

UPDATING AND VALIDATING A CURRENTLY-USED GAS DYNAMICS MODEL
USING PARAMETER ESTIMATES FOR CALIFORNIA SEA LIONS (ZALOPHUS
CALIFORNIANUS)

A Thesis

by

MATTHEW ROBERT HODANBOSI

BS, The Ohio State University, 2014

Submitted in Partial Fulfillment of the Requirements for the Degree of

MASTER of SCIENCE

in

MARINE BIOLOGY

Texas A&M University-Corpus Christi
Corpus Christi, Texas

August 2016

© Matthew Robert Hodanbosi

All Rights Reserved

August 2016

UPDATING AND VALIDATING A CURRENTLY-USED GAS DYNAMICS MODEL
USING PARAMETER ESTIMATES FOR CALIFORNIA SEA LIONS (ZALOPHUS
CALIFORNIANUS)

A Thesis

by

MATTHEW ROBERT HODANBOSI

This thesis meets the standards for scope and quality of
Texas A&M University-Corpus Christi and is hereby approved.

Andreas Fahlman, PhD
Co-Chair

Blair Sterba-Boatwright, PhD
Co-Chair

J. Derek Hogan, PhD
Committee Member

August 2016

ABSTRACT

Theoretical models are used to predict how breath-hold diving vertebrates manage O₂, CO₂, and N₂ while underwater. One recent gas dynamics model used available lung and tracheal compliance data from various species to predict O₂, CO₂, and N₂ tensions in multiple tissues of diving marine mammals.

As variation in respiratory compliance significantly affects alveolar compression and pulmonary shunt, the objective of this thesis was to evaluate changes in model output when using species-specific parameters from California sea lions (*Zalophus californianus*). I explored the effects of lung and dead space compliance on the uptake of N₂, O₂, and CO₂ in various tissues during a series of hypothetical dives.

The updated parameters allowed for increased compliance of the lungs and an increased stiffness in the trachea. When comparing updated model output with a model using previous compliance values, there was a large decrease in N₂ uptake but little change in O₂ and CO₂ levels. Therefore, previous models may overestimate N₂ tensions and the risk of gas-related disease, such as decompression sickness (DCS), in marine mammals.

Using recently-collected empirical arterial and venous P_{O₂} data, I was able to test the model output against species-specific data for the first time. This showed that lung collapse can be altered by changing physiological parameters and that model input parameters may need to vary between dives. The results of this study suggest that previous models using data that is not species-specific may inaccurately predict the risk of gas-related disease in marine mammals. Future research can use physiological parameters from other marine mammal species as they become available to best estimate the risk of DCS in those species.

DEDICATION

I would like to thank my parents, Robert and Johanna Hodanbosi, friends, and family for their unwavering support throughout the completion of my thesis.

ACKNOWLEDGEMENTS

I would like to thank Dr. Birgette McDonald and Dr. Paul Ponganis for providing a dive data set for California sea lions that was used to develop the hypothetical dive data. Thank you to the other students of the Comparative Physiology Lab, especially Alexandra Epple, for providing feedback throughout the formation of this thesis. I would to thank the Office of Naval Research for providing funding for this project (Funding for this project was provided by the Office of Naval Research (Award # N000141512221 and ONR YIP Award # N000141410563).

TABLE OF CONTENTS

CONTENTS	PAGE
ABSTRACT.....	v
DEDICATION.....	vi
ACKNOWLEDGEMENTS.....	vii
TABLE OF CONTENTS.....	viii
LIST OF FIGURES.....	x
LIST OF TABLES.....	xi
INTRODUCTION.....	1
MATERIALS AND METHODS.....	6
<i>Model</i>	6
<i>Dive Parameters</i>	6
<i>Anatomical Parameters</i>	7
<i>Physiological Parameter Estimates</i>	7
<i>Lung Collapse Comparison</i>	10
STUDY AREA.....	11
RESULTS.....	12
<i>Updated Model Parameters</i>	12
<i>Model P_{N_2} Output</i>	14
<i>Model P_{O_2} Output</i>	17
<i>Model P_{CO_2} Output</i>	18
<i>Lung Collapse Comparison</i>	18

DISCUSSION.....	21
CONCLUSION.....	27
REFERENCES	28

LIST OF FIGURES

FIGURES	PAGE
Figure 1	7
Figure 2	13
Figure 3	14
Figure 4	15
Figure 5	16
Figure 6	17
Figure 7	19
Figure 8	20

LIST OF TABLES

TABLES	PAGE
Table 1	12

INTRODUCTION

Marine mammals routinely perform foraging dives to depths and for durations that would be fatal to other mammals. The current record for most-extreme dive belongs to the Cuvier's beaked whale (*Ziphius cavirostris*), which has been recorded diving to nearly 3000 m for 137 min (Schorr et al., 2014). Marine mammal species have physiological and anatomical traits that allow them to survive repeated exposure to these high-pressure environments. One such adaptation is their ability to manage internal gases under changing pressure to minimize accumulation of N₂ and thereby the risk of symptomatic gas bubbles.

The solubility of gases is directly related to the environmental pressure (Henry's law), and when animals are exposed to higher pressure, lung gases dissolve into the blood at higher partial pressures. In a hyperbaric environment, inert gases, like N₂, dissolve into blood or tissues until the tension of the gas has been equilibrated, at which time the tissue is said to be saturated. If the environmental pressure is reduced, the tissue or blood tension will eventually exceed the ambient partial pressure and the tissue is said to be supersaturated with the gas. Once the tissue is supersaturated, the gas may come out of solution and form bubbles. The likelihood for this to happen increases with the magnitude of supersaturation (Berghage et al., 1979; Fahlman et al., 2001; Weathersby et al., 1984). If these bubbles aggregate and become large enough, they can become lodged and cause ischemia or initiate an immune response (Kayar et al., 1997). This is a condition known as decompression sickness (DCS), or "the bends". To better understand the etiology of DCS and other gas-related diseases, it is therefore important to understand how gases (both inert and metabolic) behave in tissues at changing pressures.

The rate and amount of gases exchanged between the lungs and the blood are driven in part by the compliance of the respiratory system and the surface area of the alveoli (Bostrom et

al., 2008). To minimize N₂ uptake and the risk of DCS, it has been suggested that the respiratory anatomy of marine mammals allows the alveoli to collapse at shallow depths, at which point gas exchange ceases (Scholander, 1940). Marine mammals are equipped with a relatively stiff upper respiratory system and elastic chest and alveoli compared to terrestrial mammals (Cozzi et al., 2005; Fahlman et al., 2014a, 2011; Leith, 1979; Moore et al., 2014). As an animal dives, increasing ambient pressure causes the gas-filled spaces to compress, which increases the partial pressures of the gases in the alveoli (Boyle's Law). In turn, this increase in partial pressure of the gases in the lungs causes more gas to diffuse into the blood. However, because the chest and alveoli are compliant, these structures compress earlier and push gases into the stiff conducting airways (e.g., the trachea and bronchi), which do not participate in gas exchange. This results in a pulmonary shunt that increases with depth (Kooyman and Sinnett, 1982) and eventually causes gas exchange to cease (McDonald and Ponganis, 2012). Because these physiological processes occur at elevated pressures, it is logistically challenging to study gas dynamics in live, free-diving animals.

Mathematical models are useful tools that allow researchers to study physiological systems. For example, these models may help define which variables control complex physiological responses. However, any model is a simplification of a complex system and is limited by the accuracy of its assumptions and the input parameters. Current models aimed at defining the uptake and removal of metabolic (O₂ and CO₂) and inert gases (N₂) in breath-hold diving marine vertebrates often lack species-specific knowledge of physiological responses or parameters. For example, the changes in cardiac output during diving are often assumed to correlate with changes in heart rate and have only been measured in a limited number of marine mammal species (Miedler et al., 2015; Ponganis et al., 2006, 1991, 1990). Similarly, the changes

in gas diffusion across the lung as pressure changes are not well understood. Therefore, mathematical models that utilize respiratory compliance data (i.e., the degree of compression experienced by tissues under increasing pressure) have been used to estimate diffusion rates and the resulting gas tensions in blood and tissues (Bostrom et al., 2008; Fahlman et al., 2009, 2006; Fitz-Clarke, 2009).

In marine mammals, empirical physiological data, such as compliance estimates, are challenging to collect and often not available for most species. Therefore, previous diving models used data collected from a multitude of species, including terrestrial mammals such as sheep and dogs, which differ greatly in respiratory anatomy from marine mammals (Bostrom et al., 2008; Fahlman et al., 2006; Moore et al., 2014). However, the importance of species-specific physiological parameters in modeling gas dynamics is currently not known. Recently, anatomical and physiological data have been collected from living California sea lions (*Zalophus californianus*), as well as from excised respiratory tissues. These new data allow for the use of species-specific parameters to estimate gas dynamics in an individual during a series of dives and compare species-specific data to multi-species models. This thesis will explore the significance of species-specific parameters in a diving model by comparing these with parameters previously used based on data from terrestrial mammals.

