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SHORT COMMUNICATION

In vivo measurement of lung volume in ringed seals: insights from biomedical imaging

Holly Hermann-Sorensen[1], Nicole M. Thometz[2,3], Kathleen Woodie[4], Sophie Dennison-Gibby[5] and Colleen Reichmuth[3,4,*]

ABSTRACT

Marine mammals rely on oxygen stored in blood, muscle and lungs to support breath-hold diving and foraging at sea. Here, we used biomedical imaging to examine lung oxygen stores and other key respiratory parameters in living ringed seals (*Pusa hispida*). Three-dimensional models created from computed tomography (CT) images were used to quantify total lung capacity (TLC), respiratory dead space, minimum air volume and total body volume to improve assessment of lung oxygen storage capacity, scaling relationships and buoyant force estimates. The results suggest that lung oxygen stores determined *in vivo* are smaller than those derived from postmortem measurements. We also demonstrate that, whereas established allometric relationships hold well for most pinnipeds, these relationships consistently overestimate TLC for the smallest phocid seal. Finally, measures of total body volume reveal differences in body density and net vertical forces in the water column that influence costs associated with diving and foraging in free-ranging seals.

KEY WORDS: *Pusa hispida*, Buoyancy, Computed tomography, Diving physiology, Total lung capacity

INTRODUCTION

A key question in comparative physiology is how air-breathing vertebrates remain active under water for long periods on a single breath (Butler and Jones, 1997; Ponganis, 2015; Scholander, 1940). To support diving, marine mammals rely on oxygen reservoirs compartmentalized in blood, muscle and lungs. Blood and muscle oxygen stores are well studied in marine mammals relative to lungs. This can be attributed to reduced dependence on pulmonary oxygen stores in marine mammals relative to that in terrestrial species, as well as the difficulty of obtaining quantitative measurements from living, freely diving individuals (Ponganis and Williams, 2016).

Standard metrics of respiratory function include minimum air volume (MAV) and total lung capacity (TLC). MAV is the minimum volume of air in relaxed lungs (Fahlman et al., 2011; Kooyman, 1973), while TLC refers to lung volume at maximum inhalation or when manually inflated to a standard air pressure of 30 cm H_2O or 22 mmHg (Denison et al., 1971). TLC is not easily determined in living animals; for this reason, several techniques are employed to determine other respiratory parameters, which are then used to estimate TLC (Wanger et al., 2005). These methods include nitrogen washout (Sue, 2013), whole-body plethysmography (Kooyman et al., 1972; Lenfant et al., 1970) and various respirometry approaches (Scholander, 1940). In addition, allometric scaling relationships derived from empirical measurements enable estimation of TLC from body mass when species data are not available. For marine mammals, scaling relationships reported by Kooyman (1973, 1989) are used; however, these have not been updated recently, and source data for TLC often include pooled values of mixed age classes. Even so, this approach has been used ubiquitously with the assumption that estimates of TLC will hold across a wide range of body sizes and age classes.

TLC and MAV can be measured postmortem (Burns et al., 2007; Denison et al., 1971; Fahlman et al., 2011; Kooyman and Sinnett, 1979; Lydersen et al., 1992; Mitchell and Skinner, 2011) by excising the complete respiratory tract and measuring associated water displacement in both non-inflated (resting) conditions (i.e. MAV) and inflated conditions (i.e. TLC). The difference in displacement between each condition is related to the volume of the respiratory tract. Researchers interested in mammalian diving physiology rely on these postmortem estimates despite there being little information regarding the reproducibility of *ex situ* values in living animals (Fahlman et al., 2020b), with only a few studies directed at comparing pulmonary function and positioning both *in situ* and *ex situ* (Chevalier et al., 1978; Fahlman et al., 2014; Soutiere and Mitzner, 2004; Standaert et al., 1985).

Biomedical imaging has emerged as a valuable tool to examine comparative respiratory anatomy (Denk et al., 2020; Moore et al., 2011; Ponganis et al., 1992; Smodlaka et al., 2009), including the air reservoirs within living animals such as mice (Mitzner et al., 2001), dogs (Chevalier et al., 1978) and seabirds (Nevitt et al., 2014; Ponganis et al., 2015). Air spaces can be visualized and quantified using three-dimensional reconstructions of respiratory structures in both postmortem and living, anesthetized individuals. Importantly, this approach also allows for calculation of body volume (Ponganis et al., 2015), which can be used to evaluate body density and buoyancy.

We used computed tomography (CT) imaging data obtained during routine veterinary procedures to examine *in vivo* lung volume, lung capacity and whole-body buoyant force in living ringed seals (*Pusa hispida*). Their small body size and ease of handling enabled high-resolution volumetric quantification of discrete respiratory structures, including the anatomical dead space and individual lungs, as well as whole-body volume. We report respiratory parameters for the smallest phocid species, provide insight into the applicability of allometric scaling relationships, and discuss ecological implications of our findings for free-ranging seals.

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[1]University of California Santa Cruz, Department of Ocean Sciences, 115 McAllister Way, Santa Cruz, CA 95060, USA. [2]University of San Francisco, Department of Biology, 2130 Fulton Street, San Francisco, CA 94117, USA. [3]University of California Santa Cruz, Institute of Marine Sciences, 115 McAllister Way, Santa Cruz, CA 95060, USA. [4]Alaska SeaLife Center, 301 Railway Ave, Seward, AK 99664, USA. [5]Televet Imaging Solutions, PLLC, Oakton, VA 22124, USA.

