

Heat Increment of Feeding in the Common bottlenose dolphin *(Tursiops truncatus)*

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List of abbreviations

\dot{V}_{O_2} = oxygen consumption rate

\dot{V}_{CO_2} = carbon dioxide production rate

MR = Metabolic Rate

BMR = Basal Metabolic Rate

RMR = Resting Metabolic Rate

FMR = Field Metabolic Rate

TEE = Total Energy Expenditure

HIF = Heat Increment of Feeding

LC = Locomotor Cost

RER = Respiratory Exchange Ratio

1. Abstract

With digestion comes an increase in metabolism, known as heat increment of feeding, due to the mechanical and biochemical processes that take place after the ingestion of a meal. In this study I used respirometry to measure the oxygen consumption rate pre- and post-feeding in 8 common bottlenose dolphins (*Tursiops truncatus*) under human care in Kolmarden Zoo, Sweden and Oceanografic Aquarium, Spain. I measured the post-absorptive (>15 h following the last meal) and the post-prandial (30, 60, 90 and 120 min following a meal) rate of O₂ consumption after ingestion of a predetermined quantity of food (1659-2658 kcal), which consisted of capelin and herring. The mean post-absorptive basal metabolic rate was 4.03 ± 0.81 ml O₂ min⁻¹ kg⁻¹, 1.6 times Kleiber. I observed an increase between 31-61% over BMR, which reached a peak at around 60 minutes after feeding and then decreased towards pre-feeding levels at around 120 minutes. The heat increment of feeding, as a component of energy intake, provides a valuable tool for creating energetic models and predicting prey consumption in marine mammals.

Key words: bioenergetic models, bottlenose dolphin (*Tursiops truncatus*), digestion, heat increment of feeding, marine mammals, post-prandial metabolic rate, spirometry

2. Introduction

2.1. Bioenergetic models

Understanding the energy dynamics of marine mammals offers insights into their behaviour, foraging efficiency, dive performance, and ecological impact on prey (McHuron et al., 2018; Bejarano et al., 2017; Acquarone et al., 2006; Benoit-Bird, 2004; Williams et al., 2004; Costa & Gales, 2003). Individuals must balance energy intake and expenditure, allocating resources for somatic maintenance, growth, reproduction, and physical activity to optimize fitness (Gadgil & Bossert, 1970). For diving marine mammals, that balance is more complicated, as they have to acquire more energy from prey than spent in hunting and simultaneously cope with limited oxygen availability underwater (Dunstone & O'Connor, 1979). Energetic efficiency while foraging underwater is crucial, and it requires careful management of oxygen reserves and metabolic rates (Kooyman, 1989), as the total time spent on a single dive crucially influences the types of prey that can be exploited and the efficiency with which food is obtained (Fahlman et al., 2018a). This can be further accentuated due to climate change, as this alters the distribution, abundance and structure of prey communities (Learmonth et al., 2006).

Bioenergetic models combine biotic and abiotic influences and predict the mutual relationship between energy expenditure and energy intake, varying from simple equations representing average energy spending, to detailed energy budgets for different age classes, sexes and seasons, to even more intricate models that take into account changes in the environment (Booth et al., 2023). The rate of energy intake from the environment and its expenditure in different life functions affects the animal's ability to survive, grow and reproduce. Utilizing the principles of metabolic ecology, bioenergetic models capture the mechanisms behind individual energy management and in turn can help quantify larger scale processes that impact ecological communities (Pirota, 2022). These can include overall resource consumption by populations (Delmas et al., 2017), interspecies competition (Caut et al., 2006), changing environmental conditions (Humphries et al., 2004) and trait-driven interactions between predators and prey (Werner & Peacor, 2003). As the energy acquisition from the environment is susceptible to human disturbance, bioenergetic models are important in predicting the consequences of exposure to anthropogenic stressors (Costa et al., 2016).

The acquisition of empirical measurements of individual and population level effects in marine mammals is often challenging due to their life history characteristics, as they are usually elusive, large, wide-ranging and difficult to capture animals. At the same time, as human disturbance increases in the changing oceans, so does the threat on the persistence of these species. Thus, bioenergetic modelling becomes of outmost importance in marine mammal science as a way to detect and alleviate these threats through effective management and conservation for both marine mammals and their prey (Pirodda, 2022; Bejarano et al., 2017). Previously, bioenergetic models have been used for example to analyse the energy budgets of baleen whales (Lockyer, 1981) and pinnipeds (Lavigne et al., 1982) or to predict the level of disturbance for a female pilot whale (*Globicephala melas*) that would lead to population decline (Hin et al., 2019).

2.2. Metabolic rates

The foundation of bioenergetic models lies in the energetic cost of running the animal, in other words the animal's metabolic rate, which represents the total energy expenditure (TEE) of an animal in a specific set of circumstances (Noren & Rosen, 2023). The basal metabolic rate (BMR) represents the basal energy expenditure of an animal that includes the basic physiological costs associated with survival such as maintaining blood flow and cellular respiration. In order to be measured, BMR requires specific criteria to be met; animals need to be in a resting (awake but quiescent), post-absorptive (fasting), mature (non-growing) and non-reproductive (non-pregnant, non-lactating) state, within their thermoneutral zone (Kleiber, 1975). However, meeting all the criteria is seldom possible and, in these cases, the resting metabolic rate (RMR) is used. With RMR, the definition of the conditions of the measurements is possible and as a result, they can be obtained from immature or even reproductive animals (He et al., 2023).

A more ecologically relevant measure of energy expenditure is the field metabolic rate (FMR), which is the sum of all energy used over a given duration for an individual and includes the baseline energy consumption of the animal (i.e., BMR) plus the additional energy required as the animal moves and survives in its habitat, like the locomotor cost (LC) and the cost of digestion (known as heat increment of feeding [HIF]) (Fahlman et al., 2024; Noren & Rosen, 2023; Nagy, 2005; 1994). FMRs present variability both between and within the species and are associated with year-to-year changes in the abundance and availability of prey (Costa, 2007). The most common method to measure FMR is the use of doubly labelled

water, however for marine mammals this can prove challenging as it involves capture and restraint of the individuals (Iverson et al., 2010). Nevertheless, other approaches have been used, such as accelerometer-derived activity budgets, proxies from heart-rate or counting the number of breaths, in order to make the measurement of FMR easier for the breath-hold diving and free-ranging cetaceans (Fahlman et al., 2016). As TEE, FMR provides insight into the energetic strategies used by marine mammals (Costa, 2007), however in most species it is difficult to estimate since neither BMR, HIF nor LC are known (Fahlman et al., 2024).

2.3. Heat increment of feeding

2.3.1. Definition

Early studies on humans reported an increase in metabolism after the ingestion of a meal, which was described as the ‘work of digestion’ (Kleiber, 1961). The studies that followed, which were conducted across multiple taxa, recorded a rapid post-prandial increase in metabolic rate, which reaches a peak and then slowly returns to pre-feeding levels (Secor, 2009). Several terms have been created to describe this physiological phenomenon, such as specific dynamic action (SDA), dietary induced thermogenesis (DIT), post-prandial thermogenesis (PPT), thermogenic effects of feeding (TEF), thermic effect of a meal (TEM) or heat increment of feeding (HIF) (Secor, 2009; James, 1992; Beamish & Trippel, 1990; Kleiber, 1961), which will be the term used to describe the cost of digestion in this thesis. The increase in metabolism, and therefore in oxygen consumption, is thought to be a result of the mechanical and biochemical processes that accompany the ingestion, digestion, absorption and assimilation of a meal (Secor, 2009; Blaxter, 1989), as not all of the energy obtained through food is available for growth and maintenance to the organism, but some is lost in the digestive process (D. A. Rosen & Trites, 1997).

2.3.2. Measurement

Studies have shown that HIF cannot be calculated directly by knowing the composition of the animal’s diet (Hoch, 1971), so comparisons of the post-absorptive and post-prandial metabolic rates are used to estimate the changes (Dassis et al., 2014). The metabolic rates can be determined by either direct or indirect calorimetry, which in the first case quantifies the energy spent from the measured heat production (McCollum et al., 2006; Smith & Houlihan, 1995) and in the latter, from the rates of respiratory gas exchange (Secor, 2009). Therefore,

the oxygen consumption rate (\dot{V}_{O_2}), and/or carbon dioxide production rate (\dot{V}_{CO_2}), is a common method to measure the metabolic rate (Weissman et al., 1985).

