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RESEARCH ARTICLE

Navigating under sea ice promotes rapid maturation of diving physiology and performance in beluga whales

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ABSTRACT

Little is known about the postnatal development of the physiological characteristics that support breath-hold in cetaceans, despite their need to swim and dive at birth. Arctic species have the additional demand of avoiding entrapment while navigating under sea ice, where breathing holes are patchily distributed and ephemeral. This is the first investigation of the ontogeny of the biochemistry of the locomotor muscle in a year-round Arctic-dwelling cetacean (beluga whale, *Delphinapterus leucas*). Compared with what we know about other cetaceans, belugas are born with high myoglobin content (1.56±0.02 g 100 g^{-1} wet muscle mass, *N*=2) that matures rapidly. Myoglobin increased by 452% during the first year after birth and achieved adult levels (6.91±0.35 g 100 g^{-1} wet muscle mass, *N*=9) by 14 months postpartum. Buffering capacity was 48.88±0.69 slykes (*N*=2) at birth; adult levels (84.31±1.38 slykes, *N*=9) were also achieved by 14 months postpartum. As the oxygen stores matured, calculated aerobic dive limit more than doubled over the first year of life, undoubtedly facilitating the movements of calves under sea ice. Nonetheless, small body size theoretically continues to constrain the diving ability of newly weaned 2 year olds, as they only had 74% and 69% of the aerobic breath-hold capacity of larger adult female and male counterparts. These assessments enhance our knowledge of the biology of cetaceans and provide insight into age-specific flexibility to alter underwater behaviors, as may be required with the ongoing alterations in the Arctic marine ecosystem associated with climate change and increased anthropogenic activities.

KEY WORDS: Myoglobin, Muscle acid buffering capacity, Diving capacity, Marine mammal, Cetacean, Arctic

INTRODUCTION

Over evolutionary time, the locomotor muscles of aquatic birds and mammals have become specialized to support routine prolonged apneas while swimming and diving. Among these adaptations are elevated myoglobin (Mb) content and enhanced buffering capacity compared with terrestrial counterparts (Castellini and Somero, 1981; Noren and Williams, 2000; Noren, 2004). When blood perfusion to a tissue is decreased, oxygen depletion of that area is retarded by the release of Mb-bound oxygen into the tissue (Salathe and Chen, 1993). When breath-hold duration is prolonged, glycogenolytic pathways may become increasingly important (Hochachka and Storey, 1975; Kooyman et al., 1980), during which high buffering capacity in the muscle can counteract changes in pH associated with lactic acid accumulation from anaerobic metabolism (Castellini and Somero, 1981). Although it is well known that aquatic birds and mammals have high Mb content and increased muscle buffering capacity (Castellini and Somero, 1981; Noren and Williams, 2000; Noren, 2004; Ponganis, 2015), a period of postnatal development is required before these muscle characteristics are mature (for review, see Noren et al., 2014, 2015).

The demands of hypoxia in the aquatic environment should promote rapid development of the muscle biochemistry that supports diving in marine mammals; however, this is not the case. Cetaceans (mysticetes and odontocetes), which are born directly into the ocean, can take up to 3 years to achieve mature Mb levels (Dolar et al., 1999; Noren, 2004; Noren et al., 2001, 2014). It was suggested that this prolonged maturation is associated with their protracted maternal dependency periods (Noren et al., 2001, 2014), because the variation in the duration required for muscle maturation across pinniped species (seals and sea lions) was correlated with variation in their maternal dependency periods (for review, see Noren et al., 2015). However, investigations on cetaceans are limited; the full developmental trajectory of muscle maturation has only been adequately described in three studies that had large sample sizes. Thus, what we know about the postnatal development of the muscle biochemistry of cetaceans is solely based on two delphinid species (dolphins) and a phocoenid species (porpoises) of the 88 cetacean species (recognized by the International Union for Conservation of Nature) that live in very diverse habitats (e.g. coastal to pelagic and temperate to polar). Recent research demonstrated that an Arctic pinniped (Pacific walrus, *Odobenus rosmarus divergens*) has rapid muscle maturation, despite a lengthy 2–3 year nursing interval. This rapid maturation was attributed to the requirement of the calves to transit amongst sea ice, where breathing holes are patchily distributed and ephemeral (Noren et al., 2015). Among cetaceans, there are three species [bowhead whale (*Balaena mysticetus*) and two monodontids, the narwhal (*Monodon monoceros*) and beluga whale (*Delphinapterus leucas*)] that reside in the Arctic year-round, yet the muscle maturation of these animals has yet to be explored.

Investigations of the postnatal development of diving physiology in Arctic-dwelling cetaceans is timely. Changes in the Arctic marine environment are impacting marine mammals (Derocher et al., 2004; Ferguson et al., 2005; Laidre and Heide-Jørgensen, 2005; Laidre et al., 2008), and the International Whaling Commission determined that the three year-round Arctic occupant cetaceans are 'vulnerable' (IWC, 1997) because of perturbations to the Arctic marine environment, including dramatic physical changes in regional sea ice (e.g. Steele et al., 2010) and increasing anthropogenic interests in the region. Quantifying the physiological capacities of animals improves our ability to determine the range of environmental conditions under which an animal can persist without declines in fitness (Wikelski and Cooke, 2006). For marine mammals, the

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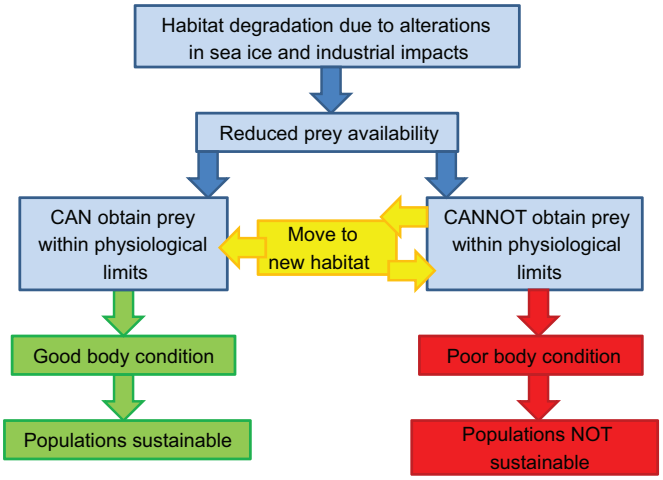

