



Cite this article: Fahlman A, Brodsky M, Wells R, McHugh K, Allen J, Barleycorn A, Sweeney JC, Fauquier D, Moore M. 2018 Field energetics and lung function in wild bottlenose dolphins, *Tursiops truncatus*, in Sarasota Bay Florida. *R. Soc. open sci.* **5**: 171280. <http://dx.doi.org/10.1098/rsos.171280>

Received: 1 September 2017

Accepted: 5 December 2017

Subject Category:

Biology (whole organism)

Subject Areas:

ecology/physiology/environmental science

Keywords:

field metabolic rate, pulmonary function test, tidal volume, diving physiology, marine mammals, spirometry

Author for correspondence:

A. Fahlman

e-mail: afahlman@whoi.edu

Field energetics and lung function in wild bottlenose dolphins, *Tursiops truncatus*, in Sarasota Bay Florida

A. Fahlman^{1,2,3}, M. Brodsky⁴, R. Wells⁵, K. McHugh⁵, J. Allen⁵, A. Barleycorn⁵, J. C. Sweeney⁶, D. Fauquier⁷ and M. Moore³

¹Fundación Oceanográfica de la Comunidad Valenciana, Gran Vía Marques del Turia 19, 46005 Valencia, Spain

²Texas A&M University-Corpus Christi, 6300 Ocean Drive, Corpus Christi, TX 78412, USA

³Woods Hole Oceanographic Institution, 266 Woods Hole Rd., MS# 50, Woods Hole, MA 02543-1050, USA

⁴Micah Brodsky, V.M.D. Consulting, Miami Shores, FL 33138, USA

⁵Chicago Zoological Society's Sarasota Dolphin Research Program, c/o Mote Marine Laboratory, 1600 Ken Thompson Parkway, Sarasota, FL 34236, USA

⁶Dolphin Quest, Oahu, 5000 Kahala Ave, Honolulu, HI 96816, USA

⁷Marine Mammal Health and Stranding Response Program, Office of Protected Resources, NOAA/National Marine Fisheries Service, 1315 East-West Highway, Room 13620, Silver Spring, MD 20910, USA

AF, 0000-0002-8675-6479

We measured respiratory flow rates, and expired O₂ in 32 (2–34 years, body mass [M_b] range: 73–291 kg) common bottlenose dolphins (*Tursiops truncatus*) during voluntary breaths on land or in water (between 2014 and 2017). The data were used to measure the resting O₂ consumption rate ($\dot{V}O_2$, range: 0.76–9.45 ml O₂ min⁻¹ kg⁻¹) and tidal volume (V_T , range: 2.2–10.41) during rest. For adult dolphins, the resting V_T , but not $\dot{V}O_2$, correlated with body mass (M_b , range: 141–291 kg) with an allometric mass-exponent of 0.41. These data suggest that the mass-specific V_T of larger dolphins decreases considerably more than that of terrestrial mammals (mass-exponent: 1.03). The average resting $s\dot{V}O_2$ was similar to previously published metabolic measurements from the same species. Our data indicate that the resting metabolic rate for a 150 kg dolphin would be 3.9 ml O₂ min⁻¹ kg⁻¹, and the metabolic rate for active animals, assuming a multiplier of 3–6, would range from 11.7 to 23.4 ml O₂ min⁻¹ kg⁻¹.

Our measurements provide novel data for resting energy use and respiratory physiology in wild cetaceans, which may have significant value for conservation efforts and for understanding the bioenergetic requirements of this species.

1. Introduction

Climate change is causing perturbations in oceanic and coastal circulation and water temperature, which can alter the spatial and temporal distribution of animals and their food resources [1]. Increased utilization of the planet's oceans by a growing human population has impacted marine ecosystems. For marine mammals known effects include increased ocean noise, increased incidence of human interactions like fishing gear entanglements and ship strikes, exposure to toxic chemicals (e.g. organochlorines such as DDT, PCBs, heavy metals, etc.), and eutrophic waste from agriculture, expanding industry and developing urban areas. Previous studies have suggested that marine predators may be valuable bio-indicators of environmental health [2]. As top-level predators, marine mammals may be particularly prone to effects of climate change and deteriorating environmental conditions; serving as the proverbial canary in the coal-mine, indicating significant changes at lower trophic levels and showing the impact those changes could have on other mammalian species, including humans, that share the same water, air and fish.

An essential aspect of understanding the ecology and distribution of any species is to define their resource and energy requirements. While considerable work has been done on the bioenergetics and respiratory physiology of seals and sea lions, technical and logistical limitations have prevented comparable measurements in wild cetaceans. One approach to estimate field metabolic rate in large whales has been to measure their breathing frequency [3–8]. The assumption is that increased breathing, as occurs during active foraging or travel, is tightly linked to the energy requirements of the activity state and can be used to assess the field metabolic rate for different activities, e.g. resting or swimming at the surface and diving. While this method is attractive in its simplicity, it assumes that the average tidal volume (V_T) and O_2 exchange fractions are known, and that they remain constant over the measurement period. This is not always the case [9], and improved knowledge of the cardiorespiratory physiology, and the temporal patterns of respiration and gas exchange following exercise or diving would significantly improve the estimate of metabolic cost [10–12]. There are also other techniques that can provide proxy estimates of field metabolic rate, such as the dilution of doubly labelled water in the body water pool, activity records or heart rate monitoring, but these techniques require careful validation to assess how changes in their relationships may change over different seasons, years, activities, life stage, etc. Still, the resting metabolic rate (RMR) is often used as a tool to provide an estimate of the minimum energy requirements for a species.

Our objective was to measure lung function and estimate RMR for common bottlenose dolphins (*Tursiops truncatus*), using breath-by-breath respirometry in wild animals. Understanding metabolic and respiratory requirements is of particular interest because it is vital to identifying and understanding the physiological limitations for survival of a species. In addition, respiratory disease is a major cause for morbidity and mortality in wild cetaceans, and increasing environmental stressors, such as exposure to pollutants, is likely to exacerbate this problem [13,14]. Despite significant advances in technology, we still know very little about the respiratory physiology of marine mammals. Because respiratory physiology is difficult to study in free-ranging dolphins, normal lung function data for wild animals are limited [15,16]. We therefore compared data collected from wild animals with data from healthy dolphins and cetaceans managed under human care [17–21] to assess if respiratory physiology measurements may provide information that is relevant to both populations.

