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Seasonal pollutant levels in littoral high-Arctic amphipods in relation to food sources and terrestrial run-off☆

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ABSTRACT

Increasing terrestrial run-off from melting glaciers and thawing permafrost to Arctic coastal areas is expected to facilitate re-mobilization of stored legacy persistent organic pollutants (POPs) and mercury (Hg), potentially increasing exposure to these contaminants for coastal benthic organisms. We quantified chlorinated POPs and Hg concentrations, lipid content and multiple dietary markers, in a littoral deposit-feeding amphipod *Gammarus setosus* and sediments during the melting period from April to August in Adventelva river estuary in Svalbard, a Norwegian Arctic Aarchipelago. There was an overall decrease in concentrations of ∑POPs from April to August (from 58 ± 23 to 13 ± 4 ng/g lipid weight; lw), Hg (from 5.6 ± 0.7 to 4.1 ± 0.5 ng/g dry weight; dw) and Methyl Hg (MeHg) (from 5 ± 1 to 0.8 ± 0.7 ng/g dw) in *G. setosus*. However, we observed a seasonal peak in penta- and hexachlorobenzene (PeCB and HCB) in May (2.44 ± 0.3 and 23.6 ± 1.7 ng/g lw). Sediment concentrations of POPs and Hg (dw) only partly correlated with the contaminant concentrations in *G. setosus*. Dietary markers, including fatty acids and carbon and nitrogen stable isotopes, indicated a diet of settled phytoplankton in May–July and a broader range of carbon sources after the spring bloom. Phytoplankton utilization and chlorobenzene concentrations in *G. setosus* exhibited similar seasonal patterns, suggesting a dietary uptake of chlorobenzenes that is delivered to the aquatic environment during spring snowmelt. The seasonal decrease in contaminant concentrations in *G. setosus* could be related to seasonal changes in dietary contaminant exposure and amphipod ecology. Furthermore, this decrease implies that terrestrial run-off is not a significant source of re-mobilized Hg and legacy POPs to littoral amphipods in the Adventelva river estuary during the melt season.

1. Introduction

Long range transport and remobilization of persistent organic pollutants (POPs) and mercury (Hg) are of environmental concern in the Arctic (AMAP, 2011, 2017; Macdonald et al., 2000). POPs bioaccumulate in lipid deposits and biomagnify in polar food webs where they can cause toxic effects (Borgå et al., 2004). The methylated form of Hg, MeHg, associates with proteins, undergoes bioaccumulation and biomagnification in aquatic food webs, with higher potency than inorganic Hg, and is also known to cause toxic effects (Kidd et al., 2012; Lavoie et al., 2013; Ruus et al., 2015).

While local sources of POPs exist in the vicinity of settlements, long-range transport is the dominant source of these semi-volatile contaminants in the Arctic (AMAP, 2004; Carlsson et al., 2018a). Mercury is

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ubiquitous in the environment, and there are important local sources in the Arctic, both natural and manmade, as well as distant sources, which contribute to the Arctic Hg load (AMAP, 2011; Drevnick et al., 2012; Schuster et al., 2018). Distant emissions of POPs and Hg reach the Arctic via long-range transport (AMAP, 1998, 2005; Macdonald et al., 2000; Pirrone et al., 2010), where they are deposited onto glaciers and permafrost, and become trapped. Hence, such cryospheric compartments may serve as terrestrial reservoirs for these persistent contaminants (Nizzetto et al., 2010). During the melt season, these contaminants may be re-mobilized in terrestrial run-off from glacial melt and permafrost thaw (secondary sources), and enter coastal environments (Blais et al., 2001; Bogdal et al., 2010; Garmash et al., 2013; Hermanson et al., 2005; Schuster et al., 2018). Because of the ongoing warming of the Arctic, terrestrial inputs of freshwater to the coastal zone in Svalbard have increased over the past four decades (Hanssen-Bauer et al., 2019). Such secondary sources of contaminants may increase annual seasonal exposure of POPs and Hg to coastal organisms in Svalbard (Kallenborn et al., 2012). Furthermore, the release of contaminants from secondary sources may effectively erode the current declining trend of POPs and Hg in top predators in the Arctic (Carlsson et al., 2018a,b; Rigét et al., 2019).

Strong seasonal changes in light intensity result in high seasonal variation in primary production (Ji et al., 2013) and associated patterns of feeding, growth and lipid storage adaptations in Arctic biota (Varpe, 2017). These inter-dependent drivers and relationships act as key controls for contaminant accumulation (Borgå et al., 2004). Seasonality in contaminant concentrations in Arctic pelagic and ice-associated invertebrates in Svalbard has also been previously documented (Borgå et al., 2004; Borgå et al., 2002; Hallanger et al., 2011; McGovern et al., 2022). However, earlier studies have not observed seasonal changes in concentrations of POPs for marine benthic invertebrates in Svalbard (Evenset et al., 2016). For Arctic coastal benthos, however, little is known about seasonal contamination, especially in relation to terrestrial run-off, even though these organisms are important contamination vectors to higher trophic level organisms in productive coastal food-webs that support important commercial and subsistence fisheries (Crossland et al., 2005; Evenset et al., 2016).

To address this knowledge gap, we investigated the role of terrestrial run-off as a secondary source of legacy POPs and Hg to littoral amphipods in the Adventelva river estuary in Svalbard, Norway. We hypothesized that the Adventelva river would be a source of remobilized contaminants, and that sediment contaminant concentrations would increase seasonally with increased runoff. In addition, we expected contaminant concentrations in the surface deposit-feeding amphipod *Gammarus setosus* (Legeżyńska et al., 2012, 2014) to be closely coupled with seasonal changes in sediment contaminant concentrations.

Sediments and amphipods were sampled monthly from April (before the melt season) to August (during the late melt season) and analyzed for concentrations of POPs (including pentachlorobenzene (PeCB), hexachlorobenzene (HCB), and polychlorinated biphenyls (PCBs)) and Hg (total Hg and MeHg (only amphipods)). To understand contamination sources, the contaminant concentrations in amphipods were assessed in relation to dietary tracers (fatty acids and stable isotopes), life history traits (lipid weight and wet weight), location, river influence (turbidity) and surface sediments.

