

RESEARCH ARTICLE

Total energy expenditure of bottlenose dolphins (*Tursiops truncatus*) of different ages

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ABSTRACT

Marine mammals are thought to have an energetically expensive lifestyle because endothermy is costly in marine environments. However, measurements of total energy expenditure (TEE; kcal day⁻¹) are available only for a limited number of marine mammals, because large body size and inaccessible habitats make TEE measurements expensive and difficult to obtain for many taxa. We measured TEE in 10 adult common bottlenose dolphins (*Tursiops truncatus*) living in natural seawater lagoons at two facilities (Dolphin Research Center and Dolphin Quest) using the doubly labeled water method. We assessed the relative effects of body mass, age and physical activity on TEE. We also examined whether TEE of bottlenose dolphins, and more generally of marine mammals, differs from that expected for their body mass compared with other eutherian mammals, using phylogenetic least squares (PGLS) regressions. There were no differences in body mass or TEE (unadjusted TEE and TEE adjusted for fat-free mass) between dolphins from the two facilities. Our results show that adjusted TEE decreased and fat mass increased with age. Different measures of activity were not related to age, body fat or adjusted TEE. Both PGLS and the non-phylogenetic linear regression indicate that marine mammals have an elevated TEE compared with that of terrestrial mammals. However, bottlenose dolphins expended 17.1% less energy than other marine mammals of similar body mass. The two oldest dolphins (>40 years) showed a lower TEE, similar to the decline in TEE seen in older humans. To our knowledge, this is the first study to show an age-related metabolic decline in a large non-human mammal.

KEY WORDS: Aging, Energetics, Field metabolic rate, Marine mammal, Metabolism, Senescence

INTRODUCTION

Marine mammals can have disproportionate effects on the structure of marine ecosystems because of their large body size and their role as apex predators (Williams et al., 2004). Understanding the daily energy requirements of marine mammals provides insight into their ranging behavior, foraging efficiency and dive performance, as well as their impact on prey populations (Acquarone et al., 2006; Bejarano et al., 2017; Benoit-Bird, 2004; Costa and Gales, 2003;

McHuron et al., 2018; Williams et al., 2004). Measurements of energy expenditure, particularly total energy expenditure (TEE; also called 'field metabolic rate'; Nagy, 2005), are essential to understand an organism's physiology and ecology (McNamara and Houston, 1996; Salzman et al., 2018). TEE has been measured in a variety of mammalian and avian species (Nagy, 1987, 2005; Pontzer et al., 2014; Simmen et al., 2015; Song and Beissinger, 2020; Speakman, 2000), but several major vertebrate groups, including cetaceans, remain poorly studied. TEE measurements of marine mammals are typically expensive, given their large body size, and their inaccessible habitats hinder the collection of repeated body fluid (i.e. blood or urine) samples required for the doubly labeled water (DLW) method. TEE data, measured using the DLW method, are available for several pinniped species (sea lions: *Neophoca cinerea*, *Zalophus californianus*, *Phocarcos hookeri*; seals: *Arctocephalus galapagoensis*, *Arctocephalus gazella*, *Halichoerus grypus*, *Mirounga angustirostris*, *Phoca vitulina*; Atlantic walrus: *Odobenus rosmarus rosmarus*), but only two cetaceans, the harbour porpoise (*Phocoena phocoena*) and the common bottlenose dolphin (*Tursiops truncatus*) (Acquarone et al., 2006; Boyd et al., 1995; Costa, 1988; Costa and Gales, 2003; Costa et al., 2013; Maresh et al., 2014; McHuron et al., 2018; Nagy et al., 1999; Rojano-Doñate et al., 2018; Sparling et al., 2008).

Over the long term (months, years), individuals must maintain a balance between energy intake and energy expenditure, and allocate energy to somatic maintenance, growth, reproduction and physical activity in a manner that maximizes their fitness (Gadgil and Bossert, 1970). TEE can change throughout an individual's lifespan, reflecting changes in energy allocation to different physiological processes. Similarly, body composition also changes throughout life. In humans, TEE and fat-free mass (FFM) decrease in adults older than 60 years (Elia et al., 2000; Manini, 2010; Speakman and Westerterp, 2010). Results regarding age-related changes in fat mass (FM) have been mixed, where FM increased (Coin et al., 2008; Kyle et al., 2001a) or decreased (Elia et al., 2000) with age in humans. Different-sized dog breeds differ in age-related changes in body composition: FM increased with age in the largest breed, but was not related with age in a medium- and small-sized breed (Speakman et al., 2003). Age-related changes in TEE and body composition are not well studied and may be species specific, and data from additional species are needed to shed light on these patterns.

Body mass, specifically FFM, is the most important factor shaping inter-specific variation in TEE (Hudson et al., 2013; Nagy, 2005; Pontzer et al., 2012, 2014; Simmen et al., 2015; Speakman, 2000). But other factors, such as thermoregulatory requirements, physical activity and organ size, are also thought to contribute to variation in daily expenditure. Variation in the size of metabolically expensive organs such as the brain and gut has been put forward as another factor resulting in inter-specific variation in TEE (Aiello and Wheeler, 1995; Isler and van Schaik, 2009; Navarrete et al.,

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2011). Cetaceans, similar to anthropoid primates, have a significantly larger encephalization quotient (a measure of relative brain size) than their sister clade the Hippopotamidae (Boddy et al., 2012), which makes energy expenditure of cetaceans an interesting point of comparison. It has also been proposed that marine mammals have an elevated TEE for their body mass (Hudson et al., 2013; Speakman and Król, 2010; Williams et al., 1993, 2001; Williams et al., 2017), and the most widely accepted hypothesis is that the elevated metabolism reflects the high cost of endothermy in a marine environment. However, other studies have questioned the generality of this hypothesis for all marine mammals (Maresh, 2014; Williams and Maresh, 2016). It has been predicted that heat loss is important only for very small marine mammals, such as sea otters (*Enhydra lutris*) and young otariid seals (Liwanag et al., 2012; Porter and Kearney, 2009), but not for larger-bodied marine mammals (Maresh et al., 2014; Porter and Kearney, 2009).

Daily physical activity is thought to contribute to intra-specific variation in TEE, and TEE has been shown to correlate with daily movement distance in some free-ranging animals, such as llamas (Riek et al., 2019), cheetahs (Scantlebury et al., 2014) and polar bears (Pagano and Williams, 2019). However, other research suggests that organisms maintain TEE homeostatically within a narrow, evolved, species-specific range such that daily expenditure is largely independent of daily physical activity (Pontzer, 2015a, 2017).