2. Material and methods

2.1. Animals

The free-ranging bottlenose dolphins were long-term residents of Sarasota Bay (Lat: 27°22'40.31" N Long: 82°35'9.40" W) and were sampled during 5–9 May 2014; 12–20 May 2015; 6–11 May 2016 and 8–12 May 2017. Juvenile and adult bottlenose dolphins of both sexes, and varying sizes (table 1) were used for the experiments. These animals were measured during periodic capture-release health assessments by the Sarasota Dolphin Research Program (SDRP), and were briefly encircled with a net,

Table 1. Year tested, animal identification (freeze-brand number), sex (M-male, F-female), body mass (M_b), straight length (SL), maximum girth (G), approximate age, breathing frequency and (N) number of breaths collected while in air or in water. Symbols ‘—’ indicate no data available.

year	animal ID	sex	M_b (kg)	length (cm)	girth (cm)	age (years)	N		f_R (breaths min^{-1})	
							air	water	air	water
2014	FB196	M	248	269	152	16	63	8	2.5	2.7
	FB268	M	227	273	147	21	72	—	2.8	1.5
	FB276	M	291	285	163	22	75	12	3.7	1.0
	FB142	M	256	274	153	22	55	14	2.7	1.8
	FB237	F	192	254	142	32	104	17	2.2	3.6
	FB239	F	117	228	113	4	—	11	2.2	2.4
	FB197	F	169	234	141	11	51	—	1.9	2.1
	FB241	F	157	242	137	7	—	28	1.3	1.1
	FB175	F	170	249	137	23	36	—	4.3	3.8
	FB133	F	167	242	138	15	60	17	2.6	2.2
	FB242	M	276	281	159	24	28	11	0.9	1.1
	FB164	M	291	262	165	25	47	16	0.8	1.9
	FB185	F	172	252	130	14	82 ^a	17 ^a	3.8	4.2
2015	FB251	F	146	240	127	>12	17	—	1.4	—
	FB245	F	110	212	114	3	104	37	3.6	4.9
	FB133	F	165	240	135	16	303	60	4.5	4.5
	FB199	F	142	236	125	13	27	25	4.8	5.6
	FB257	F	73	195	103	2	—	41	—	3.4
	FB193	F	155	250	126	31	—	19	—	3.2
	FB146	M	266	277	153	19	70	42	1.5	4.1
	FB254	M	250	273	156	26	34	10	4.0	1.6
2016	FB209	F	154	236	135	12	102	12	2.6	—
	FB255	F	81	192	108	3	15	22	2.4	1.3
	FB33	F	195	258	142	34	223	13	2.8	—
	FB259	F	100	216	112	3	1	17	—	1.2
	FB292	M	124	216	124	4	84	15	3.2	4.6
	FB294	M	95	202	114	3	46	9	4.7	1.2
	FB223	F	160	251	131	15	8	17	2.3	2.3
	FB178	M	248	272	149	21	63	19	3.7	—
	FB188	M	229	257	150	20	56	16	3.0	—
2017	FB296	M	136	228	124	4	60	61	3.3	6.5
	FB306	M	101	208	115	4	49	53	3.2	5.8

^aGas analyser not working.

examined and sampled before being released on site [2]. All spirometry trials (breath-by-breath lung function and end-expired O_2) were measured from voluntary breaths while the dolphin laid on a shaded, padded mat on the deck of a boat or was gently restrained while partially submerged in water. All work was approved by the IACUC at Texas A&M University Corpus Christi (TAMUCC-IACUC AUP#04-11) and by a research permit issued by the National Marine Fisheries Service (Scientific Research Permit No. 15543).

2.2. Respiratory flows (lung function)

The procedures and equipment used were identical to those used in our previous study on the same species under human care [19], which are briefly summarized below. Respiratory flows were measured using a custom-made Fleisch type pneumotachometer (Mellow Design, Valencia, Spain; or Micah Brodsky, V.M.D. Consulting, Miami, FL, see fig. 1 in [19]), using a low-resistance laminar flow matrix (Item # Z9A887-2, Merriam Process Technologies, Cleveland, OH). A differential pressure transducer (Spirometer Pod, ML 311, ADInstruments, Colorado Springs, CO) was connected to the pneumotachometer with two, 310 cm lengths of 2 mm I.D., firm-walled, flexible tubing. The differential pressure transducer was connected to a data acquisition system (Powerlab 8/35, ADInstruments, Colorado Springs, CO), and the data were captured at 400 Hz and displayed on a laptop computer running LabChart (v. 8.1, ADInstruments, Colorado Springs, CO). The differential pressure was used to determine flow and was calibrated using a 7.0 l calibration syringe (Series 4900, Hans-Rudolph Inc., Shawnee, KS). The signal was integrated and the flow determined as detailed previously [19].

2.3. Respiratory gas composition

The concentration of expired O_2 was subsampled via a port in the pneumotachometer and passed through a 310 cm length of 2 mm I.D., firm-walled, flexible tubing and a 30 cm length of 1.5 mm I.D. Nafion tubing, to a fast-response O_2 and CO_2 analyser (Season 2016–2017: Gemini respiratory monitor, CWE Inc.; Season 2014–2015: ML206, Harvard Apparatus, Holliston, MA, USA) at a flow rate of 200 ml min^{-1} . However, the CO_2 analyser was only working during the experiments in 2016 and we therefore do not report these data. The gas analyser was connected to the data acquisition system and sampled at 400 Hz. The gas analyser was calibrated before and after the experiment using a commercial mixture of 5% O_2 , 5% CO_2 and 90% N_2 (Product No. 17L-340, GASCO, Oldsmar, FL). Ambient air was used to check the calibration before and after each experimental trial. Mean air temperature and humidity during trials were $28.2 \pm 3.3^\circ\text{C}$ ($n = 74$, range $22.6\text{--}35.4^\circ\text{C}$) and $75.3 \pm 17.5\%$ (44–99%). The average (\pm s.e., $n = 19$) surface water temperature around the sampling boat was $26.5 \pm 0.5^\circ\text{C}$ (range: $22.7\text{--}29.7^\circ\text{C}$).

2.4. Metabolic rates

The metabolic rates were estimated as previously detailed [19], and the methods are briefly summarized here. The respiratory gas signals were phase-corrected for O_2 , to match the respirations, and the expiratory flow rate and expired O_2 content were multiplied to calculate the instantaneous volume of oxygen rate ($\dot{V}O_2$). The instantaneous $\dot{V}O_2$ was integrated over each breath to yield the total volume of O_2 exchanged during each breath. The O_2 volumes were summed for each trial period and divided by the duration of the trial to provide an estimate of the oxygen consumption rate for that time period. All dolphins were calm for at least 20 min before sampling commenced. Sampling for respiratory flow and expired gas content continued during most of the duration on the deck and up to 15 min in water. Data were selected to include sections with continuous measurements of at least 3 min. For example, if there were concerns that the flow meter did not seal correctly around the blow-hole, the breath was excluded and the duration started over. Thus, while 3 min may be a shorter duration to collect data than some studies, the total duration of measurement was considerably longer, ranging from 20 to 60 min.