Fig. 1. Flow chart showing possible responses of belugas to habitat perturbations. Outcomes for the beluga population are colored according to increasing severity, green to yellow to red.

physiological capacity for breath-hold establishes limits to foraging behaviors and habitat utilization patterns. An important factor that establishes breath-hold limits in mammals is the metabolic support at the level of the working skeletal muscle (Hochachka, 1986), as Mb content is one of the cornerstones in the aerobic dive limit calculation, particularly for cetaceans, because approximately half of their total oxygen store is found in the muscle (Kooyman, 1989; Ponganis, 2015).

In this study, we examined the ontogeny of the anaerobic (acid buffering capacity) and aerobic (Mb content) properties of the locomotor muscle (longissimis dorsi) of beluga whales that support breath-hold diving to assess age-specific diving performance (calculated aerobic dive limit, cADL; Kooyman, 1989). Dive performance limits were compared with diving behaviors of free-ranging belugas (e.g. Martin and Smith, 1992; Citta et al., 2013; Hauser et al., 2015) to ascertain how closely belugas approximate their physiological capacity, and so determine whether belugas will have the flexibility to alter foraging behaviors (dive for longer durations and to deeper depths). Predicting the resiliency of belugas to environmental perturbations is especially important because some populations of beluga are already critically endangered, such as Cook Inlet belugas (Hobbs and Shelden, 2008). Yet, it remains difficult to predict how changes in prey abundance and composition associated with the retreat of the summer sea ice will impact beluga whales (Moore and Huntington, 2008; Kovacs et al., 2011). Insights on physiological limits for breath-hold may enable scientists and managers to predict the vulnerability of marine mammals to habitat alterations before demographic consequences are realized (Williams et al., 2011; Noren et al., 2015; Fig. 1).

MATERIALS AND METHODS

Specimen and muscle collection

Data and sample collection from beluga whales [*Delphinapterus leucas* (Pallas 1776)] were approved by the University of California, Santa Cruz (UCSC) IACUC under NORES1306_A1. Straight line body length was recorded for each specimen (N=7 females and N=16 males), and each specimen was put into one of five age classes (fetus, neonate, calf, juvenile and adult) based on length, color and the presence or absence of neonatal characteristics (Table 1). The majority of the samples were collected from belugas of the Eastern Chukchi Sea (ECS) stock, with the support of the subsistence hunters shortly following an annual harvest at Point Lay, Alaska. Additional samples were collected from belugas that died at John G. Shedd Aquarium (founding animals from Churchill River in western Hudson Bay) and from dead stranded belugas from the Cooke Inlet stock in southcentral Alaska that were acquired through Alaskan stranding networks (Alaska Veterinary Pathology Services and Alaska SeaLife Center). Samples were typically collected shortly (i.e. within several hours) after death, although the stranded belugas were likely sampled many hours after death. Nonetheless, the carcasses of the stranded belugas were in good condition. In all cases, a minimum of 10 g of muscle was obtained from the major swimming muscle (longissimus dorsi) according to Noren et al. (2001). Samples were kept chilled and put into a −7°C freezer, shipped frozen to UCSC, and stored in a −80°C freezer until biochemical analyses were performed within 6 months of collection.

Muscle biochemistry

To examine the oxygen storage capacity in the muscle, Mb content (reported in g Mb 100 g^{-1} wet muscle) was determined using the procedure of Reynafarje (1963), which was adapted for marine mammals by Noren and Williams (2000). Approximately 0.5 g of thawed muscle was minced in a low ionic strength buffer (40 mmol l^{-1} phosphate, pH 6.6) and then sonicated (Sonifier Cell Disrupter Model 450, Branson Ultrasonics Corporation, Danbury, CT, USA) for 2–3 min on ice. We used a higher buffer to tissue ratio (39.25 ml buffer g^{-1} wet tissue) compared with that used previously (19.25 ml buffer g^{-1} wet tissue; Noren and Williams, 2000); the higher buffer to tissue ratio ensured the complete extraction of Mb from the tissue as muscles obtained from adult specimens were expected to have very high Mb levels based on their dark black–red color. For comparative purposes, we

Table 1. Age class delineations for the beluga whales (*Delphinapterus leucas*) in this study, based on morphological characteristics, body length and estimated age

Age class (N)	Age class characteristics	Female length (cm)	Female estimated age	Male length (cm)	Male estimated age
Fetus (1)	Full-term	–	–	150	Full-term
Neonate (2)	Umbilicus, fetal folds	–	–	159.5, 160.5	Known age (stillborn and 2 days old)
Calf (5)	Still nursing	238	2 years	172, 225, 261, 267	1–3 years
Juvenile (6)	Weaned, immature	283	6 years	307, 320, 321, 323, 324	6–8 years
Adult (9)	Sexually mature	337, 341, 347, 364, 388	13 to >20 years	337, 370, 417, 450	10 to >25 years

Estimated ages for specimen in the calf, juvenile and adult age classes were based on length at age growth curve equations for Eastern Chukchi Sea (ECS) [R. S. Suydam, Age, growth, reproduction and movements of beluga whales (*Delphinapterus leucas*), PhD thesis, University of Washington, 2009].

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analyzed the muscles (pale pink color) from neonatal specimens using both buffer to tissue ratios; the values were identical. Therefore, we recommend that the Mb content of the locomotor muscle of marine mammals be analyzed using the higher buffer to tissue ratio.

