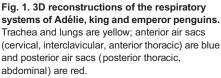
Bird ID	Sex	Age (years)	Body mass (kg)	Lung volume (ml)	Air sac volume (ml)	Lung volume (ml kg^{-1})	Air sac volume (ml kg^{-1})	Allometric volume	
								Air sac (ml kg^{-1})	Total (ml kg^{-1})
Adélie penguins									
AD143	M	24	5.14	142	1246	28	242	132	139
AD592	F	8	5.88	111	1769	19	301	131	137
AD549	F	17	3.85	97	1498	25	389	135	142
AD547	M	17	4.94	118	1320	24	267	133	139
AD539	F	18	3.35	106	946	32	282	137	144
Mean		16.8	4.63	115	1356	25	296	133	140
s.d.		6.6	1.02	17.1	305.1	4.7	56.1	2.5	2.9
N		5	5	5	5	5	5	5	5
King penguins									
KI5095	F	10.4	12.60	255	4313	20	342	123	128
KI5116	F	5.9	13.10	271	5370	21	410	122	128
KI5121	F	5.9	14.60	287	4179	20	286	121	126
KI0049	M	22.9	13.40	196	5208	15	389	122	127
Mean		11.3	13.43	252	4768	19	357	122	127
s.d.		2.60	0.85	39.7	608	2.8	54.9	0.6	0.7
N		4	4	4	4	4	4	4	4
Emperor penguins									
EM0087	F	1.5	21.60	459	7395	21	342	117	122
EM0091	F	1.5	25.45	309		12			
EM0092	F	1.5	21.00	412	7306	20	348	117	122
EM0086	M	1.5	20.00	392	7553	20	378	118	123
EM0088	F	1.5	20.90	324	7792	16	373	118	122
EM0090	M	1.5	19.80	343	7365	17	372	118	123
Mean		1.5	21.46	353	7482	18	363	118	122
s.d.		0	2.07	57.7	195.8	3.3	16.2	0.4	0.4
N		6	6	6	5	6	5	5	5

Lung volume includes both air and tissue. Lung volume was calculated at 0 cm H_2O inflation pressure; air sac volume was calculated at 30 cm H_2O pressure. For comparison, air sac and total respiratory air volumes were calculated from the allometric equations of Lasiewski and Calder (1971). ANOVA and Bonferroni comparison of means for mass-specific air sac and lung volumes revealed that only Adélie and emperor penguin mass-specific lung volumes were significantly different ($P<0.05$).
F, female; M, male; N, sample size.

Fig. 1. 3D reconstructions of the respiratory systems of Adélie, king and emperor penguins. Trachea and lungs are yellow; anterior air sacs (cervical, interclavicular, anterior thoracic) are blue and posterior air sacs (posterior thoracic, abdominal) are red.

air sacs were grouped as the posterior air sacs. In some cases, especially at higher inflation pressures, distinction of the membranous borders between adjacent air sacs was difficult.

Lung volume at 0 cm H_2O (0 kPa) inflation pressure was assumed to represent that under physiological conditions at the start of a dive (Table 1). ANOVA and *post hoc* Bonferroni comparison of means for mass-specific lung volumes revealed that only Adélie and emperor penguin values were significantly different. If the lung morphometry of these three penguin species is similar to that of the Humboldt penguin (*Spheniscus humboldti*) (Maina and King, 1987), the calculated total air volume of the lungs would be 58.7% of total lung volume, or 67 ± 10.0, 148 ± 23.3 and 227 ± 31.8 ml air in Adélie, king and emperor penguins, respectively. Tracheal volume (to tracheal bifurcation) at 0 cm H_2O (0 kPa) inflation pressure was 12 ± 3.2, 50 ± 13.3 and 72 ± 7.1 ml in Adélie, king and emperor penguins, respectively.

In terms of lung compliance and relative rigidity, it was notable that lung volume did increase with increasing inflation pressure (Fig. 2). Between 0 and 40 cm water pressure (3.92 kPa), mean lung volume increased 13.0%, 25.8% and 24.9% in Adélie, king and emperor penguins, respectively. Lung compliance, calculated from

the volume difference between 30 and 0 cm H_2O (2.94 and 0 kPa), was greater in king and emperor penguins, but values for the three species were not significantly different (ANOVA) at 0.3 ± 0.24, 1.9 ± 0.64 and 1.7 ± 1.54 ml cm^{-1} H_2O for Adélie (N=5), king (N=4) and emperor (N=5) penguins, respectively. Note that these static compliances reflect the compliance of the entire respiratory system, and not isolated lungs.

Air sac volume also increased with increasing inflation pressure (Fig. 3). Between 0 and 40 cm H_2O (3.92 kPa) pressure, mean air sac volume increased 943%, 881% and 395% in Adélie, king and emperor penguins, respectively. Visibly, the birds appeared over-distended only at 40 cm H_2O inflation pressure. Examination of solid tissue volumes (head, muscle, fat, skeleton, abdominal contents), individual respiratory volumes [trachea, lungs, anterior and posterior (posterior thoracic and abdominal) air sacs] and total body volumes revealed that changes in air sac volume were only associated with large increases in total body volume (Fig. 4). Solid tissue volume, which included abdominal organs, did not correspondingly compress with expansion of the air sacs.