2.5. Data processing and statistical analysis

All gas volumes were converted to standard temperature pressure dry (STPD, [22]). Exhaled air was assumed saturated at 37°C , inhaled air volume was corrected for ambient temperature and relative humidity.

Metabolic data are reported as the average O_2 consumption rate for an entire trial. The relationship between a dependent variable and experimental covariates was analysed using linear-mixed effects models (lme, R: A Language and Environment for Statistical Computing, R Foundation for Statistical Computing, version 3.1.0, 2014). The individual animal was treated as a random effect, which accounted for the correlation between repeated measurements on the same individual [23]. Initially, a univariate analysis was used to determine which variables to consider in a multivariate model. Variables with a $p < 0.2$, using the Wald test and log-likelihood ratio test, were considered in a multivariate model.

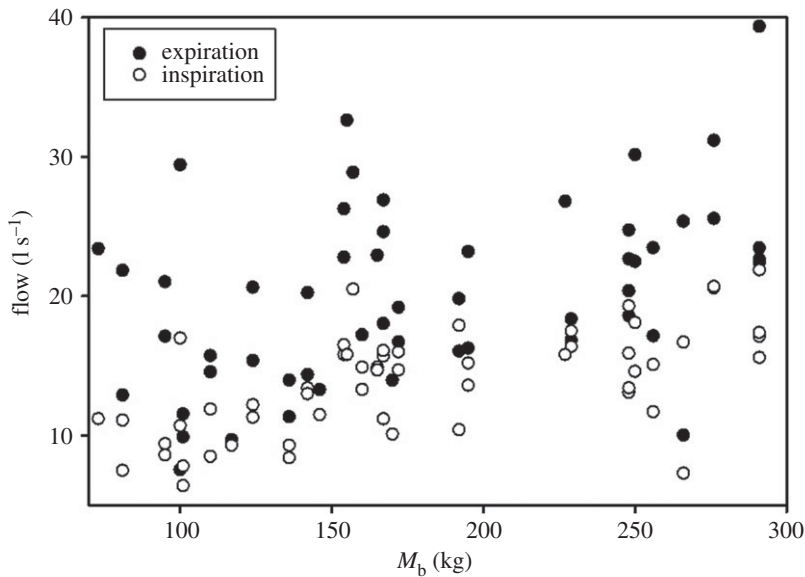


Figure 1. Expiratory or inspiratory respiratory flow (l s^{-1}) versus body mass (M_b , kg) for dolphin either in air or in water.

We used the log-likelihood ratio test and the Akaike information criterion (AIC) to determine which parameters warranted inclusion in a nested model. In this study, p -values $p \leq 0.05$ and $p < 0.01$ were considered significant and highly significant, respectively, and $p \leq 0.1$ was considered a trend. Data are presented as the mean \pm standard deviation (s.d.), unless otherwise stated.

Initially we analysed all age groups together, but we also separated animals into juveniles (less than 10 years) and adults (greater than or equal to 10 years) in order to compare allometric changes for the entire dataset and for each age class separately.

3. Results

Data from 2475 spontaneous breaths were collected over 4 years (2014–2017) from 32 male and female juvenile and adult bottlenose dolphins (table 1), living in and around Sarasota Bay, Florida. Measurements on the deck occurred over 20–60 min, and in water 15–20 min, with continuous measurements for at least 3 min.

3.1. Respiratory flows and timing

The average respiratory frequency (f_R) during trials was 2.7 ± 1.3 breaths min^{-1} (range: 0.8–6.5), and did not differ among years, sex, with body mass (M_b) or position (whether held in or out of the water; all $p > 0.1$, t -value: 0.8).

The average maximum inspiratory flow of spontaneous breaths was $13.8 \pm 3.81 \text{ s}^{-1}$ (range: 6.4–21.9 l s^{-1}), which was significantly lower than the maximum spontaneous expiratory flow of $20.0 \pm 7.51 \text{ s}^{-1}$ (range: 7.5–39.4 l s^{-1} , t -ratio = 10.9, $p < 0.01$, paired- t test). The \log_{10} -transformed inspiratory (Inspflow, $\chi^2 = 20.6$, 1 d.f., $p < 0.01$) and expiratory flow (Expflow, $\chi^2 = 14.2$, 1 d.f., $p < 0.01$) correlated with \log_{10} -transformed M_b ($\log_{10} [M_b]$, figure 1), but neither sex nor position (in-water versus out of water) affected inspiratory or expiratory flow. When separating animals into juveniles and adults, M_b did not correlate with respiratory flow ($\chi^2 = 2.9$, 1 d.f., $p > 0.05$).

There were no differences in inspiratory (5.3 ± 1.81 , range: 2.2–10.4) or expiratory (5.2 ± 1.81 , range: 2.3–10.4) V_T ($p > 0.05$, t -stat: 1.9). For all age groups, only M_b correlated with V_T ($\chi^2 = 22.8$, 1 d.f., $p < 0.01$, figure 2). When separating animals into juveniles (less than 10 years) and adults (greater than or equal to 10 years), $\log_{10} [M_b]$ warranted inclusion for adults ($p < 0.05$, $\chi^2 = 5.2$, 1 d.f.):

$$\log_{10}[V_T] = -0.18 (\pm 0.22) + 0.41 (\pm 0.17) \cdot \log_{10}[M_b], \quad (3.1)$$

The duration of the expiratory phase ($0.437 \pm 0.136 \text{ s}$) was significantly shorter than that of the inspiratory phase ($0.512 \pm 0.112 \text{ s}$), and neither inspiratory nor expiratory durations changed with sex, M_b , or whether animals were in water or out of water ($p > 0.1$ for all).