The common bottlenose dolphin (*T. truncatus*), a long-lived, highly-encephalized marine mammal, presents an opportunity to examine the effects of body mass, activity and age on daily energy requirements. The metabolic rate of common bottlenose dolphins has been measured at rest, during exercise and post-exercise (Fahlman et al., 2015, 2016, 2018a,b; Holt et al., 2015; Noren et al., 2013; Pedersen et al., 2020; van der Hoop et al., 2014; Williams et al., 1993, 2001, 2017; Yazdi et al., 1999; Yeates and Houser, 2008), but very little is known about their TEE. Previous studies relied on extrapolation from Kleiber's curve for basal metabolic rate (BMR) of terrestrial mammals or extrapolation of caloric content of consumed prey to quantify energetic requirements of dolphins and cetaceans (Bejarano et al., 2017; Benoit-Bird, 2004; Rojano-Doñate et al., 2018). To date, a single analysis (published as a conference abstract) has measured TEE using DLW in wild bottlenose dolphins in Florida (summer TEE: $6.79 \pm 1.11 \text{ W kg}^{-1}$, $N=10$; winter TEE: $4.82 \pm 0.81 \text{ W kg}^{-1}$, $N=4$) (Costa et al., 2013). Additional TEE measurements are needed to determine whether daily expenditure is elevated for common bottlenose dolphins, and marine mammals in general, relative to other taxa. Estimates of bottlenose dolphin daily energy expenditure are also needed to parametrize both intra-specific (Bejarano et al., 2017) and inter-specific bioenergetic models. These models help us to examine trophic interactions (Bejarano et al., 2017), evaluate the population-level consequences of disturbance (Pirota et al., 2018) and understand allometric scaling in marine mammals (Goldbogen et al., 2019). Moreover, bottlenose dolphins are the most common species of cetacean in human care (NMFS, 2019); thus, data on body condition and TEE can help veterinarians in marine mammal facilities refine energy requirements and adjust caloric intake.

In this study, we examined energy expenditure and body composition of 10 adult common bottlenose dolphins (*T. truncatus*) living at two marine mammal facilities: Dolphin Quest (Hawaii, USA) and the Dolphin Research Center (Florida, USA). We measured TEE using the DLW method, the gold standard for measuring TEE outside of a laboratory setting (Speakman, 1997). We investigated the relative effects of body mass, body composition, age and physical activity on TEE. Moreover, we

compared our TEE measurements with two measures of physical activity for 6 of the studied individuals. We also assessed whether TEE of bottlenose dolphins, and more generally of marine mammals, differs from that expected for their body mass compared with other eutherian mammals, using phylogenetic least squares (PGLS) regressions to control for phylogeny.

MATERIALS AND METHODS

Study species

The common bottlenose dolphin, *Tursiops truncatus* (Montagu 1821), is a long-lived, highly encephalized marine mammal found in warm temperate to tropical seas, in coastal as well as offshore waters. Age at sexual maturity varies by region, but females typically reach sexual maturity at 5–13 years and males at 9–14 years (Sergeant et al., 1973; Wells and Scott, 2018). In the wild, individuals rarely live beyond 40 years of age (Fernandez and Hohn, 1998; Mattson et al., 2006; Read et al., 1993; Sergeant et al., 1973). Old female *T. truncatus* show increased inter-birth intervals, suggesting reproductive senescence (Robinson et al., 2017). Similarly, in *Tursiops aduncus*, lactation period and inter-birth intervals increase with maternal age, while calf survival decreases with maternal age, also suggesting reproductive senescence (Karniski et al., 2018). Bottlenose dolphin males reach a larger body mass and total length than females, but patterns of growth are similar for the two sexes and no sexual dimorphism is observed regarding relative muscle mass within any life history category (Mallette et al., 2016).

Animal subjects and study sites

All animal use and methods were approved by the Duke University Institutional Animal Care and Use Committee (Protocol A017-19-01). We collected data from a total of 10 adult bottlenose dolphins (age 10–45 years; Table 1) living in natural seawater lagoons at two marine mammal facilities, Dolphin Quest (DQ), Hawaii, and Dolphin Research Center (DRC), Florida. At DQ, we collected data from 4 males (age 11.2–35 years; Table 1) between April and June 2019. The animals inhabit several natural seawater lagoons separated by floating docks and underwater barriers. The main lagoon is about 750 m² and 3.4 m deep. The other lagoons are 360 m², 190 m² and 120 m², with each of the smaller lagoons having a deepest point of ~2.4 m. During the study period, the DQ dolphins' typical routines were not altered; they were fed herring, capelin and mullet throughout the day. Three of the dolphins were born in zoological facilities, one was collected under a NOAA NMFS permit in the 1980s. During the period of measurements, average water temperature was 25–26°C in the lagoons at DQ. At DRC, we collected data from 5 males and 1 female (age 10–45 years; Table 1) between September and October 2019. DRC dolphins live in variable social groups in natural seawater lagoons (520 m² and 4.8 m deep, 540 m² and 4.8 m deep, 1000 m² and 6.0 m deep, and 2350 m² and 9.1 m deep) situated on the Gulf of Mexico. During the study period, DRC dolphins were fed according to their normal daily routine, which typically included herring, capelin and smelt, offered 3–5 times daily. During the period of measurements, average water temperature was 28°C in the lagoons at DRC.

Energy intake

To determine energy intake, the daily intake in kilograms per fish species ingested was recorded for each individual at both facilities. For dolphins at DQ, subsamples from each batch of fish were sampled via bomb calorimetry at Michelson Laboratories (Commerce, CA, USA) to estimate the caloric content (kcal) of

Table 1. Overview of measurements for each common bottlenose dolphin (*Tursiops truncatus*)

ID	Study site	Age (years)	Sex	Body mass (kg)	TEE (kcal day ⁻¹)	Body fat (%)	FFM (kg)	TBW (l)	Water turnover (l day ⁻¹)	Baseline activity	High energy behaviors (min ⁻¹)
DRC Female 1	DRC	45	F	213.6	8716	18.1	174.9	128.1	42.33	1.908	0.102
DRC Male 1	DRC	~40*	M	256.8	7028	9.8	231.7	169.6	47.26	1.138	0.189
DRC Male 2	DRC	31	M	210	13,026	12.3	184.1	134.8	39.02	1.762	0.149
DRC Male 3	DRC	29	M	265.4	18,274	6.4	248.3	181.8	46.21	1.829	0.294
DRC Male 4	DRC	22	M	257.2	20,046	8.1	236.4	173.0	49.57	1.790	0.301
DRC Male 5	DRC	~10*	M	160.9	13,004	9.7	145.3	106.4	33.26	1.791	0.123
83H1	DQ	11.2	M	142.9	6948	1.4	140.8	103.1	25.60	–	–
9FL3	DQ	~35*	M	245.8	11,526	4.7	234.1	171.4	28.55	–	–
6JK5	DQ	24.9	M	199.6	13,859	4.8	189.9	139.0	39.87	–	–
9ON6	DQ	21.5	M	193	15,105	4.2	184.8	135.3	31.69	–	–

Measurements were obtained at the two study sites: Dolphin Research Center, FL, USA (DRC) and Dolphin Quest, HI, USA (DQ). Data are presented on age, sex (F, female; M, male), body mass, unadjusted total energy expenditure (TEE), body fat percentage, fat-free mass (FFM), total body water (TBW), water turnover, baseline activity and high energy behaviors.

*Age was estimated for these individuals because they were born in the wild.

the ingested diet. For dolphins at DRC, subsamples from each batch of fish were analyzed for caloric content either by the fish supplier or by an independent laboratory (ABC Research Corporation, Gainesville, FL, USA). We corrected food intake values assuming a 90% assimilation efficiency (Lockyer, 2007; Reddy et al., 1994) and provide energy intake relative to body mass (kcal kg⁻¹).

Body mass measurements and sample collection

Body mass was measured every 2 weeks using an Altralite scale (Rice Lake Weighing Systems) and GSE 250SS indicator (GSE Scale Systems; ±0.1 kg) at DQ and a SRV713W scale (SR Instruments; ±0.1 kg) at DRC. In both places, blood and urine were collected voluntarily, without restraint. Blood was collected from the ventral fluke, urine was collected with the ventral area above the water surface.

