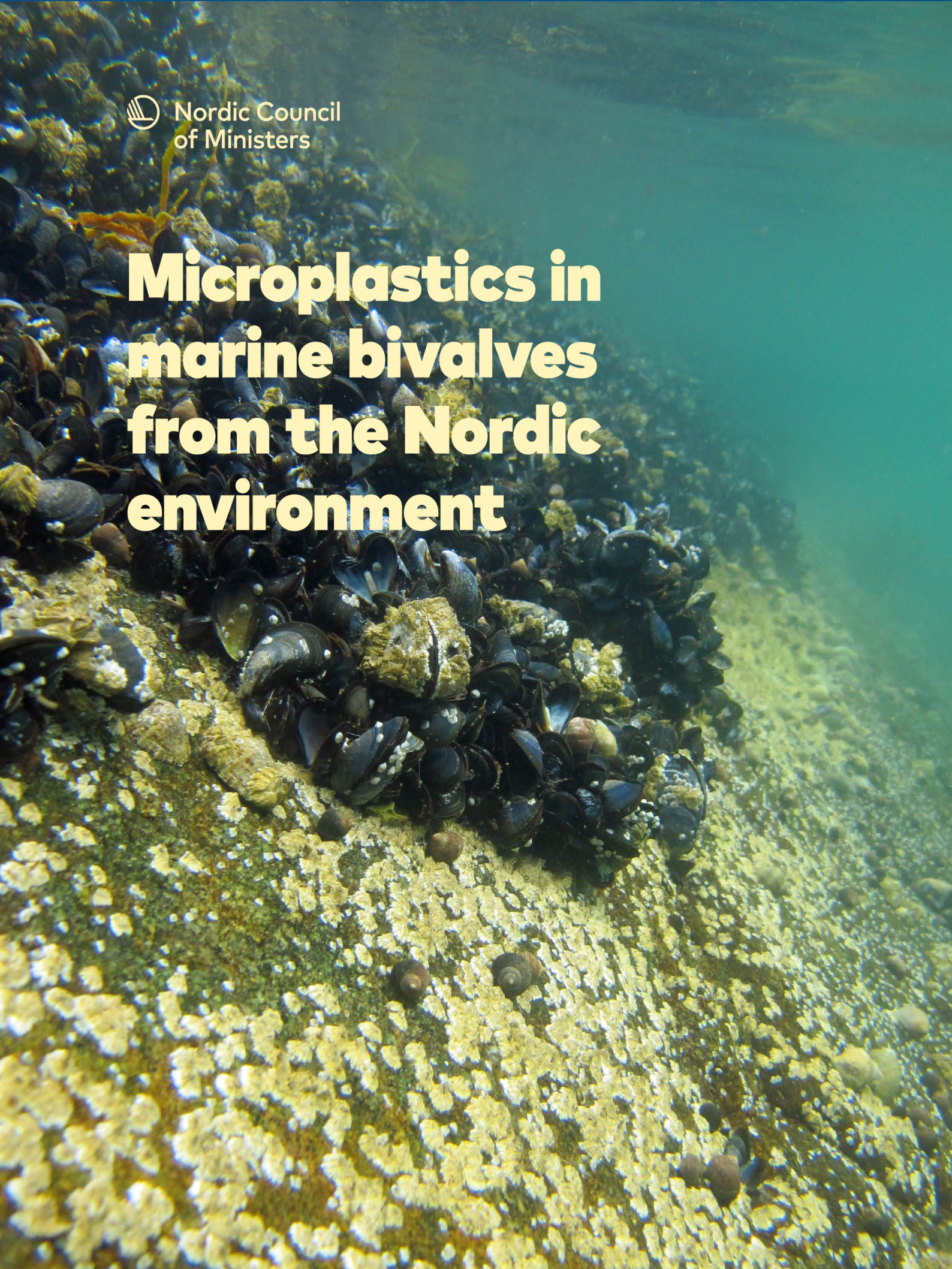
Nordic Council
of Ministers
Microplastics in marine bivalves from the Nordic environment

Contents

Preface

This report represents the results of the project "Microplastics in marine bivalves from the Nordic environment". The project was managed by the Norwegian Institiute for Water Research (NIVA) by contract from the Norwegian Environment Agency (Miljødirektoratet) and funded by the Nordic Council of Ministers (Nordisk ministerråd). Coordinator at the Norwegian Environment Agency is Runar Mathisen and the project manager at NIVA is Norman W. Green.

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Norman W. Green

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Summary

The starting point of this study was to investigate microplastics in biota across the entire Nordic marine environment. Microplastics are found in all compartments of the marine environment, and there is a call from both the scientific community and decision makers to monitor the abundance and composition of these microscopic plastic particles to understand any potential impacts upon the marine ecosystem. Previous studies of microplastics in Nordic biota have mainly been conducted in the North Sea and the Baltic Sea, and very few studies were from Skagerrak and Kattegat, as well as from the northern areas and the western areas near the Faroe Islands, Iceland and Greenland. In a pre-study conducted in 2016–2017 bivalves were suggested as suitable bioindicators for monitoring of small microplastic fraction (< 1mm). Bivalves tend to be sessile, they filter large volumes of seawater, they are relatively abundant and they are already used to monitor contaminants. Furthermore, the seafloor is considered an accumulation site for microplastics and many species of bivalves live on or near the seafloor.

In this large-scale survey of microplastics in marine bivalves from a total of 100 Nordic coastal sites were studied covering much of the Nordic marine environment ranging from Svalbard in the north, Greenland in the west, Baltic Sea in the east and the North Sea in the south. Microplastic abundance and composition were studied in five selected bivalve species that have some connection to the seafloor; the hard-bottom species of the blue mussel and closely related species (Mytilus spp) and the arctic Hiatella (Hiatella arctica), the soft bottom species of the Baltic clam (Limecola balthica), Abra nitida and Thyasira spp. Three different methods were applied; visual identification following point mode transmission Fourier transform infrared spectroscopy (μFT-IR), image scanning using automated attenuated total reflectance FT-IR (μATR-FT-IR) and pyrolysis gas chromatography mass spectrometry (Py-GCMS) of selected particles.

This study found that four out of five bivalve species contained microplastics. Hiatella arctica was not found to contain microplastics, however this is based on a very limited number of sites (n=3) covering a relatively small area. Most microplastics were detected in Mytilus spp. from highly urbanised areas, but also at some sites located close to harbours at stations on the west side of Iceland and in Bodø and Tromsø harbours in the northern part of Norway. For Mytilus spp. the two most affected sampling sites were from the Oslofjord (North Sea); Akershuskaia and Færder relative to other sites. This suggests that the Oslofjord is highly impacted by microplastic input. For the remaining bivalves, the trend was not as clear cut as it was for Mytilus spp., but it did point towards specific areas that showed higher levels of microplastics. This includes the North Sea along the west coast of Denmark and the southern part of Norway, as well as Skagerrak and Kattegat, in addition to coast off Stockholm in the Baltic Sea.

Microplastics were not found above the limit of detection (LOD) in bivalves from Svalbard, Faroe Islands or Greenland at the sites investigated. Despite not finding microplastics in bivalves from these locations, it cannot be ruled out that there are

specific point sources in these areas that are emitting microplastics. For this to be further studied, one could target possible point sources such as wastewater treatment plants that are known to release microplastics.

A combination of methods was used to point towards potential sources or the main contributors to the marine microplastics load. Visual examination provided the morphology of particles down to around 70 µm (shape, size and appearance such as colour, lack of cellular structures and so on), FT-IR gave polymeric composition, whilst pyrolysis gave information on the mass of different compounds and thereby their identity. All these methods combined can be used for inferring potential sources, at least to some extent. Microplastics derived from road-associated activity have been suggested as one of the largest sources of microplastics into the Nordic marine environment. However, not much empirical data has been available to support these estimations until now. In this study, 16 sites were dominated by rubbery fragments that are hypothesised as originating from road run-off or harbour activity, or a combination. This was based on the visual assessment (black colour, rubbery behaviour, appropriate size and sausage shape), the finding of markers for rubber using pyrolysis (indications of butadiene and isoprene), carbon black interference for the FT-IR spectrum analysed, and the similarity in appearance from the different far reaching sites suggesting a common source or pathway.

Most microplastics detected in bivalves from this study were fragments, representing 87% of the overall count, whilst fibres accounted for the remaining 13%. This contradicts previous Mytilus spp. data from the Norwegian environment. This could represent a qualitative difference in the type of microplastics released into the sea, or methodological reasons such as; higher FT-IR coverage of particles, the strict and continuously improved procedures for contamination prevention; the exclusion of sites with microplastic levels below LOD or a generally lower fibre recovery rate in this study compared to fragments.

Most microplastics found in bivalves were below 130 µm in their longest dimension, with an average of 158 µm when including only microplastics below 1000 µm. There were significant differences between particle sizes at different sites. The reasons for the significant differences in microplastic size across sites are not yet understood. This could be related to large proportions of small black particles at certain sites. All the five sites with the highest levels of black rubbery fragments (M-19, M-14, M-16, M-22 and M-15) were the sites with the smallest sized particles on average. The two sites with the highest proportion of fibres were M-10 and M-11, which were two out of the five the sites with significantly larger microplastics. Fibres tend to be longer than fragments when measured in their longest dimension. The microplastics detected in Abra nitida and Limecola balthica were smaller than the microplastics detected in Mytilus spp., which probably reflects the fact that they are smaller sized organisms.

Besides the dominant black rubbery fragments, it was also evident that marine bivalves from the Nordic environment were exposed to a wide variety of polymeric materials. Overall, 11 other polymers were detected in bivalves from the Nordic marine environment:

Based on the visual ID and point µFT-IR (*Mytilus spp., Limecola balthica and Abra nitida*)

- Polyethylene (PE)
- Polypropylene (PP)
- Semi-synthetic biobased plastics (modified cellulose)
- Epoxy plastics (e.g. paint fragments)
- Polyvinyl chloride (PVC)

Based on scanning μFT-IR (*Abra nitida and Thyasira* spp.)

- Polyacrylate
- Polyethylene (PE)
- Polydimethylsiloxane (silicone)
- Calcium stearate (a plastic additive)
- Semi-synthetic biobased plastics

Based Py-GCMS (*Mytilus* spp.)

- Polyhydroxybutyrate (PHB)
- Polylactic acid (PLA)
- Polycaprolactone (PCL)
- Polyethylene naphthalate (PEN)

Based on this extensive study as well as previous national and intersessional work, three species of bivalves living on or in the sediment could be used to monitor microplastics (> 63–1000 μm) in the Nordic environment: the hard-bottom species the common blue mussel and closely related species (Mytilus spp.) for most of the Nordic coast, the soft bottom Baltic clam (Limecola balthica) for the Baltic Sea and Abra nitida for the Norwegian coast and some parts of the North Sea. It seems that Thyasira spp. did not contain microplastics larger than 63 μm. Both Thyasira spp. and Abra nitida contained microplastics smaller than 63 μm. These species could be used for monitor microplastics smaller than 63 μm, but further method development and sampling are required.

Sammendrag

Utgangspunktet for denne studien var å undersøke forekomst av mikroplast i biota i marint miljø på tvers av Norden. Mikroplast har gjennom ulike studier blitt påvist i alle i deler av det marine miljøet. Det er et ønske fra både forskere og myndigheter å kunne overvåke mengde og type av de mikroskopiske plastpartiklene i havmiljøet for å forstå hvilken påvirkning de kan ha på det marine økosystemet. Tidligere studier av mikroplast i nordisk biota har i hovedsak foregått i Nordsjøen og i Østersjøen mens veldig få studier er utført i Skagerrak og Kattegat eller i de nordligste og vestlige områdene rundt Færøyene, Island og Grønland. I en tidligere studie fra 2016–2017 ble muslinger foreslått som egnede bioindikatorer for overvåkning av små partikler av mikroplast (< 1 mm). Dette er blant annet begrunnet med at muslinger er stedbundne, de filtrerer store mengder sjøvann, de er ganske tallrike og vidt utbredt, og de er allerede etablert som overvåkningsorganisme for miljøgifter. Dessuten lever mange av muslingene på eller i nærheten av havbunnen, som er ansett som et akkumuleringssted for mikroplast.

I denne omfattende kartleggingen er det undersøkt for mikroplast i marine muslinger fra totalt 100 ulike steder over praktisk talt hele Norden, fra Svalbard i nord til Nordsjøen i sør, og fra Grønland i vest til Østersjøen i øst. Forekomst og sammensetning av mikroplast ble undersøkt i fem forskjellige muslingarter med tilknytning til havbunnen: hardbunnsartene blåskjell og nær beslektede arter (Mytilus spp.) og den arktiske Hiatella arctica, og bløtbunnsartene Østersjømusling (Limecola balthica), Abra nitida og Thyasira spp. Tre forskjellige metoder ble benyttet for identifisering av mikroplast i muslingene; visuell identifikasjon ved hjelp av mikroskop, partikkelspesifikk analyse med Fourier transform infrarød spektroskopi (μFT-IR) og automatisert bildeskanning ved bruk av μATR-FT-IR, og pyrolysegasskromatografi massespektrometri (Py-GCMS) av utvalgte partikler.

Av de fem undersøkte artene inneholdt fire arter mikroplast over deteksjonsgrensen (LOD). Hiatella arctica inneholdt ikke mikroplast, men dette var basert på et lite antall studiesteder (n = 3) innenfor et begrenset område. Mest mikroplast ble påvist i Mytilus spp. fra urbaniserte områder, i tillegg til noen steder i nærheten av mindre urbane havneområder på vestkysten av Island og ved Bodø havn og Tromsø havn i nord-Norge. I blåskjell ble det funnet flest mikroplastpartikler i Oslofjorden (Nordsjøen), og nærmere bestemt ved Akershuskaia og ved Færder sammenlignet med de andre stedene. Dette antyder at Oslofjorden i stor grad er påvirket av tilførsler av mikroplastpartikler. For de andre muslingartene var ikke tendensen like tydelig som for blåskjell, men resultatene indikerte høyere nivåer av mikroplast. Dette inkluderte Nordsjøen representert ved vestkysten av Danmark og Sør-Norge, i tillegg til Skagerrak og Kattegat samt området utenfor Stockholm i Østersjøen.

Mikroplast ble ikke funnet over deteksjonsgrensen i de undersøkte blåskjellene fra Svalbard, Færøyene eller Grønland. Dette utelukker imidlertid ikke at det finnes punktutslipp av mikroplast i disse områdene. Det anbefales å gjøre målrettede undersøkelser av muslinger i nærheten av mulige punktutslipp slik som for eksempel renseanlegg.

