

# Composition and temporal variation in the diet of beluga whales, derived from stable isotopes

Marianne Marcoux<sup>1,2,\*</sup>, Bailey C. McMeans<sup>3</sup>, Aaron T. Fisk<sup>3</sup>,  
Steven H. Ferguson<sup>1</sup>

<sup>1</sup>Fisheries and Oceans Canada, 501 University Crescent, Winnipeg, Manitoba R3T 2N6, Canada

<sup>2</sup>Institute of Biological and Environmental Sciences, University of Aberdeen, Aberdeen AB24 2TZ, UK

<sup>3</sup>Great Lakes Institute for Environmental Research, University of Windsor, Windsor, Ontario N9B 3P4, Canada

**ABSTRACT:** The diet of individuals within a species commonly differs among sex and age classes because of differences in energy requirements and physiological needs. Belugas *Delphinapterus leucas* show a high level of sexual habitat segregation and dimorphism that could result in differences in diet between the sexes. Here, we used stable isotopes of carbon ( $\delta^{13}\text{C}$ ) and nitrogen ( $\delta^{15}\text{N}$ ) from muscle and skin samples of 88 belugas, and likely prey species, to investigate how beluga diet in Cumberland Sound (Nunavut, Canada) varied between sexes, among age classes, and over time from 1982 to 2009. Based on linear mixed-effects models, older belugas had higher  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  than younger individuals of both sexes, suggesting that older individuals feed on more benthic, higher trophic-position prey than younger individuals. We also found a strong, decreasing trend in both  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values over time, indicating either a temporal shift in beluga diet or an ecosystem-wide change in isotope values. Based on stable isotope mixing models performed on belugas sampled since 2000, both males and females fed primarily on Arctic cod *Boreogadus saida* and capelin *Mallotus villosus*. The latter is a recent invader to this ecosystem, which could explain the temporal shift in stable isotopes of the Cumberland Sound belugas.

**KEY WORDS:** Marine mammal · Monodontidae · Arctic · Time series

—Resale or republication not permitted without written consent of the publisher—

## INTRODUCTION

The energy requirements and physiological needs of individuals can change with sex and age, which can result in individual-level differences in diet and ecological interactions. For example, divergent nutritional needs are hypothesized to drive sexual segregation in several species of large herbivores (Myserud 2000). In African elephants *Loxodonta africana*, females are constrained in their movements during droughts because their dependent offspring are less mobile (Stokke & du Toit 2002), and thus they have limited access to resources. White-faced capuchins *Cebus capucinus* of different sex and reproductive status have different energy requirements and different diets (Rose 1994). Identifying such within-species differences in diet, as a result of age

and sex, is important for understanding a species' ecology and for conservation and management efforts.

The beluga *Delphinapterus leucas*, an abundant odontocete in Arctic waters, exhibits high levels of sexual segregation and dimorphism, similar to other marine mammals (Breed et al. 2006). In some populations, belugas are seasonally segregated by sex, such that, in the summer, females form large groups that can include their dependent calves, and males form separate and smaller groups (Michaud 2005, Loseto et al. 2006). Belugas can also exhibit age-related habitat segregation, with larger and smaller individuals in the Beaufort Sea using more offshore and inshore habitats, respectively (Loseto et al. 2006). Belugas are also dimorphic, with males being 1.4 times heavier than females (Stewart & Stewart 1989, Michaud 2005). Additionally, females likely have dif-

\*Email: marianne.marcoux@gmail.com

ferent energetic requirements from males during their 20 mo lactation period (Brodie 1971, Sergeant 1973). The diet of belugas throughout the Arctic is generally poorly understood, but these differences in location, size, and nutrition requirements could lead to sex- and age-related differences in diet, which has been previously shown for some beluga populations (e.g. Hudson Bay, Sergeant 1973; Beaufort Sea, Lo-seto et al. 2009).

The beluga population of Cumberland Sound, Baffin Island, Nunavut has been classified as threatened by the Committee on the Status of Endangered Wildlife in Canada, mainly because of low population size (COSEWIC 2004). The population was commercially exploited in the late 19th and early 20th centuries, which brought the population size from an estimated 8465 (SE = 426) belugas before whaling, to around 500 in the 1980s (estimated from 2 aerial surveys flown in 1985 and 1986, resulting in counts of 407 to 442 belugas, not corrected for submerged animals; Richard et al. 1990, DFO 2005). Although the population size has increased to 2017 (95% confidence limits: 1553 to 2623; DFO 2005), it is clear that the population has not yet fully recovered. Because beluga are thought to feed on Greenland halibut *Reinhardtius hippoglossoides* in the spring based on Inuit traditional knowledge (Kilabuk 1998), the emerging commercial fisheries on Greenland halibut potentially threaten the recovery of the Cumberland Sound beluga population. However, no studies have examined beluga feeding on Greenland halibut in this area.

In the present study, we use stable isotopes to evaluate the diet of belugas in Cumberland Sound. Specifically, we compared the carbon ( $\delta^{13}\text{C}$ ) and nitrogen ( $\delta^{15}\text{N}$ ) isotope values of beluga muscle and skin to a suite of potential prey items collected in Cumberland Sound to quantify diet. In addition, we investigated the influence of sex, age, and time (from 1982 to 2009) on the diet of belugas. We hypothesize that (1) male and female belugas will differ in their isotopic values as a result of differences in their energetic and dietary requirements resulting from differences in reproductive demands and (2) old individuals will have higher  $\delta^{15}\text{N}$  values as a result of feeding on higher trophic-level prey, as documented in other marine mammals (Lesage et al. 2001). Our data set covers 27 yr, which is long for a stable isotope analysis of diet in marine mammals (Hirons et al. 2001, Lesage et al. 2001, Gaden & Stern 2010), and provides the opportunity to assess the long-term feeding ecology of beluga in an ecosystem that has undergone significant changes in the past 30 yr, including

a reduction in sea ice coverage and duration (Comiso 2002, McKinney et al. 2012) and the invasion of species from more southern regions, including the capelin *Mallotus villosus* (Mallory et al. 2010, Gaston et al. 2012).

