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RESEARCH ARTICLE

High field metabolic rates of wild harbour porpoises

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ABSTRACT

Reliable estimates of field metabolic rates (FMRs) in wild animals are essential for quantifying their ecological roles, as well as for evaluating fitness consequences of anthropogenic disturbances. Yet, standard methods for measuring FMR are difficult to use on free-ranging cetaceans whose FMR may deviate substantially from scaling predictions using terrestrial mammals. Harbour porpoises (*Phocoena phocoena*) are among the smallest marine mammals, and yet they live in cold, high-latitude waters where their high surface-to-volume ratio suggests high FMRs to stay warm. However, published FMR estimates of harbour porpoises are contradictory, with some studies claiming high FMRs and others concluding that the energetic requirements of porpoises resemble those of similar-sized terrestrial mammals. Here, we address this controversy using data from a combination of captive and wild porpoises to estimate the FMR of wild porpoises. We show that FMRs of harbour porpoises are up to two times greater than for similar-sized terrestrial mammals, supporting the hypothesis that small, carnivorous marine mammals in cold water have elevated FMRs. Despite the potential cost of thermoregulation in colder water, harbour porpoise FMRs are stable over seasonally changing water temperatures. Varying heat loss seems to be managed via cyclical fluctuations in energy intake, which serve to build up a blubber layer that largely offsets the extra costs of thermoregulation during winter. Such high FMRs are consistent with the recently reported high feeding rates of wild porpoises and highlight concerns about the potential impact of human activities on individual fitness and population dynamics.

KEY WORDS: Bio-logging, Doubly labelled water, Energetics, Food intake, *Phocoena*, Respiration

INTRODUCTION

As apex predators in the marine environment, cetaceans exert a major top-down control on trophic energy cascades and facilitate nutrient recycling (Katona and Whitehead, 1988; Roman et al., 2014). Yet, specific quantification of the ecological impact of cetaceans is still largely missing because their large size and inaccessible habitats complicate measurements of energy intake and use. Estimation of energy turnover by cetaceans in the wild requires detailed knowledge of their abundance, diet and metabolic requirements. Despite the difficulty of working in the marine environment, reliable visual, acoustic and genetic methods have been developed to estimate abundance (e.g. Hammond et al., 2013; Marques et al., 2009; Palsbøll et al., 1997). Likewise, diet data have been obtained via field observations, blubber biopsies, tag data and stomach contents (e.g. Cade et al., 2016; Lesage et al., 2010; Wisniewska et al., 2016; Ross et al., 2016). However, the third requirement, assessing the field metabolic rates (FMRs) of marine animals under conditions that are ecologically relevant, has proved much more challenging.

The metabolic rate of animals scales allometrically with body mass, with an exponent that prevails across multiple terrestrial taxa, including mammals (e.g. Kleiber, 1932; Brody, 1945; Hemmingsen, 1960; Enquist et al., 1998; Savage et al., 2004). It is therefore tempting to estimate the metabolic rates of cetaceans from scaling laws developed for their better-studied terrestrial counterparts (Yasui and Gaskin, 1986; Gallagher et al., 2018). However, numerous secondary adaptations to a life in water may complicate such extrapolations. Many studies suggest that marine mammals have elevated basal metabolic rates (BMRs) compared with terrestrial mammals of similar size, mainly due to (1) the increased cost of staying warm in water (e.g. South et al., 1976; Costa and Williams, 1999; Williams et al., 2001), a medium with much higher thermal capacity and conductivity than air, and (2) the consequences of a carnivorous diet (McNab, 1986; Williams et al., 2001; Maresh, 2014). However, many BMR measurements of marine mammals fail to meet the conditions required for measuring BMR, i.e. assessing mature individuals in a non-reproductive state, post-adsorptive at rest, and within their thermoneutral zone, and thereby overestimate the BMR of marine mammals (reviewed by Lavigne et al., 1986 and Maresh, 2014).

Irrespective of the difficulties of determining BMR in marine mammals, a more ecologically relevant measure is the FMR, i.e. the average rate of energy expenditure under field conditions. FMR studies in marine mammals suggest that the scaling exponent of the relationship between body mass and FMR is lower than for terrestrial mammals (Boyd, 2002; Costa and Maresh, 2018; Maresh, 2014); field energy expenses of the smallest marine mammals appear to be higher than predicted for similar-sized terrestrial mammals, while large marine mammals have lower energetic costs than expected. This pattern may be explained by smaller marine mammals experiencing higher mass-specific heat loss in comparison to terrestrial mammals, while large marine mammals lose relatively little heat to the environment due to their low surface-to-volume ratios, and have the benefit of lower maintenance costs compared with terrestrial mammals because cetaceans do not need to use energy to support their body weight (Boyd, 2002).

Harbour porpoises are among the smallest marine mammals and yet, they live in temperate to Arctic waters and may, therefore, represent one of the metabolic extremes in the ecologically diverse

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group of cetaceans. Despite the possibility of collecting metabolic measurements from porpoises in captivity (Kanwisher and Sundnes, 1965; Otani et al., 2001; Reed et al., 2000), there is no consensus on how their metabolic rate in the wild compares with that of similar-sized terrestrial mammals. In an early study, Kanwisher and Sundnes (1965) reported that the BMR of two captive juveniles were two to three times higher than in similar-sized terrestrial mammals, and argued that the high BMR was due to the high cost of thermoregulation in water. The notion of high metabolic rates is also supported by high food intakes reported for captive individuals (Dudok Van Heel, 1962; Andersen, 1965; Myers et al., 1978; Lockyer et al., 2003; Kastelein et al., 1990, 1997a, 2018). In a similar setting as Kanwisher and Sundnes (1965), Reed et al. (2000) measured oxygen consumption rates in captive juvenile porpoises in a small tank that rendered values two times higher than the BMR predicted from similar-sized terrestrial mammals. However, Reed et al. (2000) acknowledged that these measurements on juvenile animals in a potentially stressful situation may have overestimated the BMR, and concluded that porpoises likely do not have a higher BMR than terrestrial mammals. In line with that, several studies (Yasui and Gaskin, 1986; Otani et al., 2001; Gallagher et al., 2018) on free-ranging adult harbour porpoises have concluded that both BMR and FMR are close to those predicted from the Brody–Kleiber scaling equation (Kleiber, 1932; Brody, 1945) for terrestrial mammals. It has further been argued that studies reporting elevated food intakes in captive porpoises overestimate FMR because captive animals eat more than necessary to counter boredom (Yasui and Gaskin, 1986). In contrast, a recent study on wild porpoises has revealed high feeding rates, supporting the hypothesis that porpoises do indeed have high metabolic demands (Wisniewska et al., 2016).

As highlighted in a recent discussion between Hoekendijk et al. (2018) and Wisniewska et al. (2018a), it is critical to establish what and how much porpoises eat to determine their ecological impact as top predators on the energy flow in shallow water ecosystems, to assess their potential overlap with human fisheries and to quantify the energetic consequences of disturbance from human encroachment, such as fishing, shipping and oil exploration. Here, we address that pertinent data gap by using a novel combination of captive and field studies to test the hypothesis that porpoises have metabolic rates comparable to similar-sized terrestrial mammals.

MATERIALS AND METHODS
Study design
FMRs in wild harbour porpoises were estimated using a combination of captive and field studies. First, we measured the energy requirements of captive harbour porpoises using daily food intake records of three individuals over several years. In parallel, the FMR of one captive porpoise was measured using the doubly labelled water (DLW) method (Lifson and McClintock, 1966; Speakman, 1997), and compared with its corresponding food intake to assess food assimilation efficiency. The measured daily FMR was coupled with the daily respiration rate of this porpoise, allowing the average energy turnover of a single respiration to be calculated. We then measured respiration rates of 13 free-swimming wild porpoises tagged with a sound recorder to capture the energetic costs of a range of natural behaviours. Considering that the energetic value of a respiration is related to tidal volume, which is in turn mass related, the average energy turnover calculated for the DLW-injected individual was corrected for the size of each of the tagged wild porpoises and finally combined with the number of respirations to estimate the FMR of wild porpoises.

Captive studies
Data from captive porpoises were collected at the Fjord&Bælt centre in Kerteminde, Denmark. Three adult harbour porpoises, two females and a male: Freja (born 1995), Sif (born 2004, died 2017) and Eigil (born 1994, died 2016), were housed in an outdoor net pen of 30×20 m with an average depth of 4 m. In this open water system, the animals were exposed to natural annual temperature and light fluctuations, and therefore, faced the same thermoregulatory challenges and seasonal cues as wild porpoises in the same region. The health of each animal was regularly monitored, including multiple body measurements and daily respiration counts. The three primary body measurements used to monitor body condition were (1) standard body length (BL), taken from the tip of the jaw to the notch of the tail in a straight line parallel to the body; (2) total body mass (M_b), recorded by having the animals voluntarily beach onto a scale (Kruuse model PS250); and (3) blubber thickness, measured dorsally, laterally and ventrally in three positions along the body (see Lockyer et al., 2003 for details) by means of a portable ultrasonic subdermal fat scanner (Lean Meter by Renco). Respiration counts of all animals were sampled daily during 10 min periods, with time of the day randomly chosen between 08:00 h and 16:00 h.

The captive porpoises were fed three to four times per day. Their diet consisted primarily of mackerel (*Scomber scombus*), herring (*Clupea harengus*), sprat (*Sprattus sprattus*), capelin (*Mallotus villosus*) and sand eels (*Hyperoplus lanceolatus* and *Ammodytes tobianus*). Fish composition and mass of every feeding session were systematically recorded, and converted to caloric value following the Nordic Nutrition Recommendations (Nordic Council of Ministers 2004) at the Steins Laboratory (Eurofins Steins Laboratorium, Ladelundvej 85 DK-6600 Vejen). Briefly, to convert to caloric intake, the caloric content of the fish (cal g^{-1}) was determined for a few fish per batch using bomb calorimetry. The detailed records of caloric intake over many years allowed the animal care staff to anticipate changes in energy requirements throughout the year, and evaluate caloric needs based on appetite and motivation. Despite careful monitoring of food intake, the open pen allows entry of small fish that porpoises may occasionally have consumed, resulting in a slight underestimation of food ingestion and hence, underestimation of their energy requirements.

