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Trends in the trophic ecology of St. Lawrence beluga (*Delphinapterus leucas*) over the period 1988-2012, based on stable isotope analysis

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Canada

Foreword

This series documents the scientific basis for the evaluation of aquatic resources and ecosystems in Canada. As such, it addresses the issues of the day in the time frames required and the documents it contains are not intended as definitive statements on the subjects addressed but rather as progress reports on ongoing investigations.

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ABSTRACT

The paucity of information related to the trophic ecology and diet of St. Lawrence Estuary (SLE) beluga limits our understanding of contaminant pathways, and precludes the identification of period, region and prey species likely to be the most important for their successful reproduction and survival. We used a combination of gastro-intestinal data and stable C and N isotope ratios in beluga muscle to infer their trophic position and diet over the period 1988 to 2012, and to identify potential changes in ecosystem structure or functioning over this period. When controlling for inter-annual variability, δ^{13}C and δ^{15}N values were higher in males than females in both juveniles and adults, which is consistent with sex dimorphism and differential access to prey assemblages. Trends in isotopic signatures over the study period could not be examined for calves and juveniles given small overall sample sizes. In adults, δ^{13}C values varied significantly over the study period, and in a similar way in males and females, with a slow increase from 1988 to 2002 followed by a decline of approximately 1‰ from 2003 through 2012. The decline observed in δ^{13}C values after 2002 was echoed by δ^{15}N values, but was overall modest at approximately 0.6‰ over the 10 yr period. There are several potential explanations for the observed decline in isotopic signature, including a change in beluga diet, or in the diet of some of their prey. Isotopic mixing models using 11 potential prey of SLE beluga suggested sandlance and squid, as well as capelin, herring and tomcod as important dietary sources for adult beluga. The model also suggested that groundfish species such as redfish, Atlantic cod and white hake were more important in the diet of adult males than females.

Évolution de l'écologie alimentaire des bélugas du Saint-Laurent (*Delphinapterus leucas*) de 1988-2012, d'après l'analyse d'isotopes stables

RÉSUMÉ

La rareté d'information concernant l'écologie trophique et le régime alimentaire des bélugas de l'estuaire du Saint-Laurent (ESL) limite notre compréhension des voies d'action des contaminants et prévient l'identification des périodes, régions et espèces de proies susceptibles d'être les plus importantes pour leur reproduction et leur survie. Nous avons utilisé une approche combinant des données de contenus gastro-intestinaux et de rapports d'isotopes stables de C et N de muscle de bélugas afin d'inférer leur position trophique et leur diète durant la période 1988 à 2012, et d'identifier de possibles changements dans la structure et le fonctionnement de l'écosystème durant cette période. En contrôlant pour les variations interannuelles, les rapports de δ^{13}C and δ^{15}N étaient plus élevés chez les mâles que les femelles autant pour les juvéniles que les adultes, ce qui est conséquent avec le dimorphisme sexuel de l'espèce et un accès différentiel aux divers assemblages de proies. Les tendances dans les signatures d'isotopes stable au cours de la période d'étude n'ont pu être examinées pour les veaux et les juvéniles compte tenu de la faible taille d'échantillon. Chez les adultes, les valeurs de δ^{13}C variaient de manière significative au cours de la période d'étude, et d'une manière semblable chez les mâles et les femelles, suivant une faible croissance de 1988 à 2002 suivie d'un déclin d'approximativement 1‰ de 2003 à 2012. Le déclin observé dans les valeurs de de δ^{13}C ont trouvé écho dans celles de δ^{15}N, mais étaient somme toute modeste à approximativement 0,6 ‰ sur une période de 10 ans. Il existe plusieurs explications plausibles pour le déclin observé des signatures isotopiques, telles qu'un changement dans la diète des bélugas ou de la diète de certaines de leurs proies. Un modèle de mélange isotopique reposant sur 11 proies potentielles du béluga de l'ESL suggère que le lançon et le calmar, le capelan, le hareng et le poulamon sont d'importantes ressources alimentaires pour les bélugas adultes. Le modèle suggère également que les poissons de fond tels que le sébaste, la morue Atlantique et la merluche blanche occupent une place plus importante dans la diète des mâles que celle des femelles.

INTRODUCTION

There is much speculation as to reasons for the non-recovery of St. Lawrence Estuary (SLE) beluga in spite of nearly 25 years protection from hunting (Hammill et al. 2007). Over the last few years, high mortality rates among newborn calves have added to concerns for the recovery of this population (Lesage et al. 2014; Mosnier et al. 2014), and raised questions as to physical condition and general health of mothers and calves, and the possible emergence of environmental conditions unfavorable to these segments of the population. Limiting factors to recovery probably involve habitat degradation through contamination, increased anthropogenic activities, or changes to ecosystem structure and functioning (DFO 2014). The paucity of information related to the trophic ecology and diet of SLE beluga limits our understanding of contaminant pathways, and precludes the identification of period, region and prey species likely to be the most important for their successful reproduction and survival.

Diet data for SLE beluga is 75 years-old, and comes mostly from areas that they no longer use (Vladykov 1946). In the 1930s, sandlance (*Ammodytes* sp.) and capelin (*Mallotus villosus*) were among the main prey of beluga. Squid (*Illex illecebrosus*) and Atlantic cod (*Gadus morhua*) were also consumed regularly. However, these two species are now scarce in scientific fisheries, questioning their availability to beluga. *Nereis* polychaetes, which were abundant in beluga stomachs, and American eels (*Anguilla rostrata*), which now represent a fraction of historical stocks in the SLE, were proposed as sources for contaminants such as organochlorines, polycyclic aromatic hydrocarbons, and mirex (Hickie et al. 2000), but their importance in beluga contemporary diet remains unknown. In this context, even confirmation of species ingestion might be invaluable in directing research efforts and conservation actions.

Diet composition has commonly been inferred from the analysis of prey remains in digestive tracts of predators. This approach provides information on ingested species, but is associated with several biases related mainly to the differential availability or erosion of prey hard parts during digestion (Pierce and Boyle 1991, Tollit et al. 2003). Other weaknesses are that digestive tracts only provide information on recently consumed meals (Orr and Harvey 2001), likely restricting the identification of prey species to an area near where and when the sample or animal was collected. In addition, digestive tracts of individuals found stranded on beaches are frequently empty limiting, as in the SLE beluga, the amount of information available to infer population diet and trophic ecology.

Techniques exploiting chemical tracers such as the naturally occurring carbon (C) and nitrogen (N) stable isotopes are used increasingly in ecological studies to examine trophic relationships, habitat use and migratory patterns of wildlife species (e.g., Das et al. 2003, Bustamante et al. 2006, Crawford et al. 2008). This approach is based on the demonstration that isotope ratios in tissues of a predator are correlated with those of their diet (Minagawa and Wada 1984, Caut et al. 2009). While nitrogen isotope ratios provide information on trophic position of species, carbon isotope ratios are more informative of the carbon sources they depend on. Isotope ratios may also reveal changes in the quality or abundance of inorganic sources of C and N supplying the base of the food web (Sonnerup et al. 1999), or modification in ecosystem productivity, structure or functioning (Schell 2000). In contrast with the digestive tract approach, the stable isotope technique provides diet estimates that are not affected by the differential rate of disappearance of prey in stomach contents or faeces. It bypasses the problems associated with the high frequency of empty stomachs typical of marine mammals, and estimates the assimilated, not just ingested, food. Furthermore, because the turnover time of isotopes is a function of a tissue's metabolic rate, the analysis of tissues with variable metabolic rates can provide information on diet integrated over a few days (e.g. blood plasma, liver) or a few

months (e.g. red blood cells or muscle) (Tieszen et al. 1983, Hobson and Clark 1992, Hilderbrand et al. 1996).

One drawback to the stable isotope approach is that it cannot identify which species from a given trophic level were consumed. However, in cases where prey are isotopically distinct, isotopic mass-balance mixing models can be used to infer diet composition (Phillips and Greg 2003). Recent advances in this field has allowed diet estimation to move into a Bayesian framework, where uncertainty in input parameters (e.g., predator and prey isotopic signatures and composition, isotope metabolism) can be taken into account in estimating the most probable diets (Moore and Semmens 2008; Parnell et al. 2010). However, such models require some basic knowledge of potentially important prey species in order for them to be included in the model, and for it to provide realistic diets.

In this study, we used a combination of gastro-intestinal data and stable C and N isotope ratios in beluga muscle to infer their trophic position and diet over the period 1988 to 2012, and to identify potential changes in ecosystem structure or functioning over this period.

MATERIALS AND METHODS

SAMPLE COLLECTION

Beluga samples

From 1988 to 2012, muscle tissue was collected from well-preserved to moderately-decomposed beluga carcasses (freshness codes ≤ 3, Geraci and Lounsbury 1993) found in the Estuary and Gulf of St Lawrence. Samples were taken from the side of the animal, wrapped into aluminum foil and placed in air-tight plastic bags. All samples were stored at –20°C until analysed.

