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RESEARCH ARTICLE

Body condition impacts blood and muscle oxygen storage capacity of free-living beluga whales (*Delphinapterus leucas*)

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ABSTRACT

Arctic marine ecosystems are currently undergoing rapid environmental changes. Over the past 20 years, individual growth rates of beluga whales (*Delphinapterus leucas*) have declined, which may be a response to climate change; however, the scarcity of physiological data makes it difficult to gauge the adaptive capacity and resilience of the species. We explored relationships between body condition and physiological parameters pertaining to oxygen (O_2) storage capacity in 77 beluga whales in the eastern Beaufort Sea. Muscle myoglobin concentrations averaged 77.9 mg g^{-1}, one of the highest values reported among mammals. Importantly, blood haematocrit, haemoglobin and muscle myoglobin concentrations correlated positively to indices of body condition, including maximum half-girth to length ratios. Thus, a whale with the lowest body condition index would have ~27% lower blood (26.0 versus 35.7 ml kg^{-1}) and 12% lower muscle (15.6 versus 17.7 ml kg^{-1}) O_2 stores than a whale of equivalent mass with the highest body condition index; with the conservative assumption that underwater O_2 consumption rates are unaffected by body condition, this equates to a >3 min difference in maximal aerobic dive time between the two extremes (14.3 versus 17.4 min). Consequently, environmental changes that negatively impact body condition may hinder the ability of whales to reach preferred prey sources, evade predators and escape ice entrapments. The relationship between body condition and O_2 storage capacity may represent a vicious cycle, in which environmental changes resulting in decreased body condition impair foraging, leading to further reductions in condition through diminished prey acquisition and/or increased foraging efforts.

KEY WORDS: Arctic climate change, Cetacean, Aerobic dive time, Haemoglobin, Marine mammals, Myoglobin

INTRODUCTION

Arctic marine ecosystems are undergoing rapid change, with the Arctic Ocean predicted to be free of summer sea ice within the next few decades (Stroeve et al., 2007; Wang and Overland, 2012). Long-lived Arctic vertebrates with low reproductive rates are particularly vulnerable, having evolved specialized behavioural, physiological and morphological adaptations that have enabled their survival in Arctic environments (Gilg et al., 2012). The sensitivity of a species to climate change is assessed based on its adaptive

capacity and resilience to environmental perturbations, which is determined by physiological limits, ecological traits and genetic diversity (Huey et al., 2012; Williams et al., 2008). Unfortunately, for most wild populations there is a scarcity of physiological data to predict intraspecific responses to climate change (Hetem et al., 2014; Williams et al., 2008). An understanding of physiological limits is important as any animal that routinely operates at its maximum physiological capacity may be unable to withstand stressful events such as declines in prey availability and environmental fluctuations (Costa et al., 2001).

Beluga whales (*Delphinapterus leucas*) exhibit a circumpolar distribution and are the most abundant Arctic species of toothed whales (Odontoceti), and are thus a potential indicator species for the response of Arctic marine mammals to climate change (Laidre, 2008; Laidre et al., 2015; Moore and Huntington, 2008; Tynan and DeMaster, 1997). There are over 150,000 beluga whales worldwide (Jefferson et al., 2012), with approximately 40,000 individuals belonging to the eastern Beaufort Sea beluga stock, one of Canada's largest populations (Allen and Angliss, 2015). Habitat use of Beaufort Sea beluga whales is associated with sea ice and differs by size, sex and reproductive status; large males use permanent pack ice in the Canadian Arctic Archipelago whereas small males and females select coastal and open-water habitat (Loseto et al., 2006; Richard et al., 2001). Differences in foraging strategies exist between sexes, as only male belugas venture into areas deeper than 600 m and have been documented to dive to over 500 m in Viscount Melville Sound and the Canadian Basin (Richard et al., 2001). The purpose of these deep dives is unknown, but has been hypothesized to be for finding breathing holes in heavy ice pack (Richard et al., 1997), orientation (Richard et al., 1998) and foraging in deep-water feeding areas (Harwood and Smith, 2002).

A 20 year decline in the inferred growth rates (measured as size-at-age) of individuals has recently been documented in the beluga population, which is hypothesized to result from progressive climate change (Harwood et al., 2014, 2015). Changes in body condition have also been reported in several other marine predators in the Beaufort Sea ecosystem, which have been hypothesized to reflect an increase in secondary productivity and a decrease in the availability of Arctic cod (*Boreogadus saida*), an important forage fish and the main prey of the beluga population (Harwood et al., 2015; Loseto et al., 2009). Reductions in body condition of ringed seals, *Pusa hispida* (Ferguson et al., 2017), and polar bears, *Ursus maritimus* (Stirling and Derocher, 2012), and mortality events in walrus, *Odobenus rosmarus* (Fischbach et al., 2009), have similarly been associated with climate-induced loss of sea ice. Reductions in sea ice not only affect habitat use by Arctic whales but have also facilitated the northward migration of temperate species (Bouchard et al., 2017; Falardeau et al., 2014). As a result, beluga whales may have to adopt new foraging strategies to accommodate shifts in prey abundance. Reductions in sea ice may also increase predation pressure. Already, an increase in killer whale (*Orcinus orca*)

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sightings corresponding to sea ice loss has been reported across the eastern Canadian Arctic (Higdon and Ferguson, 2009). As a long-lived species (>60 years) with a low reproductive rate, beluga whales may not be able to readily adapt to the challenges induced by climate change.

The total onboard oxygen (O_2) storage capacity of marine mammals is a determinant of their overall dive performance, which in turn affects their ability to search for prey (Kooyman, 1989). In light of the aforementioned growth rate decline in Beaufort Sea beluga whales, the overall objective of our study was to estimate blood and muscle O_2 storage capacity in whales from Inuit subsistence harvests and examine their relationship with indices of body condition to better understand the physiological response of this population to Arctic environmental change. We hypothesized that declines in body condition may have adverse physiological effects on blood and muscle O_2 stores that may negatively impact breath hold endurance and overall dive performance. To address this question, we measured blood haematocrit and haemoglobin concentrations, and myoglobin concentrations and buffering capacity in the longissimus dorsi muscle, and assessed their relationships with two indices of body condition. A second objective was to examine the potential role of the spleen in augmenting blood O_2 stores in belugas. Understanding the O_2 storage capacity of beluga whales is useful for identifying individuals within the population that are most vulnerable to environmental change and for future conservation efforts directed at other marine mammals.

