



DNA metabarcoding reveals surprisingly diverse diet in under-ice polar cod during autumn

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Abstract

Polar cod (*Boreogadus saida*) functions as a key species in the Arctic food web and is one of the most abundant Arctic fishes. Despite its ecological importance, the feeding ecology of juveniles sampled under the sea ice is unknown. In November 2019, at the onset of sea ice formation, sea-ice associated polar cod and zooplankton samples were collected in the Beaufort and Chukchi seas. Stomach contents were highly digested and largely unidentifiable by morphological analysis. DNA metabarcoding, however, facilitated identification of most prey items to the species-level. Juvenile polar cod had a broad dietary spectrum and consumed at least 45 taxa, with calanoid copepods and ostracods being the most abundant prey taxa. The most frequent prey species were the pelagic ostracod *Boroecia maxima* and the calanoid copepods *Calanus hyperboreus* and *Metridia longa*. Contrary to expectations, the upper water column in the Alaskan Arctic below consolidated ice contained moderate abundances of energy-rich epipelagic zooplankton and under-ice fauna, particularly calanoid copepods and ice amphipods, in late fall. This zooplankton may provide a critical resource for late hatchers to survive in low-temperature habitats with low energy expenditure.

Keywords Arctic cod · Beaufort Sea · *Boreogadus saida* · Chukchi Sea · Food web

Introduction

The Arctic marine ecosystem is characterized by a relatively simple food web with a limited number of trophic interactions, which increases the importance and reliance on individual trophic links (Hobson and Welch 1992; Węśławski et al. 2006). Polar cod (*Boreogadus saida* (Lepechin, 1774); Gadidae) is one of the most abundant fishes in Arctic seas (Benoit et al. 2008; Logerwell et al. 2010; Antonov et al. 2017). The species plays a key role in this ecosystem by channelling up to 75% of the energy from lower to higher trophic levels (Bradstreet 1986; Welch et al. 1992). This mid-trophic fish is one of the most fundamental lipid-rich food sources for numerous arctic seabirds and marine mammals (Bradstreet and Cross 1982; Welch et al. 1992; Harter et al. 2013).

Polar cod is widely distributed in the Alaskan Arctic Ocean (i.e. Beaufort and Chukchi seas) with large spatial and temporal variations (Jarvela and Thorsteinson 1999; Forster et al. 2020). During the open-water season, polar cod is commonly found feeding in shallow nearshore lagoons and bays (Thorsteinson et al. 1990; Cannon et al. 1991;

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Matley et al. 2013). In winter, adults typically spawn under consolidated sea ice with eggs developing under or near the ice cover, and larvae hatching in nutrient-rich waters at the start of ice break-up (Craig et al. 1982; Kono et al. 2016). Planktonic young-of-the-year live primarily in the epipelagic layer in spring and summer (Sekerak 1982; Geoffroy et al. 2016) and descend to deeper waters to join age-1 + polar cod after the first summer (Miller 1979; Geoffroy et al. 2016). Small late hatchers, however, are thought to associate with newly formed sea ice for feeding and protection, before a part of the population joins polar cod in the mesopelagic layer the following summer (Lønne and Gulliksen 1989; Geoffroy et al. 2016). An unknown proportion of the juvenile population appears to stay associated with the sea ice for 1–2 years and travel large distances with the Beaufort Gyre or the Transpolar Drift (Melnikov and Chernova 2013; David et al. 2016; Maes et al. 2025).

While the summer diet of polar cod in the Alaskan Arctic Ocean has been described (Rand et al. 2013; Gray et al. 2016; Buckley and Whitehouse 2017), the feeding habits of juveniles congregating under sea ice in autumn are understudied (Geoffroy et al. 2023). Sea-ice formation clearly influences the composition and distribution of the surface layer zooplankton community (Darnis et al. 2008; David et al. 2015; Hop et al. 2021a, b), and might hence also affect the available prey spectrum for sympagic polar cod juveniles. In general, the planktivorous polar cod mainly feeds on copepods, gammarid and hyperiid amphipods, and mysids (Lowry and Frost 1981; Kohlbach et al. 2017; Carlig et al. 2021). Young-of-the-year are thought to feed opportunistically on locally abundant prey rather than target specific prey (Bradstreet 1986), although Bouchard and Fortier (2020) reported positive selection for *Calanus glacialis* by larval and juvenile polar cod in Greenland during summer. In the Alaskan Arctic Ocean, calanoid copepods (especially *Pseudocalanus* spp.) dominate the under-ice zooplankton community (Horner and Murphy 1985), and thus are considered the main prey of larval and juvenile polar cod (Darnis et al. 2008; Bouchard and Fortier 2020).

Loss of sea-ice habitat is expected to cause significant shifts in the abundance and biomass of sea-ice associated fauna with negative cascading effects throughout the entire ice associated ecosystem (Barber et al. 2015; Kohlbach et al. 2016; Hop et al. 2021a, b). In addition, a community-wide poleward shift of boreal species is already changing the Arctic marine ecosystem (Fossheim et al. 2015; Møller and Nielsen 2020). For instance, a transition in the calanoid copepod community from Arctic *Calanus glacialis* and *C. hyperboreus* to boreal *C. finmarchicus*, coupled with a decrease in lipid availability, is expected to impact higher trophic levels (Møller and Nielsen 2020), including the lipid reserves of polar cod and winter survival of juveniles (Koenker et al. 2018a, b; Copeman et al. 2020). Geoffroy

et al. (2023) assessed the risk on the survival of larvae, juvenile and immature polar cod with a changing prey field to be high, given their narrow prey spectrum and potential mismatch dynamics related to larval emergence and prey availability depending on ice break-up timing (Søreide et al. 2010). Therefore, there is an increasing need to monitor the diet of polar cod to further the knowledge on its feeding ecology and to detect potential changes in its prey field (Buckley and Whitehouse 2017; Maes et al. 2022).

We investigated the under ice diet of juvenile polar cod in the north-eastern Chukchi and western Beaufort seas during the onset of sea ice formation using stomach content DNA metabarcoding. Furthermore, we investigated the available prey spectrum based on zooplankton samples from the same localities and compare diet composition to prey availability. We hypothesize that lipid-rich calanoid copepods and sympagic amphipods are the preferred prey of sea-ice associated polar cod juveniles. The new insights will help us better understand the resilience of sympagic polar cod at the onset of winter and to future change in a rapidly warming Arctic Ocean.

