Testing of methodology for measuring microplastics in blue mussels (Mytilus spp) and sediments, and recommendations for future monitoring of microplastics (R & D-project)

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Norwegian Institute for Water Research

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Summary

Miljødirektoratet tasked NIVA to investigate methods used for the extraction of microplastics from environmental samples of blue mussels and marine sediment. Presented here are the results of methods tested, as well as NIVAs recommendations for future monitoring of microplastics in the Norwegian environment. Based on the current literature and this study, *Mytilus* spp. appears to be a promising bioindicator for the smallest sized microplastic (<1 mm) in the water column. However, improvements are still required to optimise mussel surveys for quantitative monitoring surveys of Norwegian coastal environments. Steps required include optimising the number of individuals analysed, investigating the role of mussel size, investigating the impact of collection depth and exposure to air as well as inter-site variation between mussel populations. Blue mussels alone should not be the only environmental matrix monitored for microplastic pollution. Sediments are proposed as the final destination of most microplastics in the environment. Hence, monitoring of sediment seem to be appropriate and important for long-term trends. However, due to the complexity of sediment analysis, it might be more suitable to use sediment dwelling organisms, such as worms and/or bivalves feeding off/in the sediment. Methods for sediment sampling and further microplastic analysis require further optimisation and testing.

Four keywords	Fire emneord
1. Plastics	1. Plast
2. Anthropogenic debris	2. Menneskeskapt forsøpling
3. Contamination	3. Forurensning
4. Monitoring	4. Overvåkning

This report is quality assured in accordance with NIVA's quality system and approved by:

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Testing of methodology for measuring microplastics in blue mussels (*Mytilus spp*) and marine sediments, and recommendations for future monitoring of microplastics

R & D-project

Preface

This report presents the results from the project "*Testing of methodology for measuring microplastics in blue mussels and marine sediments, and recommendations for future monitoring of microplastics (R & D-project)*" (Utprøving av metodikk for måling av mikroplast i blåskjell og marine sedimenter, og anbefalinger for fremtidig overvåking av mikroplast (FoU-prosjekt)). The project has been run in agreement between Miljødirektoratet as client and NIVA as project managers. The client's contact has been Camilla Fossum Pettersen. Project leader at NIVA has been Marianne Olsen. Sampling of sediments was carried out by Bjørnar A. Beylich, except for one sample from Bergen which was sampled by Sondre Kvalsvik Stenberg. Blue mussels were sampled within the project MILKYS, run by NIVA for Miljødirektoratet. Analysis of microplastics in sediment has been carried out by David Eidsvoll Pettersen, Amy Lusher and Nina Bonaventura. Analysis of microplastics in blue mussels has been carried out by Amy Lusher, Inger Lise Nerland Bråte, Karine Bue Iversen and Nina Bonaventura. All statistics has been carried out by Amy Lusher, who has also written the report with support from Inger Lise Nerland Bråte, Rachel Hurley and Marianne Olsen. Marianne Olsen carried out QA of the report. NIVA appreciates the opportunity to complete this project, and acknowledge everyone involved for good cooperation.

Oslo, 05.12.2017

Marianne Olsen
Project leader

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Abbreviations

ATR	Attenuated Total Reflectance
CH_2O_2	Formic acid
dH_2O	Distilled water
EFSA	European Food Safety Authority
EPS	Expanded Polystyrene
FPA	Focal Plane Array
FT-IR	Fourier Transform InfraRed
g^{-1} (w.w.)	per gram (wet weight)
$HClO_4$	Perchloric acid
HDPE	High Density Polyethylene
HNO_3	Nitric acid
HT-GPC	High Temperature Gel Permeation Chromatography
IR-SEM-EDS	Infrared scanning electron microscope with energy dispersive spectroscopy
KOH	Potassium hydroxide
LDPE	Low Density Polyethylene
LOD	Limit of detection
LOQ	Limit of quantification
MSFD	Marine Strategy Framework Directive
NaCl	Sodium Chloride
NaI	Sodium Iodide
NOAA	National Oceanographic and Atmospheric Administration
PA	Polyamide
PA-66	Nylon
PC	Polycarbonate
PET	Polyethylene terephthalate
PMMA	Poly methyl methacrylate
PS	Polystyrene
PP	Polypropylene
PTFE	Polytetrafluorethylene
PVC	Polyvinyl Chloride
Pyr-GC-MS	Pyrolysis Gas Chromatography Mass Spectrometry
SNT	Sodium/Nitrate Thiosulfate
WWTP(s)	Wastewater Treatment Plant(s)
$ZnCl_2$	Zinc Chloride

Summary

Miljødirektoratet tasked NIVA to investigate methods used for the extraction of microplastics from environmental samples of blue mussels and marine sediment. Presented here are the results of methods tested, as well as NIVAs recommendations for future monitoring of microplastics in the Norwegian environment.

Microplastics have been identified worldwide throughout the marine environment; beaches, the water surface, the water column and benthic sediment can all contain microplastics. Both terrestrial and marine sources can contribute to the release of microplastics into the marine environment and oceanic currents can facilitate transport. There is still insufficient quantitative information on microplastics in the Norwegian marine environment, despite that there are some estimations on numbers of microplastics released into the Nordic marine environment, and a few studies have identified microplastics in surface water, sea ice, sediment samples and biota from the Nordic marine environment

Many different methods have been developed to monitor microplastics, although the lack of standardisation limits comparability. In general, water samples may be filtered, digested and separated by density depending on the proportion of organic matter. It appears the best methods to process sediment samples are sieving and density separation, and when processing biota, organic material should be digested prior to analysis. After processing, samples are most often subject to visual and chemical identification to isolate plastic particles. It is vital to carry out standardised monitoring to acquire a baseline understanding of microplastic contamination in the Norwegian environment. This project aimed to identify suitable methods for monitoring microplastics in blue mussels and sediments, and proposes an approach for future monitoring of microplastic on a regional scale.

Methods used in this study were chosen based on ease of use, cost, preservation of plastic polymers, suitability for the removal of biogenic material and the ability to efficiently separate out plastics from sediments with varying organic content and grain size. Sampling stations around the coast of Norway were chosen to represent different levels of anthropogenic influence including urban, industrial, rural and combination areas. Blue mussels were collected from 13 stations along the Norwegian coast and sediment samples were collected from four stations within the Oslofjord and Bergen harbour.

Suspected plastics were extracted from blue mussels by dissolving organic material with 10 % potassium hydroxide (KOH) solution, incubating for 24 h at 60 °C and filtering the remaining homogenate. Both sieving and density separation using saturated sodium iodide (NaI) solution were tested as extraction techniques for the analysis of microplastics in sediment samples. Suspected plastic particles were verified as plastics using a combination of visual and chemical techniques. Contamination control was carried out throughout the processing and analysis. Any presence of contamination in blank samples was accounted for in the results.

Blue mussels were efficiently analysed for microplastics presence using the alkali digestive protocol, visual identification and chemical verification using Fourier transform infrared spectroscopy (µFT-IR). As expected, sediment samples were more complex to process for microplastic analysis. Due to the varying degrees of organic content and fine grain size, only semi-quantitative data could be obtained. Presence and absence data along with form of suspected plastics was acquired but chemical verification was not completed.

Plastics were found in 76.6 % of individual blue mussels (*Mytilus spp*.), with at least one individual containing microplastics from all 13 sites. The overall average plastic load was 1.84 particles individual^{-1} (range 0 – 14.67) or 1.85 particles g^{-1} w.w. (range 0 – 24.45). Particles from blue mussels consisted of fibres (85 %), fragments (11 %) and films and foams (4 %). Most of the particles were blue in colour (39 %) and the most common polymers were semi-synthetic fibres composed of chemically altered cellulose. The concentrations of plastics per gram were generally low close to urban areas whereas the highest average concentration was seen in a rural site in Finnmark. It is however important to note that it is unclear whether this is a true representation of the quantitative plastic pollution between sites, since the results might have been affected by other environmental and methodological factors, such as the larger sized mussels collected from urban sites. Plastic fragments and fibres were identified in replicate sediment samples using both methods (sieving and density separation). The identification of beads in sediment samples however, requires chemical classification before they can be accepted due to similarities with foraminifera and colour change caused by NaI flotation.

This is the first time that microplastics have been identified in the Norwegian environment through a large scale, co-ordinated survey. Differences in levels of microplastics identified in mussels from sites around the Norwegian coast may be caused by several factors such as hydrographical and atmospheric conditions, including tidal flow and amplitude, ocean currents, freshwater flow, locality to anthropogenic inputs and atmospheric deposition. Other procedural steps may also have an impact on the results seen here such as mussel size and the overall analysis conducted.

In addition to semi-synthetic cellulosic polymers, other polymers isolated from blue mussels included polyesters, polypropylene and polyethylene, Ethylene-vinyl acetate foam and epoxy resin. Potential sources of these particles could range from textiles, general use plastics, paints, and finally, oil and tar.

Based on the current literature and this study, *Mytilus spp*. appears to be a promising bioindicator for the smallest sized microplastic (<1 mm) in the water column, due to their ecology, ease of sampling, effective sample processing and further analysis. However, improvements are still required to optimise mussel surveys for quantitative monitoring surveys of Norwegian coastal environments. Steps required include optimising the number of individuals analysed, investigating the role of mussel size, investigating the impact of collection depth and exposure to air as well as inter-site variation between mussel populations.

Blue mussels alone should not be the only environmental matrix monitored for microplastic pollution. Sediments are proposed as the final destination of most microplastics in the environment. Hence, monitoring of sediment seem to be appropriate and important for long-term trends. However, due to the complexity of sediment analysis, it might be more suitable to use sediment dwelling organisms, such as worms and/or bivalves feeding off/in the sediment. Methods for sediment sampling and further microplastic analysis require further optimisation and testing, but generally for future monitoring of sediments, it is recommended to use core samples to monitor sediment deposition of microplastics.

Sammendrag

På oppdrag fra Miljødirektoratet har NIVA undersøkt egnede metoder for mikroplastanalyse av det marine miljøet, nærmere bestemt for blåskjell og sediment. Presentert i rapporten er resultatene fra de ulike metodene som ble testet ut, i tillegg til NIVA sine anbefalinger for fremtidig overvåking av mikroplast i det norske marine miljøet.

På verdensbasis har det blitt identifisert mikroplast i alle komponenter av det marine miljøet; strender, overflatevann, vannsøylen, bentiske sedimenter og i biota. Både terrestriske og marine kilder kan bidra til utslipp av mikroplast som kan transporteres via havstrømninger. Det er fortsatt lite empirisk data tilgjengelig for mikroplast i nordisk marint miljø, selv om det eksisterer noen estimater av mikroplast-utslipp, og noen studier har påvist plast i overflatevann, havis, sedimenter og i biota.

Mange ulike metoder har blitt utviklet for å undersøke mikroplast i miljøet, men mangelfull standardisering begrenser sammenligning mellom studier. Generell tilnærming for vannprøver er filtrering, nedbrytning av organisk materiale og tetthets-separering (avhengig av mengden organisk materiale i vannprøven). Det ser ut til at den beste metoden for å opparbeide sedimentprøver er ved sikting og tetthets-separering, og for biota bør alt organisk materiale brytes ned før analyse. Etter prøveopparbeiding blir det vanligvis gjennomført en visuell analyse og en kjemisk identifisering for å isolere ut plastpartikler. Det er svært viktig at undersøkelser og analyser gjennomføres på en standardisert måte for å en forståelse av dagens mikroplast-forurensingen i det norske miljøet. Dette prosjektet har derfor som mål å identifisere egnede metoder for overvåking av mikroplast i blåskjell og sedimenter, samt å foreslå fremtidige fremgangsmåter for å overvåke mikroplast i marint miljø.

Metodene som er benyttet i denne studien ble valgt på grunnlag av brukervennlighet, kostnad, preservering av plast, evnen til å bryte ned organisk materiale og evnen til å separere ut plast fra sedimenter med ulik mengde organisk materiale og ulik kornstørrelse. Innsamlingsstasjonene langs norskekysten ble valgt basert på ulik forventet påvirkning av menneskelig aktivitet; urban påvirkning, industriell påvirkning, rurale stasjoner og såkalte kombinasjonsområder. Blåskjell ble samlet fra 13 ulike stasjoner og sedimenter fra fire ulike lokaliteter i Oslofjorden og fra Bergen byhavn.

Potensiell plast ble separert fra blåskjell ved å bryte ned organisk materiale med 10% kaliumhydroksid (KOH), med en inkuberingstid på 24 timer ved 60°C før filtrering. Sediment ble både siktet og tetthets-separert ved hjelp av en mettet løsning med natriumjodid (NaI) for å ekstrahere ut mikroplast. Potensiell plast ble verifisert som plast ved å kombinere visuelle og kjemiske teknikker. Kontamineringskontroller ble inkludert for både prøveopparbeiding og analyse. Kontaminering ble justert for i resultatbehandlingen.

Blåskjell ble effektivt analysert for mikroplast ned til 150 µm ved å bruke den alkaliske nedbrytnings-metoden KOH, etterfulgte med visuell og kjemisk identifisering (Fourier transform infrared spectroscopy - µFT-IR). Ikke uventet viste sedimentprøvene seg å være en mye mer kompleks matriks å analysere for mikroplast. På grunn av ulik mengde organisk materiale og andel finkornet sediment

var resultatene egnet for semi-kvalitativ presentasjon. Tilstedeværelse eller fravær av potensiell plast i sediment er rapportert samt hvilken form det var funnet i, men kjemisk analyse ble ikke utført.

Mikroplast ble funnet i 76.6 % av blåskjellindividene (*Mytilus spp*.), hvor minst et individ fra hver lokasjon inneholdt plast. Den gjennomsnittlige totale plastmengden funnet per individ var 1.84 partikler (spredning fra 0 – 14.67 partikler) og 1.85 partikler per gram v.v (spredning fra 0-24.45). Mikroplast funnet var fibre (85 %), fragmenter (11 %) samt film og skumplast (4 %). De fleste partiklene var blå (39 %) og mesteparten av mikroplasten funnet i blåskjellene var semi-syntetisk plastfibre (kjemisk modifisert cellulose). Konsentrasjonen av plast per gram blåskjell var generelt lav for urbane områder, mens den høyeste gjennomsnittlige konsentrasjonene av mikroplast ble funnet i rurale Finnmark. Det er derimot viktig å påpeke at det er usikkert om dette er et reelt bilde på den kvantitative plastforurensing for det gitte området, siden resultatene kan ha blitt påvirket av andre faktorer, slik som store blåskjell fra urbane lokasjoner. I sediment-prøvene ble det funnet plastfibre og plastfragmenter i replikater for begge sediment-metodene (sikting og tetthetsseparering). For sedimentene trengs det videre identifikasjon av plast-perler, såkalte «beads» før de kan bli akseptert som plast-beads på grunn av sin likhet med foraminifera og mulig fargeendring som følge av NaI-flotasjon.

Denne studien er den første stor-skala undersøkelsen av mikroplast i det norske miljøet. Forskjellene mellom mikroplastnivåer i blåskjell fra de ulike lokasjonene langs norskekysten kan ha blitt påvirket av flere forhold som hydrologiske og atmosfæriske forhold, inkludert tidevann og amplitude, havstrømninger, ferskvannspåvirkning samt lokale antropogene kilder og luftforurensing. Prosedyrepåvirkning kan derimot ikke utelukkes som forklarende årsak, som ulik størrelse på blåskjellene.

I tillegg til semi-syntetisk plast, ble det også funnet andre plasttyper i blåskjellene som polyester, polypropylen, polyetylen, etylen-vinylacetat skum og epoksy-resin. Mulige kilder til denne plastforurensingen kan være tekstiler, generelt plastbruk, maling og tjære/olje kompositter.

Basert på eksisterende litteratur i tillegg til denne studien, ser *Mytilus spp* ut til å være en lovende indikator for overvåking av den minste mikroplasten (<1 mm) i vannsøylen på grunn av deres økologi, det er lett å samle nok blåskjell for analyse og det foreligger en relativt standardisert metode for prøveopparbeiding og videre analyse. Det kreves imidlertid noe optimalisering av metoden for å kunne bruke blåskjell for kvantitativ overvåking av mikroplast i det norske miljøet. Dette inkluderer optimalisering av antall individer analysert, inkludert undersøkelse av varians innen et område, betydningen av ulik størrelse på individer, posisjon i vannsøylen og eksponering for luft.

Blåskjell bør ikke være den eneste komponenten av det marine miljøet som overvåkes for mikroplastforurensing. Sedimenter er antatt å være endepunkt for mesteparten av mikroplastpartiklene i miljøet, og derfor bør sediment-analyser også inkluderes i fremtidig overvåking. Siden det er krevende å analysere sedimenter for mikroplast kan det være mer hensiktsmessig å analysere sediment-levende organismer i stedet/i tillegg til sedimentene, slik som flerbørstemark og/eller snegl som lever av/i sedimentene. Metoder for sediment-innsamling og videre analyse trenger betydelig optimalisering og testing, men generelt anbefales det for fremtidig overvåking av sedimenter og deponering av mikroplast, at kjerneprøve blir benyttet.

1 Introduction

Microplastics in the marine environment come from the breakdown of larger plastic items and the release of plastics produced in the microscale. Beaches, the water surface, the water column, benthic sediment and biota can contain microplastics. When biota interact with microplastics growth, development and reproduction may be affected. It is vital to carry out standardised monitoring in Norway to acquire a baseline understanding of microplastic contamination in the environment. This project aimed to identify suitable methods for monitoring microplastics in blue mussels and sediments, and proposes an approach for future monitoring of microplastic on a regional scale.

1.1 Sources and distribution of microplastics to the marine environment

Microplastics have been identified worldwide throughout the marine environment. Some microplastics are released directly into the environment whereas others breakdown as a result of environmental processes. Both terrestrial and marine sources can contribute to the release of microplastics into the marine environment and oceanic transport can move microplastics over large distances. Estimations on the number of microplastics released into the Nordic marine environment have been carried out but there is a lack of empirical data to support these estimations.

Plastic production and use has amplified since being first introduced as a commercial material in the 1940s. Present day production, estimated at 322 million tonnes in 2015, shows that plastics are a ubiquitous product and dominate the consumer market (PlasticsEurope 2016). Once in the environment, plastics can degrade into smaller sizes, ranging from the macroscopic to the microscopic. Further degradation to the nanoplastic range has been monitored through laboratory studies (Lambert and Wagner 2016). Microplastic, as the term suggests, refers to a small item of plastic; defined in this document as large microplastics, 1 – 5 mm, and small microplastics <1 mm. This definition follows standard SI units, but also encompasses large microplastics (Browne 2015; Galgani et al. 2013; GESAMP 2016).

Plastic items are found in all environmental matrixes; in surface waters, the water column, beaches, the sea floor and organisms. They are regularly found on shorelines in coastal waters, offshore accumulation zones, remote tropical islands, the Arctic, the Antarctic, and deep-sea sediments (for review see GESAMP 2016). Along with multiple scientific reviews of distribution, national and regional projects have attempted to highlight sources and sinks of plastic pollution (*e.g.,* Sundt *et al.* 2014). Distribution, fate and potential impacts of plastics may be influenced by the quantities, sources and types (size, shape, density, chemical composition, colour). Buoyant plastics can be moved over large distances by ocean currents, whereas fouled or dense particles may sink and become incorporated into the sediment matrix (GESAMP 2016). Reports estimating sources of plastic waste should be treated with caution because of the level of uncertainty and extrapolations used (*e.g.,* Jambeck *et al.* 2015). Currently, reliable quantitative comparisons between sources and input loads are not possible and this represents a significant knowledge gap (UNEP 2016).

There are multiple sources and routes of entry for plastics of all sizes into the ocean**.** Environmental processes including weathering, UV-degradation, oxidation and wave action lead to fragmentation of larger plastic items (Andrady 2015). Microplastic pollution is projected to increase in the foreseeable

future with the continued environmental breakdown and fragmentation of present stocks and future production of plastic items. Numerous sampling designs and methods have been used to investigate microplastics in the marine environment, which makes comparisons between studies almost impossible due to a lack of inter-comparability (Lusher 2015). In oceans, the small size and low density of microplastics contributes to their widespread transport by ocean currents and this can complicate analysis of sources and distribution trajectories. For example, coastal mariculture and fishing activities may be a localised source of microplastics, whereas sources of microplastics in offshore fishing grounds may be harder to interpret because of the influence of oceanic distribution (Lusher *et al.* 2017a).

Traditionally, there were two broad classifications of microplastics (primary and secondary). However, as more sources and types of microplastics are identified the classifications become harder to adhere too. Originally, **primary microplastics** were defined as plastics manufactured in sizes smaller than 5 mm, and **secondary microplastics** were defined as plastics that reach the micro-scale following the breakdown of larger items once in the environment (Cole *et al.* 2011). Problems with classification of microplastics based on source arise when microplastics are formed during use or following disposal. This makes a distinction between primary and secondary microplastics difficult. Henceforth, three different classes of microplastics are described based on their origin:

1) Microplastics which are produced for use in the microsize (traditionally known as primary microplastics). This includes pellets used by plastic producers and fabricators to manufacture larger plastic products, and plastic beads and grains incorporated into cosmetics and personal care products. The release is generally from land or loss at sea during transportation.

2) Plastic materials which breakdown during use or as a by-product of maintenance resulting in microplastics. These particles may be generated from the use of larger items such as synthetic fibres produced through washing textiles and clothing, as well as airborne fibres and fragments from the breakdown of car tyres, road paints or in-use fishing gear.

3) Plastic materials which breakdown in the environment when they are no longer used for their original purpose. Marine sources include microplastic particles produced from the breakdown of abandoned, lost or otherwise discarded plastic items from fishing, shipping and recreational activities.

1.1.1 Sources of microplastics in the Nordic marine environments

Plastic production, use on land, as well transport via wastewater treatment plants to riverine systems can contribute to microplastic pollution. Estimations on the number of microplastics released into Nordic environments have been carried out but they lack empirical data.

Sources of larger plastic items can be easy to identify due to characteristic features, whereas sources for microplastics can present challenges. Three comprehensive reports have assessed the main sources of microplastics into the Danish, Norwegian and Swedish environments (Table 1). Secondary microplastics are estimated to be the biggest contributor of microplastics to the Danish environment with 5 000 to 12 200 tonnes per year, while primary microplastics account for 460 to 1 670 tonnes per year (Lassen *et al.* 2015). However, the annual input of primary microplastics to the Norwegian environment was estimated to be ~ 8 000 tonnes (Sundt *et al.* 2014). The largest source of secondary microplastics to the Norwegian environment was attributed to abrasion of car tyres and road markings with estimated annual input of ~ 5 000 tonnes. For the Swedish environment, approximately 13 000 tonnes of microplastics are estimated generated from car tyres every year and an estimated loss of 2 300 – 3 900 tonnes of granules from artificial turf per year, but the report also highlighted that there is no information on how much is entering the aquatic environment (Magnusson *et al.* 2016).

Wastewater treatment plants (WWTPs) can act as a source and transport pathway for microplastics into the environment. Most Nordic countries have sophisticated WWTPs; however, when plants are not working adequately, undergoing maintenance or during times of overflow, there may be a higher level of input of microplastics to recipient water courses. Estimated emissions for WWTPs within Nordic countries vary. For example, a recent report based on empirical data from Denmark found large variations in the level of microplastic from ten different WWTPs, ranging from 0.2 to 30 mg L^{-1}. Talvite *et al.* (2015) found that microplastic fibres collected in surface waters in the Helsinki archipelago may have originated in WWTPs close to the receiving body of water. Comparatively, approximately 3 % of the total volume of microplastics remained in effluent making the wastewater treatment process very efficient in Denmark (Vollertsen *et al.* 2017). As microplastics are retained in sludge which is often directly applied as fertilizer to agriculture, it is important to consider sludge as a potential source of microplastics in itself (Nizzetto *et al.* 2016).

Table 1. Estimated total emissions of microplastics from Nordic countries. Value is total emissions in tonnes per year (% of total).

Location	Denmark (Lassen *et al.* 2015)	Norway (Sundt *et al.* 2014)	Sweden (Magnusson *et al.* 2016)
Type 1-Primary microplastics (produced for use in the microscale)			
Raw materials for plastic production	3 – 56 (0.3 %)	Discharge: 250 (3.0 %) Spill: 200 (2.4 %)	310 – 530
Personal care products	9 – 29 (0.2 %)	40 (0.5 %)	60
Rubber granules (incl. artificial turfs)	450 – 1 580 (10.5 %)	No data	2 300 – 3 900
Marinas (incl. blasting abrasives)	0.05 – 2.5 (0.01 %)	400 (4.8 %)	No data
Paints	2 – 7 (0. 1%)	No data	No data
Pharmaceuticals	No data	No data	No data
Sludge application	No data	No data	26 (>2 mm)
Type 2- Microplastics formed through use, a by-product of maintenance and wear and tear			
Laundry and textiles	200 – 1 000 (6.2 %)	Consumer: 600 (7.1 %) Commercial: 100 (1.2 %)	180 – 2 000
Footwear	100 – 1 000 (5.7 %)	No data	No data
Cooking utensils, sponges etc.	20 – 180 (1.0 %)	No data	No data
Building materials	80 – 480 (2.9 %)	#270 (3.2 %)	No data
Paints (excl. ship paints)	150 – 810 (5.0 %)	130 (1.5 %)	130 – 250
Road markings	110 – 690 (4.1 %)	320 (3.8 %)	520
Tire abrasion	4 200 – 6 600 (55.8 %)	4 500 (53.6 %)	13 000
Ship paints	40 – 480 (2.7 %)	330 (3.9 %)	480 – 1 360
Household dust	No data	450 (5.4 %)	0.9 – 17
City dust	No data	130 (2.4 %)	No data
Waste handling and recycling	No data	~500 (4.9 %)	No data
Other uses	100 – 1 000 (5.7 %)	No data	No data
Type 3. Microplastics form through breakdown in the environment			
Fisheries and aquaculture	No data	> 1 000	4 – 226
Sewage	No data	460	No data
Plastic bags	No data	60	No data
Other	No data	No data	No data
OVERALL TOTAL	5 500-13 900		

#classed as building repair

1.1.2 Microplastics in the Nordic marine environment

Although few in number, studies have identified microplastics in surface waters, ice and sediment samples from the Nordic marine environment. Proportion of plastic items in marine litter seem to increase from the Baltic Sea towards the North Atlantic and Arctic, indicating long-range transport of plastics. There is insufficient quantitative information on microplastics in the water column, sedimentation of microplastics and biota interactions

Monitoring of microplastic in the Nordic marine environment is increasing, although most data available on plastic items refer to macroplastics. Large plastic items have been identified on shorelines; local anthropogenic activity, as well as transport on ocean currents may be a source of this pollution (MARLIN 2013; van Sebille *et al.* 2012). The contribution of plastic items to beached marine litter appears to increase from the Baltic Sea (62 %) to Skagerrak (76 %) and the eastern North Sea (71 %) and furtherstill towards the North Atlantic (88 %) and the Arctic (97 %), indicating that plastic items may be transported over long distances (Strand *et al.* 2015). Large scale mapping of sea bed litter in the Arctic and sub-arctic waters shows that most litter in offshore locations originated from fishing activities. Plastic and rubber were the second biggest class of macrodebris identified (Buhl-Mortensen and Buhl-Mortensen 2017). Temporal increases have also been observed in sea floor litter at HAUSGARTEN, a long-term ecological research station in the eastern Fram Strait (Bergmann and Klages 2012; Tekman *et al.* 2017).