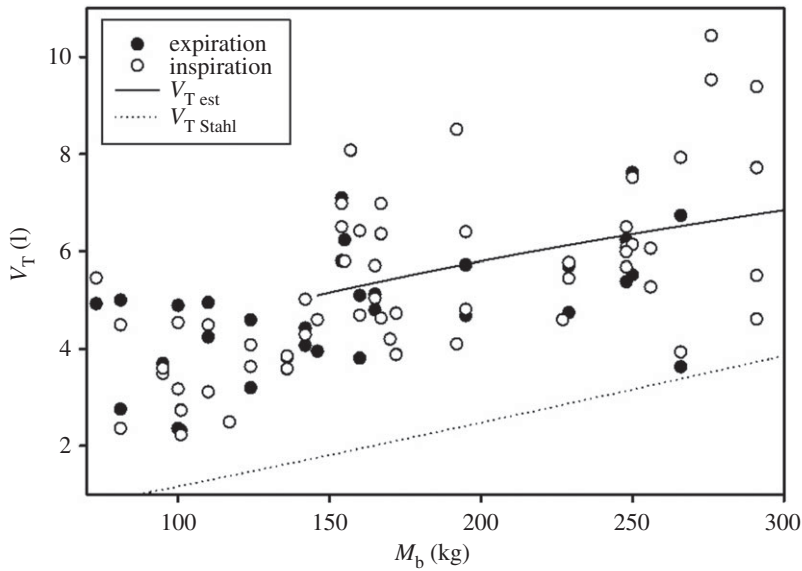


Figure 2. Inspiratory tidal volume (V_T , l) versus body mass (M_b , kg) for dolphins either in air or in water. Solid line is the estimated V_T for adult dolphins (equation (3.1)) and the dotted line the regression equation for terrestrial mammals [24].

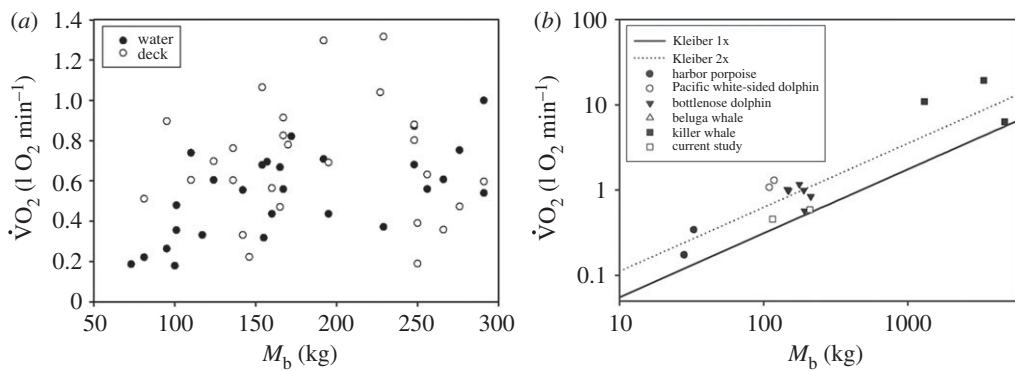


Figure 3. Rate of O_2 consumption ($\dot{V}O_2$, l O_2 min^{-1}) versus body mass (M_b , kg) for (a) dolphins in air and in water from the current study. (b) For a range of cetaceans from the following studies: harbour porpoise [25,26], Pacific white-sided dolphin [27,28], bottlenose dolphin [17–20], beluga whale [29], killer whale [30,31].

3.2. Gas exchange

The average ($n = 56$) end-expiratory O_2 was $10.4 \pm 1.4\%$, and was significantly higher in female dolphins ($10.8 \pm 1.4\%$, $n = 35$) as compared with males ($10.0 \pm 1.4\%$, $n = 21$, $p < 0.05$, Wald test). In addition, there was a trend that females or being positioned in the water had higher end-expired O_2 compared with males or animals positioned out of the water ($\chi^2 = 3.08$, 1 d.f., $p < 0.08$).

3.3. Metabolic rates

Resting $\dot{V}O_2$ (RMR) was estimated for periods during which the dolphin had remained calm for a minimum of 20 min. The sample duration used for calculating the $\dot{V}O_2$ ranged from 3 to 15 min (figure 3a). The estimated RMR varied substantially within and among animals and the mass-specific $\dot{V}O_2$ ($s\dot{V}O_2$) ranged from $0.76 \text{ ml } min^{-1} \text{ kg}^{-1}$ to $9.45 \text{ ml } O_2 \text{ min}^{-1} \text{ kg}^{-1}$ (figure 3a). There was a correlation between \log_{10} -transformed $\dot{V}O_2$ ($\log_{10}[\dot{V}O_2]$), M_b ($\log_{10}[M_b]$) and whether animals were positioned in (position = 1) or out of the water (position = 0, figure 3a, $p < 0.05$, $\chi^2 = 5.7$, 1 d.f.)

$$\log_{10}[\dot{V}O_2] = -1.15 (\pm 0.40) + 0.43 (\pm 0.18) \cdot \log_{10}[M_b] - 0.13 (\pm 0.05) \cdot \text{position}. \quad (3.2)$$

but neither year nor sex were significant.

When separating animals into juveniles and adults, both position and $\log_{10}[M_b]$ warranted inclusion for juveniles ($p < 0.01$, $\chi^2 = 9.0$, 1 d.f.)

$$\log_{10}[\dot{V}O_2] = -2.75 (\pm 0.71) + 1.25 (\pm 0.35) \cdot \log_{10}[M_b] - 0.23 (\pm 0.08) \cdot \text{position}. \quad (3.3)$$

but neither position or M_b warranted inclusion for adults ($p > 0.1$, $\chi^2 = 2.1$, 1 d.f.).

4. Discussion

We estimated RMR and measured lung function parameters across 2 age classes of bottlenose dolphins (juvenile and adult) over a 4-year period. When both age groups were analysed together, RMR and V_T rate scaled with M_b . When the groups were separated into juveniles and adults, RMR only correlated with M_b for juveniles. V_T scaled with M_b for adults with an allometric mass-exponent of 0.41 ± 0.17 . These results are similar to those measured in bottlenose dolphins under human care, suggesting that data from the latter group provide physiological information of relevance for wild dolphins.

The measured V_T collected in this study agree well with previous measurements in cetaceans [16]. The average mass-specific V_T (sV_T) recorded in the current study was 30.9 ml kg^{-1} (range: $13.6\text{--}67.5 \text{ ml kg}^{-1}$), which is similar to previous data for the bottlenose dolphin ($27.5\text{--}58.8 \text{ ml kg}^{-1}$, [9,19,32]), the harbour porpoise ($39.3\text{--}52.6 \text{ ml kg}^{-1}$ in [25]) and grey whale ($12.0\text{--}35.3 \text{ ml kg}^{-1}$, [33]). Results from these past studies are from a range of experiments with animals positioned on land or in water, and from voluntarily participating animals in human care to restrained free-ranging animals. For all data, both the respiratory flow and V_T scaled with M_b , but when separated into juveniles and adults, only V_T from the latter group correlated with size. Unlike terrestrial mammals, where the mass-exponent is close to 1 (table 1 in [24]), V_T scaled allometrically with a mass-exponent of 0.41 (equation (3.1)). Consequently, the sV_T decreased with M_b and for a 150 kg and a 300 kg dolphin was 34 ml kg^{-1} and 23 ml kg^{-1} , respectively. Similarly, the V_T as a percentage of the estimated total lung capacity (TLC_{est}) decreased from 38% for a 150 kg dolphin, to 27% for a 300 kg animal [16,34]. The V_T in the dolphin was greater when compared with terrestrial mammals of comparable size, but the relative difference decreased with size.