Materials and methods

Sampling

Environmental and biological samples were collected at 11 stations along the Chukchi–Beaufort shelf break (bottom depth ranging from 169 to 2382 m) during research expedition GO-WEST on RV *Sikuliaq* in November 2019, when sea ice was actively forming (Fig. 1; Table 1; Flores et al. 2020). Polar cod were sampled directly under the sea ice with the Surface and Under-Ice Trawl (SUIT; van Franeker et al. 2009) and with a midwater trawl at one open water station (Methot trawl; Methot 1986). The SUIT was towed over distances of 815 to 2201 m at mean speeds between 0.59 and 1.27 m s⁻¹ and under between 11 and 51 cm thick sea ice. Fish were dissected on board and whole stomachs were preserved in 96% ethanol for molecular analysis. Smaller gadids difficult to visually identify to species-level were molecularly identified with DNA barcoding to distinguish polar cod from other gadids following the protocol in Bouchard et al. (2020). Twenty-two out of 107 stomachs were used for visual analysis of the stomach content; the remaining stomachs were briefly checked for stomach fullness and used for DNA metabarcoding.

Epipelagic mesozooplankton was sampled with a 25-cm diameter CalVET system (CalCOFI Vertical Egg Tow; Smith 1985) equipped with two 150 mm mesh nets. The nets were fished vertically from 100 m depth to the surface. The volume filtered was measured with calibrated General Oceanics flowmeters mounted inside the nets. Volumes recorded

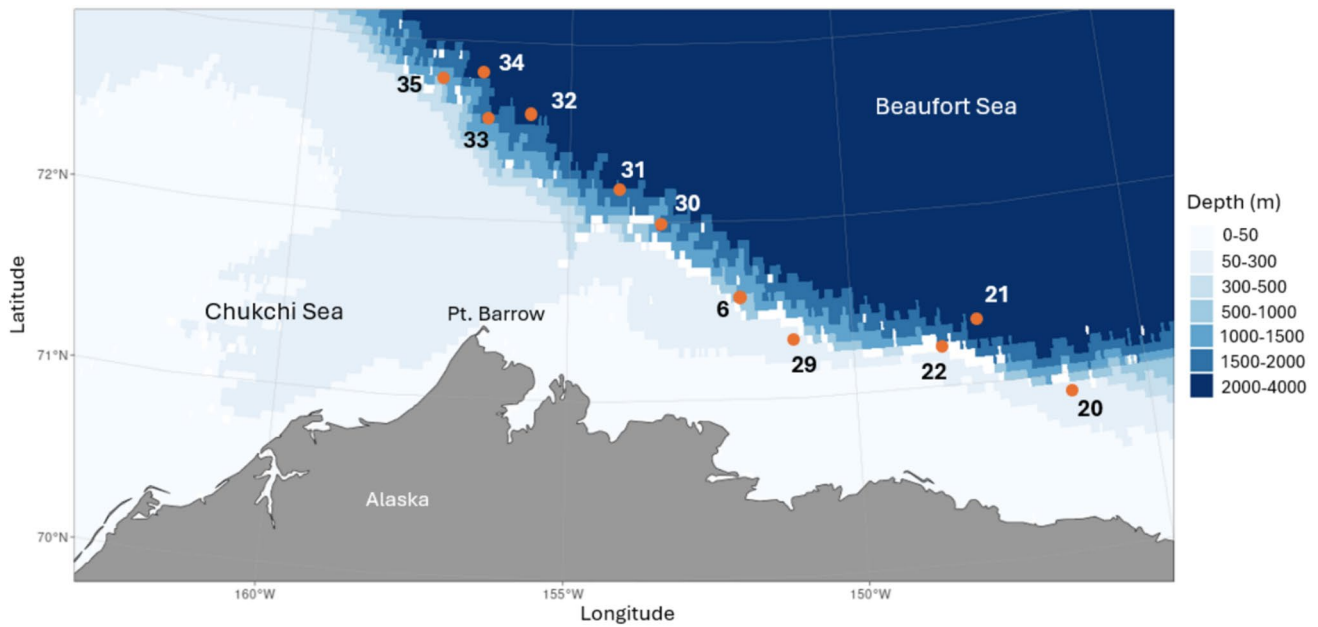


Fig. 1 Map of the 11 sampling stations where polar cod and zooplankton were collected in the Beaufort and Chukchi seas with station numbers. Station 6 was the only ice-free sampling location. Low

salinity, nutrient-rich water from the Chukchi Sea enters the western Beaufort Sea at Point Barrow, which represents the boundary between the two seas

Table 1 Sampling details of polar cod (*Boreogadus saida*) collected during research expedition GO-WEST on RV *Sikuliaq* in 2019 used for stomach content DNA metabarcoding

Area	St	Day	Lat	Lon	Gear	Sal	Chla	Ice	N	TL (min–max)	SL (min–max)
BES	6	11 Nov	71.58	–151.92	MT	28.15	0.64	0	18	71 (46–108)	59 (40–90)
BES	6	11 Nov	71.58	–151.92	SUIT	28.15	0.64	0	1	80	73
BES	20	14 Nov	70.91	–146.31	SUIT	27.63	0.59	100	9	61 (43–81)	51 (39–69)
BES	21	15 Nov	71.35	–147.81	SUIT	27.26	0.51	100	17	83 (62–142)	70 (52–119)
BES	22	15 Nov	71.24	–148.50	SUIT	27.77	0.50	100	3	71 (62–80)	60 (52–66)
BES	29	17 Nov	71.32	–151.04	SUIT	NA	0.45	100	2	71 (65–76)	61 (57–65)
BES	30	18 Nov	71.98	–153.36	SUIT	NA	0.46	90	13	78 (66–91)	67 (55–84)
BES	31	18 Nov	72.19	–154.07	SUIT	27.53	0.55	60	7	81 (75–106)	69 (63–90)
CHU	32	19 Nov	72.61	–155.72	SUIT	NA	0.52	90	5	77 (73–81)	65 (61–67)
CHU	33	19 Nov	72.58	–156.53	SUIT	26.89	0.54	100	2	78 (74–82)	64 (60–67)
CHU	34	19 Nov	72.83	–156.71	SUIT	27.02	0.46	100	2	85 (75–95)	71 (63–78)
CHU	35	20 Nov	72.79	–157.47	SUIT	27.76	0.54	70	3	88 (82–93)	73 (68–76)

Latitude (Lat) and longitude (Lon) are reported in decimal degrees. Sampling was conducted using a Surface and Under Ice Trawl (SUIT) or Methot trawl (MT). Sensor data during SUIT hauls and sea-ice properties as noted by observers are provided: Sal= salinity (PSU), Chla= chlorophyll a concentration (mg m^{-3}) at the surface (0–2 m), Ice= sea-ice coverage (%), n= number of samples. Polar cod mean total length (TL) and mean standard length (SL) with minimum and maximum (min–max) range for each station are reported in millimetres. Sampling areas include the Beaufort Sea (BES) and Chukchi Sea (CHU)

from the flowmeter were compared with volumes estimated from the distance (depth) towed at each station to detect net clogging. In practice, little clogging occurred, and the nets performed with near 100% filtration efficiency. All samples were preserved in 4% formaldehyde solution in seawater for later sorting. In the laboratory, each sample was split sequentially using a Folsom splitter until the smallest subsample contained about 200 specimens of the most abundant taxa.