2. Methods and materials

2.1. Sampling location

Two stations (200 × 150 m) were studied in the Adventelva river estuary close to Longyearbyen, a small settlement with ~2400 inhabitants on the West coast of Svalbard (Fig. 1). The City station (78°13.465′N 15°40.213′E) and the North station (78°14.455′N

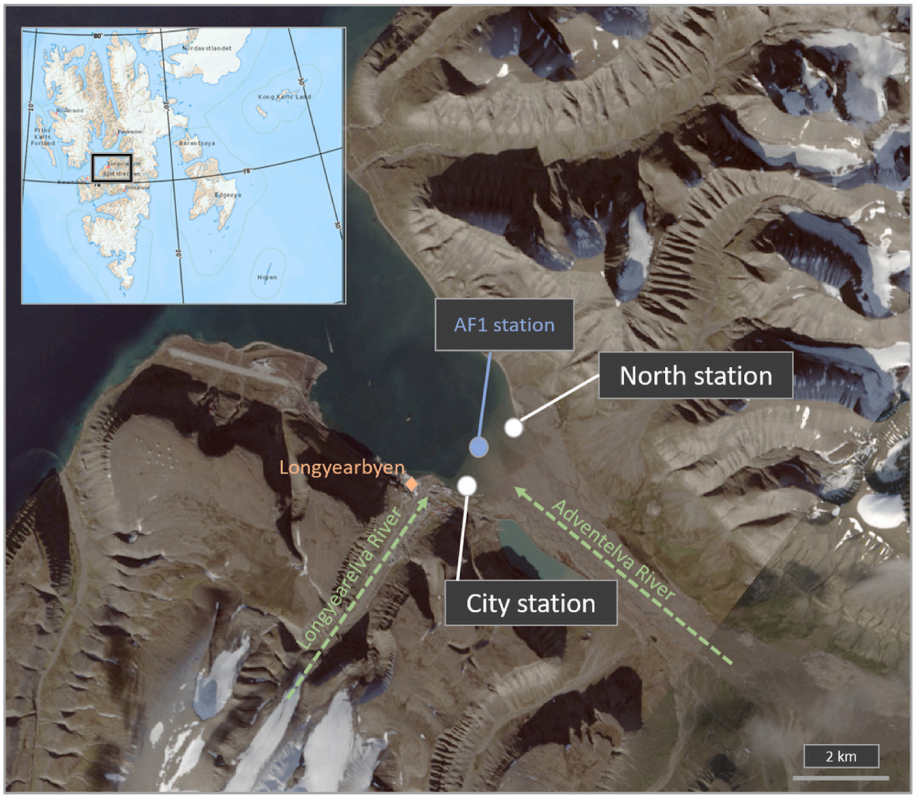

Fig. 1. Upper left corner: Map of Svalbard with Adventfjorden highlighted. Right corner: Map of Adventelva river estuary with Longyearbyen, Adventelva, Longyearelva, AF1 station, the City and North station highlighted. AF1 station was collected by McGovern et al. (2020) during the same field campaign. Courtesy of the Norwegian Polar Institute©.

15°43.244′E) are positioned in the littoral zone on both sides of the outlet of the Adventelva river, a glacier-fed braided river that gives way to tidal flats in inner Adventfjorden. Both the City and North stations receive inputs from the Adventelva river, while the City station is also impacted by the smaller, glacier fed Longyearelva River. Discharge in the Adventelva river is dominated by snowmelt in May/June, followed by a peak in glacial runoff in July/early August, giving way to increased importance of rainfall during late summer and early autumn (McGovern et al., 2020; Nowak et al., 2021). Sedimentation rates on the tidal flats in Adventfjorden during the melt season are high (~1000 $g/m^{-2}d^{-1}$ July), and resuspension and redeposition and events, driven by tides, cause mixing of sediment layers and turbid waters (Zajączkowski et al., 2007). In April 2018, both rivers were still frozen with no observed discharge to the Adventelva river estuary. The substrate at both stations includes mudflats with varying degrees of sand interspersed with larger rocks and overlaying turbid water during high tide (Wesławski et al., 2011).

2.2. Sample collection and processing

The Arctic gammarid amphipod *G. setosus* inhabits the littoral zone of Svalbard fjord systems, where it lives in close contact with the sediments (Wesławski, 1994). As one of the most abundant littoral macro-organisms in Svalbard fjords, *G. setosus* is an important food source for other macroinvertebrates, fish, and birds (Väinölä et al., 2008; Wesławski, 1990; Węsławski et al., 2018). *G. setosus* is a deposit-feeder with a life span of 2.5–4 years and typically spends 2 years in an immature state, before maturing and reproducing (Legeżyńska et al., 2014; Wesławski et al., 2002).

Sampling occurred monthly at the City and North station from April to August 2018 during low tide, when rocks, beneath which *G. setosus* can be found (Carlsson et al., 2018b; Weslawski et al., 1999), emerge in the eulittoral zone. Whole specimens of *G. setosus* were pooled to obtain sufficient biomass for each replicate. Within each station area, replicates (2–4 per station; Table S1, Supplemental Information) were collected from non-overlapping sub-areas with a 10-m radius. In May, only the City station was sampled (no specimens found at the North station). In June, while amphipods were collected for Hg and dietary markers, there was insufficient material for POPs analyses.

After collection, amphipods were placed in filtered seawater from Adventfjorden for gut evacuation (~4 °C for 12–24 h in the dark), and body size (tip of the rostrum to end of the telson) was recorded. A random subsample of amphipods from each replicate was used for species identification using a stereo microscope following Tzvetkova (1972). With the species confirmed, amphipods from each replicate were frozen for POPs, Hg, MeHg and stable isotope (SI) analysis (−20 °C), and fatty acid (FA) analysis (−80 °C; Table S1). Equal numbers of second- and third-year amphipods (11–25 mm and 26–34 mm, respectively) were assigned to each sample and analysis to minimize confounding ontogenetic differences in contamination (Borgå et al., 2004). Juveniles (individuals <10 mm;Wesławski et al. (2002)) were removed.

Surface sediments (depth: 0–1.5 cm) were collected from the station replicates using a stainless-steel spoon, and split for POPs, Hg and SI analysis (frozen at −20 °C; Table S1).

2.3. Laboratory analysis

2.3.1. Dietary markers

Stable isotopes of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$), as well as total carbon (C) and nitrogen (N) content were determined for amphipods and sediments at the University of California, Davis (UC Davis Stable Isotope Facility, USA). As carbonate removal through acidification can affect $\delta^{15}N$ values (Carabel et al., 2006; Søreide et al., 2006), two subsamples were run in parallel, one untreated sample for determination of $\delta^{15}N$ and N content, and one acidified sample for determination of $\delta^{13}C$ and organic carbon content. Amphipods and sediments were pretreated and acidified according to McGovern et al. (2018) and Harris et al. (2001), respectively, and measured using an elemental analyzer with an isotope ratio mass spectrometer. Long-term standard deviations at UC Davis are ±0.2‰ for ^{13}C and 0.3‰ for ^{15}N. Stable carbon and nitrogen isotope values are presented using delta notation, relative to international standards (Vienna PeeDee Belemnite for C, and atmospheric N for nitrogen) (Fry, 2006). Total organic carbon (TOC) content (%) in acidified sediment samples was used for TOC-normalization of contaminant concentrations.

