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RESEARCH ARTICLE

Novel locomotor muscle design in extreme deep-diving whales

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SUMMARY

Most marine mammals are hypothesized to routinely dive within their aerobic dive limit (ADL). Mammals that regularly perform deep, long-duration dives have locomotor muscles with elevated myoglobin concentrations that are composed of predominantly large, slow-twitch (Type I) fibers with low mitochondrial volume densities (V_{mt}). These features contribute to extending ADL by increasing oxygen stores and decreasing metabolic rate. Recent tagging studies, however, have challenged the view that two groups of extreme deep-diving cetaceans dive within their ADLs. Beaked whales (including *Ziphius cavirostris* and *Mesoplodon densirostris*) routinely perform the deepest and longest average dives of any air-breathing vertebrate, and short-finned pilot whales (*Globicephala macrorhynchus*) perform high-speed sprints at depth. We investigated the locomotor muscle morphology and estimated total body oxygen stores of several species within these two groups of cetaceans to determine whether they (1) shared muscle design features with other deep divers and (2) performed dives within their calculated ADLs. Muscle of both cetaceans displayed high myoglobin concentrations and large fibers, as predicted, but novel fiber profiles for diving mammals. Beaked whales possessed a sprinter's fiber-type profile, composed of ~80% fast-twitch (Type II) fibers with low V_{mt}. Approximately one-third of the muscle fibers of short-finned pilot whales were slow-twitch, oxidative, glycolytic fibers, a rare fiber type for any mammal. The muscle morphology of beaked whales likely decreases the energetic cost of diving, while that of short-finned pilot whales supports high activity events. Calculated ADLs indicate that, at low metabolic rates, both beaked and short-finned pilot whales carry sufficient onboard oxygen to aerobically support their dives.

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INTRODUCTION

Marine mammals dive on a single breath-hold, restricting access to oxygen to that which they carry within their lungs, blood and muscle (Scholander, 1940). Diving mammals display several adaptations for increasing onboard oxygen stores, such as a large blood volume, hematocrit and hemoglobin concentration (reviewed by Snyder, 1983), as well as an enhanced concentration of myoglobin within their locomotor muscles (reviewed by Kooyman and Ponganis, 1998). These animals can lower their rate of oxygen usage *via* physiological adjustments associated with the mammalian dive response (reviewed by Kooyman, 1989) and by employing energy efficient locomotion, such as prolonged glides (Williams et al., 2000). This suite of morphological, physiological and behavioral features increases the duration of a dive that can be performed aerobically, known as the aerobic dive limit (ADL) (Kooyman et al., 1980; Williams et al., 2000).

It has been hypothesized that marine mammals perform the majority of their dives aerobically (Kooyman et al., 1980; reviewed by Ponganis et al., 2011). By staying within its ADL, a diving mammal increases its foraging efficiency by decreasing the time it must spend at the surface metabolizing the lactate resulting from anaerobic metabolism (Kooyman et al., 1980). An animal's ADL is experimentally determined by measuring blood lactate levels following a dive (Kooyman et al., 1980), but because these measurements are often difficult to obtain, ADL is more often calculated by dividing an animal's total body oxygen stores by its diving metabolic rate (dMR) (Kooyman et al., 1980). The average dive duration for several species has been demonstrated to be at or below its experimentally and/or calculated ADL (cADL) (e.g. Ponganis et al., 1997; Williams et al., 1999; Feldkamp et al., 1989; Shaffer et al., 1997; Burns, 2000; Davis and Kanatous, 1999; Noren et al., 2004; Hassrick et al., 2010; Williams et al., 2011), supporting the 'aerobic diving' hypothesis of Kooyman et al. (Kooyman et al., 1980).

Marine mammals that routinely perform deep, long-duration dives, such as the Weddell seal (*Leptonychotes weddellii*) and the narwhal (*Monodon monoceros*), share a suite of specializations of the locomotor muscle that appear to contribute to extending their ADL. These characteristics include elevated myoglobin concentrations and a muscle fiber profile composed predominantly of large, slow-twitch, oxidative (Type I) fibers with low mitochondrial volume densities (V_{mt}) (Kanatous et al., 2002; Williams and Noren, 2011; Kielhorn et al., 2013). Enhanced myoglobin concentrations increase intramuscular oxygen stores while a low V_{mt} is hypothesized to decrease the rate at which those oxygen stores are utilized during a dive (Kanatous et al., 2002). Large muscle fiber size also likely lowers muscle basal metabolic rate by decreasing the relative surface area across which ions must be pumped to maintain the cell's membrane potential (Johnston et al., 2004; Jimenez et al., 2011). Collectively, these features of the

locomotor muscle of deep divers serve to increase an animal's total body oxygen stores and decrease the rate at which these stores are utilized during a dive, thereby increasing their ADL.

Recent tagging studies, however, of two groups of extreme deep-diving cetaceans have challenged the view that most marine mammals dive aerobically. Beaked whales (including *Ziphius cavirostris* and *Mesoplodon densirostris*) perform the deepest and longest average dives of any air-breathing vertebrate (835–1070 m, 46.5–58 min) (Tyack et al., 2006). These animals maintain a relatively steady and slow vertical speed (1.5 m s^{-1}) and utilize energy-efficient gliding during a dive (Tyack et al., 2006). Using the best available data at the time, Tyack et al. (Tyack et al., 2006) provided the first cADL for these animals, which ranged from 25 to 33 min. These results led the authors to hypothesize that beaked whales routinely perform foraging dives that extend well beyond their ADL; however, they did not have access to any species-specific morphological data for beaked whales that could directly inform calculations of oxygen storage capability or muscle metabolism.

Short-finned pilot whales (*Globicephala macrorhynchus*) routinely perform foraging dives with a median duration and depth of 15 min and 762 m, respectively (Soto et al., 2008). This deep-diving species also utilizes energy-saving gliding, but remarkably, at the deepest portion of the dive, can perform sprints with a vertical speed ranging from 4 to 9 m s^{-1}. It was calculated that these sprinting events represented an eightfold increase in the energetic cost required to overcome drag when compared with the mean vertical speed of 2 m s^{-1} observed during ascent and descent (Soto et al., 2008). These results suggest that their diving metabolic rate may be higher than those of other deep divers, which routinely utilize slow swim speeds (Williams and Noren, 2011; Williams et al., 2000; Tyack et al., 2006). To date, no data exist on the morphology of their locomotor muscle and no ADL has been determined for this species.

Consequently, the goals of our study were twofold. The first was to investigate the characteristics of the locomotor muscle (muscle fiber profile and size, V_{mt} and myoglobin concentration) of beaked whales (*M. densirostris*, *Mesoplodon europaeus* and *Mesoplodon mirus*) and short-finned pilot whales to gain insight into how they perform their extreme dives. The second goal was to use species-specific morphological data to estimate total body oxygen stores to determine whether these animals are capable of performing their extreme dives aerobically.

Because of the long duration of the dives, the relatively slow and steady swim speeds, and the energy-saving gliding behavior of beaked whales, we hypothesized that the locomotor muscle of these species would display characteristics similar to those observed in other deep-diving mammals to extend ADL, including high myoglobin concentration, low V_{mt}, and a high percentage of large Type I fibers. We hypothesized that the locomotor muscle of the short-finned pilot whale would also be similar in design to those of other deep divers, but that it would possess a higher percentage of fast-twitch (Type II) fibers to support their high-speed sprinting behavior. Because ADL is dependent on both the amount of oxygen stored within the body and dMR, we utilized a range of mass-scaled metabolic rates obtained from other diving mammals to calculate ADL under varying levels of activity.

MATERIALS AND METHODS

Specimens

Because of the rarity of the species investigated, this study utilized a frozen archive of high quality muscle samples from beaked and short-finned pilot whales stranded along the Virginia and North Carolina coasts. Five adult beaked whale samples were collected from individual strandings of three species, *Mesoplodon densirostris* Blaineville 1817 (N=2 males; 407–423 cm length), *M. europaeus* (Gervais 1855) (N=1 male, 1 female; 443–465 cm length) and *M. mirus* True 1913 (N=1 male; 455 cm length). Six adult short-finned pilot whale (*Globicephala macrorhynchus* Gray 1846) samples (1 male, 5 females; 387–480 cm length) were obtained during a 2005 mass stranding in North Carolina (Hohn et al., 2006). All specimens utilized in this study were of high quality [Smithsonian Institution Code 1 (alive at the time of stranding and died or was euthanized) or 2 (freshly dead) (Geraci and Lounsbury, 2005)] and were scored as being in good body condition, with no signs of emaciation or muscle degradation. Cross-sections of the epaxial locomotor muscle were collected at a thoraco-lumbar position usually within 24 h of the animal's stranding date. Muscle samples were wrapped in Saran Wrap®, placed in Ziploc® bags and frozen at −20°C until analyzed. For two individuals that stranded during the tenure of this study (the *M. mirus* adult noted above and an additional subadult female, 244 cm long *G. macrorhynchus*), samples of muscle were collected for examination at the ultrastructural level.

Specimens were collected under NOAA Stranding Agreements to The University of North Carolina, Wilmington (UNCW), and UNCW Institutional Animal Care and Use Committee protocol nos AU09-019, 2006-015 and 2003-013.