Microplastics were first reported in surface waters of the Nordic marine environment, between Tromsø and Svalbard (70 – 78 °N) in 2014 (Lusher *et al.* 2015). Cózar *et al.* (2017) have since reported that accumulation zones appear to be present north and east of Greenland, and within the Barents Sea. Microplastics reported in the Stockholm Archipelago and Baltic Sea had plastic concentrations almost ten times greater in coastal areas (Gewert *et al.* 2017). The most recent findings from Baltic Sea shows that there has not been a significant change in concentration of microplastics in plankton samples from 1991 to 2015 (mean ± SD: 0.21 ± 0.15 particles m^{-3}, n = 97, Beer *et al.* 2017), which indicates both efficient wastewater treatment and that the water column is not the final destination for microplastics.

In the Arctic, sea ice can act as a source and sink for entrained plastics, such that it accumulates floating microplastics when it freezes which are then released when it melts (Obbard *et al.* 2014; Lusher *et al.* 2015). For example, ice cores collected from the Fram Strait, contained high concentrations of microplastics; mean concentrations of 2 x 10^6 particles m^{-3} in pack ice and 6 x 10^5 particles m^{-3} in land-locked ice (Bergmann *et al.* 2016). It is also hypothesized that as sea ice retreats and shipping and fishing activity increase there may be greater input of marine pollution in to the Arctic, although baseline data on contamination from ocean transport and local input are required (Lusher *et al.* 2015).

Sedimentation of microplastics has been demonstrated as the Nordic seafloor is also polluted by plastic items, although there is insufficient quantitative information. Recent findings at HAUSGARTEN revealed high numbers of microplastics in sediments, 42 – 6 595 microplastics kg^{-1}, with the northernmost stations containing the highest quantities (Bergmann *et al.* 2017). Further studies of microplastics in sediment are required for future monitoring. This will help to understand the fate of microplastics in the ocean.

1.2 Interactions between organisms and microplastics

Marine organisms can interact with microplastics through adhesion, absorption, ventilation and ingestion. Laboratory experiments have shown negatives effects on feeding, the immune system, growth, energy levels, fecundity and reproduction.

Field and laboratory experiments have established that organisms can take up microplastics. This has stimulated a large volume of research concentring on the forms of interaction and ecotoxicological effects. Specifically, there has been a rise in attention to the effects of microplastics on marine organisms, especially those which are consumed commercially. This has been driven by concerns regarding impacts on human health (Lusher *et al.* 2017ab). All organisms have the potential to interact with microplastics present in the marine environment. Over 230 different species of marine organisms have been found to uptake plastics and microplastics *in natura* (Kühn *et al.* 2015). Excluding birds, turtles and mammals, 55 % of species have a commercial importance (Lusher *et al.* 2017a). Interacting with microplastics directly may result in adherence to external appendages, absorption, ventilation and ingestion. Organisms may also be affected if they consume prey that has previously ingested microplastics; leading to indirect contamination through secondary, or trophic, transfer. Concerns towards microplastic effects on marine biota have led to several laboratory exposures and toxicological studies, which have confirmed that a diverse array of organisms, across trophic levels, can ingest microplastics (GESAMP 2016). These studies have enabled monitoring of the uptake and distribution of microplastic within whole organisms as well as excised tissues, *e.g.,* gills, intestinal tract and liver. Microplastic interactions have been observed at an individual level as well as through secondary transfer from prey to predator (for review see GESAMP 2016). Secondary transfer is not likely to lead to microplastic accumulation because most microplastics (>150 µm) will not translocate into the tissue of their hosts (EFSA 2016).

Laboratory studies have identified some potential effects of microplastic exposure including: increased immune response, decreased food consumption, weight loss, energy depletion, decreased growth rate, decreased fecundity and impacts on subsequent generations (for review see Lusher *et al.* 2017a). Noteworthy negative effects of microplastic exposure observed under laboratory conditions often involve excessively high exposure levels (GESAMP 2016; Lenz *et al.* 2016).

1.2.1 Microplastics in Nordic biota

Studies on Nordic species interacting with microplastics are sparse, and of insufficient quantity and quality, to identify current trends or baseline levels. Blue mussels have been suggested as an appropriate indicator organism for future monitoring.

A recent report on behalf of the Nordic Council of Ministers (Bråte *et al.* 2017) has reviewed microplastic ingestion by Nordic marine biota. In summary, ingestion of plastics has been documented in 13 out of 14 fish species (a total of 5 241 fish individuals from nine different studies). In the first long-term study on microplastics in the Nordic marine environment, 814 fish (Atlantic herring, *Clupea harengus* and European sprat, *Sprattus sprattus*) were investigated, of which 20 % contained plastics with 95 % of particles <5 mm (Beer *et al.* 2017). Microplastic concentration in Baltic Sea fish remained constant over the past three decades (1987 – 2015) with no significant difference between species, locations or time of day.

Fewer studies have been carried out on invertebrates and microplastics in the Nordic environment. So far, a total of 205 blue mussels, from six studies have been investigated. In a limited study from

Svalbard, 20 % of mussels contained fibrous plastics (Sundet *et al.* 2015), whereas 68 % of mussels from the Swedish coast had ingested microplastics (Gustafsson 2015). These studies all focus on interaction through ingestion. Other forms of interaction including inhalation, adhesion to external appendages and uptake following ingestion of contaminated prey needs to be studied. Currently, the effect of microplastics on Nordic biota have not been investigated.

Unfortunately, comparisons between and within biota from the Nordic marine environment are difficult as there are a limited number of studies on the same species from different locations and different methods have been used. For future monitoring within the Nordic marine environment, it will be important to identify a suitable indicator species for microplastics, reflecting the present impact. Bråte *et al.* (2017) discussed the possibilities of identifying a monitoring species and concluded that blue mussels may be appropriate as they are already utilised in other regional, national and international monitoring programmes.

1.3 Aims and Deliverables

This report aims to identify and test suitable methods for monitoring microplastics in blue mussels and sediments. This will be accomplished by:

- Summarising the currently employed methods used to identify microplastics in blue mussels and sediments (Chapter 2)

- Detailing the approach and methods employed for sampling and analysing microplastics in blue mussels and sediments in this study (Chapter 3)

- Presenting the results on the presence of microplastics in blue mussels and sediments (Chapter 4)

- Discussing the results with regards environmental variables, influence of methodology, site specific differences and potential sources (Chapter 5)

- Evaluating the suitability of the chosen techniques used in the sampling, preparation, identification and quantification of microplastics from environmental samples (Chapter 6)

- Discussing the relevance of mussels and sediment for monitoring (Chapter 6)

- Proposing an approach for future of microplastic monitoring on a regional scale (Chapter 7)

- And finally, providing concluding remarks on the presence of microplastics in environmental samples (Chapter 8)

2 Methodological review: microplastic monitoring in the environment

Many different methods have been developed to monitor microplastics in the environment, although there is a lack of standardisation which limits comparability. Water samples may be filtered, digested and separated by density depending on the proportion of organic matter. Sieving and density separation appear to be the most appropriate methods for processing sediments, but this still needs some standardisation of methods. When processing biota, organic material should be digested, and several processing methods can be used. After processing, samples are most commonly subject to visual identification, followed by verification of polymeric material in at least a subsample of particles.

Methods used to determine the quantities and types of suspected environmental microplastics vary. This leads to concerns about whether results are a true representation of microplastic contamination or whether the results reflect the sampling procedure. There have been calls from the scientific community to standardise methodological approaches to allow for replication and better comparability between studies. This was the motivation behind the ongoing JPI-Oceans BASEMAN project. It is challenging to collect representative data from different environmental matrixes since microplastics do not behave and move as classical particle-bound environmental pollutants, and are not evenly distributed in the environment (Nuelle *et al.* 2014). Comparisons between studies are complicated by inconsistencies in methods and reporting units, the confounding patterns of spatial and temporal variability, the influence of environmental conditions and contamination control. It must be noted that handling and processing steps could alter the presence of microplastics in individual samples. For example, there may be loss of microplastics prior to animal preservation because of handling stress, physical movement, and the physiological and behavioural specificities of the sampled organism (Lusher *et al.* 2017b).

Some guidelines on sampling procedures are available (*e.g.,* MSFD guidelines, Galgani *et al.* 2013, and NOAA laboratory guidelines, Masura *et al.* 2015). Without an understanding of the variables influencing a samples collection, there is a limit to the extent of comparability. Monitoring programs need to be standardised, or intercalibrated, at regional, national and international scales.

In short, microplastics can be sampled from different environments using a variety of methods and once samples are collected they can be pre-treated to reduce their volume and/or remove organic matter by way of sieving, density separation, digestion or filtering. Most studies utilise a combination of methods. Researchers usually identify microplastic presence in samples (presence/absence, % occurrence in samples, and amount), followed by a validation step to visually accept particles based on characteristics (*e.g.,* Lusher *et al.* 2014) which should be verified through analysis of their molecular structure (Löder and Gerdts 2015).

Table 2. Examples of methods for sampling microplastics from the water column and sea surface

Method	Advantages	Limitations
Horizontally towed nets (*e.g.,* manta, Neuston, plankton net)	• Can be deployed from different sized vessels • Sample the sea surface • Flow meter allows estimation of volume filtered • Sampling can be conducted when vessel is underway • A vertically configured manta net allows deployment at high speed	• Use is weather dependant • Cannot account for environmental variables • High potential for contamination from towing if ropes are used • Volume of water filtered can only be estimated • Towing time must be limited to avoid net clogging • Under samples particles smaller than mesh size • Vessel speed must be restricted
Plankton nets	• Can be deployed from different sized vessels • Flow meter allows estimation of water volume samples • Variable depths • Not weather dependant	• Risk of sample contamination on deck • Under samples particles smaller than mesh size • Vessel speed must be restricted
Bongo nets	• Can be deployed from different sized vessels • Can be used in the water column • Paired nets can obtain replicate samples	• Risk of sample contamination on deck • Under samples particles smaller than mesh size • Vessel speed must be restricted
Underway pumps	• Sample a known volume of water • Can better control for contamination	• Intakes are small and upper limit of size may be set by any filters on the intake • Adverse sea states affect position of intake in water column
Submersible pumps	• Sample a known volume of water • Can better control for contamination	• Sampling platform needs to be stationary • Intakes are small and upper limit of size may be set by any filters on the intake
Continuous plankton recorded	• Can be used over a large distance on moving vessels	• Subsurface samples, cannot sample surface • Restricted size of intake ($1 cm^2$) underestimates larger particles
Epibenthic sledge	• Samples can be collected just above the seabed	• Difficult to accurately estimate water volume • Weather dependant • Contamination concern from deck storage • Size of particles captured is dependent on mesh size

2.1 Sampling the sea surface and water column

Water samples can be collected using different methods and it is generally recommended that samples are volume reduced before processing with sieves or by means of density separation.

Water samples are commonly collected using sampling gears which are towed horizontally, vertically or obliquely through the water column or at the water surface, or by way of pumping water onboard a vessel or sampling platform (Table 2). Nets with different mesh sizes influence the size of particles collected. Irrespective of sampling method used, surface sampling should be conducted in calm sea conditions with minimal tidal and wave interference. Samples can be separated into different size fractions by sieving or separated from biological material by way of density separation, air drying and digestion. Remaining particles can be subjected to visual examination or chemical analysis to identify and verify particles of synthetic origin.

2.2 Sampling beaches and benthic sediment

Beach and benthic sediment samples can be collected by grab, samples or by using cores. It is recommended that beach and sediment samples undergo sieving and density separation for effective processing and extraction of microplastics.

Beach samples are normally collected from the surface or using corers and sieved to reduce the volume and remove larger debris (Table 3). Benthic sediments can be sampled by taking cores or grab samples which permit an accurate assessment of the volume of sediment collected. Once collected, sediment samples must be processed to extract microplastics from the sediment matrix. This is typically performed through sieving (to separate the sediment based on size), elutriation (to separate particles based on their size, shape and density with liquid or air) or density separation (to separate particles using floating properties of different material in salt solution). Sieving helps to reduce the sample volume, where large volumes of sediment particles can impair visual identification and chemical characterisation. By using large- and fine-mesh sieves, large items of debris and fine sediment and particulate organic matter (*e.g.,* fine clays) can be removed. Furthermore, the use of a series of sieves can help to separate particle out into size classes, which gives us information on particle behaviour in environmental systems. However, if sediment samples have a large proportion of minerogenic and organic material concentrated in the same size fractions as the target microplastics (*e.g.,* sands at 63 µm – 2 mm), sieving may not be effective at isolating microplastic particles for visual identification.

Microplastics can also be separated with the use of an elutriation device. Elutriation separates lighter, smaller or less dense particles by using an upward flow of fluid or gas. This was first used in the preparation of samples for microplastic analysis by Claessens *et al.* (2013). High extraction efficiencies have been documented using this technique. This technique could potentially be complicated by the uplift of fine clay particles in complex environmental samples; however, the design of Claessens *et al.* (2013) passes the extract through a 38 µm mesh to reduce this problem. A density separation step might be required on the final extracted sample, but the volume required is much lower and costs are, therefore, reduced. Elutriation devices are effective at processing large sediment samples (*e.g.,* >1 kg) and rapidly reducing the sample volume. However, only one sample can be processed at a time, which may increase the time required to prepare large quantities of microplastic samples. Therefore, this method would be ineffective for large scale monitoring programmes.

Table 3. Examples of methods for sampling microplastics from beach and benthic sediments

Method	Advantages	Limitations
Selective sampling	• Rapid sampling on beaches • Suitable for citizen sampling initiatives • Commonly used for sampling resin pellets	• Sampling efficiency is only as good as the collector *E.g.,* size collected depends on the visual ability of the sampler
In situ sieving	• Rapid sampling on beaches • Suitable for citizen sampling initiatives • Commonly used for sampling resin pellets	• Limited to coarse mesh sizes • Unsuitable for wet sediments without using water
Grab sampling	• Easy to use • Small sampling devices can be sued from small boats	• Sediment surface may be disrupted during operation
Box coring	• Maintains water-sediment interface • Multicores allow replicates at sites	• Sediment surface may be disrupted during operation
Sediment gravity core	• Preserves sediment-water interface • Can provide record of microplastic deposition	• Small surface area • Requires heavier lifting gear on vessels

Finally, density separation can also be used to isolate microplastic particles for analysis. This technique utilises salt solutions of known densities to float particles out from the host sediment matrix. Early sediment microplastic studies utilised saturated NaCl solutions (density: 1.2 g cm^{-3}) (Hidalgo-Ruz *et al.* 2012); however, this fails to separate out many higher density plastics such as polyethylene terephthalate (PET) or polyvinyl chloride (PVC). To counteract this issue, alternative solutions have been adopted including ZnCl$_2$ (1.6 g cm^{-3}), SNT (1.46 g cm^{-3}) and NaI (1.8 g cm^{-3}). These capture most polymer types, whilst the host sediment matrix (typically >2 g cm^{-3}) is left behind. Specific separation devices that utilise density solutions have been developed, for example the Munich Plastic Sediment Separator (Imhof *et al.* 2012); however, these have been shown to exhibit extraction efficiencies as low as 13 % when processing complex environmental samples (Zobkov and Esiukova, 2017). Instead, smaller scale flotation in beakers or centrifuge tubes provides far higher extraction efficiencies and facilitates the processing of many samples simultaneously. Recently published method development has introduced a portable method to separate microplastics from different sediment types using density floatation with an extraction efficiency of 95.8 % (± SE 1.6 %; min 70 %, max 100 %) (Coppock *et al.* 2017). This method is cheap, reproducible and portable whic h may be a useful addition to monitoring programmes. Density separation is now the most commonly utilised extraction technique and is recommended by both NOAA and MSFD as the most cost effective and appropriate separation method (Galgani *et al.* 2013; Masura *et al.* 2015).

Sieving, density separation and elutriation may require a further organic matter removal step, depending on the content of organic material. Much of this material may float out at a similar density to microplastic particles or may be associated with the same size fractions. This can impair visual identification since the organic content may physically block microplastic particles in extracted samples. Several protocols have been discussed in the literature, where oxidation treatments have been shown to be the most effective (Nuelle *et al.* 2014). Remaining particles can be subjected to visual examination or chemical analysis to identify and verify particles of synthetic origin.

2.3 Sampling biota

Biotic tissues such as digestive tracts and muscle tissue can be extracted and examined using visual examination. Digestion of biotic material is recommended to prevent masking of anthropogenic material.

Biota can be sampled from the environment in many ways including trawling, nets, cages and hand collection from shore. Handling stress and physical movement may cause loss of microplastics through gut evacuation or inversion prior to preservation. Therefore, care must be taken to account for stomach inversion and it is suggested that, especially with fish, individuals which display signs of recent stomach inversion should be removed from analysis (Lusher *et al.* 2017b). When collecting blue mussels for microplastic analysis, it is important to carefully remove the byssus threads from the substrate to avoid stressing them. A recent publication on mussels from the North Sea, preserved individuals in ethanol immediately after sampling to avoid gut clearance (Beer *et al.* 2017). When mussels are stressed they close, therefore ethanol fixation might not be required if individuals are frozen straight after collection.

Any animals held in nets or traps for extended periods of time may also consume microplastics collected in the sampling device. Therefore, the time between collection and preservation should be as short as possible to minimise stress. If animals are not collected by the researchers, *e.g.,* when buying fish or shellfish from supermarkets, it can be difficult to control for contamination and sampling bias (Lusher *et al.* 2017b).

After collection, target tissues are extracted and microplastics can be isolated for biotic material by dissection, depuration, homogenization, digestion with chemicals or enzymes, saline washes, density flotation and visual inspection (Lusher *et al.* 2017b). A number of different approaches have been applied to digest biotic material including: acidic treatment (*e.g.,* HNO_3, $HClO_4$, CH_2O_2), alkaline treatments (*e.g.,* KOH and NaOH), oxidising treatments (*e.g.,* H_2O_2), and enzymatic treatments (*e.g.,* Protenase K, Lipex and Savinase). Of these protocols, KOH is perhaps the most appropriate strategy since this treatment is economically cost efficient, utilizes easily accessible chemical, requires a simple sampling procedure (Foekema *et al.* 2013; Dehaut *et al.* 2016; Kühn *et al.* 2016).

After digestion, the remaining solution can be filtered to retain resistant materials which can be further separated by density. Density separation has been recommended by MSFD and NOAA. NaCl has a density of 1.2 g cm^{-3}, is inexpensive and non-hazardous; however, it will lead to an underestimation of more dense particles. NaI and $ZnCl_2$ solutions are more dense and are therefore able to float high-density plastics.

Extracted particles can be subjected to visual examination or chemical analysis to identify and verify particles of synthetic origin.

2.4 Identification of microplastics

Researchers have many techniques at their disposal to allow accurate identification of microplastics. Steps taken to confirm particle identity can consist of both visual and chemical verification.

Once samples have been processed and prepared the quantity and type of microplastics should be ascertained. There are different techniques to do this which can be divided into visual and chemical techniques. Techniques range from simple observation under a microscope to advanced emerging techniques including focal plane array Fourier transform infrared spectrometry (FPA-FT-IR) which is capable of automatic scanning (for review see Löder and Gerdts 2015).

2.4.1 Visual identification

Visual identification, based on morphological characteristics, is an essential step when sorting samples. Plastics can be classified by their morphological characteristics including size, shape and colour. Particles can be sorted into size groups where size is typically based on the longest dimension. There are five main categories for shape: beads, fibres, fragments, foams and films.

The small size and physical heterogeneity of microplastic particles present a challenge to accurately use visual identification. However, there are some steps that can be followed to aid in visual identification (Box 1). Supporting steps for visual analysis include using a hot needle, which is fast and cheap, although it cannot provide accurate polymer identification. Knowledge of melting points can only provide a range of potential polymers. Polarised light microscopes can be used to infer the birefringent properties of suspected polymers and Nile red or Rose Bengle dyes can be used to dye suspected particles. Excluding non-plastic materials is another method which can be carried out through digestion, oven drying or freeze drying. Caution should be given when microplastics suffer embrittlement, fragmentation or bleaching, or are encrusted with biota/biogenic material. This may skew results and due to these challenges secondary analysis should be used. Visual identification is highly subjective resulting in inconsistencies between researchers. Therefore, visual identification of microplastics, especially in the smaller size range, should always be supported by secondary analyses to confirm the identity of polymeric material (Lusher *et al.* 2017b).

2.4.2 Chemical classification

There are several analytical techniques which can be used to verify suspected polymeric materials. Some technique can be used to infer resin constituents, plastic additives and dyes, whereas other can be used to infer the chemical make-up of a particle and identify polymers. These techniques require specialised equipment which can be expensive. Each have their own limitations including un-optimised techniques, size limitations and time constraints related to processing and analysis **(Figure 1).** These methods can be destructive and non-destructive, and the techniques are constantly being adapted in increase speed and ease of determination of microplastic content. Many reviews have been carried out on these developing techniques such as Lenz *et al.* (2015); Wesch *et al.* (2016) and Löder and Gerdts (2015).

In short: non-destructive vibrational techniques include Fourier Transformed Infra-Red spectrometry (FT-IR), Attenuated Total Reflectance (ATR), and Raman spectrometry. These can be stand-alone instruments or include automated scanning coupled with microspectrometry. Destructive techniques include Pyrolysis–Gas Chromatography combined with Mass Spectroscopy (Pyr-GC-MS), high temperature gel-permeation chromatography (HT-GPC) with IR detection, SEM–EDS and thermoextraction, and desorption coupled with GC-MS. Low cost options include polarized light microscopy to observe birefringent properties of polymers, or using stains including Nile Red to colour plastic polymers, or simply melting plastics using known melting points and a hot needle (De Witte *et al.* 2014; Maes *et al.* 2017; Shim *et al.* 2017).

Box 1.

Steps taken to assist in visual identification

- Form of plastics

Potential plastics may be solid or flexible, but the surface features should be uniform. There should be no visible cellular structure and particles should withstand contact or handling. Fibres should be consistent in width and exhibit no fraying or branching. Fragments may appear 'frayed' or degraded but should be consistent in structure throughout and resilient when handled using forceps. Microbeads should be shiny in texture and spherical in shape. Plastics typically exhibit a homogenous gloss or shine.

- Colours of plastics

Colours may be used to differentiate between anthropogenic debris and organic material where particles are 'unnatural' colours such as blue or bright pink. However, colour alone should not be used to identify suspected plastic particles and other physical characteristics must be considered. Typically, microplastics are homogenous in colour although there are some exceptions to this.

- The Hot Needle Test

This test is useful in cases when researchers cannot distinguish between plastic pieces and organic matter or other anthropogenic debris. In the presence of a very hot needle, plastic pieces will melt or curl. Biological and other non-plastic materials will not. It is important that the needle is sufficiently hot (*e.g.,* >200 °C) or plastics may not react. Additionally, some particle types (*e.g.,* microbeads) may not exhibit a clear reaction based on their form. Hence, it is vital that this test is used in conjunction with a thorough knowledge of microplastic characteristics and is not solely relied upon. Despite this, the hot needle test can be useful in separating plastic and non-plastic fibres, which are often difficult to visually separate. Although, semi-synthetic fibres such as rayon will not react based on their chemical composition (typically produced from organic material).
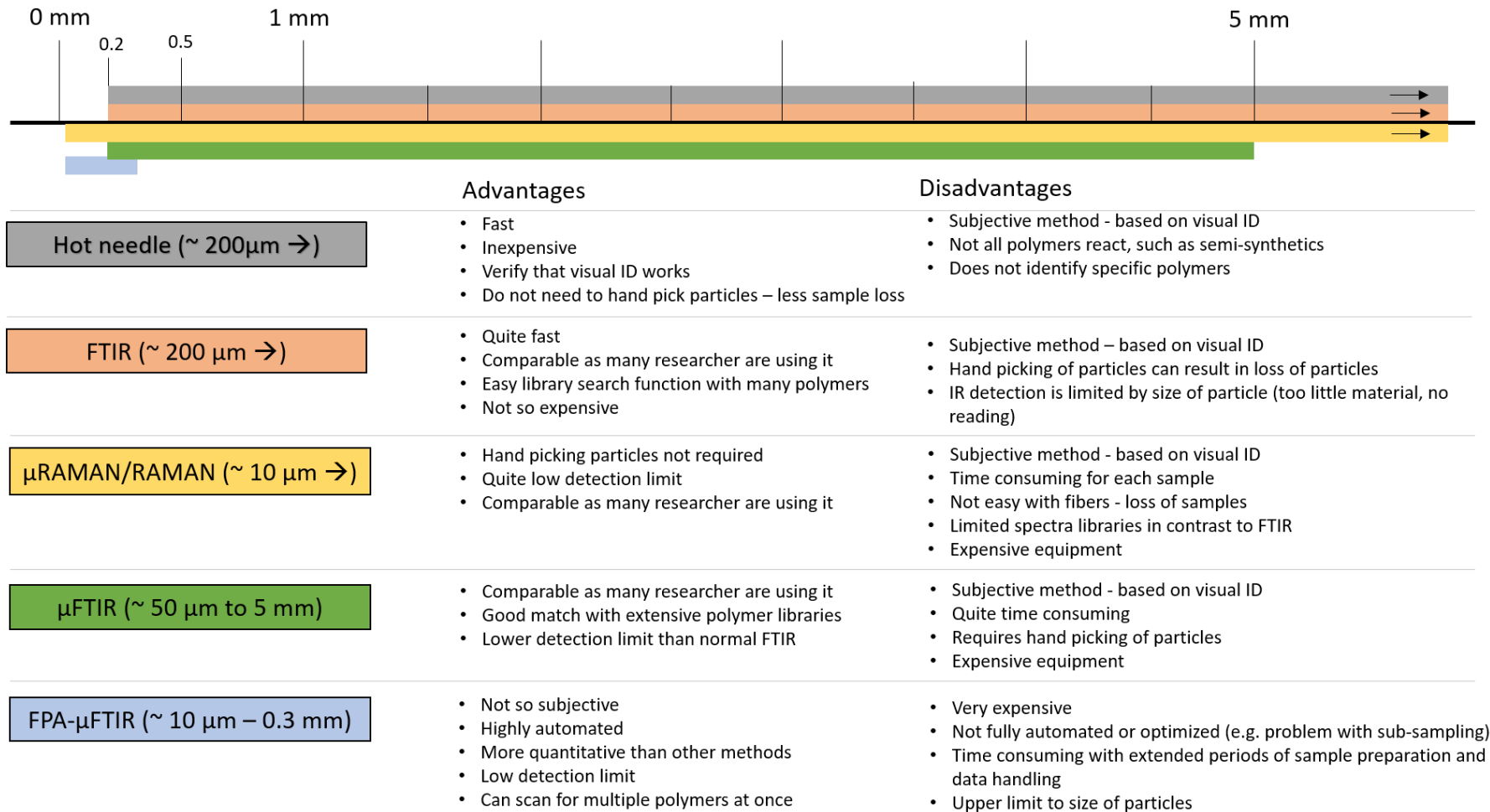

Figure 1. Different verification methods suitable for different sizes of microplastics.