MATERIALS AND METHODS
Sample collection

Samples were collected from 77 adult beluga whales (20 females, 57 males) harvested from July to early August 2012 to 2014 at Inuvialuit hunting camps at Hendrickson Island (69°50′N, 133°58′W), Kendall Island (69°49′N, 135°29′W) and East Whitefish (69°22′N, 133°37′W) in the Inuvialuit Settlement Region, Northwest Territories, Canada (Fig. 1). None of these whales were killed for the purpose of the study. Through our partnerships with community members and hunters from the Inuvialuit Settlement Region, we were granted permission to opportunistically sample tissues from their traditional subsistence hunts. However, we acknowledge a potential selection bias as subsistence hunters preferentially harvest large males (Harwood and Smith, 2002; Harwood et al., 2002), explaining the greater number of males relative to females in our dataset. As we worked on remote islands in which whales were hauled onto land by community members with no access to vehicles or machinery, we were unable to measure full girth or total body mass. Hence, sex, standard length (straight line measurement from the tip of the rostrum to the fluke notch; Sergeant and Brodie, 1969) and maximum half-girth (measured from the dorsal ridge to the approximate ventral
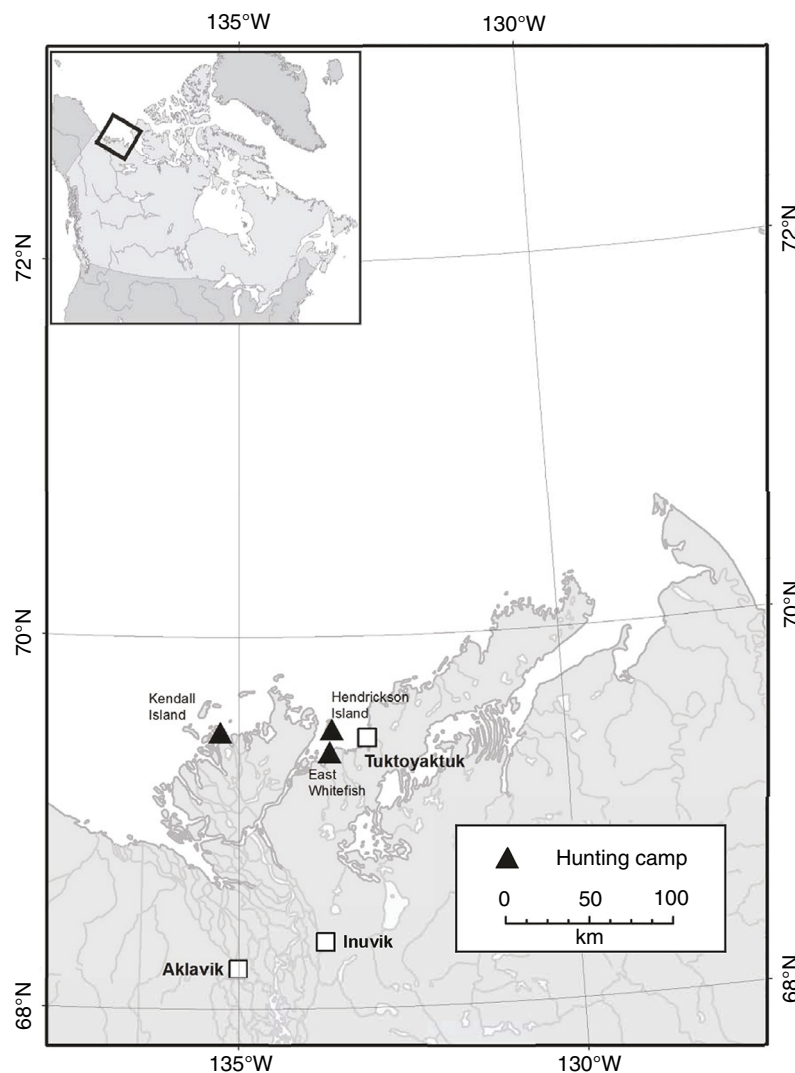

Fig. 1. Sample collection sites for beluga whale tissues. The map shows traditional Inuvialuit hunting camps (triangles) located in the Inuvialuit Settlement Region, Northwest Territories, Canada.

midline) were recorded for each specimen. Total body mass (kg) was estimated for males and females using allometric relationships for eastern Hudson Bay belugas determined by Doidge (1990) using length (cm) and assuming 2× half-girth to be equivalent to maximum full-girth (cm):

$$\text{Mass}_{\text{females}} = 10^{-3.96} \times \text{length}^{1.08} \times \text{maximum girth}^{1.71}, \quad (1)$$

$$\text{Mass}_{\text{males}} = 10^{-4.33} \times \text{length}^{2.46} \times \text{maximum girth}^{0.36}. \quad (2)$$

Age was determined by counting growth layer groups from teeth collected from lower jaws, in which one growth layer group (composed of a dark and light layer) equals 1 year (Stewart et al., 2006). Teeth were cut and growth layer groups from the longitudinal midline sections were counted in three blind replicates by one reader using a binocular microscope.

Blood analysis
Sixty whole-blood samples (∼2 ml) were collected from the carotid artery for haematocrit and haemoglobin determination. All haematocrit measurements were immediately determined on-site using a micro-haematocrit centrifuge (SpinCrit, Brown, Indianapolis, IN, USA) according to the manufacturer's instructions. Haemoglobin concentration was measured in triplicate on thawed, well-mixed samples via absorbance changes using a Biotek Synergy HT Multi-Mode Microplate Reader according to the manufacturer's instructions (Hemoglobin Colorimetric Assay Kit, Cayman Chemical). Whole blood was initially collected without an additive, but samples were well mixed prior to freezing. A comparison was completed to test whether samples treated with heparin (mixed in BD Vacutainers™ treated with sodium heparin) exhibited different haemoglobin concentrations from untreated samples from the same individuals (see Results).