After establishing a baseline metabolic rate, the animal is provided with a natural or formulated meal, so that the individual is satiated or that it corresponds to a targeted percentage of its body mass. Then the metabolic rates are calculated either continuously or at intervals, in order to create a profile of the post-prandial response, which is usually depicted as a graph with the metabolic rate plotted against the time post-feeding (Figure 1; Secor, 2009).

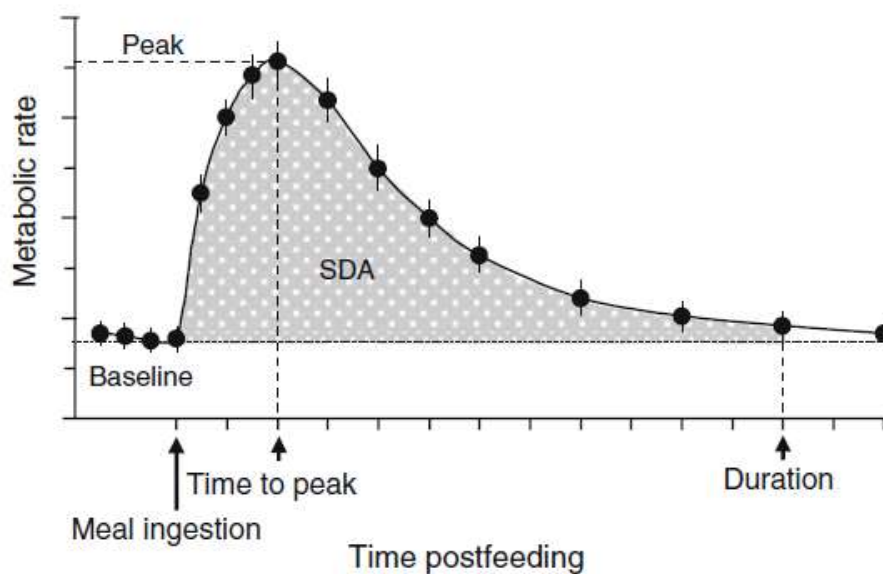


Figure 1. Hypothetical post-prandial metabolic profile of metabolic rate plotted against time post-feeding (Secor, 2009)

2.3.3. Influencing factors

The duration and magnitude of HIF can depend on several factors, such as the size and composition of the meal (Hoch, 1971; Kriss et al., 1934), the temperature difference between the animal and its prey (Wilson & Culik, 1991), as well as the age, body size, body composition and overall nutritional state of the animal (Secor, 2009; Ashwell-Erickson, 1981; Brody, 1945); along with environmental factors, like ambient temperature and gas composition (Secor, 2009). For example, in cases where the meal is large, intact or has a strong exoskeleton, more digestive effort is needed and therefore the increment is greater than in cases where the meal is small, fragmented or soft-fleshed (Secor, 2009). As for the meal composition, the energetic cost of processing food is lowest for lipids and carbohydrates and

highest for proteins (Blaxter, 1989; Poehlman & Horton, 1989). When the meal temperature is significantly lower than the animal's body temperature, extra heat must be generated by the body to elevate it. A large and cold meal will require more energy for its warming, than a smaller and warmer meal, therefore the digestive response will be greater (Berteaux, 2000).

2.4. Previous studies

The HIF or at least a component of the post-prandial metabolism has been studied in more than 250 invertebrate and vertebrate species (Secor, 2009) and a large percentage of that includes terrestrial mammals, especially ruminants (Webster, 1983; Kleiber, 1961). Nevertheless, only a few of these studies involve marine mammals, with the review of Booth et al. (2023), particularly emphasizing the lack of relevant studies in cetaceans. The studies that do exist include species such as sea otters (*Enhydra lutris*) (Costa & Kooyman, 1984), harp seals (*Phoca groenlandica*) (Gallivan & Ronald, 1981), harbour seals (*Phoca vitulina*) (Markussen et al., 1994; Ashwell-Erickson, 1981), ringed seals (*Phoca hispida*) (Parsons, 1977), South American fur seals (*Arctocephalus australis*) (Dassis et al., 2014) and Steller sea lions (*Eumetopias jubatus*) (D. A. Rosen & Trites, 1997).

2.5. Study animal

The common bottlenose dolphin (*Tursiops truncatus*, Montagu, 1821) is a globally distributed species, inhabiting a variety of coastal and open-ocean environments across tropical and temperate waters worldwide (Leatherwood et al., 1983). As the most common species of cetacean housed in managed care (NMFS, 2019), offers a valuable subject for exploring the impact of factors like body mass, activity, and age on daily energy requirements (Rimbach et al., 2021). Studies in metabolic rate have been conducted under various conditions such as rest, during and after exercise, and in animals of different ages (Rimbach et al., 2021; Pedersen et al., 2019; Fahlman et al., 2018a, 2018b; Williams et al., 2017; Fahlman et al., 2016, 2015; Holt et al., 2015; Van Der Hoop et al., 2014; Noren et al., 2013; Williams et al., 2001; Yazdi et al., 1999; Williams et al., 1993). However, little is known about the metabolic effects of digestion and only a single measurement has been performed where HIF was measured after the consumption of 1.4 kg of capelin (Yeates & Houser, 2008b).

In previous studies on feeding requirements, energetic needs and prey consumption, researchers depended on either extrapolating data from Kleiber's estimate of basal metabolic

rate ($BMR = 0.0093 M_b^{0.75}$, where BMR is in litres of O₂ per minute and M_b is the body mass in kilograms), or extrapolating the caloric content of ingested prey to estimate the energy needs of dolphins and cetaceans and formulate bioenergetic models (Rojano-Doñate et al., 2018; Bejarano et al., 2017; Benoit-Bird, 2004). For the bottlenose dolphin, Bejarano et al. (2017), developed a bioenergetic model to estimate annual energy requirements and prey biomass consumption based on model variations of FMR. Nevertheless, additional measurements of metabolic rates, cost of lactation, caloric intake and metabolization efficiency for the species could be used to improve this and related models (Bejarano et al., 2017).

2.6. Aim

Since for the bottlenose dolphin there have been studies on the basal metabolic rate and the locomotor cost but not the cost of digestion, this thesis aims to measure HIF in the species. In detail, the study aims to investigate the magnitude and duration of the increase in metabolic rate related to digestion, through indirect calorimetry by measuring the oxygen consumption and carbon dioxide production before and after the provision of a predetermined quantity of food.

3. Materials and Methods

3.1. Animals

The study was conducted in eight bottlenose dolphins housed in managed care at Kolmården Zoo, Sweden and Oceanogràfic Aquarium, Spain, from June to December 2023. The anonymous identification, year of birth, sex, and body mass of each individual animal are summarized in Table 1. The experiments were conducted using operant conditioning, with the voluntary participation of the dolphins. The animals were not restrained and had the option to participate or withdraw at any stage of the experimental trials. Before the start of the study the dolphins were desensitized to the equipment and trained for novel research-associated behaviours. Consequently, the data on respiratory variables and metabolism were gathered from animals in a calm, normal physiological state, before and after feeding. The diet consisted of thawed capelin (*Mallotus villosus*) and herring (*Clupea harengus*), and the total daily energetic intake was adjusted for each individual based on their body mass, body

condition and behaviour. The time of when each animal had eaten last on the day before each trial was recorded to account for the fasted period.

3.2. Experimental procedure

3.2.1. Respiratory flows

A custom-made Fleisch type pneumotachometer (ADM+, Valencia, Spain) with a low-resistance laminar flow matrix (Item# Z9A887-2, Merriam Process Technologies, Cleveland, OH) was placed over the blowhole of the dolphin. Differential pressure across the flow matrix was measured using a differential pressure transducer (MPX-2.5 mbar type 339/2, Harvard Apparatus, Holliston, MA) connected to the pneumotachometer with two, 400 cm lengths of 1.5 mm I.D., firm walled, flexible tubing to generate a negative deflection for exhalation (Fahlman et al., 2015).