Fatty acid (FA) analysis in amphipods was carried out at Ryerson University (Toronto, Canada). In short, total lipids in lipolyzed and homogenized amphipods were extracted (Folch et al., 1957) with chloroform:methanol (v:v/2:1). Subsamples of the total lipid extracts were used to determine lipid content gravimetrically. The FA were derivatized using the sulfuric acid in methanol (v:v/1%) method. Following derivatization, FA were quantified using a Shimadzu GC-2010 plus, with an AOC-20i/s auto sampler and twin auto injectors, together with Shimadzu LabSolutions software which identifies and quantifies FA by referencing them to retention times of FA from a series of standards (GLC 463, GLC 68E, and 23:0, NuChek Prep., Waterville, MN, USA). In total, 38 FA (Table S2) were analyzed (mean ~80% recovery and methylation efficiency using tricosanoic acid (23:0) as an internal standard).

2.3.2. Contaminant analyses

Chlorobenzenes (PeCB, HCB) and PCBs (PCB-28, -52, −101, −118, −138, −153, −180) in amphipods and sediments were analyzed at the Norwegian Institute for Water Research (NIVA; Oslo, Norway) as described, for sediments, in Johansen et al. (2021), with some adjustments made for analysis of biotic samples. In brief, organochlorines in thawed, homogenized amphipods and freeze-dried sediments were extracted with cyclohexane: isopropanol (v:v/1:1) and cyclohexane: dichloromethane (v:v/1:1), respectively, aided through ultra-sonication. Amphipod extracts were used to determine lipid weight gravimetrically until constant weight (±0.005 g) was reached. Lipid weight was used for lipid-normalization of organochlorines concentrations. Thereafter, both extracts were cleaned with sulfuric acid (96%) and further clean-up of sediment extracts with gel permeation chromatography (Agilent Technologies, 1260 infinity II equipped with a GPC column). Separation and quantification of organochlorines were carried out by a gas chromatograph connected to a triple quadrupole mass spectrometer (GC-MS/MS). The internal standard contained PCB-30, -53 and −204 and related to an eight-point calibration curve for each compound. Recoveries of certified reference materials (CRMs) NIST (1944 New York/New Jersey waterway sediment) and NIST (2974a *Mytilus edulis* tissue) were between 60 and 82%, however, PCB-28 in amphipods had a consistent low recovery mean of 45% and was not recovery corrected. PeCB was not included in the CRMs, however it is expected to have similar recoveries as HCB based on similar physical properties and was therefore kept in this study.

Analysis of total Hg (Hg) in freeze-dried and homogenized amphipods and sediments was performed at the University of Oslo (UiO; Oslo, Norway), using a direct mercury analyzer (DMA-80 Milestone). Recoveries of CRM NRC (DORM-4 fish muscle) were between 88 and 92%. Variation in Hg concentrations between duplicate amphipod and sediments samples were on average 4% (range 1–7%) and 27.5% (range 4–46%), respectively.

Analysis of MeHg in amphipods was performed at Stockholm University (SU; Stockholm, Sweden), following Braaten et al. (2014). In short, MeHg was extracted from freeze-dried and homogenized amphipods via digestion with nitric acid (30%). Following extraction, the extracts were neutralized with potassium hydroxide (45%) and buffered with sodium acetate buffer. Sodium tetraethyl borate solution was added to the extracts for ethylation, and MeHg was analyzed on a 2700 Methyl Mercury Auto-Analysis System (Tekran Corporation). Recoveries of CRMs NRC (DORM-4 fish muscle) and NRC (TORT-2 lobster

hepatopancreas) and spiked samples were between 91 and 112% and 90%, respectively. Variation in MeHg concentrations between duplicate samples was on average 24.8% (range 2–44%).

All sediment POP and Hg concentrations are reported on a dry weight (dw) basis, and where concentrations have been normalized for sediment TOC content this will been noted (TOC). All amphipod POP concentrations are presented on lipid weight (lw) basis (if not otherwise specified), while Hg and MeHg concentrations are reported on a dry weight (dw) basis.

2.4. Data treatment and statistical analyses

2.4.1. Dietary markers and wet weight

The organic C:N molar ratios in the amphipods exceeded 3.5, and ranged from 3.8 to 6.1, indicating high and variable lipid content, which can lead to bias in amphipod $\delta^{13}C$ values, since lipids are often depleted in $\delta^{13}C$ relative to proteins and carbohydrates (Post et al., 2007). To correct for the influence of lipid content on amphipod $\delta^{13}C$ values, these values were adjusted based on C:N values as described by Post et al. (2007).

Specific individual FA, as well as sums or ratios of FA proportions, are commonly used to interpret FA data as indicators of major carbon sources. For example, the relative proportion of ∑C18PUFA +22:6n-3 has been used to indicate a diet of flagellates (flagellate marker) (Falk-Petersen et al., 1998), ∑16:1n-7 + 20:5n-3 to indicate diatoms (diatom marker) (Dalsgaard et al., 2003; Søreide et al., 2008), ∑15:0 + 17:0 + 17:1 to indicate bacteria (bacteria marker) (Findlay et al., 1990; Guckert et al., 1985), 20:4n-6 indicate macroalgae (macroalgae marker), and 22:0 to indicate terrestrial material (terrestrial marker) (Dalsgaard et al., 2003). Low proportions of ∑PUFA indicate low quality carbon sources and the opposite indicate high quality (Dalsgaard et al., 2003). These FA-based dietary markers (FA markers) were used in multivariate correspondence analysis (CA) to interpret the relative importance of different carbon sources through the season and analysis of the contaminant variation in *G. setosus*.

Mean wet weight (ww) (g) per amphipod was calculated by weighing pooled amphipods and dividing with the number of specimens, intended for POP and Hg analysis, respectively.

2.4.2. Statistical analysis

All statistical analyses were performed using the software R (version 4.0.4, R core team 2021) with the α-level set at 0.05. Organochlorines were quantified above the detection limit in 78–100% of the amphipod samples, depending on the compound, and 100% of the sediment samples and all compounds were included in the data treatment. Values below the limit of detection were substituted using a distribution-based (beta distribution) multiple imputation method (Baccarelli et al., 2005). Outliers, identified by Cooks distance, were excluded from statistical analysis.

To evaluate spatial differences of SI within months, and monthly differences in contaminants (log10 (x+1) lw/ww) and SI for biota, several statistical testes were applied depending on residual distributions and variance (evaluated using diagnostic plots; Whitlock et al., 2015). For normal residual data with homogenic variance, a *t*-test was used. For normal residual data with heterogenic variance, a Welch test (*t*-test or ANOVA depending on group number) was used, followed by with a Games Howell post-hoc test if there were more than two groups. For non-normal residual data and heterogenic variance, the Kruskal-Wallis rank sum test was used (Whitlock et al., 2015). Since the North station only had 2 observations of SI for July, this month was excluded when testing within month spatial variations. Pearson's correlation coefficient or Spearman's rank test were used to evaluate correlations between concentrations of contaminants in amphipods and sediments (including TOC-normalized), monthly changes of $\delta^{13}C$ in amphipods (Whitlock et al., 2015).

