

## **Lung Mechanics in Marine Mammals**

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### **LONG-TERM GOALS**

The long term goal of this study is to develop methods to study lung physiology in live marine mammals and to use these techniques to investigate the mechanical properties of the respiratory system in different marine mammals. This effort is vital to understand how diving mammals manage inert and metabolic gases during diving and will help determine what behavioral and physiological responses increase DCS risk.

### **OBJECTIVES**

Recent theoretical studies have suggested that marine mammals commonly live with elevated blood and tissue N<sub>2</sub> levels, and that they use both physiological and behavioral means to avoid DCS [1, 2]. But what physiological variables are the most important to reduce N<sub>2</sub> levels below those that cause DCS, and how important is a link between behavior and physiology? For example, if the duration of each individual dive was extended, the repeated dives during a bout (a series of repeated dives with a short intervening surface interval) may result in accumulation of N<sub>2</sub> to levels that may cause DCS. A variety of situations, such as sonar exposure, reduction in prey abundance, predator avoidance or environmental change, may result in behavioral changes in dive pattern. Such changes could cause elevated tissue and blood N<sub>2</sub> levels that either result in DCS or force the animal to end a foraging bout prematurely to prevent the formation of inert gas bubbles. Prematurely ending a diving bout reduces foraging efficiency and could have detrimental implications for survival. While the results from

theoretical studies have to be viewed with caution, sensitivity analyses have indicated that the degree of gas exchange and cardiac output during diving are the most important variables determining  $N_2$  levels in blood and tissue, and thereby the DCS risk. However, current knowledge of how gas exchange is altered by compression of the respiratory system, possibly to the limit of collapse, is rudimentary at best.

The proposed lung collapse depth in the Weddell seal is around 30 m [3] and 70 m for the bottlenose dolphin [4]. In another study, no apparent differences in pulmonary shunt were observed between species with widely different respiratory structure [California sea lion vs. harbor seal, 5]. The results also suggested that complete cessation of gas exchange may not occur until a depth  $> 150$  m [5], even when the animal exhaled before diving. In a previous ONR funded effort, a mathematical model was developed to explain these divergent results (ONR award number N00014-07-1-1098). The results from this work implied that beaked whales commonly experience end-dive  $N_2$  levels that would cause a significant proportion of DCS cases in terrestrial mammals [1]. It was proposed that as the  $N_2$  levels increased they could eventually limit the extent of a dive bout [1, 2, 6, 7]. It was also suggested that the normal dive behavior and physiological adjustments could be important to reduce end-dive  $P_{N_2}$  [1, 6, 7].

The model results predict that the alveolar-collapse-depth, and thereby the degree of gas exchange, is greatly affected by the compliance values of the different parts of the respiratory system [8]. While results from mathematical models should be tested with empirical data, few studies have examined respiratory mechanics of live marine mammals [9, 10]. The model therefore used compliance values from an excised marine mammal lung for the lower respiratory tract, and of an excised trachea from a terrestrial animal for the upper airways [8]. To enhance our ability to predict how anthropogenic sound may interact with gas management during diving, an improved understanding of the physical properties that affect compression of the respiratory system and gas exchange is warranted.

## APPROACH

This project is separated into three aims:

**Aim 1:** To test our hypothesis that deep divers have a more compliant respiratory system that will enhance compression and collapse of the thoracic cavity, we measured the inspiratory and expiratory flow-rates during quiet breathing in addition to airway and esophageal pressures. These data will be used to calculate airway resistance, pulmonary and thoracic compliance (pressure-volume relationship). The static compliance values will be compared to the data previously determined in post-mortem marine mammals [11].

**Aim 2:** We hypothesize that species that dive deeper and for longer duration have significantly lower end-tidal  $O_2$  and higher  $CO_2$  levels, and to test that hypothesis we monitored end-tidal  $O_2$  and  $CO_2$  in anesthetized, spontaneously breathing pinnipeds.