TEE

Using the DLW method (Lifson and McClintock, 1966; Speakman, 1997), we determined TEE of 10 bottlenose dolphins (see Table S1 for information on dilution spaces and isotope depletion rates). Dolphins ingested premeasured doses (125–195 g) of DLW (6% ²H₂O, 10% H₂¹⁸O) tailored to body mass to provide sufficient initial isotopic enrichment (Speakman, 1997). Each individual provided one urine or blood sample (4 ml) prior to dosing and another three to five samples post-dose ingestion. The first post-dose ingestion was collected 4.4–7.5 h after dosing, and the remainder were collected every 2–3 days for 5–9 days. Mean duration from dosing to last urine sample was 7.5 days. Samples were kept frozen and shipped in an insulated container to Duke University for isotopic analysis.

Isotope analysis

Samples were filtered using carbon black and a 30 kDa centrifuge concentrator (Vivaspin®). Enrichments of ²H and ¹⁸O were determined using integrated cavity off-axis spectroscopy (ABB®). We used the slope–intercept method to determine the dilution spaces ND and NO and the depletion rates kD and kO for ²H and ¹⁸O, respectively (Berman et al., 2020; Pontzer, 2018; Speakman, 1997). We ran all samples in triplicate or quadruplicate, and used the average isotope enrichment for subsequent calculations. We determined total body water (TBW) from isotope dilution as:

$$TBW = (NO/1.007 + ND/1.041)/2. \quad (1)$$

The mean (±s.d.) isotope dilution space ratio was 1.016±0.030. We calculated the rate of CO₂ production (mol day⁻¹) following the

two-pool equation (Speakman, 1997) as:

$$rCO_2 = 0.455 TBW(1.007 kO - 1.041 kD). \quad (2)$$

We used CO₂ production to calculate TEE (kcal day⁻¹) using the Weir equation (Weir, 1949):

$$TEE = 22.4 rCO_2(1.1 + 3.9/FQ), \quad (3)$$

where FQ is the food quotient, which reflects the macronutrient content of the diet. We used FQ=0.8, and FFM was calculated from TBW assuming a hydration coefficient of 0.732 for FFM. We calculated FM by subtracting FFM from total body mass.

Physical activity of DRC dolphins

We collected behavioral data during the 5–9 days after the dose was administered. We conducted observations during 118 training sessions, and noted the number of behaviors with a high cost of locomotion shown per dolphin per session. We defined behaviors as having a high cost of locomotion that involved fast swimming (which leaves white water/big wake), and/or propelling more than ¾ of the body up out of the water, and considered the following behaviors as having a high cost of locomotion: dive, back dive, spiral dive, flying forward tail walk, front flip, back flip, back tail walk, forward tail walk, breach, speed swim, tummy speed swim, spiral speed swim, vertical spin, belly flop, hurdle jump, slip and slide on dock, dive over object, banana jump, beach on dock. For each individual, we observed sessions for an average of 305 ±57.7 min (range 217–384 min), and individuals showed 56.8±23.5 (range 39–91) behaviors with a high cost of locomotion. Sessions occurred both in the mornings (between 08:30 h and 11:26 h; 46.6% of sessions) and in the afternoons (between 12:01 h and 16:45 h; 53.4% of sessions). For every individual, we calculated the number of high energy tasks shown per minute as the sum of high energy tasks divided by the number of session minutes for that dolphin. To determine a baseline level of physical activity, we conducted observations outside of session times.

Baseline physical activity during the day was recorded using scan sampling (N=101 scans). For each individual, an average of 17 observations (range 16–20) was conducted during the DLW measurement period. Observations were conducted both in the mornings (between 08:43 h and 11:49 h; 40% of observations) and in the afternoons (between 12:00 h and 17:00 h; 60% of observations). Each observation lasted 10 min, and every 30 s the focal dolphin's activity level was rated on a scale from 1 to 3. Score 1 was noted when the individual was floating or showed very low

activity, score 2 was noted for regular swimming, and score 3 was noted for fast swimming or jumping. For every individual, we calculated baseline activity as the sum of all scores recorded in all observations (range 454–607) divided by the number of scan sampling points. Because studies have found energy expenditure at slow swim speeds is only slightly elevated compared with BMR, it is likely that score 1 and 2 have a similarly low cost of locomotion, compared with score 3 (Williams et al., 2017). Consequently, we calculated a second activity, in which we assumed that score 1 and 2 are relatively similar in their cost of locomotion, and assigned score 1 a value of 1.3 and score 2 a value of 1.7, and then calculated an alternative activity as the sum of all scores divided by the number of scan sampling points.

We did not conduct observations early in the mornings and late in the afternoons or during the night because the facility is closed during that time. Wild dolphins show an increase in activity around sunrise and sunset, which is associated with feeding behaviors (Miller et al., 2010; Vermeulen et al., 2015). At DRC, dolphins were fed only during the time range during which our observations occurred and thus we do not assume that activity of DRC dolphins peaked around sunrise and sunset.

Comparative TEE

To compare bottlenose dolphin TEE with data from other marine and eutherian mammals, we used published comparative datasets (Pontzer, 2015b; Pontzer et al., 2014; Simmen et al., 2015), and included additional species, such as the polar bear (Pagano and Williams, 2019), sea otter (Williams et al., 1988) and walrus (Acquarone et al., 2006) (Table S2). We were unable to include previously published (as conference abstract) TEE data for bottlenose dolphins because body mass was not reported (Costa et al., 2013). We used the phylogenetic structure of the analyzed species from a published mammalian supertree (Bininda-Emonds et al., 2007). Seven species, for which TEE data were available, were not present in the phylogenetic tree and we removed these species for the phylogenetically controlled analysis (*Canis familiaris*, *Eremitalpa namibensis*, *Lemmus trimucronatus*, *Papio anubis*, *Papio cynocephalus*, *Perognathus formosus* and *Vulpes macrotis*).

Statistical analyses

We conducted all analyses in R v.3.6.2 (<http://www.R-project.org/>). We used Welch's *t*-tests to assess study population-level differences in age, body mass and TEE. We investigated relationships between ln-transformed TEE and ln-transformed body composition variables using linear models (LMs). We did not use linear mixed models (LMMs) with population (DQ versus DRC) as a random factor because such models resulted in singularity. We assessed the relationship between body mass and water turnover using a LMM that included population (DQ versus DRC) as a random factor. We fitted all LMs using the function 'lm', and LMMs using the function 'lmer' in the R package lme4 (Bates et al., 2015). Unadjusted TEE increases with body mass (Nagy, 1987, 2005; Speakman, 2000), but larger individuals generally expend less energy per gram body mass than smaller individuals (Pontzer et al., 2016; Speakman, 2005; Tschöp et al., 2012). Different tissues have different metabolic rates, and adipose tissue has a much lower metabolic activity than FFM (Krebs, 1950). Thus, body composition, and especially FFM, affects TEE. To control for variation in FFM, we calculated an adjusted TEE for each dolphin based on a multiple regression model with TEE as the dependent variable and FFM as an independent variable (Poehlman and Toth,

1995; Pontzer, 2015b; Ravussin and Bogardus, 1989; Tschöp et al., 2012). TEE and FFM were ln-transformed for this model. Using predicted and residual TEE for each measurement, we calculated adjusted TEE as:

$$\text{Adjusted TEE} = 100(1 + \text{Residual TEE}/\text{Predicted TEE}). \quad (4)$$

An adjusted TEE of 120% indicates a measured TEE that is 20% greater than predicted and an adjusted TEE of 80% indicates a measured TEE that is 20% lower than predicted.