Because of the visible over-distention at 40 cm H_2O (3.92 kPa), the air sac volume at 30 cm H_2O (2.94 kPa) pressure was assumed to
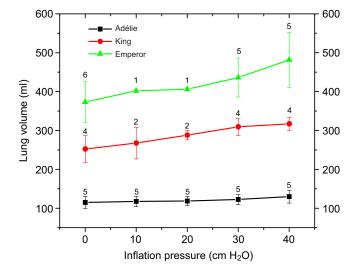

Fig. 2. Mean lung volume of Adélie, king and emperor penguins at inflation pressures of 0, 10, 20, 30 and 40 cm H_2O (0, 0.98, 1.96, 2.94, 3.92 kPa). Error bars represent ±s.e.; numbers above each data point indicate sample size (N).
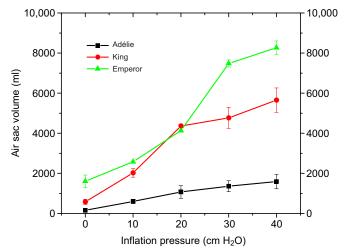

Fig. 3. Mean air sac volume of Adélie, king and emperor penguins at inflation pressures of 0, 10, 20, 30 and 40 cm H_2O (0, 0.98, 1.96, 2.94, 3.92 kPa). Error bars represent ±s.e.; N values are the same as in Fig. 2.
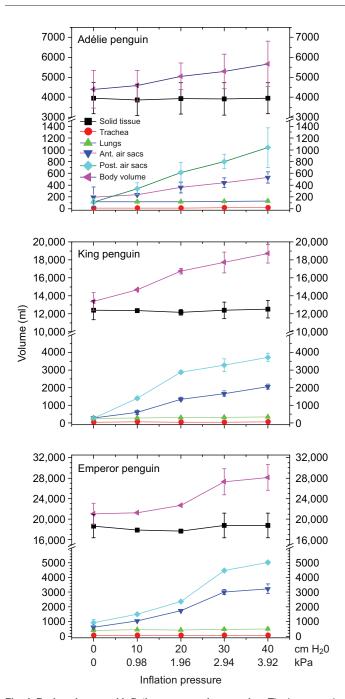
Fig. 4. Body volume and inflation pressure in penguins. The increases in the volume of the anterior (ant.) air sacs and posterior (post.) air sacs with higher inflation pressure were associated primarily with an increase in total body volume, but not with large decreases or compression of solid tissue volume, which included abdominal contents. Although lung volume increased with inflation pressure (Fig. 2), the increase was minor relative to that of the air sacs because of both the relative volume of the lungs and the approximately 100-fold greater compliance of the air sacs. Tracheal volume was only 0.1–0.2 of lung volume; changes in tracheal volume were minor and inconsistent with changes in inflation pressure. N values are as in Fig. 2. Poster air sacs were the posterior thoracic and abdominal air sacs.

be representative of the maximum air sac volume possible prior to a dive (Table 1). ANOVA revealed that these mass-specific total air sac volumes did not significantly differ among the three species, but that air sac compliance did. Air sac compliance, again a reflection of the entire respiratory system and body wall, was measured between

0 and 30 cm H_2O (0 and 2.94 kPa) inflation pressure, and was 40 ± 12.2, 140 ± 16.6 and 194 ± 11.4 ml cm^{-1} H_2O for Adélie ($N=5$), king ($N=4$) and emperor ($N=5$) penguins, respectively.

The volume of the tracheobronchial tree at 0 cm H_2O (0 kPa) inflation pressure (Table 2) was calculated from measurements of tracheal volume (to the tracheal bifurcation) and extrapulmonary bronchial volume (from the bifurcation to the lung), and volumetric morphometric data (Maina and King, 1987; Maina and Nathaniel, 2001) on intrapulmonary primary bronchi (3.56% of lung volume, Table 2). Air capillary volume (17.67% of total lung volume) and parabronchial volume (37.71% of total lung volume) were estimated similarly from the measured lung volume (Table 2). The calculated compression ratios of air sac volume to air capillary+parabronchial+ tracheobronchial volume (Table 3) were equivalent to depths of 153, 221 and 230 m in Adélie, king and emperor penguins, respectively. Calculated compression ratios of air sac volume to air capillary+ tracheobronchial volume were equivalent to depths of 344, 431 and 450 m in Adélie, king and emperor penguins, respectively.

Body mass and total body volume at each inflation pressure allowed calculation of body density, buoyancy and the percentage of the body submerged at different air sac volumes (Fig. 5). For air sac volumes measured at 0 cm H_2O pressure, 96–98% of the body would be submerged in the three species. At 30 cm H_2O (2.94 kPa) pressure, the percentage of the body submerged was 74% in all species, reinforcing the use of the air sac volumes at that inflation pressure as the maximum air sac volume prior to a dive.

DISCUSSION
Methodology
Study subjects
We chose to study Adélie, king and emperor penguins because of their availability, the previous estimates of diving air volumes in the three species (Sato et al., 2002, 2011), and the differences in routine and maximum depths among the three species. Adélie penguins typically make dives of 10–40 m, with a maximum reported depth of 180 m (Chappell et al., 1993; Norman and Ward, 1993; Watanuki et al., 1997), while emperor penguins routinely utilize the entire water column to depths of 400 m with a maximum reported depth of 564 m (Kirkwood and Robertson, 1997; Kooyman and Kooyman, 1995; Rodary et al., 2000; Wienecke et al., 2007). King penguins regularly make dives as deep as 100–250 m with a maximum reported depth of 343 m (Charrassin and Bost, 2001; Kooyman et al., 1992; Pütz and Cherel, 2005; Pütz et al., 1998).