**Aim 3:** The experimental results will be compared with data obtained from our previous hyperbaric CT studies. The combined results will be used to revise a model that predicts the extent of gas exchange for a range of species.

## WORK COMPLETED

### *Aim 1:*

Over the 2 years of the project we managed to estimate the structural properties of the respiratory system of anesthetized pinnipeds (Table 1, Fig. 1). In some animals where euthanasia was planned, we managed to measure both lung mechanics in vivo during spontaneous breathing (dynamic) and mechanical ventilation (static), and the static compliance after euthanasia. The respiratory compliance of these various measurements is summarized in Table 1.

**Table 1. Animal identification number, facility (The Marine Mammal Center, TMMC; Open Water Research Station, OWRS), group (Ph=phocid, Ot=otariid), species, body mass ( $M_b$ ), animal length, and compliance ( $C$ ,  $\text{cmH}_2\text{O}$ ) for live ( $C_{\text{live}}$ ), intact dead animals ( $C_{\text{dead}}$ ) and excised lungs ( $C_{\text{exc}}$ ).**

**Species abbreviation (Pv = *Phoca vitulina*, Ma = *Mirounga angustirostris*; Cu = *Callorhinus ursinus*; Zc = *Zalophus californianus*; Ej = *Eumetopias jubatus*). \* $P_{\text{tp}}$  all < 10 $\text{cmH}_2\text{O}$ . Compliance estimates for excised lungs are from deflation data. Animal with suspect pneumonia**

Animal ID	Facility	Group	Sex	Species	$M_b$ (kg)	Length (cm)	$C_{\text{live}}$	$C_{\text{dead}}$	$C_{\text{exc}}$	TLC <sub>est</sub>
							L • $\text{cmH}_2\text{O}$			
F97SI	OWRS	Ot	F	Ej	228	228	0.362	----	----	19.9
F97HA	OWRS	Ot	F	Ej	172	200	0.168	----	----	15.4
F00BO	OWRS	Ot	F	Ej	160	209	0.417	----	----	14.4
F00YA	OWRS	Ot	F	Ej	206	232	0.220	----	----	18.2
F03WI	OWRS	Ot	F	Ej	171	221	0.432	----	----	15.3
F03RO	OWRS	Ot	F	Ej	161	211	0.107	----	----	14.5
CSL102 44	TMMC	Ot	M	Zc	126	166	0.924*	----	----	11.6
CSL103 01	TMMC	Ot	F	Zc	12	84	----	----	0.056	1.3
CSL103 20	TMMC	Ot	M	Zc	18	106	----	0.205	----	1.9
CSL103 25	TMMC	Ot	M	Zc	107	116	----	----	0.258	9.9
CSL103 28	TMMC	Ot	M	Zc	75	146	----	----	0.197	7.2
CSL106 38	TMMC	Ot	M	Zc	49	133	0.154	0.171	----	4.8
CSL106 50	TMMC	Ot	M	Zc	17	121	0.128	----	----	1.8
CSL106 53	TMMC	Ot	M	Zc	39	140	0.295	0.247	----	3.9
NFS266	TMMC	Ot	F	Cu	53	125	0.575	----	----	5.2
HS2250	TMMC	Ph	F	Pv	8	45	----	----	0.021	0.9
HS2258	TMMC	Ph	M	Pv	10	83	0.112	----	----	1.1
HS2266	TMMC	Ph	F	Pv	10	81	0.116	----	0.087	1.1
ES3354	TMMC	Ph	F	Ma	50	124	0.300	----	----	4.9
ES3418	TMMC	Ph	F	Ma	42	129	0.140*	----	----	4.2