## MATERIALS AND METHODS

### Sampling and stable isotope analysis

Beluga samples were opportunistically collected in Cumberland Sound (65° 13' N, 66° 45' W; Fig. 1) from harvests by the local community. Muscle and skin samples were collected from each individual between 1982 and 2009 (samples collected in 14 out of the 27 yr) from May to November. For some belugas, the sex of the individuals was recorded (n = 68) based on an examination of the reproductive tract and ventral slits (Dahl et al. 2000; however, see Petersen et al. 2012 for a cautionary note about this method). In addition, an established proxy measure for the age of individuals (tooth growth layer group [GLG]; n = 76) (Lockyer et al. 2007, Luque et al. 2007) was available for some animals. Muscle and skin samples were preserved frozen in plastic bags.

The potential prey items of belugas that were collected in Cumberland Sound in April 2008 and 2009 and in August 2007 to 2009 included capelin (n = 12), Arctic char *Salvelinus alpinus* (n = 30), Greenland hali-

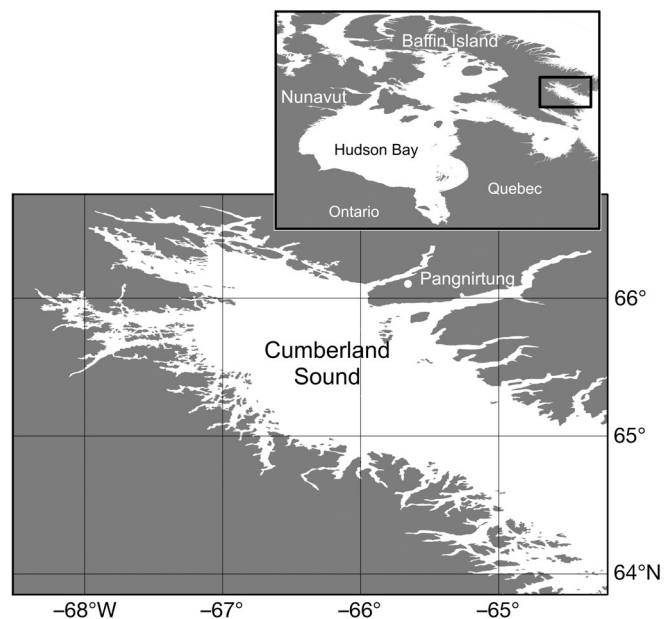


Fig. 1. Cumberland Sound, in Nunavut, Canada, where most beluga *Delphinapterus leucas* and potential prey items were sampled

but ( $n = 76$ ), shrimp *Lebbeus polaris* ( $n = 7$ ), shorthorn sculpin *Myoxocephalus scorpius* ( $n = 23$ ), and polychaetes ( $n = 7$ ). Because no samples were available for Arctic cod *Boreogadus saida* from Cumberland Sound, we included the average of values from 2 other Arctic locations (Coats Island:  $62^{\circ} 30' N$ ,  $83^{\circ} 00' W$ , 2004 to 2009,  $n = 36$ ; Davis Strait:  $65^{\circ} 00' N$ ,  $58^{\circ} 00' W$ , 2004,  $n = 10$ ). The potential prey species were chosen because of their availability in the area and because they have been identified as prey of belugas in other populations (Seaman et al. 1982, Watts & Draper 1986, Kilabuk 1998, Dahl et al. 2000, Loseto et al. 2009).

Prior to stable isotope analysis, samples were freeze-dried for 48 h and homogenized, and lipid was extracted using a 2:1 solution of chloroform:methanol as described in McMeans et al. (2009). Of each sample,  $\sim 0.5$  mg was weighed into tin capsules and run on a continuous-flow isotope-ratio mass spectrometer (Delta V Advantage, Thermo Electron) at the Great Lakes Institute for Environmental Research, University of Windsor. Stable isotopes are expressed as delta ( $\delta$ ) values where  $\delta X = 1000 \times [(R_{\text{sample}} \times R_{\text{standard}}^{-1}) - 1]$ , and  $X = {}^{15}\text{N}$  or  ${}^{13}\text{C}$ , and  $R =$  the ratio of  ${}^{15}\text{N}:{}^{14}\text{N}$  or  ${}^{13}\text{C}:{}^{12}\text{C}$ . Replicate analyses of National Institute of Standards and Technology (NIST) standard bovine muscle (NIST 8414,  $n = 37$ ) and internal lab standard (tilapia muscle,  $n = 7$ ) yielded a precision (i.e. 1 SD) of 0.15 and 0.27‰ for  $\delta^{15}\text{N}$  and 0.05 and 0.08‰ for  $\delta^{13}\text{C}$ , respectively.

### Statistical analysis

We evaluated the correlates of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values in beluga samples using a backwards-step-wise approach with linear mixed-effects models using the package nlme (Pinheiro et al. 2009) in the statistical software R (R Development Core Team 2010). The explanatory variables included in the initial model were: (1) age class (categorical variable; immature [3 to 11 GLGs] [Sergeant 1973], middle age [12 to 32 GLGs], and old [33 to 52 GLGs]), (2) sex, (3) season (categorical variable based on the seasonal migration of belugas: either *ice-covered* if the isotopic values represent the diet when the belugas were feeding in ice-covered water in the south of Cumberland Sound [samples from May to end of July], or *open water* representing the period when belugas were feeding in open water in the North of Cumberland Sound [from August to the end of November]; these dates take into account that stable isotope values reflect the diet of the previous 70 to 75 d in belugas [St Aubin et al. 1990] and that belugas move toward

the north of Cumberland Sound in June and go back toward the south of Cumberland Sound in October [Kilabuk 1998, Richard & Stewart 2009]), (4) the year (continuous variable) the sample was harvested, as well as (5) tissue sample type (skin or muscle). We also tested for the interaction between all variables. Finally, we included the ID of the individual as a random effect because the analysis included 2 samples per individual (skin and muscle). The significance of fixed and random effects was evaluated by comparing the fit of the models with and without the term of interest using maximum likelihood ratio tests ( $\chi^2$  distribution,  $df =$  the difference in the degrees of freedom between the nested models). We started with the full model and examined interactions first, then we removed the terms that did not improve the fit of the model ( $p > 0.05$ ). Lipids are enriched in carbon compared to other tissues (DeNiro & Epstein 1977, Tieszen et al. 1983). In order to investigate the effect of differences in lipid on the carbon content within and between tissues, we also investigated the effect of age class, sex, and tissues on the carbon to nitrogen (C:N) ratios of the samples using similar statistical analysis, with ID as a random effect. Normality of the data was examined using Shapiro tests (Royston 1982). Final models were inspected for the normality of residuals using normal quantile-quantile plots, and for heteroscedasticity using plots of the residuals against fitted values.