I denne studien ble det benyttet en kombinasjon av ulike metoder for å vurdere mulige kilder til mikroplast i marine miljø. Visuell undersøkelse ga partiklenes morfologi ned til ca. 70 µm (form, størrelse, farge, mangel på cellulære strukturer mm), FT-IR ga partiklenes polymersammensetning, mens pyrolyse-GCMS ga informasjon om massen til ulike stoff og dermed informasjon om polymertype. Samlet kan disse metodene til en viss grad gi indikasjon på mulig opphav til partiklene. Mikroplast fra veiavrenning har blitt foreslått som en av de største kildene til mikroplastutslipp i det nordiske miljøet. Fram til nå har det imidlertid vært svært begrenset med empiriske data som kan støtte disse estimatene. Ved 16 av de 100 undersøkte stedene var dominert av gummiaktigepartikler. Disse partiklene antas å stamme fra veiavrenning eller havneaktivitet, eller en kombinasjon. Denne antagelsen er basert på den visuelle vurderingen (svart farge, gummiaktig fremtoning, passende størrelse og pølseaktig form), funn av kjemiske markører for gummi ved bruk av py-GCMS (butadien og isopren), «carbon black» interferens for FT-IR-spekteret. Stor likhet mellom partikler fra svært ulike lokaliteter antyder at opphavet er en type kilde som ikke er lokalspesifikk.

Flesteparten av mikroplastpartiklene som ble identifisert i blåskjell (87%) var fragmenter, mens fibre utgjorde de resterende 13%. Dette skiller seg fra tidligere undersøkelser av blåskjell i Norge der fibre har vært dominerende. Dette kan skyldes faktiske forskjeller i type mikroplastpartikler som er tilført havmiljøet, men kan også ha metodiske årsaker. Eksempler på dette kan være at en høyere andel av de identifiserte partiklene er sjekket ved hjelp av FT-IR i denne studien enn i tidligere studier, prosedyrer for å forebygge forurensning av prøver under innsamling og analyse forbedres kontinuerlig, prøver med mikroplastnivåer under deteksjonsgrensen er ekskludert i denne studien eller det kan være ulik grad av fibergjenvinning i forhold til fragmenter mellom forskjellige studier.

Flesteparten av mikroplastpartiklene var mindre enn 130 µm i sin lengste dimensjon, med et gjennomsnitt på 158 µm når partikler over 1000 µm ble ekskludert. Det var signifikante forskjeller mellom partikkelstørrelser for de forskjellige stasjonene. Årsakene til dette er ikke fullt ut forstått, men det kan til dels være relatert til de høye nivåene av gummipartikler for enkelte stasjoner. De fem innsamlingsstedene der det ble funnet flest svarte gummipartikler (M-19, M-14, M-16, M-22 og M-15) hadde også de laveste gjennomsnittlige partikkelstørrelsene. De to stasjonene der det ble funnet flest fibre var blant de fem stasjonene der de største mikroplast-partiklene ble påvist. Fibre er ofte lange og den lengste dimensjonen av partiklene har derfor en tendens til å være lengre enn fragmenter. Mikroplastpartiklene som ble funnet i Abra nitida og Limecola balthica var mindre enn partiklene i Mytilus spp., noe som trolig reflekterer at disse artene er mindre i størrelse enn blåskjell.

Foruten de dominerende svarte gummipartiklene var det tydelig at muslinger i nordisk havmiljø blir eksponert for en lang rekke andre polymer-materialer. Totalt ble 11 andre polymer-typer påvist:

Basert på visuell ID og punkt µFT-IR (Mytilus spp., Limecola balthica og Abra nitida)

- Polyetylen (PE)
- Polypropylen (PP)

- Semi-syntetisk materiale (modifisert cellulose)
- Epoksyplast (f. eks. malingsfragmenter)
- Polyvinylklorid (PVC)

Basert på automatisert skanning av μFT-IR (Abra nitida og Thyasira spp.)

- Polyakrylat
- Polyetylen (PE)
- Polydimetylsiloksan (silikon)
- Kalsiumstearat (et plasttilsetningsstoff)
- Semi-syntetisk materiale

Basert på Py-GCMS (Mytilus spp.)

- Polyhydroksybutyrat (PHB)
- Polymelkesyre (PLA)
- Polykaprolakton (PCL)
- Polyetylen naftalat (PEN)

Basert på denne omfattende studien samt tidligere nasjonalt og internasjonalt arbeid, ser det ut til at tre muslingarter kan være egnet for å overvåke mikroplast (63–1000 μm) i det nordiske havmiljøet; blåskjell og nær beslektede arter (Mytilus spp.) i mesteparten av kystområdene i Norden, Østersjømusling (Limecola balthica) i Østersjøen og Abra nitida langs deler av norskekysten og Nordsjøen. Våre funn antyder at Thyasira spp. ikke tar opp mikroplast større enn 63 μm, men i både Thyasira spp. og Abra nitida ble det påvist mikroplast mindre enn 63 μm. Disse artene kan derfor være egnet til å overvåke små mikroplastpartikler < 63 μm, men mer metodeutvikling og flere datapunkter er nødvendig for å vurdere dette nærmere.

Abbreviations

Short	Full name
ACES	Department of Environmental Science and Analytical Chemistry, Stockholm University
ApN	Akvaplan-niva
ATR FT-IR	Attenuated total reflection Fourier transform infrared
CH3COOH	Acetic acid
DCC	Diamond Compression Cell
DTU	National Institute of Aquatic Resources, University of Denmark
EPS	Expanded polystyrene
FIEA	Faroe Islands Environment Agency
FT-IR	Fourier transform Infrared
Fraction A	Particles above 63µm
Fraction B	Particles below 63µm
GC-MS	Gas chromatography–mass spectrometry
GF/A	Glass microfibre filter
GINR	Greenland Institute of Natural Resources
HDPE	High density polyethylene
KOH	Potassium hydroxide
LDPE	Low density polyethylene
LOD	Limit of Detection
LOQ	Limit of Quantification
MPs	Microplastics
NEA	Norwegian Environment Agency (Miljødirektoratet)
NIVA	Norwegian Institute for Water Research
NMR	Nordic Council of Ministers (Nordisk ministerråd)

PA	Polyamide
PA6	Polyamide 6
PC	Polycarbonate
PCA	Principal Component Analysis
PE	Polyethylene
PEF	Polyethylene furanoate
PET	Polyethylene terephthalate
PLA	Polylactic acid
PMMA	Polymethyl methacrylate
PP	Polypropylene
PS	Polystyrene
PUR	Polyurethane
PVA	Polyvinyl alcohol
PVC	Polyvinyl chloride
Pyr-GC MS	Pyrolysis gas chromatography mass spectrometry
SAN	Styrene-acrylonitrile
SYKE	Finish Environment Institute
UISRC	University of Iceland – Sudurnes Research Centre
v/v	volume to volume
VKM	Norwegian Scientific Committee for Food and Environment
WWTPs	Wastewater Treatment Plants

1. Background for report

Microplastics (MPs) are found in marine environments worldwide. Marine organisms can interact with microplastics through adhesion, absorption, ventilation and ingestion (Lusher, 2015). Ingestion has been described as the primary mode of interaction between organisms and microplastics as a form of environmental contamination, however, the consequences of this interaction is still not clear. Laboratory experiments have found negative impact on feeding, growth, energy levels, fecundity and reproduction, as well as sublethal effects within immune systems (Wright et al., 2013). Unfortunately, many laboratory exposures to date use unrealistic exposure regimes, such as very high concentrations, and further research is still required to build a clear picture of the consequences of microplastic exposure.

A very recent report from the Norwegian Scientific Committee for Food and Environment (VKM), concluded that it is challenging to perform environmental risk assessment of microplastics due to data gaps and constraints within the scientific literature such as those mentioned above (VKM 2019). The microplastic levels in different environmental matrices as well as extent across large geographical regions is not fully understood, which is also hindering the development of risk assessments.

As many species have been shown to contain microplastics, biota can be used to monitor microplastics levels whilst simultaneously providing important interaction data. Organisms which live in the water column or in surface waters can provide information on buoyant plastics, as well as transitory plastics which are on route to deeper sediments, following changes related to buoyancy and density (Andrady 2017). Considering reports suggesting the sediments can be the end point for as much as 90% of microplastics (Booth et al., 2017), microplastics in organisms that live in, on or near the sediment should be investigated. Benthic organisms, or those associated with the benthic community, may therefore be suitable as a sentinel species for monitoring microplastics in the environment.

The VKM report concluded that further information is required to evaluate microplastics in the Norwegian and the Nordic environments to understand microplastic abundance and potential sources. This echoes the conclusion of a Nordic Council of Ministers (NMR) scoping project from 2017 on the status of microplastic knowledge from the Nordic marine environment (Bråte et al., 2017), where the Nordic marine environment was defined as: the Norwegian Sea, Greenland Sea, the Norwegian and Danish sector of the North Sea, Skagerrak and Kattegat, as well as the Baltic Sea. It did also include all sea areas close to Greenland (south, east and north, but not west), sea areas north and north-east of Svalbard, and coastal sea areas north-east of Varangerhalvøya (Figure 1) (Bråte et al., 2017).

Several criteria for defining bioindicators were suggested when monitoring for microplastics in the Nordic marine environment (Bråte et al., 2017) including:

- Species should be abundant in the environment and easy to sample

- Ethical considerations (e.g. not use species that are threatened or protected)
- Cost of sampling/analysing the biota – sampling simultaneously for other pollutants, rapid sample process to increase the number of samples that can be analysed
- Species should be commercially and/or ecologically important
- Can be comparable to global studies (global range)
- Should be abundant in the Nordic area
- Known to contain microplastics

In the absence of agreed target species or tissues to monitor, most microplastic studies in the Nordic area so far have used fish stomachs; however, several issues have been raised with using fish stomachs for monitoring purposes based on the abovementioned criteria (among others). These include the complexity of the sample matrix, the stage of gut clearance (retention time) when sampled, their motile behaviour and the potential for ingestion of plastics in the trawl during sampling. Marine bivalves, such as Mytilus spp., were suggested to be more suitable than fish when it comes to standardisation of sampling and analysis (Bråte et al., 2017). Mytilus spp. have been identified as a suitable species for monitoring microplastics in the environment (Dehaut et al., 2016; Beyer et al., 2017; Lusher et al., 2017a; Bråte et al., 2018; Li et al., 2019), particularly suited to studying the finer (<1mm) waterborne faction of microplastics (Lusher et al., 2017a, Bråte et al., 2018). Due to the size preferences of bivalves, they should, however, not be used as the only bioindicator for marine plastic pollution. Other species, such as the Northern Fulmar sea bird (Fulmarus glacialisor) or larger benthic species, might be better suited for plastics larger than 1 mm.

In the 2017 scoping project, it was also found that most microplastic studies were conducted in species from the North Sea and the Baltic Sea, and very few studies had been performed using biota from Skagerrak, Kattegat, north in the Nordic area, west and north of the Faroe Islands, Iceland and Greenland (Bråte et al., 2017).

1.1 Aims and deliverables

The overall aims of this study were to:

- primarily investigate spatial trends across the Nordic marine environment in microplastic abundance and composition using bivalves as bioindicators;
- assess the use of multiple bivalve species to monitor microplastics in the marine environment; and

- infer potential sources of microplastics found in indicator organisms.

The results from this investigation will be used to assess the differences in abundance and composition of microplastics across the entire Nordic water regions, as well as provide insights into gradients of presumed sources or transport vectors (e.g. ocean currents). The focus for the selection of species were those that live on, in or in the vicinity of the bottom sediments. The aim of the investigation was also to help guide authorities as to how microplastics might be routinely monitored. The investigation was also meant to provide a basis for presentation of results in more scientific fora. The programme was carried out between June 2018 and November 2019, with the final report ready at the end of November.

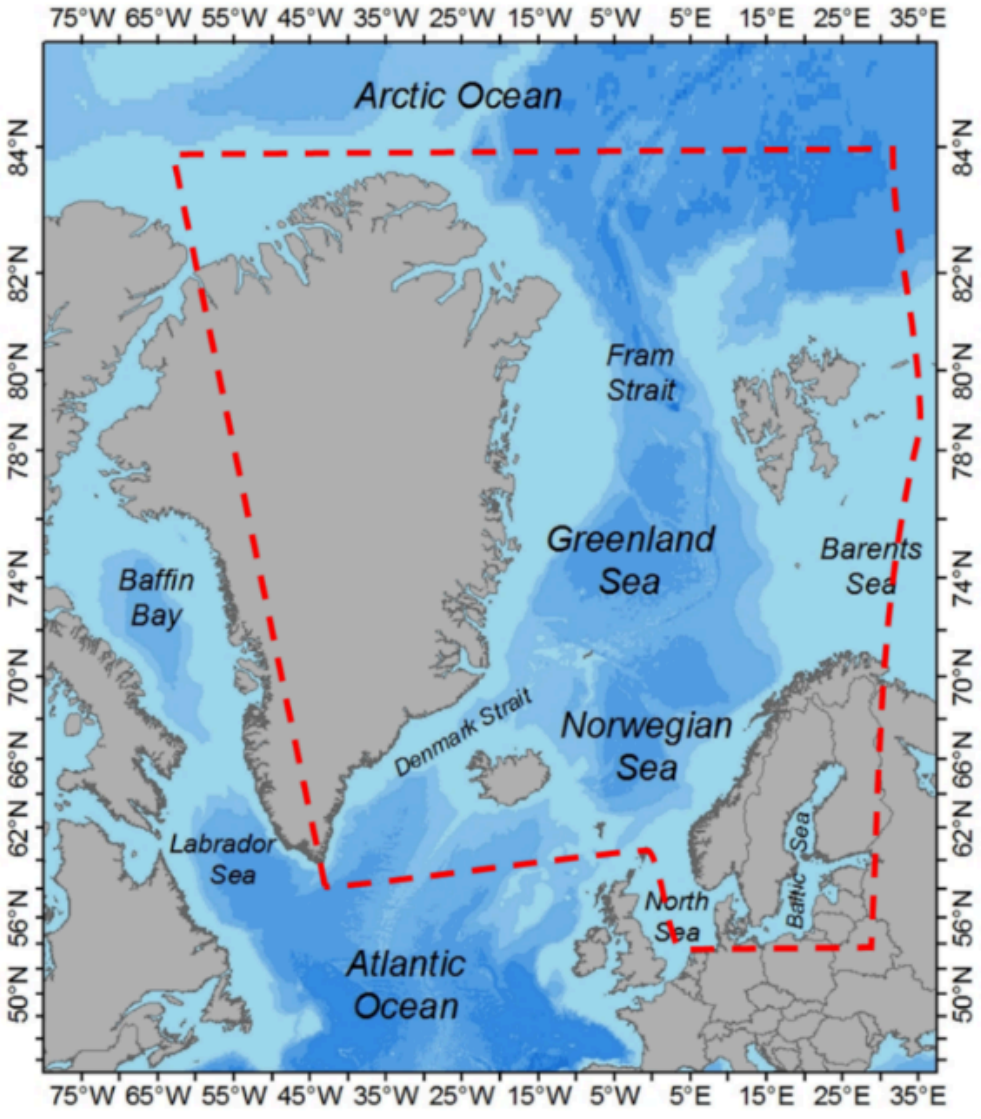

Figure 1: The Nordic Environment as defined in the Nordic Council of Ministers (NMR) scoping project on microplastics (Bråte et al., 2017).

