FT-IR UNDERSØKELSE

Microplastics in polychaetes from the Norwegian Continental Shelf

Miljødirektoratet

Report No.: 2018-1274, Rev. 01
M-1222|2018
Document No.: 236843
Date: 2019-02-05

Project name:	FT-IR undersøkelse
Report title:	Microplastics in polychaetes from the Norwegian Continental Shelf
Customer:	Miljødirektoratet, Brattørkaia 15B, 7010, Trondheim, Norway
Customer contact:	Eivind Farmen
Date of issue:	2019-02-05
Project No.:	10112475
Organization unit:	Environmental Monitoring
Report No.:	2018-1274, Rev. 01 M-1222|2018
Document No.:	236843

DNV GL AS Region Norway
5373,Region Norway
P.O. Box 300,,
Tel: + 47 67 57 99 00
CNORDNV11

Applicable contract(s) governing the provision of this Report:

Objective:

Analysis of amount and type of microplastics in polychaetes in sediments from the Norwegian Continental Shelf (NCS)

Prepared by:	Verified by:	Approved by:
Thomas Møskeland Thomas Møskeland Marine biologist	Jens Laugesen for Jens Laugesen Chief specialist	Tormod Glette Tor Jensen Vice President - Head of Section
Heidi Knutsen for Heidi Knutsen Project engineer (NGI)	AP Hans Peter Arp Senior specialist (NGI)	
Jakob B. Cyvin Jakob Bonnevie Cyvin Master's student (NMBU)	A Pettersen Arne Pettersen Head of section (NGI)	

DNV GL Distribution:

☒ OPEN. Unrestricted distribution, internal and external.

☐ INTERNAL use only. Internal DNV GL document.

☐ CONFIDENTIAL. Distribution within DNV GL according to applicable contract.*

☐ SECRET. Authorized access only.

*Specify distribution:

Keywords:
Microplastic, sediment, polychaetes, biota

Rev. No.	Date	Reason for Issue	Prepared by	Verified by	Approved by
01	2019.02.05	Two new samples added. Updated numbers of particles pr. Individual due to underestimation in rev.00. Did not extrapolate in those cases where not all particles where counted due to high numbers of particles on filter.	Heidi Knutsen (NGI) and Thomas Møskeland	Jens Laugesen	Tor Jensen

Table of contents

ACKNOWLEDGEMENTS

This study presents sampling and analysis of microplastics in polychaetes. The sampling area covers large scale geographical areas on the Norwegian Continental Shelf (NCS). All samples are taken in conjugation with regional monitoring surveys on the NCS carried out on behalf of the Oil & Gas industry. This study had therefore not been initiated without the good will from Oil & Gas operators, especially Equinor and ConocoPhillips Norway.

The Norwegian Environment Agency saw this as a good opportunity to acquire knowledge of microplastic abundance on the NCS and therefore funded the project.

The Norwegian Geotechnical Institute (NGI) has put in own effort, assisted through the projects FANTOM (RCN, 231736/F20) and JPI Oceans WEATHER-MIC (RCN, Project Grant 257433/E40), for the development of the analytical protocols and execution of the analysis and reporting. In a relatively short period they have managed to deliver results. A special thanks to Prof. Hans Peter Arp and Heidi Knutsen for the cooperation, as well as Veronica Castro Bustelo for lab assistance. Also, a special thanks to Jakob Cyvin at NMBU (Norwegian University of Life Sciences) whose substantial contribution to this report was done in partial fulfilment of the Masters Project tentatively entitled, "Microplastics in Polychaetes from the Norwegian Continental Shelf".

Høvik 5th of February 2019

Thomas Møskeland – project manager DNV GL

1 EXECUTIVE SUMMARY

During the regional environmental sediment monitoring campaign of the Norwegian Continental Shelf (NCS) on behalf of the oil & gas industry in 2017, 35 sediment samples from a large geographical area were sampled with a van Veen grab (surface area of 0.15 m^2), covering the central North Sea, northern North Sea and the Barents Sea. The sediment samples were processed and analyzed for microplastics (between 45 µm and 5 mm) at NGI environmental laboratory, which is described in DNV GL (2018a and 2018b).

During the sediment monitoring in 2017, biological samples from 12 stations were sampled. In this follow up study, polychaetes of the *Owniidae* family from these 12 stations were analysed for microplastics (between 45 µm and 5 mm, or between 26 µm and 5 mm) at NGI. These polychaetes are thin, cylindrical, segmented worms that live in flexible tubes that are burrowed in the sediment. The flexible tubes are composed of sand grains or shell fragments glued together in an overlapping fashion. This project has focused on analysis of the worm and the tube combined.

The analysed polychaetes have been found in stomach-content of haddock, different species of flatfish and in small Atlantic hookear sculpin. Their presence in different fish species makes the concentration of microplastic in the *Owiniidae* relevant for more than the benthic fauna itself, as they are potential vectors for trophic transfer.

After chemical digestion and density separation, microplastic particles were identified in polychaetes by micro FT-IR analysis. Total microplastics are defined as total plastic polymers, paint and rubber particles. The lower estimate is based on those confirmed by FT-IR using the applied quality index (≥ 0.6 match score with reference library). The conservative estimate is those that were confirmed by FT-IR (the lower estimate) as well as particles whose FT-IR spectra did not correspond to anything in the library with a sufficient quality index, as there is a possibility these unknown particles could include weathered microplastics. Note that this "conservative estimate" is considered similar, but somewhat different value than the "potential maximum microplastic" value given in the previous reports (DNV GL 2018a and DNV GL 2018b), as the "conservative estimate" is more influenced by partially oxidized biological (polychaetes) particles. Therefore, emphasis is placed on the lower estimate values for final reporting, as presented in Table 1-1.

Table 1-1 Overview of microplastic concentrations in *Owniidadae* in the units of items/individuals and items/g wet weight (ww).

Location	**Items/indvidual** Average ± SD (min-max)		**Items/g ww** Average ± SD (min-max)	
	Lower estimate	Conservative estimate	Lower estimate	Conservative estimate
All areas (n = 12)	**5 ± 4** (0,96 - 14)	77 ± 67 (17 - 250)	**1628 ± 1660** (14 - 5333)	49613 ± 117347 (685 - 417333)
Central North Sea (n = 8)	**4 ± 2** (2 - 9)	93 ± 79 (17 - 250)	**1570 ± 1845** (14 - 5333)	68094 ± 142995 (685 - 417333)
Northern North Sea (n = 2)	**12 ± 3** (10 - 14)	51 ± 3 (49 - 53)	**2765 ± 1288** (1855 - 3676)	11766 ± 3231 (9481 - 14051)
Barents Sea (n = 2)	**2 ± 2** (1 - 3)	41 ± 20 (27 - 55)	**724 ± 748** (194 - 1253)	13538 ± 11416 (5465 - 21611)

Each individual sample contained microplastic. The average MP concentration was highest in the Northern North Sea, followed by the Central North Sea and the Barents Sea, though not significantly, as there were only two samples from the Northern North Sea and the Barents Sea compared to eight samples from the central North Sea. It is difficult based on these results, and the results for the sediments from the same stations (DNV GL 2018b) to see any clear regional difference in microplastic abundances in polychaetes at present stage, although the results could indicate relatively higher concentrations in the Northern North Sea.

A comparison of the lower estimate of number of plastics items identified with FT-IR in sediment (DNV GL, 2018b) and polychaeta samples from the same stations along the NCS were used to project Biota-Sediment accumulation factors (BSAF) of microplastic based on the ratio of these concentrations. These BSAF values ranged from 34 to 2142. Though the BSAF results are considered very preliminary, and probably subject to change with follow-up analysis, it is evident that the number of positively identified microplastics (according to the applied procedure) are consistently larger in polychaetes (per g ww) than in sediments (per g dw). Though more data is considered needed to confirm these estimates, if they are confirmed this would imply that microplastics are enriched in polychaetes at a per g basis, implying poor excretion rates. The results also account for the general observation that less than a gram of polycheates was required to find microplastics, whereas for a sediment sample ca. 600 g or more was required. Further analysis should be done to validate whether the MPs in the polychaetes are accumulated in the biological tissues or in the surrounding tube structure.

A variety of microplastics were found in the samples. The most frequently found plastic polymers in polychaetes were polyacrylamide, chlorinated PP and PE, PVC, nylon, paint, rubber and others (most libraries belonging to commercial, tradename polymers of unconfirmed composition, plasticizers and additives). There were several reoccurring plastic types in sediments and polychaetes, in particular: polyacrylamide, and chlorinated PP and PE. Major differences are PVC was more commonly detected in the polychaete samples, whereas paint and rubber were more abundant in sediment samples.

In general, there is very little information in the microplastic literature regarding benthic fauna. In addition, differences in sampling and analytical methodologies make comparisons with previous studies difficult, although magnitude-scale comparisons may be reasonable. Bour et al. (2018) have reported microplastic abundance in benthic fauna from the inner Oslo Fjord in Norway, which was in the same range as this study, though the polymer composition was different (mostly PE and PP in the Oslo Fjord).

The main conclusions are as follows:

- Microplastics were identified in all polychaete samples analysed, with an overall mean concentration of 5 ± 4 items/individual, corresponding to 1628 ± 1660 items/g wet weight. The results highlight the widespread distribution of microplastics in benthic fauna of the NCS, and that microplastics can be transferred to polychaetes, where they may accumulate in concentrations compared to the surrounding sediments. This provides a direct route for microplastics to transfer further up in the benthic food chain. Further research is needed to assess potential risks or ecotoxic effects of the concentration levels presented in this study. Mechanistic pathways for microplastic accumulation (e.g. accumulation in the guts, or in the surrounding tube structures) should also be investigated.
- The most frequently occurring plastics in polychaetes were polyacrylamide, additives, plasticizers, PET, nylon and paint. At present, the wide variety of plastic types identified makes it difficult to pinpoint a particular source. Supplementary analyses could identify sources and transport routes of these types

of particles, as part of an initiative to monitor or curtail increasing concentrations. This would also minimize statistical uncertainties.

Further analysis is planned to differentiate concentrations between microplastic concentrations within the *Owiniidae* themselves and their surrounding tubes. Additional subsamples of relevant sediment samples may be obtained. Particle morphology, in particular in terms of microplastic fibers vs granules, may also be investigated as part of this future work.

