Characterizing respiratory capacity in belugas (*Delphinapterus leucas*)

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ABSTRACT

We measured respiratory flow, breath duration, and calculated tidal volume (V_T) in nine belugas (*Delphinapterus leucas*, mean measured body mass: 628 ± 151 kg, n = 5) housed in managed care facilities. Both spontaneous (resting at station) and trained maximal respirations (chuffs) were measured. The mean ($\pm$ s.d.) inspiratory V_T for spontaneous breaths (16.7 ± 4.7 l, range: 7.5-18.7 l) was larger than those predicted based on respiratory scaling equations from terrestrial mammals and was $32 \pm 10\%$ of estimated total lung capacity (TLC$_{est}$) based on an equation from static measurements made on a range of cetaceans and pinniped lungs, and $52 \pm 18\%$ of estimated vital capacities (V_C, mean: 27.7 ± 8.9 l, range: 16.7-40.3 l) based on respiratory measurements obtained during trained maximal respirations. Expiratory flow ($\dot{V}_{exp}$, spontaneous: 26.1 ± 5.5 l s^{-1}, chuff: 66.8 ± 22.5 l s^{-1}) was significantly higher as compared with inspiratory flow ($\dot{V}_{insp}$, spontaneous: 22.3 ± 4.6 l s^{-1}, chuff: 30.1 ± 8.4 l s^{-1}), and the maximal expiratory flow recorded was 212 l s^{-1}. The breath duration was shorter for forced breaths (Expiration: 518 ± 101 ms; Inspiration: 905 ± 170 ms; Total: 1423 ± 227 ms) as compared with spontaneous breaths (Expiration: 995 ± 176 ms; Inspiration: 1098 ± 219 ms; Total: 2093 ± 302 ms). These data provide baseline estimates of the respiratory capacity of belugas.

1. Introduction

The unique niches of polar marine mammals combined with their apex position within these ecosystems make them potential indicators species for monitoring the effects of climate change, which includes both increasing ocean temperatures and decreasing sea ice, on ecosystem health (Moore, 2008; Tynan and DeMaster, 1997). Due to their completely aquatic habitat beluga (*Delphinapterus leucas*), or white whales, may be particularly vulnerable to these ecosystem alterations, which may include changes in prey diversity, availability and location (Perry et al., 2005). These potential changes in prey characteristics, frequency and distribution could translate into the need for beluga to increase both foraging duration and distance in order to obtain enough

prey for survival. Deeper and longer foraging bouts will in turn require longer dive times in between respirations (Martin and Smith, 1999), which may or may not exceed the physiologic capability of belugas. Thus, understanding the respiratory and cardiovascular traits required by marine mammals to manage life in an extreme environment, the physiological constraints imposed on these animals, and how these limitations may affect physiology and survival are crucial.

Marine mammals are exposed to a wide range of water pressure while foraging for food, and are thought to have physiological traits that prevent, or at least reduce, the occurrence of pressure-related problems, e.g., pulmonary barotrauma or decompression sickness (Fahlman et al., 2017; García-Párraga et al., 2018). Scholander (1940) proposed that marine mammals have conducting airways that resist

compression, while the alveolar space and chest are compliant and easily compressed. Ridgway et al. (1969) were the first to provide evidence of the proposed chest compliance of cetaceans by using remote videography of bottlenose dolphins to document remarkable thoracic compression from 50 to 300 m in depth. Combined, these traits would help prevent pulmonary barotrauma, or lung squeeze, as water pressure progressively compresses the highly compliant chest and alveolar space.

Theoretical modeling work indicates that the structural properties (compliance) of the lungs and conducting airways dictate how pressure affects compression of the conducting airways and alveolar space (Bostrom et al., 2008; Fahlman et al., 2009, 2011; Fitz-Clarke, 2007). This variation in compliance alters how pressure affects lung compression, transpulmonary pressures, and the risk for lung squeeze. In addition, it has been suggested that deep diving species have smaller lungs and lower total lung capacity (TLC), which would help prevent excessive uptake of N_2 and reduce the risk of DCS (Piscitelli et al., 2010; Scholander, 1940). However, recent work on bottlenose dolphins indicates that there are no differences in resting tidal volume (V_T) in shallow and deep diving ecotypes (Fahlman et al., 2018a, c). In addition, a recent hypothesis suggests that the cardiorespiratory responses may provide a means to favor O_2 and CO_2 exchange over N_2 during diving (García-Párraga et al., 2018). Thus, a better understanding of the respiratory requirements will improve our understanding of the respiratory limitations to diving.

Measurements of respiratory function in voluntarily participating cetaceans have been performed in a few studies (see references in Fahlman et al., 2017). As there are differences in ecology, anatomy (specifically in the structure of the respiratory passages and lung morphology), and physiology within different species of cetaceans (Fahlman et al., 2017; Hooker et al., 2012; Kielhorn et al., 2013; Piscitelli et al., 2010, 2013; Reidenberg and Laitman, 2008; Wislocki and Belanger, 1940), it is likely that respiratory function also differs. In addition, an improved understanding of the respiratory physiology, diving lung volumes and changes during diving are important to provide better input to modeling efforts. More detailed investigations into the respiratory physiology of multiple species is necessary to establish similarities or differences in respiratory function and, if possible, generalize findings (Wislocki and Belanger, 1940). Thus, this investigation aims to widen the understanding of odontocete respiratory function by measuring breath durations, respiratory flow, and calculating tidal volumes during resting and maximal respiratory effort breaths in voluntarily participating belugas.