3 Study on presence of microplastics in blue mussels and sediments from Norway

This study was devised to allow testing of techniques for processing samples of bivalves (*Mytilus spp.*) and benthic sediments, as well as reporting on the levels of microplastics in the chosen matrixes from different geographical sites. Methods were chosen based on ease of use, cost and suitability for the removal of biogenic material or the efficiency to separate sediments from different matrixes with varying organic content and grain size. Potassium hydroxide was chosen as an appropriate method for processing blue mussels. Sieving and density separation were chosen to test their suitability with different sediments. Sampling stations around the coast of Norway were chosen to represent different levels of anthropogenic influence including urban, industrial, rural and combination areas. After sample processing, extracted particles were subjected to visual and chemical identification.

3.1 Choice of methods

3.1.1 Bivalves

When choosing the most suitable processing technique for bivalves, two important criteria were considered. The chemical must (1) dissolve as much organic material as possible, whilst (2) preserving any plastic items in the sample.

Potassium hydroxide (KOH) was chosen as the most appropriate methods for processing blue mussels, *Mytilus spp.,* from the Norwegian coast. KOH was chosen based on cost, ease of use and speed of achieving results. This decision was made based on the published research (*e.g.,* Foekema *et al.* 2013, Dehaut *et al.* 2016, Kühn *et al.* 2017) as well as experience within NIVA. Other available methods use chemicals and enzymes which are more expensive and their impact or alteration on different polymers have not been thoroughly tested. At time of investigation and writing, the scientific community is pursuing KOH as an appropriate method for preparing mussel samples for microplastic analysis. KOH dissolves organic content to a high degree and the treatment does not appear to have significant effect on plastics (based on colour, shape, weight and chemical characterization). Dehaut *et al.* (2016) did not observe any significant effects on a range of polymers including high and low-density polyethylene (HDPE, LDPE), polyamides (PA), polycarbonate (PC), polymethyl-methacrylate (PMMA), polypropylene (PP), polystyrene and expanded polystyrene (PS, EPS), polytetrafluorethylene (PTFE) and polyvinyl chloride (PVC); although there was some colour and shape alteration to polyethylene terephthalate (PET).

3.1.2 Sediments

In this study, two extraction techniques were tested to isolate microplastics from sediment samples. **Sieving** and **density** separation were trialled, whereas elutriation was not considered based on the time required to process individual samples and the small sample volume to be tested. Sieving and density separation represent cost- and time-effective processing techniques. The aim was to test the efficacy of these approaches for use in environmental monitoring schemes where sample processing must be cheap, use readily available reagents and apparatus, and produce samples that facilitate quick

and accurate microplastic identification. As four replicates were sampled at each site, two replicates were analysed for each method to account for sediment heterogeneity.

3.2 Choice of sampling stations

Sample stations were located along the Norwegian coast, from the Swedish boarder in the south to the Russian border in the north **(Figure 2).** Using the knowledge acquired through NIVA's long-term monitoring programmes, *e.g.*, MILKYS – where blue mussels are used as an indicator of environmental contamination, stations were chosen to be representative of the coast of Norway with a focus on highly polluted or reference locations distribution along the Norwegian coast. Stations were separated into four categories: (1) urban; (2) industrial; (3) rural; and, (4) areas with a combination of environmental impacts. It must be noted that as microplastic distribution and behaviour in the environment is not fully understood, these sites were chosen with a lot of uncertainty about the range of possible anthropogenic impact.

- Category 1, *urban*, is defined on proximity to a large city. Strong anthropogenic influences are found close to large urban areas and it is suspected that the more urbanised the city the more microplastic present such as impact from WWTPs. Stations in this category include Bergen city port, Akershuskaia and Gåsøya in the inner Oslofjord, and Ramtonholmen at Røyken in Oslofjorden.

- Category 2, *industrial*, is defined as an area with industrial impact but not necessarily close to a large city. Stations in this category include Kvalnes and Byrkjenes in Sørfjorden.

- Category 3, *rural*, defined as areas not close to urban centres or local industrial impacts, the direct and local anthropogenic impact appears to be small, but they may be affected by long-transported microplastic or non-treated local sewage discharges. Stations in this category include Skallneset, Måløy and Singlekalven in Hvaler.

- Category 4, *combination*, is defined as rural areas where there may be uncertainty about sources and loads of microplastics. These areas have uncertain anthropogenic impact. Based on the locations these are likely to receive multiple sources which are difficult to characterize related to size of locations, catchment area etc. Stations in this category include Solbergstrand in Oslofjorden, Ørland in the outer Trondheimsfjord, Bodo and Lille Terøy in the Hardangerfjord. For example, Solbergstrand may be influenced by sources of microplastics from local recreation, or densely populated urban areas in Drøbak and surrounding areas in the Inner Oslofjord. Lille Terøy at the mouth of the Hardangerfjord is a seemingly rural area but may be influenced by high tourism at certain times of year. Ørland in the outer Trondheimsfjord has an unequivocal influence from Trondheim as Norway's third largest city and airport run-off is suspected as another source of microplastics. Bodø is close to the airport so there could be a mix of local, urban and long-range microplastics in the area.
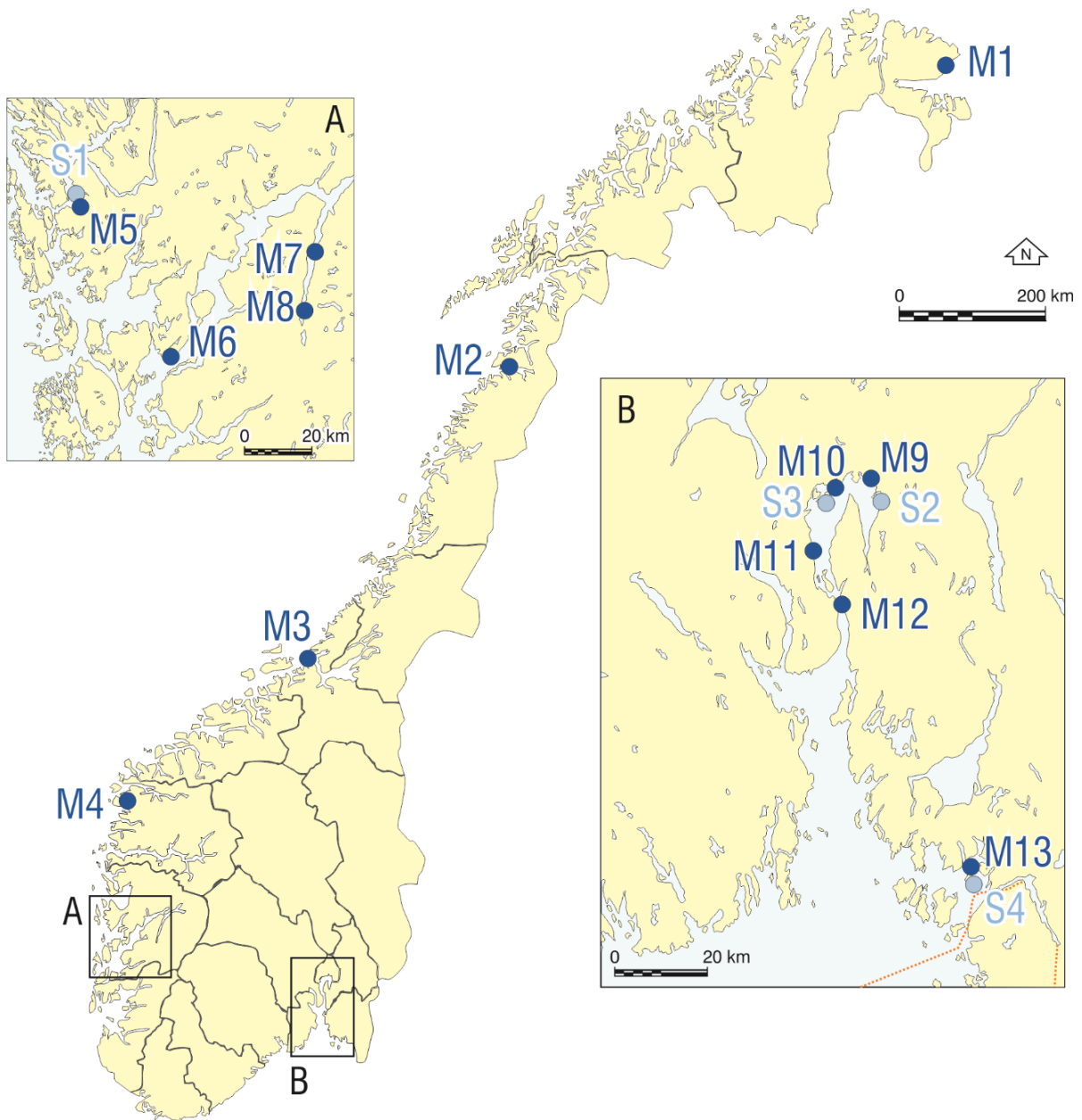
Figure 2. Sample locations of blue mussels, *Mytilis spp.,* (M1-M13) and benthic sediment (S1-S4) collected from around Norway. Sample labels correspond to **Table 4** (mussels) and **Table 5** (sediment).

3.3 Sample collection

Blue mussels were collected from 13 stations and sediment samples were collected from four stations.

3.3.1 Bivalves

Blue mussels, *Mytilus spp.*, were collected during August – November in 2016 and 2017 during the MILKYS sampling programme. By sampling during this period, researchers could ensure that their condition would not be affected by spawning. Mussels were collected according to NIVA procedure 17221. In short, mussels were collected using different techniques depending on their position in the water column. Mussels on the shoreline and intertidal were generally collected by hand. Those that were submerged were collected by snorkling. Two exceptions are mussels from Akershuskia which were collected using a metal rake from the quayside and mussels from Byrkjenes which were collected by snorkelling from a submerged branch (**Table 4**). A maximum of 20 individuals which were representative of each population (2 – 6 cm in length) were collected per site. Only alive and not obviously damaged individuals were collected. Individuals were frozen (- 20 °C) as soon as possible after collection.

3.3.2 Sediments

Four sites were chosen to assess microplastics in benthic sediments **(Figure 2; Table 5).** Benthic sediments were collected during the MILKYS sampling programme in 2017, except from the sediment collected from Bergen (S1). For S2 – S4, a box corer was used to collect the sediment samples. On retrieval a corer was inserted into the box core to extract a depth profile of sediment. The top 0 – 5 cm of sediment were divided into 1 cm increments and stored in separate containers. If over laying water was present in the sampling equipment, it was siphoned off and stored for separate analysis. This procedure was similar to that described in Martins *et al.* (2017). The only sediment collected in a different manner was Bergen (S1). Here, four replicate grab samples were collected with a Van Veen grab. The top 0 – 2 cm of sediment was sampled from the grab using a metal spoon and stored in a glass jar. All samples were stored frozen (- 20 °C) until processing.

Table 4. Mussel stations sorted by geographical location. Each category has been defined based on potential sources of anthropogenic impact.

Site	Station name (Region)	Area	Category	Location (WGS84)	Substrate	Position (depth)	Collection method	Comment
M1	Skallneset (Finnmark)	North	Rural	70,1372 N 30,34175 E	Rock	Shoreline, intertidal (0 m)	Hand	Near national park Very exposed to the sea.
M2	Bodø (Norland)	North	Combi.	67,41271 N 14,62193 E	Concrete pier	Subsurface (0-1 m)	Hand	Exposed area. Some rope and plastic surfaces. 20 km from Bodø port
M3	Ørland, outer Trondheimsfjord (Sør-Trøndelag)	Central	Combination	63,65186 N 9,56386 E	Rock and sand	Shoreline, intertidal (0 m)	Hand	Close to airport, urban and rural areas boat harbour
M4	Måløy (Sogn og Fjordane)	Central	Rural	61,93098N 5,05241E	Pontoon	Subsurface (0.2 -1.2 m)	Hand	
M5	Bergen city port (Hordaland)	West	Urban	60,40080N 5,30352E	Rock	Shoreline, intertidal (0 m)	Hand	
M6	Lille Terøy (Hordaland)	West	Combination	59,98400N 5,75450E	Pontoon	Subsurface (0-0.5)	Hand	Mouth of Hardangerfjord FW from high rain and river flushing
M7	Kvalnes, Sørfjorden (Hordaland)	West	Industrial	60,22050N 6,60200E	Rock and sand	Intertidal, subsurface (0-1 m)	Snorkelling	Metal industry FW from high rain and river flushing

M8	Byrkjenes, Sørfjorden (Hordaland)	West	Industrial	60,08383N 6,55050E	Attached to submerged branch	Subsurface, possible exposure (0-1 m)	Snorkelling	Metal industry Large FW influence from river
M9	Akershuskaia (Oslo)	East	Urban	59,90533N 10,73633E	Quayside (cement with tyre fender)	Subsurface (0-1 m)	Metal rake with net	Boat traffic. Alna river.
M10	Gåsøya, Bærum (Akershus)	East	Urban	59,85133N 10,58900E	Rock	Subsurface (0-1 m)	Snorkelling	5-6 km northeast of VEAS WWTP
M11	Ramtonholmen, Røyken, Oslofjorden (Buskerud)	East	Urban	59,74450N 10,52283E	Rock and sand	Subsurface (1-2 m)	Snorkelling	About 5 km south of VEAS WWTP
M12	Solbergstrand (Akershus)	East	Combination	59,61550N 10,65150E	Sand and Rock	Intertidal (0-1m)	Hand	Mouth of Oslofjord FW stream
M13	Singlekalven, Hvaler (Ostfold)	East	Rural	59,09500N 11,13667E	Sandy bottom with some rocks	Subsurface (0.5-1.5m)	Snorkelling	National park River drainage from Glomma.

Table 5. Sediment stations sorted based on geographical location. Each category has been defined based on potential anthropogenic impact.

Site	Station name (Region)	Area	Category	Location (WGS84)	Comment
S1	Bergen Harbour (Hordaland)	West	Urban	60,39585 N 5,26790 E	
S2	Bekkelaget/ Alna (Oslo)	East	Urban	59,89445 N 10,74723 E	Near mouth of Alnaelva river, inner Oslofjord
S3	Gåsøya, Indre Oslofjord (Akershus)	East	Urban	59,79485 N 10,52085 E	5-6 km northeast of VEAS, inner Oslofjord
S4	Singlekalven, Hvaler (Østfold)	East	Rural	59,09123 N 11,13203 E	National park

3.4 Extraction of suspected plastics from samples

Extraction of suspected plastics from blue mussels involved dissolution of organic content with 10 % KOH, incubation 24 h at 60°C and filtering the remaining homogenate. Both sieving and density separation with saturated NaI solution were tested as extraction techniques for the analysis of microplastics in sediment samples.

3.4.1 Mussels

Mussels were processed in a clean laboratory environment to reduce sources of contamination. Firstly, individuals were measured with callipers before opening. Soft tissue was excised from the shells and weighed (g, w.w.). Secondly, mussels were placed in individual glass beakers before a premade solution of 10 % KOH was added. Beakers were sealed with aluminium foil and placed in an incubator for 24 h at 60 °C with continuous agitation (145 rpm). After samples were removed from the incubator and cooled, the homogenate was filtered under vacuum onto glass microfibre filters (GF/D, 2.7 µm) (**Figure 3**). Filter papers were assessed for the presence of suspected plastics following the steps described in **Section 3.5**
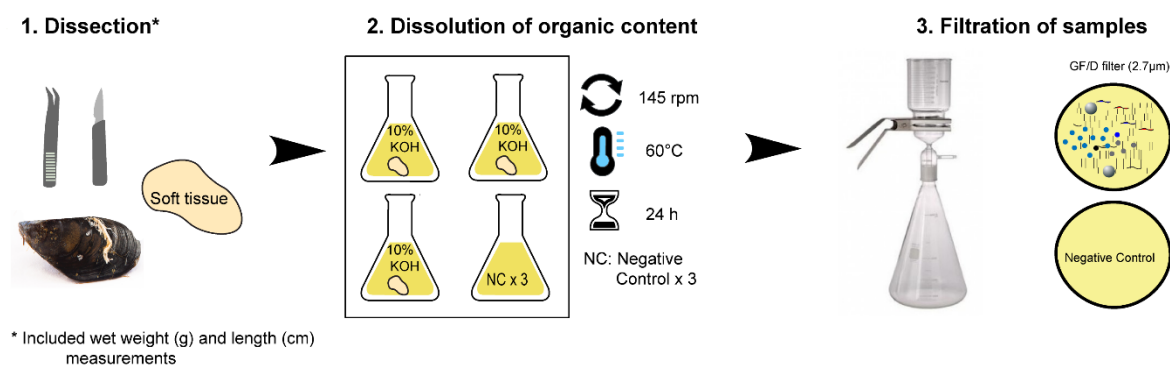
Figure 3. Schematic diagram of mussel sample preparation

3.4.2 Sediments

Two extraction techniques were tested for the analysis of microplastics in sediment samples. Both approaches were tested on replicate samples from four sample locations. Two replicates per location were analysed for each method to account for sediment heterogeneity.

Approach 1: Sieving
Sediment samples were defrosted at room temperature prior to separation. The samples were then wet sieved through a stack of sieves with sequentially finer mesh sizes: 5 mm, 1.0 mm, 500 µm, 250 µm, 100 µm, 50 µm **(Figure 4).** Each sample was placed into the uppermost sieve (5 mm mesh size) and washed through using a constant flow of water. This was delivered using a hose with a 38 µm filter to prevent potential laboratory contamination of microplastics from the water supply. The sediments retained on each sieve were carefully washed into separate petri dishes and dried at 60 °C for 24 hours. Each sample was then visually assessed **(Section 3.5)** and microplastic particles were removed with forceps and counted.
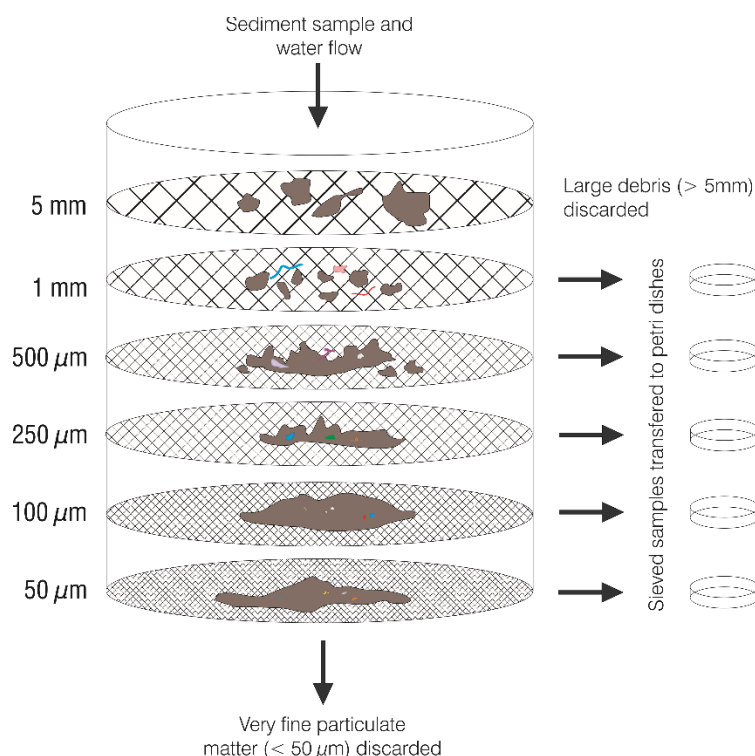
Figure 4. Schematic diagram of sediment separation using wet sieving

Approach 2: Density separation
Defrosted samples were placed into clean, pre-washed glass jars. A saturated NaI density solution (1.8 g cm^{-3}) was added to the container and each was filled to the top. The containers were closed with a tight seal lid and agitated vigorously for 1 minute. The samples were then left for 24 hours to allow the fine particulate matter to settle out of suspension. The floating material was then decanted into a vacuum filter and passed through glass microfibre filter papers (GF/D). The filter papers were transferred to separate petri dishes and dried at room temperature for 3 days (**Figure 5**). Each filter paper was then visually assessed and microplastic particles were identified **(Section 3.5).**
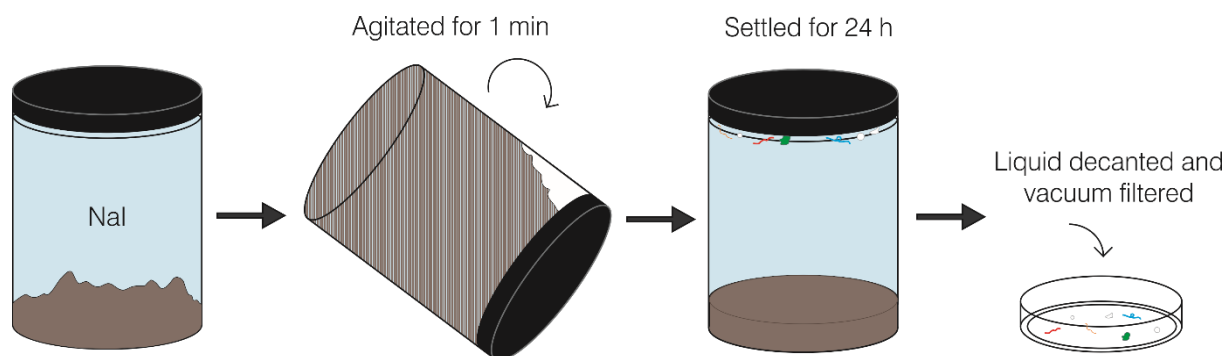
Figure 5. Schematic diagram of sediment separation using density

3.5 Verification of plastics

Potential anthropogenic particles were verified as plastics using a combination of visual and chemical techniques. Results were adjusted according to the verification methods.

3.5.1 Visual

All samples on separate filter papers were visually inspected for the presence of potential particles. As this study focused on particles >150 μm the microscope was set at 30 x magnification. Image analysis software (Infinity Analyse) was used to photograph and measure the dimension of individual particles. All particles were recorded in the corresponding excel spread sheet.

Mussel samples were much clearer than sediment samples. This allowed researchers to circle suspected particles. Suspected plastics in sediment samples were individually extracted from the filter papers for further visual inspection. All suspected particles were assessed on based on their visual characteristics, as discussed in **Section 2.4.1.** Buddy checks were performed throughout the visual analysis to check the visual accuracy of the researchers.

To ensure for contamination control, petri dish lids were only open when required. If they were opened a control blank was performed for the same duration to check for airborne contamination.

3.5.2 Chemical

A subsample of all visually identified plastics were subjected to chemical analysis by ATR using a ThermoScientific Nicolet iS50 FT-IR. Specifically, all suspected plastics from four sites (Bergen, Lille Terøy, Akershuskia and Gåsøya) were analysed with μFT-IR.

In short, each suspected plastic particle was flattened and held in place using a diamond compression cell. Each prepared sampled was then exposed to a beam of infrared light (4 000 – 400 cm^{-1}). The infrared absorption spectrum was recorded using 50 scans and automatically compared against library spectra to obtain the chemical characterisation of the sample. Polymer identification was verified based on the % match. Only spectra matched greater than 70 % were accepted.

3.6 Laboratory blanks and controls

Contamination control was carried out throughout the sampling: during collection, processing and analysis. Any presence of contamination in blank samples was accounted for in the results.

Steps taken to avoid contamination included:

- Use of a clean laboratory with reduced access. All researchers using the laboratory wore cotton clothing including cotton laboratory coats. The laboratory was thoroughly cleaned down and dusted between use.

- Filtered (0.22 µm) dH20 water was used for preparation of 10 % KOH and for all washing of glassware.

- During mussel processing, three blanks were run per day. These consisted of filtered water processed with 10 % KOH in the same way as mussels.

- Mussels were inspected for contamination of tissue surfaces before they were dissolved.

- When samples were exposed to air during microscope work a wet filter paper was left exposed for the same duration.

3.7 Statistical analysis

To investigate whether there was any statistical difference between the number of microplastics observed at each site, several potential categorical variables were considered. As with most microplastic research, the data collected did not conform to homogeneity of variance, therefore, non-parametric statistical analyses were performed. Kruskal-Wallis was carried out to see if statistical differences occurred between categorical variables including site, category and geographical location. Results are presented for microplastics individual $^{-1}$ and microplastics g^{-1} (w.w). Where significant differences were observed, pairwise Mann-Whitney tests were carried out.

4 Results

Plastics were found in 76.6 % of individual blue mussels (*Mytilus spp.*), with at least one individual containing microplastics from all 13 sites. The overall average plastic load was 1.84 particles per individual (range 0 – 14.67) or 1.85 per gram w.w. (range 0 – 24.45). Particles from blue mussels consisted of fibres (85 %), fragments (11 %) and films and foams (4 %). Most of the particles were blue in colour (39 %). Based on µFT-IR, visual identification accounted for 88 % match. The most common polymers were semi-synthetic fibres composed of cellulose. Concentrations of plastics per gram were generally low close to urban areas whereas the highest concentration was seen in a rural site in Finnmark. Plastic fragments and fibres were identified in replicate sediment samples using both methods. The identification of beads in sediment samples requires chemical classification before they can be accepted due to similarities with foraminifera and colour change caused by NaI flotation.

The results section will first discuss the identification of potential contamination in blank samples and how the results have been adjusted accordingly. Results from the presence of plastics in blue mussels (*Mytilus spp.*) and sediments will be discussed separately in Section 4.2 and Section 4.3 respectively. In each subsection, the composition of plastics found through visual identification will be followed by a discussion of the polymers confirmed by chemical classification (µFT-IR). Finally, the results will focus on the data acquired and its usefulness for monitoring programmes. All results are mean ± SD unless otherwise stated.