The samples were centrifuged at −4°C and 28,000 ***g*** for 50 min (Sorvall RC – 5C Plus superspeed refrigerated centrifuge, DuPont Instruments). The clear supernatant was extracted and then bubbled at room temperature with pure CO for approximately 8 min. To ensure a complete reduction, 0.02 g of sodium dithionite was added. The absorbance of each sample was read at room temperature at 538 and 568 nm on a spectrophotometer (Shimadzu UV–visible recording spectrophotometer UV–160, Shimadzu Corporation, Kyoto, Japan). All samples were run in triplicate alongside a muscle sample obtained from a harbor porpoise (*Phocoena phocoena*), which served as a control because the Mb content of this specimen has been determined previously (Noren et al., 2014).

To examine a component of the anaerobic capacity of the muscle, we explored the ability of the muscle to buffer against lactic acid. The muscle buffering capacity (β) due to non-bicarbonate buffers was determined using procedures of Castellini and Somero (1981) and adapted by Noren (2004). Briefly, thawed samples (approximately 0.5 g) were minced in 10.0 ml normal saline solution (0.9% NaCl), and sonicated (Sonifier Cell Disrupter Model 450, Branson Ultrasonics Corporation) for 3 min on ice. Samples were maintained at 37°C by immersion of the test flask in a warm water bath and titrated with 0.2 mol l^{-1} NaOH. Buffering capacity was measured in slykes (μmoles of base required to raise the pH of 1 g wet muscle mass by one pH unit, over the range of pH 6.0 to 7.0). Changes in pH were measured using an accumet basic pH/mV/°C meter (AB15+, Fisher Scientific) with an accumet liquid-filled, glass body single-junction combination pH Ag/AgCl Electrode (13-620-285, Fisher Scientific) and separate ATC probe (13-620-19, Fisher Scientific). All samples were run in triplicate alongside a muscle sample obtained from a harbor porpoise (*P. phocoena*), which served as a control because the acid buffering capacity of this specimen has been determined previously (Noren et al., 2014).

Modeling breath-hold limits

The cADL was determined by dividing calculated total body oxygen stores by estimates of diving metabolic rate following methods described in Kooyman (1989). The cADL accurately predicts the experimentally determined aerobic dive limit (ADL; Kooyman, 1989; Kooyman and Ponganis, 1998) when estimates of body oxygen stores and metabolic rate are reliable (Ponganis et al., 1997). We used species-specific oxygen storage data and a diving metabolic rate typical of odontocetes. Details of the assumptions are provided below.

The calculations for the oxygen storage capacity of the blood in liters are as follows:

$$\text{Arterial } O_2 = (0.33 \times \text{BV} \times m)(\text{Hb} \times 0.00134)(0.95 - 0.20 \text{ saturation}), \quad (1)$$

$$\text{Venous } O_2 = (0.67 \times \text{BV} \times m)(\text{Hb} \times 0.00134) \times [0.95 \text{ saturation} - (0.05 \times 0.95 \text{ saturation})], \quad (2)$$

where 0.33 and 0.67 are the estimated proportions of arterial and venous blood, respectively (Lenfant et al., 1970), BV is blood volume, m is body mass, Hb is hemoglobin, and 0.00134 is the oxygen binding capacity of Hb (l O_2 g^{-1} Hb; Kooyman, 1989). Body mass values were derived from Gompetz models that analyzed sex-specific body mass as a function as age from data collected from a successful breeding program in an aquarium (S.R.N. and C. P. Poll, unpublished data). This provided details for newborn body mass (63 kg), and maximum adult female (754 kg) and maximum adult male (1016 kg) body mass, as well as age-specific body mass for each sex. Age-specific values for Hb were derived from an equation of age versus Hb content in beluga whales (S.R.N. and C. P. Poll, unpublished data). The proportion of saturation and depletion of arterial and venous oxygen reserves is described in detail in Ponganis (2011).

The age-specific BV values used in Eqns 1 and 2 were estimated according to:

$$\text{BV} = 813\ \text{Hb} - 38.6, \quad (3)$$

where BV is in ml kg^{-1} and age-specific Hb (g ml^{-1}) values were derived as described for Eqns 1 and 2. Eqn 3 was used by Noren et al. (2002) to estimate age-specific BV values for bottlenose dolphins, and we found that BV estimates from Eqn 3 based on measured Hb for adult belugas in a study by Ridgway et al. (1984) had an average error of <1% compared with the measured BV of these animals.

The calculation for the oxygen storage capacity of the muscle in liters is as follows:

$$\text{Muscle } O_2 = (\text{Mb} \times 0.00134)(m \times p), \quad (4)$$

where Mb is in g 100 g^{-1} wet muscle mass as determined in this study, 0.00134 is the oxygen binding capacity of Mb (l O_2 g^{-1} Mb; Kooyman, 1989), m is body mass and p is the proportion of body mass appropriated to muscle. Previous cADL calculations for beluga whales (Shaffer et al., 1997) assumed a value of 0.36 for p based on data from bottlenose dolphins (*Tursiops truncatus*), where 36% of body mass was represented by muscle [H. W. Goforth, Glycogenetic responses and force production characteristics of a bottlenose dolphin (*Tursiops truncatus*) while exercising against a force transducer, PhD thesis, University of California at Los Angeles, 1986]. However, beluga whales have more adipose tissue than most cetaceans and have been found to have only 15.9% muscle mass (Sergeant and Brodie, 1969). Therefore, p in our study was ascribed the value of 0.159. Admittedly, the assumption that the proportion of muscle mass is static throughout ontogeny could overestimate the muscle oxygen stores of immature belugas because allometric data from harbor porpoises indicated that the proportion of muscle mass increased from 26% at birth to 33% by physical maturity (McLellan et al., 2002). Sex-specific differences in muscle biochemistry could not be tested because of limited samples sizes; thus, Mb levels were assumed to be similar across sex as has been assumed in previous studies on cetaceans (Noren et al., 2002, 2014). Moreover, no sex-specific differences in Mb levels were found in another sexually dimorphic species, the northern elephant seal (*Mirounga angustirostris*; P. H. Thorson, Development of diving in the northern elephant seal, PhD thesis, University of California, Santa Cruz, 1993).

