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ORIGINAL PAPER

Sourcing fatty acids to juvenile polar cod (*Boreogadus saida*) in the Beaufort Sea using compound-specific stable carbon isotope analyses

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Abstract Juvenile polar cod (*Boreogadus saida*) are often found in close association with sea ice and represent an important trophic link in the Arctic food web. However, the proportional contribution of sea ice algal production via the sympagic food web to the diet of polar cod is unknown. To estimate the proportional contribution of fatty acids (FAs) from sea ice-derived particulate organic matter (i-POM) to the diet of juvenile polar cod, we used FA profiling and compound-specific stable carbon isotope analysis of individual FAs from juvenile polar cod collected from three regions in the Beaufort Sea. The $\delta^{13}C$ values of the FAs 14:0, 16:4n-1, 18:0, 20:5n-3 and 22:6n-3 in the polar cod were found to most strongly resemble pelagic POM rather than i-POM. Results from isotope-mixing models using diatom FA markers indicated that the proportional contribution of FAs from i-POM to juvenile polar cod was $\leq$2 %, which suggests that juvenile polar cod had not sourced their FAs from i-POM. Thus, changes in sea ice coverage due to environmental change may not affect juvenile polar cod in regard to nutrients such as FAs but may still affect their populations by reducing critical shelter from predators.

Keywords Polar cod · *Boreogadus saida* · Sea ice · Fatty acids · Compound-specific stable isotopes

Introduction

Sea ice is a defining feature of the marine ecosystem in the Arctic and serves as an important habitat for many species of primary producers, fish and marine mammals (Gosselin et al. 1997; Laidre et al. 2008). Reductions in the extent and duration of seasonal sea ice could cause changes in the supply and source of primary production that could propagate throughout the marine food web (ACIA 2004; Piepenburg 2005; Budge et al. 2008). Sea ice algae and pelagic phytoplankton are the two main sources of primary production in the Arctic (Horner and Schrader 1982). Although the contribution of sea ice algae to total primary production is unknown, it is thought to range from 4 to 26 % in seasonally ice-covered waters and may be as high as 57 % in perennially ice-covered waters (Gosselin et al. 1997; Sakshaug 2004). Determining the contribution of sea ice algae to the marine food web in the Arctic will help to predict how changes in sea ice extent and conditions could affect food web dynamics for higher trophic level organisms such as polar cod (*Boreogadus saida*).

Polar cod is a widely distributed and abundant species, which inhabits the circumpolar Arctic seas (Renaud et al. 2012). This species is thought to facilitate energy and nutrient transfer in the Arctic by serving as a trophic link between sympagic amphipods and pelagic copepods and higher trophic level organisms such as ringed seals (*Phoca hispida*), narwhal (*Monodon monoceros*) and beluga

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whales (*Delphinapterus leucas*) (Bradstreet and Cross 1982; Craig et al. 1982; Bluhm and Gradinger 2008; Darnis et al. 2012). In Lancaster Sound, Canada, alone it is estimated that birds and marine mammals annually consume 148,000+ metric tons of polar cod (Welch et al. 1992).

Sea ice is thought to provide polar cod, especially juveniles, with shelter and protection from predators (Gradinger and Bluhm 2004). However, it is currently unknown whether sea ice algae is an important component of the sympagic food web that supports polar cod. Previous research has indicated that in addition to shelter, sea ice may provide an important source of nutrients for multiple species of juvenile invertebrates (Gradinger et al. 2009). Some studies have shown polar cod sometimes consume higher proportions of sympagic rather than pelagic macrofauna (Lønne and Gulliksen 1989; Renaud et al. 2012). This suggests that juvenile polar cod are not selective feeders and may feed upon a wide variety of locally abundant items and thus may rely on sympagic invertebrates when using a sea ice habitat (Lønne and Gulliksen 1989; Walkusz et al. 2011).