At least one tooth (or the entire jaw when possible) was also collected for age determination. Ages were estimated from growth layer groups (GLGs) of dentine in teeth, using a longitudinal midline section or half tooth, and high-resolution (4800 dpi optical resolution, 24-bit color) digital imagery (Epson scanner Perfection V500 photo) to allow for magnification, light and contrast adjustments. One GLG was assumed to be deposited each year (Stewart et al. 2006; Lockyer et al. 2007; Luque et al. 2007)[1].

Isotope ratios were examined separately for newborn calves or young-of-the-year (YOY) (< 1 GLG), juveniles (1–7 GLGs), and the two sex classes in the case of adults (8+ GLGs). Beluga neonates (also referred to as newborns) were identified on the basis of size, the presence of an umbilical cord, and/or embedded teeth, with neonatal dentinal layer. Total standard length (Brodie 1971; Robeck et al. 2005; Suydam 2010) was used in combination with carcass observation date, and signs of emergence of teeth (Brodie 1971) to identify one- or two-year old calves potentially misclassified as newborn or juveniles. Total length (Brodie 1971; Robeck et al. 2005; Suydam 2010) was used in combination with stranding date, and signs of emergence of teeth (Brodie 1971), to identify one- or two-year old calves potentially misclassified as newborn or vice and versa. Age 8 was used as the minimum for adulthood, or sexual maturity in

[1] While the traditional annual deposition rate of 2 GLGs was recently upheld based on an analysis of data on growth and reproduction of Cumberland Sound and captive beluga (Brodie et al. 2013), evidence in support of this hypothesis was criticized on a number of accounts during a workshop on age estimation in monodontids (Tampa Convention Center, Nov. 2011).

females (Robeck et al. 2005; Suydam 2010). The same age was applied for adulthood in males, although males are expected to become sexually mature a few years later than females (reviewed in Lesage and Kingsley 1995).

Potential prey

Approximately 60 species of fish and invertebrates were sampled between 1995 and 2010, and analyzed for stable isotope ratios to characterize the structure of the St. Lawrence ecosystem (Lesage et al. 2001), the diet of the Gulf rorquals (Gavrilchuk et al. 2014) and SLE rorquals (V. Lesage[2], unpublished data), Estuary and Gulf harp seals (Hammill et al. 2005), and SLE beluga (this study). While most samples were 10–40 cm in length, as these appeared to be prey size generally targeted by beluga (Vladykov 1946) and pinnipeds (Hammill and Stenson 2000) smaller and larger specimens were also collected. Potential prey for SLE beluga were identified based on stomach contents data from SLE beluga sampled in the 1930s and 1983-2012 (see Appendix A). Additional species were included based on diet of beluga from other regions (Seaman et al. 1982, Heide-Jørgensen and Teilmann 1994, Hobbs et al. 2008), diet of seals feeding in the St. Lawrence (Hammill and Stenson 2000), prey distribution and relative abundance in the Estuary and northwestern Gulf of St. Lawrence.

The primary source for open-water samples were bottom-trawl surveys for groundfish and shrimps, or oceanographic and plankton surveys conducted during the ice-free period (late April to late October) in the Estuary and northern Gulf of St. Lawrence. Additional specimens of spawning and littoral species were obtained during near-shore dives and from hand-collection by local partners at tidal fishing weirs along both the north and south shores of the SLE. Zooplankton were collected from vertical tows using a 1 m diam x 3 m long Bongo net with 333 µm mesh. Zooplankton were kept alive overnight to allow clearance of gut contents and was separated according to size and species. Zooplankton, larger invertebrates and fishes were stored at –20°C until analyses. Depending on size, either a muscle sample (fishes and larger invertebrates including decapods and Euphausiacea) or the entire organism (other crustaceans) were analysed for stable isotope signatures.

SAMPLE PREPARATION AND ANALYSES

Accounting for lipids and lipid-extraction

Over the last 25 years, thoughts on the necessity or not to remove lipids from tissues prior to isotopic determination have greatly evolved. Lipids are ^{13}C depleted relative to protein (DeNiro and Epstein 1977, McConnaughey and McRoy 1979), and may introduce biases in our interpretation of trophic ecology (e.g. Kiljunen et al. 2006, Murry et al. 2006). Until recently, lipid extraction prior to isotope analyses was the accepted approach. Over the last 10 years, studies on a variety of freshwater or marine invertebrates and vertebrates documented an enrichment in $\delta^{15}N$ as a result of lipid extraction (e.g. Pinnegar and Polunin 1999, Sotiropoulos et al. 2004, Murry et al. 2006, Sweeting et al. 2006, Mintenbeck et al. 2008), leading to a growing consensus as to the necessity to determine $\delta^{13}C$ and $\delta^{15}N$ from separate sample aliquots, i.e., $\delta^{13}C$ from lipid-extracted sample and $\delta^{15}N$ from bulk tissue (Sotiropoulos et al. 2004; Kiljunen et al. 2006; Logan et al. 2008; Lesage et al. 2010). When not possible, recommendations were to lipid-correct $\delta^{13}C$ values when tissues were not lipid-extracted, and to correct $\delta^{15}N$ values for lipid-extracted tissues (e.g., Alexander et al. 1996; Fry et al. 2003; McConnaughey and McRoy

[2] Fisheries and Oceans Canada, Mont-Joli, QC CANADA

1979; Kiljunen et al 2006; Post et al. 2007; Logan et al. 2008). However, model applicability across taxa and tissues has been questioned, leading to the recommendation and development of taxa - and tissue-specific models (e.g., Kiljunen et al. 2006, Sweeting et al. 2006, Bodin et al. 2007, Post et al. 2007, Smyntek et al. 2007, Mintenbeck et al. 2008, Logan et al. 2008; Lesage et al. 2010).

In this study, $\delta^{13}C$ and $\delta^{15}N$ values were determined from separate sample aliquots only for beluga muscle, and for a number of years (i.e., 1997, and 2009 through 2012). Beluga samples collected from 1998 through 2008, and all of the prey samples, which were mostly collected and analyzed in the late 1990s and early 2000s, were lipid-extracted prior to isotopic analysis and thus, required correction of $\delta^{15}N$ values. Conversely, beluga muscle collected in 1988 through 1993 was not lipid-extracted prior to isotopic determination and thus, required $\delta^{13}C$ corrections. Correction factors specific to beluga muscle and prey tissue were developed based on examination of lipid-extraction effects on C and N isotope ratios of muscle tissue for beluga (N = 63) and a subset of 10 fish species (N = 97 individuals) (Appendix B).

All samples were placed in aluminum cups, freeze-dried to a constant mass, and ground to a powder. Lipids were extracted from ~0.2 g of dried material and a solvent consisting of a mixture of chloroform and methanol (2:1 v/v). Tissues were placed in in a glass tube with 10 ml of the solvent mixture, sonicated for 10 min, and stored overnight at 4°C with gentle shaking. The sample was centrifuged for 10 min and supernatant removed (Folch et al. 1957). The extraction procedure was repeated 3 times, after which a test for complete extraction was performed using sulfophosphovanillin (Barnes and Blackstock 1973). Following lipid removal, samples were dried by evaporation, rinsed with distilled water, dried overnight at 50°C, and powdered again.

Isotope analysis

Samples were analyzed for carbon and nitrogen isotope ratios using a Continuous Flow Stable Isotope Mass Spectrometer coupled to a Carla Erba Elemental Analyzer (CHN EA1110 or CHNS-O EA1108); Environmental Isotope Laboratory, University of Waterloo, and Isotope Tracer Technologies Inc., Waterloo, Ontario, Canada). Isotope ratios are expressed in delta (δ) notation as parts per thousand (or per mil, ‰) differences from a standard ($R_{standard}$), i.e. carbonates from Vienna Pee Dee Belemnite (PDB) limestone (using the Vienna PDB scale) for $\delta^{13}C$ and atmospheric N for $\delta^{15}N$, following:

$$\delta X = [(R_{sample}/R_{standard}) - 1] \times 1000 \quad (1)$$

where X is ^{13}C or ^{15}N and R is the corresponding $^{13}C/^{12}C$ or $^{15}N/^{14}N$ ratio. Analytical error based on replicate analyses of samples (n = 54) and laboratory standards was 0.1‰ for both $\delta^{13}C$ and $\delta^{15}N$. Values for C:N ratios are reported based on uncorrected percentage element data.

