Coupled changes between the H-Print biomarker and $\delta^{15}$N indicates a variable sea ice carbon contribution to the diet of Cumberland Sound beluga whales.

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Abstract

Sub-Arctic habitats are being exposed to increasingly long periods of open water as sea ice continues to decline in thickness and extent. Some hypothesise that this will result in a reduction, and maybe total loss of sea ice derived (sympagic) carbon supply; however, the impact of such change on ecosystems requires further investigation. Here, we used the H-Print biomarker approach that utilises well-defined indicators of both sympagic and phytoplanktic carbon, in combination with stable isotopes ($\delta^{15}$N), to study the effect of reducing sympagic carbon availability on beluga whales (Delphinapterus leucas) in the sub-Arctic ecosystem of Cumberland Sound. Our data show that decreasing $\delta^{15}$N in belugas was negatively correlated with pelagic carbon (H-Print) within their diet. We also identified a statistically significant ($R^2 = 0.82; P = <0.01$) change point in the proportion of sympagic/pelagic carbon within beluga around the year 2000, signified by consistently reducing $\delta^{15}$N, coupled with increasing pelagic carbon composition. This observed shift from sympagic to pelagic contribution to diet is likely to remain a feature of the Cumberland Sound ecosystem during the projected reduction of sea ice.

Key-words – Highly branched isoprenoid (HBI), H-Print, Delphinapterus leucas (beluga whale), IP$_{25}$, Arctic ecosystem, sea ice, nitrogen isotopes, ecosystem
Introduction

The ecological responses to ongoing trends of global warming are already clearly visible (Arrigo et al. 2008; Grebmeier et al. 2006; Kortsch et al. 2012; Walther et al. 2002) and are likely to continue to be influenced by the ongoing borealisation of Arctic ecosystems (Kortsch et al. 2012). One important characteristic of such modifications in sub-Arctic ecosystems is a predicted transition away from sympagic (ice associated) primary production, in favour of more pelagic production (Grebmeier et al. 2006). Clearly, if sea ice disappears completely, sympagic carbon will be withdrawn altogether. Such shifts will likely provide newly extended habitat to non-native species that are able to take advantage of the increased duration of open water associated with declining sea ice cover (Huse and Ellingsen 2008). Compounded by this shifting primary production source, and the introduction of non-native species, ecosystems can be forced to undergo reorganisation of community structure, often creating new energy pathways (Kortsch et al. 2012), which can have important consequences for the flow of energy to upper trophic level organisms. While the stimulus for community reorganisation typically involves relatively gradual alteration to the physical environment, the associated community response can be far more abrupt. For example, a rapid reorganisation of the benthic community was triggered around north Svalbard in 2000 in response to steadily increasing sea surface temperature since 1980 (Kortsch et al. 2012).

The ecosystem within Cumberland Sound in the Canadian Arctic represents a changing sub-Arctic system that provides habitat for typical higher trophic level Arctic species including ringed seal (*Pusa hispida*), beluga whale (*Delphinapterus leucas*), Greenland shark (*Somniosus microcephalus*) and polar bear (*Ursus maritimus*). In addition to the potential impacts of climate change, summarised above, the sedentary population of beluga whales in Cumberland Sound faced a further challenge due to commercial exploitation up until the 1980s, resulting in this now relatively small population being classified as
threatened (Cosewic 2004). Gaining an understanding of how the Cumberland Sound ecosystem might be changing, and how this might impact beluga is therefore an even more pressing research target. Previous studies exploring changes in the ecosystem of Cumberland Sound have used stable isotopes of carbon (δ\(^{13}\)C) and nitrogen (δ\(^{15}\)N) to gather information on beluga diet. Using this approach, it was suggested that Arctic cod (Boreogadus saida), with seasonal contributions from Greenland halibut (Reinhardtius hippoglossoides), represented the main prey in the diet of these belugas (Marcoux et al. 2012). However, Marcoux et al. (2012) identified a 0.08‰ yr\(^{-1}\) decrease in δ\(^{15}\)N between 1982 and 2009 with a concomitant decrease in δ\(^{13}\)C values (0.01‰ yr\(^{-1}\)) in beluga over the same time period. Interpretation of these changes could potentially provide information on how the Cumberland Sound ecosystem might be changing. However, a number of factors can complicate the interpretation of bulk stable isotope data such as the potential influence of tissue fractionation (Newsome et al. 2010) and the challenges associated with distinguishing between, for example, changes in the isoscape that can alter the isotopic composition of the prey base (Graham et al. 2010), versus a change in predator diet. A better understanding of the underlying source of carbon over the last 30 years in Cumberland Sound is therefore required to help identify whether decreasing beluga δ\(^{13}\)C and δ\(^{15}\)N values reflect a change in diet and/or foraging habit of beluga as the ecosystem undergoes change.