Mean ( $\pm$ SD)					
SSL	183 $\pm$ 28	217 $\pm$ 12	0.284 $\pm$ 0.138		
CSL	55 $\pm$ 43	126 $\pm$ 25	0.375 $\pm$ 0.373	0.208 $\pm$ 0.038	0.170 $\pm$ 0.104
NFS	53	125	0.575		
HS	9 $\pm$ 1	70 $\pm$ 21	0.114 $\pm$ 0.003		0.054 $\pm$ 0.047
ES	46 $\pm$ 6	126 $\pm$ 4	0.220 $\pm$ 0.113		

Modifications of the equipment, including custom-made fittings that prevented turbulent flow in the tubing, in the second year allowed us to accurately measure flow-rates, airway and esophageal pressures during voluntary breathing and mechanical ventilation (Fig. 1).

**Aim 2:** We used a fast response gas analyzer to record end-expiratory O<sub>2</sub> and CO<sub>2</sub>, which allowed measurement of end-tidal respiratory gas composition (ML206, AD Instruments). In addition, we used an O<sub>2</sub> dilution/re-breathing experiment to estimate residual volume in 3 animals that underwent a terminal anesthesia procedure. Our experimental protocol involved sampling of animals that underwent a planned clinical procedure, therefore specific measurements were only available in some animals and the distribution of various measurements was therefore uneven.

**Aim 3:** Analysis of the data for *Aim 1* is complete and a paper almost ready for submission. A graduate student has been identified that will help revise the mathematical model with new parameters for alveolar compression and gas exchange. We will run the model with dive data and arterial and venous P<sub>O<sub>2</sub></sub> for California sea lions provided by Drs. Gitte McDonald and Paul Ponganis. The estimated blood P<sub>O<sub>2</sub></sub> will be compared with the measured data and determine the accuracy of the model.

## RESULTS

**Aim 1:** Data for respiratory compliance have been analyzed. Figure 1 shows a representative pressure-volume curve for the lung and chest in 3 different sea lions. Table 1 summarizes the static compliance for all animals where data were collected is a representative A representative figure is show and are reported in Table 1.

### *Static P-V relationship in live animals*

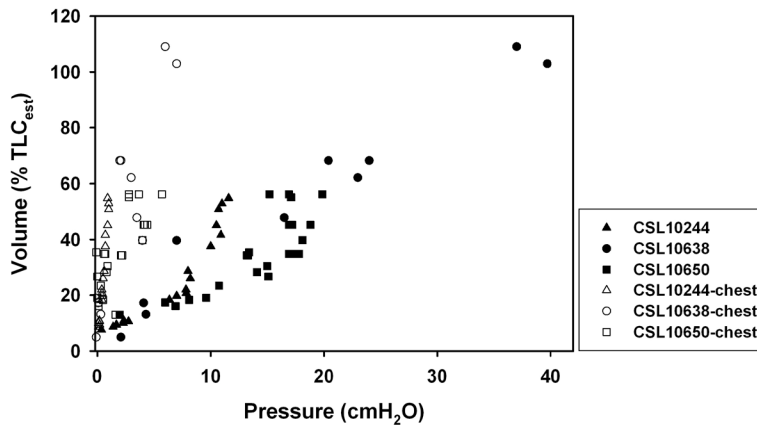
Morphometrics and static P-V data were collected from 3 otariid and 2 phocid species (Table 1). In all animals, the chest wall compliance (C<sub>CW</sub>) was greater than the lung compliance (C<sub>L</sub>, Fig. 1). The sC<sub>L</sub> was not different in otariids (n = 11, 0.617  $\pm$  0.498 cmH<sub>2</sub>O<sup>-1</sup>) as compared to phocids (n = 4, 1.078  $\pm$  0.493 cmH<sub>2</sub>O<sup>-1</sup>, one-way ANOVA, F = 0.790, P = 0.393, Fig 2). There was a significant correlation between animal size (body mass) and specific lung compliance (sC<sub>L</sub> = -0.0051 *body mass* + 1.25, r<sup>2</sup> = 0.57), but this relationship was due to the significantly lower specific lung compliance in captive Steller sea lions (0.252  $\pm$  0.131 cmH<sub>2</sub>O<sup>-1</sup>) as compared with wild animals (1.065  $\pm$  0.414 cmH<sub>2</sub>O<sup>-1</sup>, one-way ANOVA, F = 21.2, P = 0.0005). There was great variability in the specific chest compliance (sC<sub>CW</sub>) within and among species (3.40  $\pm$  2.50 cmH<sub>2</sub>O, Fig. 3) and there were no significant difference among species (one-way ANOVA P > 0.1).