The pneumotachometer was calibrated using a 7.0 litre calibration syringe (Series 4900, Hans-Rudolph Inc, Shawnee, KS). The signal was integrated and the flow determined by calibrating the pneumotachometer using the syringe immediately before and after each trial, through a series of pump cycles at various flow speeds (Fahlman et al., 2015) to calibrate the differential pressure and flows for the expiratory and inspiratory phases to be determined.

3.2.2. Respiratory gas composition

Respiratory gases were subsampled via a port in the pneumotachometer and passed through a 400 cm length of 5 mm I.D., firm walled, flexible tubing and a 30 cm length of 1.5 mm I.D. Nafion tubing, to fast-response O₂ and CO₂ analysers (ML206, Harvard Apparatus, Holliston, MA, USA) at a flow rate of 200 ml min⁻¹ with a response time for a 90% change to equilibrium for O₂ and CO₂ of 67 ms and 94 ms, respectively (Fahlman et al., 2015). A mathematical correction for the response time was used as detailed in Allen et al.(2022). The gas analysers were connected to the data acquisition system (Powerlab 8/35, ADInstruments, ColoradoSprings, CO, USA), sampled at 2000 Hz and displayed on a laptop computer running LabChart (v7.3.7, ADInstruments). The respiratory gas signals were phase-corrected to match the respirations, to account for the lag resulting from gas flow through the tubing.

The expiratory flow and expired O₂ and CO₂ concentrations were multiplied to calculate the instantaneous oxygen consumption rate (\dot{V}_{O_2} , litre O₂ min⁻¹) and carbon dioxide production

rate (\dot{V}_{CO_2} , litre CO_2 min^{-1}). The instantaneous \dot{V}_{O_2} and \dot{V}_{CO_2} were integrated to yield the total volume of O_2 and CO_2 exchanged for each breath. The volumes were summed for each breath during the trial period and divided by the duration of the trial to provide an estimate of the \dot{V}_{O_2} and \dot{V}_{CO_2} for that time period. The duration of \dot{V}_{O_2} and \dot{V}_{CO_2} measurements varied between 5 and 7 minutes, depending on the amount of time each animal was trained for.

The gas analyser was calibrated before the experiment using a commercial mixture of 4% O_2 , 5% CO_2 and balance AR (Arcal23, Airliquide). Ambient air was used to check the calibration before and after each experimental trial. Daily air temperature and humidity were recorded using a digital hygrometer and thermometer, with the means being $21.64 \text{ }^\circ\text{C} \pm 4.42 \text{ }^\circ\text{C}$ and $55.33 \pm 15.53 \%$ respectively. The average pool temperature in Kolmården Zoo was $23.56 \pm 0.31 \text{ }^\circ\text{C}$, in Oceanogràfic Aquarium $22.45 \pm 2.21 \text{ }^\circ\text{C}$, whereas across both facilities $22.8 \text{ }^\circ\text{C} \pm 1.9 \text{ }^\circ\text{C}$. Temperatures were within the thermoneutral zone of 5-25 $^\circ\text{C}$ for bottlenose dolphins (Williams et al., 2001).

3.2.3. Temporal metabolic response of digestion

Each trial session consisted of one post-absorptive measurement and one or more post-prandial measurements. Following an overnight fast, the dolphin was positioned at the side of the pool and was allowed to spontaneously breathe through the pneumotachometer for a period between 5-7 minutes, during which the respiratory flows and expired gas composition were measured (Figure 2). Following this post-absorptive measurement, the dolphin was given a predetermined quantity of food that varied between 1 and 1.5 kg of capelin and herring, which corresponded to 1659-2658 kcal. Metabolic measurements were then repeated after 30, 60, 90 and 120 minutes, without additional food given between trials. For some trials a single post-feeding measurement was taken, whereas for others, up to three consecutive measurements were made without the provision of food in-between. For all individuals, a minimum of three trials per animal and caloric intake were obtained. For two individuals, two different caloric intake levels were tested, 2658 kcal, and 1798 kcal.



Figure 2. A custom- made pneumotach was used to sample breathing

3.3. Data analysis and statistics

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

The statistical analysis was performed using R version 2023.12.1.402 (R Core Team, 2023) in the RStudio integrated development environment (RStudio Team, 2023). First, the mean BMR was calculated from the \dot{V}_{O_2} of the fasted trials. Then, for each animal, the peak metabolic rate was calculated as the highest value of \dot{V}_{O_2} observed after feeding, across all time points. HIF was computed as the difference between the peak metabolic rate and the BMR for each group of energy intakes (Table 2). The Specific Dynamic Action scope (SDA scope) (McCue, 2006) was calculated to allow for easier comparisons between studies (Appendix 1), by dividing the peak metabolic rate by the BMR (Table 2). The respiratory exchange ratio (RER) was calculated as $\dot{V}_{CO_2} \dot{V}_{O_2}^{-1}$. The Kleiber ratio was calculated as the measured \dot{V}_{O_2} divided by the estimated BMR from Kleiber's equation for BMR for terrestrial mammals ($BMR = 0.0093 M_b^{0.75}$, where BMR is in litres of O₂ per minute and M_b is the body mass in kilograms; Kleiber, 1961).

In addition, a generalized additive model (GAM) (mgcv package, v1.8-34; Wood, 2011) was used to investigate the relationship between oxygen consumption and various predictor variables. The variables time, energy intake, age, body mass and pool temperature were included as smooth terms, representing potentially nonlinear relationships, whereas sex as a parametric term. Each animal was accounted for as a random effect to accommodate potential clustering within individuals.

In this study p -values ≤ 0.05 were considered as significant. Data are presented as the mean \pm standard deviation, unless otherwise stated.

4. Results

Data from 50 trials were collected for 2 female and 6 male bottlenose dolphins, aged between 5 and 40 years old (mean age: 19.8 ± 15.35 years).

4.1. Pre- and post-fasting metabolic rates

The BMR (Table 1) was determined following a minimum 15-hour overnight fasting period and found to average 0.75 ± 0.12 ml O₂ min⁻¹ (mass corrected mean: 4.03 ± 0.81 ml O₂ min⁻¹ kg⁻¹). The average peak metabolic rate after digestion was calculated as 1.06 ± 0.22 ml O₂ min⁻¹ (mass corrected mean: 5.65 ± 1.1 ml O₂ min⁻¹ kg⁻¹).

From the dolphins participating in the study, one was a juvenile (five years old) and seven were adults (10-40 years old). Therefore, for the juvenile it was assumed that the post-absorptive value represents the RMR, whereas for the adults the BMR. For the juvenile the mass specific RMR was 3.61 ml O₂ min⁻¹ kg⁻¹ which was not significantly different as compared with the adults 4.03 ± 0.81 ml O₂ min⁻¹ kg⁻¹ (One-sample t-test, $df = 6$, $t = 2.176$, $p = 0.072$). The peak metabolic rate after feeding for the juvenile was 5.8 ml O₂ min⁻¹ kg⁻¹, which did not differ significantly from the respective peak metabolic rate for the adults which was 5.57 ± 1.26 ml O₂ min⁻¹ kg⁻¹ (One-sample t-test, $df = 6$, $t = 0.654$, $p = 0.537$).

Table 1. Animal ID, sex (M: Male, F: Female), year of birth (YOB, * represents the estimated year if YOB is unknown), body mass, energy intake, total daily energetic intake, energy intake as % of the total daily energetic intake, basal metabolic rates, post-prandial metabolic rates and estimates of HIF. The number of observations for each animal is listed in parenthesis.