Muscle histochemistry and fiber diameter

Standard histochemical techniques, which have been successfully utilized in previous studies of cetacean muscle (e.g. Dearolf et al., 2000; Etnier et al., 2004; Cotten et al., 2008; Kielhorn et al., 2013), were used. Approximately 1 cm^3 blocks of m. longissimus dorsi were cut from a position just ventral to the superficial tendon (Pabst, 1990), coated with Optimal Cutting Temperature compound (Sakura Finekek, Torrance, CA, USA), rapidly frozen in isopentane cooled by liquid nitrogen, and sectioned at 10 µm thickness in a cryostat (Leica Microsystems, Wetzlar, Germany) at −19°C.

Tissue sections were stained for (1) myosin-ATPase using acidic (43.5 mmol l^{-1} barbital acetate, 43.5 mmol l^{-1} HCl, pH 4.2) and alkaline (53 mmol l^{-1} NaCl, 53 mmol l^{-1} glycine, 45 mmol l^{-1} NaOH, 32 mmol l^{-1} $CaCl_2$, pH=10.4) preincubations (Hermanson and Hurley, 1990), (2) succinate dehydrogenase (SDH) (Nachlas et al., 1957) and (3) α-glycerophosphate dehydrogenase (α-GPDH) (Wattenberg and Leong, 1960). For the myosin-ATPase assay, a range of preincubation pHs, both acidic and alkaline, were tested for each of the study groups. The preincubation pH chosen was that which provided the most obvious differentiation between fiber types. Sections were viewed using a brightfield microscope (Olympus BX60, Center Valley, PA, USA) at ×20 magnification and digital micrographs were collected using a SPOT RT color camera (Diagnostic Instruments, Sterling Heights, MI, USA). For myosin ATPase-stained sections, fibers were classified as Type I or Type II (Brooke and Kaiser, 1970), and for SDH- and α-GPDH-stained sections, fibers were classified as oxidative, intermediate or glycolytic (Peter et al., 1972). Percentage of fiber type by area was determined using a Mertz-curvilinear grid system (Dearolf et al., 2000). The grid was placed over the digital micrograph and the number of points residing within each of the different muscle fiber types or white space was counted (Dearolf et al., 2000). Percent area was calculated by dividing the count for each fiber type by the total fiber count. A minimum of 500 fibers was counted for each of the histological techniques utilized.

Fiber diameter was determined by digitally tracing the outline of fibers in Adobe Photoshop (version 7.0, Adobe Systems, San Jose, CA, USA). Micrographs of myosin ATPase-stained fibers using the alkaline preincubation were selected and 10 fibers of each fiber type

that were approximately round in cross-sectional area were arbitrarily chosen. Average mean fiber diameter was determined using the 'Measure' tool of Image Pro Plus (version 6.0, MediaCybernetics, Rockville, MD, USA) (Dearolf et al., 2000). Data are reported as means ± s.d.

Myoglobin concentration

Myoglobin concentration was determined following the method of Reynafarje (Reynafarje, 1963). Frozen muscle samples weighing ~0.5 g were cleaned of connective tissue and blotted dry before being diluted in ice-cold 0.04 mol l^{-1} phosphate buffer (39.25 ml of buffer for every 1 g of muscle). Tissue was homogenized (PowerGen125, Fisher Scientific, Pittsburgh, PA, USA) and centrifuged (Beckman J2-21M/E Centrifuge, Brea, CA, USA) at 28,000 ***g*** at 4°C for 50 min. The supernatant was bubbled with carbon monoxide for 8 min and 0.04 g of sodium dithionite was added to ensure complete reduction of the myoglobin. The sample was then bubbled with carbon monoxide for another 2 min before absorption readings were taken at 538 and 568 nm with a spectrophotometer (Ultrospec® 3000, Pharmacia Biotech, Piscataway, NJ, USA). The difference in absorbance at the two wavelengths was used to determine myoglobin concentration using the equations provided by Reynafarje (Reynafarje, 1963). Three muscle samples were analyzed for each individual, and three spectrophotometric measurements were obtained for each of the replicates from an individual specimen.

Mitochondrial and lipid volume density

Following primary fixation in 0.5% paraformaldehyde and 2.5% glutaraldehyde solution in phosphate buffered saline, pH 7.4 for at least 24 h, muscle fascicles of *M. mirus* and the subadult *G. macrorhynchus* were placed in a secondary fixative of 1% osmium tetroxide and 0.8% potassium ferricyanide in 0.2 mol l^{-1} cacodylate buffer for 2 h. Fascicles were then dehydrated in ethanol and embedded in 100% Spurr epoxy resin. Sections were cut at 90 nm thickness using an ultramicrotome (Reichert Ultracut E, Depew, NY, USA) placed on Formvar-coated copper grids, and post-stained with uranyl acetate and lead citrate (Reynolds, 1963). Sections were examined with a Phillips CM-12 transmission electron microscope (FEI, Hillsboro, OR, USA) and film negatives were digitized using a scanner (MicroTek Scanmaker 1900, MicroTek Lab, Sante Fe Springs, CA, USA) prior to being processed in Adobe Photoshop (version 7.0).

Volume fraction of mitochondria per unit volume of muscle fiber (V_{mt}) was determined by point-counting at least 100 total mitochondria in the transmission electron micrographs. Mitochondria were further categorized as either subsarcolemmal or interfibrillar to calculate their relative volume densities. Subsarcolemmal mitochondria were those juxtaposed to the sarcolemmal membrane (i.e. with no intervening myofibrils) and were used to determine subsarcolemmal V_{mt} ($V_{mt,s}$). All other mitochondria were defined as interfibrillar and were used to determine interfibrillar V_{mt} ($V_{mt,i}$). The volume density of lipid droplets (V_{li}) within the muscle fibers was determined using similar methods. For beaked whales, V_{mt} could be determined separately for Type I and Type II fibers. For short-finned pilot whales, fibers could not be differentiated at the ultrastructural level; thus, mitochondrial volume densities reported for this species are for all fiber types combined.

Calculated aerobic dive limit

To permit comparison across species, ADL was determined for a beaked whale and a short-finned pilot whale using species-specific data on oxygen stores scaled to a body mass of 1000 kg. An individual's total body oxygen stores were calculated by combining the oxygen storage capacities of its locomotor muscle, blood and lungs. This value was then divided by a variety of mass-scaled dMRs to determine cADLs under varying levels of exercise.

Total oxygen stores of the locomotor muscle, defined here to include the epaxial, hypaxial and abdominal muscles (Arkowitz and Rommel, 1985; Dolar et al., 1999), were calculated using mean myoglobin (Mb) concentrations obtained in this study and muscle masses estimated using the mean percentage of total body mass (TBM) composed of locomotor muscle mass (LMM). LMM/TBM (%) was determined by measuring LMM during necropsy of animals included in this study and other closely related animals of the same genus (dissected by authors or provided by Dr J. G. Mead, Smithsonian Institution). Total oxygen stores within the locomotor muscle (ml O_2) were calculated assuming an oxygen binding capacity of 1.34 ml O_2 g^{-1} Mb (Ponganis et al., 2011) using the following equation:

$$\text{Muscle } O_2 = [\text{Mb}] \times 1.34 \times \text{LMM}, \quad (1)$$

where [Mb] is myoglobin concentration in g Mb g^{-1} muscle and LMM is in g.

Blood oxygen stores were estimated from hemoglobin (Hb) concentrations collected from stranded beaked and short-finned pilot whales (provided by C. A. Harms, North Carolina State University, and M. Brodsky, Marine Mammal Conservancy). Mass-specific total blood volume (TBV; ml kg^{-1}) was determined using adult average hemoglobin concentration as described by Snyder (Snyder, 1983):

$$\text{TBV} = 813 \times [\text{Hb}] - 38.6, \quad (2)$$

where [Hb] is hemoglobin concentration in g Hb ml^{-1}. It was assumed that arterial volume represented one-third and venous volume two-thirds of total blood volume (Lenfant et al., 1970). Arterial blood stores were assumed to be 95% saturated at the beginning of a dive and 8% saturated at the end of the dive (Ponganis et al., 2011). Venous O_2 stores were assumed to be 5 volume% less than arterial oxygen stores (Lenfant et al., 1970). The oxygen binding capacity of hemoglobin was assumed to be 1.34 ml O_2 g^{-1} Hb (Ponganis et al., 2011). From hemoglobin concentration and the oxygen binding affinity, the capacitance coefficient (β_{BO_2}; ml O_2 l^{-1} blood) was calculated using the following equation from Davis and Kanatous (Davis and Kanatous, 1999):

$$\beta_{BO_2} = [\text{Hb}] \times 1.34, \quad (3)$$

where [Hb] is hemoglobin concentration in g Hb l^{-1}. Arterial and venous blood oxygen stores (ml O_2) were calculated as:

$$\text{Arterial } O_2 = \tfrac{1}{3}\text{TBV} \times 0.95 \times \beta_{BO_2}, \quad (4)$$

$$\text{Venous } O_2 = \tfrac{2}{3}\text{TBV}\,(\text{Arterial } \beta_{BO_2} - 50), \quad (5)$$

where TBV is in liters and β_{BO2} is in ml O_2 l^{-1} blood.