Recently, it has been shown that the analysis of certain highly branched isoprenoid (HBI) diatom lipids within Arctic animals can provide information on the source of organic carbon to an individual’s diet (Brown and Belt 2012b; Brown et al. 2014c) and is achieved by measuring the relative abundances of several individual HBIs with known source-species associations. For example, a contribution from sympagic (ice associated) carbon is determined on the basis of the presence of the mono-unsaturated HBI IP\(_{25}\) ("Ice Proxy with
25 carbons” (Belt et al. 2007)), together with a structurally related di-unsaturated HBI (Fig.1; IIb), both of which have been shown to be produced by certain Arctic sea ice diatoms (Belt et al. 2007, Belt and Müller 2013, Brown et al. 2014b). In contrast, some pelagic diatoms produce a range of other HBI isomers, typically tri-unsaturated isomers (e.g. Fig. 1; IIIa-d) that are different from those produced by sea ice diatoms (e.g. Belt et al. 2001, 2008, Brown and Belt 2016). Accordingly, determination of the relative distributions of such HBIs are considered to represent an HBI-fingerprint or ‘H-Print’ that is characteristic of the ecological conditions of the environment in which the source diatoms lived. On this basis, the H-Print is capable of providing well-defined end-member signatures of sympagic and pelagic carbon sources. Expressed as pelagic versus total HBIs (Brown et al. 2014a), higher H-Print values thus represent an increased proportion of pelagic carbon; conversely, lower H-Prints represent increased sympagic carbon. As a recent application of this technique, Brown et al. (2014a) analysed more than 300 ringed seal H-Prints from Cumberland Sound between 1990 and 2011, to show that the composition of carbon available to ringed seals varied in direct relation to changes in sea ice extent. In the current study, we hypothesised that if the observed annual decrease in δ15N of beluga in Cumberland Sound is related to changes in the ecosystem, rather than artefacts of potential fractionation or changing isoscapes, beluga should also convey a contrasting trend in the H-Print signature, indicative of changing composition (sympagic/pelagic) of carbon consumed. Specifically, we predicted that if reducing sea ice is responsible for driving a change in Cumberland Sound towards increased pelagic productivity, we would observe increasing H-Print values that are consistent with increasing pelagic contributions to the food web. In contrast, the absence of a relationship between H-Print and δ15N would likely indicate additional and/or different drivers behind the observed changes in beluga stable isotope values.
Materials

Geographical setting - Cumberland Sound, Nunavut, Canada, lies on the southeast coast of Baffin Island at approximately 65°N, 65°W and is a large inlet (250 km long and 80 km wide) with numerous glacial fjords. Cumberland Sound is characterised by seasonally variable sea ice cover with the presence of a polynya in winter (Hannah et al. 2009), which preserves open water conditions in the southeast (Fig. 2). For a more detailed description of the region see Richard et al. (2009).

Satellite-linked telemetry – Two male and five female beluga whales, between 315 – 370 cm in length, were tagged with SPLASH tags near Pangnirtung (66°16’18’’ N, 67°05’90’’ W) between 2006 and 2009 (Watt et al. 2016) to gauge the sedentary nature of whales. Methods for the capture and release of whales were previously described by Orr et al. (2001). GPS location data was obtained using the ARGOS system (CLS America). Only location data with accuracy of < 500 m were used. The transmission duration of location data varied for individual whales (2006/7 = 226 d; 2007 = 104/109 d; 2008/9 = 4/222/246/249 d) and, collectively, provide data for all seasons defined in Cumberland Sound: summer = July-September (daily transmissions); autumn = October-November (transmissions every 4 days); winter = December-May (transmissions every 4 days) (Richard et al. 2009). For visual comparison of telemetry data to sea ice, MODIS Aqua Surface Reflectance Bands 1, 3 and 4 were retrieved manually, courtesy of the NASA EOSDIS Land Processes Distributed Active Archive Center (LP DAAC), USGS/Earth Resources Observation and Science (EROS) Center, Sioux Falls, South Dakota, http://oceancolor.gsfc.nasa.gov/WIKI/OCSSW(2f)Ancillary(2f)SeaIce.html#Monthly_Data_