As empirical physiological data become available for marine mammal species, it is important to compare the model output to these data. Recently, researchers were able to collect venous and arterial P_{O_2} data from free-ranging, diving California sea lions (McDonald and Ponganis, 2013, 2012). These data allowed the researchers to estimate when lung collapse occurred during a series of dives and to develop a regression line relating maximum dive depth and depth of lung collapse for each dive. In the current project, the model output included lung

volume and depth at each time-step of a dive series. Therefore, I was able to determine the depth at which lung collapse occurred during a modeled dive and compared that to the empirical data. Then, I altered two input physiological parameters, diving lung volume and degree of bradycardia (i.e., decrease in heart rate), to determine how changes in these parameters impact lung collapse depth. This allows us to better understand the physiological adaptations marine mammals utilize to mitigate gas uptake during a series of dives.

This gas dynamics model also has the capability to predict how marine mammals will interact with their environment. One scenario of particular interest is the eco-physiological impact of climate change on diving marine mammals. Marine mammals often occupy the highest trophic levels within their ecosystems, which are shown to be more affected by climate change than organisms occupying lower trophic levels (Both et al., 2009). Since California sea lions prey on marine fishes of low- and mid-trophic levels, they are highly influenced by the behavior of these lower trophic level species (Edwards and Richardson, 2004). It has been reported that marine fishes will change latitude (i.e., move towards the poles) or change mean depth in response to increasing water temperatures (Perry et al., 2005; Roessig and Woodley, 2004). Since marine mammal foraging bouts at depth are not always successful (Austin et al., 2006), California sea lions may have to alter their diving behavior in order to avoid decreasing their foraging success rate.

Some potential alterations to diving behavior that California sea lions could perform are increasing dive depth, time spent at depth during a dive, or decreasing the interval of time at the surface between dives. Additionally, California sea lions could shift their prey preferences to species that are easier to catch, which could have an impact on their physiology as a whole, and on their metabolism and respiratory quotient (i.e., the amount of O₂ inhaled versus the amount of

CO₂ exhaled) in particular (Krogh and Lindhard, 1920). By investigating gas tension output of the revised model, I was able to determine if California sea lions have the physiological capabilities to alter their diving behavior without drastically increasing the chance of running out of oxygen or developing DCS during a foraging bout.

MATERIALS AND METHODS

Model

The model described in this thesis is an updated version of the model used by Fahlman et al. (2009) which combined a gas dynamics (Fahlman et al., 2006) and lung compression model (Bostrom et al., 2008). The combined model estimates changes in pulmonary, blood, and tissue O₂, CO₂, and N₂ levels during breath-hold diving. The body is divided into 5 different compartments where gases were exchanged: blood, brain, central circulation, fat, and muscle. These compartments are grouped as fast (blood, brain, central circulation), intermediate (muscle), and slow (fat) based on their tissue-time constants and blood flow (Fahlman et al., 2009). In the current effort, these stores are unchanged. The updated model parameters are based on respiratory compliance data collected from anesthetized California sea lions, as well as from excised tissues from deceased specimens (Fahlman et al., 2014a; Moore et al., 2014).

Dive Parameters

A simulated dive bout (i.e., a series of repeated dives with a short intervening surface interval) was created that consisted of 6 dives, with an ascent and decent rate of 1.5 m·sec⁻¹, to a pressure of 30.1 atmospheres (ATA; 291 m), and a 400 sec surface interval (

Figure 1). An initial surface time of 400 sec was included to allow the initial gas levels to stabilize prior to the start of the dive bout. This dive series was based on the parameters observed in a previously published dive data collected from a free-ranging female California sea lion (McDonald and Ponganis, 2013).

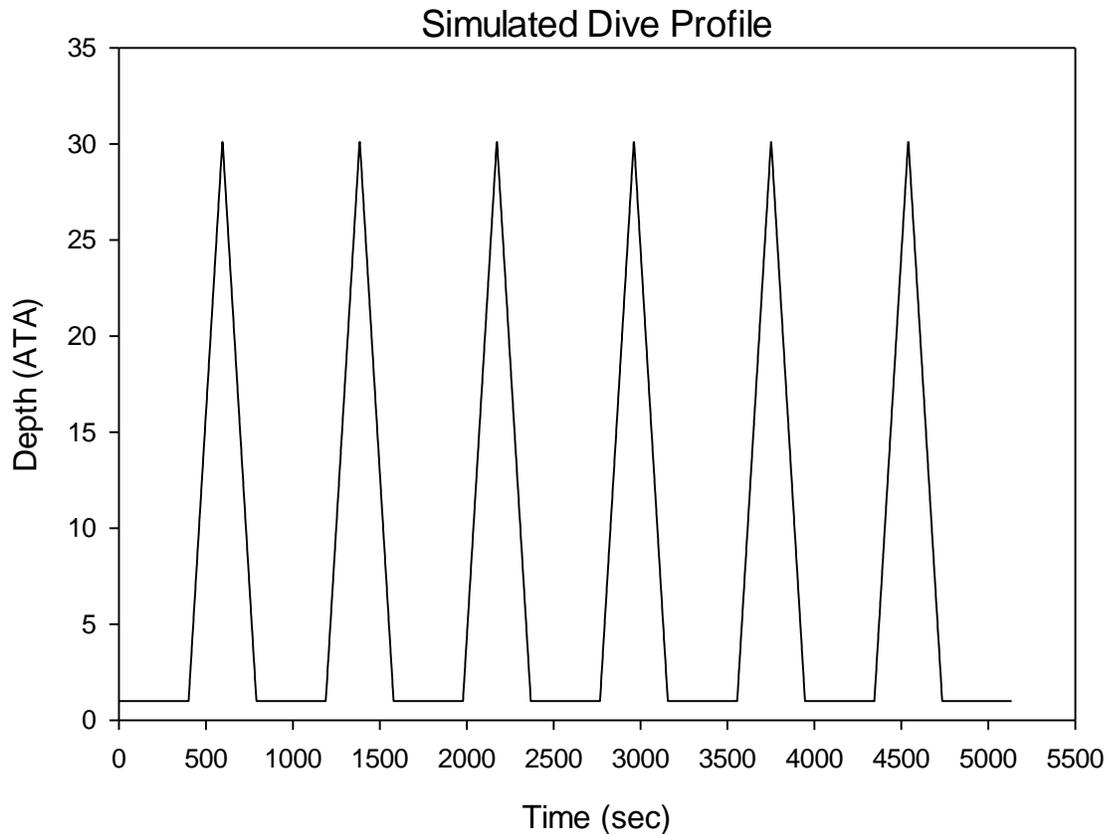


Figure 1. A simulated dive profile used in the model to estimate tissue-specific gas tensions at depth.

Anatomical Parameters

Body mass was estimated as the mean body mass of the male California sea lions from which the updated parameter estimates originated (64 kg, N=3; Fahlman et al., 2014). Diving lung volume (i.e., the volume of gas in the lungs as the animal initiates a dive) was estimated as the mean of previously published diving lung volumes (percentage of total lung capacity [TLC]) collected from forced dived California sea lions (48% TLC, N=2; Kooyman and Sinnett, 1982). The volume of the dead space (i.e., trachea and bronchi) was estimated at 5% TLC based on previously published data (Kooyman and Sinnett, 1979; Moore et al., 2014).

Physiological Parameter Estimates

Data collected on the pressure-volume (P-V) relationship from the lungs of 3 live adult California sea lions with no signs of respiratory illness while under anesthesia (Fahlman et al., 2014a) and excised conducting airways (Moore et al., 2014), as well as pulmonary shunt, were fit to the following equations. The P-V relationship for the alveolar space was:

$$V_{A,N} = a(1 + e^{-c(-b-P_{A,S})})^{-1} \quad (1)$$

where a , b , c , are fitting parameters, $P_{A,S}$ is the structural pressure of alveoli and $V_{A,N}$ is the normalized alveolar volume ($V_A \cdot TAC^{-1}$; Bostrom et al., 2008). The fitting parameters can be interpreted as follows: parameter a is the maximum normalized volume of lung expansion, parameter b is the pressure value at which the inflection point of the curve occurs, and parameter c represents how quickly the lungs can go from fully inflated to uninflated. To estimate tracheal compliance, P-V data from 3 California sea lions were fitted to the following equation:

$$V_{D,N} = (1 - P_{D,S}(0.0981Kp)^{-1})^{-1/n} \quad (2)$$

where Kp and n are fitting parameters that define the stiffness of the tracheal walls, $P_{D,S}$ is the structural pressure of dead space and $V_{D,N}$ is the normalized dead space volume ($V_D \cdot V_{D,o}^{-1}$; Bostrom et al., 2008). Previously published pressure-shunt data collected from 2 California sea lions (Kooyman and Sinnett, 1982) were fit to the following pulmonary shunt equation:

$$\text{Shunt} = 1 - (g \cdot (DV_A \cdot V_A^{-1})^{-h}) \quad (3)$$

where g and h are fitting parameters that define how quickly the lungs collapse, where DV_A is the estimated alveolar volume at depth and V_A is the maximum alveolar volume (Fahlman et al., 2009). The parameters were estimated using either nonlinear mixed effects models or nonlinear least squares models. For equations 1 and 2, the use of individual animals as levels of a random grouping factor was tested. The fitting parameters of the models were tested both as fixed values across all individuals, or permitted to vary individually by animal. Corrected Akaike information criteria (AICc; Burnham and Anderson, 2003) was used to determine the optimal choices of fixed versus random parameters.

