

## RESEARCH ARTICLE

# The significance of respiration timing in the energetics estimates of free-ranging killer whales (*Orcinus orca*)

Marjoleine M. H. Roos\*, Gi-Mick Wu and Patrick J. O. Miller

## ABSTRACT

Respiration rate has been used as an indicator of metabolic rate and associated cost of transport (COT) of free-ranging cetaceans, discounting potential respiration-by-respiration variation in  $O_2$  uptake. To investigate the influence of respiration timing on  $O_2$  uptake, we developed a dynamic model of  $O_2$  exchange and storage. Individual respiration events were revealed from kinematic data from 10 adult Norwegian herring-feeding killer whales (*Orcinus orca*) recorded with high-resolution tags (DTAGs). We compared fixed  $O_2$  uptake per respiration models with  $O_2$  uptake per respiration estimated through a simple 'broken-stick'  $O_2$ -uptake function, in which  $O_2$  uptake was assumed to be the maximum possible  $O_2$  uptake when stores are depleted or maximum total body  $O_2$  store minus existing  $O_2$  store when stores are close to saturated. In contrast to findings assuming fixed  $O_2$  uptake per respiration, uptake from the broken-stick model yielded a high correlation ( $r^2 > 0.9$ ) between  $O_2$  uptake and activity level. Moreover, we found that respiration intervals increased and became less variable at higher swimming speeds, possibly to increase  $O_2$  uptake efficiency per respiration. As found in previous studies, COT decreased monotonically versus speed using the fixed  $O_2$  uptake per respiration models. However, the broken-stick uptake model yielded a curvilinear COT curve with a clear minimum at typical swimming speeds of 1.7–2.4 m s<sup>-1</sup>. Our results showed that respiration-by-respiration variation in  $O_2$  uptake is expected to be significant. And though  $O_2$  consumption measurements of COT for free-ranging cetaceans remain impractical, accounting for the influence of respiration timing on  $O_2$  uptake will lead to more consistent predictions of field metabolic rates than using respiration rate alone.

**KEY WORDS:** Metabolic rate, Oxygen uptake, Respiration timing, Cetacean, Respiration rate, *In situ*

## INTRODUCTION

Cetacean populations can have a significant influence on marine ecosystems (Jefferson et al., 1991; Estes et al., 1998; Plagányi and Butterworth, 2009), and estimates of their energetic requirements at sea are essential to assess their prey intake and role within food webs. Cetacean metabolic rates have also been studied to give insight on reproduction costs (New et al., 2013), migration costs (Rodríguez de la Gala-Hernández et al., 2008), activity costs (Goldbogen et al., 2011) and potential impacts of disturbance (Christiansen et al., 2014; Villegas-Amtmann et al., 2015).

However, direct measurement of field metabolic rate (FMR) of free-ranging cetaceans remains challenging, if not unfeasible for most species.

Early respirometry studies on captive cetaceans and pinnipeds showed that respiration rate increased with increasing  $O_2$  consumption rate ( $\dot{V}_{O_2}$ ) (Scholander and Irving, 1941; Hampton et al., 1971; Hampton and Whittow, 1976). Respiration rate was therefore considered as a reliable metric for metabolic rate (MR; Yazdi et al., 1999; Sumich, 2001) and has been used widely (Irving et al., 1941; Scholander and Irving, 1941; Spencer et al., 1967; Olsen et al., 1969), including in key studies on free-ranging cetaceans (Sumich, 1983; Dolphin, 1987; Blix and Folkow, 1995; Rodríguez de la Gala-Hernández et al., 2008; Williams and Noren, 2009; Christiansen et al., 2014, Villegas-Amtmann et al., 2015).

For animals with discrete respirations, MR (or  $\dot{V}_{O_2}$ ) can be calculated as:

$$\dot{V}_{O_2} = \Sigma T_{O_2} = \Sigma V_T E_{O_2}, \quad (1)$$

where  $T_{O_2}$  is the amount of  $O_2$  exchanged per respiration (l), and is the product of tidal lung volume ( $V_T$ , l respiration<sup>-1</sup>) and the percentage of  $O_2$  extracted from inhaled air ( $E_{O_2}$ ) (Wartzok, 2002).  $\dot{V}_{O_2}$  can therefore be estimated by summing  $T_{O_2}$  across multiple respirations over a specified time period.

In studies that derive FMR from respiration rate, the observed respiration rate is typically multiplied by single average  $V_T$  and fixed  $E_{O_2}$  values, estimated from captive animals. These FMR estimates are then linked to behaviours such as feeding, travelling and resting (Dolphin, 1987; Blix and Folkow, 1995), or speed (Sumich, 1983; Blix and Folkow, 1995; Rodríguez de la Gala-Hernández et al., 2008; Williams and Noren, 2009; Christiansen et al., 2014). Correlations between respiration rate and activity level like travel speed tend to be very weak (Williams and Noren, 2009; Christiansen et al., 2014). A crucial assumption made in those studies is that every respiration has a constant level of  $V_T$  and  $E_{O_2}$  (Sumich, 1983; Dolphin, 1987; Blix and Folkow, 1995; Rodríguez de la Gala-Hernández et al., 2008; Williams and Noren, 2009; Christiansen et al., 2014). However, studies on cetaceans have shown that  $E_{O_2}$  can vary greatly between respirations (Olsen et al., 1969; Ridgway et al., 1969; Wahrenbrock et al., 1974; Sumich, 1994, 2001; Kriete, 1995; Fahlman et al., 2015, 2016). Also,  $V_T$  within an individual is not necessarily constant and has been shown to fluctuate (Spencer et al., 1967; Olsen et al., 1969; Wahrenbrock et al., 1974; Gallivan et al., 1986; Kriete, 1995; Fahlman et al., 2015, 2016). This oversight concerning variability in  $E_{O_2}$  and  $V_T$  decreases the potential accuracy of respiration rate as a metric for cetacean MR.

Over sufficiently long time scales,  $O_2$  consumption by body metabolism must be balanced by  $O_2$  uptake. However, on shorter time scales, significant deviations between  $\dot{V}_{O_2}$  and FMR are expected to occur as breath-hold divers deplete stored  $O_2$  to different amounts between respirations (Goldbogen et al., 2012). Net gain of

Sea Mammal Research Unit, Scottish Oceans Institute, University of St Andrews, St Andrews, Fife KY16 8LB, UK.

\*Author for correspondence (marjoleineroos@gmail.com)

 M.M.H.R., 0000-0002-0854-8770

**List of symbols and abbreviations**

BMR	basal metabolic rate ( $\text{l O}_2 \text{ s}^{-1}$ )
$C_d$	drag coefficient
COT	cost of transport ( $\text{J kg}^{-1} \text{ m}^{-1}$ )
$E_{\text{O}_2}$	oxygen extraction (%)
FMR	field metabolic rate ( $\text{l O}_2 \text{ s}^{-1}$ )
$k$	slope of $\text{MR}_L$ versus $U^3$ ( $\text{l O}_2 \text{ m}^{-3} \text{ s}^2$ )
MR	metabolic rate ( $\text{l O}_2 \text{ s}^{-1}$ )
$\text{MR}_L$	locomotion component of metabolic rate ( $\text{l O}_2 \text{ s}^{-1}$ )
$P_{\text{O}_2}$	oxygen partial pressure
$P_T$	mean thrust power (W)
$Re$	Reynolds number
SPL	sound pressure level (dB re. $1 \mu\text{Pa}$ )
TBO	total body oxygen store (l)
$T_{\text{O}_2}$	oxygen uptake per respiration (l)
$U$	speed through the water ( $\text{m s}^{-1}$ )
$\dot{V}_{\text{O}_2}$	oxygen consumption rate ( $\text{l O}_2 \text{ s}^{-1}$ )
$V_T$	tidal lung volume (l)

$\text{O}_2$  decreases as the number of consecutive respirations increases, as  $\text{O}_2$  stores [blood (haemoglobin), muscle (myoglobin) and respiratory tract] are replaced, and the partial pressure of  $\text{O}_2$  ( $P_{\text{O}_2}$ ) in the blood approaches that of inhaled air (Boutillier et al., 2001; Fahlman et al., 2008, 2016). Apnoea duration influences  $P_{\text{O}_2}$  in the body and therefore the expected efficiency of  $E_{\text{O}_2}$  from inhaled air at the end of every dive. A prolonged apnoea, as occurs during longer dives, causes a larger  $\text{O}_2$  storage depletion compared with that from shorter dives. The uptake efficiency increases swiftly for short dives and flattens for longer dives when the maximum possible uptake is approached (Sumich, 1994, 2001; Parkes et al., 2002; Wilson et al., 2003; Fahlman et al., 2008). A higher MR, dependent upon activity level, causes the  $\text{O}_2$  store to deplete faster than a lower MR. Therefore, a higher MR causes a greater  $\text{O}_2$  flux, and thus a greater expected  $E_{\text{O}_2}$ , than after an equivalent apnoea with a lower MR.

In this study, we developed an alternative approach to estimate FMR from respiration events. Instead of assuming a fixed  $E_{\text{O}_2}$  per respiration, we applied a simple  $\text{O}_2$ -uptake function, in which  $E_{\text{O}_2}$  decreases as body  $\text{O}_2$  stores become more saturated. For simplicity, we set  $V_T$  to be constant. The fact that over a sufficiently long time scale  $\dot{V}_{\text{O}_2}$  and FMR should balance provides a tool to compare models that assume fixed versus variable  $T_{\text{O}_2}$  by correlating  $\dot{V}_{\text{O}_2}$  with underwater activity over an appropriate time period. This study focused on the aerobic metabolism of free-ranging killer whales, *Orcinus orca* (Linnaeus 1758). Killer whales are ‘single-breathers’ that tend to continually dive between respirations, making it possible to detect respirations using depth recordings, in contrast to some deep-diving cetaceans like sperm whales (*Physeter macrocephalus*), which can remain passive at the surface during recovery periods. Though physiological data on killer whales are difficult (if not impossible) to collect at sea, some valuable physiological data have been collected on captive specimens (Table 1). Still, there exists a lack of solid values on energetic requirements of killer whales. The few studies that have been conducted on this species involving direct measurements of MR are based on a small number of animals and datasets (Kasting et al., 1989; Kriete, 1995; Worthy et al., 2014). Studies on the FMR of free-ranging animals have relied on respiration rates, assuming a constant  $E_{\text{O}_2}$ . However, respiration rates correlated weakly with speed derived from killer whale surfacing positions (Kriete, 1995; Williams and Noren, 2009). Moreover, the relationship between cost of transport (COT) and speed is expected to be parabolic with a

clear minimum at the optimal speed. This expected minimum in the killer whale COT curve has not been observed in previous studies (Williams and Noren, 2009). By including continuous data on respiration timing and underwater activity, both recorded by an animal-attached tag (Johnson and Tyack, 2003; Miller et al., 2010), a model of  $\text{O}_2$  exchange and  $\text{O}_2$  stores enabled a re-examination of predicted  $E_{\text{O}_2}$  dynamics. The objective was to investigate the potential influence of respiration timing, in addition to respiration rate, on killer whale FMR estimates using this model.