To determine the diet of belugas, we used mixing models for stable isotopic data within a Bayesian framework based upon a Gaussian likelihood with Dirichlet prior mixtures SIAR package (Parnell & Jackson 2010) in R version 2.12.2 (R Development Core Team 2010). Before performing this analysis, we examined the differences in  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values between males and females, as well as between tissue samples, using multivariate analysis of variance including ID as a fixed factor (MANOVA with Pillai's trace test). Since both sex and tissue type had a significant effect on  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values (sex:  $F_{2,49} = 27.9$ ,  $p < 0.0001$ ; muscle:  $F_{2,49} = 26.6$ ,  $p < 0.0001$ ), we analyzed skin and muscle samples separately and treated males and females as different groups in the mixing models for stable isotopic data analysis. Muscle diet-tissue discrimination factors (mean  $\pm$  SD) were  $1.52 \pm 0.42$  and  $2.31 \pm 1.02$  for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ , respectively while skin diet-tissue discrimination factors were  $2.29 \pm 0.59$  and  $2.57 \pm 0.52$  for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ , respectively. Since there are no published values for diet-tissue discrimination factors in beluga skin and muscle, discrimination factors included in mixing models were obtained by taking the average

Table 1. *Delphinapterus leucas*. Beluga samples available for analysis. GLG: tooth growth layer group

		Muscle	Skin
Sex	Male	40	38
	Female	28	22
	Unknown	8	8
GLGs	3–11	14	13
	12–32	32	26
	33–52	16	13
	not available	14	16
Year	1982–1989	15	14
	1990–1999	26	21
	2000–2009	35	33
Month	May	4	6
	Jun	7	4
	Jul	38	35
	Aug	9	7
	Sep	2	2
	Oct	0	0
	Nov	2	3
	not available	14	11

of previously published values from other marine mammals fed on known, high-protein diets (which can affect discrimination factor; Gannes et al. 1997), and included: harp seals *Pagophilus groenlandicus*, harbor seals *Phoca vitulina* and ringed seals *Phoca hispida* (Hobson et al. 1996), killer whale *Orcinus orca* (Caut et al. 2011), and bottlenose dolphins *Tursiops truncatus* (Fernández et al. 2011). Because we found an effect of year on stable isotope values (see 'Results'), we only included beluga samples from 2000 and after in the diet analysis to correspond with when prey samples were obtained.

Table 2.  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  (mean  $\pm$  1 SD) of the 7 prey species included in the mixing model to estimate diet as well as of beluga *Delphinapterus leucas* males and females

Species	n	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)
Capelin <i>Mallotus villosus</i>	12	$-19.52 \pm 0.26$	$13.83 \pm 0.47$
Arctic char <i>Salvelinus alpinus</i>	30	$-18.64 \pm 0.57$	$14.06 \pm 0.64$
Arctic cod <i>Boreogadus saida</i>			
Coats Island	36	$-19.26 \pm 0.65$	$14.81 \pm 0.92$
Davis Strait	8	$-20.47 \pm 0.32$	$13.49 \pm 0.48$
Average	44	$-19.86 \pm 0.48$	$14.15 \pm 0.70$
Halibut <i>Reinhardtius hippoglossoides</i>	76	$-18.83 \pm 0.27$	$16.88 \pm 0.65$
Shorthorn sculpin <i>Myoxocephalus scorpius</i>	23	$-17.28 \pm 0.76$	$15.38 \pm 1.13$
Red shrimp <i>Lebbeus polaris</i>	7	$-18.23 \pm 0.71$	$13.94 \pm 0.40$
Polychaetes	7	$-16.46 \pm 0.63$	$11.68 \pm 0.93$
Beluga female (skin)	22	$-18.01 \pm 0.29$	$16.58 \pm 0.54$
Beluga female (muscle)	28	$-18.31 \pm 0.33$	$16.67 \pm 0.94$
Beluga male (skin)	38	$-17.87 \pm 0.33$	$16.76 \pm 0.67$
Beluga male (muscle)	40	$-18.02 \pm 0.31$	$17.40 \pm 1.28$

Table 3. *Delphinapterus leucas*. Predictors of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  (‰) obtained from linear mixed-model analyses. Fixed effects were removed by a backwards-step-wise process. For  $\delta^{15}\text{N}$ , the p-values for both sex and growth layer group (GLG) could not be evaluated because the interaction between these terms remained in the final model

$\delta^{13}\text{C}$	Estimate	SE	$\chi^2$	df	p
Intercept	22.65	10.91			
Tissue, skin	0.20	0.040	19.33	1	<0.0001
Age, immature	-0.19	0.091	16.03	2	0.0003
Age, old	0.29	0.094	16.03	2	0.0003
Year	-0.020	0.005	12.44	1	0.0004
ID			14.41	1	0.0001
$\delta^{15}\text{N}$	Estimate	SE	$\chi^2$	df	p
Intercept	278.57	27.22			
Tissue, skin	-212.44	24.21			
Year	-0.13	0.014			
Year:Tissue, skin	0.11	0.012	36.1	1	<0.0001
Age, immature	-0.61	0.21	8.13	2	0.017
Age, old	-0.034	0.22	8.13	2	0.017
ID			17.99	1	<0.0001

## RESULTS

Muscle and skin samples were collected from 88 individuals. A summary of the data is provided in Table 1. The length (mean  $\pm$  SD) of all belugas was 381.5 cm  $\pm$  60.44 cm and the GLG was 22.6  $\pm$  12.64. Average values for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  in muscle and skin samples of females and males are provided in Table 2.