End-dive tissue gas tension differences were calculated using the following equation:

$$\text{Percent difference} = [(old - new) \cdot old^{-1}] \cdot 100\% \quad (4)$$

where *old* is the gas tension value from the model run using the old parameters and *new* is the gas tension value from the model run using the new parameters. The values were collected 3 seconds after surfacing to allow the lung gasses to stabilize prior to analysis. Because all fast-loading tissues had similar tissue-time constants and gas tension outputs, a single representative tissue (i.e., brain) was chosen for analysis of this tissue type.

All model fitting was performed using R version 3.2.1 (R Core Team, 2015) including the nlme package version 3.1-124 (Pinheiro et al., 2016) and the MuMIn package version 1.15.6 (Barton, 2015). The physiological modeling was performed using MATLAB (R2010a) version 7.10.0.499 (The MathWorks Inc., 2010).

Lung Collapse Comparison

A single simulated dive was created based on the dive profiles collected by McDonald and Ponganis (2013, 2012), with an ascent and decent rate of $1.5 \text{ m}\cdot\text{sec}^{-1}$, to a pressure of 30.1 atmospheres (ATA; 291 m), and 2000 sec initial surface time before the dive bout (Figure 2). Mass was estimated as the mean published mass of individuals sampled in a study using free-ranging individuals (80 kg, N=7; McDonald and Ponganis, 2013). Minimum lung volume before lung collapse occurred was set to 0.01% of diving lung volume. A sensitivity analysis was performed on the model to determine how variations in two physiological parameters, diving lung volume and degree of bradycardia (i.e., decrease in cardiac output), affected the depth of lung collapse. Lung compliance values were set to species-specific values presented below. Then, surface cardiac output ($\text{CO}_{\text{surface}}$) was set to previously published values for California sea lions ($\text{CO}_{\text{surface}} = 3.0 \text{ ml}\cdot\text{kg}^{-1}\cdot\text{s}^{-1}$; Ponganis et al., 1991). Diving lung volumes were varied between 40% and 100% of estimated total lung capacity. Next, diving bradycardia was varied so that CO_{dive} was between 20% and 100% of $\text{CO}_{\text{surface}}$. Metabolic rate was constant at 100% of estimated surface metabolic rate. These values were chosen based on previously published diving heart rate values in California sea lions (Elsner et al., 1964).

The resulting output of the model was compared to empirical data presented on the relationship between maximum dive depth and lung collapse depth presented by McDonald and Ponganis (2012). Based on the regression line for these data and the chosen maximum dive depth, the target lung collapse depth was 22.6 ATA. The range of observed collapse depths for dives of all depths was 16.0-27.0 ATA (McDonald and Ponganis, 2012).

STUDY AREA

My project utilized pre-existing empirical data collected on California sea lions. In 2010 and 2011, researchers captured lactating California sea lions on San Nicolas Island, CA, USA to collect physiological data while free diving (McDonald and Ponganis, 2013, 2012). The P-V relationships were collected on anesthetized and deceased individuals at the Marine Mammal Center in Sausalito, CA, USA (Fahlman et al., 2014a).

RESULTS

Updated Model Parameters

The alveolar compliance equation (Equation 1) with the lowest AICc included parameter a as a fixed factor amongst all animals and parameters b and c as random factors between each animal. Alveolar compliance was greater using the updated parameter estimates (Table 1; Figure 2). The tracheal compliance equation (Equation 1) with the lowest AICc included parameters Kp and n as fixed factors amongst all animals. Tracheal compliance was less using the updated parameter estimates (Table 1; Figure 2). Pulmonary shunting was slightly more efficient using the updated parameter estimates (Table 1; Figure 3).

Alveolar Compliance Equation (Eq. 1)		
Parameter	Former Value	Updated Value
A	1.11	1.03±0.07
B	1.23	1.76±0.27
C	1.34	1.80±0.14
Tracheal Compliance Equation (Eq. 2)		
Parameter	Former Value	Updated Value
Kp	-12.78	-12.54±11.90
N	0.907	4.24±2.46
Pulmonary Shunt Equation (Eq. 3)		
Parameter	Former Value	Updated Value
g	-1.06	-1.07±0.01
h	0.17	0.21±0.01

Table 1. The previous and updated parameter estimate values used for the equations used in the gas dynamics model.

Compliance Estimates

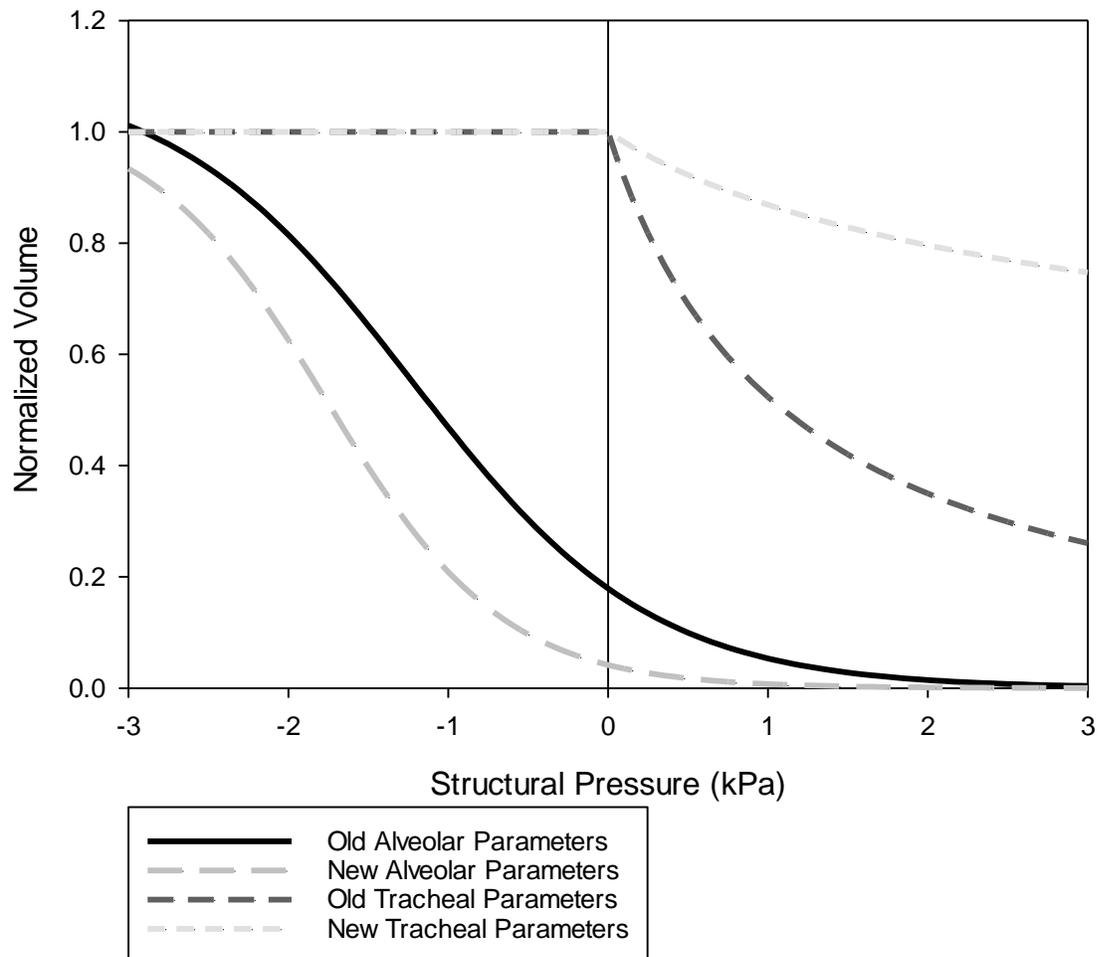


Figure 2. Normalized lung volume estimates using the former parameter estimates (darker lines) and the updated parameter estimates (lighter lines).