**MATERIALS AND METHODS****Data**

Animal experiments were carried out under permits issued by the Norwegian Animal Research Authority (permit no. 2004/20607 and S-2007/61201), in compliance with the ethical use of animals in experimentation. The research protocol was approved by the University of St Andrews Animal Welfare and Ethics Committee and the WHOI Institutional Animal Care and Use Committee.

For this study, we used 50.8 h of data recorded by digital acoustic recording tags (DTAGs; Johnson and Tyack, 2003) attached using suction cups to five adult male and five adult female free-ranging Norwegian herring-feeding killer whales between 2005 and 2009. Continuous acoustic and sensor recordings of the DTAG are synchronous (Johnson and Tyack, 2003), so the relative timing of sounds and motion can be determined precisely.

Characteristics information was collected for all tagged individuals, which were categorized into age–sex classes according to body size, as  $\text{O}_2$  storage and use were expected to scale with body size in this sexually dimorphic species. For age–sex class, adult-sized animals with a tall dorsal fin were defined as adult males and those without a tall dorsal fin were classified as adult females. Though some of the latter could have been sub-adult males, their body size was smaller than that of adult males, which was the primary reason for sex categorization. Individual tag records ranged from 1.67 h to almost 12.5 h in length (Table S1). Some whales were tagged simultaneously and data records of four whales included time periods in which they were experimentally exposed to sonar (Table S1) (Miller et al., 2011), which probably affected the speed of these individuals during some periods of their tagging record (Miller et al., 2014).

**Data processing**

DTAG data were processed and analysed using MATLAB® (v7.5.0.342R2007b, MathWorks) and RStudio® (v0.98.994, The R Foundation for Statistical Computing). Pressure, 3-axis magnetometer and 3-axis accelerometer measurements at 50 Hz sampling rate were converted to depth (m), magnetic field ( $\mu\text{T}$ ) and acceleration ( $g$ ), respectively, using calibration values, and condensed to a sampling rate of either 10 Hz for tags deployed in 2005 and 2006 or 5 Hz for tags deployed in 2009. Animal pitch, roll and heading data were derived from magnetometer and accelerometer values after correcting for tag orientation on the whale (Johnson and Tyack, 2003), which changed gradually and/or swiftly during seven suction-cup tag deployments.

**Respiration events and rates**

DTAG depth data enable detection of surfacings, which are respiration events (Miller et al., 2010). Surfacing were automatically detected using depth criteria to define the start and end of each dive (see Miller et al., 2010, for details). The detected surfacings were manually checked by inspecting the dive profile, and acoustic recordings in rare cases of uncertainty.

**Table 1. Values of all parameters used for the O<sub>2</sub> model for both male and female killer whales**

Parameter	Units	Male	Female	Source
Body length	m	6.65	5.40	Kriete (1995) <sup>1</sup>
Body mass	kg	3913	2800	Kriete (1995) <sup>2</sup>
Body surface area	m <sup>2</sup>	22.90	17.00	Fish (1998) <sup>1</sup>
V <sub>T</sub>	l	258.5	149.0	Kriete (1995) <sup>3</sup>
Lung O <sub>2</sub> store	l	31.1	22.7	Noren et al. (2012)
Muscle O <sub>2</sub> store	l	57.0	41.4	Noren et al. (2012)
Blood O <sub>2</sub> store	l	49.2	35.8	Noren et al. (2012)
Maximum TBO <sup>4</sup>	l	137.3	99.9	
BMR	l O <sub>2</sub> s <sup>-1</sup>	0.1050	0.0731	Kriete (1995)
BMR range in sensitivity analyses	l O <sub>2</sub> s <sup>-1</sup>	0.090–0.2814	0.070–0.1974	See Materials and methods
Fixed T <sub>O<sub>2</sub></sub>	l respiration <sup>-1</sup>	22.3	11.9	Kriete (1995) <sup>5</sup>
Max. T <sub>O<sub>2</sub></sub>	l respiration <sup>-1</sup>	25.52	13.68	Kriete (1995) <sup>5</sup>
Max. T <sub>O<sub>2</sub></sub> range in sensitivity analyses	l respiration <sup>-1</sup>	17.18–54.16	10.58–31.22	Kriete (1995)
Slope of MR versus U <sup>3</sup>	l O <sub>2</sub> m <sup>-1</sup> s <sup>-1</sup>	0.006115	0.004922	Present study
Slope range in sensitivity analyses	l O <sub>2</sub> m <sup>-1</sup> s <sup>-1</sup>	0.0031–0.0795	0.0025–0.0618	See text

V<sub>T</sub>, tidal lung volume; TBO, total body O<sub>2</sub> store; BMR, basal metabolic rate; T<sub>O<sub>2</sub></sub>, O<sub>2</sub> uptake per respiration; MR, metabolic rate; U, speed.

<sup>1</sup>Extrapolation of data from Kriete (1995) or Fish (1998).

<sup>2</sup>Body mass was estimated by Kriete (1995) for her study animals using the equation by Bigg and Wolman (1975) plus 20% as a correction factor for captive killer whales.

<sup>3</sup>V<sub>T</sub> in the present study was assumed to be equal to the maximum V<sub>T</sub> found by Kriete (1995), who expected that measured maximum V<sub>T</sub> better reflected true V<sub>T</sub>. Therefore, this maximum V<sub>T</sub> was used in all calculations.

<sup>4</sup>Maximum TBO was estimated through accumulation of maximum lung, muscle and blood O<sub>2</sub> store capacities.

<sup>5</sup>Kriete (1995) found a mean E<sub>O<sub>2</sub></sub> (O<sub>2</sub> extraction from inhaled air) of 41.15% and 38.10% of maximum V<sub>T</sub> for males and females, respectively, representing a T<sub>O<sub>2</sub></sub> of 22.3 and 11.9 l O<sub>2</sub> respiration<sup>-1</sup>, respectively. For all calculations, it was assumed that the proportion of O<sub>2</sub> in air is 0.2095.

<sup>6</sup>Maximum E<sub>O<sub>2</sub></sub> values per respiration per sex were derived from data collected by Kriete (1995), who measured a mean E<sub>O<sub>2</sub></sub> (for maximum V<sub>T</sub>) during high activity levels of 47.1% and 43.8% for males and females, respectively.

Differences in respiration rate between sexes were investigated using generalized estimating equations (GEE). Respiration rates were modelled using a Gaussian distribution of errors and a one-step autoregressive correlations structure (AR1). The model structure was selected using the quasi-Akaike information criteria (QIC) (Pan, 2001).

### Speed measurements from flow noise

Speed through water or swimming speed (*U*) was estimated by merging analyses of kinematic measurements and the sound pressure levels (SPLs) of the recorded low-frequency flow noise, as done previously by other authors (Goldbogen et al., 2006; Simon et al., 2009). The low-pass frequency filter, specified per tag record, ranged from 200 to 350 Hz. Kinematic speed was estimated for entire tag records by dividing the depth change rate over 1 s intervals by the sine of the instantaneous body pitch angle. A pitch angle threshold for these calculations was introduced per individual and per tag period, ranging from 45 to 65 deg, depending on data quantity and pitch angle occurrence and distribution. Flow noise SPLs and kinematic speed estimates from the sensor data were synchronized over 1 s intervals.

An exponential least-squares regression between kinematic speed and flow noise SPLs was fitted to data from depths >10 m, as surfacing events produced high noise levels unrelated to *U*. Separate regressions were performed for each tag record and periods between tag movements within tag records containing body pitch angles larger than the pitch angle threshold and a SPL range larger than 10 dB. For tag periods not meeting these requirements, the speed–SPL regression of the preceding period was applied. In the event of a gradual tag movement, average regression parameter values from the previous and following tag period without tag movements were used (Table S2). When either the previous or following period without tag movement, or both, did not contain large enough pitch angles or a SPL range larger than 10 dB, but the gradual movement period did, this latter period was handled as a period without tag

movement (Table S2). The regression was then used to calculate *U* at 1 s intervals from the SPLs throughout each tag record. Again, to avoid overestimation errors in *U* calculation due to the elevated SPL caused by surfacings, *U* estimates during respiration events and the 3 s preceding them were replaced by the last estimate before that interval. *U* estimates for the 3 s after the respiration event were replaced with the following estimate.

Differences in *U* between sexes were investigated using GEEs with correlation structure ‘independence’. Model structure was selected using QIC.

### Estimated MR in relation to speed

Size-dependent FMR of the swimming whales was estimated by summing basal metabolic rate (BMR) and locomotion costs (MR<sub>L</sub>) at a 1 s resolution. BMR was assumed to be equal to the standard metabolic rates quantified by Kriete (1995) (Table 1). A sex-specific relationship between U<sup>3</sup> and energetic cost was fitted by combining equations used previously by Fish (1998) and Guinet et al. (2007).

The Reynolds number (*Re*) was modelled as a function of animal length (*L*, in m), the swimming speed (*U*, in m s<sup>-1</sup>) and kinematic viscosity (*v*) of seawater (1.044×10<sup>-6</sup> m<sup>2</sup> s<sup>-1</sup>):

$$Re = LU/v. \quad (2)$$

A relationship between *Re* and the drag coefficient (*C<sub>d</sub>*) exclusively for killer whales, provided by Fish (1998), was used to estimate *C<sub>d</sub>*, as done previously by Guinet et al. (2007):

$$C_d = 6.35 Re^{-0.3719}. \quad (3)$$

Mean thrust power (*P<sub>T</sub>*, W) needed to overcome drag was estimated through the following equation as proposed by Fish (1998):

$$P_T = 0.5 \rho S U^3 C_d, \quad (4)$$

where  $\rho$  is seawater density ( $1026 \text{ kg m}^{-3}$ ) and  $S$  is the body surface area in  $\text{m}^2$ .

$\text{MR}_L$  was estimated by correcting  $P_T$  for a propulsive efficiency ( $\eta$ ) of 0.8 (fig. 5 in Fish, 1998):

$$\text{MR}_L = P_T/\eta. \quad (5)$$

$\text{MR}_L$  was modelled as  $k \cdot U^3$ , where  $k$  is a coefficient ( $1 \text{ O}_2 \text{ m}^{-3} \text{ s}^2$ ) determined using morphometrics according to sex (Table 1).  $\text{MR}_L$  was derived for  $U$  of  $0\text{--}10 \text{ m s}^{-1}$ . A linear function, with the intercept set at BMR, was calculated for both 3913 kg males ( $\text{MR}=0.006115 \times U^3 + 0.1050$ ) and 2800 kg females ( $\text{MR}=0.004922 \times U^3 + 0.0731$ ).