Source.
**Sample collection**

**Beluga whale** – Opportunistic sampling of 142 beluga whales was carried out in Cumberland Sound between 1982 and 2009 (samples obtained for 18 of 27 years) by Inuit hunters as part of their subsistence harvests (Table 1). Sampling was carried out in accordance with the community-based monitoring program coordinated by Fisheries and Oceans Canada in Winnipeg, Manitoba, Canada. Of the 142 whales, 95% were collected during summer (Fig. 3). The remaining 8 samples were collected during autumn (n = 2) and winter (n = 6). Overall, beluga length ranged from 194 – 514 cm, which was correlated (r = 0.72) with the age of whales (1 – 47 yr), as determined by counting growth layer groups in extracted teeth (Scheffer and Myrick 1980). Samples were relatively evenly distributed across all ages (0-10y = 21%; 0-20y = 36%; 20-30y = 21%; 30-40y = 18%; 40-50y = 4%) with only slight bias towards males (male:female sex ratio = 1:0.8) (Table 1, Fig. 3). The bias towards whales sampled during the summer provided an ideal opportunity for the long-term inter-annual comparison required. However, the sampling resolution was insufficient to permit robust assessment of potential variability in H-Print and δ15N data in relation to biometric variables (e.g. age, weight). Sub-samples of whale liver were frozen onsite in a freezer at -20°C and then shipped to Fisheries and Oceans Canada where they were stored at -30°C. Liver was chosen since it is known to be metabolically active (Vander Zander et al. 2015), resulting in relatively short turnover (~weeks – 1 month). This relatively rapid turnover has previously enabled analysis on seasonal scales in a temporal study of over 300 ringed seals from Cumberland Sound (Brown et al. 2014a). Accordingly, liver tissue was used for all samples in this study.

**Beluga prey** – In Cumberland Sound, a range of marine fish are potentially available as prey for beluga. These include, but are not limited to, Greenland halibut (Reinhardtius hippoglossoides), capelin (Mallotus villosus), Arctic cod (Boreogadus saida), Greenland cod
(Gadus ogac), gelatinous snailfish (Liparis fabricii), Arctic alligatorfish (Ulcina olrikii), Arctic char (Salvelinus alpinus) and several species of sculpin (Richard et al. 2009). Of these, liver samples of two key species were available for comparison with beluga; Greenland halibut and capelin sampled within Cumberland Sound. While belugas from other regions are also known to target redfish (Sebastes marinus) and shrimp (Pandalus borealis) as well as squid (Quakenbush 2015), samples were not available for these species. Greenland halibut were sampled in the southern region of Cumberland Sound during the summer open water period (August 2012; n = 21) and in the northern region in the winter when landfast ice had formed (April 2012; n = 44). All fish were sampled from scientific longlines set either from the Nunavut Government research vessel, the Nuliajuk (summer), or through ice holes by Inuit fishermen (winter). Longlines consisted of 400 – 2000 m length of base rope with 200-2000 gangions and size 14-16 Mustad Duratin circle hooks. Lines were set at depths ranging from 400 –1100 m and soaked on average for 12 hours. On hauling of the lines, all Greenland halibut were measured (Fork length; FL) and liver tissue sampled and immediately stored frozen at -20°C prior to stable isotope analysis. Capelin (n = 17) were sampled using dip nets in open water from a small boat in July 2015. Fish ranged in length from 9 to 11.5 cm. Additional capelin (n = 5) were also recovered from the stomach of a single harp seal (Pagophilus groenlandicus; ARPG-15-00-13). Of the 17 capelin sampled, biomarker data were only used from 8 individuals since HBIs were below the limit of accurate quantitation in some. All fish liver tissue samples were freeze dried (-45°C; 20 Pa; 72 h), ground using a mortar and pestle and, following homogenisation, halibut were further sub-sampled and analysed for nitrogen stable isotopes with additional sub-samples being sent to Plymouth University for analysis of HBIs.
Analysis of stable isotopes – Prior to stable carbon ($\delta^{13}$C) and nitrogen isotope ($\delta^{15}$N) analysis on beluga whale and Greenland halibut liver samples, lipids were removed using a 2:1 chloroform:methanol solvent following the Bligh and Dyer (1959) method. Subsequently, 400-600 µg of tissue was weighed into tin capsules where $\delta^{13}$C and $\delta^{15}$N values were measured by a Thermo Finnigan DeltaPlus mass-spectrometer (Thermo Finnigan, San Jose, CA, USA) coupled with an elemental analyzer (Costech, Valencia, CA, USA) at the Chemical Tracers Laboratory, Great Lakes Institute for Environmental Research, University of Windsor. Analytical precision, assessed by the standard deviation of replicate analyses of two standards (NIST 1577c, n=7; NIST 8414, n=46) and an internal lab standard (tilapia muscle, n=53), were all ≤ 0.1‰ for $\delta^{15}$N and $\delta^{13}$C. Instrumentation accuracy was assessed from NIST standards 8573 and 8547 for $\delta^{15}$N and $\delta^{13}$C (n=19). The mean differences from the certified values were all ≤ 0.1‰ for $\delta^{15}$N and $\delta^{13}$C.

