

Pulmonary Function and Resting Metabolic Rates in California Sea Lions (*Zalophus californianus*) on Land and in Water

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Abstract

Respiratory flow, expired O₂, and CO₂ were measured during voluntary participation while spontaneously breathing in 13 confirmed healthy, male California sea lions (*Zalophus californianus*; body mass [M_b] range: 49 to 130 kg). Expiratory and inspiratory flow (\dot{V}_{exp} and \dot{V}_{insp}), tidal volume (V_{Texp} and V_{Tinsp}), and breath durations (T_{exp} , T_{insp} , and T_{tot}) were collected on land (lying down in sternal recumbency and sitting up) and floating in water to test the hypothesis that lung function changes with body position and on land versus in water. For sea lions on land, no differences were seen in any of the lung function values when comparing lying down versus sitting up. However, when comparing animals on land versus in water, both T_{exp} and T_{insp} decreased and \dot{V}_{exp} and \dot{V}_{insp} increased, while the V_{Texp} and V_{Tinsp} remained the same. The resting mass-specific V_T (25.1 ± 1.7 ml kg⁻¹) in the current study was approximately 24 to 30% of the estimated total lung capacity. We also measured breath-by-breath gas uptake to determine the O₂ consumption rates ($\dot{V}O_2$) and CO₂ production rates ($\dot{V}CO_2$) during rest on land and in water. There were no differences in $\dot{V}O_2$ or $\dot{V}CO_2$ on land as compared with water, and the average estimated values were 0.58 ± 0.22 l O₂ min⁻¹ (range: 0.24 to 1.01 l O₂ min⁻¹) and 0.50 ± 0.19 l CO₂ min⁻¹ (range: 0.22 to 0.89 l CO₂ min⁻¹), respectively, which agrees with results from other studies on otariids. Additionally, the allometric mass-exponent for V_T and $\dot{V}O_2$ were 1.13 to 1.20 and 0.86, respectively. These data are the first reported estimates of metabolic rate and lung function in confirmed healthy California sea lions.

Key Words: tidal volume, breath duration, diving physiology, respiratory flow, pulmonary function

Introduction

Eco-physiologists have been particularly interested in understanding the cardiorespiratory traits that allow marine mammals to manage O₂ while diving. Most physiological studies have focused on assessing the resting or field metabolic rate (Ponganis, 2011), while work on cardiorespiratory physiology, even though it is a crucial component for understanding energy use, has been limited (Ponganis et al., 1990, 1991, 1997; Fahlman & Madigan, 2016; McKnight et al., 2019). Cetaceans and sirenians are fully aquatic, while pinnipeds spend varying time on land and in water. Due to the effect of gravity on land, we would expect that these different lifestyles may have resulted in differences in the functional properties of the respiratory system in these groups. A number of studies have investigated the respiratory physiology in marine mammals, but there is still limited understanding of within and between species differences in lung mechanics and function (for reviews, see Kooyman, 1973; Piscitelli et al., 2010; Fahlman et al., 2017). A few studies have investigated lung function in pinnipeds (Kooyman et al., 1971; Kerem et al., 1975; Matthews, 1977; Reed et al., 1994; Fahlman & Madigan, 2016), but we are not aware of any study that has compared the differences on land and in water in this group.

The aquatic breathing pattern in cetaceans and sea lions are opposite that of terrestrial mammals and begin with an exhalation followed by an inspiration and a long respiratory pause. Marine

mammals that exhibit the aquatic breathing pattern have a lower respiratory frequency (f_R) and higher tidal volume (V_T) as compared with land mammals to maintain the same alveolar ventilation (Mortola & Sequin, 2009; Fahlman et al., 2017). It has been hypothesized that the aquatic breathing pattern, with an inflated lung between breaths, is favorable for buoyancy and/or gas exchange (Mortola & Sequin, 2009). Furthermore, in cetaceans and pinnipeds, the vital capacity (V_C) is close to the total lung capacity (TLC), and the minimum air volume (MAV) is only around 7% of TLC (Kooyman, 1973; Piscitelli et al., 2010; Fahlman et al., 2011), while in land mammals it is considerably greater. However, recent work indicates that cetaceans seldom have a V_T that is close to V_C , even following exercise or a static breath-hold (Fahlman et al., 2016, 2019b). In addition, most marine mammals have reinforced airways, which are theorized to aid in alveolar compression collapse during diving (Scholander, 1940; Bostrom et al., 2008; Moore et al., 2014). Alveolar compression results in a pulmonary shunt, which is the morphological trait most often proposed to reduce the risk of diving-related problems such as decompression sickness or the bends (Scholander, 1940; Kooyman et al., 1970; Denison et al., 1971; Kooyman & Sinnott, 1982; Bostrom et al., 2008). The reinforced airways also explain how some species are able to generate very high expiratory flow (Kooyman, 1973; Kooyman et al., 1975; Kooyman & Cornell, 1981). However, little is still known about the respiratory physiology of most marine mammal species despite its importance in gas exchange and implications during diving.