The Adélie and king penguins, all long-term inhabitants at SeaWorld, were adults ranging in age from 5.9 to 24 years, with body masses typical of their species (Table 1). The emperor penguins were all approximately 1.5 years old, had already undergone the molt to adult plumage, and had arrived in the SeaWorld Penguin Encounter at about 5 months of age. Body masses at the time of the study were typical of young emperor penguins (Kooyman and Ponganis, 1994).

Technique
The compliance of avian air sacs has long complicated measurement of air sac volume. Spontaneous ventilation and air sac volume change with anesthesia and body position; in addition, air sac volume differs between spontaneous and artificial ventilation (Hawkins et al., 2013; King and Payne, 1964; Malka et al., 2009; Scheid et al., 1974). Shrinkage/expansion of cast materials, injection/inflation pressures and differences in abdominal mass can all contribute to potential error and variation in measurements (Powell, 2015; Scheid and Piiper, 1969). Although inert gas

Table 2. Estimated volumes of the tracheobronchial (TB) trees, air capillaries and parabronchi in Adélie, king and emperor penguins

Bird ID	Trachea +extrapulmonary bronchi volume (ml)	Lung primary bronchi volume (ml)	Total TB tree volume (ml)	Air capillaries volume (ml)	Parabronchi volume (ml)	'Rigid' air volume (ml)	Air capillaries +TB tree volume (ml)
Adélie penguins							
AD143	14.7	5.2	19.9	25.6	54.7	100.2	45.5
AD539	10.4	3.8	14.2	18.7	40.0	72.9	32.9
AD549	9.1	3.5	12.6	17.5	37.3	67.5	30.1
AD547	17.7	4.8	22.5	23.9	50.9	97.3	46.4
AD592	13.7	3.9	17.6	19.3	41.1	77.9	36.8
Mean	14.3	4.2	18.5	21.0	44.8	83.1	38.3
s.d.	5.73	0.71	6.24	3.53	7.54	14.73	7.33
N	5	5	5	5	5	5	5
King penguins							
K10049	63.3	9.8	73.1	48.4	103.3	224.8	121.5
K15095	67.1	10.2	77.3	50.7	108.2	236.3	128.0
K15116	44	9.7	53.7	48.2	102.9	204.9	102.0
K15121	36.5	7.3	43.8	36.0	76.9	156.7	79.8
Mean	52.7	9.2	62.0	45.9	97.9	205.7	107.8
s.d.	14.81	1.34	15.90	6.63	14.16	35.10	21.71
N	4	4	4	4	4	4	4
Emperor penguins							
EM0086	77.2	15.8	93.0	78.6	167.8	339.5	171.7
EM0087	73.6	16.9	90.5	84.1	179.5	354.2	174.7
EM0088	71.0	12.2	83.2	60.8	129.7	273.8	144.0
EM0090	94.7	13.1	107.8	65.0	138.8	311.6	172.8
EM0091	76.3	12.0	88.3	59.4	126.7	274.3	147.6
EM0092	73.7	14.9	88.6	74.0	158.0	320.7	162.7
Mean	77.8	14.2	91.9	70.3	150.1	312.3	162.2
s.d.	8.59	2.04	8.42	10.12	21.59	33.13	13.42
N	6	6	6	6	6	6	6

The extrapulmonary tracheobronchial volumes (from the most caudal point of the occipital bone to lung) were measured from CT scan data at 0 cm H_2O inflation pressure. The intrapulmonary primary bronchi volumes, the air capillary volumes and the parabronchial volumes were determined from lung volumes measured from CT scan data at 0 cm H_2O inflation pressure, and from prior lung morphology data (Maina and King, 1987; Maina and Nathaniel, 2001). Primary bronchi, air capillary and parabronchial volumes were assumed to be 3.56%, 17.67% and 37.31% of total lung volume, respectively. 'Rigid' air volume is the sum of volumes of the total TB tree, air capillaries and parabronchi.

washout techniques have been used to measure air sac volume during steady-state ventilation (Scheid et al., 1974), such techniques do not provide the largest volumes that might be attained during a maximum inspiration prior to the dive of a penguin. This is particularly significant in regard to the maximum capacity of the abdominal air sacs, which is considered to greatly exceed the typical volume during normal respiration (Scheid, 1979).

Therefore, in order to try to assess maximum inspiratory air sac volume in penguins, we chose to use the CT scan approach and to measure air sac and lung volumes at different inflation pressures while the penguins were anesthetized, apneic and in the supine (dorsal recumbency) position. Although the supine position is associated with a decrease in volume of the abdominal air sacs

because of their compression by the abdominal contents (King and Payne, 1964; Malka et al., 2009; Nevitt et al., 2014), this was the position preferred by the clinical veterinary staff. The supine position has also been often recommended in veterinary texts because the prone (sternal recumbency) position has been considered to restrict respiration in anesthetized birds (Jaensch et al., 2002; Malka et al., 2009). We also felt that positive pressure inflation to a pressure as high as 40 cm H_2O (3.92 kPa) would overcome this effect. In addition, differences in body position of anesthetized hawks resulted in only about a 5% change in lung volume determined by CT scan analysis (Malka et al., 2009).