2. Methods

2.1 Bivalve species, their distribution and ecology

Based on ecological criteria, see for instance Beyer et al., (2018), marine bivalves were chosen as the taxonomic target group, with a special focus on *Mytilus* spp. In total, NIVA identified five bivalves that live either in or in close proximity to sediments: *Mytilus spp.*, *Limecola balthica* (formerly called *Macoma balthica*), *Abra nitida, Thyasira spp.*, and *Hiatella arctica*. In addition to criteria relating to the feasibility of microplastics analyses and avoidance of contamination, the species chosen for this study have a geographical distribution throughout the Nordic study area and are comparable in their general biology and ecology, while they represent different life strategies and feeding modes. They also occupy different habitats, which in turn affect their exposure to microplastics. By studying these five species it allowed comparisons of microplastic contamination between species living in, on and above benthic sediments (Table 1). Furthermore, these species have been also routinely collected for scientific purposes, leading to good knowledge of where to find them and knowledge regarding their biology. Bivalves are easy to collect, process and analyse, and many laboratory studies of microplastic have been conducted using these organisms. The five selected species occupy different habitats, which may influence their exposure to microplastics, enabling comparisons of microplastic contamination of specimens living in, on and above marine sediments.

Table 1: Bivalve species included in the current study and description of their ecology.

Species	Habitat	Feeding mode
Mytilus spp.	Hard substrate	Suspension filter feeder
Limecola balthica	In/on sediment	Siphon feeding on sediment/ water column
Abra nitida	In/on sediment	Detritus feeder
Thyasira spp.	In sediment	Suspension feeding, bacteria farming
Hiatella	Hard substrate	Suspension filter feeder

Blue mussel (*Mytilus edulis*), a very common cosmopolitan bivalve, is distributed throughout the North Atlantic and along the coast of the White Sea. The more boreal *M. edulis* may be confused with the Mediterranean *M. galloprovincialis*, or the Pacific *M. trossulus*, and in some locations along northern European coasts two or all three species co-occur and/or produce hybrids (Oliver et al., 2010). Collected individuals along the Norwegian coast has also been seen not to be entirely restricted to *M. edulis* (Brooks & Farmen 2013), and may include *M. trossulus*, and *M. galloprovincialis*. Hence, in this report, these species are referred to collectively as

Mytilus spp (Figure 2). Along the west coast of Svalbard *Mytilus* spp. have been observed since the early 2000s and have spread in the region, with circumstantial evidence for reproductive activity and recruitment of young individuals in recent years (Mathiesen et al., 2017; Leopold et al., 2019). The shell is inequilateral and roughly triangular in shape, but shell shape varies considerably with environmental conditions. The shell colour varies from purple or blue to brown. *Mytilus* spp. are variable in size, from populations not exceeding 20–30 mm in length to the largest specimens measuring up to 20 cm (Marlin, 2019). *Mytilus* spp. live in intertidal areas attached to rocks and other hard substrates with a strong and slightly elastic fibrous structure located in the foot, the so-called byssal threads, which are secreted by byssal glands. *Mytilus* spp. are suspension filter feeders and ingest phytoplankton (dinoflagellates, small diatoms, flagellates, various unicellular algae), zoospores, other protozoans, and detritus, filtered from the surrounding water. They also play a vital role in the removal of bacteria, and also toxins from the water column. Indigestible materials can be rejected as pseudofaeces (Kiørboe *et al.*, 1980).

Figure 2: Mytilus spp.
Source: Wikipedia. Photograph: Clark University, MA, USA.

Limecola balthica (Figure 3), commonly called the Baltic clam or Baltic tellin, is a small infaunal clam in the family Tellinidae. *Limecola balthica* lives in the northern parts of both the Atlantic and Pacific oceans, and also extends to the Subarctic both in North America and in Europe. The European distribution ranges from southern France north to the White Sea and Pechora Sea and also includes the inner brackish parts of the Baltic Sea (Strelkov *et al.*, 2007). *Limecola balthica* is an euryhaline species, i.e. it is adapted to a wide range of salinities, down to 3–4‰ (10% of ocean salinity). It usually lives in the intertidal or shallow subtidal zone, in estuaries and on

tidal flats (Oliver *et al.*, 2010). In the brackish Baltic Sea, it lives submerged down to water depths of >100m (Strelkov *et al.*, 2007). The shells are smooth, relatively flat, oval or somewhat trigonal in shape, and less than 30 mm long (Denisenko *et al.*, 2003). The shell colour is polymorphic, varying between individuals and localities and can vary between white, pink, yellow and orange. Concentric growth rings indicating the age of the specimen are often clearly visible. Living buried in the mud or silt, they extend two narrow siphons to the surface of the seafloor. Through the siphons, they feed on organic matter on the sediment surface or the overlaying water.

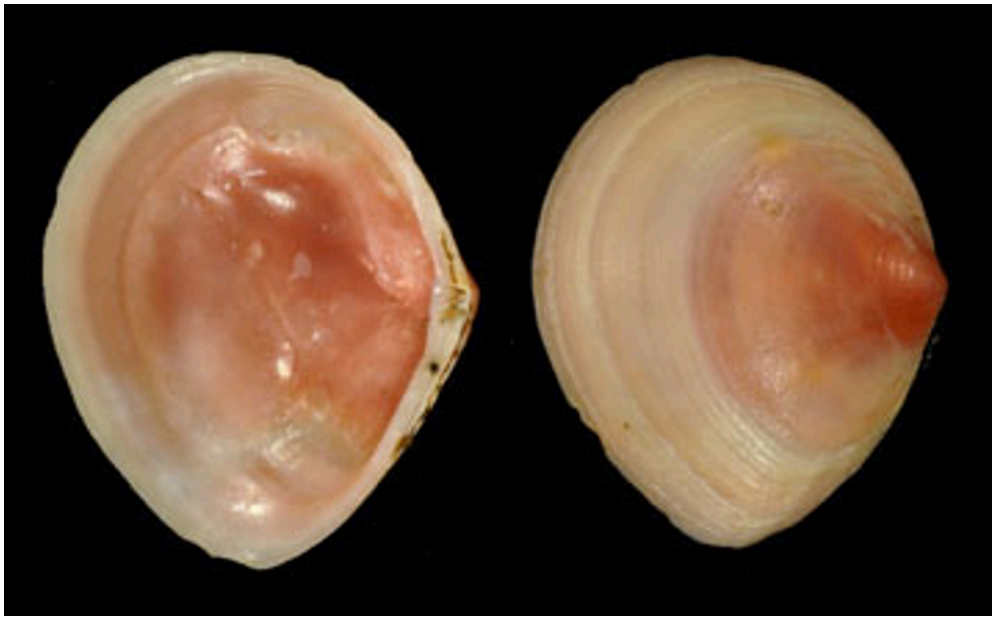

Figure 3: Limecola balthica
Source: Collection and photo Natural History Museum Rotterdam (NMR 36199).
Photograph: Natural History Museum, Rotterdam,
the Netherlands.

Abra nitida, (Figure 4), is a marine clam in the family Semelidae, distributed all along the Norwegian coast. The specimens are small (approximately 20 mm in length and <15 mm in height), and have thin asymmetrical shells in a glossy, pearly-white colour, sometimes translucent and scattered with small specks. *Abra nitida* inhabits self-made burrows in mud, sandy mud, silty sand and muddy gravel in the sublittoral zone (down to 183 m depth) (Marlin 2019). where it mainly feeds on detritus. They are considered an important food source for flat fish (Harbo 2001).

Figure 4: Abra nitida

Source: Oliver, P. G., Holmes, A. M., Killeen, I. J. & Turner, J. A. (2016). Marine Bivalve Shells of the British Isles. Amgueddfa Cymru - National Museum Wales. Available from: http://naturalhistory.museumwales.ac.uk/britishbivalves.

Thyasira spp. (Figure 5), is a genus of small globular bivalves with thin and fragile shells. Several species co-occur in Nordic waters, e.g. *T. flexuosa, T. dunbari* and *T. gouldi,I* and are very similar in morphology. *T. gouldi* has a pan-arctic distribution and are found along the north coast of Norway, and around the coast of Greenland. They inhabit a small chamber in the top few centimetres of soft mud or sand-mud sediments rich in organic matter (Marlin 2019). The family Thyasiridae contains symbiotic and asymbiotic species that live beneath the seabed surface. Feeding strategies may vary from suspension feeding, deposit feeding to bacteria farming. Sulfur-oxidizing symbiotic bacteria are 'farmed' along burrow linings and then collected with the bivalve foot (a variation of bivalve deposit-feeding called pedal feeding) (Zanzerl *et al.*, 2019).

Figure 5: Thyasira spp.
Source: Oliver, P. G., Holmes, A. M., Killeen, I. J. & Turner, J. A. (2016). Marine Bivalve Shells of the British Isles. Amgueddfa Cymru - National Museum Wales. Available from: http://naturalhistory.museumwales.ac.uk/britishbivalves.

Hiatella arctica, (Figure 6), also called the 'wrinkled rock borer', has a thick shell with an irregular, but generally almost rectangular, shape. It is cosmopolitan species found from pole to pole and from the intertidal zone down to 800 m depth. The bivalves attach to hard substrates such as rocks, kelp hapterons (holdfasts) or crevices, but are also able to bore themselves into soft rock (Rees & Dare, 1993). Their development involves a relatively long pelagic phase in the larval stage. *Hitaella arctica* is a suspension feeder (Denisenko *et al.*, 2003).

Figure 6: Hiatella arctica

Source: Wikipedia. Photograph: Jan Johan ter Poorten; modified by Tom Meijer.

2.2 Location and sampling of bivalves

In total, 100 sites were sampled; collected from the east coast of Greenland and coastal waters of Iceland, Faroe Islands, Norway (including Svalbard), Denmark, Sweden and Finland. The samples were partly from new sampling (2018–2019) and partly from stored samples (2013–2018). The selection of sample locations was largely driven by the availability of bivalve samples and field sampling schedules from the summer of 2018 and early spring of 2019 of the participating research entities. The target species was to have a wide geographical distribution with some overlap in the distributions of other target bivalve species. The overlap was important to provide a possible means to compensate for different feeding strategies in order to make a pan regional assessment. The choice of samples and locations was based on:

- geographical distribution in Nordic marine environment
- availability of archived samples and on-going field work
- choice of indicator organisms (bivalves)
- transects to investigate supposed pollution gradient
- some stations with same bivalves to investigate differences in uptake of microplastic and provide a possible way to "normalize" data across a large region.

In total, 100 samples (i.e. sites) were used (Table 2, from Figure 7 to Figure 11). Except for *Mytilus* spp. collected in shallow water (<2m), samples were collected from soft bottom sediment.

As mentioned above, the natural distributions of these target species are not entirely overlapping which complicated a pan-regional assessment. To address this issue, at some sites two target species were analysed in order to compare the composition of microplastics in the attempt to make a broader regional assessment. Details concerning the samples can be found in Table 16 in Appendix 7.1.

Possible differences in contaminant uptake between *Mytilus* spp. were assumed to be small and they were not taken into account. This species was collected from shallow water (0–2 depth) by hand during the period August to October 2018, except for three samples of small individuals (<5mm) derived from stored grab samples taken during the years 2014–2015 at sampling depths that ranged from 50 to 61 m.

All but three samples of *Limecola balthica* were collected during field work conducted in September 2018 and January 2019. Only three samples were collected from stored grab samples taken during the years 2015 and 2016. The sampling depths ranged from 16 to 32 m.

All but three samples for *Abra nitida* were derived from stored grab samples taken during the years 2013–2017, and for a few of these samples, *Abra* spp. and *A. longicallis* were also included. The three samples were collected using a Van Veen grab during field work in August–September 2018 and were classified as *Abra nitida*. The sampling depths ranged from 27 to 426 m.

All but five samples for *Thyrasira* spp. were derived from stored grab samples taken during the years 2013–2017. These samples included *T. sarsii*, *T. obsulata*, *T. equalis* and *T. gouldi*. The five samples were collected using a Van Veen grab during field work in August–September 2018 and were classified as *Thyrasira* spp. The sampling depths ranged from 27 to 423 m.

The domination of Norwegian sites regarding *Abra nitida* and *Thyasira* spp. was mainly due to the costs of sampling of new specimens from a broader range of sites. The samples used in current study were already sampled but not yet analysed. The use of historical samples was also to test if such samples, which were preserved in ethanol, are suitable for studying microplastics occurrence.

The three *Hiatella arctica* samples were derived from store grab samples taken in 2014 with a depth range of 148–167 m.

Table 2: Number of bivalves sites and distribution (see also from Figure 7 to Figure 11 and more detailed description in Table 16 in Appendix 7.1.).