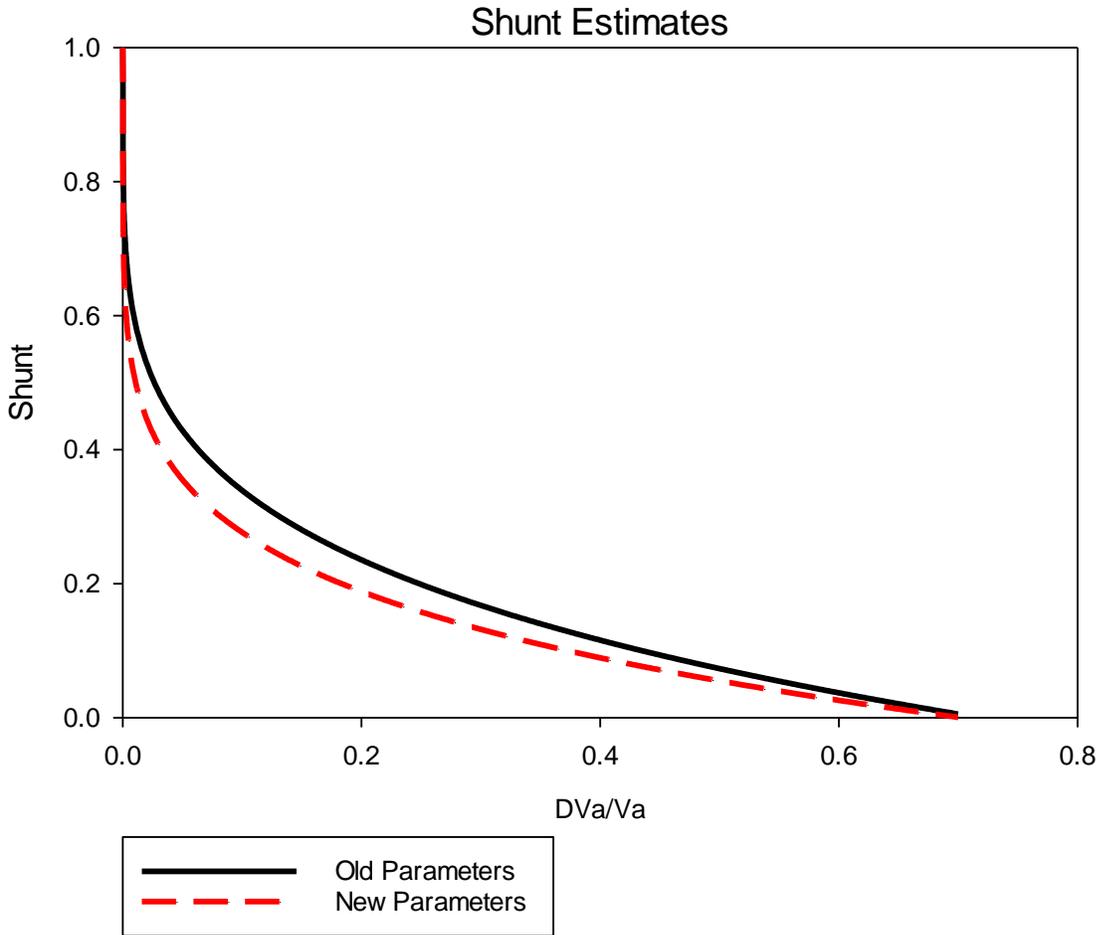


Figure 3. Shunt estimates using the former parameter estimates (black) and the updated parameter estimates (red).

Model P_{N_2} Output

Using a simulated dive data set, the model output using the updated parameter estimates had lower P_{N_2} estimates than the model output using the former parameter estimates in all tissue types: fast-loading tissues (e.g., brain, Figure 4), intermediate-loading tissues (e.g., muscle, Figure 5), and slow-loading tissues (e.g., fat, Figure 6). The end-dive P_{N_2} between old and new model outputs decreased by $51.2 \pm 0.1\%$ for brain, $39.6 \pm 0.4\%$ for muscle, and $22.6 \pm 7.7\%$ for fat.

Brain PN₂

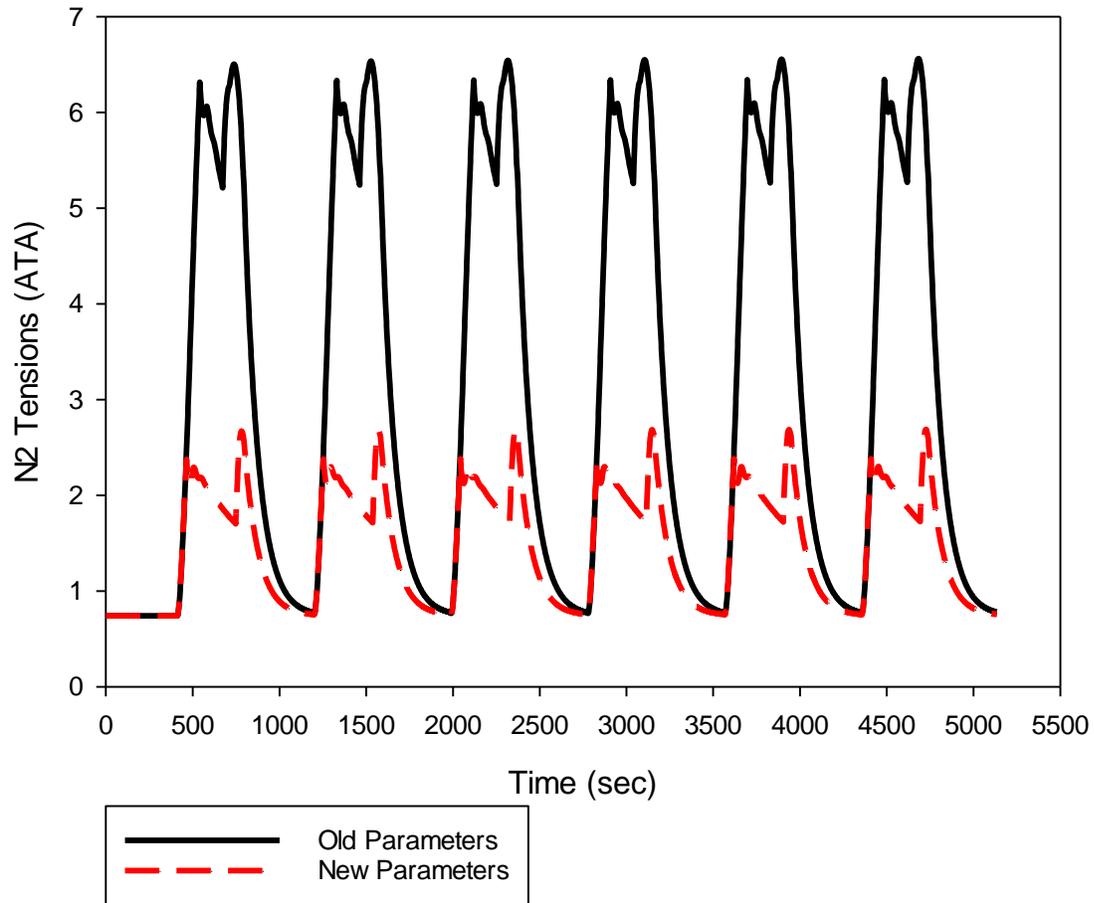


Figure 4. Brain (a fast-loading tissue) P_{N_2} estimates using the former parameter estimates (black) and the updated parameter estimates (red) using a simulated dive profile from a California sea lion.

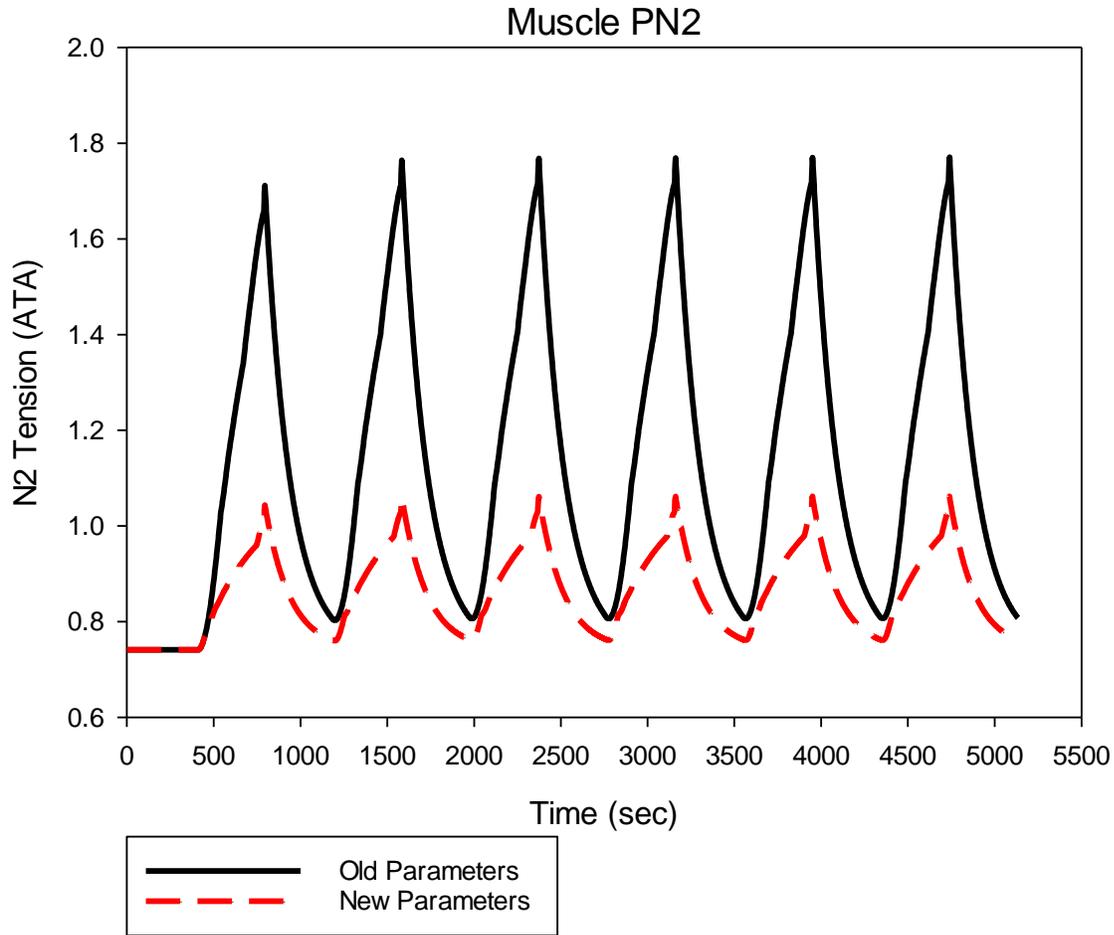


Figure 5. Muscle (an intermediate-loading tissue) P_{N_2} estimates using the former parameter estimates (black) and the updated parameter estimates (red) using a simulated dive profile from a California sea lion.