To avoid redundancy among variables used for hypothesis testing, a subset of the dietary tracers (FA markers and SI) was included in statistical analyses. $\delta^{13}C$ was excluded because the FA makers provide more detailed information on specific food sources utilized. Variance inflation factors (VIF), which measure the extent of multicollinearity, identified the macroalgae FA marker as highly collinear with other FA markers, and this marker was therefore excluded. All other FA markers were retained (VIF <2).

Linear regression models were used to analyze ($\log_{10}$ (x + 1) transformed) Hg and MeHg (ng/g dw) in amphipods during reverse model selection. Explanatory variables included life history traits (mean ww per amphipod), between station differences (station), river influence (turbidity (NTU) at a nearby station (AF1; Fig. 1) sampled during the same field campaign (McGovern et al., 2020)), and FA-based dietary markers (flagellate-, bacteria-, diatom-, and terrestrial markers (expressed as % of total FA: % TFA)). Continuous explanatory variables were standardized with the mean of the variable (not those on a percentage basis) and the FA markers were scaled (each value was subtracted by the mean and divided by the sd).

Multivariate analysis was performed using the 'vegan' package (Oksanen et al., 2017) in R. This package provides tools for ordination and constrained analyses including constrained correspondence analysis (CCA), principal component analysis (PCA) and redundancy analysis (RDA). CCA was performed on FA compositional profiles (% TFA) in amphipods and with the explanatory variables Month, $\delta^{13}C$ (‰) and $\delta^{15}N$ (‰) in amphipods. PCA and RDA were conducted on ($\log_{10}$ (x + 1) transformed) POP concentrations in amphipods (ng/g lw) for variation exploration and hypothesis-testing by constraining the ordination with explanatory variables, respectively (Greenacre et al., 2013). Continuous explanatory variables were standardized with the mean of the variable (not those on a percentage basis) and the FA markers were scaled. In the PCA and RDA, explanatory variables included between station differences (station), river influence (turbidity) and FA-based dietary markers (flagellate-, bacteria-, diatom-, and terrestrial marker (% TFA)). To account for the variance from life history traits (lipid content and mean ww per amphipod), the ordination was constructed on lipid-normalized concentrations with the variance from weight of amphipods as a conditioned variable (covariate). During model selection using CCA and RDA, constraining variables were selected using forward model selection with a double-stopping criterion to prevent overestimation of the variance explained and inflated type 1 error (Blanchet et al., 2008).

Since replicates analyzed for response variables (SI, FA, POPs, Hg, MeHg; Table S1) differed, not all replicates could be included in all statistical analysis, and therefore during hypothesis testing, the total number was limited by the variable with the lowest replication.

3. Results

3.1. Seasonality in contaminant concentrations

3.1.1. Amphipods

HCB was the dominant contaminant in *G. setosus* (n = 22; 14.3 ± 8.2 ng/g lw), followed by PCB-153 (7.4 ± 5.2 ng/g lw). Within the study period, the ∑chlorobenzenes and ∑PCBs ranged from 1.3 to 27.2 and 4.5–70.3 ng/g lw, respectively (Fig. 2A and B and Table S3). Chlorobenzene concentrations had a concave pattern, increasing from April to May (∑ in May; 26.1 ± 1.4 ng/g lw; Welch's *t*-test; lw: p = 0.08, ww: p = 0.04), and then decreased to August (Welch's *t*-test; lw & ww p = <0.001; Fig. 2A and Table S3). In contrast, PCB concentrations declined from April (∑ 34.9 ± 23 ng/g lw) to August (∑ 8.2 ± 2.1 ng/g lw; Welch's *t*-test; lw p = 0.001, ww p = 0.02; Fig. 2B and Table S3).

Over the study period, Hg and MeHg in *G. setosus* (n = 33) ranged from 16 to 30 and 1–39 ng/g dw, respectively. Hg and MeHg concentrations typically decreased from April (24.3 ± 3.6 and 21.7 ± 8.5 ng/g dw, respectively) to August (17.5 ± 1.5 and 3.6 ± 2.7 ng/g dw, respectively; Fig. 2C and D and Table S3. However, May, June and July concentrations kept rather constant with a slight increase (Welch's

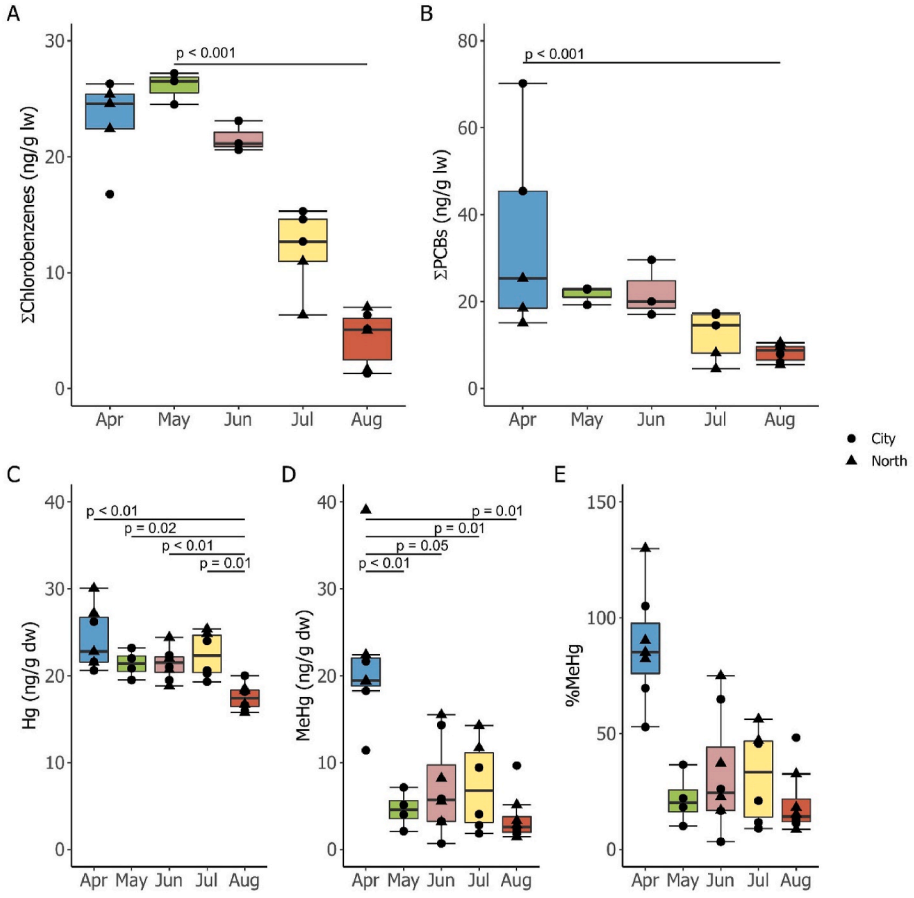

Fig. 2. Boxplots of concentrations of (A) ∑Chlorobenzenes (PeCB and HCB), (B) ∑PCBs (PCB-28, -52, −101, −118, −138, −153 and −180), (C) Hg and (D) MeHg by month (color) and station (symbol) in amphipods. POPs are reported in ng/g lw and Hg/MeHg in ng/€w. (E) Boxplot of the proportion of total Hg present as MeHg (%MeHg) by month (color) and station (City – circle, North - triangle) in amphipods. POPs; n = 22 and Hg/MeHg; n = 33. Box plots show the five-number summary of a set of data: including the minimum score, first (lower) quartile, median, third (upper) quartile, and maximum score. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

