Microplastic accumulation by tube-dwelling, suspension feeding polychaetes from the sediment surface: A case study from the Norwegian Continental Shelf

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ABSTRACT

Sediment samples (0–1 cm) and tube-dwelling polychaetes from the Norwegian Continental Shelf and the Barents Sea were collected, including areas close to oil and gas installations and remote locations. Microplastics (≥ 45 μm) were found in quantifiable levels in 27 of 35 sediment samples, from 0.039 to 3.4 particles/g_{dw} (dw = dry weight); and in 9 of 10 pooled polychaete samples, from 11 to 880 particles/g_{ww} (ww = wet weight). Concentrations were significantly higher in tube-dwelling polychaetes than sediments from the same locations (p<0.0097) by orders of magnitude. To quantify this factor increase in polychaetes, a Biota-Sediment Particle Enrichment Factor (BSPEF) is introduced, which ranged from 100 to 11000 g_{dw}/g_{ww} (280–31000 g_{dw}/g_{dw}). Higher microplastic levels were observed in polychaete tube than in soft tissue (n=4). The feeding behavior and life cycle of tube-dwelling polychaetes could have an important influence on the transport, distribution and food-chain dynamics of microplastics on the seafloor.

1. Introduction

Microplastics are distributed throughout the oceans, including remote areas such as deep sea sediments (Bergmann et al., 2017; Van Cauwenberghe et al., 2013) and Antarctica (Zarfl and Matthies, 2010), and they are found in a myriad of different marine organisms, ranging from zooplankton to whales (Cole et al., 2014; Nelms et al., 2019). The presence of these microplastics has caused concern because of the mechanical hazards to respiratory organs and the circulatory system (Browne et al., 2008; Cox et al., 2019; Farrell and Nelson, 2013; GESAMP, 2015; Kögel et al., 2019), as well as the potential leaching of additives (Vered et al., 2019). Recently it has been found that the presence of nano- and microplastics can adversely alter invertebrate populations in sediment (Redondo-Hasselerharm et al., 2020).

Due to the wide range of properties microplastics can have, including composition, size, shape, surface chemistry and degree of biofouling, different distributional patterns are expected to occur among water, sediment and biota as a function of these properties. For instance, low-density, non-colloidal polyolefin plastics will float under still conditions, whereas non-colloidal microplastics which are denser than water, such as nylon, polyethylene terephthalate (PET) and polyvinylchloride (PVC) will tend to sink and accumulate in sediments. However, low-density particles may sink over time due to biofouling (microorganism growth) and aggregation with denser particles (Bour et al., 2018; Galloway et al., 2017; Lobelle and Cunliffe, 2011; Morét-Ferguson et al., 2010; Van Cauwenberghe et al., 2013). The distribution and settling processes of microplastic in the marine environment is also impacted by ocean currents. Floating plastics have the potential to undergo

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long-range transport via currents and other hydrodynamic processes, while currents beneath the sea surface can transport less buoyant plastic. Globally, large-scale convergence zones of microplastic have been found at five oceanic gyres (North Atlantic, South Atlantic, South Indian, North Pacific and South Pacific) (Nerland et al., 2014).

Ocean sediments are hypothesized to be the major sink of microplastics (Van Cauwenberghe et al., 2015b). According to a review of microplastics in the marine environment from the year 2012 (Hidalgo-Ruz et al., 2012), values for microplastic abundances ranged from 0.21 to more than 77000 items/m^2 in sediment, which is several orders of magnitude higher than reported abundances at the sea surface (from 8×10^{-5} to 5 items/m^2), although this difference may also be affected by differences in sampling and analytical methodology.

Many unknowns remain about the impacts of microplastic on benthic ecosystems (Galloway et al., 2017). Though there is concern because the size range of microplastic makes them especially available for small-sized deposit and suspension feeders (Graham and Thompson, 2009; Van Cauwenberghe et al., 2015a). Sediment-dwelling organism's (infauna) exposure to microplastics is therefore of interest. Benthic infauna generally consists of low trophic level organisms, such as worms and mud crabs. In addition to affecting these organisms, the consumption of benthic infauna by higher trophic level organisms (e.g. haddock (Schückel et al., 2010) and flatfishes (Yeung and Yang, 2014)), introduces microplastics to the upper levels of the food chain.

With respect to understanding the effects of microplastics on sediment-dwelling organisms, polychaetes are particularly interesting, as they are worms that burrow or build tubes in sediments, and are found in most marine environments. Jang et al. (2018) investigated polychaetes that were able to burrow in abandoned expanded polystyrene (EPS) buoys at the coast of South Korea as their habitat, where they would both consume and produce a significant amount of microplastics. It was estimated that a single EPS dwelling polychaete can produce hundreds of thousands of microparticles per year. Further, Wright et al. (2013) have reported significantly reduced energy reserves by up to 50% for polychaetes maintained in sediments spiked with microplastics (unplasticized polyvinylchloride, UPVC) in laboratory tests. The results indicate that depleted energy reserves arise from a combination of reduced feeding activity, longer gut residence times of ingested material and inflammation (Wright et al., 2013).

Mathalon and Hill (2014) found microplastic in polychaete faecal casts and sediments from beaches along Nova Scotia. A recent study by Bour et al. (2018) investigated microplastics in polychaetes from the inner Oslo Fjord in Norway. They estimated one to two particles per individual. However, to the best of our knowledge, the presence of microplastics in sediment-dwelling polychaetes from deep-sea sediments has not been investigated. Taylor et al. (2016) found microplastics in several deep-sea organisms, including hermit crabs and sea pens, collected in the mid-Atlantic and Indian Oceans. Graham and Thompson (2009) showed through feeding trials that benthic sea cucumbers ingest significant amounts of plastic fragments. Still, information on microplastic in benthic organisms is scarce, and inconsistent reporting on concentration regarding size-distribution and methodology makes it difficult to compare different studies. Further, there is very little information comparing sediment concentrations of microplastic with benthic dwelling organisms, to evaluate if microplastic concentrations are higher in biota compared to surrounding sediments.