The most abundant taxa were identified, staged, counted, and weighed. Each larger subsample was examined to identify, count, and weigh the larger, less abundant taxa. Blotted preserved individual wet mass was determined for larger taxa, while individual wet masses obtained from earlier studies in the Western Arctic (e.g., Coyle and Pinchuk 2002; Coyle et al. 2008; Pinchuk and Eisner 2017) were used to estimate biomass of small (< 2 mm) taxa.

In addition to vertical mesozooplankton tows, under-ice macrofauna was sampled with the SUIT, which was equipped with a 150 µm zooplankton net. The zooplankton net frequently collected large amounts of slush ice that filled up to one third of the smaller mesh net. Mesozooplankton catches from these tows were not considered quantitative as the large amounts of ice could have disrupted small animals. Macrofauna (> 0.5 cm) were still collected, and their species composition was analyzed in relative terms in order to avoid bias in total abundances due to unquantifiable differences in the filtration efficiency of the net due to ice clogging.

DNA metabarcoding

Stomach contents were homogenized, and DNA was extracted using the NucleoSpin® Tissue kit (Macherey–Nagel) following the manufacturer's instructions. Because stomachs were small, the entire stomach content was used for DNA extraction without subsampling. The DNA metabarcoding library was prepared according to the protocol described in Maes et al. (2022). In brief, the universal primers *mlCOIintF* and *jhHCO2198* (Leray et al. 2013) were used to amplify a fragment of the mitochondrial cytochrome oxidase subunit I (*COI*) gene. Negative controls were included in all PCR runs to detect cross-contamination. PCR products with at least 20 ng DNA were pooled and paired-end sequenced with a 600-cycle kit on the MiSeq Sequencing System (Illumina, Inc.) at the Genomics Core of the KU Leuven, Belgium.

Filtering, taxonomy assignment and data analysis

Raw reads were demultiplexed using the bcl2fastq v2.16 tool integrated in the Illumina platform. The data were processed using R package DADA2 v1.16 (Callahan et al. 2016), which creates amplicon sequence variants (ASVs). Raw forward and reverse reads were filtered and trimmed using the following parameters: maxEE = 1 (maximum number of expected errors allowed in a read), maxN = 0 (removes reads with ambiguous nucleotides), trimLeft = 30 (removes first 30 bp of each read), and truncLen = 290, 250 (truncates the forward and reverse reads to 290 and 250 bp, respectively). Filtered and trimmed reads were merged using the mergePairs function. Chimeric sequences were removed with the removeBimeraDenovo function. The following criteria were used for taxonomic assignment in BOLD: (1) a taxon was assigned if the barcode matched a single locally occurring taxon in the database with ≥ 97% sequence similarity level; (2) if the barcode matched more than one taxon with ≥ 97% sequence similarity level, a taxon was assigned at the genus-level. We excluded species that were identified as within-laboratory contaminations (i.e. study species from the home laboratory). Taxa seen at least twice in at least 1%

of the samples were retained and samples with less than 20 reads in total were discarded from the final dataset using R package phyloseq v1.26.1 and following the recommendations of McMurdie and Holmes (2013). Sequence reads were analysed based on frequency of occurrence (% FOO) and relative read abundance (% RRA). Frequency of occurrence was estimated as the proportion of individuals containing a particular prey taxon.

Alpha diversity indices (i.e. observed species, Shannon and Simpson diversity) were calculated per sampling area (Beaufort and Chukchi seas) using R package phyloseq. Wilcoxon rank-sum tests (Mann–Whitney) were used to test whether the observed numbers of ASVs differed either between sampling areas, with fish length or ice coverage. Multivariate analyses were conducted using both RRA and presence/absence (PA) data. All downstream analyses (after demultiplexing) were conducted in R v4.1.2.

Visual diet analysis

Stomach contents were gently removed from 22 polar cod, and ingested prey items were visually examined under a microscope. Prey items were identified to the lowest taxonomic level and developmental stage possible. Degree of digestion of prey was recorded according to the following scale: (1) fresh prey, (2) slightly digested prey, (3) well digested prey, and (4) highly digested prey. Total prey wet mass was determined by multiplying a mean individual prey taxon wet mass obtained from zooplankton data by the number of that specific prey taxon in the gut.

Statistical analysis

We analysed the spatial structure of diet composition, epipelagic mesozooplankton and under-ice macrofauna community structure using non-metric multidimensional scaling (NMDS) plots. NMDS plots were generated based on Euclidian distance matrices calculated from $\log(x + 1)$ – transformed %RRA from diet data and relative abundance data from CalVET and SUIT catches, respectively (Aitchison 1982). Environmental variables (temperature, salinity, chlorophyll a, ice concentration, and ice thickness) were plotted on the NMDS plots if statistically significant ($p < 0.05$) using the envfit function from R package vegan v2.5.7 (Oksanen et al. 2013). To assess whether diet composition and prey distribution datasets followed a similar spatial structure, we analysed potential correlations between all three combinations of these distance matrices with the Mantel test. Analysis of similarity (ANOSIM; Oksanen et al. 2013) was used to test for significant differences in the diet composition between the pre-defined categories daylight condition (day, night, twilight) and geographical domain (Western Beaufort or Chukchi seas).

Results

Environmental and biological data

The upper 100 m of the water column were highly stratified across the study area with a cold ($< 2\text{ }^{\circ}\text{C}$) and low saline ($< 30\text{ PSU}$) Polar mixed layer (PML) extending to $\sim 30\text{ m}$ depth. Below the PML, there was an intermediate halocline layer formed by the remnant Bering/Chukchi Summer Water and Bering/Chukchi Winter Water with wide ranges of temperature (from -1 to $4\text{ }^{\circ}\text{C}$) and salinity (from 30 to 33 PSU) extending to $\sim 200\text{ m}$ depth. The halocline layer overlaid warmer ($> 0\text{ }^{\circ}\text{C}$), highly saline (33–34 PSU) Atlantic Deep Water that impinges on the Chukchi and Beaufort slopes. Modest amounts of chlorophyll *a* (mean 0.129 ± 0.023 [95% CI] mg m^{-3}) were observed in the upper 0–50 m layer. Small phytoplankton made up the majority (70%) of the chlorophyll *a* biomass. Polar cod ranged from 43 to 142 mm total length (TL) without any evidence for spatial patterns (Table 1). Based on age and growth estimates by Melnikov and Chernova (2013), most polar cod were likely young-of-the-year (TL $< 75\text{ mm}$) or age-1 + polar cod (TL $> 75\text{ mm}$). Four specimens were identified as Arctic cod *Arctogadus glacialis* with DNA barcoding and excluded from subsequent analyses.