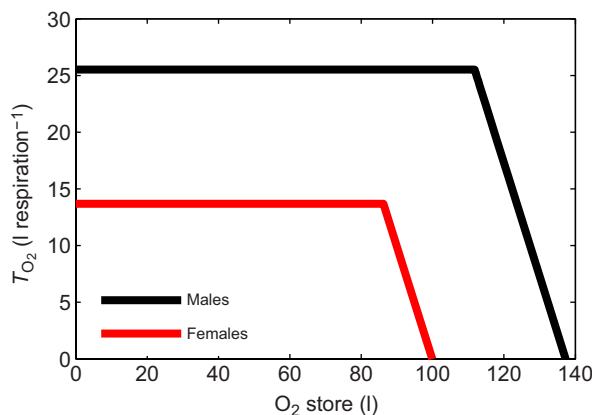
### O<sub>2</sub> uptake models

To estimate each individual whale's O<sub>2</sub> store dynamically in time, by which the  $T_{O_2}$  is estimated per respiration, an O<sub>2</sub> exchange model was established combining activity level indicators from the tag data with existing information on killer whale physiology and energetics (Table 1):

$$\text{TBO}_t = \text{TBO}_{t-1} - \text{MR}_{t-1} + T_{O_2,t}, \quad (6)$$

where  $\text{TBO}_t$  represents the total O<sub>2</sub> stored (l) in the lungs, blood and muscle together at time  $t$ ,  $\text{MR}_{t-1}$  is the total O<sub>2</sub> consumption (l) based upon BMR and  $\text{MR}_L$  during the preceding second, and  $T_{O_2,t}$  is the O<sub>2</sub> (l) taken up if the whale respired at time  $t$ , or zero if no respiration occurred.  $T_{O_2,t}$  was modelled in three different ways: model 1, a constant value from the literature; model 2, a constant fitted to the data to achieve a balanced O<sub>2</sub> budget over each animal's entire tag-recording period; and model 3, a broken-stick O<sub>2</sub>-uptake function in which O<sub>2</sub> uptake depends upon the store at the time of the respiration. If at the time of respiration the O<sub>2</sub> store is lower than maximum TBO minus maximum  $T_{O_2}$ , then  $T_{O_2}$  is the maximum possible uptake. Otherwise,  $T_{O_2}$  is maximum TBO minus the existing O<sub>2</sub> store (Fig. 1), which realistically caps the O<sub>2</sub> uptake according to the maximum TBO.

The model was initialized at the first second after the first breathing bout of at least six respirations within each tag record, at which point TBO was assumed to be saturated at the maximum TBO value (Table 1).



**Fig. 1. A broken-stick O<sub>2</sub>-uptake function for male and female killer whales as used in the O<sub>2</sub> model (model 3).** Here, O<sub>2</sub> uptake per respiration ( $T_{O_2}$ ) is a function of the O<sub>2</sub> store at the time of each respiration. The maximum O<sub>2</sub> uptake per respiration was set at 25.52 and 13.68 l for males and females, respectively. The maximum total body O<sub>2</sub> store capacity was set at 137.3 and 99.9 l for males and females, respectively (Table 1).

### Relationship between whale activity and O<sub>2</sub> uptake

Using all models,  $\dot{V}_{O_2}$  (estimated at a 1 s resolution) was calculated over successive 15 min time intervals to model against  $U^3$  over the same 15 min intervals. The 15 min time duration was considered to be sufficient to even out O<sub>2</sub> store fluctuations during typical dives, but short enough to capture temporal variation in underwater activity levels. To eliminate potential influence by biased start and stop points by filtering, both the first and last 15 min interval was excluded from analyses. Because O<sub>2</sub> consumption and uptake should be balanced over 15 min intervals, the average  $\dot{V}_{O_2}$  and average activity level ( $U^3$ ) should be linearly related (Eqn 4). We therefore regressed O<sub>2</sub> uptake as a function of  $U^3$ . Because BMR was fitted as a constant in the model, we regressed ( $\dot{V}_{O_2}$ –BMR) against  $U^3$  through the origin.

We used a GEE model with whale identity as random factor and a Gaussian family of distribution for the residuals. Correlation structure AR1 was included to account for auto-correlation among 15 min intervals. The model included sex as a factor.

### Sensitivity analyses

Because of uncertainty in the parameter values, the sensitivity of the results of model 3 to the slope of  $\text{MR}_L$  versus  $U^3$  ( $k$ ), BMR and the maximum  $T_{O_2}$  was tested by varying these parameters one at a time according to values derived from other studies where possible.

The slope of  $\text{MR}_L$  versus  $U^3$  was varied over a range of values (Table 1), with the highest value extracted from results by Williams et al. (1993), who found a relatively high MR for swimming captive bottlenose dolphins (*Tursiops truncatus*) compared with other studies on swimming cetacean MR. Because there are no published estimates below the values used, the lower limit of the sensitivity analyses was set at half the value (Table 1).

The values of BMR used were from Kriete (1995), who obtained measurements on just one adult male and one adult female killer whale. Upper limits for the sensitivity analysis of this parameter (Table 1) were derived from the equation by Kasting et al. (1989), which yielded relatively high BMR values compared with other studies on cetacean BMR. The lower limits were set according to recently found BMR values by Worthy et al. (2014), which are relatively low compared with other cetacean BMR study outcomes.

Both the upper and lower limits for the sensitivity analyses of  $T_{O_2}$  values for both sexes were derived from Kriete (1995), who measured a maximum and minimum  $\dot{V}_T$  for both male and female killer whales which were multiplied by the percentage of O<sub>2</sub> in air (20.95%; Table 1).

Averages of the values initially used in model 3 and the upper and lower limit were also tested for all parameters to strengthen sensitivity analysis outcomes.

### Metabolic COT calculations

Estimated  $\dot{V}_{O_2}$  over the 15 min intervals using the different O<sub>2</sub>-uptake models were multiplied by the conversion factor of  $20.1 \text{ kJ l}^{-1} \text{ O}_2$  to derive estimates in J. These values were divided by the speed measurements over the same 15 min interval to derive non-mass-specific COT ( $\text{J m}^{-1}$ ). Finally, the non-mass-specific COT estimates were divided by the body mass per sex to calculate the mass-specific COT ( $\text{J kg}^{-1} \text{ m}^{-1}$ ) for the different O<sub>2</sub>-uptake models.

## RESULTS

### Respiration rate

The mean±s.d. respiration rate was  $1.54 \pm 0.22$  respirations  $\text{min}^{-1}$  and ranged from 1.08 to 2.18 respirations  $\text{min}^{-1}$  (Table S3). The

**Table 2.** Relationships between modelled  $O_2$  uptake over 15 min intervals estimated through the  $O_2$  model with fixed  $O_2$  uptake (model 1), estimated fixed  $O_2$  uptake (model 2), and fluctuating  $O_2$  uptake according to the broken-stick  $O_2$ -uptake function (model 3), versus  $U^3$  over 15 min intervals for individuals and sexes

	No. of 15 min intervals	Model 1		Model 2		Estimated $T_{O_2}$	Model 3	
		$r^{2*}$	RMSE	$r^{2*}$	RMSE		$r^2$	RMSE
<b>Female</b>								
05_316a	7	-46.174	0.2317	-7.440	0.0345	4.19	0.990	0.0027
05_321b	4	-175.528	0.0548	-4.696	0.0033	3.99	0.952	0.0010
05_322a	11	-19.391	0.1605	-5.139	0.0440	5.94	0.997	0.0028
06_313s	6	-2.773	0.0789	0.201	0.0115	3.77	0.976	0.0015
06_327s	19	-131.023	0.2069	-31.440	0.0422	4.89	0.981	0.0038
All females	47	-26.313	0.2061	-4.371	0.0350	4.56±0.88 <sup>‡</sup>	0.899	0.0112
<b>Male</b>								
05_320b	5	-126.019	0.4561	-5.325	0.0230	5.04	0.784	0.0095
05_322b	9	-14.945	0.4057	0.531	0.0152	4.87	0.908	0.0053
06_327t	20	-82.653	0.4973	-3.816	0.0255	4.76	0.912	0.0051
09_144a	44	-53.733	0.4559	-12.276	0.0693	6.88	0.938	0.0143
09_144b	47	-19.924	0.4812	-8.803	0.1317	8.92	0.907	0.0262
All males	125	-27.400	0.4507	-3.675	0.0499	6.09±1.80 <sup>‡</sup>	0.954	0.0161

RMSE, root mean squared error.

\*Negative  $r^2$  values show that the fit of the model is worse than the fit of a horizontal line at the mean estimated  $O_2$  uptake (null hypothesis) because the intercept is set at BMR and the locomotion component of MR ( $MR_L$ ) estimates lie well above this value.

<sup>‡</sup>Estimated fixed  $T_{O_2}$  values ( $\pm$ s.d.) for sexes were derived as the average of individuals within the sex.

highest respiration rate was observed for one of the males, while the lowest rate was detected for one of the females (Table S3). Matching the previous finding of Miller et al. (2010), males exhibited a mean respiration rate ( $1.57\pm 0.23$  respirations  $\text{min}^{-1}$ ) that did not differ significantly from that of the females ( $1.47\pm 0.20$  respirations  $\text{min}^{-1}$ ; two-tailed Wald test:  $P=0.053$ ).

#### Speed from flow noise measurements

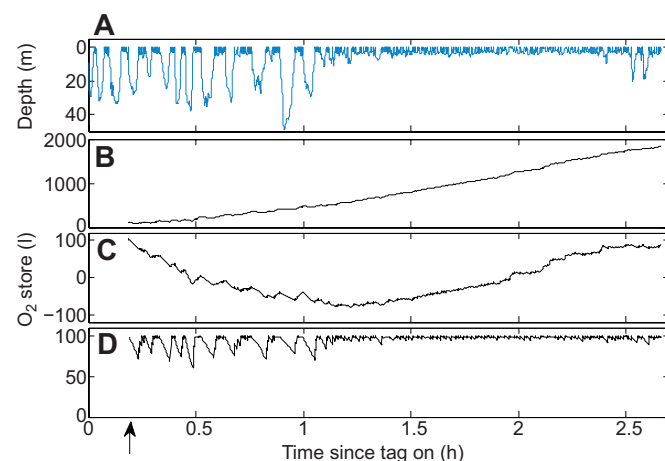
The mean  $\pm$  s.d.  $U$  for all whales was  $1.89\pm 0.61$   $\text{m s}^{-1}$ , and ranged from 0.69 to  $4.05$   $\text{m s}^{-1}$ , with both extremes being males (Table S3). The range of  $U$  estimates for females was somewhat smaller, ranging from 0.72 to  $2.80$   $\text{m s}^{-1}$  (Table S3). The mean  $\pm$  s.d.  $U$  for females ( $1.72\pm 0.59$   $\text{m s}^{-1}$ ) and males ( $1.96\pm 0.61$   $\text{m s}^{-1}$ ) was not significantly different (two-tailed Wald test:  $P=0.680$ ).

#### $O_2$ model using fixed $O_2$ uptake (model 1)

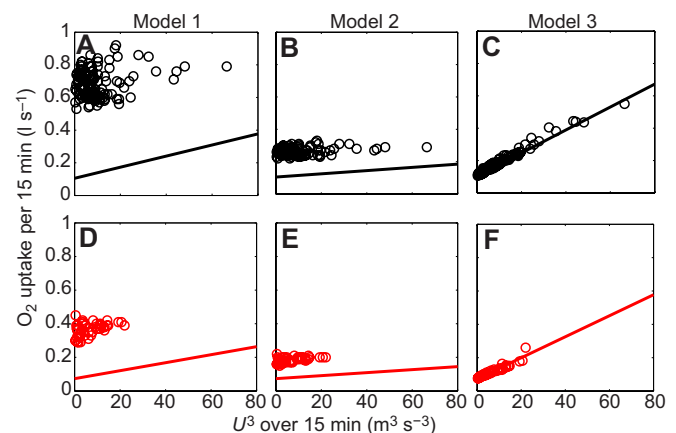
Using the literature value for a fixed  $T_{O_2}$  (model 1), no correlation between  $\dot{V}_{O_2}$  and  $U^3$  over the 15 min intervals was found for any of the individual whales or either sex when setting the intercept at BMR (Table 2). The fixed  $T_{O_2}$  resulted in an unrestrained and unrealistically high  $O_2$  store for all individuals, well exceeding their maximum TBO, meaning there was no balance between  $O_2$  use and uptake over the tag period (e.g. Fig. 2B). The fitted GEE model lay well below all data points for both sexes (Fig. 3A,D).