*Author for correspondence (coll@ucsc.edu)

H.H-S., 0000-0001-7345-2129; N.M.T., 0000-0002-5035-5219; K.W., 0000-0003-3270-4099; C.R., 0000-0003-0981-6842

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MATERIALS AND METHODS

Subjects and animal handling

One female and three male subadult ringed seals, *Pusa hispida* (Schreber 1775), were evaluated. Age was estimated from the length, mass and overall development of each individual at intake for rehabilitative care at the Alaska SeaLife Center (Seward, AK, USA). Length and mass were determined within 1 week of the CT procedure. Standard length (linear distance from nose to tail) was either directly measured on the day of the CT procedure or measured from full-body scans. Animal mass was obtained via a platform scale (W.C. Redmon Co., Peru, IN, USA; or Ohaus SD751, Ohaus Corp., Parsippany, NJ, USA). Two individuals (PH1701 and PH1804) presented with verminous pneumonia at intake and were treated with anti-helminthic drugs during rehabilitation, with resolution prior to imaging. Thus, the scans included in this study represent healthy individuals cleared of parasites, with no clinical evidence for lungworm infection present at the time of the scans.

Seals were briefly restrained at the Alaska SeaLife Center and given a pre-anesthetic intramuscular injection of midazolam (0.2–0.5 mg kg^{-1}) and butorphanol (0.24–0.7 mg kg^{-1}) (see Woodie et al., 2020). Following sedation, a single lumen central venous catheter (16–18 g, 13–15 cm) was placed in the epidural vertebral sinus flushed with heparinized saline and capped as in Goertz et al. (2008). Patency of the soft catheter was ensured prior to transport to the nearby imaging facility. Prior to the CT procedure, propofol (2–3 mg kg^{-1}) was administered intravenously via the catheter to allow for intubation and inflation of an endotracheal tube cuff. Seals were maintained on oxygen and isoflurane gas for the duration of the procedure. Full inflation of the cuff prevented air leakage around the tube. Supplemental intravenous propofol was titrated incrementally to facilitate intentional apneic intervals during scanning with manual, intermittent, positive-pressure ventilation prior to and following each imaging series. A non-steroidal anti-inflammatory medication (meloxicam, 0.2–0.5 mg kg^{-1}), and broad-spectrum antibiotic (cefazolin, 10–20 mg kg^{-1}) were administered intravenously via the catheter. Following the CT procedure, sedation was reversed with separate injections of intramuscular or intravenous naltrexone (2 mg naltrexone per 1 mg butorphanol) and intravenous flumazenil (1 mg flumazenil per 20 mg midazolam). The endotracheal tube was removed after regular spontaneous respirations resumed. Following extubation, seals were returned to the Alaska SeaLife Center where they resumed normal eating and activity within an hour. The duration of anesthesia was less than 1 h from propofol induction to recovery and extubation.

CT scans were performed with a GE 16 Light Speed Scanner, GE 16 Bright Speed Scanner (General Electric Healthcare, Chalfont St Giles, Bucks, UK), or a Siemens 32/64 Somatom GO-UP Scanner (Siemens, Munich, Germany). Modified thorax protocols (Table S1; S.D.-G., unpublished) were used to obtain optimized images of the full respiratory tract with slice thickness of 0.625–2.5 mm. An initial scan was obtained on two seals (PH1802 and PH1804) in sternal recumbency without lung inflation during apnea, with the pressure gauge of the anesthesia circuit at 0 mmHg. This condition was defined as the resting, relaxed position of the lungs when the seal was out of water. All seals were scanned in sternal recumbency with lungs hyperinflated to a pressure of 30 mmHg. To test for replicability of lung volume at a given pressure, variation in volume within inflation conditions, and the difference in volume as a result of patient position, one seal (PH1802) received additional scans in both dorsal and sternal recumbency at inflation pressures of both 30 and 37 mmHg.

Animal handling activities including rescue, rehabilitation and diagnostic CT procedures were authorized under NOAA's Marine Mammal Health and Stranding Response/Research Program 18786, Stranding Agreement SA-AKR-2019-01, and marine mammal research permit 18902. Research was approved by the Institutional Animal Care and Use Committees at the University of California Santa Cruz and the Alaska SeaLife Center.

Volumetry

To determine key respiratory parameters at known lung inflation conditions, DICOM images from CT series were imported to 3D Slicer (Fedorov et al., 2012; https://www.slicer.org/) and converted into closed-surface three-dimensional models. Anatomical structures were manually separated into volumetric segmentations of trachea, bronchi, and left and right lungs based on tissue attenuation (Fig. 1). Tracheal volume was defined from the image immediately caudal to the laryngeal cartilages extending to the image of the cranial margin of the carina. Bronchial volume was defined as the region from the carina to distal portions of the cranial and caudal lobar bronchi. Bronchioles were too diffuse to manually trace, so their volume is included in the volume of the lungs. TLC included both the tissue and air spaces of the left and right lungs, in addition to the bronchioles. The inclusion of the tissue and air spaces is in line with other studies (Lydersen et al., 1992) with which we compared our values. The volume of each segment was calculated in cubic centimeters and converted to milliliters.