The gross, histologic, and radiographic anatomy of the sea lion lung has been well described (Denison et al., 1971; Denison & Kooyman, 1973; Dennison et al., 2009), but there is limited published information available with regard to function (Denison et al., 1971; Kerem et al., 1975; Matthews, 1977; Fahlman et al., 2011, 2014). Studies involving excised lungs have estimated the TLC, airway structure, and alveolar emptying as well as inflation and deflation pressures (Lenfant et al., 1970; Denison et al., 1971; Fahlman et al., 2011, 2014). Previous studies have investigated lung function in the sea lion in or out of water (Kerem et al., 1975; Matthews, 1977; Fahlman & Madigan, 2016), but none of these specifically investigated whether there were differences in lung function on land or in water. In a previous study of southern sea lions (*Otaria flavescens*), it was suggested that laying on land may have affected the reported V_T and resting O_2 consumption rate ($\dot{V}O_2$) (Fahlman & Madigan, 2016). It was suggested that the weight of the animal on the chest while in sternal recumbency could have

altered lung function and gas exchange, and that it possibly increased the metabolic cost of breathing (Fahlman & Madigan, 2016). It is known that both posture and water immersion alters gas exchange, respiratory flow, and volumes in man (Prefaut et al., 1976, 1978; Lundgren & Miller, 1999). We, therefore, measured lung function and gas exchange in and out of water to evaluate how breathing and metabolism changes for a semi-aquatic mammal in the two different environments where they spend their life.

In addition to improving basic eco-physiology, there is a need for understanding normal respiratory function and energy use in marine mammals. Not only does this add to understanding basic physiology within and between species to better evaluate how the environment may have shaped adaptations, but this may also help improve veterinary medicine. Respiratory disease is a significant cause of morbidity and mortality in marine mammals, and understanding baseline lung function in healthy animals could provide novel ways to assess respiratory health in populations in human care as well as in the wild (Dierauf & Gulland, 2001; Borque-Espinosa et al., in press). In the present study, we provide data for lung function and breath-by-breath gas uptake in California sea lions (*Zalophus californianus*) housed in managed care. These data allowed us to assess lung function (V_T , f_R , respiratory flow [\dot{V}], and total breath duration [T_{tot}]) and resting $\dot{V}O_2$ changes with animal size and position (laying and sitting on land, and floating in water) within a single species.

Methods

Study Animals

Thirteen healthy (8 neutered and 5 juvenile/not neutered), male California sea lions, managed under human care at the U.S. Navy Marine Mammal Program (MMP), participated under voluntary control in a total of 77 experimental trials (March to April 2016: $n = 24$; November 2016: $n = 53$; Table 1). The health status of the sea lions included in this study was determined based on a complete physical examination and blood analysis (CBC and chemistry panel). Additionally, lung/chest radiographs were performed to determine that each animal was free of pulmonary disease.

Each animal was weighed (± 0.5 kg), and the straight length was measured the week of the experimental trial (Table 1). All sea lions were housed in open-water, netted enclosures in San Diego Bay, California (USA). The mean daily air temperature was $22.8 \pm 4.3^\circ\text{C}$ (range: 15.0 to 35.0°C), and there were significant differences between the temperature in March/April ($19.9 \pm 2.9^\circ\text{C}$, $n = 28$, t value 6.6, $df = 86$ d of measurement, $p < 0.01$) and

Table 1. Details on research subjects

Animal ID	Age (y)	Length (cm)	M_b (kg)	$\dot{V}O_2$ (l min ⁻¹)	$\dot{V}CO_2$ (l min ⁻¹)
ZC1	34	174	129.8 ± 2.9	0.69 ± 0.14 ⁷	0.57 ± 0.13
ZC2*	4	154	52.0 ± 0.2	0.24 ¹	0.23
ZC3	34	182	110.0 ± 9.0	0.86 ± 0.21 ⁵	0.79 ± 0.17
ZC4	15	167	83.5 ± 1.1	0.49 ± 0.07 ¹¹	0.38 ± 0.07
ZC5	15	189	102.1 ± 1.6	0.96 ± 0.07 ¹¹	0.83 ± 0.07
ZC6	14	184	98.0 ± 0.9	0.55 ± 0.05 ⁴	0.49 ± 0.06
ZC7	9	204	94.6 ± 1.9	0.59 ± 0.05 ⁴	0.48 ± 0.05
ZC8*	4	156	54.1 ± 3.3	0.24 ± 0.04 ⁶	0.22 ± 0.03
ZC9	6	144	49.1 ± 2.0	0.45 ± 0.06 ¹¹	0.34 ± 0.04
ZC10*	5	161	64.7 ± 1.2	0.73 ± 0.06 ²	0.66 ± 0.06
ZC11	5	144	57.6 ± 3.4	0.46 ± 0.04 ¹¹	0.43 ± 0.04
ZC12*	4	144	52.8 ± 5.6	0.40 ± 0.04 ¹⁰	0.36 ± 0.04
ZC13*	4	146	55.8 ± 5.3	0.61 ± 0.163 ³	0.55 ± 0.14

Animal ID, age (y), straight length (cm), average (\pm SEM) body mass (M_b , kg), O₂ consumption ($\dot{V}O_2$), and CO₂ production ($\dot{V}CO_2$) rates for 13 healthy, male California sea lions (*Zalophus californianus*) participating in the study. Superscript numbers are the numbers of repeated measurements for measuring $\dot{V}O_2$ and $\dot{V}CO_2$. The $\dot{V}O_2$ was used as a comparative estimate of metabolic rate. *Juvenile animals not neutered at the time of the study.

November (25.3 ± 3.9°C; $n = 60$). The mean daily humidity and ambient pressure were, respectively, 55 ± 18% (range: 13 to 100%; $n = 88$) and 101.7 ± 0.2 kPa (range: 101.3 to 101.9 kPa; $n = 86$). The mean water (\pm SD) temperature in March was 16.3 ± 0.4°C ($n = 30$); in April it was 17.6 ± 0.4°C ($n = 31$); and in November it was 21.2 ± 1.0°C ($n = 31$).