The breathing strategy of adult terrestrial mammals involves a high respiration frequency f_R , low V_T , and a brief respiratory pause following expiration. Aquatic mammals, on the other hand, have a lower f_R , a greater V_T and a respiratory pause that often lasts for several seconds to minutes following inhalation [16,35]. It has been hypothesized that the aquatic breathing strategy may enhance buoyancy management [35], or help keep the arterial partial pressure of CO_2 (P_{ACO_2}) at levels similar to that of land mammals [36]. Minute ventilation, the product of V_T and f_R , is regulated to deliver O_2 to and remove CO_2 from the alveoli. In terrestrial mammals, V_T (1.04) scales isometrically while f_R (-0.26) is inversely related to M_b , resulting in a mass-exponent for minute volume that scales with the metabolic rate [24]. Thus, the allometric changes in minute ventilation in terrestrial mammals are governed by an f_R that decreases with M_b . The data reported in the current study suggest that the decrease in mass-specific minute ventilation with size is governed by a reduction in V_T , while f_R seems to remain constant. This agrees with other data reported for cetaceans, where the mass-exponent for f_R was not different from 0 (see data on cetaceans in table 1 in [35]). Thus, as mass-specific RMR decreases with size, the reduction in minute ventilation is caused by a reduction in mass-specific V_T . Variation in V_T is more efficient to alter alveolar ventilation as it reduces the dead space ventilation. Consequently, the allometric changes in cetaceans may be an evolutionary consequence that helps reduce the work of breathing and enhances gas exchange in the aquatic environment.

The basal metabolic rate (BMR) of an animal is an estimate of the energetic cost of the basic functions required to sustain life, and it is measured under highly specific and controlled conditions, e.g. adult animals in a thermoneutral environment. In field situations, it is logistically challenging to meet these conditions, and RMR often serves as a proxy for BMR. Relatively few measurements of RMR have been made in marine mammals, and most of these have been made on animals in managed care or with animals held under quasi-natural conditions [17–21,25,37–43]. Many of the older studies concluded that the mass-specific RMR is considerably higher in aquatic species than in their terrestrial counterparts. Possible explanations for the higher RMR in dolphins include increased energetic demands due to thermoregulation or a high protein diet [44–47]. More recent studies, where the sample techniques have been refined for marine mammals, have measured RMRs that are at or near basal levels [18,21,27,29,30]. Thus, there is currently considerable controversy whether estimated BMR/RMR in marine mammals is truly elevated as compared to terrestrial species, and criticisms include limitations with experimental design (e.g. duration of measurements) and analysis of collected

data [48–50]. Studies have shown that variation in RMR is affected by body condition, the thermal environment, desensitization through training and psychological state [18,19,29,30,51,52]. These past studies indicate that experimental design and conditions may significantly alter the results and could explain the large variability in RMR reported across published studies. In past studies, the mass-specific RMR for bottlenose dolphins in water ranged from 3.0 to 7.0 ml O₂ min⁻¹ kg⁻¹ (M_b range 125–250 kg, [10,17–20,53]), and were in the same range as the values reported in the current study (figure 3*b*).

In the current study, the data from all age groups indicate that RMR scaled allometrically with a mass-exponent that was significantly lower than those reported for terrestrial mammals (equation (3.2), [54,55]). In land mammals, BMR in adults is well known to scale allometrically with body size over several orders of magnitude, but may vary greatly within species or a narrow weight range. For example, the mass-specific value may vary 2- to 3-fold, and the variation explained by M_b is often low [56,57]. When the data were separated into juveniles and adults, RMR only correlated with M_b for the juveniles (figure 3*a*). The mass-exponent for juveniles was greater than the adult value in terrestrial mammals (equation (3.3)).

There are a number of possible reasons for our findings. First, the measurements in the current study do not fulfil the criteria for BMR; we studied juvenile and adult animals and had no control over whether the animals were pre- or post-prandial. This may increase the variation, but we should also expect the mass-specific RMR to be higher as younger animals tend to have greater energetic requirements as they allocate considerable energy for growth. Second, breath-by-breath respirometry was used in the current study, which is experimentally challenging [19]. These limitations have been discussed previously, and it is unlikely that limitations in the experimental design would have significantly affected the results. Factors such as stress and personality are known to alter metabolic rate, and while stress often causes hypermetabolism, some species respond with a reduction in metabolic rate [56,58,59]. This may explain the low RMR in the current study, and wild dolphins may respond by reducing metabolic rate in response to any stress associated with capture. Fourth, in the current study the dolphins had been restrained for at least 20 min before measurements began, followed by repeated measurements ranging from 3 to 15 min. In some previous studies, the resting period ranged from 40 s to 4.5 min [17,19,20]. As the duration of measurement of RMR is important [21,27,60], it is possible that these extended measurement periods helped calm the animals and reduce the RMR. In fact, studies where the dolphins were resting for 10–20 min in the respirometer have reported values that are similar to those predicted by Kleiber [18,21,29,52]. Consequently, appropriately designed metabolic studies on marine mammals managed under human care provide RMR values that are similar to those in wild populations.

Studies indicate that RMR is about 30–40% of daily energy requirement [61,62], and understanding a species' metabolic cost is important for assessing energy flow within populations and ecosystems. For marine mammals, estimated or measured RMR values have been used in bioenergetics models [63–67]. Results vary greatly among models with varying assumptions, and the largest variation is generally caused by uncertainty in energy requirements and diet [64]. The ingestion rates for animals under human care have been used to provide estimates of energy requirements, but these studies are likely to overestimate metabolic rates (see references in [50]). A number of studies have measured RMR in dolphins under human care [17–21,68], but the current study is the first to provide estimates for wild animals. Our results indicate that RMR overlaps (RMR in current study for all animals: 3.7 ± 1.8 ml O₂ min⁻¹ kg⁻¹, range: 0.8–9.4 ml O₂ min⁻¹ kg⁻¹) with those in previous studies for animals under human care (3.0–7.4 ml O₂ min⁻¹ kg⁻¹) [17–19,21]. These results are similar to the values estimated by Kleiber's equation and provide a measured value that can be used in bioenergetics models for dolphins [69]. As RMR did not vary with M_b (range: 141–291 kg) in adult dolphins, the average $\dot{V}O_2$ (592 ml O₂ min⁻¹) can be used for an estimate of the RMR for this M_b range. For a 150 kg dolphin under the seasonal water temperature conditions of our study (spring in Sarasota Bay, FL), the RMR would be 3.9 ml O₂ min⁻¹ kg⁻¹. Assuming a multiplier of 3–6 for the daily metabolic rate of active animals [63,70], the metabolic rate would range from 11.7 to 23.4 ml O₂ min⁻¹ kg⁻¹ (126–252 MJ kg⁻¹ yr⁻¹). This value is similar to the field metabolic rates estimated from a bioenergetics model in dolphins [63].