Species	Baltic Sea	Denmark	Norwegian coast	Faroe Islands	Iceland	Greenland	Total
Abra nitida	0	3	28	0	0	0	**31**
Thyasira spp.	0	5	15	0	0	0	**20**
Limecola balthica	10	0	4	0	0	0	**14**
Mytilus spp.	3	4	11	3	7	4	**32**
Hiatella arctica	0	0	3	0	0	0	**3**
Total	**13**	**12**	**61**	**3**	**7**	**4**	**100**

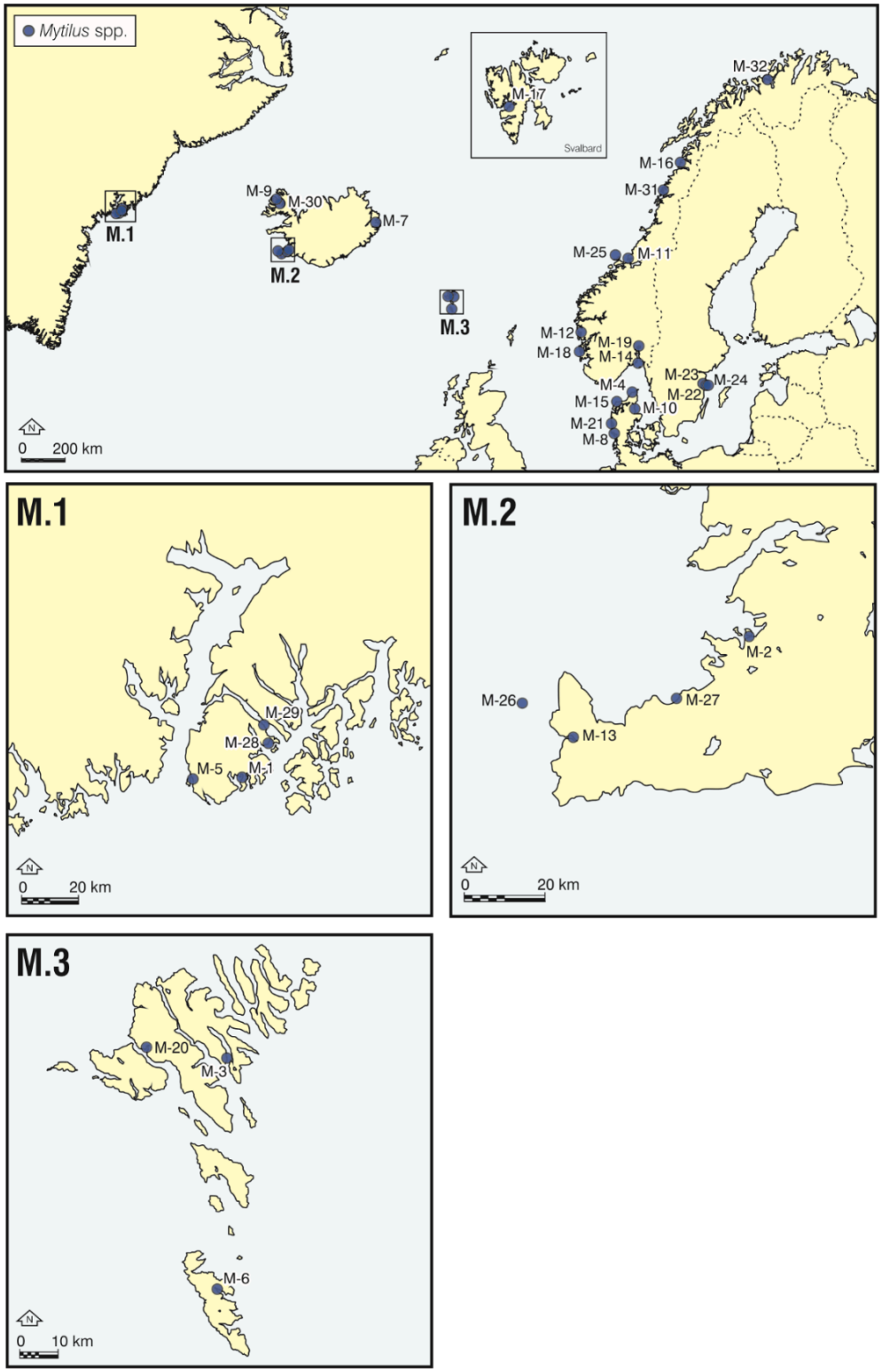

Figure 7: Sites where blue mussel and closely related species (*Mytilus* spp.) were sampled during the period 2014–2018. Detailed maps show east coast of Greenland (M.1), southwest coast of Iceland (M.2) and the Faroe Islands (M.3). Further information is available in Table 16 in Appendix 7.1.

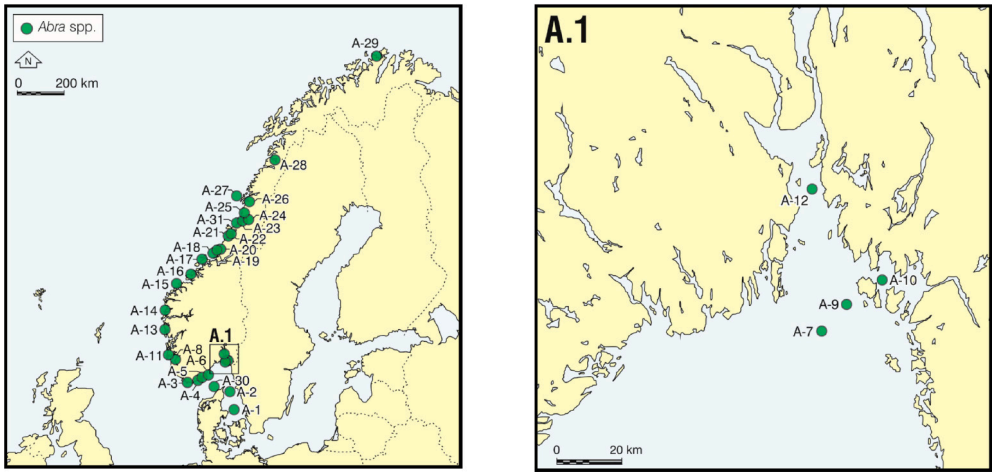

Figure 8: Sites where the bivalve *Abra nitida* were sampled during the period 2013–2018. Detailed map shows the Oslofjord (A.1). Further information is available in Table 16 in Appendix 7.1.

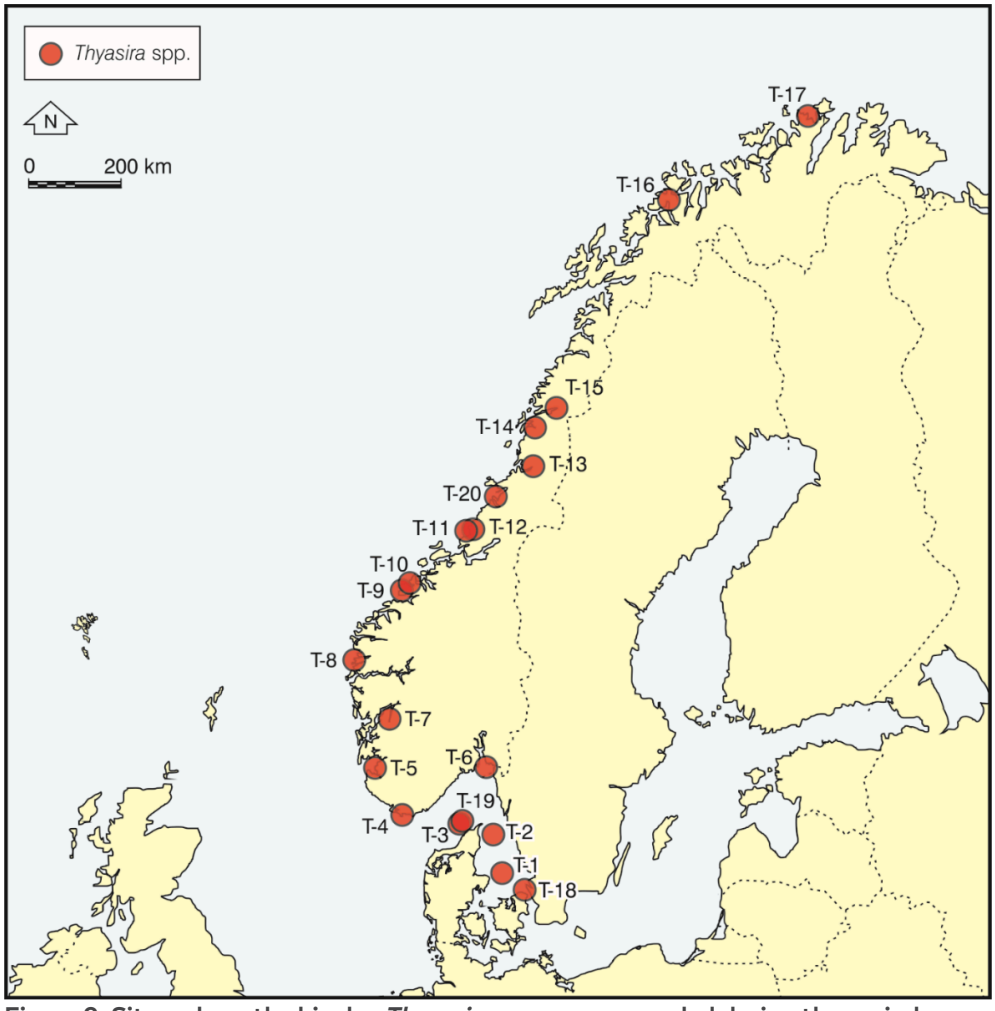

Figure 9: Sites where the bivalve *Thyrasira* spp. were sampled during the period 2014–2018. For further information is available in Table 16 in Appendix 7.1.

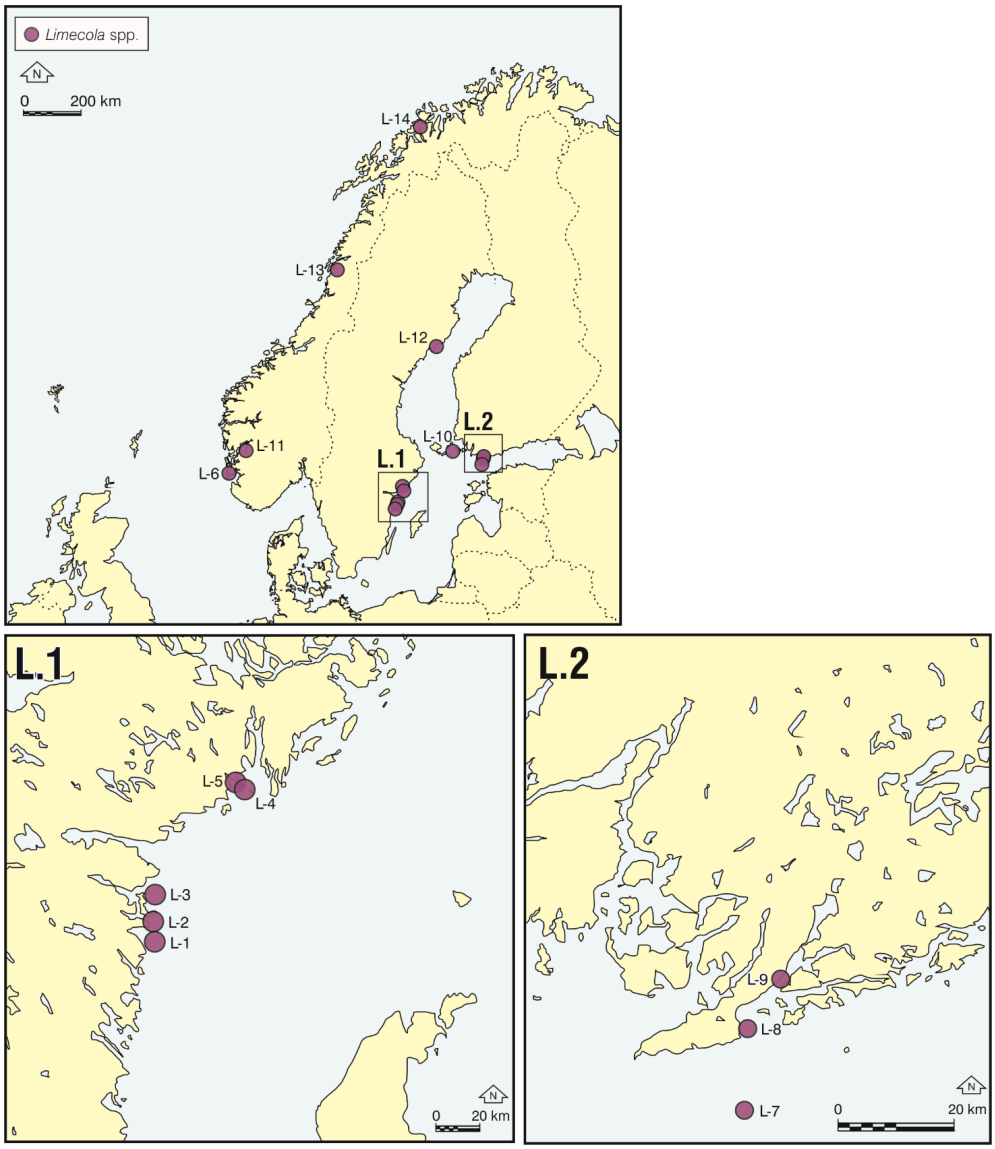

Figure 10: Sites where the bivalve *Limecola balthica* were sampled during the period 2015–2019. Detailed maps show east coast of Sweden near Stockholm (L.1) and the south coast of Finland (L.2). Further information is available in Table 16 in Appendix 7.1.

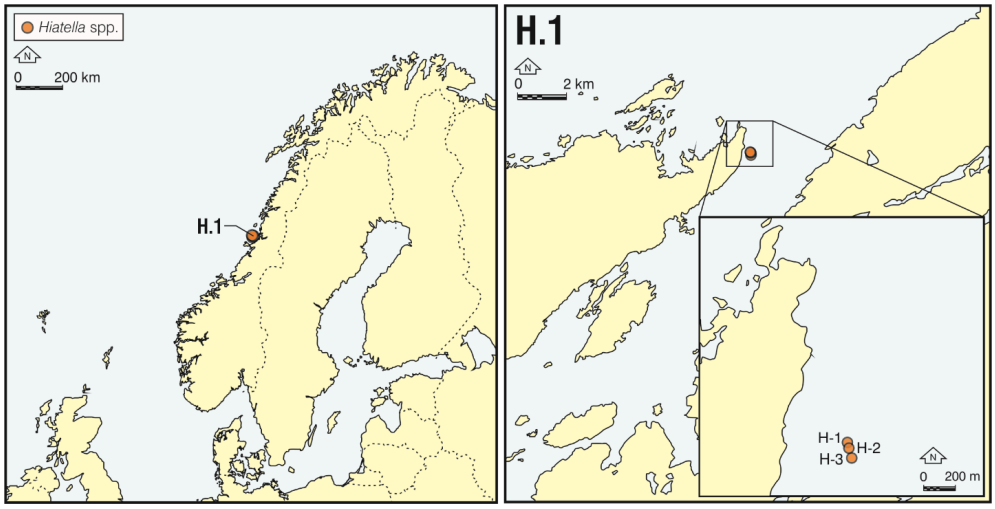

Figure 11: Sites where the bivalve *Hiatella arctica* were sampled in 2014. Detailed map shows the west coast of Norway, north of Rørvik (H.1). Further information is available in Table 16 in Appendix 7.1.

2.3 Sample preparation

Different sizes of individuals were samples, which had to be prepared for analysis in slightly different ways. Large individuals (*Mytilus* spp.) followed validated methods with minor modifications (Bråte et al., 2018). Smaller individuals, which included *Mytilus* spp. (<15 mm), *Thyasira* spp., *Abra nitida*, *Limecola balthica* and *Hiatella arctica* were processed in two fractions: Fraction A (> 63 µm) and Fraction B (< 63 µm).