As the birds did appear over-distended in both the neck and abdomen at the highest inflation pressure, those air sac volumes

Table 3. Estimation of maximum safe depth threshold for avoidance of barotrauma ('squeeze') in penguin lungs based on compression ratios of air sac volume to 'rigid' air volume

Species	Air sac:rigid volume		Air sac:AC+TBT		DAV:rigid volume		DAV:AC+TBT	
	Ratio	Depth (m)	Ratio	Depth (m)	Ratio	Depth (m)	Ratio	Depth (m)
Adélie	16.3	153	35.4	344	12.0	110	26.1	251
King	23.1	221	44.1	431	7.3	63	13.9	129
Emperor	24.0	230	46.0	450	9.4	84	18.1	171

Air sac volume is the mean value in each species from CT scan data in Table 1. This estimation assumes there is no compression at depth of the air capillaries, parabronchi or tracheobronchial tree. A similar calculation was performed for the ratio of air sac volume to the volume of the air capillaries and the tracheobronchial tree from Table 2. For comparison, ratios and depths are also calculated with the diving air volume estimated at the end of dives from swim speed–buoyancy calculations in each of these species (Sato et al., 2002, 2011). Because diving air volume includes air sac, lung, airway and feather layer air, the resulting ratios and depths are probably overestimates of safe depth thresholds if there is no compression of lung air volumes or tracheobronchial structures at depth. AC, air capillary; TBT, tracheobronchial tree; DAV, diving air volume.
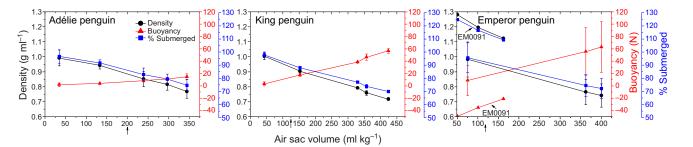
Fig. 5. Body density decreased and buoyancy increased as air sac volume increased with inflation pressure in the three species. These parameters were calculated on the basis of the known body mass of the birds and the total body volume and air sac volume measured at the different inflation pressures (Fig. 4). The percentage of the body submerged changed from >96% at the lowest mean air sac volume to 74% at the mean air sac volume at 30 cm H_2O (2.94 kPa) inflation pressure. Notably, the heaviest emperor penguin (EM0091) was more negatively buoyant at all air sac volumes measured [at as high as 20 cm H_2O (1.96 kPa) inflation pressure in that bird]. N values are as in Fig. 2, except that EM0091 is graphed separately in this figure. For comparison, arrows indicate end-of-dive air volume (respiratory air and feather layer air) estimated from buoyancy–velocity calculations in diving penguins (Sato et al., 2002, 2011).

were assumed to be above maximum inspiratory capacity. At 30 cm H_2O (2.94 kPa) pressure, the birds did not appear distended and the solid tissue masses of the birds were not significantly compressed; consequently, those 30 cm H_2O values were used as the maximum air sac volumes possible prior to a dive. In addition, the density, buoyancy and percentage of the body submerged were not unreasonable at this inflation pressure.

Lung volume

Lung volumes at 0 cm H_2O (kPa) were assumed to be representative of that at the start of a penguin's dive because the avian lung is considered relatively rigid, with the lung of the duck changing less than 2% in volume during spontaneous respiration (Macklem et al., 1979). As we had hypothesized, mass-specific lung volume was least in the deepest-diving species, although differences in mean mass-specific lung volumes were only significant between Adélie and emperor penguins (Table 1). In addition, the mass-specific lung volume of a Humboldt penguin was previously determined to be 30 ml kg^{-1} (Maina and King, 1987; Maina and Nathaniel, 2001), about 50% greater than those of the king and emperor penguins in this study. The Humboldt penguin routinely makes dives of <20 m with a maximum reported depth of only 54 m (Culik, 2001; Luna-Jorquera and Culik, 1999). This 30 ml kg^{-1} value in the Humboldt penguin is also supported by recent CT determinations of lung volume (Nevitt et al., 2014).

Smaller mass-specific lung volumes in deeper diving species would allow for lower air capillary and parabronchial volumes, and, thus, greater protection against barotrauma in the deeper divers. However, these differences in lung volume are probably most related to differences in body mass, metabolic rate and gas exchange requirements (Fig. 6). The regression equation for the data in Fig. 6, based upon previously collected data (Maina and Nathaniel, 2001) and the lung volumes in this study, was nearly identical to that determined in a prior allometric study (Lasiewski and Calder, 1971). Thus, although a larger body size and relatively smaller lung volume confer an advantage for deeper diving penguins in the avoidance of barotrauma, these differences among the species are probably most attributable to body mass and metabolic rate.

It is unknown whether greater protection against lung barotrauma is afforded by a further reduction in parabronchial and/or air capillary volumes in the deeper diving species. In the shallow-diving Humboldt penguin, the relative volumes of the parabronchi and air capillaries were among the lowest of the eight species examined (Maina and Nathaniel, 2001). The larger body sizes of

king and emperor penguins and the 'normal' metabolic scope (maximal O_2 consumption rate) of emperor penguins (Kooyman and Ponganis, 1994) are consistent with this possibility.