2 INTRODUCTION

The increase in plastic production and use since the mid-1950s has been accompanied by an increase in plastic litter in the ocean (GESAMP, 2016). Thus, concern about the widespread distribution of plastic and microplastic debris in the oceans has grown rapidly in recent years. A literature review by Hidalgo-Ruz et al., (2012), reported microplastic concentrations in sediments (0.21 to more than 77 000 items m^{-2}) which are substantially higher than those from sea surface samples ($8 * 10^{-5}$ to 5 items m^{-2}). In both long-term studies (from 1976 to 2015 by Courtene-Jones et al., 2019) and measurements of microplastic concentration in 2017 (Bour et al., 2018), benthic fauna is found to ingest microplastics.

Depending on their origin, microplastic can be categorized as primary or secondary microplastic (Andrady, 2017). Primary microplastic include production pellets/powders and microbeads used for cosmetic formulations and industrial abrasives, whereas secondary microplastic are typically derived from fragmentation of larger plastic items either during use of products or due to weathering degradation of their litter (Andrady, 2017). Most microplastic in the marine environment are thought to be secondary microplastic (Barnes et al., 2009; Hidalgo-Ruz et al., 2012).

Weathering of microplastic can lead to changes in the polymers chemical-physical properties, which further affect the sinking behaviour. For instance, floating microplastics with a density less than the surrounding seawater can accumulate encrusting foulants (e.g. algae), which have a ballasting effect over a period of time, increasing their apparent density, causing them to sink in the water column and ending up in deep water or in the sediment (Andrady, 2017). The presence of microplastic in sediments could cause negative effects, such as increased microplastic ingestion by soft-bottom fauna.

As described in an earlier report by DNV GL and NGI (2018a and 2081b), sediment samples from the Norwegian Continental Shelf (NCS) have been sampled by DNV GL on behalf of the oil & gas industry and analysed for microplastic by the Norwegian Geotechnical Institute (NGI). In addition, samples of soft-bottom fauna were sampled from many of the same stations. Twelve of these samples were analysed for microplastic > 45 µm (> 0.045 mm), and the results are presented in this report. As it has become common to define microplastic as plastic particles < 5 mm in diameter, this was the upper size limit in the present investigation.

3 MATERIAL AND METHODS

3.1 Sampling locations

Benthic fauna from 12 stations were sampled along the NCS. The locations of the samples are shown in Figure 3-1 below. Some detailed station information is presented in Table 3-1.

Table 3-1. General information of the sampling stations.

Sampling station	Region	Direction[1] (°)	Distance[2] (m)	Depth (m)	Sediment characteristic	TOC (%)
Reg-02	Central North Sea	n.r	n.r	68	Fine sand (MDΦ = 2.79)	0.31
Reg-04	Central North Sea	n.r	n.r	71	Fine sand (MDΦ = 2.75)	0.32
Reg-06	Central North Sea	n.r	n.r	72	Fine sand (MDΦ = 2.87)	0.33
Reg-09	Central North Sea	n.r	n.r	66	Fine sand (MDΦ = 2.66)	0.19
Reg-14	Central North Sea	n.r	n.r	80	Fine sand (MDΦ = 2.74)	0.24
VAL-02	Central North Sea	74	500	76	Fine sand (MDΦ = 2.82)	
VAL-15	Central North Sea	254	500	76	Fine sand (MDΦ = 2.80)	0.42
ULA-06	Central North Sea	45	250	71	Fine sand (MDΦ = 2.86)	0.28
KV-01	Northern North Sea	140	250	187	Fine sand (MDΦ = 2.68)	0.25
VI-30	Northern North Sea	330	250	330	Silt (MDΦ = 5.6)	0.4
KRT-14	Barents Sea	n.r	n.r	440	Silt and clay (MDΦ=5.70)	1.34
KF2-6	Barents Sea	85	900	242	Silt and clay (MDΦ=4.05)	1.76

1) Direction from an oil & gas installation 2) Distance from an oil & gas installation
n.r. = not registered, MDΦ = Median Grain diameter

All samples were sampled with a van Veen grab with a total surface area of 0.15 m^2. All biological samples were taken from 0.1 m^2 from this grab (0.05 m^2 are used for chemical analysis). In general, 5 replicates were sampled from each location, meaning that they represented a total surface area of 0.5 m^2. Five replicas were analyzed and presented in this report.

In the field, the samples were sieved with sieves with diameter down to 1 mm, transferred to buckets and conserved with a solution of formaldehyde and hexamine. A coloring agent was added (pink Bengal) which makes it easier to sort the animals in the laboratory. All animals were sorted by picking out all animals by use of tweezers and magnifying glass and divided into 5 main groups, where polychaetas was one main group, and each group was stored in small glass containers with 70 % ethanol at DNV GLs accredited Biology lab. The glass containers containing polychaetas were delivered to NGI which performed the analysis, meaning they identified and picked out *Oweniidae* species, see chapter below.

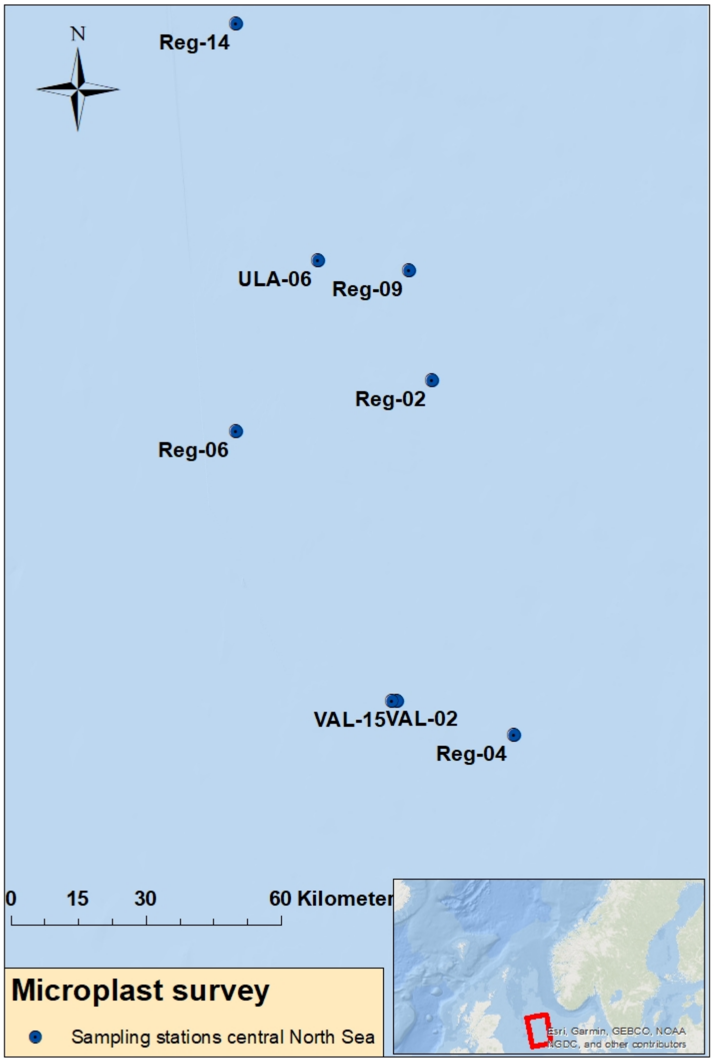

Figure 3-1. Overview of sampling stations. Central North Sea

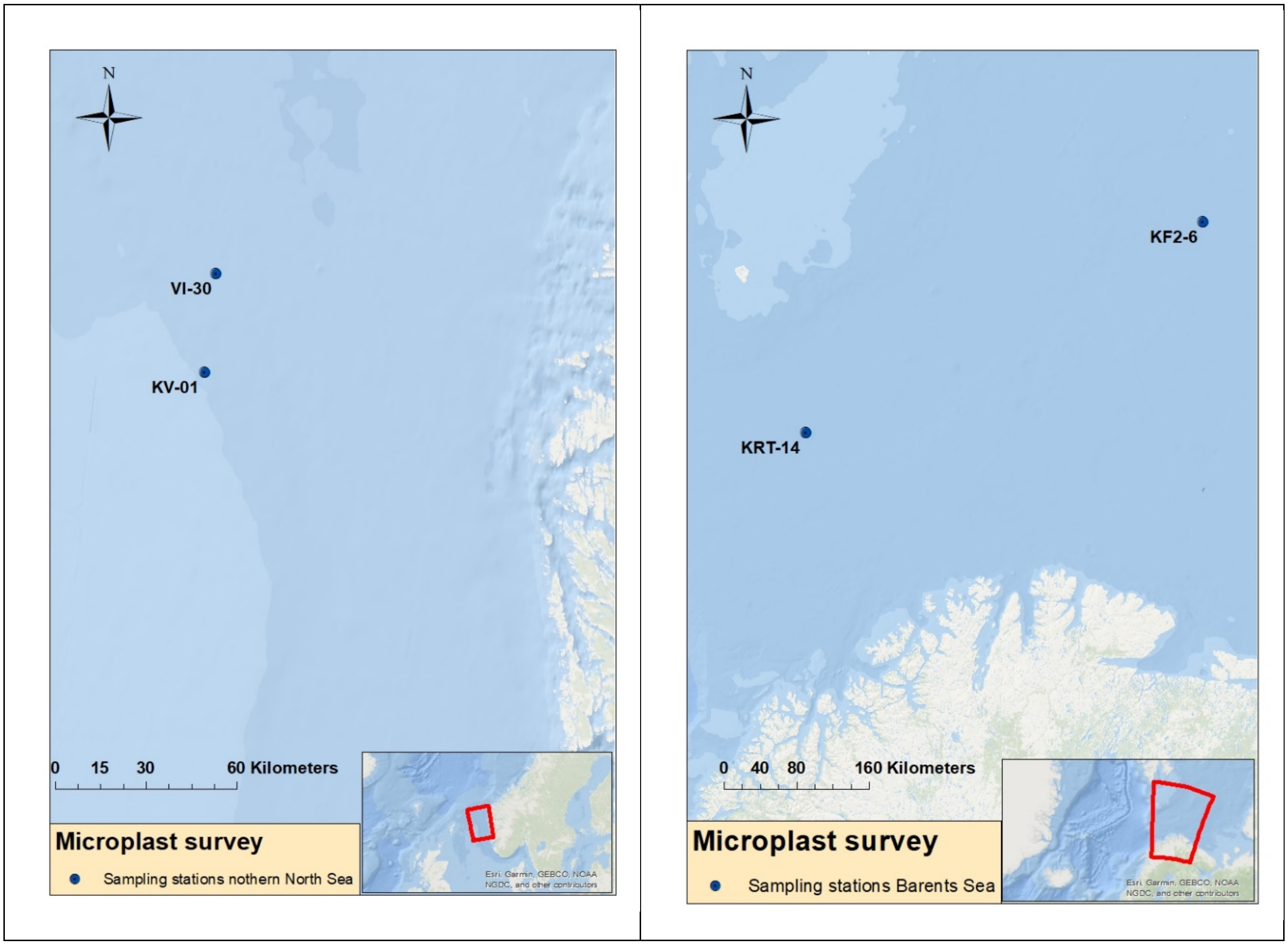

Figure 3-1 continues. Overview of sampling stations, northern North Sea (left) and Barents Sea (right).