The calculation for the oxygen storage capacity of the lung in liters is as follows:

$$\text{Lung } O_2 = \text{TLV} \times 0.50 \times 0.15, \quad (5)$$

where TLV is total lung volume. Because of a lack of data on how lung volume changes with age in beluga whales, we assumed

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a constant mass-specific lung volume as in previous studies (e.g. Noren et al., 2014), where TLV in liters was calculated from an allometric regression for marine mammals, in which TLV=0.1×$m^{0.96}$ (Kooyman, 1989) and body mass (m) is in kg. Diving lung volume was assumed to be 50% of TLV (based on data from dolphins; see Ponganis, 2011), with 15% representing the oxygen extracted during the dive (Kooyman, 1989). Future research on how lung volume changes with maturation is warranted.

The rate at which oxygen stores are depleted is the metabolic rate of the animal. Across air-breathing, diving vertebrates, including three odontocetes, which included beluga (Noren et al., 2012), shallow diving emperor penguins (*Aptenodytes forsteri*; Ponganis et al., 2010) and freely diving Weddell seals (*Leptonychotes weddelli*; Castellini et al., 1992; Ponganis et al., 1993), a cADL that assumed an oxygen consumption rate of 2× Kleiber basal metabolic rate (BMR; Kleiber, 1975) best approximated experimentally determined ADLs. Thus, we assumed a diving metabolism of 2×BMR, as used for other cetaceans (Noren et al., 2002, 2014), to estimate age-specific maximum breath-hold limits and bottom times at various dive depths, where BMR (l O_2 min^{-1}) is 0.0101×$mass^{0.75}$ (Kleiber, 1975) and mass is in kg. Admittedly, immature belugas could have elevated diving metabolism, but how diving metabolism changes with age in cetaceans is unknown. Meanwhile, data collected from Weddell seals (*L. weddelli*) demonstrated that experimentally determined ADLs for pup, yearling and adult Weddell seals were best approximated by cADLs that assumed a diving metabolism of 4×BMR, 2×BMR and 1×BMR, respectively (for a review of these data, see Schreer et al., 2001). Thus, we also provide cADLs based on 4×BMR, as might be the case for immature belugas.

To estimate age- and sex-specific submergence times at given depths (d), a swim speed (s) of 2.0 m s^{-1} was assumed. This assumption is based on maximum swim speed (swim speed range: 0.25–2.0 m s^{-1}) estimated for diving belugas (Hauser et al., 2015) and the average of the maximum descent and maximum ascent rates (descent rate range: 1.43–2.20 m s^{-1}; ascent rate range: 1.23–1.84 m s^{-1}) measured for diving belugas (Martin and Smith, 1992). Using the maximum ascent and descent rate provided the best estimate of maximum dive depth because beluga whales increase ascent and descent speeds with increasing dive depth (Heide-Jørgensen et al., 1998; Martin and Smith, 1999; Laidre et al., 2003; Hauser et al., 2015). In addition, this speed approximates the minimum cost of transport speed measured for dolphins (*T. truncatus*), which by definition elicited the lowest oxygen consumption rate (Williams et al., 1993). The equation used is as follows:

$$\text{Bottom time} = \text{cADL} - (s \times 2d), \quad (6)$$

where cADL is in seconds, s is the ascent and descent speed and d is dive depth. Submerged search time was calculated for depths of 200 and 300 m, as ECS belugas typically feed at 200–300 m, where cod reside in the water column (Citta et al., 2013; Hauser et al., 2015). Belugas have also been observed to have extended occupancy times at 200–400 m and >400 m (Hauser et al., 2015); thus, submergence times at 400 m, as well as the maximum achievable dive depths, which assume a touch and go dive with zero bottom time, were calculated. Subsurface submergence time, as is required for traveling below sea ice, assumed no transit time to depth, and was therefore equivalent to the cADL.