2. Materials and methods

2.1. Study subjects

Pulmonary function testing was conducted at three managed care facilities maintaining belugas (*Delphinapterus leucas*) in their animal collections. A total of nine (3 male, 6 female) whales of varying ages (from 8 to > 35 years) were included in the study (Table 1); five from SeaWorld San Antonio (San Antonio, Texas, USA), two from Oceanogràfic (Valencia, Spain), and two from Vancouver Aquarium (Vancouver, British Columbia, Canada). The study protocols were accepted at each facility, as well as with Texas A&M University–Corpus Christi's Institutional Animal Care and Use Committee (AUP 04-14), and all trials were carried out in accordance with the National Institutes of Health guide for the care and use of Laboratory animals (NIH Publications No. 8023, revised 1978).

2.2. Morphometrics

Body mass (M_b, kg) was provided through direct measurements from SeaWorld, or was estimated using an equation by Doidge (1990b) from straight length (SL in cm, $M_b = 10^{-3.84} \, SL^{2.58}$) based on wild

populations. We did not use separate equations for males and females including girth as the reported exponents differ in the same report (Doidge, 1990b). The SL was measured in straight line from the tip of the rostrum to the fluke notch. Maximal girth (G, cm) was measured at the animal's widest point (The Commitee on Marine Mammals American Society of, M., 1961). Using date of birth or other time references from wild caught individuals, the age is reported/estimated for the middle of the trial period (February 2015) and was used for analysis. The sexual maturity of Animal S-4 was unknown, although likely mature, and all other animals were presumed sexually mature adults (Brodie, 1971; Burns and Seaman, 1986; Robeck et al., 2005). Variables for each animal are summarized in Table 1.

2.3. Equipment

Data was collected using a custom-designed Fleisch style pneumotachometer (Micah Brodsky, V.M.D. Consulting, Miami, FL). The bottom of the device contained a medical grade soft silicon interface creating a seal with the skin surrounding the blowhole of the animal (Fig. 1A). A low resistance custom-made flow cell (Laminar Matrix Part Z9A887-2, Meriam Process Technologies, Cleveland, OH), was placed inside the plastic housing of the pneumotachometer (Fig. 1B). The differential pressure on either side of the flow-cell was relayed through firm walled tubing (310 cm in length, 2 mm internal diameter, McMaster-Carr, Elmhurst, IL) to a differential pressure transducer (MPX type 339/2, Harvard Apparatus, Holliston, MA, USA). The signal was passed through an amplifier (TAM-A Transducer Amplifier Module, Hugo Sachs Elektronik, Harvard Apparatus, Holliston, MA), sampled at 400 Hz using a PowerLab A/D converter (Series 8/35, ADInstruments, Colorado Springs, CO), and was transferred to a laptop (Toughbook 31, Panasonic, Newark, NJ) running LabChart (Version 8, ADInstruments, Colorado Springs, CO). To prevent baseline drift, condensation in the sample tubing was prevented by keeping the equipment in the ambient atmosphere for at least one hour prior to collection, and the lines were cleared using dry pressurized air immediately before each experimental trial.

2.4. Procedure

Experimental trials took place between February 21, 2014 and February 5, 2016, between 9:00 and 16:45 local time (Table 2).

All experiments were performed through voluntary participation by animals, which had been previously conditioned to the behavioral requirements necessary to complete experimental trials. Study animals were able to end a trial at any time merely by swimming away.

At the beginning of an experimental trial, each beluga stationed with their head facing the trainer. The pneumotachometer was placed over the blowhole, assuring that there were no leaks around the base of the pneumotachometer. Animals were trained to maintain their own buoyancy at the surface with minimal activity. Along with spontaneous tidal breaths, the animals were trained to perform a forceful expiratory behavior, known as a "chuff", which was used to approximate the vital capacity (V_C).

2.5. Calibration

The relationship between respiratory flow and differential voltage was determined before and after each trial, using a 7.01 calibration syringe (4900 Series, Hans Rudolph, Shawnee, KS). The pneumotachometer was placed at the opening of the syringe and air was forced through the flow cell using a range of flow rates in both directions. At high flows (> 60 l s^{-1}), the relationship between differential pressure and flow became non-linear (Fahlman et al., 2015). Therefore, separate calibration factors were applied for each respiratory event (expiratory breath, inspiratory breath, expiratory chuff, inspiratory chuff) by converting raw voltage to flow (l s^{-1}) (Epple, 2016). However, due to the

Table 1

Animal ID (ID), facility (Sea World-SW, Vancouver Aquarium-VA, Oceanografic-Oce), date of birth (DOB, year/month/day), age (years), straight length (SL, cm), maximum girth (G, cm), measured (M_b), estimated body mass (Mb$_{est}$, Doidge, 1990b) during trial period, average ($\pm$ sd) breathing frequency (f_R) during 5 min focal observations and estimate total lung capacity (TLC$_{est}$ = 0.135 $M_b^{0.92}$, Fahlman et al., 2011; Kooyman, 1973). The values in parenthesis in the $M_{b\text{-est}}$ column are the equation estimate increased by 22%, which represents the mean error of the animals with measured M_b. *Estimated date of birth for wild caught animals. + Animal is at least 35 years old. Variables for which a measurement could not be obtained are listed as "NA". § Body mass determined during necropsy. $^+$Indicates M_b used to estimate TLC$_{est}$.