DATA ANALYSES

Isotopic model

Diet composition was estimated using a multi-source, two-isotope Bayesian mixing model (Parnell et al. 2010). This type of model explicitly accounts for uncertainty in input parameters, such as discrimination factor and predator and prey isotopic signatures, and express diet estimates in terms of probability distributions of prey contributions (Moore and Semmens 2008; Parnell et al 2010). These models require information on isotopic signatures of the predator and each of their potential prey, as well as the degree of metabolism (or trophic discrimination) of

each of the isotopes associated with their deposition in a particular tissue. Trophic discrimination factor for cetacean muscle tissue is undefined. However, a captivity study on phocids indicate that metabolic fractionation of C and N isotopes result in an enrichment of the muscle δ^{13}C and δ^{15}N values of 1.3‰ (SD=0.3‰) and 2.4‰ (SD=0.5‰), respectively (Hobson et al. 1996). These values were assumed to be similar for beluga muscle, and were used as discrimination factors. Isotopic models also require that prey be isotopically distinct, or that prey with similar isotopic signatures be grouped (Phillips et al. 2005). Diagnostic plots associated with the SIAR package in R (R Development Core Team, 2009) allow identification of prey with strong reversed contributions. These sources were combined *a posteriori* for each iteration by adding the negatively correlated dietary proportions of the model, and recomputing posterior probability distributions (Parnell et al. 2013). Based on the combination of historical and more recent digestive tract data (Appendix 1), seal diet data, prey distribution and relative abundance in the Estuary, 11 species were considered in the isotopic model as potential prey of SLE beluga (Table 2). Rainbow smelt (*Osmerus mordax*) differed isotopically between the Upper and Lower estuary and thus, were considered as two separate sources in the model.

Statistical analysis

Differences in isotope signature between bulk and lipid-extracted samples were examined for each isotope separately using paired t-tests and linear regressions, to predict and correct for significant effects when appropriate.

Differences in δ^{13}C and δ^{15}N values among sex and age class (YOY, juvenile, adult) were examined using a mixed-model ANOVA with sampling year as a random effect to control for potential inter-annual variability.

Trends in isotopic signature over the study period were examined using a Generalized Additive Model (GAM), to which a smoother was applied to sampling year, while including significant effects as covariates (e.g., sex or age) (Hastie and Tibshirani 1986, 1990). Penalized cubic regression splines was chosen as the smoothing parameter to get the best possible fit while incorporating the least amount of error (Wood 2006). The optimal degree of smoothing was determined using a generalized crossed-validation method to estimate the smoothing parameter, taking into account residual deviance and effective degrees of freedom (edf) (R package mgcv).

The R command to execute the GAM model is given by:

```
gam(y ~ s(x, k=10) + factor(sex), gamma=1.4, bs="cr")
```

where y is the response variable and x is the predictive variable ‘year’, k is the basis dimension for the smooth, bs specifies the class of smoother, here the penalized cubic regression ‘cr’ spline. A gamma of 1.4 was used to reduce chance of overfitting (Wood 2006).

RESULTS

INTRASPECIFIC AND TEMPORAL TRENDS

When controlling for inter-annual variability, δ^{13}C values varied in a different way between sexes depending on age classes (interaction sex vs age: $F_{[1,199]} = 6.01$, $p = 0.0145$); values were identical in YOY regardless of sex, but higher in males than females in both juveniles and adults (Table 1). Similarly, δ^{15}N values were consistently higher in males than females (sex effect:

$F_{[1,199]}$ = 24.7, p < 0.0001; NS interaction). In addition, δ^{15}N values varied among age classes, with the highest values observed in YOY (age effect: $F_{[1,199]}$ = 224.7, p < 0.0001; Table 1).

Trends in isotopic signatures over the study period could not be examined for calves and juveniles given small overall sample sizes (Table 1). In adults, δ^{13}C values varied significantly over the study period (*edf* = 4.50, *F* = 40.5, deviance explained 65%, p < 0.0001), and in a similar way in males and females, with a slow increase from 1988 to 2002 followed by a decline of approximately 1‰ from 2003 through 2012 (Figures 1 and 2). While δ^{15}N values also varied significantly over the study period, deviance explained by the model when taking into account sex differences in δ^{15}N values was small (*edf* = 2.8, *F* = 5.6, deviance explained 27%, p = 0.0006). The decline observed in δ^{13}C values after 2002 was echoed by δ^{15}N values, but was overall modest at approximately 0.6‰ over the 10 yr period (Figures 1 and 2).

DIET COMPOSITION ESTIMATED FROM ISOTOPIC MIXING MODELS

Of the 11 potential prey included in the isotopic mixing model, two groundfish species (Atlantic cod and white hake) and three pelagic forage fish (capelin, herring and tomcod) largely overlapped in isotopic signatures (Figure 3). In addition, diagnostic plots indicated negatively correlated contributions of sandlance and squid; thus contributions of these sets of species were combined for each iteration of the model, and posterior probability distributions recalculated.

The prey group composed of sandlance and squid was the most important dietary source for adult beluga females (Figure 4a), with mean contributions varying from 16 to 42% depending on years. Capelin, herring and tomcod were the second most important dietary source with a stable mean contribution to the diet of 25 to 28% over the study period. The enrichment in δ^{13}C values in the early phase of the study period and depletion following 2003 were attributed mainly to the prey group composed of sandlance and squid (Figure 5).

Model outputs were not as clear for adult male beluga. There was no real dominant prey species in the diet (Figure 4b). While the most important prey for adult males were the same as those identified for adult females, redfish, Atlantic cod and white hake, three demersal fish contributed more significantly to the diet of males than females, with average contributions of 21 to 29% (vs. 18 to 23% for females) depending on year. Temporal patterns in δ^{13}C values were not clearly echoed in diet contribution for any of the prey groups in the case of adult males (not shown).

DISCUSSION

Ontogenic dietary shifts and differences in diet in size-dimorphic species are well-documented in marine mammals and other species. Differences in body size, diving capabilities or food handling ability between age classes (Scholander et al. 1942, Kleiber 1961) may result in animals feeding at different trophic levels as they mature (life-history omnivory; Werner and Gilliam 1984, Sprules and Bowerman 1988). Many species of fish become more piscivorous with age, which would increase their trophic position relative to juvenile conspecifics. In marine mammals, lower prey capture or handling skills and diving ability in young individuals (Le Boeuf et al. 1996, Horning and Trillmich 1997) likely promote feeding at lower trophic levels and reduce resource overlap with older animals. In SLE beluga, differences in isotopic C and N were observed among age classes, with YOY being particularly enriched in δ^{15}N relative to juveniles or adults (Table 2). Variability about the mean was high for δ^{15}N values of YOY, probably as a result of the variable amount of milk ingested by the calf prior to death, i.e.,

stillborn, newborn, or older suckling calves. Milk is synthesized by the female at least in part from body reserve and remobilization of proteins, which leads to an enrichment of the nursing calf relative to mother tissues (e.g., Lesage et al. 2001).

Body size dimorphism between male and female beluga may also lead to differences in trophic positions. Adult males of SLE beluga had higher δ^{15}N values and thus occupied higher trophic positions than adult females, and may have consumed a greater proportion of food resources hardly accessible to, or too large for females (Kooyman 1989, Boyd and Croxall 1996). The ^{13}C-enrichment of adult males relative to females was consistent with occupation of a higher trophic position, but also with a larger dependency on benthic or demersal species, or with differences in distributions among the two sexes. During summer, adult females are observed most frequently in the ^{13}C depleted Upper Estuary, whereas adult males concentrate mainly in the deep channel of the ^{13}C-enriched Lower Estuary (Tan and Strain 1979; Michaud 1993), where they may have access to different prey communities compared to adult females. Recent diet data from digestive tracts is currently too scarce to examine sex differences in diet or prey size selection. The limited diet data available from the 1930s and from sites no longer used by belugas do not support the hypothesis of more intense feeding of males on benthic or demersal resources (Vladykov 1946). While the isotopic mixing model suggests a greater importance of these demersal prey in the diet of adult males, posterior probability distributions greatly overlapped among the two sexes.

Given the 2-3 mo turnover rate of C and N isotope in muscle tissue of small and large mammals (Tieszen et al. 1983; Hilderbrand et al. 1996), isotopic signatures of beluga sampled in different years are expected to be independent, i.e., there should not remain traces of the previous-year diet isotopic signature in the tissue. It follows that the decrease in δ^{13}C values observed in adult males and females, starting approximately in 2003, likely reflects a progressive but sustained change in their trophic position, either as a result of a change at the base of the food web or in the diet of their prey, in beluga distribution, or in the importance of particular prey. A similar decline in mean δ^{13}C values over approximately the same period was observed in four baleen whales from the Gulf of St Lawrence (Gavrilchuk et al. 2014). The Suess effect could cause a decline in δ^{13}C values of the oceanic dissolved inorganic carbon (DIC) pool through a decrease in δ^{13}C of atmospheric CO_2 (Friedli et al. 1986; Keeling et al. 1996). However, the mean decrease in DIC of 0.03‰ per year reported in the North Atlantic Ocean (Sonnerup et al. 1999) is largely insufficient to explain the decline observed in the baleen whales or SLE beluga over the period of concern, particularly considering their their position in the food web and values for trophic enrichment (see Gavrilchuk et al. 2014).

A change at the base of the food web, such as a decrease in primary productivity, a change in phytoplanktonic species composition or in water mass characteristics towards more saline waters may also lead to a decline in δ^{13}C values of DIC or primary producers. However, given the observed attenuation of isotopic variation with trophic position (Gavrilchuk et al. 2014), it is unlikely that isotopic changes at the base of the food web can be the main cause for the 1‰ decline in δ^{13}C of SLE beluga. While there are no time series monitoring changes in isotopic signature of primary producers or primary or secondary consumers in the Estuary, two studies conducted 15 years apart and using the same collection methodology and sampling station indicate no clear shift in isotopic signature for the vast majority of the species examined (Plourde and Lesage[3], Unpublished data).

