

Lung Mechanics in Marine Mammals

Andreas Fahlman
Department of Life Sciences
Texas A&M- Corpus Christi
6300 Ocean Dr., Unit 5892
Corpus Christi, TX 78412
phone: +1-361-825-3489 fax +1-361-825-2025 email: andreas.fahlman@tamucc.edu

William Van Bonn
A. Watson Armour III Center for Animal Health and Welfare
John G. Shedd Aquarium
1200 S. Lake Shore Drive
Chicago IL 60605
phone: 312-692-2746 email: bvanbonn@sheddaquarium.org

Stephen Loring
Department of Anesthesia, Critical Care and Pain Medicine
Beth Israel Deaconess Medical Center
Boston, Massachusetts
phone: 617-667-3092 email: sloring@bidmc.harvard.edu

Award Number: N00014-12-1-0269

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 N₂ 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 N₂ 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 N₂ 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 N₂ 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

Report Documentation Page				Form Approved OMB No. 0704-0188	
Public reporting burden for the collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden, to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to a penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number.					
1. REPORT DATE 30 SEP 2013		2. REPORT TYPE		3. DATES COVERED 00-00-2013 to 00-00-2013	
4. TITLE AND SUBTITLE Lung Mechanics in Marine Mammals				5a. CONTRACT NUMBER	
				5b. GRANT NUMBER	
				5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S)				5d. PROJECT NUMBER	
				5e. TASK NUMBER	
				5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) Texas A&M - Corpus Christi, Department of Life Sciences, 6300 Ocean Dr, Unit 5892, Corpus Christi, TX, 78412				8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)				10. SPONSOR/MONITOR'S ACRONYM(S)	
				11. SPONSOR/MONITOR'S REPORT NUMBER(S)	
12. DISTRIBUTION/AVAILABILITY STATEMENT Approved for public release; distribution unlimited					
13. SUPPLEMENTARY NOTES					
14. ABSTRACT					
15. SUBJECT TERMS					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT Same as Report (SAR)	18. NUMBER OF PAGES 7	19a. NAME OF RESPONSIBLE PERSON
a. REPORT unclassified	b. ABSTRACT unclassified	c. THIS PAGE unclassified			

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: We will measure the inspiration ($\dot{V}_{insp}$) and expiration flow rates ($\dot{V}_{exp}$) during quiet breathing in addition to airway (P_{air}) and esophageal (P_{eso}) 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 (Fahlman and Moore, unpublished observation). We hypothesize that deep divers have a more compliant respiratory system that will enhance compression and collapse of the thoracic cavity.

Aim 2: We will monitor end-tidal O_2 and CO_2 in anesthetized, spontaneously breathing marine mammals. We hypothesize that species that dive deeper and for longer duration have significantly lower end-tidal O_2 and higher CO_2 levels.

Aim 3: The experimental results will be compared with data obtained from our previous and on-going 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:

In the second year, additional experiments were conducted 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.

Table 1. Number of samples in each category

Species	Static compliance		End-tidal		Re-breathing
	Live animal	Dead Intact	O ₂	CO ₂	
California Sea Lion	4	2	5	5	1
Northern fur seal	1	1	1	1	1
Harbor seal	1	-	2	2	1
Elephant seal	1	-	2	2	-

Modifications of the equipment allowed us to accurately measure flow-rates, airway and esophageal pressures during voluntary breathing and mechanical ventilation (Fig. 1).

Aim 2: In the second year we also used a fast response visible spectrum absorption O₂ (Oxigraf X2004) and infrared CO₂ (Servomex Model 15050) analyzer, which allowed measurement of end-tidal respiratory gas composition (ML206, AD Instruments, Fig. 1). In addition, we used an O₂ dilution/re-breathing experiment to estimate residual volume in 3 animals that underwent a terminal anesthesia procedure (Table 1). 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 is therefore uneven.

Aim 3: Analysis of the data collected from the first year is almost complete and the analysis of the data from the second year is underway. As we have collected respiratory compliance data in both live animals and then in the excised lungs after the animal was euthanized, we plan to compare how well these data compare. We will then compare these data to our previous data from excised lungs collected at Woods Hole Oceanographic Institution. In addition, we also collected data to estimate residual volume in live animals. The procedure was possible in animals that were anesthetized and ventilated on O₂, using the O₂-dilution method. The estimated RV will be compared to those from excised lungs using the water displacement method {Fahlman, 2011 #92}.