DNA metabarcoding and taxonomic assignments

In total, 36 out of 107 stomachs (33.6%) were empty based upon visual inspection during sampling. The content of 85 stomachs was analysed using DNA metabarcoding (and the content of the remaining 22 stomachs was analysed visually). Illumina MiSeq sequencing provided a total of 6,812,522 paired-end reads and 799 ASVs of the COI gene. From the total number of paired-end reads, 78,132 and 338,895 reads were discarded because of potential within-laboratory contamination and lack of database matches, respectively. Additionally, 5,686,520 *Boreogadus saida* reads were excluded from the entire dataset. A total of 29 samples were removed due to the low number of total reads (< 20 reads), including 20 stomachs that were considered empty based on visual inspection. Three samples were removed because of missing metadata. The final dataset contained 287,465 reads and 362 ASVs from 53 polar cod stomachs. Detailed information on the filtering steps, ASVs obtained for each taxon and number of reads obtained for each individual sample can be found in Supplementary Tables S1, S2 and S3, respectively.

Most taxonomic assignments (44 out of 49) were certain at the species-level; five taxa were identified at the

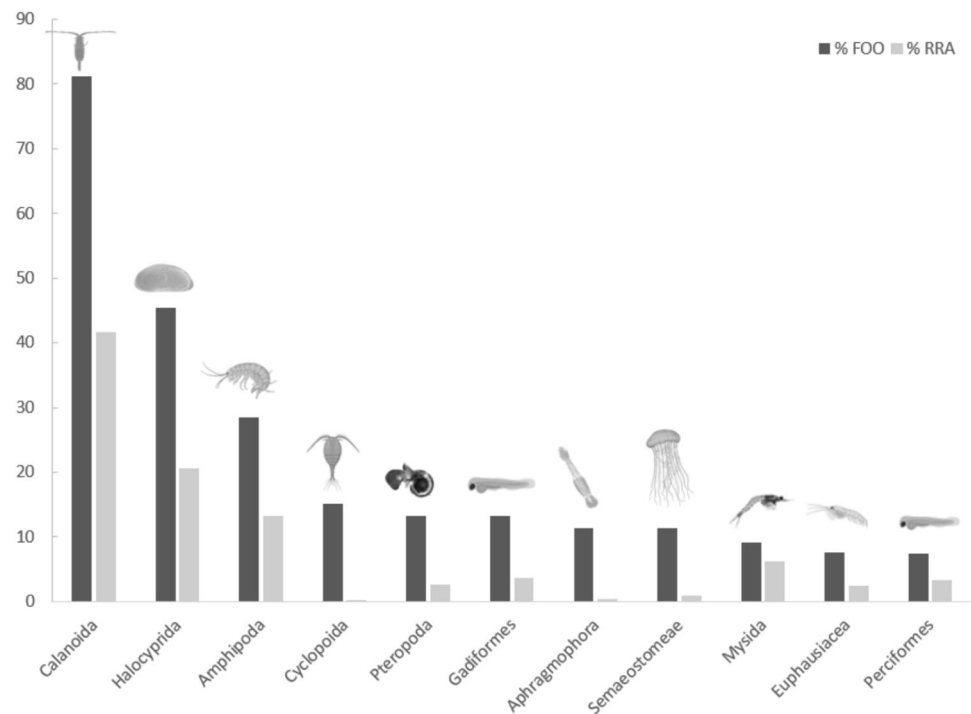
genus-level. A DNA barcode belonging to the decapod *Hyas* sp. matched with both *H. coarctatus* and *H. lyra-tus*, hence species-level identification was inconclusive. However, during previous field sampling in the Chukchi Sea only *H. coarctatus* was collected (Weems, UAF, pers. comm.). Likewise, while some *Acartia* sp. barcodes unambiguously matched *A. longiremis*, for others the species-level identification was unclear (*A. longiremis* or *A. hudsonica*). One *Anisakis* sp. barcode, a parasitic nematode, matched *A. simplex* and *A. typical*, which could not be resolved based on either similarity of sequences or tree-based identification in BOLD. The molecular identification of jellyfish from the family Semaestomeae was particularly challenging. Most barcodes belonging to the genus *Cyanea* were not assigned to a species with certainty. Tree-based identification in BOLD suggests that *Cyanea* barcodes probably belong to *C. tzetlinii* or *C. capillata*, but due to uncertainty we retained them as genus-level identifications. Furthermore, one *Aurelia* sp. barcode could not be assigned to the species-level but matched with 99.7% similarity to an unidentified *Aurelia* sp. barcode from the Russian Arctic region.

Polar cod diet based on molecular data

The diet of polar cod consisted of at least 45 species belonging to eight phyla. Polar cod fed on a broad spectrum of prey items with calanoid copepods as the predominant order in terms of both frequency of occurrence and relative read abundance (FOO = 81.1%, RRA = 41.6%), followed by pelagic ostracods (FOO = 45.3%, RRA = 20.5%) and sea-ice associated amphipods (FOO = 28.3%, RRA = 13.1%) (Fig. 2). At the species-level, the pelagic ostracod *Boroecia maxima* (FOO = 45.3%, RRA = 20.5%) and the calanoid copepod *Calanus hyperboreus* (FOO = 45.3%, RRA = 15.6%), were most common, followed by *Metridia longa* (FOO = 39.6%, RRA = 6.0%) (Table 2). These prey species were consumed by juvenile polar cod of all sizes (Supplementary Table S4). Polar cod that consumed fish (presumably eggs and/or larval stages) ranged in length from 76 to 108 mm TL. A total of four fish species were detected: Arctic cod *Arctogadus glacialis* (FOO = 11.3%, RRA = 3.5%), stout eelblenny *Anisarchus medius* (FOO = 5.7%, RRA = 3.3%), marbled eelpout *Lycodes ravidens* (FOO = 1.8%, RRA $\leq 0.1\%$), and walleye pollock *Gadus chalcogrammus* (FOO = 1.8%, RRA $\leq 0.1\%$) (Supplementary Table S5). Similarly, the mysid shrimp *Neomysis rayii* was only found in larger polar cod ranging in size from 76 to 82 mm TL. The parasitic tapeworm *Diphyllobothrium schistochilos* and parasitic nematode *Anisakis* sp. were found in two and one polar cod stomachs, respectively.