Segments of the respiratory tract were considered with respect to whether surfaces were available for oxygen exchange. Anatomical dead space (the portion not in contact with gas exchange surfaces) was defined as the volume of the trachea plus the volume of the bronchi; this measure is not equivalent to respiratory dead space (Fowler, 1948; Rossier and Bühlmann, 1955) as the bronchioles could not be partitioned from the tissues of the lungs in this study. MAV was characterized here as the lung volume in the non-inflated (resting) condition, with inflation pressure of 0 mmHg. Because individuals were measured out of water, we presume this metric will differ somewhat from MAV values obtained from seals resting in water at the surface (Fahlman et al., 2020a). TLC was determined as the volume of the inflated lungs at 30 mmHg. This pressure is higher than the standard of 22 mmHg (30 cm H_2O) used to measure TLC in other mammalian studies (Denison and Kooyman, 1973; Denison et al., 1971; Kooyman and Sinnett, 1982; Loring et al., 2016; Moore et al., 2011; Weibel, 1973), but was necessary for the clinical diagnostic protocol. Specifically, the ringed seals' lungs were hyperinflated to ensure that no atelectasis (partial or full collapse) or scarring of lung tissue was present. To obtain the proportion of blubber that contributed to total body volume, blubber was segmented and quantified for the two animals for which we had

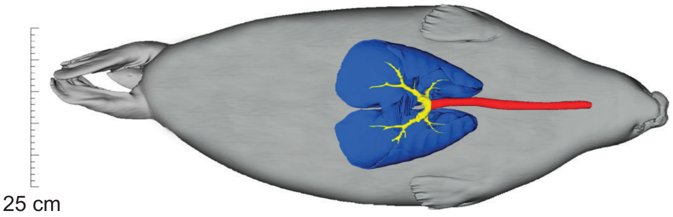

Fig. 1. 3D reconstruction of ringed seal PH1802 at a lung inflation pressure of 30 mmHg. Ventral side of animal shown with body contour in gray. The trachea is red, bronchi are yellow and lungs are blue. See Movie 1 for 3D reconstruction in both non-inflated and inflated conditions. (color online).

whole-body scans (PH1802 and PH1804). Further, surface area to volume ratio (SA:V) was directly measured for seal PH1802.

Allometry

To compare our results with those of other marine mammals, we considered the commonly used allometric scaling equation: TLC=$0.1M_b^{0.96}$ (Kooyman, 1989) (where M_b is body mass). We also determined another scaling equation specific to pinnipeds. The TLC data included in the pinniped-only allometric plot either were collected empirically or could be calculated from empirically reported mass-specific total lung oxygen stores. We evaluated our primary measure of mean TLC as a function of mean total body mass for the subadult ringed seals in our study. We then compared our results with expected values from these allometric relationships to determine whether total body mass is a reliable indicator of total lung capacity for ringed seals.

Body density and buoyant force

CT data were further used to estimate the whole-body buoyant force of two seals (PH1802 and PH1804) at specific lung inflation pressures, as in Ponganis et al. (2015). Body density was calculated by dividing body mass by total body volume and comparing this with the density of seawater. Total body volume (ml) was determined by segmentation of the CT data as described above. Whole-body buoyant force (N) was calculated for each seal at each lung inflation pressure:

$$\text{Buoyant force} = \boldsymbol{g} \times M_b \times (\rho_{\text{seawater}}/\rho_{\text{total body}}), \tag{1}$$

where $\boldsymbol{g}$ is the acceleration of gravity at 9.807 m s^{-2}, M_b is body mass in kg, ρ_{seawater} is the density of seawater at 10°C in g ml^{-1}, and $\rho_{\text{totalbody}}$ is the calculated density of the seal's body in g ml^{-1}. The corresponding downward (gravitational) force (N) was also determined:

$$\text{Downward force} = (\boldsymbol{g} \times M_b). \tag{2}$$

Net (total) force was determined by subtracting the downward force (Eqn 2) from the buoyant force (Eqn 1). Buoyant force was only calculated in the inflated condition for seal PH1804, as there was no full-body scan available in the non-inflated condition. Buoyant force was calculated in both inflated and non-inflated conditions for seal PH1802, and total body volume was compared at the level of the whole animal relative to changes in the respiratory tract volume.

RESULTS AND DISCUSSION

Volumetric measurements

Primary comparisons of respiratory structures and volumetric analyses were made at lung inflation pressures of 0 and 30 mmHg in sternal recumbency (Table 1). When inflated to 30 mmHg, TLC ranged from 870 to 2271 ml, resulting in mass-specific values between 52 and 92 ml kg^{-1}. The right lung was larger than the left in all individuals in the inflated condition, with an average size difference of 6.3%. For two individuals measured in the non-inflated condition, MAV was 564 and 886 ml, with mass-specific values of 22 and 32 ml kg^{-1}. Lung volume for these individuals increased by a factor of 2.5 when fully inflated.

Maximum respiratory tract volume was 904–2323 ml. This was equivalent to 11% and 18% of total body volume for the two seals with full-body scans (PH1804 and PH1802). The anatomical dead portion of the respiratory tract changed little with inflation for two individuals with comparable non-inflated and inflated scans (PH1503 and PH1802). These seals exhibited similar increases in tracheal volume (∼15%) and negligible increases in bronchi volume (∼1%) from non-inflated to inflated conditions. Thus, while the volume of the total respiratory tract changed by an average factor of 2.5 when the lungs were inflated, most of this difference was due to changes in lung volume.