Doubly labelled water
The FMR of one captive harbour porpoise, Freja, was estimated using the DLW method on three occasions (December 2014, August 2015 and February 2016). An initial blood sample was collected from a fluke vein to determine background isotope values. A weighed 11–12 ml dose of sterile ^{2}H and ^{18}O water (34% and 66% enrichment, respectively; Cambridge Isotope Laboratories) was then injected into the fluke vein. The amount of ^{18}O and ^{2}H injected was selected to provide an initial enrichment of at least 220 ppm. To ensure complete equilibrium, we waited 3 h to collect the equilibrium blood sample to measure initial ^{18}O and ^{2}H levels. During the first experiment, blood samples were collected 3, 5 and 7 days post DLW injection to identify the most appropriate study duration (post values less than 50% of initial enrichment and greater than 20% background). From this, we selected a study duration of 5 days to ensure that values would remain above 20% background values even if metabolic rate increased at other times of the year. All blood samples were centrifuged at 3000 rpm within 30 min of collection. Serum was frozen at −20°C in sealed glass vials and sent to Iso-Analytical Limited (www.iso-analytical.com) for analysis.

Body composition (% lipid) was estimated using the labelled water technique (Iverson et al., 1993; Webb et al., 1998), assuming a

hydration state of 73% in lean tissue (Pace and Rathbun, 1945) and 10% in fat (Lockyer, 1995; Lockyer and Kinze, 2003). CO_2 production was calculated using the Speakman 2-pool equation as it has been determined to be the most accurate for animals >10 kg (Sparling et al., 2008). Daily energy expenditure (or daily FMR) was calculated from CO_2 production using a respiration quotient (RQ) value of 0.8, with its corresponding conversion factor of 20.1 kJ $l^{-1}O_2$. There are limited data on the RQ of harbour porpoises. Reed et al. (2000) and Boutilier et al. (2001) reported RQs of juvenile harbour porpoises that varied from 0.6 to above 1. Given that other marine mammals with similar high fat and high protein diets present RQs that range from 0.71 to 0.87 (e.g. Kooyman et al., 1981; Feldkamp, 1987; Boily and Lavigne, 1995), we estimated the RQ value for harbour porpoises to be 0.8.

Energetic value of a single breath

During the final DLW measurement (February 2016), the number of ventilations of the DLW-injected animal (Freja) and the other captive harbour porpoises were visually monitored for 20 min intervals over 24 h. During the following summer, total respirations were again visually monitored in Freja and Sif over 24 h to investigate how respiration rate varied over the course of a day and between subsequent years. Animals were temporarily marked and a few weak lights were on during the night to aid in porpoise identification and accurate data collection. To calculate the average energy turnover of a single respiration, the daily FMR measured on Freja was divided by her daily respiration count. Although tidal volume may vary with each breath, we assumed that this variation would be normally distributed when measured over long periods (24 h) and therefore, the average is considered a reliable estimate.

To apply this value to other harbour porpoises, it was necessary to adjust for the size of the animal, as tidal volume varies with size. This required first estimating the relationship between size and lung volume. Total lung capacity (TLC) is related to mass, but no specific equation has been derived for harbour porpoises. Kooyman (1989) presented a general equation describing the relationship between TLC and body mass for marine mammals, $TLC=0.1 \times M_b^{0.96}$. However, this equation may be problematic in harbour porpoises, whose body mass varies by more than 15% during the year as they alter their blubber layer (Lockyer, 1995; Kastelein et al., 2018). While lean body mass should be a more reliable measure, lean body mass estimates were not available for all the wild harbour porpoises in this study. We therefore used standard body length to estimate mass using equations estimated from a dataset of porpoise morphometric measurements obtained from bycaught and stranded porpoises from our study population (Eqns 1 and 2, see Table S1, Fig. S1 for more details):

$$M_{b,\,male} = 0.0006 \times BL^{2.25}, \tag{1}$$

$$M_{b,\,female} = 0.0001 \times BL^{2.67}. \tag{2}$$

Where BL is standard body length in cm and M_b is total body mass in kg.

By using length to estimate mass, we avoided the potential confounding effect of seasonal variation in body condition. Assuming that tidal volume scales in relation to total body mass in the same way as TLC, we created a parameter to adjust the energetic value of a respiration obtained from Freja ($E_{resp,\,Freja}$) to that of a porpoise $E_{resp,\,i}$ with different calculated mass ($M_{b,\,i}$) using the scaling exponent from Kooyman (1989):

$$E_{resp,\,i} = E_{resp,\,Freja} \times \left(\frac{M_{b,\,i}}{M_{b,\,Freja}}\right)^{0.96}. \tag{3}$$

This adjusting parameter was validated using the annual food intake and respiration rates of the other two captive harbour porpoises. Assuming that food intake relates to body mass with the same scaling exponent as FMR, and that respiration rate relates inversely, we modified Eqn 3 to predict food intake and respiration rate. We substituted $E_{resp,\,Freja}$ for either the food intake or respiration rate of Freja, and compared the prediction with the empirical data. We found that the estimated annual food intake and respiration rates differed from the actual measurements by less than 10%.

Field studies

From 2012 to 2016, we tagged 13 harbour porpoises that were incidentally caught in pound nets in the inner Danish waters (see Wisniewska et al., 2016 for details). The porpoises were lifted onto a small boat, and standard body length, girth and blubber thickness were measured to assess body condition. Handling duration was less than 15 min. Girth and blubber thickness were only collected if the animal responded well to handling (see Eskesen et al., 2009 for details). A high-resolution sound and movement recording digital tag (DTAG-3, 74×154×46 mm; Johnson et al., 2009) was attached with suction cups 5 cm behind the blowhole before the animal was lowered into the water and released. The DTAG sampled 16-bit stereo audio at 500 kHz (clip level of 179 dB re. 1 µPa), and contained a pressure sensor, tri-axial accelerometer and magnetometer sampling at 250 or 625 Hz (16 bit). The tags were set to detach passively, and recovered with the aid of Argos satellite location and/or VHF tracking.

Respiration data

Only periods where the tag did not slide from the initial tagging position were used for the analysis. Sound files were examined aurally and visually using an audit tool (www.soundtags.org) displaying spectrograms of 5 s data segments (Hamming window, FFT size 512, 75% overlap) that allow the identification of respirations. The beginning and end of each respiration (exhalation+inhalation) were clearly detectable because the tag was close to the blowhole, and manually marked for each animal. For each respiration, we calculated duration and energy flux density (EFD) [RMS of the sound pressure level data+$10\log_{10}$(respiration duration)] to analyse their frequency distribution and explore potential multimodal variations, i.e. whether there were different types of respirations in terms of the studied parameters. Respiration duration and EFD varied little within an individual and in most porpoises were normally distributed with mean duration varying from 0.52 to 0.76 s and EFD from 119 to 130 dB re. 1 μPa^2 s among individuals. Neither duration nor EFD showed a relationship to the size of the individuals. Given the normal distribution of these respiration characteristics, it was not possible to reject the hypothesis that for each porpoise all respirations came from the same distribution, indicating that there were no distinct respiration modes. Analysis of the temporal pattern of the respirations revealed that tagged porpoises had lower respiration rates during the first hour of deployment compared with the remainder of the deployment. We therefore removed the first hour of data for all tagged porpoises to avoid any effects of handling on the resulting respiration rates.

Estimation of FMR in free-ranging porpoises

Daily respiration counts were estimated for each wild porpoise deployment with estimation accuracy depending on the deployment duration. To estimate the potential upper and lower limit on daily respiration counts, we performed a sensitivity analysis to estimate the 95% variation interval of the total number of respirations for

each deployment. For each deployment duration, we took 1000 random samples of that specific duration in all deployments longer than the variation interval we wanted to estimate. From the 1000 samples, we computed the median and the 0.025 and 0.975 quantiles, and estimated the percentage of variation by dividing the difference between the quantiles and the median by the median number of respirations. We then computed both the mean and the median of the resulting percentage of variation for the lower and upper limit of each deployment duration, and used them to estimate the variation limits of each daily respiration rate estimate. For this method, we assumed that respiration variation between individuals was the same; we did not consider age or sex because of the lack of power. Assuming that the distribution of mass-specific tidal volume is the same for ventilations in captive and wild porpoises, we calculated the mass-corrected respiration energy turnover for each individual using Eqn 3, and combined it with its daily respiration count to estimate the daily FMR of each wild porpoise.

DTAG data processing was performed in MATLAB R2013b (MathWorks) and data handling was done in R (version 3.3.2) using custom tools.

Animal ethics

Captive porpoises are kept by the Fjord&Bælt centre under permit no. SVANA-610-00084 from the Danish Ministry of Food, Agriculture and Fisheries, and 1996-3446-0021 from the Danish Forest and Nature Agency (under the Danish Ministry of the Environment). Their care and all experiments were approved by the IACUC committee of Aarhus University and are in strict accordance with the recommendations of the Danish Ministry of Food, Agriculture and Fisheries (issuing the permit to keep the animals), the Danish Ministry of the Environment (issuing permit to take wild animals) and the Danish Council for Experiments on Animals. Handling and tagging of wild porpoises were carried out under permission issued to J.T. from the Danish Forest and Nature Agency (NST-3446-00016) and the Animal Welfare Division (Ministry of Justice, 2010-561-1801).

RESULTS
Energy intake in captive harbour porpoises

Compilation of data on captive animals from 2009 to 2017 showed that at the time of the study, Freja, Sif and Eigil were adult porpoises with body lengths of 156, 153 and 146 cm, respectively. Food intake of the three porpoises displayed an annual cyclic pattern with 95% of the data ranging from 15.5 to 31.3 MJ day^{-1}. Food intake typically increased steeply in mid-July, reaching a peak in mid-November. Thereafter, it decreased, reaching a minimum during April (Fig. 1). When females were pregnant, their energy intake differed from the typical pattern, with lower food intake at the beginning of pregnancy (September to February) and higher than usual at the end of pregnancy (March to July) (see Sif's food intake in Fig. 1A). In addition, food intake gradually decreased over the nine years of collected data, resulting in a decrease in annual caloric intake of 20% for both Freja (23 years old) and Sif (14 years old), potentially related to ageing. Blubber thickness measured at standard body locations fluctuated in a similar pattern to that of food intake, but delayed in time (Fig. 1B). At the mid-dorsal position (D3), 95% of the blubber thickness values reached a maximum during January and February (up to 38 mm), and a minimum during August and September (down to 17 mm). These patterns coincide with annual fluctuations in water temperature, where 95% of the data reached minimums near 0°C in January and February, and maximums near 20°C in July and August (Fig. 1).