The final model examining the correlates of  $\delta^{13}\text{C}$  values included the fixed factors age class, year, and tissue type as well as the random factor ID (Table 3). Old belugas had higher  $\delta^{13}\text{C}$  than young ones (Fig. 2a). The  $\delta^{13}\text{C}$  values decreased with year, averaging 0.01‰ yr<sup>-1</sup> (Fig. 3a). Skin tissue samples had higher  $\delta^{13}\text{C}$  values than muscles samples. The final model examining the correlates of  $\delta^{15}\text{N}$  values included the factors age class, year, and tissue type, as well as the interaction between year and tissue type (Table 3). Similarly to  $\delta^{13}\text{C}$  values,  $\delta^{15}\text{N}$  values in old individuals were higher than values in young ones (Fig. 2b). The year had a strong negative effect on  $\delta^{15}\text{N}$ , with values decreasing by an average of 0.8‰ over 10 yr (Fig. 3b). Contrary to the

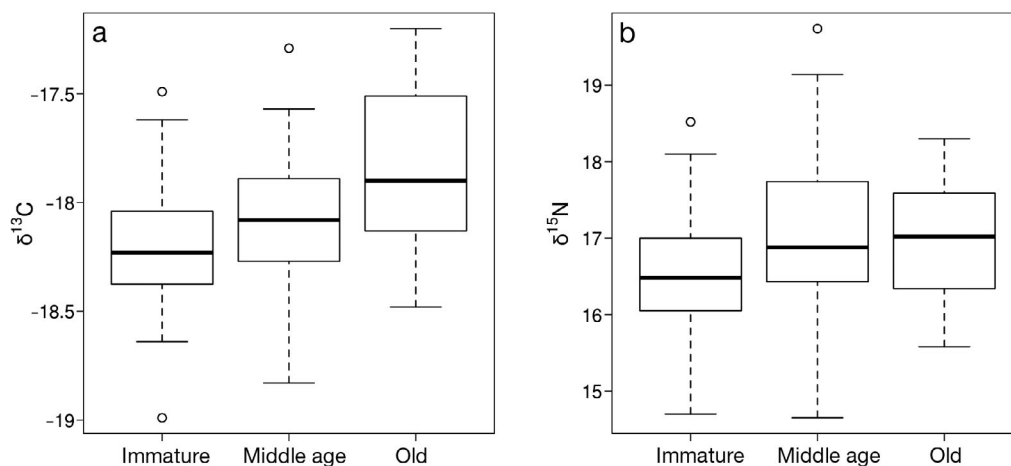


Fig. 2. *Delphinapterus leucas*. (a)  $\delta^{13}\text{C}$  and (b)  $\delta^{15}\text{N}$  values of muscle and skin samples from females and males grouped by age classe based on tooth growth layer groups (GLGs: immature 3–11 GLGs, middle age 12–32 GLGs, and old 33–53 GLGs). Box-and-whisker plots: outside edges of the box = first and third quartiles, middle line = median, whiskers = 2 SD, and dots = outliers

results for  $\delta^{13}\text{C}$  values, skin tissue samples had lower  $\delta^{15}\text{N}$  values than muscle samples. The random effect ID explained 62 % of the variance in  $\delta^{13}\text{C}$  and 57 % of the variance in  $\delta^{15}\text{N}$ . The C:N ratio was only influenced by the tissue sample type ( $\chi^2 = 135.24$ ,  $df = 1$ ,  $p < 0.001$ ), where skin samples had higher C:N ratios than muscle samples (average  $\pm$  S.D:  $3.67 \pm 0.16$  and  $3.32 \pm 0.09$ , for skin and muscle respectively).

The stable isotope values from the 7 potential prey items are shown in Table 2. Greenland halibut had the highest  $\delta^{15}\text{N}$  values, while polychaetes had the lowest (16.9 and 11.7‰, respectively). For  $\delta^{13}\text{C}$  values, polychaete worms had the highest value, while capelin had the lowest value (–16.5 and –19.9‰, respectively; Fig. 4). Because red shrimp and Arctic

char had similar isotopic values, they were grouped for the analysis of diet. Mixing-model estimates of dietary contributions based on both muscle and skin samples identified cod as having the highest mean dietary proportional contribution to the diet of both males and females (contribution ranging from 0.37 to 0.50) followed by capelin (contribution ranging from 0.28 to 0.39). For both models, the isotopic value for capelin and cod overlapped, making it difficult for the models to differentiate between the 2 prey items (indicated by coefficients of correlation between capelin and cod generated by diagnostic plots in SIAR varying from –0.53 to –0.93). However, cod nitrogen values were marginally significantly higher than capelin nitrogen values ( $t$ -test:  $t = 1.86$ ,  $p = 0.06$ ).

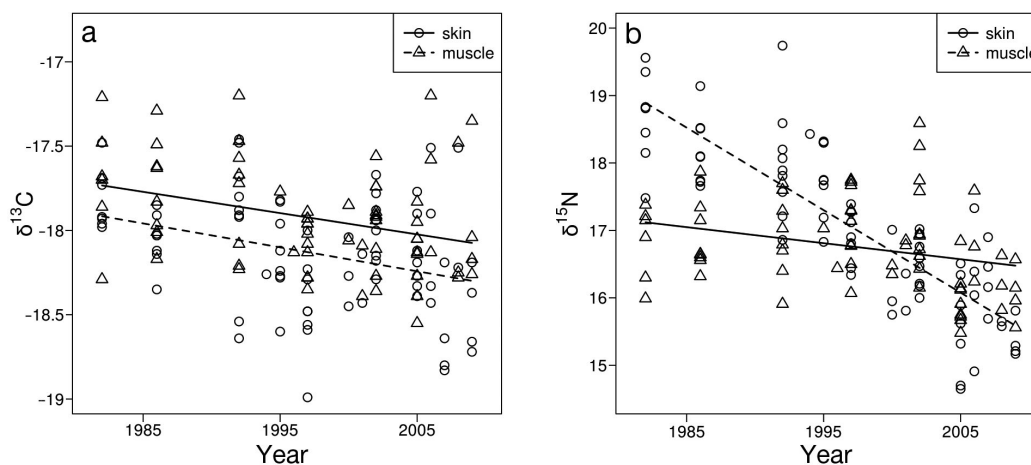


Fig. 3. *Delphinapterus leucas*. Variation in (a)  $\delta^{13}\text{C}$  and (b)  $\delta^{15}\text{N}$  values from muscle and skin samples across years. Lines of best fit from a simple linear regression with year as the independent variable ( $\delta^{13}\text{C}$ , skin:  $R^2 = 0.11$ ,  $p = 0.007$ , muscle:  $R^2 = 0.12$ ,  $p = 0.002$ ;  $\delta^{15}\text{N}$ , skin:  $R^2 = 0.08$ ,  $p = 0.02$ , muscle:  $R^2 = 0.65$ ,  $p < 0.001$ . The analysis in the text and Table 3 includes other independent variables in a linear mixed model)