5. Conclusion

Numerous studies have tried to identify the energetic requirements of marine mammals, and recently interest as to how anthropogenic disturbances may alter these requirements has increased. For models that attempt to predict how disturbances may alter population levels, an understanding of the

eco-physiology of a study species is crucial. These data are important for helping ecologists understand the flow of energy between different trophic levels. However, there is limited understanding about the physiology of marine mammals and how physiological constraints limit survival. The data presented in this paper provide estimates on the energy requirements and respiratory physiology in two age classes (3–34 years of age) of briefly restrained wild bottlenose dolphins that had been free-ranging just prior to measurement. These data will help improve estimates from bioenergetics models and contribute to our understanding of how a changing environment may alter survival in this species.

Ethics. All work was approved by the IACUC at Texas A&M University Corpus Christi (TAMUCC-IACUC AUP#04-11) and by a research permit issued by the National Marine Fisheries Service (Scientific Research Permit No. 15543).

Data accessibility. The model and datasets used in this study are freely available at the following link: <https://osf.io/6wjh8/> [71].

Authors' contributions. A.F. conceived of the study, designed the experiments, collected and analysed the data, carried out the statistical analysis and drafted the paper. R.W. coordinated the dolphin health assessments, arranged for funding, and holds the National Marine Fisheries Service Scientific Research Permit (No. 15543) and Mote Marine Laboratory IACUC approvals for the work. M.B. designed and built components of the testing equipment. M.M., J.C.S., R.W. and M.B. helped develop the testing methodology. K.M., J.A. and A.B. assisted in coordinating sampling, handling, and collection and verification of data from health assessments. J.C.S. and D.F. provided veterinary care for dolphins sampled in the field, and helped coordinate sampling in the field. All authors helped revise the paper and gave final approval for publication.

Competing interests. We have no competing interests.

Funding. Funding for this project was provided by the Office of Naval Research (ONR YIP Award # N000141410563, Dolphin Quest, Inc., and Woods Hole Oceanographic Institution).

Acknowledgements. A special thanks to all the more than 140 staff, students, volunteers and collaborating researchers of the Sarasota Dolphin Research Program for their support of more than 40 research projects during each health assessment. Thanks to Alexandra Epple, Teresa Lorenzo, Julie van der Hoop and Austin Allen for help in collecting the data.

References

- Brierley AS, Kingsford MJ. 2009 Impacts of climate change on marine organisms and ecosystems. *Curr. Biol.* **19**, R602–R614. (doi:10.1016/j.cub.2009.05.046)
- Wells RS *et al.* 2004 Bottlenose dolphins as marine ecosystem sentinels: developing a health monitoring system. *EcoHealth* **1**, 246–254. (doi:10.1007/s10393-004-0094-6)
- Rodriguez de la Gala-Hernandez S, Heckel G, Sumich JL. 2008 Comparative swimming effort of migrating gray whales (*Eschrichtius robustus*) and calf cost of transport along Costa Azul, Baja California, Mexico. *Can. J. Zool.* **86**, 307–313. (doi:10.1139/Z07-141)
- Armstrong AJ, Siegfried WR. 1991 Consumption of Antarctic krill by minke whales. *Antarc. Sci.* **3**, 13–18. (doi:10.1017/S09594102091000044)
- Blix AS, Folkow LP. 1995 Daily energy expenditure in free living minke whales. *Acta Physiol. Scand.* **153**, 61–66. (doi:10.1111/j.1748-1716.1995.tb09834.x)
- Christiansen F, Rasmussen MH, Lusseau D. 2014 Inferring energy expenditure from respiration rates in minke whales to measure the effects of whale watching boat interactions. *J. Exp. Mar. Biol. Ecol.* **459**, 96–104. (doi:10.1016/j.jembe.2014.05.014)
- Folkow LP, Blix AS. 1992 Metabolic rates of minke whales (*Balaenoptera acutorostrata*) in cold water. *Acta Physiol. Scand.* **146**, 141–150. (doi:10.1111/j.1748-1716.1992.tb09402.x)
- Sumich JL. 2001 Direct and indirect measures of oxygen extraction, tidal lung volumes and respiratory rates in a rehabilitating gray whale calf. *Aquat. Mam.* **27**, 279–283.
- Ridgway SH, Scronce BL, Kanwisher JW. 1969 Respiration and deep diving in the bottlenose porpoise. *Sci.* **166**, 1651–1654. (doi:10.1126/science.166.3913.1651)
- Fahlman A, van der Hoop J, Moore MJ, Levine G, Rocho-Levine J, Brodsky M. 2016 Estimating energetics in cetaceans from respiratory frequency: why we need to understand physiology. *Biol. Open* **5**, 436–442. (doi:10.1242/bio.017251)
- Fahlman A, van der Hoop J, Moore MJ, Levine G, Rocho-Levine J, Brodsky M. 2017 Response to 'On the importance of understanding physiology when estimating energetics in cetaceans'. *Biol. Open* **6**, 307–308. (doi:10.1242/bio.023143)
- Folkow LP, Blix AS. 2017 On the importance of understanding physiology when estimating energetics in cetaceans. *Biol. Open* **6**, 306. (doi:10.1242/bio.023929)
- Venn-Watson S *et al.* 2015 Adrenal gland and lung lesions in Gulf of Mexico common bottlenose dolphins (*Tursiops truncatus*) found dead following the Deepwater Horizon oil spill. *PLoS ONE* **10**, e0126538. (doi:10.1371/journal.pone.0126538)
- Smith CR *et al.* 2017 Slow recovery of Barataria Bay dolphin health following the Deepwater Horizon oil spill (2013–2014), with evidence of persistent lung disease and impaired stress response. *End. Spec. Res.* **33**, 127–142. (doi:10.3354/esr00778)
- Smith CR *et al.* 2012 Pulmonary ultrasound findings in a bottlenose dolphin *Tursiops truncatus* population. *Dis. Aquat. Organ.* **101**, 243–255. (doi:10.3354/dao02537)
- Fahlman A, Moore MJ, Garcia-Parraga D. 2017 Respiratory function and mechanics in pinnipeds and cetaceans. *J. Exp. Biol.* **220**, 1761–1763. (doi:10.1242/jeb.126870)
- Yazdi P, Kilian A, Culik BM. 1999 Energy expenditure of swimming bottlenose dolphins (*Tursiops truncatus*). *Mar. Biol.* **134**, 601–607. (doi:10.1007/s002270050575)
- Yeates LC, Houser DS. 2008 Thermal tolerance in bottlenose dolphins (*Tursiops truncatus*). *J. Exp. Biol.* **211**, 3249–3257. (doi:10.1242/jeb.020610)
- Fahlman A, Loring SH, 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)
- Williams TM, Friedl WA, Haun JE. 1993 The physiology of bottlenose dolphins (*Tursiops truncatus*): heart rate, metabolic rate and plasma lactate concentration during exercise. *J. Exp. Biol.* **179**, 31–46.
- Noren DP, Holt MM, Dunkin RC, Williams TM. 2013 The metabolic cost of communicative sound production in bottlenose dolphins (*Tursiops truncatus*). *J. Exp. Biol.* **216**, 1624–1629. (doi:10.1242/jeb.083212)
- Quanjer PH, Tammeling GJ, Cotes JE, Pedersen OF, Peslin R, Yernault J-C. 1993 Lung volumes and forced ventilatory flows. *Eur. Respir. J.* **6**, 5–40. (doi:10.1183/09041950.00051693)
- Littell RC, Henry PR, Ammerman CB. 1998 Statistical analysis of repeated measures data using SAS procedures. *J. Anim. Sci.* **76**, 1216–1231. (doi:10.2527/1998.7641216x).
- Stahl WR. 1967 Scaling of respiratory variables in mammals. *J. Appl. Physiol.* **22**, 453–460.
- Reed JZ, Chambers C, Hunter CJ, Lockyer C, Kastelein R, Fedak MA, Boutilier RG. 2000 Gas exchange and heart rate in the harbour porpoise, *Phocoena phocoena*. *J. Comp. Physiol. B.* **170**, 1–10. (doi:10.1007/s003600050001)