#### $O_2$ model using estimated fixed $O_2$ uptake (model 2)

The estimated fixed values of  $T_{O_2}$  (model 2) for females and males ranged between 3.77 and 5.94  $\text{l O}_2$  respiration $^{-1}$ , and 4.76 and 8.92  $\text{l O}_2$  respiration $^{-1}$ , respectively (Table 2). All these estimated values



**Fig. 2.** Example of a time series plot of tagged female 05\_316a. Shown are the dive profile (A) and  $O_2$  store estimated over the entire tag record using the  $O_2$  model with fixed  $O_2$  uptake per respiration from the literature (model 1, B), estimated fixed  $O_2$  uptake (model 2, C), and fluctuating  $O_2$  uptake according to the broken-stick  $O_2$ -uptake function (model 3, D). Note that approximately 0.2 h after tag-on time (indicated by the arrow on the x-axis), the total body  $O_2$  store (TBO) was assumed to be saturated after a respiration bout of at least 6 respirations, and the model was initiated.



**Fig. 3.**  $O_2$  uptake versus  $U^3$  for tagged male and female killer whales. Both  $O_2$  uptake and speed ( $U$ ) were averaged over 15 min intervals for male (black,  $N=5$ ) and female (red,  $N=5$ ) killer whales.  $O_2$  uptake was estimated using the  $O_2$  model with fixed  $O_2$  uptake (model 1: A, males; D, females), optimized fixed  $O_2$  uptake (model 2: B, males; E, females), and fluctuating  $O_2$  uptake according to the broken-stick  $O_2$ -uptake function (model 3: C, males; F, females). Solid lines represent the relationship between  $O_2$  uptake and  $U^3$  fitted with an intercept set at basal metabolic rate (BMR).

**Table 3. Relationships between modelled O<sub>2</sub> uptake (model 3: broken-stick model) versus U<sup>3</sup> over 15 min intervals for male and female killer whales**

	r <sup>2</sup>	RMSE	Slope
<b>Males</b>			
<i>k</i>			
0.080	-7.620	0.4669	0.0046
0.043	-3.840	0.4181	0.0049
0.006*	0.954	0.0161	0.0071
0.005	0.955	0.0130	0.0058
0.003	0.954	0.0079	0.0035
BMR			
0.281	0.909	0.0228	0.0070
0.193	0.945	0.0177	0.0071
0.105*	0.954	0.0161	0.0071
0.098	0.954	0.0161	0.0071
0.090	0.954	0.0160	0.0071
Max. T <sub>O<sub>2</sub></sub>			
54.16	0.957	0.0155	0.0071
39.84	0.957	0.0156	0.0071
25.52*	0.954	0.0161	0.0071
22.29	0.949	0.0169	0.0071
19.06	0.923	0.0206	0.0069
<b>Females</b>			
<i>k</i>			
0.062	-9.534	0.2114	0.0060
0.033	-3.523	0.1758	0.0081
0.005*	0.899	0.0112	0.0061
0.004	0.936	0.0067	0.0049
0.002	0.937	0.0033	0.0024
BMR			
0.197	0.780	0.0174	0.0064
0.135	0.793	0.0182	0.0071
0.073*	0.899	0.0112	0.0061
0.072	0.896	0.0113	0.0064
0.070	0.899	0.0111	0.0064
Max. T <sub>O<sub>2</sub></sub>			
31.22	0.946	0.0075	0.0059
22.45	0.945	0.0076	0.0059
13.68*	0.899	0.0112	0.0061
12.06	0.833	0.0157	0.0068
10.43	0.806	0.0172	0.0069

N=5 males, 125 intervals; N=5 females, 47 intervals.

Sensitivity analysis summary statistics are given for different slopes of MR<sub>L</sub> versus U<sup>3</sup> (*k*), BMR and maximum T<sub>O<sub>2</sub></sub> per sex. The intercept was set at BMR. Only one of the parameters was varied at a time, while the two other parameters kept their original value.

\*Original values for parameters as used in model 3 initially.

were considerably lower than the values used by Williams and Noren (2009). The mean±s.d. estimated fixed T<sub>O<sub>2</sub></sub> found for males (6.09±1.80 l O<sub>2</sub> respiration<sup>-1</sup>) was somewhat higher (two-tailed *t*-test: *P*=0.137) than the mean±s.d. estimated value found for females (4.56±0.88 l O<sub>2</sub> respiration<sup>-1</sup>; Table 2). Under model 2, there was a moderate correlation between estimated  $\dot{V}_{O_2}$  and U<sup>3</sup> for only one female and one male (Table 2). When grouping animals by sex, there was no relationship between  $\dot{V}_{O_2}$  and U<sup>3</sup> (Table 2, Fig. 3B,E). Although by definition the accumulated  $\dot{V}_{O_2}$  over each time series was constrained to be equal to the accumulated MR, O<sub>2</sub> stores estimated under model 2 resulted in an unrealistically temporary excess of the estimated maximum TBO, and/or an unrealistically negative O<sub>2</sub> store for all individuals (e.g. Fig. 2C).

### O<sub>2</sub> model including the broken-stick O<sub>2</sub>-uptake function (model 3)

The model of O<sub>2</sub> uptake fluctuating per respiration according to O<sub>2</sub> store (model 3) led to a strong association between  $\dot{V}_{O_2}$  and U<sup>3</sup> for all

individuals (Table 2). This association was weaker for all females grouped than for females individually, whereas for males the opposite was true (Table 2). Of the females, 05\_322a had overall a higher  $\dot{V}_{O_2}$ . The data point representing the highest speed and  $\dot{V}_{O_2}$ , which shows the greatest deviation, belonged to this particular female (Fig. 3F).

MR and  $\dot{V}_{O_2}$  were better balanced for all individuals when applying T<sub>O<sub>2</sub></sub> as a function of O<sub>2</sub> store, without exceeding the estimated TBO capacity. Nor were negative O<sub>2</sub> store values obtained using this model. When the animals respired often in sequence after a longer dive, the first couple of respirations were sufficient to replenish O<sub>2</sub> stores. As the O<sub>2</sub> stores became more saturated, T<sub>O<sub>2</sub></sub> decreased (e.g. Fig. 2D).

### Sensitivity analyses

#### Slope of MR<sub>L</sub> versus U<sup>3</sup> (*k*)

The  $\dot{V}_{O_2}$ -U<sup>3</sup> correlation was sensitive to the diverse energetic parameter values derived from the literature (Table 3, Fig. 4A,D).

As expected, the occurrence of maximum T<sub>O<sub>2</sub></sub> respirations increased when increasing the slope of MR<sub>L</sub> versus U<sup>3</sup> and vice versa. Average T<sub>O<sub>2</sub></sub> decreased when decreasing the slope of MR<sub>L</sub> versus U<sup>3</sup> (e.g. Fig. S1).

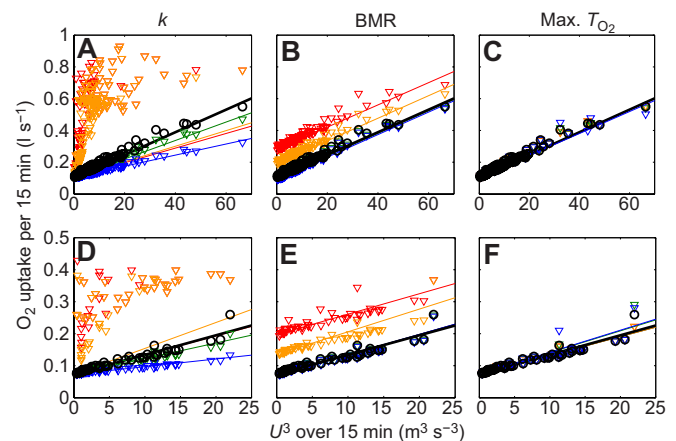
#### BMR

Varying BMR according to BMRs derived from other studies caused only a minor change of the  $\dot{V}_{O_2}$ -U<sup>3</sup> correlation (Table 3, Fig. 4B,E). Moreover, BMR values used originally in model 3 gained the highest r<sup>2</sup> values (Table 3).

When applying tested BMR values that were higher than the used values, the occurrence of maximum T<sub>O<sub>2</sub></sub> respirations increased and vice versa. Average T<sub>O<sub>2</sub></sub> decreased with decreasing BMR (e.g. Fig. S2).

#### Maximum T<sub>O<sub>2</sub></sub>

The  $\dot{V}_{O_2}$ -U<sup>3</sup> correlation became tighter when increasing the maximum T<sub>O<sub>2</sub></sub>, especially for females (Table 3, Fig. 4C,F).



**Fig. 4. Killer whale O<sub>2</sub> uptake per 15 min interval.** Uptake was estimated using fluctuating O<sub>2</sub> uptake according to the broken-stick O<sub>2</sub>-uptake function (model 3) versus U<sup>3</sup> per sex (top: males, N=5; bottom: females, N=5), with different values for the slope of the locomotion component of metabolic rate (MR<sub>L</sub>) versus U<sup>3</sup> (*k*) (A,D), BMR (B,E) and maximum T<sub>O<sub>2</sub></sub> (C,F) implemented for sensitivity analyses of the model. The results of the parameter values tested (triangles) during the sensitivity analyses are colour coded: red, highest; orange second highest; green, second lowest; and blue, lowest. Solid lines in associated colours represent regressions fitted between O<sub>2</sub> uptake (estimated using the different parameter values) and U<sup>3</sup>. The original model outcomes are coloured black.