Body composition and daily energy expenses

Using the DLW technique, we measured FMR and body condition of Freja, a non-reproductive adult female (on contraception), in December 2014, August 2015 and February 2016. Although her mass and % lipid changed significantly throughout the year, lean body mass was stable across seasons (Table 1). Similarly, estimated FMR was surprisingly stable for the three measurement periods, with the variation between measurements (range 1–14%, Table 1) being less than the estimated precision of the DLW method (Sparling et al., 2008; Dalton et al., 2014). When DLW FMR estimations and food intake (assuming 90% assimilation efficiency; Goodman-Lowe et al., 1999; Lockyer, 2007) were compared, the FMR exceeded caloric intake during February and August. The difference was greatest in August, and in contrast, caloric intake exceeded FMR during December when porpoises were still building up their blubber layer (Fig. 1B).

### Ventilations in captive porpoises

Daily respiration counts for Freja, Sif and Eigil following the DLW measurement in February 2016 were 3761, 3423 and 4263 (2.6, 2.4 and 3.0 respirations min^{-1}), respectively. Respiration rates measured in summer 2017 were within 10% of these numbers and decreased by about 23% at night compared with daytime (Fig. 2). Respiration counts over 10 min periods taken daily between 2010 and 2015, and in 2017, showed an average of 3.0 (range 2.4–3.6), 3.1 (2.5–4.0) and 3.3 (2.7–4.0) respirations min^{-1} for Freja, Sif and Eigil, respectively. Eigil became ill in late 2014, and hence, only data from before his illness were included. Respiration rates estimated from daily 10 min intervals (generally collected between 10:00 h and 15:00 h; Fig. S2) were slightly higher than those calculated during 24 h observations (Fig. S3), reflecting the effect of higher daytime respiration rates, likely influenced by specific dynamic action/heat increment of feeding (SDA/HIF) or/and human activity around the pool. The seven years of respiration data also provided the opportunity to investigate annual patterns in respiration rates. All three individuals tended to breathe at lower rates during warmer months (decrease ranged from 9 to 12%, Fig. 1 and Fig. S3), with little variation between years (Fig. S4), similar to the 10% decrease observed in the daily respiration rate from winter 2016 to summer 2017.

We estimated the average energetic value of a single respiration for Freja to be 5.2 kJ by dividing her FMR, estimated from DLW, by the number of respirations over 24 h during the same period. After adjusting this energy value for their different masses (Eqn 3), the daily FMRs of Sif and Eigil were estimated to be 17.2 MJ day^{-1} ($\dot{V}_{O_2}$: 4 ml O$_2$ kg^{-1} min^{-1}) and 17.7 MJ day^{-1} ($\dot{V}_{O_2}$: 13.0 ml O$_2$ kg^{-1} min^{-1}), respectively. To reduce the effect of seasonal changes in body condition, mass was estimated using Eqns 1 and 2 for all three porpoises.

Ventilations in wild porpoises

Ventilation data were obtained from 13 wild porpoises of both sexes and various age classes and lengths (Table 2; Fig. 3). Deployment duration, after removing the first deployment hour, ranged from 5.8 to 38.5 h.

Average daily number of respirations in wild porpoises ranged from 2824 to 6396 (Table 2). The sensitivity analysis performed to assess the potential range of variation in the estimated daily respiration counts showed that the 95% variation interval of daily respiration could range from 4 to 22% at the lower end, and from 6 to 17% at the higher end (Table S2).
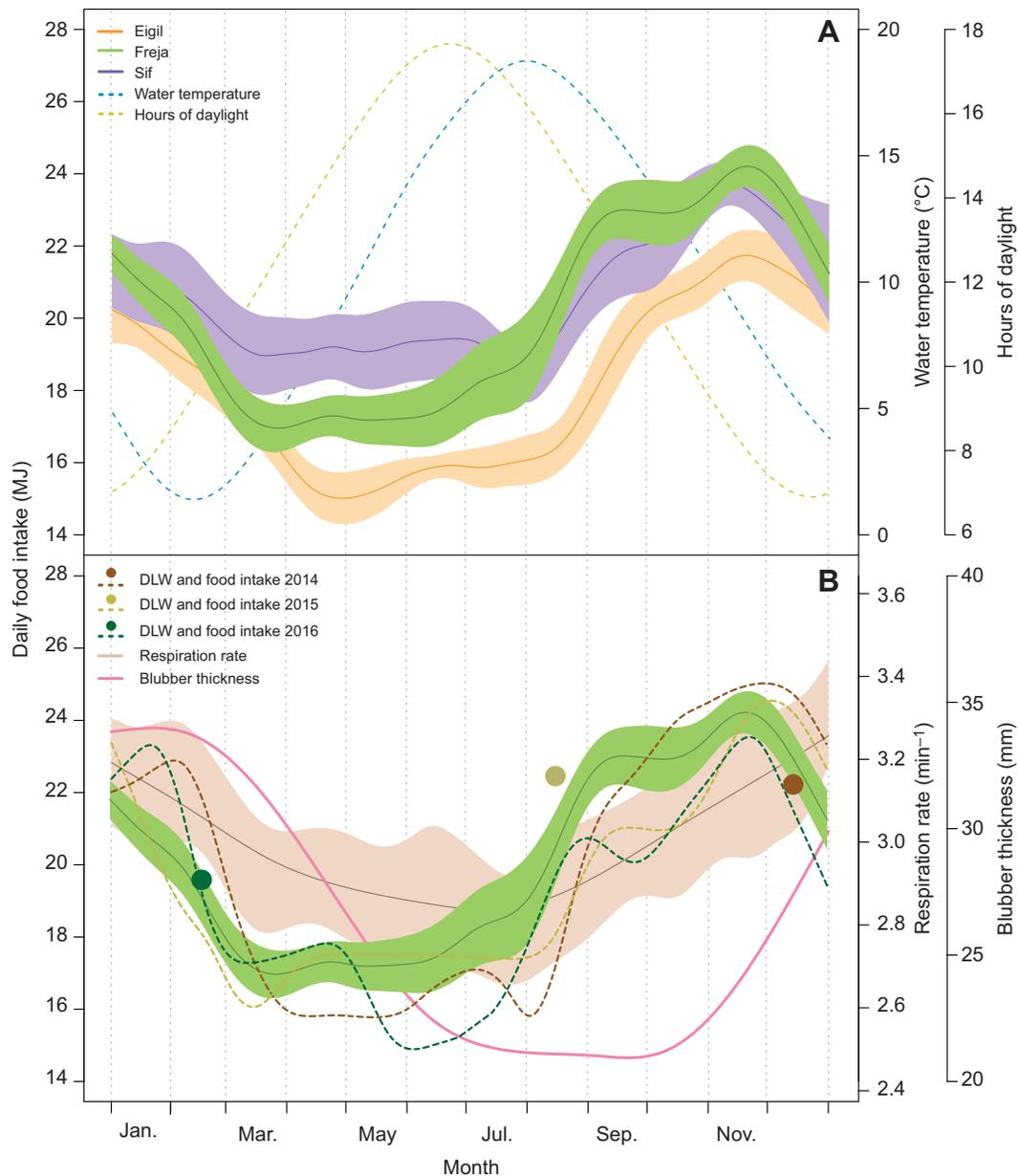
Fig. 1. Daily food intake and respiration rates from the three captive harbour porpoises across the seasons from 2009 to 2017. (A) Average daily food intake. Darker lines denote the smoothed mean for each animal, while the shaded regions around them show the s.e.m. Sif was pregnant in 2013 and 2014, increasing her average food intake between March and July. Annual number of daylight hours and water temperature are plotted for comparison. (B) Average daily food intake, respiration rate and blubber thickness at a mid-dorsal location (D3, see Lockyer et al., 2003, for details) for Freja. Food intake data are overlaid with DLW measurements, indicated with coloured dots (each colour a different year). The coloured dashed lines represent specific food intake for Freja during those years. In both panels, food intake values are corrected assuming a 90% assimilation efficiency (Goodman-Lowe et al., 1999; Lockyer, 2007).

Wild porpoise respiration rates (mean=2.9; range=2.0–4.4 respirations min^{-1}) were similar to those of captive porpoises (Fig. 2, Table 2). However, the daily pattern differed from captive porpoises with the wild porpoises having night-time respiration rates 9% higher than daytime respiration rates (Wilcoxon signed-ranks test, Z=4, P-value=0.007) (Figs 2 and 3). Both captive and wild porpoises displayed high temporal variability in respiration rate (Figs 2 and 3); but the wild porpoises also showed greater inter-individual variability probably because of differences in size and fluctuating activity levels from feeding and non-feeding (Figs 2 and 3). Despite the temporal variability in respiration rate, daily respiration rates (day^{-1}) in the wild individual with a deployment length of about 38 h, showed less than 10% difference over the

2 days, demonstrating small fluctuations in the daily respiration rate across days.

Estimation of FMR in free-ranging porpoises
Estimated FMR of wild porpoises ranged from 7.8 to 31.0 MJ day^{-1} (or 8.8 to 20.1 ml O_2 kg^{-1} min^{-1}) (Table 2). The tagged animals included both juvenile animals as well as some adults, leading to a wide range in body mass (26 to 76 kg, Table 2). Considering only the five adults, the average FMR was 21.7 MJ day^{-1} (12.1 ml O_2 kg^{-1} min^{-1}), similar to the average FMR of captive porpoises (18.2 MJ day^{-1}). Over the full dataset, the log-transformed daily FMRs and individual body mass presented a positive linear relationship with a scaling exponent of 0.9

Table 1. Total body composition and FMR estimates for Freja during the three DLW measurements

	Dec 2014	Aug 2015	Feb 2016
Water temperature (°C)	4	17	3
M_b (kg)	64	56	65
TBW (l)	31	31	30
Lean body mass (kg)	43	42	41
Fat proportion (%)	33	24	37
FMR (MJ day^{-1})	22.2	22.4	19.6
$\dot{V}_{O_2}$ (ml O$_2$ kg^{-1} min^{-1})	12.0	13.8	10.4

TBW, Total body water; M_b, body mass; $\dot{V}_{O_2}$, oxygen consumption.