Lung oxygen stores were estimated using the relationships between total lung capacity (TLC) and body mass for delphinids (Gentry and Kooyman, 1986) and deep-diving kogiids, as it has been demonstrated that kogiid and ziphiid lung mass scale similarly with total body mass (Piscitelli et al., 2010). Diving lung volume (DLV; ml) was assumed to be 100% of TLC (Kooyman, 1989), alveolar O_2 concentration was assumed to be 100% upon the initiation of the dive, and alveolar O_2 extraction was assumed to be 15% (Lenfant et al., 1970). Lung oxygen stores (ml O_2) were calculated as:

$$\text{Lung } O_2 = \text{DLV} \times 0.15. \quad (6)$$

As dMRs have not yet been determined for the species included in this study, a suite of theoretical dMRs was devised. Previously

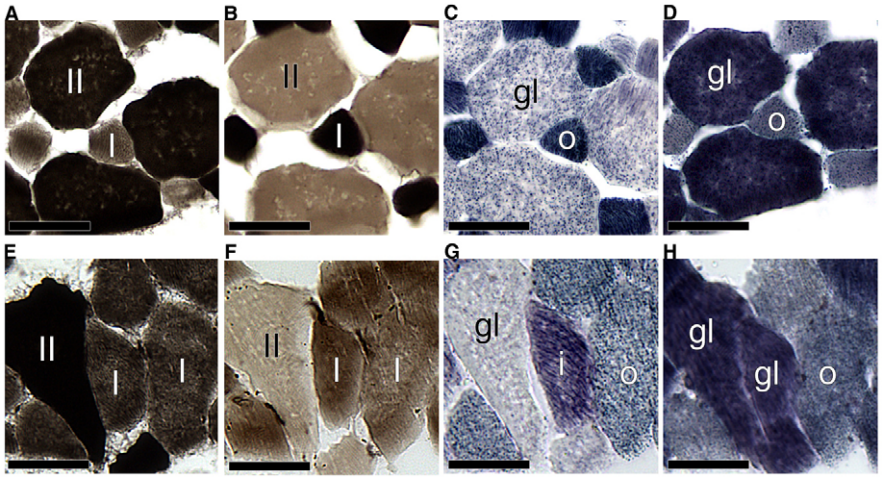

Fig. 1. Serial cross-sections of the m. longissimus dorsi of *Mesoplodon densirostris* (A–D) and *Globicephala macrorhynchus* (E–H). Scale bars, 50 µm. Muscle sections stained for the alkaline (A,E) and acidic (B,F) preincubations of myosin ATPase were used to distinguish Type I and II fibers. Muscle sections stained for succinate dehydrogenase (C,G) and α-glycerophosphate dehydrogenase (D,H) were used to distinguish glycolytic (gl), oxidative (o) and intermediate (i) fibers.

determined metabolic rates for the Weddell seal and bottlenose dolphin (*Tursiops truncatus*) include values for resting and varying levels of underwater activity (Davis and Kanatous, 1999; Williams et al., 2004; Williams et al., 1993). Metabolic rates were assumed to scale to body mass (e.g. Kleiber, 1975; Weibel et al., 2004) to correct for differences in body mass between the specimens in this study and those from which the metabolic rate was originally measured (*sensu* Tyack et al., 2006). Diving MR was also determined by calculating basal metabolic rate as predicted by Kleiber (Kleiber, 1975), as well as calculating the animal's cost of transport (COT_{TOT}; $J\,kg^{-1}\,m^{-1}$) from Williams (Williams, 1999):

$$COT_{TOT} = 7.79TBM - 0.29, \quad (7)$$

where TBM is in kg. COT_{TOT} was multiplied by the animal's swim speed (assuming a caloric equivalent of $4.8\,kcal\,l^{-1}\,O_2$ and a conversion factor of $4.187\times10^3\,J\,kcal^{-1}$) to calculate dMR (Williams and Noren, 2011; Tyack et al., 2006; Soto et al., 2008).

RESULTS

Muscle fiber profile, fiber diameter and myoglobin concentration

In beaked whales, all staining methods distinguished only two fiber types (Fig. 1A–D). Myosin ATPase stains demonstrated that the majority of the muscle (~80% by area) was composed of Type II fibers (Table 1). This finding was supported by the results of the metabolic stains (SDH and α-GPDH), with ~80% of the muscle by area composed of glycolytic fibers (Table 2). The global mean fiber diameter of the Type II fibers was approximately two times larger than that of the Type I fibers (Fig. 1A–D, Table 3).

In short-finned pilot whales, myosin ATPase stains displayed two distinct fiber types (Fig. 1E,F, Table 1). Although intermediate-staining fibers have been previously identified in marine mammal studies using the myosin ATPase assay (Dearolf et al., 2000; Kielhorn et al., 2013), none of the pHs tested in this study resulted in further differentiation of fiber types in the short-finned pilot whales. Type I fibers comprised approximately two-thirds of the fiber area, so it was expected that the SDH and αGPDH stains would result in fiber profiles with approximately two-thirds oxidative fibers. However, SDH-stained sections distinguished three distinct fiber types (oxidative, intermediate and glycolytic; Fig. 1G), each representing approximately one-third of the fiber area (Table 2). In contrast, α-GPDH-stained sections distinguished only two fiber types (oxidative and glycolytic; Fig. 1F) with approximately two-thirds of the muscle area staining as glycolytic fibers (Table 2).

A comparison of serial sections (Fig. 1) revealed that approximately one-half of the fibers classified as Type I by myosin ATPase staining methods (i.e. roughly 33% of the total profile) stained darkly for SDH and lightly for α-GPDH, as would be expected for slow-twitch, oxidative fibers. The remaining Type I fibers, however, were intermediately stained for SDH and darkly stained for α-GPDH, suggestive of a slow-twitch fiber with both high oxidative and high glycolytic capacities. Such fibers have been previously described as slow-twitch, oxidative, glycolytic (SOG) fibers in other mammals (Whitmore, 1982; Suzuki and Hayama, 1991). In adult short-finned pilot whales, SOG fibers comprised ~33% of the total fiber population (Table 2). The global mean fiber diameters of all fiber types were similar (Fig. 1, Table 3).

The m. longissimus dorsi of beaked whales displayed relatively high myoglobin concentrations ranging from 6.92 to $8.57\,g\,Mb\,100\,g^{-1}$ muscle across species (Table 3), whereas the mean myoglobin concentration of this epaxial muscle in the short-finned pilot whale was $6.82\pm0.43\,g\,Mb\,100\,g^{-1}$ muscle (Table 3).

Mitochondrial and lipid volume density

To perform volume density measurements, a goal was set to count at least 100 lipid droplets as well as 100 of each type of mitochondria

Table 1. Mean (±s.d.) fiber type by area of m. longissimus dorsi using alkaline and acidic preincubations of myosin ATPase staining

	Alkaline myosin ATPase		Acidic myosin ATPase	
Species	% Type I	% Type II	% Type I	% Type II
Mesoplodon spp.	19.7±7.8	80.3±7.8	21.0±11.2	79.0±11.2
M. densirostris	16.9±8.5	83.1±8.5	20.5±12.3	79.5±12.3
M. mirus	18.7±6.6	81.3±6.6	17.7±8.1	82.3±8.1
M. europaeus	23.1±6.0	76.9±6.0	23.3±11.2	76.7±11.2
Globicephala macrorhynchus	62.2±10.9	37.8±10.9	65.5±12.1	34.5±12.1

Mesoplodon spp. represents combined species mean.

Table 2. Mean (±s.d.) fiber type percentage of m. longissimus dorsi using succinate dehydrogenase (SDH) and α-glycerophosphate dehydrogenase (α-GPDH) staining techniques

	SDH			α-GPDH	
Species	% Oxidative	% Intermediate	% Glycolytic	% Oxidative	% Glycolytic
Mesoplodon spp.	21.5±9.6	–	78.5±9.6	18.5±7.9	81.5±7.9
M. densirostris	21.4±9.2	–	78.6±9.2	16.0±7.8	84.0±7.8
M. mirus	17.7±5.7	–	82.3±5.7	19.5±7.9	80.5±7.9
M. europaeus	23.2±10.9	–	76.8±10.9	20.9±7.3	79.1±7.3
Globicephala macrorhynchus	35.7±17.1	31.5±16.3	32.8±10.9	32.5±14.6	67.5±14.6

Mesoplodon spp. represents combined species mean.

Table 3. Global mean (±s.d.) fiber diameter and myoglobin concentration of the m. longissimus dorsi

	Diameter (μm)			
Species	Type I fibers	Type II fibers	II:I	[Mb] (g Mb 100 g^{-1} tissue)
Mesoplodon spp.	39.1±11.3	79.6±11.5	2.0	7.34±0.87
M. densirostris	30.1±3.0	70.0±7.5	2.3	6.92±0.44
M. mirus	40.2±5.5	77.1±26.2	1.9	8.57±0.10
M. europaeus	47.6±12.4	90.5±10.1	1.9	7.41±1.09
Globicephala macrorhynchus	66.7±13.4	66.8±13.3	1.0	6.82±0.43

Mesoplodon spp. represents combined species mean.

within the muscle fibers. However, in both *M. mirus* and *G. macrorhyncus*, lipid and subsarcolemmal mitochondria were too rarely encountered within the fibers to meet this objective (Table 4).

In *M. mirus*, V_{mt} differed between the two fiber types (Table 4). V_{mt} of Type I fibers was ~3.5 times higher than in Type II fibers. Interfibrillar mitochondria contributed the greatest proportion to total V_{mt} in both fiber types. In Type I fibers, volume densities of subsarcolemmal mitochondria and lipid were approximately five times greater than in Type II fibers.