**Table 4. Equations and regression statistics for the correlations between O<sub>2</sub> uptake versus level of activity, and corresponding cost of transport versus *U* per sex in killer whales**

	Equation		<i>r</i> <sup>2</sup>	RMSE	App. optimal <i>U</i> (m s <sup>-1</sup> )
<b>Model 1</b>					
$\dot{V}_{O_2}$					
Females	0.03272× <i>U</i> +0.236		0.233	0.0353	
Males	0.02333× <i>U</i> +0.536		0.028	0.0840	
COT		AIC			
Females	0.547× <i>U</i> <sup>2</sup> −2.627× <i>U</i> +4.078	5.91	0.791	0.2446	2.2–2.7
	1.962× <i>U</i> <sup>-0.8773</sup>	0.715	0.805	0.2338	–
Males	0.425× <i>U</i> <sup>2</sup> −2.658× <i>U</i> +5.096	3.24	0.847	0.2403	3.0–3.5
	2.861× <i>U</i> <sup>-0.9277</sup>	1.19	0.847	0.2393	–
<b>Model 2</b>					
$\dot{V}_{O_2}$					
Females	0.01254× <i>U</i> +0.090		0.233	0.0135	
Males	0.00637× <i>U</i> +0.146		0.028	0.0230	
COT		AIC			
Females	0.210× <i>U</i> <sup>2</sup> −1.007× <i>U</i> +1.563	−84.26	0.791	0.0937	2.2–2.7
	0.7518× <i>U</i> <sup>-0.8773</sup>	−89.45	0.805	0.0896	–
Males	0.116× <i>U</i> <sup>2</sup> −0.726× <i>U</i> +1.392	−321.24	0.847	0.0656	3.0–3.5
	0.7813× <i>U</i> <sup>-0.9277</sup>	−323.29	0.847	0.0654	–
<b>Model 3</b>					
$\dot{V}_{O_2}$					
Females	0.00601× <i>U</i> <sup>3</sup> +0.0731		0.904	0.0111	
Males	0.00769× <i>U</i> <sup>3</sup> +0.1050		0.970	0.0140	
COT					
Females	0.191× <i>U</i> <sup>2</sup> −0.751× <i>U</i> +1.161		0.864	0.0323	1.7–2.2
Males	0.109× <i>U</i> <sup>2</sup> −0.470× <i>U</i> +0.944		0.713	0.0404	1.9–2.4

Model 1: O<sub>2</sub> model using fixed *T*<sub>O<sub>2</sub></sub>; model 2: O<sub>2</sub> model using optimized fixed *T*<sub>O<sub>2</sub></sub>; model 3: O<sub>2</sub> model including a broken-stick O<sub>2</sub>-uptake function. O<sub>2</sub> uptake ( $\dot{V}_{O_2}$ ) was estimated by the different model alternatives; level of activity was represented by speed (*U*, m s<sup>-1</sup>); cost of transport (COT) was measured as J kg<sup>-1</sup> m<sup>-1</sup>.  $\dot{V}_{O_2}$ , COT and *U* were all averaged per 15 min interval. *N*=5 males, *N*=5 females.

The frequency of occurrence of maximum *T*<sub>O<sub>2</sub></sub> was decreased by increasing this parameter value. Varying maximum *T*<sub>O<sub>2</sub></sub> did not affect the frequency of occurrence of *T*<sub>O<sub>2</sub></sub> values lower than the lowest tested value (e.g. Fig. S3). The low occurrence of *T*<sub>O<sub>2</sub></sub> values between the lowest tested and other tested *T*<sub>O<sub>2</sub></sub> values was equally distributed, with frequency decreasing with an increasing difference between the lowest tested and other tested *T*<sub>O<sub>2</sub></sub> values (e.g. Fig. S3). Thus, though varying maximum *T*<sub>O<sub>2</sub></sub> did not significantly change the overall results, O<sub>2</sub> uptake per respiration was limited by the set maximum *T*<sub>O<sub>2</sub></sub>, especially for the lower tested values (e.g. Fig. S3).

In summary, the sensitivity analyses demonstrated that, over the range of parameter values tested, the O<sub>2</sub> model with variable uptake (model 3) was especially sensitive to changes in the slope of MR<sub>L</sub> versus *U*<sup>3</sup>. The results for females were somewhat more sensitive to parameter value changes than were results for males. Nonetheless, including the broken-stick O<sub>2</sub>-uptake function in the O<sub>2</sub> model always yielded a better fit than using a fixed uptake per respiration (model 1).

### Metabolic COT

For models 1 and 2, the relationship between COT and speed was best modelled by a power function with no minimum for both sexes (Table 4, Fig. 5B,D). In contrast, COT estimated by model 3 with fluctuating *T*<sub>O<sub>2</sub></sub> was best represented by a second-order polynomial equation, with an underlying mechanism that revealed the expected U-shaped curve with a broad yet distinct minimum at a speed between 1.7 and 2.2 m s<sup>-1</sup> for females and between 1.9 and 2.4 m s<sup>-1</sup> for males (Fig. 5F). These estimated minimum COT speeds were the lowest for both sexes compared with results of model 1 and 2 (Table 4, Fig. 5F).

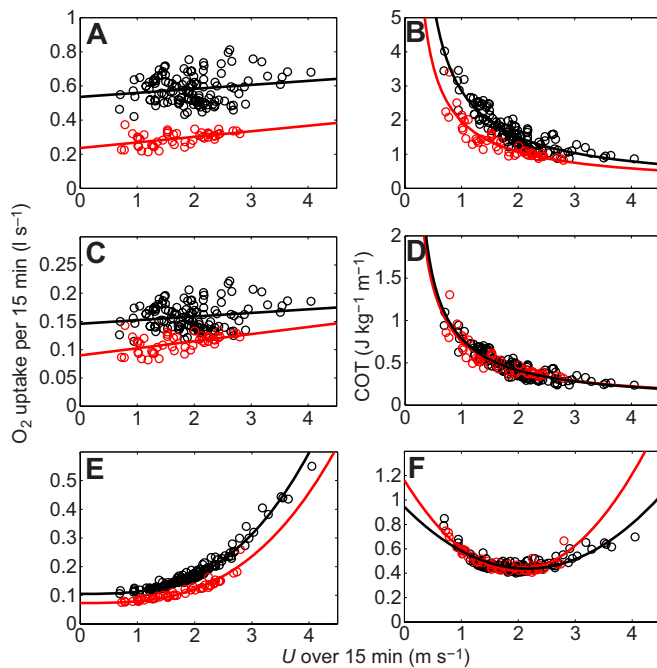
Minimum mass-specific COT estimated with fixed *T*<sub>O<sub>2</sub></sub> (model 1) was the highest (approximately 0.8 J kg<sup>-1</sup> m<sup>-1</sup> for both sexes,

closely matching the results of Williams and Noren, 2009), while minimum COT estimated by optimized fixed *T*<sub>O<sub>2</sub></sub> (model 2) was the lowest (approximately 0.2 J kg<sup>-1</sup> m<sup>-1</sup> for both sexes; Fig. 5B,D,F). Estimated minimum COT according to model 3 was 0.44 and 0.42 J kg<sup>-1</sup> m<sup>-1</sup> for male and female killer whales, respectively. Minimum COT estimated using model 2 and 3 revealed no difference between sexes (Table 4, Fig. 5D,F).

Correlation between  $\dot{V}_{O_2}$  and *U* over 15 min intervals, estimated with either model 1 or 2, was weak when fitting a simple linear regression as done by Williams and Noren (2009), especially for males (Table 4, Fig. 5A,C).  $\dot{V}_{O_2}$  estimated using model 3 showed a strong non-linear relationship with increasing linear speed for both sexes as anticipated (Table 4, Fig. 5E).

### DISCUSSION

In this study, we developed an alternative approach to the counting of respirations as a means to estimate O<sub>2</sub> uptake in free-ranging cetaceans. Rather than assuming that each respiration delivers an equivalent amount of O<sub>2</sub> (*T*<sub>O<sub>2</sub></sub>) to the body, we implemented a simple O<sub>2</sub>-uptake function (broken-stick; Fig. 1), which more realistically limits O<sub>2</sub> uptake from respirations when body O<sub>2</sub> stores are predicted to be more fully saturated. O<sub>2</sub> uptake is predicted to increase linearly with *U*<sup>3</sup>, but as has been found in previous studies (Williams and Noren, 2009; Christiansen et al., 2014), relationships between activity levels (quantified as *U*<sup>3</sup>) and  $\dot{V}_{O_2}$  were not apparent or were very weak when *T*<sub>O<sub>2</sub></sub> was assumed to be constant. In contrast, we found strong associations between predicted  $\dot{V}_{O_2}$  and underwater activity over 15 min intervals when uptake could vary across respirations using the broken-stick uptake function. The fixed *T*<sub>O<sub>2</sub></sub> derived from Kriete (1995), as used by Williams and Noren (2009) (model 1), overestimated the predicted



**Fig. 5.**  $O_2$  uptake and mass-specific cost-of-transport (COT) estimations over 15 min intervals as a function of  $U$  in killer whales.  $O_2$  uptake (left) was estimated through the  $O_2$  model with fixed  $O_2$  uptake (model 1, A), optimized fixed  $O_2$  uptake (model 2, C), and fluctuating  $O_2$  uptake according to the broken-stick  $O_2$ -uptake function (model 3, E). COT (right) was estimated through model 1 (B), model 2 (D) and model 3 (F). Estimations for tagged male ( $N=5$ ) and female ( $N=5$ ) killer whales are presented by black and red circles, respectively. Solid lines show fitted regressions (see Table 4).

energetic requirements of both sexes, especially those of male killer whales. This could have been due to the parameter values used in the model rather than the fact that  $T_{O_2}$  was fixed. However, model 2, which balanced  $\dot{V}_{O_2}$  and MR over the entire data recording period by specifying the value of  $T_{O_2}$ , still caused unrealistic maximum TBO excesses and deficits in modelled  $O_2$  stores and no association between estimated activity level ( $U^3$ ) and  $\dot{V}_{O_2}$ . This indicates that respiration rate alone is not a reliable indicator of energy requirements in killer whales. Allowing  $T_{O_2}$  to vary with  $O_2$  store at the time of each respiration (model 3) led to more stable fluctuations in the modelled  $O_2$  store. A much stronger association between estimated  $\dot{V}_{O_2}$  and  $U^3$  was found for model 3, as expected, as that model includes a mechanism to constrain  $O_2$  uptake by the MR predicted from BMR and estimated speed. Our results therefore demonstrate that the approach of applying an  $O_2$ -uptake function can overcome the weak relationships between activity levels and  $\dot{V}_{O_2}$  found when  $T_{O_2}$  is fixed per respiration.

An important outcome of the present study is that by using the broken-stick  $O_2$ -uptake function we were able to describe a U-shaped COT curve with a clear minimum at an intermediate speed (Fig. 5F). In contrast, the COT curve based upon a fixed uptake per respiration was monotonically decreasing (Fig. 5B,D). Our model led to a much lower predicted FMR and COT than previous studies have predicted for killer whales (Kriete, 1995; Williams et al., 2004a; Guinet et al., 2007). However, because key parameter values such as true  $MR_L$  and BMR remain indeterminate for now, our focus was on the improved predictive power of a varying  $O_2$ -uptake model, rather than the specific MR or COT estimates. Our approach, however, could be useful to estimate FMR in the future, when more accurate estimates of parameters such as  $MR_L$  and BMR become available.

These results were for the resolution of 15 min intervals, which are relevant for MR variations at relatively fine time scales. This could include describing the relative FMR associated with different behaviours or the effects of disturbance events of relatively short duration. However, if one were interested in estimating the lifetime  $\dot{V}_{O_2}$  of a whale, fitting a fixed  $T_{O_2}$  to 24 h or longer intervals would probably suffice. Further work could determine at what temporal scale the timing of respirations improves estimates of MR.