4.1 Correction of microplastic concentrations

As airborne contamination cannot be discounted, the presence of microplastics settling on wet filter papers and those appearing in procedural blanks were monitored and corrected for. Three replicate procedural blanks were processed in the same way to samples. When suspected plastic particles were found in the procedural blanks, the average per day (split by colour, and shape) were taken from the results of the corresponding samples. Procedural contamination was therefore accounted for based on particle shape and colour. Four samples did not have any blanks run due to processing errors, therefore to account for contamination in these samples the average from all previous blanks were taken **(Table 6).** The number controlled for in the blank was based on the colour and shape, not the overall average for the blank replicates. Limit of detection (LOD) and limit of quantification (LOQ) are commonly used within classical chemistry as a detection limit. LOD calculated as three times standard deviation and LOQ is calculated as ten times standard deviation. These values have been calculated **(Table 6)** but have not been used for the result correction. Common practise within the microplastics research community is to adjust results based on particles identified in blanks. Furthermore, microplastics do not behave like classical pollutants, for example with patchy distribution and low solubility. Until further research is carried out on microplastic behaviour in environmental matrices LODs and LOQs were not used in this analysis. This method may be adopted in the future as more research is carried out.

Table 6. Identification of particles in blank samples. Limit of detection (LOD) is x3 standard deviation (St. dev) and limit of quantification (LOQ) is x10 standard deviation (St. dev).

Blank ID		A	B	C	D	E	F*
Samples processed the same day		*M2, M4*	*M7, M9*	*M6, M12*	*M5*	*M8, M10*	*M1, M3, M11, M13*
	Rep. 1	1	3	1	0	0	-
	Rep. 2	0	0	0	0	1	-
	Rep. 3	1	1	0	0	0	-
Fragments	**Mean**	0.67	1.33	0.33	0	0.33	0.53
	St.dev	0.58	1.53	0.58	0	0.58	0.65
	LOD	1.73	4.58	1.73	0	1.73	1.96
	LOQ	5.77	15.28	5.77	0	5.77	6.52
	Rep. 1	8	4	0	0	2	-
	Rep. 2	3	1	1	0	2	-
	Rep. 3	1	1	0	0	1	-
Fibres	**Mean**	4.00	2.00	0.33	0	1.67	1.60
	St.dev	3.61	1.73	0.58	0	0.58	1.30
	LOD	10.82	5.20	1.73	0	1.73	3.90
	LOQ	36.06	17.32	5.77	0	5.77	12.98

* did not have blanks, used the mean of all blanks

4.2 Mussel samples

Results of microplastics in mussel samples have been divided into sections. First, the results of visual identification corrected for blanks are presented; followed by chemical classification of particles subjected to FTIR. The results are discussed based on microplastics per individual and microplastics gram w.w.$^{-1}$.

4.2.1 Quantification of the shape, size and colours of plastics identified in blue mussels

A total of 616 particles were identified from all mussel samples (n = 252) using visual identification. Of the 575 particles that were measured (93 %), sizes of plastics ranged from 0.15 mm (detection limit) to 8.01 mm, with an average size of 0.95 ± 0.93 mm. Based on the classification of plastics, 66 % were classified as small microplastics (<1 mm), 32 % were classified as large microplastics (1 mm – 5 mm) and 2 % were mesoplastics (5 mm – 2.5 cm) (**Figure 6A).** Fibres were the most common form of plastics identified (85 %), followed by fragments (11 %) and other (foams and films, 4 %) **(Figure 6B)**. Colour was used to support visual analysis **(Figure 7).** Blue particles were most prevalent in colour (39 %). Other particle colours identified articles were black, grey, red, pink, orange, green, yellow, white, transparent and mixed colours (for example fibres that was blue on one side and transparent on the other side). However, colour is a subjective/ambiguous identification parameter which can be influenced by the individual observer. Therefore, results presented here are merely to show the visual range of particles found.
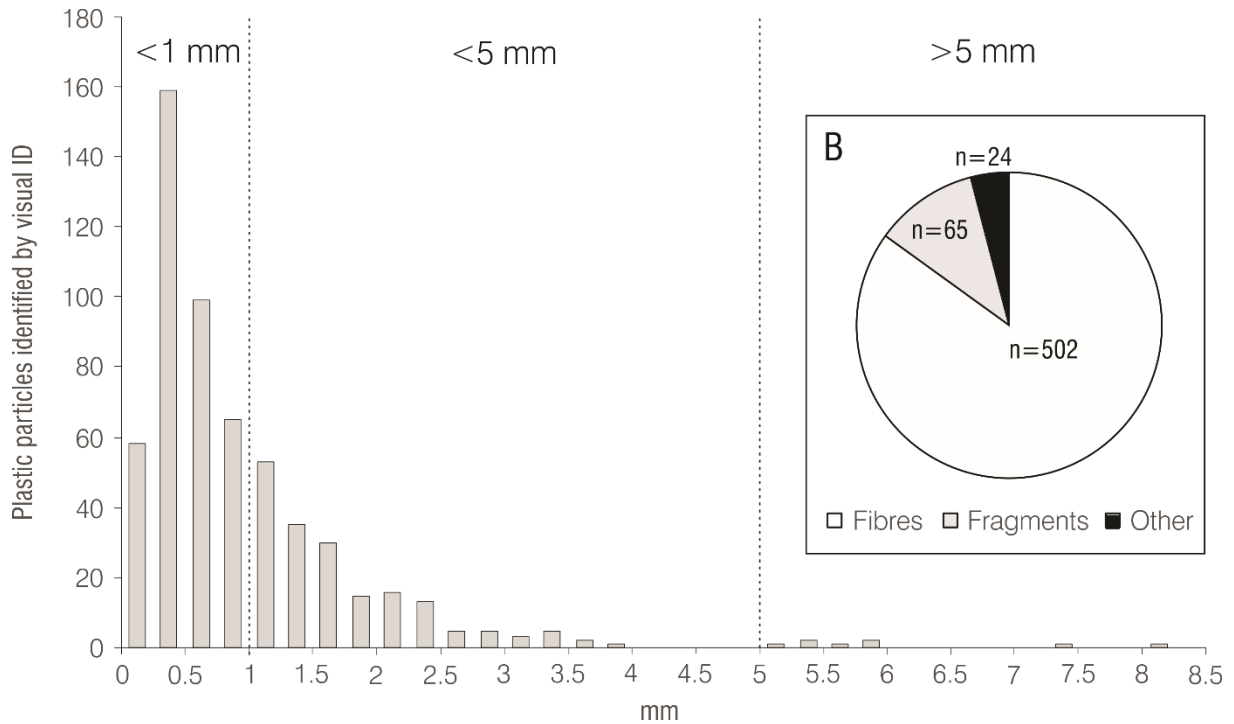
Figure 6. Size distribution (A) and composition (B) of plastics particles extracted from blue mussels (*Mytilus spp*.) from Norway.
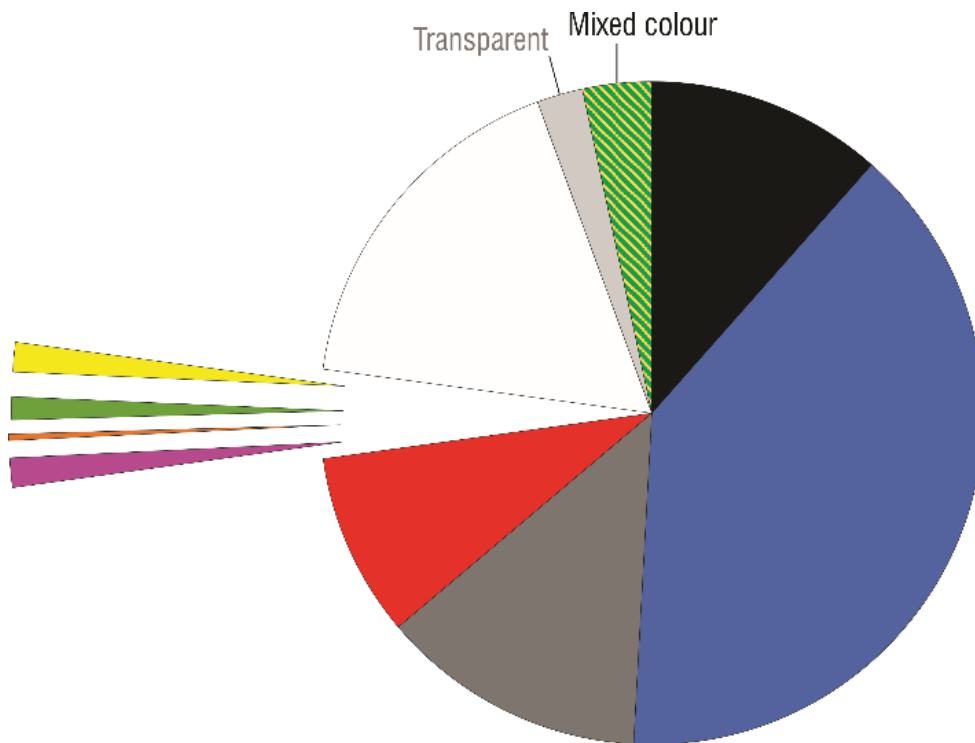

Figure 7. Colour spectra of plastic particles identified through visual identification

Table 7. Average number of plastics, per individual and per g w.w, extracted from blue mussels. Results are displayed as mean (range, SD). N: number of individuals; %: percentage ingestion

Site	Station name	Area	Influence	N	%	MP individual $^{-1}$	MP g w.w^{-1}
M1	Skallneset	North	Rural	20	95	4.29 (0 – 10.81, 2.97)	9.69 (0 – 24.44, 6.23)
M2	Bodø	North	Combination	20	90	1.39 (0 – 4.68, 1.10)	1.55 (0 – 5.60, 1.45)
M3	Ørland	Central	Combination	20	55	0.75 (0 – 3.00, 0.90)	0.30 (0 – 1.26, 0.38)
M4	Måløy	Central	Rural	20	90	1.35 (0 – 3.35, 1.20)	1.95 (0 – 11.52, 2.71)
M5	Bergen	West	Urban	20	60	0.95 (0 – 3.00, 0.99)	0.29 (0 –0.87, 0.33)
M6	Lille Terøy	West	Combination	20	65	1.48 (0 – 3.67, 1.43)	0.39 (0 – 1.21, 0.38)
M7	Kvalnes	West	Industrial	20	60	1.77 (0 – 14.67, 3.35)	2.04 (0 – 24.45, 5.40)
M8	Byrkjenes	West	Industrial	20	90	2.28 (0 – 5.35, 1.67)	1.69 (0 – 3.71, 1.19)
M9	Akershuskaia*	East	Urban	20	90	1.92 (0 – 6.00, 1.80)	0.43 (0 – 1.49, 0.40)
M10	Gåsøya	East	Urban	20	65	1,571 (0 – 4.67, 1.61)	0.25 (0 – 0.74, 0.25)
M11	Ramtonholmen	East	Urban	12	100	2,294 (0 – 4.93, 1.44)	0.50 (0.05 – 1.24, 0.33)
M12	Solbergstrand	East	Combination	20	45	1,3525 (0 – 9.67, 2.31)	2.64 (0– 17.58, 4.58)
M13	Singlekalven	East	Rural	20	100	2,732 (0 – 8.53, 1.97)	1.81 (0.16 – 10.52, 2.27)
TOTAL		-	-	**252**	**76.6**	**1.84 (0 – 14.67, 2.06)**	**1.85 (0 – 24.45, 3.74)**

* Many black particles (visually resembling the EVA foam/oil based particles), smaller than the detection limit, were observed in several of individuals from Akershuskaia.
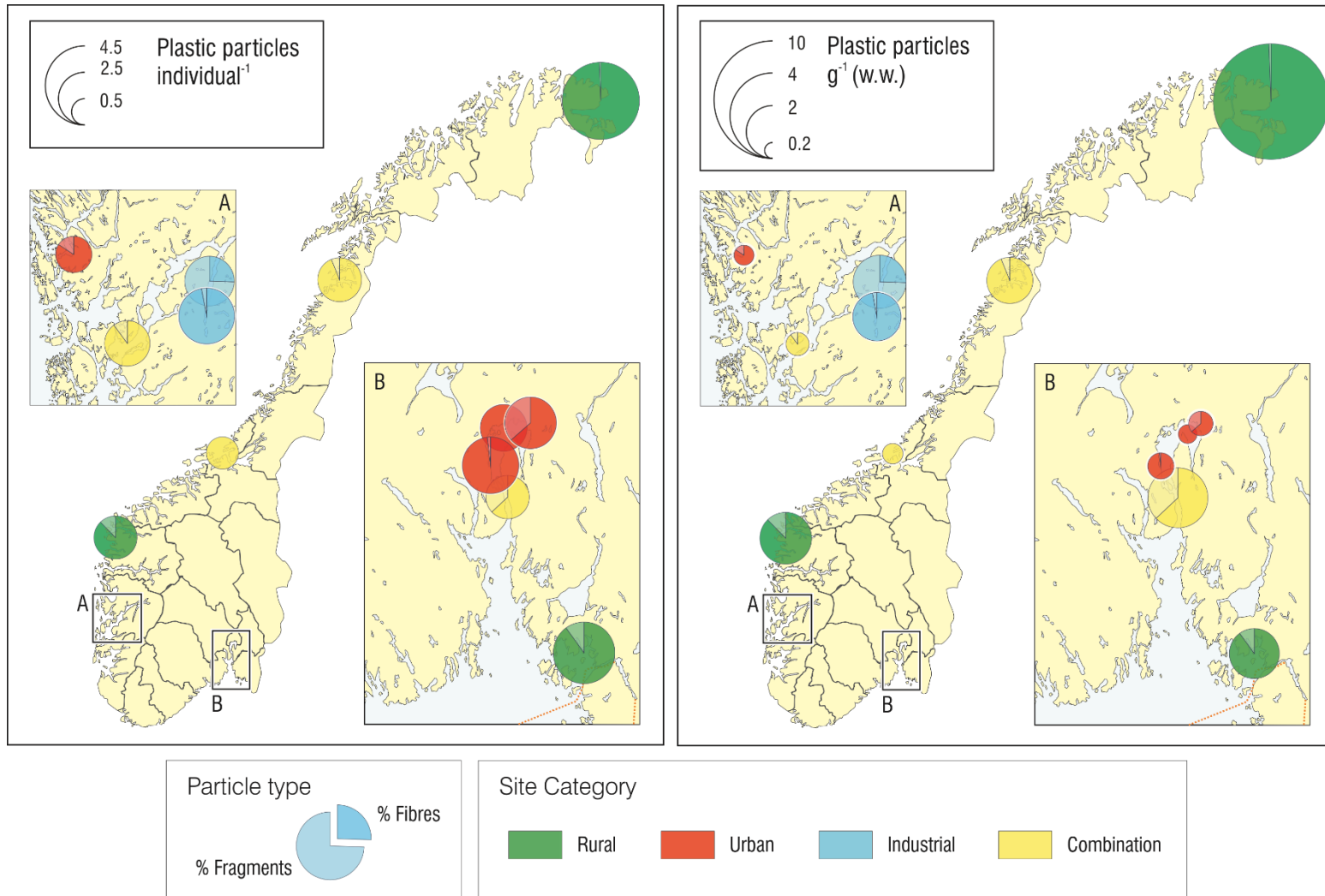
Figure 8. Levels of plastics extracted from blue mussels (*Mytilus spp.)* from locations around the Norwegian coast. Values are reported as the average number of plastics particles per individual and the average number of particles per gram of tissue (w.w.)

4.2.2 Visual identification of microplastics in blue mussels

Suspected plastic particles were identified in mussels from all 13 investigated locations (**Table 7**). At least one individual per site contained plastic particles. The percentage of ingestion (number of individuals containing plastic particles) ranged from 45 % (Site M12, Solbergstrand) to 100 % (Site M11, Ramtonholmen; and M13, Singlekalven). **Figure 8** demonstrates the spatial variability of the results which includes an apparent hotspot in Finnmark. In total, 193 out of 252 individuals (76.6 %) had ingested plastics.

There was a clear difference in the weight of individuals collected per site which affected the results **(Figure 9).** Mussels from urban locations were larger than mussels from rural and industrial locations. It was necessary to account for differences in size and standardise the results. As weight and length are significantly correlated (R = 0.89, *P* <0.001) it was deemed appropriate to use weight as a proxy for size of organisms. There were some differences in the results when they are presented both as microplastics per individual and microplastics g^{-1} w.w. Both sets of results showed that there were some significant differences in the grouping of data based on geographical location as well as the assigned categories based on anthropogenic influence (**Table 8**). The following results are discussed in terms of microplastics per individual and microplastics g^{-1} (w.w.).
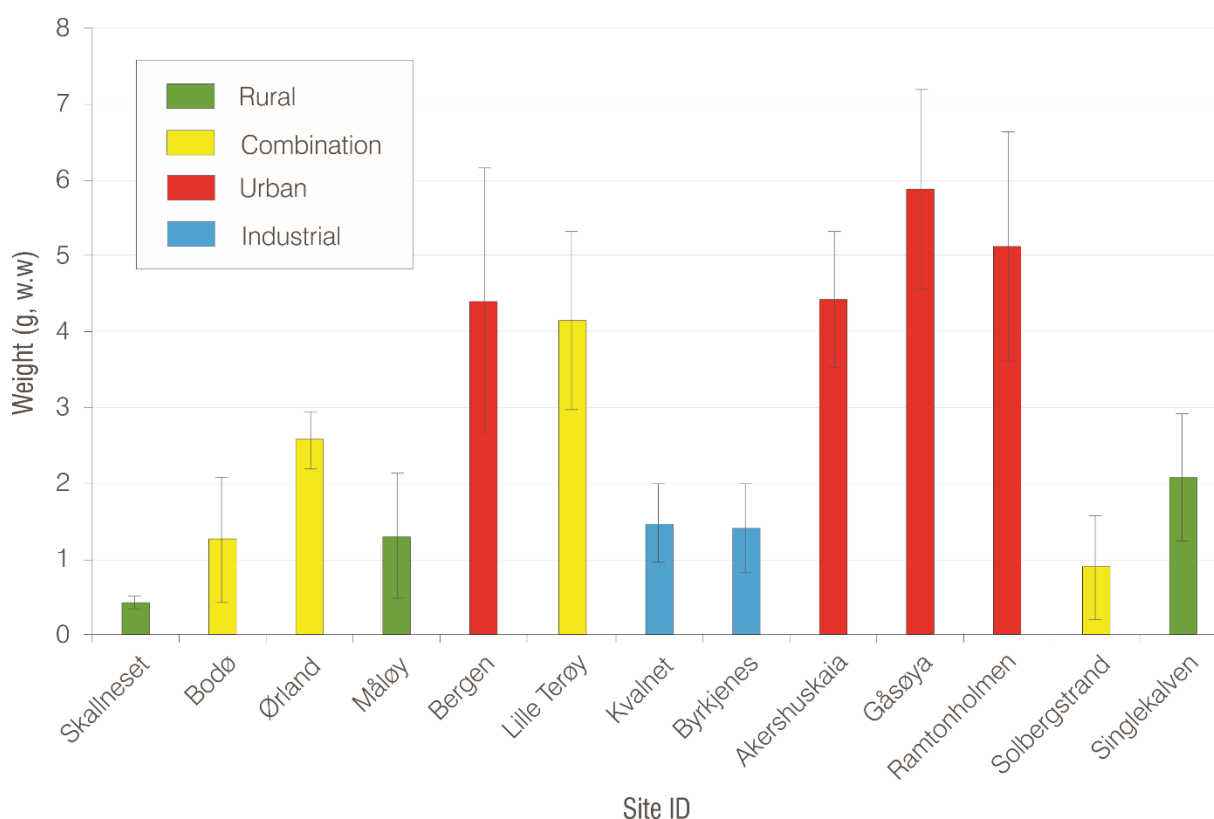

Figure 9. Average weight of blue mussel individuals collected at each of the 13 sites around the Norwegian coast. Data displayed are mean (g w.w.) ± SD.

Table 8. Results of Kruskal-Wallis tests showed a significant difference (P <0.05) for both microplastics per individual and microplastics per gram w.w.

	DF	Microplastics individual $^{-1}$		Microplastics g $^{-1}$ (w.w)	
		H- value	P value	H- value	P value
Site	12	20.95	0.001	83.67	0.001
Location	3	16.54	0.001	37.40	0.001
Category	3	13.22	0.001	52.03	0.001

Results per individual: Overall, the average plastic load per individual was 1.84 particles (± 2.06 SD). The highest level of ingestion was observed at site M1, Skallneset, (4.29 ± 2.97 SD) whereas the lowest level of ingestion was observed in M3, Ørland, (0.75 ± 0.90 SD) **(Figure 10).** When results were grouped by assigned category, it appeared that rural sites had significantly more microplastics than industrial and urban sites. Sites with a combination of anthropogenic influences had a similar number of microplastics as individuals from industrial sites. Geographically, northern sites had higher numbers of microplastics per individual than other locations. Specifically, the site with the highest number of microplastics per individuals, M1, Skallneset, was in the most northerly coastal location in the Barents Sea.

Results per g (w.w): Comparatively, the average plastic load g^{-1} w.w. was 1.85 particles (± 3.74 SD). The highest level of ingestion was observed at site M1, Skallneset, (9.69 ± 6.23 SD) whereas the lowest level of ingestion was observed in M10, Gåsøya, (0.25 ± 0.25 SD) **(Figure 11).** It appears that site, location and assigned category may have influenced the average number of particles ingested when standardised by g^{-1} w.w. (**Table 8**). Differences between the assigned categories appear to be more pronounced when displayed as plastics g^{-1} w.w. Rural sites were significantly different from all other sites. Individuals from industrial and combination sites had similar numbers of microplastics, as did urban and combination sites. Geographically, northern sites had higher numbers of plastics g w.w.$^{-1}$. Sites from the west coast and the east coast did not appear to be different.

It is important to note that at some sites, there were smaller particles that fell below the detection limit and could not be include in the results. For example, many individuals from Akershuskaia contained several small unidentified particles that resembled EVA foam. As these could not be confirmed through either visual or chemical identification their identity remains uncertain. However, due to similarities with EVA foam this highlights the need to develop more sophisticated analysis to prevent a possible underestimation of the smaller microplastics.

In addition to the internal plastics, external plastics were also detected on mussels: a PP (confirmed by FT-IR) particle resembling a piece of rope was attached to the byssus thread of one of the mussels from Lille Terrøy.

Varying numbers of pearls were observed in individual mussels throughout this survey. It is not uncommon to find pearls in environmental samples, as pearls are formed when an irritant is present in the mantle of the individual, such as grains of sand. Whether pearl formation is trigged by the substrate organisms were exposed to, or anthropogenic particles such as microplastics, will require further investigation (For more information refer to Box 2 in Section 7.1.5).
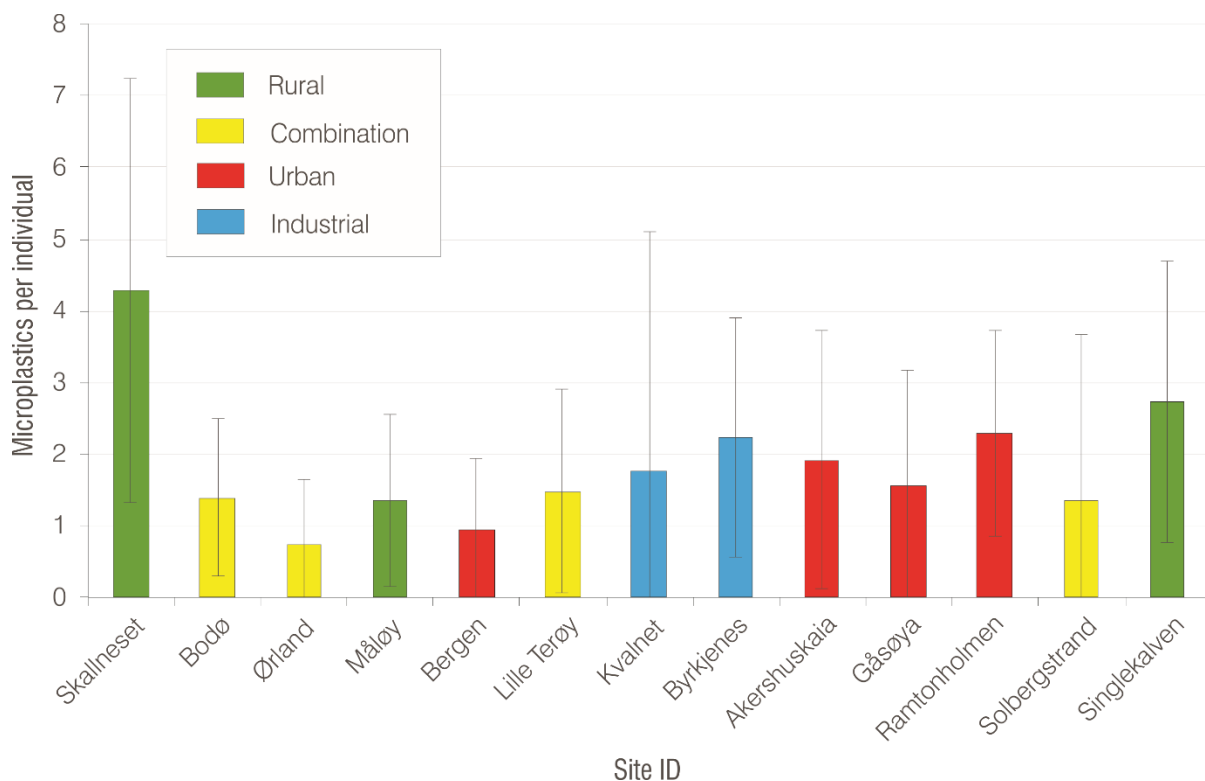
Figure 10. Average number of plastics identified per individual from 13 sites around the Norwegian coast. Data displayed are mean ± SD.
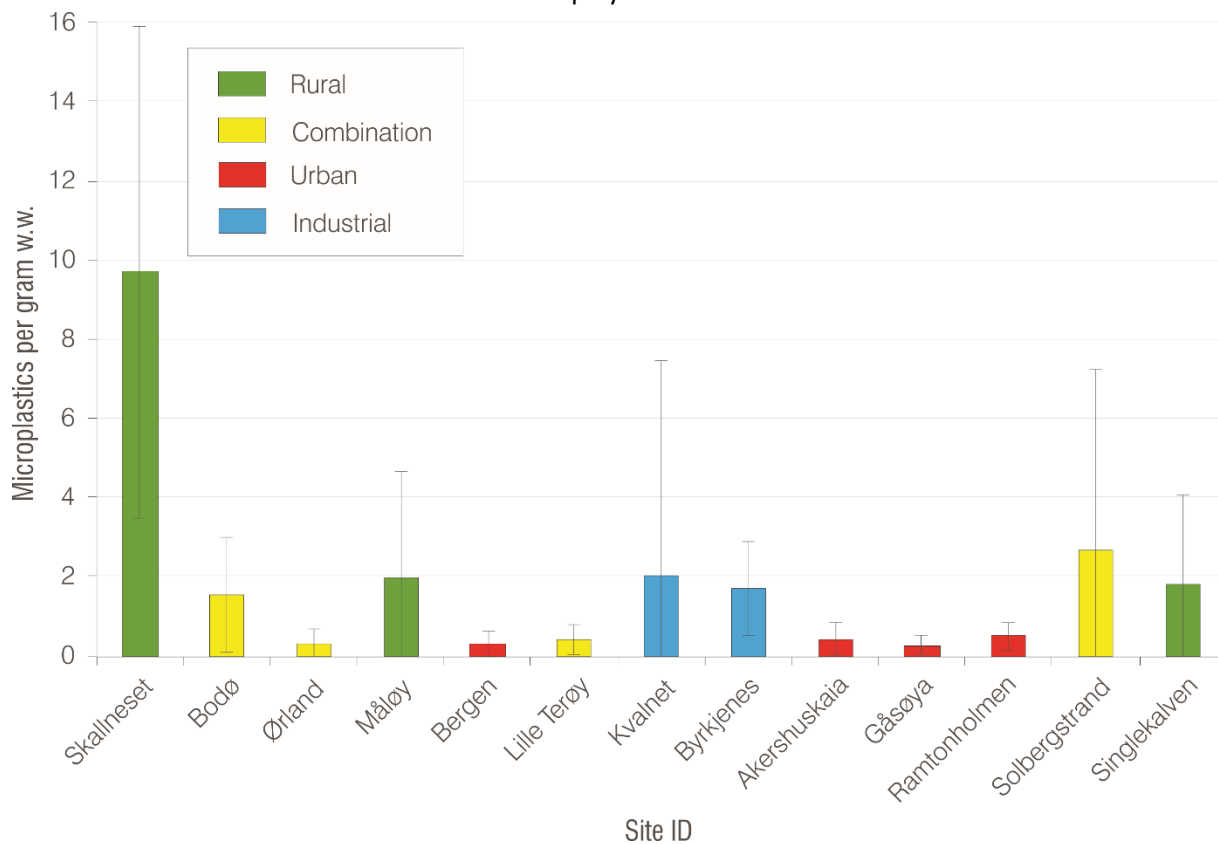

Figure 11. Average number of plastics identified per gram w.w. from 13 sites around the Norwegian coast. Data displayed are mean ± SD.