In the subadult short-finned pilot whale, V_{mt} was determined for all three fiber types combined as these could not be differentiated at the ultrastructural level (Table 4). As in the beaked whale, interfibrillar mitochondria were the predominant mitochondrial type within the fiber, with subsarcolemmal mitochondria comprising only 12% of the total mitochondrial volume density. Lipid appeared to comprise less than 1% of the total muscle volume.

Calculated aerobic dive limit

In both beaked and short-finned pilot whales (assumed body mass of 1000 kg), locomotor muscle oxygen stores contributed the largest percentage to total body oxygen stores, followed by blood and lungs (Table 5). The mass-specific total body oxygen store of the short-finned pilot whale was ~79% of that of the beaked whale.

These total body oxygen stores were used to calculate ADL using a range of dMRs (supplementary material Table S1). At low dMRs, the average dive duration of both cetaceans fell within their cADL. For example, for dMRs scaled from those measured (Williams et al., 2004) or estimated (Davis and Kanatous, 1999) for Weddell seals, both deep-diving cetaceans (*Mesoplodon* spp. and *G. macrorhyncus*) would routinely dive within their cADL. Above these dMRs, cADLs fell below average dive duration. The cADLs produced using COT_{TOT} (Williams, 1999) and average swim speed of *M. densirostris* (Tyack et al., 2006) and *G. macrorhynchus* (Soto et al., 2008) were approximately half the average dive duration of each study group (Fig. 2). For both study groups, at extremely high levels of exertion (Williams et al., 1993), the cADL falls far below average dive duration (Fig. 2).

DISCUSSION

Previous studies of the locomotor muscles of deep-diving marine mammals have demonstrated that these species share a suite of adaptations that increase onboard oxygen stores while slowing the rate at which these stores are utilized, thus extending ADL. Their locomotor muscles display elevated myoglobin concentrations and are composed predominantly of large Type I fibers (reviewed by Kooyman and Ponganis, 1998; Kanatous et al., 2002; Lestyk et al., 2009; Williams and Noren, 2011; Kielhorn et al., 2013). V_{mt} are also lower in deep divers than in shallow divers or athletic terrestrial species (Kanatous et al., 2002; Kielhorn et al., 2013). The results of this study indicate that beaked whales and short-finned pilot whales do not uniformly display these characteristics and that each possesses a novel fiber profile compared with those of other deep divers.

It is important to note that this study did rely upon high quality, freshly stranded specimens. Care was taken to select only individuals

Table 4. Mean (±s.d.) mitochondrial and lipid volume densities of m. longissimus dorsi for an adult beaked whale and a subadult short-finned pilot whale

Species	Fiber type	V_{mt} (%)	$V_{mt,s}$ (%)[a]	$V_{mt,i}$ (%)	V_{li} (%)
Mesoplodon mirus (adult)	Type I	4.4±2.4	0.5±0.6	4.0±0.6	1.1±0.7
	Type II	1.2±0.7	0.1±0.2	1.1±0.7[a]	0.2±0.3[a]
	Combined fibers	2.4±2.2	0.3±0.4	2.1±2.0	0.5±0.7
Globicephala macrorhynchus (subadult)	Combined fibers	7.3±4.6	0.9±1.8	6.4±3.7	0.6±0.7[a]

Total mitodochondrial (V_{mt}), subsarcolemmal mitodochondrial ($V_{mt,s}$), interfibrillar mitodchondrial ($V_{mt,i}$) and lipid (V_{li}) volume densities are based upon counts of 100 of each item except where noted[a].

Table 5. Estimated total body oxygen stores for 1000 kg *Mesoplodon* spp. and *Globicephala macrorhynchus*

	Specimen		
	Mesoplodon spp.	*G. macrorhynchus*	Source
Muscle			
Locomotor muscle mass	48% TBM	32% TBM	Present study
Myoglobin (g Mb 100 g^{-1} muscle)	7.3	6.8	Present study
O_2 combining capacity (ml O_2 g^{-1} Mb)	1.34	1.34	Ponganis et al., 2011
Total O_2 in muscle (l)	46.8 (53.8%)	28.9 (42.3%)	
Blood			
Hemoglobin (g dl^{-1})	22.9	19.6	C. Harms, unpublished data; M. Brodsky, unpublished data
Blood volume (l)	147.6	120.3	Snyder, 1983
Capacitance coefficient (ml O_2 l^{-1})	306.9	262	Davis and Kanatous, 1999
Arterial volume (l)	49.2	40.1	Ponganis et al., 2011
Venous volume (l)	98.4	80.2	Ponganis et al., 2011
Hemoglobin combining capacity (ml O_2 g^{-1} Hb)	1.34	1.34	Ponganis et al., 2011
Arterial O_2 stores (l)	13.1	9.1	Ponganis et al., 2011
Venous O_2 stores (l)	23.8	16.0	Lenfant et al., 1970
Total O_2 stores in blood (l)	36.9 (42.5%)	25.1 (36.7%)	
Lungs			
Lung volume (l)	21	95.6	Piscitelli et al., 2010; Gentry and Kooyman, 1986
Diving lung volume (DLV; l)	21	95.6	Kooyman, 1989
Alveolar O_2 extraction	15% DLV	15% DLV	Kooyman, 1973
Total O_2 in lungs (l)	3.2 (3.7%)	14.3 (21.0%)	
Total O_2 in body (ml kg^{-1})	86.9	68.3	

TBM, total body mass.

in good body condition, with no gross evidence of emaciation or muscle degradation; however, they do not necessarily represent a random sample of these populations. Such a limitation is inherent in this study, as there are currently no other sources of tissue available for these protected species.

Locomotor muscle morphology of extreme deep divers

Beaked whales

The large fiber size, low V_{mt} and high myoglobin concentration of the beaked whale muscle are characteristics similar to those observed in other deep-diving species. The myoglobin concentration of beaked whale locomotor muscle is among the highest measured for any mammal, and *M. mirus* has the highest value (8.57±0.10 g Mb 100 g^{-1} tissue) published for any cetacean species to date. This high myoglobin concentration, along with a large locomotor muscle mass (almost 50% of total body mass), provides beaked whales with an extremely high muscle oxygen storage capacity (Table 5).

However, unlike other deep-diving mammals, the muscle displayed a novel fiber profile, composed of ~80% Type II fibers (Tables 1, 2). This fiber profile is comparable to that of mammalian sprinters (Williams et al., 1997; Gunn, 1978) and high-performance, steady-swimming fish (e.g. tuna) (Ellerby et al., 2000) with an enhanced glycolytic capacity. Why would a slow-swimming beaked whale build its muscle like that of a sprinter, which relies upon

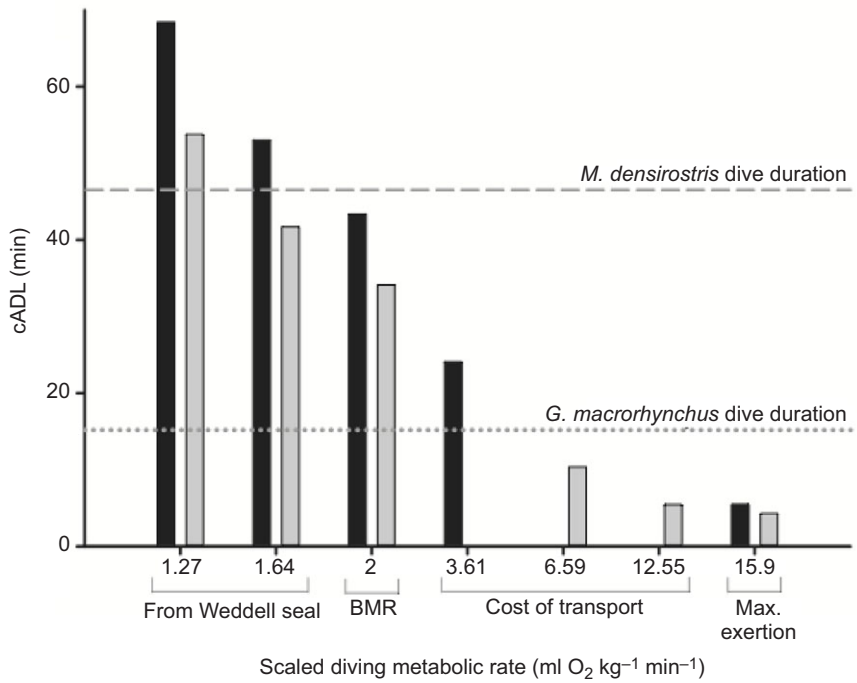

Fig. 2. Calculated aerobic dive limit (cADL) for *Mesoplodon* spp. (black bars) and *Globicephala macrorhynchus* (gray bars) assuming a 1000 kg body mass. Diving metabolic rates, including basal metabolic rate (BMR), were obtained from the literature (see supplementary material Table S1). Average dive durations for *M. densirostris* and *G. macrorhynchus* were obtained from Tyack et al. (Tyack et al., 2006) and Soto et al. (Soto et al., 2008), respectively.

glycolytic metabolism to power burst activity, while having an extremely high muscle oxygen storage capacity? Although it may seem counter-intuitive, we hypothesize that the morphology of the locomotor muscle of beaked whales is a novel design (*sensu* Lauder, 1982) that increases the animal's total body oxygen stores, while limiting the rate of oxygen consumption during a dive.