3.2 Polychaetes

In this project, the polychaete (benthic worm) family Oweniidae was investigated for microplastic content. The family Oweniidae contains a lot of morphologically quite similar species of polychaetes. Therefore, the use of *Owiniidae* is more correct than the names of the single species. We have investigated mainly *Galathowenia oculata* (classification in Table 3-2) and other Oweniidae collected from the Norwegian continental shelf (NCS), that look morphologically quite similar to other species present (e.g. *Owenia Fusiformis*). Most of the individuals are *Galathowenia oculata* (DNV GL pers. comm.). These polychaetes are thin, cylindrical, segmented worms that live in a flexible tube made of sand, and are borrowed in the sand, with their anterior ends just protruding from above the surface. The tube is composed of sand grains or shell fragments glued together in an overlapping fashion (Oug et al., 2011), as illustrated in Figure 3-2. This project has focused on analysis of the worm and the tube combined.

Table 3-2. Galathowenia oculata classification (Zachs, 1923).

Phylum	**Annelida**
Class	Polychaeta
Order	Sabellida
Family	Oweniidae
Genus	Owenia
Species	*Galathowenia oculata*

The family *Owiniidae* are registered at moderate sediment depths and in continental slopes (Fauchald, 1977). An estimate of their dispersion range is from the intertidal zone to depths of up to 4500m (Tyler, 2018). This indicates that they are widely distributed. The *Owenia fusiformis* is widespread around the British Isles and in the Northern Sea and in the Barents sea (https://www.marlin.ac.uk/species/detail/1703). The density of the *Owenia fusiformis* is reported to be up to 4660 individuals per m^2 sediment. The spatial distribution is reported "throughout northwest Europe, the Mediterranean, the Indian Ocean and the Pacific" (Tyler, 2018). The growth cycle is largest in summer, low in autumn and negligible in winter, starting up again in April; their age can reach four years (Rouse & Pleijel, 2001). Information about the *Galathowenia oculata* (previously called *Myriochele oculata*) is scarce, but it has a wide distribution in the northern hemisphere, according to World Register of Marine Species (http://www.marinespecies.org/aphia.php?p=taxdetails&id=146950#distributions).

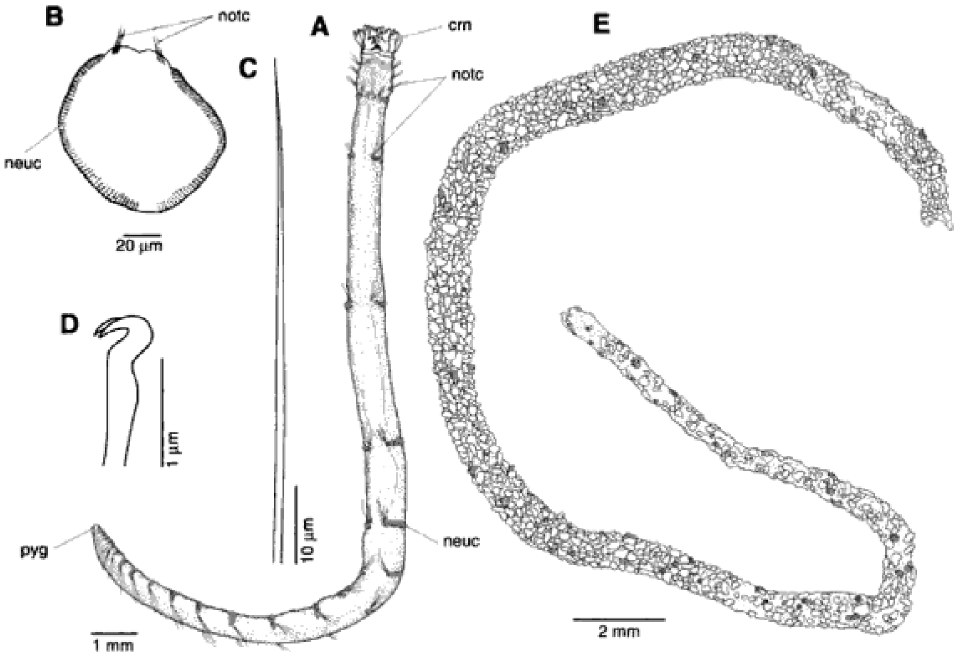

Figure 1.97 Family Oweniidae. *Owenia fusiformis*. **A**, entire animal, dorsal view. **B**, transverse section of body showing chaetal distribution. **C**, capillary notochaeta from parapodium of chaetiger 10. **D**, neurochaetal hook from parapodium of chaetiger 10. **E**, tube. **crn**, crown; **neuc**, neurochaetal 'patch'; **notc**, notochaetae; **pyg**, pygidium. [A. Murray]

Figure 3-2. Illustration of the Oweniidae family (source: Anderson, 2001).

Galathowenia oculata and other Oweniidae are important when considering trophic transfer, i.e. the transfer of contaminants from low-food chain organisms to higher food-chain organisms. They have been found in stomach-content of Haddock, different species of flatfish and in small Atlantic hookear sculpin (Schückel, Ehrich, Kröncke and Reiss, 2010; Yeung & Yang, 2014; Källgren, Pedersen & Nilssen, 2014). Their presence in different fish species makes the concentration of microplastic in the *Owiniidae* relevant for more than the benthic fauna itself, as they are potential vectors for trophic transfer.

Owenia fusiformis feeds on sediments around itself and can filter water (Rouse & Pleijel, 2001). According to Glasby (2000), particles less than 200 µm can be ingested, which indicates that they can ingest microplastic particles < 200 µm. Feeding strategies for other Oweniidae than the O. *fusiformis* are unknown according to Glasby (2000).

3.3 Sample preparation and analytics

3.3.1 Polychaete – microplastic separation

The polychaete samples (preserved with 70 % ethanol in glass jars) were delivered to NGI by DNV GL, and were stored at 2 – 4 ºC until processing. The samples were processed and analysed for microplastics (45 µm – 5 mm or between 26 µm and 5 mm) at NGI's Environmental laboratory, as described in the following.

3.3.2 Sample preparation

The first step of sample preparation was to select five polychaetes of the *Owniidadae* family from each of the stations and transfer them to pre-weighed 26 µm steel mesh filters (SS316 Grade - TWILL - Woven-n Wire Mesh, Warrington UK), which were wrapped around the polychaetes and sealed with a preweighed steel wire/tea bag as illustrated in Figure 3-3. The sealed polychaetes were then rinsed with milliQ-water in a glass beaker to remove ethanol and left at 60 ºC over night for determination of dry weights.

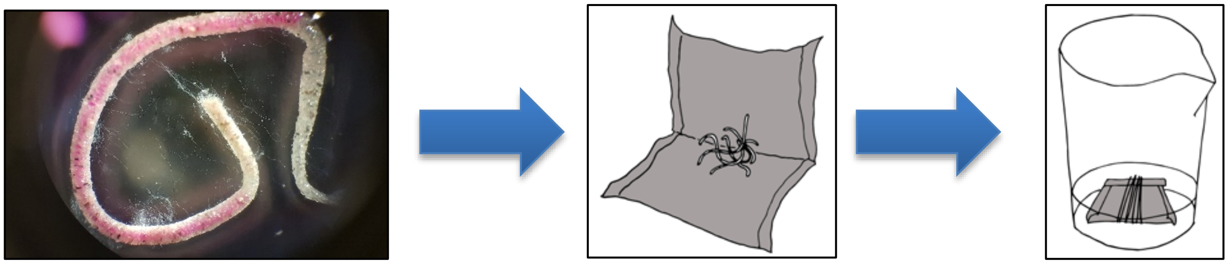

Figure 3-3. Illustration of sample preparation (drawings by Kjersti S. Moen, 2018).

Wet-weight was calculated as shown in Formula 1, based on the dry matter content (obtained from four samples of 15, and two of 30 *Oweniidaes* in each sample).

$$DM\% = \frac{m_{dry}}{m_{wet}} * 100\% \qquad \textit{Formula 1}$$

Where:

DM% = dry matter content

m_{dry} = weight of sample (g) after drying at 60 °C over night

m_{wet} = weight of sample (g) before drying

3.3.3 Chemical oxidation

A two-step chemical digestion process was performed for removal of organic material. The first step involves dissolving organic polymers, such as chitin and cellulose, using a mixture of NaOH, urea and thiourea (Zhang et al., 2013). During this step, some of the organic material dissolves and is rinsed through the steel mesh "tea bag" filter (steel mesh) or is otherwise partially dissolved to facilitate oxidation in the second step.

The second step involves digesting of the remaining sample with 30% H_2O_2 and NaOH. Initial tests with this digestion method indicated that it can successfully remove organic solids like paper and cotton (98 ± 4 % sample digestion), yet it is relatively harmless to the plastics tested (4% maximum sample digestion, for PET fibres). The digestion step was done at least once and repeated up to three times depending on the amount of organic matter present (Figure 3-4). Further details and the validation of this procedure will soon be published (Olsen et al., in prep.).

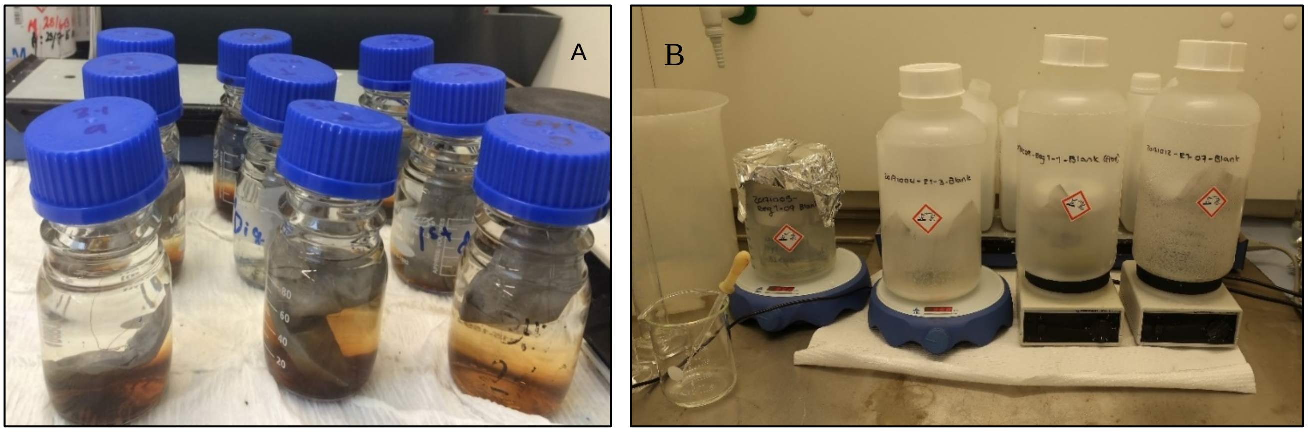

Figure 3-4. Chemical digestion step 1 (A) and 2 (B).