ANOVA; Hg and MeHg $p < 0.001$, followed by Games Howell post-hoc test, all months compared to August $p < 0.05$; all other comparisons $p = 0.34$–1 for Hg and all months compared to April $p <\leq 0.05$; all other comparisons $p = 0.53$–1 for MeHg). The proportion of total Hg present as MeHg (%MeHg) ranged from 3 to 130% (Fig. 2E and Table S3), and with a seasonal mean of 40 ± 32%.

3.1.2. Sediments

HCB was the dominant contaminant in sediments (n = 16; 0.07 ± 0.02 ng/g dw and 5.7 ± 1.9 ng/g TOC), followed by PCB-52 (0.04 ± 0.02 ng/g dw and 3.6 ± 2.3 ng/g TOC), and PCB-153 (0.03 ± 0.03 ng/g dw and 2.8 ± 2.5 ng/g TOC). Values for ∑POPs ranged between (0.08–0.55 ng/g dw and 5.2–43.4 ng/g TOC; Figure S1A-I and Table S4). Hg concentrations in sediments (n = 33) ranged from 21 to 76 ng/g dw (1.5–6.2 μg/g TOC) over the study period (Figure S1J and Table S4).

TOC content in sediments ranged from 0.4 to 1.9% over the study period with highest values in June at the City station and lowest values in July at the North station (Figure S2).

3.1.3. Correlations between sediments and amphipods

POP concentrations in sediments (both before and after TOC-normalization) and *G. setosus* were not significantly correlated (Pearson correlation; −0.14–0.48 $p > 0.05$; n = 16, includes both stations). However, within stations, PCB-101, -118, −138, −153 and −180 in *G. setosus* and sediments at the North station were correlated (Pearson correlation; 0.8–0.89 $p < 0.05$). Meanwhile, for the remaining compounds at the North station and for all the compounds at the City station, there were no significant correlations (Pearson correlation; −0.5–0.7 $p > 0.05$).

Concentrations of Hg in sediments were not correlated with Hg or MeHg in *G. setosus* (n = 33; includes both stations; Spearman's rank order correlation; −0.12–0.01 $p > 0.05$, respectively). However, TOC-normalized sediment Hg concentrations did correlate with Hg concentrations in *G. setosus* (Spearman's rank order correlation; 0.46–0.6, $p = 0.007$ and < 0.001, respectively; Fig. 4D). Although, within stations, a significant correlation between Hg in *G. setosus* and TOC-normalized Hg in sediments was only observed for the North station (Spearman's rank order correlation; 0.66, $p = 0.01$).

3.2. Seasonality in total lipid and wet weight

Total lipid content (% ww) in *G. setosus* displayed a concave pattern with highest values in June and July (Figure S3B) and ranged between 0.9 and 2.6% over the study period. Mean ww per amphipod (g) for both POP and Hg analysis ranged between 0.1 and 0.35 g and also showed a concave pattern over the study period (Figure S3C & D).

3.3. Seasonality in amphipod food sources

G. setosus SI values (n = 28 excluding July) were not significantly different between stations within months (T-test, Welch *t*-test; $p > 0.05$), with the exception of $\delta^{15}N$ values in August (Kruskal-Wallis; $p = 0.02$), and therefore the stations were pooled. The $\delta^{15}N$ values decreased from April to August (Spearman's rank order correlation = −0.85, $p < 0.0001$; n = 34). Whereas $\delta^{13}C$ displayed a concave pattern of higher values in May, June and July and lower in April and August (Figure S3A and Table S5), ranging from −18.9 to −22.0‰ (Welch ANOVA; $p = 0.03$, followed by Games Howell post-hoc test, June/August $p = 0.04$ and May/August $p = 0.07$; all other comparisons $p = 0.1$–0.8; n = 34).

The dominant FA in *G. setosus* (n = 23) throughout the study period were 16:0, 16:1n-7c, 18:1n-9c, 20:5n-3 and 22:6n-3, which together constituted almost 70% of the total FA (Table S2). The first two CA axes

(CA1 and CA2) for the analysis of *G. setosus* FA profiles (% TFA) accounted for 60% of the total variation in the data set (Fig. 3A). Canonical correspondence analysis (CCA) showed that the significant explanatory variable month explained 54% of the total constrained variation in the model (CCA, permutation tests 999, $p = 0.001$).

The pattern of FA markers showed a similar concave shape along CA2, as observed for $\delta^{13}C$ values, and a seasonal pattern with April and August differing from May, June and July. In May, June and July, there were higher contributions of the diatom marker (Σ16:1n-7, C16PUFA and 20:5n-3), with the greatest contribution in June, and higher proportions of bacteria and flagellate markers (Σ15:0 + 17:0 + 17:1 and ΣC18PUFA + 22:6n-3 respectively) in April and August. Proportions of terrestrial marker 22:0 increased throughout the season (Fig. 3A & S4B).

3.4. Drivers of contaminant seasonality in amphipods

The first two axes of the PCA of POP concentrations in *G. setosus* ($n = 22$) accounted for 91% of the total variation in the dataset (Fig. 3B). The RDA showed that the significant explanatory variables diatom and terrestrial markers explained 40% of the variation, each explaining 13.5% and 14.8%, respectively (RDA, permutation tests 999, $p = 0.001$). All POPs increased along PC1, while the proportional terrestrial marker decreased along PC1, thus a relative increase in terrestrial-derived carbon sources in *G. setosus*' diet, were associated with decreased POP concentrations (Figs. 3B & 4). However, a seasonal concave pattern is seen for the chlorobenzenes, with highest mean concentration in May (26.1 ± 1.4 ng/g lw), coinciding with a relative increase in the contribution of diatoms to amphipod diet (Figs. 2A & 4A).

Hg in *G. setosus* ($n = 22$) could not be explained by the explanatory variables included in the current study ($R^2 = 0.26$, $p = 0.127$). However, for MeHg, the bacterial marker was the only significant explanatory variable and explained 17% variation, with higher MeHg concentrations in *G. setosus* associated with higher dietary contributions of bacteria ($R^2 = 0.2$, $p = 0.05$; Fig. 4C).