| Animal ID	Facility	Sex	DOB	Age	SL (cm)	G (cm)	M_b (kg)	$M_{b\text{-est}}$ (kg)	f_R (breaths min^{-1})	TLC$_{est}$ (l)
S-1	SW	F	1986/01/01*	29	312	NA	562	393	2.7 ± 0.4[6]	46
S-2	SW	F	2000/07/24	15	338	263	612	484	2.3 ± 0.2[5]	49
S-3	SW	M	1987/08/13	27	384	231	891	672	2.6 ± 0.5[6]	70
S-4	SW	M	2007/07/23	8	330	202	510	455	2.1 ± 0.7[5]	42
S-5	SW	F	1986/01/01*	29	NA	224	565	NA	2.2 ± 0.3[6]	46
O-1	Oce	F	1996*	19	385	238	NA	677 (826$^+$)	2.8 ± 0.5[30]	65$^+$
O-2	Oce	M	1983*	35$^+$	382	228	NA	663 (809$^+$)	3.0 ± 0.5[30]	64$^+$
V-1	VA	F	1995/07/23	20	356	240	560§	553 (675$^+$)	1.5 ± 0.4[15]	45$^+$
V-2	VA	F	1986*	29	381	248	645§	659 (804$^+$)	1.6 ± 0.5[15]	64$^+$
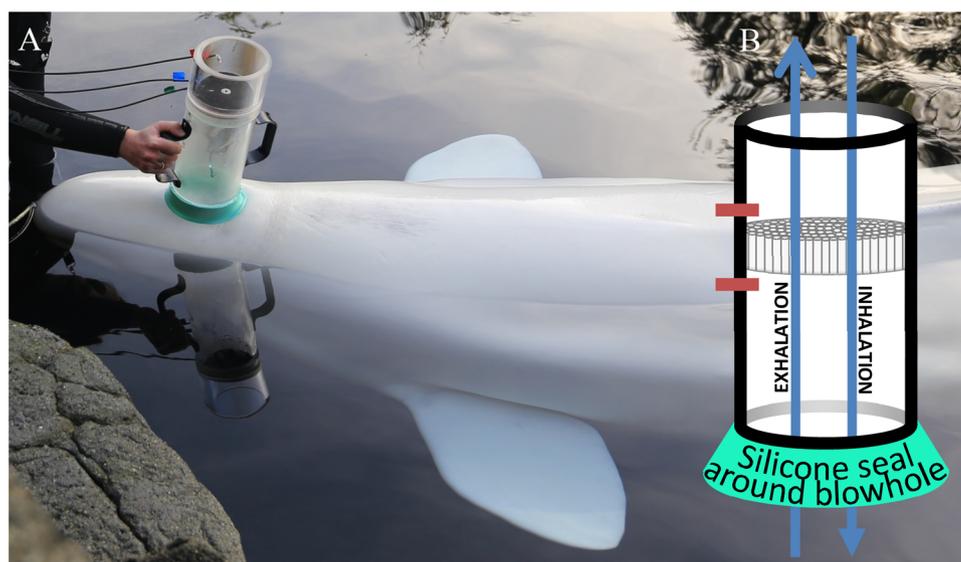
Fig. 1. Experimental setup and equipment used for research trials. Panel A shows how the pneumotachometer was placed over the blowhole while the animal rested at the surface. As the animal breathed through the flow cell, pressure was relayed to the data acquisition unit and computer. Panel B shows a schematic of the pneumotachometer. The silicone interface creates an airtight seal between the pneumotachometer and the animal's blowhole. Air (blue arrows) passes through the flow cell (gray piece), which creates slight resistance resulting in different air pressures on each side of the flow cell. Ports on each side of the flow cell (red) are connected to a pressure transducer. The difference in pressure between the two ports is calibrated to yield flow. Figure obtained from Epple (2016).

Table 2

Animal ID (ID), number of trials (# trials), spontaneous breaths (# Spontaneous), and chuffs (# chuffs).

Animal ID	# trials	# Spontaneous	# Chuffs
S-1	10	221	33
S-2	12	217	25
S-3	12	162	37
S-4	11	139	33
S-5	9	222	23
O-1	15	121	53
O-2	9	172	26
V-1	7	158	15
V-2	9	196	31

inability to consistently match the high flow rates of all chuffs (> 120 l s^{-1}), it is possible that our recorded maximal exhalation overestimates the true value. After separate calibration factors were applied, the combined flow was filtered using a low-pass filter with a cut-off frequency of 10 Hz and a transition width (sharpness) of 50 Hz to minimize spurious data points. Finally, the flow was integrated to estimate V_T.

2.6. Breath characteristics

Respiratory frequency (f_R) was calculated for each trial by dividing

the total number of consecutive breaths by the total time elapsed from the first breath of the trial to the last. If a trial consisted of multiple sections, an average of those sections was taken to represent the entire trial. For comparison, non-trial f_R were measured for each animal using a five-minute duration while the animals were not actively swimming (Table 1).