(R^2=0.64, P-value=0.001, Fig. 4), which did not differ significantly from values estimated for marine mammals (~0.5; Boyd, 2002; Maresh, 2014) or terrestrial mammals (~0.7; Nagy, 2005). Comparison with the FMR predicted by the Nagy (2005) equation for mammals demonstrated that the estimated FMR of wild porpoises exceeded Nagy's predictions by an average of 28%, although this difference increases to 41% when only adults are considered (Table 2, Fig. 4). The difference between our FMR estimates for adults and the FMR predicted when only terrestrial carnivores were considered (using equation provided in Maresh, 2014) was 20% higher on average (Fig. 4). Wild porpoise FMRs were 3–4 times higher than the BMR predicted from Kleiber's equation (Kleiber, 1932) (Table 2).

DISCUSSION

Direct and indirect respirometry measurements from sea otters and small pinnipeds have led to the notion that small marine mammals have elevated FMRs to counter a high mass-specific heat loss in water (Hurley and Costa, 2001; Sparling, 2004; Yeates et al., 2007). For one of the smallest cetaceans, the harbour porpoise, some studies suggest that FMRs are similar to those of similar-sized terrestrial mammals (Yasui and Gaskin, 1986; Otani et al., 2001; Gallagher et al., 2018), but this conflicts with the high food intakes (Andersen, 1965; Dudok Van Heel, 1962; Kastelein et al., 1990, 1997a, 2018; Lockyer et al., 2003; Myers et al., 1978) and high metabolic rates (Kanwisher and Sundnes, 1965) reported for captive porpoises, and the recent reports of high feeding rates in wild porpoises (Wisniewska et al., 2016). To address this knowledge

gap, we here combine unique data from captive and wild harbour porpoises to test the hypothesis that harbour porpoises have elevated FMRs.

Our results reject the hypothesis that captive porpoises have a metabolic rate that is the same as similar-sized terrestrial mammals. Rather, we show that their FMR is higher than predicted from both terrestrial mammals in general and mammalian carnivores on land. Previous studies have speculated that captive porpoises overeat due to boredom, leading to an overestimation of the FMR of wild individuals if extrapolating from captive conspecifics (Yasui and Gaskin, 1986). However, the body composition and blubber thickness data do not support this notion; captive porpoises in our study had fat contents of 24–37% of body weight, and blubber thickness in the mid-dorsal area of 17–38 mm (Kastelein et al., 2018; Lockyer et al., 2003), closely resembling measurements from wild individuals for the same seasons and body lengths (Lockyer, 1995; Lockyer and Kinze, 2003; McLellan et al., 2002). In principle, captive porpoises could still have an elevated food intake and a low FMR if their assimilation capacities were poor compared with wild conspecifics. However, daily FMRs, as shown by the DLW results, are sometimes slightly higher than daily energy acquisition (Fig. 1), supporting high food assimilation efficiencies, as found in other marine mammals (e.g. Goodman-Lowe et al., 1999; Lockyer, 2007; Williams et al., 2004). These findings show that the captive porpoises used in this study are not fed more than necessary to meet their energetic demands, and demonstrate that captive porpoises in sea pens have approximately two times higher FMR than previously predicted for wild conspecifics (Fig. 4) (Yasui and Gaskin, 1986; Otani et al., 2001; Gallagher et al., 2018).

It has been hypothesized that the main driver of the high metabolic rate of small marine mammals is elevated heat loss in water (e.g. Kanwisher and Sundnes, 1965; Yeates et al., 2007). It follows that fluctuations in water temperature would result in seasonal fluctuations in the metabolic rate of porpoises. However, despite the large annual water temperature fluctuations at the surface, from below 0 to more than 20°C, in the inner Danish waters, the FMR measured with DLW in one captive porpoise was relatively stable over different seasons and water temperatures (Fig. 1, Table 1), and respiration rates for the three animals varied less than 15% across seasons. FMR variation was less than 14%, which was lower than the
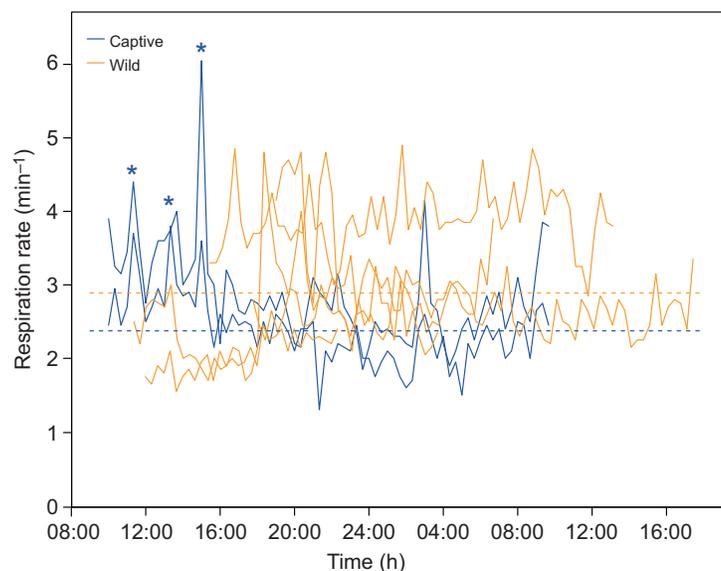
Fig. 2. Daily variation in respiration rate of adult captive and wild porpoises. The estimates are based on 20 min intervals over 24 h for captive porpoises Freja and Sif (blue solid lines), and over 24, 22, 17, 11 and 12 h, for the five adult wild porpoises (orange solid lines). For comparative purposes, only adult wild porpoises are displayed; see Table 2 and Fig. 3 for mean respiration rate and respiration pattern of all wild porpoises. Mean daily respiration rate for captive and wild porpoises are indicated by dashed lines of the same colour. The three peaks (*) in respiration rate between 10:00 h and 16:00 h in the captive porpoises coincide with the after-feeding periods, probably representing the effect of SDA/HIF.

Table 2. Morphometrics and respiration rate data for 13 tagged wild harbour porpoises

ID	Age and sex	BL (cm)	Estimated M_b (kg)	Tag on time (h)	Daily respiration rate (day^{-1})	Respiration rate (min^{-1})	Estimated FMR (MJ day^{-1})	FMR/ BMR$_{Kleiber}$	FMR/ FMR$_{TM}$	$\dot{V}_{O_2}$ (ml O$_2$ kg^{-1} min^{-1})
hp16_316a	Juvenile male	113	26	38.5	6396	4.4	15.3	4.48	1.81	20.1
hp13_102a	Juvenile male	114	27	22.7	4917 (4693–5239)	3.4	12.0 (11.4–12.8)	3.46	1.40	15.5
hp15_267a	Juvenile male	121	31	27.0	2824 (2626–3024)	2.0	7.8 (7.3–8.4)	2.05	0.83	8.8
hp12_272a	Juvenile female	122	31	20.9	3314 (3177–3525)	2.3	9.3 (8.9–9.9)	2.41	0.97	10.4
hp13_170a	Juvenile male	123	32	14.3	3407 (3128–3779)	2.4	9.8 (9.0–10.9)	2.49	1.01	10.6
hp14_226b	Juvenile male	126	34	20.7	4132 (3961–4395)	2.9	12.5 (12.0–13.3)	3.05	1.24	12.9
hp15_096a	Juvenile male	128	35	10.5	3533 (3049–3958)	2.5	11.1 (9.5–12.3)	2.63	1.07	11.0
hp14_305a	Adult male	133	38	24.3	3774 (3578–4008)	2.6	12.8 (12.2–13.6)	2.86	1.16	11.7
hp13_145a	Juvenile female	135	41	5.8	4183 (3242–4897)	2.9	15.3 (11.8–17.9)	3.22	1.31	12.9
hp15_160a	Adult female	145	49*	22.2	5620 (5379–5960)	3.9	24.6 (23.6–26.1)	4.50	1.83	17.2
hp12_293a	Adult female	163	68‡	16.7	3775 (3529–4115)	2.6	22.3 (20.9–24.4)	3.23	1.32	11.4
hp16_264a	Adult female	163	68‡	10.9	2955 (2550–3311)	2.1	17.5 (15.1–19.6)	2.53	1.03	9.0
hp15_117a	Adult female	170	76*	12.0	4703 (4134–5218)	3.3	31.0 (27.29–34.4)	4.12	1.69	14.2
Mean all animals (N=13)		135	43	19	4118	2.9	15.5	3.2	1.28	12.8
Mean adults (N=5)		155	60	17	4165	2.9	21.7	3.4	1.41	12.7

Estimated body mass was calculated based on Eqns 1 and 2. Daily FMR and 95% variation limits for each wild porpoise were estimated based on daily respiration rate and the mass-corrected average respiration turnover calculated using Eqn 3 and the sensitivity analysis. FMR$_{TM}$ was calculated based on Nagy (2005) FMR equation for mammals, and BMR$_{Kleiber}$ on Kleiber (1975). The digits in the individual IDs indicate the year and Julian day of tag deployment. Animals are sorted according to increasing standard body length (BL).
*Potentially pregnant females, mass was estimated from length and is therefore likely an underestimation.
‡Females accompanied by a calf, potentially lactating. Note that hp16_316a was the longest deployment and, because of the method used to calculate variation interval of daily respiration rates, it was not possible to use any other deployment to estimate the variation interval.

estimated precision of the DLW method, and much lower than the expected heat loss variation. Despite the rather stable FMR, we observed large fluctuations in the food intake of captive porpoises (Lockyer et al., 2003; Kastelein et al., 1997a, 2018), with energy acquisition increasing around mid-July, and peaking during mid-November (more than 50% increase relative to the minimum) after which it decreased again during late winter and spring (Lockyer et al., 2003). The increase in food intake was followed by a similar increase in blubber mass of up to 10 kg (Kastelein et al., 2018), while lean body mass remained stable (Table 1). The progressive increase in blubber thickness shows that porpoises eat more than needed to meet their FMR during late summer and autumn, and that the surplus calories are invested in thickening their blubber layer probably to counter dropping water temperatures during late autumn and winter. Once they reach a blubber layer that is approximately twice as thick as during summer, they progressively reduce caloric intake, eventually ingesting less energy than they use and hence, consuming some blubber energy stores to meet their metabolic demands during spring and early summer. From this pattern, we suggest that porpoises adjust their food intake, and consequently blubber thickness, to largely offset varying thermoregulatory costs from changing water temperatures, thereby having constant heat loss over different seasons. This hypothesis is supported by the approximate doubling in blubber thickness layer from summer to winter to match an approximate doubling in the body to water thermal gradient (Fig. 1), resulting in the same heat loss, if all other factors remain constant (Schmidt-Nielsen, 1983).