4. Discussion

4.1. Contaminant burden in amphipods

The overall ΣPOP concentrations in our study for *G. setosus* are low, and are within, or are one to two orders of magnitude lower, than other Arctic benthic invertebrates (Svalbard; Evenset et al., 2016; Hop et al., 2001; White Sea area; Muir et al., 2003) and fish (cod and sculpin from Svalbard) (Evenset et al., 2016). Amphipods are several orders of magnitude lower than glaucous gulls from Svalbard, which are high up in the food change (Sagerup et al., 2009; Table S6). Hg and MeHg concentrations in *G. setosus* from this study are within the same order of magnitude as those observed for littoral gammarid amphipods from the Canadian Arctic and sub-Arctic (Van Der Velden et al., 2012;Table S6). %MeHg in *G. setosus* from the current study are similar to those reported for other invertebrate primary consumers (Morel et al., 1998; Ruus et al., 2015). In the Arctic, %MeHg shows a wide range in zooplankton and changes seasonally (Ruus et al., 2015; Stern et al., 2005).

4.2. Seasonality in contaminant burden in amphipods and sediments

Assuming that diet is the primary exposure route for contaminants such as POPs and Hg (Boese et al., 1990; Kaag et al., 1997; Lawrence et al., 2001; Tsui et al., 2004), and that deposit-feeding amphipods have a diet based on organic matter deposited in the sediments (Legeżyńska

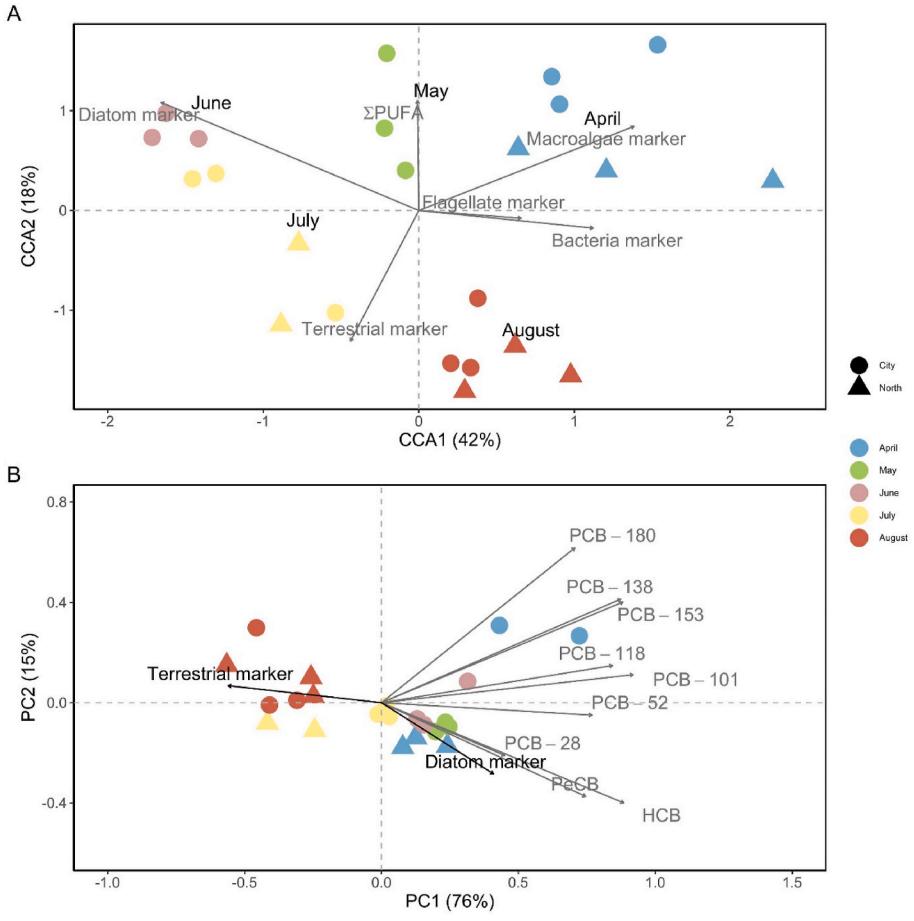

Fig. 3. (A) Constrained correspondence analysis (CCA) plot of fatty acid (FA) compositions (% total fatty acid (TFA)) in amphipods ($n = 23$). The site scores represent individual amphipod samples. The significant categorical variable month is shown as black centroids and the dietary FA markers (% TFA) are visible as grey arrows. (B) Principal component analysis (PCA) of POP concentrations ($\log_{10}(x+1)$ ng/g lw) (visible as black arrows) in amphipods ($n = 22$) with significant continuous dietary explanatory variables, terrestrial and diatom FA markers (% TFA), passively placed on top of the ordination as black vectors. The site scores represent individual amphipod samples. Color and symbol represent month (April – blue, May – green, June – purple, July – yellow, August – red) and station (City – circle, North – triangle), respectively. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