Traditional stomach content analysis has been used to determine the frequency and relative occurrence of macrofauna in the diet of polar cod (Lowry and Frost 1981; Lønne and Gulliksen 1989; Walkusz et al. 2011; Christiansen et al. 2012; Renaud et al. 2012) but this approach cannot estimate the proportional contribution of sea ice-derived fatty acids (FAs). FA biomarkers have been used to study sources of diet for several decades. There are inherent differences in FA biosynthesis and storage patterns among organisms, and FAs can be conservatively transferred from diet to consumer (Dalsgaard et al. 2003; Budge et al. 2006; Iverson 2009). For example, diatoms are capable of synthesizing the FA 16:4n-1 in large amounts, which allows it to be used as a biomarker of that source when identified in a consumer (Ackman et al. 1968; Budge et al. 2008; Søreide et al. 2010). Certain FAs from marine algae are conservatively transferred through the food chain and their stable carbon isotope ratios (^{13}C:^{12}C—expressed as $\delta^{13}C$ values) can be used to track the contribution of primary production to higher trophic levels (i.e., Budge et al. 2008). A number of studies have shown that sea ice algae can have significantly higher $\delta^{13}C$ values relative to pelagic phytoplankton at both the bulk (i.e., total organic carbon—TOC) and compound-specific (i.e., FA) levels (Hobson et al. 2002; Søreide et al. 2006; Budge et al. 2008; Gradinger 2009; Gradinger et al. 2009; Wang et al. 2013). Thus, the proportional contribution of sea ice-derived particulate organic matter (i-POM) FAs, relative to pelagic POM (p-POM) FAs can be estimated using FA biomarkers in conjunction with compound-specific stable carbon isotope analyses of individual FAs (Budge et al. 2008). We aimed to determine the $\delta^{13}C$ values of individual FAs of juvenile polar cod from the Beaufort Sea and compare them with values of i-POM and p-POM to estimate the proportional contribution of i-POM FAs to their diet. We hypothesized that in addition to providing shelter from predators, juvenile polar cod also rely on sea ice habitat for FAs.

Materials and methods

Study site and sample collection

Juvenile polar cod samples used in this study were collected between August 12th and September 14th, 2011 from three stations in different regions of the Beaufort Sea: Eastern Beaufort (EB10, 70.5619 N and −146.1066 W), Central Beaufort (CB32, 70.8096 N and −151.632 W) and Western Beaufort (WB14, 71.3442 N and −152.0087 W). Pelagic samples were collected using an Isaacs-Kidd Midwater Trawl (IKMT, 3 mm mesh), with mouth dimensions 1.5 m wide by 1.8 m high. Unfortunately, not enough is known about the spawning locations or the movements of larval polar cod to be absolutely certain that the samples we used in our study were never near the McKenzie River. Although uncertainty exists, we are confident that the polar cod sampled in our study were not likely in close contact with the McKenzie River because the distance from the McKenzie River to our closest sampling location is approximately 450 km. Benthic gears included both otter trawls (OT) and plumbstaff beam trawls (PSBT). OT (38 mm codend mesh, 19 mm codend liner) were 9.1 m. The PSBT (7 mm body mesh, 4 mm codend liner mesh) had a 4.7-m headrope and a 4.6-m footrope. The mouth of the net was held open with a 3-m-long pipe. Sampling depths at stations EB10, CB32 and WB14 were 41, 16 and 90 m, respectively. Samples from CB32 were taken using a PBST ($n = 10$). Samples from WB14 were taken with an OT ($n = 15$), and from EB10 using both an IKMT ($n = 6$) and OT ($n = 2$). Polar cod samples were euthanized, according to an approved UAF Institutional Animal Care and Use (IACUC) protocol (134765-9), by placing the fish in a 130 mg/L solution of tricaine methanesulfonate until gill movement ceased. Fish samples were then frozen at −20 °C until analysis. Total length (TL), weight and age class were determined, and sample stomachs and otoliths were removed prior to freezing.

Fish aging

One otolith from each juvenile polar cod was cross-sectioned through the otolith's nucleus. The surface of the otolith sections was ground with a grinder-polisher

(BUEHLER MetaServ 250) until the growth rings were visible under a microscope (Leica with V3 software application). Once the growth rings were visible, otoliths were illuminated with transmitted light and photographed. Two readers used the photographs to independently count annual growth rings and to assign an age class. If age class estimates from the two readers were incongruent, the readers counted the number of growth rings together until an age class was agreed upon.