The number of ASVs was neither significantly different between polar cod from the Chukchi and Beaufort seas, nor between size classes, stations with varying sea-ice

Fig. 2 Frequency of occurrence (% FOO; dark grey) and relative read abundance (% RRA; light grey) of most common prey orders detected by DNA metabarcoding in polar cod stomachs in the Alaskan Arctic Ocean



coverage, topographic regions (continental shelf, continental slope and deep-water stations) or size classes of polar cod (Supplementary Fig. S1). The Shannon diversity index suggests that prey composition (based on number of reads) was evenly distributed between areas, size classes, and stations with varying sea-ice coverage. The observed richness (i.e. number of ASVs) was higher, but not significantly different, for polar cod sampled on the continental slope and in deep water stations compared to the continental shelf. The ostracod *B. maxima* and calanoid copepods *C. hyperboreus*, *P. glacialis* and *M. longa* were the most common prey items in all areas (deep water, continental slope, and continental shelf), although the frequency of occurrence of prey items varied (Supplementary Table S6). Ice-associated amphipods *Apherusa glacialis* and *Gammarus wilkitzkii* and copepod *C. glacialis* were only found in the diet of polar cod sampled in western stations in the Beaufort Sea (Table 2). Pteropods (i.e. *Clione limacina* and *Limacina helicina*) were only detected in a few stomachs from the western stations, whereas they were more frequently found in polar cod sampled in the Chukchi Sea (Table 2).

Additional dietary data based on visual analysis

Sixteen out of 22 polar cod stomachs visually analysed contained prey. Morphological identification, however, was difficult due to high digestion levels (average degree of digestion 3.4) (Table 3). Three taxa were identified to the species-level: *Pareuchaeta glacialis* (copepodite stages IV and V, and adult females), *Metridia longa* (copepodite stage

V and adult females) and *Calanus hyperboreus* (copepodite stage V). The remaining prey was classified as copepod and mysid fragments, gammarid amphipods and *Pseudocalanus* spp. (copepodite stage V).

Prey availability

A total of 34 epipelagic mesozooplankton taxa were recorded from the CalVET tows. Epipelagic mesozooplankton abundance was low at most stations (mean 70 ± 9 [95% CI] ind m^{-3}), except at the only ice-free Station 6 sampled at the beginning of the cruise, where epipelagic mesozooplankton were nearly twice as abundant as the average across all other stations (Fig. 3a). Small (<2 mm TL) widespread boreal neritic copepods *O. similis*, *Pseudocalanus* spp. and oceanic *Microcalanus* spp. predominated numerically at all stations, comprising over 75% of the zooplankton community. In contrast, large (>2 mm TL) Arctic copepods *C. hyperboreus*, *P. glacialis*, and *M. longa*, and chaetognaths *Parasagitta elegans* comprised the bulk of the zooplankton biomass at all stations (mean 91 ± 33 [95% CI] $mg\ m^{-3}$) (Fig. 3b). Arctic copepods *Chiridius obtusifrons* and *Heterorhabdus norvegicus* occurred in small numbers at deep stations pointing to the potential influence of deep Atlantic water. Ostracods (likely *Boroecia maxima*) were recorded in all tows, albeit in small quantities. Larger zooplankton such as scyphozoans, juvenile hyperiids (*Themisto libellula*), ice amphipods (*Onisimus* spp., *Eusirus holmii*, *Apherusa glacialis*), krill (*Thysanoessa raschii* and *T. inermis*), and nearshore mysids (*Neomysis rayii*, *Acanthomysis*

Table 2 Overview of prey taxa found in the polar cod stomachs in the Beaufort (BES) and Chukchi (CHU) seas identified by DNA metabarcoding

Phylum	Order	Species	% FOO			% RRA			
			BES	CHU	ALL	BES	CHU	ALL	
Chaetognatha	Aphragmophora		14.0	0	11.3	0.5	0	0.4	
		<i>Parasagitta elegans</i>	7.0	0	5.7	0.5	0	0.4	
Cnidaria	Leptothecata		4.7	0	3.8	1.8	0	1.5	
	Semaeostomeae		12	10	11.3	1.2	<0.1	0.9	
	<i>Chrysaora melanaster</i>		9.3	10	9.4	1.0	0	0.9	
Nemertea	Monostilifera		2.3	0	1.9	2.0	13.1	1.6	
Mollusca	Pteropoda		7.0	40	13.2	0.1	13.1	2.5	
		<i>Clione limacina</i>	0	30	5.7	<0.1	12.4	2.3	
		<i>Limacina helicina</i>	7.0	20	9.4	0.1	0.6	0.2	
Arthropoda*	Amphipoda*		34.9	0	28.3	16.1	0	13.1	
		<i>Apherusa glacialis</i>	20.9	0	17.0	9.1	0	7.4	
		<i>Gammarus wilkitzkii</i>	11.6	0	9.4	2.5	0	2.0	
	Balanomorpha		2.3	0	1.9	1.6	0	1.3	
	Calanoida*		81.4	80	81.1	45.6	24.4	41.6	
		<i>Acartia longiremis</i>	9.3	0	7.5	2.8	0	2.3	
		<i>Calanus glacialis</i>	16.3	0	13.2	4.4	0	3.5	
		<i>Calanus hyperboreus</i>*	48.8	30	45.3	16.8	10.1	15.6	
		<i>Epilabidocera longipedata</i>	7.0	0	5.7	2.3	0	1.9	
		<i>Eurytemora herdmani</i>	9.3	0	7.5	1.2	0	1.0	
		<i>Pseudocalanus acuspes</i>	14	10	13.2	0.4	0.4	0.4	
		<i>Pseudocalanus minutus</i>	7.0	10	7.5	0.8	0.2	0.7	
		<i>Pseudocalanus mimus</i>	11.6	0	9.4	1.0	0	0.8	
		<i>Pseudocalanus newmani</i>	7.0	10	7.5	<0.1	0.2	0.1	
		<i>Paraeuchaeta glacialis</i> *	34.9	20	32.1	8.0	10.1	8.4	
		<i>Heterorhabdus norvegicus</i>	14	0	11.3	<0.1	0	0.2	
		<i>Metridia longa</i> *	39.5	40	39.6	7.0	1.8	6.0	
		<i>Scolecithricella minor</i>	2.3	20	5.7	<0.1	0.1	<0.1	
		Cyclopoida		14	20	15.1	<0.1	0.7	0.1
		Cyclopoida	<i>Oithona similis</i>	14	20	15.1	<0.1	0.7	0.1
		Decapoda		2.3	0	1.9	0.3	0	0.2
	Euphausiacea		9.3	0	7.5	2.9	0	2.4	
		<i>Thysanoessa raschii</i>	7.0	0	5.7	2.8	0	2.3	
<i>Boroecia maxima</i>		44.2	50	45.3	21.1	18.0	20.5		
Harpacticoida		4.7	0	3.8	<0.1	0	<0.1		
Mysida*	<i>Neomysis rayii</i>	2.3	40	9.4	<0.1	32.7	6.2		
Chordata	Gadiformes		9.3	30	13.2	1.7	11.0	3.5	
		<i>Arctogadus glacialis</i>	7	30	11.3	1.7	11.0	3.5	
	Perciformes		9.3	0	7.4	4.1	0	3.3	
	<i>Anisarchus medius</i>	7.0	0	5.7	4.1	0	3.3		