After chemical digestion, the steel mesh was opened and the extracts added to filtered $ZnCl_2:CaCl_2$-solution (density > 1.52 g/cm^3) in a separation funnel (Figure 3-5) and left for 30 min to 1.5 hours for visual density separation, in which particles with a density less than the separation fluid ($ZnCl_2:CaCl_2$-solution) will float, such as plastics and other low-density materials that are resilient to the digestion method, like bitumen, charcoal and some forms of natural organic matter (Figure 3-5).

The floating material was collected and concentrated on a steel mesh filter with a diameter of 13 mm (pore size either 26 µm or 45 µm - specified for each sample in the results part) by filtering under vacuum and flushed with methanol. To ensure that all particles had been transferred, the steel mesh was thereafter cleaned in an ultrasonic bath submerged in methanol. The methanol solution, containing particles from the filter, was thereafter filtered onto another filter.

The extracted particles were analysed for their polymer composition by FT-IR analysis, see chapter below.

Figure 3-5. Density separation

3.3.4 Polymer identification

Plastic polymers were identified and characterised using micro FT-IR spectroscopy. The micro FT-IR system used was a Perkin Elmer Spotlight 200 FT-IR microscope, equipped with a Frontier FT-IR spectrometer. The system consists of a microscope, spectrometer, PC, stage controller and a joystick.

Figure 3-6 Spotlight 200 – microscope and frontier IT system

Prior to analysis, the Spotlight 200 was set up, and the microscope was focused as described in the Spotlight 200 User's Guide. The scan parameters were set to the following settings:

Table 3-3. FT-IR scan parameters setup

Resolution	8 or 16 cm^{-1}
Wave number range	4 000 – 600 cm^{-1}
Number of accumulations	4 for the sample image

The steel mesh filters containing the sample material, were analysed in transmittance mode. In transmittance mode, the infrared radiation penetrates the particle before arriving at the detector, giving an infrared spectrum of the entire volume of the particle. This mode works best with thin or translucent particles. According to the manufacturer, the micro FT-IR system ensures spectra from sample areas down to the diffraction limit of 10 µm.

The obtained IR spectra were compared with libraries of polymer spectra available through Perkin-Elmer, namely "Polymer", "ATR-Spectra", "Transmission-Spectra" and "Fluka". Particle identification is done through the software, which compares the obtained spectrum with those in the spectrum libraries, which includes a wide variety of plastic polymers, organic substances, salts and minerals, many of which are highly unlikely to be a major component of marine samples. Samples with quality index < 0.7 and ≥ 0.6 were considered individually for acceptance, whereas matches with scores > 0.7 were all accepted. Though there is no established quality index for what is considered accepted/rejected (or known/unknown), a value of 0.6 was considered acceptable to allow for weathering/surface coating of polymers, as well as the presence of additives. In cases where there were several matches above 0.6, usually the best match was chosen. Exceptions were done when the spectra with the highest quality index was different from several other spectra with a similar score. For instance, if there was one match with "polymer a" with a quality index of 0.91 from one library and diverse matches for "polymer b" were present from all libraries, with scores ranging between 0.8-0.9, "polymer b" was chosen. Spectra were manually checked to ensure that IR peaks were visually distinguishable above noise. If they were not, the spectra were rejected regardless of the quality index and not considered further. Matches with a score less than 0.6 were rejected and were denoted as "unknown" particles in the report. The identified items were categorized into the groups shown in Table 3-4.

Table 3-4. Categories for classification of particles. The colouring in the table corresponds to the colouring in the pie charts (Figure 4-2).

Particle Category	Description
Unknown**	Particles identified by FT-IR, but with a quality index < 0.6
Mineral	Particles with no organic chemical bond visible in the IR spectrum (such as inorganic salts, glass, etc.)
Paint*	Particles identified to be composed of oxy-resins, adhesives, or paint additives such as epoxy resin, phenoxy resin, particles containing organo-tin, bisphenols, etc.
Petro-Pyro	Typical petroleum substances, such as hydrocarbon resins, petroleum products, etc.
Plastic*	Commercial synthetic polymers, or a weathered derivative thereof, such as oxygenated polymers; not included are semi-synthetics derived from biopolymers like cellulose, such as rayon, viscose, cellophane, etc
Rubber*	Particles identified as rubbers, polymers used as rubbers (e.g. SBR, silicon rubber), or resins containing rubber compounding agents
Organic	Particles identified as organic macromolecules like cellulose, rayon, chitin, proteins, or in general particles containing organic carbon molecular bonds, that do not fit into any of the above categories

**The lower microplastic concentration estimates reported are defined as total plastic polymers, paint and rubber.*
***The conservative microplastic concentrations reported are defined as total plastic polymers, paint, rubber, as well as unknown particles, as some of the unknown particles could in fact be plastics, but with a low match score with the reference library, i.e. due to weathered surface of the particles.*

Particles identified as plastic were further subdivided into plastic type in Table 3-5. In case of blends, the main polymer in the composition was chosen (with the exception of PE:PP).

Table 3-5. Plastic polymer categories used in this report

Plastic Category	Description
PE	Polyethylenes (E.g. LDPE,HDPE, LLDPE, etc.)
PE-chlorinated	Chlorinated polyethylenes
PE-chlorosulfonated	Chlorosulfonated polyethylenes
PE-oxidized	Oxidized polyethylenes
PE:PP	Blends of polyethylene:polypropylene
PP	Polypropylenes
PET	Polyesters, polyethylene terephthalates
PS	Polystyrenes
PTFE	Polytetrafluoroethylenes
PP-chlorinated	Chlorinated polypropylenes
Polyacrylamide	Polyacrylamides
PMMA	Polymethylmethacrylate and other polyacrylates
PU	Polyurethane foams
PVF	Polyvinyl fluorides
PVC	Polyvinyl chlorides
Melamine	Melamines (all resin blends)
Nylon	Polyamides (e.g. nylon)
EVA	Ethylene vinyl acetate resins
Others	Synthetic polymers not belonging to the above list

3.4 Quality control

There are several limitations to the method used in terms of identifying all microplastic particles in a sample. These include density (below 1.52 kg/L), particle size (26 or 45 µm - 5 mm) and chemical digestion limitations (recalcitrant particles other than plastic). Precautions were taken throughout the analysis method to account for laboratory contamination through the use of blanks and keeping the sample closed to the laboratory atmosphere as much as possible. To reduce airborne contamination, several contamination prevention strategies were performed, such as thorough washing of the equipment with MilliQ or ultrasonic cleaning in MilliQ water, proper sealing of the samples with aluminium foil as much as possible etc. The response of the FT-IR was periodically checked by sampling pure microplastic reference materials, and ensuring they had a good signal above the base line (well-resolved peaks, and quality score >0.93). Method blank samples were prepared and analysed by FT-IR to evaluate polymer contamination resulting from the preparation and analytical procedure, and to correct for this.

There are also limitations with regard to the FT-IR analysis used. In literature, it is common to use a quality index of 0.7 as the limit (e.g. Obbard et al., 2014). However, in agreement with the procedure used in this report, Obbard et al. (2014) individually inspected and interpreted any matches with scores < 0.7 but ≥ 0.6, while any matches with quality index ≥ 0.7 were accepted. This method was used as it can be difficult to obtain spectra with high score if the plastics have been present in the environment for a considerable time, such as for the sediment samples from the NCS. Weathering of the polymers affect their surface and thereby their spectra, which makes comparison with reference spectra more difficult. As such a score limit of 0.7 could lead to an underestimation of plastics, as particles with a lower score in fact could be plastics. At the same time, the score limit of 0.6 could also lead to an overestimation of plastics if the limit is not conservative enough, as the uncertainty increases with decreasing score.

The number of a specific type of particles (e.g. PTFE, glass, organic) in the analysed polychaete samples (n_p) were corrected due to the number of counted particles of the same FT-IR spectra in the method blanks as shown in Formula 2.

$$n_p = n_{p,sample} - n_{p,blank} \qquad \textit{Formula 2}$$

Where:

$n_{p,sample}$ = number of particles, p, identified with FT-IR to belong to one of the categories in Table 3-4 and Table 3-5in the sample.

$n_{p,blank}$ = number of particle, p, identified with FT-IR to belong to the categories in Table 3-4 and Table 3-5 in the blanks. This value for different particle type was obtained using data from different method blanks. The value used in Formula 2 one would be corrected for the number of sample filters. For example, if three sample filters were used for $n_{p,sample}$, then the three x the average number of particles per method blank filter were used as $n_{p,blank}$.

For unknown, organic, mineral and petro-pyro particles, the individual type was not included in calculating n_p; however, for plastic, paint and rubber, this was first done for individual particle type (e.g. PE, PET, phenoxy resin, etc.). Then, the n_p for all plastic, paint and rubber particles was calculated by summing the n_p values for individual types (as listed in Table 3-5).

Based on the positively identified particles, lower estimates of microplastic concentration were calculated per kg wet polychaetes and per individual. In addition, conservative microplastic concentration estimates were calculated with the inclusion of particles categorized as unknown (match score < 0.6 with reference library), as these potentially could be highly weathered plastic particles.Note that this "conservative estimate" is considered similar, but somewhat different value than the "potential maximum microplastic" value given in the previous reports (DNV GL 2018a and 2018b), as the "conservative estimate" as it is more influenced by partially oxidized biological particles.

3.5 Chemicals and solvents

All chemicals used during solution preparation are listed below.