2.3.1 Large bivalves

Mytilus spp. were the only bivalve species to be processed using the modified standard procedure (n= 29 sites, 545 individuals). Between 14 and 20 individuals were processed per site. In addition, three *Mytilus* spp. sites were analysed together with the other four taxonomic groups and termed 'small bivalves' (see Section 2.3.2 and Table 16 in Appendix 7.1). All samples which were stored frozen, were then defrosted and their lengths were measured (cm) with callipers before opening. Soft tissue was dissected out before being weighed (g, w.w.) and placed in a pre-rinsed, clean glass beaker. A premade, filtered solution of 10% KOH was added to each beaker with a ration of 1:10 (biota: KOH, v/v). Beakers were sealed with aluminium foil and placed in an incubator for 24 h at 60 °C with continuous agitation (120 rpm). Samples were removed from the incubator and allowed to cool before being filtered under vacuum onto glass microfibre filter papers (GF/A, pore size, 1.6µm) (Figure 12). Filter papers were dried before being visually inspected for the presence of suspected microplastics plastics following the steps described in Section 2.4.1.

Mytilus spp. dry weight was calculated from additional individuals not previously analysed for microplastics an additional 1–3 individuals (see Table 19 in Appendix 7.3 for exact numbers). Four sites did not have a enough surplus individuals (M-5, M-8, M-17 and M-22). For these sites, the overall mean of all sites was calculated (84.35% w. w) and used to convert the wet weight of the individuals to dry weight, as

presented in Table 20 in Appendix 7.3.

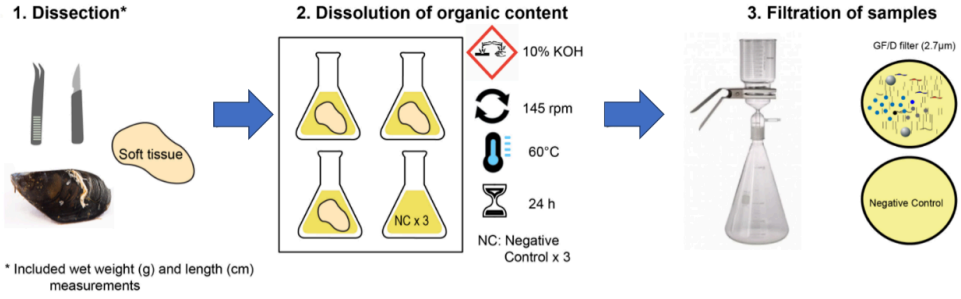

Figure 12: Flow of sample preparation of *Mytilus* spp. (figure reproduced from Bråte et al., 2018).

2.3.2 Small bivalves

Four other bivalve species, *Thyasira spp., Abra nitida, Limecola balthica*, *Hiatella arctica,* in addition to three sites with very small *Mytilus* spp., were processed for microplastic analysis (Table 3).

The species were too small for tissue dissection (Figure 13) and had to be incubated with KOH as whole organisms, followed by a 5% acetic acid (CH_3COOH) treatment to fully dissolve whole and/or debris of shell as well as other calcium carbonate ($CaCO_3$) based structures (e.g. shell remains) in the sample. Digestion of bivalve shells, mainly consisting of $CaCO_3$, occurs at pH <6, with increased solubility when exposed to lower pH. Removal of $CaCO_3$ debris is crucial as presence of shell and/or pearl formation may interfere with the FT-IR scanning of the sample in the later stages of analysis. A few large specimens of *Limecola balthica* which in some cases were large in size (between 3–10 individuals, ranging from 0.004 g w.w. up to 6.051 g w.w. – see in Table 21 in Appendix 7.6.1). The larger *Limecola balthica* had a thick shell, and therefore the shell was removed from the solution as soon as the soft tissue had detached.

Table 3: Number of individuals of *Thyasira* spp., *Abra nitida*, *Limecola balthica*, *Hiatella arctica* and *Mytilus* spp. from the Nordic environment processed as small bivalves, therefore splitting them in to Fraction A and Fraction B for analysis.

Species	No of sites	No of ind.
Abra nitida	31	589
Limecola balthica	14	233
Thyasira spp.	20	480
Hiatella arctica	3	17
Mytilus spp.	3	58

Figure 13: Selection of images taken during sample processing. A: *Thyasira* spp. in a glass sample container, preserved in ethanol. B: *Abra nitida* in a glass sample container preserved in ethanol and stained with rose bengal. Individuals selected for sample processing; C: *Thyasira* spp., D: *Abra nitida*, E and F: *Limecola balthica* and G: *Hiatella arctica*.

The stored samples of *Thyasira* spp., *Abra nitida* and *Hiatella arctica*, and to a lesser degree, *Limecola balthica* were preserved in formalin and then stored in ethanol. No evaporation of the excess ethanol was performed prior to weighing the sample. In some cases, this led to difficulties in determining the wet weight of some samples due to the ethanol and small tissue content. To avoid any external sample contamination during processing of the samples, the bivalve species were weighed in glass beakers prior to the addition of KOH. The same sample preparation was also performed for smaller *Mytilus* spp. from three sites (M-25, M-31 and M-32). Since *Limecola balthica* were larger, the shells were removed manually after KOH treatment in a Laminar Flow, Class II cabinet to avoid any external contamination, see Table 16 to Table 19 in Appendix 7.1.

Digestion of the soft tissue using KOH was performed for the bivalves as previously described in Section 2.4.1. The processing of the small bivalves is described in Figure 14. In short, soft tissue was digested after incubation of the whole individual in 10% KOH at 40°C on a shaking table at 125 rpm, leaving only shell debris, sediments, and potential microplastics remaining after <24h. The pH of the KOH solution was 13.5–14.5 after 24h of incubation. To further dissolve all shell debris and other calcium carbonate structures, the pH was adjusted to 4.3–5.0, by first adding 1:1 (v/v) of 10% acetic acid (a novel method that was developed for this project), followed by addition of a 1:1 – 5:7 ratio of 5% acetic acid to increase the solubility of calcium carbonate. The samples where then incubated at 40°C (and sometimes 60°C depending on the amount of organic matter), at 125 rpm from 1 up to 12 hours. Some polymers can be affected at 60°C (Dehaut *et al.*, 2016), therefore it was aimed to keep the temperature at 40 °C whenever possible. Only one sample had to be incubated at 60°C.

Following digestion, the samples were filtered, as described in Figure 14, and were separated into two fractions. The samples were split across two different filters papers during filtering to improve both the visual inspection and µFT-IR steps (single point mode for Fraction A: >63 µm and µATR imaging for Fraction B: <63 µm). In short, the samples for Fraction B were first passed through a 63 µm stainless steel mesh and filtered onto a Cellulose Nitrate (CN) filter (25 mm, 8 µm), using a glass vacuum filtering system (Ø17 mm; Sterlitech, USA). All remaining debris on the 63 µm steel mesh were then filtered onto a Whatman GF/A filter (pore size 1.6 µm; Ø 45 mm), using a Nalgene filtering system (such as for Fraction A). The two filters (GF/A and CN) represent particle sizes above 63 µm (Fraction A) and below 63µm (Fraction B), respectively. The pre-filtration of Fraction B was performed to limit the presence of sand and/or silt particles on the CN filter. Such particles may damage the germanium crystal used by the FT-IR instrument during µATR imaging.

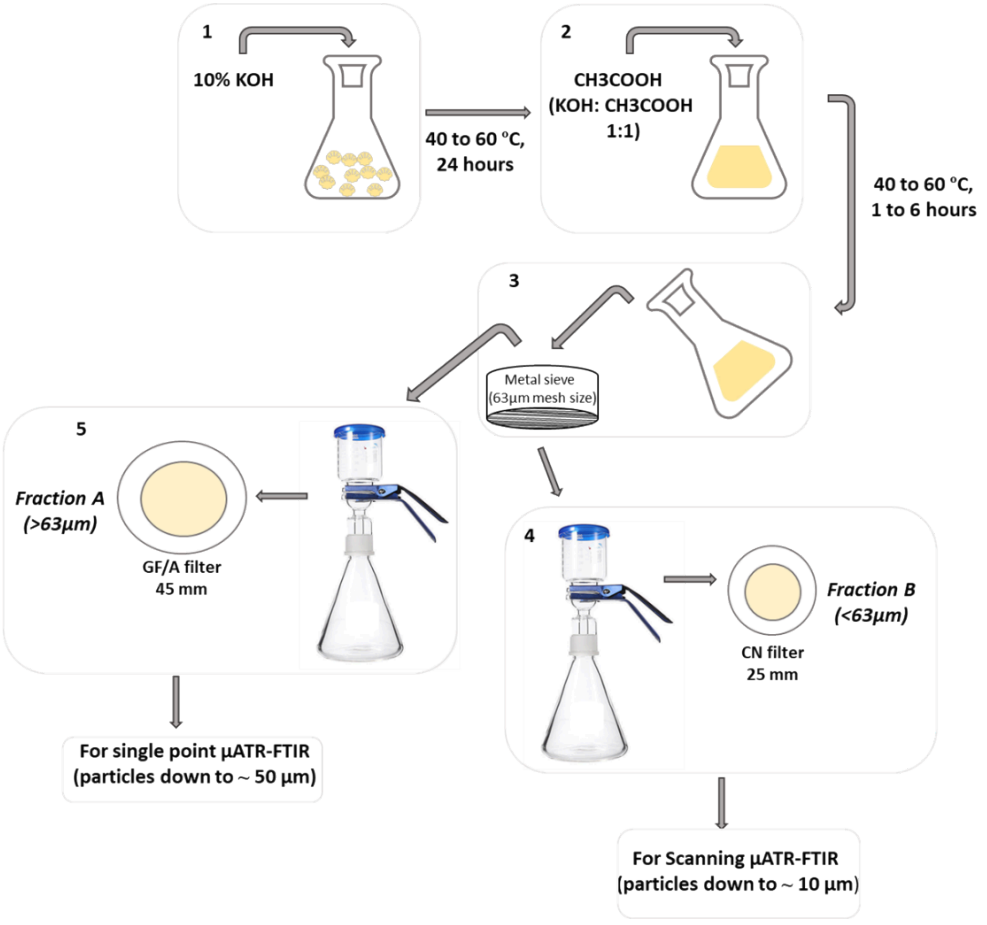

Figure 14: Sample preparation of small bivalves *Thyasira* spp., *Abra nitida, Limecola balthica, Hiatella arctica* and *Mytilus* spp.

2.4 Microplastic analysis

The main goal for MP analysis is to determine if particles smaller than 0.5 cm found in the samples, are composed of plastic polymers or simply natural particles such as sand grains or organic material. Since one method is not enough to tell us everything we need to know (what is it made of, how does it look, and where are they from), several of methods are typically applied. In this study the main methods applied were microscope investigations, infrared scanning of particles and mass estimation.

2.4.1 Mytilus spp. and Fraction A of small bivalves

Visual ID

All *Mytilus* spp. (n=558) and the small bivalves were analysed for Fraction A (particles larger than 63µm) using visual identification. All filter papers were visually inspected for the presence of potential microplastics. The lower size limit of detection for visual ID was ~ 50µm. A Nikon SM2 745 T stereomicroscope with image

analysis software (Infinity Analyse v.6.5.6) was used to photograph and measure (both longest dimension and shortest dimension in µm) of individual particles. The selection of particles was made following internal NIVA protocols which were developed from Lusher *et al.,* (2014) and are described in detail in an earlier report (Lusher *et al.*, 2017a). All microplastics were recorded with a description of their morphology (fibre or fragment), size and colour.

µFT-IR (single point)

Following visual analysis and physical characterisation of suspected microplastic, 100% of particles from each sample were subjected to further chemical characterisation using µFT-IR analysis, except for black rubbery fragments (see section below). This was performed on a PerkinElmer Spotlight 400 µFT-IR spectrometer. To improve the quality of the spectra generated, particles were prepared for analysis using a diamond compression cell (DCC) accessory. Particles were carefully transferred from filter papers to the DCC with use of extra fine micro forceps. The DCC was used to compress particles to a thin, homogenous thickness. The DCC was then loaded onto the µFT-IR microscope stage for analysis. Measurements were obtained in transmission mode and at 4 cm^{-1} spectral resolution for the range 4000 to 600 cm^{-1}. Spectra were produced from a composite of 2 co-scans. Background measurements were taken before each batch of particles was analysed. Library matching was performed in the Spectrum 10 software (v. 10.6.2). Each spectrum was compared to several different libraries available at NIVA: PerkinElmer ATR Polymers library, STJapan Polymers ATR library, BASEMAN library (Primpke *et al.*, 2018), and several in-house libraries including reference polymers, different textile materials, and potential sources of laboratory contamination. All spectra were manually inspected to ensure that the library matches were acceptable.

Black rubbery fragments

Black rubbery fragments are sometimes present in microplastic samples. These are typically suspected to be derived from tyres or other rubber composite products, as they contain carbon black as a filler. All black rubbery fragments identified within the samples were first subject to physical characterisation during the visual analysis step. This included noting particles that were both deep black in colour and highly elastic when handled with micro forceps. Additionally, the occurrence of 'sausage'-shaped particles was recorded, which is also often associated with black rubbery fragments (Vogelsang *et al.*, 2018). A small subsample of these particles was tested using single point µFT-IR in transmission mode (see above) to identify spectra indicative of the presence of carbon black within the particle. Carbon black absorbs most of the infrared light during FT-IR analysis, particularly in transmission mode which measures the light transmitted through the entire thickness of each particle. The resulting spectra for particles suspected to contain carbon black is characterised by complete absorption of the IR beam. These criteria were used to define 'black rubbery fragments' in this project.

The difficulties noted for analysing black rubbery fragments using FT-IR may be partially reduced by using the ATR mode (attenuated total reflectance). The penetration depth of the ATR approach is small (1–2 µm), so less of the IR light is

absorbed by the carbon black. This has been demonstrated by Vollertsen and Hansen (2016) for fragments taken from car tyres. Based upon this, a subsample of particles was initially testing using the μATR imaging mode on the PerkinElmer Spotlight 400 FT-IR following the method outlined in Section 2.4.2. Unfortunately, the particles from this study were too small to obtain reliable spectra. For this reason, samples were instead submitted for targeted py-GCMS analysis, which is described in Section 2.5.