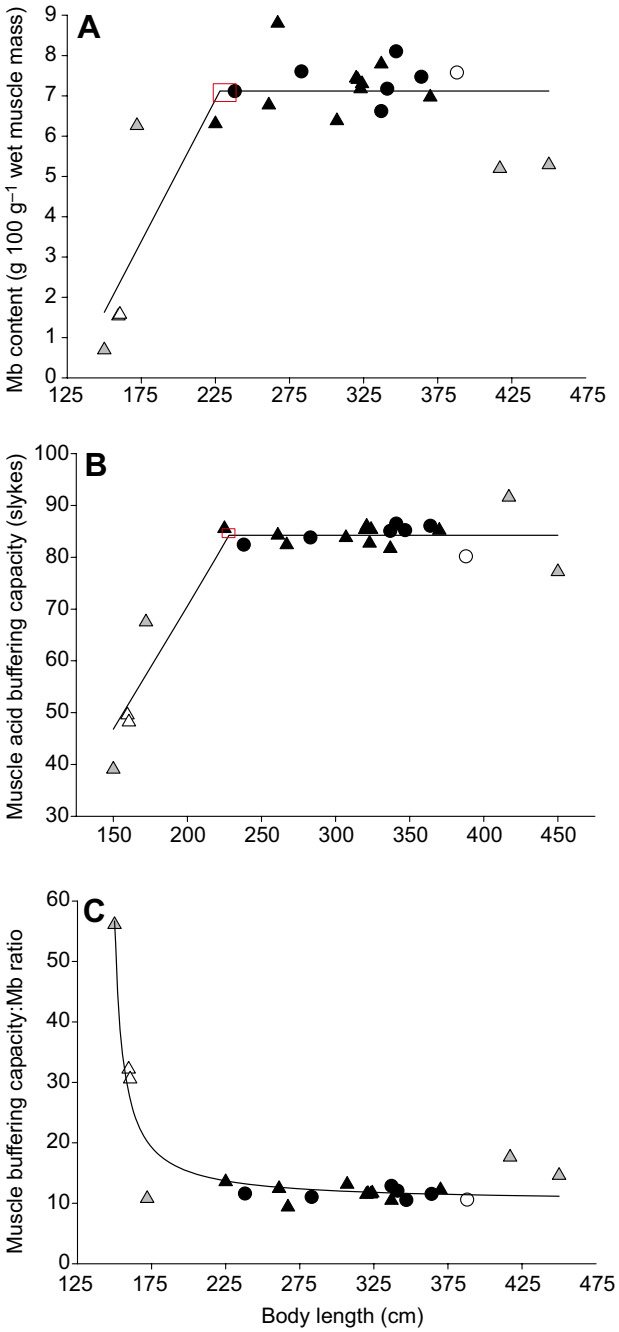

Fig. 2. Muscle biochemistry of the major locomotor muscle (longissimus dorsi) of beluga whales. (A) Myoglobin (Mb) content, (B) buffering capacity and (C) buffering capacity to Mb ratio of the major locomotor muscle, plotted against straight body length. Each symbol represents the mean for an individual specimen that was analyzed in triplicate. Symbols are colored according to their origin: subsistence hunt, black; stranding, gray; and aquarium, white. Males (N=16) and females (N=7) are denoted by triangles and circles, respectively. The solid lines represent the linear increases in muscle Mb content and buffering capacity with body length (our index of age), until an asymptote for mature muscle biochemistry was achieved. Red boxes show the upper and lower 90% confidence intervals for data on both the x- and y-axes. Until belugas achieved a body length of 228 cm, Mb content increased according to 0.07×length−8.93 (N=5, r^2=0.61, $F_{3,19}$=19.542, P≤0.0001) and buffering capacity increased according to 48.0×length−0.25 (N=5, r^2=0.89, $F_{3,19}$=72.172, P≤0.0001). Details on the segmented regression analysis are described in Materials and methods. Buffering capacity to Mb ratio decreased according to 8.32+[(1.94 length)(−143.99+length)−1] (r^2=0.9226, $F_{2,20}$=119.1935, P<0.0001).

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Statistics

Because of limited sample sizes, sex-specific differences in muscle biochemistry were not examined. This is typical of studies on cetaceans because obtaining samples is extremely difficult (for review, see Noren et al., 2014). Thus, as in previous studies, data were combined for analyses. The relationships between body length (x) and muscle biochemistry (y; either Mb content or buffering capacity) were examined using segmented regression analysis (SegReg, www.waterlog.info) to identify the breakpoint in these relationships. The breakpoint represents the body length at which muscle biochemistry plateaus and the muscle has matured. The selection of the best breakpoint and function type was based on maximizing the statistical coefficient of explanation, and performing tests of significance across seven types of function (http://www.waterlog.info/pdf/regtxt.pdf). Plots displaying regression analyses of body length versus buffering capacity to Mb ratio were performed using Sigma Plot (Jandel Scientific 10.0).

Age class analyses enabled us to interpret our results in terms of life history stage for comparison with previous studies. Therefore, Mb content and buffering capacity were compared across age classes (calf, juvenile and adult) by a one-way analysis of variance in combination with an all-pairwise multiple comparison procedure (Holm–Sidak method) with a significance level of $P<0.05$ (Sigma Stat 3.5, Jandel Scientific). A sample size of 2 precluded the inclusion of the neonate age class in the ANOVA; thus, the neonate age class was compared with each of the other age classes using Student's t-tests. A sample size of 1 excluded the fetus from all age class comparisons. Values are reported as means±1 s.e.m.

RESULTS

Muscle biochemistry

Beluga whales are not born with mature muscle biochemical properties that support breath-hold diving (Fig. 2, Table 2). The muscle biochemistry of the major locomotor muscle increased linearly with length as the animals grew and matured until a breakpoint occurred at a straight body length of 228 cm, where a plateau in both muscle Mb content and buffering capacity was achieved (Fig. 2A,B). Based on the ECS growth curve equation [R. S. Suydam, Age, growth, reproduction and movements of beluga whales (*Delphinapterus leucas*), PhD thesis, University of Washington, 2009], a 228 cm-long animal is approximately 1.2 years old. This is in agreement with our age class comparisons, which demonstrated that the muscle biochemistry of 1–3 year old belugas is similar to that of juveniles and adults, in terms of both Mb content ($F_{2,17}=0.207$, $P=0.815$) and buffering capacity ($F_{2,17}=1.383$, $P=0.278$). Only the neonatal age class, which was composed of a full-term stillborn and a 2 day old animal, had significantly lower levels compared with all of the older age classes ($P<0.05$; Table 2). For animals ≥228 cm in length ($N=18$), a plateau of 7.12±0.21 g 100 g^{-1} wet $muscle^{-1}$ and 84.16±0.71 slykes represented mature levels for Mb content and buffering capacity, respectively (Fig. 2A,B).

It should be noted that the mature Mb level found here is double the concentration found previously for beluga whales (Noren and Williams, 2000). This discrepancy may be due to differences in sampling location. In the present study, muscle samples were specifically taken from the midbelly of the longissimus dorsi. This site was chosen because it represents the highest Mb levels, as Mb concentrations in cetaceans are heterogeneous across and within muscle groups (Harrison and Davis, 1998). Although Noren and Williams (2000) ascribed the same sampling criteria, there was some ambiguity about the location from which the beluga whale muscle samples were taken because those particular samples were obtained opportunistically.