Replicate scans in sternal recumbency at 37 mmHg for seal PH1802 showed that TLC varied by 5% (74 ml) between scans. Lung volume varied similarly between 30 and 37 mmHg, with an increase of 5% (75 ml) at the higher inflation pressure. When hyperinflated to 37 mmHg, lung volume was 13% (194 ml) greater in dorsal recumbency than in sternal recumbency.

Full-body scans for seal PH1802 were evaluated in both inflated and non-inflated conditions to determine changes in respiratory tract volume and total body volume. The increase in respiratory tract volume was 815 ml. In contrast, total body volume in the inflated condition increased by only 446 ml, equivalent to a 2% increase in body volume. The directly measured surface area of this seal was 58,193 cm^2 and its total body volume was 25,004 cm^3, resulting in a SA:V of 2.3:1. For the two seals for which total blubber volume could be measured from CT scans, blubber by total body volume was 33% (PH1804) and 49% (PH1802).

Allometric relationships

The scaling equation we determined for pinnipeds using previously published values (TLC=$0.1M_b^{0.98}$) is remarkably close to the classic relationship reported by Kooyman (1989) for marine mammals (Fig. 2). Indeed, the Kooyman (1989) equation falls within the 95% confidence interval of the pinniped-only equation, suggesting that this offset is not significant. Source data for the pinniped-only relationship are provided in Table S2 (Burns et al., 2007; Falke et al., 2008; Kooyman and Sinnett, 1982; Lenfant et al., 1970; Lydersen et al., 1992; Reed et al., 1994). The ringed seals in this study are the smallest pinnipeds for which TLC data are now available. When compared with the scaling relationships described above, our *in vivo* measurements obtained from ringed seals are about 27% lower than predicted.

Body density and net buoyant force

Body density and buoyant force were calculated from the measured total body volume of one individual (PH1804) in the inflated condition, and another individual (PH1802) in both non-inflated and inflated conditions. Taking body mass into account, both seals exhibited similar body density irrespective of inflation condition. Individual PH1802 was denser than seawater (1.027 g ml^{-1} at 10°C) at both 0 mmHg (1.052 g ml^{-1}) and 30 mmHg (1.033 g ml^{-1}) inflation conditions, whereas seal PH1804 was less dense than seawater (0.989 g ml^{-1}) in the inflated condition. Based on these measurements PH1802 had negative net vertical forces in both non-inflated (−6.0 N) and inflated (−1.3 N) conditions. In contrast, PH1804 had a positive net force of 7.8 N in the inflated condition.

Physiological and ecological considerations

The anatomical dead space of ringed seals comprised only 3% of total respiratory tract volume and changed little between non-inflated and inflated conditions. This negligible change can be attributed to the rigid hyaline cartilage reinforcement of the trachea (Smodlaka et al., 2009), which aids in lung collapse while diving by allowing compressed air from the lungs to be stored within this non-compliant compartment (Kooyman, 1973). The largest volume measured of the air-filled respiratory tract – including dead space and TLC – was 2.3 l. We found that TLC was three times smaller in

Table 1. Respiratory volume for ringed seals, shown with measures of body volume and corresponding body density and vertical forces

Individual	PH1701[a]	PH1503[a]	PH1804[b]	PH1802[c]	Range
Sex	F	M	M	M	
Age (months)	15.6	43	16.7	25.8	
Mass (kg)	14.9	27.5	20.5	26.2	
ST length (cm)	–	90	81.5	86	
Non-inflated (0 mmHg)					
Trachea volume (ml)	–	39.9	–	26.2	26.2–39.9
Trachea volume (ml kg^{-1})	–	1.4	–	1.0	1.0–1.4
Bronchi volume (ml)	–	9.9	–	9.3	9.3–9.9
Bronchi volume (ml kg^{-1})	–	0.4	–	0.4	–
Left lung volume (ml)	–	465	–	262	262–465
Right lung volume (ml)	–	421	–	302	302–421
Total lung volume (ml)	–	886	–	564	564–886
Total lung volume (ml kg^{-1})	–	32.2	–	21.5	21.5–32.2
Total respiratory tract volume (ml)	–	936	–	599	599–936
Total body volume (ml)	–	–	–	24,913	–
Body density (g ml^{-1})	–	–	–	1.052	–
Buoyant force (N)	–	–	–	250.7	–
Downward force (N)	–	–	–	256.7	–
Net (total) force (N)	–	–	–	−6.0	–
Inflated (30 mmHg)					
Trachea volume (ml)	23.1	43.1	34.6	32.2	23.1–43.1
Trachea volume (ml kg^{-1})	1.6	1.6	1.7	1.2	1.2–1.7
Bronchi volume (ml)	11.2	9.1	13.7	10.3	9.1–13.7
Bronchi volume (ml kg^{-1})	0.8	0.3	0.7	0.4	0.3–0.8
Left lung volume (ml)	426	1108	912	650	426–912
Right lung volume (ml)	444	1163	977	723	444–1163
Total lung capacity (ml)	870	2271	1890	1372	870–2271
Total lung capacity (ml kg^{-1})	58.4	82.6	92.2	52.4	52.4–92.2
Diving lung volume (l)	0.4	1.1	0.9	0.7	0.4–1.1
Usable lung O_2 (l)	0.1	0.2	0.1	0.1	0.1–0.2
Diving lung O_2 store (ml kg^{-1})	4.4	6.2	6.9	3.9	3.9–6.9
Total respiratory tract volume (ml)	904	2323	1938	1415	904–2323
Total body volume (ml)	–	–	20,719	25,359	20,719–25,359
Body density (g ml^{-1})	–	–	0.989	1.033	0.989–1.033
Buoyant force (N)	–	–	208.7	255.4	–
Downward force (N)	–	–	200.9	256.7	–
Net (total) force (N)	–	–	7.8	−1.3	−1.3–7.8
Difference in respiratory tract (%)	–	148	–	136	–
Difference in total body volume (%)	–	–	–	2	–
Difference in total body volume (ml)	–	–	–	446.6	–