#### Speed from flow noise as an activity metric to estimate $MR_L$

Speed derived from DTAG data showed the potential to function as an activity metric in the developed  $O_2$  model. Speeds derived through flow noise SPL are approximate and the reliability of the measurements depends in part on tag placement and the proportion of diving behaviour including large body pitch angles. In addition, speed could not be derived during noisy surfacing events. Investigating the influence of ocean ambient noise, noise generated by oscillation of the flukes (dependent upon tag placement) and noise generated by the water surface recorded for logging whales on speed estimates would improve estimates of this relationship. Speed could also be measured more directly using fly-wheel speed sensor tags, rather than acoustic tags (Watanabe et al., 2011).

Speed through water ( $U$ ) was then used to estimate locomotion costs ( $MR_L$ ), which are predicted to increase as a function of  $U^3$ , as hydrodynamic drag is predicted to increase as a function of  $U^2$  (Vogel, 1994) and multiplication by speed converts drag force to power (work per unit time). The precise relationships between  $MR_L$  and  $U^3$  should be examined more thoroughly. Our derivation of expected  $MR_L$  followed the analysis of Guinet et al. (2007), but yielded total MR values that were substantially lower than other predictions (Williams et al., 2004a) of killer whale FMR. Fahlman et al. (2016) also found that estimates of MR using respiration rate and constant  $O_2$  uptake were as much as 2–5 times higher than when using measured variation in  $O_2$  uptake. We used the same BMR estimates as Williams et al. (2004a), so the difference in our estimates arises from the difference between BMR and FMR, which we assumed only included  $MR_L$ . Similar assumptions have been made for large marine mammals for which FMR measurements were possible (Williams et al., 2004b). We estimated  $MR_L$  to be quite small because of the very low  $C_d$  of this streamlined animal (Fish, 1998), but we neglected other metabolic requirements, e.g. overcoming active drag due to swimming motions and thermoregulation, which could cause MR to be greater than we estimated. It remains unclear whether extrapolations of FMR to killer whales from measurements of other marine mammals (Williams et al., 2004a) are accurate, which should be a focus of future research.

The results of this study were partially based upon the physiological parameters applied to the  $O_2$  model for which exact values regarding killer whales remained uncertain. Sensitivity analyses showed that the  $O_2$  model was most sensitive to variation in the slope of  $MR_L$  versus  $U^3$  for the range of parameter values tested. Fish (1998) found a theoretical  $C_d$  for swimming killer whales which was 3.8 times lower than the  $C_d$  calculated through Eqn 3. If this lower  $C_d$  from Fish (1998) were true, then using Eqn 3 would have yielded a  $\dot{V}_{O_2}$  that was 3.8 times too large, and the overestimation of energetic requirements based on respiration rate would have been even more drastic. However, Guinet et al. (2007), using a similar theoretical approach to that of Fish (1998) to define free-ranging killer whale MR, estimated a relationship between MR and speed (see fig. 3 in Guinet et al., 2007). For a swim speed of



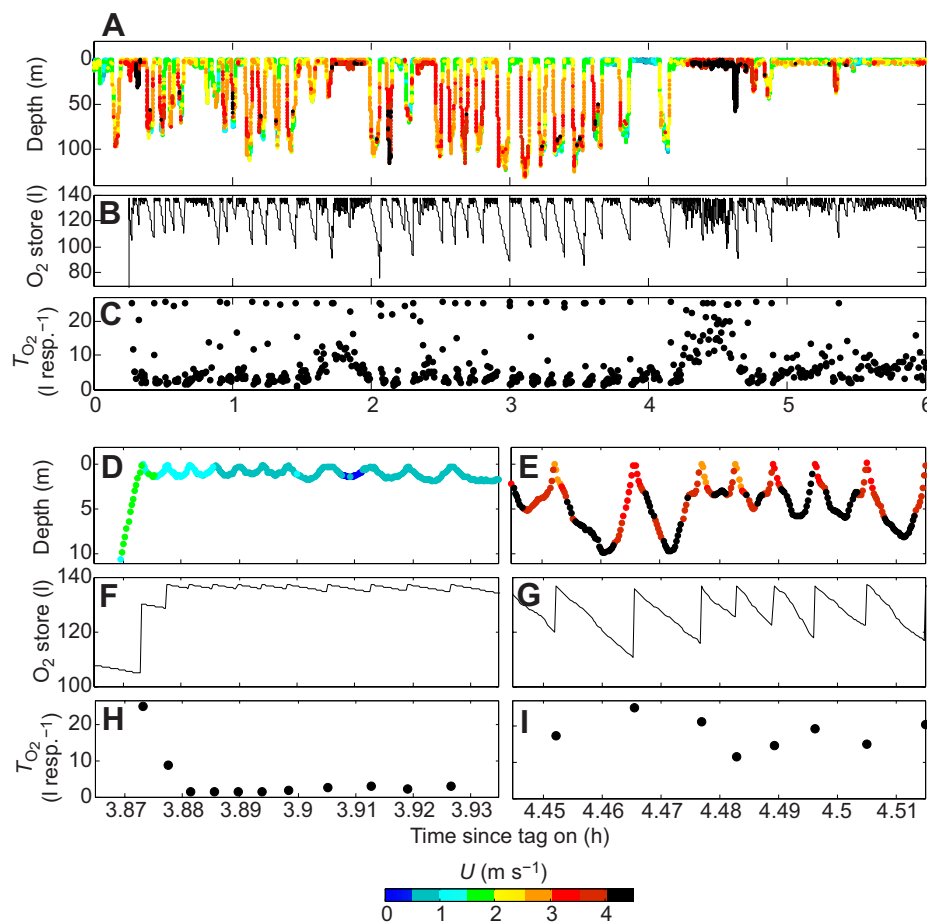
4.7 m s<sup>-1</sup> in females, this relationship predicted a  $\dot{V}_{O_2}$  that was 1.6 times higher than the  $\dot{V}_{O_2}$  estimated using model 3 of the present study for the same speed. This indicates once more the uncertainty in predicting actual MR in killer whales. During the sensitivity analyses, using extrapolated MR from data collected on other cetacean species (Otani et al., 2001; Shaffer et al., 1997; Williams et al., 1993, 2004b; O'corry-Crowe, 2009) in model 3 resulted in a higher MR than approximated using the estimated slope of MR<sub>L</sub> versus  $U^3$ . Other than for the two highest tested values of the slope of MR<sub>L</sub> versus  $U^3$ , the correlation between  $\dot{V}_{O_2}$  and  $U^3$  was not greatly affected, which suggests that our conclusions are robust to uncertainty in the values of parameters we needed to employ in our study.

### Influences of sex and activity level on respiration behaviour

Differences in the correlation between  $\dot{V}_{O_2}$  and  $U^3$  between sexes and between individuals of the same sex could be due to individual differences in physiology and behaviour. Foraging or hunting strategies will be decisive for respiration timing (Thompson et al., 2003). Species that are social and behave in groups, such as killer whales, are expected to be partly constrained by the decision making of conspecifics with diverse physiological abilities regarding body size. In all social groups to which the 10 tagged individuals belonged, at least one juvenile or calf was present. Miller et al. (2010) suggested that to maintain group cohesion, larger individuals could perform under their physiological limit (physiological compromise), or that hunting and foraging roles could be assigned to individuals according to physiological capacities (division of labour). The physiological compromise hypothesis was supported

by our result that the O<sub>2</sub> stores of the 10 adult killer whales estimated through model 3 were never fully depleted. Female energetic requirements are higher during lactation (Fedak and Anderson, 1982; Costa et al., 1986; Noren et al., 2011) and when the calf is swimming in the echelon position because of the increase in drag (Noren, 2008), factors not included in our model.

Animals make behavioural decisions, driven by their physiology and ecological context, which shape their observable diving and respiration behaviours (Fedak and Thompson, 1993; Thompson et al., 1993). During high-level activity (porpoising at higher speeds), we found that respirations were relatively equally separated in time, and respiration intervals tended to be longer than those during surfacing periods between dives (Fig. 6E). Also,  $T_{O_2}$  was greater than during low activity (Fig. 6H,I). This observation could be explained by characteristics of the O<sub>2</sub>-uptake model in combination with behavioural state. While porpoising, the animal is driven by the motivation to move fast (in this case as a response to sonar; Miller et al., 2014) and therefore respire in the most efficient way to reduce the number of surfacings, which lead to increased surface drag (Hertel, 1966; Costa and Williams, 1999). Thus, this male killer whale changed its respiration behaviour in a manner that appears to functionally increase  $T_{O_2}$  during a period of high-speed travel. High average  $T_{O_2}$  depends upon apnoea duration and MR. However, during lower activity periods,  $T_{O_2}$  was maximal for the first couple of respirations after a dive and decreased gradually according to O<sub>2</sub> store replenishment (e.g. Fig. 6F,H). A possible purpose of these later respirations could be delayed CO<sub>2</sub> offload rather than O<sub>2</sub> uptake after long dives (Boutilier et al., 2001; Wilson et al., 2003; Fahlman et al., 2008).



**Fig. 6. Time-series plots of tagged male killer whale 09\_144a.** Shown are the dive profile (A), O<sub>2</sub> store estimated with fluctuating O<sub>2</sub> uptake according to the broken-stick O<sub>2</sub>-uptake model (model 3; B) and O<sub>2</sub> uptake per respiration (C) for the first half of the tagging record. Enlarged parts of the tag record represent a low activity period (D) with corresponding O<sub>2</sub> store estimated through model 3 (F) and  $T_{O_2}$  (H), and a high activity period (porpoising at high speed; E) with corresponding O<sub>2</sub> store estimated through model 3 (G) and O<sub>2</sub> uptake per respiration (I). Colours in the dive profile indicate speed. Note that maximum total body O<sub>2</sub> store and maximum O<sub>2</sub> uptake for a male killer whale were set at 137.3 and 25.52 l, respectively.

Noren et al. (2012) concluded that apnoea of 13.3 min in an adult male killer whale did not cause a rise in blood lactate levels. The maximum apnoea durations observed in our study were 6.1 and 5.3 min for males and females, respectively; it was assumed that lactate accumulation was not an issue. Nevertheless, it is hypothesized that anaerobic metabolism would be valuable to consider for future  $O_2$  models concerning short-term anaerobic sprints or cetaceans that perform longer duration or high-activity dives that may exceed their diving lactate threshold.

### Metabolic COT

COT has been derived from respiration rate in various studies on free-ranging cetaceans in which it was generally assumed that swimming effort was reflected in respiration rate (Sumich, 1983; Rodríguez de la Gala-Hernández et al., 2008; Williams and Noren, 2009; Christiansen et al., 2014). Williams and Noren (2009) and Christiansen et al. (2014) both found a weak linear correlation between respiration rate and speed, and argued that the absence of a minimum COT was caused by the lack of high-speed observations. In our study, COT estimated through the  $O_2$  models with fixed  $T_{O_2}$  (Fig. 5B,D; model 1 and 2) also monotonically decreased with increasing speed. However, when COT was estimated by the  $O_2$  model with the broken-stick  $O_2$ -uptake function, the expected curvilinear relationship between MR and speed was found, showing a clear minimum for speeds of approximately 1.7–2.2 m s<sup>-1</sup> for females and 1.9–2.4 m s<sup>-1</sup> for males. The speed of 1.7 and 2.0 m s<sup>-1</sup> estimated from flow noise in the present study for females and males, respectively, fell within these predicted optimal COT speed ranges. However, optimal COT speed depended somewhat on the slope between  $MR_L$  versus  $U^3$ , which remained uncertain for this study.