[3] Fisheries and Oceans Canada, Mont-Joli, QC

Some of the ^{13}C-depletion observed in beluga following 2003 could also result from a progressive shift of beluga distribution toward more ^{13}C-depleted habitats, such as the Upper Estuary, the Saguenay River or the Gulf of St Lawrence (Tan and Strain 1979; Lesage et al. 2001). The distribution of stranded carcasses does not necessarily reflect the area used by an individual prior to its death and thus, cannot be used to examine this question. Aerial survey data may help address this question, but have not so far been examined in this perspective.

The progressive ^{13}C-depletion observed in SLE beluga over the last 10 years of the study could also result from a change in their diet or in that of some of their prey. Isotopic discrimination during metabolism and thus enrichment in the heavier isotope is expected to be at least 2 to 3 times higher for ^{15}N than ^{13}C (Caut et al. 2009; Newsome et al. 2010). If the 1‰ decrease in δ^{13}C observed over the period 2003-2012 was related to a decrease in trophic position, a concomitant 2-3‰ decrease in δ^{15}N values would be expected; however, this is not observed. Assuming that the observed change in carbon isotope signature is related to a change in beluga diet and not in that of their prey, this suggests that beluga continued to feed approximately at the same, although slightly lower trophic position (mean reduction in δ^{15}N value of 0.5‰), but that the relative importance of prey with different carbon origins has changed. In other words, the decline in beluga δ^{13}C values may reflect a decline in the contribution of ^{13}C-enriched prey to their diet, or an increased contribution of marine or catadromous/anadromous species but of similar trophic positions.

The isotopic mixing models suggest that sandlance and possibly squid, which are both relatively depleted in δ^{13}C compared to other potential prey, might be responsible for the depletion of SLE beluga over the past decade. While this may be the case, there is currently no data on the abundance of these preys to corroborate results obtained through the isotopic mixing model. The similarity among prey sources in terms of isotopic signature, their high number in the model, and isotopic variability are all factors that might reduce the power of isotopic mixing models in determining the relative importance of dietary items (Parnell et al. 2010).

The temporal change in the carbon isotopic signature of SLE beluga may also resulted from a change in the diet of some of their prey over the period 2003-2012. However, observation of a change in δ^{13}C values concomitantly in beluga from the SLE and in blue, fin and humpback whales from the Gulf of St. Lawrence requires that the prey items affected are common to the two ecosystems and marine mammal species groups. There is only fragmentary isotopic data for the recent years and more would be needed to cover the various potential prey species for the beluga and the baleen whales to determine whether this is a plausible explanation for the observed patterns. The change would need to be substantial in order to create a one-trophic level effect (1‰) change in the prey and marine mammal predator.

It is also possible that other prey not included in the model, or for which we have little data, may be responsible for the depletion in δ^{13}C values of adult beluga. A study of the Gulf of St. Lawrence ecosystem structure over the past several decades has documented major changes at various levels in the system, including increases in the abundance of small demersal fish and fall Atlantic herring in the northwestern Gulf of St. Lawrence (Plourde et al. 2014). Changes observed in the isotopic signature of St Lawrence beluga are inconsistent with an increase of these species in the diet, unless a new influx of fish populations to the SLE has recently taken place. In contrast, other species such as the striped bass has also increased in abundance in the St. Lawrence over the past decade. This anadromous species, which is likely to have a more marine (^{13}C-depleted) signature, could inflate the δ^{13}C value of beluga if preyed upon more heavily as it increased in the system. However, there is currently no indication that this species is important in the beluga diet.

This study identified sex differences in the isotopic signatures of SLE beluga which are indicative of differences in diet or access to prey assemblages. The sustained declined in carbon isotopic signature observed in both adult female and male beluga indicate that this population is facing a changing structure in the SLE ecosystem, which has effects on their diet or habitat use. Whether this change in their trophic ecology is positive or negative for their health, reproduction and survival is not known. Means to investigate body condition in wild beluga in parallel to trophic markers may help further our understanding of the beluga trophic ecology and resource needs.

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Table 1. Isotopic signature (in ‰, with SD in parentheses) according to age and sex classes for St Lawrence beluga found dead in 1988–2012.

Age class	Sex	N	$\delta^{13}C$	$\delta^{15}N$
Newborn	F	17	-16.92 (0.50)	17.68 (1.05)
	M	15	-16.92 (0.50)	17.96 (1.07)
Juvenile	F	10	-17.40 (0.36)	15.23 (0.68)
	M	11	-17.15 (0.49)	15.50 (0.52)
Adult	F	100	-17.22 (0.43)	15.24 (0.45)
	M	71	-16.89 (0.34)	15.74 (0.64)

Table 2. Mean isotopic values for potential prey species of St Lawrence beluga.

Name_s	n	$\delta^{13}C$	$\delta^{15}N$	SD $\delta^{13}C$	SD $\delta^{15}N$
Ammodytes spp.	17	-18.75	11.18	0.61	0.30
Clupea harengus harengus	41	-18.24	13.54	0.89	0.41
Gadus morhua	7	-18.33	15.09	0.46	0.91
Illex illecebrosus	3	-19.34	12.85	0.12	0.25
Mallotus villosus	116	-18.52	13.12	0.87	0.96
Microgadus tomcod	12	-18.21	12.78	0.27	0.37
Nereis virens	6	-13.29	10.83	0.37	0.33
Osmerus mordax (Lower)	17	-15.27	12.91	1.59	0.85
Osmerus mordax (Upper)	8	-19.17	15.22	0.62	0.60
Sebastes sp.	6	-18.95	13.70	0.26	0.30
Urophycis tenuis	3	-17.80	16.41	0.28	0.51
Gadus ogac	2	-16.10	14.44	0.05	0.16

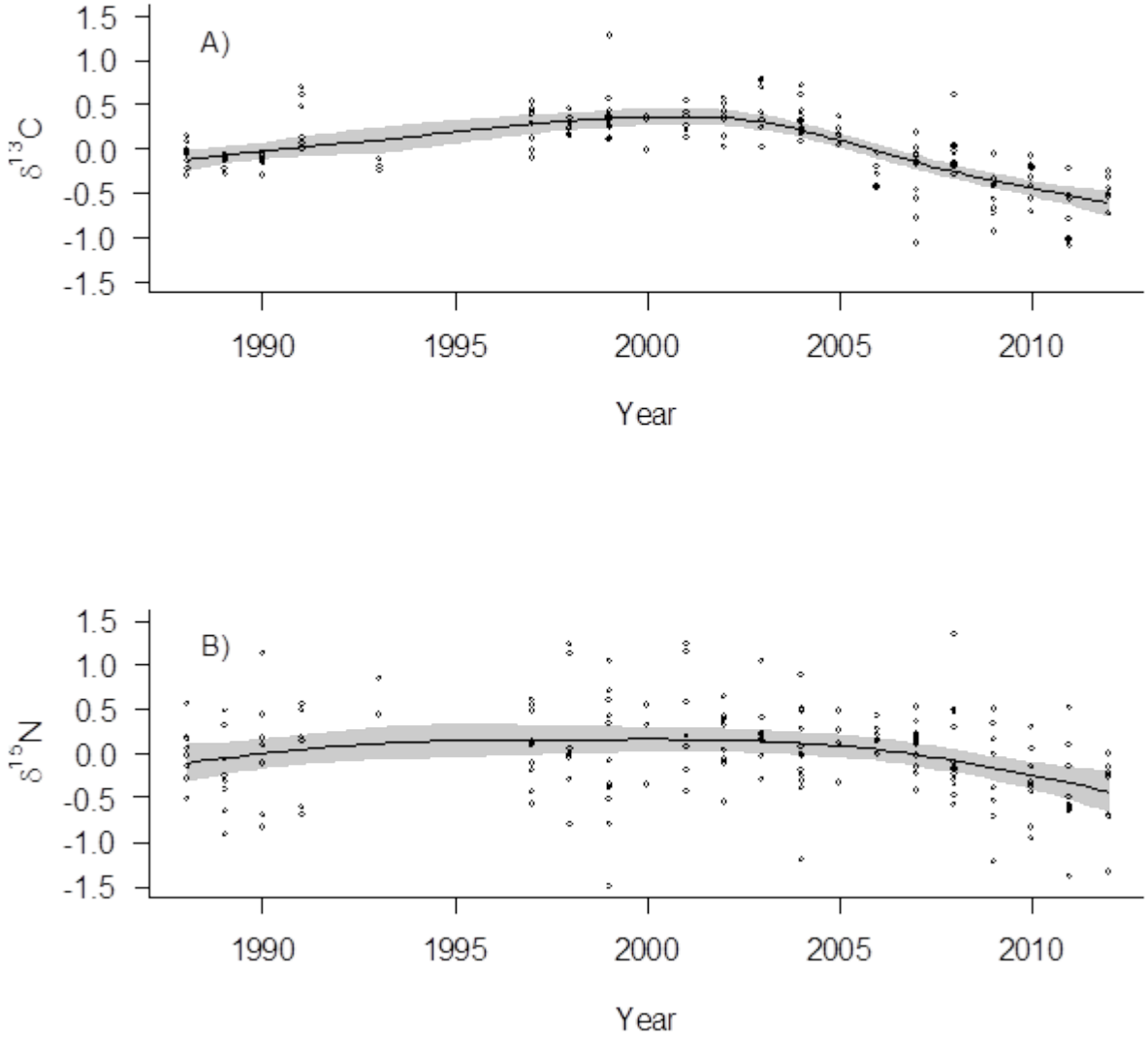

Figure 1. Trends in δ13C (A) and δ15N values (B) in adult beluga over the study period, i.e., 1988 to 2012, estimated using Generalized Additive Models and penalized cubic regression splines as a smoother (curve). The y-axis shows deviations from mean δ13C values and the 95% credibility interval (shaded area). Each dot represents the isotopic value for a sampled beluga.