Diving lung volume is estimated as 50% of total lung capacity. Usable lung O_2 was calculated based on 15% oxygen extraction efficiency.
CT scanner model: [a]GE 16 Slice Light Speed, [b]GE 16 Slice Bright Speed, [c]Siemens 32/64 Somatom GO-UP.

our ringed seals measured *in vivo* than in ringed seal lungs assessed postmortem (Lydersen et al., 1992). This could be due in part to constraints of lung inflation within an enclosed body cavity versus when the respiratory tract is excised. While developmental differences may confound comparisons across age classes, our mass-specific estimates of TLC were also smaller than measures obtained from the excised lungs of adult seals (Lydersen et al., 1992), suggesting that postmortem measurements may overestimate lung capacity.

Given the hyperinflation applied during prescribed veterinary assessments, our measurements provide an upper bound of TLC. Notably, we found little difference in TLC at pressures of 30 and 37 mmHg, indicating that the lungs reached maximum expansion in both conditions. While normally measured at a standard pressure of 22 mmHg, the TLC values reported are likely biologically relevant as they capture full inflation of the lungs within the body cavity; however, they may not be physiologically accurate as a result of hyperinflation. We found that subject positioning had a greater influence on lung volume than inflation pressure, highlighting the differential effects of gravity and recumbency on TLC estimates obtained out of water. Measurements conducted in dorsal recumbency allowed for more complete expansion of the lungs and chest wall and more accurate assessment of TLC.

We measured MAV that was about 40% of TLC. This is much higher than values based on excised respiratory tracts in other marine mammals, which indicate MAV is 0–16% of TLC (Fahlman et al., 2011; Kooyman and Sinnett, 1979). Although it is a common metric, MAV can be difficult to compare across studies. Here, MAV was measured in living, apneic seals when lungs were relaxed in the non-inflated condition. Other studies have defined MAV as the volume of relaxed lungs when transpulmonary pressure is zero (Kooyman and Sinnett, 1979), a condition that can only be achieved postmortem. MAV has also been related to both functional residual capacity (FRC, the air volume remaining after a passive exhalation) and residual volume (RV, the air volume remaining after forceful exhalation) in living animals (Fahlman et al., 2011). Our definition of MAV most closely aligns with FRC; therefore, comparisons to

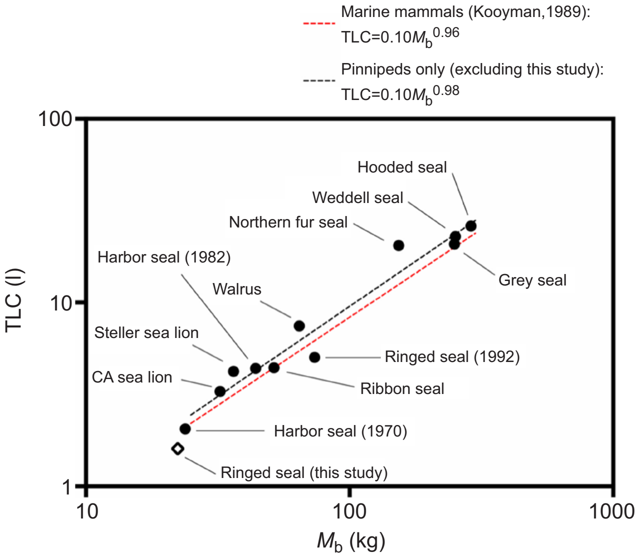

Fig. 2. Logarithmic plot of mean total lung capacity (TLC) as a function of total body mass (M_b). Kooyman's (1989) allometric scaling equation for marine mammals, TLC=0.1$M_b^{0.96}$ (red dashed line) is plotted with the pinniped-only relationship determined in this study, TLC=0.1$M_b^{0.98}$ (black dashed line). Source data for pinniped-only line are provided in Table S2. Ringed seals from this study are shown as a group mean (n=4).

postmortem studies of other marine mammals may not be appropriate.

Diving lung volume (DLV) is commonly estimated at 50% of TLC for pinnipeds, with an oxygen extraction efficiency of 15% (Kooyman, 1973; Kooyman and Sinnett, 1982; Kooyman et al., 1971). As direct measurements exist for only a few species (Kooyman et al., 1971; Ponganis, 2011), we often rely on these assumptions to quantify mass-specific DLV. For our ringed seals, the traditional assumptions yield a DLV ranging from 0.4 to 1.1 liters and corresponding mass-specific DLV from 3.9 to 6.9 ml kg^{-1}. Similar to TLC, these values for DLV in immature ringed seals are lower than previously reported for adult ringed seals (Lydersen et al., 1992), and more similar to values reported for harbor seal pups (Burns et al., 2005). Although the assumptions outlined above can be useful in estimating DLV when empirical data are lacking, much remains to be learned about how respiratory capacity including DLV may change across ontogeny.