Animal ID	Sex	YOB	Body mass (kg)	Energy intake (kcal)	Average total daily energetic intake (kcal)	Energy intake as % of total daily energetic intake	BMR (L O ₂ min ⁻¹)	MR (L O ₂ min ⁻¹) at time (min) after feeding and (n)=number of trials				HIF (L O ₂ min ⁻¹)
								30	60	90	120	
Tt1	M	1985*	185.6	2658	7627	34.85	0.68 ± 0.04 (2)	0.99 ± 0.30 (2)	0.91 (1)	-	0.89 (1)	0.31
Tt2	M	2014	188.3	2658	16365	16.24	0.70 ± 0.24 (4)	0.80 ± 0.28 (4)	1.21 ± 0.47 (4)	-	1.11 ± 0.16 (4)	0.51
Tt3	M	2013	177.7	2658	11199	23.73	0.95 ± 0.21 (8)	0.91 ± 0.41 (4)	1.38 ± 0.25 (4)	-	1.34 ± 0.18 (4)	0.43
				1798	9799	18.35		1.17 ± 0.37 (3)	1.28 ± 0.15 (3)	-	1.10 ± 0.08 (3)	
Tt4	M	2006	172	2658	7136	37.25	0.75 ± 0.18 (7)	0.83 ± 0.18 (4)	0.78 ± 0.13 (3)	-	0.64 ± 0.13 (3)	0.08
				1798	7440	24.17		0.91 ± 0.18 (3)	0.97 ± 0.29 (3)	-	0.85 ± 0.19 (3)	

Tt5	F	1985*	200.6	2658	10804	24.6	0.57 ± 0.23 (4)	0.56 ± 0.16 (3)	0.75 ± 0.28 (4)	-	0.56 ± 0.07 (3)	0.18
Tt6	F	1983	156.6	1798	9227	19.49	0.72 ± 0.11 (9)	-	0.61 (1)	0.94 (1)	0.75 ± 0.16 (7)	0.21
Tt7	M	2018	149.7	1659	9407	17.64	0.54 ± 0.12 (7)	0.54 (1)	-	0.87 (1)	0.58 ± 0.13 (5)	0.33
Tt8	M	1985	240.9	2221	11436	19.42	0.89 ± 0.18 (8)	1.25 (1)	-	1.05 (1)	0.93 ± 0.21 (3)	0.36

4.2. Temporal changes in metabolic rate following a meal

The metabolic rate increased by an average of 21.4% 30 min following a meal, continued to increase to a maximum of 33.2% observed at 60 min (Table 2), and then decreased towards the basal value (Figure 3).

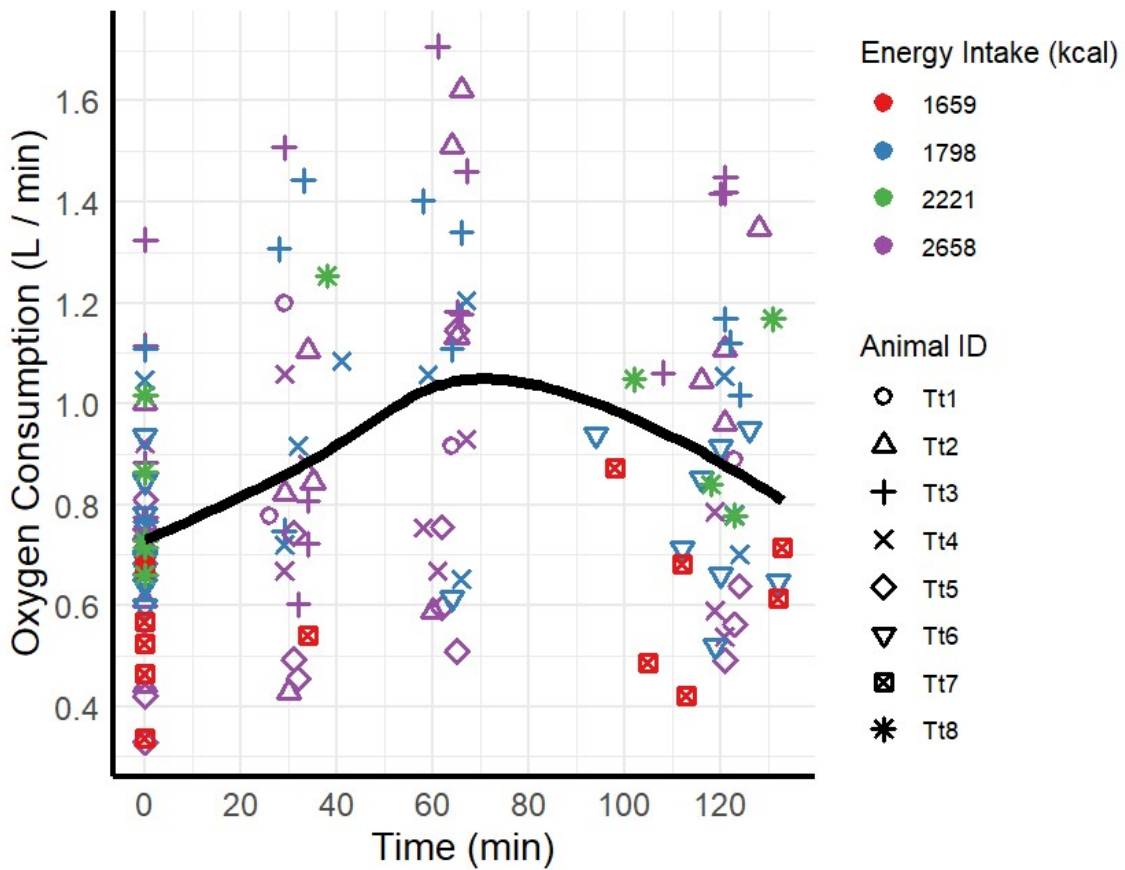


Figure 3. Oxygen consumption in relation to time since last feed. The symbols represent the different individuals and the colours the energy intakes. Based on the mean oxygen consumption across each time point, the black line represents the predicted rate of oxygen consumption across time.

Table 2. Percentages of oxygen consumption in relation to time since last feed.

Time since last feed (min)	Mean % increase over BMR
30	21.39
60	33.2
90	30.68
120	26.95

4.3. Metabolic responses to varying energy intake levels

The relative increase in metabolism (% of HIF) for each respective group was also calculated. Five individuals were provided with an energy intake of 2658 kcal and had a mean maximum increase of 41.2 % over BMR across all time points, one individual with 2221 kcal had a maximum increase of 40.5%, three individuals fed with 1798 kcal had a mean increase of 31.5% and the juvenile individual who was provided with 1659 kcal had a maximum increase of 61.1% over RMR (Table 3). The % of the provided energy intake as it corresponds to the total daily energetic intake of each individual is shown in table 1. The SDA scope varied between 1.3 to 1.6 for the different energy intakes.

Table 3. HIF and SDA scope estimates grouped by energy intake amounts. In parenthesis the number of individuals that were tested with each caloric intake is represented.

Energy intake (kcal)	HIF (L O ₂ min ⁻¹)	HIF % increase in metabolism over BMR	SDA scope
2658 (5)	0.3 ± 0.18	41.19 ± 22.61	1.41
2221 (1)	0.36	40.45	1.4
1798 (3)	0.26 ± 0.07	31.54 ± 2.84	1.31
1659 (1)	0.33	61.11	1.61
Mean	0.31	43.57	1.43

4.4. Generalized Additive Model (GAM)

A GAM was fitted to examine the significance of oxygen consumption and predictor variables. The model was constructed using a Gaussian family with an identity link function and had the following formula:

oxygen consumption \sim s (time, k=5) + s (energy intake, k=4) + s (age, k=4) + s (body mass, k=4) + s (pool temperature, k=4) + sex + s (animal, bs="re")

For the parametric coefficients (Appendix 2), the results showed that the intercept was significantly different from zero ($\beta = 0.6838$, $SE = 0.1464$, $t = 4.672$, $p < 0.001$). The effect of sex (coded as 0 for females and 1 for males) was not statistically significant ($\beta = 0.1906$, $SE = 0.1744$, $t = 1.093$, $p = 0.277$).

Regarding the smooth terms (Figure 4), the results showed statistical significance for the time (edf = 2.63, Ref.df = 2.699, $F = 6.479$, $p < 0.001$). Similarly, the term for animal, which was modelled as a random effect, was highly significant (edf = 3.29, Ref.df = 5.000, $F = 10.435$, $p < 0.001$). The rest of the smooth terms, i.e., energy intake, age, body mass, pool temperature were not statistically significant (Appendix 4).