Fatty acid and compound-specific stable carbon isotope analysis

Laboratory methods for extracting lipids from polar cod samples followed Folch et al. (1957) and Budge et al. (2006) for the preparation of FA methyl esters (FAMEs), which are standard and routine methods. Briefly, each fish sample was homogenized and then 2:1 chloroform/methanol was used to extract lipids. FAMEs were prepared through acidic transesterification using H_2SO_4 in methanol and quantified using temperature-programmed gas liquid chromatography on a Perkin Elmer Autosystem II Capillary FID gas chromatograph fitted with a 30 m × 0.25 mm id column coated with 50 % cyanopropyl-methylpolysiloxane (DB-23) and linked to a computerized integration system (Varian Star software) according to Iverson et al. (2002). $\delta^{13}C$ values of individual FAs within the mixture of FAMEs were analyzed by routing the effluent from a GC (Trace GC Ultra, Bremen, Germany) through a combustion interface (Finnigan GC combustion III, Bremen, Germany) linked to a continuous flow stable isotope ratio mass spectrometer (IRMS) (Thermo Finnigan Delta V, Bremen, Germany). FAMEs for analysis using IRMS were separated using the same GC column and method described above for FID analyses of FAMEs. Addition of a methyl group derived from methanol used during transesterification can potentially result in FAMEs with slightly different values from FAs present in the source material. Free FA (FFA) standards 16:0 and 18:0 were transesterified with the same reagents described above to correct for the contribution of carbon from this methyl group and determine any kinetic isotope effects. Thin layer chromatography confirmed the purity of the FFA prior to transesterification, and the $\delta^{13}C$ values for FFA were determined using an elemental analyzer (EA) (Costech ECS4010) routed to an IRMS. The $\delta^{13}C$ values of these FFA that had then been transesterified into their respective FAMEs were then also measured using the GC-IRMS system described above. There was no evidence of a kinetic isotope effect associated with transesterification, which is expected for a reaction that goes to completion (Rieley 1994); therefore, the difference between the $\delta^{13}C$ values for FFAs and FAMEs with the same chain length was due to the added methyl group. The proportional contribution of this methyl group to a given FA depends on its chain length; thus, an average $\delta^{13}C$ value for this added methyl group was calculated using the following equation:

$$\delta^{13}\mathrm{C}\ (n+1)\left[\delta^{13}\mathrm{C}_{\mathrm{FAME}}\right]-n\left[\delta^{13}\mathrm{C}_{\mathrm{FFA}}\right]$$

where n is the number of carbon atoms in the FFA (Abrajano et al. 1994). We then calculated an average $\delta^{13}C$ value (−47.8 ‰) for the methyl-derived carbon based on the difference between the $\delta^{13}C$ values of the corresponding FFA and their respective FAME and used this to correct our FAME data by rearranging the above equation. All stable carbon isotope ratios of FAMEs are expressed in delta (δ) notation as follows:

$$\delta^{13}\mathrm{C}\ (‰) = \left(R_{\mathrm{sample}}/R_{\mathrm{standard}}-1\right) * 1000$$

where the $\delta^{13}C$ value is the stable carbon isotope ratio expressed in per mil (‰) or parts per thousand relative to the stable carbon isotope ratio of an international standard (Vienna Pee Dee Belemnite—VPDB). R_{sample} and R_{standard} are the ^{13}C:^{12}C of the sample and standard, respectively. The $\delta^{13}C$ values were calibrated using a standard mixture of eight n-alkanoic acid ethyl and methyl esters (supplied by Indiana University Stable Isotope Reference Materials), where the r^2 of the measured versus expected relationship was >0.99. Analytical precision was tracked using a C16 FA laboratory standard, which was analyzed after every tenth sample, which was 0.8 ‰ (representing the 1 SD of 14 analyses of the C16 FA standard interspersed during the sample run).

Data analysis

Bray–Curtis similarity matrices and permutational multivariate analysis of variance (PERMANOVA; McArdle and Anderson 2001) were used to investigate the spatial, age and weight variation associated with polar cod samples using the 42 FA present in proportions >0.1 % using PRIMER v6 (Primer-E Ltd). A non-metric multidimensional scaling (nMDS) plot was used to visualize differences between FA profiles of samples. Similarity percentages routines (SIMPER) were performed to determine the FAs that contributed most to the observed differences. Data were standardized, and log(1 + X) transformed prior to analysis. We also used a Bayesian multisource stable isotope-mixing model (Stable Isotope Analysis in R—SIAR, Parnell et al. 2010) to estimate the contribution of i-POM and p-POM FAs to juvenile polar cod. Three FAs were used as our mixing model sources (16:4n-1, 20:5n-3 and 22:6n-3). FA $\delta^{13}C$ values from i-POM and p-POM used in our SIAR model came from