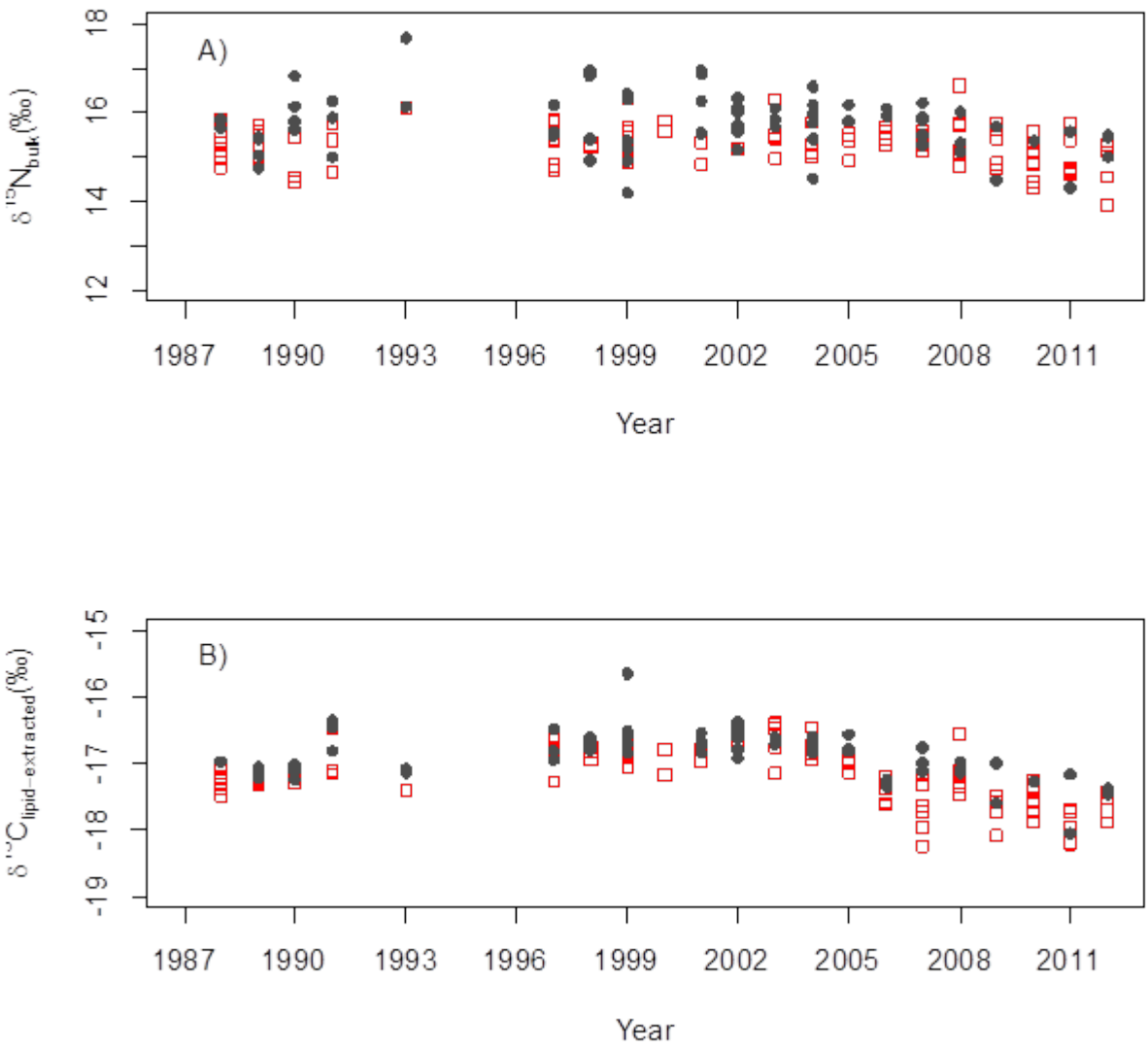

Figure 2. Trend in carbon (A) and nitrogen (B) isotopic ratios of male (black dots) and female (red square) adult beluga over the study period, i.e., from 1988 to 2012.

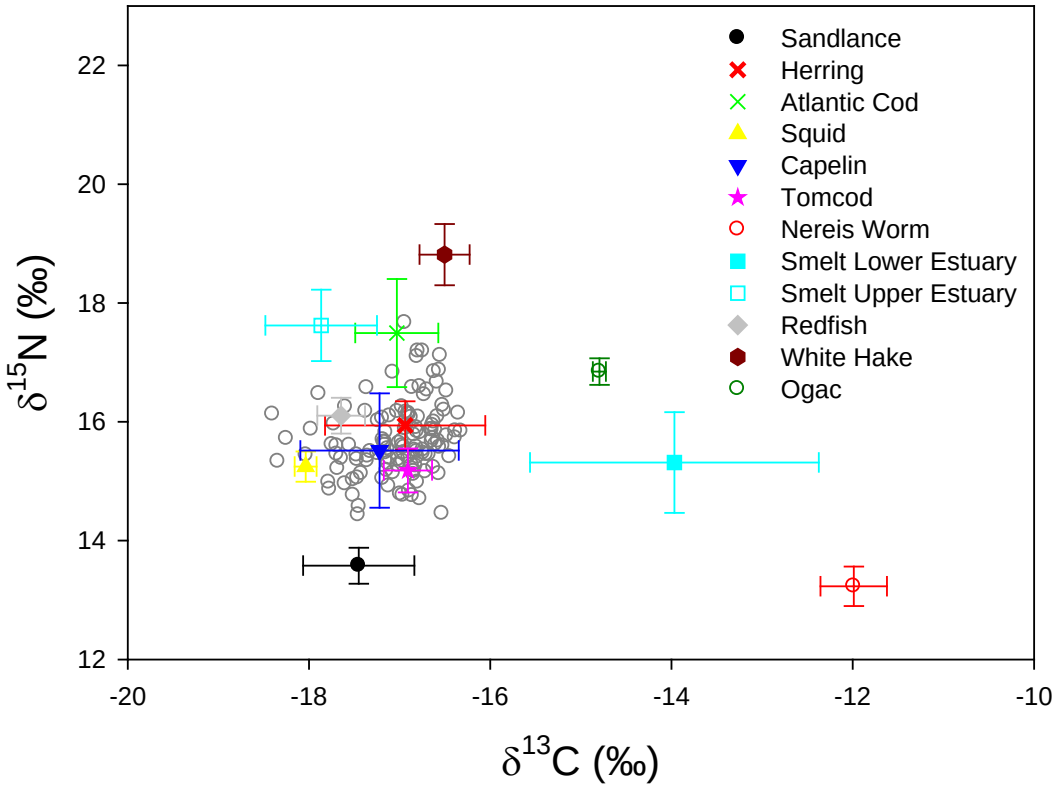

Figure 3. Adult beluga isotopic signature (grey symbols) in relation to the mean (± SD) signature they would have if their diet was constituted purely of each of the listed prey.