Muscle collection and myoglobin analysis
Approximately 10 g of longissimus dorsi muscle from the dorsal ridge area of 75 individuals was collected. All samples were placed in cryovials, immediately frozen in dryshippers, and stored at cryogenic temperature (ca. −150°C). Because of a dryshipper failure, samples from 18 belugas briefly thawed but were immediately frozen at −20°C for 2 weeks before being stored at −80°C until analysis. We subsequently used this incident to test for differences between these individuals and the 57 specimens continuously stored at cryogenic temperatures (see Results).

Myoglobin concentration was determined for ∼0.5 g muscle samples using methods modified from Reynafarje (1963) and Noren and Williams (2000). An absorbance scan (500–700 nm in 1 nm steps) was conducted using an Ultrospec 70 spectrophotometer (Biochrom Ltd, Cambridge, UK). Peaks were verified using a myoglobin standard from equine muscle (M0630-1G, Sigma-Aldrich). To account for potential variability in muscle water content, subsamples were oven-dried at 70°C for 24 h and percentage water content was determined gravimetrically. Myoglobin concentration was subsequently calculated following Reynafarje (1963) and corrected to a water content of 75%. All analyses were conducted in duplicate.

Spectral deconvolution has been shown to improve the accuracy of myoglobin concentration determinations by separating additive peak components using a modified algorithm for haeme proteins (Masuda et al., 2008). We employed a non-linear, iterative curve-fitting algorithm (Völkel and Berenbrink, 2000) using SigmaPlot

12.0 software that used the optical spectra (between 500 and 700 nm) of known concentrations of pure carbonyl myoglobin, carbonyl haemoglobin and reduced cytochrome c to assess their contributions to the measured spectra of the diluted CO-equilibrated and reduced tissue extracts produced by the Reynafarje (1963) method. Pure carbonyl myoglobin was obtained by reducing a small quantity of crystalline horse skeletal muscle metmyoglobin (Sigma M0630) with dithionite in extraction buffer that was equilibrated with CO. Carbonyl haemoglobin was obtained by lysing a few drops of human blood from a finger prick in 3 volumes of water followed by further dilution in extraction buffer. After centrifugation, the clear supernatant was equilibrated with CO. The concentrations of these standard solutions were obtained using extinction coefficients of 14.7 and 13.4 1 mmol^{-1} cm^{-1} at 540 nm for myoglobin and haemoglobin, respectively (Masuda et al., 2008). The spectrum of a 1 mmol l^{-1} solution of reduced horse skeletal muscle cytochrome c [which does not bind CO at physiological pH (Butt and Keilin, 1962)], in a 1 cm path length cuvette from 500 to 700 nm was interpolated from data in Margoliash and Frohwirt (1959). A spectrum of diluted milk was used to mimic the sloping baseline absorption spectra of samples where some protein precipitation seemed to have occurred. The measured millimolar concentrations in the cuvette were converted to milligrams per gram of wet muscle (corrected to 75% water content) using the dilution factor of 20 ml g^{-1} wet muscle during extraction and the assumed relative molecular mass of myoglobin and haemoglobin subunits of 17,000 g mol^{-1}, as in Reynafarje (1963).

Muscle buffering capacity
The buffering capacity of ∼0.5 g longissimus dorsi samples was determined in duplicate following the procedures of Castellini and Somero (1981). The initial pH of the homogenate equilibrated to 37°C in a water bath was recorded using an Accumet Basic AB 15 pH meter equipped with an Accumet 13-620-96 Micro glass combination pH electrode (Fisher Scientific); 40 µl aliquots of 0.2 mol l^{-1} NaOH were sequentially added to the sample, which was then mixed and the pH recorded (per aliquot) until a change of 1 unit had been observed (between pH 6 and 7).

Spleen mass
Whole spleens from 69 whales were removed and weighed using a portable field balance (Ohaus compact series CS2000). Visual inspection of the dissected spleens revealed they were largely devoid of blood, suggesting they were contracted (Cabanac, 2002; Cabanac et al., 1997).

Indices of body condition
We employed maximum half-girth measurements and an approach similar to that used by George et al. (2015) for bowhead whales (Balaena mysticetus) to determine the body condition of individual whales as previously described in Choy et al. (2017). Girth measurements have been recommended for this purpose based on health evaluations and necropsies of beluga carcasses from the St Lawrence River, as this variable was positively correlated with the scaled mass index (Larrat, 2014). Accordingly, a body condition index (BCI) was calculated for each whale from the residuals of the best-fitting model for predicting maximum half-girth with length, age and sex as predictors. In addition, we used maximum half-girth to length (GL) ratios as these are commonly used as a BCI in other marine mammals (Sato et al., 2002; Trites and Jonker, 2000) and may allow for better comparisons across different studies to identify long-term trends or population differences.

Statistical analyses

Multiple linear regression models were used to assess the relationships among BCI, sex, age and mass with haemoglobin concentration, haematocrit, myoglobin concentration, buffering capacity and spleen mass. For each dependent variable, two models were assessed using either maximal half-girth residuals or maximal GL ratios as indicators for body condition. In addition, spleen mass was included as a predictor variable for haemoglobin concentration and haematocrit. Model selection was based on Akaike's information criterion corrected (AICc) for small sample size using the R package AICcmodavg (https://CRAN.R-project.org/package=AICcmodavg). In addition, the log-likelihood ratio test was used to compare the goodness of fit using the R package lm test (Zeileis and Hothorn, 2002; https://cran.r-project.org/web/packages/lmtest/index.html). Model selection was completed using forward selection by fitting each variable to the null model with significant parameters that resulted in improvement in the model fit selected for inclusion. AICc weights were calculated based on the remaining models. Plots of residuals were used to ensure that assumptions for normality, linearity and homogeneity of variance were met. Models were assessed for multicollinearity among our predictors by calculating variance inflation factors (VIF<3 in all models) to determine which variables are highly related (Zuur et al., 2007, 2010).

All statistical analyses were conducted using R 3.2.5 (http://www.R-project.org) and significance was judged at $\alpha=0.05$. Data are reported as means±1 s.e.m. All raw data are tabulated in Table S1.