The thermo-neutral zone of harbour porpoises has not been measured; however, our findings imply that it changes seasonally, as in other small endotherms living in habitats with significant ambient temperature fluctuations, such as the arctic fox (Hart, 1956). The low variability in porpoise FMRs within a large range of water temperatures suggests that by building a thick blubber layer during the colder months, and even potentially changing their blubber heat conductivity (Koopman, 2007; Samuel and Worthy, 2004), porpoises can lower their lower critical temperature to counter the increased temperature gradient. Having a thick blubber layer all year round would increase drag and buoyancy, and therefore cost of transport, as well as perhaps the risk of overheating in warmer months, while a constant thin blubber layer would impose extremely high thermoregulatory costs in the colder months. Thus, the dynamic insulation strategy of porpoises may reflect a trade-off between the costs of keeping warm and the cost of increased drag and buoyancy.

Both captive and wild porpoises undergo the same seasonal blubber thickness fluctuations (Fig. 1, Lockyer and Kinze, 2003; McLellan et al., 2002), indicating that natural changes in weight and blubber thickness in wild, healthy porpoises are not primarily driven by temporal changes in food availability or calorific content, but rather by environmental or physiological drivers of appetite over the season. Given the temporal correlation between water temperature, energy intake and blubber thickness, and that light level fluctuations are independent of local climate fluctuations and will affect water temperature, we speculate that the changes in appetite are likely light
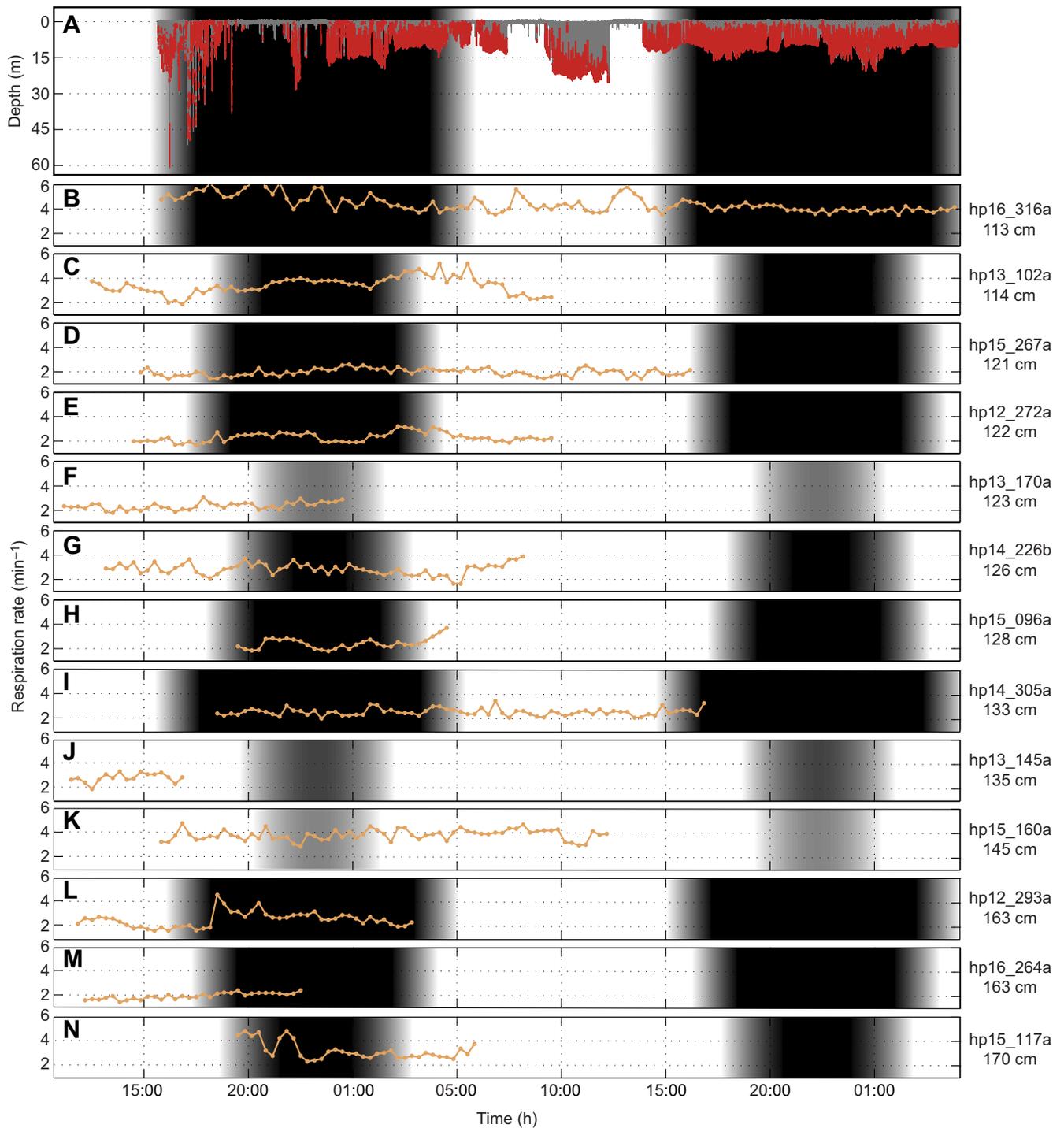
Fig. 3. Respiration rate pattern for all 13 tagged wild harbour porpoises. (A) Dive profile from the longest DTAG deployment (hp16_316a). Individual prey capture attempts (echolocation buzzes) are marked in red. (B–N) Orange lines show respiration rate (min^{-1}) averaged over 20 min periods for all 13 wild porpoises as recorded by the attached tag. The shaded area represents twilight (grey) and night (black). Animals are sorted according to increasing standard body length (indicated below ID), following the same order as Table 2.

driven (Loudon, 1994; Mrosovsky and Sherry, 1980); a topic that warrants further investigation.

The identical blubber thickness fluctuations in captive and wild porpoises also imply that both seek to stabilize FMRs over the seasonal fluctuations in water temperature. This then begs the question of whether captive and wild porpoises have similar FMRs,

or if wild porpoises have higher FMRs owing to higher activity levels from having to catch live prey. Our data suggest that captive and wild porpoises have similar FMRs (Tables 1 and 2, Fig. 4). While captive porpoises had higher respiration rates during the day (when feeding sessions occurred, Fig. 2) and wild porpoises displayed higher respiration rates at night (Figs 2 and 3), when they
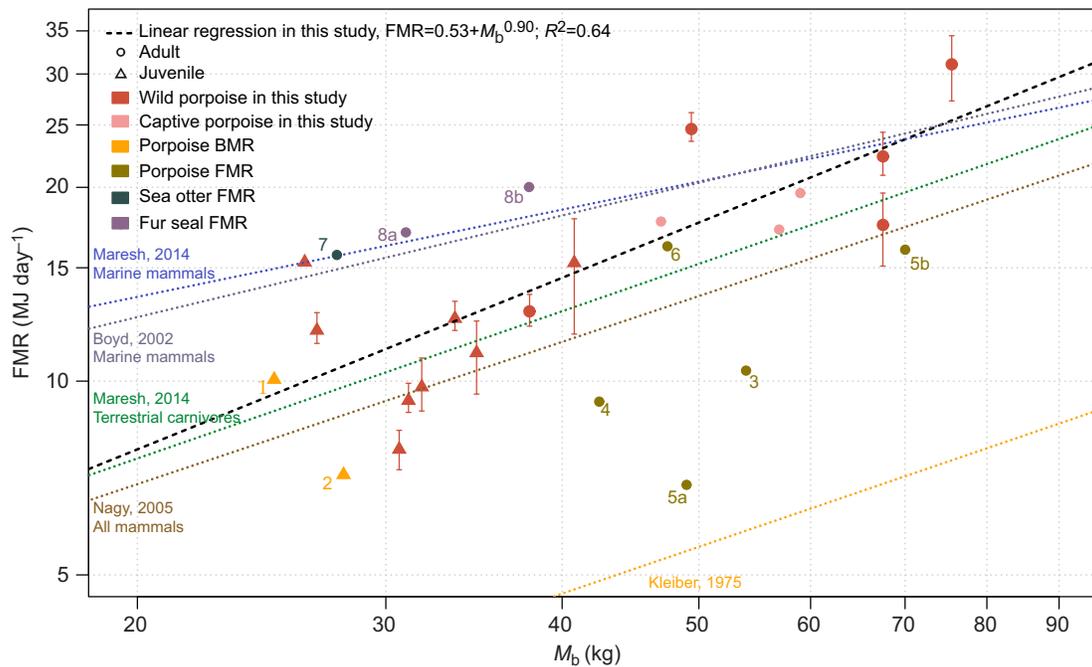
Fig. 4. Logarithmic relationship between mass and metabolic rate for harbour porpoises and other small marine mammals. The dashed black line shows the relationship from porpoises in this study. Previously proposed relationships are plotted with coloured dashed lines (citation indicated in the same colour). Previously published estimates of BMR and FMR from a variety of marine mammals are included. Numbers indicate the citation: [1]Kanwisher and Sundnes (1965); [2]Reed et al., 2000; [3]Yasui and Gaskin, 1986; [4]Otani et al., 2001; [5]Gallagher et al., 2018: [a]estimate for an adult male and [b]estimate for a pregnant and lactating female; [6]Kastelein et al., 2018; [7]Yeates et al., 2007; [8]Jeanniard-du-Dot et al., 2017: [a]estimate for Antarctic fur seal and [b]estimate for northern fur seal. See also fig. 2 in Kastelein et al., 2018.

primarily feed (Wisniewska et al., 2016), the average daily respiration rates were similar between captive and wild porpoises of similar size (Fig. 2). Given the different activity level of captive and wild porpoises, similar FMRs may indicate that the less active captive porpoises spend more energy on thermogenesis, while free-ranging porpoises can offset the cost of keeping warm by using heat produced from higher levels of muscle activity. However, the conclusion that FMRs in captive and wild porpoises are similar is critically dependent on the assumption that average tidal volume and oxygen uptake per breath is the same for porpoises of the same size. For captive porpoises, these assumptions are supported by the fact that the estimated FMRs for Sif and Eigil using mass, the size-adjusted average respiration turnover and ventilation counts are within 10% of their food intake. Remarkably similar results are obtained when using the published data from a captive adult male porpoise studied by Kastelein et al. (2018) (Fig. 4). However, because of the different lifestyles of captive and wild porpoises, the assumptions of equal tidal volume and oxygen uptake distributions between captive and wild porpoises may not be correct. Previous studies on porpoises and other cetaceans show that tidal volume increases after exercise (Ridgway et al., 1969; Fahlman et al., 2016). Wild individuals are therefore, if anything, likely to employ larger tidal volumes, especially after an intense feeding period, leading to higher oxygen use per breath, and therefore, to our underestimation of the average FMR of wild harbour porpoises.