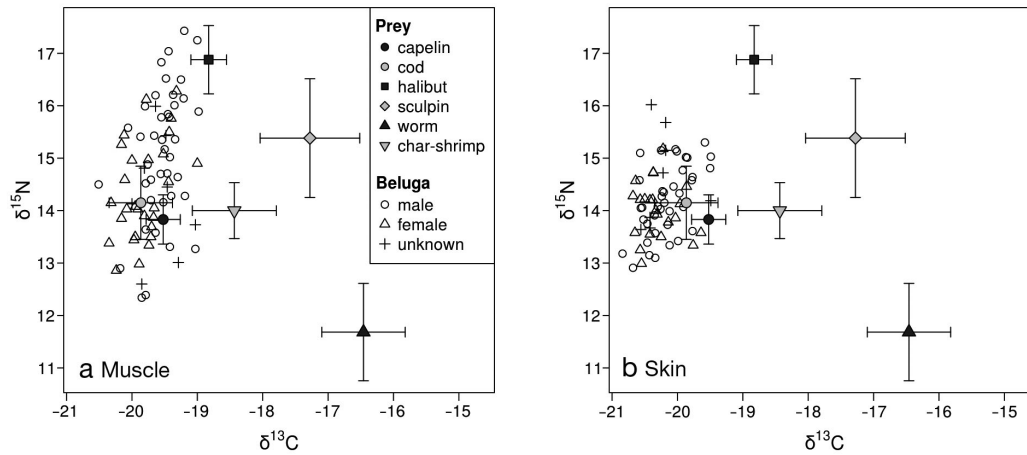


Fig. 4. *Delphinapterus leucas*. Scatterplots of isotopic values of potential prey of beluga included in the diet analysis from (a) muscle and (b) skin samples. Means  $\pm$  SD. In addition, female, male, and unknown-sex belugas are shown corrected for trophic discrimination by subtracting 1.52‰ and 2.31‰ for muscle carbon and nitrogen, respectively, as well as 2.29‰ and 2.57‰ for skin carbon and nitrogen. See Table 2 for scientific names of prey

Models based on muscle and skin samples gave slightly different results for the prey of secondary importance. For males, all the other prey contributed <0.1 to the diet (Fig. 5a,b). For females, the model using muscle samples did not identify other important prey (Fig. 5a), while the model using skin samples identified the group char-shrimp as prey of secondary importance (contribution of 0.12; Fig. 5b).

## DISCUSSION

The results from our mixing-model analysis of diet, examining either skin or muscle samples, suggest that the diet of belugas was mainly composed of Arctic cod and capelin. Results from Cumberland Sound

therefore agree with the observation that cod dominates the diet of belugas in the Beaufort Sea and Svalbard (Dahl et al. 2000, Loseto et al. 2009). Capelin is also an important food source for belugas in Western Hudson Bay and Svalbard (Watts & Draper 1986, Dahl et al. 2000). Greenland halibut was not one of the major prey items for belugas, based on the stable isotope mixing models. Given that beluga samples were collected between May and November and that the turnover rate of beluga skin is around 70 to 75 d (St Aubin et al. 1990), the diet reconstructed from skin samples is likely to represent diet from approximately March to September. Our data therefore do not allow us to infer the fall and winter diet of belugas, when some halibut move to shallower water (<500 m; Peklova et al.

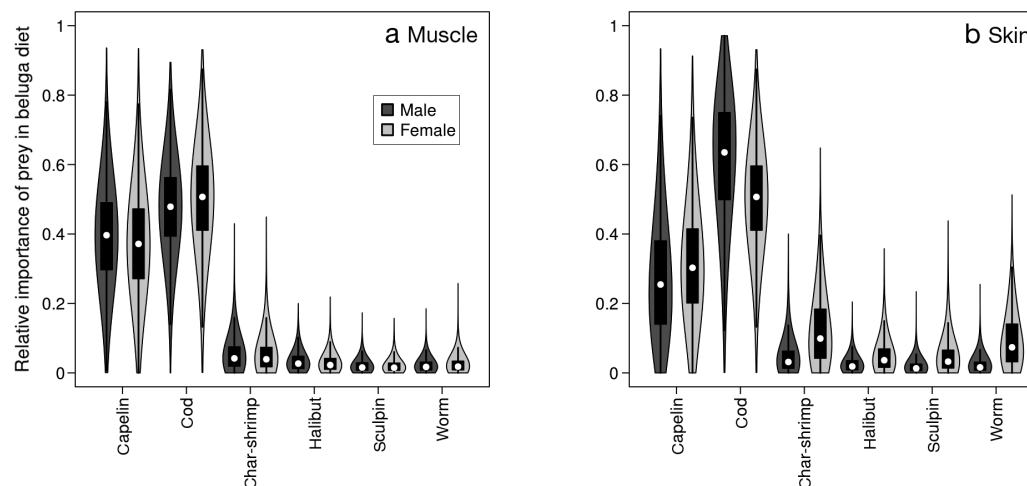


Fig. 5. *Delphinapterus leucas*. Violin plot of the likely contribution of each prey in male and female beluga diet based on (a) muscle and (b) skin samples. Thin lines: the extent of all data points, black boxes: second and third quantiles (0.25–0.75), and white dots: median values. A rotated kernel density is added at each side of the black boxes. See Table 2 for scientific names of prey

2012) and when belugas may feed on halibut. In addition, we did not detect a seasonal trend in the isotope values of belugas over the portion of the year covered by our sample. Limitations of diet analysis using mixing models include the sensitivity of the model to the diet-tissue discrimination factor values entered in the analysis as well as the choice and number of potential prey in the model (Bond & Diamond 2011). In addition, we used an average isotope value for cod from Coats Island and Davis Strait because we did not have samples from Cumberland Sound. It is possible that stable isotope values for cod in Cumberland Sound differ from the average value from Coats Island and Davis Strait. Given the general limitations of this type of analysis, results from our mixing model should be treated as exploratory rather than definitive (Parnell et al. 2010).