The MMP houses and cares for a population of California sea lions in San Diego Bay, is accredited by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC), and adheres to the national standards of the U.S. Public Health Service Policy on the Humane Care and Use of Laboratory Animals and the Animal Welfare Act. The MMP's animal care and use program is routinely reviewed by an Institutional Animal Care and Use Committee (IACUC) and the Department of Defense Bureau of Medicine and Surgery. The animal use and care protocol for MMP California sea lions in support of this Office of Naval Research-funded study (ONR YIP Award #N-000141410563) was approved by the MMP's IACUC (Approval No. 117-2016), the IACUC at Texas A&M University–Corpus Christi (TAMUCC-IACUC AUP #04-11), and the U.S. Navy's Bureau of Medicine and Surgery (BUMED NRD-1024). The health status of the sea lions included in this study was determined based on a complete physical examination and blood analysis (CBC and chemistry panel). Additionally, lung/chest radiographs were performed to determine that each animal was free of pulmonary disease.

Respiratory Flow Measurements

The sea lions were trained to voluntarily place their nose and mouth into a custom rigid face mask until an air-tight seal was achieved. The face mask was lined with soft, padded neoprene to avoid irritation with the skin (Figure 1A). In an earlier study, we ensured that the mask sealed against the snout by performing a leak test (Fahlman & Madigan, 2016). The leak test was done by having the sea lion breathing while laying in water, with the interface between the snout and the face mask submerged in water. This test ensured that no bubbles were seen during exhalation or water inside the mask during inhalation. In the current study, this leak test was not repeated, but we paid careful attention throughout each trial to make sure that the mask was properly placed to avoid any air leaks. The animals were allowed to breathe spontaneously while positioned either sitting up on land (Figure 1A & B), laying down in sternal recumbency on land (Figure 1C), or while floating horizontally in water (Figure 1D).

Expired (\dot{V}_{exp}) and inspired (\dot{V}_{insp}) flow were measured using a pneumotachometer (3813 series, 0-800 l min⁻¹; Hans-Rudolph Inc., Shawnee, KS, USA) placed inside a custom-made face mask. The maximum dead-space of the mask was approximately 200 ml and varied slightly depending on how much of the snout was placed inside the mask. The pneumotachometer was connected to a differential pressure transducer (Spirometer Pod, ML 311; ADInstruments, Colorado Springs, CO,