Total body O2 stores and calculated aerobic dive limits

As BCI was found to have a significant effect on both haemoglobin and myoglobin concentration, and because these variables are expected to have additive effects on overall body O$_2$ storage capacity, we estimated the volume of usable O$_2$ stored in the lungs, blood and muscle tissues of each individual following recommendations outlined in Ponganis et al. (2011). To better isolate the effects of BCI on blood and muscle storage capacity, we repeated these analyses with haemoglobin and myoglobin concentrations predicted by their linear regression models with BCI (Fig. 2A,C); the average body mass for beluga whales (861.1 kg; Table 1) was used for these calculations to avoid confounding effects of mass in ensuing comparisons.

Oxygen stores in the lungs were estimated based on total lung capacity (TLC). TLC (in litres) was calculated from body mass (kg) and the allometric equation for marine mammals (Fahlman et al., 2011; Kooyman, 1973):
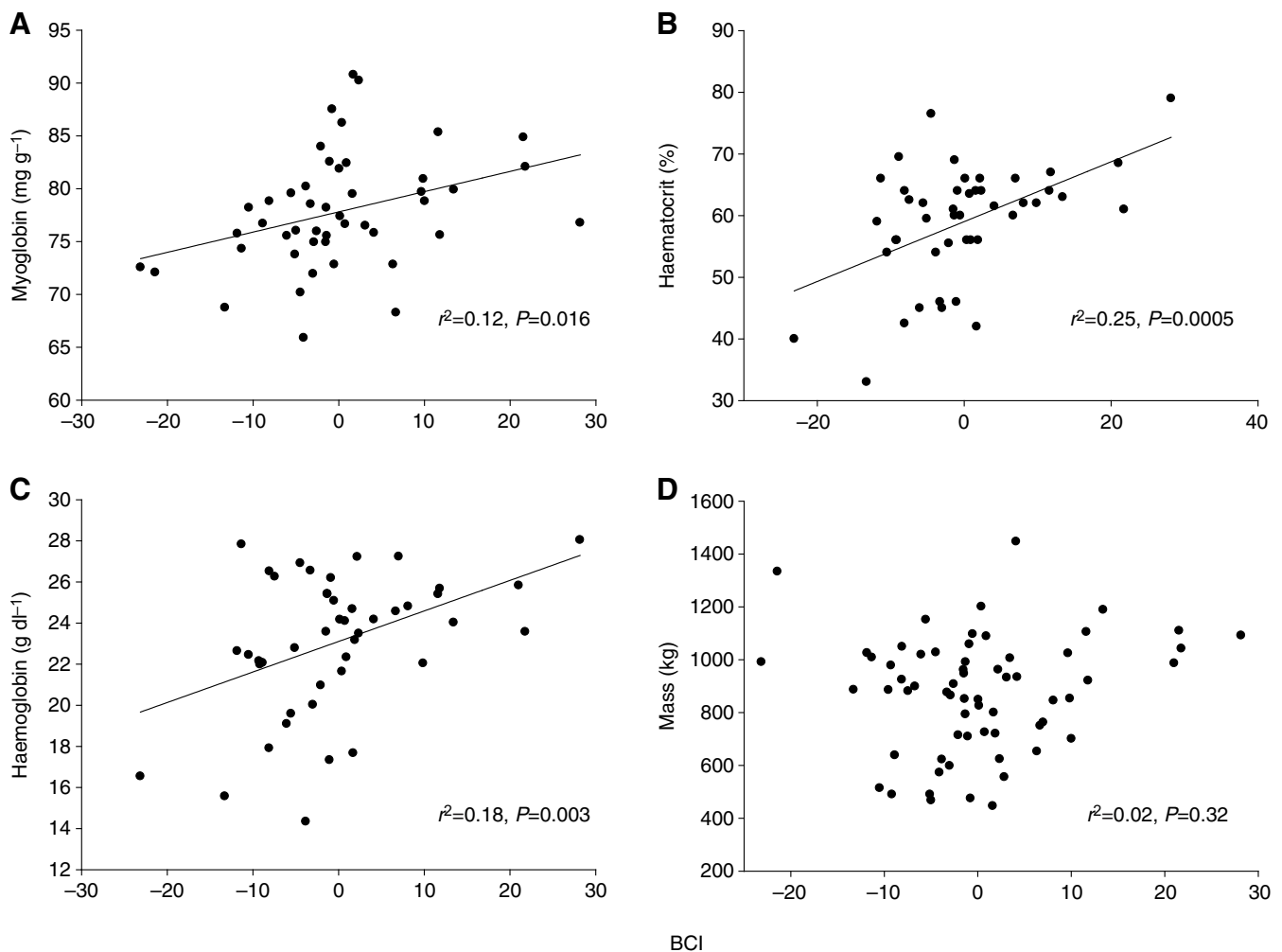
$$TLC = 0.135 \times mass^{0.92}. \tag{3}$$

Fig. 2. Effects of body condition index (BCI) on blood and muscle O$_2$ storage capacity. Relationship between BCI based on the residuals of maximum half-girth and (A) muscle myoglobin concentration (*n*=47), (B) blood haematocrit (*n*=46), (C) haemoglobin concentration (*n*=46) and (D) total body mass (*n*=64) of Beaufort Sea beluga whales.

Table 1. Biological data and physiological parameters of O$_2$ storage capacity for male (*n*=57) and female (*n*=20) Beaufort Sea beluga whales

Sex	Age (years)	Length (cm)	Maximum half-girth (cm)	Mass (kg)	Haematocrit (%)	Haemoglobin (g dl^{-1})	Myoglobin (mg g^{-1})	Myoglobin (mg g^{-1}) (corrected)	Buffering capacity (Slykes)	Spleen mass (g)
F	41.1±3.1 (14)	372.1±5.3 (20)	103.0±2.1 (20)	599.8±27.8 (20)	55.0±2.1 (12)	21.2±1.0 (12)	83.2±2.1 (15)	77.8±1.5 (15)	72.4±1.5 (15)	134.9±16.4 (18)
M	28.9±1.1 (50)	**419.6±3.5** (57)	**117.1±1.7** (57)	**952.7±21.8** (57)	59.6±1.3 (48)	23.4±0.4 (48)	84.1±0.9 (42)	78.0±0.8 (42)	74.0±0.7 (42)	202.1±13.7 (51)
All	31.5±1.3 (64)	407.3±3.8 (77)	113.4±1.6 (77)	861.1±25.0 (77)	58.7±1.1 (60)	23.0±0.4 (60)	83.9±0.8 (57)	77.9±0.7 (57)	73.6±0.7 (57)	184.6±11.5 (69)

Data are means±1 s.e.m. with sample size in parentheses. 'Corrected' refers to myoglobin concentrations that have been determined using spectral deconvolution. Where differences between sexes were significant, based on a two-sample *t*-test, the higher mean is in bold.