Additionally, because the maturation of Mb content and muscle buffering capacity occurs simultaneously, it might be argued that the ontogenetic increase in Mb drives the increase in buffering capacity because Mb contributes to the buffering capacity of muscle. An analysis of the relationship between body length (index of age) and the ratio of buffering capacity to Mb content demonstrated that the smallest (youngest) belugas had the highest ratio of buffering capacity to Mb content, implying that muscles with low Mb levels had a proportionately greater buffering capacity (Fig. 2C). This indicates that Mb is not the predominant buffering agent of the muscle, confirming similar findings in other studies (Castellini and Somero, 1981; Noren, 2004; Noren et al., 2014, 2015).

Modeling breath-hold limits

A result of the increase in Mb content with maturity (Table 2) is an increase in the mass-specific muscle oxygen store, which translates into an increase in total body oxygen stores with age (Fig. 3). As the quantity of Mb increased with age, the relative contribution of the different oxygen storage compartments (lung, blood and muscle) to total body oxygen stores changed (Fig. 3). The increased total body oxygen stores, combined with increased body size and the accompanying lower mass-specific metabolic rate with body size resulted in increased dive capacity (cADL) with age, and an ability to exploit deeper depths (Table 3). Likewise, increased breath-hold capacity enabled longer search times at depth (Fig. 4).

DISCUSSION

An important factor that sets breath-hold limits for diving marine mammals is the metabolic support at the level of the working

Table 2. Development of muscle biochemistry in beluga whales

Age class (N)	% Adult length	Mb (g 100 g^{-1} wet muscle)	% Adult Mb	Statistics for comparison with neonates	Buffering capacity (slykes)	% Adult buffering capacity	Statistics for comparison with neonates
Fetus (1)	40%	0.70[1]	10%	–	39.08	46%	–
Neonate (2)	43%	1.56±0.02[2]	23%	–	48.88±0.69	58%	–
Calf (5)	62%	7.05±0.47[3]	102%	$t=-7.062$, d.f.=5, $P<0.001$	80.44±3.28	95%	$t=-5.732$, d.f.=5, $P=0.002$
Juvenile (6)	84%	7.22±0.18[4]	104%	$t=-17.465$, d.f.=6, $P<0.001$	84.51±0.51	100%	$t=-35.004$, d.f.=6, $P<0.001$
Adult (9)	–	6.91±0.35	–	$t=-6.978$, d.f.=9, $P<0.001$	84.31±1.38	–	$t=-11.558$, d.f.=9, $P<0.001$

[1]Myoglobin (Mb) value used to obtain calculated aerobic dive limit (cADL) for newborns; [2]Mb value used for 6 month olds; [3]Mb value used for 1–3 year old calves; [4]Mb value used for 3–10 year old animals.

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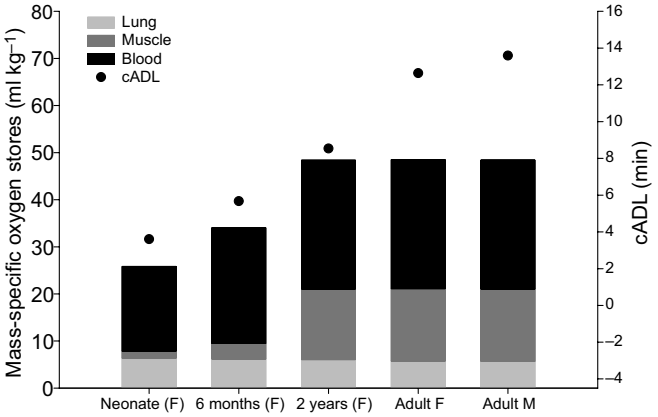

Fig. 3. Total body oxygen stores of a female (F) neonate, 6 month nursing calf and 2 year old newly weaned calf, and of a maximum-sized adult female and male. Total body oxygen stores are divided into lung, muscle and blood values. cADL, calculated aerobic dive limit. See Materials and methods for details of the assumptions for these calculations.

skeletal muscle (Hochachka, 1986; Castellini and Somero, 1981). Prior to this study, the muscle biochemistry that supports breath-hold diving in immature Arctic-dwelling cetaceans had not been described (for reviews, see Noren and Williams, 2000; Noren et al., 2014). We found that belugas have low Mb content and buffering capacity at birth compared with their adult counterparts (Table 2), but Mb levels of neonatal belugas are higher than levels measured in all other neonatal cetaceans examined to date (see Noren et al., 2014, for review), with the exception of the deep-diving Gervais' beaked whale (*Mesoplodon europaeus*; B. P. Velten, A comparative study of the locomotor muscle of extreme deep-diving Cetaceans. MSc thesis, University of North Carolina at Wilmington, 2012). In addition, unlike other cetaceans (for review, see Noren et al., 2014) and the majority of pinnipeds (for review, see Noren et al., 2015), maturation of the muscle biochemistry of belugas occurs prior to the age of weaning. Adult levels were evident in belugas as small as 228 cm in length (Fig. 2A,B), representing 14 month old animals [R. S. Suydam, Age, growth, reproduction and movements of beluga whales (*Delphinapterus leucas*), PhD thesis, University of Washington, 2009], while weaning does not occur until 2–3 years of age [R. S. Suydam, Age, growth, reproduction and movements of beluga whales (*Delphinapterus leucas*), PhD thesis, University of Washington, 2009; Matthews and Ferguson, 2015]. Interestingly, Pacific walrus (*Odobenus rosmarus divergens*) also demonstrate rapid muscle maturation despite a lengthy 2–3 year nursing interval (Noren et al., 2015). Rapid muscle maturation in belugas and walruses could represent convergent evolution; immature animals of pagophilic species must have adequate Mb content and buffering capacity to transit under sea ice, which has patchily distributed and ephemeral breathing holes.