Lipid extraction and purification - Extraction of HBI lipids from liver sub-samples (0.1-1.6 g) was carried out using established techniques (Belt et al. 2012; Brown et al. 2014a). An internal standard (9-octylheptadec-8-ene (9-OHD); 10 µL; 2 µg mL$^{-1}$) was added to enable the quantification of HBIs (if required at a later date) according to Belt et al. (2012). Samples were saponified in a methanolic KOH solution (~ 5 mL H$_2$O:MeOH, 1:9; 20% KOH) for 60 mins (70°C). Hexane (3 x 4 mL) was added to the saponified solutions, which were then vortexed (1 min) and centrifuged (2 min; 2500 revolutions per minute).

Supernatant solutions containing non-saponifiable lipids (NSLs) were transferred to clean vials with glass pipettes and dried (N$_2$ stream) to remove traces of H$_2$O and MeOH. NSLs were then re-suspended in hexane (1 mL) and fractionated, providing non-polar (5 mL hexane) lipids using column chromatography (SiO$_2$; 0.5 g), while more polar lipids (e.g., cholesterol) were retained on the columns.
Lipid analysis - Analysis of purified non-polar lipid extracts containing HBIs was carried out using gas chromatography – mass spectrometry (GC-MS) according to Belt et al. (2012). Total ion current (TIC) chromatograms were used to determine the retention time and mass spectra of HBIs and these were compared with those of authentic standards and published literature for identification purposes.

Lipid quantification - HBIs were quantified by measurement of the mass spectral intensities of the molecular ion for each HBI in selective ion monitoring (SIM) mode (i.e. m/z 350.3 for Ice Proxy with 25 carbons (IP25), m/z 348.3 for IIb, m/z 346.3 for IIIa-d). The analytical intensities of individual HBIs were then normalised according to totals derived from all 6 HBIs. The resulting distribution provides the basis for the H-Print (Brown et al. 2014c) which is defined here as the ratio of the HBI contributions from planktonic diatoms (Σ IIIa-d) vs. those from sea ice diatoms (Σ IP25 and IIb) according to Brown et al. (2014a) and is further modified (Eq.1) to provide normalised H-Print values within the range 0 – 100%.

\[
H - \text{Print}\% = \frac{\left( \Sigma \text{IIIa} + \text{IIb} + \text{IIId} \right)}{\left( \Sigma \text{IP25} + \text{IIb} + \text{IIIa} + \text{IIId} \right)} \times 100
\]

(1)

Numerical analysis – Numerical analyses were carried out in RStudio v0.99.441 (R-Core-Team 2016). NMDS was used to assess if the variability in the relative abundances of the HBIs (H-Print) could be used to identify differences in sympagic/pelagic carbon within Cumberland Sound animals. perMANOVA (9999 permutations) was used to test the significance of Bray-Curtis dissimilarities for beluga sex and year of sampling and similarly (and all available species), with respect to HBI content. ANOVA was used to compare the H-Print of spring and summer halibut. The Student’s t–test for two samples was used to compare mean stable isotope values between species. Correlation of H-Print and δ^{15}N were performed using Pearson’s product-moment correlation (r). Local Polynomial Regression Fitting from
the [stats] package used a locally weighted least squares fit to smooth temporal $\delta^{15}$N and H-Print data prior to using linear regression to test the relationship between H-Print and $\delta^{15}$N, dependent upon sampling year ($R^2$). Broken stick regression applied a linear regression model with a user-defined change-point determined as the most significant change in slope established through testing multiple iterations ranging between 1996 and 2003.
Results

Highly branched isoprenoid lipids in Cumberland Sound animals – All beluga and fish liver extracts contained HBI lipids, with the same six isomers being present in every animal sampled (Fig. 1). Variability in the relative proportion of each HBI isomer was evident between individual animals. For example, the sea ice diatom biomarker IP$_{25}$ and the co-produced di-unsaturated HBI (IIb) ranged from 4-26% and 8-55%, respectively, in beluga, while in halibut they ranged from 0.6-8% and 21-53% of the total HBIs. In contrast, the phytoplanktic diatom derived HBIs (IIIa-d) were more abundant in capelin, collectively representing more than 76% of the total HBIs in these fish. This variation was explored using NMDS which did not detect significant differences in HBI composition between male and female beluga (perMANOVA, Pseudo-$F =$ 1.4, $P =$ 0.26), but did find that beluga HBI composition varied, to some extent, between sampling year (perMANOVA, Pseudo-$F =$ 3.3, $P =$ 0.045) and, more significantly, between the four species sampled (perMANOVA, Pseudo-$F =$ 56.4, $P <$ 0.001) (Fig.4). Since this multivariate ordination supported previously identified trends in the variable distribution of HBIs, with NMDS1 reflecting the contribution of sea ice (IP$_{25}$ and IIb) and phytoplanktic (IIIa-d) input (Fig. 4), the H-Print was considered to provide an accurate univariate representation of multidimensional HBI data.