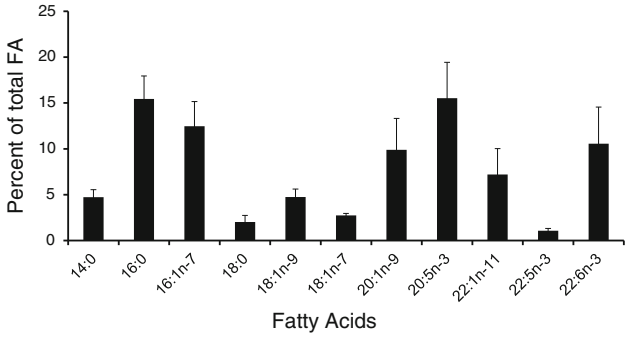

Fig. 1 The percentage composition of fatty acids in analyzed juvenile polar cod (*Boreogadus saida*, $n = 32$) that yielded reliable $\delta^{13}C$ values. Means ± 1SD

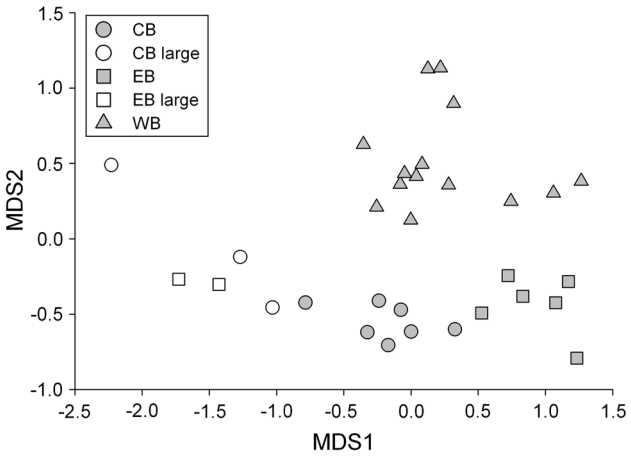

Fig. 2 nMDS plot of 42 fatty acids present in proportions >0.1 % in juvenile polar cod (*Boreogadus saida*, $n = 32$) collected in 2011 from the central (CB), eastern (EB) and western (WB) Beaufort Sea. Large fish between 73 and 99 mm. Small fish <58 mm. 2-D stress = 0.09

analyses of samples collected from the Bering Sea in 2010 (Wang et al. 2013). We also conducted a SIAR-mixing model using the 16:4n-1 and 20:5n-3 $\delta^{13}C$ values from sea ice algae and pelagic phytoplankton collected from waters off of Barrow, Alaska for comparison (Budge et al. 2008). Trophic enrichment factors and concentration dependencies were assumed to be zero in the model (Budge et al. 2011).

Results

There were no significant differences in fish TL (ANOVA, $P = 0.3$), weight (ANOVA, $P = 0.1$) or age class (ANOVA, $P = 0.1$) between the three regions. Average TL of fish from the WB14, CB32 and EB10 were 52.9 ± 3.4, 58.6 ± 23.1 and 46.9 ± 18.8 mm, respectively (mean ± 1SD). Samples from the WB14, CB32 and EB10 weighed 0.6 ± 0.1, 1.6 ± 1.9 and 0.7 ± 1.0 g, respectively (mean ± 1SD). Ages of individuals were estimated to be age 0 ($n = 26$) and age 1 ($n = 7$). A total of 72 FAs were identified in the juvenile polar cod samples analyzed and of those 14:0, 16:0, 16:1n-7, 18:0, 18:1n-9, 18:1n-7, 20:1n-9, 20:5n-3, 22:1n-11 and 22:6n-3 made up, on average, 86 % of the overall FA composition (Fig. 1) (see also Supplemental Data file for a complete data set associated with these samples). The FAs with the highest overall proportions were 16:0 and 20:5n-3 (15 and 16 % of the total FAs, respectively). For individuals from all three regions, monounsaturated FA (MUFA) was present in the highest proportions (44 ± 9.1 %), followed by polyunsaturated FA (PUFA; 32 ± 6.8 %) and saturated FA (SAT; 23 ± 3.4 %). The ratio of MUFA to PUFA was 1.4 when regions were combined. The largest juvenile polar cod samples (>73 mm) had a MUFA to PUFA ratio of 2.7, which was the highest ratio recorded while the smallest fish (<73 cm) had a MUFA to PUFA ratio of 1.2. Variability in juvenile polar cod FA profiles between regions and size class is shown in the nMDS plot (Fig. 2). FA profiles were significantly different between regions (PERMANOVA, $P < 0.001$). Within the central and eastern Beaufort regions, FA profiles were significantly different between size classes (PERMANOVA, $P < 0.001$). There was a significant interaction between region and size class ($P < 0.004$). The FAs that contributed to 47.2 % of the dissimilarity between size classes were 22:6n-3, 20:1n-7, 22:1n-11, 22:1n-9, 20:1n-9 and 20:5n-3.