Relative to predictions based on scaling relationships, the immature ringed seals in this study had lower than expected lung capacity. This was also the case for adult ringed seals measured postmortem (Lydersen et al., 1992), suggesting the relatively small TLC values obtained here are not explained by methodology or ontogeny. Rather, the deviation of ringed seal lungs from common scaling relationships may be explained by their compact body size and extensive blubber stores. One of the novel aspects of this work was our ability to directly measure SA:V in one individual. This metric is rarely empirically determined but is relevant to aspects of thermoregulation, hydrodynamics and energetics. To compensate for large SA:V and associated heat loss in polar waters, ringed seals have considerable blubber reserves that may comprise half their body volume. Although serving different primary functions, the relative volume of both lungs and blubber have important effects on buoyancy in the smallest phocid.

Seals must manage dynamic buoyant forces and associated energetic costs while diving (Watanabe et al., 2006; Williams et al., 2000). The imaging approach employed here enabled a variety of volumetric measurements relevant to evaluating constraints on diving. Despite high blubber content (48% of body volume), seal PH1802 had a net negative (sinking) force in both inflated and non-inflated lung conditions. In contrast, seal PH1804 had lower blubber volume (33% of body volume), but exhibited a net positive (buoyant) force in the inflated lung condition. These somewhat surprising results were driven by relatively small differences in overall body size (mass and volume) and body density, although in absolute terms, both seals were almost neutrally buoyant in seawater. For reference, some penguins have a net positive force of +15 to 50 N (Ponganis et al., 2015), while larger seal species exhibit net negative forces from −15 to −132 N (Beck et al., 2000; Webb et al., 1998). In comparison, the net vertical forces on the ringed seals were relatively small (−6 to +7 N) and could likely be adjusted by changes in lung volume at the start of a dive. These near-neutral values are physiologically advantageous as they should limit the overall cost of foraging, diving and moving through the marine environment (Adachi et al., 2014; Miller et al., 2012; Nousek-McGregor et al., 2014; Richard et al., 2014; Sato et al., 2013).

We conclude that *in vivo* measurements of lung capacity in ringed seals are smaller in both absolute and mass-specific terms relative to postmortem assessments. Further, total body mass consistently underestimates TLC in this species when considered in the context of established allometric relationships. This deviation likely results from their small, compact body size and exceptional blubber stores. Biomedical imaging can provide accurate quantification of specific regions of the respiratory tract, as well as additional measures of total body and blubber volume that have important ecological implications for free-ranging individuals.

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Competing interests

The authors declare no competing or financial interests.

Author contributions

Conceptualization: H.H.-S., N.M.T., C.R.; Methodology: H.H.-S., K.W., S.D.-G.; Formal analysis: H.H.-S.; Investigation: H.H.-S.; Resources: C.R.; Data curation: H.H.-S., K.W.; Writing - original draft: H.H.-S., N.M.T., C.R.; Writing - review & editing: H.H.-S., N.M.T., K.W., S.D.-G., C.R.; Visualization: H.H.-S.; Supervision: C.R.; Project administration: N.M.T., C.R.; Funding acquisition: N.M.T., C.R.

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Data availability

Original DICOM data are available from the Dryad digital repository (Hermann-Sorensen et al., 2020): https://doi.org/10.7291/D1R68J

Supplementary information

Supplementary information available online at https://jeb.biologists.org/lookup/doi/10.1242/jeb.235507.supplemental

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Table S1. Modified thoracic CT imaging protocols for ringed seal cases shown in Table 1.

	GE 16 Slice Light Speed	**GE 16 Slice Bright Speed**	**Siemens 32/64 Somatom GO-UP**
Scan type	Helical full	Helical full	Helical full
Rotation time (s)	0.5	0.5	1.5, 1.0 (opt 0.8)
Beam collimation (mm)	20	20	-
Thickness (mm)	0.6250 – 2.50	0.6250 – 2.50	2.0
Spiral pitch factor	1.35	1.35	0.8
Speed (mm/rotation)	27.5	27.5	17.89
Reconstruction interval	matched to slice thickness	matched to slice thickness	2.0
Gantry tilt	0	0	0
Gantry period	-	0.60	-
Scan FOV	Large	Large	Whole body
kVp	120	120	130
Xray tube current (mA)	100 – 440	100 – 440	40 – 42
Noise index	11.57	11.57	15
Dose reduction	260	260	On - 3
Algorithm	Standard, bone plus	Standard, bone plus	Br40, Br60
Reformats	Full	Full	Full

Table S2. Lung oxygen stores for marine carnivores, showing the ringed seals in this study with comparative data for other species. Species for which total lung capacity (TLC) is available are plotted in Figure 2. Diving lung volume (DLV) O_2 store calculated based on assumption that DLV is 50% of TLC for pinnipeds. Usable O_2 for gas exchange is estimated as 15% of DLV (Kooyman, 1989), and reported as mass-specific diving lung volume oxygen store (DLV O_2 store). Data are provided for individuals when possible (n=1), or as grouped mean values (n>1).