2.7. Data processing and statistical analysis

Expiratory duration (T_{exp}), inspiratory duration (T_{insp}), total breath duration (T_{tot}), maximal expiratory flow ($\dot{V}_{exp}$), maximal inspiratory flow ($\dot{V}_{insp}$), expiratory tidal volume (V_{Texp}), and inspiratory tidal volume (V_{Tinsp}) were identified for each breath using LabChart. T_{exp} was defined as the duration from the start of the exhalation until the flow returned to zero, and T_{insp} as the duration from the end of expiration until the flow again returned to zero. $\dot{V}_{exp}$ and $\dot{V}_{insp}$ were identified using calibrated and filtered flow, and V_{Texp} and V_{Tinsp} were calculated through integration of their respective flow curve (Fahlman et al., 2015). The mean for each respiratory variable was calculated for each animal by averaging the respective variable during spontaneous breaths, as well as during chuffs.

For each animal the mean volume of the three largest chuffs was used as an estimate of the V_C. The relative volume of the V_C exchanged per spontaneous breath was determined by dividing the V_T by V_C or the estimated total lung capacity (TLC$_{est}$). The TLC$_{est}$ was estimated from

the M_b, based on an equation from static measurements made using excised lungs from a range of cetacean and pinniped species ($TLC_{est} = 0.135\ M_b^{0.92}$) (Fahlman et al., 2011; Kooyman, 1973). To mass-correct data, we used the measured M_b, or for those without a measured value we used $M_{b\text{-est}}$ as determined from the equation relating straight length (SL) to $M_{b\text{-est}}$ (Doidge, 1990b). We also mass-corrected V_T (sV_T), and $\dot{V}$ ($s\dot{V}$) by dividing the variable by M_b or $M_{b\text{-est}}$.

All gas volumes were converted to standard temperature pressure dry (STPD, Quanjer et al., 1993). Exhaled air was assumed saturated at 37 °C, inhaled air volume was corrected for ambient temperature and relative humidity (Fahlman et al., 2015). Only breaths that consisted of both a single expiration and single inspiration and during which the pneumotachometer maintained a proper seal with the animal were included in statistical analysis.

Simple paired comparisons of expired and inspired V_T, $\dot{V}$, T were analyzed using paired t-tests. The relationship between a dependent variable (e.g. mean, T_{exp}, T_{insp}, T_{tot}, $\dot{V}_{exp}$, $\dot{V}_{insp}$, V_{Texp}, V_{Tinsp}, and V_C) and experimental covariates (length, $M_b/M_{b\text{-est}}$, breath type [spontaneous/chuff]) was analyzed using generalized linear-mixed effects models (GLM, lme, R: A Language and Environment for Statistical Computing, R Foundation for Statistical Computing, Version 3.3.3, 2016). 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). Best models were chosen by the log-likelihood ratio test (LL), and/or the Akaike information criterion (AIC) against the null model (AIC_{null}) and significant parameters assessed by the t-value between the estimate and its standard error. In this study P-values ≤ 0.05 were considered as significant and $P \leq 0.1$ were considered a trend. Data are presented as the mean ± standard deviation (s.d.), unless otherwise stated.

3. Results

A total of 94 trials, consisting of 1,884 respirations (1,608 breaths and 276 chuffs) from nine individuals were used for the analysis.

3.1. Body mass

The difference in measured and estimated ($M_{b\text{-est}}$) M_b for the 4 belugas where SL was measured suggested that the equation underestimated $M_{b\text{-est}}$ by a mean (± s.d.) of 22 ± 8%. We therefore added 22% to the $M_{b\text{-est}}$ of belugas from the other facilities where no weights were measured (Table 1). Two additional M_b's were obtained at post mortem examination, 23 months post-mortem, after trials ended, where $M_{b\text{-est}}$ was 1% lower during the trial as compared with the measured M_b at necropsy in beluga V-1. For animal V-2, $M_{b\text{-est}}$ was 2% lower during the trial as compared with during necropsy (Table 1). We therefore used the measured SL during the trial period to calculate $M_{b\text{-est}}$ to mass-correct V_T and $\dot{V}$ for these two individuals (Table 1).

3.2. Respiratory variables

The mean T_{exp}, T_{insp}, and T_{tot} decreased by 48%, 18%, and 32%, respectively, during forced breaths (T_{exp}: 518 ± 101 ms; T_{insp}: 905 ± 170 ms; T_{tot}: 1423 ± 227 ms) as compared with spontaneous breaths (T_{exp}: 995 ± 176 ms, GLM, $\chi^2 = 334$, df = 1, P < 0.01; T_{insp}: 1098 ± 219 ms, $\chi^2 = 506$, df = 1, P < 0.01; T_{tot}: 2093 ± 302 ms, $\chi^2 = 396$, df = 1, P < 0.01, Fig. 2).