2.4.2 Fraction B of small bivalves

All the small bivalves were analysed for Fraction A (particles larger than 63μm) as described in the section "visual ID". Some sites where further investigated with a 'deep-dive' into Fraction B (smaller than 63μm) with a total of 15 samples and 5 blanks. This comprised the use of a large germanium ATR crystal, which can isolate an area of 500 μm^2 for FT-IR imaging. This differs from the single point mode used for >50 μm particles in this study in two respects. Firstly, the imaging capacity conducts FT-IR measurements for each pixel within a defined area, removing any visual bias. Secondly, it can be operated at very fine spatial resolution, permitting for the analysis of very small microplastic particles that cannot be detected through visual identification methods. Three sites with *Thyasira* spp. (site T1, T2 and T6 with triplicates from each site i.e. pooled samples of 10 individuals) and two sites of *Abra nitida*, (A10 and A12 also in triplicates of 10 pooled individuals) were selected for this analysis.

Sample preparation

A cellulose nitrate (CN) filter was mounted onto a metal holder fitted for the ATR imaging mode. A visual image is first taken of the area that will be scanned (Figure 15 A). The μATR crystal was lowered into contact with the sample to collect a chemical image. When comparing the visual image with the chemical image it was noted that the area analysed was not exactly the same. This may be due to movement of the instrument. Prior to the collection of spectral data, a background measurement was taken to account for CO_2 and water vapour levels in the analytical atmosphere. An initial pre-scan of a small area (10 x 10 μm) was also performed to ensure a good contact had been made with the sample.

Data collection

SpectrumIMAGE (v.R1.8 for Spotlight 400) was used to collect and interpret the spectral data. For each sample, an area of 500 x 500 μm in the centre of the filter was scanned. The total sample diameter was ~17 mm (circle), giving a total of 0.32% of the filter that was scanned. Within this area, 6400 individual spectra were collected. FT-IR imaging was performed at a spatial resolution of 6.25 μm and a spectral resolution of 4 cm^{-1} for the range 4000–700 $cm^{-1,}$ with an interferometer speed of 2.2 cm s^{-1}. Spectra were produced from a composite of 8 co-scans. Particles can be detected using this imaging technique provided that they are resolved across at least 2 pixels.

Data treatment

In total across 15 samples and 5 blanks, 128,000 spectra were obtained for Fraction B. The data were expressed as average absorbance and represented by a chemical image as illustrated in Figure 15 B. First, the data were corrected for atmospheric interference. Due to the high number of spectra collected, all spectra could not be inspected individually. Therefore, a PCA (principal component analysis) was performed, giving a PCA chemical image, as illustrated with Figure 15 C, which depicts the mixed PCA with all components viewed simultaneously. This PCA method enables the structures lying in the data set to be highlighted in a standardised way. The spectra that accounted for most of the variation in the dataset was automatically set as PCA1, the spectrum representing the second most variation was PCA2, and so on. The different PCAs were, however, manually inspected to see if they did cover the particles of interest. The ones that looked to be artefacts where 'hidden' from the view and others more relevant were chosen. Overall, a minimum of three and a maximum of five factors per sample were used.

For each of the structures (or principal components) that were found – for example structure 2 in T2_rep1 illustrated in Figure 15 D – a spectrum was obtained from the region in the imaged area that represented the strongest signal. This spectrum was composed of several spectra conjoined to reduce noise. The representing spectra obtained from a specific particle is illustrated in Figure 15 E. The spectrum obtained from the ATR imaging scan was transferred to Spectrum IR 10 for library matching analysis, as was performed for single point mode. The spectra were also investigated by an analytical chemist

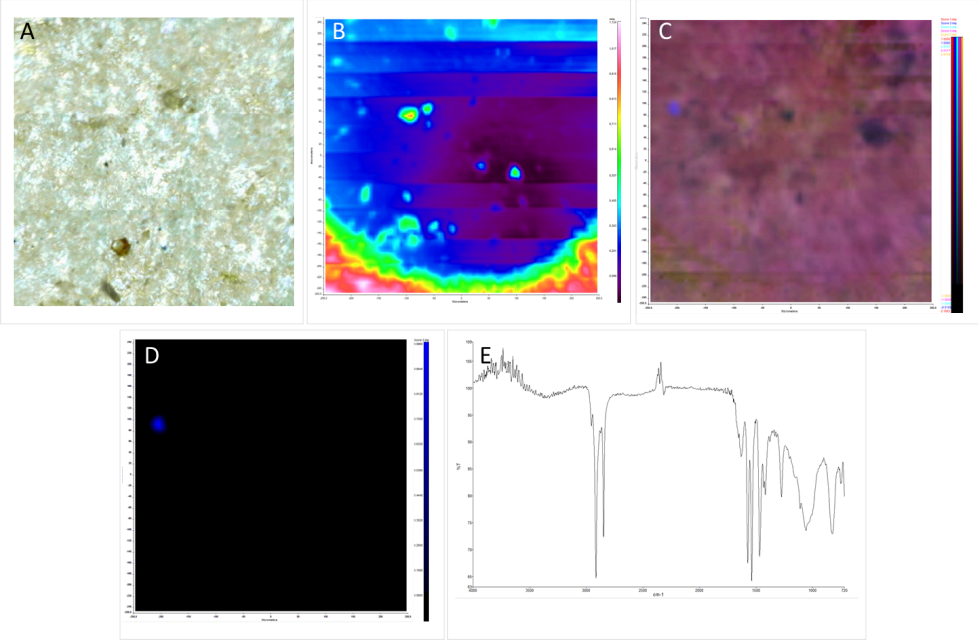

Figure 15: Flow of analysis method for Fraction B (<63 µm). A: T2_rep1. Visible image taken with the software of the approximate site of analysis. Size: 500x500 µm. B: Chemical image (spectral image) after atmospheric correction for T2_rep1. The red and green edge is the edge of the germanium crystal. C: Combined chemical image (spectral image) after PCA for T2_rep1.D: Single chemical image (spectral image) of structure 2 after PCA for T2_rep1. E: Representing spectrum obtain from structure 2 after PCA exemplified for T2_rep1. The spectrum does match with calcium stearate.

2.5 Pyrolysis gas chromatography mass spectrometry of selected Mytilus spp. samples

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Pyrolysis Gas Chromatography Mass Spectrometry (Py-GCMS) is an analytical technique used for measuring the chemical composition of organic samples. Samples are first 'pyrolyzed' – heated up to a high temperature in an inert atmosphere or vacuum – in a pyrolysis unit. The gases produced during this heating are captured and transferred to the GCMS, which analyses polymers and other complex organic molecules. This can be used to both indicate the presence or quantify the masses of different plastic polymers in a sample.

2.5.1 Targeted and non-targeted approach

Ten samples of *Mytilus* spp. were sent to Eurofins for pyrolysis gas chromatography mass spectrometry (Py-GCMS). Two approaches to analysis were performed: targeted and non-targeted approach. The targeted approach was used to specifically identify the presence of rubbers and eight common polymer types.

For the targeted rubber analysis, the pyrolysis was performed with calibration curves for polybutadiene and polyisoprene. These are used as standards to help

identify target compounds, which in this case were used to identify the presence of rubber compounds that could indicate potential tyre rubber or other rubber composites. This was based upon the occurrence of small black rubbery fragments, described in Section 2.4.1. In addition, the following eight polymers were targeted to provide an overview of common polymer types associated with microplastic contamination:

- Polyethylene (PE)
- Polypropylene (PP)
- Polystyrene (PS)
- Polyvinyl chloride (PVC)
- Polyethylene terephthalate (PET)
- Polyamide 6 (PA6)
- Polymethyl methacrylate (PMMA)
- Polycarbonate (PC)

For both targeted and non-targeted approaches, a subsample of the filter papers produced for the microplastic analysis (shown in Figure 14) was taken by slicing a quarter of the total filter paper area. This was folded and sealed in aluminium foil for transport to Eurofins. This subsampling was necessary as there are limits of the total amount of glass fibre filter that can be dissolved during the sample preparation stage for Py-GCMS analysis. To improve the outcome of the Py-GCMS analysis for qualitatively indicating the presence of rubber compounds, the area of the filter paper that had the highest density of black rubbery fragments (identified during visual analysis) was selected for subsampling.

At Eurofins, each subsample was further subdivided so a total of one eighth of the original filter paper was subjected to analysis. This was placed into a 10 µl sample cup and digested with tetramethylammonium-hydroxide. The samples were analysed in parallel. A total of 22 samples, including blanks, were pyrolyzed at 600 °C in the pyrolysis unit before the resulting gases were analysed in the GCMS at Eurofin.

The raw data that was produced was first converted to netCDF using the data conversion package provided by the GCMS vendor (i.e. Agilent) at Eurofins and then provided to NIVA for further data treatment. The raw data in netCDF format were imported into a Matlab programming environment. To identify the presence of polymers that were not included in the targeted approach (see above list), a further 19 commonly detected polymers were selected. Their exact mass and 3–5 characteristic fragments of the monomers that can be detected using GCMS was detailed. All the samples, including the blanks, were screened for these 19 polymers using the recorded fragments in our list. We only considered a polymer to be 'potentially present' in the samples if we found the mass of the monomer and at least three fragments of that polymer in the sample data. Finally, the height of the

peak of the detected polymers were used as an indicator of their levels in the samples. It should be noted that these are tentative identifications. For further confirmation of their presence in the samples, internal standards are required. For this 'tentative indication' assessment, polymers that were identified above the levels observed in the blanks, were marked with +, ++, or +++ depending on the peak heights of the polymers in the pyrogram data generated by the GCMS.

2.6 Quality assurance and quality control (QA/QC)

The main goal of quality assurance and quality control is to ensure that the methods used to investigate microplastics in environmental samples are giving us trustworthy results. Many different considerations are important in this aspect. One of the main challenges with microplastic studies is that plastic fibres are very common all around us, even in the laboratory, and therefore it is important to ensure that these fibres do not end up in our samples, and if they do, that the results take that into account.

2.6.1 Lab contamination prevention, procedural blanks and LOD and LOQs

All sample processing was carried out in a cleaned laboratory with a HEPA filtered air inflow (H13 rating) and restricted access (following NIVA protocols). A decontamination process is performed before entry into the main laboratory space. All filtration of all bivalves occurred in a laminar flow cabinet that protects the samples from airborne contamination. The RO water used for washing and making of solutions were filtered (Millipak membrane filter; 0.22 µm), and all solutions, KOH and $CaCO_3$, used for digestion were also filtered prior to use (GF/A filter; pore size 1.6 µm).

Limit of detection (LOD) is the lowest concentration that can be detected by an instrument, and limit of quantification (LOQ) the lowest value that can be quantified. Since the method used for microplastic analysis in biota is very sensitive to contamination, procedural blanks are important. Three procedural blanks were performed to monitor procedural contamination on each day of processing, to allow for specific corrections per day. These procedural blanks were treated following an identical procedure to the biota samples, however only containing the solutions and no biota. The results were corrected by subtracting the average of the three blanks carried out on the corresponding day. Overall 54 particles were found in the 46 blanks, two particles classified as fragments and the remaining 44 were fibres. All of the fibres were composed of cellulosic material, one of the fragments were cellulosic and other was blue composed of PP, possibly derived from a bottle cap. Due to the domination of fibres, the LOD and LOQ were merged and not separated by fibres and fragments such as in earlier investigations, since the fragments did not contribute much to the overall results.

Procedural blanks can be used to determine the LOD and LOQs. There is no standardized approach to calculate LOD and LOQs for microplastic samples. Therefore, the following approach was applied.

The LODs and LOQs were calculated for the total number of blanks (n=46 for

bivalves; n=19 for Fraction A) by calculating the average microplastic number in the blanks + (3 x St.dev.), while the LOQ was set to three times the LOD. This gave a LOD of 2.77 particles and a LOQ of 8.31 (Table 4).

Table 4: Procedural blanks with corresponding LOD and LOQ for this current study for *Mytilus* spp.
All but two microplastics (MPs) were cellulosic fibres (n=54).

Average no. of MPs per blank	St.Dev	Max	Min	LOD	LOQ	n_{blanks}
0.43	0.78	3.00	0.00	2.77	8.31	46

2.6.2 Visual ID

To reduce the subjectivity by different researchers performing the visual ID, one person performed all visual analyses. Furthermore, all samples were blind labelled to prevent the observer being biased by sample locations. To ensure for contamination control, petri dish lids were only open when necessary for analysis.

2.6.3 Recovery tests

Recovery tests can be applied to understand how much, and what types, of microplastics are obtained from environmental samples with the method used. This gives an idea of how much and what kind of microplastics are likely to be extracted from environmental matrices. However, it is important to note that recovery tests are often based on virgin reference materials that have not been out in the environment and therefore tend to behave differently. This can result in the recovery tests showing an underestimation in particle recovery. On the other hand, virgin particles are easier to identify chemically, such as by FT-IR, than weathered particles since they do not have a coating of organic material, are oxidized, or so on, which can lead to an *over*estimation in the recovery test. All together it is therefore likely that the recovery test is not giving the full picture of the recovery, but it can be used as an indicator of the method applied and its accuracy for different types of microplastics.

For this current study two different recovery tests were performed, one for KOH method used for *Mytilus* spp. and one for KOH plus acetic acid used for the small bivalves.

Mytilus spp. -KOH

Reference particles were directly spiked into the samples to ensure efficient transfer of material. The test was done with or without mussel tissue, at different temperatures (60 or 40°C) and for different incubation times (24, 48 or 72 hours). The mussels used were commercially available mussels that had been depurated (gut cleared). The reference microplastics were counted onto a spatula under a Nikon SMZ 745T stereomicroscope at 20x magnification. They were then placed

directly into each control sample. The spatula was inspected for any residual particles, which were washed into the control sample if present. All beakers were spiked with four types of reference particles available in-house at NIVA, in the same beaker with ten particles of each type (Figure 16 and Figure 17):

1. **PET fibres** – Orange polyester fibres (101–2194 µm in length; IQR: 493–992 µm) were produced by washing blankets ('Skogsklocka', IKEA, Norway) in a clean washing machine system (Candy Smart, model no. CS 1692D3-S). Fibres were filtered from the laundry effluent and dried before use. Density: ~0.96 to 1.45 g/cm3 (Zhao *et al.,* 2018)

2. **Tyre particles** between 250–500 µm were obtained from Genan, Denmark. This material is generated during the recycling of end-of-life passenger car tyre. The material was sieved to obtain the fraction 250–500 µm and purified to remove residual metal and fibre contamination from the tyre recycling process. Approximate density: 1.16 g/cm3

3. **PVC fragments** were obtained from Goodfellow, UK. These were sieved to isolate particles between 150–250 µm. Approximate density: 1.38 g/cm3

4. **PET fragments** were also obtained from Goodfellow, UK. These were sieved to obtain the 250–300 µm fraction. Approximate density: 1.38 g/cm3

In general, the highest recovery rate for the four polymers tested were obtained in presence of biota, with an average recovery of 74 ± 10% (ranging between 58 to 92%), when removing the polyester (PET) fibres (see appendix). The test also displayed that tyre particles had the highest recovery rate (81 ± 15%) across all treatments tested (w./wo biota, temperature and incubation time). The highest loss of reference materials was identified for the PET fibres, displaying a high variability of recovery across the different treatments tested. The inconsistent number of fibres recovered amongst the different treatments suggests other factors such as precision during polymer spike-in, polymers stuck to the incubation vessel, insufficient rinsing and/or filtering of the samples may introduce the source of variability. Fibres have previously been suggested to be more challenging to recovery in both lab-based recovery tests (Thiele *et al.*, 2019). For the two other fragments tested, PVC and PET, the particles were recovered at 71 ± 7% and 67 ± 8%, respectively across all biota treatments.