Species	**Age**	**Mass (kg)**	**Sample (n)**	**TLC (L)**	**DLV (L)**	**DLV O_2 store (mL kg^{-1})**	**Method**	**Reference**
Phocids								
Ringed seal *Pusa hispida*	subadult	26.2	1	1.37	0.69††	3.9 [j]	in vivo [f]	A
		20.5	1	1.89	0.94††	6.9 [j]	in vivo [f]	A
		27.5	1	2.27	1.14††	6.2 [j]	in vivo [f]	A
		14.9	1	0.87	0.44††	4.4 [j]	in vivo [f]	A
	adult	73.7†	50	5.04 ± 1.24†	2.52††	6.8 [j]	postmortem [a]	B
Harbor seal *Phoca vitulina*	mixed	23.8†	5	2.05†	1.03††	6.5 [j]	postmortem [a]	C
	neonatal	10.3 ± 0.4†	395			5.3 [k]	postmortem [a]	D
	nursing pup	24.9 ± 1.5†				5.3 [k]	postmortem [a]	D
	weaned pup	28.9 ± 0.5†				5.3 [k]	postmortem [a]	D
	yearling	33.1 ± 0.5†				-	postmortem [a]	D
	adult	52.1 ± 1.6†				12.2 [k]	postmortem [a]	D
	subadult	44	1	4.4			in vivo [c]	L
	subadult	40	1	4			in vivo [c]	L
	subadult	36	1	3.6			in vivo [c]	L
	subadult	52	1	5.1			in vivo [c]	L
	subadult	48	1	4.8			in vivo [c]	L
Grey seal *Halichoerus grypus*	pup	40.1 ± 1.3	10			4.1[k]	in vivo [c]	E
	yearling	51.6 ± 2.7†	10			4.1[k]	in vivo [c]	E
	adult female	191.5 ± 6.0†	10			4.1[k]	in vivo [c]	E
	adult	250†	4	20.81†	10.4††	6.2 [j]	in vivo [c]	F

Species	Age	Mass (kg)	Sample (n)	TLC (L)	DLV (L)	DLV O_2 store (mL kg^{-1})	Method	Reference
Ribbon seal *Histriophoca fasciata*	adult	51.7[†]	4	4.42[†]	2.2[††]	6.4[j]	postmortem[a]	C
Harp seal *Pagophilus groenlandicus*	neonatal	10.3 ± 1.1[†]	40				postmortem[e]	G
	nursing pup	29.4 ± 1.1[†]					postmortem[e]	G
	weaned pup	36.6 ± 1.1[†]					postmortem[e]	G
	yearling	29.3 ± 1.6[†]					postmortem[e]	G
	adult	115.4 ± 4.7[†]					postmortem[e]	G
Hooded seal *Cistophora cristata*	adult female	252.1 ± 17.9[†]	6	(22.9)		6.8[k]	postmortem[e]	G
	nursing pup	38.5 ± 12.5[†]	2	(1.4)		2.8[k]	postmortem[e]	G
	weaned pup	48.1 ± 2[†]	6	(2.0)		3.1[k]	postmortem[e]	G
Weddell seal *Leptonychotes weddellii*	pup	106	1			4.1[†, k]	in vivo[c]	H
		137	1			4.1[†, k]	in vivo[c]	H
		124	1			4.1[†, k]	in vivo[c]	H
		107	1			4.1[†, k]	in vivo[c]	H
	subadult	260	1	(22.2)	11.1	6.4[j]	*in vivo*[b, h]	I
		345	1	(23.6)	11.8	5.1[j]	*in vivo*[b, h]	I
		261	1	(32.6)	16.3	9.4[j]	*in vivo*[b, h]	I
	adult	425[†]	4	Scruffy	14	4.1[†, j]	*in vivo*[b]	J
					14	4.1[†, j]	*in vivo*[b]	J
					11	4.1[†, j]	*in vivo*[b]	J
					20	4.1[†, j]	*in vivo*[b]	J
					7	4.1[†, j]	*in vivo*[b]	J
					20	4.1[†, j]	*in vivo*[b]	J
				Gentle Ben	13	4.1[†, j]	*in vivo*[b]	J
					7	4.1[†, j]	*in vivo*[b]	J
					13	4.1[†, j]	*in vivo*[b]	J

Species	**Age**	**Mass (kg)**	**Sample (n)**	**TLC (L)**	**DLV (L)**	**DLV O_2 store (mL kg^{-1})**	**Method**	**Reference**
				Ringo	8	4.1[†, j]	*in vivo* [b]	J
					5	4.1[†, j]	*in vivo* [b]	J
					13	4.1[†, j]	*in vivo* [b]	J
					6	4.1[†, j]	*in vivo* [b]	J
Otariids								
Northern fur seal *Callorhinus ursinus*	mixed	153.5[†]	4	20.45[†]	10.2[††]	10.0 [j]	postmortem [a]	C
Steller sea lion *Eumetopias jubatus*	mixed	36.3[†]	4	4.22[†]	2.1[††]	8.7 [j]	postmortem [a]	C
	neonatal	22[†]	1	1.96[†]	1.0[††]	6.7 [j]	postmortem [a]	C
Odobenids								
Walrus *Odobenus rosmarus*	mixed	64.5[†]	3	7.45[†]	3.7[††]	8.7 [j]	postmortem [a]	C
Mustelids								
Northern sea otter *Enhydra lutris lutris*	adult	27.6[†]	3	9[†]	5.7	31.0 [j]	postmortem [a]	C
Southern sea otter *Enhydra lutris nereis*	neonatal	2.6 ± 0.18[†]	5			42.7 ± 3.09 [k]	postmortem[i]	K
	small pup	4.4 ± 0.30[†]	2			48.7 ± 7.31 [k]	postmortem[i]	K
	large pup	8.5 ± 0.62[†]	4			41.6 ± 4.56 [k]	postmortem[i]	K
	juvenile	12.2 ± 0.99[†]	3			41.6 ± 1.60 [k]	postmortem[i]	K
	adult	19.9 ± 1.01[†]	6			31.1 ± 2.30 [k]	postmortem[i]	K