In this study, sediment and polychaetes that build and reside in tubes in sediment (tube-dwelling polychaetes), were sampled from the Norwegian Continental Shelf and the Barents Sea (Fig. 1), at areas nearby oil and gas activities as well as remote areas, and assessed for the presence of microplastic with a consistent method for sediment and polychaete analysis. To our knowledge, this is the first study comparing microplastic levels in polychaetes and surrounding sediments using a consistent method. To investigate the potential enrichment of microplastic in polychaetes relative to sediment from the same sampling stations, a new Biota-Sediment Particle Enrichment Factor (BSPEF) is introduced for the first time, and is defined as the particle concentration in biota divided by the particle concentration in the surrounding sediment. An initial comparison of microplastic abundance in polychaete soft tissues vs. tubes was also conducted.

2. Materials and methods

2.1. Site description and sampling method

In 2017, sediment samples (n = 35) and marine polychaetes (collected from 10 stations, 9 of which were the same as the sediment samples) from the Norwegian Continental Shelf (NCS) were sampled opportunistically as part of the annual sediment survey on behalf of the oil and gas industry, which compares stations from potentially influenced sites with sites that should not be influenced by this industry. As the objective of this study was to investigate different regions of the Norwegian Continental Shelf as opposed to differences between sampling stations per se, replicates from the stations were not included. Sampling stations were spread over three regions: the central North Sea, the northern North Sea and the Barents Sea (Fig. 1). These regions could be contaminated by anthropogenic marine activities (e.g. oil and gas, mining, fishing and shipping) as well as long-range transport sources via ocean currents. Site- and sediment specific information such as sampling station names, coordinates and sampling depth, sediment median grain diameter and total organic content (TOC), as well as an overview of the general large-scale currents in the sampling regions are provided in the Supplementary Information (SI, Tables S1–S4 and Figs. S1–S4).

Sediment samples were collected with van Veen grab samplers with surface area 0.15 m^2, except for one sample (EKO-14), from the central North Sea, with a surface area 0.10 m^2. The top 0–1 cm layer was collected by opening the top of the van Veen grab and scraping the top of the sediment sample with a metal spoon. The sediment sample was then placed in glass jars, preserved with 5% formaldehyde (Carlo Erba Erbapharm, France), stored and shipped cold (4 °C) to the laboratory, where they were further stored cold until analysis. The sampling strategy was in accordance with national standards (NS-EN ISO 5667-19:2004, 16665:2013 and 16260:2012), as described in the Norwegian Environment Agency's guidelines for environmental monitoring of offshore petroleum activities (NEA, 2020).

Of the 30 samples collected from the northern and central North Sea, 15 stations were established by the development of the annual sediment survey by the Norwegian Environmental Agency and relevant authorities to be unaffected by the oil and gas activities (reference stations) and thus represent the natural state of the region where there is oil and gas activity (NEA, 2020). As there is no oil and gas activity in the Barents Sea, the 5 samples from this region were also denoted as reference samples (for detailed information, see the SI).

Most sampled polychaetes belonged to the Oweniidae family, where the main species investigated were *Galathowenia oculata, Galathowenia fragili* and *Owenia borealis*. As the species in the Oweniidae family contain several morphological similarities, it is challenging to differentiate between species, which could be a potential source of variance. The benthic fauna samples were taken by pooling 5 separate 0.15 m^2 van Veen grab samples to obtain 5 L of sediment. The samples were sieved with 1 mm sieves during fieldwork, transferred to glass jars and conserved with a solution of formaldehyde and hexamine (Carlo Erba Erbapharm, France). After sorting at DNV GL's accredited Biology lab, polychaetes were preserved with 70% ethanol (Antibac AS, Norway) and shipped to NGI's lab for microplastic analysis, where they were stored at 4 °C until analysis.

2.2. Chemicals

Density separation fluid was made by mixing ZnCl$_2$ (VWR International, 97%) and CaCl$_2$ (VWR International, 90–98% purity) to make a ZnCl$_2$:CaCl$_2$-solution based on a weight ratio of 4.4: 3.6: 2 kg (ZnCl$_2$:

Fig. 1. Overview of the sampling stations in the central and northern North Sea, and the Barents Sea. More detailed information including more localized maps with station names are provided in the SI.

CaCl$_2$:MilliQH$_2$O), which had a density of $\geq$1.52 g/cm^3 (Hudgins, 1964). The chemicals used for the chemical digestion were Urea (Sigma Aldrich, Germany, $\geq$98%), Thiourea (Merck, Germany, $\geq$98%), NaOH pellets (Merck, Germany, 99–100%) and 50% H$_2$O$_2$ (VWR International, Germany, analytical grade) which was diluted. Sodium dodecyl sulfate (SDS; VWR International, Germany, $\geq$99%) was used as cleaning agent. Further information is provided in the SI (section S2.1).

2.3. Sediment density separation

The formaldehyde in the sample containers was decanted through a 45 μm steel mesh filter (#300 Mesh – 0.045 mm Aperture – 0.04 mm Wire Diameter – SS316 Grade – Woven Wire, the Mesh Company, Warrington UK). Subsamples of approximately 100 g sediment were dried at 60 °C for two days, for estimation of dry matter content (DM%). Density separation was carried out in an in-house designed density separator referred to as the Bauta Microplastic-Sediment Separator (BMSS, Fig. 2). It is based on a similar design as presented by Imhof et al. (2012). Filtered (Whatman GF/D filters), high-density ZnCl$_2$:CaCl$_2$-solution ($\rho \geq 1.52$ g/cm^3) was used as separation fluid. Note that a mix of ZnCl$_2$ and CaCl$_2$ was used in order to reach the desired density while using less ZnCl$_2$ (Hudgins, 1964), due to concerns of corrosion, cost and environmental emissions of Zn^{2+}.