Frequency of occurrence (% FOO) and relative read abundance (% RRA) are given per sampling region (BES, CHU) and all individuals combined (ALL). Frequency of occurrence and relative read abundance on order level is based on all species detected. Prey taxa at the species-level are provided if present in > 5% of all polar cod stomachs. The two most common species overall are marked in bold. Phyla/orders/species also found during visual analysis are marked with an asterisk. The list with all prey taxa is provided in Supplementary Table S5

pseudomacropsis) were sometimes abundant in under-ice macrofauna from SUIT tows (Fig. 3c). NMDS plots showed no distinct grouping in relative diet composition, but with few exceptions the ordinations of all three NMDS plots

followed an east–west trend, which weakly coincided with a trend from daytime to nighttime sampling (Fig. 4). ANOSIM indicated a correlation between relative diet composition and sampling region ($r=0.318$; $p=0.046$), but there

Table 3 Summary of stomach content of polar cod based on visual identification per station with n = total number of individuals, TL total length (cm) and RFI recognizable food items

Station	6	21	22	30	Total
N	1	8	3	10	22
Average TL	75	79	78.3	70.8	75.4
Average weight	2.4	3.4	3.6	2.1	2.7
Digestion degree	4	2.3	3.7	2.5	3.4
RFI	1	33	10	13	56
n empty stomachs	0	3	0	3	6
Stomach content					
Average number of recognizable food items ($N \cdot \text{ind}^{-1}$)					
Gammarid amphipod	0	0	0.3	0	0.05
Copepod fragments	0	0	0.7	0.3	0.2
<i>Calanus hyperboreus</i>	0	0	0	0.1	0.05
<i>Pseudocalanus</i> spp.	0	0	0	0.1	0.05
<i>Pareuchaeta glacialis</i>	1	0.9	2.3	0.6	1.0
<i>Metridia longa</i>	0	3.3	0	0.1	1.2
Mysidae fragments	0	0	0	0.1	0.05
Frequency of occurrence (% FOO)					
Gammarid amphipod	0	0	33.3	0	4.5
Copepod fragments	0	0	66.7	20.0	18.2
<i>Calanus hyperboreus</i>	0	0	0	10.0	4.5
<i>Pseudocalanus</i> spp.	0	0	0	10.0	4.5
<i>Pareuchaeta glacialis</i>	100	62.5	33.3	50.0	54.5
<i>Metridia longa</i>	0	37.5	0	10.0	18.2
Mysidae fragments	0	0	0	10.0	4.5

Both average weight and average stomach content weight are given in gram. Details on morphological measurements are also provided per station. Frequency of occurrence (% FOO) is given per station and for the total number of fish. For station codes see Table 1

was no significant correlation with daytime in any dataset ($p > 0.05$). None of the environmental variables (temperature, salinity, chlorophyll a, ice concentration and ice thickness) were identified as statistically significant, except for salinity in the CalVET samples, although no clear pattern could be observed (Fig. 4). Ice cover at the sampling stations was at least 60% (station 31), but usually ranged from 90 to 100%. The ice formation stage was consistent across most stations, classified as young gray ice, with thickness ranging from roughly 10 to 50 cm and snow cover varying from 0 to 15 cm. The Mantel test showed that the spatial structures of the relative diet composition and the relative macrofauna abundances from SUIT catches were significantly correlated with each other ($r = 0.353$; $p = 0.016$), unlike between relative diet composition and relative mesozooplankton abundances from CalVET net catches ($p > 0.05$). There was, however, a significant correlation between the spatial structure of the relative abundances of under-ice macrofauna and relative abundances of epipelagic mesozooplankton ($r = 0.458$; $p = 0.001$).

Discussion

This first DNA metabarcoding study of stomach contents from sympagic juvenile polar cod caught during sea ice formation in the Alaskan Arctic Ocean revealed a broad dietary spectrum with at least 45 prey taxa. In general, our results are largely in accordance with previous under-ice diet studies in the Alaskan Arctic Ocean which have shown that polar cod primarily feeds on pelagic copepods and sea-ice associated amphipods (Bradstreet and Cross 1982; Craig et al. 1982; Cross 1982). However, the pelagic ostracod *Boroecia maxima*, which dominated our dataset numerically, has been rarely reported in previous studies, indicating a potential under sampling of small and easily digested taxa by traditional morphological diet studies (Bradstreet and Cross 1982; Bouchard and Fortier 2020). The digestion rates of ostracods, especially eggs and larval stages, are likely faster than those of larger crustacean prey, and may explain why they went undetected during visual inspection of the stomach samples.

Calanoid copepods, such as *Calanus* spp. and *Metridia* spp., were the second dominating prey group in our dataset and were also common and widespread in the epipelagic zooplankton community as sampled with CalVET tows. The presence of calanoid copepods in the epipelagic water column in late autumn is further proof that at least part of the *Calanus* population remains active in the surface layer water during winter (Barth-Jensen et al. 2022). Several prey taxa (i.e. pteropods, chaetognaths, mysids and fishes) were only detected in larger-sized polar cod individuals (> 70 mm TL). Notably, the amphipods *Themisto* sp. and *Onisimus* sp. were abundant in the under-ice macrofauna samples of the SUIT, but were consumed by few polar cod ($< 5\%$). The gape width of most sampled polar cod (mean gape width 5 mm; estimated with results from Carlig et al. 2021) may have been insufficient to feed on these large (up to 60 mm) amphipods. Conversely, larger (approximately > 100 mm TL) polar cod consume more *Themisto* sp. and *Onisimus* sp. (Lønne and Gulliksen 1989; Renaud et al. 2012). The occurrence of other under-ice fauna sampled by the SUIT, such as *Apherusa glacialis* and *Calanus glacialis*, was well reflected in the diet composition.