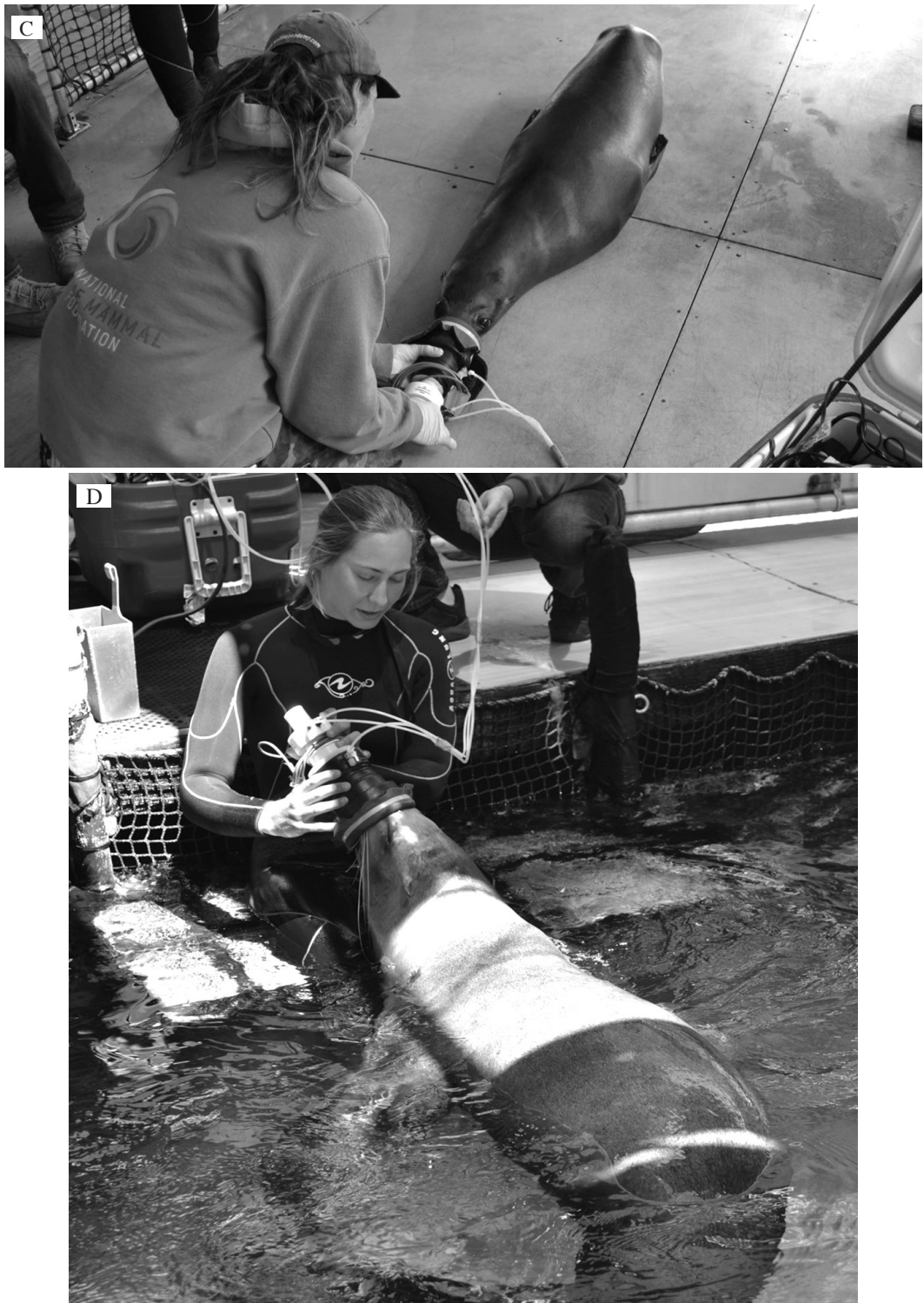


Figure 1. Images showing the (A) face mask and how breaths were measured in several positions: (B) sitting up on land, (C) laying down (sternal recumbency) on land, and (D) floating in the water.

USA) via a 310-cm length of 2-mm inner diameter, firm-walled, flexible tubing. The pneumotachometer was calibrated for linearity and flow using a 7-L calibration syringe (Series 4900; Hans-Rudolph Inc.) immediately before and after each trial through a series of pump cycles at various flow rates. The pump cycles allowed the relationship between differential pressure and flows for the inspiratory and expiratory phases to be determined. To avoid spurious peaks, the reported maximal inspiratory and expiratory flows were the average flows over 20 ms—10 ms on either side of the maximal recorded inspiratory or expiratory flow.

The differential pressure transducer was connected to a data acquisition system (Powerlab 8/35; ADInstruments), and the data were captured at 400 Hz using a laptop computer running *LabChart*, Version 8.1 (ADInstruments). Data were continuously collected during each trial, and the \dot{V}_{exp} and \dot{V}_{insp} integrated to estimate expiratory (V_{Exp}) and inspiratory (V_{Ins}) V_{IS} , respectively.

Respiratory Gas Composition and Metabolic Rates
Respiratory gases were subsampled via a port in the pneumotachometer and passed through a 310-cm length of 2-mm inner diameter, firm-walled, flexible tubing and a 30-cm length of 1.5-mm inner diameter Nafion tubing to fast-response O₂ and CO₂ analyzers (Gemini respiratory monitor; CWE Inc., Ardmore, PA, USA) at a flow rate of 200-ml min⁻¹. The gas sampling line was placed close to the snout, allowing gas sampling of expired gas. The flow rate also helped renew the gas in the face mask and reduced the dead space. The flow to the gas analyzer was corrected for the analysis of respiratory flow and volumes. The gas sensors were connected to the data acquisition system and sampled at 400 Hz. The gas analyzers were calibrated before and after the experiment using a commercial mixture of 5% O₂, 5% CO₂, and 90% N₂, certified accurate to at least 0.01% (Product #17L-340; Gasco, Oldsmar, FL, USA).

The expiratory flow and end-expired gas concentrations were used to estimate the resting \dot{V}_{O_2} and CO₂ production rates (\dot{V}_{CO_2}), where the former was used as a comparative estimate of metabolic rate against other studies and species. The respiratory gas data were phase corrected to account for the delay caused by the flow in the sample line. The expiratory flow-rate (\dot{V}_{exp}) and expired O₂ and CO₂ content were multiplied to calculate the instantaneous \dot{V}_{O_2} and \dot{V}_{CO_2} . The instantaneous \dot{V}_{O_2} and \dot{V}_{CO_2} were integrated over each breath to yield the total volume of O₂ and CO₂ exchanged during each breath. The volume was summed for each trial period and divided by the duration of the trial to provide an estimate of the \dot{V}_{O_2} or \dot{V}_{CO_2} for that time period.

Data Processing and Statistical Analysis

All gas volumes were converted to standard temperature pressure dry (STPD; Quanjer et al., 1993). Exhaled air was assumed saturated at 37°C, and inhaled air volume was corrected for ambient temperature and relative humidity.

The relationship between a dependent variable (\dot{V}_{exp} , \dot{V}_{insp} , V_{Exp} , V_{Ins} , T_{exp} , T_{insp} , T_{tot} , end-expired O₂, CO₂, \dot{V}_{O_2} , and \dot{V}_{CO_2}) and experimental covariates (position, M_b , season, air or water temperature, and breath number) was analyzed using linear-mixed effects models ('lme,' R, Version 3.1.0; R Core Team, 2014). The individual animal was treated as a random effect, which accounted for the correlation between repeated measurements on the same individual (Littell et al., 1998). Initially, dependent variables were selected for inclusion in a multivariate model if the univariate analysis had a p value < 0.2 (Wald's test). Best models were chosen by the Akaike information criterion (AIC) against nested models, and significance was determined using the Likelihood ratio test (LRT). In this study, $p \leq 0.05$ values were considered as significant, and $p \leq 0.1$ values were considered to be a trend. Multiple comparisons were done using the Tukey test in the multcomp library. Data are presented as the mean \pm standard deviation (SD) unless otherwise stated.

Results

We only analyzed complete breaths that began with an exhalation and ended with an inhalation (see Figure 1 in Fahlman & Madigan, 2016). Single exhalations or inhalations were removed. Initially, we analyzed the difference between inhaled and exhaled flow, volume, and duration (see "Lung Function" section). We confirmed that the dead space of the face mask did not significantly alter end-expired gas composition or lung function as neither variable changed systematically throughout the research trial ($p > 0.3$ for all using GLM). Next, we focused on the effect of position (laying or sitting on land or floating in water) on lung function ($V_{\text{I}}, f_{\text{R}}, T_{\text{tot}}$, and \dot{V} ; see "Lung Function on Land vs in Water at Different Body Positions") and gas exchange (end-expired gas content and metabolic rate; see "Respiratory Gases and Metabolic Rate on Land vs in Water").