Our lung volume measurements suggest a third possible mechanism of protection against barotrauma. We expect that the penguin lung is relatively rigid as in other birds during spontaneous ventilation, but the lung expansion we observed at positive inflation pressures suggests to us that some compression of the lung may occur at depth. This is especially so in the deeper diving king and emperor penguins, in which lung volume increased by 25% at the highest inflation pressure, and lung compliance was approximately six times greater than in the shallower diving Adélie penguin. This expansion of the penguin lung during inflation presumably occurred primarily in the parabronchi and air capillaries because, as measured in the Humboldt penguin, the primary bronchi constitute less than 4% of the total lung volume, while the parabronchi/air capillaries constitute about 95% of the air volume in the lung (Maina and Nathaniel, 2001). For these reasons and the assumed greater rigidity and resistance to compression of air capillaries, we
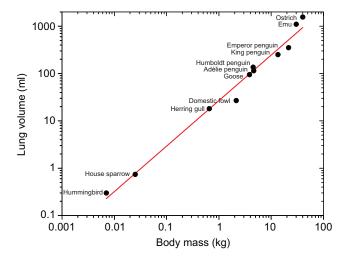

Fig. 6. Logarithmic plot of lung volume versus body mass from data in this study and those of other birds (Maina and Nathaniel, 2001). The regression line formula (log lung volume=1.42877+0.96172 log body mass; r^2=0.98, P<0.05) is nearly identical to that of Lasiewski and Calder (1971). Species names not listed in the text include the emu (*Dromiceius novaehollandiae*), goose (*Anser anser*), domestic fowl (*Gallus gallus*), herring gull (*Larus argentatus*), house sparrow (*Passer domesticus*) and hummingbird (*Colibri coruscans*).

suggest that compression and a reduction in the volume of the parabronchi may afford further protection against pulmonary barotrauma in deep-diving penguins.

Air sac volume

Air sac volume did not plateau but continued to increase with greater inflation pressure. As already reviewed, we considered the values at 30 cm H_2O (2.94 kPa) inflation pressure to represent a maximum air sac volume. These air sac values (Table 1) were approximately 2.2, 2.9 and 3 times the allometrically predicted values for these Adélie, king and emperor penguins, respectively (Lasiewski and Calder, 1971).

These measured values were also greater than even the highest calculated diving air volumes (respiratory and plumage air) in free-diving birds for any of the three species (Sato et al., 2002, 2011). However, the air volume calculations in free-diving penguins raise several questions. First, diving air volume estimations might underestimate the start-of-dive air volume if penguins exhaled prior to the final ascent, the time period of the data from which the calculations were performed. The variability in estimated air volumes, especially for deeper dives of emperor penguins, was consistent with this possibility (Sato et al., 2011).

One might also question whether these air sac volumes, determined during positive pressure ventilation in our study, are representative of an air volume that could be spontaneously inspired prior to a dive. Positive pressure ventilation is known to increase measured respiratory volumes by 30–70% in a paralyzed, ventilated duck versus a spontaneously breathing duck (Scheid and Piiper, 1969; Scheid et al., 1974). However, air volumes during spontaneous ventilation are not necessarily maximal inspiratory volumes, and penguins do dive on inspiration. In addition, in the free-diving lesser scaup, respiratory air volumes were about twice the value predicted by allometric equations (Stephenson, 1995). Even the total respiratory air volume of the spontaneously breathing duck was about 35% greater than that predicted by allometric equations (Lasiewski and Calder, 1971; Scheid et al., 1974). And lastly, although we do not know how penguins might accomplish this, perhaps for deep dives there might also be an avian equivalent of 'lung packing' in human divers (Lindholm and Lundgren, 2009).

The percentage of the body submerged at different air sac volumes was also supportive of air sac volumes as large as those measured at 30 cm H_2O (2.94 kPa). Based on our calculations and measurements, about 74% of the body would be submerged at these high air sac volumes in all of the species. Although observations of penguins at the surface prior to their deepest dives have not been documented, emperor penguins, even at rest in an ice hole, can, at times, float considerably above the water (Fig. 7).

Baroprotection: potential mechanisms

The mechanisms contributing to the avoidance of barotrauma to the 'rigid' lungs of diving birds are unresolved. The structure and composition of the air capillaries, parabronchi and surrounding tissue as well as attachments to the chest wall contribute to the overall rigidity of the avian lung (Maina et al., 2010a,b; Maina, 2007a,b). Lung volume changed less than 2% during spontaneous respiration in the duck (Jones et al., 1985). Furthermore, although the parabronchi do have some compliance, there is no evidence, albeit under mild compression, that the air capillaries collapse (Macklem et al., 1979). Collapse of the air capillaries has also long been considered unlikely because of their probable inability to re-expand secondary to their small diameter and radius of curvature (Duncker, 1974, 1972). Given that lung air volume is about 10% of the total respiratory air volume (Duncker, 1974; Powell, 2015;

Fig. 7. Emperor penguins floating at rest in an ice hole near the ice edge at Cape Washington, Antarctica. A considerable percentage of the body can be above the water surface, consistent with calculations of body density, buoyancy and the percentage of the body submerged (Fig. 5). The potential contribution of changes in the air volume of the feather layer in such situations is unknown.

Scheid, 1979), it has been previously postulated that some compensatory mechanism must occur in birds diving deeper than 90–100 m (Croll et al., 1992).

A larger ratio of air sac-to-lung volume has been proposed as a possible protective mechanism in diving birds (Croll et al., 1992). Indeed, based on morphometry data, total air volume in the lung of the Humboldt penguin is less than in non-diving species: about 59% compared with 65–75% of lung volume (Duncker, 1972; Maina and Nathaniel, 2001). More importantly, it is the volume of the relatively rigid parabronchi and, especially, the non-collapsing air capillaries of the lung that is most critical in this ratio and in the avoidance of lung squeeze. Air capillary and parabronchial volumes were about 18% and 38% of total lung volume, respectively, in the Humboldt penguin (Maina and King, 1987; Maina and Nathaniel, 2001). As a percentage of total lung volume, air capillary volume in the penguin was in the lower range of values of seven species, while parabronchial volume was the lowest, except for that of the ostrich (*Struthio camelus*).