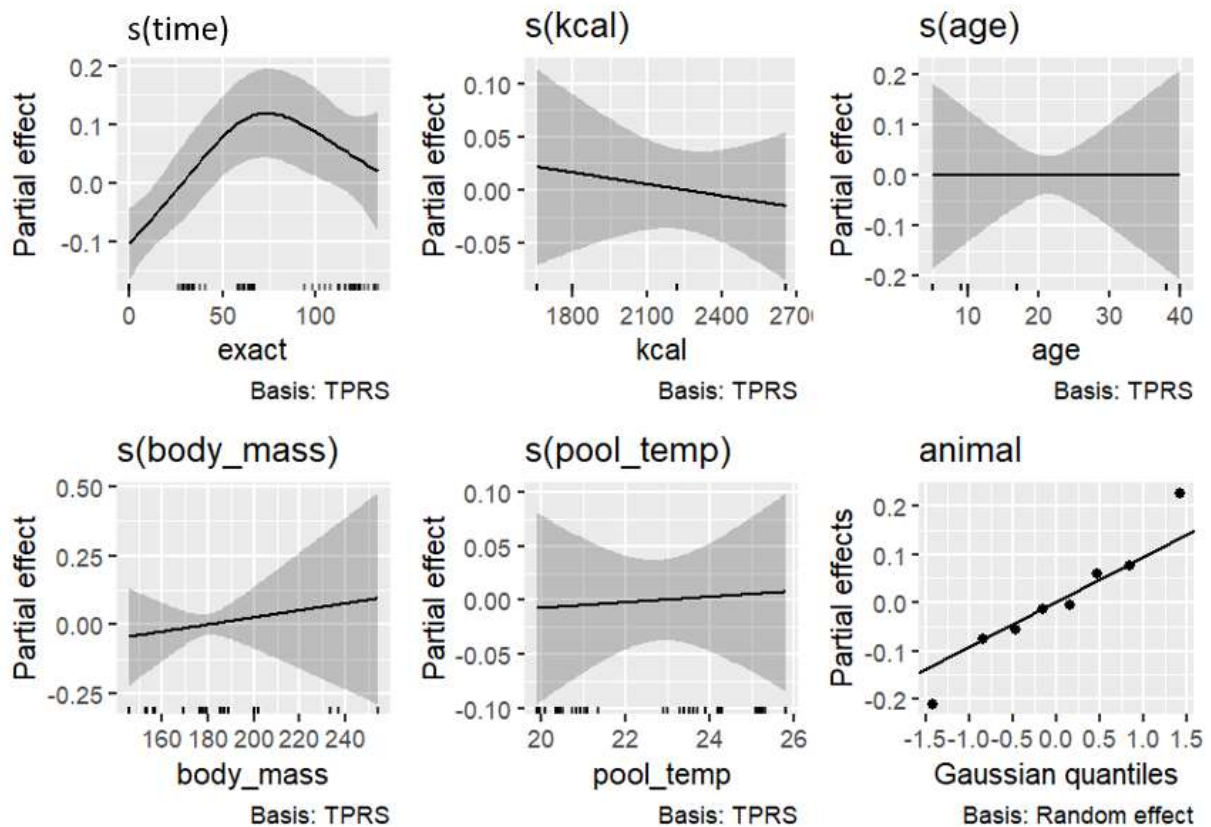


Figure 4. Partial effects of smooth terms of the GAM

The model accounted for about 43.9% of the variation in oxygen consumption, as reflected by an adjusted R-squared value of 0.439. Additionally, it explained 48.4% of the deviance.

4.5. Breathing frequency & Tidal volume

During the fasted period the breathing frequency (Figure 5) was calculated as 2.55 ± 1.02 breaths per minute and the tidal volume (Figure 6) as 4.12 ± 1.39 L. At the post-fasted period the breathing frequency as calculated as 2.65 ± 1.24 breaths per minute and the tidal volume as 4.68 ± 1.95 L (see Appendix 4 for all measurements).

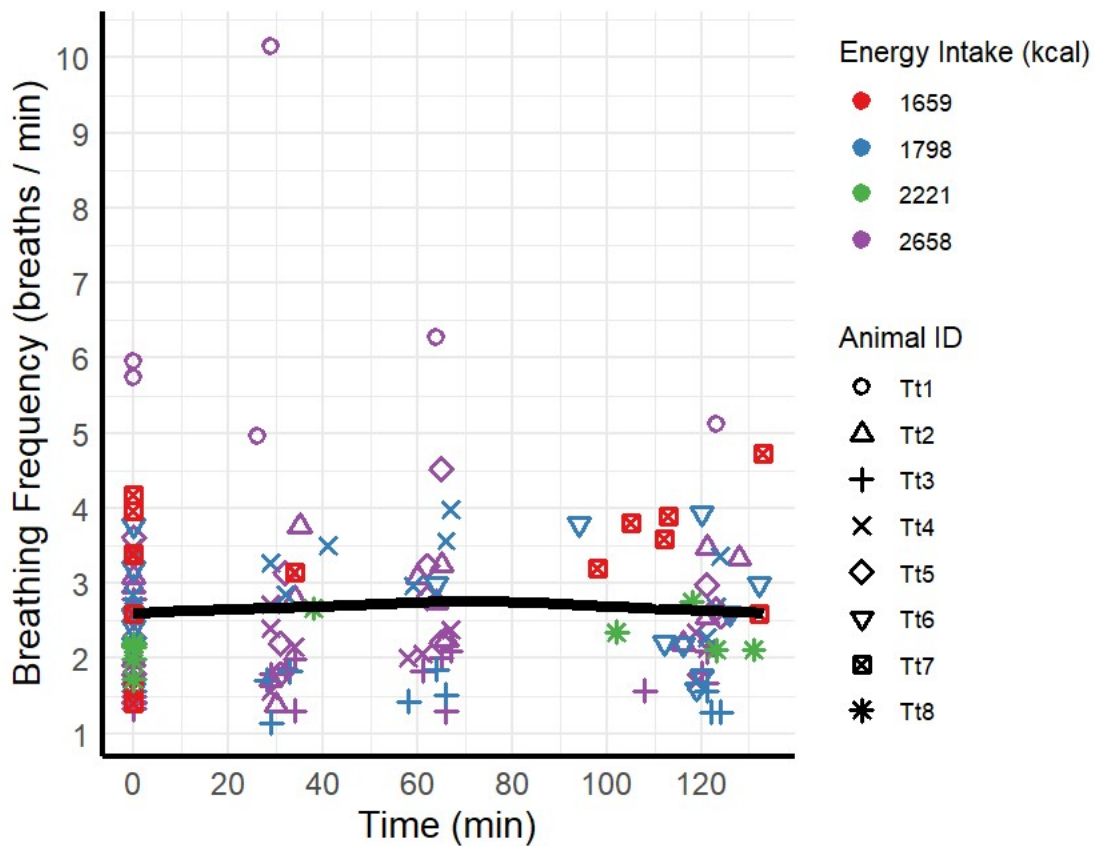


Figure 5. Breathing frequency in relation to time since last feed. The symbols represent the different individuals and the colours the energy intakes. The black line represents the breathing frequency across the measured time points.