As cetaceans inhale immediately before diving (Ridgway et al., 1969), diving lung volume was assumed to equal TLC, and exploitable lung O$_2$ stores (l) were calculated by multiplying this value by an alveolar O$_2$ extraction efficiency of 15% that assumes a fractional O$_2$ concentration of 0.20 upon submergence and a value of 0.05 at the end of the dive (Kooyman, 1973):

$$\text{Lung O}_2 \text{ stores} = \text{TLC} \times 0.15. \tag{4}$$

Muscle O$_2$ stores (l) were calculated using body mass (kg) and myoglobin (Mb; g 100 g^{-1}) concentrations determined from spectral deconvolution based on the equation:

$$\text{Muscle O}_2 \text{ stores} = (\text{mass} \times 0.159) \times (\text{Mb} \times 0.00134), \tag{5}$$

where 0.159 is the proportion of muscle mass in beluga whales (Sergeant and Brodie, 1969) and 0.00134 is the oxygen binding capacity of myoglobin (l O$_2$ g^{-1}) (Kooyman, 1989).

To calculate blood stores, blood volume (BV; ml kg^{-1}) was estimated based on measurements of beluga whales in Ridgway et al. (1984):

$$\text{BV} = \text{mass} \times 0.127. \tag{6}$$

Total blood stores were determined assuming an initial arterial oxygen saturation of 95% and final arterial saturation of 20%, and an initial venous oxygen content that is 5 vol% (5 ml O$_2$ dl^{-1}) less than the initial arterial oxygen content and a final venous oxygen content of zero (Ponganis et al., 2011). We also assumed 0.001341 O$_2$ g^{-1} to be the oxygen binding capacity of haemoglobin (Hb in g ml^{-1}; Kooyman, 1989), and 0.33 and 0.67 as the estimated proportions of arterial (l) and venous blood (l) (Lenfant, 1970):

$$\text{Arterial O}_2 = (0.33 \times \text{BV} \times \text{mass}) \times (\text{Hb} \times 0.00134)$$
$$\times (0.95 - 0.20 \text{ saturation}), \tag{7}$$
$$\text{Venous O}_2 = (0.67 \times \text{BV} \times \text{mass})$$
$$\times (\text{arterial O}_2 \text{ content} - 5 \text{ vol\%}). \tag{8}$$

We employed 2× basal metabolic rate (Kleiber, 1975) to calculate the aerobic dive limit (cADL) of our whales, as this has been suggested as the best approximation of diving metabolic rate (DMR) for odontocetes (Noren and Suydam, 2016; Noren et al., 2002). Using body mass (kg), DMR (ml O$_2$ kg^{-1} min^{-1}) was estimated as:

$$\text{DMR} = 2 \times 10.13 \times \text{mass}^{-0.25}. \tag{9}$$

Using total mass-specific O$_2$ stores (ml O$_2$ kg^{-1}) and DMR (ml O$_2$ kg^{-1} min^{-1}), cADL (min) was then estimated as follows:

$$\text{cADL} = \frac{\text{Total mass} - \text{specific O}_2 \text{ stores}}{\text{DMR}}. \tag{10}$$

RESULTS

Myoglobin concentrations calculated following the method of Reynafarje (1963) (83.9±0.8 mg g^{-1}; Table 1) were higher than those determined using spectral deconvolution (77.9±0.7 mg g^{-1}; paired *t*-test, t_{56}=22.10, *P*<0.001); as the latter method has been suggested to better correct for residual haemoglobin contamination in the muscle (Masuda et al., 2008), it was used for subsequent O$_2$ storage calculations. Myoglobin concentrations determined from intermittently thawed samples (*n*=18) were significantly lower (by ~10%) than those of samples that had been continuously stored at cryogenic temperatures (*n*=57; two-sample *t*-test, t_{73}=−4.53, *P*<0.0001), and hence the former were removed from further

analyses. Buffering capacity averaged 73.6±0.7 Slykes and was positively correlated with myoglobin concentration (n=57; $F_{1,55}$=15.77, r^2=0.21, P<0.0001). Blood haematocrit averaged 58.7±1.1% while haemoglobin concentrations averaged 23.0±0.4 g dl^{-1} (n=60). Haemoglobin concentrations in blood samples that were frozen with or without heparin did not differ (paired t-test, t_{24}=0.21, P=0.84). Blood haemoglobin concentrations significantly increased with haematocrit percentage (n=60; $F_{1,58}$=92.85, r^2=0.62, P<0.0001) across all whales.

In comparison to females, male beluga whales were larger in body length (paired t-test, t_{75}=−7.13, P<0.0001; Table 1), maximum half-girth (t_{75}=−4.43, P<0.0001) and total body mass (t_{75}=−8.74, P<0.0001), though females were older (t_{62}=4.50, P<0.0001). There was no difference in GL ratios (t_{72}=−0.62, P=0.54) between sexes. The model of best fit for maximum half-girth included sex, age and length as predictors (Table S2; $F_{3,60}$=21.9, r^2=0.52, P<0.0001; Table 2). As noted earlier, in addition to the GL ratio, the residuals from this best-fit model for maximum half-girth were used henceforth as a BCI. Mean measurements for additional physiological parameters of O$_2$ stores are given in Table 1.