### *Whole animal-dead*

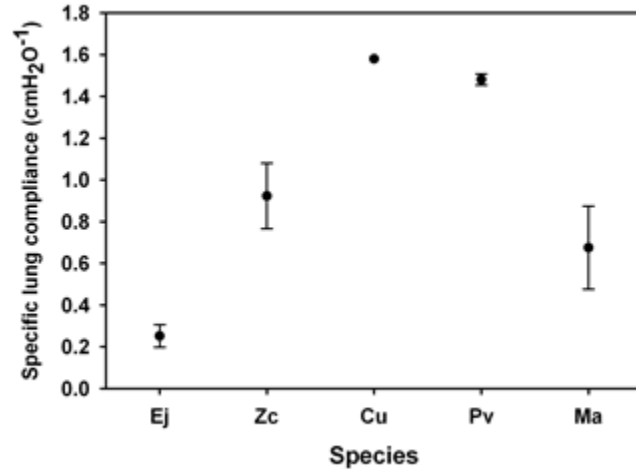
Lung compliance was estimated in 3 intact California sea lions (CSL10320, CSL10638, CSL10653) post mortem. In two of these, lung compliance data had also been measured before euthanasia and there was good agreement between the measured lung compliances in the live and dead animals (Fig. 4, CSL10638 and CSL10653).

### *Excised lungs*

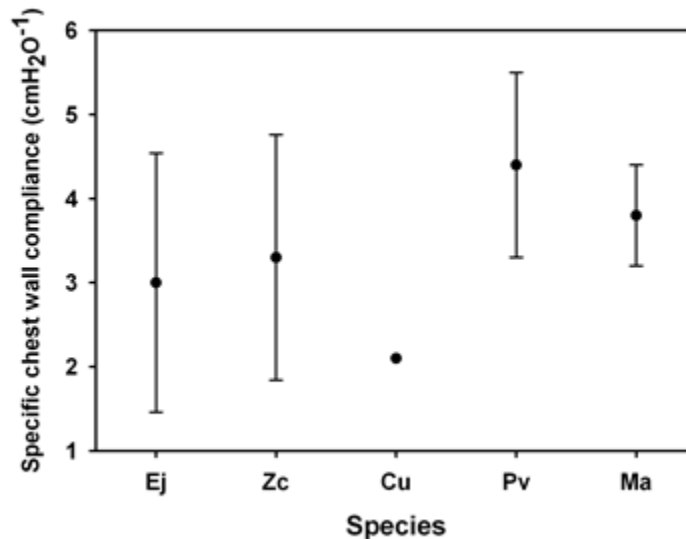
The P-V relationship from excised lungs from 2 harbor seals (HS2250 and HS2266) and 4 California sea lions (CSL10301, CSL10325, CSL10328, and CSL10320, Table 1) were examined. The  $C_L$  was lower in lungs from smaller animals, but the  $sC_L$  was similar in all but one animal (CSL10301), where it was twice as high. The pressure volume-relationships of the excised lungs from two harbor seals (HS2266 and HS2255) of similar size were identical (Fig. 4). The curve from the live HS2266 was slightly shifted to the right (Fig. 5), but had similar slope.