Thoracic pooling of blood has also been proposed as a possible protective mechanism in diving birds (Kooyman et al., 1999). Certainly, blood capillaries constitute a large percentage of the gas exchange tissue in the penguin lung (Maina and Nathaniel, 2001). However, recent studies of the compliance and architecture of avian pulmonary blood capillaries (Watson et al., 2008; West, 2009; West et al., 2010) would argue against expansion of pulmonary blood capillaries into the air capillary space. Alternatively, if there were venous sinuses in the parabronchial mucosal walls of penguins, distention of these veins during dives might act to fill space and protect against barotrauma in the parabronchi.

Another potential mechanism of decreasing parabronchial volume at depth is through smooth muscle contraction and a decrease in the diameter of the parabronchial lumen. Such constriction is feasible given that smooth muscle cells do underlie the parabronchial endothelium (Duncker, 1972; Scheid, 1979; Welsch and Aschauer, 1986). It is notable that parasympathetic stimulation via the vagus nerve resulted in parabronchial constriction in both ducks and geese (Barnas et al., 1978; King and Cowie, 1969).

For the avoidance of pulmonary barotrauma, the volume of the tracheobronchial tree must also be considered in addition to that of the air capillaries and parabronchi of the lung. Just as alveolar compression in mammals at depth forces air into the more rigid bronchi and trachea (Kooyman et al., 1970; Scholander, 1940),

compression of penguin air sacs presumably forces air into the bronchi and trachea as well as into the lung. And tracheal volumes of birds are 4.5 times greater than those of mammals (Hinds and Calder, 1971).

Despite movement of air from alveoli to the trachea and bronchi in diving seals, tracheal volume does decrease at depth; tracheal diameter decreased by 50% during simulated dives in a pressure chamber to 306 m depth (Kooyman et al., 1970). Penguin tracheas may also decrease in volume although the biomechanical properties of their tracheas and bronchi have not been investigated. There are also other potential compensatory mechanisms to avoid tracheal barotrauma. Submucosal veins in the walls of cetacean and sea turtle tracheas have been postulated to become engorged at depth, thus decreasing tracheal volume and providing protection (Cozzi et al., 2005; Davenport et al., 2013, 2009, 2014; Ninomiya et al., 2005). It is unknown whether such submucosal venous plexuses exist in the tracheal walls of penguins. It is notable, however, that engorgement of venous plexuses in the middle ears of king penguins is thought to provide baroprotection to the ear at depth just as it does in marine mammals (Odend'hal and Poulter, 1966; Sadé et al., 2008).

Because of the importance of the volumes of the air capillaries, parabronchi and tracheobronchial tree in the avoidance of pulmonary barotrauma, we calculated the ratio of maximum air sac volume to the summed volumes of those three components of the penguin respiratory system.

Ratio of air sac volume to air capillary+parabronchial+tracheobronchial volume

The air sac volume to air capillary+parabronchial+tracheobronchial volume ratios in all three species of penguins (Table 3) do not account for the prevention of barotrauma to the rigid air capillaries at the maximum dive depths reported for these birds. On a theoretical basis, compression of the maximal inspiratory air sac volume [30 cm H_2O (2.94 kPa) inflation pressure] to a volume less than that of the air capillaries, parabronchi and tracheobronchial tree would require depths of only 153, 221 and 230 m in Adélie, king and emperor penguins, respectively. These depths are less than the maximum reported depths of these animals, and far less than many routine dives of king and emperor penguins. Similar ratio analyses utilizing the diving air volumes from buoyancy–swim speed calculations resulted in even shallower 'safe' depth thresholds for the prevention of barotrauma (Table 3).

How then is barotrauma prevented in the 'rigid' avian lung? We propose several potential mechanisms based upon the reviews in previous paragraphs. First, the parabronchi may be more compliant than thought, and may decrease in volume because of compression by increased ambient pressure during a dive. Second, the parabronchi may be constricted at depth through parabronchial smooth muscle contraction induced by the intense parasympathetic activation required to produce heart rates as low as 10 beats min^{-1} during deep dives (Barnas et al., 1978; Wright et al., 2014). Third, similar to seals, the trachea may be compressed to some degree in deep-diving penguins. And fourth, if large submucosal veins exist in the walls of the parabronchi, bronchi or trachea, blood engorgement of those veins could occupy space and provide baroprotection at depth. As evidenced by the calculation of the ratio of air sac volume to air capillary+tracheobronchial volume in Table 3, a decrease in volume equivalent to that in the parabronchi would greatly increase the 'safe' depth threshold in all three species and account for pulmonary baroprotection in almost all dives.

In addition, it is possible that air capillary and parabronchial volumes in deeper diving penguin species are smaller (as a percentage of lung volume) than in the shallow-diving Humboldt penguin. Lung morphometry studies would be extremely valuable in this regard.

Although these processes may contribute to the avoidance of pulmonary barotrauma in penguins, there is still the unresolved question of how penguins avoid excess nitrogen absorption at depth and minimize the risk of decompression sickness and nitrogen narcosis. Our data and proposals do not directly address this issue. As already stated, the available evidence does not support the occlusion of air capillaries by engorgement of pulmonary blood capillaries at depth. There are several alternative possibilities. First, with documented heart rates as low as 10 beats min^{-1}, the resulting reduction in cardiac output through the lung should lessen nitrogen absorption (Wright et al., 2014). Second, intrapulmonary shunting may occur at depth, especially at low heart rates. Intrapulmonary shunts, estimated at 28% in emperor penguins at rest, decreased by 50% during the tachycardia prior to diving (Meir and Ponganis, 2009). It is possible the percentage shunt increases even more during the severe bradycardias at maximum depth in emperor penguins. Although pathways and mechanisms for such shunting are not known (Makanya and Djonov, 2009; West et al., 1977; Woodward and Maina, 2008), it is notable that a relatively large blood volume has been reported in the penguin lung (Maina and King, 1987; Maina and Nathaniel, 2001).