We determined age-related changes in body mass, FFM, FM, body fat percentage, adjusted TEE and energy intake, and the relationship between energy intake and expenditure using LMMs. We included age as a fixed factor and population (DQ versus DRC) as a random factor. In mammals, body mass increases with age until an asymptote is reached (Sebens, 1987; West et al., 2001), and in many species a quadratic relationship between age and body mass has been reported, where body mass decreases again at old age (Nussey et al., 2011; Pépin et al., 1996; Tafani et al., 2013). Because the relationships between age and body mass, FFM, FM, body fat percentage and adjusted TEE are likely non-linear, we included age and its quadratic term to assess age-related changes in body mass, FFM, FM, body fat percentage and adjusted TEE. Because of non-significance of the term, we removed the quadratic term of age in the models assessing age-related changes in FFM and body fat percentage.

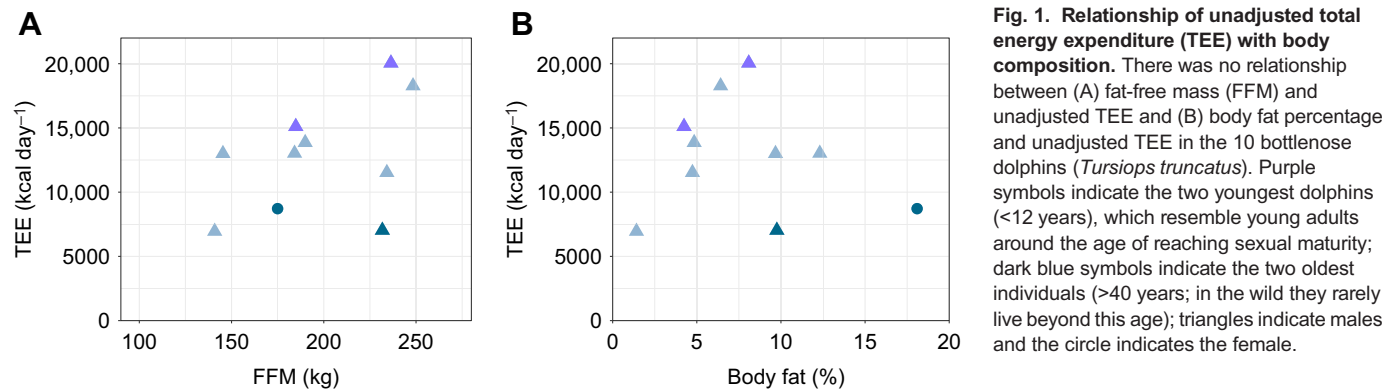
We assessed the relationship of two measures of activity, (1) baseline activity level and (2) high energy behaviors, with age and body fat percentage using linear models. We examined the relationship between Adjusted TEE and two measures of activity using LMs, which included one measure of activity (baseline activity or high energy behaviors) as fixed factor.

We used PGLS regression to assess the association between body mass and TEE after controlling for phylogenetic relatedness between species. We used the 'pgls' function in the caper package to perform PGLS regression (<https://CRAN.R-project.org/package=caper>). We accounted for polytomies using the function 'multi2di' in the R package ape (Paradis and Schliep, 2019). We fitted a model using a maximum-likelihood (ML) estimate for Pagel's lambda (Pagel, 1999). We included habitat (marine versus terrestrial) as an explanatory variable to assess whether marine mammals have a similar or an elevated TEE compared with that of similarly sized terrestrial mammals. In addition, we also fitted two linear models (one including all TEE data and one excluding the 7 species that were not present in the mammalian supertree) using habitat as an explanatory variable. We report model results as estimates±s.e.

RESULTS

We did not find statistically significant differences in body mass (Welch two sample *t*-test: $t=1.197$, d.f.=6.35, $P=0.27$) between dolphins at DRC (mean±s.d. mass 227.3±40.3 kg, range 160.9–265.4 kg) and dolphins at DQ (195.3±42.1 kg, range 142.9–245.8 kg; Table 1). Further, we did not find statistically significant differences in age ($t=0.894$, d.f.=7.65, $P=0.40$; Table 1), unadjusted TEE ($t=0.540$, d.f.=7.91, $P=0.60$; Table 1) or adjusted TEE ($t=0.344$, d.f.=7.76, $P=0.74$).

FFM, FM and body fat percentage were not related to unadjusted TEE [LMs: FFM: estimate±s.e. 0.73 ± 0.59 , d.f.=8, $t=1.24$, $P=0.25$, 95% confidence interval (CI) -0.63 – 2.08 , $R^2=0.06$; Fig. 1A; FM: 0.10 ± 0.15 , $t=0.63$, $P=0.55$, d.f.=8, 95% CI -0.26 – 0.45 , $R^2=-0.07$; body fat percentage: 0.08 ± 0.18 , $t=0.43$, $P=0.68$, d.f.=8, 95% CI



$-0.34-0.50$, $R^2=-0.10$; Fig. 1B). Water turnover increased with body mass (LMM: 0.11 ± 0.04 , d.f.=7.34, $t=2.67$, $P=0.03$, $R^2=0.69$).

Age-related changes in adjusted TEE and body composition

Body mass showed a quadratic relationship with age (LMM: age 11.36 ± 3.59 , d.f.=6.1, $t=3.16$, $P=0.02$; age^2 -0.17 ± 0.07 , d.f.=6.2, $t=-2.59$, $P=0.04$, $R^2=0.69$; Fig. 2A). FFM also showed a quadratic relationship with age (age 11.40 ± 3.85 , d.f.=7.0, $t=2.96$, $P=0.02$; age^2 -0.18 ± 0.07 , d.f.=7.0, $t=-2.56$, $P=0.04$, $R^2=0.69$). Neither FM nor body fat percentage showed a quadratic relationship with

age (both $P>0.14$). FM increased with age (0.52 ± 0.13 , d.f.=7.1, $t=4.12$, $P=0.004$, $R^2=0.87$; Fig. 2B), while body fat percentage did not vary with age (0.18 ± 0.09 , d.f.=7.1, $t=2.10$, $P=0.07$, $R^2=0.70$; Fig. 2D). Adjusted TEE showed a quadratic relationship with age (age 5.57 ± 3.25 , d.f.=6.1, $t=1.71$, $P=0.14$; age^2 -0.13 ± 0.06 , d.f.=6.2, $t=-2.22$, $P=0.07$, $R^2=0.61$; Fig. 2C). Energy intake also decreased with age (-0.36 ± 0.14 , d.f.=7.2, $t=-2.49$, $P=0.04$, $R^2=0.69$; Fig. 3A), consistent with our measures of TEE, but the relationship between energy intake and expenditure did not reach statistical significance (0.14 ± 0.10 , d.f.=7.0, $t=1.39$, $P=0.21$, $R^2=0.65$; Fig. 3B).