The magnitude of this potential underestimation can be assessed by comparing the estimated energy turnover per breath for Freja with calculated lung volumes. Using an energy equivalent of 20.083 kJ l^{-1} of oxygen and assuming an average oxygen absorption of 45% (Reed et al., 2000), an animal-specific average

tidal volume can be estimated by accounting for the 21% O_2 content in air. From the average energy turnover of 5.2 kJ breath^{-1} determined for Freja, we estimate an average tidal volume of 2.7 litres. This value resembles results found in other studies of harbour porpoises (Kanwisher and Sundnes, 1965; Kastelein et al., 1997b; Kooyman and Sinnett, 1979) and represents ∼55% of the approximated total lung capacity (TLC) in a 56 kg adult based on Kooyman's (1989) equation: TLC=0.1×$M_b^{0.96}$. In comparison, the vital capacity of cetaceans has been estimated to be ∼80% of their TLC (Fahlman et al., 2015; Kooyman, 1973; Kooyman et al., 1981; Olsen et al., 1969). If tidal volumes in wild porpoises always reach vital capacity, we could be underestimating the energy turnover of each respiration by up to 30% in the most extreme of scenarios. Thus, wild porpoises could potentially have FMRs that are up to 45% higher than similar-sized conspecifics in captivity, further enforcing the finding that their FMRs are significantly higher than in terrestrial mammals of similar size.

Another possible source of error in wild porpoises that in turn may overestimate actual FMR relates to the potential effects of tagging. The addition of a tag on the body increases the drag and therefore, cost of transport (Wilson et al., 1986), leading to a higher FMR for the same behaviour (Geertsen et al., 2004). The DTAG-3 has a smooth hydrodynamic form intended to minimise drag, and a frontal area of 22 cm^2, less than 5% of the frontal area of any of the tagged porpoises. Yet, computational fluid modelling suggests that impeded water flow around the suction cups can create added drag and lift (Fiore et al., 2017). In a captive setting, tags affect swimming speed and O_2 consumption, with tagged animals swimming slower, and thereby reducing drag, or increasing their O_2 consumption when maintaining speed (van der Hoop et al., 2014, in review). Unfortunately, the extent of this effect is difficult to estimate on a free-ranging swimming animal.

We observed greater variability in the FMR estimations of wild porpoises in comparison to captive porpoises. Daily energy requirement estimations can be affected by variables such as growth, reproductive status and environmental conditions, as well as dive depth and duration, and activity level. Wild porpoises could only be tagged from March to November when pound nets are used, and therefore we were limited when evaluating seasonal changes in the FMR of wild porpoises. Yet, we did not observe any temporal pattern in FMR estimates along the 9 months in which animals were tagged, which is consistent with the relatively stable FMR of captive porpoises over season. Part of the variation could be explained by different age groups, and particularly within adults by their reproductive status. Pregnant females showed higher respiration rates than the lactating ones, matching the increase in energy intake that we and Blanchet et al. (2008) have observed in pregnant captive porpoises. Higher variability of FMR in wild animals could also be related to their much more dynamic lifestyle. Wild individuals target different prey and employ varying foraging strategies (Wisniewska et al., 2016), and are exposed to different external natural and anthropogenic stressors, such as boats and other human activities (Wisniewska et al., 2018b), that may temporarily increase their metabolic rate. Additionally, we do not know whether some of our tagged animals were sick or had any breathing anomalies. Harbour porpoises more than 1 year old often carry a substantial amount of lungworms and other parasites (Lockyer and Kinze, 2003). For instance, we observed that hp16_316a had a remarkably high respiration rate in comparison to the rest of the juveniles, resulting in a high FMR estimate (Table 2, Fig. 4).

Collectively, we conclude that previous FMR estimates for free-ranging porpoises (Yasui and Gaskin, 1986; Otani et al., 2001; Gallagher et al., 2018) are too low. Our FMR estimates are 40% higher than for terrestrial mammals of the same size, and 20% when comparing with terrestrial carnivores. Two equations have been advanced to estimate the FMR of marine mammals from mass (Boyd, 2002; Maresh, 2014), but these equations are unfortunately not phylogenetically corrected. Our results reveal that the estimated FMRs of wild adult porpoises (without the potential maximal 45% underestimation) are on average lower than predicted by these equations, but higher than predicted for a terrestrial mammal of similar size using both the equation from Nagy (2005) for all terrestrial mammals and from Maresh (2014) for carnivores only (Fig. 4). From these data, we cannot resolve whether the elevated FMRs stem from the carnivorous lifestyle of porpoises (McNab, 1986; Williams et al., 2001) and/or the relatively large heat loss from their small bodies in cold water (Kanwisher and Sundnes, 1965); the answer may well be a mix of the two. While porpoise FMRs estimated in this study are indeed higher than for terrestrial mammals, they are apparently not as high as those of small semi-aquatic marine mammals, such as sea otters (Yeates et al., 2007) and fur seals (Jeanniard-du-Dot et al., 2017) (Fig. 4). This discrepancy may of course stem from our potential underestimation of FMR related to tidal volume, and/or from differences in lean versus total body mass across these clades. However, perhaps more likely, the lower FMR estimates of porpoises may reflect that they, as fully aquatic mammals, have lower costs of transport by using lift-based propulsion compared with pinnipeds and sea otters that fully or partially employ the less efficient drag-based propulsion (Williams, 1999) and have poorer insulation. It would therefore seem that inclusion of more cetaceans in scaling studies of marine mammal metabolic rates may make the slopes deviate less from Kleiber scaling than currently presumed based on Boyd (2002) and Maresh (2014), and that small pinnipeds (mainly otariids) may require more

food than small cetaceans do per unit of mass. As such, it may be argued that scaling of FMR across different taxa is increasingly meaningless, and that we should start doing comparisons within and between clades rather than assuming that size alone can explain differences in FMR across all marine mammals with very different evolutionary trajectories (White et al., 2009).

From an ecological point of view, the FMR estimates found for free-ranging porpoises indicate that a minimum of 15 kJ of fish per minute, equivalent to 3 g of high-energy fish (5.6 kJ g^{-1}, Steimle and Terranova, 1985), need to be consumed by an adult porpoise to meet its energetic needs, implying an average daily ration of more than 8% of its body weight. This is consistent with daily food intakes of porpoises in captive settings (Lockyer et al., 2003; Kastelein et al., 1997a, 2018) where an adult porpoise eats around 1 metric tonne of fish per year. Given that porpoises in inner Danish waters appear to rely heavily on prey species that range from 3 to 10 cm (Wisniewska et al., 2016), equivalent to 0.5 to 12 g using the weight–length relationship of Coull et al. (1989), such individuals need to catch approximately 500 fish of ∼10 cm or more than 10,000 fish of ∼3 cm per day to meet their energetic requirements. These numbers are consistent with the rates of prey captures reported by Wisniewska et al. (2016, 2018a), highlighting the important ecological impact that porpoises as predators may have in the energy cascades of coastal ecosystems. Moreover, the high energy requirements and small prey choice of porpoises in inner Danish waters imply that they must spend a significant portion of their time feeding, potentially leaving little room for compensation of lost foraging opportunities due to anthropogenic disturbances (MacLeod et al., 2007; Wisniewska et al., 2018a,b). This may well be different in other areas where larger and more energetic prey are caught.

Harbour porpoises depend on blubber, both as an energy store and for insulation against the cold marine environment (Lockyer, 2007; MacLeod et al., 2007). We have shown that they rely on building a blubber layer thick enough to fully or partially offset the extra cost of thermoregulation during winter. If so, we predict that Greenlandic porpoises should have thick blubber layers year round, with less variation between summer and winter. To accumulate blubber, energy gains must exceed energy expenses. However, the period over which energy gain and expenditure must be balanced is size-dependent. Small animals, such as harbour porpoises, need to eat frequently; their fat stores in relation to their mass-specific metabolic rates is proportionally smaller than in larger marine mammals (Boyd, 2002), and therefore, the energy equilibrium must be more tightly balanced in time. A decrease in the foraging rates of harbour porpoises due to anthropogenic disturbance will reduce energy acquisition, producing a quick mobilization of energy storage (Kastelein et al., 1997a; Lockyer et al., 2003; MacLeod et al., 2007) that could directly affect their blubber thickness and hence, insulation capabilities. Loss of foraging opportunities could be particularly critical during the period when porpoises thicken their blubber layer to offset the decrease in water temperature or when water temperatures are the lowest. Reduced insulation will increase the cost of thermoregulation, resulting in higher FMR, as observed in sea otters, where changes in fur thermal insulation capacity doubled their metabolic rate (Costa and Kooyman, 1982). A continued decrease in energy intake may evoke a negative spiral of continuous mobilization of blubber energy stores and greater thermoregulatory costs that will facilitate the ultimately lethal incidence of parasites and pneumonia, complete functional depletion of energy stores and consequent death by starvation or hypothermia. Given the calorific value of blubber (38 MJ kg^{-1}; Brody, 1945) and an FMR of ∼20 MJ day^{-1}, a fasting porpoise will lose ∼1 kg of blubber every

2 days. Thereby, the 10–20 kg of blubber that adult porpoises have, depending on season (Table 1), will deplete in less than 20 to 40 days. This estimate does not include the rapidly additive costs of thermoregulation related to the continuous loss of insulation, and the finding that half of the lipid mass in harbour porpoises is structural blubber that will not be mobilized during starvation (Koopman et al., 2002). It is therefore plausible that a porpoise in cold water can starve to death in a week (Kastelein et al., 1997a), highlighting the importance of understanding the potential energetic effects of impacts of human activities on the fitness of individual porpoises and population dynamics.