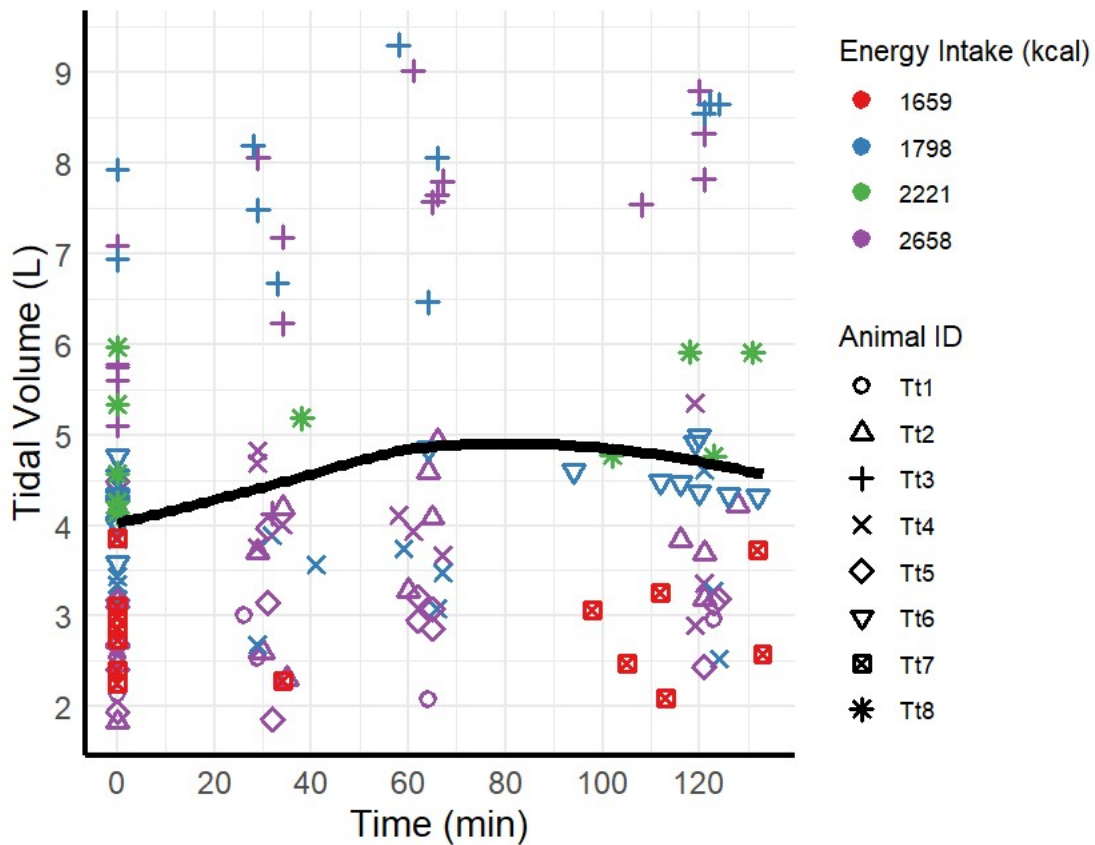


Figure 6. Tidal volume in relation to time since last feed. The symbols represent the different individuals and the colours the energy intakes. The black line represents the tidal volume across the measured time points.

4.6. Respiratory exchange ratio

The mean respiratory exchange ratio (Figure 7) was calculated as 0.793 ± 0.099 during the fasted trial, 0.780 ± 0.084 at 30 minutes, 0.784 ± 0.092 at 60 minutes and 0.815 ± 0.076 at 120 minutes.

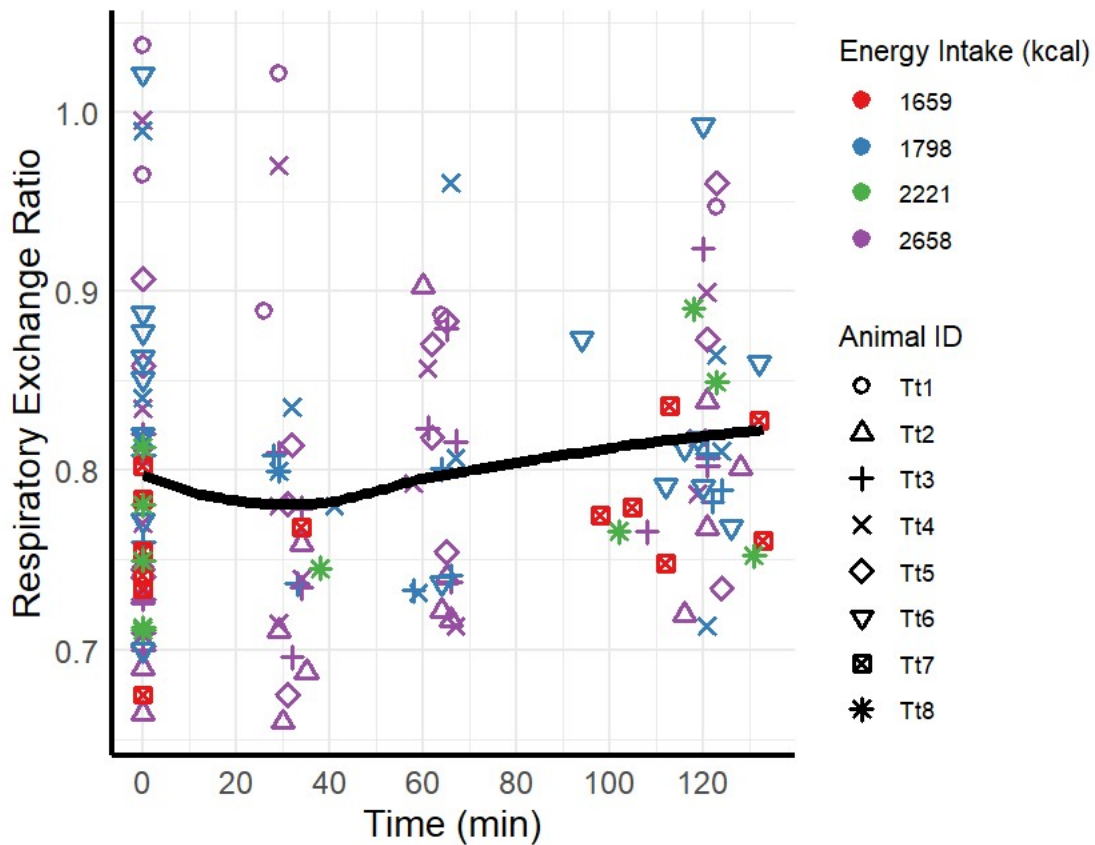


Figure 7. RER in relation to time since last feed. The symbols represent the different individuals and the colours the energy intakes. The black line represents the RER across the measured time points.

4.7. Kleiber's ratio

The measured \dot{V}_{O_2} was calculated as 1.6 times higher than the estimated BMR from Kleiber's equation for terrestrial mammals (Table 4).

Table 4. Animal ID, measured \dot{V}_{O_2} , estimated BMR from Kleiber's equation for terrestrial mammals and Kleiber's ratio.

Animal ID	Measured \dot{V}_{O_2} (L O ₂ min ⁻¹)	Estimated BMR (L O ₂ min ⁻¹)	Kleiber's ratio
Tt1	0.68	0.47	1.45

Tt2	0.7	0.47	1.49
Tt3	0.95	0.45	2.11
Tt4	0.75	0.44	1.7
Tt5	0.57	0.5	1.14
Tt6	0.72	0.41	1.76
Tt8	0.89	0.57	1.56
Mean	0.75 ± 0.12	0.47 ± 0.04	1.6 ± 0.28

5. Discussion

In the present study I measured the metabolism in bottlenose dolphins after an overnight fast and at 30, 60, 90 and 120 minutes after the ingestion of a meal. The mean BMR was calculated as $4.03 \pm 0.81 \text{ ml O}_2 \text{ min}^{-1} \text{ kg}^{-1}$ and the mean peak metabolic rate after feeding as $5.65 \pm 1.1 \text{ ml O}_2 \text{ min}^{-1} \text{ kg}^{-1}$. The \dot{V}_{O_2} was estimated as 1.6 times Kleiber. Depending on the calories ingested I observed an increase between 31-61% over RMR, which reached a peak at around 60 minutes after feeding and returned to pre-feeding levels at around 120 minutes. The study provides results on the metabolic effect of feeding in multiple individuals, and agrees with the single measurement in the same species by Yeates & Houser (2008) and the result presented in the rough toothed dolphin (*Steno bredanensis*) (Fahlman et al., 2024).

Metabolic rates

The measurement of the BMR has to satisfy specific conditions, which require the individual to be mature but non-productive, post-prandial (fasted), in its thermoneutral zone and resting but not sleeping (Kleiber, 1961). In the current study, the conditions were satisfied for all

individuals but one, the juvenile, that was potentially still in a non-mature and growing state. So when excluding the juvenile, the measured BMR was within the range of 3.0 to 7.0 ml O₂ min⁻¹ kg⁻¹, established by previous studies on the species (Fahlman et al., 2018a; Fahlman et al., 2016; Yazdi et al., 1999; Ridgway & Patton, 1971). Several studies on marine mammals have reported elevated BMRs in comparison to their similarly sized terrestrial counterparts and even though others dispute it, an estimated BMR of bottlenose dolphins has been calculated as 1.96 ± 0.45 times Kleiber. However, measurement of the BMR of cetaceans has proven difficult and only five species of odontocetes have been studied to date (Noren & Rosen, 2023). According to the review of Noren & Rosen (2023), comparatively smaller species of odontocetes, like the harbor porpoise (*Phocoena phocoena*) and the Pacific white-sided dolphin (*Lagenorhynchus obliquidens*) show higher values (2.45 x Kleiber and 3.39 x Kleiber, respectively), whereas the killer whale (*Orcinus orca*) lower (1.69 x Kleiber) when compared to the bottlenose dolphin. The explanation could be found in the fact that smaller species inhabiting cold temperate waters, might require a high metabolism to achieve thermal neutrality (Noren & Rosen, 2023). Another hypothesis suggests that the higher metabolic rates reported for marine mammals are a result of the longer small intestines required for their specific type of marine carnivory (Williams et al., 2001). Here, the metabolic rates from the fasted animals were, on average, 1.6 times higher than those from similarly sized terrestrial mammals (Kleiber, 1961). Because the methods used across various studies are not always the same, as for example flow-through respirometry used in Yeates & Houser (2008), van der Hoop et al. (2014) and Yazdi et al. (1999) or breath by breath respirometry used in Allen et al. (2022) and Pedersen et al. (2019), sometimes different results are reported in the same species. So, additional measurements such as the ones provided from this study, can help determine if the discrepancies are due to methodological reasons or if the variation comes from other parameters, such as the breathing frequency (Fahlman et al., 2024).