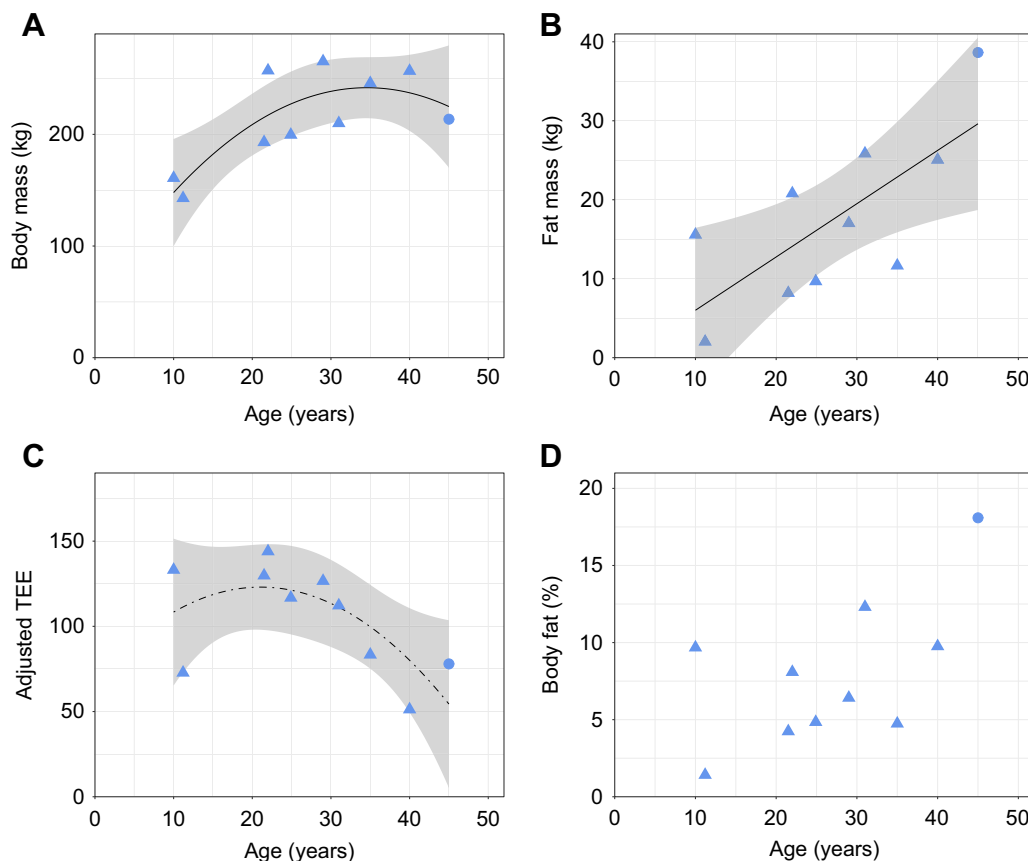


Fig. 2. Age-related changes in body composition and TEE. Age-related changes in (A) body mass, (B) fat mass, (C) Adjusted TEE and (D) body fat percentage in 10 bottlenose dolphins (*T. truncatus*). The black line in A indicates the quadratic regression line $y=51.0+11.36(\text{age})-0.17(\text{age}^2)$ ($R^2=0.69$); the black line in B shows the linear regression line $y=2.27+0.52(\text{age})$ ($R^2=0.87$); and the dashed line in C shows the quadratic regression line $y=65.64+5.57(\text{age})-0.13(\text{age}^2)$ ($R^2=0.61$; line is dashed because the P -value for the quadratic term of age was 0.07). Shading represents the 95% confidence interval (CI). Triangles indicate males and the circle indicates the female.

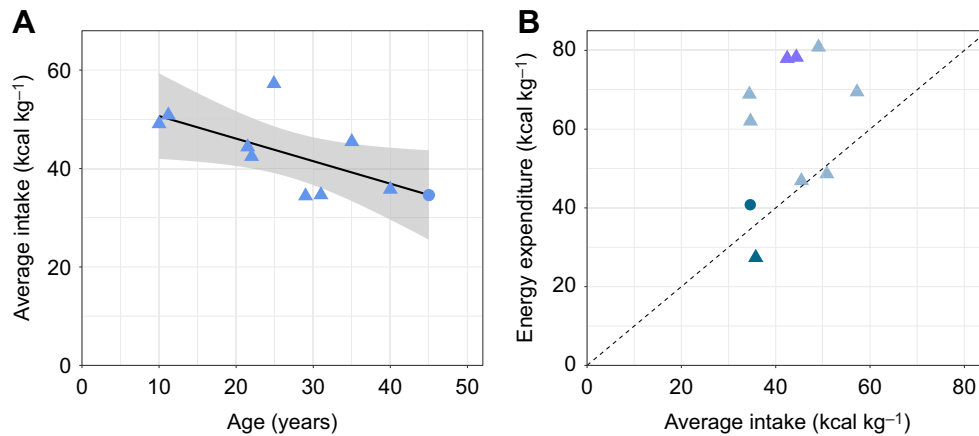


Fig. 3. Relationship of energy intake with age and energy expenditure. (A) Age-related change in energy intake per kilogram of body mass (during the week of TEE measurement) in 10 bottlenose dolphins (*T. truncatus*). The black line indicates the linear regression line, shading represents the 95% CI. (B) Energy expenditure per kilogram of body mass versus energy intake per kilogram of body mass. The dashed line indicates the regression line when energy intake would equal expenditure. Purple symbols indicate the two youngest dolphins (<12 years), which resemble young adults around the age of reaching sexual maturity; dark blue symbols indicate the two oldest individuals (>40 years; in the wild they rarely live beyond this age); triangles indicate males and the circle indicates the female. Food intake values were corrected assuming a 90% assimilation efficiency (Lockyer, 2007; Reddy et al., 1994).

Activity and TEE

There was little variation with regard to baseline activity level. However, one of the two individuals that was >40 years (DRC Male 1) had a much lower baseline activity level (1.13) compared with all other individuals (1.67–1.90; Table 1, Fig. 4). Neither total activity score (the sum of all scores) nor baseline activity level (total activity score divided by number of scores) was related to age (total activity: -3.11 ± 1.61 , d.f.=4, $t=-1.94$, $P=0.13$, $R^2=0.43$; baseline activity level: -0.01 ± 0.03 , d.f.=4, $t=-0.60$, $P=0.58$, $R^2=0.07$; Fig. 4A) or body fat percentage (total activity: 0.36 ± 6.84 , d.f.=4, $t=0.05$, $P=0.96$, $R^2<0.01$; baseline activity: 0.02 ± 0.03 , d.f.=4, $t=0.46$, $P=0.67$, $R^2=0.04$; Fig. 4C). Using the alternative activity score yielded the same results, and neither baseline activity level (-0.003 ± 0.004 , d.f.=4, $t=-0.78$, $P=0.48$, $R^2=0.10$) nor total activity score was correlated with age (-1.60 ± 1.98 , d.f.=4, $t=-0.81$, $P=0.46$, $R^2=0.12$) or body fat percentage (total activity: -2.43 ± 6.41 , d.f.=4, $t=-0.38$, $P=0.72$, $R^2=0.03$; baseline activity level: 0.01 ± 0.01 , d.f.=4, $t=0.39$, $P=0.72$, $R^2=0.03$). The rate of high energy behaviors per minute was not related to age (-0.001 ± 0.003 , d.f.=4, $t=-0.38$, $P=0.72$, $R^2=0.03$; Fig. 4B) or body fat percentage (-0.02 ± 0.01 , d.f.=4, $t=-2.52$, $P=0.07$, $R^2=0.56$; Fig. 4D).