4.2.3 Chemical identification of microplastics in blue mussels

Subsamples of representative particles from four sites were subjected to chemical identification. µFT-IR was used to confirm the identify of plastic polymers by matching their IR spectra to a polymer library. A total of 126 particles were subjected to µFT-IR analysis. This was 20 % of the total particles[1] visually identified in the study. Based on percentage match, only 14 of the 126 particles were rejected as spectra indicated they were not plastic (**Table 9**). A range of polymers were identified including polyethylene, polyester (*incl.* polyethylene terephthalate, PET), acrylic, polypropylene (PP), polyamide (PA), epoxy resin (with bisphenol A), cellulose-based polymers and oil/tar compounds. The most commonly identified polymers were those of cellulosic origin, including rayon and cellophane **(Figure 12)**. Cellulosic plastics, commonly referred to as semi-synthetic plastics, are produced by chemically altering the molecular structure of organic material such as wood pulp and sugars (**Figure 13**). PET is a polyester which is often used for textiles, PP is commonly used for packaging materials and ethylene-vinyl acetate (EVA) is foam with multiple applications (**Figure 14**). A wide array of polymers were found at Akershuskaia, M10, which could be due to a high impact of secondary microplastics because of high anthropogenic activity. The other sites around the Norwegian coast were dominated by synthetic fibres **(Figure 15).**
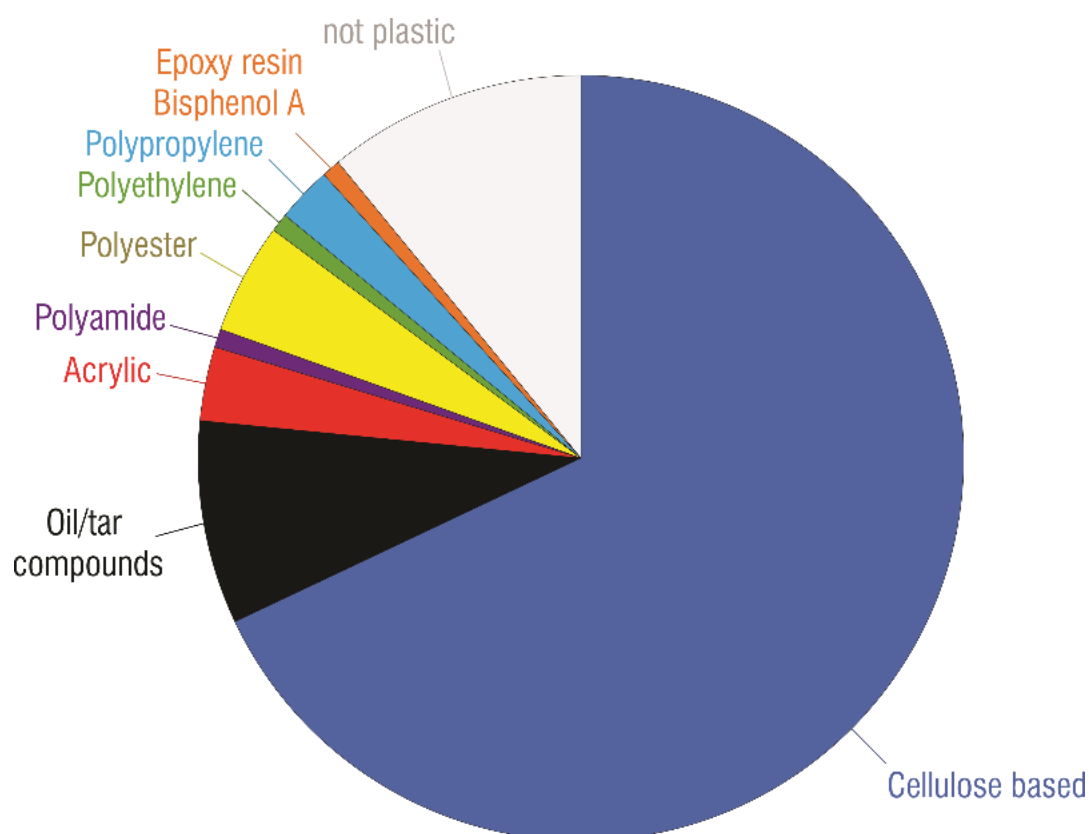
Figure 12. Polymers and non-plastic items identified using FT-IR

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[1] Raw plastic count was 616 before data corrected for blanks.
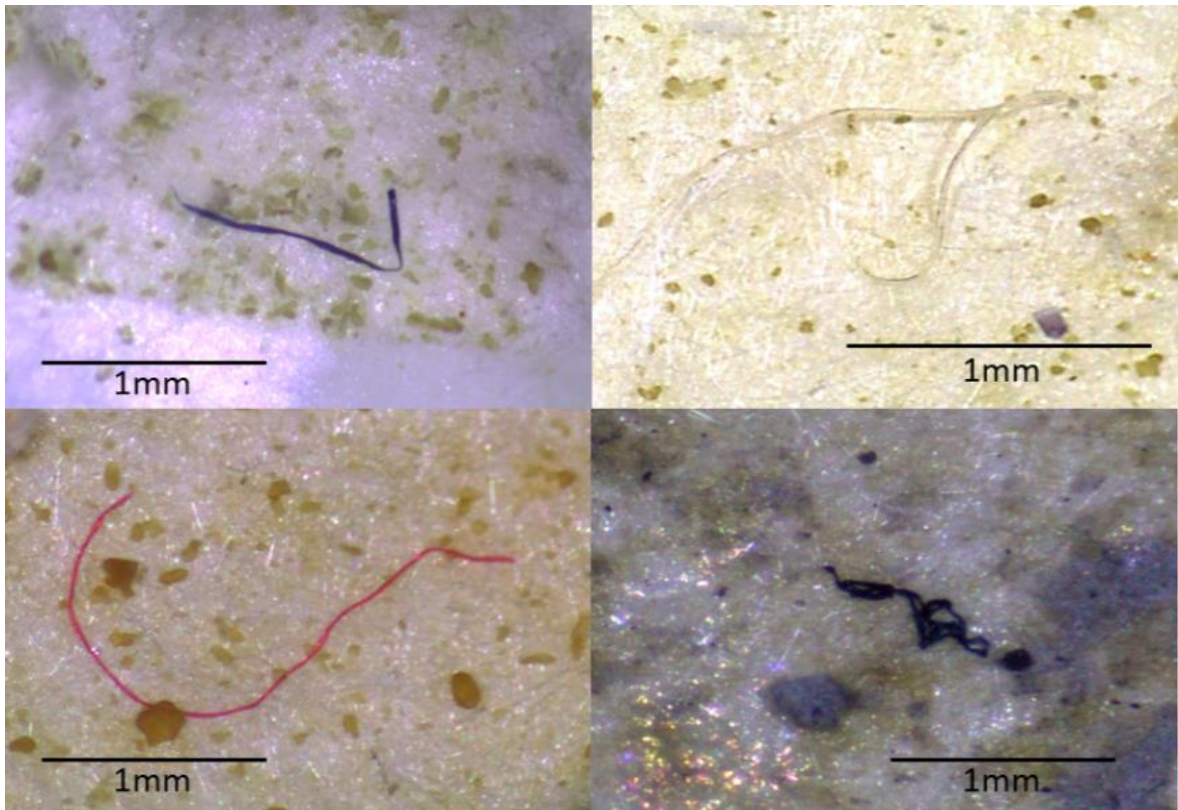
Figure 13. Examples of semi-synthetic (cellophane/viscose rayon) fibres identified in blue mussels (*Mytilus spp.)* from Norway**.**
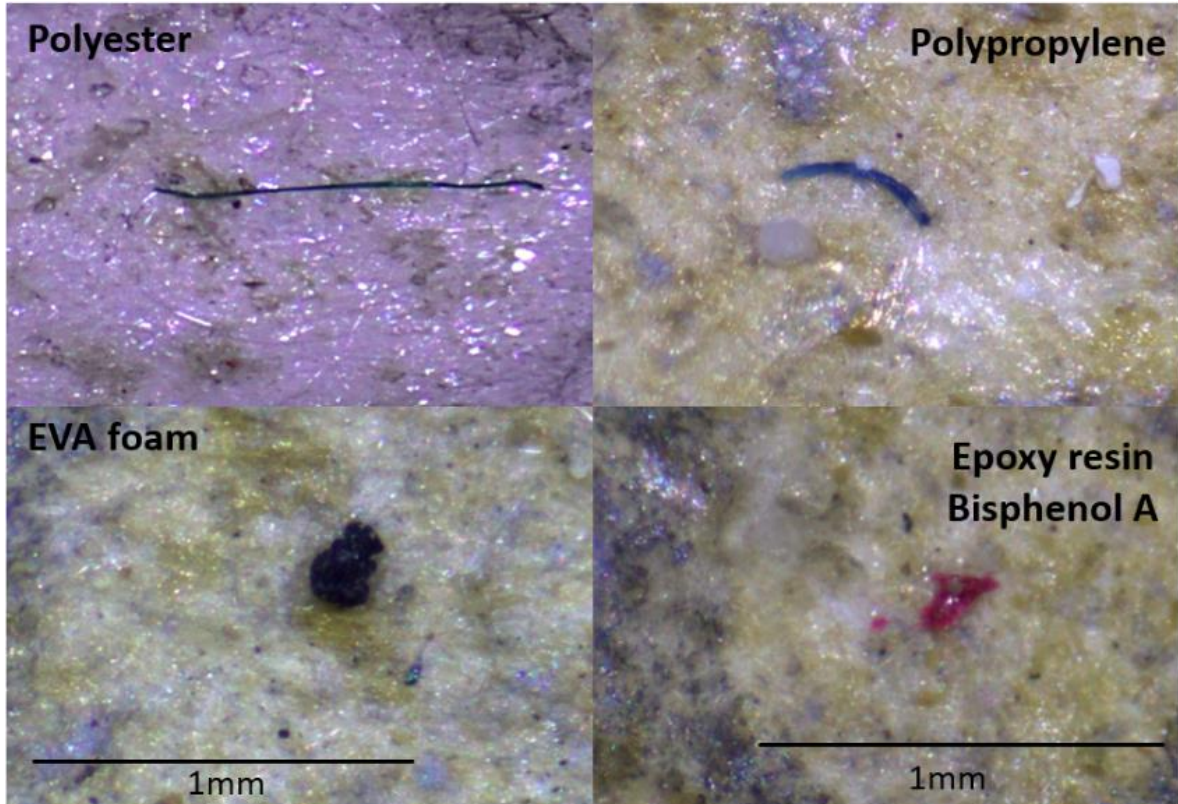
Figure 14. Examples of other polymers identified in blue mussels (*Mytilus spp.)* from Norway.

4.2.4 Comparison between visual and chemical results

The accuracy of NIVA research scientists to visually identify microplastics >150 µm from biota samples was high (>80 % accuracy, **Table 9**). An average of 11 % of the visually identified plastics, were reclassified as non-plastics which was predominantly the mineral travertine, a form of limestone.

Table 9. Results from µFT-IR analysis.

	Total	Not plastic	Confirmed plastic	Correct identification
M5	24	3	21	88%
M6	16	2	14	88%
M9	51	9	42	82%
M10	35	0	35	100%
Total	**126**	**14**	**112**	**89%**

Figure 15. Plastics identified from a subsample of particles across four sample stations

4.3 Sediment samples

Sediment samples were prepared using two approaches: *Sieving* and *Density* separation. **Figure 16** shows how samples were processed across the four replicates. Analysis was focused on the top 0 – 2 cm from each site. For S2, S3 and S4, two replicates were prepared by sieving and two replicates were prepared by density separation. For S1 only two replicates were prepared by density separation.

- S1. Four replicate samples were collected with a Van Veen grab. Only the top 0 – 2 cm of sediment was collected. Two replicates were prepared by density separation. The remaining two replicates were not processed because sieving had already been deemed an unsuitable method due to time constraints and extraction efficiencies.
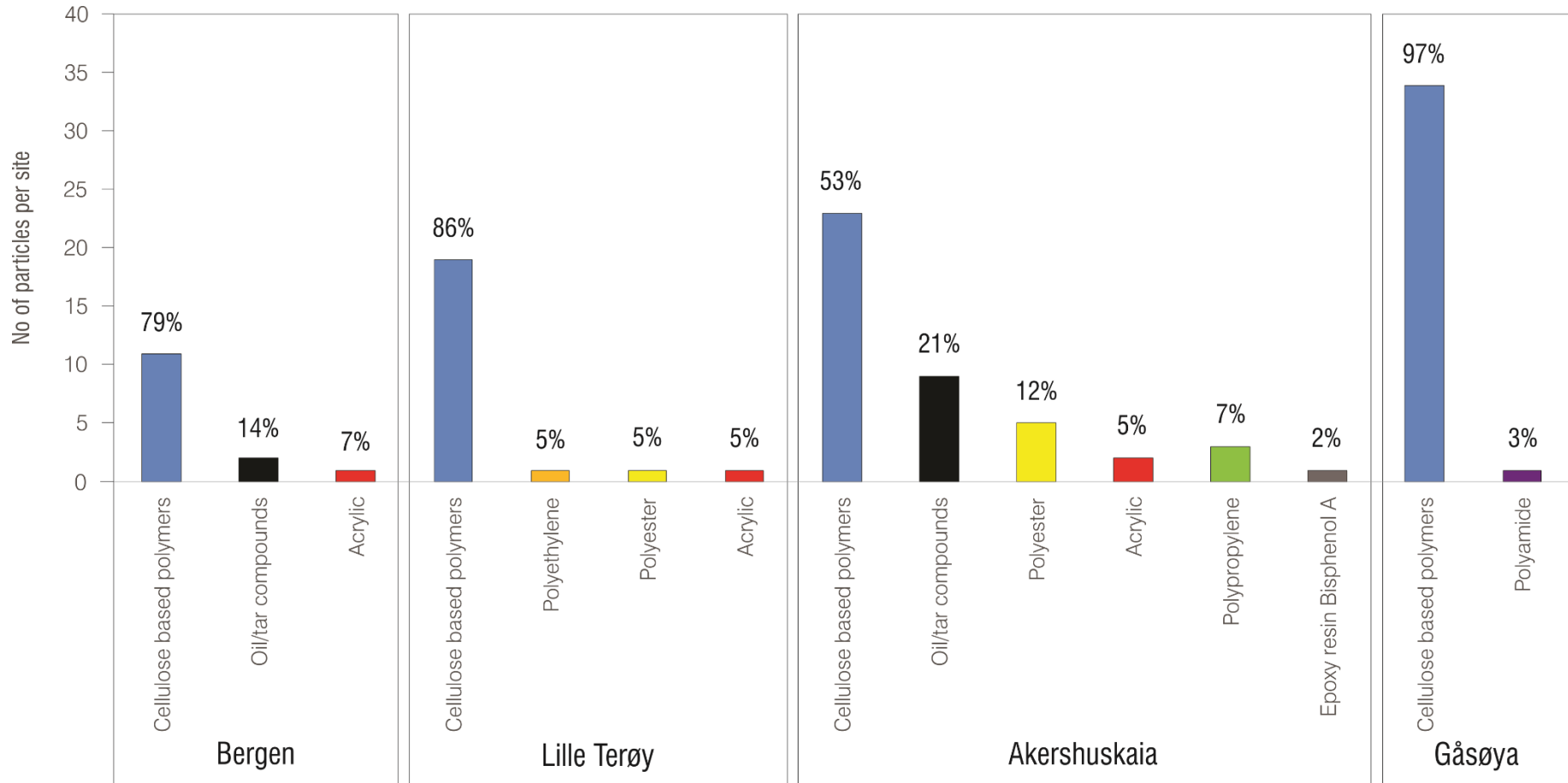
- S2 and S4: Four replicates samples were collected from box cores. Each core was divided into 1 cm depth bands. Only the top two depth segments (0 – 1 cm, 1 – 2 cm) were processed. Two replicates were processed by density and two replicates were processed by sieving. During sieving the segments 0 – 1 cm and 1 – 2 cm were processed together.

- S3: Four replicates samples were collected from box cores. Each core was divided into 1 cm depth bands. In all but one of the replicated the top two depth segments (0 – 1 cm, 1 – 2 cm) were processed. Two replicates were processed by density and two replicates were processed by sieving. During sieving the segments 0 – 1 cm and 1 – 2 cm were processed together. Replicate three which was processed for density was fully analysed (0 – 5 cm) using the density method.
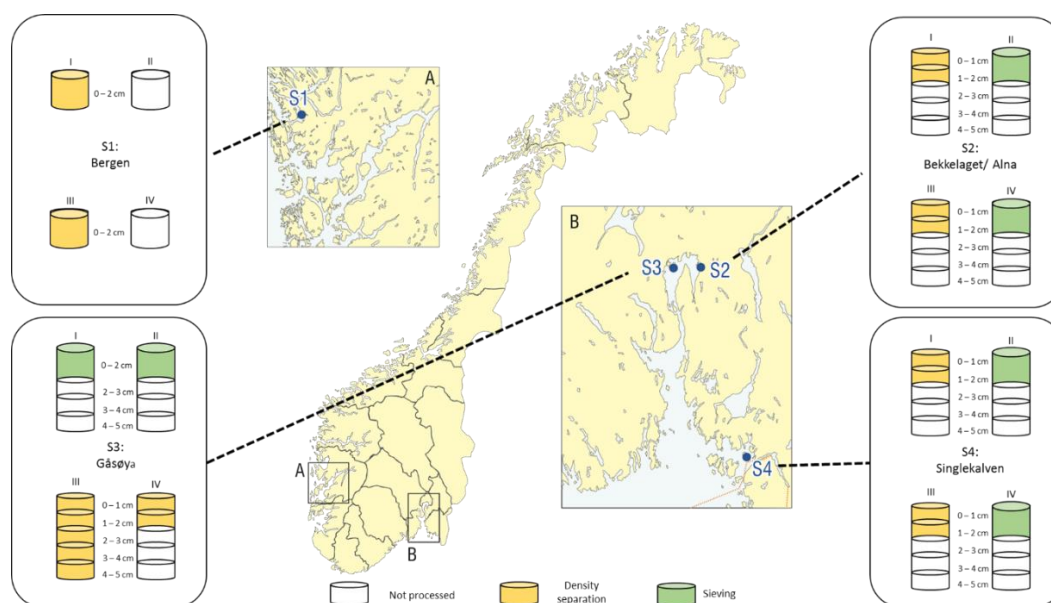

Figure 16. Division of analysis between sediment collected from the coast of Norway. Samples were prepared by sieving (green) and density separation (orange).

4.3.1 Visual identification of microplastics in sediments

A summary of the two methods tested to extract microplastics from sediment is presented below. The detailed results for visual identification is presented in the Appendix 1.

Approach 1: Sieving:
Replicates from three sites were assessed by sieving (S2, S3, S4). There was a large number of fine particles and inorganic material which hindered the identification (Appendix 1A). The results which were obtainable suggest that there was a mixture of fibres and fragments across all samples:

- S1: Samples were not processed by sieving as it the approach was deemed inappropriate due to high organic matter content.

- S2: Both replicates had suspected plastics. These included large amounts of fibres and some fragments. Due to the clay particles it was extremely difficult to separate them from the sediment matrix.

- S3: Both replicates had suspected plastics. These included large amounts of fibres and some fragments. Some beads were identified. Due to the particles clay particles it was extremely difficult to separate them from the sediment matrix.

- S4: Both replicates had suspected plastics. These included large amounts of fibres and some fragments. Due to the particles clay particles it was extremely difficult to separate them from the sediment matrix.

Approach 2: Density:
Each depth section separated by density with NaI was super-saturated with salt and clay particles. This made visual identification almost impossible (Appendix 1B). To correct for this, samples were soaked in distilled water for four hours before re-filtering them onto multiple filer papers (**Figure 17**).

- S1: Two replicate samples were processed by density separation (0 – 2 cm). There was a large amount of fine particulate organic matter in both the NaI extractions. Fibres, fragments and beads were extracted.

- S2: Two replicate samples were processed by density separation. There was a large amount of fine particulate organic matter in both the NaI extractions. Fibres, fragments and beads were extracted. Both replicates were rehydrated in distilled water to remove salt crystals before rinsing and filtering a second time.

- S3: Two replicate samples were processed by density separation. There was a large amount of organic matter in both the NaI extractions. Fibres, fragments and beads were extracted. Both replicates were rehydrated in distilled water to remove salt crystals before rinsing and filtering a second time. This site contained floating fine particulate organic matter as well as foraminifera which made the differentiation between suspected beads and biota impossible without further analytical techniques.

- S4: Two replicate samples were processed by density separation. There was a large amount of organic matter in both the NaI extractions. The samples were not rinsed in distilled water due to time constraints. Fibres, fragments and beads were extracted. Additionally, there was a large amount of crystalline salt which obscured and impeded the analysis **(Figure 18).**
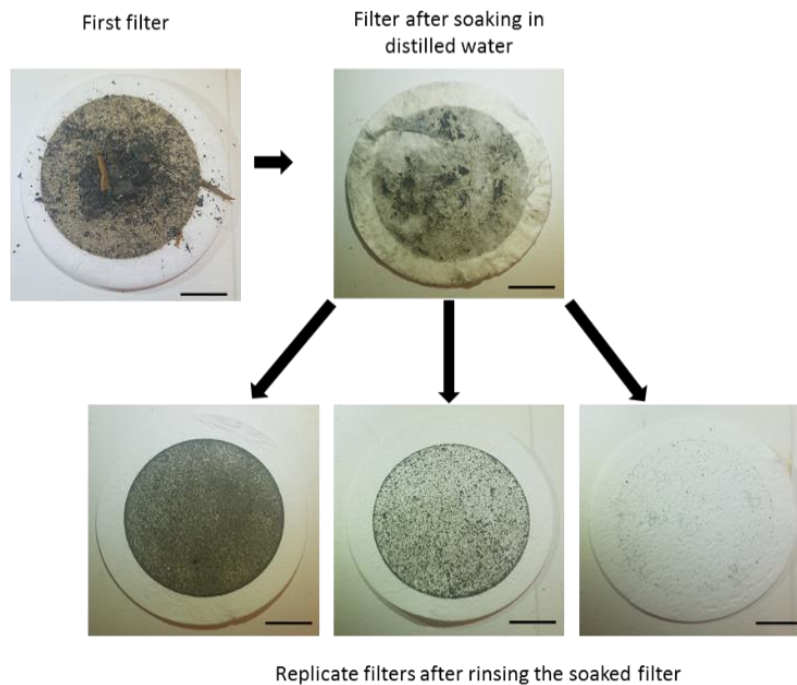
Figure 17. Example of filter papers after NaI floatation, soaking in distilled water and rinsing onto subsequent filter papers. Scale bar represents 10 mm.
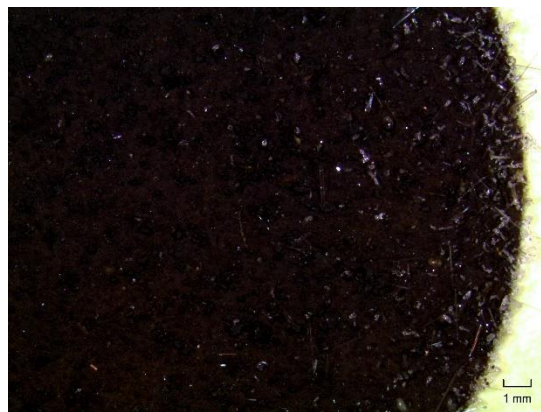
Figure 18. Crystalline salt on a filter paper

4.3.2 Chemical identification of microplastics in sediments

Chemical classification of plastics extracted from sediments has not been carried out. It has therefore not been possible to verify the identification of plastic particles for the sediment samples.

5 Discussion of results

This is the first time that microplastics have been identified in the Norwegian environment through a large scale, co-ordinated survey. Mussels from around the Norwegian coast were found to ingest microplastics. Mussels from rural locations had more microplastics than those from urban and industrial locations. Differences in the levels of microplastics identified in mussels from sites around the Norwegian coast may be influenced by hydrographical and atmospheric conditions, including tidal flow and amplitude, ocean currents, freshwater flow, locality to anthropogenic inputs and atmospheric deposition. Other procedural steps may have had an impact on the results, such as mussel size and the overall analysis conducted. Polymeric compounds identified in mussels include semi-synthetic cellulose based polymers, polyesters, polypropylene and polyethylene. Potential sources of these particles could range from general use plastics, textiles, paints, cosmetics, and finally, oil and tar.