For microplastic quantification, approximately 500 g wet sediment was made into a homogenized slurry with 100 g ZnCl$_2$:CaCl$_2$-solution ($\rho\sim$1.52 g/cm^3) until no clumps were visible. After filling the BMSS with ZnCl$_2$:CaCl$_2$-solution until the constriction of the glass column, homogenized sediment-ZnCl$_2$:CaCl$_2$-slurry was introduced gradually with a spatula from the top. The glass column was covered with aluminium foil to prevent airborne contamination. After 30 min of stirring with a bottom-fitted propeller at 40 rpm, the sample was left at least over-night for density separation until the ZnCl$_2$:CaCl$_2$-solution was visibly clear.

By attaching the separation chamber and raising the level of the ZnCl$_2$:CaCl$_2$-solution over the shut-off valve, floating material ($\rho \leq 1.52$ g/cm^3) was collected in the separation chamber. After closing the shut-off valve and lowering the fluid level to the glass column by opening the upper air-vent (Fig. 2), the separation chamber was closed, dismantled and fastened in an inversed position for vacuum-filtration onto 45 μm

Fig. 2. Sketch of the custom-designed Bauta Micoplastic-Sediment Separator (BMSS).

stainless steel mesh filter. Particles that stuck to the constriction of the glass column were rinsed off and into the ZnCl$_2$:CaCl$_2$-solution, raised into the remounted separation chamber, and the filtration process was repeated until no particles attached to the glass walls were visible. After flushing with Milli-Q water several times, the concentrated filtrate was enclosed by folding the 45 μm steel mesh filter into an envelope, and secured with a steel wire (Alloy Wire Co. Ltd). Finally, the samples were dried at 60 °C overnight, and weighed before treatment by chemical digestion. Note that this method has been validated with a microplastic spiking protocol (see section 2.8 and the SI).

2.4. Chemical digestion

Chemical digestion was performed using a two-step dissolution oxidation method as described by Olsen et al. (2020). In brief, the first step involves soaking the sample in a mixture of 8% NaOH, 6.5% thiourea and 8% urea in water in a ratio of 40 ml per 0.1 g dry weight sample and storing at −20 °C for 40 min to allow dissolution of cellulosic materials (Jin et al., 2007; Olsen et al., 2020). In the second step, oxidation is performed using 30% H$_2$O$_2$ and 1.0% NaOH, by first adding H$_2$O$_2$ at ratios of 30 mL per 0.1 g dry weight sample aliquots of 0.75 mL 10M NaOH per 0.1g of sample (Olsen et al., 2020). Initial tests with the digestion method indicated that it can successfully remove organic solids like paper and cotton (98 $\pm$ 4% sample digestion), yet is relatively harmless to the plastics tested (4% maximum sample digestion, for PET fibres) (Olsen et al., 2020). The digestion step was done at least once and repeated up to four times depending on a visual assessment of organic material remaining and weight reduction from the previous step.

The weight of the remaining sample includes all particles with a density $\leq$1.52 g/cm^3, a size $\geq$45 μm, and with resilience to the chemical digestion process. In addition to microplastics, this could include other materials such as soot, char and porous glass/carbonates. Therefore, further analysis with Fourier-Transform Infrared spectroscopy (FT-IR microscopy) was performed to determine material composition (described below).

2.5. Extraction of microplastic from polychaetes

Five polychaetes from each station of the Oweniidae family from the central North Sea and the Barents Sea were selected. In the Northern North Sea this polychaete family was not found, and therefore another, unidentified species of tube-dwelling polychaete was selected, that appeared to be larger. The polychaetes were selected from the formaldehyde solution with tweezers and wrapped in 45 μm steel mesh filters and sealed with steel wire for analysis of total body microplastic concentrations (sample weight ~0.008 g wet weight). The sealed samples were then rinsed with MilliQ-water and left at 60 °C overnight before treatment by the same two-step chemical digestion method as described for the sediments.

Density separation of polychaete samples was performed after the chemical digestion step, with the use of a separation funnel. The funnel was filled with ZnCl$_2$:CaCl$_2$-solution and the chemically oxidized polychaete samples were transferred by opening the steel mesh and transferring with a spatula. After 40 min to 2 h of density separation, the floating material was collected and concentrated on a steel mesh filter with a diameter of 13 mm (pore size of 45 μm) by filtering under vacuum followed by rinsing with MilliQ and methanol. To remove any residue remaining on the steel mesh filter, it was placed in a glass beaker with MilliQ and placed in an ultrasonic bath for 30 min (60W). Loosened particles were filtered onto a separate 13 mm steel mesh filter (pore size of 45 μm). After drying at 60 °C overnight in sealed aluminium containers, the two filters with extracted microplastics were analysed with FT-IR microscopy and corrected due to method blanks (SI section S2.3.2).