$\delta^{13}C$ values were determined for FAs 14:0, 15:0, 16:4n-1, 18:0, 20:5n-3, 22:5n-3 and 22:6n-3 (see also Supplemental Data). Of these FAs only 14:0, 16:4n-1, 18:0, 20:5n-3 and 22:6n-3 have corresponding FA $\delta^{13}C$ values in i-POM and p-POM (Fig. 3). The $\delta^{13}C$ values of the individual FAs in polar cod were not significantly different between regions (ANOVA: $P > 0.06$). FA 16:4n-1 had the highest variation between samples with a SD of 1.7 ‰. All other reported FAs had very low variability with SD < 1.0 ‰. Because no significant differences were detected between regions in terms of TL, weight or $\delta^{13}C$ values of each of the FAs, we pooled all of the data across regions to examine any ontogenetic trends. In relation to both TL and weight, the $\delta^{13}C$ values of 20:5n-3 decrease initially by ~3 ‰ and then appear to increase (Fig. 4). There appears to be subtle ontogenetic trends for this FA in juvenile polar cod with a decrease to minimum $\delta^{13}C$ values occurring between 50 and 80-cm TL (Fig. 4). When the data were split into two size classes (polar cod <73 and >73 mm), a number of FAs (16:0, 18:0, 20:1n-9, 20:5n-3, 22:1n-11, 22:1n-9, 22:1n-7 and 22:6n-3) showed significant (Spearman Rank Correlation with $p < 0.0045$ Bonferroni correction) differences in FA relative abundance

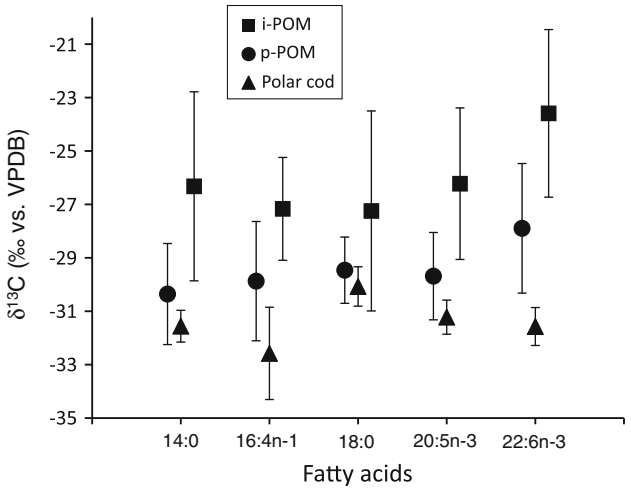

Fig. 3 $\delta^{13}C$ values of fatty acids (means ± 1SD) measured juvenile polar cod (*Boreogadus saida*, 14:0 $n = 30$, 16:4n-1 $n = 14$, 18:0 $n = 32$, 20:5n-3 $n = 32$ and 22:6n-3 $n = 32$) and i-POM and p-POM from the Bering Sea (Wang et al. 2013)

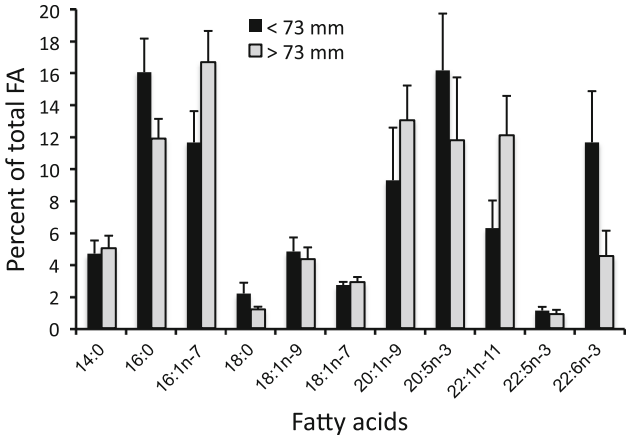

Fig. 5 FAs from juvenile polar cod (*Boreogadus saida*) showing significant differences in relative abundance between two size classes (<73 mm $n = 27$ and >73 mm $n = 5$)