5.1 Influence of environmental variables

Based on the results there were considerable differences in the numbers of microplastics per individual as well as per gram, between sites and their assigned categories. It is very likely that environmental variables influence the distribution of microplastics within the water column, such as hydro-chemical, hydro-physical conditions, atmospheric condition and atmospheric deposition. These variables probably affect the different sample sites along the Norwegian coast.

5.1.1 Ocean currents

Ocean currents, including the movement of water into and out of a region will influence the number of microplastics. Microplastics might be transported into a region from offshore sources or trapped in a region because of circulating currents. It is important to understand local circulation at each site. Ocean circulation appears to have a strong influence at Skallneset. There is a back-eddy present in the Barents Sea, this means that water that enters the region, such as coastal currents and the water from the North Atlantic, circulate leading to an accumulation of anthropogenic materials (van Sebille *et al.* 2012). Conversely, strong ocean currents can act to flush out anthropogenic material from a region, as described in **Section 5.1.2**. Bodø, Ørland, Måløy, Bergen and Lille Terøy are exposed to the North Atlantic and reduced numbers of microplastics may be related to flushing from the ocean currents.

5.1.2 Tidal flow and amplitude

Many of the sites chosen for this study experience varying degrees of tidal influence (**Table 10**). Sites with high tidal flow and amplitude are expected to experience a reduced number of microplastics due to the rapid turnover of water. This can be seen in sites on the west coast which are heavily influenced by the North Atlantic and have a high tidal amplitude. Sites such as Bodø, Ørland, Måløy, Bergen and Lille Terøy have between 1 − 2 m tidal amplitude, experience flushing and had low levels of microplastics. There is one exception to this, Skallneset experiences the highest tidal amplitude (~2.5 m) but its location in the Barents Sea appears to be more affected by oceanographic circulation. Areas with low tidal flow an amplitude, such as those situated in Sørfjorden and the inner Oslofjord are not likely to be influences by tidal flow and other environmental variables should be considered.

Table 10. Tidal flushing and amplitude for site locations.

Site	Station name	Area	Tidal amplitude (m)	Level of tidal flushing	Comment
M1	Skallneset	North	~2.5	Low	Back eddy in Barents Sea
M2	Bodø	North	~2	High	Exposed to North Atlantic
M3	Ørland	Central	~2	High	Exposed to North Atlantic
M4	Måløy	Central	~1.5	High	Exposed to North Atlantic
M5	Bergen	West	1	High	
M6	Lille Terøy	West	1	High	
M7	Kvalnes	West	~1	Low	Freshwater flushing
M8	Byrkjenes	West	~1	Low	Freshwater flushing
M9	Akershuskaia	East	0.2 – 0.3	Low	
M10	Gåsøya	East	0.2 – 0.3	Low	
M11	Ramtonholmen	East	0.2 – 0.3	Low	
M12	Solbergstrand	East	0.2 – 0.3	Low	Freshwater flushing from Oslofjord
M13	Singlekalven	East	0.2 – 0.3	Low	Freshwater flushing from Glomma river

5.1.3 Freshwater flushing

Freshwater flowing into a location can contribute to the input but also the dispersal and removal of plastic pollution. High levels of freshwater input encourage water turn-over and thus flushing from a location. Fjords often have fast surface water turn-over due to the input of rivers even though stagnation may occur in deeper, bottom waters. This may explain why sites including Bodø, Bergen, Lille Terøy and Singlekalven had low numbers of microplastics. Oslofjord is normally considered to be a semi-enclosed fjord in view of environmental pollution, however this may not be the case for microplastics. The sites in Oslofjord (Akershuskaia, Gåsøya and Ramtonholmen) were expected to have high levels of microplastics related to location of anthropogenic inputs, such as boat harbours, road run off and input of wastewater. However, high numbers of microplastics were not recovered from mussels in these sites compared to those from other locations. It is possible that the flow of surface water out of the fjord may be rapid. It must be mentioned that mussels from these sites were larger and this may have impacted the results (as discussed in **Section 5.2.2**)

5.1.4 Locality to sources of input

Urban areas were expected to have high levels of microplastics due to their relative distance from sources of anthropogenic release. This included urban and road run-off from areas with large

populations, riverine transport of litter and release of microplastics through WWTPs. The results of this study revealed that in fact the reverse was the case. Mussels located near urban populations (Bergen, Akershuskaia, Gåsøya and Ramtonholmen) had lower levels of microplastics, but they were larger in size. This shows that there may be additional environmental variables affecting the presence and distribution of microplastics. Microplastics may not be retained in the fjord for an extended period and any microplastics released from WWTPs may be transported out the fjords very quickly in surface waters. Another possibility is that the mussel sites analysed are not located close to wastewater treatment plant (WWTP) effluent outflow. Despite this, many semi-synthetic particles were identified in mussel samples in Oslofjord. It is possible that these particles originate from the washing of synthetic materials **(Section 4.3.2)**. Finally, particles extracted from mussels at Akershuskaia had the largest variety of polymers which suggests that there is a high variety of plastic pollution sources.

5.1.5 Atmospheric deposition

Atmospheric deposition of pollutants is of concern. Areas such as the Northern coast of Norway are known for having high levels of contaminant deposition (Green *et al.* 2012). As microplastics can also be transported atmospherically microplastics may be observed in higher numbers in areas with high deposition. Interestingly, Skallneset had the highest level of microplastics in the study and the Barents Sea has also been identified as an area of high contaminant deposition.

5.2 Influence of methodology

During monitoring surveys, methods need to be controlled to understand whether differences observed between sites are a true representation of microplastic contamination. The limited number of replicates used in the sediment study prevents an assessment of the methodology. However, there was an appropriate amount of repetition in the mussel survey to allow some comparisons. In this study, it was observed that some of the methodology steps may have influenced the final results obtained from mussel samples. This highlights the importance of standardising collection parameters. Chosen methods are evaluated in **Section 6.** This study accounted for temporal variation by sampling mussels from a similar time frame (2016-2017) as well as conducting the same method of extraction and analysis across all sites. However, the sampling was not standardised, including depth of collection and size of individuals.

5.2.1 Depth at collection

Organisms that are collected from the shoreline are exposed to airborne particles as well as waterborne particles whereas organisms collected from submerged locations are predominantly exposed to particles in the water column. Furthermore, the salinity and retention time of water, and subsequently the retention of microplastics could be very different between surface waters and deeper waters.

5.2.2 Size of organisms

Organisms of different sizes were analysed in the study. Size may influence the number of microplastics retained in digestive tracts despite correcting for weight. More information is needed on this to confirm the speculations.

5.3 Site specific differences observed

Considering the points raised in **Section 5.1** and **Section 5.2**, significant differences between sites, based on particles per individual and particles per gram, were observed which may be related to site specific pollution.

M1, Skallneset –   This rural area is remote from large areas of urban activity and close to Varangerhalvøya national park. It is however very exposed to the Barents Sea. Mussels were collected from the shoreline in an intertidal area meaning mussels would be exposed to microplastics present both in the water column as well as those in surface waters and the water-air interface. Mussels from this site were identified as having significantly higher levels of macroplastics than other sites. This could be related to **atmospheric deposition** of airborne microplastics, low **tidal flow** and **amplitude** as well as **limited circulation** caused by the back-eddy present in the region. For example, the continued input of contaminates and sea water from further afield, such as transport from the North Atlantic, enters the Barents Sea and are not released due to the water currents. Contaminants therefore remains accumulating, which could be linked to the 6th gyre as proposed by van Sebille *et al.* (2012) and Cozar *et al.* (2017). The tidal flow and amplitude is smaller in this location than sites on the west coast. This could explain the large number of microplastics identified in a rural, remote site.

M2, Bodø –   This site is in a remote location of the fjord with little boat traffic (20 km from the port) and freshwater input. Mussels were sampled from an exposed area from concrete, rope and plastic surfaces. Whether the plastic surfaces are a source of microplastics are unknown, but there is no indication of this in the results. The fjord has a high turn-over of water and could experience **freshwater flushing**. This could explain why the values of microplastics found in mussels from this site were not high.

M3, Ørland – This site is in a small boat harbour close to a military airport base which could suggest a high level of anthropogenic impact, however there is a high **tidal flow** in the area which could move material out of source locations quickly. There is considerable tidal flushing with a tidal amplitude of 1.5 m. This could explain the low average number of microplastics recorded.

M4, Måløy – This site is exposed to the North Atlantic. There is a high tidal amplitude (~ 1.5 m) so a rapid turn-over of water would occur in this area. The number of microplastics were relatively high and may be explained by the influx of particles through ocean transport. As previously discussed, tidal flushing would remove particles from an area, it is not possible to explain the high levels in this area.

M5, Bergen – This site is in the outer fjord and it could therefore experience **freshwater flushing** and **tidal flushing** as water movement out of the fjord and is flushed by the North Atlantic. The high turn-over of water (1 m tidal amplitude) may explain the low numbers of microplastics being retained in this location.

M6, Lille Terøy – This site, as with other sites on the west coast of Norway, is situation in the outer fjord and would experience **tidal flushing** (1 m tidal amplitude). There is also a large **freshwater input** during times of high rain fall and river flushing. The combined effect of freshwater flushing and tidal mixing might explain the low levels of microplastics observed in this location.

M7 and M8, Kvalnes and Byrkjens – Results from both these industry sites are indistinguishable. Although both are situated inside Sørfjorden, it is likely that Byrkjens experiences more freshwater influence due to the locality to a river. Mussels in this area had relatively high levels of microplastics

which could be explained by **low tidal flushing** and **limited water circulation** in the fjord causing microplastics to be retained the in region. Furthermore, Kvalnes mussels were collected from the intertidal and are therefore could be to atmospheric deposition, whereas Byrkjens mussels were submerged.

M9, Akershuskaia – Results from urban locations might represent different feeding behaviours and sources. Interestingly, urban areas had the lowest numbers of microplastics when standardised to g^{-1}. There is a **low tidal amplitude** in the Oslofjord. Organisms were collected from the subsurface and not exposed to air as readily as those on the west coast. If the **freshwater flow** is rapid out of the fjords, which is suspected due to its location near to the Alna river, then these organisms might not be exposed to high quantities of microplastics in the surface waters, even with more sources of urban contamination (including road run-off and boat traffic).

M10, Gåsøya – Like Akershushaia, this urban location might experience different sources of microplastics. There is a **low tidal amplitude** in the Oslofjord. Organisms were collected from the subsurface and not exposed to air as readily as those on the west coast. If the **freshwater flow** is rapid out of the fjords, which is suspected due to its location near to the Alna river, then these organisms might not be exposed to high quantities of microplastics in the surface waters, even with more sources of urban contamination. Gåsøya is located north east of VEAS WWTP, and with the southward directional flow of water it is unlikely they are affected by microplastic input from the WWTP.

M11, Ramtonholmen – like Akershushaia and Gåsøya, this urban location might experience different sources of microplastics. There is a **low tidal amplitude** in the Oslofjord. Organisms were collected from the subsurface and not exposed to air as readily as those on the west coast. If the **freshwater flow** is rapid out of the fjords, which is suspected due to its location near to the Alna river, then these organisms might not be exposed to high quantities of microplastics in the surface waters, even with more sources of urban contamination. Ramtonholmen is located 5 km south of VEAS WWTP and may be influenced by microplastics released from WWTP. The outlow from WWTP outlet is below the surface and buoyant particles may not reach the feeding zone of mussels, however animals were collected from the subsurface and may have been influenced. However, low numbers of microplastics identified in these mussels suggest that there may be a local effect from fjord flushing of the rivers. Microplastics are not retained in fjord for an extended period of time and it could be that WWTPs release microplastics but they are transported out the fjords very quickly, and particles are found in areas outside the fjord instead.

M12, Solbergstrand – This location further away from urban areas on exit of the fjord may experience local accumulation area as the water flow slows on exit of the fjord. The level of microplastics in Solbergstrand were greater than sites in the inner Oslofjord. Solbergstrand is in the mouth of Oslofjord and close to a freshwater stream. Individuals were collected from intertidal so exposed to **water-flow** as well as **atmospheric deposition.**

M13, Singlekalven – This is an area of high freshwater input as it is at the mouth of Norway's largest river. It is likely that there is a high contribution of microplastics from land based sources. **Low tidal flow**, **reduced water circulation** and **atmospheric deposition** of airborne particles to air water interface may explain the high numbers of particles identified in mussels from this area.

5.4 Possible sources of microplastics found in Norwegian mussels

Based on qualitative data, qualified speculations regarding source of microplastic contamination to mussels can be made. Some of the qualitative results are also discussed in **Section 4.2.3.**

5.4.1 Semi-synthetics

Semi-synthetic cellulosic polymers were the most common plastics found in blue mussels (*Mytilus spp.*) in the Norwegian environment. Cellophane® was the most common commercial semi-synthetic cellulosic polymer identified using µFT-IR. Cellophane® is a brand name for viscose rayon, which was the first manufactured fibre. Natural cellulose is much more dense than synthetic cellulose (Whelan 1994), and the weight of dry clean viscose rayon is $1.5 - 1.53$ g cm^{-3} (Ellis and Smith 2009). However, with a density greater than water ($1.02 - 1.029$ g cm^{-3}) one would expect viscose rayon to sink. However, rayon absorbs water which leads the material to swell, and swelling might impact the bouncy. In addition, rayon fibres have a high aspect ratio (due to their long length in relation to diameter) that gives them a high surface area and changes their physical properties in relation to bulk material (Steinmann and Saelhoff 2016). This again might affect the floating properties of the material. Behaviour and fate of semi-synthetic fibres in the complex marine environment is not understood and further research is needed. However, based on the results of this study, these particles are available for blue mussels to ingest them.

Chemical alteration of wood to textile fibres is thoroughly reviewed in Shen *et al.* (2010). These alterations make them semi-synthetic cellulosic fibres more durable in nature, unlike un-altered cellulose. Therefore, material origin might not be appropriate when deciding whether they are considered an environmental issue, when the physical properties, such as durability, can be more important. Semi-synthetic polymers as a form of environmental pollution lack vital information as their fate and effects in the environment are unknown. The production volume of synthetic cellulose fibres is higher than all other synthetic organic polymer (Shen *et al.* 2010). It is therefore vital that their presence in the environment implications for biota are understood. Currently, there is not enough knowledge to exclude cellulosic polymers from environmental results. Furthermore, the interactions between semi-synthetic polymers and environmental pollutants (as well as chemicals added in the production), their sorption and leakage of chemicals to/from cellulosic polymers, are much less studied than other synthetic polymers. Nevertheless, some chemical modifications of cellulosic material include, for example, enhanced metal-binding ability (reviewed in O'Connell *et al.* 2008).

5.4.2 Polyester

Polyesters are a group of polymers, commonly used in textiles. Polyethylene terephthalate (PET) was found in this study and has been regularly identified in other environmental matrices including Atlantic cod (*Gadus morhua*) from the Norwegian environment (Bråte *et al.* 2016). PET has a high density (1.38 g cm $^{-3}$) and is likely to sink in the water column exposing organisms that are feeding subsurface.

5.4.3 Acrylic

Acrylic (also known as polymethyl methacrylate, PMMA) is commonly used in textiles and paints. It was found in mussels from three out of four stations tested by FT-IR. This polymer has also been found in Atlantic cod (*Gadus morhua*) from the Norwegian environment (Bråte *et al.* 2016). With a density of

around 1.18 g cm^{-3} acrylic is likely to sink in the water column exposing organisms that are feeding subsurface.

5.4.4 Polypropylene and polyethylene

Polypropylene (PP) and polyethylene (PE) are the two most common polymers in terms of production and those found in the marine environment. It was therefore not surprising that they were identified in this study. The sources of these polymers are broad and widespread, ranging from general use plastics (plastic bags and bottles, clothing) to primary microplastics incorporated into consumer products. PP and PE have low densities and float in sea water. However, biofouling facilitates sinking by reducing buoyancy, and it is therefore not surprising to find them in blue mussels which could be exposed feeding in surface waters as well as submerged locations.

5.4.5 Polyamides

Polyamides (PA), including Nylon, have a density of 1.15 g cm^{-3}. PA is commonly used in textile products. This polymer is likely to float in the water column however, biofouling is known to facilitate sinking by reducing buoyancy, and it is therefore not surprising to find them in blue mussels which could be exposed feeding in surface waters as well as submerged locations.

5.4.6 Ethylene Vinyl Acetate and Epoxy Resin

Mussels from Akershuskaia contained particles made of ethylene vinyl acetate (EVA) and epoxy resin (with bisphenol-A) which is not often found in microplastic surveys. To the authors knowledge, this is the first time both polymers have been identified in blue mussels. The origins of these polymers are unknown, but foamed rubber or "*skumgummi*" in Norwegian, often consists of EVA foam and it has wide range of applications and it is hard to establish the source of this pollution. EVA foam is a thermoplastic commonly used in running shoe midsoles, children's toys and in cycle tyres (Wang *et al.* 2012). The density of clean EVA foam, 0.93 g cm^{-3} (Hashim *et al.* 2017), enables the polymer to float in seawater and can be bioavailable for filtrating organisms. Bisphenol A is a common component in epoxy resin and it is a well-known endocrine disruptor, as reviewed in *e.g.,* Rubin (2011). It has also been found negative effects on metabolism for marine mussels exposed to bisphenol A (reviewed in Canesi and Fabbri 2015). Density of bisphenol A based epoxy resin is around 1.17 g cm^{-3}.

5.4.7 Oil/tar compounds

The category oil/tar compounds, was identified by FT-IR-library match as "*parking lot tar*". Petroleum has a density of around 0.82 - 0.92 g cm^{-3}, thereby floating in water. Unfortunately, it was impossible to identify specific chemical composition of these compounds which hindered the identification of potential sources. Further investigation is needed.

6 Evaluation of methods used

Mussels appear to be promising bioindicators for the smallest sized microplastic (<1 mm), due to their ecology, ease of sampling, the standardised sample processing and further analysis. However, with improvements blue mussel surveys can also be used for quantitative monitoring surveys of the Norwegian coastal environment. The choice of sampling sites for mussels should be carefully considered, as should the number of individuals, their position on the shoreline, depth and method of collection. Methods for sediment collection and analysis require further testing to optimised monitoring. Other biota could be considered including sediment dwelling bivalves and worms. Extraction of plastics from biota using 10 % KOH is an appropriate method. Classifying plastics based on their shape, size and polymer type is appropriate for monitoring surveys, but colour should not be used as the primary identification parameter due to the subjectivity of visual colour. If used, more robust colour identification should be implemented.

This section will discuss the effectiveness of methods used for the preparation of mussels using digestion and the preparation of sediments using sieving and density separation. The sections are divided into sampling, extraction and interpretation of results. For the mussel method to be fully quantitative, some improvements are needed: when sampling gut clearance should be avoided (fixate in ethanol), the same sized mussels should be used from each site (might not be enough to standardise toward grams), they should be from the same position in the water column (tidal or inter-tidal) and also if basing the results on visual analysis, it is important to include buddy check or having the same person doing all the visual identification.

6.1 Sampling

6.1.1 Choice of sampling sites

Using a site classification can help investigate the level of contamination at different locations. However, site classification is not yet implemented within the field of microplastic research. It is important to consider many potential sources of plastic contamination when making these assessments, as discussed in **Section 5**. Based on the findings in this report, it is too early to conclude if the classifications used here are appropriate for assessing microplastic load in the marine environment. Furthermore, it might be appropriate to change the categories used (

Table 11). The sampling site in Bodø should be re-classified as a rural location because it is an isolated location, far from a fishing port with a high turn-over of water. Singlekalven should be reclassified as a combination site because it is in a rural area but influenced heavily by the Glomma river and the Oslofjord.

As highlighted in **Section 5** sites can be influenced by many different environmental variables and it is therefore important to understand what could be affecting microplastic loading in organisms and sediments. It is vital that interspatial (site) differences are further investigated. This means that differences between shoreline, subsurface sampling, and populations within a site should be investigated. Exposed organisms may be encounter microplastics through atmospheric deposition, accumulating in surface waters and the water column. Whereas individuals collected from submerged location will only be exposed to microplastics in the water column.

Table 11. Recommended updates to site classification based on anthropogenic influence.

Site	Station name	Original classification	Updated classification	Comments
M1	Skallneset	Rural	Rural	-
M2	Bodø	Combination	Rural	Far from fishing port, isolated etc. Lots of water movement in area
M3	Ørland	Combination	Combination	Even though this site is rural, there is an unknown impact from the military airbase but far from urban location
M4	Måløy	Rural	Rural	-
M5	Bergen	Urban	Urban	-
M6	Lille Terøy	Combination	Combination	Far from sources but large water mixing
M7	Kvalnes	Industrial	Industrial	-
M8	Byrkjenes	Industrial	Industrial	-
M9	Akershuskaia	Urban	Urban	
M10	Gåsøya	Urban	Urban	
M11	Ramtonholmen	Urban	Urban	
M12	Solbergstrand	Combination	Combination	
M13	Singlekalven	Rural	Combination	Far from urban but close to the mouth of Norway's largest river

6.1.2 Number of individuals/replicates collected

Mussels: The choice of 20 individuals has provided an appropriate comparison of contamination for some of the sites. Due to the different variance between microplastic content in mussel individuals for different sites more data is needed. Further power analysis can inform about the number of individuals required. To accurately recommend the sample size required, more data is needed on several populations from the same site to study the inter-site variation. It will be important to use mussels that are the same size as it might not be enough to standardise toward grams due to differences in *e.g.,* growth, development and stage of reproduction. Also, it is important to note that there may be a difference in microplastic uptake and thereby spread in the presented data between sub-species of *Mytilus*. NIVA has investigated the abundance of different mussel species (*Mytilus edulis*, *Mytilus trossulus* or hybrids) along the Norwegian shoreline (Brooks and Farmen, 2013) which could be incorporated into future monitoring programmes. Both ICES (2013) and MSFD (Galgani *et al.* 2013) recommend 50 individuals per species.

Sediments: Due to cost and time limitations, the number of replicates used in this report were not sufficient to acquire robust data to compare microplastics level between sites. However, based on previously published research, going forward it would be advisable to collect a minimum of three replicates per site for future investigations (Martins *et al.* 2017).

6.1.3 Collection and preservation methods

Mussels: In this study mussels were collected from different depth zones: shorelines, intertidal and submerged. It is very likely that this has influenced the results due to exposure duration to atmospheric as well as waterborne plastics. It is important for future monitoring programmes to standardise the collection method for better comparisons. Collection of mussels from shoreline appears to be appropriate to aquire the required sample size in a short period of time without the need for additional expenses in sampling platforms. It is extremely important that all individuals are collected form the same depth. Byssus threads should be cut carefully to prevent stress on mussels. Mussels collected should be the same size to account for age and reproductive stage. By freezing mussels as soon as possible after collection, individuals were persevered effectively for later analysis. Since carrying out this research, Beer *et al.* (2017) recommended that organisms are preserved in ethanol. Therefore, for future sampling regimes, preservation in ethanol is advised even though, when removed from water, mussels typically close their shells.

Sediments: Sampling for sediment using a core would be the most appropriate method for monitoring microplastics deposition to benthic sediments. It was observed that using a composite grab sample impeded the results whereas a core has the potential to allow a discussion of the distribution of microplastics though sediment layers. This method has been shown as an effective tool for monitoring benthic sediments in Ireland (Martin *et al.* 2017). Furthermore, using a core can allow for an understanding of plastic deposition rate in sediments and may present a historical record of plastics in undisturbed sediments. Slicing should be done as soon as possible to avoid mixing of sediment layers, if slicing needs to be done in the laboratory then cores should be transported vertically to avoid disturbance. Any overlaying water, sediment-water interface, can be syphoned and retained for analysis of microplastics. It is recommended that samples are frozen as soon as possible after collection. This will preserve them for later analysis. Sediment traps could be considered as an additional method to monitor presence of microplastics.

6.2 Extraction protocols

6.2.1 Mussel preparation in 10 % KOH

KOH was sufficient at dissolving mussel tissues as this had been previously tested in early laboratory experiments by the researchers involved (Bråte *et al.* submitted; Lusher *et al.* 2015). In future monitoring programs, it is recommended that KOH is used as it is a cheap and effective technique which is now being pursued as the most suitable method for monitoring biota.

Since Dehaut et al (2016) conducted a wide degradation study, this study conducted a small recovery test of the most common polymers: PP, PET, PS, PA-66 and LDPE (five replicates for each polymer). The same test was carried out on 100 % viscose and a viscose mixture (50 % viscose, 46 % cotton and 4 % elastin). There was a 100 % recovery of these polymers when they were exposed to 10 % KOH in the same way as the mussel samples.

Cellulose acetate (CA) was the only polymer found to be destroyed by KOH digestion (Dehaut *et al.* 2016). CA and cellulosic fibres are similar in many ways, and used to be considered the same textile. There are differences in production making rayon more heat-resistant than CA. Prior to the current study, semi-synthetic cellulosic polymers had not been tested for alterations by KOH treatment. This is the first-time viscose has been tested with KOH. The increased heat-resistance in rayon might explain why rayon did not disintegrate during the KOH process, as expected for CA. However, there were observations where viscose had leached its dye. During testing, 100 % viscose was more bleached than the mixture of viscose and cotton. As seen from **Figure 19,** there appeared to be some instances where dye leached from cellulosic fibres following extraction from blue mussels. Several transparent and white cellulosic fibres were identified in blue mussels; whether this was their original colour prior to KOH treatment is unknown. It is possible that these fibres may have leached their colour during KOH treatment. Transparent fibres were hard to identify, and it is possible some fibres may have been overlooked. Since such a high proportion of cellulosic polymers were identified, the alteration of semi-synthetic material by alkaline dissolving methods should be assessed. Another option is to use other filter papers with an increased contrast, to be more certain about finding the transparent/white fibres in the sample. It is also challenging to chemically distinguish between semi-synthetic cellulosic fibres and naturally occurring cellulose fibres through µFT-IR for example (Comnea-Stancu *et al.* 2017). Despite this, the results from the cellulosic particles are included since the KOH treatment degraded any natural cellulose fibres.

Figure 19. Example of cellophane/rayon fibre with colour seeming to be leaching of after KOH-treatment.

6.2.2 Preparation of sediment samples

Sediment replicates were first prepared by wet sieving. Sieving was not effective as a large proportion of the sediment matrix was in the same size fraction as the target microplastic particles (*i.e.,* large volumes of sandy material). It was observed that sample volume (~ 30 g) and organic matter content were high, which lead to physical obscuration of the sample therefore complicating the analysis. Some sediments were dried to obtain dry weight before sieving. This lead to aggregations of fine particulate matter which impeded sieving.