Respiratory O₂ stores

In regard to O_2 stores, contrary to our hypothesis, the mass-specific air sac volumes measured at 30 cm H_2O inflation pressure were greater in deeper divers. Prior measurements of diving air volumes, whether in simulated or free dives, had always resulted in smaller mass-specific total air volumes and respiratory O_2 stores in deeper divers (Kooyman et al., 1973; Ponganis et al., 1999; Sato et al., 2002, 2011). The greater respiratory air volumes in the deeper diving penguins also contrast with the pattern observed in marine mammals, in which respiratory air volumes were decreased in deeper divers (Ponganis et al., 2011).

Although start-of-dive respiratory volumes are still not documented in penguins, if initial air sac volumes for deep dives are near the maximum limit we suggest, the respiratory O_2 store and total body O_2 store would increase significantly. In the emperor penguin, for example, based on the difference between 117 ml air kg^{-1} [mean maximum end-of-dive value in free-diving birds (Sato et al., 2011)] and 381 ml air kg^{-1} (lung volume+air sac volume; Table 1), total body O_2 stores would increase from 68 to 119 ml O_2 kg^{-1}, and the respiratory O_2 store would increase from 33% to 61% of total.

Body density and buoyancy

The CT scan volume measurements and the body masses of the birds allowed novel calculations of body density, buoyancy and percentage of the body submerged for different air sac volumes in live penguins. Most such measurements and calculations in diving birds have involved analyses of carcass materials and assumptions as to respiratory and feather air volumes. Body density and buoyancy of ducks and guillemots were calculated to be near 0.7 g ml^{-1} and 3–5 N, respectively (Lovvorn et al., 1999; Lovvorn and Jones, 1991). Similar buoyancies were estimated in a variety of alcids and the little blue penguin (*Eudyptula minor*) (Watanuki et al., 2006). In another study including auks, loons, cormorants and spheniscid penguins, body density and buoyancy were calculated to be 0.99–1.03 g ml^{-1} and 3–5 N, respectively (Wilson et al., 1992). The estimates in the spheniscid penguin utilized a respiratory air

volume of 160 ml kg^{-1}, and a feather layer air volume of 200 ml kg^{-1}. In contrast, estimates of feather layer air volume in Adélie penguins were much smaller, near 17 ml kg^{-1}, and maximum respiratory air volume was also greater, near 200 ml kg^{-1} (Kooyman et al., 1973; Sato et al., 2002, 2006). There is clearly a large range of estimated values in these difficult-to-measure variables. We suggest that 3D reconstructions from CT scans provide another approach that may allow accurate estimation of total body density and buoyancy at measured respiratory air volumes.

At our suggested upper limit for air sac volume, body density was near 0.7 g dl^{-1} for all three species and buoyancy was near 15 N for Adélie penguins and 50 N for king and emperor penguins. Although such buoyancies, especially for king and emperor penguins, are higher than typically estimated for small penguins, buoyancy is greater in larger animals (Miller et al., 2004; Skrovan et al., 1999), and emperor and king penguins are much larger birds than those other avian species in which buoyancy has been previously estimated. In addition, greater buoyancy could probably be rapidly diminished by a quicker descent and faster stroke rate at the start of deeper dives (Williams et al., 2012). Lastly, field observations of floating penguins (Fig. 7) were consistent with our calculated percentage of the body submerged at the surface, again suggesting that such large air volumes and buoyancies are at times possible.

It was also remarkable that the heaviest emperor penguin in the study (EM0091, 4–5 kg greater in mass than the other emperor penguins) was negatively buoyant even at an air sac volume of 162 ml kg^{-1} (Fig. 5). This was the air sac volume at 20 cm H$_2$O (1.96 kPa) inflation pressure, the highest inflation pressure used in this animal. Presumably, near-neutral buoyancy would occur at higher air sac volumes in this bird. Notably, EM0091 also had the lowest fat volume (1628 ml versus 3613±1609 ml for the other five birds, CT analysis data not shown). Such high body densities and low buoyancies demonstrate the potential confounding effects that changes in body mass and composition may have on buoyancy throughout the annual cycle of an emperor penguin or even during a foraging trip. For example, the additional 2.5 kg of food in the stomach of an emperor penguin returning from a foraging trip (Sato et al., 2011) will not only limit expansion of the air sacs but also increase the mass and body density of the bird.

Conclusions

CT scan analyses and 3D anatomical reconstructions of live penguins have allowed determination of lung volume, air sac volume, total body volume, body density and buoyancy in Adélie, king and emperor penguins. The lung volume of penguins scaled allometrically with those of other birds, and resulted in a decreased risk of pulmonary barotrauma in deeper diving species because of the lower lung, parabronchial and air capillary volumes in these larger animals. [The last two volumes were calculated on the basis of previously published morphometric data (Maina and King, 1987; Maina and Nathaniel, 2001)].

The air sac volume at 30 cm H$_2$O (2.94 kPa) was assumed to be the maximum air sac volume prior to a deep dive. Although two to three times allometric predictions of resting air sac volume and greater than estimated end-of-dive air volume, these values were consistent with prior studies of respiratory air volumes in free-diving ducks. In addition, the body volumes, densities and buoyancies determined at this inflation pressure were reasonable.