The recovery rate of polymers was comparable to previously published studies (Karami *et al.*, 2017; Thiele *et al.*, 2019).

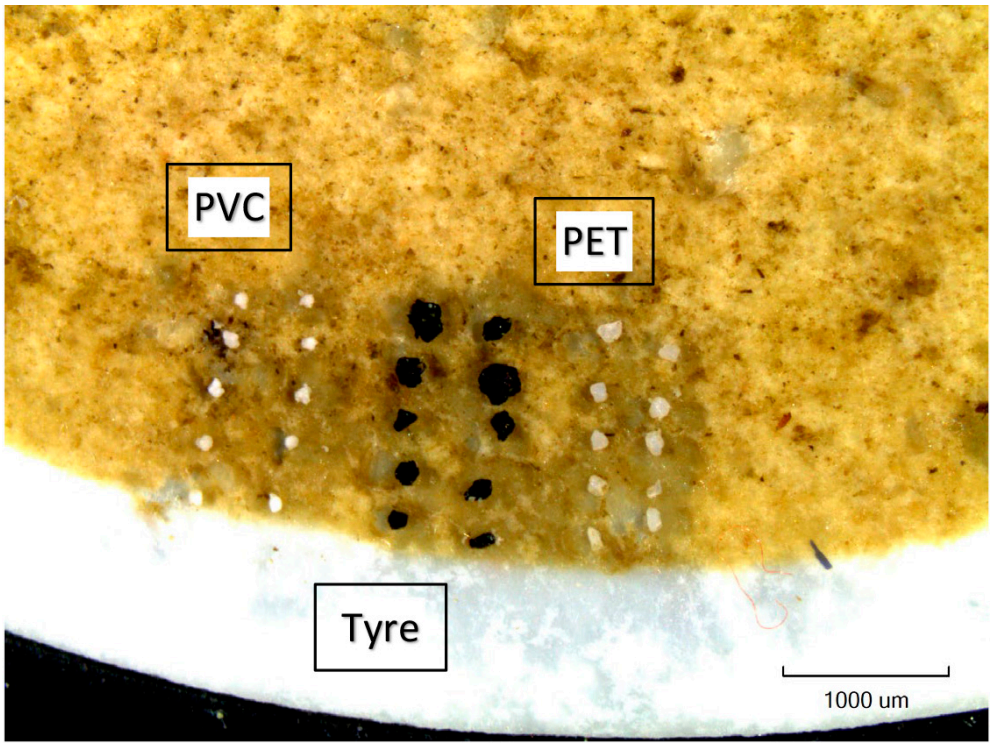

Figure 16: Illustration from recovery test with *Mytilus* spp. tissue present. Ten particles of PVC fragments, tyre particles and PET fragments. One PET fibre is also present in the lower right corner.

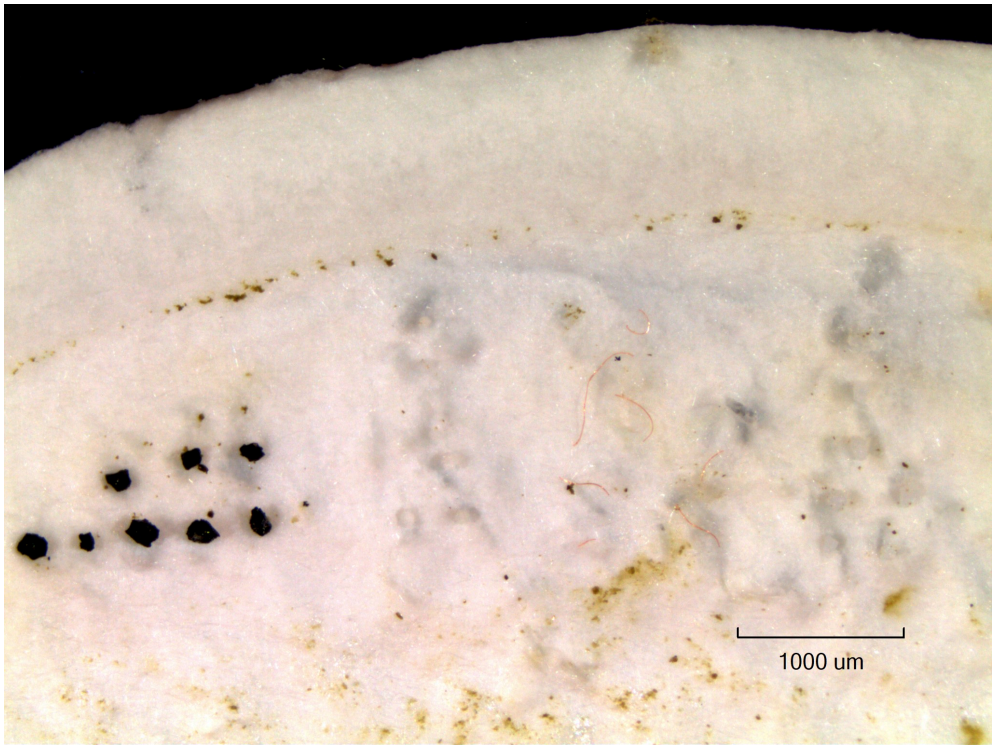

Figure 17: Illustration from recovery test with *Mytilus* spp. tissue present and seven PET fibres.

Other bivalves – KOH + acetic acid

A qualitative recovery test (non-numerical) was applied to test effects of sample treatment (method used for the smaller bivalves) on spiked reference materials. In this test, however, no biotic material was present. This was to see the 'worst case scenario' of the treatment with no 'protection' by biotic material. Acetic acid was chosen as a digestion agent as it has previously shown in various 'chemical resistant tables' to have little/no corrosion on polymers when tested at a maximum concentration of 5% at 40 degrees, incubated for up to 30 days. Both KOH and acetic acid can be corrosive on polymers, but concentrations of 10% KOH and 5% acetic acid had no visual qualitative effect (inspection of FT-IR spectra) on seven reference polymers Table 5 (see also Table 18 in Appendix 7.2). Since no effects were anticipated, and due to the previous KOH test applied for KOH, the current recovery test was not based on numerical particle recovery, but rather spiked based on mg (and sometimes particle numbers were given), and the results were investigated by inspecting the FT-IR data. Based on this recovery test, no qualitative effects were found on the tested polymers.
The particles tested were[1] :

1. **PP pellets**, 3 mm – 0.153 gram (5 particles). Approximate density: 0.83 to 0.85 g/cm3

2. **PA-66** (nylon) pellets – 0.07 gram (5 particles). Density: 1.13 g/cm3 (Zhao *et al.,* 2018)

3. **LDPE** – 0.142 gram (5 particles). Approximate density: ~ 917 to 0.93 g/cm3

4. **PET fibres** – see bivalve recovery test for info of the particles. 0.0067 gram (unknown particle number)

5. **PET fragments** between 150 – 250 µm; 0.014 gram (unknown particle number). Approximate density: 1.38 g/cm3

6. **PVC fragments** between 150 and 250 µm; 0.029 gram (unknown particle number). Approximate density: 1.38 g/cm3[3]

7. **Tyre particles** see bivalve recovery test for info of the particles. 0.0175 gram (unknown particle number). Approximate density: 1.16 g/cm3

1. Majority of densities from Zhao *et al.,* 2018, the rest from technical information following the reference materials.

Table 5: Polymers tested for impact of KOH and acetic acid.

No	Abbreviations	Shape	Recovered	FT-IR assessment (Impact yes/no)
1.	PP	Fragments	Yes	No
2.	PA66	Fragments	Yes	No
3.	LDPE	Fragment	Yes	No
4.	PET	Fibres	Yes	No
5.	PET	Fragment	Yes	No
6.	PVC	Fragment	Yes	No
7.	Rubber (Tyre)	Fragments	Yes	No

3. Results

3.1 Mussels (Mytilus spp.)

When studying microplastics in marine organisms, some of the important objectives are to address the distribution of microplastics in a region, especially areas that are most impacted. In this study, microplastics were found in Mytilus spp. (which included the common blue mussel) from 11 out of 29 sites. These sites where either in areas with a lot of human activities, or near harbours. Akershuskaia, in the inner Oslofjord, had the highest number of microplastics.

3.1.1 Quantitative microplastics results

A total of 545 mussels were investigated for microplastics, spread across 29 sites in the Nordic environment. Mussels with microplastics above the LOD were found in individuals at 11 out of 29 sites when expressing results as microplastics (MPs) per individual (Figure 18, Table 7). In Table 7, the yellow shading highlights individuals above the LOD (2.77 MPs per individual), whilst green highlights values above the LOQ (8.31 MPs per individual). All values below the LOD were sat to 0. For the full dataset with levels also below the LOD, see Table 19 in Appendix 7.3.

Sites exceeding the LOD with average values of microplastic per individual above 1 (after blank correction), in descending order were (mean ± SD):

- M-19: Akerhuskaia from the inner Oslofjord (North Sea; Norway), with an average of > 61 (± SD 75) MPs per individual
- M-14: Færder in outer Oslofjord (North Sea; Norway) with an average of 6 (±13) MPs per individual
- M-16: Bodø harbour (Norwegian Sea; Norway) with an average of 3.1 (±5.6) MPs per individual
- M-22: Outside Stockholm (Baltic Sea; Sweden) with an average of 1.98 (±3.84) MPs per individual
- M-15: Hanstholm (North Sea; Denmark) with an average of 1.1 (±3.48) MPs per individual

The other sites with *Mytilus* spp. individuals containing microplastics above LOD had an average of less than 1 MPs per individual:

- M-21: Hvite Sande (North Sea; Denmark)
- M-4: Hirtshals (Skagerrak; Denmark)
- M-9: Bolungarvik harbour, (Norwegian Sea; Iceland)
- M-11: Outer Trondheimsfjorden (Norwegian Sea; Norway)
- M-18: Singlekalven, Outer Oslofjord (North Sea; Norway)
- M-10: DKE1, Øster Hurup (Kattegat; Denmark)

Overall, the *Mytilus* spp. results indicate that urbanized areas, not surprisingly, had the highest levels of microplastics. The highest microplastics levels in mussels were observed at Akerhuskaia (M-19) in the Oslofjord, with an average of more than 61 MPs per individual. This high average value is despite the exclusion of a large number of particles (>100) characterised as black rubbery fragments that exceeding the upper threshold for quantification and full characterisation. Based on the statistical analysis (Table 6), M-19 was significantly different from half of the other sites: M-16, M-22, M-4, M-9 and M-18. These high levels in mussels from Akershukaia were also seen in previous years, as published in Lusher *et al.*, 2017a and Bråte *et al.*, 2018. The site with the second highest microplastic values were Færder (M-14) from the Outer Oslofjord; however, this site was not significantly different from any other site. Despite this, it seems that the Oslofjord, and especially Akerhuskaia, is of special concern when it comes to microplastic pollution, particularly given that this finding has persisted across several investigations. In addition to urban diffuse sources, point sources of microplastics may also be the cause for these elevated levels at these sites. For more in depth-discussion see section 3.1.5. Other sites with relatively high values of microplastics, despite not being significantly higher than other sites, were found in mussels from Bodø harbour from the Norwegian sea, in the Baltic Sea outside of Stockholm and Hanstholm at the west coast of Denmark (Skagerrak). The rest of the sites with mussels containing microplastics above LOD, are relatively urban sites, with a possible exception of Bolungarvik harbour at the west coast of Iceland which is impacted by harbour activity alone.

Microplastics were not found above LOD in mussels from Svalbard, Faroe Islands nor Greenland at the sites investigated. Similar overall results were also found when expressing the results as microplastics per gram d.w. tissue (Figure 45 in Appendix 7.4).

Table 6: Results from a Tukey-Kramer HSD test on log transformed data of the number of microplastics (MPs) at the different sites. Levels not connected by same letters are significantly different. For example, site M-19 is significantly different from M-16, M.22, M-4, M-21, M-9 and M-18.

Site	Country	Name			Mean log MPs
M-19	Norway	Akerhuskaia	**A**		4.39
M-14	Norway	Færder	**A**	**B**	3.01
M-15	Denmark	Hanstholm	**A**	**B**	2.29
M-16	Norway	Bodø harbour		**B**	2.21
M-22	Sweden	Outside Stockholm		**B**	2.00
M-10	Denmark	Øster Hurup	**A**	**B**	1.39
M-4	Denmark	Hirtshals		**B**	1.30
M-11	Norway	Outer Trondheims-fjorden	**A**	**B**	1.30
M-21	Denmark	Hvite Sande		**B**	1.29
M-9	Iceland	Bolungarvik harbour		**B**	1.10
M-18	Norway	Singlekalven		**B**	1.10

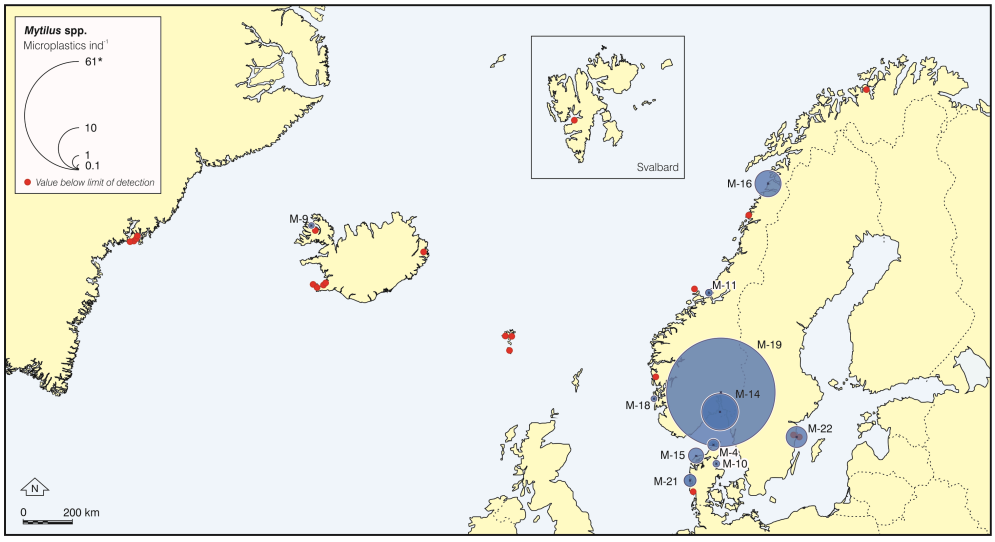

Figure 18: Average number of microplastics (MPs) per individual of *Mytilus* spp. Blue colour indicates *Mytilus* spp. individuals at sites with values above the LOD (2.77 MPs per individual) and the size is relative to the number of MPs. Red sites were studied, but values were below the LOD. For more information of sites see appendix. Asterisk * refers to upper quantification limit for particles at site M-19

Table 7: Microplastics (MPs) per individual above the LOD. Yellow colour indicates values above the LOD (2.77 MPs per individual) and green above the LOQ (8.31 MPs per individual). All values below the LOD were set to 0. The values are blank corrected.