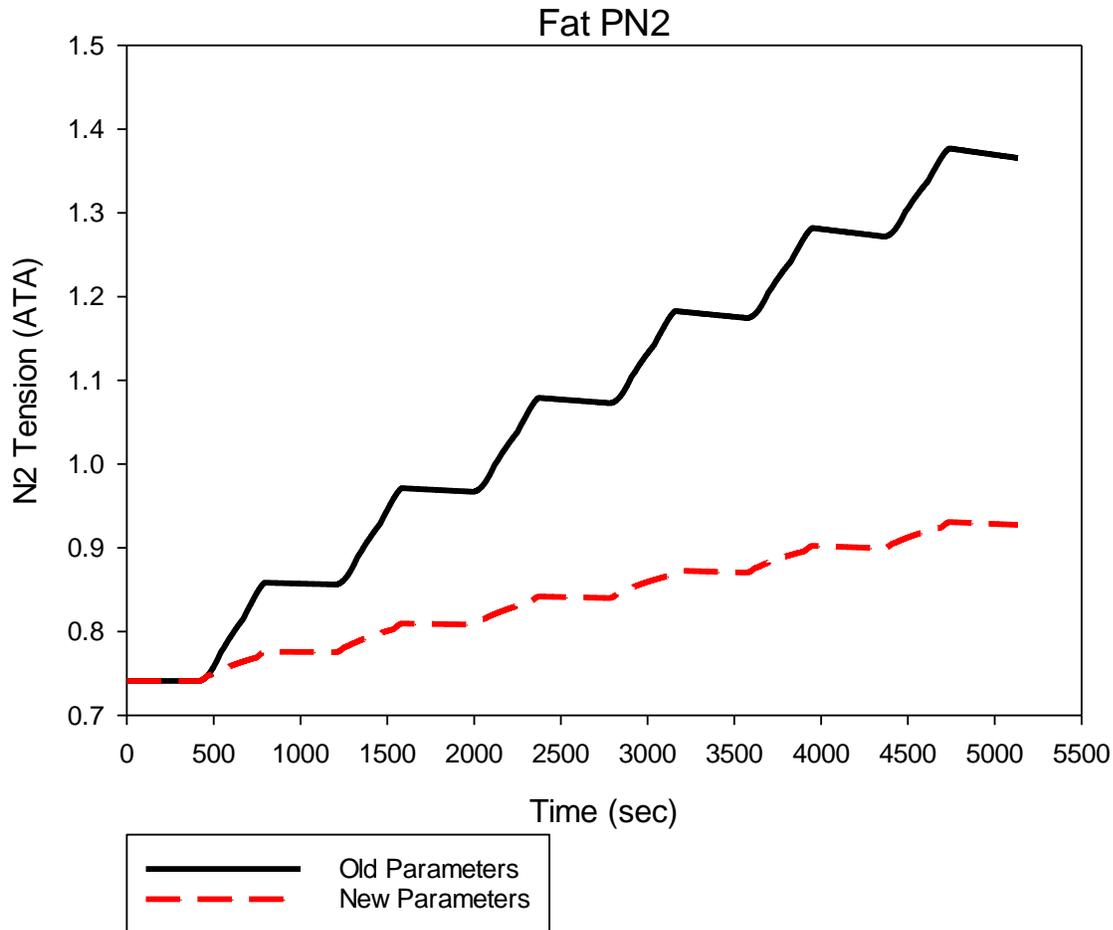


Figure 6. Fat (a slow-loading tissue) P_{N₂} estimates using the former parameter estimates (black) and the updated parameter estimates (red) using a simulated dive profile from a California sea lion.

Model P_{O₂} Output

The model output using the updated parameter estimates had similar P_{O₂} estimates to the model output using the former parameter estimates in all tissue types: fast-loading tissues (e.g., brain), intermediate-loading tissues (e.g., muscle) and slow-loading tissues (e.g., fat), and never reached a value of 0 ATA during the dive series. The end-dive P_{O₂} decreased by <0.1% for brain, 5.6±0.1% for muscle, and <0.1% for fat (Figure 7).

Model P_{CO_2} Output

Also, the model output using the updated parameter estimates had similar P_{CO_2} estimates to the model output using the former parameter estimates in all tissue types: fast-loading tissues (e.g., brain), intermediate-loading tissues (e.g., muscle) and slow-loading tissues (e.g., fat). The end-dive P_{CO_2} decreased by $1.5 \pm 0.1\%$ for brain, $0.5 \pm 0.1\%$ for muscle, and $0.3 \pm 0.1\%$ for fat (Figure 7).

Lung Collapse Comparison

Using previously reported diving lung volumes for California sea lions led to lung collapse occurring much earlier in a dive than expected based on the empirical data. However, varying diving lung volume with other diving parameters led to a lung collapse depth similar to that predicted by the empirical data (collapse depth=22.6 ATA; Figure 8). Lines with a CO_{dive} between 20% and 33% $CO_{surface}$ intersected the predicted collapse depth at diving lung volumes between 90% and 100% TLC. Diving lung volume had to be larger than 60% TLC to see lung collapse depth values within the range presented by McDonald and Ponganis (2012). Also, changes in diving lung volume had a greater effect on lung collapse depth than changes in cardiac output.

End Dive Gas Tension Differences

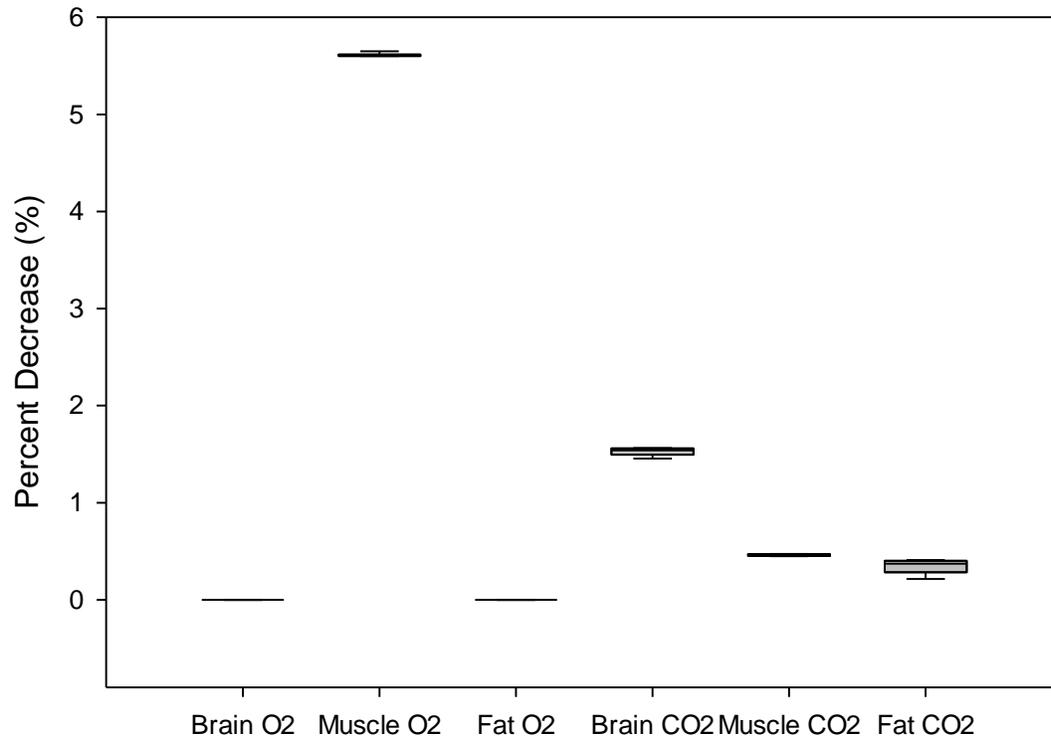


Figure 7. End-dive P_{O_2} and P_{CO_2} differences ($[\text{old}-\text{new}]/\text{old} \times 100\%$) between model runs with old and new parameters for fast-loading (brain), intermediate-loading (muscle), and slow-loading (fat) tissues.