Our results suggest age segregation in the diet and/or habitat use of belugas. Carbon isotope values in both skin and muscle samples were significantly greater in older belugas relative to values in younger belugas; the C:N ratio did not vary with age class and does not explain this relationship. In general, benthic or inshore species tend to have higher  $\delta^{13}\text{C}$  values compared to pelagic or offshore species (France 1995). These results suggest that older belugas either inhabit different areas or feed more on benthic species than younger individuals. The  $\delta^{15}\text{N}$  values from old belugas were higher than in younger individuals, providing evidence that old belugas feed at higher trophic levels than younger conspecifics. This difference might also result from belugas of different age classes feeding on the same prey species but selecting different sizes of prey. However, our sample size did not allow us to test this hypothesis. Thus, both  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  provided evidence of age segregation in Cumberland Sound belugas. Similarly, belugas in the eastern Beaufort Sea and in the St. Lawrence River showed differences in diet based on size (a proxy of age) (Lesage et al. 2001, Loseto et al. 2009). Alternative explanations to the differences in isotopic values among ages include physiological processes such as growth, pregnancy, lactation, and fasting; however, these processes have been only rarely investigated (Newsome et al. 2010).

Nitrogen isotope values in our beluga samples showed a decreasing trend with year, indicating a shift in beluga diet, or changes in the ecosystem that have resulted in a shift in the isotope values of the prey base. Over the period of our study,  $\delta^{15}\text{N}$  in beluga muscle and skin samples decreased by an average of  $0.08\text{‰ yr}^{-1}$ . Anecdotal observations suggest that capelin are becoming more abundant in Arctic

regions, representing a potential new prey resource for belugas. This phenomenon has been observed by examining the diets of Arctic birds such as thick-billed murres *Uria lomvia* in southern Hudson Bay, where capelins are replacing Arctic cod in their diet (Mallory et al. 2010, Gaston et al. 2012). Capelin has relatively lower values of  $\delta^{15}\text{N}$  compared to other fish species sampled in our study such as Arctic cod and Greenland halibut. A possible increase in the proportion of capelin in the diet of belugas over the years of this study, relative to a declining proportion of Greenland halibut or Arctic cod in their diet (see Kelly et al. 2010 for diet in the 1980s), could explain the strong negative effect of year on  $\delta^{15}\text{N}$  values.

Over the study period (1982 to 2009), values of  $\delta^{13}\text{C}$  decreased by  $0.01\text{‰ yr}^{-1}$  in both muscle and skin samples. A similar trend in  $\delta^{13}\text{C}$  values has been observed in beluga liver in the Hudson Bay region from 1984 to 2008, with a decrease of  $0.026\text{‰ yr}^{-1}$  (Gaden & Stern 2010). Gaden & Stern (2010) explain this decrease by an increase in the amount of time spent foraging in more offshore regions, or by a shift toward feeding more on pelagic sources. However, decreasing  $\delta^{13}\text{C}$  values are also similar to the oceanic  $^{13}\text{C}$  Suess effect caused by the addition of anthropogenic  $\text{CO}_2$  depleted in  $^{13}\text{C}$  (Gruber et al. 1999, Sonnerup et al. 1999, Körtzinger et al. 2003). Thus, changes in beluga  $\delta^{13}\text{C}$  values might simply reflect ecosystem changes as observed under the Suess effect in other oceans. Regardless, the potential impact of the likely increasing capelin on belugas in Cumberland Sound and on Arctic ecosystems warrants further study. Either through direct or indirect effects, larger capelin populations could increase contaminant accumulation (McKinney et al. 2012) and change the quantity and quality of nutrients and fats (Thiemann et al. 2008, Leu et al. 2011) moving through food webs.

Skin samples had higher values of  $\delta^{13}\text{C}$  than muscle samples, while muscle samples were more enriched in  $\delta^{15}\text{N}$  compared to skin samples. Skin and muscle tissues are expected to have different isotope values because of differences in turnover rates (Tieszen et al. 1983, Hobson & Clark 1992). Tissues with shorter turnover rates provide insight into diet over shorter time scales, whereas tissues with longer turnover rates provide insight into diet over longer time scales. The estimated complete turnover rate for the skin of a captive beluga and a bottlenose dolphin was between 70 and 75 d (Hicks et al. 1985, St Aubin et al. 1990). There is no available turnover rate data for beluga muscle, thus interpreting differences in isotope values between the two tissues is difficult.

Results from published studies on marine mammals show no consistent difference between the carbon and nitrogen values in skin and muscle samples (Hobson et al. 1996, Abend & Smith 1997, Dehn & Follmann 2007, Horstmann-Dehn et al. 2012). Since diet analysis and isotopic comparison based on muscle and skin samples gave slightly different results in our study, we suggest that multiple tissues should be investigated when available. However, a better understanding of the metabolic properties of different tissues is crucial to fully interpret diet analysis of multiple tissues.

*Acknowledgements.* We thank the Inuit hunters of Pangnirtung for collecting samples of beluga skin and muscle from their subsistence hunt. We thank Gregg Tomy, Anna Hussey, Brent Young, Blair Dunn, Gary Stern, Rob Stewart, Magely Chambellant, and Kyle Elliott for sample collection and preparation and laboratory work. Two anonymous reviewers provided useful comments. Funding for this project was provided in part by the Ocean Tracking Network (OTN), a Natural Science and Engineering Research Council (NSERC) Network grant to A.T.F. and S.H.F., and Fisheries and Oceans Canada to S.H.F.