H-Print values were calculated for each individual animal using Eq.1 (Fig. 3). Overall, beluga exhibited greater variation in H-Print values than halibut (20-87% and 35-75%, respectively). Since season was not a significant predictor of H-Print for halibut sampled during either April or August (one-way ANOVA, $F_{1,52} =$ 0.42, $P =$ 0.52), these data were combined for comparison to summer beluga H-Prints. The mean combined spring and summer halibut H-Print values (51%) were found to be significantly higher ($t =$ -4.04, 154 d.f., $P <$ 0.01) than those of beluga (45%). Capelin had the highest mean H-Print (83%), with individuals ranging from 76 to 92%. Summer samples (n = 46) of previously published
Cumberland Sound ringed seal liver H-Prints (Brown et al. 2014a) were re-expressed here using Eq.1 for comparison to beluga and fish liver (Fig. 5). Seals contained some of the lowest measured H-Print values and, overall, ranged from 14 to 59%. Mean seal H-Prints (30%) were found to be significantly lower than both beluga ($t = 6.98$, 188 d.f., $P < 0.01$) and halibut ($t = 11.49$, 98 d.f., $P < 0.01$).

Stable carbon and nitrogen isotope composition of animals – Mean beluga isotopic compositions ($\delta^{15}N = 16.6 \pm 0.6\%o$) were significantly higher than those of ringed seals ($\delta^{15}N = 15.7 \pm 1.1\%o$; $t = 13.3$, 155 d.f., $P < 0.01$), halibut ($\delta^{15}N = 15.2 \pm 0.7\%o$; $t = 13.3$, 155 d.f., $P < 0.01$) and capelin ($\delta^{15}N = 13.5 \pm 0.4\%o$; $t = 10.22$, 106 d.f., $P < 0.01$) (Table 1). $\delta^{15}N$ values for capelin were obtained from literature values for summer caught capelin in Cumberland Sound and ranged from 12.9 to 13.8\%o (Dennard et al. 2009; Marcoux et al. 2012; McMeans et al. 2013; Morris et al. 2016). $\delta^{13}C$ values of lipid extracted beluga liver ranged $-16.8$ to $-18.9\%o$ and were comparable to those of skin and muscle reported previously (Marcoux et al. 2012; supplementary figure).

Variation in carbon source versus $\delta^{15}N$ – To test the hypothesis that changes in the carbon composition of beluga diet reflect the variability in the $\delta^{15}N$ of beluga, we compared individual H-Prints (carbon composition) with $\delta^{15}N$. Variability in H-Prints was significantly correlated to $\delta^{15}N$ with a reasonably strong inverse relationship ($r = -0.53$; $P < 0.01$) between them (Fig. 5). ANOVA identified that sampling year was a significant predictor of H-Print ($F_{1,140} = 3.94$, $P = 0.49$), so we also investigated whether a temporal co-variation may be evident in the H-Print and $\delta^{15}N$ of beluga. To achieve this, we first derived representative intra-annual H-Prints and $\delta^{15}N$, by applying a weighted (least squares) smooth to both time series data. A 2nd degree polynomial fit was then selected as it was considered to provide the
best representation of the observed coupled variability. Linear regression analysis of the
extracted smoothed data revealed that H-Print was a significant predictor of the trend
observed in δ¹⁵N values over the last 30 years (R² = 0.82; P < 0.01) (Fig. 6). The change in
the δ¹⁵N previously observed in the late 1990s was further investigated with broken stick
regression (Fig. 7). The most significant change-point (2000) in both beluga H-Print and δ¹⁵N
values (P < 0.01) was determined using multiple iterations as a function of year (1996–
2003).
Discussion