Table 3-6. List of chemicals

Chemicals	Molecular formula	Manufacturer/ Distributor	Purity (%)
Zinc Chloride	$ZnCl_2$	VWR International	97
Calcium Chloride	$CaCl_2$	VWR International	90-98
Hydrogen peroxide	30 % H_2O_2	VWR International	Analytical grade
Urea	$CO(NH_2)_2$	Sigma Aldrich	≥ 98
Thiourea	CH_4N_2S	Merck K GaA	≥ 98
Sodium hydroxide	NaOH	Merck K GaA	99 – 100
Sodium dodecyl sulphate	$CH_3(CH_2)11OSO_3Na$	Sigma Aldrich	≥ 99 (Chromatography)

3.5.1 Preparation of $ZnCl_2:CaCl_2$-solution

The $ZnCl_2:CaCl_2$-solution was prepared by combining distilled water, $ZnCl_2$ and $CaCl_2$ in this exact order. The ratio by weight was 4.4: 3.6: 2 kg ($ZnCl_2:CaCl_2:H_2O$) (Hudgins, C. M., 1964). As the reaction is exothermic, the carboys were placed in a tub filled with ice under a fume hood. Precipitates and impurities were centrifuged and filtered out by first placing the salt solution in Nalgene centrifugation vials, rotating at 4000 RPM for 10 minutes and then filtering the supernatant through a Whatman GF/D filter using a high-pressure filtration apparatus. Finally, the density of the solution was controlled by dividing the weight of filtered $ZnCl_2:CaCl_2$ solution in a 100-mL volumetric flask by the volume.

3.5.2 Preparation of solutions used for chemical digestion

The NaOH, urea, thiourea solution was made according to Olsen et al. (in prep). The 30 % H_2O_2 was made by dilution of 50 % H_2O_2 with Milli-Q water.

4 RESULTS

4.1 Quality control

As part of method development, two different types of steel mesh filters were used for the filtration of microplastic prior to FT-IR analysis, 26 µm and 45 µm. The advantage of the 26 µm is that a larger population of particles are collected on the filter; the advantage of 45 µm is that this was the filter size used for a parallel study on microplastics in sediments obtained from the same area as the polychaetes in this study (DNV GL, 2018a). Five blanks for the 45 µm mesh were run, and three blanks for the 26 µm mesh. The results are presented in Table 4-1. No statistical difference in the total number of plastic particles in the blanks was evident in the 45 µm and 26 µm blank samples; however, the variety of particles on the 26 µm filter was more diverse.

Table 4-1. Average abundance (± standard deviation) of particles in blanks within each defined FT-IR category for plastics.

Plastic particles identified in blanks*	45 µm (n = 5)	26 µm (n = 3)
PE	0.8 ± 1.3	0.0 ± 0.0
PP	1.8 ± 1.5	0.3 ± 0.6
PET	1.4 ± 1.5	1.3 ± 2.3
PS	0.0 ± 0.0	0.3 ± 0.6
Polyacrylamide	2.2 ± 3.5	0.7 ± 1.2
PMMA	0.2 ± 0.4	0.0 ± 0.0
PU	0.2 ± 0.4	0.0 ± 0.0
Melamine	0.4 ± 0.9	0.0 ± 0.0
Nylon	0.4 ± 0.5	1.0 ± 1.7
Ion exchange resins	0.4 ± 0.5	2.0 ± 2.0
Plastic (other)	0.4 ± 0.5	2.7 ± 3.1
Rubber (other)	0.0 ± 0.0	0.3 ± 0.6
Phenoxy Resin	0.0 ± 0.0	0.7 ± 1.2
Paint (Zonyl)	0.0 ± 0.0	2.0 ± 3.5
All particles	0.82 ± 0.94	0.87 ± 0.89

**Microplastics are herein defined as total plastic, paint and rubber particles*

It is noteworthy that the contamination in the method blanks for this experiment were the highest encountered in our lab to date. It is suspected the main contamination source is cross-contamination from samples. These blanks were conducted after sample processing was initiated, explicitly to check for this. Future work planned with these samples will try to prevent this cross contamination.

4.2 Identified microplastics

4.2.1 Microplastic concentrations

Total microplastics are herein defined as total plastic, paint and rubber particles (Table 3-5). The lower estimate of total microplastic concentrations are based on those confirmed by FT-IR using the applied quality index (≥ 0.6 match score with reference library). The conservative microplastic concentrations are those that are confirmed by FT-IR (lower estimate) as well as particles whose FT-IR spectra did not correspond to anything in the library with a sufficient quality index (the category "unknown"). They could be highly weathered plastic, plastic composites etc., but they could also be partially digested polychaeta particles. As such, we do not have sufficient evidence to conclude on the identity of these particles.

The microplastic concentrations are presented in Table 4-2 and Figure 4-1 for the samples collected in this study. Emphasis is placed on the lower estimate value for final reporting, as most of the unknown particles likely are partially digested Polychaeta.

Table 4-2. Range of microplastic concentrations in each sample. Lower estimate: microplastic confirmed by FT-IR (plastic, paint and rubber with quality index >0.6), presented in bold. Conservative estimate: those confirmed by FT-IR and particles whose FT-IR spectra did not correspond to anything in the library ("unknown" quality index < 0.6, possible plastic, e.g. highly weathered plastic).

Sample	**Location**	**Filter size**	**Items/indvidual** Average ± SD (min-max)		**Items/g ww** Average ± SD (min-max)	
			Lower estimate	Conservative estimate	Lower estimate	Conservative estimate
Reg1-02	Central North Sea	26	**9.3**	154	**563**	9343
Reg1-04		45	**2.8**	89	**713**	22699
Reg1-06		45	**2.5**	19	**519**	4040
Reg1-09		45	**1.8**	17	**339**	3223
Reg1-14		45	**1.6**	77	**14**	685
Val-02		26	**4.5**	101	**3250**	72083
Val-15		45	**4.0**	34	**1827**	15347
ULA-06		26	**3.2**	250	**5333**	417333
KV-01	Northern North Sea	45	**14**	53	**3676**	14051
VI-30		45	**9.6**	49	**1855**	9481
KF2-6	Barents Sea	26	**0.96**	27	**194**	5465
KRT-14		26	**3.2**	55	**1253**	21611
All areas			**5±4** (0,96-14)	77±67 (17-250)	**1628±1660** (14-5333)	49613±117347 (685-417333)
Central North Sea			**4±2** (2-9)	93±79 (17-250)	**1570±1845** (14-5333)	68094±142995 (685-417333)
Northern North Sea			**12±3** (10-14)	51±3 (49-53)	**2765±1288** (1855-3676)	11766±3231 (9481-14051)
Barents Sea			**2±2** (1-3)	41±20 (27-55)	**724±748** (194-1253)	13538±11416 (5465-21611)

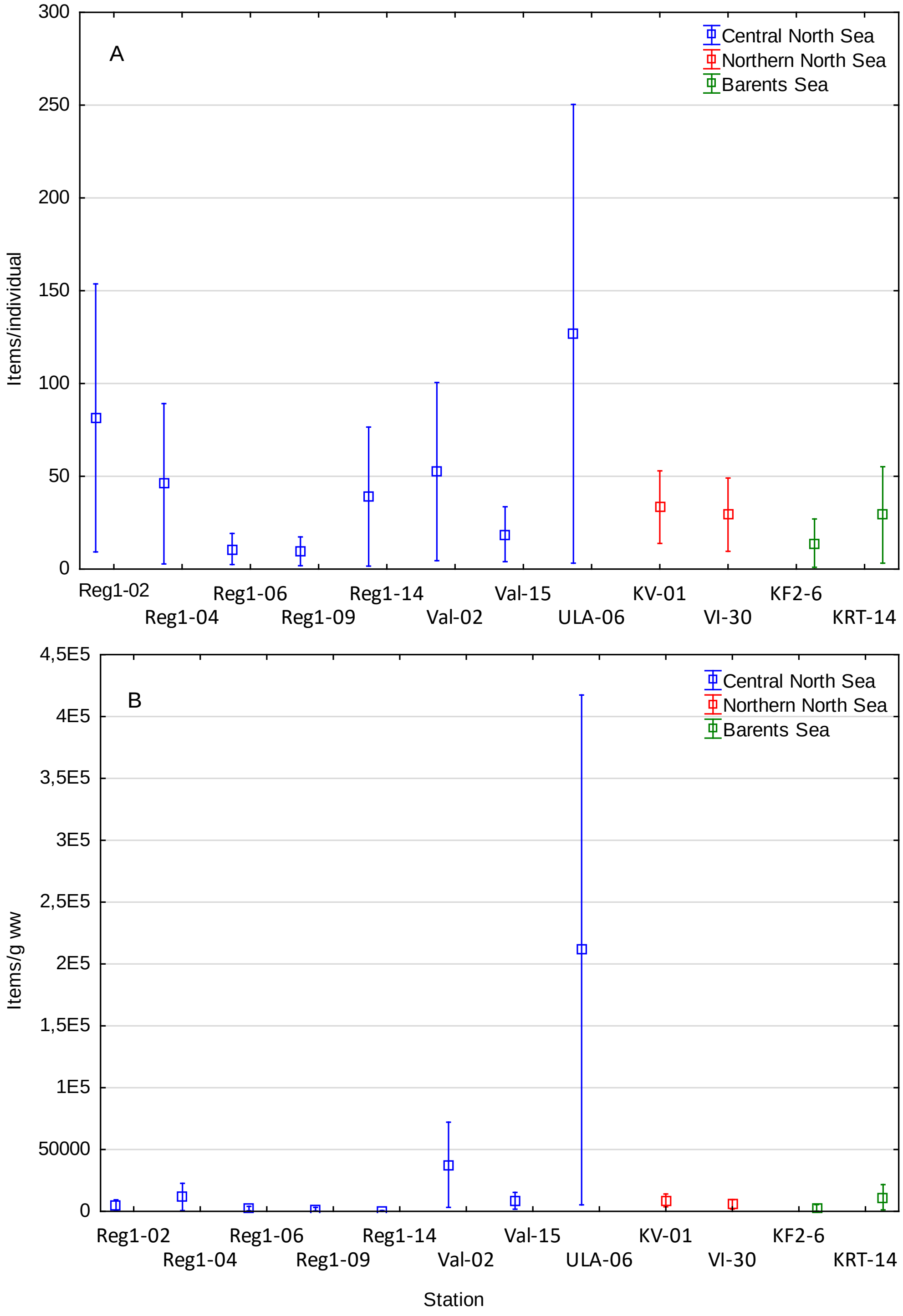

Figure 4-1. Microplastic concentration range (A: items/individual; B: items/g wet weight). Boxes indicate median concentrations, while whiskers show lower and conservative concentration estimates. Blue: the central North Sea, red: Northern North Sea and green: Barents Sea.