Two features of the Type II fibers, large diameter and low V_{mt}, can function to decrease the muscle's metabolic rate. The 'optimal fiber size' hypothesis, suggested by Johnston et al. (Johnston et al., 2004) for fish muscle and experimentally demonstrated in invertebrate muscle by Jimenez et al. (Jimenez et al., 2011), states that large muscle fibers have a decreased basal metabolic cost compared with smaller fibers. The smaller surface-area-to-volume ratio in the larger fibers decreases the number of energy-utilizing, ionic pumps required to maintain the cell membrane potential (Johnston et al., 2004; Jimenez et al., 2011). The Type II fibers of beaked whales are extremely large (~80 μm), falling within the size range of muscle fibers previously observed in other deep-diving mammals (Kanatous et al., 2002; Williams and Noren, 2011; Kielhorn et al., 2013).

The V_{mt} of the Type II fibers is also extremely low. While Type II fibers have been commonly described as having a much lower V_{mt} than aerobically poised Type I fibers in a number of vertebrates (e.g. Williams et al., 1997; Hoppeler et al., 1987; Mathieu-Costello et al., 1992), to the best of our knowledge the V_{mt} for beaked whale Type II fibers (1.2%) is the lowest reported for any mammal. This value is more similar to that of the white muscle of the relatively inactive black sea bass (*Centropristis striata*; 1.2%) or southern flounder (*Paralichthys lethostigma*; 1.0%) (Burpee et al., 2010) and falls just below that predicted for a sedentary mammal of similar size (Fig. 3), suggesting that these cells have a very low rate of oxygen usage (Kanatous et al., 2002).

As the locomotor muscle fiber profile of beaked whales is similar to that of sprinting vertebrates, we hypothesize that these cetaceans utilize their muscle in a similar fashion (reviewed by Williams et al., 1997; Dickson, 1996), powering routine locomotion aerobically with the small proportion of muscle composed of Type I fibers. As characteristics of the Type II fibers do not appear to adapt them to use the oxygen bound within the cell at a high rate, we hypothesize that they instead provide a large, metabolically inexpensive oxygen store within the locomotor muscle, supplying oxygen to the Type I fibers during routine swimming. In *M. mirus*, the V_{mt} of Type I fibers (4.4%) was over three times higher than that of the Type II fibers, indicating that these fibers have a higher aerobic capacity. However, this V_{mt} value is still much lower than that of athletic, terrestrial species or shallow-diving marine mammals and is only slightly higher than that observed in the m. longissimus dorsi of the deep-diving Weddell seal (Kanatous et al., 1999; Kanatous et al., 2002; Hoppeler et al., 1987) (Fig. 3).

Further support for our hypothesis comes from the small size of the Type I fibers (~39 μm), which are much smaller than the Type I fibers of other deep-diving mammals (Kanatous et al., 2002; Williams and Noren, 2011; Kielhorn et al., 2013) and more similar in size to those of a mouse (Pathi et al., 2012). The small size of these fibers likely decreases the diffusion distance for oxygen as it moves from the Type II fibers to the mitochondria within the Type I fibers, where it is utilized during a dive. The Type I fibers are also uniformly distributed within the muscle cross-section, often being completely surrounded by the larger Type II fibers (Fig. 1A–D). The Type I fibers of beaked whales have much more lipid present compared with the Type II fibers (Table 4) and the locomotor muscle of the Weddell seal (Kanatous et al., 2002). Lipid has been hypothesized to be the main aerobic fuel source in Weddell seals

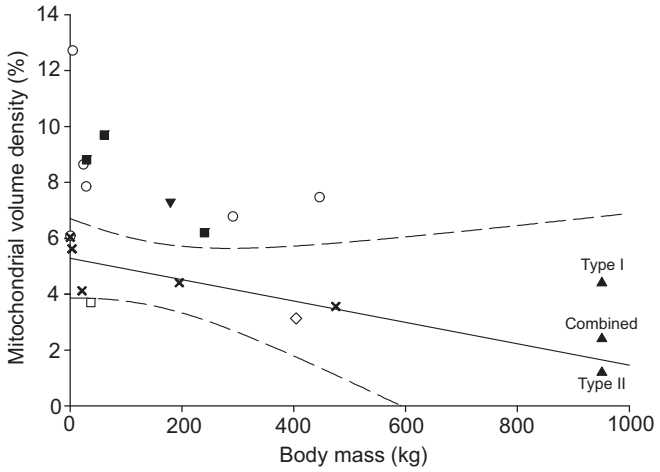

Fig. 3. Mitochondrial volume density for mammals ranging from white rats (open circle) to beaked whales (*Mesoplodon mirus*; closed triangle) (after Kanatous et al., 2002). The regression line (r^2=0.56) and 95% confidence intervals apply only to sedentary animals (×) using data from Weibel et al. (Weibel et al., 2004). Terrestrial athlete data (open circles) are from Weibel et al. (Weibel et al., 2004); shallow-diving pinnipeds data (black squares) are from Kanatous et al. (Kanatous et al., 1999); deep-diving pinnipeds data (open diamond) are from Kanatous et al. (Kanatous et al., 2002); sprinting athlete data (open square) are from Williams et al. (Williams et al., 1997); adult *M. mirus* (black triangles) and subadult *Globicephala macrorhynchus* data (black upside-down triangle) are from the present study.

(Kanatous et al., 2002), and the results of this study suggests that lipid likely serves the same role in the Type I fibers of beaked whales.

Although this morphology suggests a novel design to decrease the energetic cost of diving, it also provides beaked whales with an enhanced glycolytic capacity compared with other deep divers. This enhanced glycolytic capacity may be utilized to power burst swimming, although high-swim speeds have not been previously observed in these species (Tyack et al., 2006). An enhanced glycolytic capacity may also be used if these animals deplete their oxygen stores and must rely on anaerobic metabolism to fuel portions of their extreme dives (see Calculated aerobic dive limit section, below).

Short-finned pilot whales

The locomotor muscle of the short-finned pilot whale possesses both a high myoglobin concentration and a large muscle fiber size, characteristics that prolong ADL as would be predicted for a deep-diving mammal (Table 3) (Kanatous et al., 2002; Williams and Noren, 2011; Kielhorn et al., 2013). Like other deep-diving mammals, they also have a muscle fiber profile composed predominantly of Type I fibers. However, approximately one-half of these slow-twitch fibers varies from the accepted fiber-type classification scheme by having both high oxidative and glycolytic capacities. This fiber type has never been reported in any diving mammal and is rarely observed in terrestrial animals (Whitmore, 1982; Suzuki and Hayama, 1991). In the present study, as in Whitmore (Whitmore, 1982) and Suzuki and Hayama (Suzuki and Hayama, 1991), the two different forms of the Type I fibers observed in the short-finned pilot whale had similar staining properties in the myosin ATPase stains (i.e. they could not be differentiated), but these fibers were capable of being differentiated from each other based on their metabolic properties, as demonstrated by SDH and α-GPDH staining. To the best of our knowledge, this is the first study to report SOG fibers consistently across individuals and composing a large percentage of the overall fiber profile within the locomotor muscle of any mammal.

The presence of SOG fibers may be a reflection of the relatively high, but variable, activity level of these animals, which are capable of performing high-speed sprints at depth (Soto et al., 2008). Together, the SOG and Type II fibers comprised approximately two-thirds of their locomotor muscle fiber profile. This unique fiber-type profile suggests that short-finned pilot whales, like beaked whales, have an enhanced capacity to utilize anaerobic metabolism during a dive. Such capability may be required during high activity periods, such as sprints (e.g. Williams et al., 1997; reviewed by Dickson, 1996), or should the locomotor muscle have to rely on anaerobic metabolism due to the depletion of onboard oxygen stores during a dive.

The V_{mt} of the m. longissimus dorsi of the short-finned pilot whale was determined from a subadult as fresh tissue from an adult specimen was not available during the course of this study. The V_{mt} of this subadult's locomotor muscle was much higher than that of either the deep-diving Weddell seal (Kanatous et al., 2002) or beaked whales and is more comparable to that of similarly sized terrestrial athletes and shallow-diving pinnipeds (Kanatous et al., 1999; Weibel et al., 2004) (Fig. 3). This characteristic may be a reflection of the high activity levels of these animals. This value, however, may overestimate the V_{mt} of adult short-finned pilot whales, as V_{mt} has been shown to decline across ontogeny in Weddell seals (Kanatous et al., 2008). Other characteristics of this subadult's muscle, such as myoglobin and fiber-type profile, closely reflected those of the adult specimens (supplementary material Table S2); however, because it is not known how V_{mt} may change across ontogeny in cetaceans, more data are required before conclusions can be drawn about the aerobic capacity of short-finned pilot whales.