H-Print analyses of Arctic ecosystems – Here, we demonstrate that the biomarker-based H-Print represents a useful addition to the existing experimental approaches used for studying food web dynamics in polar environments. Developmentally, the application of various source-specific HBI lipids as tracers of sympagic and pelagic food sources within Arctic (and other) ecosystems has evolved following the first identification of these biomarkers in sea ice (Belt et al. 2007, 2013; Brown et al. 2011), sediments (Belt et al. 2007) and lower trophic position animals (Brown and Belt 2012a). Thus, initial studies represented somewhat qualitative reports, whereby the presence of certain HBIs such as IP_{25} provided binary evidence for the consumption of sea ice derived organic matter (Brown and Belt 2012b). Subsequent investigations initially employed the analysis of simple bivariate relationships between specific HBIs in higher trophic position animals (Megson et al. 2014) and these were taken further through the use of the H-Print approach, which enabled more complex multivariate HBI relationships to be deciphered using PCA. The value of multidimensional approaches for demonstrating sympagic and pelagic carbon partitioning across multiple trophic levels in Arctic foodwebs has recently been demonstrated (Brown et al. 2014c, 2015). Further, by expressing the H-Print as a univariate ratio of pelagic and sympagic HBIs, Brown et al. (2014a), demonstrated how a modified H-Print could be used to identify temporal changes to the underlying carbon reaching ringed seals from Cumberland Sound. However, such a modification potentially suffers from the same poor definition of end member values common to some other proxy methods, including carbon stable isotopes (Bouillon et al. 2011), with the clear and reproducible identification of unique values representative of sea ice and phytoplankton, being particularly problematic. Since our ordination analysis provides further support of the capability of the univariate H-Print to accurately represent sympagic/pelagic carbon (Fig. 4), we propose the re-expression of all H-
Print biomarker ratios as percentages, such that sympagic (0%) and pelagic (100%) end-member values are necessarily constrained (Eq. 1). In doing so, this now provides clearly defined end-member values which offer important benefits for attempting to determine the contribution of these two carbon sources in a mixed carbon pool. One particular benefit is clearly illustrated here in the interpretation of H-Prints in Cumberland Sound animals (Fig. 5). For example, H-Print values for capelin in our study (76-92%) were close to the newly-defined pelagic carbon end-member value of 100%, which is consistent with a previous assessment of stable isotope and fatty acid data by McMeans et al. (2013) who showed capelin diet was dominated (98%) by phytoplanktic carbon. In addition, pan-Arctic comparisons can be made using Eq. 1 to re-express all previously published HBI data from a range of Arctic locations (Fig. 8).

Variation in carbon source versus δ15N – We hypothesised that if the observed change in δ15N values of beluga was related to changes in the ecosystem, beluga H-Print signatures would likely exhibit a coupled trend. Our analyses indicated that, while variability was present in beluga δ13C values, this was relatively small. Since factors other than changes in feeding habit can influence isotopic analysis, as suggested previously, the changes in δ13C observed here could not be attributed exclusively to changes in the ecosystem. In contrast, H-Print values had much greater variability, enabling a clearer identification of a significant coupling between beluga δ15N values and H-Print, with the H-Print being responsible for the majority of the variability in δ15N of beluga whales over the 30 year sampling period. Similarly to δ13C, the interpretation of variability in beluga δ15N values alone can be complicated and might, at least in part, be impacted by changes in the isoscape (Graham et al. 2010) or metabolic fractionation (Newsome et al. 2010). In contrast, variability in the H-Print is a reflection of changes in sympagic/pelagic algal species composition and is indicative of changing composition (sympagic/pelagic) of carbon consumed. Therefore, we attribute this
coupled change, observed between two independent variables (H-Print and \(\delta^{15}\)N), to a shift in
the underlying carbon reaching Cumberland Sound beluga. Alternatively, the variability
could be due to the changes in diet associated with seasonal migratory paths of beluga.
However, since aerial surveys (Richard et al. 2009) and satellite-linked telemetry data (Watt
et al. unpublished data), further supported by our own observations, suggest that this
community of beluga whales likely remain within Cumberland Sound year round, we do not
believe this to be the case. Instead, this population appears to only migrate between the
northern and southern sectors of Cumberland Sound, probably following recurrent polynyas
(Richard et al. 2009), moving into shallow fjords to the north during summer and deeper
waters in the south in winter. This somewhat sedentary behaviour of Cumberland belugas
means their diet is largely governed by the availability of prey within Cumberland Sound at
any given time. For instance, in line with the impact of reducing sea ice (Arrigo et al. 2008;
Huse and Ellingsen 2008), the recent increases in more transient Atlantic/sub-Arctic prey
entering the Sound (e.g. capelin; McKinney et al. 2012, Ulrich 2013) could potentially
modify the composition of the typical prey present in the Sound. Such modification of the
available prey biomass could result in a change in beluga diet, similar to that seen for sea
birds in Hudson Bay (Gaston et al. 2003). In an environment such as Cumberland Sound, it is
possible that the impact of transient species on prey availability could be somewhat less for
predatory species with a wider geographical range. For example, over the same sampling
period, Cumberland Sound ringed seals, although broadly associated to the same region,
exhibited lower H-Prints and more variable \(\delta^{15}\)N when compared to those from beluga,
consistent with a diverse omnivorous diet of sympagically associated prey both within and
outside of the Sound (Yurkowski et al. 2016). Therefore, one possible explanation for the
change in carbon source reaching beluga could be related to reducing sea ice (Perovich and
Richter-Menge 2009) and the associated transition towards increased in-situ pelagic primary
production in these circumstances (Grebmeier et al. 2006). Alternatively, prey species may also be transferring a pelagic carbon signature from the north Atlantic/sub-Arctic ecosystems into Cumberland Sound in the same way that increases in transient species to Cumberland Sound provides a mechanism for the transfer of allochthonous contaminants (McKinney et al. 2012). What is clear, however, is that changes in available carbon are leading to a diminishing proportion of sympagic carbon contribution to beluga.