(Fig. 5). The most notable feature from this analysis was the larger amount of 20:1 and 22:1 FAs in the larger (>73 mm) polar cod, which are FA biomarkers for calanoid copepods. The SIAR modeling of $\delta^{13}C$ values of FAs from juvenile polar cod relative to the same FAs from i-POM and p-POM from the Bering Sea (Wang et al. 2013) and sea ice algae and pelagic phytoplankton (Budge et al. 2008) showed that juvenile polar cod derived ≤2 % of their FAs from i-POM (Table 1).

Table 1 SIAR isotope-mixing model estimates of i-POM in juvenile polar cod using $\delta^{13}C$ values of fatty acids 16:4n-1, 20:5n-3 and 22:6n-3

Fatty acids	i-POM (%)
16:4n-1, 20:5n-3, 22:6n-3[a]	1.2 ± 1.1
16:4n-1, 20:5n-3[a]	2.0 ± 2.0
20:5n-3[a]	1.8 ± 1.7
16:4n-1, 20:5n-3[b]	2.0 ± 1.9

Means ± 1SD

[a] i-POM and p-POM values from the Bering Sea (Wang et al. 2013)

[b] Sea ice algae and pelagic phytoplankton values from waters off of Barrow, Alaska (Budge et al. 2008)

Discussion

Our stable carbon isotope-mixing models using FAs estimated a negligible (≤2 %) proportional contribution of the FAs from i-POM (Table 1). In addition, the relatively high levels of FAs 20:1n-9 and 22:1n-11 in the juvenile polar cod specimens suggest the consumption of calanoid copepods (Falk-Petersen et al. 1987), which are a common pelagic prey item for some polar cod (Lowry and Frost 1981; Bradstreet and Cross 1982; Lønne and Gulliksen

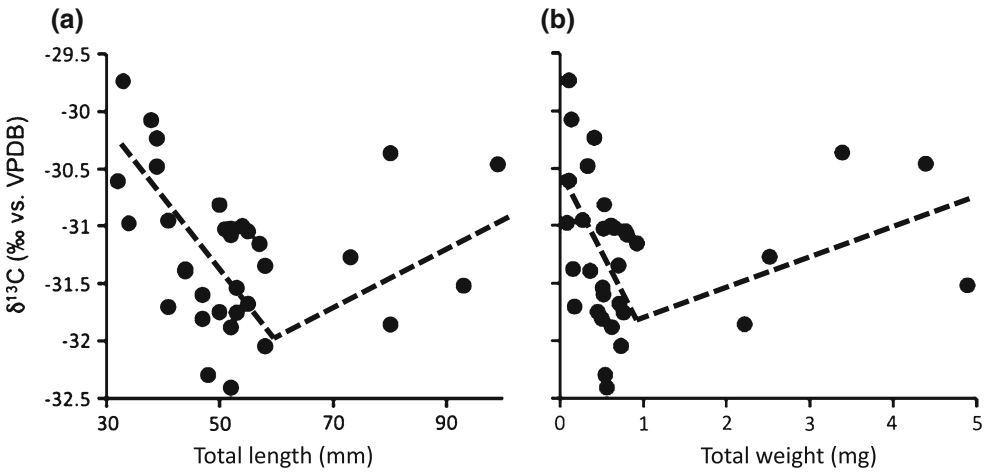

Fig. 4 $\delta^{13}C$ values of fatty acid 20:5n-3 versus (**a**) length (**b**) and weight for juvenile polar cod (*Boreogadus saida*, $n = 32$)

1989; Christiansen et al. 2012; Renaud et al. 2012; Rand et al. 2013). While FAs from i-POM can compose a proportion of the diet of copepods (Søreide et al. 2006; Tamelander et al. 2008), our analyses of $\delta^{13}C$ values of FAs in juvenile polar cod are considerably lower than any values for the corresponding FAs found in i-POM and are more congruent with the range of values found in p-POM from seasonally ice-covered waters (Fig. 3).