Nonetheless, underdeveloped muscle physiology and body size act synergistically, resulting in low diving capacities for immature marine mammals. Estimated mass-specific muscle oxygen stores for neonatal and 6 month old belugas were only 10% and 22% that of fully grown adult belugas (Fig. 3), resulting in cADLs of approximately 28% and 45% that of fully grown adults (Table 3). As a result, the foraging behaviors of lactating females could be constrained if the dependent calves accompany their mothers to depth. Additionally, despite having achieved mature Hb (S.R.N. and C. P. Poll, unpublished data) and Mb levels, the breath-hold capacity of 2 year olds is only 74% and 69% of the capacity of larger adult female and male counterparts, respectively. Small body size continues to limit the breath-hold capacity of juveniles until full body size is attained (Table 3). Indeed, larger marine mammals dive for longer durations than smaller marine mammals (Schreer and Kovacs, 1997) because the oxygen carrying capacity of the body increases with body mass by the power of 1.0 while oxygen consumption increases with body mass by the power of 0.75 (Kleiber, 1975). These theoretical assumptions are supported by data obtained from free-ranging belugas; larger belugas dive for longer durations than smaller belugas (Martin and Smith, 1999). Moreover, the variability in age-specific diving capacity is even more pronounced when a higher diving metabolism is assumed for immature belugas (Table 3), as growing, immature marine mammals typically have higher mass-specific metabolic rates than adults (for review, see Schreer et al., 2001).

Table 3. Calculated aerobic dive limit (cADL) and maximum dive depth achievable within these estimated breath-hold limits in relation to age, sex and diving metabolism for neonatal to 10 year old belugas

	Female				Male			
	2×BMR		4×BMR		2×BMR		4×BMR	
Age (years)	cADL (min)	Max. depth (m)	cADL (min)	Max. depth (m)	cADL (min)	Max. depth (m)	cADL (min)	Max. depth (m)
0	3.61	216	1.80	108	3.61	216	1.80	108
0.5	5.67	340	2.84	170	6.00	360	3.00	180
1	8.54	512	4.27	256	8.87	532	4.43	266
2	9.30	558	4.65	279	9.38	563	4.69	281
3	9.91	594	4.95	297	9.84	591	4.92	295
4	10.46	627	5.23	314	10.34	620	5.17	310
5	10.82	649	5.41	325	10.71	643	5.36	321
6	11.13	668	5.56	334	11.05	663	5.52	331
7	11.33	680	5.66	340	11.31	679	5.66	339
8	11.48	689	5.74	344	11.58	695	5.79	347
9	11.59	695	5.79	348	11.81	708	5.90	354
10	11.67	700	5.84	350	12.01	721	6.01	360
At max. mass	12.58	755	6.29	377	13.53	812	6.77	406

Ten years is the approximate age when both sexes achieve maximum body size. BMR, metabolic rate, calculated according to Kleiber (1975). See Materials and methods for the assumptions made for these calculations and the references that support these assumptions.

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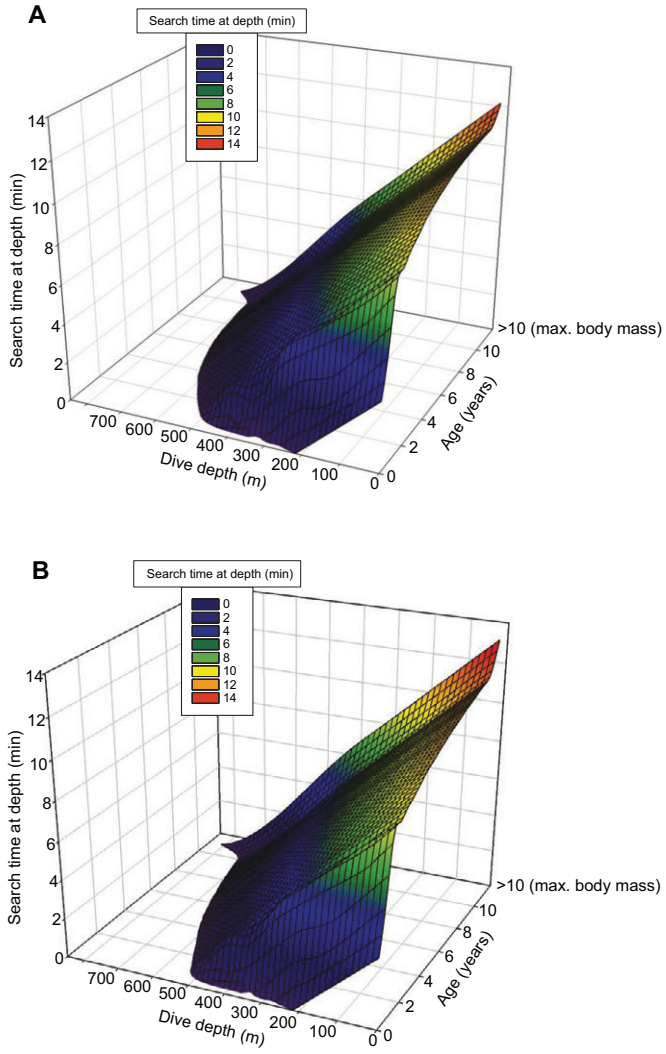

Fig. 4. Theoretical search time in relation to age and dive depth for beluga whales. (A) Females; (B) males. Age- and sex-specific submerged search times just below the surface (no transit time) and at 200, 300 and 400 m depth. Maximum dive depth assumes a touch and go dive, with no search time at depth. A diving metabolism of 2× Kleiber basal metabolic rate and a swim speed of 2 m s^{-1} were assumed. See Materials and methods for details of the assumptions for these calculations.