26. Karandeeva OG, Matisheva SK, Spapunov VM. 1973 Features of external respiration in the delphinidae. In *Morphology and ecology of marine mammals: seals, dolphins and porpoises* (eds KK Chapskii, VE Soklov), pp. 196–206. New York, NY: John Wiley & Sons.
27. Rechsteiner EU, Rosen DAS, Trites AW. 2013 Energy requirements of Pacific white-sided dolphins (*Lagenorhynchus obliquidens*) as predicted by a bioenergetic model. *J. Mam.* **94**, 820–832. (doi:10.1644/12-MAMM-A-206.1)
28. Ohizumi H, Terasawa F, Kitamura M, Oshita I, Iwasawa L, Kawaguchi K. 2009 Respiratory gas exchange and metabolic rate of captive Pacific whitesided dolphins (*Lagenorhynchus obliquidens*) under stationary condition. In *Proc. 18th Biennial Conf. on the Biology of Marine Mammals, Quebec City, Canada*.
29. Rosen DAS, Trites AW. 2013 Resting metabolic rate of a mature male beluga whale (*Delphinapterus leucas*). *Aquat. Mam.* **39**, 85–88. (doi:10.1578/AM.39.1.2013.85)
30. Worthy GAJ, Worthy TAM, Yochem PK, Dold C. 2013 Basal metabolism of an adult male killer whale (*Orcinus orca*). *Mar. Mam. Sci.* **30**, 1229–1237. (doi:10.1111/mms.12091)
31. Kriete B. 1995 *Bioenergetics in the killer whale, Orcinus orca*. Vancouver, Canada: The University of British Columbia.
32. Irving L, Scholander PF, Grinnell SW. 1941 The respiration of the porpoise, *Tursiops truncatus*. *J. Cell. Comp. Physiol.* **17**, 145–168. (doi:10.1002/jcp.1030170203)
33. Wahrenbrock EA, Maruscha GF, Elsner R, Kenney DW. 1974 Respiration and metabolism in 2 baleen whale calves. *Mar. Fish. Rev.* **36**, 3–9.
34. Kooyman GL. 1973 Respiratory adaptations in marine mammals. *Am. Zool.* **13**, 457–468. (doi:10.1093/icb/13.2.457)
35. Mortola JP, Limoges M-J. 2006 Resting breathing frequency in aquatic mammals: a comparative analysis with terrestrial species. *Respir. Physiol. Neurobiol.* **154**, 500–514. (doi:10.1016/j.resp.2005.12.005)
36. Mortola JP, Sequin J. 2009 End-tidal CO₂ in some aquatic mammals of large size. *Zool.* **112**, 77–85. (doi:10.1016/j.zool.2008.06.001)
37. Williams TM, Fuiman LA, Horning M, Davis RW. 2004 The cost of foraging by a marine predator, the Weddell seal *Leptonychotes weddellii*: pricing by the stroke. *J. Exp. Biol.* **207**, 973–982. (doi:10.1242/jeb.00822)
38. Kooyman GL, Kerem DH, Campbell WB, Wright JJ. 1973 Pulmonary gas exchange in freely diving Weddell seals (*Leptonychotes weddellii*). *Respir. Physiol.* **17**, 283–290. (doi:10.1016/0034-5687(73)90003-0)
39. Fahlman A, Svård C, Rosen DAS, Jones DR, Trites AW. 2008 Metabolic costs of foraging and the management of O₂ and CO₂ stores in Steller sea lions. *J. Exp. Biol.* **211**, 3573–3580. (doi:10.1242/jeb.023655)
40. Reed JZ, Chambers C, Fedak MA, Butler PJ. 1994 Gas exchange of captive freely diving grey seals (*Halichoerus grypus*). *J. Exp. Biol.* **191**, 1–18.
41. Gallivan GJ. 1981 Ventilation and gas exchange in unrestrained harp seals (*Phoca groenlandica*). *Comp. J. Biochem. Physiol. A* **69**, 809–813. (doi:10.1016/0300-9629(81)90175-4)
42. Gallivan GJ, Best RC. 1980 Metabolism and respiration of the Amazonian manatee (*Trichechus inunguis*). *Physiol. Zool.* **53**, 245–253. (doi:10.1086/physzool.53.3.30155787)
43. Kasting NW, Adderley SAL, Safford T, Hewlett KG. 1989 Thermoregulation in beluga (*Delphinapterus leucas*) and killer (*Orcinus orca*) whales. *Physiol. Zool.* **62**, 687–701. (doi:10.1086/physzool.62.3.30157921)
44. Irving L. 1969 Temperature regulation in marine mammals. In *The biology of marine mammals* (ed. HT Andersen), pp. 147–174. New York, NY: Academic Press.
45. Kanwisher J, Sundness G. 1965 Physiology of a small cetacean. *Hval. Skrift.* **48**, 45–53.
46. Kanwisher JW, Ridgway SH. 1983 The physiological ecology of whales and porpoises. *Sci. Am.* **248**, 110–120. (doi:10.1038/scientificamerican.0683-110)
47. Ridgway SH. 1972 Homeostasis in the aquatic environment. In *Mammals of the sea: biology and medicine* (ed. SH Ridgway), pp. 590–748. Springfield, IL: Charles C Thomas Publisher.
48. Innes S, Lavigne D. 1991 Do cetaceans really have elevated metabolic rates? *Physiol. Zool.* **64**, 1130–1134. (doi:10.1086/physzool.64.4.30157960)
49. Kasting N. 1991 Reply to technical comment, do cetaceans really have elevated metabolic rates? *Physiol. Zool.* **64**, 1135–1136. (doi:10.1086/physzool.64.4.30157961)
50. Lavigne DM, Innes S, Worthy GAJ, Kovacs KM, Schmitz OJ, Hickie JP. 1986 Metabolic rates of seals and whales. *Can. J. Zool.* **64**, 279–284. (doi:10.1139/z86-047)