An initial investigation was done to compare tube vs. inner soft tissue concentrations of microplastic. For this purpose, polychaetes from two
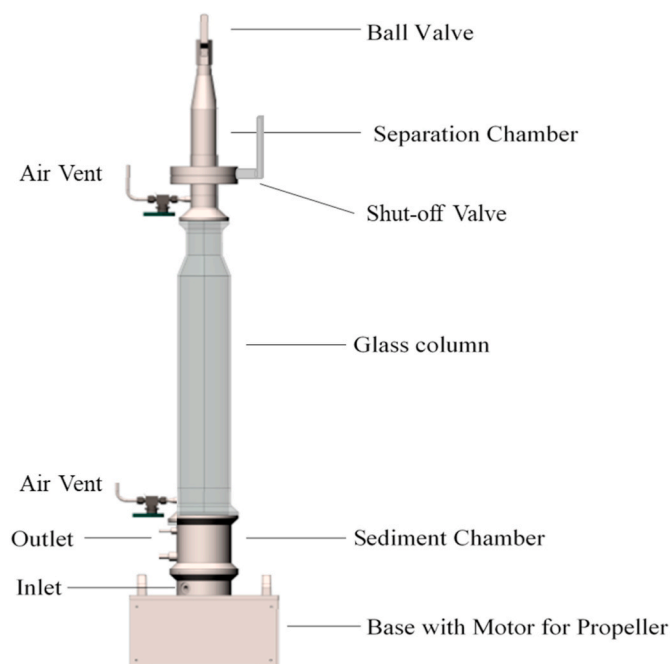
regions were used: the central North Sea (reference stations Reg-1, Reg-2 and Reg-6; soft tissues and tubes analysed in parallel, where each parallel consisted of 6 polychaetes (2 per station pooled together)) and the Barents Sea (soft tissues and tubes analysed in parallel, each sample consisting of 5 polychaetes from station KF2-6). Tubes were separated from the worms' soft tissue by holding the worms between two fingers (Nitrile gloves) and dragging the tissue gently out of the tube with tweezers. Separated soft tissue and tube samples (n = 4) were then enveloped in separate 45 μm steel mesh filters and sealed with steel wires for analysis as described above.

2.6. Biota-Sediment Particle Enrichment Factor

To interpret the results and to investigate the potential enrichment of microplastic in polychaetes relative to the surrounding sediment, a new Biota-Sediment Particle Enrichment Factor (BSPEF) was defined as the particle concentration in biota, $c_{p,biota}$ (particles$_p$/g$_{biota}$ or mg$_p$/g$_{biota}$), divided by the particle concentration in the surrounding sediment, $c_{p,sed}$ (particles$_p$/g$_{sed}$ or mg$_p$/g$_{sed}$).

$$BSPEF\ (g_{sed}/g_{biota}) = c_{p\ biota}\ /\ c_{p,sed} \qquad (1)$$

The BSPEF can be based on either dry weights (dw) or wet weights (ww) of biota, relative to dw of sediment, and could also be applied to soils. It is noted that the equation for the BSPEF (equation (1)) is similar to Biota-Sediment Accumulation Factor (BSAF); however, the new term BSPEF is introduced as BSAF is restricted to single organic compounds at steady-state, not for particles such as microplastics.

2.7. FT-IR analysis

To identify and quantify microplastics, a micro Fourier Transform Infrared (FT-IR) imaging system was used (Perkin Elmer Spotlight 200i FT-IR microscope, wave number range: 4000-600 cm^{-1}, 8-16 cm^{-1} resolution, 4 accumulations) in transmittance mode. The steel mesh filters with polychaetes (13 mm diameter, 45 μm porosity) were analysed directly. For sediment samples, subsamples were transferred manually onto 13 mm steel-mesh filters for analysis in transmittance mode, or onto a 13 mm gold plate for analysis using the micro-ATR crystal mode. Particles large enough to be picked up by tweezers were analysed by the Frontier ATR assembly. Approximately 200 unique particles per sample were randomly selected and analysed with FT-IR. Results were then extrapolated to the whole sample based on the ratio of FT-IR analysed particles and the total number of particles in the sample. Therefore, the larger the total particles in the sample, the larger extrapolation bias.

FT-IR spectra were identified by comparison with reference spectra in libraries from Perkin-Elmer (namely "Polymer", "ATR-Spectra", "Transmission-Spectra" and "Fluka"). Particles with quality index or match score ≥0.7 (70%) were accepted as verified polymers. The particles were categorized according to the groups in Table S7 (unknown, mineral, oxy-resin, petro-pyro, plastic polymer, rubber and organic).

2.8. Method limitations and quality control

The method presented is suitable for microplastics with a density <1.52 g/cm^3 (density separation solution), particle diameter ≥45 μm (pore size of filters used) and resistance to the digestion process (Olsen et al., 2020). The FT-IR analysis is dependent on matches with library spectra above 0.7; these were individually checked, and dubious matches were removed (e.g. spectra with low signal-to-noise ratio were manually removed). Potential contamination resulting from the different steps in the methods applied were accounted for using method blanks, which were analysed using the same protocol as environmental samples. For sediments and polychaetes, method blanks (n = 9 and n = 5, respectively) were run in between samples to control for contamination and used to correct the microplastic concentrations. For sediments, spiked blanks (n = 8), to quantify recovery rates, were prepared by spiking weighed amounts reference PET powder (75–300 μm diameter), PE fibres (38 μm diameter) and PET pellets (3–5 mm diameter) into sediments recovered after density seperation (i.e. "clean", microplastic seperated sediment). The recoveries were on average 77 ± 19%, and this was used for recovery correction of the microplastic dry weights. No meaningful way of spiking "clean" polychaetes was tenable, so no recovery standards were made for this sample type. Field blanks were not acquired, though as some sediments and polychates appeared free of microplastics (after method blank correction), field sampling was assumed as a minor contamination source. For future sampling campaigns, field blanks initially free of microplastics (i.e. deeper sediment samples from the same stations as the surface samples, originating from a time prior to plastic production) should be included as quality control samples. The impurities collected on the method blank steel mesh filters contributed to 0.001 g$_{dw}$; samples that contained less than this after digestion are reported as < LOQ. Particle counts of specific types of microplastic that were zero or did not exceed the number seen in method blanks are resported as not detected "n.d.". See the SI for further details (S2.2.5). The protocol for microplastic quantification in polychaetes was compared with the quality score system by Hermsen et al. (2018), and resulted in a score of 15 out of 20 (for details, see the SI section S2.3.4), which is high compared to other studies, which according to Hermsen et al. (2018) range from 0 to 15, and average 8.

2.9. Data analysis

Statistical analysis (pearson's correlations and ANOVA Tukey HSD tests) was performed with Statistica v. 13.1 (©1984–2016 by Statsoft, Tulsa, USA). The significance level was set at p = 0.05.