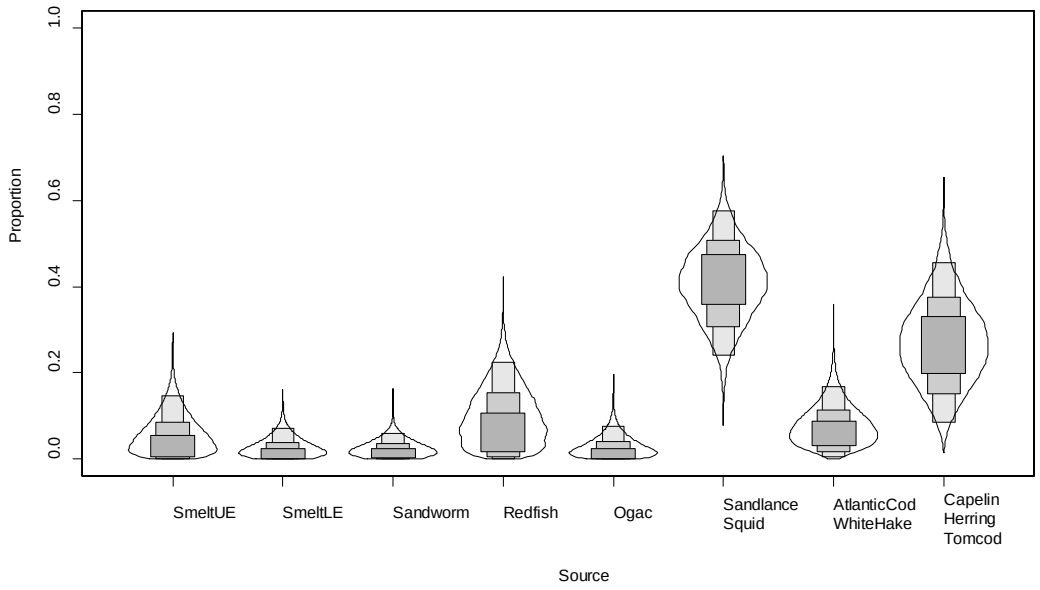

Figure 4a. Diet of adult female beluga inferred from isotopic mixing models. Contributions are presented as 50% (inner box), 75%, and 95% (outer box) credibility intervals, with the shape of their density distribution (violin plot).

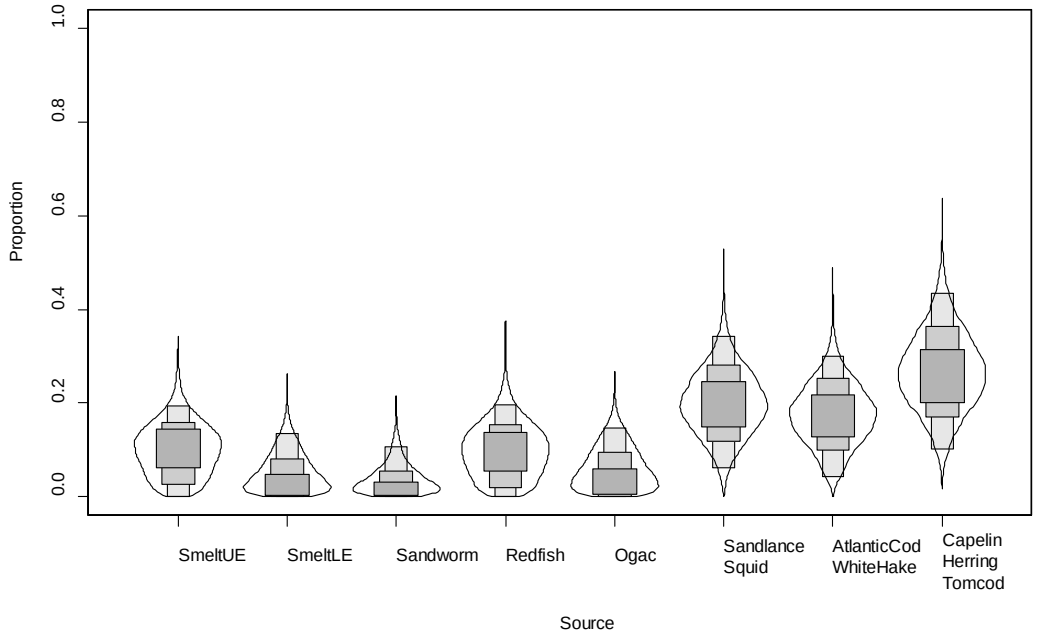

Figure 4b. Diet of adult male beluga inferred from isotopic mixing models. Contributions for each of the prey groups are presented as in figure 4a.

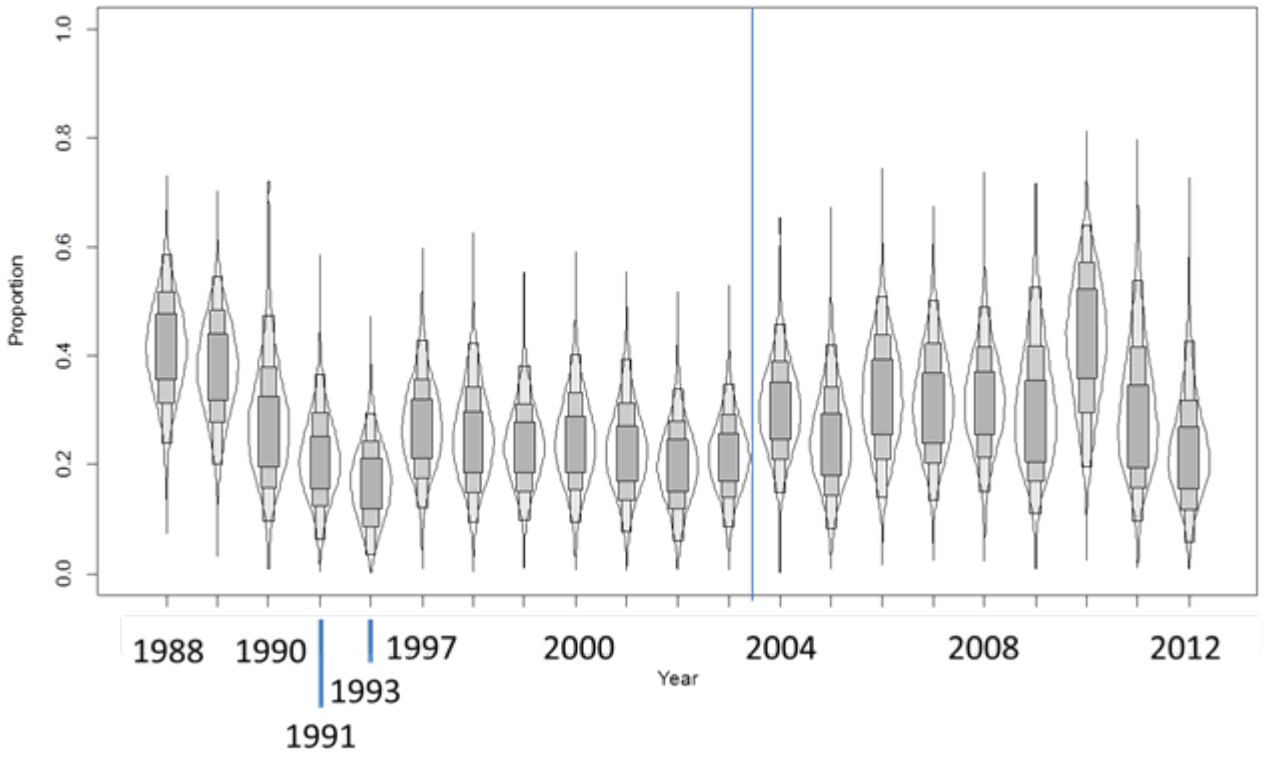

Figure 5. Evolution of the proportion of sandlance and/or squid in the diet of adult female beluga over the period 1988-2012. The contribution of sandlance for each year are presented as 50% (inner box), 75%, and 95% (outer box) credibility intervals, with the shape of their density distribution (violin plot).

APPENDIX A

STOMACH CONTENTS

As part of a concurrent study (V. Lesage, S. Lair, P. Béland, L.M. Measures, D. Martineau, S. Turgeon, Unpublished data[4]), digestive tracts were collected from beluga found dead in the St Lawrence Estuary between 1983 and 2012, and judged to be in condition to warrant a complete necropsy at the St-Hyacinthe veterinary school. Prior to 2007, contents were collected opportunistically, when prey remains were obvious in the stomachs or intestine. Starting in 2007, digestive tracts were collected systematically and their contents processed using standard protocols (Hammill et al. 2005). Contents were sorted using fine mesh sieves and identified to the lowest taxon possible using otoliths and bones and internal collections.

Sixteen fish and five invertebrate species or groups were identified in the digestive tract of 27 beluga that were found dead between 1989 and 2012 (Table A1). These results contrast with those obtained by Valdykov (1946) during June through November, mainly from the Manicouagan banks (n = 89) where beluga no longer are found, with a few individuals coming from near Les Escoumins (n = 16) and Rivière Ouelle (n = 2). Given the small sample size in our study, no attempt was made at quantifying diet composition.

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Table A1. Occurrence of species (% of digestive tracts with prey remains) in the diet of beluga collected mainly at the Manicouagan Bank in 1938-1939 (Vladykov 1946) and in various areas of the Estuary and northern Gulf of St. Lawrence from beluga found dead in 1989−2012 (V. Lesage et al.[5], Unpublished data). Species selected as part of the isotopic mixing model are underlined.