**Figure 1. Relationship between transpulmonary (airway pressure minus esophageal pressure) or chest wall pressure (esophageal pressure minus ambient pressure) and inspired volume expressed as a percent of estimated total lung capacity [ $TLC = 0.135 \times Mb^{0.92}$ , 11, 12] for 3 individual California sea lions (*Zalophus californianus*).**



**Figure 2.** Average specific lung compliance [the lung compliance divided by the estimated residual volume ( $RV$ ), where  $RV$  was assumed equal to the Minimum Air Volume which was an average 7% of total lung capacity in excised lungs [11]] for captive Steller sea lions ( $Ej = Eumetopias\ jubatus$ ), California sea lion ( $Zc = Zalophus\ californianus$ ), Northern fur seal ( $Cu = Callorhinus\ ursinus$ ), harbor seal ( $Pv = Phoca\ vitulina$ ) and elephant seal ( $Ma = Mirounga\ angustirostris$ ).



**Figure 3.** Average specific chest compliance (the lung compliance divided by the estimated residual volume ( $RV$ ), where  $RV$  was assumed equal to the Minimum Air Volume which was an average 7% of total lung capacity in excised lungs [11]) for captive Steller sea lions ( $Ej = Eumetopias\ jubatus$ ), California sea lion ( $Zc = Zalophus\ californianus$ ), Northern fur seal ( $Cu = Callorhinus\ ursinus$ ), harbor seal ( $Pv = Phoca\ vitulina$ ) and elephant seal ( $Ma = Mirounga\ angustirostris$ ).

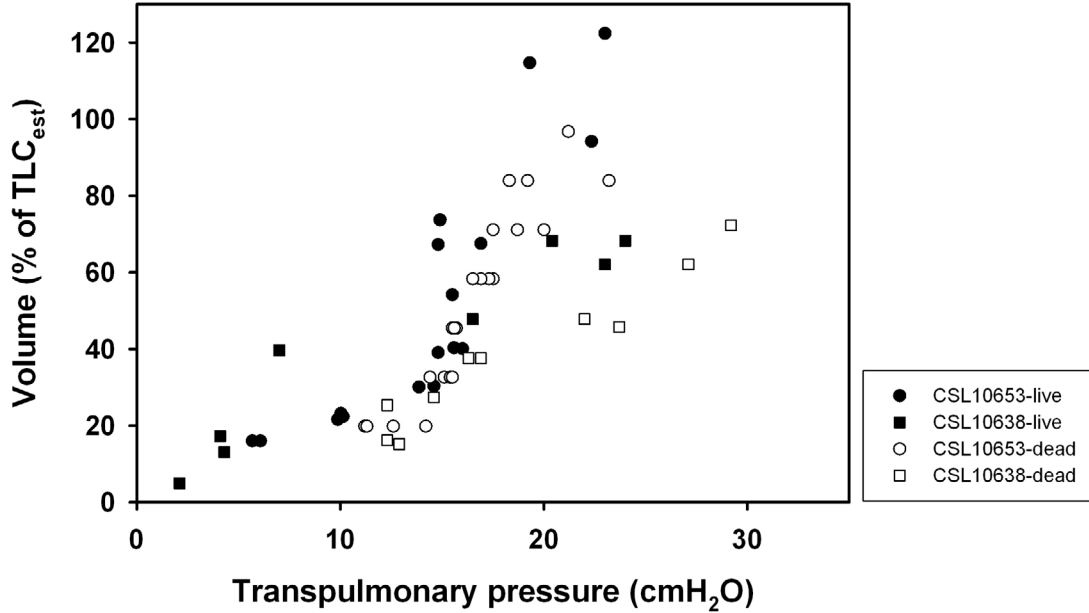


Figure 4. Relationship between transpulmonary (airway pressure minus esophageal pressure) and inspired volume expressed as a percent of estimated total lung capacity [ $TLC = 0.135 \times Mb^{0.92}$ , 11, 12] for alive or deceased California sea lions (*Zalophus californianus*).