Effects of age, sex, body mass and BCI on blood and muscle oxygen storage parameters

The best model for myoglobin concentration was BCI alone, with higher myoglobin concentrations found in belugas in better condition (Table S2; Fig. 2A; $F_{1,45}$=6.32, r^2=0.12, P=0.016). Mean muscle water content was 73.5±0.2% and was unaffected by body condition (n=57; r^2=0.04, P=0.12). Haematocrit was also best predicted by BCI (Fig. 2B; $F_{1,43}$=14.25, r^2=0.25, P=0.0005), and significantly increased with body condition. The model of best fit for haemoglobin concentration included BCI, sex, age and the

interactions of age and sex as predictors ($F_{4,40}$=5.29, r^2=0.35, P=0.002); haemoglobin concentration increased with BCI (Fig. 2C) but decreased with age in females (r^2=0.66, P=0.01). There was no relationship between total body mass and BCI (r^2=0.02, P=0.32; Fig. 2D). Muscle proton buffering capacity and spleen mass were unaffected by BCI. Buffering capacity was best fitted by the null model, and was not related to sex, age or any of the morphometric parameters. Log spleen mass was best fitted by age+mass (Table 2; $F_{2,54}$=4.74, r^2=0.15, P=0.013). We noted that for both sexes, spleens comprised the same percentage of total body mass (~0.02%).

The relationships between physiological parameters pertaining to O$_2$ stores and body condition were also supported by models fit using GL ratios (Fig. S1). The best predictor for myoglobin concentration was the GL ratio alone, with higher myoglobin concentrations found in belugas in better condition (Table 3; $F_{1,45}$=5.13, r^2=0.10, P=0.028). The best-fit model for haematocrit was the GL ratio ($F_{1,43}$=14.91, r^2=0.26, P=0.0004), while that for haemoglobin concentration included GL ratio, sex, age and the interactions of age and sex as predictors ($F_{4,40}$=5.49, r^2=0.35, P=0.001).

Using average values for body mass, myoglobin and haemoglobin concentration (Table 1), and the standard framework for estimating the usable O$_2$ stores of marine mammals (see Materials and Methods), the mean O$_2$ storage capacity of our Beaufort Sea whales was 58.7 ml kg^{-1}, with greater O$_2$ stores in blood (30.3 ml O$_2$ kg^{-1} or 51.6% of total O$_2$ stores) relative to muscle (16.6 ml O$_2$ kg^{-1}; 28.3%) and lungs (11.8 ml O$_2$ kg^{-1}; 20.1%). Based on the significant relationships between BCI and both myoglobin and haemoglobin concentration, and controlling for body mass, the whale with the lowest BCI was predicted to have ~12% lower muscle (15.6 versus 17.7 ml kg^{-1}) and ~27% lower

Table 2. Multiple linear regression models for maximum half-girth and O$_2$ storage parameters in Beaufort Sea beluga whales

Dependent	Predictors	Value	Standard error	t	P
Maximum half-girth	Intercept	4.00	16.97	0.24	0.81
	Length	0.21	4.6×10^{-2}	4.58	<0.0001
	Age	0.48	0.14	3.42	0.001
	Sex	10.33	4.16	2.48	0.016
Haematocrit (%)	Intercept	59.03	1.23	47.85	<0.0001
	BCI	0.49	0.13	3.78	<0.001
Haemoglobin (g dl^{-1})	Intercept	27.59	3.10	8.89	<0.0001
	BCI	0.13	4.53×10^{-2}	2.77	0.008
	Age	−0.16	7.28×10^{-2}	−2.22	0.032
	Sex	−4.94	3.52	−1.40	0.17
	Age×sex	0.19	9.16×10^{-2}	2.10	0.042
Myoglobin (mg g^{-1})	Intercept	77.81	0.75	104.36	<0.0001
	BCI	0.19	0.08	2.51	0.016
Buffering capacity (Slykes)	Intercept	73.04	0.75	96.85	<0.0001
log[Spleen mass]	Intercept	5.08	0.31	16.44	<0.0001
	Age	−1.29×10^{-2}	5.62×10^{-3}	−2.28	0.027
	Mass	5.00×10^{-4}	2.7×10^{-4}	1.89	0.064
Haematocrit (%)	Intercept	6.55	13.63	0.48	0.63
	GL	189.48	49.06	3.86	<0.001
Haemoglobin (g dl^{-1})	Intercept	15.86	5.41	2.93	0.006
	GL	53.93	18.70	2.89	0.006
	Age	−0.23	0.07	−3.21	0.003
	Sex	−6.85	3.46	−1.98	0.054
	Age×sex	0.22	0.09	2.43	0.020
Myoglobin (mg g^{-1})	Intercept	59.03	8.33	7.08	<0.0001
	GL	68.11	30.08	2.27	0.028

Predictor values are provided along with their associated standard errors, t-statistics and P-values. Models were fitted with either body condition index (BCI) or maximum half-girth to length (GL) ratio. Results are presented for the most parsimonious model based on Akaike's information criterion corrected (AICc). Spleen mass was measured in g.

Table 3. Longissimus dorsi muscle myoglobin concentration and mass-specific total body O_2 stores of adult beluga whales from different populations and other marine mammal species

Species		Myoglobin (mg g^{-1})	Mass-specific total body O_2 stores (ml O_2 kg^{-1})	Reference
Beluga whale	Beaufort Sea	77.9±0.7 (57) 83.9±0.8 (57)*	58.7	This study
	Chukchi Sea	69.1±3.5 (9)*	~50	Noren and Suydam, (2016)
	Unspecified	34.4±3.9 (5)*	51.9 (1)	Noren and Williams, (2000); Noren et al., (2012)
Narwhal (*Monodon monoceros*)		78.7±9.9 (3)*	74.5±5.1 (3)	Williams et al. (2011)
Short-finned pilot whales (*Globicephala macrorhynchus*)		68.2±1.8 (6)*	68.3 (1)	Velten et al. (2013)
Beaked whales (*Mesoplodon* spp.)		73.4±3.9 (5)*	86.9 (1)	
Northern elephant seals (*Mirounga angustirostris*)		75±0.9 (59)*	27.1 to 47.0	Hassrick et al. (2010)
Arctic hooded seals (*Cystophora cristata*)		94.8±8.9 (14)*	89.5±3.0 (14)	Burns et al. (2007)
Harp seals (*Phoca groenlandica*)		85.9±12.5 (9)*	71.6±3.4 (6)	

Data are means±1 s.e.m. with sample size in parentheses.
*Myoglobin concentrations determined by the Reynafarje (1963) method.

blood (26.0 versus 35.7 ml kg^{-1}) O_2 stores than the whale with the highest BCI.