For spontaneous breaths, there was large variation in the expired and inspired V_T (Fig. 3), but there were no differences in the mean (± s.d.) V_{Tinsp} (16.2 ± 4.7 l) or V_{Texp} (15.5 ± 3.9 l, $\chi^2 = 1.8$, df = 1, P = 0.17), but $\dot{V}_{exp}$ (26.1 ± 5.5 l s^{-1}, Fig. 4) was significantly higher as compared with $\dot{V}_{insp}$ (22.3 ± 4.6 l s^{-1}, $\chi^2 = 12.1$, df = 1, P < 0.01). When animal was included as a random factor, neither M_b nor SL were important to explain V_T or $\dot{V}$. However, V_{Tinsp}, $\dot{V}_{exp}$ and $\dot{V}_{insp}$ increased
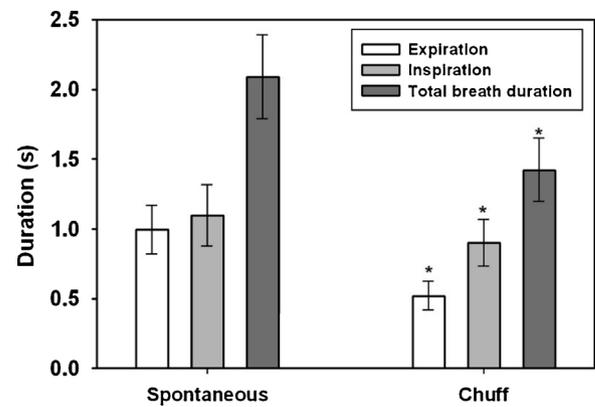
Fig. 2. Mean expiratory, inspiratory, and total breath duration (s) during spontaneous breaths and maximal respiratory efforts (chuffs). Error bars represent standard deviation (n = 9). *duration significantly different between spontaneous breaths and chuffs (P < 0.05).
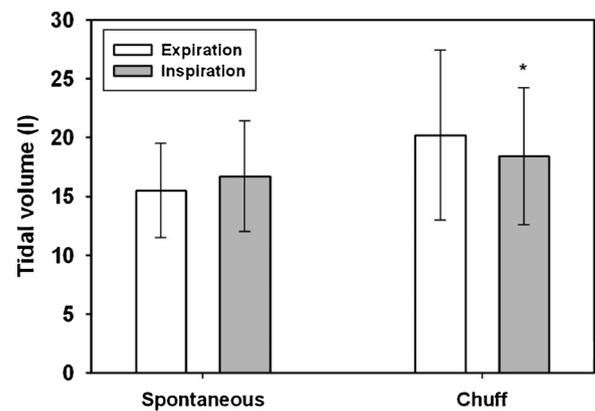
Fig. 3. Mean expiratory and inspiratory volumes exchanged during breaths and chuffs. Error bars represent standard deviation (n = 9). *volume significantly different between spontaneous breaths and chuffs (P < 0.05).
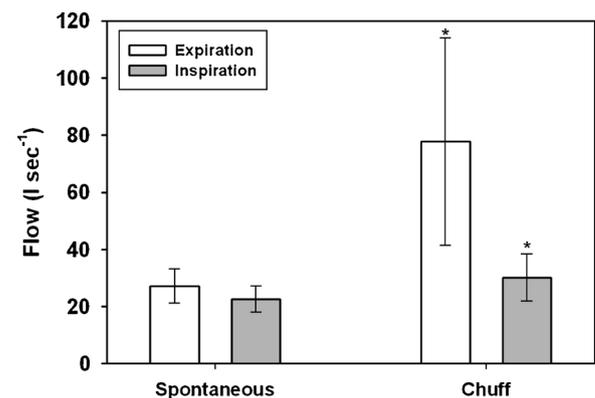
Fig. 4. Mean expiratory and inspiratory flow rates during spontaneous breaths and maximal respiratory efforts (chuffs). Error bars represent standard deviation (n = 9). *flow significantly different between spontaneous breaths and chuffs (P < 0.05).

during chuffs (V_{Tinsp}: 18.8 ± 6.4 l; $\dot{V}_{exp}$: 66.8 ± 22.5 l s^{-1}; $\dot{V}_{insp}$: 30.1 ± 8.4 l s^{-1}) as compared to spontaneous breaths (V_{Tinsp}: 16.2 ± 4.7 l, $\chi^2 = 20.6$, df = 1, P < 0.01; $\dot{V}_{exp}$: 26.1 ± 5.5 l s^{-1}, $\chi^2 = 1349$, df = 1, P < 0.01; $\dot{V}_{insp}$: 22.3 ± 4.6 l s^{-1}, $\chi^2 = 342$, df = 1, P < 0.01). In addition, $\dot{V}_{exp}$ was higher as compared with $\dot{V}_{insp}$ both for spontaneous breaths ($\chi^2 = 158$, P < 0.01) and chuffs ($\chi^2 = 374$, P < 0.01).

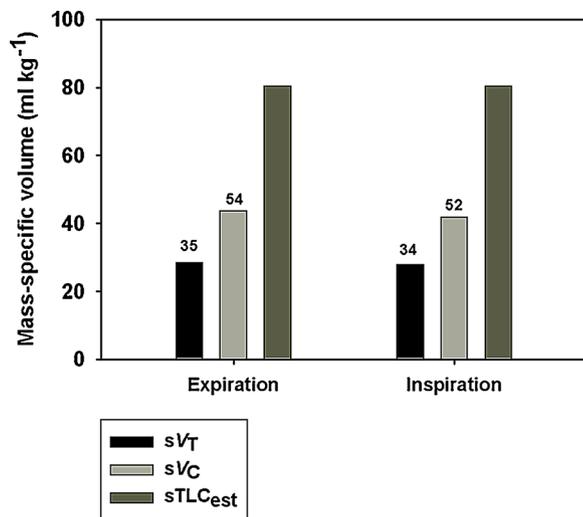Similarly, mass specific measurements, sV_{Tinsp} (28.8 ± 11.8 ml

Fig. 5. Measured mass-specific expiratory and inspiratory tidal volume (sV_T), vital capacity (sV_C), and estimated mass-specific total lung capacity ($sTLC_{est}$). TLC_{est} was calculated from body mass based on static measurements of excised lungs from a range of cetacean and pinniped species (Fahlman et al., 2011; Kooyman, 1973). The values above the bars are the percentage of $sTLC_{est}$.