There was no relationship between adjusted TEE and total activity score (0.35 ± 0.27 , d.f.=4, $t=1.32$, $P=0.26$, $R^2=0.26$) or baseline activity (85.48 ± 47.16 , d.f.=4, $t=1.81$, $P=0.14$, $R^2=0.40$; Fig. 4E). Using the alternative activity score also showed no relationship between adjusted TEE and total activity score (-0.11 ± 0.33 , d.f.=4, $t=-0.35$, $P=0.75$, $R^2=0.02$) or baseline activity (221.3 ± 109.9 , d.f.=4, $t=2.01$, $P=0.11$, $R^2=0.45$). There was no relationship between high energy behaviors and adjusted TEE (183.1 ± 186.3 , d.f.=4, $t=0.98$, $P=0.38$, $R^2=0.16$; Fig. 4F).

Comparative TEE

Both the PGLS and the non-phylogenetic linear regression indicated that marine mammals have an elevated TEE compared with terrestrial mammals (PGLS: -0.699 ± 0.222 , $t=-3.14$, $P=0.002$, d.f.=102, adjusted $R^2=0.90$; linear regression: -0.901 ± 0.156 , $t=-5.78$, $P<0.0001$, d.f.=102, adjusted $R^2=0.97$; linear regression including the 7 species excluded for PGLS: -0.900 ± 0.158 , $t=-5.72$, $P<0.0001$, d.f.=109, adjusted $R^2=0.97$; Fig. 5A). TEE of bottlenose dolphins fell 17.1% below the regression line for

marine mammals, similar to that of harbour porpoises (*P. phocoena*) and Galápagos fur seals (*A. galapagoensis*) which fell 14.3% and 11.4% below the regression line for marine mammals (Fig. 5B), respectively. However, TEE of several terrestrial mammals fell closer to the regression line for marine mammals than to the regression line for terrestrial mammals. For example, TEE of the polar bear (*Ursus maritimus*) fell 34.5% above the regression line for terrestrial mammals, and TEE of the gray wolf (*Canis lupus*) and the African wild dog (*Lycaon pictus*) fell 30.8% and 36.4% above the regression line for terrestrial mammals, respectively (Fig. 5B).

DISCUSSION

TEE measurements are available only for a limited number of marine mammals, and only for two cetaceans. Moreover, age-related changes in TEE and body composition have rarely been evaluated in cetaceans. Here, we report that adjusted TEE and body composition of bottlenose dolphins change with age. Body mass and FFM showed a quadratic relationship with age, suggesting that body mass and FFM decrease in older dolphins. Such an age-related loss of body mass was not reported in a cross-sectional study in wild bottlenose dolphins (Read et al., 1993). But a longitudinal study reported inter-individual variation in aging rates in US Navy bottlenose dolphins (Venn-Watson et al., 2020), and serum creatinine levels decrease with increasing age, indicating a decrease in muscle mass in the species (Venn-Watson et al., 2011). Muscular senescence has also previously been reported in wild Weddell seals (*Leptonychotes weddellii*) and Atlantic spotted dolphins (*Stenella frontalis*) (Hindle et al., 2009; Sierra et al., 2013). Thus, a decrease in FFM at old age, as reported here, might indicate muscular senescence, similar to sarcopenia occurring in old humans (Larsson et al., 2019).

To date, few studies have investigated whether animals show age-related changes in body composition and TEE. Our results show that dolphin FM increased with age: young adults (individuals younger than 20 years) had a FM of 8.8 kg (5.5% body fat) and individuals older than 40 years had a FM of 31.8 kg (13.9% body fat). Although not significant in our sample ($P=0.07$), likely because of the small sample size, body fat percentage showed an increasing trend with age (Fig. 2D). Adjusted TEE showed the opposite pattern and decreased with age, where the two individuals older than 40 years

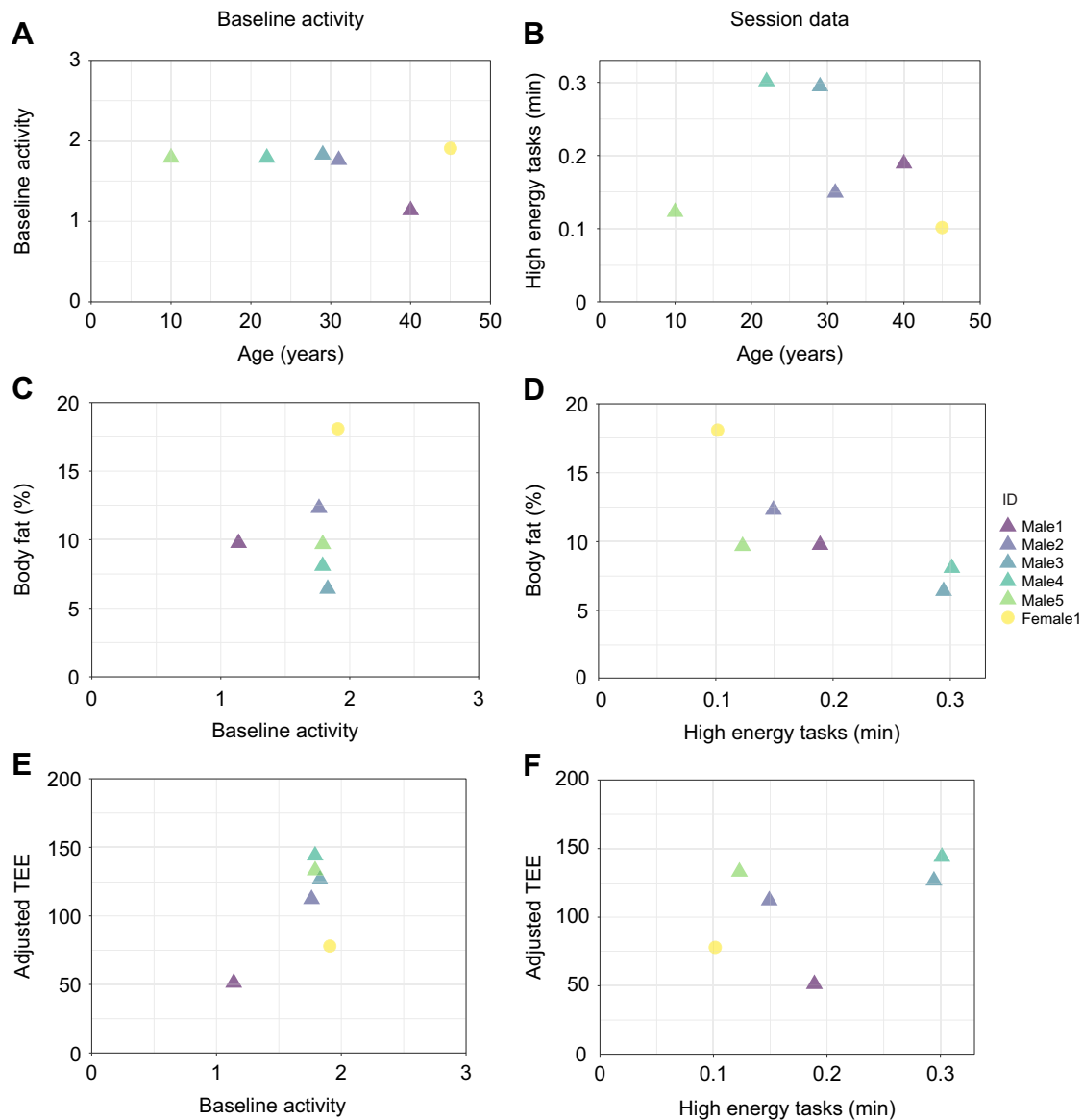


Fig. 4. Relationship between activity and age, body composition and TEE. Relationship of (A,C,E) baseline activity (total activity score divided by number of observations) and (B,D,F) high energy behaviors with age, body fat percentage and adjusted TEE in 6 bottlenose dolphins (*T. truncatus*) living at DRC. No significant relationships were found. Triangles indicate males and the circle indicates the female.