3. Results and discussion

3.1. Microplastic concentrations in sediments

Microplastic (defined as the sum of plastic polymer, oxy-resin and rubber particles with FT-IR quality index ≥0.7) were found in 27 of 35 sediment samples, ranging from 0.50 to 46 mg/kg$_{dw}$ (39–3400 particles/kg$_{dw}$), with an overall abundance of 4.7 ± 9.3 mg/kg$_{dw}$ corresponding to 370 ± 690 particles/kg$_{dw}$ (Fig. 3 and Table S12). Overall, there were significantly higher microplastic levels in the samples close to oil and gas installations (8.4 ± 13 mg/kg$_{dw}$, n = 15), compared to reference stations (2.0 ± 3.8 mg/kg$_{dw}$, n = 20; p < 0.045). However, comparing oil and gas areas within specific sampling regions, the difference was not significant (p > 0.05).

The range of reported microplastic concentrations in sediment vary widely in the literature (Table 1) For meaningful comparisons it is important to define specific methodological conditions, such as the density of the solution used in the separation process, methods of plastic identification and the size range of microplastics quantified. For example, Bergmann et al. (2017) reported substantially higher microplastic concentrations in deep sea sediments from the HAUSGARTEN Observatory west of Svalbard, which includes particles >10 μm, compared to this study (Table 1). However only 11% of the particles in the HAUSGARTEN study were >50 μm (Bergmann et al., 2017) which results in an average number of 495 particles/kg$_{dw}$, thus in the same range as this study (370 ± 690 particles/kg$_{dw}$).

According to ecotoxicity studies conducted on sediment and soil organisms (Besseling et al., 2013; Green et al., 2016; Redondo-Hasselerharm et al., 2020; Wright et al., 2013), effects were seen at concentrations higher than 0.05%. Thus, the sediment concentrations up to 46 mg/kg$_{dw}$, or 0.0046% per sediment$_{dw}$ in this study, nor in the others reviewed, are high enough to cause effects observed in these previous studies. However, the concentrations of microplastic in sediment are expected to rise in the future and may reach levels were community
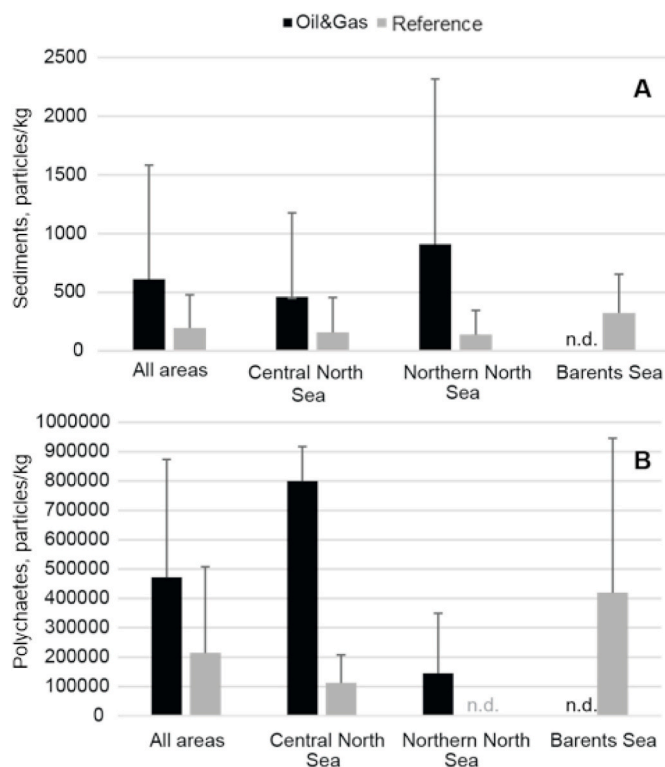
Fig. 3. Monitoring results for A) average microplastic particles per kg sediment (dry weight) and B) average microplastic particles/kg polychaete (wet weight). The results are presented to differentiate oil and gas areas (black, sediment n = 15, polychaete n = 4) from reference areas (light grey, sediment n = 20, polychaete n = 6) within the three sampling regions. Raw data presented in the SI. The error bars represents the standard deviation. n.d. = not detected (because samples not available).

effects have been observed; thus, future monitoring and exposure prevention measures are still warranted.

3.2. Composition of microplastics in sediments

The relative composition of particles categorized according to Table S7 is illustrated in Fig. 4 (detailed information in Table S15).

Overall, microplastics (defined as plastic polymers, oxy-resin and rubber particles) contributed to $12 \pm 17\%$ of the analysed particles after the sample work-up, whereas unknown, organic and petro-pyro particles contributed to $73 \pm 28\%$, $7 \pm 12\%$ and $7 \pm 14\%$, respectively. Thus, the unknown particles (whose FT-IR spectra did not correspond to anything in the library with a sufficient quality index ≥ 0.7) dominated the samples. These could include highly weathered microplastic particles and plastic composites, which would result in a negative bias in microplastic concentrations if such materials were identified as unknowns. The majority of unknowns are more likely other materials including incompletely digested organic material, coal, charcoal and/or bitumen with a density less than the separation fluid ($\rho \sim 1.52$ g/cm^3), resilience to the applied digestion method, and exhibiting complex FT-IR spectrums relative to the pure material FT-IR spectrums in the library. The dominance of unknown particles could also lead to positive biases if any are misidentified as microplastic.

Synthetic rubber, PE-chlorinated (chlorinated polyethylene, whose FT-IR resembles PVC, therefore this is used to mean both) and PET (polyethylene terephthalate) were the most frequently encountered microplastics in the samples (Table S15). Rubber material from car tires has been suggested as a major source of microplastic in the marine environment (Sundt et al., 2014). PVC and PET are also commonly used plastics (Geyer et al., 2017).