Fig. 6. Representative flow-volume curves for 2 chuffs for animal V-2 (Table 1). The chuffs begin to the right and move to the left as the lung empties, which are shown with positive flow. The blue diamond indicates the beginning of the first chuff.

kg^{-1}), $s\dot{V}_{exp}$ (100.5 ± 35.7 ml kg^{-1} s^{-1}), $s\dot{V}_{insp}$ (45.7 ± 14.7 ml kg^{-1} s^{-1}) for chuffs were higher as compared with spontaneous sV_{Tinsp} (25.5 ± 8.6 ml kg^{-1}, χ^2 = 19.5, df = 1, P < 0.01), $s\dot{V}_{exp}$ (39.8 ± 10.4 ml kg^{-1} s^{-1}, χ^2 = 1334, df = 1, P < 0.01), and $s\dot{V}_{insp}$ (34.5 ± 11.4 ml kg^{-1} s^{-1}, χ^2 = 283, df = 1, P < 0.01). Both chuffs (χ^2 = 384, df = 1, P < 0.01) and spontaneous breaths (χ^2 = 148, 1 df, P < 0.01), $\dot{V}_{exp}$ were higher when compared with $\dot{V}_{insp}$.

The mean V_C for expiration and inspiration were 28.4 ± 8.7 l, (range: 15.0–43.5 l) and 27.7 ± 8.9 l (range: 16.7–40.3 l), respectively. The mean mass-specific V_C (sV_C) was 43.7 ± 17.4 ml kg^{-1} (range: 18.6–78.7 ml kg^{-1}) for expiration, and 41.8 ± 14.4 ml kg^{-1} (20.7–63.1 ml kg^{-1}) for inspiration. Neither V_C (χ^2 = 0.65, P = 0.42), nor sV_C (χ^2 = 1.73, P = 0.19) differed during expiration or inspiration. The V_C for expiration and inspiration was, respectively, 54 ± 21% and 52 ± 18% of the estimated mass-specific total lung capacity ($sTLC_{est}$) by dividing TLCest by M_{b-est} (Table 1). The mean sV_{Texp} and sV_{Tinsp} were, respectively, 35% ± 9% (range: 12–49%) and 34% ± 10% (range: 14–50%) of $sTLC_{est}$ (Fig. 5).

Flow–volume curves for two chuffs are presented in Fig. 6. By convention, expiratory flow rates are positive. There was no clear dependence of expiratory flow-rate on lung volume during chuffs, and expiratory flows are nearly constant over most of the V_C.

3.3. Breathing frequency

The mean (± s.d) f_R during trials was 4.8 ± 2.1 breaths min^{-1} (range: 2.9–9.6 breath min^{-1}), which was higher than f_R measured for each animal through visual observations during periods when the animals were free swimming, but not active (2.3 ± 0.5 breaths min^{-1}, range: 1.6–3.0 breaths min^{-1}, paired t-test, t-value = 3.36, P < 0.01, Table 1).

4. Discussion

The cetacean respiratory system varies anatomically both compared to terrestrial mammals and also between cetacean species (Piscitelli et al., 2013). A growing number of physiologic studies have begun to lay the foundation for understanding how unique anatomical properties translate into functional differences (Fahlman et al., 2018a, 2015; Fahlman et al., 2018c; García-Párraga et al., 2018; Kooyman and Cornell, 1981; Kooyman et al., 1975; Olsen et al., 1969; Reed et al.,
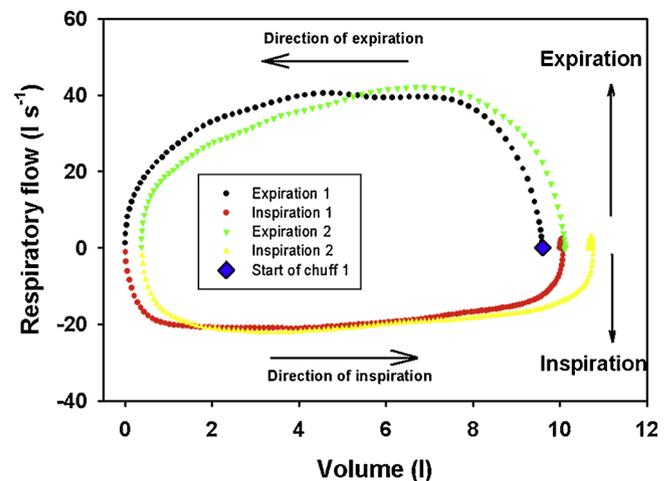
2000; Spencer et al., 1967; Wahrenbrock et al., 1974). In this study we measured the respiratory capacity in a medium sized odontocete, the beluga, with the intent to compare baseline lung function with other species and our results confirm that the V_T is significantly higher as compared with terrestrial mammals (Stahl, 1967), and similar to that reported in other marine mammal species (Fahlman et al., 2017; Piscitelli et al., 2013). The results also support previous conclusions, which indicate that cetaceans have unusually high V_T and sustained $\dot{V}$ with $\dot{V}_{insp}$ being more limited than $\dot{V}_{exp}$. The spontaneous V_T was approximately 50% of V_C but only 35% of TLCest, possibly suggesting that TLC_{est} may be lower in this deeper diving species than predicted by the equation developed for near-shore, presumably shallow-diving, individuals (Fahlman et al., 2011, 2015; Kooyman, 1973). This agrees with the suggestion that deep diving cetaceans have relatively smaller lungs and lower TLC as compared with near-shore, shallow diving individuals (Piscitelli et al., 2010; Scholander, 1940). An alternative explanation is that the belugas hold back with an apparatus placed over the blow-hole, despite several sessions to desensitize them before data collection. Thus, if normal V_T is closer to V_C it would be approximately 50% of TLCest (Fig. 5).