DISCUSSION

Marine mammals are sentinels for Arctic ecosystem change, with sea ice loss predicted to reduce available habitat, body condition, foraging success and even survival (Bluhm and Gradinger, 2008; Gilg et al., 2012; Kovacs et al., 2011; Moore and Huntington, 2008). Several effects associated with changes in sea ice and environmental conditions have been recently documented in the Beaufort Sea beluga population, including changes in individual growth rates (Harwood et al., 2014), habitat use and migration patterns (Hauser et al., 2017; Hornby et al., 2016), composition of prey species (Choy et al., 2017; Loseto et al., 2018) and body condition (Choy et al., 2017). To further investigate these effects, we measured physiological parameters of blood and muscle O_2 storage capacity and their relationships with BCI and GL ratios, in order to better understand the potential impacts of Arctic climate change. Although both models for BCI and GL ratios were significant predictors of haemoglobin and myoglobin concentration, age and sex were significant predictors of maximum half-girth. As GL ratios do not account for the effects of age and sex, we have focused our discussion on results obtained using BCI.

With a mean concentration of 77.9 mg g^{-1} [83.9 mg g^{-1} using the Reynafarje (1963) method], beluga whales have some of the highest myoglobin concentrations reported in marine mammals (Table 3). These values are higher than previous estimates for beluga whales, possibly because our samples were stored immediately at cryogenic temperatures. Notably, our values overlap with those of narwhals (*Monodon monoceros*; Williams et al., 2011). This similarity may be expected as the recently determined primary structures of beluga whale myoglobin (GenBank accession numbers: KT726933.1, KT191276.1 and XM_022599904.1) only differ from those of narwhal at a single site (47Lys→Arg), and hence myoglobin proteins from the two species exhibit the same high electrostatic net surface charge that seems to determine maximal tissue myoglobin concentrations in diving mammals (Mirceta et al., 2013).

Blood haematocrit (58.7±1.1%) and haemoglobin (23.0 ±0.4 g dl^{-1}) concentrations were also at the upper end of previous measurements for the species (mean range haematocrit: 49.3– 59.0%; haemoglobin: 18.0–22.3 g dl^{-1}; Cornell et al., 1988; Hedrick and Duffield, 1991; MacNeill, 1975; Noren et al., 2018;

Norman et al., 2012; Norman et al., 2013; St. Aubin and Geraci, 1989; St. Aubin et al., 2001). This finding may in part be due to a bias in sample collection, as subsistence hunters preferentially harvest large males (Harwood and Smith, 2002; Harwood et al., 2002), which tended to show higher average haemoglobin and haematocrit values than females. By contrast, muscle buffering capacities from our belugas (73.6±0.7 Slykes) were similar to or slightly lower than those previously measured from beluga whales (74.2–84.3 Slykes; Noren, 2004; Noren and Suydam, 2016). The relatively high buffer values for belugas may be particularly important in extending dive time during stressful events (e.g. evading predators, searching for breathing holes) when anaerobic by-products accumulate in the muscle. Notably, the positive correlation we found between muscle myoglobin concentration and buffering capacity is not observed in interspecific comparisons of cetaceans, though was also found in bottlenose dolphins (*Tursiops truncatus*; Noren, 2004). Spleen mass was not related to male/female differences, BCI, GL ratios or blood parameters of beluga whales. The spleen mass of beluga whales (0.02% of body mass) is comparable to that of other cetaceans and therefore probably does not serve a significant blood storage role in this group of mammals (Cowan and Smith, 1999; Berta et al., 2015).

Our results indicate that haemoglobin concentrations decreased with age in female beluga whales. This observation may be an indication of physiological senescence, which was only seen in the females because of their higher mean ages compared with males. Age-related decreases in blood haemoglobin and haematocrit have been previously reported in bottlenose dolphins over 35 years of age (Venn-Watson et al., 2011). Captive beluga males were also found to have significantly higher haematocrit and haemoglobin levels than females; however, the effect of sex became non-significant once age was accounted for in a multivariable model (Norman et al., 2013). This age effect may in part underlie the slightly higher haemoglobin concentrations of males relative to females, and it would be interesting to see whether this and other blood parameters also decrease with age in male belugas.

Our average mass-specific O_2 store estimates for Beaufort Sea beluga whales (58.7 ml kg^{-1}) are similar to those of other deep-diving delphinoids, such as short-finned pilot whales (*Globicephala macrorhynchus*; Table 3), but higher than previous estimates for captive belugas (51.0 ml kg^{-1}; Shaffer et al., 1997; Noren et al., 2012) and wild belugas from the Chukchi Sea population (~50 ml kg^{-1}; Noren and Suydam, 2016). The high body O_2

stores estimated in this study are notable given that venous O_2 stores appear to have been overestimated by ~19% in the Noren and Suydam (2016) study as a result of a misinterpretation in their eqn 2 [briefly, their provided equation assumes that at the start of a dive, venous blood O_2 saturation is 5% lower (i.e. by ~1 ml O_2 dl^{-1}) than arterial blood O_2 saturation, as opposed to venous blood O_2 content being 5 vol% (5 ml O_2 dl^{-1}) lower than arterial blood O_2 content, as outlined by Kooyman (1989) and Ponganis (2011)]. Mass-specific total body O_2 stores of belugas in our study are lower than those previously estimated in narwhals (74.5 ml kg^{-1}; Williams et al., 2011); however, muscle stores in narwhals were calculated assuming a fractional muscle mass similar to that of the more distantly related bottlenose dolphins (0.36; Goforth, 1986), which is more than twice the value found in beluga whales (0.159; Sergeant and Brodie, 1969). As such, our results suggest that blood and muscle parameters pertaining to beluga diving physiology are more similar to those of narwhals than previously appreciated, and that this specialized physiology for prolonged diving and navigating ice habitat may make both species particularly sensitive to climate change.