Diving Lung Volume versus Lung Collapse Depth

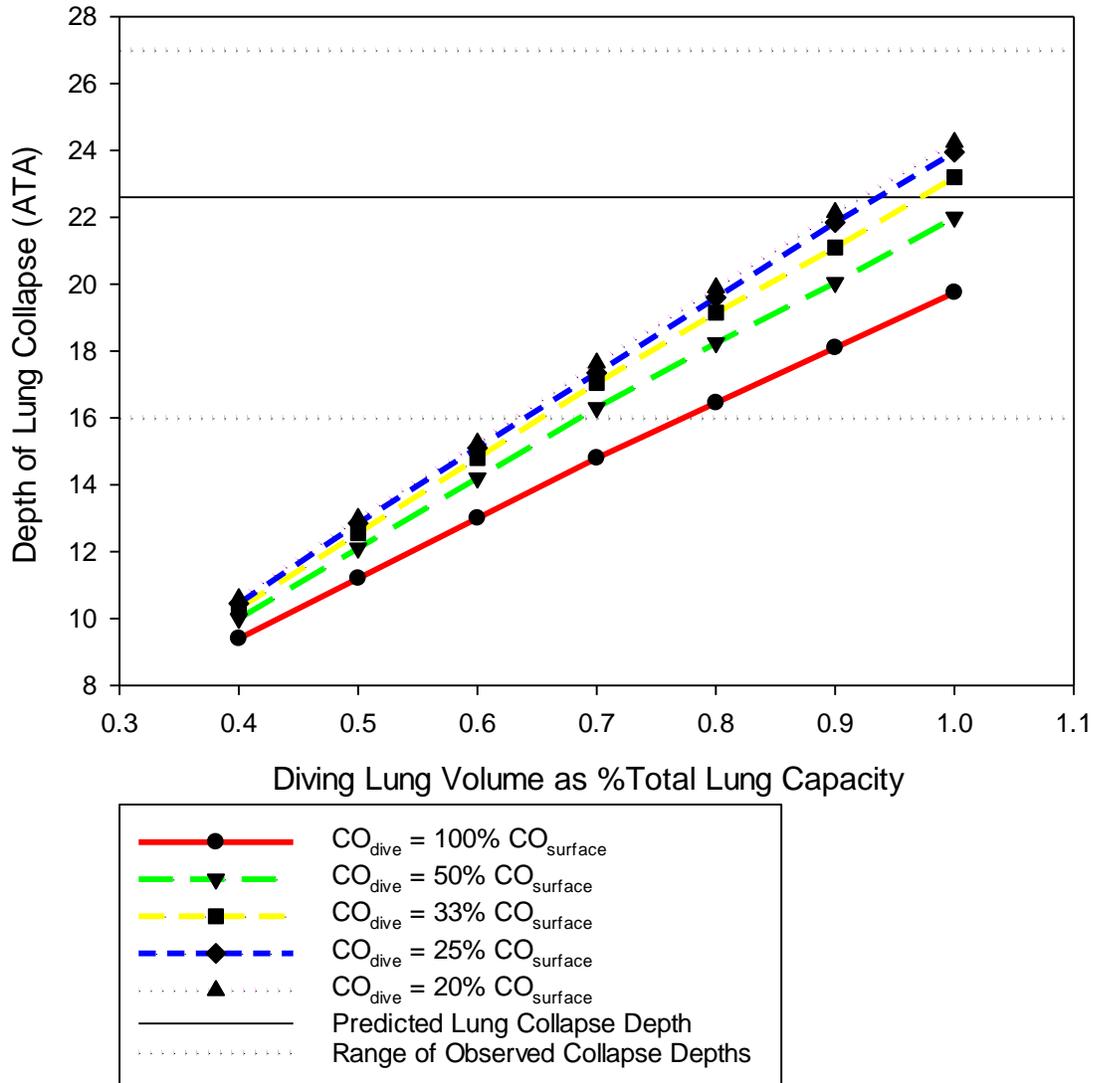


Figure 8. Points represent dive parameter sets used in the model to estimate lung collapse for a single dive to 30.1 ATA. The predicted lung collapse depth (horizontal black line) was selected based on the regression line $y=0.62 \cdot \text{depth (m)} + 35.66 \text{ m}$, then converted to ATA. Black dotted lines indicate range at which lung collapse was observed for all dives by McDonald and Ponganis (Figure 2; 2012)

DISCUSSION

In this thesis, I present a model to estimate gas exchange during breath-hold diving. This is the first attempt to quantify gas exchange using species-specific parameters for a marine mammal's respiratory system. Updated parameter estimates from species-specific data show large differences in N_2 tensions in all tissue types as compared to the previous result that did not use species-specific compliance estimates. In addition, there was almost no difference in CO_2 and O_2 tensions between the updated and previous models. New input parameters were based on data collected from excised tissues from deceased animals or live animals under anesthesia, while previous input parameters were based solely on excised tissues. Due to the lack of empirical data on the physiology of diving marine mammals, many factors in the model are likely over-simplified. Despite these limitations, the updated model provides interesting results that can help generate hypotheses as to the mechanics of gas exchange during diving.

Scholander (1940) proposed that to avoid exposure to extreme N_2 tensions, marine mammals have rigid upper-airways and flexible alveoli that collapse as the animal dives. However, research has shown that the conducting airways do experience some degree of compression (Kooyman et al., 1970). Tracheal compliance estimates used in previous models were based on data from terrestrial mammals (Aljuri et al., 1999). Respiratory compliance data for marine mammals are sparse but available for a few species (Fahlman et al., 2015, 2014a; Leith, 1979; Moore et al., 2014; Olsen et al., 1969). The tracheal compliance estimates for California sea lions show a greater degree of stiffness than the estimates used in previous models (Bostrom et al., 2008; Fahlman et al., 2009). The new data used in the updated gas exchange model support Scholander's hypothesis, that the stiff upper-airways are used as a reservoir for gases while the alveolar space collapses in order to limit gas exchange at high pressures. The

increased stiffness of the dead space and the increased compliance of the alveoli in the updated model allow the alveoli to collapse at a lower pressure than previously estimated. Thus, gas exchange occurs over a reduced pressure range, decreasing the amount of gases that enter the body.

Because models are constrained by their input parameters, it is crucial to use the most accurate and reliable data available. The new data used to estimate the parameters in this model came from animals at a rehabilitation facility (Fahlman et al., 2014a; Moore et al., 2014). One potential problem with using physiological data from animals undergoing rehabilitation is that they may have suffered from respiratory disease when they were tested. However, only animals without any signs of pulmonary disease were selected for these respiratory compliance studies (Fahlman et al., 2014a; Moore et al., 2014).

In addition, tracheal compliance parameters were estimated using excised tracheas that were taken from deceased animals that had either asphyxiated during fisheries interactions or had been euthanized for reasons other than being used for this research project (Fahlman et al., 2014a, 2011; Moore et al., 2014). Since the tracheas were no longer interacting with surrounding tissues, such as the lungs and the pleural cavity, these data may not reflect tracheal compliance in live, free-diving individuals. Therefore, the compliance may differ in the excised tracheas than tracheas in a complete respiratory system. Thus, imaging studies of live animals may help to improve our understanding of the mechanical properties of the respiratory system in marine mammals and how it behaves under pressure.

The mathematical formulas used in a model are also important to the realism of the output of the model. Because little is known about the physiology of gas exchange in the lungs of diving marine mammals, many of the equations used in the model are based on simplified,

theoretical equations. For example, since the gas concentration gradient in the lungs is not well understood, it is assumed that gases are mixed ubiquitously throughout the lungs in this model. However, previous studies in humans have shown that inhaled gases are not mixed uniformly throughout the lungs (Fowler, 1949; Krogh and Lindhard, 1917; Roelsen, 1938). In addition, lung gas concentrations are immediately changed to surface values once the animal reaches the surface in the model, which does not reflect the effect of alveolar ventilation and alternating alveolar gas concentrations during recovery of a free-diving animal (Kooyman et al., 1971). Therefore, the model may underestimate the time needed for tissues to off-gas at the surface.

In the current effort, the parameters in the mathematical formulas were allowed to be fixed amongst all individuals or vary between them. In the alveolar compliance equation, parameter a was fixed while parameters b and c were random (Equation 1). This makes sense biologically, because parameter a represents the maximum possible normalized volume, which should be around 1 for all individuals, and parameters b and c determine the compliance of the lungs, which can vary between individual animals. In the tracheal compliance equation, both parameters were fixed (Equation 2). It would make sense biologically for these parameters to be random, but there were so few data points for each animal that the accuracy of the values was greater when all data were fit together instead of individually for each animal.

In the updated model, the decreased N_2 tension output for all tissue types indicate a lower risk of gas-related diseases in California sea lions than would be estimated using the previous model parameters (Fahlman et al., 2009). In addition, the results of the current study imply that the risk of gas-related diseases in California sea lions is generally low. Thus, this species may be able to significantly change their dive behavior in response to anthropogenic disturbances, such

as naval sonar or environmental change, while minimally increasing the risk of gas bubble disease.