Overall, sensitivity analyses demonstrated that the conclusions of this study were not dependent on any specific parameters in the implemented  $O_2$  model. Only for the highest values of  $k$ , for which a wide range was tested during the sensitivity analysis, was there no relationship between  $\dot{V}_{O_2}$  and  $U^3$ . For other studies inferring a curvilinear correlation between  $\dot{V}_{O_2}$ , or respiration rate, and speed, a clear minimum COT was found as well (Sumich, 1983; Williams et al., 1993; Kriete, 1995; Yazdi et al., 1999; Otani et al., 2001). Consequently, it can be concluded that it was not just the lack of high-speed observations in previous studies of free-ranging killer whale FMR (Williams and Noren, 2009) that led to monotonically decreasing COT curves. The other key factor is that the assumption of fixed  $T_{O_2}$  within broad activity categories used by Williams and Noren (2009) fails to capture higher levels of  $O_2$  uptake per respiration which were predicted to occur when whales travelled at higher speeds in our study.

### Physiological aspects of the $O_2$ model

During the present study, BMR referred to the basal metabolic rate. According to the equation established by Kasting et al. (1989), BMR would be 2.7 times higher than the values used for both sexes. Also, during other studies it was concluded that killer whale BMR was higher than predicted by the Kleiber (1975) regression (T. M. Williams, Mammalian Physiology Lab UCSC, personal communication). Guinet et al. (2007) estimated BMR using the equation provided by Motani (2002), taking into account an elevated BMR according to body mass, and obtained values similar to those used in the present study for both sexes. Extrapolated killer whale BMR measured by Worthy et al. (2014) yielded values considerably lower than those used here for both sexes. Nevertheless, the sensitivity analyses indicated that this uncertainty did not change the main conclusions of our study.

Despite uncertainties regarding  $E_{O_2}$  percentages in cetaceans, precise fixed  $E_{O_2}$  values (and  $V_T$  values) per respiration were used in free-ranging cetacean energetics studies using respirations. Assigned  $E_{O_2}$  values showed a large range between different baleen species from 11% for grey (*Eschrichtius robustus*) and humpback whales (*Megaptera novaeangliae*) (Sumich, 1983; Dolphin, 1987) to 45% for minke whales (*Balaenoptera acutorostrata*) (Blix and Folkow, 1995; Christiansen et al., 2014). Williams and Noren (2009) employed a fixed  $E_{O_2}$  of 41% and 38% for adult male and female killer whales, respectively. Kriete (1995) applied a fixed  $E_{O_2}$  per activity level, ranging from 35% to 47% and 33% to 44% for adult male and female killer whales, respectively. Also, large variations in  $E_{O_2}$  within respiratory cycles existed for captive specimens, ranging from 8% to over 80% (Irving et al., 1941; Olsen et al., 1969; Ridgway et al., 1969; Sumich, 1994; Kriete, 1995; Fahlman et al., 2016), depending on activity, respiration interval and respiration number in a sequence. Moreover, the proportion of  $O_2$  that diffuses from the lungs into the blood during apnoea can vary (Olsen et al., 1969; Kriete, 1995), with excess  $O_2$  remaining in the lungs being exhaled before inhalation. To account for this concern, mean instead of maximum  $E_{O_2}$  values were taken from Kriete (1995) and multiplied by  $V_T$  to estimate the maximum  $T_{O_2}$ .

The broken-stick  $O_2$ -uptake function predicts the  $T_{O_2}$  depending upon the  $O_2$  store at the time of each respiration. This represents a rather crude and unrealistic mechanism of how  $O_2$  exchange actually occurs in the lungs. However, we sought to assess the potential benefit of using an uptake function which has the key features of maximum  $O_2$  uptake per respiration ( $T_{O_2}$ ) when body stores are low, and very low  $T_{O_2}$  when body stores are more fully saturated. Derivation of a truly accurate uptake function was not the goal of our study, but we recommend that a more realistic uptake function could be predicted using a gas-exchange model that includes the different body tissues and lung collapse (Fahlman et al., 2006), which could further improve predictions of  $\dot{V}_{O_2}$  in free-ranging cetaceans.

### Conclusions and future work recommendations

This study showed that an  $O_2$  model including an  $O_2$ -uptake function has the potential to be a significant improvement over using fixed  $T_{O_2}$  (respiration rate) to derive consistent MR estimates from longitudinal observations of respiration times and underwater activity level in free-ranging killer whales. The assumption that respiration rate alone is an appropriate proxy for the level of MR, which is the foundation of an important part of fundamental research on free-ranging cetacean energetics, should be re-evaluated. However, it should be stressed that the presented  $O_2$  model including a broken-stick  $O_2$ -uptake function can be substantially improved with additional data. One important constraint of the presented model is the paucity of concrete measurements on  $\dot{V}_{O_2}$  in relation to speed. The model should be validated through quantifying kinematics,  $\dot{V}_{O_2}$  and respiration-by-respiration  $E_{O_2}$  and  $V_T$  to the greatest extent possible with captive killer whales (Fahlman et al., 2016). Also, the inclusion of lactate accumulation and  $CO_2$  in the gas-exchange model would be helpful for predicting alternative functions of respirations than  $O_2$  uptake alone. Future versions of the proposed model could become useful tools to quantify the metabolism of free-ranging cetaceans and inform sustainable marine ecosystem management.

### Acknowledgements

The data were collected as part of the 3S collaboration to study the effects of sonar on cetaceans. The authors wish to thank all the parties who helped to arrange and carry out the field work in which the data were collected. Thanks to Dave Thompson

for helpful input on oxygen uptake functions and T. Williams and A. Fahlman for their useful comments on the manuscript.

### Competing interests

The authors declare no competing or financial interests.

### Author contributions

M.M.H.R. developed the approach, processed and analysed the data, carried out the statistical analysis and prepared the manuscript. G.-M.W. helped with statistical analyses and revised the manuscript. P.J.O.M. conceived the study, developed the approach, supervised all components of the study and revised the manuscript. All authors gave final approval for publication.

### Funding

The 2005 fieldwork expenses were co-funded by a US–Canada Fellowship to P.J.O.M., Ocean Life Institute and National Geographic Society grants to Ari Shapiro, and WWF Sweden funding to Tiu Similä. The 2006 and 2009 data were collected as part of the 3S collaboration with funding from the US Office of Naval Research (awards N00014-08-0984, N00014-14-1-0390), the Norwegian Ministry of Defence, the Netherlands Ministry of Defence, and WWF, Norway. Funding for data analysis and manuscript preparation was from the US Office of Naval Research (award N00014-14-1-0390).

### Supplementary information

Supplementary information available online at <http://jeb.biologists.org/lookup/suppl/doi:10.1242/jeb.137513/-/DC1>