RESULTS

Aim 1: We successfully collected lung compliance data from 7 animals, 4 California sea lions, 1 elephant seal, one harbor seal and 1 Northern fur seal. These data will be combined with the data set of 12 animals, 1 elephant seal and 11 California sea lions, collected in the previous year. In some animals, we were able to collect data from the live animal and then from the excised lung after the animal had been euthanized. Figure 1 shows a representative sample of raw data collected from a sea lion during

mechanical ventilation. Figure 2-4 shows analyzed data for the relationship between airway and esophageal pressures and volume during mechanical breathing in an anesthetized Northern fur seal (Fig. 2), Pacific harbor seal (Fig. 3), and California sea lion (Fig. 4). These data indicate that the chest is much more compliant than the lung and agrees with the suggestion that the marine mammal chest provides little resistance to collapse during deep dives.

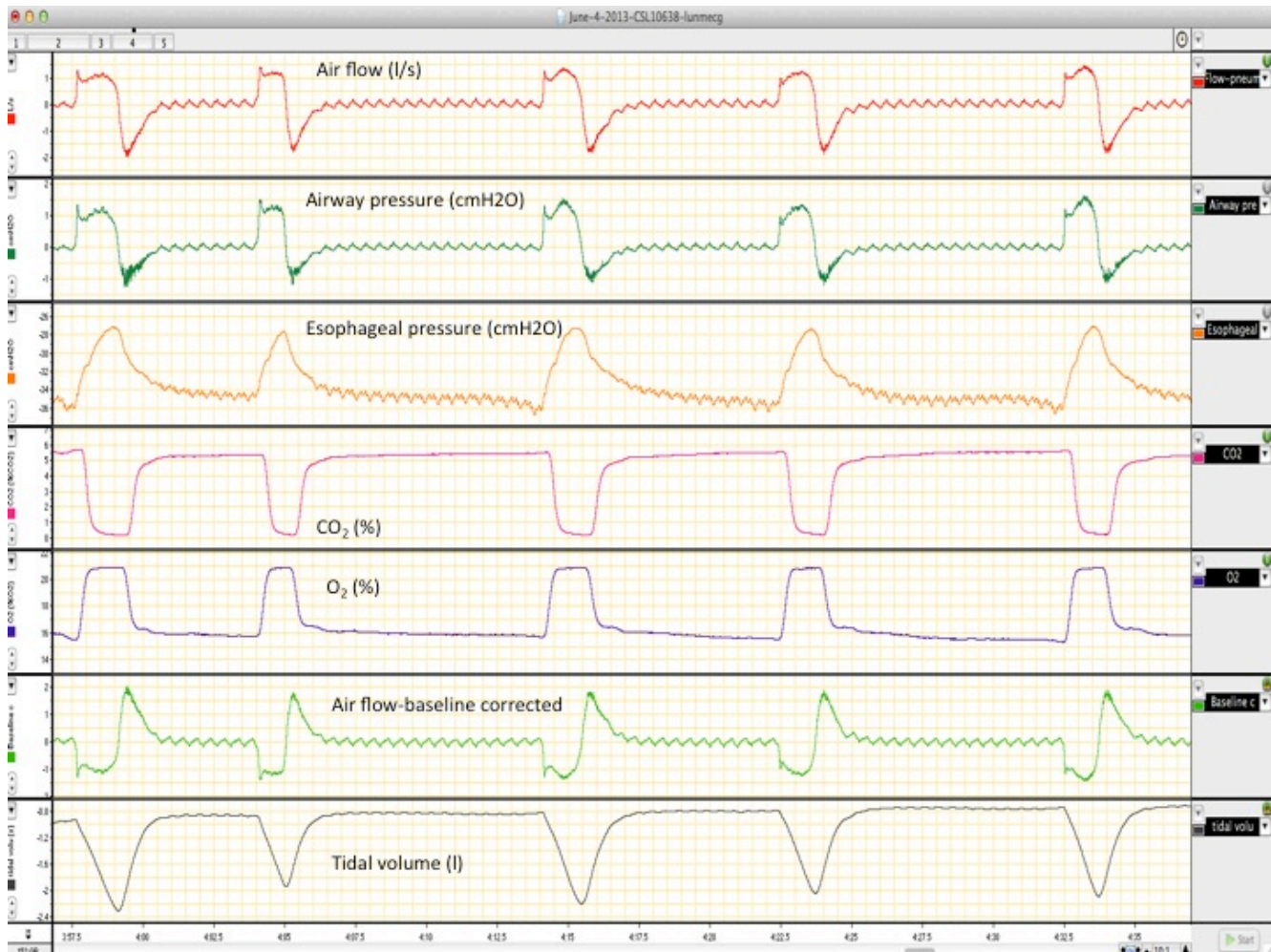


Figure 1. Raw data during mechanical ventilation in an anesthetized California sea lion (CSL10638)