expended 48.8% and 22.1% less energy than predicted, after accounting for FFM. Age-related changes in dolphins stand in contrast to findings in some other species. Several long-lived species, such as thick-billed murres (*Uria lomvia*) and tufted capuchins (*Sapajus apella*), do not show an age-related decline in TEE (Edwards et al., 2017; Elliott et al., 2014). Similarly, naked mole-rats (*Heterocephalus glaber*), a long-lived rodent (maximum lifespan >27 years), show no age-related changes in body mass, BMR, body fat percentage or FFM (O'Connor et al., 2002). However, changes in body composition in the form of muscular senescence have been reported in several long-lived marine mammals, including bottlenose dolphins (Hindle et al., 2009; Sierra et al., 2013; Venn-Watson et al., 2011, 2020). Dolphins in this study show age-related changes in TEE and FFM that are similar to those reported in humans, where TEE and FFM decrease in adults older than 52 or 60 years (Elia et al., 2000; Manini, 2010; Speakman and Westerterp, 2010). Results regarding age-related changes in FM have been mixed in humans, where FM has been

found to increase (Coin et al., 2008; Kyle et al., 2001a,b) and decrease (Elia et al., 2000; Speakman and Westerterp, 2010) with age. In dogs, FM increased with age in Great Danes, but was not related to age in smaller breeds such as Labradors and Papillons (Speakman et al., 2003). The same study found that FFM increased with age in Papillons but was not related to age in the other two breeds (Speakman et al., 2003). Japanese macaques (*Macaca fuscata*) show large inter-individual variation in body fat percentage in captivity, with adults having a higher percentage than young individuals, but age-related changes in body fat were not evident in adults (Hamada et al., 2003).

An age-related increase in FM, as reported in this study (Fig. 2B), could be caused by an increase in energy intake, a decline in TEE (Johnstone et al., 2005; Speakman and Westerterp, 2010; Speakman et al., 2003), or a combination of the two. Energy intake decreased with age in our sample (Fig. 3A). A similar age-related decrease in energy intake has previously been reported in bottlenose dolphins (Reddy et al., 1994), and energy intake of three adult harbour

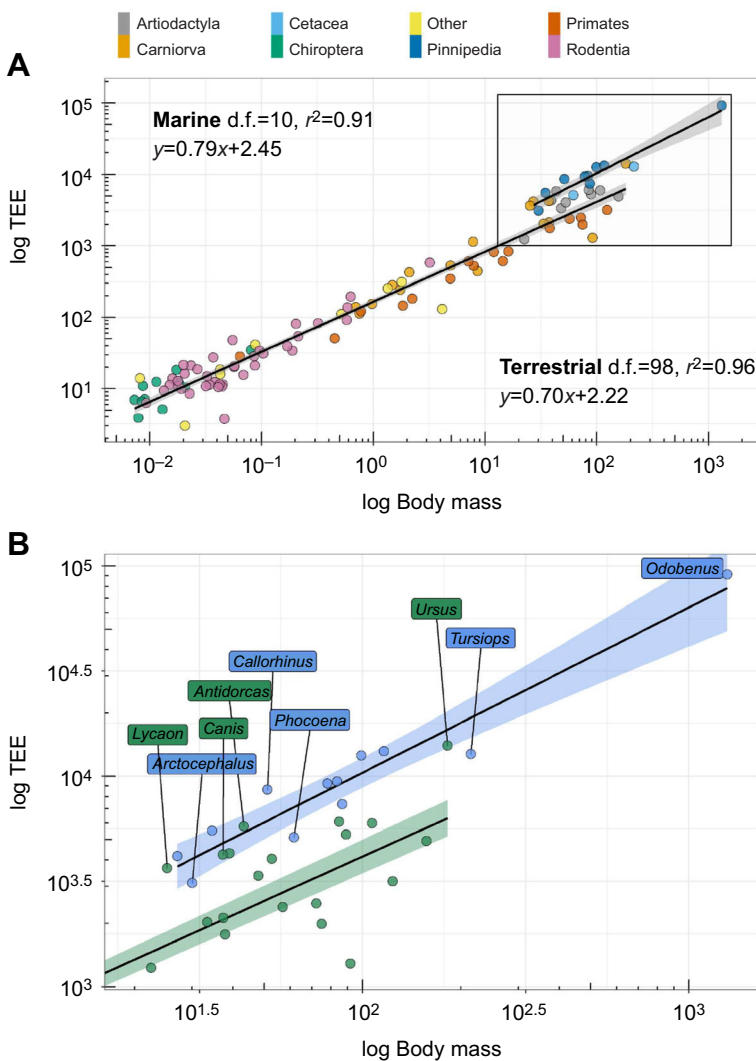


Fig. 5. Body mass and TEE in eutherian mammals. (A) TEE (kcal day⁻¹) versus body mass (kg) for eutherian mammals ($n=112$ species). Separate linear regression lines are presented for terrestrial ($n=100$ species) and marine ($n=12$ species) mammals. Shading represents the 95% CI for the linear regressions. The s.e. is smaller than the symbols for the species. (B) Inset from A in more detail, showing marine mammals in blue and terrestrial mammals in green. Specific taxa of marine and mammals are highlighted to allow inter-specific comparisons.

porpoises (*P. phocoena*) decreased by 20% over a 9 year period, potentially related to aging (Rojano-Doñate et al., 2018). Thus, the age-related increase in FM does not seem to be the result of excessive energy intake (Fig. 3B). We did not measure BMR for this study, but BMR is known to make up a large percentage of TEE in avian and mammalian species (30–86%; Drent and Daan, 1980; Ricklefs et al., 1996; Nagy et al., 1999; Speakman, 2000; Speakman et al., 2003) and thus an age-related modulation of basal metabolism might have a large effect on TEE. However, the extent to which BMR explains variation in TEE of cetaceans is still unclear. Additional studies assessing physiological alterations associated with aging need to be conducted in more species, preferably using longitudinal data rather than cross-sectional data, to understand the causes of these age-related changes.

Limitations of our study include the small number of dolphins measured, especially females ($N=1$), and the lack of very young individuals. The small sample size likely also explains why we did not find the expected positive relationship between FFM and TEE. Uncertainty in the ratio of TBW to FFM (hydration coefficient) and its variation with age might limit the accuracy of isotope-based body composition measurements. It is uncertain whether the low TEE and low body fat percentage of one individual (83H1) are accurate measurements or whether an error occurred during the data collection period (e.g. some of the DLW dose was not ingested).