#### LITERATURE CITED

- Abend AG, Smith TD (1997) Differences in stable isotope ratios of carbon and nitrogen between long-finned pilot whales (*Globicephala melas*) and their primary prey in the western north Atlantic. *ICES J Mar Sci* 54:500–503
- Bond AL, Diamond AW (2011) Recent Bayesian stable-isotope mixing models are highly sensitive to variation in discrimination factors. *Ecol Appl* 21:1017–1023
- Breed GA, Bowen WD, McMillan JI, Leonard ML (2006) Sexual segregation of seasonal foraging habitats in a non-migratory marine mammal. *Proc R Soc B Biol Sci* 273:2319–2326
- Brodie PF (1971) A reconsideration of aspects of growth, reproduction, and behaviour of the white whale (*Delphinapterus leucas*) with reference to the Cumberland Sound, Baffin Island, population. *J Fish Res Board Can* 28:1309–1318
- Caut S, Laran S, Garcia-Hartmann E, Das K (2011) Stable isotopes of captive cetaceans (killer whales and bottlenose dolphins). *J Exp Biol* 214:538–545
- Comiso JC (2002) A rapidly declining perennial sea ice cover in the Arctic. *Geophys Res Lett* 29:1956
- COSEWIC (Committee on the Status of Endangered Wildlife in Canada) (2004) COSEWIC assessment and update status report on the beluga whale *Delphinapterus leucas* in Canada. COSEWIC, Ottawa
- Dahl TM, Lydersen C, Kovacs KM, Falk-Petersen S, Sargent J, Gjertz I, Gulliksen B (2000) Fatty acid composition of the blubber in white whales (*Delphinapterus leucas*). *Polar Biol* 23:401–409
- Dehn LA, Follmann EH (2007) Comparison of stable carbon and nitrogen isotope ratios in muscle and epidermis of subsistence-harvested bowhead, beluga and gray whales. North Pacific Research Board, Fairbanks, AK
- DeNiro MJ, Epstein S (1977) Mechanism of carbon isotope fractionation associated with lipid synthesis. *Science* 197: 261–263
- DFO (Fisheries and Oceans Canada) (2005) Recovery potential assessment of Cumberland Sound, Ungava Bay, Eastern Hudson Bay and St. Lawrence beluga populations (*Delphinapterus leucas*). *Can Sci Advis Sec Sci Advis Rep* 36:1–14. DFO, Ottawa
- Fernández R, García-Tiscar S, Begoña Santos M, López A, Martínez-Cedeira J, Newton J, Pierce G (2011) Stable isotope analysis in two sympatric populations of bottlenose dolphins *Tursiops truncatus*: evidence of resource partitioning? *Mar Biol* 158:1043–1055
- France RL (1995) Carbon-13 enrichment in benthic compared to planktonic algae: foodweb implications. *Mar Ecol Prog Ser* 124:307–312
- Gaden A, Stern GA (2010) Temporal trends in beluga, narwhal and walrus mercury levels: links to climate change. In: Ferguson SH, Loseto LL, Mallory ML (eds) *A little less Arctic: top predators in the world's largest northern inland sea, Hudson Bay*. Springer, Fairbanks, AK, p 197–216
- Gannes LZ, O'Brien DM, Martínez del Rio C (1997) Stable isotopes in animal ecology: assumptions, caveats, and a call for more laboratory experiments. *Ecology* 78: 1271–1276
- Gaston AJ, Smith PA, Provencher JF (2012) Discontinuous change in ice cover in Hudson Bay in the 1990s and some consequences for marine birds and their prey. *ICES J Mar Sci* 69:1218–1225
- Gruber N, Keeling CD, Bacastow RB, Guenther PR and others (1999) Spatiotemporal patterns of carbon-13 in the global surface oceans and the oceanic Suess effect. *Global Biogeochem Cycles* 13:307–335
- Hicks BD, St. Aubin DJ, Geraci JR, Brown WR (1985) Epidermal growth in the bottlenose dolphin, *Tursiops truncatus*. *J Invest Dermatol* 85:60–63
- Hirons A, Schell D, Finney B (2001) Temporal records of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  in North Pacific pinnipeds: inferences regarding environmental change and diet. *Oecologia* 129:591–601
- Hobson KA, Clark RG (1992) Assessing avian diets using stable isotopes I: turnover of  $^{13}\text{C}$  in tissues. *Condor* 94: 181–188
- Hobson KA, Schell DM, Renouf D, Noseworthy E (1996) Stable carbon and nitrogen isotopic fractionation between diet and tissues of captive seals: implications for dietary reconstructions involving marine mammals. *Can J Fish Aquat Sci* 53:528–533
- Horstmann-Dehn L, Follmann EH, Rosa C, Zelensky G, George C (2012) Stable carbon and nitrogen isotope ratios in muscle and epidermis of arctic whales. *Mar Mamm Sci* 28:E173–E190
- Kelly TC, Loseto LL, Stewart REA, Yurkowski M, Ferguson SH (2010) Importance of eating capelin: unique dietary habits of Hudson Bay beluga. In: Ferguson SH, Loseto LL, Mallory ML (eds) *A little less Arctic: top predators in the world's largest northern inland sea, Hudson Bay*. Springer, Dordrecht, p 53–70
- Kilabuk P (1998) A study of Inuit knowledge of southeast Baffin beluga. Nunavut Wildlife Management Board, Iqaluit
- Körtzinger A, Quay PD, Sonnerup RE (2003) Relationship between anthropogenic  $\text{CO}_2$  and the  $^{13}\text{C}$  Suess effect in the North Atlantic Ocean. *Global Biogeochem Cycles*