Air sac volumes at 30 cm H$_2$O (2.94 kPa) inflation pressure resulted in air sac to air capillary+parabronchial+tracheobronchial volume ratios that would not prevent pulmonary barotrauma in the

deepest dives of all three species and in many dives of king and emperor penguins. This suggests that prevention of pulmonary barotrauma in deep-diving penguins requires a decrease in parabronchial and/or tracheobronchial volume. Such a volumetric decrease might be achieved through compression, smooth muscle constriction and/or venous engorgement.

If air sac volume prior to the deepest dives of penguins is equivalent to the values at 30 cm H$_2$O (2.94 kPa) inflation pressure, the magnitude and distribution of the body O$_2$ stores would change significantly. In the emperor penguin, the total body O$_2$ store would increase from 68 to 119 ml O$_2$ kg^{-1}, and the respiratory store from 33% to 61% of total.

Although the maximum air sac volume prior to a dive remains unknown, higher values, as measured in this study, would afford greater baroprotection and a larger O$_2$ store. The increase in buoyancy due to larger air volumes was not exceptional or unrealistic, and would be further reduced with descent to depth. Further evaluations of baroprotective mechanisms and the size of the respiratory O$_2$ store await future investigation of species-specific lung morphometry, start-of-dive air volumes and body buoyancy, and the possibility of air exhalation during dives.

MATERIALS AND METHODS

For CT procedures, penguins were transported and maintained before/after scans in a refrigerated truck. After induction of general isoflurane-O$_2$ anesthesia with mask induction and intubation (Ponganis et al., 2009, 2007), the birds were maintained under intermittent manual ventilation and hyperventilated prior to each scan so that they were apneic for the duration of the scan (about 1 min). Inflation pressure during the scan was managed with the use of the adjustable pressure-limiting valve (APL or pop-off valve) of the anesthesia circuit and monitored with use of a pre-calibrated pressure gauge. Inflation pressures were 0, 10, 20, 30 and 40 cm H$_2$O (0, 0.98, 1.96, 2.94 and 3.92 kPa). After extubation and recovery from anesthesia, each penguin was returned to its transport box and the refrigerated truck.

CT scans of the Adélie penguins were performed using a GE 64 CT scanner (General Electric Healthcare, Chalfont St Giles, Bucks, UK), and those of the king and emperor penguins with a Somoton Emotion 6 (Siemens, Munich, Germany). Imaging was performed using pediatric lung protocols (80–120 kV, ~100 mA). Between 948 and 970, 0.625 mm thick slices were acquired from each Adélie penguin; between 664 and 854, 1.25 mm thick slices were acquired from each king penguin, and between 854 and 1051, 1.25 mm slices from each emperor penguin.

The imaging data were manually segmented using Amira software (FEI, Burlington, MA, USA) based on tissue radiodensity and a priori knowledge (Scadeng et al., 2007). The datasets were divided into the following structures: lungs, trachea, anterior air sacs (interclavicular, anterior thoracic, cervical), posterior air sacs (abdominal, posterior thoracic) and solid tissue (fat, muscle, internal organs, skeleton, skin, head). Tracheal volume was measured from the most caudal point of the occipital bone (skull) to the tracheal bifurcation. The volume of an individual structure was calculated based on known voxel volume×number.

Data were analyzed and graphed in Excel$^®$ and Origin$^®$. Lung and air sac compliances were calculated as the difference in volume between 0 and 30 cm H$_2$O (0 and 2.94 kPa). After testing for normality of distribution (Shapiro–Wilk), species differences for mass-specific lung volumes, mass-specific air sac volumes, and lung and air sac compliances were evaluated with one-way ANOVA and Bonferroni comparison of means, with a significance level of $P<0.05$. All data are expressed as means±s.d. unless otherwise stated.

Body density was calculated by dividing body mass by the total body volume determined through the CT scan analysis. This total body volume did not include the feather layer volume, and, therefore, the result might be considered to be a maximum body density. The feather volume was excluded because of the unknown effects of both anesthesia and the relatively warm ambient temperature in the scanner room on feather erection and the volume of the feather layer. In addition, in the Adélie penguin,

feather layer air volume has been determined to be only 17 ml kg^{-1} (Kooyman et al., 1973); this would have little effect on total body volume as it would, on average, add less than 80 ml to the ≥4000 ml body volumes of the Adélie penguins in this study (Fig. 4).

As previously described (Sato et al., 2002), buoyant force (N) was calculated as g×body mass×[($\rho_{seawater}/\rho_{total\ body}$)−1], where g=9.807 N, body mass is in kg, $\rho_{seawater}$ is the density of seawater at 10°C (=1.027 g ml^{-1}) and $\rho_{total\ body}$ is the density (g ml^{-1}) of the penguin body measured from the body volume CT scan and body mass. The percentage of the body submerged at the surface=($\rho_{object}/\rho_{seawater}$)×100.

All CT scan procedures and transport/import of emperor penguins were conducted under UCSD IACUC protocol S11064, an Antarctic Conservation Act Permit, USDA Import Certificate and APHIS approval, and New Zealand Biosecurity (MAF)/EPA permits.

Author contributions
P.J.P., M.S. and J.S.L. conceived the study. P.J.P. and J.S.L. conducted the penguin transport. M.S. directed and analyzed CT scans; P.J.P. and J.S.L. directed/conducted transport/anesthesia for scans. P.J.P. analyzed the data. P.J.P., M.S. and J.S.L. reviewed and wrote the paper.

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