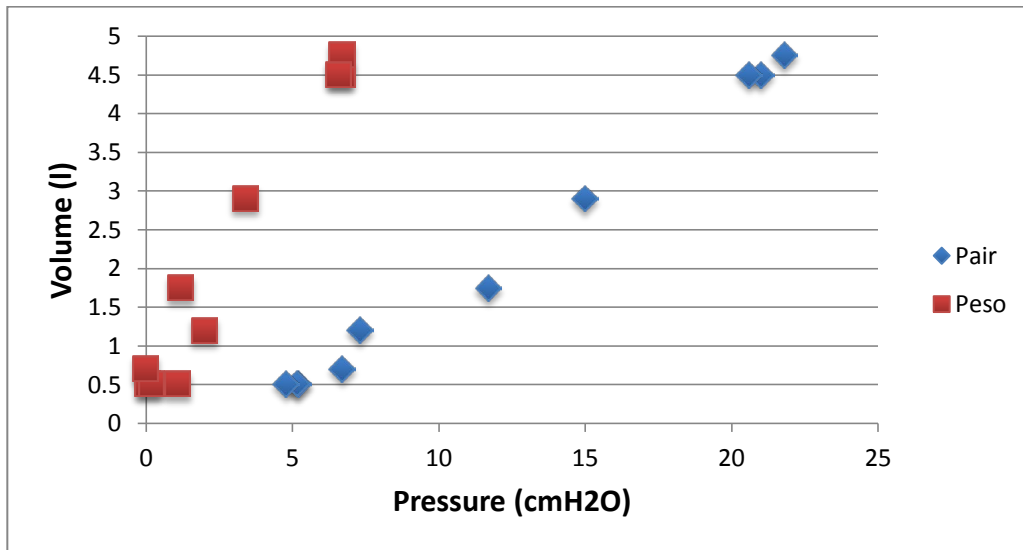


Figure 2. Raw data from mechanical ventilation of a Northern fur seal (NFS266).

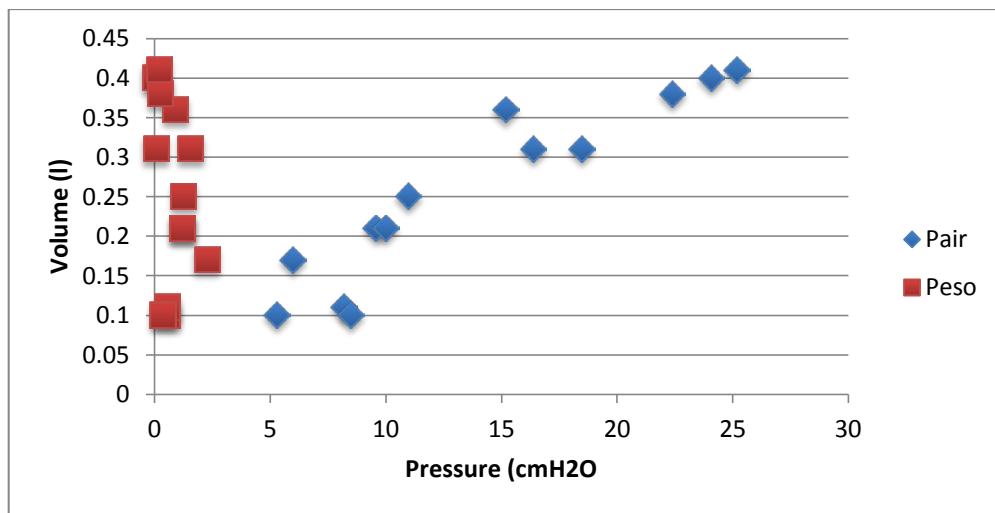


Figure 3. Raw data from mechanical ventilation of a Pacific Harbor seal (HS2328).