- 17:1005–1025
- Lesage V, Hammill MO, Kovacs KM (2001) Marine mammals and the community structure of the Estuary and Gulf of St Lawrence, Canada: evidence from stable isotope analysis. *Mar Ecol Prog Ser* 210:203–221
- Leu E, Søreide JE, Hessen DO, Falk-Petersen S, Berge J (2011) Consequences of changing sea-ice cover for primary and secondary producers in the European Arctic shelf seas: timing, quantity, and quality. *Prog Oceanogr* 90:18–32
- Lockyer C, Hohn AA, Doidge DW, Heide-Jørgensen MP, Suydam R (2007) Age determination in belugas (*Delphinapterus leucas*): a quest for validation of dentinal layering. *Aquat Mamm* 33:293–304
- Loseto LL, Richard P, Stern GA, Orr J, Ferguson SH (2006) Segregation of Beaufort Sea beluga whales during the open-water season. *Can J Zool* 84:1743–1751
- Loseto LL, Stern GA, Connelly TL, Deibel D and others (2009) Summer diet of beluga whales inferred by fatty acid analysis of the eastern Beaufort Sea food web. *J Exp Mar Biol Ecol* 374:12–18
- Luque SP, Higdon JW, Ferguson SH (2007) Dentine deposition rates in belugas (*Delphinapterus leucas*): an analysis of the evidence. *Aquat Mamm* 33:241–245
- Mallory ML, Gaston AJ, Gilchrist HG, Robertson GJ, Braune BM (2010) Effects of climate change, altered sea-ice distribution and seasonal phenology on marine birds. In: Ferguson SH, Loseto LL, Mallory ML (eds) *A little less Arctic: top predators in the world's largest northern inland sea, Hudson Bay*. Springer, Dordrecht, p 179–196
- McKinney MA, McMeans BC, Tomy GT, Rosenberg B and others (2012) Trophic transfer of contaminants in a changing Arctic marine food web: Cumberland Sound, Nunavut, Canada. *Environ Sci Technol* 46:9914–9922 doi:10.1021/es302761p
- McMeans BC, Olin JA, Benz GW (2009) Stable-isotope comparisons between embryos and mothers of a placental-trophic shark species. *J Fish Biol* 75:2464–2474
- Michaud R (2005) Sociality and ecology of the odontocetes. In: Ruckstuhl KE, Neuhaus P (eds) *Sexual segregation in vertebrates: ecology of the two sexes*. Cambridge University Press, Cambridge, p 303–326
- Mysterud A (2000) The relationship between ecological segregation and sexual body size dimorphism in large herbivores. *Oecologia* 124:40–54
- Newsome SD, Clementz MT, Koch PL (2010) Using stable isotope biogeochemistry to study marine mammal ecology. *Mar Mamm Sci* 26:509–572
- Parnell A, Jackson A (2010) *siar: stable isotope analysis in R*. R Foundation for Statistical Computing, Vienna. Available at: <http://CRAN.R-project.org/package=siar>
- Parnell AC, Inger R, Bearhop S, Jackson AL (2010) Source partitioning using stable isotopes: coping with too much variation. *PLoS ONE* 5:e9672
- Peklova I, Hussey NE, Hedges KJ, Treble MA, Fisk AT (2012) Depth and temperature preferences of the deepwater flatfish, Greenland halibut *Reinhardtius hippoglossoides* in an Arctic marine ecosystem. *Mar Ecol Prog Ser* 467:193–205
- Petersen SD, Tenkula D, Ferguson SH, Kelley T, Yurkowski DJ (2012) Sex determination of belugas and narwhals: understanding implications of harvest sex ratio. *Can Sci Adv Sec Res Doc* 2012/19. Fisheries and Oceans Canada, Ottawa
- Pinheiro J, Bates D, DebRoy S, Sarkar D, R Core team (2009) *nlme: linear and nonlinear mixed effects models*. R Foundation for Statistical Computing, Vienna. Available at: <http://CRAN.R-project.org/package=nlme>
- R Development Core Team (2010) *R: a language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna. Available at: [www.R-project.org](http://www.R-project.org)
- Richard P, Stewart DB (2009) Information relevant to the identification of critical habitat for Cumberland Sound belugas (*Delphinapterus leucas*). *Can Sci Adv Sec Res Doc* 2008/085. Fisheries and Oceans Canada, Ottawa
- Richard PR, Orr JR, Barber DG (1990) The distribution and abundance of belugas, *Delphinapterus leucas*, in eastern Canadian subarctic waters: a review and update. In: Smith TG, St Aubin DJ, Geraci JR (eds) *Advances in research on the beluga whale, Delphinapterus leucas*. *Can Bull Fish Aquat Sci* 224:23–38
- Rose L (1994) Sex differences in diet and foraging behavior in white-faced capuchins (*Cebus capucinus*). *Int J Primatol* 15:95–114
- Royston JP (1982) An extension of Shapiro and Wilk's *W* test for normality to large samples. *J R Stat Soc C Appl Stat* 31:115–124
- Seaman GA, Lowry LF, Frost KJ (1982) Foods of belukha whales (*Delphinapterus leucas*) in Western Alaska. *Cetology* 44:1–19
- Sergeant DE (1973) Biology of white whales (*Delphinapterus leucas*) in western Hudson Bay. *J Fish Res Board Can* 30:1065–1090
- Sonnerup RE, Quay PD, McNichol AP, Bullister JL, Westby TA, Anderson H (1999) Reconstructing the oceanic <sup>13</sup>C Suess effect. *Global Biogeochem Cycles* 13:857–872
- St Aubin DJ, Smith TG, Geraci JR (1990) Seasonal epidermal molt in beluga whales, *Delphinapterus leucas*. *Can J Zool* 68:359–367
- Stewart BE, Stewart REA (1989) *Delphinapterus leucas*. *Mamm Species* 336:1–8
- Stokke S, du Toit JT (2002) Sexual segregation in habitat use by elephants in Chobe National Park, Botswana. *Afr J Ecol* 40:360–371
- Thiemann GW, Iverson SJ, Stirling I (2008) Polar bear diets and Arctic marine food webs: insights from fatty acid analysis. *Ecol Monogr* 78:591–613
- Tieszen LL, Boutton TW, Tesdahl KG, Slade NA (1983) Fractionation and turnover of stable carbon isotopes in animal tissues: implications for  $\delta^{13}\text{C}$  analysis of diet. *Oecologia* 57:32–37
- Watts PD, Draper BA (1986) Note on the behavior of beluga whales feeding on capelin. *Arct Alp Res* 18:439