The remaining half of the samples were subjected to density separation using NaI. Density helped to reduce the number of samples to be visually analysed (one sample per replicate), and reduced the amount of minerogenic material. Salt crystals formed in many of the samples and they had to be soaked. After soaking and rinsing, an increased number of filter papers had to be analysed therefore increasing the time required for analysis. There was often still a layer of fine organic particulates and clays which impaired the ease of visual identification. Hence, an organic matter removal step is necessary, in addition to density separation, to produce samples that are quick and easy to visually assess for microplastic contamination.

6.3 Particle analysis

Visual and chemical steps should be used to effectively identify microplastics. Since the visual detection limit is 150 µm, this is an additional consideration that needs to be highlighted regarding sample size. The smallest microplastics might be less patchy distributed than the larger microplastics. Due to the high cost, time consuming and still developing techniques for working with the smallest microplastics (such as FPA-FT-IR), it is currently not possible to analyse all samples for the smallest microplastics. However, it is possible to (with method development) to aim for running sub-samples of mussels through FPA-FT-IR, and thereby get better understanding of also the smallest sized microplastics concerning site variation.

6.3.1 Quality control: buddy checks

Prior to starting the visual analysis of mussel samples in this report, a buddy-check test was carried out to check for inter-person variation. Ten samples were blind tested by five researchers at NIVA which have varying levels of expertise in the field of microplastics. The results showed that there were considerable differences between observers and therefore only the observers which were not significantly different in observational skills conducted the visual analysis for this study. It is recommended that buddy checks are carried out routinely to ensure observers neither underestimate or overestimate plastic presence in samples.

6.3.2 Quality control: contamination monitoring

Correcting for procedural contamination requires further investigation. Different methods are suggested to quality control data. These include (1) correcting for blanks by removing the average reported value, and (2) using a similar approach such as LOD and LOQ which is used for traditional analytical chemistry.

From the results presented here, further information is required to understand the impact of correcting data based on presence of particles in procedural blanks. Since it is not possible to process all samples from all sites on the same day there will be different levels of background contamination. Therefore, it appears to be appropriate to correct samples processed on different days based on their respective procedural blanks. This highlights the importance of daily procedural blanks. As seen from **Figure 20**, the average results from the different sites are, to some extent, influenced by the blank corrections. For example, it appears that station M4, Måløy, has the larger difference between raw data and corrected data which is seen when data is presented both as per individual and per gram.

Whether it is appropriate to use either of these approaches or alternatives, is not yet agreed upon and should be further investigated and discussed between researchers and stakeholders.
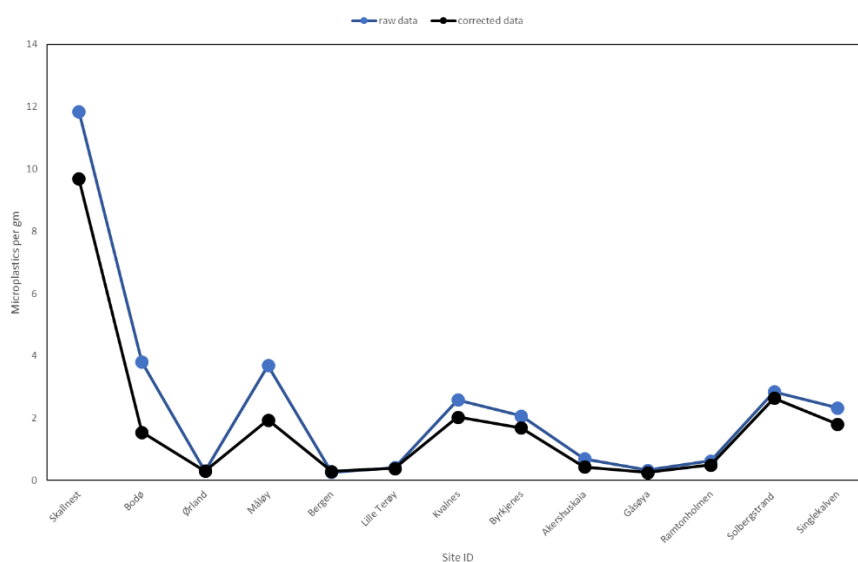
A)
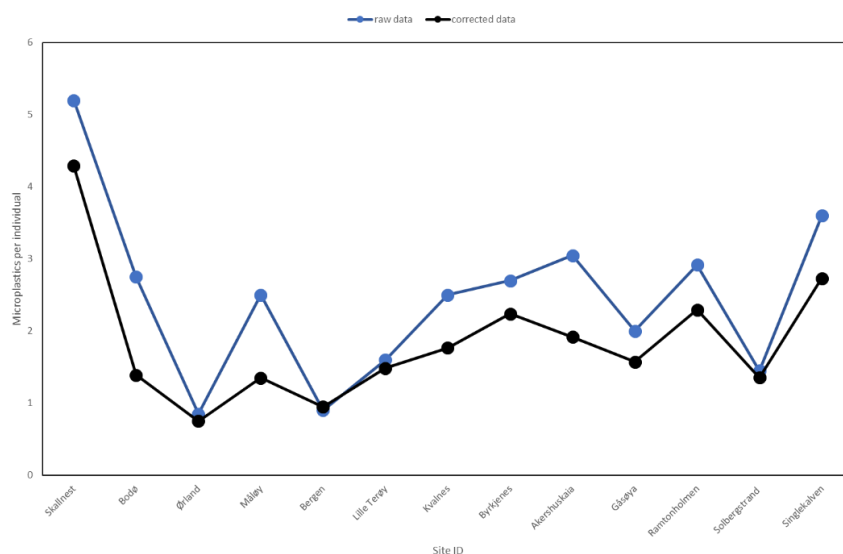
B)

Figure 20. Effect of correction for contamination in procedural blanks. Data displayed is (A) per individual and (B) per gram. Raw values are represented with the blue line and corrected data is represented with the black line.

6.3.3 Visual identification

Visual identification is necessary in nearly all methods used to identify plastic presence in environmental samples (see **Section 2.4**). Visual identification has a high proportion of subjectivity and should be supported with additional steps including, buddy checks (**Section 6.3.2**) and further analytical tests (**Section 6.3.4**). However, when using visual identification based on the particle morphology it is important the researcher is experienced with separating anthropogenic material from natural particles by using the particle characteristics discussed below:

Classifying plastics based on their **shape** is appropriate for monitoring surveys. It allows differentiation between fibrous, spherical and fragmented particles. Fragmented particles can be further divided into foams and films, but this does not appear to be necessary when presenting the data as foams and films are still fragments of larger particles. Spherical particles such as beads when identified constitute to a third category, but beads often require further analysis due to their similarity to foraminifera. Particle shape informs environmental fate and is relevant when considering the behaviour of particles in environmental settings as well as the potential for biotic interactions. Furthermore, shape may be used to infer potential sources.

Classifying plastics based on **size** is appropriate for monitoring surveys. Using different size categories allows further insight into the risk of biotic interactions as well the behaviour of plastics in the environment.

Colour should not be used as the primary identification parameter due to similarities of some colours to natural, biotic material. In this study, it was observed that viscose fibres leached their dye and become transparent. Similar transparent fibres were found in blue mussels. Transparent fibres were hard to identify on the white filter papers, and therefore there is a likelihood of an underestimation of transparent viscose fibres in the mussels.

6.3.4 Chemical identification

Classification of particles to **polymer** level is necessary as it confirms visual identification. Polymer type can also allow the segregation of particles into traditional polymers (thermoplastics and thermosets) as well as semi-synthetic plastics derived from natural materials.

The definition and terms used when discussing polymer science, can lead to confusion (Jenkins *et al.* 2009). Simplified, all plastics are polymers but far from all polymers are considered plastics. Polymers are divided into two main groups when considering origin: natural polymers and synthetic polymers. *Natural polymers* are found in nature and have not undergone any alterations by humans (for example cellulose, proteins, starch, and natural rubber) while *synthetic polymers* have been manufactured in laboratories (*e.g.,* PE, PP, PET and semi-synthetic cellulosic-materials such as rayon). Many international advisory boards do not include semi-synthetic particles due to the unknown effects. However, it is recommended that cellulosic fibres are included in monitoring surveys due to their large occurrence in biotic (observed in this study) and abiotic (other published research, i.e. Lusher *et al.* 2014) samples.

In addition, 11 % of particles identified during visual analysis were found to be non-plastics following chemical characterisation. In this report, results have not been corrected based on misidentification. The reason for not doing so is that the four sites analysed with µFT-IR had a similar misinterpretation

of plastics. This would suggest that a 11 % adjustment would be appropriate across all sites and thereby the relative difference between the sites would not be affected.

6.4 Reporting units

Mussels: Until standardised reporting units have been agreed up, it is important to report using multiple expressions of microplastic contamination. This will increase comparability with other studies to examine the true impact of plastic ingestion. As shown in **Figure 8**, the plastic concentrations can vary quite significantly between individuals when standardised by weight, meaning it is important to consider this factor. It is therefore important to standardise the results to microplastics per gram (w.w) to allow for better comparability.

Sediments: As this report was unable to successfully quantify the number of plastic particles in sediment samples, not comment can be made on the reporting units used here. However, a general observation is that most sediment studies report quantities of microplastics as particles kg^{-1}d.w. Where possible studies should report values volumetrically as particles m^{-3}. This would be relevant for core samples where the volume of sediment is known.

6.5 Evaluation of blue mussels and sediments as monitoring matrix

Blue mussels have been proven as a promising indicator organism for other contaminants (Beyer *et al.* 2017), especially reflecting the current discharges. OSPAR recommends blue mussels as a suitable monitoring species because they are sessile, robust, have large stocks for repeated sampling and reflect the local conditions (OSPAR 2012). Information on how microplastics behave in the environment is still lacking to fully evaluate the role of mussels as an indicator organism for microplastics. However, based on this study, mussels feeding in the water column could be appropriate to monitor waterborne particles, especially smaller microplastics (< 1 mm). This is supported by the observations by Karlsson *et al.* (2017), that mussels contained more microplastics than surrounding waters, suggesting they could accumulate microplastics. The method used here to collect mussels and to extract microplastics from mussels are cheap, fast and reproducible. Based on the KOH method, 40 samples (40 individuals) plus three controls could be processed per day. Weekly sample preparation ranged from 80 – 120 individuals. This was an efficient processing time suitable for long-term monitoring studies. Mussels could be used to look for differences within the water column. For example, mussels from coastal, intertidal locations could be compared to those feeding in the subsurface waters. As microplastics have different properties (density, buoyancy *etc.*) focusing on one part of the water column might not be sufficient and therefore monitoring should target both surface and subsurface locations. Despite this, blue mussels alone are probably not sufficient to monitor microplastics within all environmental matrices or all water bodies Abundance and distribution of mussels is limited by salinity, and mussels may not be present in estuaries, inner parts of fjords or river outlets with low salinity. Microplastics in the water column will eventually reach the sediment. It will be therefore appropriate to consider monitoring sediments as well as sediment dwelling organisms as supplementary monitoring tools.

Sediments are proposed as the final destination of microplastics in the environment. Following loss of buoyancy and density changes (incl. biofouling), microplastics can be deposited in sediments. Hence, monitoring of sediment could be appropriate and important for monitoring long-term trends, as well as tracking historic settling of microplastics. For comparison between sites, it is important to consider

the sedimentation rates, which is often not known. However, sediment traps can be a tool for calculating sedimentation rates of particles in general as well as present microplastic settling rates. When sampling sediment from cores or grabs for recently settled microplastics, surface sediments (0 – 1 or 0 – 2 cm) seem appropriate. However, sediments are complicated matrices and the most advanced methods for sampling and preparation are time-consuming. Based on the methods used in this report, sediment extraction was time consuming and complex. The current method is not recommended but the following alteration is suggested to improve effectiveness. Based on the volume of fine particulate organic matter and minerogenic material, it is crucial to add further processing steps to effectively extract microplastics from marine sediment matrices. A density extraction process is recommended to isolate microplastics from sediments; however, organic material also present hinders this procedure. Much of this organic material is very fine, so an initial sieving step to remove material <38 µm or <50 µm may help to reduce the problem. Alternatively, an organic matter removal step could be performed. This will reduce the number of aggregates composed of sediments, organic material and, potentially, microplastics in addition to removing fine particulate organic material. Using these steps together will help to drastically reduce the number of non-plastic particles that are extracted, and significantly improve the ease and accuracy of visual identification or chemical characterisation.

Due to the complexity of sediment analysis, it might therefore be more suitable to use sediment dwelling organisms such as polychaetes (*e.g., Hediste diversicolor*) and worms (*e.g., Arenicola marina,* or other similar species available at a specific site with same ecology) and/or sediment-dwelling bivalves (e.g., *Aequipecten opercularis*) feeding on/in the sediment (as discussed in Bråte *et al.* 2017). This way the same KOH method could be utilised. For *A. marina*, which has a tough skin, it is not possible to digest the whole organism, however the digestive tract can be isolated and processed in a similar manner to blue mussels.

Fish do not appear to be the most appropriate phylum for monitoring of microplastics in the marine environment, as it is likely that they have a fast turn-over of plastics through their digestive tract. Furthermore, fish are more motile and would be less suitable for monitoring specific sites. Mussels probably rapidly egest most microplastic particles (Karlsson *et al.* 2017) but compared to fish they are smaller and can therefore be preserved. Mussels are also sessile and more suitable than fish to reflect the impact of present discharges. It must be noted that biota may not the final destination of plastic particles, given that they have the ability to egest microplastic.

Therefore, blue mussels in combination with monitoring of other environmental matrices would be advised to get a broader picture of microplastic pollution in the ocean, such as sediment samples and/or sediment dwelling organisms.

7 Future monitoring of microplastics in the environment

Both sediment and mussels seem to be appropriate for future monitoring of microplastics in the environment. Since mussels alone may not be suitable for sampling all compartments of the marine environment, it will be important to consider other bivalves, or sediment dwelling organisms to investigate microplastics in the sediment matrix. In future monitoring programs of biota, it is recommended that KOH is used with the improvements suggested, as it is a cheap and effective techniques which is now being pursued as the most suitable method for monitoring biota. To fully understand the number of individuals required for robust monitoring of microplastic pollution, more studies are required. For future monitoring with sediments, a core would be the most appropriate method for monitoring sediment deposition of microplastics.

The purpose of future monitoring of microplastics in the environment need to be clearly defined, and the most appropriate matrices and indicator-organisms should be chosen according to the aim.

The purpose of monitoring should aim to:

- investigate the abundance of microplastic loads entering and accumulating in the environment
- investigate the fate of microplastics in the environment
- improve knowledge of the uptake of microplastics into food webs

Based on the experience gained through this study, in addition to published literature, an approach for future microplastic monitoring in the environment is proposed. This includes using bivalve molluscs and sediment samples, complemented by other environmental matrices such as sediment dwelling organisms (*e.g.,* bivalves or polychaetes) and supporting parameters. Supporting parameters are important both for the comparison between sites and to understand the factors influencing the level of contamination. A PCA would be beneficial to identify influencing parameters before deciding upon the final supporting parameters. Methods for sediment analysis need to be developed further.

When present, mussels reflect water concentrations and hence present discharges, and are appropriate for monitoring microplastic uptake into food webs by sessile filtering organisms in surface waters. Other organisms reflecting deep waters where resuspended particles may occur, should be identified. Also, sediment dwelling organisms should be identified reflecting the exposure from sediment contamination of microplastics. For long-term monitoring, the frequency of repeated sampling should be adjusted to reflect the temporal variation. Currently, there have been too few studies of the Nordic marine environment to set a frequency for monitoring surveys hence a yearly sampling regime is suggested until temporal variation is clearer.

7.1 Monitoring of bivalves

KOH treatment of bivalves is rapid, easy, does not degrade most polymers and can be used to compare with other studies since many researchers are currently utilising this method. However, from collecting samples and for further analysis, several precautions should be taken including sampling, extraction, analysis and reporting.

7.1.1 Sampling

Sampling sites: Researchers should consider anthropogenic impact and try to "classify" the stations to the best of their knowledge. On choosing sampling sites, the differences between site locations should be are fully understood, or at least, enough environmental variables should be investigated to monitor interspatial changes. The sites should be different enough so that any pronounced differences can be properly inferred.

Location of individuals: Researchers should understand the variation between populations within the same site. Locations should be chosen to represent the population within the site. Individuals should be sampled from the same position in the water column (tidal or inter-tidal) and be comparative for the whole study.

Number of individuals: Number of individuals is still unsure; 20 individuals might be sufficient. Both ICES (2013) and MSFD (Galgani *et al.* 2013) recommend 50 individuals per species.

Size and condition of individuals: A standardised size of individuals should be targeted to account for variability. It might not be enough to standardise towards weight alone. Further investigation is still required here. Should samples at the same time of year to avoid spawning, or spawning periods should be accounted for where there are spatial differences in reproduction

Contamination control: Samplers should avoid using clothes made from semi-synthetic or synthetic material.

Limit handling stress: Handle mussels with care; carefully remove byssus. Individuals should be preserved (for example in ethanol) to avoid gut clearance.

Supporting parameters: it is suggested that the following parameters are measured or registered

- Biological parameters (size and weight)
- Water depth
- Salinity
- Temperature
- Tides
- Exposure
- Currents

7.1.2 Extraction

KOH-treatment of blue mussels is rapid, easy, does not degrade most polymers and can be used to compare with other studies since many researchers are utilising this method at current time. Important steps should include:

Bivalve characteristics: Researchers should measure length (mm) and weight (g) of all individuals. Dry weight is preferable (not done in this survey). Mussels can be re-hydrated before KOH-treatment.

Contamination control steps: Researchers should carry out all work in a clean enclosed laboratory and use glassware instead of plastic. They should wear only cotton laboratory coats. Procedural blanks should be conducted on each day of sample preparation. Researchers should check for external contamination on the body of the mussel and remove if present. All solutions should be pre-filtered. Filter papers should be checked for contamination before use. When Petri dishes are opened, researchers should add additional controls (wet filter paper) and expose them to air for the same amount of time as the sample itself. If contamination is observed, results should be adjusted.

7.1.3 Particle analysis

Visual characteristics: Particles should be described in terms of size and shape**.** Researchers should take image of all suspected plastic particles and measure the length (longest dimension).

Chemical characteristics: Researchers should carry out chemical characterisation of as many particles as possible (this project found that 11 % of the suspected particles were not plastics).

Quality assurance: Researcher should include "buddy checks" as a part of the QAs/QCs.

7.1.4 Reporting units

It is important to consider all possible reporting unites. It is recommended that the number of microplastics are reported as both **particles individual^{-1}** and **particles gram^{-1}**. Researchers should also report size, type of particle, colour (somewhat subjective) and polymer type.

7.1.5 Further research questions required for blue mussel/bivalve monitoring

There are still several research questions that should be addressed to identify the most suitable monitoring programme using bivalves including size range, depth of collection, site selection, blank correction and impact of microplastics on individuals.

Size range: Ideally, individuals with different size ranges should be sampled from all sites to see if there are any differences between mussel sizes within a population, or if the results from this report is a true quantitative picture of microplastics in blue mussels from different sites.

Depth of collection: Individuals from different depths and of different sizes from the same locations along a 50 m transect should be investigated. This will allow to researchers to understand whether different flows of water in an area influence exposure. Such as organisms on the surface, in the intertidal area or those subsurface. Intertidal and on surface could be exposed to location specific

atmospheric deposition, which is presumed to be higher near the Barents see based on other chemical pollutant studies.

Site selection: More sample locations are needed are required around the coast. Sampling should be coordinated with large monitoring programs such as the MILKYS programme to avoid unnecessary cost

Blank correction: Procedural contamination should be avoided. W contamination does occur it is important to establish the correct protocol on how to correct for this. Whether it is correct to adjust by subtracting the mean of the blanks for a specific day (based on type of particle or polymer), or more appropriate to implement corrections based on LOD and LOQs still requires investigation.

Influence of environmental variables: There is a need to look into atmospheric deposition (KLIMA report 2012). Currently airborne microplastics from atmospheric deposition cannot be discounted or disregarded as a source. Furthermore, the occurrence and abundance of microplastics should be investigated with relation to ocean circulation models.

Effect of microplastic uptake: Microplastic uptake might have effects on organisms which have been observed in the laboratory, but it is important to consider that the presence of microplastics could have on individuals. For example, the presence of microplastics as an irritant might have a role in pearl formation in in wild mussels (see Box 2).

Box 2: Pearls in mussels

During the analysis, varying quantities of pearls were identified in individual mussels.

A pearl, composed of calcium carbonate, is a hard object produced within mollusc mantles. They are likely formed when an irritant microscopic object becomes trapped within the mantle folds, and could also be a result of parasite infection.

Pearls do not dissolve in KOH solution, and therefore a lot of pearls were found during mussel sample preparation. Pearls, which are visible to the human eye, are a quite well-known phenomenon. Very small pearl – just in the early formation phase –around 10 μm were also identified. Currently, there is no research on the role of environmental microplastics on pearl formation. Therefore, it is suggested that further research projects address this.

During the current analysis, the amount of pearls found in some mussel samples was problematic. Pearls were rolling around on filter papers causing movement of suspected plastic particles. Therefore, in many of the samples, pearls were removed from the original sample to avoid disturbance. It is therefore not possible at this stage to draw any conclusions from the number of pearls identified in individual mussels. Whether pearls can encapsulate microplastics is unknown. This is a significant knowledge gap that should be addressed, and currently NIVA is doing so.

It will be important to investigate whether more contaminated areas (either by microplastics or other environmental pollutants) contain more pearls. A continuation of the current survey could be carried out and researchers are recommended to count the number of pearls identified per individual. Any variation within and between sites could then assessed.

7.2 Monitoring of sediments:

7.2.1 Sampling

Sampling sites: Researchers consider anthropogenic impact and try to "classify" the stations to best of their knowledge. On choosing sampling sites, the differences between site locations should be fully understood, or at least, enough environmental variables should be investigated to monitor interspatial changes. Sites should be different enough so that any pronounced differences can be properly inferred.

Methods of collection: Sediment samples should be collected in cores and divided into 1 cm depth segments. This will allow researchers to not only monitor the top sediments, but also the deposition, and distribution within the sediment matrix. When a core is taken it is very likely that the water-surface interface remains intact. This will allow reserchers to look at the presence of microplastics in the overlaying water, which could show resuspension or settling of microplastics. If samples are collected without an interface it is recommended additional cores are collected.

Number of replicates: the number of replicates to be used per site is still unsure but a minimum of three replicates should be collected taken.

Contamination control: Samplers should avoid using clothes made from semi-synthetic or synthetic material.

Supporting parameters: it is suggested that the following parameters are measured or registered

- Water depth
- Tides
- Currents
- Grain size
- TOC
- Sediment depth
- Distance to

7.2.2 Extraction

It is recommended that core slices are processed using a combination of sieving and density separation to allow for a complete analysis of plastic presence, both in depth distribution as well as size distribution. For highly organic sediments, an organic matter removal step is advisable.

Contamination control steps: Researchers should carry out all work in a clean enclosed laboratory and use glassware instead of plastic. They should wear only cotton laboratory coats. Procedural blanks should be conducted for each day preparing samples. All solutions should be pre-filtered. If contamination is observed, results should be adjusted for this.

7.2.3 Particle analysis

Visual characteristics: Particles should be described in terms of size and shape**.** Researchers should take image of all suspected plastic particles and measure the length (longest dimension)

Chemical characteristics: Researchers should carry out chemical characterisation of as many particles as possible.

Quality assurance: Researcher should include "buddy checks" as a part of the QAs/QCs.

7.2.4 Reporting units

It is important to consider all possible reporting units. It is recommended that the number of microplastics are reported as both **particles kg^1** and **particles m^{-3}**. Researchers should also report size, type of particle, colour (somewhat subjective in some cases) and polymer type.

7.2.5 Further research questions required for sediments

There are still several research questions that should be addressed to identify the most suitable monitoring programme for marine sediments including site selection, sediment characteristics, influence of environmental variables and blank correction.

Site selection: More sample locations are required around the coast. Sampling should be coordinated with large monitoring programs such as the MILKYS programme to avoid unnecessary cost

Sediment characteristics: Different grain size and organic matter content could influence the efficiency of extraction techniques. It is important to carry out more rigorous testing for processing and extraction of microplastics from a variety of different sediment matrixes.

Influence of environmental variables: There is a need to investigate deposition of plastics to sediments and their subsequent redistribution within sediment matrix either because of environmental mixing and/or bioturbation by sediment dwelling organisms.

Blank correction: Procedural contamination should be avoided. When it does occur, it is important to establish the correct protocol on how to correct for blanks. Whether it is correct to adjust by subtracting the mean of the blanks for a specific day (based on type of particle or polymer), or more appropriate to implement corrections based on LOD and LOQs requires further investigation.

8 Conclusions

This report examines the use of mussels and sediments at monitoring tools for the presence of microplastics in the marine environment. In addition, the report includes an assessment of methods used and proposes future monitoring and research requirements. As such, the report provides a basis for assessing microplastics in the marine environment with respect to marine biota and sediment matrixes. The main conclusions were:

- A digestion protocol using 10 % KOH is efficient at extracting microplastics from blue mussels, and from this semi-quantitative data as well as qualitative data were obtained. However, some improvements and further investigations are required for the method to be fully quantitative.

- Density separation alone is not appropriate to extract microplastics from complex sediment matrixes.

- Sieving is not an appropriate method on its own to extract microplastics from complex sediment matrixes.

- Procedural contamination is expected and therefore appropriate steps must be taken to account for this.

- There is a need to establish how to standardise the correction of background contamination.

- A combination of visual and chemical analysis should be conducted to accurately identify plastic particles, including QA/QC such as buddy controls.

- Blue mussels appear to be a promising tool for monitoring small waterborne microplastics at coastal locations in the marine environment.

- Other biota should be considered if sampling in locations void of blue mussels, such as sedimentary areas and offshore locations.

- Further method development is required to identify the most suitable procedure for sediment analysis. Currently, the use of core samples appears to be most promising.

9 References

Andrady, A.L., 2015. Persistence of plastic litter in the oceans. In: *Anthropogenic Litter*. Springer, Cham, Switzerland, 57-72.

Beer, S., Garm, A., Huwer, B., Dierking, J. and Nielsen, T.G., 2017. No increase in marine microplastic concentration over the last three decades–A case study from the Baltic Sea. *Science of The Total Environment*. [Available online 19 October 2017, doi: 10.1016/j.scitotenv.2017.10.101].

Bergmann, M. and Klages, M., 2012. Increase of litter at the Arctic deep-sea observatory HAUSGARTEN. *Marine Pollution Bulletin*, 64(12), 2734-2741.

Bergmann, M., Peeken, I., Beyer, B., Krumpen, T., Primpke, S., Tekman, M.B. and Gerdts, G., 2016. Vast Quantities of Microplastics in Arctic Sea Ice—A Prime Temporary Sink for Plastic Litter and a Medium of Transport. In: *MICRO 2016: Fate and Impact of Microplastics in Marine Ecosystems: From the Coastline to the Open Sea*, Elsevier, 75.