English name	Scientific name	*Vladykov 1938-39* N = 107	This study N = 27
Fishes			
American sand lance	*Ammodytes sp.*	54	4 (1)
Capelin	*Mallotus villosus*	50	15 (4)
Atlantic herring	*Clupea harengus*	2	11 (3)
Rainbow smelt	*Osmerus mordax*	1	11 (3)
Gadidae (Unspecified)			26 (7)
Atlantic cod, Ogac	*Gadus morhua*, *Gadus ogac*	42	33 (9)
Fourbeard rockling	*Enchelyopus cimbrius*		4 (1)
Haddock	*Melanogrammus aeglefinus*	2	
White hake	*Urophycis tenuis*	1	18 (5)
Silver hake	*Merluccius bilinearis*		4 (1)
Atlantic tomcod	*Microgadus tomcod*	17	4 (1)
Winter flounder	*Pseudopleuronectes americanus*	6	4 (1)
Smooth flounder	*Liopsetta putnami*	5	4 (1)
Witch flounder	*Glyptocephalus cynoglossus*	1	
Smooth & Thorny skate	*Raja senta & R. radiata*	6	
Snailfish	*Liparis sp.*	4	4 (1)
Ocean pout/*Lycodes* sp.	*Macrozoarces americanus*	2	7 (2)
Sculpins/Cottidae	*Myoxocephalus* sp.	33	11 (3)
Lumpfish	*Cyclopterus lumpus*	1	4 (1)
Daubed shanny	*Lumpenus maculatus*		4 (1)
Redfish	*Sebastes* sp.		22 (6)
Marlin-spike (Macrouridae)	*Nezumia bairdii*		7 (2)
Sea lamprey	*Petromyzon marinus*	2	
Atlantic sturgeon	*Acipenser oxyrhinchus*	3	
American eel[a]	*Anguilla rostrata*[a]		4[a] (1)
Atlantic salmon	*Salmo salar*	1	
Invertebrates			
Northern shortfin squid	*Illex illecebrosus*	33	18 (5)
Northern Atlantic octopus	*Bathypolypus bairdii*	20	11 (3)
Gasteropods	*Buccinum undatum*	9	7 (2)
Decapods	Decapoda	65	18 (5)
Amphipodes	Amphipoda	35	
Lamellibranches	Genders *Cystodaria/Mesodesma*	35	
Polychaetes	*Neanthes (Nereis) virens*	60	59 (16)

[a] Otolith too eroded to be 100% sure

[5] Fisheries and Oceans Canada, Mont-Joli, QC CANADA

APPENDIX B

LIPID EFFECTS

Effect of lipid extraction on δ13C and δ15N values of muscle tissue was examined for 63 beluga and 97 individuals from 10 fish species (Table B1). Lipid-extraction had a significant and positive effect on $\delta^{13}C$ value of muscle, with a mean enrichment of 0.56‰ in fish (SD=0.60‰; 95% CI = 0.44–0.68‰, paired *t*-test: $t_s = 9.2$, $df = 96$, $p < 0.0001$), and of 0.67‰ in beluga (SD=0.60‰, 95% CI = 0.52–0.82‰, paired *t*-test: $t_s = 9.1$, $df = 62$, $p < 0.0001$). The magnitude of the effect in beluga (i.e., ($\delta^{13}C_{lipid\text{-}free}$ - $\delta^{13}C_{bulk}$) could be predicted from the $\delta^{13}C$ value of untreated sample, so that $\delta^{13}C_{lipid\text{-}free}$ = -0.71448 x $\delta^{13}C_{bulk}$ - 12.24354 - $\delta^{13}C_{bulk}$ (R^2_{adj} = 0.66, mean error = 0.33‰, maximum error (95% CL) = 0.68‰). Although the relationship in fish was significant (i.e., slope different from 1; $p < 0.0001$), $\delta^{13}C$ value of untreated samples were poor predictors of the magnitude of the effect of lipid extraction in $\delta^{13}C$ values (R^2_{adj} = 0.37).

Lipid extraction also had a significant and positive effect on $\delta^{15}N$ values of fish tissue and beluga muscle, with a mean enrichment of 0.71‰ in fish (SD=0.30‰; 95% CI = 0.65–0.77‰, paired *t*-test: $t_s = 23.1$, df = 96, p < 0.0001), and of 0.25‰ in beluga (SD=0.36‰, 95% CI = 0.16–0.35‰, paired *t*-test: $t_s = 5.6$, df = 61, p < 0.0001). This effect was also revealed through linear regression and was predictable with a R^2_{adj} of 0.92 and 0.93 for fish and beluga muscle, respectively (Figure B2; B3). The slope was marginally insignificantly different from 1 in fish and beluga muscle (both $P = 0.06$). Applying the regression for predicting $\delta^{15}N$ values of untreated muscle ($\delta^{15}N_{bulk}$ = -0.03238 + 0.94704 x $\delta^{15}N_{lipid\text{-}extracted}$) reduced the mean error to ± 0.22‰, and maximum error (95% *CL*) to ± 0.58‰ in fish. However, the use of the regression did not reduce the mean error for beluga muscle and thus, a simple 0.25‰ correction was applied to $\delta^{15}N$ values of lipid-free tissue in this case to account for lipid extraction.

Table B1. Fish species included in the analysis for the effect of lipid extraction on isotope ratios.

Common name	Scientific name	N	Length (cm)
American sandlance	*Ammodytes sp.*	3	10-15
Atlantic cod	*Gadus morhua*	24	11-38
Atlantic herring	*Clupea harengus harengus*	2	23-24
Capelin	*Mallotus villosus*	10	12-16
Cunner	*Tautogolabrus adspersus*	3	19-23
Longhorn sculpin	*Myoxocephalus octodecemspinosus*	10	16-27
Thorny skate	*Amblyraja radiata*	13	unknown
White hake	*Urophycis tenuis*	18	13-?
Winter flounder	*Pseudopleuronectes americanus*	5	30-34
Winter skate	*Leucoraja ocellata*	9	unknown

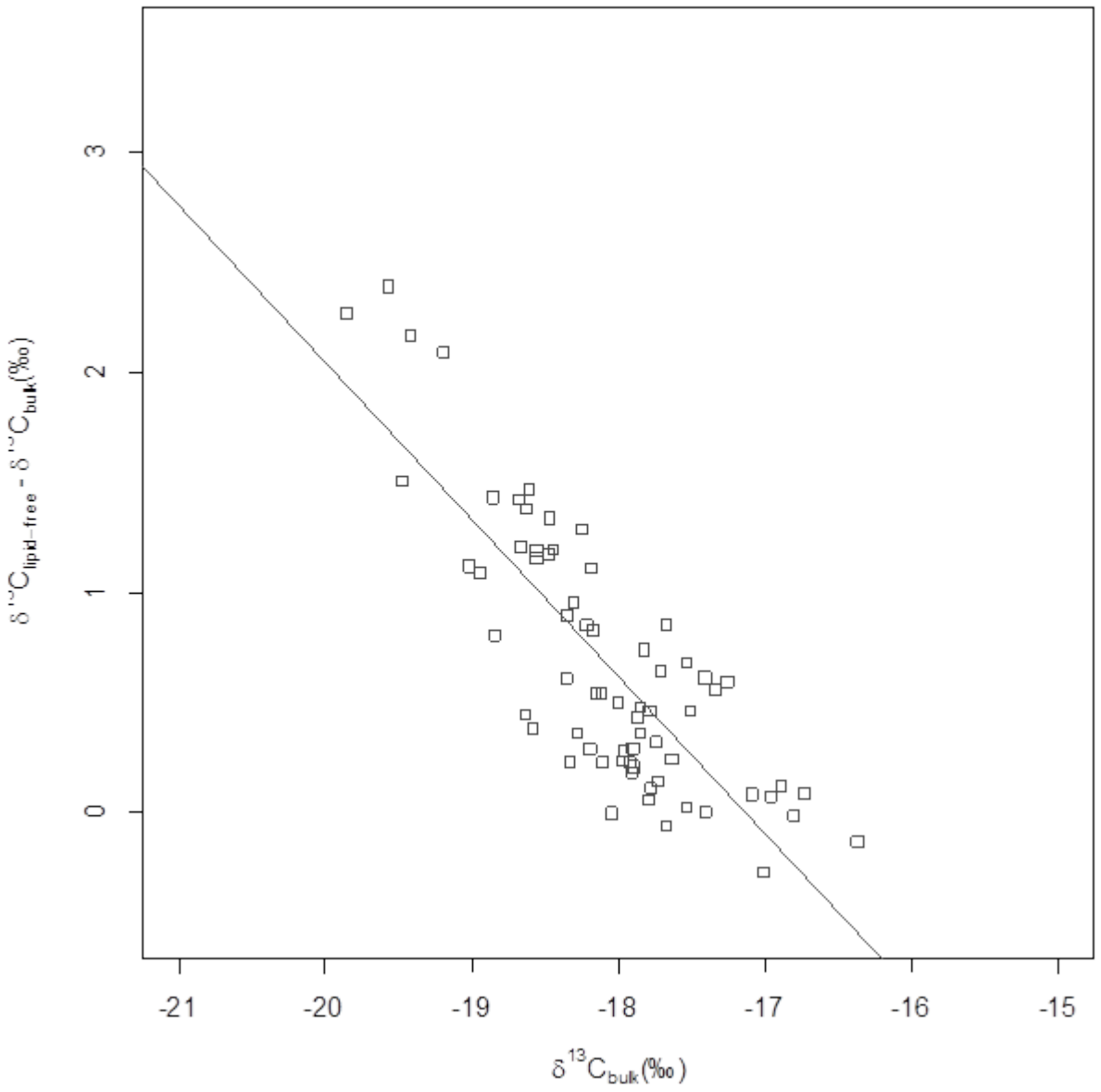

Figure B1. Relationship between the magnitude of the difference between δ13C values of lipid-free and untreated muscle samples, and δ13C value of bulk muscle tissue in beluga. Regression coefficients for predicting lipid-free δ13C values from δ13C values of untreated samples are presented in the text of the Appendix.