Calculated aerobic dive limit

Both beaked whales and short-finned pilot whales in this study stored the largest percentage of their total body oxygen stores in their locomotor muscle, a trend that has previously been observed in other cetaceans studied to date (Noren and Williams, 2000; Williams and Noren, 2011). Beaked whales store a larger percentage of their total oxygen stores within their locomotor muscle than do short-finned pilot whales. Approximately 48% of the total body mass of beaked whales was locomotor muscle, compared with 30–36% in other marine mammals (e.g. Lenfant et al., 1970; Goforth, 1986; Davis and Kanatous, 1999, McLellan et al., 2002), including short-finned pilot whales (32%). For both beaked whales and short-finned pilot whales, blood provided the second largest oxygen store. Short-finned pilot whales store a larger percentage of their total oxygen stores within their lungs compared with beaked whales, which have very small estimated lung volumes (Table 5) (Piscitelli et al., 2010).

There are a number of potential sources of bias in the body oxygen stores estimated in this study. Calculations of locomotor muscle oxygen stores were based upon myoglobin measurements from a single site within the epaxial locomotor muscle. Although it has been demonstrated that no significant difference in myoglobin concentration exists within the epaxial muscle of some odontocetes (Noren and Williams, 2000), other studies have shown slight variation in myoglobin concentration both within [11% (Polasek and Davis, 2001)] and across [15–20% (Dolar et al., 1999); 3–16% (Polasek and Davis, 2001)] the locomotor muscles of pelagic delphinids. The myoglobin concentration used for beaked whales was the mean value for all three species, thus this estimation is not the highest that could be obtained for beaked whales. Only including the oxygen stores of the locomotor muscles presents a conservative estimate of the animal's muscle oxygen stores that likely provides a slight underestimation of the animal's total muscle oxygen stores. The calculations also relied on limited data currently available on hemoglobin concentrations to estimate blood volume. Finally, lung oxygen stores were included in these estimations; however, because both of these groups of animals dive to great depths, lung collapse likely results in lung oxygen stores being negligible during a deep dive (reviewed by Davis and Kanatous, 1999).

Although absolute values for total body oxygen stores are dependent upon a number of assumptions, the overall pattern observed in this study is clear: the beaked whale had a greater mass-specific oxygen storage capacity ($86.9\,ml\,O_2\,kg^{-1}$) than the short-finned pilot whale ($68.3\,ml\,O_2\,kg^{-1}$). This value for beaked whales is among the highest mass-specific body oxygen store determined for a marine mammal, second only to the Weddell seal (Davis and Kanatous, 1999; Ponganis et al., 2011). The similar mass-specific oxygen store yet much larger average adult body mass of the beaked whale (by approximately twofold) strongly suggest that the ADL of beaked whales exceeds that of Weddell seals.

In this study, estimated body oxygen stores were used to calculate ADL using a range of scaled dMRs (Fig. 3). This approach to calculating ADL was taken because evidence exists for variability in the level of bradycardia (Hindle et al., 2010; Noren et al., 2012) and metabolic rate of diving marine mammals (Hurley and Costa, 2001; Williams et al., 2004; Fahlman et al., 2008) that may depend on dive duration. Thus, in the wild, marine mammals are likely to experience a range of metabolic rates within and between individual dives that can affect their ADL (reviewed in Ponganis et al., 2011).

The extreme dive performance of beaked whales led Tyack et al. (Tyack et al., 2006) to suggest that these animals were not capable of performing their long-duration dives aerobically, and thus must routinely dive beyond their ADL. Those authors used the best data on oxygen stores in deep-diving mammals available at the time and calculated an ADL that was approximately half the length of a routine dive of their study animals. Results from this study permit estimates of cADL based on species-specific morphological features and demonstrate, for the first time, that at low dMRs, beaked whales carry sufficient onboard oxygen stores to support aerobic metabolism near to, or exceeding, their average dive durations (Fig. 2) (Tyack et al., 2006). The novel morphology of beaked whale locomotor muscle, along with their routine slow swim speeds and use of energy-efficient gliding during a dive (Tyack et al., 2006), suggests that beaked whales experience relatively low dMRs naturally.

At low dMRs, short-finned pilot whales also possess sufficient onboard oxygen stores to maintain aerobic metabolism during their average dive durations. However, these animals display faster swim speeds during a dive than beaked whales and are capable of performing high-speed sprints (Soto et al., 2008). This difference in dive behavior may indicate that these animals routinely experience relatively higher dMRs than those of the beaked whales. Thus, lower dMRs may not accurately reflect those naturally experienced by short-finned pilot whales.

At higher dMRs, cADLs for both cetaceans fall short of their average dive durations (Fig. 2) (Tyack et al., 2006; Soto et al., 2008). Should these cetaceans experience similar dMRs naturally, they would routinely dive beyond their cADL and rely upon anaerobic metabolism to fuel their extreme dives. The enhanced glycolytic capacity of the locomotor muscle of both whales suggests that they would be capable of doing so.

Previous tagging studies of beaked whales, however, have yet to demonstrate a correlation between dive duration and surface time following a long-duration dive (Baird et al., 2006), indicating that these animals do not require prolonged surface periods to process a lactate debt if it is incurred during a dive. If beaked whales rely upon glycolytically produced ATP during a deep, long foraging dive,

their short surface duration may indicate that they are capable of aerobically metabolizing lactate during the dive, or, as previously suggested by Tyack et al. (Tyack et al., 2006), that they can utilize the subsequent series of shallower dives to metabolize lactate. It has been demonstrated that Weddell seals that dive beyond their ADL are capable of removing excess blood lactate during subsequent shorter dives, as long as they are performed within the seal's ADL (Castellini et al., 1988). Beaked whales may utilize a similar strategy so that lactate produced during the dive is removed from the system in a fashion that minimizes the animal's surfacing period.

Using the cost of transport calculation (Eqn 7) allows dMR to be estimated using swim-speed data obtained from free-ranging animals during tagging studies (Tyack et al., 2006; Soto et al., 2008). This estimate of COT_{TOT} most likely overestimates diving metabolic costs as it was determined from marine mammals swimming at the water's surface (Williams et al., 1997) rather than from animals experiencing a dive response. It also assumes a constant swim speed, and thus does not incorporate energetic savings due to the use of efficient locomotion during a dive, such as gliding. Behaviorally, both groups of whales utilize prolonged periods of gliding during their dives (Tyack et al., 2006; Soto et al., 2008). Williams et al. (Williams et al., 2000) estimated that gliding in Weddell seals led to an energetic savings of 27.7%. If this energy savings is applied to the animals in this study, then cADLs determined using COT_{TOT} at average swim speeds of 1.15 and 2.1 m s^{-1} for beaked and short-finned pilot whales, respectively, are extended to 33.3 min for beaked whales and 14.2 min for short-finned pilot whales. These adjusted values are still slightly short of the animal's average dive duration.

The species-specific morphological data obtained in this study for both beaked and short-finned pilot whales, in combination with the insight into dive behavior obtained through tagging studies (Tyack et al., 2006; Soto et al., 2008), permit us to predict the level of energy expenditure of each of these extreme, deep-diving animals. Beaked whales likely routinely experience lower dMRs than the more active short-finned pilot whales. At low dMRs, both species appear capable of diving aerobically. At higher dMRs, dive durations extend beyond their respective cADLs, and both species possess locomotor muscle with an enhanced glycolytic capacity that can support anaerobic metabolism. Determining whether these extreme divers routinely stay within their ADL requires more direct insight into their dMRs.

Conclusions

Tagging studies have offered us unprecedented insights into the extreme diving behavior of beaked and short-finned pilot whales (Tyack et al., 2006; Soto et al., 2008), but until now we lacked data on the functional morphology of their locomotor muscle or species-specific estimates of total body oxygen stores. The slow-swimming beaked whales displayed a novel fiber profile for a deep diver, dominated by large, Type II fibers with an extremely low V_{mt}. This 'sprinter's' profile, coupled with some of the highest myoglobin concentrations yet reported for a cetacean, appears to increase muscle oxygen stores while decreasing the rate at which these stores are used during a dive. We hypothesize that beaked whales utilize the small cross-sectional area of Type I fibers to power routine swimming, while their Type II fibers serve as an inexpensive oxygen store within the muscle.

The locomotor muscle of the short-finned pilot whale, which can sprint at depth, was predominantly composed of Type I fibers, but also possessed a highly glycolytic profile due to the presence of SOG fibers. This novel fiber type has never before been identified in the locomotor muscle of a marine mammal. Unlike other deep divers, it appears that short-finned pilot whales also possess relatively high V_{mt}, suggesting a high aerobic capacity of their locomotor muscle.

These species-specific morphology data, coupled with data from the tagging studies, permit us to estimate the total body oxygen stores of these animals and hypothesize the energetic costs they may experience during their extreme dives. Using this approach, we have demonstrated that each diver can remain within its cADL during routine dives at low dMRs, but both beaked whales and short-finned pilot whales likely exceed their cADLs at higher dMRs. Accurate metabolic rate measurements of free-diving cetaceans, although logistically difficult to obtain, would add invaluably to our understanding of the energetics of extreme diving.