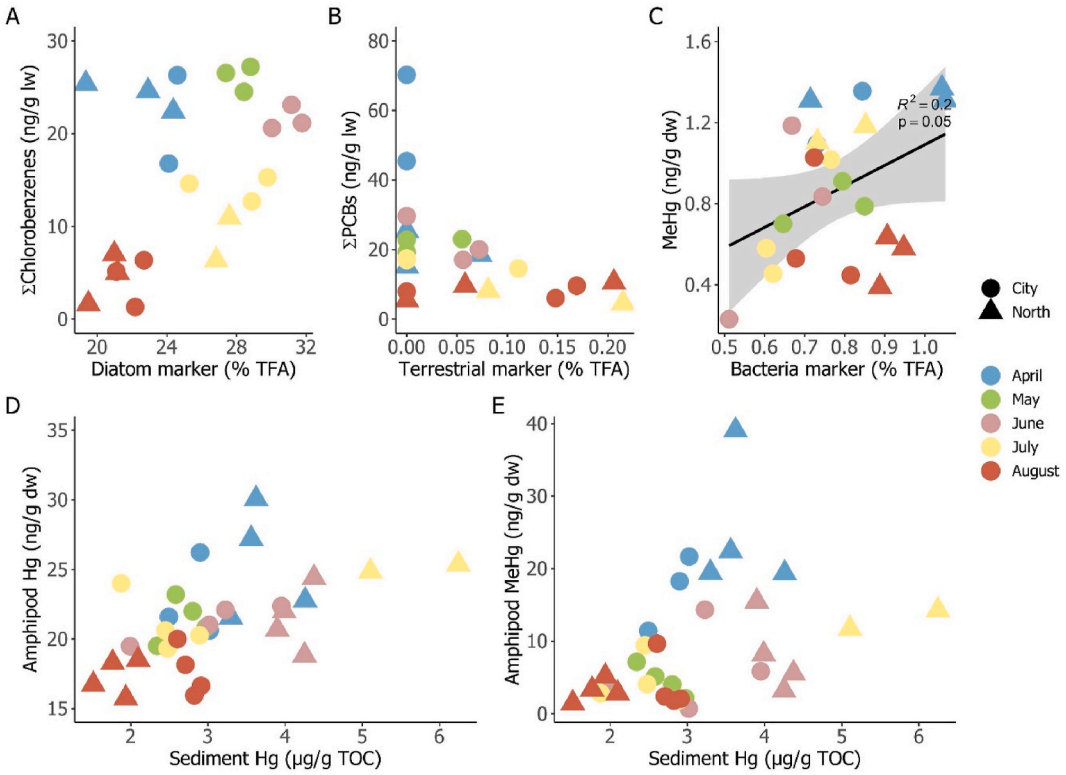

Fig. 4. Scatterplot of concentrations (ng/g lw) of (A) ∑Chlorobenzenes (PeCB and HCB) and (B) ∑PCBs (PCB-28, -52, −101, −118, −138, −153 and −180) by diatom and terrestrial FA marker (% total fatty acid (TFA)), respectively, in amphipods (n = 22). (C) Linear regression of MeHg concentration (log 10 + 1 ng/g dw) and bacteria FA marker (% TFA) in amphipods ($R^2 = 0.2$, p = 0.05, n = 22). The grey area is the 95% confident interval. Scatterplot of (D) Hg concentration (ng/g dw) in amphipods and sediment Hg (µg/g TOC) and ϵ MeHg concentration (ng/g dw) in amphipods and sediment Hg (µg/g TOC; n = 33). Color and symbol represent month (April – blue, May – green, June – purple, July – yellow, August – red) and station (City – circle, North - triangle), respectively. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

et al., 2012, 2014), contaminant concentrations in *G. setosus* were expected to reflect seasonal concentration patterns of sediment-adsorbed POPs and Hg. However, the amphipods were only partially predicted by the sediment's concentrations, as observed at the North station for PCB-101, -118, −138, −153 and −180 in sediments as well as TOC-normalized Hg in sediments.

A partial (or complete) mismatch could suggest that amphipods were not in a steady state with the sediments, or that processes such as bioavailability, elimination, partial retention, biomagnification etc. (Borgå et al., 2004) are taking place, perhaps distorting direct time-for-time correlations. Contaminant mismatch between the environment and biota has been reported elsewhere, e.g. Hallanger et al. (2011) found a seasonal (May–October) mismatch between dissolved POPs in the water phase and POP concentrations in zooplankton in Kongsfjorden. Furthermore, Josefsson et al. (2011) observed no correlation between a surface-deposit feeding amphipod with water or sediment POP concentrations. A similar lack of correlation has also been observed for Hg and MeHg concentrations between sediments and biota (Buckman et al., 2019; Lawrence et al., 2001; Taylor et al., 2012; Taylor et al., 2019). Recent experimental work has shown that MeHg concentrations in deposit feeding amphipods tend to be more strongly correlated with MeHg concentrations in the water than in the sediments (Buckman et al., 2019), and that the main source of Hg to amphipods tends to be settling pelagic organic material (Jonsson et al., 2014). Therefore, the lack of correlation between sediments and deposit-feeding amphipods, which feed on freshly deposited material from the pelagic zone, is likely because the bulk surface sediment samples analyzed in this study are not representative of the pelagic organic material that these amphipods utilize (Buckman et al., 2019). This is likely to be the case in our Arctic estuarine study system, where glacier meltwater delivers high loads of terrestrial inorganic and organic particulate matter into the coastal environment (Johansen et al., 2021; Zajączkowski et al., 2007), where they settle out and can dilute or bury the contaminant signal from recently deposited pelagic material.

4.3. Seasonality in carbon sources

In Adventfjorden, the spring phytoplankton bloom typically occurs in May (Wiedmann et al., 2016), and is dominated by diatoms (Hegseth, 1998), which result in seasonal biomass fluxes of phytoplankton to the benthic community (Grebmeier et al., 1988; Thompson et al., 2008). Indeed, a parallel study confirmed a spring phytoplankton bloom in May, with observations of enriched $\delta^{13}C$ values in pelagic particulate organic matter, alongside depleted nutrient concentrations (McGovern et al., 2020). We observed the highest mean $\delta^{13}C$ values in May, coinciding with this spring bloom, and suggesting that these surface-deposit feeding amphipods were utilizing freshly deposited marine carbon (McGovern et al., 2020; Peterson et al., 1987). Results of FA analysis further suggest that this marine carbon utilization was largely based on diatoms, as diatom FA marker had the highest relative contribution in *G. setosus* in May–July, coinciding with the enrichment in $\delta^{13}C$ values (Peterson et al., 1987).

Meanwhile, depletion in $\delta^{13}C$ values alongside increasing proportions of bacterial FA marker and decreased relative proportions of PUFA in G. *setosus* in June, July and August, illustrate the shift away from diatoms to lower quality, "re-worked" carbon sources later in the summer (Dalsgaard et al., 2003). The increased contributions of the macroalgae and flagellate markers in April, July and August, suggest that flagellate- and macroalgae-derived detritus are relied on both before and after the spring bloom (Legeżyńska et al., 2012; Søreide et al.,

2008). Furthermore, increased contributions of the FA 22:0 alongside depleted $\delta^{13}C$ values indicate that, in addition to flagellates and macroalgae, terrestrial material was also utilized by *G. setosus* in July and August when terrestrial run-off strongly influences the Adventelva river estuary (McGovern et al., 2020).

These findings agree with previous studies, which have documented macroalgae-derived detritus and dinoflagellates in the gut contents of *G. setosus* (Legeżyńska et al., 2012), as well as observed terrestrial organic matter assimilation by various estuarine fauna, including mysids and amphipods (Dunton et al., 2012; Dunton et al., 2006; Harris et al., 2018). The strong seasonal variation in resource utilization by *G. setosus* also highlights their connectivity to the pelagic environment and potentially associated contaminant pathways.

4.4. Drivers of seasonality in contaminant burden in amphipods

4.4.1. Winter

Contaminant concentrations were high in amphipods in April, when ice covered much of the intertidal zone (pers. obs.; E. Skogsberg). This finding agrees with previous studies which have observed higher POPs and Hg concentrations in the dissolved phase in winter when sea-ice and cold temperatures trap compounds in the water column and prevent volatilization to the atmosphere (Hargrave et al., 2000; Sommar et al., 2010). High dissolved concentrations in the water column then allow for increased food web uptake.

Uptake of Hg, however, is also affected by Hg speciation, with MeHg known to most rapidly bioaccumulate and biomagnify in aquatic food-webs (Lavoie et al., 2013). We observed the highest MeHg concentrations in amphipods in April, alongside the highest observed %MeHg, potentially suggesting that methylation was occurring in the sediments (Fitzgerald et al., 2007), or through the winter months before ice melt (Hines et al., 2012). Furthermore, our results highlight the connection between amphipod MeHg concentrations and food-web dynamics. Contributions of bacterial FA marker alone were significant in explaining amphipod MeHg concentrations, indicating that in April, while background MeHg concentrations may have been higher, the concentrations in amphipods may also be a result of reliance on the microbial based food-web, which has a greater number of trophic levels and thus allows for increased biomagnification (Jonsson et al., 2017).