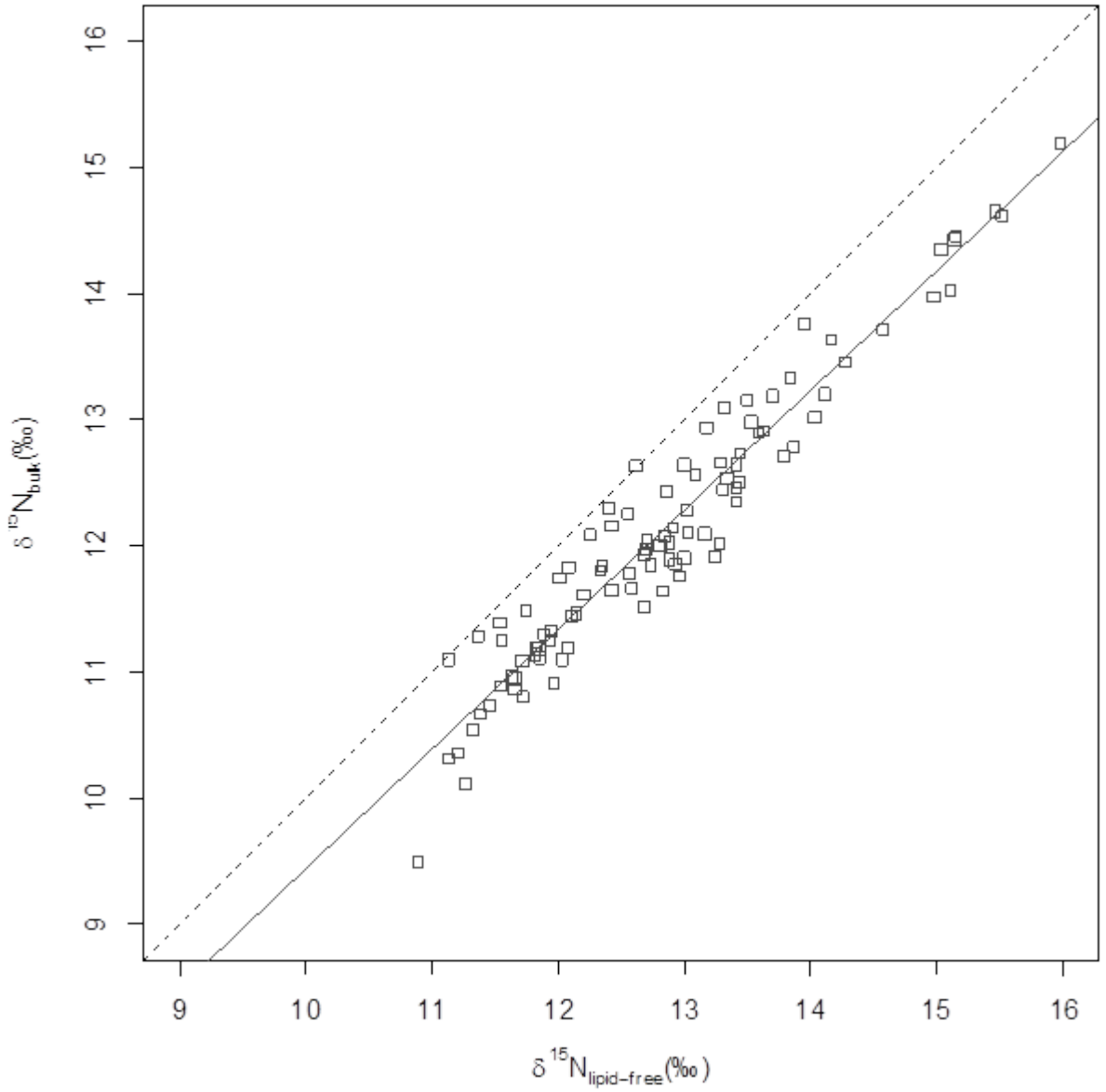

Figure B2. Effects of lipid extraction from fish muscle samples (n=97) on δ15N values. The dotted line corresponds to no difference between lipid-extracted and non-extracted samples. Regression coefficients for predicting δ15N values of untreated samples from lipid-extracted samples (solid line) are presented in the text of the Appendix.

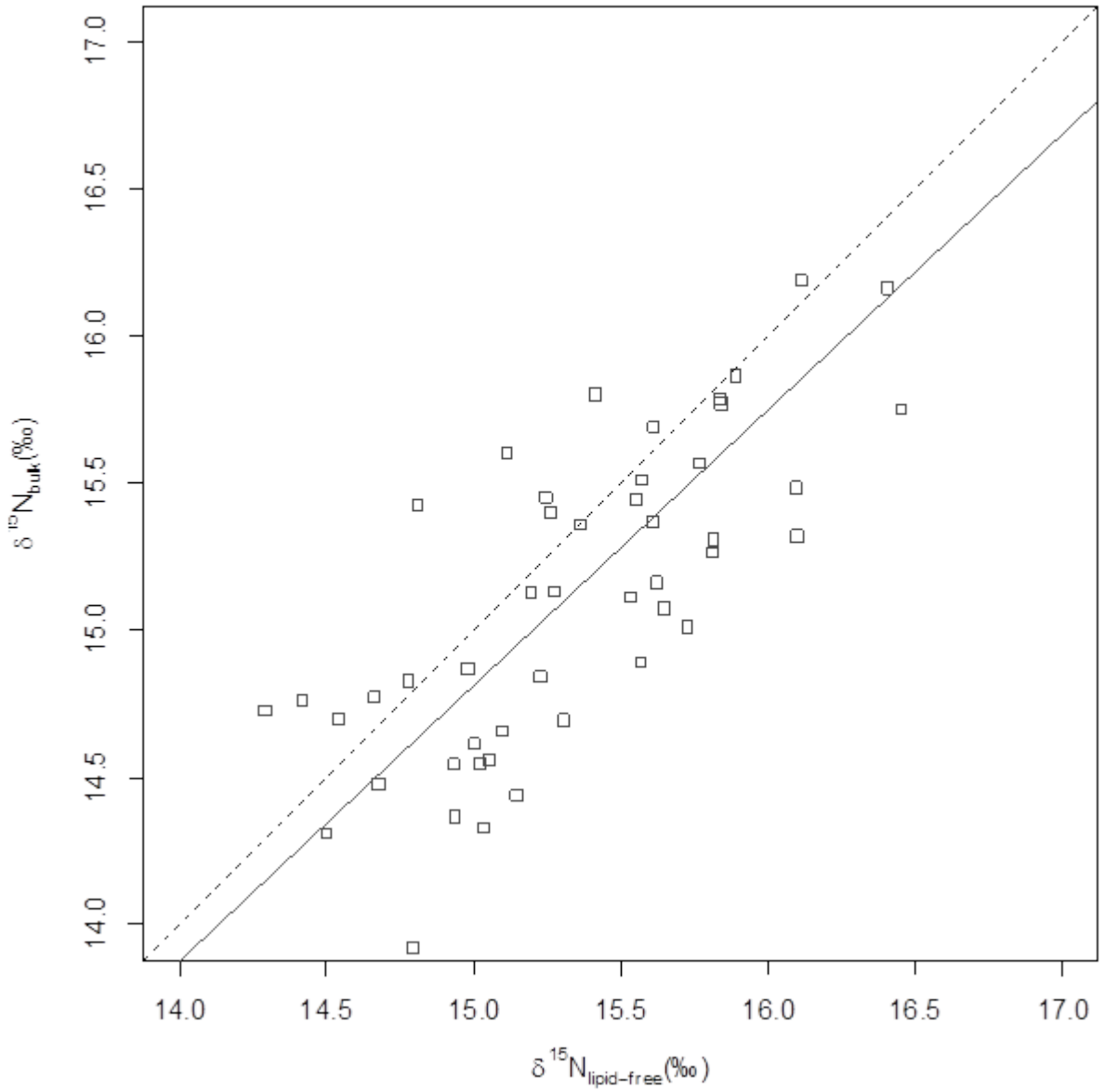

Figure B3. Effects of lipid extraction from beluga muscle samples (N = 63) on δ15N values. The dotted line corresponds to no difference between lipid-extracted and non-extracted samples. Regression coefficients for predicting δ15N values of untreated samples from lipid-extracted samples (solid line) are presented in the text of the Appendix.