51. Rosen DA, Winship AJ, Hoopes LA. 2007 Thermal and digestive constraints to foraging behaviour in marine mammals. *Phil. Trans. R. Soc. B* **362**, 2151–2168. (doi:10.1098/rstb.2007.2108)
52. Rechsteiner EU, Rosen DAS, Trites AW. 2013 Seasonal resting metabolic rate and food intake of captive Pacific white-sided dolphins (*Lagenorhynchus obliquidens*). *Aquat. Mam.* **39**, 241–252. (doi:10.1578/AM.39.3.2013.241)
53. Ridgway SH, Patton GS. 1971 Dolphin thyroid: some anatomical and physiological findings. *Zeitschrift für vergleichende Physiologie* **71**, 129–141. (doi:10.1007/BF00297974)
54. Darveau C-A, Suarez RK, Andrews RD, Hochachka PW. 2002 Allometric cascade as a unifying principle of body mass effects on metabolism. *Nature* **417**, 166–170. (doi:10.1038/417166a)
55. Kleiber M. 1987 *The fire of life: an introduction to animal energetics*, p. 453, 5th edn. Huntington, NY: R. E. Krieger.
56. Careau V, Thomas D, Humphries MM, Réale D. 2008 Energy metabolism and animal personality. *Oikos* **117**, 641–653. (doi:10.1111/j.0030-1299.2008.16513.x)
57. Speakman JR, Król E, Johnson MS. 2004 The functional significance of individual variation in basal metabolic rate. *Physiol. Biochem. Zool.* **77**, 900–915. (doi:10.1086/427059)
58. Gorr TA. 2017 Hypometabolism as the ultimate defence in stress response: how the comparative approach helps understanding of medically relevant questions. *Acta Physiologica* **219**, 409–440. (doi:10.1111/apha.12747)
59. Rabasa C, Dickson SL. 2016 Impact of stress on metabolism and energy balance. *Curr. Opin. Behav. Sci.* **9**, 71–77. (doi:10.1016/j.cobeha.2016.01.011)
60. Hayes JP, Speakman JR, Racey PA. 1992 Sampling bias in respirometry. *Physiol. Zool.* **65**, 604–619. (doi:10.1086/physzool.65.3.30157972)
61. Ricklefs RE, Konarzewski M, Daan S. 1996 The relationship between basal metabolic rate and daily energy expenditure in birds and mammals. *Am. Nat.* **147**, 1047–1071. (doi:10.1086/285892)
62. Speakman JR, Ergon T, Cavanagh R, Reid K, Scantlebury DM, Lambin X. 2003 Resting and daily energy expenditures of free-living field voles are positively correlated but reflect extrinsic rather than intrinsic effects. *Proc. Natl Acad. Sci. USA* **100**, 14 057–14 062. (doi:10.1073/pnas.2235671100)
63. Bejarano AC, Wells RS, Costa DP. 2017 Development of a bioenergetic model for estimating energy requirements and prey biomass consumption of the bottlenose dolphin *Tursiops truncatus*. *Ecol. Modell.* **356**, 162–172. (doi:10.1016/j.ecolmodel.2017.05.001)
64. Winship AJ, Trites AW, Rosen DAS. 2002 A bioenergetic model for estimating the food requirements of Steller sea lions *Eumetopias jubatus* in Alaska, USA. *Mar. Ecol. Prog. Ser.* **229**, 291–312. (doi:10.3354/meps229291)
65. Noren SR, Udevitz MS, Jay CV. 2012 Bioenergetics model for estimating food requirements of female Pacific walrus *Odobenus rosmarus divergens*. *Mar. Ecol. Prog. Ser.* **460**, 261–275. (doi:10.3354/meps09706)
66. Noren DP. 2011 Estimated field metabolic rates and prey requirements of resident killer whales. *Mar. Mam. Sci.* **27**, 60–77. (doi:10.1111/j.1748-7692.2010.00386.x)
67. McHuron EA, Costa DP, Schwarz L, Mangel M. 2017 State-dependent behavioural theory for assessing the fitness consequences of anthropogenic disturbance on capital and income breeders. *Method. Ecol. Evol.* **8**, 552–560. (doi:10.1111/2041-210X.12701)
68. Noren DP, Holt MM, Dunkin RC, Williams TM. 2017 Echolocation is cheap for some mammals: dolphins conserve oxygen while producing high-intensity clicks. *J. Exp. Mar. Biol. Ecol.* **495**, 103–109. (doi:10.1016/j.jembe.2017.07.002)
69. Croll DA, Kudela R, Tereshy BR. 2006 Ecosystem impact of the decline of large whales in the North Pacific. In *Whales, whaling, and ocean ecosystems* (eds JA Estes, DP DeMaster, DF Doak, TM Williams, RL Brownell), pp. 200–212. Los Angeles, CA: University of California Press.
70. Costa D. 2009 Energetics. In *Encyclopedia of marine mammals* (eds WF Perrin, B Würsig, JGM Thewissen), pp. 383–391, 2nd edn. Amsterdam, The Netherlands: Academic Press.
71. Fahlman A, Brodsky M, Wells R, McHugh K, Allen J, Barleycorn A, Sweeney JC, Fauquier D, Moore M. 2017 Data deposited: Field energetics and lung function in wild bottlenose dolphins, *Tursiops truncatus*, in Sarasota Bay Florida. OSF Repository. (<https://osf.io/6wjh8/>)