In addition, it has been shown that energy requirements decrease with age, when corrected for body mass (Rimbach et al., 2021). Rechsteiner et al. (2013) reported that the resting metabolic rate of juvenile Pacific white-sided dolphins was 1.5 higher than that of the adults. In the current study, no statistically significant difference was recorded. However, it needs to be noted that there was a difference in the size of the sample with only one juvenile compared to seven adults. The highest metabolic rates, both BMR and peak metabolic rate were recorded in the youngest individual of the adult age class, who was 10 years old at the time of the study. This could be explained by the fact that bottlenose dolphins undergo multiple growth

phases; an early period of rapid growth, followed by a decrease in growth velocity and a period of sustained but slower growth, and then a rapid increase in growth at around the age of sexual maturity, which according to modelled approaches occurs at around 10 years old (McFee et al., 2010). Nevertheless, it is worth noting that in the current study a considerable amount of individual variation was observed. Indeed, in similar studies, individual differences are often apparent, as a result of differences in personality or stress levels (Careau et al., 2008).

Lung function parameters

Breath by breath respirometry allows the recording of other respiratory variables such as the breathing frequency and the tidal volume. In fact, a way to estimate the FMR in large whales is by their breathing frequency, based on the assumption that increased breathing is associated with the energy requirements of the activity performed by the animal at that time, i.e., travelling or foraging (Fahlman et al., 2018a). This assumption is also reliant on the fact that the average tidal volume and O₂ exchange fractions are known and constant during the measurement, which is not always the case (Ridgway et al., 1969), so the values provided here can help improve the estimates on the respiratory physiology for the species but also for marine mammals in general.

The average breathing frequency was calculated as 2.55 ± 1.02 breaths per minute and 2.65 ± 1.24 breaths per minute, at the pre- and post- fasted trials respectively, which is similar to the value of 2.7 ± 1.3 breaths per minute established for wild bottlenose dolphins in Sarasota Bay by Fahlman et al. (2018a). The estimations for the tidal volume, 4.12 ± 1.39 L for the fasted trial and 4.68 ± 1.95 L for the post-fasted trials, compare well with the results of 5.3 ± 1.8 L of the aforementioned study as well (Fahlman et al., 2018a). In the smaller rough toothed dolphin, the breathing frequency and tidal volume measurements were found to be lower than the ones of bottlenose dolphins, both in fasted and post-fasted periods (Fahlman et al., 2024).

The respiratory exchange ratio, given by the CO₂ produced divided by the O₂ consumed, is another parameter that can provide useful information as an indicator of metabolic balance on the substrate being metabolized. For carbohydrates its value is the highest (1.0), whereas for lipids the lowest (0.7) (Wrightington, 1942). In this study it varied from 0.793 ± 0.099 during the fasted trial, to 0.780 ± 0.084 at 30 minutes, 0.784 ± 0.092 at 60 minutes and finally 0.815 ± 0.076 at 120 minutes. In previous studies on the species it was found to range between 0.67

to 0.81 (Fahlman et al., 2015). For the fasted rough toothed dolphins, the mean RER was 0.76 ± 0.04 and 0.74 ± 0.07 after feeding (Fahlman et al., 2024). The described results are in agreement with the fact that marine mammal diet is high in proteins and lipids and low in carbohydrates (Wells et al., 2013).

Heat increment of feeding

The mean HIF recorded was 43% higher than the resting metabolic rate. The 8 individuals were given a diet of 1 to 1.5 kg of capelin and herring, which correspond to 1659 - 2658 kcal. For 2658 kcal the increase in HIF was $41 \pm 2 \%$, for 2221 kcal 40%, for 1798 kcal $32 \pm 3 \%$ and for 1659 kcal 61%. Literature suggests that the amount of the meal ingested affects HIF (Costa, 2009). In fact, the results show a trend of a higher meal intake resulting in a higher HIF %, with the exception however –even though not statistically significant- of the lower amount of calories resulting in the highest HIF percentage difference. This could be explained by the fact that the individual that was tested with the lowest amount, was the youngest of the group and therefore could be a sign of the elevated energetic requirements, as younger animals tend to allocate considerable energy for growth (Fahlman et al., 2018a). In addition, it was only one animal that was tested with this particular amount of calories, so the limited sample may have an effect on the results.

On that note it was interesting to investigate the difference the amount of calories can induce on the same individuals. Here, I was able to test this with only two dolphins that were initially given an amount of 2658 kcal and later repeated the process with 1798 kcal. While I did not find a significant difference when comparing the total post-fasted metabolic rate between the two caloric intakes, future studies should focus more in depth on that aspect. One potential explanation for the discrepancy could be the fact that there might not be considerable difference between the two quantities. In the study of Rosen & Trites (1997), Steller sea lions were fed with 2 and 4 kg of herring, resulting in a small but statistically significant difference in HIF between the two meals ($12.4 \pm 0.9\%$ increase in HIF for the larger meal size, whereas $9.9 \pm 0.9\%$ for the smaller meal size) . In the current study, due to constraints regarding the dolphins' total diets, such a change was not possible, allowing room for a difference of 0.5 kg between the meals. This knowledge could provide interesting information about individuals in the wild, as it could explain if the size of the meal could potentially become a constraint if it raises energetic requirements. Increased metabolism due to digestion can impact foraging ability by increasing metabolic overhead, thereby decreasing available aerobic dive time, so

there could be a point when the cost of digestion makes the energetic efficiency of foraging no longer profitable (Rosen et al., 2007).

In general, the results presented here are similar to the study of Yeates & Houser (2008), where a 220 kg bottlenose dolphin that consumed 1800 kcal had an increase between 20% 25 min after the ingestion of the meal to 40% 60 to 100 minutes after feeding. To provide a frame of reference, the three individuals that ingested the same amount of calories in this study, who had a mean body mass of 168.77 kg, reached a maximal post-prandial increase of about 31% between 60 and 90 min after feeding. The fasted and post-prandial metabolism has been investigated in another odontocete species the rough-toothed dolphin, where 1-2 hr after the consumption of a meal, the metabolic rate showed an increase of 29% (Fahlman et al., 2024). In contrast to the previous study, for the bottlenose dolphins, I was able to conduct repeated measurements without providing food in-between and as a result acquire more points on the curve describing the temporal response and differentiate between one and two hours. However, I acknowledge that it was not possible to perform a controlled experiment where animals would remain fasted between the first and later measurements.

In other species, HIF has been measured as 4.7–9.0% of gross energy intake in harbor seals consuming herring (Markussen et al., 1994), in harp seals 11–13% of total energy intake when eating herring (Gallivan & Ronald, 1981), and in Steller sea lions it varied from 9.9% to 12.4% of the total intake, depending on the meal size (Rosen & Trites, 1997). Since in relevant studies there are differences in how HIF is expressed, one common way to compare within them is the SDA scope (McCue, 2006). For the bottlenose dolphin it was calculated to average between 1.3 and 1.6 depending on the caloric intake. When comparing with other marine mammal species, in otariids it was found to be higher, between 1.6 and 2.1 (Dassis et al., 2014; Liwanag, 2010; Rosen & Trites, 1997), whereas in phocids it has a broader range of variation from 1.3 to 9 (Markussen et al., 1994; Barbour, 1993; Ashwell-Erickson, 1981; Gallivan & Ronald, 1981; Parsons, 1977). In general, the results for the bottlenose dolphin seem to be on the lower end in comparison to the other marine mammal species described. Unlike bottlenose dolphins, these species spend considerable amounts of time outside the water, subjecting themselves to a wider thermal gradient and therefore may utilize different mechanisms to achieve thermoregulation, which could include thermal substitution. In fact, it has been reported that the heat produced during digestion could be used to offset costs

associated with thermoregulation, especially in sea otters (Costa & Kooyman, 1984), however this hypothesis was not supported when studying the Steller sea lion (Rosen & Trites, 2003).