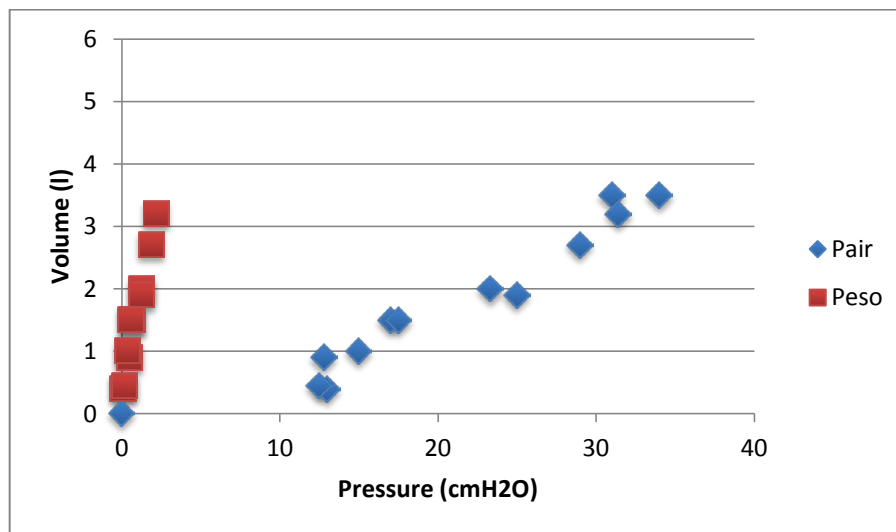


Figure 4. Raw data from mechanical ventilation of a California sea lion (CSL10638).

Aim 2: We collected continuous end-tidal O₂ and CO₂ 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 PCO₂ levels without apparent problems. In addition, O₂-dilution data were collected with the aim to estimate residual volume.

Aim 3: The data analysis is underway (see Figure 2-4). The analyzed data from live and deceased pinnipeds will be compared with our previous data set in excised lungs from odontocetes and phocids. These data will be used to update our model that predicts the air volumes in the upper (conducting airways) and lower (alveolar space) airways.

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_{N₂} 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.

REFERENCES

1. Hooker, S.K., R.W. Baird, and A. Fahlman, *Could beaked whales get the bends? Effect of diving behaviour and physiology on modelled gas exchange for three species: Ziphius cavirostris, Mesoplodon densirostris and Hyperoodon ampullatus*. Respiratory Physiology and Neurobiology, 2009. **167**: p. 235-246.

2. Zimmer, W.M.X. and P.L. Tyack, *Repetitive shallow dives pose decompression risk in deep-diving beaked whales*. Marine Mammal Science, 2007. **23**: p. 888-925.
3. Falke, K.J., et al., *Seal lung collapse during free diving: evidence from arterial nitrogen tensions*. Science, 1985. **229**(August 9): p. 556-557.
4. Ridgway, S.H. and R. Howard, *Dolphin lung collapse and intramuscular circulation during free diving: evidence from nitrogen washout*. Science, 1979. **206**(4423): p. 1182-1183.
5. Kooyman, G.L. and E.E. Sinnett, *Pulmonary shunts in Harbor seals and sea lions during simulated dives to depth*. Physiological Zoology, 1982. **55**(1): p. 105-111.
6. Fahlman, A., et al., *Estimating the effect of lung collapse and pulmonary shunt on gas exchange during breath-hold diving: the Scholander and Kooyman legacy* Respiratory Physiology and Neurobiology, 2009. **165**(28-39).
7. Fahlman, A., et al., *Deep diving mammals: Dive behavior and circulatory adjustments contribute to bends avoidance*. Respiratory Physiology & Neurobiology, 2006. **153**(1): p. 66-77.
8. Bostrom, B.L., A. Fahlman, and D.R. Jones, *Tracheal compression delays alveolar collapse during deep diving in marine mammals*. Respiratory Physiology and Neurobiology, 2008. **161**: p. 298-305.
9. Leith, D.E., *Comparative mammalian respiratory mechanics*. The physiologist, 1976. **19**: p. 485-510.
10. Olsen, C.R., F.C. Hale, and R. Elsner, *Mechanics of ventilation in the pilot whale*. Respiration Physiology, 1969. **7**: p. 137-149.