Lung Function

For a total of 3,522 spontaneous breaths from the 13 sea lions, the average (\pm SEM) \dot{V}_{exp} (3.01 ± 0.47 l s⁻¹; $n = 13$) was significantly higher than \dot{V}_{insp} (2.36 ± 0.33 l s⁻¹, paired t test, t value = 3.85, $df = 12$, $p < 0.01$), but there were no differences between expiratory (1.96 ± 0.23 l) and inspiratory V_{IS} (2.05 ± 0.24 l, t value = 1.72, $df = 12$, $p > 0.1$).

The average (\pm SEM) T_{tot} was 2.38 ± 0.13 s, where T_{exp} (1.06 ± 0.07 s) was significantly shorter as compared with T_{insp} (1.29 ± 0.07 s, t value = 3.67, $df = 12, p < 0.01$).

Lung Function on Land vs in Water at Different Body Positions

Neither season nor temperature warranted inclusion in any model for lung function ($p > 0.1$ for all). There were no differences in lung function (\dot{V}_{exp} , \dot{V}_{insp} , V_{Texp} , V_{Tinsp} , T_{exp} , T_{insp} , T_{tot}) for animals sitting or laying on land ($\chi^2 < 1.5$, $df = 1, p > 0.6$ for all variables), and, therefore, we pooled these data on land.

There were no differences in V_{Texp} (2.2 ± 0.2 l, $\chi^2 = 1.9$, $df = 1, p > 0.1$) or V_{Tinsp} (2.0 ± 0.2 l, $\chi^2 = 2.8$, $df = 1, p > 0.05$) when measured in water or on land (Figure 2A). The V_T was between 26 to 30% of the estimated TLC ($TLC_{est} = 0.135 M_b^{0.92}$) (Kooyman, 1973; Fahlman et al., 2011), and both V_{Tinsp} and V_{Texp} correlated with M_b with mass-exponents close to 1 (Figure 2A; Table 2). The measured average (\pm SEM) mass-specific V_T for all animals on land and in water was 25.1 ± 1.7 ml kg^{-1} .

Both average (\pm SEM) \dot{V}_{exp} (water: 4.5 ± 0.8 l s^{-1} ; land: 2.6 ± 0.4 l s^{-1}) and \dot{V}_{insp} (water: 3.2 ± 0.5 l s^{-1} ; land: 2.1 ± 0.3 l s^{-1}) were greater when animals were submerged in water compared to when they were on land (Figure 2B; Table 2). Similarly to V_T , both \dot{V}_{exp} and \dot{V}_{insp} increased with M_b (Table 2).

The T_{exp} (water: 0.81 ± 0.06 s; land: 1.26 ± 0.08 s, $\chi^2 = 11.5$, $df = 1, p < 0.01$), T_{insp} (water: 1.22 ± 0.13 s; land: 1.43 ± 0.06 s, $\chi^2 = 4.1$, $df = 1, p < 0.05$), and T_{tot} (water: 2.03 ± 0.17 s; land: 2.69 ± 0.12 s, $\chi^2 = 6.9$, $df = 1, p < 0.01$) were greater when animals were on land as compared to when they were in water. The average (\pm SEM) f_R was 6.5 ± 0.8 breaths min^{-1} and did not differ on land or in water ($\chi^2 = 2.3$, $df = 1, p > 0.1$), and did not change with M_b ($p > 0.1$, F-ratio = 0.66).

Respiratory Gases and Metabolic Rate on Land vs in Water

The average (\pm SEM, $n = 13$; Table 1) end-expiratory CO_2 and O_2 were $6.2 \pm 0.2\%$ and $14.0 \pm 0.29\%$, respectively, and neither correlated with M_b (F-ratio < 0.5 , $r^2 < 0.05$, $p > 0.1$). The average $\dot{V}CO_2$, $\dot{V}O_2$, and respiratory quotient (RQ) were 0.51 ± 0.05 l CO_2 min^{-1} , 0.59 ± 0.05 l O_2 min^{-1} , and 0.87 ± 0.01 (range: 0.77 to 0.94; Table 1 &

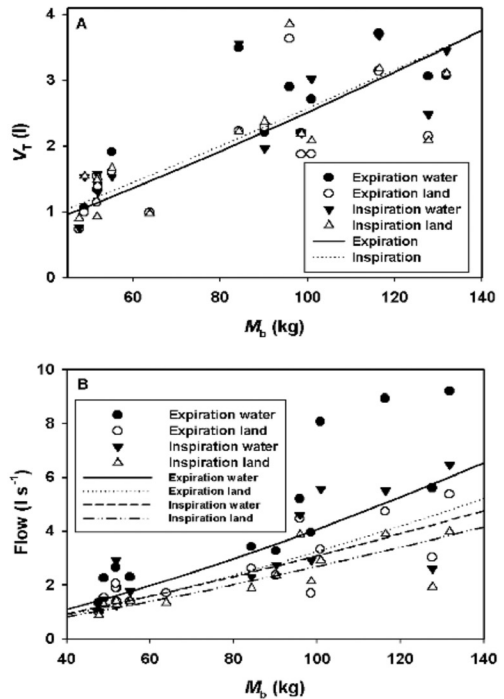


Figure 2. Expired and inspired (A) tidal volume (V_T) and (B) respiratory flow in relation to body mass (M_b) in California sea lions in water and on land. Regression lines are based on results in Table 2.