Our results prompt the need for further evaluation of juvenile polar cod's assumed role in the transfer of nutrients from sympagic ecosystems. Renaud et al. (2012) have previously questioned whether polar cod receive considerable amounts of nutrients from the sympagic environment and have pointed out that the studies documenting polar cod in association with sea ice (Lønne and Gulliksen 1989; Gradinger and Bluhm 2004) have observed abundances that pale in comparison to pelagic schools of polar cod (Welch et al. 1993; Crawford and Jorgenson 1996; Matley et al. 2012). Regardless, ice-associated polar cod may still be an integral component of the diet of higher trophic level sympagic organisms, even though large segments of juveniles may not receive shelter from sea ice (Bluhm and Gradinger 2008).

Juvenile polar cod have been observed in close association with and are thought to receive shelter from sea ice (Lønne and Gulliksen 1989; Gradinger and Bluhm 2004), which has led to discussion concerning their role in relation to the sympagic food web (Darnis et al. 2012). Age-0 polar cod in the Beaufort Sea have also been found in water likely resulting from ice-melt (Parker-Stetter et al. 2011). Unfortunately, very little is known about the diet of juvenile polar cod despite their importance in the polar food web (Bradstreet and Cross 1982). The few studies that have examined the diets of ice-associated polar cod have indicated that they consume higher proportions of sympagic prey species than pelagic conspecifics (Bradstreet and Cross 1982; Craig et al. 1982; Lønne and Gulliksen 1989; Renaud et al. 2012), but sympagic fauna do not dominate their diets (Renaud et al. 2012). However, the relative importance of dietary sympagic prey in polar cod appears to be influenced by the type of sea ice in which they inhabit (e.g., first-year or multiyear ice). The stomachs of polar cod sampled from first-year ice have been found to have higher proportions of pelagic crustaceans such as calanoid copepods than polar cod collected near multiyear ice; those inhabiting multiyear ice had a more diverse diet that contained higher proportions of sympagic organisms (Renaud et al. 2012). Even in these different sea ice environments, there is evidence to suggest that pelagic fauna can dominate polar cod diets (Lønne and Gulliksen 1989; Renaud et al. 2012).

While the role of juvenile polar cod in the sympagic food web remains uncertain, stomach content analyses from throughout the Arctic indicate that polar cod are generalist feeders that consume locally abundant prey, with their diet of copepods, shrimp, mysids and amphipods being influenced by prey availability (Bradstreet et al. 1986; Lønne and Gulliksen 1989; Walkusz et al. 2011; Christiansen et al. 2012). Our compound-specific stable carbon isotope analyses of individual FAs does not appear to support this conclusion due to the nonsignificant differences in the $\delta^{13}C$ values of FAs (14:0, 15:0, 16:4n-1, 18:0, 20:5n-3, 22:5n-3 and 22:6n-3) between regions of the Beaufort Sea and the overall low variability ($\sim$1 ‰) of the $\delta^{13}C$ values of examined FAs. One explanation for this small variability in the $\delta^{13}C$ values of FAs in juvenile polar cod is limited prey availability caused by mechanical feeding restrictions (i.e., gape size limitations). Fish diets have been shown to change with size (Robb and Hislop 1980; Martell and McClelland 1994; Rand et al. 2013) and one possible reason for this shift is a larger gape, which increases the size and types of prey that can be consumed (Robb and Hislop 1980; Mehner et al. 1998). While few studies have directly compared the diets of different size classes of polar cod, the literature that has made such comparisons indicates that fish length is related to the types of consumed prey (Lowry and Frost 1981; Lønne and Gulliksen 1989; Ajiad and Gjøsæter 1990; Walkusz et al. 2011; Renaud et al. 2012). In the Canadian Beaufort Sea, polar cod larvae were found to primarily consume small species of copepods, while juveniles targeted larger and heavier species of copepods (Walkusz et al. 2011). Prior research in the Beaufort Sea has indicated significant differences in the diets between adult and juvenile polar cod with adults consuming higher proportions of mysids, gammarid amphipods and large copepods (Lowry and Frost 1981; Craig et al. 1982; Walkusz et al. 2011). Renaud et al. (2012) also found evidence of gape limitations in pelagic polar cod taken near Svalbard, where small polar cod ($\leq$8 cm) fed primarily on *Calanus* copepod species and larger individuals consumed higher proportions of the hyperiid amphipod *Themisto* species. Fish also seem to be an important part of adult polar cod in the Beaufort Sea (Rand et al. 2013). Thus, a small gape, that limits prey accessibility, could explain developmental differences in stomach contents of juvenile polar cod and the low variability of and the nonsignificant differences of the $\delta^{13}C$ values of FAs in this study. Gape size may be important, but it may also relate to the ability of a certain sized fish to catch a prey item. Our data derived from smaller (i.e., <8 cm) fish indicates that the proportion of calanoid copepods in the diet of juvenile polar cod, evident from the relative abundance of 20:1 and 22:1 FAs (Fig. 5), increased between the two size classes we examined (<73 and >73 mm).