Significance of diminishing sympagic carbon – In Cumberland Sound we observed considerable overlap in carbon source composition (H-Print) within many of the beluga and halibut samples (Fig. 4 and 5). Since these traditionally pelagic (beluga) and epibenthic (halibut) predators also appeared, in some cases, to have a coupled overlap in δ15N, it is possible that this could be indicative of the changes in the underlying sympagic/pelagic carbon composition, and overall ecosystem structure. Indeed, under the influence of climate change, modification of the composition of available prey biomass will likely result in some degree of reorganisation of the Cumberland Sound foodweb, as observed elsewhere (Grebmeier et al. 2006; Kortsch et al. 2012; Walther et al. 2002). The consequence of any such change is likely to be observed in the supply of energy to higher trophic levels. The opportunistic predatory nature of beluga (Kelley et al. 2010) means they are likely to be particularly good indicators of ecosystem modification, especially since they are not considered particularly sensitive to changes in availability of pelagic prey species (Laidre et al. 2008). The coupled variability observed here in both H-Print and δ15N of beluga provide evidence in support of a changing ecosystem. Whether this change is due to a shifting prey base, or changes in dietary preference remains to be seen, and our understanding will be improved by subsequent detailed analysis of the Cumberland Sound foodweb. Overall, the temporal changes in carbon source we observe are consistent with increasing climate change driven range-shifts of pelagic prey species, including those of beluga prey (e.g. capelin; Huse
and Ellingsen 2008). While beluga are also known to prey upon Greenland halibut (Quakenbush et al. 2015), the extent of overlapping in δ\textsubscript{15}N values and H-Prints we observed between beluga and halibut could suggest the occurrence of potential ecosystem reorganisation. The impact of such change might even result in an increase in competition for a common prey between predators, including beluga and Greenland halibut. We hypothesise such a reorganisation of the ecosystem could be expected to occur in response to increasingly extended open water periods associated to the warming climate, although further analyses are required to test this. While we observed changes in the underlying carbon available to the ecosystem we note that, both in the past as well as at present, sympagic carbon remains an important feature that has contributed to the Cumberland Sound ecosystem throughout our study period.

Conclusions

At the outset of this study, we aimed to identify if changes in carbon utilisation within the ecosystem could represent a viable explanation of decreasing δ\textsubscript{15}N of beluga whales in Cumberland Sound since 1982. Our combined biomarker (H-Print) and isotope data show that changes in the proportion of sympagic and pelagic carbon in beluga whales accounted for almost all of the variability in δ\textsubscript{15}N. Further, we identified that, while the sympagic component of beluga carbon had varied over time, this carbon still played an important role in the ecosystem, and continued to be channelled into beluga throughout the study period. That said, the identification of a significant change-point around 2000, indicated a steady decline in the amount of sympagic carbon reaching beluga. This trend of reducing sympagic contribution to the ecosystem is likely to remain a feature of Cumberland Sound during the predicted further reduction of sub-Arctic sea ice.
Acknowledgements

Biomarker research was funded by the award of a Research Project Grant (RPG-2014-021) from the Leverhulme Trust. Additional field funding was provided through the Government of Nunavut and the Ocean Tracking Network, Fisheries and Oceans Canada, Species at Risk and the Nunavut Wildlife Management Board. All field work was conducted under DFO License to Fish for Scientific Purposes, and prior approval was obtained from the Freshwater Institute Animal Care Committee (FWI-ACC-06-07-010, FWI-ACC-07-08-038, FWI-ACC-08-09-008) and followed approved protocols. We thank the many dedicated people in the research field camps, Jack Orr in particular, for the handling and instrumenting of the beluga whales and the Hunters and Trappers Organization in Pangnirtung, Nunavut, Canada. We also thank the crew of the Nuliajuk and Peter and Robbie Kilabuk for sampling Greenland halibut. We are grateful to Professor Waite and an anonymous reviewer for their very helpful comments during review.
**Figure and table legends**

Table 1. Summary (mean ± 1 standard deviation) of biometric, H-Print and δ¹⁵N data for species analysed.

Fig. 1. Structures of sea ice diatom (top) and phytoplanktic diatom (bottom) highly branched isoprenoids (HBIs) measured in Cumberland Sound animals for calculation of the H-Print using Eq. 1.

Fig. 2. Map of southern Baffin Island and Davis Strait showing March 2012 sea ice extent obtained from surface reflectance (bands 1, 3 and 4) using NASA’s Moderate Resolution Imaging Spectrometer (MODIS Aqua). Dotted line represents the median March sea ice extent for 1980-2010. Coloured circles represent locations (ARGOS) of tagged beluga whales in Cumberland Sound (accuracy of <500m) between 2006 and 2009 described in the methods. Inset: Map of North Canada and Greenland with red box showing position of Cumberland Sound, southern Baffin Island and Davis Strait.