Table 2. Results for generalized linear models for voluntary breaths

Dependent variable	b_0	Position (land)	$\log[M_b]$	r^2	χ^2	p
$\log(V_{Texp})$	-2.00 ± 0.28	--	$\log(1.20 \pm 0.15)$	0.71	16.2	< 0.01
$\log(V_{Tinsp})$	-1.85 ± 0.30	--	$\log(1.13 \pm 0.16)$	0.66	14.8	< 0.01
$\log(\dot{V}_{exp})$	-2.21 ± 0.30	-0.10 ± 0.02	$\log(1.41 \pm 0.16)$	0.80	16.0	< 0.01
$\log(\dot{V}_{insp})$	-2.09 ± 0.32	-0.06 ± 0.02	$\log(1.29 \pm 0.17)$	0.73	13.7	< 0.01
$\log(\dot{V}O_2)$	-1.89 ± 0.45	--	$\log(0.86 \pm 0.24)$	0.51	9.2	< 0.01
$\log(\dot{V}CO_2)$	-1.83 ± 0.46	--	$\log(0.79 \pm 0.24)$	0.45	7.7	< 0.01

Multivariate GLM including log10-transformed (Log) expired and inspired tidal volume (l, $\log[V_{Texp}]$, $\log[V_{Tinsp}]$), flow (l s^{-1} , $\log[\dot{V}_{exp}]$, and $\log[\dot{V}_{insp}]$), O_2 consumption rate (l O_2 min^{-1} , $\log[\dot{V}O_2]$), and CO_2 production rate (l CO_2 min^{-1} , $\log[\dot{V}CO_2]$). Results show intercept (b_0), position (land = 1, water = 0), log10-transformed body mass ($\log[M_b]$), residual r-squared (r^2), χ^2 for log-likelihood ratio test, and p value from the likelihood ratio test.

Figure 3), respectively. There were no differences in $\dot{V}CO_2$ (water: 0.46 ± 0.07 l CO₂ min⁻¹; land: 0.53 ± 0.06 l CO₂ min⁻¹; $\chi^2 = 2.3$, $df = 1$, $p > 0.1$), $\dot{V}O_2$ (water: 0.51 ± 0.08 l O₂ min⁻¹; land: 0.62 ± 0.07 l O₂ min⁻¹; $\chi^2 = 2.1$, $df = 1$, $p > 0.1$), or RQ (water: 0.90 ± 0.03 ; land: 0.86 ± 0.01 , $\chi^2 = 2.7$, $df = 1$, $p > 0.1$) on land or in water. The corresponding average (\pm SD) mass-specific $\dot{V}CO_2$ ($s\dot{V}CO_2$) and $\dot{V}O_2$ ($s\dot{V}O_2$) were 6.4 ± 2.0 ml CO₂ min⁻¹ kg⁻¹ and 7.3 ± 2.1 ml O₂ min⁻¹ kg⁻¹, respectively. Both $\dot{V}CO_2$ and $\dot{V}O_2$ correlated with M_b with a mass-exponent close to 0.75 (Table 2; Figure 3). The Kleiber ratio was estimated by dividing the observed metabolic rate (Table 1) by the predicted basal metabolic rate (BMR, 1 O₂ min⁻¹) using Kleiber's (1961) equation (BMR = $0.00993 M_b^{0.75}$). The Kleiber ratio ranged from 1.1 to 3.8, and the overall average was 2.2 ± 0.6 for all data.

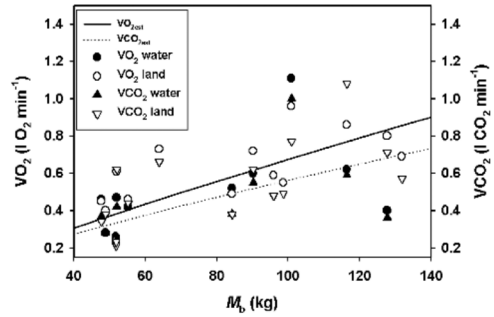


Figure 3. Oxygen consumption rate ($\dot{V}O_2$) and carbon dioxide production rate ($\dot{V}CO_2$) in relation to M_b for California sea lions in water and on land. Regression lines are based on results in Table 2.