### References

- Bigg, M. A. and Wolman, A. A.** (1975). Live-capture killer whale (*Orcinus orca*) fishery, British Columbia and Washington, 1962–1973. *J. Fish. Res. Board Can.* **32**, 1213–1221.
- Blix, A. S. and Folkow, L. P.** (1995). Daily energy expenditure in free living minke whales. *Acta Physiol. Scand.* **153**, 61–66.
- Boutilier, R. G., Reed, J. Z. and Fedak, M. A.** (2001). Unsteady-state gas exchange and storage in diving marine mammals: The harbor porpoise and gray seal. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **281**, R490–R494.
- Christiansen, F., Rasmussen, M. H. and 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.
- Costa, D. P. and Williams, T. M.** (1999). Marine mammal energetics. In *Biology of Marine Mammals* (ed. J. E. Reynolds and S. A. Rommel), pp. 176–217. Washington: Smithsonian Institution Press.
- Costa, D. P., Le Boeuf, B. J., Huntley, A. C. and Ortiz, C. L.** (1986). The energetics of lactation in the Northern elephant seal, *Mirounga angustirostris*. *J. Zool.* **209**, 21–33.
- Dolphin, W. F.** (1987). Dive behavior and estimated energy expenditure of foraging humpback whales in southeast Alaska. *Can. J. Zool.* **65**, 354–362.
- Estes, J. A., Tinker, M. T., Williams, T. M. and Doak, D. F.** (1998). Killer whale predation on sea otters linking oceanic and nearshore ecosystems. *Science* **282**, 473–476.
- Fahlman, A., Olszowka, A., Bostrom, B. and Jones, D. R.** (2006). Deep diving mammals: dive behavior and circulatory adjustments contribute to bends avoidance. *Respir. Physiol. Neurobiol.* **153**, 66–77.
- Fahlman, A., Svärd, C., Rosen, D. A. S., Jones, D. R. and Trites, A. W.** (2008). Metabolic costs of foraging and the management of O<sub>2</sub> and CO<sub>2</sub> stores in Steller sea lions. *J. Exp. Biol.* **211**, 3573–3580.
- Fahlman, A., Loring, S. H., Levine, G., Rocho-Levine, J., Austin, T. and Brodsky, M.** (2015). Lung mechanics and pulmonary function testing in cetaceans. *J. Exp. Biol.* **218**, 2030–2038.
- Fahlman, A., van der Hoop, J., Moore, M. J., Levine, G., Rocho-Levine, J. and Brodsky, M.** (2016). Estimating energetics in cetaceans from respiratory frequency: why we need to understand physiology. *Biol. Open* **5**, 4, 2016, 436–442.
- Fedak, M. A. and Anderson, S. S.** (1982). The energetics of lactation: accurate measurements from a large wild mammal, the grey seal (*Halichoerus grypus*). *J. Zool.* **198**, 473–479.
- Fedak, M. A. and Thompson, D.** (1993). Behavioural and physiological options in diving seals. In *Marine Mammals: Advances in Behavioural and Population Biology*, Vol 66 (ed. I. L. Boyd), pp. 333–348. Oxford: Clarendon Press.
- Fish, F. E.** (1998). Comparative kinematics and hydrodynamics of odontocete cetaceans: morphological and ecological correlates with swimming performance. *J. Exp. Biol.* **201**, 2867–2877.
- Gallivan, G. J., Kanwisher, J. W. and Best, R. C.** (1986). Heart rates and gas exchange in the Amazonian Manatee (*Trichechus inunguis*) in relation to diving. *J. Comp. Physiol. B Biochem. Syst. Environ. Physiol.* **156**, 415–423.
- Goldbogen, J. A., Calambokidis, J., Shadwick, R. E., Oleson, E. M., McDonald, M. A. and Hildebrand, J. A.** (2006). Kinematics of foraging dives and lunge-feeding in fin whales. *J. Exp. Biol.* **209**, 1231–1244.
- Goldbogen, J. A., Calambokidis, J., Oleson, E., Potvin, J., Pyenson, N. D., Schorr, G. and Shadwick, R. E.** (2011). Mechanics, hydrodynamics and energetics of blue whale lunge feeding: Efficiency dependence on krill density. *J. Exp. Biol.* **214**, 131–146.
- Goldbogen, J. A., Calambokidis, J., Croll, D. A., McKenna, M. F., Oleson, E., Potvin, J., Pyenson, N. D., Schorr, G., Shadwick, R. E. and Tershy, B. R.** (2012). Scaling of lunge-feeding performance in rorqual whales: mass-specific energy expenditure increases with body size and progressively limits diving capacity. *Funct. Ecol.* **26**, 216–226.
- Guinet, C., Domenici, P., de Stephanis, R., Barrett-Lennard, L., Ford, J. K. B. and Verborgh, P.** (2007). Killer whale predation on bluefin tuna: exploring the hypothesis of the endurance-exhaustion technique. *Mar. Ecol. Prog. Ser.* **347**, 111–119.
- Hampton, I. F. G. and Whittow, G. C.** (1976). Body temperature and heat exchange in the Hawaiian spinner dolphin, *Stenella longirostris*. *Comp. Biochem. Physiol. A Physiol.* **55**, 195–197.
- Hampton, I. F. G., Whittow, G. C., Szekerczes, J. and Rutherford, S.** (1971). Heat transfer and body temperature in the Atlantic bottlenose dolphin, *Tursiops truncatus*. *Int. J. Biometeorol.* **15**, 247–253.
- Hertel, H.** (1966). *Structure, Form, Movement*. New York: Reinhold.
- Irving, L., Scholander, P. F. and Grinnell, S. W.** (1941). The respiration of the porpoise, *Tursiops truncatus*. *J. Cell. Comp. Physiol.* **17**, 145–168.
- Jefferson, T. A., Stacey, P. J. and Baird, R. W.** (1991). A review of killer whale interactions with other marine mammals: predation to co-existence. *Mammal. Rev.* **21**, 151–180.
- Johnson, M. P. and Tyack, P. L.** (2003). A digital acoustic recording tag for measuring the response of wild marine mammals to sound. *IEEE J. Oceanic Engng.* **28**, 3–12.
- Kasting, N. W., Adderley, S. A. L., Safford, T. and Hewlett, K. G.** (1989). Thermoregulation in beluga (*Delphinapterus leucas*) and killer (*Orcinus orca*) whales. *Physiol. Zool.* **62**, 687–701.
- Kleiber, M.** (1975). *The Fire of Life: An Introduction to Animal Energetics*. Huntington, NY: Krieger Pub Co.
- Kriete, B.** (1995). *Bioenergetics in the killer whale, Orcinus orca*. PhD thesis. University of British Columbia.
- Miller, P. J. O., Shapiro, A. D. and Deece, V. B.** (2010). The diving behaviour of mammal-eating killer whales (*Orcinus orca*): variations with ecological not physiological factors. *Can. J. Zool.* **88**, 1103–1112.
- Miller, P. J. O., Antunes, R., Alves, A. C., Wensveen, P., Kvadsheim, P., Kleivane, L., Nordlund, N., Lam, F. P., van IJsselmuide, S., Visser, F. et al.** (2011). *The 3S Experiments: Studying the Behavioural Effects of Naval Sonar on Killer Whales (Orcinus orca), Sperm Whales (Physeter macrocephalus), and Long-Finned Pilot Whales (Globicephala melas) in Norwegian Waters*. St Andrews: Scottish Oceans Institute.
- Miller, P. J. O., Antunes, R. N., Wensveen, P. J., Samarra, F. I. P., Alves, A. C., Tyack, P. L., Kvadsheim, P. H., Kleivane, L., Lam, F.-P. A., Ainslie, M. A. et al.** (2014). Dose-response relationships for the onset of avoidance of sonar by free-ranging killer whales. *J. Acoust. Soc. Am.* **135**, 975–993.
- Motani, R.** (2002). Swimming speed estimation of extinct marine reptiles: energetic approach revisited. *Paleobiology* **28**, 251–262.
- New, L. F., Moretti, D. J., Hooker, S. K., Costa, D. P. and Simmons, S. E.** (2013). Using energetic models to investigate the survival and reproduction of beaked whales (family Ziphiidae). *PLoS ONE* **8**, e68725.
- Noren, S. R.** (2008). Infant carrying behaviour in dolphins: costly parental care in an aquatic environment. *Funct. Ecol.* **22**, 284–288.
- Noren, S. R., Redfern, J. V. and Edwards, E. F.** (2011). Pregnancy is a drag: hydrodynamics, kinematics and performance in pre- and post-parturition bottlenose dolphins (*Tursiops truncatus*). *J. Exp. Biol.* **214**, 4151–4159.
- Noren, S. R., Williams, T. M., Ramirez, K., Boehm, J., Glenn, M. and Cornell, L.** (2012). Changes in partial pressures of respiratory gases during submerged voluntary breath hold across odontocetes: Is body mass important? *J. Comp. Physiol. B Biochem. Syst. Environ. Physiol.* **182**, 299–309.
- O’corry-Crowe, G. M.** (2009). Beluga Whale (*Delphinapterus leucas*). In *Encyclopedia of Marine Mammals*, 2nd edn (ed. W. F. Perrin, B. Würsig and J. G. M. Thewissen), pp. 108–112. San Diego, CA: Academic Press.
- Olsen, C. R., Hale, F. C. and Elsner, R.** (1969). Mechanics of ventilation in the pilot whale. *Respir. Physiol.* **7**, 137–149.
- Otani, S., Naito, Y., Kato, A. and Kawamura, A.** (2001). Oxygen consumption and swim speed of the harbor porpoise *Phocoena phocoena*. *Fish. Sci.* **67**, 894–898.
- Pan, W.** (2001). Akaike’s information criterion in generalized estimating equations. *Biometrics* **57**, 120–125.
- Parkes, R., Halsey, L. G., Woakes, A. J., Holder, R. L. and Butler, P. J.** (2002). Oxygen uptake during post dive recovery in a diving bird *Aythya fuligula*: implications for optimal foraging models. *J. Exp. Biol.* **205**, 3945–3954.
- Plagányi, É. E. and Butterworth, D. S.** (2009). Competition with Fisheries. In *Encyclopedia of Marine Mammals*, 2nd edn (ed. W. F. Perrin, B. Würsig and J. G. M. Thewissen), pp. 268–275. San Diego, CA: Academic Press.

- Ridgway, S. H., Scronce, B. L. and Kanwisher, J. (1969). Respiration and deep diving in the bottlenose porpoise. *Science* **166**, 1651–1654.
- Rodríguez de la Gala-Hernández, S. R., Heckel, G. and Sumich, J. L. (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.
- Scholander, P. F. and Irving, L. (1941). Experimental investigations on the respiration and diving of the Florida manatee (*Trichechus manatus*). *J. Cell. Comp. Physiol.* **17**, 169–191.
- Shaffer, S. A., Costa, D. P., Williams, T. M. and Ridgway, S. H. (1997). Diving and swimming performance of white whales, *Delphinapterus leucas*: An assessment of plasma lactate and blood gas levels and respiratory rates. *J. Exp. Biol.* **200**, 3091–3099.
- Simon, M., Johnson, M., Tyack, P. and Madsen, P. T. (2009). Behaviour and kinematics of continuous ram filtration in bowhead whales (*Balaena mysticetus*). *Proc. R. Soc. B Biol. Sci.* **276**, 3819–3828.
- Spencer, M. P., Gornall, T. A., III and Poulter, T. C. (1967). Respiratory and cardiac activity of killer whales. *J. Appl. Physiol.* **22**, 974–981.
- Sumich, J. L. (1983). Swimming velocities, breathing patterns, and estimated costs of locomotion in migrating gray whales, *Eschrichtius robustus*. *Can. J. Zool.* **61**, 647–652.
- Sumich, J. L. (1994). Oxygen extraction in free-swimming gray whale calves. *Mar. Mamm. Sci.* **10**, 226–230.
- Sumich, J. L. (2001). Direct and indirect measures of oxygen extraction, tidal lung volumes and respiratory rates in a rehabilitating gray whale calf. *Aquat. Mamm.* **27**, 279–283.
- Thompson, D., Hiby, A. R. and Fedak, M. A. (1993). How fast should I swim? Behavioural implications of diving physiology. In *Marine Mammals: Advances in Behavioural and Population Biology*, Vol 66 (ed. I. L. Boyd), pp. 349–368. Oxford: Clarendon Press.
- Thompson, D., Moss, S. E. W. and Lovell, P. (2003). Foraging behaviour of South American fur seals *Arctocephalus australis*: Extracting fine scale foraging behaviour from satellite tracks. *Mar. Ecol. Prog. Ser.* **260**, 285–296.
- Villegas-Amtmann, S., Schwarz, L. K., Sumich, J. L. and Costa, D. P. (2015). A bioenergetics model to evaluate demographic consequences of disturbance in marine mammals applied to gray whales. *Ecosphere* **6**, 183.
- Vogel, S. (1994). *Life in Moving Fluids: The Physical Biology of Flow*, 2nd edn. Princeton, NJ: Princeton University Press.
- Wahrenbrock, E. A., Maruschak, G. F., Elsner, R. and Kenney, D. (1974). Respiration and metabolism in two baleen whale calves. *Mar. Fish. Rev.* **36**, 3–9.
- Wartzok, D. (2002). Breathing. In *Encyclopedia of Marine Mammals*, 2nd edn (ed. W. F. Perrin, B. Würsig and J. G. M. Thewissen), pp. 152–156. San Diego, CA: Academic Press.
- Watanabe, Y. Y., Sato, K., Watanuki, Y., Takahashi, A., Mitani, Y., Amano, M., Aoki, K., Narazaki, T., Iwata, T., Minamikawa, S. et al. (2011). Scaling of swim speed in breath-hold divers. *J. Anim. Ecol.* **80**, 57–68.
- Williams, R. and Noren, D. P. (2009). Swimming speed, respiration rate, and estimated cost of transport in adult killer whales. *Mar. Mamm. Sci.* **25**, 327–350.
- Williams, T. M., Friedl, W. A. and Haun, J. E. (1993). The physiology of bottlenose dolphins (*Tursiops truncatus*): heart rate, metabolic rate and plasma lactate concentration during exercise. *J. Exp. Biol.* **179**, 31–46.
- Williams, T. M., Estes, J. A., Doak, D. F. and Springer, A. M. (2004a). Killer appetites: assessing the role of predators in ecological communities. *Ecology* **85**, 3373–3384.
- Williams, T. M., Fuiman, L. A., Horning, M. and Davis, R. W. (2004b). The cost of foraging by a marine predator, the Weddell seal *Leptonychotes weddellii*: Pricing by the stroke. *J. Exp. Biol.* **207**, 973–982.
- Wilson, R. P., Simeone, A., Luna-Jorquera, G., Steinfurth, A., Jackson, S. and Fahlman, A. (2003). Patterns of respiration in diving penguins: Is the last gasp an inspired tactic? *J. Exp. Biol.* **206**, 1751–1763.
- Worthy, G. A. J., Worthy, T. A. M., Yochem, P. K. and Dold, C. (2014). Basal metabolism of an adult male killer whale (*Orcinus orca*). *Mar. Mamm. Sci.* **30**, 1229–1237.
- Yazdi, P., Kilian, A. and Culik, B. M. (1999). Energy expenditure of swimming bottlenose dolphins (*Tursiops truncatus*). *Mar. Biol.* **134**, 601–607.