As presented in Figure 4-1 and Table 4-2, the positively identified (lower estimate for all areas) MP concentration was 5 ± 4 items/individual, corresponding to 1628 ± 1660 items/g wet weight. Further, average MP concentrations were higher in the Northern North Sea compared to the Central North Sea and the Barents Sea. It is unclear whether the differences are significant, as only two samples from the Northern North Sea and the Barents Sea were analysed, compared to eight samples from the central North Sea. Further, it is important to mention that most polychaetes in the samples from the Central North Sea and the Barents Sea were mainly Oweniidae (*Galathowenia oculata*, see section 3.2), whereas the samples from the Northern North Sea were mixed samples with different polychaetes species (see picture of sample KV-01 in Figure 4-2). This could be of importance with regards to their relatively higher microplastic concentrations compared to the Central North Sea and the Barents Sea. As we do not know the specific species composition in the mixed samples (KV-01 and VI-30), it is difficult to determine the possible uptake into higher trophic levels. However, it is known that the gut content in different fish species in the Northern Sea contains a variety of polychaete species. For instance, Yeung and Yang (2014) found polychaetes to be the dominant family of prey in Flatfish in the Northern Bering Sea. In addition, Schûckel et al. (2010) reported that Haddock in the Northern Sea pray on various polychaetes (Schûckel et al., 2010).

Figure 4-2 Mixed sample of polychaetes from station KV-01 (Northern North Sea) on a 45 µm steel mesh filter.

Sample Reg1-02, Val-02 and Ula-06 were analyzed at 26 µm steel mesh filters, whereas the others from the Central North Sea were analyzed at 45 µm filters. This could explain their relatively higher microplastic concentrations compared to the others from the same region. The difference between lower and conservative microplastic concentration was especially large for sample ULA06 (Table 4-2 and Figure 4-1) due to a relatively high amount of unknown particles (quality index < 0.6).

4.2.2 Sample composition

The percentage of each identified particle category in the digested polychaete samples (ref Table 3-4) is listed in Table 4-3. Pie charts with average relative compositions for each particle category are shown in Figure 4-3. As previously mentioned, the lower microplastic concentration estimates are based on the amount of total plastic polymers, paint and rubber particles, whereas conservative estimates in addition include the unknown particles (i.e. particles with a match score < 0.6 with the reference library). The majority of particles were classified as unknown or organic (on average: 70 % and 21 %, respectively). Regarding the presence of organic materials, this implies that the digestion method was not fully effective at removing 100 % of all organic material, which shows the importance of performing FT-IR for identification of plastic particles.

The most frequently found plastic polymers were polyacrylamide, chlorinated PP and PE, PVC, nylon, paint, rubber and others (most libraries belonging to commercial, tradename polymers of unconfirmed composition, plasticizers and additives) (see Table 4-3).

Table 4-3. Percent composition of particles in the samples, as classified by FT-IR, after chemical digestion and density separation. Unknown particles represent particles with FT-IR spectra which did not correspond to anything in the reference library (quality index score < 0.6 with the library). The colours in the table correspond to the colouring in Figure 4-3.

Sample	Filter size	Location	Unknown	Mineral	Organic	Paint	Petro-pyro	Plastic	Rubber	Most frequent plastics
Reg1-02	26	Central North Sea	81 %	0 %	13 %	0 %	0 %	5 %	0 %	Others*, PP-chlorinated, PE-chlorinated
Reg1-04	45		78 %	4 %	16 %	0 %	0 %	3 %	0 %	Others*, PVC, PE-chlorinated
Reg1-06	45		53 %	3 %	37 %	0 %	0 %	8 %	0 %	Polyacrylamide, Others*, PVC
Reg1-09	45		49 %	2 %	43 %	0 %	0 %	6 %	0 %	Others*, PVC, PE
Reg1-14	45		84 %	4 %	10 %	1 %	0 %	1 %	0 %	Others*, PVC, PP-chlorinated
Val-02	26		79 %	2 %	15 %	0 %	0 %	3 %	1 %	PET, Rubber, Others*
Val-15	45		61 %	3 %	28 %	0 %	0 %	8 %	1 %	Others*, PET, PVC
ULA-06	26		91 %	3 %	6 %	0 %	0 %	1 %	0 %	PP-chlorinated, PE-chlorinated, Paint
KV-01	45	Northern North Sea	55 %	1 %	24 %	6 %	0 %	14 %	0 %	Others*, Paint, PVC
VI-30	45		57 %	2 %	27 %	4 %	0 %	9 %	0 %	Others*, Paint, PE-chlorinated
KF2-6	26	Barents Sea	74 %	4 %	19 %	0 %	0 %	2 %	1 %	Polyacrylamide, Rubber
KRT-14	26		73 %	3 %	19 %	0 %	0 %	4 %	0 %	Nylon, Others*, PP

**Mostly library matches belonging to commercial, tradename polymers of unconfirmed composition at the present time*

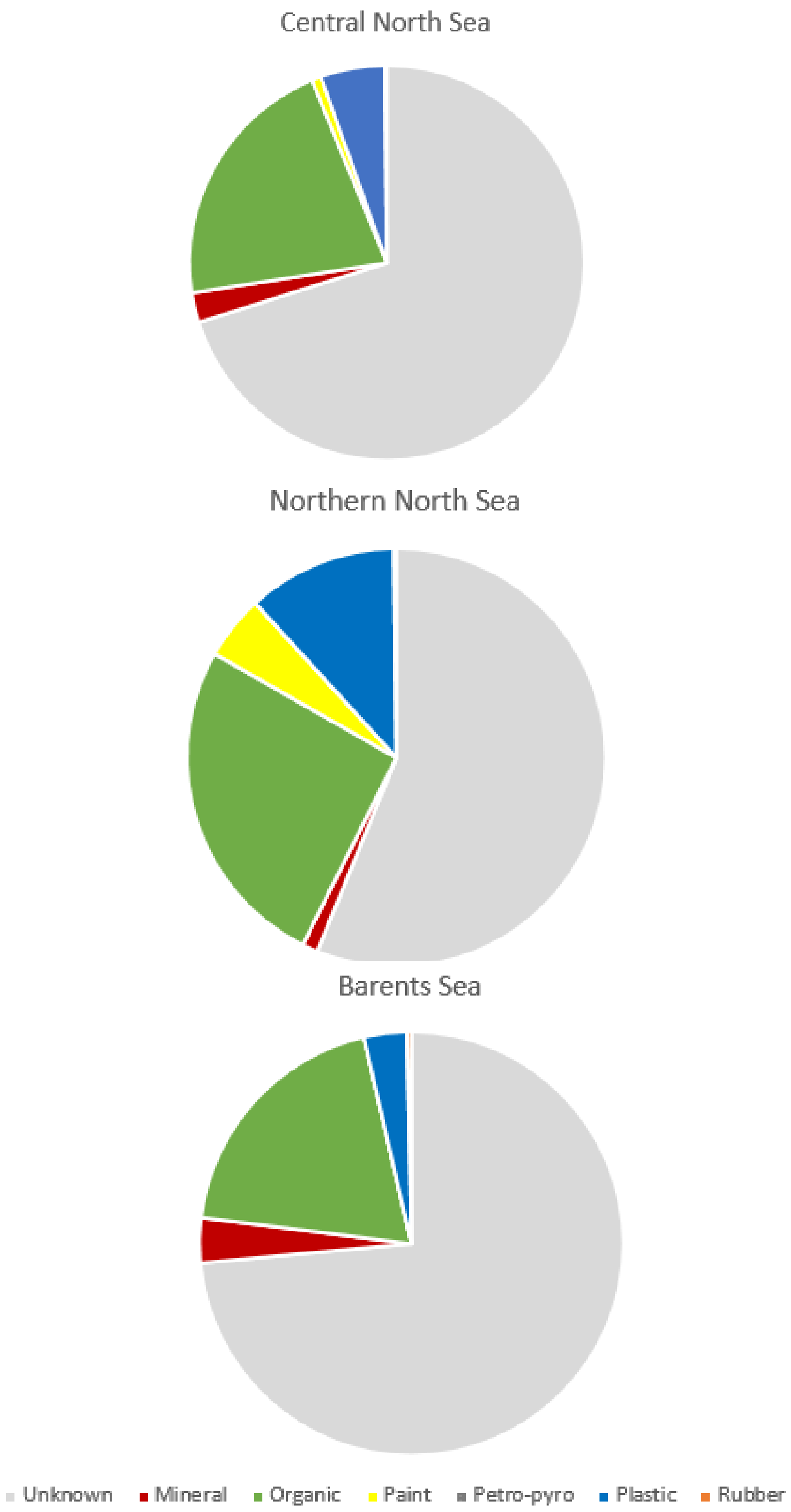

Figure 4-3. Average percentage composition of unknown (match score < 0.6 with the FT-IR library), mineral, organic and plastic (plastic polymers, paint and rubber) (petro-pyro was not identified) in samples from the central North Sea, Barents Sea and the Northern North Sea.

5 DISCUSSION

5.1 Accuracy of FT-IR data and evaluation of the method

As described in the results section, a relatively large quantity of the analyzed particles was classified as unknown due to low or no match score (score < 0.6) with reference spectra in the library database provided by the manufacturer. In addition to the fact that this may have been particles missing in the library, the low score could be caused by other factors, such as the large abundance of partially digested organic tissue in the worms that survived the digestion process. The digestion method was not fully validated for complete digestion of worms, though visually the worms were ca. 90 % digested.

Another cause of low scores are highly oxidized polymers and other materials, not conforming to reference spectra. The chemical oxidation may have also oxidized the surface of some polymers to some extent, which would affect the FT-IR spectra. Finally, there are uncertainties associated with the actual FT-IR apparatus. E.g. to obtain high quality spectra in transmission mode, it is best with samples that are ideally <50 microns thick which sit as flat on the window as possible. Verification of reported findings can be assisted through replicate analysis and using different methods. Analysis using a higher quality index cutoff, such as 0.7, would result in a fewer number of microplastics and larger proportion of unknowns; though, increase the confidence of the lower MP concentration estimates. A similar result would occur if a stricter blank correction was done, e.g. by setting the quantification limit as double the number of particles observed in the blanks.

5.2 Microplastic in polychaetes

5.2.1 Geographical distribution

Each individual sample contained microplastic. The average MP concentration was highest in the Northern North Sea, followed by the central North Sea and the Barents Sea (see Figure 5-1), but there were only two samples from the Northern North Sea and the Barents Sea, compared to eight samples from the central North Sea. Also considering the uncertainties in the analysis, the average MP concentration in the Central North Sea and the Barents Sea appear quite similar. It is difficult based on these results and the results from a similar study investigating microplastic in marine sediments (DNV GL, 2018b) to see any clear regional difference in microplastic abundances in polychaetes at present stage.