This individual had a much lower TEE compared with a similar aged individual (DRC Male 5), but at the same time they had a comparable body mass and FFM.

We found little inter-individual variation in baseline activity, which was very similar for 5 out of 6 dolphins for which activity data were available (Fig. 4). We found that neither baseline activity nor the number of high energy behaviors per minute varied with age or an individual's body fat percentage, and that adjusted TEE did not change with any measure of activity. Limitations of our study include the lack of accelerometer data, observational data of 24 h activity, or measures of swim speed and leap heights, both of which have been shown to influence metabolic rate in bottlenose dolphins (Williams et al., 2017; Yazdi et al., 1999). We think that our measurements of activity are reflective of the relative differences in activity level between subjects, but it is possible that our measures do not accurately reflect variation in daily activity among individuals, causing the similarity in activity level between individuals and the lack of correspondence between activity level and TEE in dolphins. TEE has been shown to correlate with daily movement distance in some free-ranging animals, such as llamas (Riek et al., 2019), cheetahs (Scantlebury et al., 2014) and polar bears (Pagano and Williams, 2019). However, many studies show that physical activity is unrelated to TEE in humans, non-human primates and rodents (Edwards et al., 2017; Perrigo, 1987; Pontzer,

2015b, 2017; Pontzer et al., 2014; Rimbach et al., 2018), and these studies suggest that physical activity is a relatively poor predictor of TEE both within and between species (Pontzer, 2015b, 2017). Research on more species is required to determine whether the discrepancy between studies that found an association of TEE and activity and those that did not might be due to phylogenetic or ecological differences between the studied taxa (e.g. carnivores versus herbivores) or aspects of the habitat (e.g. extreme thermal environments). However, we note that TEE of human populations with demanding lifestyles (e.g. hunter-gatherers and forager-horticulturalists) is similar to that of sedentary industrialized populations (Pontzer, 2017; Pontzer et al., 2016, 2018; Urlacher et al., 2019, 2021), and that physically active populations of wild primates have similar TEE to primate populations in captivity (Pontzer et al., 2014). Similarly, comparisons of captive versus wild or free-ranging populations of pandas, kangaroos and sheep also show no difference in TEE (Munn et al., 2013; Nie et al., 2015). Further, laboratory studies in rodents and birds often show no effect of increased physical activity on TEE (O'Neal et al., 2017; Pontzer, 2015a). Clearly, the relationship between TEE and physical activity can be complex, with the body apparently adjusting to differences in habitual activity, and warrants further study.

Both the analysis controlling for phylogeny and the non-phylogenetic linear regression indicated that marine mammals have an elevated TEE compared with terrestrial mammals (Fig. 5). We found that bottlenose dolphins expended on average 17.1% less energy than other marine mammals of a similar body mass (Fig. 5B). Similarly, harbour porpoises (*P. phocoena*) and Galápagos fur seals (*A. galapagoensis*) used 14.3% and 11.4% less energy than expected for their size. It is important to note that the sampled marine mammal species to date under-represent several marine mammal taxa – sirenians, mysticetes, most odontocetes, and many otariids and phocids. Life history differences (e.g. carnivores versus herbivores and locomotory mode) likely account for some differences within the broad group of 'marine mammals' and additional data are needed (Williams et al., 2020). It has been proposed that marine mammals have a high energy expenditure because homeothermy is costly in a marine environment. In support of this notion, some studies report that the BMR of bottlenose dolphins is 2.3 times those predicted by scaling equations for terrestrial mammals (Williams et al., 2001), and TEE has been reported to be 7 times BMR (Costa et al., 2013). However, there is large variation in BMR and resting metabolic rate (RMR) values of bottlenose dolphins, and of studies which met BMR criteria, several reported values (0.90–1.68 W kg⁻¹) relatively close to values predicted by Kleiber's scaling equation for terrestrial mammals (Noren et al., 2013; Pedersen et al., 2020; van der Hoop et al., 2014; Yeates and Houser, 2008), while others reported higher values (2.25–2.48 W kg⁻¹) (Williams et al., 1993, 2001, 2017). Thus, there is uncertainty regarding whether BMR/RMR of bottlenose dolphins is truly elevated compared with that of terrestrial mammals. The results from our study show a low TEE of 2.91 W kg⁻¹ (DQ: 2.95±0.75 W kg⁻¹ and DRC: 2.98±1.03 W kg⁻¹) compared with previously reported values of TEE in the same species collected in Florida during summer (6.79 ±1.11 W kg⁻¹, when water temperatures were comparable to water temperatures in this study) and winter (4.82±0.81 W kg⁻¹; Costa et al., 2013). Thus, it is unlikely that differences in TEE between the two studies stem from differences in the thermal environments and resulting thermoregulatory needs. TEE data reported here add to a small but growing set of measurements for cetaceans and other marine mammals. Additional cetacean TEE studies will improve

our understanding of how TEE compares with that of terrestrial mammals and how it scales with size, thus improving bioenergetic models in ecology and life history and informing conservation outcomes (Bejarano et al., 2017; Goldbogen et al., 2019).

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Competing interests

The authors declare no competing or financial interests.

Author contributions

Conceptualization: A. Allen, B.H., H.S., H.P.; Methodology: H.P., E.G.; Investigation: A. Amireh, A. Allen, E.G., C.K., H.S.; Resources: A. Allen, B.H., E.G., H.P.; Data curation: R.R., A. Allen; Writing - original draft: R.R.; Writing - review & editing: R.R., A. Amireh, A. Allen, B.H., E.G., C.K., H.S., H.P.; Visualization: R.R.; Supervision: A. Allen, B.H., H.P.; Project administration: H.P., A. Allen, H.S.; Funding acquisition: B.H., H.P., A. Allen

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Table S1. Overview of dilution spaces (ND and NO), dilution space ratio (ND/NO), deuterium depletion rate (kD) and ^{18}O depletion rate (kO) for each individual.

ID	Study site	Mass (kg)	ND (mol)	NO (mol)	ND/NO	kD (d ⁻¹)	kO (d ⁻¹)
DRC Female 1	DRC	213.6	7373.32	7180.98	1.027	0.3030	0.3332
DRC Male 1	DRC	256.8	9819.65	9460.29	1.038	0.2540	0.2748
DRC Male 2	DRC	210	7746.81	7572.01	1.023	0.2658	0.3032
DRC Male 3	DRC	265.4	10464.52	10197.03	1.026	0.2331	0.2705
DRC Male 4	DRC	257.2	9949.78	9715.54	1.024	0.2629	0.3059
DRC Male 5	DRC	160.9	6121.60	5968.10	1.026	0.2868	0.3324
83H1	DQ	142.9	5909.35	5807.58	1.018	0.2287	0.2562
9FL3	DQ	245.8	9704.76	9771.22	0.993	0.1552	0.1802
6JK5	DQ	199.6	8016.22	7782.69	1.030	0.2625	0.3006
9ON6	DQ	193	7851.49	7525.00	1.043	0.2130	0.2530

Table S2. Body mass and total energy expenditure (TEE) in eutherian mammals studied with doubly labeled water. Average values of mass and TEE are reported per species. In cases where more than one reference is presented, we averaged data from all included references.

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