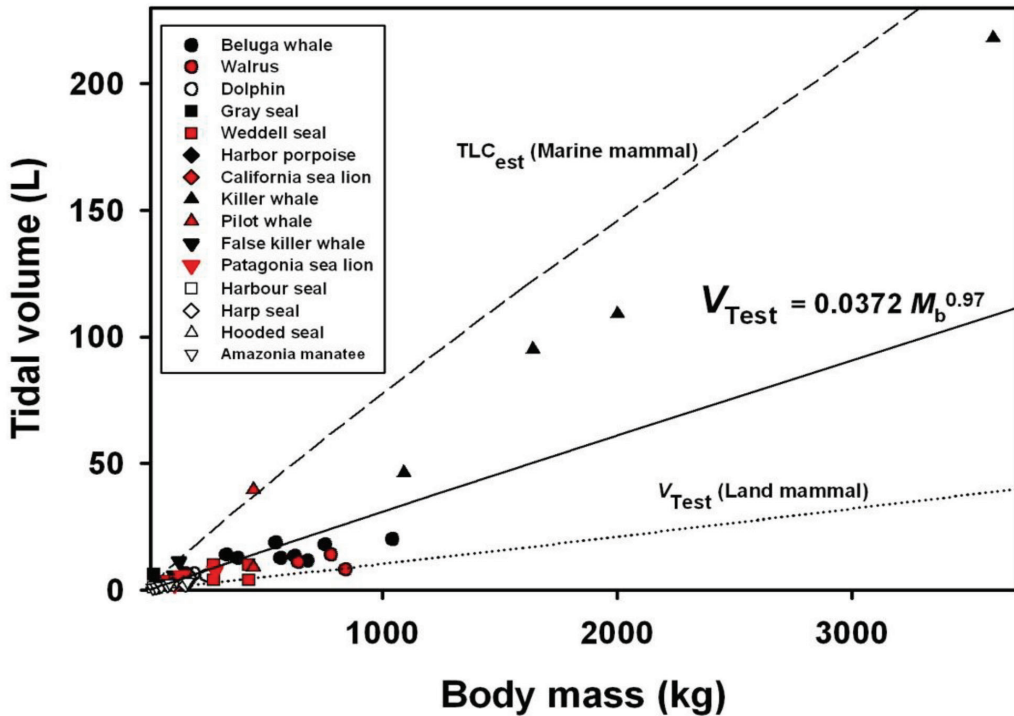


Figure 4. The relationship between measured resting tidal volume (V_T) and M_b in a number of marine mammal species. Different marine mammals are represented by colored symbols, and the solid line and equation are the allometric fit to the data. For comparison, the relationship between estimated V_T (V_{Test} , dotted line; V_{Test} [ml] = $7.69 M_b^{1.04}$) and M_b is shown for terrestrial animals (Stahl, 1967), and the relationship between estimated total lung capacity (TLC_{est} , broken line; TLC_{est} [l] = $0.135 M_b^{0.92}$) and M_b is shown for marine mammals (Kooyman, 1973; Fahlman et al., 2011). *References:* Gray seal (Reed et al., 1994), bottlenose dolphin (Fahlman et al., 2015b, 2018a, 2018b, 2019b), Weddell seal (Kooyman et al., 1971), harbour porpoise (Reed et al., 2000), California sea lion (Kerem et al., 1975; Matthews, 1977), pilot whale (Olsen et al., 1969), killer whale (Kasting et al., 1989), beluga whale (Kasting et al., 1989; Fahlman et al., 2019a), walrus (Fahlman et al., 2015a), southern sea lion (Fahlman & Madigan, 2016), harbor seal (Craig & Päsche, 1980), harp seal (Gallivan, 1981), hooded seal (Päsche, 1976), Amazonian manatee (Gallivan & Best, 1980), and false killer whale (Piscitelli et al., unpub. obs.).

Discussion

Our data indicate that the respiratory timing and flow differs on land and in water to keep V_T the same in the two media. In addition, the measured V_T during spontaneous/voluntary breaths was higher than those predicted from terrestrial mammals (Figure 4), and both V_T and \dot{V} changed with M_b . The estimated resting $\dot{V}O_{2S}$ and $\dot{V}CO_{2S}$ did not differ in water or on land and were similar to those measured in similar-sized Steller sea lions (*Eumetopias jubatus*) and California sea lions in water (Hurley & Costa, 2001; Fahlman et al., 2008, 2013), and Steller and southern sea lions on land (Rosen & Trites, 1997, 1999; Fahlman & Madigan, 2016). Thus, the results presented herein represent the first measurements of $\dot{V}O_2$, $\dot{V}CO_2$, and lung function for confirmed healthy California sea lions.

A number of past studies have attempted to assess respiratory function and metabolic rate on restrained, semi-restrained, or trained sea lions participating voluntarily (Kerem et al., 1975; Matthews, 1977; Rosen & Trites, 1997, 1999; Hurley & Costa, 2001; Fahlman et al., 2013; Fahlman & Madigan, 2016). In previous work, we measured lung function and metabolic rate in southern sea lions laying in sternal recumbency on land (Fahlman & Madigan, 2016). In the sea lions, it was proposed that laying in sternal recumbency may have affected lung function as compared with sitting or floating in water (Fahlman & Madigan, 2016). In the current work, we did not see any differences in \dot{V} or V_T when animals were in sternal recumbency or sitting up supported by their pectoral flippers. Consequently, the increased weight on the chest while laying down did not affect respiratory function in the sea lions. In water as compared with air, the \dot{V} increased, the T_{tot} decreased, and the V_T remained the same (see “Results” and Table 2). Thus, the sea lions reduced \dot{V} on land with longer T_{tot} s to achieve the same V_T as when submerged in water, possibly suggesting that California sea lions alter breathing strategy in water vs on land. This suggests that the increased hydrostatic pressure in water helped increase \dot{V} , while on land the sea lions increased T_{tot} to maintain constant V_T and alveolar ventilation.