While the f_R was higher during trials than during periods of minimal activity, this could indicate that the whales hyperventilated or adjusted the breathing strategy (increased f_R and decreased V_T) while resting next to the trainer. While we cannot rule out the possibility that the elevated f_R is evidence of hyperventilation, future work monitoring end-expired CO_2 could help resolve this issue. However, the consistency in results between belugas, both within and between facilities, does not support the suggestion that the animals were hyperventilating. In addition, the high flow rates reported here should be considered carefully as the extreme value may be an overestimate as the calibration required for accurate measurements at very high flow rates had high variability. We propose that this inherent error, was in part, due to the design limitations of the pneumotachometer. The pneumotachometers were designed to minimize dead space while simultaneously promoting laminar flow. The necessity for limited dead space prevents true laminar flow from ever being established in a pneumotachometer of this general design and size, therefore we know that true laminar flow was not achieved (Finucane et al., 1972). Evidences for this hypothesis was that at high flow rates, the flow calibrations became non-linear and differed significantly for expiration and inspiration during maximal respiratory efforts. Some turbulence is unavoidable, which could result in an overestimated flow rate (Grenvik et al., 1966). Despite these limitations, these results provide us with an initial estimate of the respiratory

capacity of belugas.

All belugas exhibited high respiratory flow over most of the lung volume; similar results have been observed in other cetaceans during both *in vivo* studies (Fahlman et al., 2018a, 2015; Fahlman et al., 2018c; Gans, 2013; Kooyman and Cornell, 1981) and using excised lungs (Kooyman and Sinnett, 1979), indicating that this is likely a conserved trait of cetacean respiration. The maximal peak expiratory flow rate (PEFR; $212 \, l \, s^{-1}$) was similar to that previously reported for a gray whale calf ($202 \, l \, s^{-1}$, Kooyman et al., 1975), but higher than that for the killer whale ($180 \, l \, s^{-1}$, Spencer et al., 1967), dolphin ($162 \, l \, s^{-1}$, Fahlman et al., 2018a, 2015; Fahlman et al., 2018c; Kooyman and Cornell, 1981), and harbor porpoise ($61 \, s^{-1}$, Reed et al., 2000). When the $\dot{V}_{exp}$ was expressed as V_C's per second, the maximal flow was $6.4 \, V \, s^{-1}$ (range: 1.9–6.4 $V_C \, s^{-1}$) which is comparable to previously reported values (Kooyman and Cornell, 1981; Spencer et al., 1967). During expiration, there was a rapid increase to a relatively constant $\dot{V}_{exp}$ followed by a rapid decline (Fig. 6). Similar to data in dolphins and sea lions (Fahlman et al., 2015; Fahlman and Madigan, 2016; Kerem et al., 1975; Kooyman and Cornell, 1981; Matthews, 1977), the variations in flows from chuff to chuff suggest that the $\dot{V}_{exp}$ were effort dependent over most of the V_C.

Our results indicated that the V_T of chuffs was larger than that of spontaneous breaths, which agrees with previous work in dolphins (Fahlman et al., 2015). In some previous studies the authors reported that most expiratory volumes were larger than the inspiratory volumes (Fahlman et al., 2015; Kooyman and Cornell, 1981). However, separate calibration factors for spontaneous and forced breaths improved the problems with non-linearity at higher flow rates. Consequently, the high PEFR values reported here may represent the upper limits of respiratory capacity of this species and highlights the importance of doing these studies in trained animals as it allows for controlled studies and observation of a larger range of flow than may be possible in wild animals.

Our results show that $\dot{V}_{insp}$ were lower and less variable as compared with $\dot{V}_{exp}$, typically associated with inspiratory flow restrictions. While these differences could be biased by studying trained animals under spontaneous control, this observation agrees with those of other cetaceans both in alive animals (Fahlman et al., 2015; Kooyman and Cornell, 1981; Reed et al., 2000) and from excised lungs (Kooyman and Sinnett, 1979). Thus, we propose that these results represent true inspiratory limitations and that $\dot{V}_{insp}$ is lower than $\dot{V}_{exp}$.

Previous data has indicated large differences in V_T both between and within cetacean species (Fahlman et al., 2018a, 2015; Fahlman et al., 2018c; Gans, 2013; Irving et al., 1941; Kooyman and Cornell, 1981; Olsen et al., 1969; Ridgway et al., 1969), and this research reinforces those results. Spontaneous V_{Tinsp} (mean: $16.7 \pm 4.7 \, l$, range: 8.8–23.0 l) were similar, though slightly larger, than those previously reported for belugas (12.6–14.0 l, Kasting et al., 1989). All animals had measured V_T's that were greater (mean: $156 \pm 88\%$, range: 9–312%) than those predicted based on terrestrial mammals (Stahl, 1967). This confirms that allometric scaling of respiratory variables using equations from terrestrial mammals do not appear to be good predictors for marine mammals (Fahlman et al., 2017). Mathematical models used to understand how marine mammals partition gases during diving are dependent on accurate estimates of the diving lung volume (Bostrom et al., 2008; Fahlman et al., 2009). Thus, our results highlight the importance of species specific studies since models developed for terrestrial species cannot be accurately extrapolated, particularly for use with cetaceans.