PAM (polyacrylamide) was the most frequent microplastic in sample Reg-7 and EKO-21 (central North Sea), as well as in sample KV-14 (northern North Sea). EKO-21 also had the highest microplastic concentrations in this study (Table S12). PAM is an environmentally persistent and soluble polymer (Hennecke et al., 2018; Jop et al., 1997), commonly used as a viscosity enhancer in enhanced oil and gas recovery (Xiong et al., 2018). This could explain its presence in sediment. It should be mentioned that it is disputed in the community whether soluble polymers should be defined as microplastics or not (Arp and Knutsen, 2019; Hartmann et al., 2019). Several studies have, however, reported PAM particles in sediments analysed for microplastics (Constant et al., 2019; de Jesus Piñon-Colin et al., 2018; Ramírez-Álvarez et al., 2019), as well as in other environmental samples (e.g. fish and seal scats (Nelms et al., 2018) and turtles (Duncan et al., 2019)). The origin of these particles could be insoluble PAM from low pH synthesis, cross-linked structures of PAM or flocculated PAM composites (Arp and Knutsen, 2019).

In addition to the frequently encountered PE-chlorinated polymers (density approx. 1.2–1.5 g/cm^3), there were many other microplastics with densities exceeding that of seawater (1.02–1.03 g/cm^3), such as

Table 1
Abundance of microplastics in sediments.

Location	Particle size (n)	Microplastic concentration particles/kg$_{dw}$	Methodological conditions	Reference
Deep sea sediment, Norwegian Continental Shelf incl. the Barents Sea	*45 μm-5 mm(35)*	*370 ± 690*	*Separation fluid: ρ=1.52 g/cm^3; pore size of filter: 45 μm; chemical digestion: 30% H$_2$O$_2$[a]; identification of microplastic: FT-IR analysis*	*This study*
Deep sea sediment from HAUSGARTEN observatory, North Atlantic, west of Svalbard	>10 μm-5mm(9)	4356 ± 675	Separation fluid: ρ = 1.7–1.8 g/cm^3; pore size of filter: 20 μm; chemical digestion: Fenton's reagent (FeSO$_4$ in combination with 30% H$_2$O$_2$); identification of microplastic: FT-IR analysis	Bergmann et al. (2017)
Deep sea sediment from, Norwegian Continental Shelf	>5 μm-5 mm(10)	120 ± 97(23–391)	Separation fluid: ρ~1.6 g/cm^3; pore size of filter: 5 μm; chemical digestion: 30% H$_2$O$_2$; identification of microplastic: visual microscopy and Raman spectroscopy.	NGU (2018)
Deep sea sediment from Atlantic Ocean and Mediterranean Sea	35 μm-1 mm(11)	12[b]	Separation fluid: ρ~1.6 g/cm^3; pore size of sieve: 35 μm; chemical digestion: 30% H$_2$O$_2$; identification of microplastic: visual microscopy and Raman spectroscopy.	Van Cauwenberghe et al. (2013)
Harbor sediment, southern North Sea, IJmuiden in the Netherlands	<5 mm(3)	44 ± 55	Separation fluid: ρ = 1.2 g/cm^3; pore size of filter: 0.7 μm; chemical digestion: 30% H$_2$O$_2$; visual analysis using light microscope	Karlsson et al. (2017)
Harbor sediment near the outflow of a water treatment plant in Oslo, Norway	45 μm-5 mm(3)	670 ± 720	Same as this study.	Olsen et al. (2020)

[a] Two-step digestion protocol: 1) 8% NaOH:6.5% thiourea:8% urea, 2) 30% H$_2$O$_2$:1% NaOH (see section 2.4).
[b] Overall, an average abundance of 0.5 particles per 25 cm^2 was found in the top cm of sediment, corresponding to 12 particles/kg assuming a bulk sediment density of 1.70 g/cm^3.
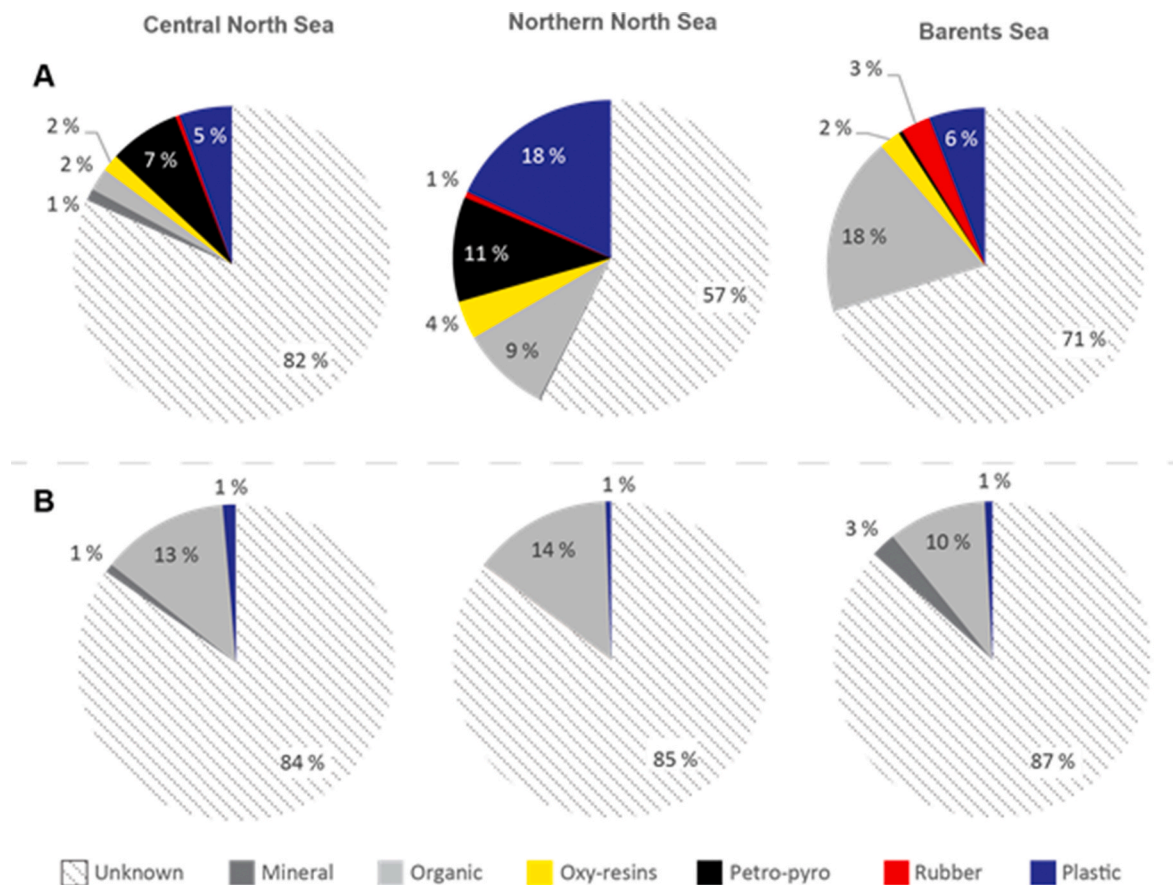
Fig. 4. Average percentage composition of unknown (match score < 0.7 with the FT-IR library), mineral, organic, petro-pyro, plastic, oxy-resin and rubber particles, for the central and northern North Sea and Barents Sea, with a comparison of microplastic found in A: sediments, and B: polychaetes.