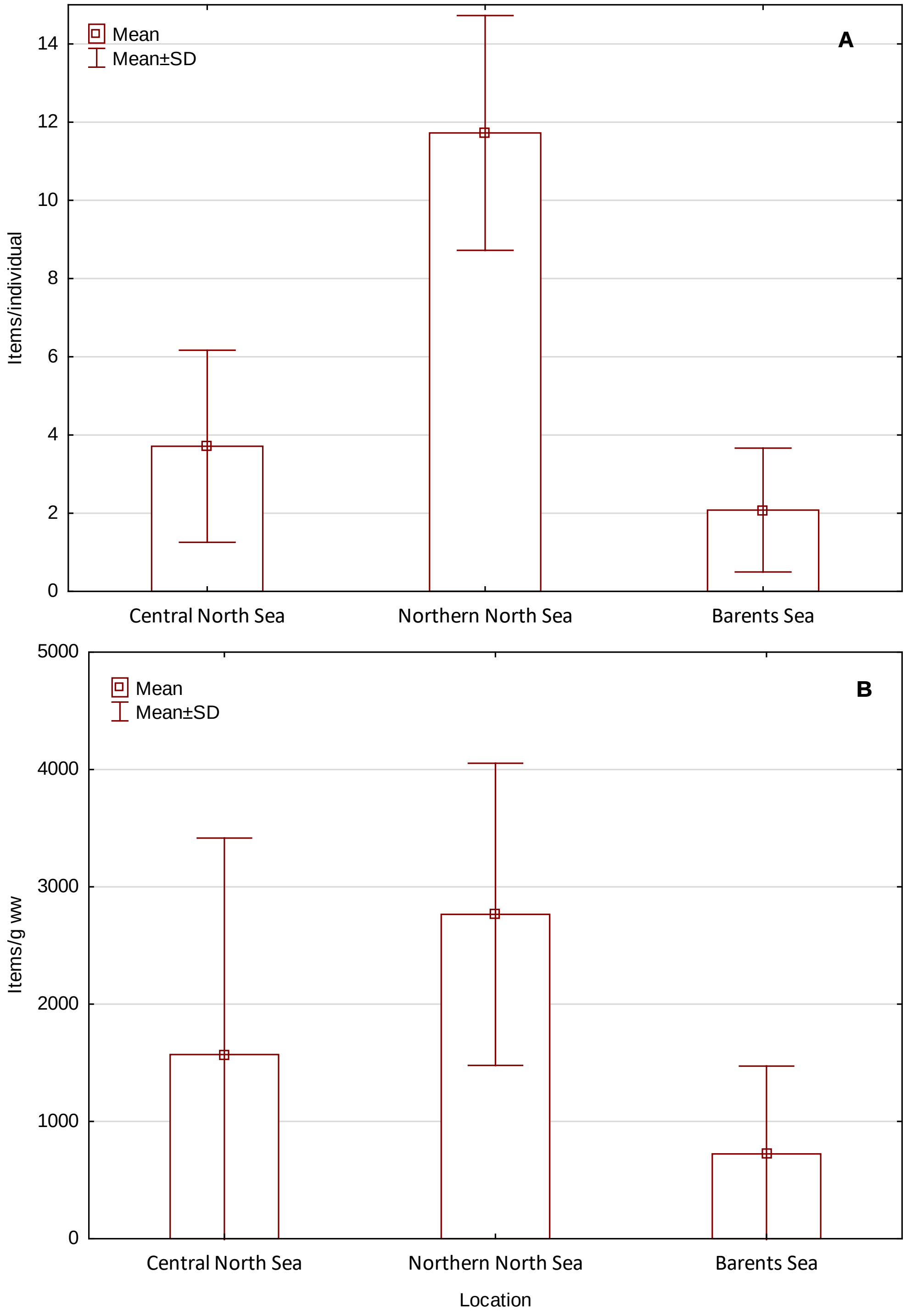

Figure 5-1. Average microplastic concentrations (items/individual). A = lower estimate (microplastic defined as plastic polymers, paint and rubber). B = conservative estimate (lower estimate + particles categorized as unknown due to low match score with FT-IR spectra in the library) NB! Different scaling on the y-axis.

5.2.2 Comparison of microplastic in polychaete and sediment, and speculations of origins

Microplastic concentrations in sediment samples from the same stations discussed in this report, except for station KV-01, have been given in DNV GL (2018a and 2018b). A comparison of the lower estimate for number of plastics items identified with FT-IR in sediment- and polychaeta samples from the same stations along the NCS are shown in Table 5-1. Also shown in this table is the projected Biota-Sediment accumulation Factor (BSAF) of microplastics based on the ratio of this concentration:

BSAF (g dw/ g ww) = Items in polycheate (g ww)/Items in sediment (g dw).

Table 5-1. A comparison of the number of microplastics identified in polychaeta and sediments with FT-IR, as well as the derived estimated BSAF values.

Sample/station	Location	Polychaetes Items/g ww (lower estimate)	Sediments lower estimate Items/g dw (DNV GL, 2018b)	Sediments Max Items/g dw (DNV GL, 2018b)	Items min BSAF (g dw / g ww)[1]	Items max BSAF (g dw / g ww)[2]
Reg1-02	Central North Sea	563	<LOD	0.38	1482	-
Reg1-04	Central North Sea	713	<LOD	9.2	77	-
Reg1-06	Central North Sea	519	0.54	9.7	54	961
Reg1-09	Central North Sea	339	0.029	0.73	465	11700
Reg1-14	Central North Sea	14	0.05	0.42	34	286
Val-02	Central North Sea	3250	0.75	7.5	435	4334
Val-15	Central North Sea	1827	<LOD	4.8	381	-
ULA-06	Central North Sea	5333	3.10	29.0	184	1720
VI-30	Northern North Sea	1855	3.4	8.8	211	550
KF2-6	Barents Sea	194	0.071	0.94	207	2737
KRT-14	Barents Sea	1253	0.2	0.59	2142	6264
All areas Average ± SD (min-max)					515 ± 674 (34 - 2142)	3569 ± 3869 (286 - 11700)

1) Based on the potential maximum plastic items in sediment, lower estimate in biota; 2) based on the lower estimate in sediment and lower estimate in biota.

Though the results in Table 5-1 are considered very preliminary, and probably subject to change with follow-up analysis (e.g. with analysis of replicate samples from the same stations), it is interesting to discuss these preliminary results for their implications to the bioaccumulation of microplastics in sediments and to polychaetes. As evident, the number of positively identified microplastics (according to the applied procedure) are consistently larger in polychaetes (per g ww) than in sediments (per g dw), with minimum BSAF values ranging from 34 to 2142 (at Reg 1-14 and KRT-14, respectively). Though more data is considered needed to confirm these estimates, if they are confirmed this would imply that microplastics are enriched in polychaetes at a per g basis, implying poor excretion rates. However, the uncertainty regarding these numbers has to be taken into account. Nevertheless, this does account for the general observation that less than a gram of polychaetes was required to find microplastics, whereas for a sediment sample ca. 600 g or more were required (DNV GL, 2018a). Further analysis should be done to validate whether the MPs in the polychaetes are accumulated in the biological tissues or in the surrounding tube structure.

In Table 5-2 a comparison of the two most frequent types of microplastics are compared in polychaetes and sediments.

Table 5-2. Comparison of the most abundant plastics identified in sediment- and polychaete samples from the same stations. Plastics are herein defined as plastic polymers, paint and rubber.

Sample / station	Most frequent plastic identified in polychaete	Most frequent plastic identified in sediment
Reg1-02	Others*, PP-chlorinated, PE-chlorinated	Polyacrylamide, Paint
Reg1-04	Others*, PVC, PE-chlorinated	n.d.
Reg1-06	Polyacrylamide, Others*, PVC	Paint, PP-chlorinated
Reg1-09	Others*, PVC, PE	PE-chlorosulfonated, Paint
Reg1-14	Others*, PVC, PP-chlorinated	Others*, Paint
Val-02	PET, Rubber, Others*	Rubber,
Val-15	Others*, PET, PVC	n.d.
ULA-06	PP-chlorinated, PE-chlorinated, Paint	Others*, Rubber
KV-01	Others*, Paint, PVC	n.a.
VI-30	Others*, Paint, PE-chlorinated	Paint, Rubber
KFT2-6	Polyacrylamide, Rubber	PE-chlorinated, Paint
KRT-14	Nylon, Others*, PP	PE:PP, Paint

*n.d. = not detected; n.a. = not analyzed; *others: library matches belonging to commercial, tradename polymers of unconfirmed composition at the present time.*

As evident from the table above, a variety of microplastics were found in the samples. Looking at the table as a whole, there were several reoccurring plastic types in sediments and polychaetes, in particular: polyacrylamide, and chlorinated PP and PE. Major differences are PVC was more commonly detected in the polychaetes samples, whereas paint (e.g. phenoxy resins) and rubber were more abundant in sediment.

Polyacrylamides are commonly used as a flocculant in water and wastewater treatment, as a soil conditioner, viscosity modifier and friction reducer in both enhanced oil recovery and high volume hydraulic fracturing. Any presence in sediments may be within sediment flocks, or due to low water-soluble polyacrylamide mixtures. It is noted that many of the findings, such as on regional stations and in the Barents Sea, are far away from Oil & Gas installations.

Chlorinated PEs are used as major and minor components in a wide assortment of applications in industry, including as process aid in rigid PVC foam applications as a partial replacement for acrylics. Applications include cable and wire coverings.

Nylon was the most abundant plastic polymer in the polychaeta sample from station KRT-14. Nylon is a family of synthetic polymers, based on polyamides. It is a thermoplastic material that can be melted into fibers, films or shapes. One of its uses include as monofilaments in fishing lines (it was also identified in Val-15, but not as the most abundant polymer).

As evident by Table 5-2, PET was not one of the most abundant MPs identified in sediment samples, but it was one of the most frequent plastic identified in polychaetes from station Val-02 and Val-15. PET is the most common thermoplastic polymer resin of the polyester family and is used in a variety of products, such as fibers for clothing and containers for liquids and foods. The PET particles identified in polychaetes from station Val were mostly fibers. Interestingly, no MPs were identified in sediment from Val-15 (with a match score ≥ 0.6 with the reference library), which could be due to uncertainties in the method (i.e. difficult to transfer fibers from the original sediment samples to the investigated subsamples which were analyzed with FT-IR), or it could be that these polymers were not present in the sediment samples.