LIST OF SYMBOLS AND ABBREVIATIONS

ADL	aerobic dive limit
cADL	calculated aerobic dive limit
COT_{TOT}	cost of transport
DLV	diving lung volume
dMR	diving metabolic rate
Hb	hemoglobin
LMM	locomotor muscle mass
Mb	myoglobin
SDH	succinate dehydrogenase
SOG	slow-twitch, oxidative, glycolytic
TBM	total body mass
TBV	total blood volume
TLC	total lung capacity
V_{li}	lipid volume density
V_{mt}	mitochondrial volume density
$V_{mt,i}$	interfibrillar mitochondrial volume density
$V_{mt,s}$	subsarcolemmal mitochondrial volume density
α-GPDH	α-glycerophosphate dehydrogenase
β_{BO_2}	blood capacitance coefficient

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AUTHOR CONTRIBUTIONS

B.P.V. helped with the design of the study, performed assays, collected and analyzed the data, wrote the paper and produced the figures. R.M.D. and S.T.K. were involved in design of the study and provided help with assay performance. W.A.M. was involved in design of the study and sample collection. D.A.P. designed the study, helped analyze and collect the data, and helped write the paper. All authors discussed the results and commented on the manuscript.

COMPETING INTERESTS

No competing interests declared.

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REFERENCES

Arkowitz, R. and Rommel, S. (1985). Force and bending movement of the caudal muscles in the short-finned pilot whale. *Mar. Mammal Sci.* **1**, 203-209.

Baird, R. W., Webster, D. L., McSweeney, D. J., Ligon, A. D., Schorr, G. S. and Barlow, J. (2006). Diving behaviour of Cuvier's (*Ziphius cavirostris*) and Blainville's (*Mesoplodon densirostris*) beaked whales in Hawai'i. *Can. J. Zool.* **84**, 1120-1128.

Brooke, M. H. and Kaiser, K. K. (1970). Muscle fiber types: how many and what kind? *Arch. Neurol.* **23**, 369-379.

Burns, J. M. (1999). The development of diving behavior in juvenile Weddell seals: pushing physiological limits in order to survive. *Can. J. Zool.* **77**, 737-747.

Burpee, J., Bardsley, E., Dillaman, R., Watanabe, W. and Kinsey, S. (2010). Scaling with body mass of mitochondrial respiration from the white muscle of three behaviorally distinct teleost fishes. *J. Comp. Physiol. B* **180**, 967-977.