Fig. 3. Count plots of beluga whale annual and seasonal sampling used for δ¹⁵N and HBI analysis on this study. Top: number of whales sampled each year. Middle: number of whales sampled in each month (combined across all sampling years). Bottom: Age distribution of whales sampled (combined for all years and months of sampling). For all plots, dark grey = females, light grey = males, white = undetermined sex.

Fig. 4. Non-metric Multidimensional Scaling (NMDS) ordination plots with vectors plotting the linear correlation of NMDS scores of individual HBIs (vector length scaled by the strength of the correlation). Animals grouped to the left of NMDS1 are most influenced by
the sympagic HBIs (IP\textsubscript{25} and IIb) and those grouped to the right are most influenced by the pelagic HBIs (IIIa and IIIb). An indication of the carbon source is therefore defined on this basis. Top: Beluga sampled as part of this study with polygons grouping animals of the same sex. Middle: Beluga sampled as part of this study with polygons grouping individuals according to year of sample collection. Additional colouring shows animals collected before (orange) and after (green) the year 2000. Bottom: All animals analysed in this study, with ringed seals from Brown et al. (2014a), with polygons grouping species.

Fig. 5. H-Print and $\delta^{15}$N of all samples analysed in this study. Additional ringed seal H-Prints were re-calculated according to Eq. 1 here using data from summer caught seals reported in Brown et al. (2014a). For all species, samples are a composite from all years sampled (Table 1). Main: Biplot of H-Print and $\delta^{15}$N with sympagic and pelagic end-member carbon sources defined by H-Print (0\% and 100\% respectively) based on the known source of HBI lipids. Shaded ellipses represent multivariate t-distributions with 95\% confidence. Green dashed and blue solid lines are lines of best fit for Pearson Product-moment correlations of $\delta^{15}$N versus H-Print for beluga whales and halibut respectively with their corresponding r values ($P = < 0.01$). Top: H-Print boxplot distributions of biplot data. Right: $\delta^{15}$N boxplot distributions of biplot data.

Fig. 6. Regression of smoothed (Local Polynomial Regression Fitting using a locally weighted least squares fit) H-Print and $\delta^{15}$N of Cumberland Sound beluga whales. Shaded area is ±1 standard error.

Fig. 7. Broken stick regression, with a user-defined change-point determined as the most significant change in slope established through testing multiple iterations ranging between
1996 and 2003, of H-Print (Top) and δ^{15}N (Bottom) of Cumberland Sound beluga whales. A statistically significant change-point (vertical dashed lines) was located at the year 2000 ($P = 0.01$) for both H-Print and δ^{15}N.

Fig. 8. Schematic representation of underlying carbon source across the full range of H-Print values. Mean Cumberland Sound beluga (cross) and ringed seal (star) H-Prints are shown in context against published highly branched isoprenoid data from other organisms which are re-expressed here as H-Prints using equation 1. Brown and Belt (2012b); Brown et al. (2013a); Brown et al. (2013b); Brown et al. (2015); Brown et al. (2014b); Brown and Belt (2016).

Supplementary figure. Variation in δ^{13}C values of Cumberland Sound beluga whale liver samples across years. Line of best fit from linear regression with year as the independent variable ($R^2 = 0.03$, $p = 0.06$). Shaded area is ±1 standard error.
References


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Mean ± 1SD: Delphinapterus leucas

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Mean ± 1SD: Mallotus villosus

Min: 8, Max: 47, Median: 9

Mean ± 1SD: Reinhardtius hippoglossoides

Min: 0, Max: 94, Median: 64

* Literature values for Cumberland Sound

Mean ± 1SD

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Mean ± 1SD: Delphinapterus leucas

Min: 5, Max: 142, Median: 13, Mean ± 1SD: 19.5 ± 11.3

Mean ± 1SD: Mallotus villosus

Min: 3, Max: 9, Median: 3, Mean ± 1SD: 13.5 ± 0.4*

Mean ± 1SD: Reinhardtius hippoglossoides

Min: 4, Max: 94, Median: 14, Mean ± 1SD: 15.2 ± 0.7

* Literature values for Cumberland Sound

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Fig. 1

Sympagic diatom source

Pelagic diatom source

IP$_{25}$

IIb

IIIa

IIIb

IIIc

IIId

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Disclaimer: This is a pre-publication version. Readers are recommended to consult the full published version for accuracy and citation.
Disclaimer: This is a pre-publication version. Readers are recommended to consult the full published version for accuracy and citation.
100% sympagic
100% pelagic

“Disclaimer: This is a pre-publication version. Readers are recommended to consult the full published version for accuracy and citation.”