rubber, PET, phenoxy-resin and PS, which are expected to sink to the seafloor. However, there were low density plastics as well, like PP, PE or PE-oxidized that appeared in both reference samples (Reg-9, Reg-14, SC3-4, KRT-14 and GRS-2) and near oil and gas areas (e.g. KV-2, VI-1, Table S15). The appearance of low density polymers indicates enhanced sinking behaviour due to a combination of processes including weathering, biofouling, flocculation with e.g. biota, faecal matter and/or clay minerals (Galloway et al., 2017). Previous studies have shown that the accumulation of biofilm on plastic affects its sinking behavior, in addition to the effects of UV-light and mechanical abrasion (Andrady, 2011; Galloway et al., 2017; Singh and Sharma, 2008; Woodall et al., 2014).

In agreement with this study, Bergmann et al. (2017) found PE-chlorinated to be one of the most abundant particles in Arctic deep-sea sediment. The second most abundant was polyamide, which was not found here. Protocols used for FT-IR spectra library comparisons were not identical, which may affect the comparison. Other dominant plastic types reported in marine sediments from the aforementioned literature include PE and PP (polypropylene) in MAREANO sediments from the NCS (NGU, 2018). According to a review of 68 microplastic studies by Hidalgo-Ruz et al. (2012), the most common plastic polymers identified in the marine environment (including the sea surface) were PE, PP and PS. The dominant microplastics in harbor sediments near a water treatment plant in Norway were PE, PP, PVC along with rubbers, and oxy-resins (Olsen et al., 2020). These types of plastics are the most abundant produced (Geyer et al., 2017).

Sample ULA-6 from the central North Sea was one of the samples with the highest microplastic concentration. However, the majority of particles in this sample (and GYDA-21) were clear to white granules of approximately the same shape and size (100–500 μm, see photograph in Fig. S6), with unknown material composition (i.e. FT-IR quality index

<0.7, often as "polyphenyl ether 'poly(2,5-dimethyl-1,4-phenylene-3,3'-dioxo-5,5'-biindol-2,2'diyl) 2/40'"), which are therefore categorized as unknown in this study. Such particles were much less frequent in the reference samples compared to near oil-and-gas installations.

3.3. Microplastic concentrations in polychaetes

Microplastics $\geq$45 μm were found in 9 of 10 polychaete samples, with total body concentrations from 11 to 880 particles/g_{ww} (0.10–1.9 particles/individual), with overall abundances of (mean $\pm$ SD) 320 $\pm$ 350 particles/g_{ww}, corresponding to 0.78 $\pm$ 0.59 particles/individual (Fig. 3 and Table S13). On weight basis, the concentrations were significantly higher in polychaetes than in sediments from the same regions (p < 0.0097).

The numbers of microplastic particles per individual in soft tissues and tubes were comparable (central North Sea, CNS: 0.36 and 0.30; Barents Sea, BS: 0.30 and 1.4 in soft tissue and tube, respectively). However, at a per g_{ww} basis, the concentrations were from 6 to 11 times higher in the tubes (CNS: 84 vs 390 paricles/g_{ww}; BS: 140 vs 1400 particles/g_{ww}; for tissue and tube respectively, see Table S14). Even though there was substantial variance, these preliminary results imply polychaetes may favourably select certain microplastic particles as building materials for their tubes; it would be of interest to pursue this hypothesis in a follow-up study.

In general, information on the occurrence of microplastic in benthic fauna is relatively scarce compared to pelagic species. In addition, differences in sampling and analytical methodologies make comparisons with previous studies difficult. However, as previously mentioned, Bour et al. (2018) investigated microplastics (>40 μm) in polychaetes (*Hediste diversicolor* and *Sabella pavonina*) from the inner Oslo Fjord in Norway.

Their estimate of one to two particles per individual is in the same range as this study. In another study, *Hediste diversicolor* from three sites across South Devon, UK, were investigated for plastic-like particles >10 μm, based on visual microscopy (Hodgson, 2018). The average number of plastic-like particles ranged from 0.4 to 0.7 per individual, with a tendency of higher microplastic levels at sites with higher levels of potential contamination (Hodgson, 2018). By comparison, levels of microplastics have been reported in mussels (*Mytilus edulis*) from the southern North Sea ranging from 0 to 0.4 per individual (average 0.2 ± 0.18) (Karlsson et al., 2017).

3.4. Composition of microplastics in polychaetes

The relative composition of each particle category (Table S7) is illustrated in Fig. 4 (Table S16 for more details). Overall, total body microplastics (plastic polymers, oxy-resins and rubbers) contributed to only $1 \pm 1\%$ of the analysed particles, whereas unknown and organic particles from incomplete digestion contributed mostly (85 ± 8 and $13 \pm 7\%$, respectively).