In sediment samples, rubbers and paint were frequently detected. These were not as abundant in the polychaetes. Further follow up analysis should be performed to get more data on sediments and polychaetes, but based on the results so far it indicates that either i) different types of plastic particles would have different BSAF values (e.g. paint particles had low BSAF values, whereas PET and PVC had higher ones), and therefore the most frequent sediment particles are not the most frequent particles found in polychaetes, ii) differences may arise from the *Oweniidae* filtering water from the bioturbation layer in addition to sediments, iii) sampling artifacts if microplastic concentrations are heterogenous, v) sub-sampling artifacts for FT-IR analysis (entire worked up worm samples were analyzed for FT-IR, whereas only subsamples of the concentrated sediment samples were used) and vi) some combination of the above.

In addition to the abovementioned categories, there was another type of particle that was visually common in many sediment samples, but could not be characterized conclusively by FT-IR. This particle was found in sediment- and polychaeta samples (see DNV GL, 2018b). In agreement with the results from sediment samples, these particles were white / clear granules of approximately the same shape and size (100 - 500 µm). They were found in many of the samples with the highest abundance of "maximum microplastic concentrations", such as Ula-06, Reg1-14 and Val-02.

In DNV GL (2018b), the sediment sample from station ULA-06 from the central North Sea, was reported as one of the samples with highest microplastic concentration (42-384 mg/kg and 324-2 996 mg/m^2, corresponding to 3 141-29 020 items/kg and 25 330 – 234 031 items/m^2). When considering items/g concentrations, this was also the station with highest MP concentrations for the polychaetas. However, the most frequent plastic polymer varied between sediment- and polychaeta samples. E.g. for station ULA-06, the most frequent plastic polymer in sediment was "others" (mostly plasticizers and additives), whereas the most frequent plastic in polychaetes from the same station was chlorinated PP and –PE. Nevertheless, both the sediments and polychaetes had several particles of near identical spectra, where the closest match in the library was for the polyphenyl ether "POLY(2,5-DIMETHYL-1,4-PHENYLENE-3,3'-DIOXO-5,5'-BIINDOL-2,2'-DIYL) 2/40", though usually with scores between 0.4 – 0.6. We cannot conclude from this spectrum if it is in fact a polyphenyl ether, it is just noted for now as a common particle in sediment and polychaete samples that cannot yet be identified.

5.2.3 Comparison with literature

Due to little information about microplastics in *Owiniidae* in the literature, is it difficult to directly compare our results with other studies. In general, there is very little information in the literature regarding benthic fauna at al. In addition, differences in sampling and analytical methodologies make comparisons with previous studies difficult, although magnitude-scale comparisons may be reasonable. The few studies that could be found so far are presented here.

Arenicola marina, a polychaete from another family, has in laboratory tests shown to get significantly reduced energy reserves by up to 50 % when maintained in sediments spiked with microplastics (unplasticized polyvinylchloride, UPVC) (Wright et al., 2013). It was suggested that 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). The investigation was done with microplastic concentrations overlapping those in the environment (using the Wadden Sea as example).

Bour et al. (2018) also found benthic fauna to ingest microplastics in the wild. Their estimates of concentration from the inner Oslo Fjord in Norway was one to four MP particles per individual (Bour et al., 2018), which is in the same range as the lower estimates reported in this study (average 5 ± 4 items/individual, ranging from 1 to 14, Table 4-2). Bour et al. (2018) extracted eight polymer typologies from the organisms in the study, where PE and PP were the most abundant, and the MPs presented a variety of shape and sizes. Hence, the results for the Norwegian Coastal Shelf and the Oslo Fjord are similar in terms of abundance per individual, though not plastic type.

Courtene-Jones et al., (2019) conducted a study of two deep-sea benthic invertebrates (*Ophiomusium lymani* and *Hymenasterpellucidus,* both starfish) that were sampled by a span of four decades, 1976 – 2015, collected from 'Gage Station M' at a depth of 2200 m in the Rockall Trough (57.300°N, 10.383°W), west of the United Kingdom. They reported consistent concentrations over all years, at 1.96 ± 0.66 to 4.61 ± 3.62 microplastics/g w.w. This is considerably lower than the concentrations reported here for Oweniidae. This is partly due to method differences (e.g. here the entire sample was looked at, whereas in Courtene-Jones et al., (2019) particles deemed potential microplastics via a dissecting microscope were transferred for analysis); though, it could also be due to different uptake rates. Regarding polymer composition, Courtene-Jones et al., (2019) reported PET and polyamide (nylon) fibers as the most abundant in all years, here PET (most typically present as fibers) was the most abundant in the samples from the station VAL-02, and polyamide (i.e. nylon) the most abundant in KRT-14. PE, paint and PVC were also found in various samples both here and in Courtene-Jones et al. (2019). A large difference is the absence of chlorinated PE and polyacrylamide in the analysis by Courtene-Jones et al. (2019).

6 CONCLUSION AND FURTHER RECOMMENDATIONS

The results from this study highlights the widespread distribution of MPs in benthic fauna of the Norwegian Continental Shelf, and that microplastics can be transferred to polychaetes, where they may accumulate in concentration compared to the surrounding sediments. Regarding the potential hazard of microplastics, this provides a direct route for microplastics to transfer further up in the benthic food chain. Further research is needed to assess potential risks or ecotoxic effects of the concentration levels presented in this study; or to explore mechanistic pathways for microplastic accumulation (e.g. accumulation in the guts, or in the surrounding tube structures). The most frequently found plastic polymers in polychaetes were polyacrylamide, chlorinated PP and PE, PVC, nylon, paint, rubber and others (most libraries belonging to commercial, tradename polymers of unconfirmed composition, plasticizers and additives).

It is recommended to include further replicate analyses and to include additional subsamples of relevant polychaete and sediment samples, in order to increase the number of replicates and particles analysed in both media. Particle morphology, in particular in terms of microplastic fibers vs granules may also be investigated as part of future work. Further analysis is also recommended to differentiate concentrations between microplastic concentrations within the *Owiniidae* themselves or their surrounding tubes.

Additionally, the most abundant types of microplastic identified in this study, and in the parallel sediment study, gives indications of what types of microplastic are causing the most contamination within Norwegian Coastal Shelf sediments and polychaetes. Future work could also identify sources and transport routes of these types of particles, as part of an initiative to monitor or curtail increasing concentrations.

7 REFERENCES

Anderson, Don T. (2001). Polychaetes and allies: The southern synthesis. Fauna of Australia. Vol 4a. Polychaeta, Myzostomida, Pogonophora, Echiura, Sipuncula. Bulletin of Marine Science, 68(2), 180-206.

Andrady, A. L., 2017. The plastic in microplastics: A review. *Marine Pollution Bulletin*. 119, 12-22.

Barnes, D. K. A., Galgani, F., Thiompson, R. C. and Barlaz, M., 2009. Accumulation and fragmentation of plastic debris in global environments. *Philos Trans. R. Soc. B.* 364, 1985-1998.

Bour, A., Avio, C. G. Gorbi, S., Regoil, F. & Hylland, K. (2018). Presence of microplastics in benthnic and epibenthic organisms: Influence of habitat, feeding mode and tropic level. *Environmental Pollution, 243*, 1217-1225. DOI: 10.1016/j.envpol.2018.09.115

Courtene-Jones, W., Quinn, B., Ewins, C., Gary, S. F. & Narayanaswamy, B. E. (2019). Consistent microplastic ingestion by deep-sea invertebrates over the last four decades (1976)-2015), a study form the North East Atlantic. *Environmental Pollution. 244*, 503-512. DOI: 10.1016/j.envpol.2018.10.090

DNV GL (2018a). Microplastics in sediments on the Norwegian Continental Shelf. Report no: 2018-0050, Rev.01. Date: 2018-02-23.

DNV GL (2018b). Identification of polymer type of potential microplastic in offshore sediments, Rev. 00. Draft date (in prep.): 2018-10-01.

Fauchald, K. (1977). The Polychaete Worms, Definitions and Keys to the Orders, Families and Genera. Natural History museum of Los Angeles County, Science Series 28. Downloaded from: http://www.vliz.be/imisdocs/publications/123110.pdf (22.11.2018).

GESAMP, 2016. Sources, fate and effects of microplastics in the marine environment: part two of a global assessment. A report to inform the Second United Nations Environment Assembly. GESAMP Working group 40 2nd phase. Joint Group of Experts on the Scientific Aspects of Marine Environmental Protection. Published by the International Maritime

Hidalo-Ruz, V., Gutow, L., Thompson, R. C. and Thiel, M., 2012. Microplastics in the Marine Environment: A Review of the Mehods Used for Identification and Quantification. *Environ. Sci. Technol.* 46, 3060-3075.

Oug, E., Cochrane, S. K. J., Sundet, J. H., Norling. & Nilsson, H. C. (2011). Effects of the invasive red king crab (Paralithodes camtschaticus) on soft-bottom fauna in Varangerfjorden, northern Norway. *Marine Biodiversity under Change, 41*, 467-479. DOI: 10.1007/s12526-010-0068-6

Rouse, G. W. & Pleijel. (2001). *Polychaetes.* New York: Oxford University Press.

Schückel, S., Ehrich, S., Kröncke, I. & Reiss, H. (2010). Linking prey composition of haddock Melanogrammus aeglefinus to benthic prey availability in three different areas of the northern North Sea. *Journal of Fish Biology, 77*, 98-118. DOI: 10.1111/j.1095-8649.2010.02657.x

Tyler, L. BIOTIC - Biological Traits Information Catalogue (- http://www.marlin.ac.uk/biotic/browse.php?sp=4410, accessed November 2018).

Wright,, S. L., Rowe, D., Thompson, R. C., and Galloway, T. S. (2013). Microplastic ingestion decreases energy reserves in marine worms. *Current Biology, 23*(23), R1031-R1033. DOI: 10.1016/j.cub.2013.10.068

Yeung, C. & Yang, M. (2014). Habitat and infauna prey availability for flatfishes in the northern Bering Sea. *Polar Biology, 37*, 1769-1784. DOI: 10.1007/s00300-014-1560-4

Zachs, I. (1923). Sur un nouveau Ammocharidae (Myriochele oculata n.sp.) provenant de l'expedition du Prof. Derguine dans la Mer Blanche en 1922. Trav.Soc.Nat.Petrograd (1), Comptes Rendu, 53: 171-174, figs.

bout DNV GL

DNV GL is a global quality assurance and risk management company. Driven by our purpose of safeguarding life, property and the environment, we enable our customers to advance the safety and sustainability of their business. We provide classification, technical assurance, software and independent expert advisory services to the maritime, oil & gas, power and renewables industries. We also provide certification, supply chain and data management services to customers across a wide range of industries. Operating in more than 100 countries, our experts are dedicated to helping customers make the world safer, smarter and greener.