While it would be expected to see an allometric relationship between V_T, $\dot{V}$ and M_b, the current data set was unable to provide a statistical justification for inclusion of M_b in the multivariate analysis. One reason could be that we used a prediction equation from free ranging whales from Canada (Doidge, 1990a, b) to estimate M_b for 4 of the animals. It has been suggested that belugas in managed care may easily

become overweight in relation to their wild counterparts (Kastelein et al., 1994), and a previous study reported discrepancies between the weight gain and growth patterns of managed care belugas compared with their wild counterparts (Robeck et al., 2005). Thus, equations to estimate M_b based on wild animals may not provide accurate estimates for individuals in managed care. However, an equation estimating M_b from SL using managed care animals (Robeck et al., 2015) provided similar $M_{b\text{-est}}$ for this data chohort, so this reason is unlikely. Another possible reason that M_b might not be included is that we did not have any variation in our estimated or measured M_b, and therefore the model was unable to distinguish variation between individuals and M_b. For this reason, we also reported and analyzed the mass-specific values, and our sV_{Tinsp} (range: 11.0–41.6 ml kg^{-1}) for spontaneous breaths was similar to those reported previously for the beluga (mean: 32.0 ml kg^{-1}, range: 21.3–42.0 ml kg^{-1}, Kasting et al., 1989), the harbor porpoise (37.5 ml kg^{-1}, Reed et al., 2000), bottlenose dolphin (mean: 30.8 ml kg^{-1}, range: 3.8–37.8 ml kg^{-1}, Fahlman et al., 2018a, 2015; Fahlman et al., 2018c), and the pilot whale (20.0–87.8 ml kg^{-1}; Olsen et al., 1969). The sV_C (Fig. 5), on the other hand, varied between individuals, with the greatest being lower than values previously reported for the Atlantic bottlenose dolphin (94.0 ml kg^{-1}, Fahlman et al., 2015), the Pacific bottlenose dolphin (92.6 ml kg^{-1}, Kooyman and Cornell, 1981), the pilot whale (88.9 ml kg^{-1}, Olsen et al., 1969), the harbor porpoise (62.1 ml kg^{-1}, Scholander, 1940), and the killer whale (57.9 ml kg^{-1}, Kasting et al., 1989), but greater than those reported for a gray whale (35.3 ml kg^{-1}, Wahrenbrock et al., 1974). It is likely that some of these V_C's may be underestimated, as the animals in some of these studies were not trained to produce maximal breaths upon cue.

It has been shown that cetaceans are able to exchange ~80 to 90% of their TLC (Fahlman et al., 2015; Irving et al., 1941; Kooyman and Cornell, 1981; Olsen et al., 1969; Scholander, 1940). In the current study, we estimated V_C from the 3 highest respiratory efforts, and assuming that the functional residual capacity is very small as in other species (Fahlman et al., 2011; Kooyman and Sinnett, 1979; Piscitelli et al., 2010), V_C is an approximate estimate of TLC. However, V_{Tinsp} was only 32% of TLC_{est} which is lower than those measured in the bottlenose dolphin (~45%, Fahlman et al., 2015) and harbor porpoise (~40%, Reed et al., 2000). However, V_{Tinsp} was 52% of V_C and we propose two explanations. First, we cannot assure that all animals provided true maximal respiratory effort, but the consistency between animals in the relative volume for V_C (the three highest chuffs) suggest that the maximal values were similar but does not assure that these were maximal efforts. Second, it is possible that the equation for TLC_{est} does not agree well with deep diving species as V_T was only 32% of TLC_{est}. However, it is also possible that the large percentage exchange of TLC previously quoted (80–90%) represents the upper limits of cetacean physiologic capacity, rather than that being the percentage exchanged during routine exertion.

These results provide baseline data on the respiratory durations, $\dot{V}$, and V_T's exchanged in nine resting belugas. This basic understanding of the functional properties of the beluga pulmonary system is antecedent to facilitate future understanding of how changes in polar ecosystems may impact belugas, based on their respiratory physiology and diving capabilities; and the role they play in the fitness of this species in their environment. The custom-designed pneumotachometers used in this and our other studies are based largely on equipment commonly used for pulmonary function testing (PFT) in humans. In human medicine, PFT is frequently used to diagnose and monitor disease (Crapo, 1994; Miller et al., 2005). With the development of healthy baseline measurements, PFT could also be used as a non-invasive way to investigate cetacean respiratory health (Gans, 2013; Van Elk et al., 2001). In addition, the data reported in this study will help improve theoretical modeling studies that try to understand the physiological limitations to diving and define the limits of diving in marine mammals (Fahlman et al., 2009, 2018b; Hodanbosi et al., 2016; Hooker et al., 2009). Such theoretical work provides predictions concerning how man-made

changes to the environment may alter the survivability of marine species, and provide important information for management of conservation efforts. Thus, an improved understanding about the eco-physiology of marine species has the potential to help conserve wild populations.

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