The output of this updated model indicates that species-specific parameters are important to best estimate the physiological processes involved with a dive. Empirical data on the anatomy of different marine mammals is crucial in estimating how variations in gas exchange parameters affect blood and tissue gas levels. Once species-specific anatomical data are available, the model input can be adjusted to improve the potential physiological impact of changes in the environment, such as anthropogenic noise or climate change. Species that appear especially prone to decompression sickness (DCS), such as Cuvier's beaked whales (Fahlman et al., 2014b; Fernández et al., 2005; Houser et al., 2001; Jepson et al., 2003) are of particular interest, as the output of the updated model suggests that previous blood and tissue P_{N_2} estimates may be overestimated.

Although there is no evidence that California sea lions are susceptible to DCS, they make a great model species for marine mammal diving physiology. While recent reports have indicated that California sea lions may experience gas bubble disease, this may have been secondary to barotrauma (Van Bonn et al., 2013, 2011). Due to their life history traits, in particular the capability of this species to spend extended periods of time on land, researchers often choose this species for studies that require tagging and handling compared to other marine mammals. Therefore, more physiological data are available on California sea lions than species that are prone to DCS, such as beaked whales. Until more physiological data become available from cetaceans, data from California sea lions can be used to inform the model and provide estimates on the risk of DCS in other diving marine mammal species.

In order to better estimate the effects of lung collapse on gas dynamics, a critical next step is to continue to update the model with parameters not already included. For example, chest wall compliance data should be measured to assure that it does not have an effect on internal pressure and affect the collapse depth of the alveoli. By adding physiological parameters not currently included in the model, and comparing the updated model to empirical data, the accuracy of the model can continuously be increased. The process of updating and vetting the model will allow us to estimate how changes in diving behavior will affect gas tensions in marine mammals. These new estimates can then be used to estimate the risk different marine mammal species face in response to various environmental stressors or ecological changes.

The depth of lung collapse during a single dive in our model varied with diving lung volume and the degree of bradycardia (Figure 8). In particular, increasing the diving lung volume and degree of bradycardia parameters in the model increased the depth of lung collapse. This suggests that California sea lions may be able to plan how deep they will dive before initiating the dive by manipulating their heart rate or inhaling a specific amount of air. Recent research supports this idea, as McDonald and Ponganis found a positive relationship between depth of lung collapse and maximum dive depth ($r^2 = 0.76$; see figure 2 of McDonald and Ponganis, 2012). Similar findings have been reported in Adélie, king, and emperor penguins and suggested for Steller's sea lions (Sato et al., 2011, 2002; Svård et al., 2009). However, data suggests Antarctic fur seals dive with a constant lung volume (Hooker et al., 2005). An important next step in this area of study is to determine how variations in lung collapse depth affect gas tensions in tissues. In order to better estimate gas tensions, it may be crucial to update the model to allow for diving lung volume and cardiac output to vary between dives.

The findings of my research have important ecological implications as well. Marine mammals have to manage both oxygen stores and nitrogen tensions during a series of dives. The output of the model suggests that California sea lions do not reach their aerobic dive limit, when the body runs out of oxygen and switches from aerobic metabolism to anaerobic metabolism (Kooyman et al., 1980). Also, model output shows a much lower end-dive P_{N_2} value for slow-loading tissues than previously estimated (Figure 6). Therefore, if prey species move deeper in the water column in response to increasing ambient water temperatures (Perry et al., 2005; Roessig and Woodley, 2004), it appears that California sea lions may be able to alter their diving behavior (e.g., increase diving lung volume to perform deeper dives) without greatly increasing the risk of developing gas-related disease. However, an important next step is to determine how changing dive behavior impacts the metabolic costs of this species and potential effects this would have on the fecundity of individuals.

CONCLUSION

In conclusion, the work presented in this thesis is a first attempt to quantify gas exchange using species-specific parameters for a marine mammal's respiratory system. Modeling gas uptake in different tissue types using species-specific parameter estimates for California sea lions led to lower N₂ tension output in all tissue types as compared to previous estimates. However, there was little difference in O₂ and CO₂ tension output compared to previous estimates. A sensitivity analysis on diving lung volume and cardiac output using this model also provides evidence that California sea lions may be capable of planning how deep they will dive before initiating the dive by manipulating their heart rate or inhaling a specific amount of air. Because the O₂ tensions do not reach 0 ATA during the dive series, this species may be able to alter their dive behavior in response to anthropogenic disturbances or environmental change without reaching their aerobic dive limit. The lower N₂ tensions in the updated model suggest that changes in diving behavior would have caused a lower risk of gas bubble disease than previously estimated. Until specific-specific physiological data become available for cetaceans, data from California sea lions can be used to inform the model and provide the most realistic estimates on the risk of DCS in diving marine mammal species.

REFERENCES

- Aljuri, N., Freitag, L., Venegas, J.G., 1999. Modeling expiratory flow from excised tracheal tube laws. *J. Appl. Physiol.* 87, 1973–1980.
- Austin, D., Bowen, W.D., McMillan, J.I., Boness, D.J., 2006. Stomach temperature telemetry reveals temporal patterns of foraging success in a free-ranging marine mammal. *J. Anim. Ecol.* 75, 408–420. doi:10.1111/j.1365-2656.2006.01057.x
- Barton, K., 2015. MuMIn: Multi-Model Inference (R package).
- Berghage, T.E., David, T.D., Dyson, C. V., 1979. Species differences in decompression. *Undersea Biomed. Res.* 6, 1–13.
- Bostrom, B.L., Fahlman, A., Jones, D.R., 2008. Tracheal compression delays alveolar collapse during deep diving in marine mammals. *Respir. Physiol. Neurobiol.* 161, 298–305. doi:10.1016/j.resp.2008.03.003
- Both, C., Van Asch, M., Bijlsma, R.G., Van Den Burg, A.B., Visser, M.E., 2009. Climate change and unequal phenological changes across four trophic levels: Constraints or adaptations? *J. Anim. Ecol.* 78, 73–83. doi:10.1111/j.1365-2656.2008.01458.x
- Burnham, K.P., Anderson, D.R., 2003. Model selection and multimodel inference: a practical information-theoretic approach. Springer Science & Business Media.
- Cozzi, B., Bagnoli, P., Acocella, F., Costantino, M.L., 2005. Structure and biomechanical properties of the trachea of the striped dolphin *Stenella coeruleoalba*: evidence for evolutionary adaptations to diving. *Anat. Rec. A. Discov. Mol. Cell. Evol. Biol.* 284, 500–510. doi:10.1002/ar.a.20182
- Edwards, M., Richardson, A.J., 2004. Impact of climate change on marine pelagic phenology and trophic mismatch. *Nature* 430, 881–884. doi:10.1038/nature02808

- Elsner, R.W., Franklin, D.L., Van Citters, R.L., 1964. Cardiac Output during Diving in an Unrestrained Sea Lion. *Nature* 202, 809–810.
- Fahlman, A., Hooker, S.K., Olszowka, A., Bostrom, B.L., Jones, D.R., 2009. Estimating the effect of lung collapse and pulmonary shunt on gas exchange during breath-hold diving: the Scholander and Kooyman legacy. *Respir. Physiol. Neurobiol.* 165, 28–39.
doi:10.1016/j.resp.2008.09.013
- Fahlman, A., Loring, S.H., Ferrigno, M., Moore, C., Early, G., Niemeyer, M., Lentell, B., Wenzel, F., Joy, R., Moore, M.J., 2011. Static inflation and deflation pressure-volume curves from excised lungs of marine mammals. *J. Exp. Biol.* 214, 3822–3828.
doi:10.1242/jeb.056366
- Fahlman, A., Loring, S.H., Levine, G., Rocho-Levine, J., Austin, T., Brodsky, M., 2015. Lung mechanics and pulmonary function testing in cetaceans. *J. Exp. Biol.* 218, 2030–2038.
doi:10.1242/jeb.119149
- Fahlman, A., Loring, S.H., Shawn, P., Haulena, M., Trites, A.W., Fravel, V.A., Bonn, W.G. Van, Trumble, S.J., 2014a. Inflation and deflation pressure-volume loops in anesthetized pinnipeds confirms compliant chest and lungs. *Front. Physiol.* 5, 433–439.
doi:10.3389/fphys.2014.00433
- Fahlman, A., Olszowka, A., Bostrom, B., Jones, D.R., 2006. Deep diving mammals: Dive behavior and circulatory adjustments contribute to bends avoidance. *Respir. Physiol. Neurobiol.* 153, 66–77. doi:10.1016/j.resp.2005.09.014
- Fahlman, A., Tikuisis, P., Himm, J.F., Weathersby, P.K., Kayar, S.R., 2001. On the likelihood of decompression sickness during H₂ biochemical decompression in pigs. *J. Appl. Physiol.* 91, 2720–2729.