Bergmann, M., Wirzberger, V., Krumpen, T., Lorenz, C., Primpke, S., Tekman, M. B., and Gerdts, G. (2017). High Quantities of Microplastic in Arctic Deep-Sea Sediments from the HAUSGARTEN Observatory. *Environmental Science and Technology*, 51(19), 11000-11010.

Beyer, J., Green, N.W., Brooks, S., Allan, I.J., Ruus, A., Gomes, T., Bråte, I.L.N. and Schøyen, M., 2017. Blue mussels (*Mytilus edulis* spp.) as sentinel organisms in coastal pollution monitoring: A review. *Marine Environmental Research,* 130, 338-365.

Bråte, I.L.N., Huwer, B., Thomas, K.V., Eidsvoll, D.P., Halsband, C., Almroth, B.C. and Lusher, A., 2017. Micro-and macro-plastics in marine species from Nordic waters. Nordic Council of Ministers, Copenhagen, 101p.

Brooks, S.J. and Farmen, E. 2013. The distribution of the mussel *Mytilus* species along the Norwegian coast. *Journal of Shellfish Research*, 32, 1-6.

Browne, M.A., 2015. Sources and pathways of microplastics to habitats. In: *Anthropogenic Litter*. Springer, Cham, Switzerland, 229-244.

Buhl-Mortensen, L. and Buhl-Mortensen, P., 2017. Marine litter in the Nordic Seas: distribution composition and abundance. Marine Pollution Bulletin. [Available online 31 August 2017, doi: 10.1016/j.marpolbul.2017.08.048].

Canesi, L. and Fabbri, E. (2015). Environmental effects of BPA: focus on aquatic species. *Dose Response*, 13(3), 1559325815598304.

Claessens, M., Van Cauwenberghe, L., Vandegehuchte, M.B. and Janssen, C.R., 2013. New techniques for the detection of microplastics in sediments and field collected organisms. *Marine Pollution Bulletin*, 70(1), 227-233.

Cole, M., Lindeque, P., Halsband, C. and Galloway, T.S. 2011. Microplastics as contaminants in the marine environment: a review. *Marine Pollution Bulletin*, 62(12), 2588-2597.

Comnea-Stancu, I.R., Wieland, K., Ramer, G., Schwaighofer, A. and Lendl, B., 2017. On the identification of rayon/viscose as a major fraction of microplastics in the marine environment: discrimination between natural and manmade cellulosic fibers using fourier transform infrared spectroscopy. *Applied Spectroscopy*, *71*(5), 939-950.

Cózar, A., Martí, E., Duarte, C.M., García-de-Lomas, J., Van Sebille, E., Ballatore, T.J., Eguíluz, V.M., González-Gordillo, J.I., Pedrotti, M.L., Echevarría, F. and Troublè, R., 2017. The Arctic Ocean as a dead end for floating plastics in the North Atlantic branch of the Thermohaline Circulation. *Science Advances*, 3(4), e1600582.

De Witte, B., Devriese, L., Bekaert, K., Hoffman, S., Vandermeersch, G., Cooreman, K. and Robbens, J., 2014. Quality assessment of the blue mussel (*Mytilus edulis*): Comparison between commercial and wild types. *Marine Pollution Bulletin*, 85(1), 146-155.

Dehaut, A., Cassone, A.L., Frere, L., Hermabessiere, L., Himber, C., Rinnert, E., Riviere, G., Lambert, C., Soudant, P., Huvet, A. and Duflos, G., 2016. Microplastics in seafood: benchmark protocol for their extraction and characterization. *Environmental Pollution,* 215, 223-233.

EFSA (EFSA Panel on Contaminants in the Food Chain), 2016. Statement on the presence of microplastics and nanoplastics in food, with particular focus on seafood. *EFSA Journal*, 14(6), 4501, 30 pp. doi: 10.2903/j.efsa.2016.4501

Ellis, B. and Smith, R., 2009. *Polymers: A Property Database*, 2nd ed., CRC Press, Boca Raton, Florida.

Foekema, E.M., De Gruijter, C., Mergia, M.T., van Franeker, J.A., Murk, A.J. and Koelmans, A.A., 2013. Plastic in North Sea fish. *Environmental Science and Technology*, 47(15), 8818-8824.

Galgani, F., Hanke, G., Werner, S., Oosterbaan, L., Nilsson, P., Fleet, D., et al. 2014. *Guidance on Monitoring of Marine Litter in European Seas*. JRC, 1–128. Available online at: http://publications.jrc.ec.europa.eu/repository/bitstream/JRC83985/lb-na-26113-en-n.pdf.

GESAMP 2016. Sources, fate and effects of microplastics in the marine environment: part two of a global assessment. (Kershaw, P.J., and Rochman, C.M., eds). (IMO/FAO/UNESCO-IOC/UNIDO/WMO/IAEA/UN/UNEP/UNDP Joint Group of Experts on the Scientific Aspects of Marine Environmental Protection). Rep. Stud. GESAMP No. 93, 220 pp.

Gewert, B., Ogonowski, M., Barth, A. and MacLeod, M., 2017. Abundance and composition of near surface microplastics and plastic debris in the Stockholm Archipelago, Baltic Sea. *Marine Pollution Bulletin*, 120, 292-302.

Green, N.W., Skogen, M., Aas, W.,. Iosjpe, M., Måge, A., Breivik, K., Yakushev, E., Høgåsen, T., Eckhardt, S., Ledang, A.B., Jaccard, P.F., Staalstrøm, A., Isachsen, P.E. and Frantzen, S., (2012). Tilførselsprogrammet 2012. Overvåking av tilførsler og miljøtilstand i Barentshavet og Lofotenområdet. Norsk institutt for vannforskning, NIVA, Report.

Gustafsson, L. (2016). En undersökning om förekomsten av mikroplast i västsvenska blåmusslor (*Mytilus edulis*). Master Thesis. Available online at: https://issuu.com/cillidill/docs/en_unders__kning_om_f__rekomsten_av.

Hashim, F., Ismail, H., and Rusli, A. 2017. Comparison of properties of ethylene vinyl acetate/natural rubber/mengkuang leaf fibre (EVA/NR/MLF) and ethylene vinyl acetate/epoxidized natural rubber/mengkuang leaf fibre (EVA/ENR-50/MLF) thermoplastic elastomer composites. *Polymer Testing*, 61, 1-7.

Hidalgo-Ruz, V., Gutow, L., Thompson, R.C. and Thiel, M. 2012. Microplastics in the marine environment: a review of the methods used for identification and quantification. *Environmental Science and Technology*, 46(6), 3060-3075.

ICES, 2015. OSPAR Request on development of a common monitoring protocol for plastic particles in fish stomachs and selected shellfish on the basis of existing fish disease surveys. *ICES Special Request Advice,* 1, 1-6.

Imhof, H.K., Schmid, J., Niessner, R., Ivleva, N.P. and Laforsch, C., 2012. A novel, highly efficient method for the separation and quantification of plastic particles in sediments of aquatic environments. *Limnology and Oceanography: Methods*, 10(7), 524-537.

Jambeck, J.R., Geyer, R., Wilcox, C., Siegler, T.R., Perryman, M., Andrady, A., Narayan, R. and Law, K.L. 2015. Plastic waste inputs from land into the ocean. *Science*, 347(6223), 768-771.

Jenkins, A., Kratochvíl, P., Stepto, R., et al. 2009. Glossary of basic terms in polymer science (IUPAC Recommendations 1996). *Pure and Applied Chemistry,* 68(12), 2287-2311.

Karlsson, T.M., Vethaak, A.D., Almroth, B.C., Ariese, F., van Velzen, M., Hassellöv, M. and Leslie, H.A., 2017. Screening for microplastics in sediment, water, marine invertebrates and fish: method development and microplastic accumulation. *Marine Pollution Bulletin,* 122(1-2), 403-408.

Kühn, S., Van Werven, B., Van Oyen, A., Meijboom, A., Rebolledo, E.L.B. and Van Franeker, J.A., 2017. The use of potassium hydroxide (KOH) solution as a suitable approach to isolate plastics ingested by marine organisms. *Marine Pollution Bulletin*, 115(1), 86-90.

Lambert, S. and Wagner, M. 2016. Characterisation of nanoplastics during the degradation of polystyrene. *Chemosphere*, 145, 265–268.

Lassen, C., Hansen, S. F., Magnusson, K., Hartmann, N. B., Rehne Jensen, P., Nielsen, T. G., and Brinch, A. (2015). Microplastics: Occurrence, effects and sources of releases to the environment in Denmark. Copenhagen. Danish Environmental Protection Agency.

Lenz, R., Enders, K., Stedmon, C.A., Mackenzie, D.M. and Nielsen, T.G., 2015. A critical assessment of visual identification of marine microplastic using Raman spectroscopy for analysis improvement. *Marine Pollution Bulletin,* 100(1), 82-91.

Lenz, R., Enders, K. and Nielsen, T.G., 2016. Microplastic exposure studies should be environmentally realistic. *Proceedings of the National Academy of Sciences*, 11(29), E4121–E4122.

Löder, M.G. and Gerdts, G., 2015. Methodology used for the detection and identification of microplastics—A critical appraisal. In Marine anthropogenic litter (pp. 201-227). Springer International Publishing.

Lusher, A. 2015. Microplastics in the marine environment: distribution, interactions and effects. In: *Anthropogenic Litter*. Springer, Cham, Switzerland, 245-307.

Lusher, A.L., Burke, A., O'Connor, I. and Officer, R., 2014. Microplastic pollution in the Northeast Atlantic Ocean: validated and opportunistic sampling. *Marine Pollution Bulletin*, 88(1), 325-333.

Lusher, A.L., Tirelli, V., O'Connor, I., and Officer, R., 2015. Microplastics in Arctic polar waters: the first reported values of particles in surface and sub-surface samples. *Scientific Reports,* 5, 14947. doi:10.1038/srep14947

Lusher, A.L., Hollman, P.C.H. and Mendoza-Hill, J.J., 2017a. Microplastics in fisheries and aquaculture: status of knowledge on their occurrence and implications for aquatic organisms and food safety. FAO Fisheries and Aquaculture Technical Paper. No. 615. Rome, FAO. 2017.

Lusher, A., Welden, N., Sobral, P. and Cole, M. 2017b. Sampling, isolating and identifying microplastics ingested by fish and invertebrates. *Analytical Methods,* 9, 1346-1360.

Maes, T., Jessop, R., Wellner, N., Haupt, K. and Mayes, A.G., 2017. A rapid-screening approach to detect and quantify microplastics based on fluorescent tagging with Nile Red. *Scientific Reports,* 7, 44501. doi: 10.1038/srep44501.

Magnusson, K., Eliasson, K., Fråne, A., Haikonen, K., Hultén, J., Olshammar, M., Stadmark, J., Voisin, A. and Miljöinstitutet, S., 2016. Swedish sources and pathways for microplastics to themarine environment. Swedish Environmental Protection Agency, Report number: C 183.

MARLIN 2013. Litter monitoring and raising awareness. Final report of Baltic marine litter project MARLIN (http://www.cbss.org/wp-content/uploads/2012/08/marlin-baltic-marine-litterreport.pdf)

Martin, J., Lusher, A., Thompson, R.C. and Morley, A., 2017. The deposition and accumulation of microplastics in marine sediments and bottom water from the Irish continental shelf. *Scientific Reports*, 7, 10772. doi: 10.1038/srep10772.

Masura, J., Baker, J.E., Foster, G.D., Arthur, C. and Herring, C., 2015. *Laboratory methods for the analysis of microplastics in the marine environment: recommendations for quantifying synthetic particles in waters and sediments.*

O'Connell, D. W., Birkinshaw, C., and O'Dwyer, T. F., 2008. Heavy metal adsorbents prepared from the modification of cellulose: A review. *Bioresource Technology*, 99(15), 6709-6724.

Nizzetto, L., Futter, M. and Langaas, S., 201. Are agricultural soils dumps for microplastics of urban origin? *Environmental Science and Technology,* 50(20), 10777–10779.

Nuelle; M.T., Dekiff, J.H., Remy, D. and Fries, E. 2014. A new analytical approach for monitoring microplastics in marine sediments. *Environmental Pollution,* 184, 161-169.

Obbard, R.W., Sadri, S., Wong, Y.Q., Khitun, A.A., Baker, I. and Thompson, R.C., 2014. Global warming releases microplastic legacy frozen in Arctic Sea ice. *Earth's Future*, 2(6), 315-320.

OSPAR, 2012. JAMP [Joint Assessment and Monitoring Programme] Guidelines for Monitoring Contaminants in Biota. OSPAR Commission, ref. no. 99-02e. 122 pp.

PlasticsEurope. 2016. Plastics – the Facts 2016. (available at www.plasticseurope.org). Accessed 1 November 2016.

Shen, L., Worrell, E. and Patel, M.K., 2010. Environmental impact assessment of man-made cellulose fibres. *Resources, Conservation and Recycling,* 55(2), 260-274.

Shim, W.J., Hong, S.H. and Eo, S.E., 2017. Identification methods in microplastic analysis: a review. *Analytical Methods,* 9(9), 1384-1391.

Steinmann, W. and Saelhoff, A.K., 2016. Essential Properties of Fibres for Composite Applications. *In: Fibrous and Textile Materials for Composite Applications* (pp. 39-73). Springer Singapore.

Strand, J., Tairova, Z., Danielsen, J., Hansen, J.W., Magnusson, K., Naustvoll, L. and Kirk Sørensen, T., 2015. *Marine Litter in Nordic waters*, Temanord 2015:521. Nordic Council of Ministers. https://doi.org/10.6027/TN2015-521

Sundet, J.H., Herzke, D. and Jenssen, M., 2015. Forekomst og kilder av mikroplastikk i sediment, og konsekvenser for bunnlevende fisk og evertebrater på Svalbard. Sluttrapport Sysselmannen. RIS-prosjekt nr. 10495.

Sundt, P., Schulze, P.-E. and Frode, S., 2014. Sources of microplastics-pollution to the marine environment. Norwegian Environment Agency Report no: M-321.

Talvitie, J., Heinonen, M., Pääkkönen, J.-P., Vahtera, E., Mikola, A., Setälä, O. and Vahala, R., 2015. Do wastewater treatment plants act as a potential point source of microplastics? Preliminary study in the coastal Gulf of Finland, Baltic Sea. *Water Science and Technology,* 72(9), 1495–504.

Tekman, M. B., Krumpen, T., and Bergmann, M., 2017. Marine litter on deep Arctic seafloor continues to increase and spreads to the North at the HAUSGARTEN observatory. *Deep Sea Research Part I: Oceanographic Research Paper*s, 120, 88-99.

UNEP, 2016. Marine plastic debris and microplastics – Global lessons and research to inspire action and guide policy change. United Nations Environment Programme, Nairobi.

van Sebille, E., England, M.H. and Froyland, G., 2012. Origin, dynamics and evolution of ocean garbage patches from observed surface drifters. *Environmental Research Letters,* 7(4), 44040.

Vollertsen, J. and Aviaja, A., 2017. Microplastic in Danish wastewater Sources, occurrences and fate. Danish Environmental Protection Agency. Report No. 1906

Wang, L., Hong, Y. and Li, J.X., 2012. Durability of running shoes with ethylene vinyl acetate or polyurethane midsoles. Journal of Sports Science, 30, 1787–92.

Wesch, C., Bredimus, K., Paulus, M. and Klein, R., 2016. Towards the suitable monitoring of ingestion of microplastics by marine biota: A review. *Environmental Pollution,* 218, 1200-1208.

Whelan, T., 1994. *Polymers Technology Dictionary*, Chapman and Hall, London, 493pp.

Zobkov, M. and Esiukova, E., 2017. Microplastics in Baltic bottom sediments: Quantification procedures and first results. *Marine Pollution Bulletin*, 114(2), 724-732.

Appendix

Breakdown of results from sediment analysis (A)- Density, (B) Sieving

Appendix 1A
Breakdown of results from sediment analysis using sieving

Table I. Results of sieving sediment from the top 0 – 2 cm from Site 2. Replicate 2.

Sieve size	Anthropogenic material	Sediment
5 mm	Nothing retained	Nothing retained
1 mm	Nothing retained	Nothing retained
500 µm	Nothing retained	Nothing retained
250 µm	Fibres, small fragments	Nothing retained
100 µm	Fibrous aggregations	Nothing retained
50 µm	Small fragments and fibres	Fine particulates

Table II. Results of sieving sediment from the top 0 – 2 cm from Site 2. Replicate 4.

Sieve size	Anthropogenic material	Sediment
5 mm	Nothing retained	Nothing retained
1 mm	Fibers	Some clay particulates
500 µm	Nothing retained	Nothing retained
250 µm	Fragments	Nothing retained
100 µm	Fibres and fragments	Some fine particulates
50 µm	Fibrous aggregations, possible fragments	Fine particulates

Table III. Results of sieving sediment from the top 0 – 2 cm from Site 3. Replicate 2.

Sieve size	Anthropogenic material	Sediment	Comment/Suggestion
5 mm	Nothing retained	Nothing retained	
1 mm	Nothing retained	Clay aggregations	Further processing needed
			Drying sediment before sampling compromised the sample.
500 µm	Fibres, fragments, beads	Nothing retained	
250 µm	Fibres, fragments, beads	Nothing retained	
100 µm	Fibres	Clay particulates	
50 µm	Nothing retained	Clay particulates	

Table IV, Results of sieving sediment from the top 0 – 2 cm from Site 3. Replicate 4.

Sieve size	Anthropogenic material	Sediment
5 mm	Nothing retained	Nothing retained
1 mm	Fibres and fragments	Nothing retained

Sieve size	Anthropogenic material	Sediment
500 μm	Nothing retained	Nothing retained
250 μm	Nothing retained	Nothing retained
100 μm	Nothing retained	Nothing retained
50 μm	Fibres and fragments	Fine particulates

Table V. Results of sieving sediment from the top 0 – 2 cm from Site 4. Replicate 2.

Sieve size	Anthropogenic material	Sediment	Comment/Suggestion
5 mm	Nothing retained	Particles retained	
1 mm	Nothing retained	Particles retained	Not possible to separate potential plastics due to large amount of sediment particles
500 μm	Nothing retained	Particles retained	Not possible to separate potential plastics due to large amount of sediment particles
250 μm	Fibres and fragments	Nothing retained	
100 μm	Nothing retained	Nothing retained	
50 μm	Small fibres, possible fragments	Particles retained	

Table VI. Results of sieving sediment from the top 0 – 2 cm from Site 4. Replicate 4.

Sieve size	Anthropogenic material	Sediment	Comment/Suggestion
5 mm	Nothing retained	Nothing retained	
1 mm	Nothing retained	Nothing retained	Not possible to separate potential plastics due to large amount of sediment particles
500 μm	Nothing retained	Nothing retained	Not possible to separate potential plastics due to large amount of sediment particles
250 μm	Nothing retained	Nothing retained	
100 μm	Fibres and fragments	Sediment retained	
50 μm	Nothing retained	Sediment retained	

Appendix 1B
Breakdown of results from sediment analysis using density

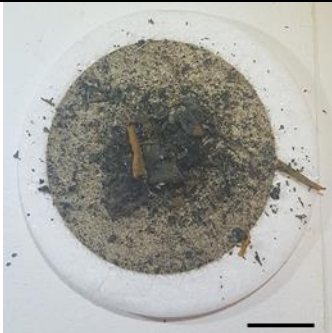**Table VII.** Density separation results from S1. R1

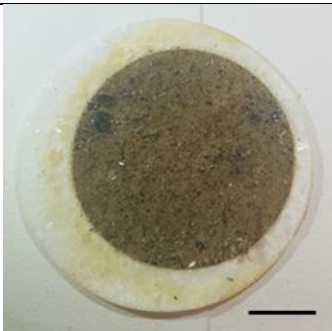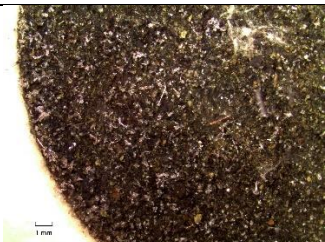| Depth section	Image of first extraction	Number of filter papers after soaking	Number of particles extracted	Comment
0-1 1-2		3	Fibres, fragments and beads	Lots of floating organic matter

Table VIII. Density separation results from S1. R3

Depth section	Image of first extraction	Number of filter papers after soaking	Number of particles extracted	Comment
0-1 1-2		5	Many fibres and fragments	Worms were also extracted from the sediment matrix. Lots of floating fine particulate organic matter

Table IX. Density separation results from S2. R1

Depth section	Image of first extraction	Number of filter papers after soaking	Number of particles extracted	Comment
0-1		11	Many fibres and fragments	

1-2		11		Many fibres and fragments

Table X. Density separation results from S2. R3
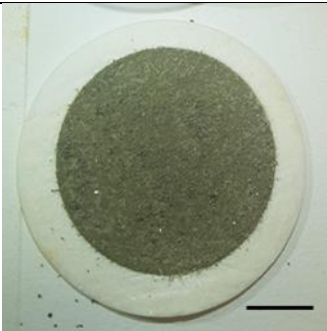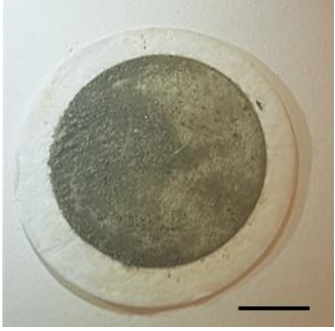
Depth section	Image of first extraction	Number of filter papers after soaking	Number of particles extracted	Comment
0-1		2	Fibres: 6 Fragments: 1 Beads: 3	
1-2		6	Fibres: 31 Fragments: 5	Still has lots of fine particulates of clay and organic matter. Fine coating on filter papers. Too difficult to differentiate.

Table XI. Density separation results from S3. R1

Depth section	Image of first extraction	Number of filter papers after soaking	Number of particles extracted	Comment
0-1		Did not soak	Fibres: 5	Crystalline salt. Could not differentiate. Too much organic matter and clay particles
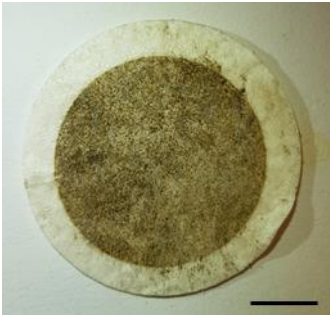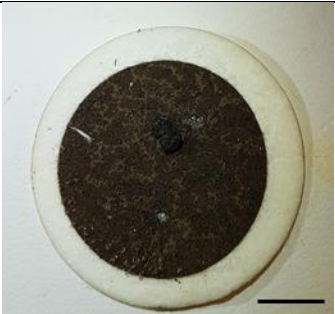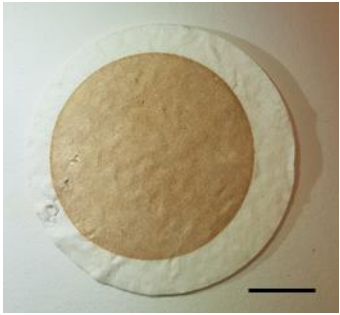
1-2		4	Fibres: 8 Fragments: 2 Beads: 1	Lots of clay particulates

Table XII. Density separation results from S3. R3

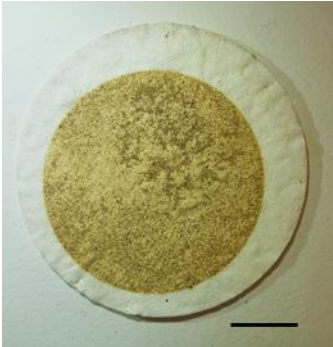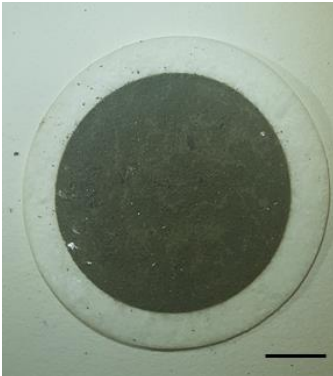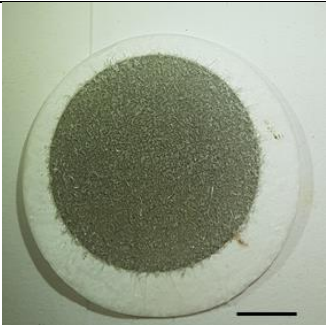| Depth section	Image of first extraction	Number of filter papers after soaking	Number of particles extracted	Comment
0-1		5	Fibres: 17 Fragments: 11 Beads: 14	There was a mixture of fibres, fragments and bead, as well as foraminifera. This made distinction between beads and foraminifera visually challenging, it is therefore recommended these samples be subjected to further analysis.
1-2		1	Fibres: 3 Fragments: 1 Beads: 3	Not rinsed. Clear but still lots of clay
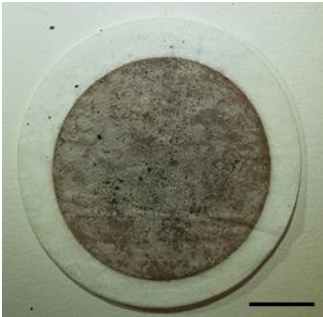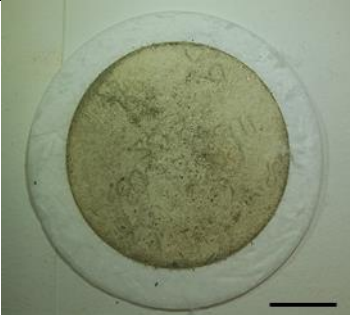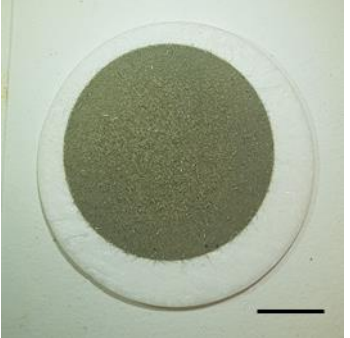
2-3		1	Fibres: 2 Fragments: 1 Beads: 0
3-4		6	Fibres: 26 Fragments: 5 Beads: 18
4-5		5	Fibres: 18 Fragments: 7 Beads: 4

Table XIII. Density separation results from S4 R1

Depth section	Image of first extraction	Number of filter papers after soaking	Number of particles extracted	Comment
0-1		Did not soak		Too much particulate organic material. Fine layer of crystalline salt.

| 1-2 | | Did not soak | Some fibres | Too much particulate organic material. Fine layer of crystalline salt. |

Table XIV. Density separation results from S4 R3

+	Image of first extraction	Number of filter papers after soaking	Number of particles extracted	Comment
0-1		Did not soak	Fibres: 6 Fragments:2 Beads: 3	
1-2		Did not soak	Some fibres	Too much particulate organic material

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