Conclusion

Harbour porpoises have FMRs that are elevated in comparison to terrestrial mammals of the same size, even when comparing with terrestrial carnivores, but lower than similar-sized marine pinnipeds and mustelids. We find that harbour porpoises seem to have fairly stable high energy turnovers despite seasonal fluctuations in water temperature. By regulating their energy intake, porpoises adjust the thickness of their blubber layer over the course of the year to largely offset the extra thermoregulatory costs associated with decreasing water temperature. During autumn, they eat more than is needed to meet metabolic demands, using the surplus energy to increase their blubber layer. However, during warmer seasons, they eat less than metabolically necessary and lose unnecessary insulative blubber that incurs drag. High FMRs are consistent with the recently uncovered high feeding rates of porpoises in the same area, highlighting concerns about the potential impacts of human activities on the fitness of individuals and population dynamics due to loss of foraging opportunities.

References

Andersen, S. (1965). L'alimentation du marsouin (Phocaena phocoaena L.) en captivité. *Vie Milieu* **16**, 799-810.

Blanchet, M.-A., Nance, T., Ast, C., Wahlberg, M. and Acquarone, M. (2008). First case of a monitored pregnancy of a harbour porpoise (*Phocoena phocoena*) under human care. *Aquat. Mamm.* **34**, 9-20.

Boily, P. and Lavigne, D. M. (1995). Resting metabolic rates and respiratory quotients of gray seals (Halichoerus grypus) in relation to time of day and duration of food deprivation. *Physiol. Zool.* **68**, 1181-1193.

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.

Boyd, I. L. (2002). Energetics: consequences for fitness. In *Marine Mammal Biology: An Evolutionary Approach* (ed. A. R. Hoelzel), p. 432. Blackwell Science.

Brody, S. (1945). *Bioenergetics and Growth: With Special Reference to the Efficiency Complex in Domestic Animals*. Oxford: Reinhold Publishing Corporation.

Cade, D. E., Friedlaender, A. S., Calambokidis, J. and Goldbogen, J. A. (2016). Kinematic diversity in Rorqual whale feeding mechanisms. *Curr. Biol.* **26**, 2617-2624.

Costa, D. P. and Kooyman, G. L. (1982). Oxygen consumption, thermoregulation, and the effect of fur oiling and washing on the sea otter, Enhydra lutris. *Can. J. Zool.* **60**, 2761-2767.

Costa, D. P. and Maresh, J. L. (2018). Energetics. In *Encyclopedia of Marine Mammals* (ed. B. Würsig, J. G. M. Thewissen and K. Kovacs), pp. 329-335. Elsevier.

Costa, D. P. and Williams, T. M. (1999). Marine mammal energetics. In *The Biology of Marine Mammals* (ed. J. E. Reynolds and S. A. Rommel), pp. 176-217. Washington, DC: Smithsonian Institution Press.

Coull, K. A., Jermyn, A. S., Newton, A. W., Henderson, G. I. and Hall, W. B. (1989). *Length/Weight Relationships for 88 Species of Fish Encountered in the North East Atlantic*. Scottish Fisheries Research Report Number 43. ISSN 0308 8022. Scottish Fish. Res. Rep. 81.

Dalton, A. J. M., Rosen, D. A. S. and Trites, A. W. (2014). Season and time of day affect the ability of accelerometry and the doubly labeled water methods to measure energy expenditure in northern fur seals (Callorhinus ursinus). *J. Exp. Mar. Bio. Ecol.* **452**, 125-136.

Dudok Van Heel, W. H. (1962). Sound and Cetacea. *Netherlands J. Sea Res.* **1**, 407-507.

Enquist, B. J., Brown, J. H. and West, G. B. (1998). Allometric scaling of plant energetics and population density. *Nature* **395**, 163-165.

Eskesen, I. G., Teilmann, J., Geertsen, B. M., Desportes, G., Riget, F., Dietz, R., Larsen, F. and Siebert, U. (2009). Stress level in wild harbour porpoises (*Phocoena phocoena*) during satellite tagging measured by respiration, heart rate and cortisol. *J. Mar. Biol. Assoc. UK* **89**, 885-892.

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**, 436-442.

Feldkamp, S. D. (1987). Swimming in the California sea lion: morphometrics, drag and energetics. *J. Exp. Biol* **131**, 117-135.

Fiore, G., Anderson, E., Garborg, C. S., Murray, M., Johnson, M., Moore, M. J., Howle, L. and Shorter, K. A. (2017). From the track to the ocean: using flow control to improve marine bio-logging tags for cetaceans. *PLoS ONE* **12**, e0170962.

Gallagher, C. A., Stern, S. J. and Hines, E. (2018). The metabolic cost of swimming and reproduction in harbor porpoises (*Phocoena phocoena*) as predicted by a bioenergetic model. *Mar. Mammal Sci.* **34**, 875-900.

Geertsen, B. M., Teilmann, J., Kastelein, R. A., Vlemmix, H. N. J. and Miller, L. A. (2004). Behaviour and physiological effects of transmitter attachments on a captive harbour porpoise (*Phocoena phocoena*). *J. Cetacean Res. Manag.* **6**, 139-146.

Goodman-Lowe, G. D., Carpenter, J. R. and Atkinson, S. (1999). Assimilation efficiency of prey in the Hawaiian monk seal (*Monachus schauinslandi*). *Can. J. Zool.* **77**, 653-660.

Hammond, P. S., Macleod, K., Berggren, P., Borchers, D. L., Burt, L., Cañadas, A., Desportes, G., Donovan, G. P., Gilles, A., Gillespie, D. et al. (2013). Cetacean abundance and distribution in European Atlantic shelf waters to inform conservation and management. *Biol. Conserv.* **164**, 107-122.

Hart, J. S. (1956). Seasonal changes in insulation of the fur. *Can. J. Zool.* **34**, 53-57.

Hemmingsen, A. M. (1960). *Energy metabolism as related to body size and respiratory surfaces, and its evolution*. Report of the Steno Memorial Hospital and Nordisk Insulin Laboratorium (Copenhagen) **9**, 1-110.

Hoekendijk, J. P. A., Spitz, J., Read, A. J., Leopold, M. F., Fontaine, M. C. (2018). Resilience of harbor porpoises to anthropogenic disturbance: must they really feed continuously? *Mar. Mammal Sci.* **34**, 258-264.

Hurley, J. A. and Costa, D. P. (2001). Standard metabolic rate at the surface and during trained submersions in adult California sea lions. *J. Exp. Biol.* **204**, 3273-3281.

Iverson, S. J., Bowen, W. D., Boness, D. J. and Oftedal, O. T. (1993). The effect of maternal size and milk energy output on pup growth in grey seals (*Halichoerus grypus*). *Physiol. Zool.* **66**, 61-88.

Jeanniard-du-Dot, T., Guinet, C., Arnould, J. P. Y., Speakman, J. R. and Trites, A. W. (2017). Accelerometers can measure total and activity-specific energy

expenditures in free-ranging marine mammals only if linked to time-activity budgets. *Funct. Ecol.* **31**, 377-386.

Johnson, M., Aguilar de Soto, N. and Madsen, P. T. (2009). Studying the behaviour and sensory ecology of marine mammals using acoustic recording tags: a review. *Mar. Ecol. Process Ser.* **395**, 55-73.

Kanwisher, J. and Sundnes, G. (1965). Physiology of a small cetacean. *Hvalrad. Skr.* **48**, 45-63.

Kastelein, R. A., Bakker, M. J. and Dokter, T. (1990). The medical treatment of 3 stranded harbour porpoises (Phocoena phocoena). *Aquat. Mamm.* **15**, 181-202.

Kastelein, R. A., Hardeman, J. and Boer, H. (1997a). Food consumption and body weight of harbour porpoises. In *The Biology of the Harbour Porpoise* (ed. A. J. Read, P. R. Wiepkema and P. E. Nachtigall), pp. 217-233. Woerden, The Netherlands: De Spil Publishers.

Kastelein, R. A., Vaughan, N., Groenenberg, H. J., Boekholt, H. A. and Schreurs, V. V. A. M. (1997b). Respiration in harbour porpoises. In *The Biology of the Harbour Porpoise* (ed. A. J. Read, P. R. Wiepkema and P. E. Nachtigall), pp. 203-215. Woerden, The Netherlands: De Spil Publishers.

Kastelein, R. A., Helder-Hoek, L. and Jennings, N. (2018). Seasonal changes in food consumption, respiration rate, and body condition of a male harbor porpoise (*Phocoena phocoena*). *Aquat. Mamm.* **44**, 76-91.

Katona, S. and Whitehead, H. W. (1988). Are cetaceans ecologically important? *Ocean. Mar. Biol. Annu. Rev.* **26**, 553-568.

Kleiber, M. (1932). Body size and metabolism. *Hilgardia* **6**, 315-353.

Kleiber, M. (1975). *The Fire of Life: an Introduction to Animal Energetics*, 2nd edn. Kreiger, Huntington.

Koopman, H. N. (2007). Phylogenetic, ecological, and ontogenetic factors influencing the biochemical structure of the blubber of odontocetes. *Mar. Biol.* **151**, 277-291.

Koopman, H. N., Pabst, D. A., McLellan, W. A., Dillaman, R. M. and Read, A. J. (2002). Changes in blubber distribution and morphology associated with starvation in the Harbor Porpoise (*Phocoena phocoena*): evidence for regional differences in blubber structure and function. *Physiol. Biochem. Zool.* **75**, 498-512.