- Fahlman, A., Tyack, P.L., Miller, P.J.O., Kvadsheim, P.H., 2014b. How man-made interference might cause gas bubble emboli in deep diving whales. *Front. Physiol.* 5, 13.
doi:10.3389/fphys.2014.00013
- Fernández, A., Edwards, J.F., Rodríguez, F., Espinosa de los Monteros, A., Herráez, P., Castro, P., Jaber, J.R., Martín, V., Arbelo, M., 2005. “Gas and fat embolic syndrome” involving a mass stranding of beaked whales (family Ziphiidae) exposed to anthropogenic sonar signals. *Vet. Pathol.* 42, 446–457. doi:10.1354/vp.42-4-446
- Fitz-Clarke, J.R., 2009. Lung compression effects on gas exchange in human breath-hold diving. *Respir. Physiol. Neurobiol.* 165, 221–228. doi:10.1016/j.resp.2008.12.006
- Fowler, W.D., 1949. Lung Function Studies. III. Uneven pulmonary ventilation in normal subjects and in patients with pulmonary disease. *J. Appl. Physiol.* 2, 283–299.
- Hooker, S.K., Miller, P.J.O., Johnson, M.P., Cox, O.P., Boyd, I.L., 2005. Ascent exhalations of Antarctic fur seals: a behavioural adaptation for breath-hold diving? *Proc. Biol. Sci.* 272, 355–363. doi:10.1098/rspb.2004.2964
- Houser, D.S., Howard, R., Ridgway, S., 2001. Can Diving-induced Tissue Nitrogen Supersaturation Increase the Chance of Acoustically Driven Bubble Growth in Marine Mammals? *J. Theor. Biol.* 213, 183–195. doi:10.1006/jtbi.2001.2415
- Jepson, P.D., Arbelo, M., Deaville, R., Patterson, I.A.P., Castro, P., Baker, J.R., Degollada, E., Ross, H.M., Herráez, P., Pocknell, A.M., Rodríguez, F., Howie, F.E., Espinosa, A., Reid, R.J., Jaber, J.R., Martin, V., Cunningham, A.A., Fernández, A., 2003. Gas-bubble lesions in stranded cetaceans. *Nature* 425, 575–576. doi:10.1038/425575a
- Kayar, S.R., Aukhert, E.O., Axley, M.J., Homer, L.D., Harabin, A.L., 1997. Lower decompression sickness risk in rats by intravenous injection of foreign protein. *Undersea*

- Hyperb. Med. 24, 329–335.
- Kooyman, G.L., Davis, R.W., Wahrenbrock, E. a, Castellini, M. a, Sinnett, E.E., 1980. Aerobic and anaerobic metabolism during voluntry Diving in Weddell Seals:evidence of preferred pathways from blood chemistry and behaviour. *J. Comp. Physiol.* 138, 335–346.
- Kooyman, G.L., Hammond, D.D., Schroeder, J.P., 1970. Bronchograms and tracheograms of seals under pressure. *Science* 169, 82–84. doi:10.1126/science.169.3940.82
- Kooyman, G.L., Kerem, D.H., Campbell, W.B., Wright, J.J., 1971. Pulmonary function in freely diving Weddell seals, *Leptonychotes weddelli*. *Respir. Physiol.* 12, 271–282.
doi:10.1016/0034-5687(73)90003-0
- Kooyman, G.L., Sinnett, E.E., 1982. Pulmonary shunts in harbor seals and sea lions during simulated dives to depth. *Physiol. Zool.* 55, 105–111.
- Kooyman, G.L., Sinnett, E.E., 1979. Mechanical properties of the harbor porpoise lung, *Phocoena phocoena*. *Respir. Physiol.* 36, 287–300.
- Krogh, A., Lindhard, J., 1920. The Relative Value of Fat and Carbohydrate as Sources of Muscular Energy. *Biochem. J.* 14, 290–363.
- Krogh, A., Lindhard, J., 1917. The volume of the dead space in breathing and the mixing of gases in the lungs of man. *J. Physiol.* 51, 59–90.
- Leith, D.E., 1979. Comparative mammalian respiratory mechanics. *Am. Rev. Respir. Dis.* 19, 485–510.
- MathWorks Inc., T., 2010. MATLAB version 7.10.0 (R2010a). Natick, Massachusetts.
- McDonald, B.I., Ponganis, P.J., 2013. Insights from venous oxygen profiles: oxygen utilization and management in diving California sea lions. *J. Exp. Biol.* 216, 3332–41.
doi:10.1242/jeb.085985

- McDonald, B.I., Ponganis, P.J., 2012. Lung collapse in the diving sea lion: hold the nitrogen and save the oxygen. *Biol. Lett.* 8, 1047–9. doi:10.1098/rsbl.2012.0743
- Miedler, S., Fahlman, A., Valls Torres, M., Alvaro Alvarez, T., Garcia-Parraga, D., 2015. Evaluating cardiac physiology through echocardiography in bottlenose dolphins: using stroke volume and cardiac output to estimate systolic left ventricular function during rest and following exercise. *J. Exp. Biol.* 218, 3604–3610. doi:10.1242/jeb.131532
- Moore, C., Moore, M., Trumble, S., Niemeyer, M., Lentell, B., McLellan, W., Costidis, A., Fahlman, A., 2014. A comparative analysis of marine mammal tracheas. *J. Exp. Biol.* 217, 1154–66. doi:10.1242/jeb.093146
- Olsen, C.R., Hale, F.C., Elsner, R., 1969. Mechanics of ventilation in the pilot whale. *Respir. Physiol.* 7, 137–149. doi:10.1016/0034-5687(69)90001-2
- Perry, A.L., Low, P.J., Ellis, J.R., Reynolds, J.D., 2005. Climate change and distribution shifts in marine fishes. *Science* 308, 1912–1915. doi:10.1126/science.1111322
- Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D., Heisterkamp, S., B.V., W., 2016. Linear and Nonlinear Mixed Effects Models (R package). doi:http://dx.doi.org/10.18637/jss.v067.i01
- Ponganis, P.J., Kooyman, G.L., Zornow, M.H., 1991. Cardiac output in swimming California sea lions, *Zalophus californianus*. *Physiol. Zool.* 64, 1296–1306.
- Ponganis, P.J., Kooyman, G.L., Zornow, M.H., Castellini, M.A., Croll, D.A., 1990. Cardiac output and stroke volume in swimming harbor seals. *J. Comp. Physiol. B* 160, 473–482. doi:10.1007/BF00258974
- Ponganis, P.J., Stockard, T.K., Levenson, D.H., Berg, L., Baranov, E.A., 2006. Cardiac output and muscle blood flow during rest-associated apneas of elephant seals. *Comp. Biochem. Physiol. - A Mol. Integr. Physiol.* 144, 105–111. doi:10.1016/j.cbpa.2006.02.009

- R Core Team, 2015. R: A Language and Environment for Statistical Computing.
- Roelsen, E., 1938. Fractional analysis of alveolar air after inspiration of hydrogen as a method for the determination of the distribution of inspired air in the lungs . *Acta Med. Scand.* 95, 452–482.
- Roessig, J., Woodley, C., 2004. Effects of global climate change on marine and estuarine fishes and fisheries. *Rev. Fish Biol. Fish.* 14, 251–275. doi:10.1007/s11160-004-6749-0
- Sato, K., Naito, Y., Kato, a, Niizuma, Y., Watanuki, Y., Charrassin, J.B., Bost, C., Handrich, Y., Le Maho, Y., 2002. Buoyancy and maximal diving depth in penguins: do they control inhaling air volume? *J. Exp. Biol.* 205, 1189–1197.
- Sato, K., Shiomi, K., Marshall, G., Kooyman, G.L., Ponganis, P.J., 2011. Stroke rates and diving air volumes of emperor penguins: implications for dive performance. *J. Exp. Biol.* 214, 2854–63. doi:10.1242/jeb.055723
- Scholander, P.F., 1940. Experimental investigations on the respiratory function in diving mammals and birds. *Hvalrad. Skr.* 22, 1–131.
- Schorr, G.S., Falcone, E.A., Moretti, D.J., Andrews, R.D., 2014. First long-term behavioral records from Cuvier’s beaked whales (*Ziphius cavirostris*) reveal record-breaking dives. *PLoS One* 9, e92633. doi:10.1371/journal.pone.0092633
- Svärd, C., Fahlman, A., Rosen, D.A.S., Joy, R., Trites, A.W., 2009. Fasting affects the surface and diving metabolic rates of steller sea lions *Eumetopias jubatus*. *Aquat. Biol.* 8, 71–82. doi:10.3354/ab00211
- Van Bonn, W., Dennison, S., Cook, P., Fahlman, A., 2013. Gas bubble disease in the brain of a living California sea lion (*Zalophus californianus*). *Front. Physiol.* 4, 1–6. doi:10.3389/fphys.2013.00005

- Van Bonn, W., Montie, E., Dennison, S., Pussini, N., Cook, P., Greig, D., Barakos, J., Colegrove, K., Gulland, F., 2011. Evidence of injury caused by gas bubbles in a live Marine Mammal: Barotrauma in a California sea lion *Zalophus californianus*. *Dis. Aquat. Organ.* 96, 89–96. doi:10.3354/dao02376
- Weathersby, P.K., Homer, L.D., Flynn, T.E., 1984. On the likelihood of decompression sickness. *J. Appl. Physiol. Respir. Environ. Exerc. Physiol.* 57, 815–825.