The most frequent polymers in the central North Sea and the northern North Sea were as with the sediments chlorine-containing polymers (PE-chlorinated and PVC) and PAM, as well as a variety of other polymers (PE, PE:PP, PET, phenoxy resins and nylon). PET and PP were the most frequent polymers in the Barents Sea. Bour et al. (2018) extracted eight polymer types from marine organisms from the inner Oslo Fjord in Norway, where the low density polymers PE and PP were the most abundant in the polychaetes. Similar for sediments, this composition largely reflects plastic production (Geyer et al., 2017) and diverse processes, including interaction with organic matter like biofilm and faecal matter transport, that can lead to the sinking of low-density plastics (Bour et al., 2018; Galloway et al., 2017; Lobelle and Cunliffe, 2011; Morét-Ferguson et al., 2010; Van Cauwenberghe et al., 2013).

3.5. Biota-Sediment Particle Enrichment Factor

To investigate the potential enrichment of microplastic in polychaetes relative to sediment from the same stations along the NCS and the Barents Sea, projected Biota-Sediment Particle Enrichment Factors (BSPEF, Table 2) were calculated based on the ratio of microplastic in polychaetes to sediment. It is underscored as an important caveat that the results in Table 2, including for the BSPEF, uniquely apply to the definition of microplastic used in this study and the methodological limitations (e.g. size, density and FT-IR detection) used here.

The BSPEF values in Table 2 ranged from 100 to 11000 g_{dw}/g_{ww} (280–31000 g_{dw}/g_{dw}). To our knowledge, these are amongst the first reported enrichment values for marine, suspension-feeding benthic species. Karlsson et al. (2017) reported that microplastic levels in filter-feeding blue mussels (on a per g_{dw} basis) were approximately a factor 1000 higher than observed in sediment from the same area. Though this may have been biased by using a 1.2 g/cm^3 density solution, it is within range of what we observed for polychaetas in this study.

In most cases, the composition of microplastic in polychaetes was different than in the sediments. Therefore, the relatively high BSPEF values do not indicate accumulation of specific polymer types. Rather this describes the net accumulation of particles within sediment and polychaetes that can be classified and detected as microplastics. In general, expanding the definition and method limitation of microplastics in this study to include other types of particles, sizes and densities of particles, the concentrations obtained in this study in both sediments and biota would increase; but the impact on the resulting BSPEF values would be difficult to extrapolate.

Nevertheless, the consistent finding of large BSPEF values indicate substantial enrichment, which has interesting implications regarding the possible role of studied polychaetes on the transport dynamics of microplastics. The relatively higher abundance of microplastics in polychaetes than in sediment could be related to suspension-feeding (Riisgård and Larsen, 2010). In sediment, they stretch out their palps over the surface to collect organic-rich material falling down from the water column, which could include microplastics. In this way their diet consists more so of particulates above the sediment surface, than from within the sediment surface used to derive these BSPEF. Further these tube-dwelling polychaetes use these suspended sediments as both food and as the building blocks of their tube.

As the preliminary results of 6–11 times higher microplastic content in tube than in soft tissue, it is hypothesized that the majority of microplastic in the studied polychaetes are in the tube rather than in the soft tissue. In a recent study by Piazzolla et al. (2020), visual inspection showed microplastic particles within the tube structure of the polychaete *Sabella spallanzanii* as a result of its building process. Further research is needed to validate these results, and if validated to identify the mechanisms through which this occurs. Nevertheless, for food chain transfer considerations, both the Oweniidae soft tissue and the tube are consumed, and the use of these BSPEF would therefore be relevant for food-chain dynamics. Additionally, these BSPEF could have relevance for the fate-and-transport of microplastics at the sediment-water interface, as they influence horizontal microplastic distribution on the sediment surface and also burial deeper into sediment as being to some extent influenced by the presence and feeding-behaviour of tube-dwelling polychaetes. Important follow-up questions are if this enrichment in polychaetes is observed in other geographical areas and for other benthic species. On a more methodological level, it is of interest to see how well these findings and BSPEF values are replicated across different methods of quantifying microplastic, or types/definitions of microplastic. In addition, studies that link emission source with sediment sinks, in combination with fate and transport models that account for oceanic currents and particle settling processes, should be performed to better establish in which regions microplastics accumulate most.

CRediT authorship contribution statement

Heidi Knutsen: Writing - original draft, Writing - review & editing, Conceptualization, Methodology, Validation, Formal analysis, Investigation, Visualization. **Jakob Bonnevie Cyvin:** Conceptualization,

Table 2

Concentrations of microplastic ($\geq$45 μm) of varying composition in polychaetes and sediments sampled from the same stations along the Norwegian Continental Shelf. Derived Biota-Sediment Particle Enrichment Factors (BSPEF) for microplastic particles are also provided for this entire class of microplatic. SD = standard deviation.

Location area	Station	Polychaetes Particles/g_{ww} mean $\pm$ SD (n)	Sediments Particles/g_{dw}	BSPEF g_{dw}/g_{ww}	BSPEF g_{dw}/g_{dw}
Central North Sea	Reg-4	190 ± 17 (2)	n.d.	–	–
	Reg-6	200 ± 79 (2)	0.98	200	560
	Reg-9	51 ± 12 (2)	0.076	670	1900
	Reg-14	11 ± 15 (2)	0.11	100	280
	VAL-15	880 (1)	0.089	9900	28000
	ULA-6	720 ± 854 (2)	1.9	380	1000
Northern North Sea	VI-30	n.d. (1)	34	–	–
Barents Sea	KRT-14	48 ± 67 (2)	0.054	890	2500
	KF2-6	790 ± 1100 (2)	0.071	11000	31000

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.marenvres.2020.105073.

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