Perhaps the most significant finding was that BCI and GL ratios were positively correlated with haematocrit, haemoglobin and myoglobin concentrations in beluga whales (Fig. 2; Fig. S1), and were better predictors of these physiological O_2 storage parameters than other biological variables such as age, sex and body mass. Considering that haemoglobin and myoglobin concentrations are central factors determining exploitable blood and muscle O_2 stores, these differences are predicted to significantly impact total body O_2 storage capacity and hence physiological dive performance. Indeed, based on observed differences in myoglobin and haemoglobin concentrations alone, we calculate that – independent of body mass – differences in blood and muscle O_2 stores between the two BCI extremes are 9.7 and 2.1 ml O_2 kg^{-1}, respectively (Fig. 3). Further assuming that lung O_2 stores are unaffected by BCI (11.8 ml O_2 kg^{-1}), we estimate that mass-specific total O_2 stores of a whale with the lowest observed BCI are >18% lower than those of a whale of equivalent body mass with the highest BCI (53.4 versus 65.2 ml O_2 kg^{-1}), which equates to a >3 min difference in calculated aerobic dive limit (14.3 versus 17.4 min). However,

several lines of evidence suggest that this predicted reduction in aerobic dive limit is a conservative estimate. First, a BCI based on maximum half-girth incorporates changes in blubber thickness as well as muscle mass (George et al., 2015); therefore, observed declines in maximum half-girth may to some extent be associated with catabolism of lean muscle tissues and hence a decrease in muscle mass that would further reduce muscle O_2 storage capacity, in addition to the observed decrease in myoglobin concentration. This contention is supported by studies on harbour porpoises (*Phocoena phocoena*), where epaxial muscle mass is lost during starvation, hypothesized to be the result of protein catabolism and dehydration (Koopman, 2001; Stegall et al., 1999); however, mean muscle water content was unaffected by body condition in our study, suggesting that muscle dehydration was not contributing to poor body condition in our whales. Second, beluga whales with reduced body condition presumably also have elevated diving metabolic rates that could potentially further compromise dive duration. For example, mass-specific diving metabolic rates of nutritionally stressed Steller sea lions (*Eumetopias jubatus*) were significantly (>10%) higher than pre-trial controls, which corresponded to an overall decrease in foraging efficiency during a dive bout (Gerlinsky et al., 2014). Finally, a recent study on captive beluga whales suggests that use of interspecific allometric equations obtained on nearshore marine mammals may overestimate values for total lung capacity in deep-diving beluga whales (Fahlman et al., 2019a). If true, this would result in lower estimates of mass-specific lung O_2 stores in the present study and thereby lead to relatively larger impacts of muscle and blood O_2 store reductions on estimates of aerobic dive limits. In addition, the end-expired O_2 content of the first breath after a dive has been measured to decrease with breath-hold duration in bottlenose dolphins (13.2% to 5.4%: Ridgway et al., 1969; 15.5% to 4.2%: Fahlman et al., 2019b), which suggests there are errors associated with assuming a static O_2 exchange fraction (Fahlman et al., 2019b). More precise physiological measurements on beluga whales may show similar variation in end-expired O_2 content that is associated with dive duration and allow more precise estimates of maximal usable lung O_2 stores. A potential caveat is that nutritional stress was also accompanied by increased blood volume in Steller sea lions (Gerlinsky et al., 2014); if this also occurs in beluga, it would act to counter the above aspects.

Considering there has been a 20 year decline in growth rates (Harwood et al., 2015, 2014), and that summer body condition of Beaufort Sea beluga is affected by prey abundance and environmental factors (Choy et al., 2017), there may be accompanying changes in foraging ability of beluga whales. Arctic cod, a major food source of beluga whales, display a size-class gradient with depth, with peak biomass in the Canadian Beaufort Sea occurring between 350 and 500 m (Majewski et al., 2016). As energy density also increases with fork length of Arctic cod (Harter et al., 2013), beluga whales in poor body condition may be less able to attain the depths with the greatest biomass of the largest and most energy dense prey, leading to reduced caloric consumption. Reductions of lipid reserves due to inadequate consumption of prey may also lead to further energy deficits as a result of increases in thermoregulatory and foraging costs, as has been proposed previously for other species of marine mammals (Rosen et al., 2007). In summary, belugas in better physical condition may fare better under stressful circumstances, such as evading predators or ice entrapments, as they are predicted to have maximal aerobic dive times that are at least >20% longer than those of whales with the lowest BCI values. The relationship between
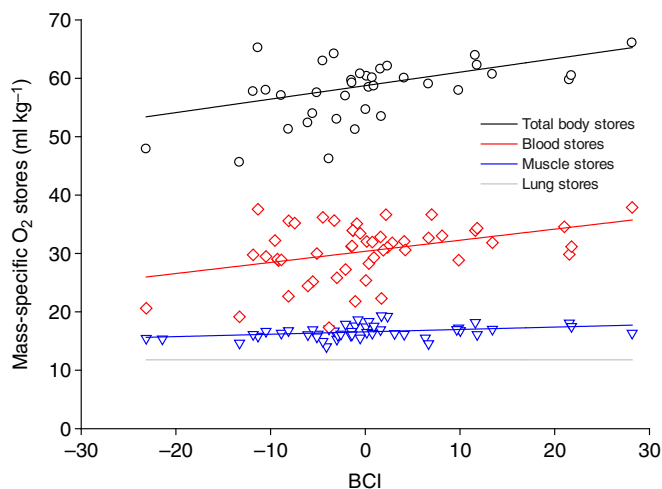

Fig. 3. Relationships between BCI and O_2 stores. BCI based on the residuals of maximum half-girth, and predicted (solid lines) versus individually calculated estimates for lung, muscle (triangles; *n*=57), blood (diamonds; *n*=60) and total O_2 stores (circles; *n*=45) of Beaufort Sea beluga whales (see Discussion for details).

body condition and O_2 storage capacity may represent a vicious cycle in beluga whales, in which environmental changes resulting in decreased body condition impair diving ability, leading to further reductions in condition through diminished prey consumption and/ or increased foraging efforts, and a heightened mortality risk due to predation and ice entrapment.

Author contributions

Conceptualization: E.S.C., K.L.C.; Methodology: E.S.C., K.L.C., M.B.; Software: M.B.; Validation: K.L.C.; Formal analysis: E.S.C., M.B.; Investigation: E.S.C., M.B.; Resources: K.L.C.; Writing - original draft: E.S.C.; Writing - review & editing: E.S.C., K.L.C., M.B., J.D.R., L.L.L.; Visualization: E.S.C.; Supervision: K.L.C., J.D.R., L.L.L.; Project administration: K.L.C., L.L.L.; Funding acquisition: E.S.C., K.L.C., L.L.L.

Supplementary information

Supplementary information available online at
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