Limitations and future perspectives

As it has already been mentioned there were limitations within this study. Even though the number of dolphins involved was higher than in any previous related research, due to the individual variation observed, future studies could incorporate an even larger sample to investigate the degree of this variation. To obtain an accurate sample in the limited time available all individuals were fed with the same -commonly followed for this species under human care- diet of capelin and herring. This could prove as a limitation as existing literature suggests that the difference in HIF is also dependent on the composition of the meal. Future research could factor that in and investigate different types of food, for example only capelin or only herring, providing variability in the proportion of proteins, carbohydrates and lipids they contain. Likewise, the quantity of the fish given should be further varied, preferably with higher amounts, to examine the upwards limits of the increase in HIF. The fish given to the dolphins were thawed but still kept on ice before the start of the fasted trial, so it is important to note this difference in temperature as well. While I was able to conduct continuous measurements without the provision of food, the first trial of the post-absorptive oxygen consumption was always followed by a meal. Thus, a control trial with measurements at all same time points without providing any energy intake initially, could contribute interesting information. Finally, to understand the complete scope of energy losses from the ingested energy to net energy, it is essential to combine these data with other parameters of the energy flow, that is for example the apparent digestive efficiency.

Conclusion

Multiple studies have tried to investigate the energetic requirements of marine mammals, particularly considering human disturbances that can affect them. Models that aim to predict how disturbances may alter population levels, require understanding of the eco-physiology of the study species in order to quantify the flow of energy within the organism and between different trophic levels. Nevertheless, there is limited knowledge of physiology of marine mammals and how related constraints affect survival. The data presented in this study provide estimates on the energy requirements and respiratory physiology in multiple individuals of bottlenose dolphins under human care in different stages of the digestion process. These data

will help improve estimates from bioenergetics models and contribute to our understanding of how a changing environment may alter survival in this species.

6. Societal and ethical considerations

All procedures were approved by the Animal Care Committee of Fundacion Oceanogràfic (OCE-32-23). The participation of the animals in the experiment was voluntary using operant conditioning. The equipment used was non-invasive and every trial was conducted with the cooperation of the animal caretakers at the facilities, who knowing the behaviour of the animals, were able to recognize any potential signs of stress and could stop the procedure at any time if needed. The research required the animals to be in a calm state, so they had been trained and desensitized to the equipment beforehand. Spirometry through pulmonary function testing is used as a diagnostic tool to assess lung health in cetaceans (Borque-Espinosa et al., 2020; Fahlman et al., 2015), therefore the behaviour trained and utilized in this study has direct benefits in maintaining the welfare of the dolphins under human care.

Climate change is a topic that holds increased significance in current times. Already by the mid-1990s, scientists started to highlight the possibility of climate change inducing irreversible effects on marine mammals (Tynan & DeMaster, 1997). Today, the concern for how human activities may affect the behaviour of individuals across species continues to increase (Chudzińska et al., 2024). Human disturbance in the marine environment includes changes in prey resources, ocean acidification, fishing gear, plastics and other contaminants, as well as anthropogenic noise (Davies et al., 2009), making marine mammals vulnerable to behavioural changes that are depended on their movement ecology, reproductive strategy and body size (Keen et al., 2021). In order to mitigate human disturbance, action is being taken by including the conservation and sustainable use of the oceans, seas and marine resources in the United Nation's Sustainable Development Goals. This study will hopefully contribute towards fulfilling the target of this goal by increasing scientific knowledge and enhancing conservation. In order to conserve marine mammals, first the understanding of how climate change affects them, especially at population levels, is needed (Gulland et al., 2022). As bioenergetics function as the primary link between physiological processes and the ecological patterns of populations (Yodzis & Innes, 1992), this study can contribute in bridging that gap, by providing more data which will improve estimates of wild populations.

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Appendix

Appendix 1: SDA scope of different species

	Species	SDA scope	Data source
Otariids	South American fur seal <i>(Arctocephalus australis)</i>	1.6	(Dassis et al., 2014)
	Northern fur seal <i>(Callorhinus ursinus)</i>	1.6	(Liwanag, 2010)
	Steller sea lions <i>(Eumetopias jubatus)</i>	1.7 to 2.1	(D. A. Rosen & Trites, 1997)
Phocids	Northern elephant seals <i>(Mirounga angustirostris)</i>	1.4 to 1.6	(Barbour, 1993)
	Harp seal <i>(Phoca groenlandica)</i>	1.4 to 1.7	(Gallivan & Ronald, 1981)
	Ring seal <i>(Phoca hispida)</i>	1.8 to 2	(Parsons, 1977)

	Habor seal <i>(Phoca vitulina)</i>	1.3 to 9	(Ashwell-Erickson, 1981; Markussen et al., 1994)
Sea otters	Sea otter <i>(Enhydra lutris)</i>	1.5	(Costa & Kooyman, 1984)
Odontoceti	Bottlenose dolphin <i>(Tursiops truncatus)</i>	1.3 to 1.6	This study

Appendix 2. Parametric coefficients of the GAM.

Term	Estimate	Std. Error	t value	Pr (> t)
Intercept	0.6838	0.1464	4.672	<0.001 ***
sex (Male)	0.1906	0.1744	1.093	0.277

Appendix 3: Approximate significance of smooth terms in the GAM.

Smooth Term	edf	Ref.df	F	p-value
s(time)	2.263	2.699	6.479	0.00053***
s(energy intake)	1.000	1.000	0.253	0.61611
s(age)	1.000	1.000	0.000	0.99046

s(body mass)	1.000	1.000	0.242	0.62347
s(pool temperature)	1.000	1.000	0.033	0.85560
s(animal)	3.290	5.000	10.435	< 0.001***

Appendix 4: Fasted and post-fasted breathing frequency and tidal volume values

Animal ID	Fasted		Post-fasted							
			30 min		60 min		90 min		120 min	
	Breathing frequency (breaths/min)	Tidal volume (L)	Breathing frequency (breaths/min)	Tidal volume (L)	Breathing frequency (breaths/min)	Tidal volume (L)	Breathing frequency (breaths/min)	Tidal volume (L)	Breathing frequency (breaths/min)	Tidal volume (L)
Tt1	5.84 ± 0.15	2.33 ± 0.28	7.56 ± 3.67	2.77 ± 0.33	6.28	2.07	-	-	5.11	2.96
Tt2	2.47 ± 0.64	2.55 ± 0.56	2.42 ± 1.09	3.20 ± 0.89	2.84 ± 0.44	4.23 ± 0.72	-	-	2.89 ± 0.62	3.74 ± 0.43
Tt3	1.72 ± 0.51	6.06 ± 1.18	1.66 ± 0.32	6.85 ± 1.39	1.72 ± 0.31	7.98 ± 0.95	-	-	1.61 ± 0.29	8.33 ± 0.48
Tt4	2.54 ± 0.53	3.84 ± 0.55	2.63 ± 0.67	3.92 ± 0.72	2.83 ± 0.82	3.67 ± 0.36	-	-	2.42 ± 0.57	3.67 ± 1.09
Tt5	2.47 ± 0.80	3.07 ± 1.07	2.37 ± 0.69	2.99 ± 1.06	3.19 ± 0.98	3.02 ± 0.15	-	-	2.74 ± 0.22	2.92 ± 0.42
Tt6	2.82 ± 0.74	4.23 ± 0.34	-	-	2.99	4.86	3.79	4.60	2.46 ± 0.74	4.62 ± 0.33

Tt7	2.93 ± 1.10	2.89 ± 0.53	3.14	2.28	-	-	3.20	3.07	3.72 ± 0.76	2.83 ± 0.65
Tt8	2.05 ± 0.42	5.04 ± 0.92	2.67	5.18	1.43 ± 0.11	4.81 ± 0.30	2.35	4.77	2.59 ± 0.47	5.33 ± 0.57

Appendix 5. Breathing frequency and tidal volume in relation to body mass