Additionally, during cold winter periods, when organisms are consuming low quality food sources, experience starvation and low growth, it is typical to find higher contaminant levels compared to the summer months when somatic growth rates are higher resulting in growth dilution (Frantzen et al., 2011; Moreno et al., 2014; Nyberg et al., 2015; Wright et al., 1999).

4.4.2. Snowmelt and diatoms

The utilization of diatoms by *G. setosus* coincided with a seasonal peak (in May) in concentrations of chlorobenzenes in *G. setosus*, suggesting that the spring bloom was a source of these contaminants. Settling phytoplankton represent an important pathway for the vertical flux of both POPs and Hg to the benthic habitat due to high bioconcentration rates in phytoplankton (Everaert et al., 2015; Söderström et al., 2000; Zaferani et al., 2020).

Furthermore, concurrent with the phytoplankton spring bloom in May in Adventfjorden in 2018 (McGovern et al., 2020), the Adventelva and Longyearelva rivers started running, suggesting that terrestrial contamination from secondary sources may contribute to the overall contaminant load in surface waters (Blais et al., 2001; Bogdal et al., 2010; Bogdal et al., 2009; Kallenborn et al., 2012). However, runoff during the early melt season is mainly comprised of snowmelt from the previous winter's precipitation, rather than permafrost thaw or glacial meltwater (Nowak et al., 2021). Thus, phytoplankton in the estuary, close to the river outlets in May, were likely exposed to contaminants from snowmelt and the associated "seasonal reservoir" of contamination from long-range transport (dry and wet) and/or local emission deposited during the winter season (Cabrerizo et al., 2019). Phytoplankton may subsequently sequester this contamination and transport it to the benthic community during periods of high biotic uptake and sedimentation, as is typical during the spring bloom (Everaert et al., 2015; Nizzetto et al., 2012). Similarly, in alpine lakes, elevated PCB concentrations have been observed in fish and the water column in spring (Nellier et al., 2015; Perga et al., 2017) and in the Canadian Arctic, snowmelt in rivers displayed higher concentrations of POPs compared to later in the season (Cabrerizo et al., 2019).

In addition to POPs, dissolved Hg has also been measured in higher concentrations during the snowmelt period in many Arctic rivers (Dommergue et al., 2010; Douglas et al., 2017; Mu et al., 2019), including the Adventelva river (Poste, unpublished data). In our study, Hg and MeHg in amphipods did not display the same seasonal peak with snowmelt in May as the chlorobenzenes, but decreased from April to May, remained consistent through May, June and July, and decreased again in August. Positive relationships between Hg in *G. setosus* and TOC-normalized sediment Hg concentrations, and higher contributions of diatom FA marker to the diet in May, June and July, suggest that large inputs of setting phytoplankton from the spring bloom may represent an Hg-enriched source of deposited organic matter to the sediments (Outridge et al., 2007; Zaferani et al., 2020), leading to sustained Hg concentrations in amphipods selectively feeding on these diatoms in the weeks that follow.

4.4.3. Contaminant dilution

With the hypothesis that increasing terrestrial run-off would deliver re-mobilized legacy contaminants to the coast (Blais et al., 2001; Kallenborn et al., 2012), amphipods were expected to increase in contamination from April to August. While we did observe strong seasonal changes in contaminant burden for POPs and Hg in amphipods, the overall seasonal decrease in contaminants was unexpected. The overall decrease in *G. setosus'* contaminant concentrations from April, with no terrestrial run-off, to August, after several months of terrestrial run-off, coincided with a dietary shift from diatoms to terrestrial material, as seen for zooplankton from a recent study in Isfjorden (McGovern et al., 2022). This suggests that terrestrial material is less contaminated than marine phytoplankton, causing a seasonal 'dilution effect' in *G. setosus*, which could be a result of less contamination in the ambient environment later in the season (seasonal 'dilution effect' of the estuary). Indeed, Arctic rivers have been shown to contain declining POP concentrations (dissolved and particulate) as the melting season progresses (Cabrerizo et al., 2019).

For Hg a recent study from Svalbard found terrestrial inputs to be associated with Hg contamination in the sediments (Kim et al., 2020), and preliminary results from a parallel study indicate that concentrations of inorganic Hg in the Adventelva and Longyearelva rivers were higher than in the fjord, with a seasonal transition from dissolved to particulate-associated Hg from spring snowmelt to summer flow with high glacier meltwater contribution (Poste, unpublished data). Despite evidence of a terrestrial-Hg source to the fjord during the melt season, and results suggesting amphipod utilization of terrestrial carbon sources in July and August, we did not observe a concurrent seasonal increase in these amphipods. These finding suggest other physical processes are at play that may remove Hg from the water column or limit its bioavailability.

Furthermore, Svalbard rivers transport large amounts of inorganic particles from glacial melt and terrestrial organic material to the coast where they contribute to substantial sedimentation rates in river estuaries (Zajączkowski, 2008). Because Hg and POPs have a high affinity for organic and inorganic particles (Black et al., 1988; Carlberg et al., 1986; Larsson et al., 1992; Pierard et al., 1996), high sedimentation rates are an effective removal-pathway for these contaminants from the water column to the sediments, where they can become quickly buried (Kirk et al., 2012). In addition, increased temperatures during summer, amplified in this turbid estuary by high absorption of solar radiation by

suspended particulate matter, may facilitate net volatilization of POPs and Hg to the atmosphere (Cabrerizo et al., 2019; Douglas et al., 2012). Furthermore, although amphipod lipid content and ww were not significant predictors of contaminant concentrations in *G. setosus*, they may still contribute to the overall seasonal contaminant decrease, through growth dilution and/or removal through molting, both of which increase with higher temperature for amphipods (O'Callaghan et al., 2020; Pöckl, 1992).

5. Conclusions

We observed a peak in chlorobenzene concentrations in May, alongside high dietary reliance on diatoms, indicating that settling phytoplankton is likely a contaminant source of snow and ice-melt derived chlorobenzenes to *G. setosus*. Overall, amphipod POPs and Hg concentrations decreased through the melt season, likely due to enhanced volatilization with increased temperatures, as well as removal from the water column through sedimentation. Furthermore increased particle-bound Hg, a seasonal dilution of POPs with river influence in the estuary, which impact amphipod contamination indirectly via lowering concentrations in food sources, likely contributed to the seasonal contaminant decrease. Our study reveals that mobilization and transport of legacy POPs and Hg from melting glaciers and thawing permafrost does not necessarily lead to increased concentrations in littoral amphipods during the melt season. Contaminant concentrations in *G. setosus* appear to respond to a range of seasonal changes in our study system, related to contaminant exposure and amphipod ecology. Finally, our results highlight the importance of understanding the role of seasonality in costal contamination cycling and food web accumulation.

Credit author statement

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2022.119361.

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