In terrestrial mammals, V_T correlates with M_b with a mass-exponent that is close to 1 (Stahl, 1967); and for land mammals, the mass-specific V_T is about 9 ml kg⁻¹ for M_b s, ranging from 50 to 120 kg, or approximately 13% of TLC_{est} ($TLC_{est} = 0.054 M_b^{1.06}$, where TLC_{est} is in l and M_b is in kg) (Stahl, 1967). In marine mammals, V_T is approximately 32 to 43% of TLC_{est} in animals ranging in M_b from 20 to 3,600 kg (Figure 4; Kooyman, 1973; Fahlman et al., 2017). In the current study, V_T was between 26 to 30% of

TLC_{est} , and the allometric mass-exponent close to unity (Table 2). The measured mass-specific V_T (sV_T) in the current study (25.1 ± 1.7 ml kg⁻¹) appears to be at the lower end compared to results for resting juvenile California sea lions in water (19 to 69 ml kg⁻¹, $M_b = 32$ to 46 kg) (Kerem et al., 1975; Matthews, 1977), but similar to those in adult southern sea lions resting on land (20 to 40 ml kg⁻¹) (Fahlman & Madigan, 2016) and other marine mammal species (Mortola & Sequin, 2009; Fahlman et al., 2017). While V_T appears to be considerably higher in marine as compared with terrestrial mammals, Figure 4 reveals that the V_T of spontaneous breaths of resting marine mammals is not close to TLC_{est} or V_C . Consequently, while the residual volume is low, and some species appear to be able to exchange a large proportion of their TLC in a single breath (Fahlman et al., 2017), most breaths are considerably lower than V_C . This has significant consequences as it has long been assumed that most breaths are equal or close to the V_C .

While working with trained individuals helps reduce the stress caused by exposure to new experimental procedures or restraints, it may introduce a potential bias in that the trainer may inadvertently alter the behavior of the test subject. Similar to past studies (Fahlman et al., 2015b, 2019a; Fahlman & Madigan, 2016), we used the average f_R (\pm SEM) during the research trials and compared these to historical data (from 2014 until 2017) from focal observations ($f_R = 5.2 \pm 0.6$ breaths min⁻¹; $n = 19$) while they were undisturbed. These data confirmed that the experimental procedure did not alter the f_R of the animals (t test, $p = 0.11$, t value = 1.66, $df = 32$) and were similar to those reported in undisturbed resting adult and juvenile California sea lions. We, therefore, propose that the lung function data presented herein represent normal values in California sea lions.

The $\dot{V}O_2$ reported in the current study did not differ on land nor in water. Some studies argue that the elevated metabolic cost reported for marine mammals as compared with the predicted basal metabolic rate (BMR) from Kleiber’s equation for terrestrial mammals reflects increased thermoregulatory cost in water (Irving, 1969; Hurley & Costa, 2001). Other studies have reported resting $\dot{V}O_{2S}$ in both pinnipeds and cetaceans that are closer to predicted basal levels than terrestrial mammals (Rea & Costa, 1992; Webb et al., 1998; Kohin et al., 1999; Noren, 2002; Yeates & Houser, 2008; Worthy et al., 2013; Fahlman et al., 2018a, 2018b), and it has been argued that the higher values may stem from failure to adhere to the strict definitions when measuring BMR (Innes & Lavigne, 1991). It is possible that the lack of a difference

on land and in water may reflect the short duration of these trials, which in most cases lasted less than 5 min after a 2- to 3-min period for the sea lion to settle down. In addition, we did not conduct our measurements in post-absorptive animals, and digestion is known to increase the $\dot{V}O_2$ (Rosen & Trites, 1997). If digestion elevated the $\dot{V}O_2$, it may have raised the metabolic rate to counter any thermal heat loss in water. This, in addition to the short measurement period, may explain why the reported $\dot{V}O_2$ was 2.2 times higher than the value estimated from Kleiber's equation. Despite these limitations, the estimated mass-specific resting $\dot{V}O_{2S}$ (sRMR: 4.1 to 10.5 ml O₂ min⁻¹ kg⁻¹) in the current study were similar to those measured in Steller and California sea lions in water (Steller sea lions: 7.4 to 9.2 ml O₂ min⁻¹ kg⁻¹; California sea lions: 5.7 to 10.4 ml O₂ min⁻¹ kg⁻¹) (Hurley & Costa, 2001; Fahlman et al., 2013), and Steller and southern sea lions in air (Steller sea lions: 3.0 to 9.5 ml O₂ min⁻¹ kg⁻¹; southern sea lions: 4.1 to 10.5 ml O₂ min⁻¹ kg⁻¹) (Rosen & Trites, 1997, 1999; Fahlman & Madigan, 2016). It is also possible that the current and past studies in sea lions reflect behavioral or physiological differences between otariids and phocids/cetaceans.

The data presented in the current study enhance our understanding of the physiological capacity of California sea lions. With current projections of climate change, improved understanding of marine mammal eco-physiology will be a crucial and increasingly important topic for proper mitigation and conservation efforts. In addition, the results presented in the current study provide baseline data that may help to better understand, diagnose, and treat respiratory problems in pinnipeds. For example, baseline lung function of healthy marine mammals is useful for understanding and diagnosing respiratory health and the impact of disease caused by pollution such as oil spills or run-off from urbanization (Schwacke et al., 2014; Borque-Espinosa et al., 2019). Respiratory disease is a common problem in both wild and managed care pinnipeds, and disease symptoms are often masked until the animal is severely affected. Pulmonary function testing (PFT) is commonly used to assess lung health in humans (Crapo, 1994; Miller et al., 2005) and may be a useful diagnostic tool in marine mammal veterinary medicine (Van Elk et al., 2001; Borque-Espinosa et al., 2019). Thus, the objectives with the current study were to obtain data on baseline lung function and metabolic rate from diagnostically healthy California sea lions breathing spontaneously.

We conclude that both the measured V_T and metabolic rates are within the range of those measured in other otariids of similar size; the reported data likely represent valid estimates for this

species. Our data provide improved understanding of the respiratory capacity of sea lions and confirm measurements from other marine mammals that the V_T is higher as compared to similar-sized terrestrial mammals, or about 24 to 30% of the TLC_{est} . In addition, the respiratory flow and breath durations differ on land and in water but in opposite direction to result in similar V_{Is} .

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