Kooyman, G. L. (1973). Respiratory adaptations in marine mammals. *Am. Zool.* **13**, 457-468.

Kooyman, G. L. (1989). *Diverse Divers: Physiology and Behaviour. Zoophysiology*, Vol. 23. Springer.

Kooyman, G. L. and Sinnett, E. E. (1979). Mechanical properties of the harbor porpoise lung, Phocoena phocoena. *Respir. Physiol.* **36**, 287-300.

Kooyman, G. L., Castellini, M. A. and Davis, R. W. (1981). Physiology of diving in marine mammals. *Annu. Rev. Physiol.* **43**, 343-356.

Lavigne, D. M., Innes, S., Worthy, G. A. J., Kovacs, K. M., Schmitz, O. J. and Hickie, J. P. (1986). Metabolic rates of seals and whales. *Can. J. Zool.* **64**, 279-284.

Lesage, V., Morin, Y., Riox, È., Pomerleau, C., Ferguson, S. H. and Pelletier, É. (2010). Stable isotopes and trace elements as indicators of diet and habitat use in cetaceans: predicting errors related to preservation, lipid extraction, and lipid normalization. *Mar. Ecol. Prog. Ser.* **419**, 249-265.

Lifson, N. and McClintock, R. (1966). Theory of use of the turnover rates of body water for measuring energy and material balance. *J. Theor. Biol.* **12**, 46-74.

Lockyer, C. (1995). Aspects of the morphology body fat condition and biology of the Harbour porpoise, Phocoena phocoena, in British Waters. *Rep. Int. Whal. Commn.*, 199-209.

Lockyer, C. (2007). All creatures great and smaller: a study in cetacean life history energetics. *J. Mar. Biol. Assoc. UK* **87**, 1035-1045.

Lockyer, C. and Kinze, C. (2003). Status, ecology and life history of harbour porpoise (Phocoena phocoena), in Danish waters. *NAMMCO Sci. Publ.* **5**, 143-176.

Lockyer, C., Desportes, G., Hansen, K., Labberté, S. and Siebert, U. (2003). Monitoring growth and energy utilisation of the harbour porpoise (Phocoena phocoena) in human care. *NAMMCO Sci. Publ.* **5**, 107-120.

Loudon, A. S. I. (1994). Photoperiod and the regulation of annual and circannual cycles of food intake. *Proc. Nutr. Soc.* **53**, 495-507.

MacLeod, C. D., Santos, M. B., Reid, R. J., Scott, B. E. and Pierce, G. J. (2007). Linking sandeel consumption and the likelihood of starvation in harbour porpoises in the Scottish North Sea: could climate change mean more starving porpoises? *Biol. Lett.* **3**, 185-188.

Maresh, J. L. (2014). Bioenergetics of marine mammals: the influence of body size, reproductive status, locomotion and phylogeny on metabolism. PhD Thesis, University of California, Santa Cruz.

Marques, T. A., Thomas, L., Ward, J., DiMarzio, N. and Tyack, P. L. (2009). Estimating cetacean population density using fixed passive acoustic sensors: an example with Blainville's beaked whales. *J. Acoust. Soc. Am.* **125**, 1982-1994.

McLellan, W. A., Koopman, H. N., Rommel, S. A., Read, A. J., Potter, C. W., Nicolas, J. R., Westgate, A. J. and Pabst, D. A. (2002). Ontogenetic allometry and body composition of harbour porpoises (*Phocoena phocoena*, L.) from the western North Atlantic. *J. Zool.* **257**, 457-471.

McNab, B. K. (1986). The influence of food habits on the energetics of Eutherian mammals. *Source Ecol. Monogr.* **56**, 1-19.

Mrosovsky, N. and Sherry, D. F. (1980). Animal anorexias. *Science* **207**, 837-842.

Myers, W. A., Horton, H. C., Heard, F. A., Jones, A., Winsett, G. and McCulloch, S. A. (1978). The role of recorded data in acclimatizing a harbour porpoise (*Phocoena phocoena*). *Aquat. Mamm.* **6**, 54-64.

Nagy, K. A. (2005). Field metabolic rate and body size. *J. Exp. Biol.* **208**, 1621-1625.

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.

Pace, N. and Rathbun, E. N. (1945). Studies on body composition: III. The body water and chemically combined nitrogen content in relation to fat content. *J. Biol. Chem.* **158**, 685-691.

Palsbøll, P. J., Allen, J., Bérubé, M., Clapham, P. J., Feddersen, T. P., Hammond, P. S., Hudson, R. R., Jørgensen, H., Katona, S., Larsen, A. H. et al. (1997). Genetic tagging of humpback whales. *Nature* **388**, 767-769.

Reed, J. Z., Chambers, C., Hunter, C. J., Lockyer, C., Kastelein, R., Fedak, M. A. and Boutilier, R. G. (2000). Gas exchange and heart rate in the harbour porpoise, *Phocoena phocoena*. *J. Comp. Physiol. B* **170**, 1-10.

Ridgway, S. H., Scronce, B. L. and Kanwisher, J. (1969). Respiration and deep diving in the bottlenose porpoise. *Science* **166**, 1651-1654.

Roman, J., Estes, J. A., Morissette, L., Smith, C., Costa, D., McCarthy, J., Nation, J. B., Nicol, S., Pershing, A. and Smetacek, V. (2014). Whales as marine ecosystem engineers. *Front. Ecol. Environ.* **12**, 377-385.

Ross, S. D., Andreasen, H. and Andersen, N. G. (2016). An important step towards accurate estimation of diet composition and consumption rates for the harbor porpoise (*Phocoena phocoena*). *Mar. Mammal Sci.* **32**, 1491-1500.

Samuel, A. M. and Worthy, G. A. (2004). Variability in fatty acid composition of bottlenose dolphin (*Tursiops truncatus*) blubber as a function of body site, season, and reproductive state. *Can. J. Zool.* **82**, 1933-1942.

Savage, V. M., Gillooly, J. F., Woodruff, W. H., West, G. B., Allen, A. P., Enquist, B. J. and Brown, J. H. (2004). The predominance of quarter-power scaling in biology. *Funct. Ecol.* **18**, 257-282.

Schmidt-Nielsen, K. (1983). *Animal Physiology: Adaptation and Environment*. Cambridge, Scholander: Cambridge University Press.

South, F. E., Luecke, R. H., Zatzman, M. L. and Shanklin, M. D. (1976). Air temperature and direct partitional calorimetry of the California sea lion (Zalophus Californianus). *Comp. Biochem. Physiol. A Physiol.* **54**, 27-30.

Sparling, C. E. (2004). Metabolic rates of captive grey seals during voluntary diving. *J. Exp. Biol.* **207**, 1615-1624.

Sparling, C. E., Thompson, D., Fedak, M. A., Gallon, S. L. and Speakman, J. R. (2008). Estimating field metabolic rates of pinnipeds: doubly labelled water gets the seal of approval. *Funct. Ecol.* **22**, 245-254.

Speakman, J. R. (1997). *Double Labelled Water: Theory and Practice*. Chapman and Hall.

Steimle, F. W., Jr. and Terranova, R. J. (1985). Energy equivalents of marine organisms from the continental shelf of the temperate Northwest Atlantic. *J. Northwest Atl. Fish. Sci.* **6**, 117-124.

van der Hoop, J. M., Fahlman, A., Hurst, T., Rocho-Levine, J., Shorter, K. A., Petrov, V. and Moore, M. J. (2014). Bottlenose dolphins modify behavior to reduce metabolic effect of tag attachment. *J. Exp. Biol.* **217**, 4229-4236.

Webb, P. M., Crocker, D. E., Blackwell, S. B., Costa, D. P. and Boeuf, B. J. (1998). Effects of buoyancy on the diving behavior of northern elephant seals. *J. Exp. Biol.* **201**, 2349-2358.

White, C. R., Blackburn, T. M. and Seymour, R. S. (2009). Phylogenetically informed analysis of the allometry of mammalian basal metabolic rate supports neither geometric nor quarter-power scaling. *Evolution* **63**, 2658-2667.

Williams, T. M. (1999). The evolution of cost efficient swimming in marine mammals: limits to energetic optimization. *Philos. Trans. R. Soc. B Biol. Sci.* **354**, 193-201.

Williams, T. M., Haun, J., Davis, R. W., Fuiman, L. A. and Kohin, S. (2001). A killer appetite: metabolic consequences of carnivory in marine mammals. *Comp. Biochem. Physiol.* **129**, 785-796.

Williams, T. M., Estes, J. A., Doak, D. F. and Springer, A. M. (2004). Killer appetites: assessing the role of predators in ecological communities. *Ecology* **85**, 3373-3384.

Wilson, R. P., Grant, W. S. and Duffy, D. C. (1986). Recording devices on free-ranging marine animals: does measurement affect foraging performance? *Ecology* **67**, 1091-1093.

Wisniewska, D. M., Johnson, M., Teilmann, J., Rojano-Doñate, L., Shearer, J., Sveegaard, S., Miller, L. A., Siebert, U. and Madsen, P. T. (2016). Ultra-high foraging rates of harbor porpoises make them vulnerable to anthropogenic disturbance. *Curr. Biol.* **26**, 1441-1446.

Wisniewska, D. M., Johnson, M., Teilmann, J., Siebert, U., Galatius, A., Dietz, R. and Madsen, P. T. (2018a). High rates of vessel noise disrupt foraging in wild harbour porpoises (Phocoena phocoena). *Proc. R. Soc. B* **285**, 20172314.

Wisniewska, D. M., Johnson, M., Teilmann, J., Rojano-Doñate, L., Shearer, J., Sveegaard, S., Miller, L. A., Siebert, U. and Madsen, P. T. (2018b). Response to "Resilience of harbor porpoises to anthropogenic disturbance: must they really feed continuously?". *Mar. Mammal Sci.* **34**, 265-270.

Yasui, W. Y. and Gaskin, D. E. (1986). Energy budget of a small Cetacean, the harbour Porpoise, *Phocoena phocoena* (L.). *Ophelia* **25**, 183-197.

Yeates, L. C., Williams, T. M. and Fink, T. L. (2007). Diving and foraging energetics of the smallest marine mammal, the sea otter (*Enhydra lutris*). *J. Exp. Biol.* **210**, 1960-1970.