Nearly 37% of the stomachs were empty, which is somewhat in contrast with the hypothesis that at least a fraction of the young-of-the-year associate with newly formed sea ice for feeding. Empty stomachs were previously reported for age-1 + polar cod (Lønne and Gulliksen 1989; Matley et al. 2013) but not yet for young-of-the-year (Walkusz et al. 2011). The benefits of protection under the sea-ice cover and delayed migration of late hatchers to deeper water with their larger conspecifics might outweigh the energetic costs of reduced feeding. A similar mechanism

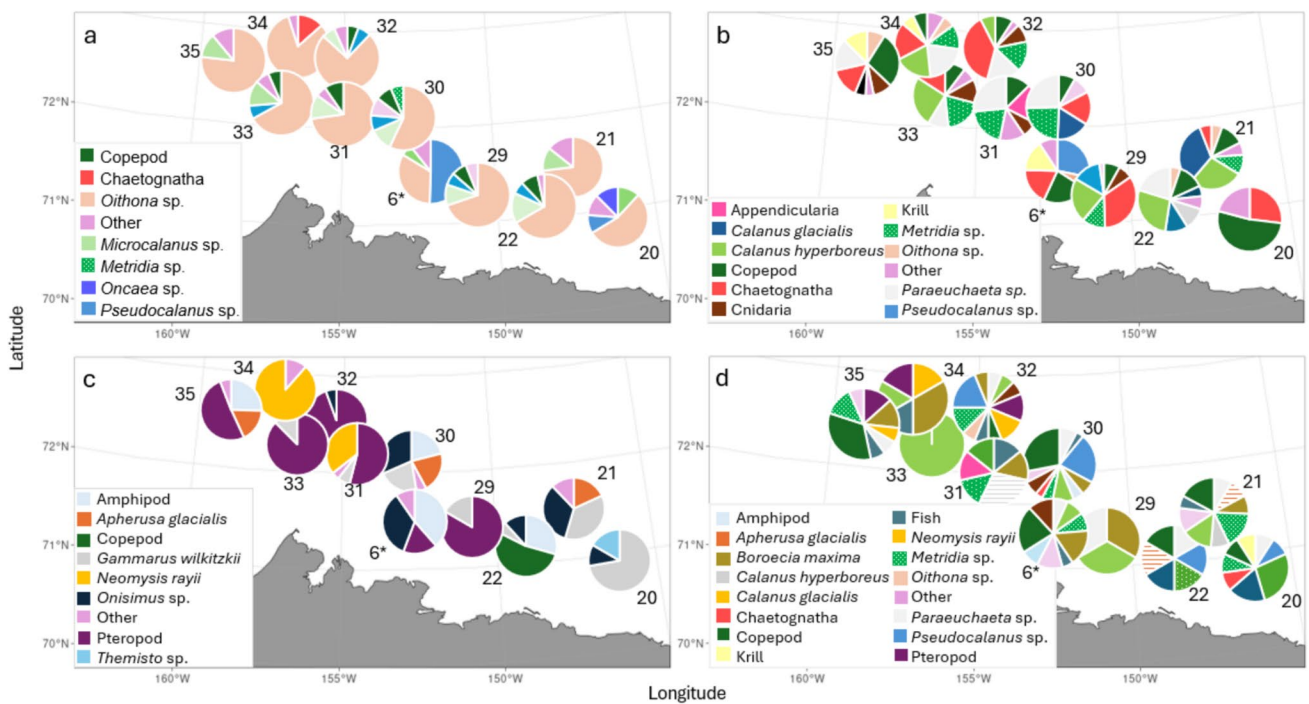


Fig. 3 **a** Abundance (ind m^{-3}) and **b** biomass (mg m^{-3}) of zooplankton taxa in the upper 100 m layer at 11 sampling stations recorded from the CalVET tows in the Chukchi and Beaufort seas. **c** Relative under-ice fauna composition (>0.5 cm) from SUIE tows of the top 2 m under the sea ice. **d** Frequency of occurrence of prey in polar cod (*Boreogadus saida*) stomachs based on DNA metabarcoding. Only

taxa representing over 5% of the prey composition per station are shown individually, all others taxa are grouped in higher taxonomic categories (i.e. Amphipod, Chaetognatha, Copepod, Cnidaria, Fish, Krill or Pteropod). Taxon colours are consistent across all four sub-figures. Station numbers are provided with station 6 marked with an asterisk (*) as the only ice-free sampling location

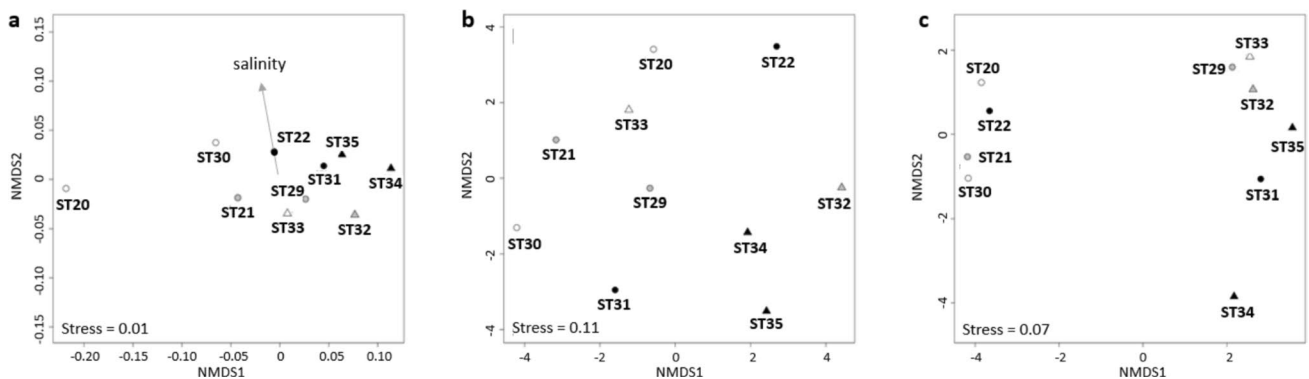


Fig. 4 Nonmetric Multi-Dimensional Scaling (NMDS) plots showing the similarity between sampling sites in a 2-dimensional ordination according to **a** relative zooplankton composition from CalVET net sampling of the top 100 m of the water column, **b** relative diet composition determined by metabarcoding, and **c** relative under-ice fauna composition from SUIE sampling of the top 2 m under the

sea ice. Sampling sites were marked according to geographical area (circles=Western Beaufort Sea, triangles=Chukchi Sea), and daylight conditions during SUIE sampling (white=daylight, grey=twilight, black=night). Statistically significant environmental variables ($p < 0.05$) are shown on the NMDS plot

has been observed in schooling polar cod (Hop et al. 1997). Preliminary energetic analyses showed that the lipid densities of these polar cod were very low (Louise Copeman, pers. comm.).