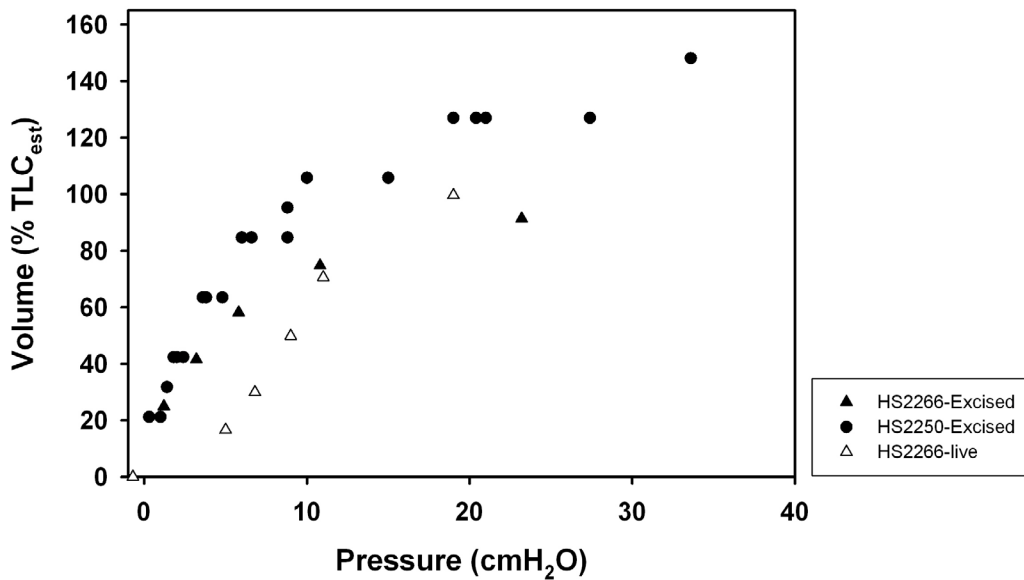


Figure 5. Relationship between transpulmonary (airway pressure minus esophageal pressure) and inspired volume expressed as a percent of estimated total lung capacity [ $TLC = 0.135 \times Mb^{0.92}$ , 11, 12] for an excised lung and live harbor seal (*Phoca vitulina*). Triangle-HS2266; Circle-HS2250.

**Aim 2:** We collected continuous end-tidal O<sub>2</sub> and CO<sub>2</sub> data in all animals both during voluntary and mechanical ventilation (Fig. 1). These data suggest that sea lions may experience relatively (as compared to terrestrial mammals) high end-tidal PCO<sub>2</sub> levels without apparent problems. In addition, O<sub>2</sub>-dilution data were collected with the aim to estimate residual volume. These data are still being analyzed.

**Aim 3:** The data analysis is completed and the respiratory compliance data for the California sea lion will be used to re-parameterize a gas exchange model [13]. Dive data and measured venous and arterial P<sub>O<sub>2</sub></sub> data from California sea lions have been obtained from Drs. Gitte McDonald and Paul Ponganis and will be used to determine how well the revised model predicts alveolar compression and blood gas tensions.

## IMPACT/APPLICATIONS

This work is intended to enhance our understanding of how the respiratory system responds during diving in marine mammals. The results will provide information that will allow us to provide species specific pressure-volume parameters for the airways. These data will enable more realistic predictions of how the lungs compress to the limit of collapse and improve our understanding how marine mammals manage gases during diving.

The results can be used to determine how changes in dive behavior, including those from man-made interference, affect blood and tissue P<sub>N<sub>2</sub></sub> levels. Thus, our results will enhance the fundamental understanding and interpretation of avoidance of the effect of anthropogenic sound, and enable knowledgeable decisions about sonar deployment, related training exercises and responses to NGO concerns. This should be of value to the US Navy Marine Mammal Program.

## RELATED PROJECTS

A ONR YIP award to the PI will continue this work on awake cetaceans (ONR award No. N000141410563).

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