References: A: this study; B: Lydersen et al., 1992; C: Lenfant et al., 1970; D: Burns et al., 2005; E: Noren et al., 2005; F: Reed et al., 1994; G: Burns et al., 2007; H: Burns and Castellini, 1996; I: Falke et al., 2008; J: Kooyman et al., 1971; K: Thometz et al., 2015; L: Kooyman and Sinnett, 1982. Values represented within parentheses were back-calculated from diving lung volumes.

[a] Inflation of excised lungs via water displacement; [b] Nitrogen washout; [c] Author calculated or used values based on allometric equations established in other studies; [d] Author calculated based on allometric equations established from study itself; [e] Used combination of inflation of excised lungs and calculations from allometric equations established from study itself; [f] *In vivo* biomedical imaging; [g] Whole body plethysmography; [h] Pneumotachograph; [i] Used body size, lung mass, and empirical measures of lung volume to calculate TLC and DLV; [j] Value calculated here from usable O_2 and body mass. Mass-specific DLV O_2 store from southern sea otters used to calculate mass-specific DLV O_2 store for northern sea otters; [k] Value reported in original publication referenced; [†] Published value was either a mean with no individual value available or was given as a range with only one value for TLC calculated; in these cases, sample size is given; [††] Value calculated here based on assumption that DLV for pinnipeds is 50% of TLC, with 15% of DLV being usable O_2 for oxygen exchange (Kooyman, 1989). Usable O_2 (liters) for northern sea otters calculated from mass-specific DLV of southern sea otter adults measured in Thometz et al., 2015

Table S2 References

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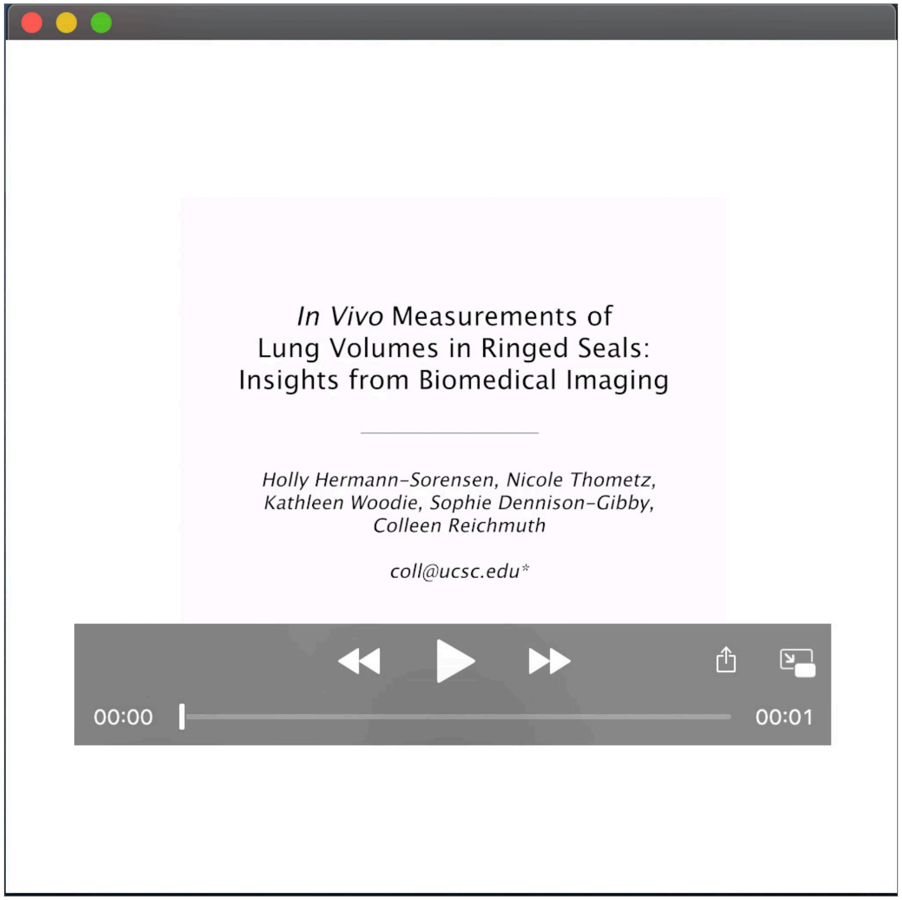

In Vivo Measurements of
Lung Volumes in Ringed Seals:
Insights from Biomedical Imaging

Holly Hermann-Sorensen, Nicole Thometz,
Kathleen Woodie, Sophie Dennison-Gibby,
Colleen Reichmuth

*coll@ucsc.edu**

Movie 1. Three-dimensional reconstructions of ringed seal PH1802 at both inflated (0 mmHg) and inflated (30 mmHg) lung inflation pressures. The trachea is red, bronchi are yellow, and lungs are blue.