Pre-winter zooplankton assemblages in the Arctic Ocean are strongly associated with hydrodynamic and sea-ice conditions (Horner and Murphy 1985; Darnis et al. 2008; Ershova et al. 2021). The resulting spatial variability

of the zooplankton community is reflected in the east–west change of diet composition, epipelagic mesozooplankton and under-ice macrofauna community structure of our datasets (Fig. 4). In agreement with previous studies, small *Pseudocalanus* spp., but also the small copepods *Oithona similis* and *Microcalanus* spp. comprised numerically at least 75% of the epipelagic mesozooplankton community. Despite its abundance, *O. similis* was detected in only 16.1% of the stomachs, consistent with Walkusz et al. (2011). In contrast, the ostracod *B. maxima* was only detected in small quantities in all zooplankton catches of the CalVET net, yet, it was found in nearly half of the investigated stomachs using DNA metabarcoding. From October to February, *B. maxima* is mainly concentrated in the upper water layer (0–50 m) (Chavtur et al. 2015). Its avoidance of warm Atlantic Water during the polar night to avoid grazing by predatory plankton, as well as a year-round breeding season, are considered an adaptation to reduce winter mortality (Chavtur and Bashmanov 2007). Polar cod were likely feeding on ostracods, possibly eggs or larval stages closely associated with sea ice and therefore largely absent in the epipelagic mesozooplankton samples. The dominance of *B. maxima*, *A. glacialis* and *C. glacialis* in the diet supports the notion that the under-ice habitat was the main feeding ground of polar cod and explains why the spatial structure of the diet correlated better with the community structure of under-ice macrofauna than with that of epipelagic mesozooplankton.

Based on the under-ice diet composition of polar cod, several other interesting observations on the zooplankton community composition were made. The mysid *Neomysis rayii* is considered a boreal sublittoral species (Petryashev 1989, 2002), but was detected in roughly 10% of the stomachs sampled offshore over deep water and along the continental slope. Moreover, *N. rayii* was also surprisingly abundant in SUIT catches over the continental slope (> 1000 m water depth; Flores et al. 2020). These observations provide further evidence for cross-shelf transport of coastal water towards the basin and its influence on biological communities (Smoot and Hopcroft 2017). The presence of walleye pollock (*Gadus chalcogrammus*), presumably small juveniles, in one stomach was unexpected as the cold temperatures and ice coverage in the northern Bering and Chukchi seas constitute a migration barrier that normally prevents young walleye pollock from advancing poleward (Koenker et al. 2018a, b). However, it agrees with observations of unusually high abundances of juvenile walleye pollock over much of the Chukchi Sea shelf in the summer of 2019 (Levine et al. 2023). Finally, the DNA of the tapeworm *Diphyllobothrium schistochilos*, a well-known parasite of Arctic seals and previously thought to be absent from the Beaufort Sea (Maltsev 1998; Schaeffner et al. 2018), was detected in two polar cod stomachs.

While the advantages of dietary metabarcoding are well-established, particularly the taxonomic information on prey species at the species-level, the discarding of ASVs without taxonomic match, occurrence of multiple ASVs for the same taxon and biases in both diversity estimates and quantitative assessments of metabarcoding data requires attention. Here, 43% of the ASVs were discarded due to a lack of taxonomic match in the database. These ASVs might be the result of potential sequencing artifacts (Drake et al. 2021) or belong to species for which the COI barcode has not yet been included in the reference database (Questel et al. 2021). In addition, the presence of cryptic species complicates efforts to include all species in reference databases (Tempestini et al. 2018). Several studies have reported the presence of cryptic species in marine invertebrates such as copepods, amphipods and polychaetes, which are known prey taxa of polar cod (Crouch et al. 2020; Tempestini et al. 2018). Furthermore, for some taxa, multiple ASVs were present, which might be the result of genetic variability within the species, heteroplasmy (i.e. the presence of more than one type of mitochondrial DNA within an individual), or a technical artefact (i.e. sequencing error, PCR amplification bias) (Callahan et al. 2017). To address these limitations in future studies, expanding the COI reference database, particularly for marine invertebrates, is strongly recommended. Investigating the extent of intraspecific variation in COI sequences of specific species can also clarify the origin of multiple ASVs. To account for potential biases in diversity estimates, we recommend to analyse the net samples with metabarcoding, similar to the mock communities suggested by Ershova et al. (2023). The metabarcoding data of the zooplankton in the net relative to their real proportions in the sample would provide a measure of the extent of potential bias, which could be extrapolated to the dietary metabarcoding data.

Our results show that, in the surface layers in the Alaskan Arctic, energetically rich prey species such as calanoid copepods, mysids, and ice amphipods might provide the energy needed for late hatchers to survive in combination with low energy expenditure in the low-temperature habitat during late autumn. Accelerated inflow of Atlantic water in the Arctic and further regression of the ice cover will gradually replace Arctic specialists with sub-Arctic and boreal generalists, at least on Arctic shelves (Ravelo et al. 2020). These changes will likely favour the Arctic-boreal *Pseudocalanus* sp. and may be detrimental to Arctic pelagic copepods and ostracods like *C. glacialis* and *B. maxima* (Darnis et al. 2008; Geoffroy et al. 2023). The diverse and opportunistic feeding behaviour of polar cod juveniles may be advantageous under changing environmental conditions. However, it remains uncertain how a decline of lipid-rich prey will impact polar cod body condition, which may influence the energy flow across the entire food web (Geoffroy et al. 2023).

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Author contributions SMM conceptualized the study. FM and HF designed the research project. SMM, AP, FM, RtB, and HF collected the samples. SMM, BH and AP created the data. SMM and AP analyzed the data and interpreted the findings. SMM wrote the first draft of the manuscript. All authors contributed to the interpretation of the findings and manuscript revision.

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Data availability The data and accompanying R script are publicly available in the public repository Zenodo (doi: 10.5281/zenodo.15100365).

Declarations

Competing interests The authors declare no competing interests.

Animal ethics and welfare Polar cod were sampled and processed according to and within national and international guidelines for the use and care of animals. Sampling in US waters was approved by the Institutional Animal Care and Use Committee of the University of Alaska Fairbanks.

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