Mechanical feeding restrictions may also partially explain the observed developmental relationship between size and weight and the $\delta^{13}C$ values of FAs 20:5n-3

(Fig. 4). We speculate that the patterns could represent a 'washing out' of a maternal (egg) signal followed by an influence of increasing gape sizes from ~60 mm TL. We estimated the possible maternal influences on the $\delta^{13}C$ values of FA 20:5n-3 to determine if those values suggest a contribution of sea ice algal-derived nutrients. A trend line (linear extrapolation) fitted to the $\delta^{13}C$ values of FA 20:5n-3 versus TL (Fig. 4) compared to the estimated TL_0 values is only 0.5 ‰ lower than the $\delta^{13}C$ values of 20:5n-3 (−26.9 ‰) of adults from offshore of Cooper Island, AK (Budge et al. 2008). The $\delta^{13}C$ values of FA 20:5n-3 in adult polar cod from Cooper Island were interpreted as indicating a proportional contribution of 8 % from sea ice algae (Budge et al. 2008). This suggests that a potential link between juvenile polar cod and sympagic primary production might be mediated through a maternal influence of FA composition.

A complication with our study was the unknown lipid turnover rate of FAs in juvenile polar cod. The samples in this study were collected in August and early September so it is possible that even if the sampled polar cod had received considerable amounts of sea ice algal-derived FAs that these FAs could have turned over prior to sampling. We are unaware of any published lipid turnover rates in polar cod. Even though FA turnover rates have not been determined, the polar cod samples in Budge et al. (2008) were also collected in August and their two-end member (sea ice algae and pelagic phytoplankton) mixing model results indicated a contribution of sea ice-derived FAs. Budge et al. (2008) estimated that polar cod from Barrow received 61–95 % and 6–62 % of FAs 16:4n-1 and 20:5n-3 from sympagic sources, respectively, and Cooper Island samples received an estimated 22–94 % of FA 16:4n-1 and up to 32 % of FA 20:5n-3 from sympagic sources. This suggests that even though juvenile polar cod used in our study were sampled 1–2 months after sea ice was last present (Fig. 1), their FAs may not have completely turned over. Further research is needed to understand the rate and processes of FA turnover in polar cod. It was not possible for us to collect livers from these small cod. However, a future experimental feeding study could include sampling livers from a pooled set of individuals for stable isotope analysis to address tissue turn over.

In addition to unknown lipid turnover in polar cod, we made assumptions that i-POM and p-POM $\delta^{13}C$ values from the Bering Sea (Wang et al. 2013) were similar to those in the Beaufort Sea and there was no fractionation in FAs 16:4n-1, 20:5n-3 and 22:6n-3. The lack of site-specific end members could lead to inaccurate model estimates. For example, the $\delta^{13}C$ values of the FA 16:4n-1 and 22:6n-3 from our polar cod samples are outside the range of both end members (Fig. 4). However, we conducted several permutations of the SIAR-mixing model (Table 1), both with and without these FAs and also with the end member values previously used in Budge et al. (2008) to estimate the proportional contribution of sea ice-derived FAs relative to pelagic-derived FAs. All of these permutations estimated that i-POM contributed ≤2 % of the FAs examined. In regard to fractionation, $\delta^{13}C$ values of 20:5n-3 and 22:6n-3 from Steller's and Spectacled Eider adipose tissue did not differ from the $\delta^{13}C$ values of these FAs in their diet (Budge et al. 2011).

In conclusion, our study suggests that juvenile polar cod from the across the Beaufort Sea directly sourced negligible amounts of sea ice-derived FAs. A relationship between both age and weight and the $\delta^{13}C$ values of the FA 20:5n-3 suggests a possible maternal (egg) influence on the $\delta^{13}C$ values of this FA from juvenile polar cod, indicating a possible indirect link between sea ice production and juvenile polar cod. Thus, changes in sea ice coverage due to environmental change may not directly affect juvenile polar cod with respect to nutrients such as FAs but may still affect their populations by reducing critical habitat used as shelter from predators.

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