Ultimately, the physiological capacity for breath-hold defines the limits to performance for subsurface transit between breathing holes in the ice, vertical travel to prey patches and the benthos, submerged search time for prey, as well as submergence tactics used to avoid predation (e.g. from killer whales, *Orcinus orca*). Although there are data on the at-sea swimming, diving and habitat use of beluga whales (e.g. Martin and Smith, 1992, 1999; Martin et al., 1993, 1998, 2001; Heide-Jørgensen et al., 1998, 2001; Kingsley et al., 2001; Citta et al., 2013; Hauser et al., 2014, 2015), these studies only provide a glimpse of their capabilities and behaviors within the context of their current ecosystem. Knowledge of age- and sex-class limits for maximum dive duration and depth (Table 3), as well as the amount of submergence time at a given depth (Fig. 4), can support predictions of how beluga whales may respond to alterations in prey availability due to species turnover associated with the borealization of fish communities (Fossheim et al., 2015) and increased predation as pagophobic mammal-eating killer whales increase their presence in the Arctic as sea ice declines (Higdon et al., 2012). When comparing our theoretical assessment of maximum dive duration and depth with the foraging behaviors of free-ranging beluga whales (e.g. Martin and Smith, 1992, 1999; Martin et al., 1993, 1998, 2001; Heide-Jørgensen et al., 1998, 2001; Kingsley et al., 2001; Citta et al., 2013; Hauser et al., 2014, 2015), we gain insight into how closely belugas are operating at their physiological capacity. Our estimates for maximum dive capacity (assuming a diving metabolism of 2× Kleiber BMR; Table 3) for a mature 754 kg female and 1016 kg male were 12.58 and 13.53 min, respectively (Table 3), and approximated the mean dive duration of 13.1 min recorded for free-ranging beluga whales (Martin and Smith, 1992) but were less than the maximum dive duration of free-ranging beluga whales (21–22.9 min; Martin and Smith, 1992; Citta et al., 2013). Moreover, the maximum dive depth that a mature 754 kg female and 1016 kg male could achieve within these theoretical breath-hold limits (assuming a swim speed of 2.0 m s^{-1}) was 755 and 812 m (Table 3). These depths are about 150 m shallower than maximum depths recorded for free-ranging adult belugas (Hauser et al., 2015). Based on this theoretical assessment, beluga may have little flexibility to alter foraging behaviors because marine mammals that operate at their physiological limits cannot increase dive duration or depth (Costa et al., 2001), which is how marine mammals typically respond to decreased prey availability (Feldkamp et al., 1989; Crocker et al., 2006; Melin et al., 2008).

Admittedly, the diving behavior of belugas varies according to region (Hauser et al., 2015), so maximum performance may not always be required for foraging success. For example, when beluga whales forage on Arctic cod, they target the water column at 200–300 m, where cod are most abundant (Citta et al., 2013; Hauser et al., 2015). Assuming a swim speed of 2.0 m s^{-1}, newly weaned 2 year olds and the largest adults are afforded 4–6 and 8–10 min of search time at these depths, respectively, before needing to transit to the surface to take a breath (Fig. 4). Clearly, search time is influenced by the maturity and size of the body oxygen stores, making newly weaned belugas competitively disadvantaged compared with older, larger animals. As sea-surface temperatures continue to rise with global climate change, the spatial distribution of fish communities will continue to change (Fossheim et al., 2015). Belugas may need to adapt to new dietary regimes (Loseto et al., 2009). If belugas must increase dive depth to 400 m in order to forage, newly weaned belugas will only be afforded 2.5 min of search time before having to return to the surface to breathe; this is less than half the search time available to adult belugas (Fig. 4). Limited breath-hold capacity in combination with inexperience could make newly weaned belugas particularly susceptible to starvation when faced with changes in the ecosystem. Indeed, immature marine mammals are disproportionately affected during prey-limited periods (Trillmich and Limberger, 1985; DeLong et al., 1991).

One mechanism to minimize intraspecific competition, particularly during resource-limited periods, is for the habitat to be partitioned according to life history class. This has been observed in sexually dimorphic pinnipeds, such as gray (*Halichoerus grypus*; Beck et al., 2003) and southern elephant (*Mirounga leonina*; McIntyre et al., 2010) seals. This has also been shown in beluga whales; smaller sized belugas and females with calves use shallow, nearshore open water habitats, medium-sized males and large females select ice-edge habitats offshore, and the largest males select heavy sea ice concentrations in deep offshore water (Loseto et al., 2006, 2008; Citta et al., 2013; Hauser et al., 2014, 2015). Although age- and sex-class segregation is currently serving to minimize intraspecific competition in belugas, the geographic range

of polar cetaceans is expected to be greatly reduced as sea ice contracts (Tynan and DeMaster, 1997; Harwood, 2001; Simmonds and Isaac, 2007). As sea ice recedes further offshore, cod (*Boreogadus saida*), which is the primary prey of belugas across populations and habitats (Kleinenberg et al., 1964; Heide-Jørgensen and Teilmann, 1994; Dahl et al., 2000; Boltunove and Belikov, 2002; Loseto et al., 2009; Marcoux et al., 2012), will follow because they are pagophilic. Indeed, there is evidence that when sea ice cover is low, belugas are further offshore (Heide-Jørgensen et al., 2010). This range shift may be problematic for immature belugas and females with calves that prefer nearshore habitats and are disadvantaged when competing for resources with larger animals that have greater dive capacities.

Conclusions

This is the first study to describe the development of the muscle biochemistry that supports breath-holding in an Arctic-dwelling cetacean. Compared with other cetaceans, belugas are born with a high Mb content that matures rapidly to support their existence among sea ice, where entrapment can occur. By quantifying the physiology that supports breath-hold foraging, we gained insight into age- and sex-specific habitat utilization patterns. Large variation in body size within this species makes immature belugas and adult lactating females competitively disadvantaged compared with adult males. Investigations like this provide a valuable a tool to define critical habitat and predict demographic consequences of habitat perturbations.

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Competing interests

The authors declare no competing or financial interests.

Author contributions

S.R.N. developed the approach and secured the funding for the research, performed the laboratory and data analyses, and prepared the manuscript. R.S. secured the funding for sample collection, collaborated with the Alaskan subsistence hunters to secure the muscle samples from the beluga whales and revised the manuscript.

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