Castellini, M. A., Davis, R. W. and Kooyman, G. L. (1988). Blood chemistry regulation during repetitive diving in Weddell seals. *Physiol. Zool.* **61**, 379-386.
Cotten, P. B., Piscitelli, M. A., McLellan, W. A., Rommel, S. A., Dearolf, J. L. and Pabst, D. A. (2008). The gross morphology and histochemistry of respiratory muscles in bottlenose dolphins, *Tursiops truncatus*. *J. Morphol.* **269**, 1520-1538.
Davis, R. W. and Kanatous, S. B. (1999). Convective oxygen transport and tissue oxygen consumption in Weddell seals during aerobic dives. *J. Exp. Biol.* **202**, 1091-1113.
Dearolf, J. L., McLellan, W. A., Dillaman, R. M., Frierson, D., Jr and Pabst, D. A. (2000). Precocial development of axial locomotor muscle in bottlenose dolphins (*Tursiops truncatus*). *J. Morphol.* **244**, 203-215.
Dickson, K. A. (1996). Locomotor muscle of high-performance fishes: what do comparisons of tunas with ectothermic sister taxa reveal? *Comp. Biochem. Physiol.* **113A**, 39-49.
Dolar, M. L. L., Suarez, P., Ponganis, P. J. and Kooyman, G. L. (1999). Myoglobin in pelagic small cetaceans. *J. Exp. Biol.* **202**, 227-236.
Ellerby, D. J., Altringham, J. D., Williams, T. and Block, B. A. (2000). Slow muscle function of Pacific bonito (*Sarda chiliensis*) during steady swimming. *J. Exp. Biol.* **203**, 2001-2013.
Etnier, S. A., Dearolf, J. L., McLellan, W. A. and Pabst, D. A. (2004). Postural role of lateral axial muscles in developing bottlenose dolphins (*Tursiops truncatus*). *Proc. R. Soc. Lond. B* **271**, 909-918.
Fahlman, A., Svärd, C., Rosen, D. A. S., Jones, D. R. and Trites, A. W. (2008). Metabolic costs of foraging and the management of O_2 and CO_2 stores in Steller sea lions. *J. Exp. Biol.* **211**, 3573-3580.
Feldkamp, S. D., DeLong, R. L. and Antonelis, G. A. (1989). Diving patterns of California sea lions, *Zalophus californianus*. *Can. J. Zool.* **67**, 872-883.
Gentry, R. L. and Kooyman, G. L. (eds) (1986). *Fur Seals: Maternal Strategies on Land and at Sea.* Princeton, NJ: Princeton University Press.
Geraci, J. R. and Lounsbury, V. J. (2005). *Marine Mammals Ashore: A Field Guide For Strandings*, 2nd edn. Baltimore, MD: National Aquarium in Baltimore.
Goforth, H. W., Jr (1986). Glycogenolytic responses and fore production characteristics of a bottlenose dolphin (*Tursiops truncatus*), while exercising against a force transducer. PhD thesis, University of California, Los Angeles, CA, USA.
Gunn, H. M. (1978). Differences in the histochemical properties of skeletal muscles of different breeds of horses and dogs. *J. Anat.* **127**, 615-634.
Hassrick, J. L., Crocker, D. E., Teutschel, N. M., McDonald, B. I., Robinson, P. W., Simmons, S. E. and Costa, D. P. (2010). Condition and mass impact oxygen stores and dive duration in adult female northern elephant seals. *J. Exp. Biol.* **213**, 585-592.
Hermanson, J. W. and Hurley, K. J. (1990). Architectural and histochemical analysis of the biceps brachii muscle of the horse. *Acta Anat.* **137**, 146-156.
Hindle, A. G., Young, B. L., Rosen, D. A. S., Haulena, M. and Trites, A. W. (2010). Dive response differs between shallow- and deep-diving Steller sea lions (*Eumatopias jubatus*). *J. Exp. Mar. Biol. Ecol.* **394**, 141-148.
Hohn, A. A., Rotstein, D. S., Harms, C. A. and Southall, B. L. (2006). Report on marine mammal unusual mortality event UMESE0501Sp: multispecies mass stranding of pilot whales (*Globicephala macrorhynchus*), minke whales (*Balaenoptera acutorostrata*), and dwarf sperm whales (*Kogia sima*) in North Carolina on 15-16 January 2005. NOAA Technical Memorandum NMFS-SEFSC-537.
Hoppeler, H., Kayar, S. R., Claassen, H., Uhlmann, E. and Karas, R. H. (1987). Adaptive variation in the mammalian respiratory system in relation to energetic demand: III. Skeletal muscles: setting the demand for oxygen. *Respir. Physiol.* **69**, 27-46.
Hurley, J. A. and Costa, D. P. (2001). Standard metabolic rate at the surface and during trained submersions in adult California sea lions (*Zalophus californianus*). *J. Exp. Biol.* **204**, 3273-3281.
Jimenez, A. G., Dasika, S. K., Locke, B. R. and Kinsey, S. T. (2011). An evaluation of muscle maintenance costs during fiber hypertrophy in the lobster *Homarus americanus*: are larger muscle fibers cheaper to maintain? *J. Exp. Biol.* **214**, 3688-3697.
Johnston, I. A., Abercromby, M., Vieira, V. L. A., Sigursteindóttir, R. J., Kristjánsson, B. K., Sibthorpe, D. and Skúlason, S. (2004). Rapid evolution of muscle fibre number in post-glacial populations of Arctic charr *Salvelinus alpinus*. *J. Exp. Biol.* **207**, 4343-4360.
Kanatous, S. B., DiMichele, L. V., Cowan, D. F. and Davis, R. W. (1999). High aerobic capacities in the skeletal muscles of pinnipeds: adaptations to diving hypoxia. *J. Appl. Physiol.* **86**, 1247-1256.
Kanatous, S. B., Davis, R. W., Watson, R., Polasek, L., Williams, T. M. and Mathieu-Costello, O. (2002). Aerobic capacities in the skeletal muscles of Weddell seals: key to longer dive durations? *J. Exp. Biol.* **205**, 3601-3608.
Kanatous, S. B., Hawke, T. J., Trumble, S. J., Pearson, L. E., Watson, R. R., Garry, D. J., Williams, T. M. and Davis, R. W. (2008). The ontogeny of aerobic and diving capacity in the skeletal muscles of Weddell seals. *J. Exp. Biol.* **211**, 2559-2565.
Kielhorn, C. E., Dillaman, R. M., Kinsey, S. T., McLellan, W. A., Gay, D. M., Dearolf, J. L. and Pabst, D. A. (2013). Locomotor muscle profile of a deep (*Kogia breviceps*) versus shallow (*Tursiops truncatus*) diving cetacean. *J. Morphol.* (in press).
Kleiber, M. (1975). *Fire of Life: An Introduction to Animal Energetics.* New York, NY: Krieger.
Kooyman, G. L. (1973). Respiratory adaptations in marine mammals. *Am. Zool.* **13**, 457-468.
Kooyman, G. L. (1989). *Diverse Divers: Physiology and Behavior.* New York, NY: Springer.
Kooyman, G. L. and Ponganis, P. J. (1998). The physiological basis of diving to depth: birds and mammals. *Annu. Rev. Physiol.* **60**, 19-32.
Kooyman, G. L., Wahrenbrock, E. A., Castellini, M. A., Davis, R. W. and Sinnett, E. E. (1980). Aerobic and anaerobic metabolism during voluntary diving in Weddell seals: evidence of preferred pathways from blood chemistry and behavior. *J. Comp. Physiol. B* **138**, 335-346.
Lauder, G. V. (1982). Historical biology and the problem of design. *J. Theor. Biol.* **97**, 57-67.
Lenfant, C., Johansen, K. and Torrance, J. D. (1970). Gas transport and oxygen storage capacity in some pinnipeds and the sea otter. *Respir. Physiol.* **9**, 277-286.
Lestyk, K. C., Folkow, L. P., Blix, A. S., Hammill, M. O. and Burns, J. M. (2009). Development of myoglobin concentration and acid buffering capacity in harp (*Pagophilus groenlandicus*) and hooded (*Cystophora cristata*) seals from birth to maturity. *J. Comp. Physiol. B* **179**, 985-996.
Mathieu-Costello, O., Agey, P. J., Logemann, R. B., Brill, R. W. and Hochachka, P. W. (1992). Capillary-fiber geometrical relationships in tuna red muscle. *Can. J. Zool.* **70**, 1218-1229.
McLellan, W. A., Koopman, H. N., Rommel, S. A., Read, A. J., Potter, C. W., Nicolas, J. R., Westgate, A. J. and Pabst, D. A. (2002). Ontogenetic allometry and body composition of harbour porpoises (*Phocoena phocoena*, L.) from the western North Atlantic. *J. Zool.* **257**, 457-471.
Nachlas, M. M., Tsou, K. C., De Souza, E., Cheng, C. S. and Seligman, A. M. (1957). Cytochemical demonstration of succinic dehydrogenase by the use of a new p-nitrophenyl substituted ditetrazole. *J. Histochem. Cytochem.* **5**, 420-436.
Noren, S. R. and Williams, T. M. (2000). Body size and skeletal muscle myoglobin of cetaceans: adaptations for maximizing dive duration. *Comp. Biochem. Physiol.* **126A**, 181-191.
Noren, S. R., Cuccurullo, V. and Williams, T. M. (2004). The development of diving bradycardia in bottlenose dolphins (*Tursiops truncatus*). *J. Comp. Physiol. B* **174**, 139-147.
Noren, S. R., Williams, T. M., Ramirez, K., Boehm, J., Glenn, M. and Cornell, L. (2012). Changes in partial pressures of respiratory gases during submerged voluntary breath hold across odontocetes: is body mass important? *J. Comp. Physiol. B* **182**, 299-309.
Pabst, D. A. (1990). Axial muscles and connective tissues of the bottlenose dolphin. In *The Bottlenose Dolphin* (ed. S. Leatherwood and R. R. Reeves), pp. 51-67. San Diego, CA: Academic Press.
Pathi, B., Kinsey, S. T., Howdeshell, M. E., Priester, C., McNeill, R. S. and Locke, B. R. (2012). The formation and functional consequences of heterogeneous mitochondrial distributions in skeletal muscle. *J. Exp. Biol.* **215**, 1871-1883.
Peter, J. B., Barnard, R. J., Edgerton, V. R., Gillespie, C. A. and Stempel, K. E. (1972). Metabolic profiles of three fiber types of skeletal muscle in guinea pigs and rabbits. *Biochemistry* **11**, 2627-2633.
Piscitelli, M. A., McLellan, W. A., Rommel, S. A., Blum, J. E., Barco, S. G. and Pabst, D. A. (2010). Lung size and thoracic morphology in shallow- and deep-diving cetaceans. *J. Morphol.* **271**, 654-673.
Polasek, L. K. and Davis, R. W. (2001). Heterogeneity of myoglobin distribution in the locomotory muscles of five cetacean species. *J. Exp. Biol.* **204**, 209-215.
Ponganis, P. J., Kooyman, G. L., Winter, L. M. and Starke, L. N. (1997). Heart rate and plasma lactate responses during submerged swimming and trained diving in California sea lions, *Zalophus californianus*. *J. Comp. Physiol. B* **167**, 9-16.
Ponganis, P. J., Meir, J. U. and Williams, C. L. (2011). In pursuit of Irving and Scholander: a review of oxygen store management in seals and penguins. *J. Exp. Biol.* **214**, 3325-3339.
Reynafarje, B. (1963). Simplified method for the determination of myoglobin. *J. Lab. Clin. Med.* **61**, 138-145.
Reynolds, E. S. (1963). The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. *J. Cell Biol.* **17**, 208-212.
Scholander, P. F. (1940). Experimental investigations on the respiratory function of diving mammals and birds. *Hvalraadets Skr.* **22**, 1-131.
Shaffer, S. A., Costa, D. P., Williams, T. M. and Ridgway, S. H. (1997). Diving and swimming performance of white whales, *Delphinapterus leucas*: an assessment of plasma lactate and blood gas levels and respiratory rates. *J. Exp. Biol.* **200**, 3091-3099.
Snyder, G. K. (1983). Respiratory adaptations in diving mammals. *Respir. Physiol.* **54**, 269-294.
Soto, N. A., Johnson, M. P., Madsen, P. T., Diaz, F., Dom'nquez, I., Brito, A. and Tyack, P. (2008). Cheetahs of the deep sea: deep foraging sprints in the short-finned pilot whales off Tenerife (Canary Islands). *J. Anim. Ecol.* **77**, 936-947.
Suzuki, A. and Hayama, S. (1991). Histochemical classification of myofiber types in the triceps surae and flexor digitorum superficialis muscle of Japanese macaques. *Acta Histochem. Cytochem.* **24**, 323-328.
Tyack, P. L., Johnson, M., Soto, N. A., Sturlese, A. and Madsen, P. T. (2006). Extreme diving of beaked whales. *J. Exp. Biol.* **209**, 4238-4253.
Wattenberg, L. W. and Leong, J. L. (1960). Effects of coenzyme Q_{10} and meadione on succinic dehydrogenase activity as measured by tetrazolim salt reduction. *J. Histochem. Cytochem.* **8**, 296-303.
Weibel, E. R., Bacigalupe, L. D., Schmitt, B. and Hoppeler, H. (2004). Allometric scaling of maximal metabolic rate in mammals: muscle aerobic capacity as determinant factor. *Respir. Physiol. Neurobiol.* **140**, 115-132.
Whitmore, I. (1982). Oesophageal striated muscle arrangement and histochemical fibre types in guinea-pig, marmoset, macaque and man. *J. Anat.* **134**, 685-695.
Williams, T. M. (1999). The evolution of cost efficient swimming in marine mammals: limits to energetic optimization. *Philos. Trans. R. Soc. Lond. B* **354**, 193-201.
Williams, T. M. and Noren, S. R. (2011). Extreme physiological adaptations as predictors of climate-change sensitivity in the narwhal, Monodon monoceros. *Mar. Mamm. Sci.* **27**, 334-349.
Williams, T. M., Friedl, W. A. and Haun, J. E. (1993). The physiology of bottlenose dolphins (*Tursiops truncatus*): heart rate, metabolic rate and plasma lactate concentration during exercise. *J. Exp. Biol.* **179**, 31-46.
Williams, T. M., Dobson, G. P., Mathieu-Costello, O., Morsbach, D., Worley, M. B. and Phillips, J. A. (1997). Skeletal muscle histology and biochemistry of an elite sprinter, the African cheetah. *J. Comp. Physiol. B* **167**, 527-535.
Williams, T. M., Davis, R. W., Fuiman, L. A., Francis, J., Le Boeuf, B. J., Horning, M., Calambokidis, J. and Croll, D. A. (2000). Sink or swim: strategies for cost-efficient diving by marine mammals. *Science* **288**, 133-136.
Williams, T. M., Fuiman, L. A., Horning, M. and Davis, R. W. (2004). The cost of foraging by a marine predator, the Weddell seal *Leptonychotes weddellii*: pricing by the stroke. *J. Exp. Biol.* **207**, 973-982.

Table S1. Metabolic rate (MR) conditions that were used to calculate aerobic dive limits of *Mesoplodon* spp. and *Globicephala macrorhynchus*

Species	Animal condition at time of measurement	Source
Leptonychotes weddellii (387.4 kg individual)	Lowest MR after dive <23 min	Williams et al., 2004
Leptonychotes weddellii (450 kg individual)	Calculated based on organ MR of rat/human scaled to weight of seal	Davis and Kanatous, 1999
Mammal	Basal MR	Kleiber, 1975
Mesoplodon densirostris	Calculated using COT and average vertical velocity of 1.15 m s^{-1}	Williams et al., 2011; Tyack et al., 2006
Globicephala macrorhynchus	Calculated using COT and average vertical velocity of 2.1 m s^{-1}	Williams et al., 2011; Soto et al., 2008
Globicephala macrorhynchus	Calculated using COT and average sprint speed of 4 m s^{-1}	Williams et al., 2011; Soto et al., 2008
Tursiops truncatus (145 kg individual)	Pushing against a load cell at full exertion	Williams et al., 1993

COT, cost of transport.

Table S2. Mean (±s.d.) percent of Type I fibers by area and myoglobin concentration for a subadult *Globicephala macrorhynchus* (used for mitochondrial volume density measurement) and relative percentage of mean adult value for each measurement

	Subadult *G. macrorhynchus*	% of mean adult value
% Type I fibers (alkaline myosin ATPase)	67.3±9.9	108
% Type I fibers (acidic myosin ATPase)	68.3±10.7	104
[Mb] (g Mb 100 g^{-1} tissue)	6.88±0.29	100