Country	Norway						Sval-bard	Iceland							Faroe Islands			Greenland				Denmark					Sweden		
Site	M-11	M-12	M-14	M-16	M-18	M-19	M-17	M-26	M-27	M-30	M-2	M-13	M-7	M-9	M-3	M-20	M-6	M-28	M-29	M-1	M-5	M-8	M-21	M-10	M-15	M-4	M-22	M-23	M-24
Ind 1	0	0	22.67	0	0	4.00	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ind 2	3.67	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ind 3	0	0	44.67	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	4.00	0	0	0	5.67	0	0
Ind 4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ind 5	0	0	17.67	17.30	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	13.34	3.67	0	0	0
Ind 6	0	0	6.67	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ind 7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	7.34	3.67	0	0	0
Ind 8	0	0	28.67	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	11.67	0	0
Ind 9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ind 10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	9.67	0	0
Ind 11	0	0	0	0	0	164.00	0	0	0	0	0	0	0	0	0		0	0	0	0	0	0	0	0	0	0	0	0	0
Ind 12	0	0	0	8.30	0	148.00	0	0	0	0	0	0	0	0	0		0	0	0	0	0	0	4.00	0	0	0	0	0	0
Ind 13	0	0	0	8.30	0	148.00	0	0	0	0	0	0	0	0	0		0	0	0	0	0	0	3.00	0	0	3.67	0	0	0
Ind 14	0	0	0	3.30	0	148.00	0	0	0	0	0	0	0	0	0		0	0	0	0	0	0	0	0	0	0	0	0	0
Ind 15	0	0	0	10.30	0	148.00	0	0	0	0	0	0	0	0	0		0	0	0	0		0	0	0	0	0	4.67	0	0
Ind 16	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		0	0	0	0		0	0	4	0	0	0	0	0
Ind 17	0	0	0	0	0	148.00	0	0	0	0	0	0	0	0	0		0	0	0	0		0	0	0	0	0		0	0
Ind 18	0	0	0	14.30	3.00	148.00		0	0	0	0	0	0	0	0		0	0	0	0		0		0	0	0		0	0
Ind 19		0	0	0	0	11.00		0	0	0	0	0	0	0	0		0	0	0			0		0	0	0		0	0
Ind 20		0	0	0	0	148.00		0	0	0	0		0	0	0		0	0				0		0	0			0	0
No of ind	18	20	20	20	20	20	15	20	20	20	20	19	20	20	20	10	20	20	19	18	14	20	17	20	20	19	16	20	20
Mean MPs	0.20	0.00	6.02	3.14	0.15	60.75	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.15	0.03	0.12	0.10	0.00	0.00	0.08	0.05	0.00	0.65	0.20	1.034	0.58	1.98	0.00	0.00
St.Dev	0.87	0.00	13.02	5.64	0.71	75.30	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.71	0.08	0.15	0.16	0.00	0.00	0.15	0.12	0.00	1.46	0.94	3.48	1.41	3.84	0.00	0.00
Max	4	0	45	17	3	164	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	4	4	13	4	12	0	0
Min	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Average blank (3 reps)	0.33	0.33	0.33	0.70	0.00	2.00	2.00	0.00	0.00	0.00	0.00	2.00	0.00	2.00	0.66	0.70	0.66	0.00	0.00	0.66	0.66	0.00	0.00	0.00	0.66	0.33	0.33	0.33	0.00

3.1.2 Influence of *Mytilus* spp. size and number of microplastics

Mytilus spp. size, in terms of both length and weight, varied between the sites (Figure 19). It is an advantage when performing monitoring to aim for sampling mussels that are approximately the same size. However, due to large variation in *Mytilus* spp. size between different Nordic sample areas, it was difficult to define a size for sample collection. The largest mussels processed were mussels from one site in the Faroe Islands (M-20), while M- 14, M-15 and M-19 also were relatively large. All of these sites had mussels with microplastic levels above the LOD, except from M-20. The smallest mussels were from the Baltic Sea (M-22, 23 and 24), where it is challenging to sample mussels suitable for monitoring since they are small and not highly abundant in number. For the five sites that contained the highest number of microplastics (more than 1 MPs per individual), i.e. M-19, M-14, M-16, M-22 and M-15, the number of microplastics per individual was plotted against the size of the mussels (gram w.w and length in mm) to see if there were any impact of size of the individual on microplastic content. Based on this analysis, Figure 20 A and B**,** it was evident that there was no impact of *Mytilus* spp. size (weight nor length) on the microplastic levels. This suggests that *Mytilus* spp. size is not the driving factor for microplastic intake, rather the level of exposure is likely to be a more important factor.

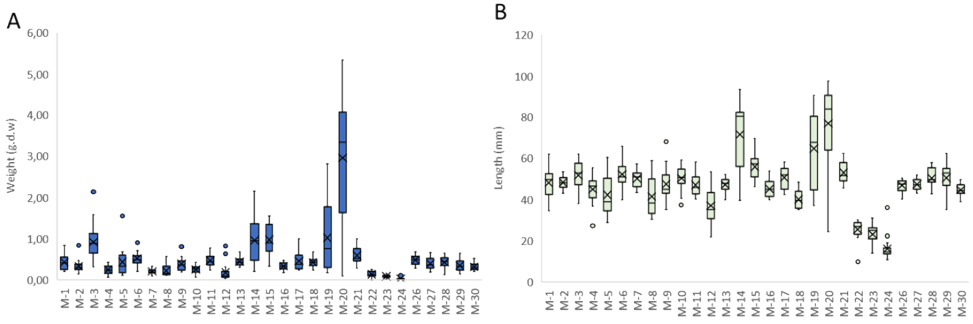

Figure 19: Box and whisker plots of *Mytilus* spp. Data displaying, A: dry weight, and B: length of individuals. Outliers are represented as dots. Most of the sites are based on n=20 individuals, for full information see Table 20 and Table 21 in Appendix 7.3.

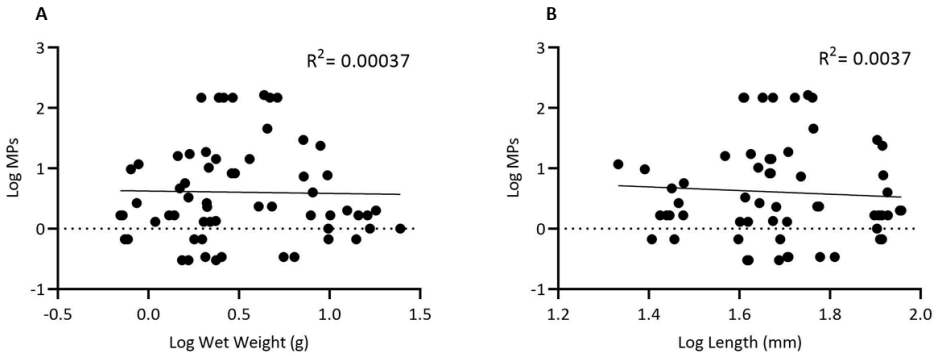

Figure 20: Microplastics (MPs) presented as log-MPs in mussels from the five sites (M-19, M-14, M-16, M-22 and M-15) above LOD that exceeded 1 MPs per individual plotted against A: log wet weight (g) and B: log length (mm). R^2: R-squared - Coefficient of determination. See site locations in Figure 7.

3.1.3 Microplastic morphology (size, shape and polymeric composition)

Another important aspect of microplastic studies is to understand what type of microplastics are found, their characteristics; their shape (round, irregular, fibres), their size and what material they are made of. In this way it may be possible to come closer to understand the pollution source. Most of the microplastics found in this study were small black fragments or sausage-shaped particles, probably composed of rubber and suggested to be derived from road-run off or harbour activities. As for most other MP studies, more MP were found in the smallest analysed size classes.

Most microplastics detected in this study were fragments, accounting for 87% of the overall number, whilst fibres accounted for the remining 13% (Table 8 and Figure 21). Only two sites were dominated by fibres: site M-11 in the Outer Trondheimsfjord (Norway) and M-10 from Øster Hurup (Denmark). This contradicts previous *Mytilus* spp. data from the Norwegian environment (Lusher *et al.*, 2017a; Bråte *et al.*, 2018) and other international studies that have also found that fibres dominate in biota samples (Rezania *et al.*, 2018). There might be several reasons for this difference. This could represent a qualitative difference in the type of microplastics released into the sea, such as fibre release from WWTPs. This is, however, not likely to be the case since so many sites had a domination of fragments and, to our knowledge, no specific large-scale measures for a reduction in fibre release have been implemented. More credible reasons are therefore:

- A higher FT-IR coverage with 100% of the particles subjected to FT-IR analysis[2] leading to improved identification (and exclusion from the dataset) of more natural fibres such as cotton or wool which typically visually resemble plastic fibres during visual analysis

2. Excluding rubbery particles.

- This could be a reflection of the strict, and continuously improved procedures for contamination prevention during analysis

- The exclusion of sites with microplastic levels below the LOD. A general observation was that fibres dominated in sites with microplastic values lower than the LOD

- A generally lower recovery rate in this study, as seen from section 2.6.3, for fibres than for fragments

The size of microplastics detected in mussels from the Nordic environment ranged from 34 µm to 7386 µm (average: 300 µm), Table 9. Most microplastics found in mussels were below 130 µm in their longest dimension (Figure 22) with an average of 158 µm when including only microplastics below 1000 µm ($n_{particles}$=571) and excluding particles above 1000 µm ($n_{particles}$=33). We believe that 158 µm as an average represents a superficial assessment of microplastics found in this study based upon the significant skew in the particle size distribution A left skewed size distribution is in accordance with other microplastic studies, including the previous studies of microplastics in mussels from the Norwegian environment (Lusher *et al.*, 2017a and Bråte *et al.*, 2018). One study carried out in England saw that there was a significant difference between the fragment size in mussels and surrounding sea water, compared to the surrounding sediment, where sediments contained larger fragments. However, there was no significant difference between the size of fibres found in mussels compared to sediment (Scott *et al.*, 2019). The microplastic size in an organism may not always directly proportional to what is in their surrounding environment, which is an issue that needs to be further investigated.

There were significant differences between microplastic sizes at different sites. Site M-7, M-21, M-11, M-4, M-10 and M-9 had significantly larger microplastics than the other sites (with the exception of M-18). Site M-19 (Akershuskaia) and M-22 (Outside Stockholm) had significantly smaller sized particles than the rest (with the exception of site M-18) (Table 10). The reasons for the significant differences in microplastic size across sites are not understood. This could be related to large proportions of small black particles at certain sites. All the five sites with the highest levels of black rubbery fragments (M-19, M-14, M-16, M-22 and M-15) are the sites with the smallest sized particles. The two sites with the largest proportion of fibres were M-10 and M-11, which were two out of five sites with the significantly larger microplastics. Fibres are often long and tend to be longer than fragments in their longest dimension.

Table 8: Shape of microplastics (MPs) across the different sites exceeding the LOD ($n_{particles}$=601).

Site	Country	Name	Fragments (%)	Fibres (%)
M-15	Denmark	Hanstholm	100	0
M-22	Sweden	Outside Stockholm	98	2
M-14	Norway	Færder	94	6
M-19	Norway	Akerhuskaia	91	9
M-16	Norway	Bodø harbour	90	10
M-18	Norway	Singlekalven	75	25
M-21	Denmark	Hvite Sande	67	33
M-9	Iceland	Bolungarvik harbour	63	37
M-4	Denmark	Hirtshals	45	55
M-11	Norway	Outer Trondheims-fjorden	25	75
M-10	Denmark	Øster Hurup	11	89
Total			**87**	**13**

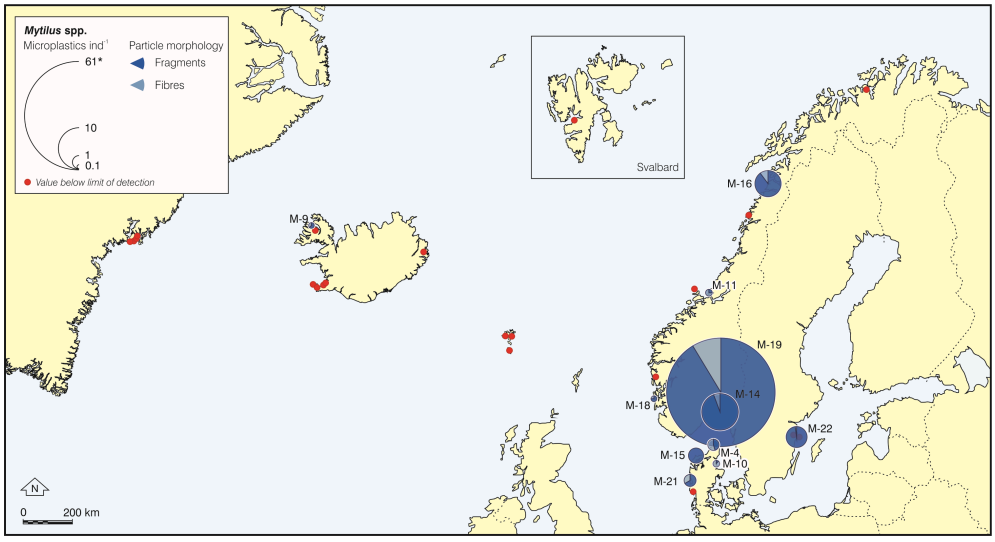

Figure 21: Morphology of microplastics (MPs) found in *Mytilus* spp. from the Nordic environment. Dark blue colour illustrates fragments, while lighter blue indicates fibres. Asterisk * refers to upper quantification limit for particles at site M-19.

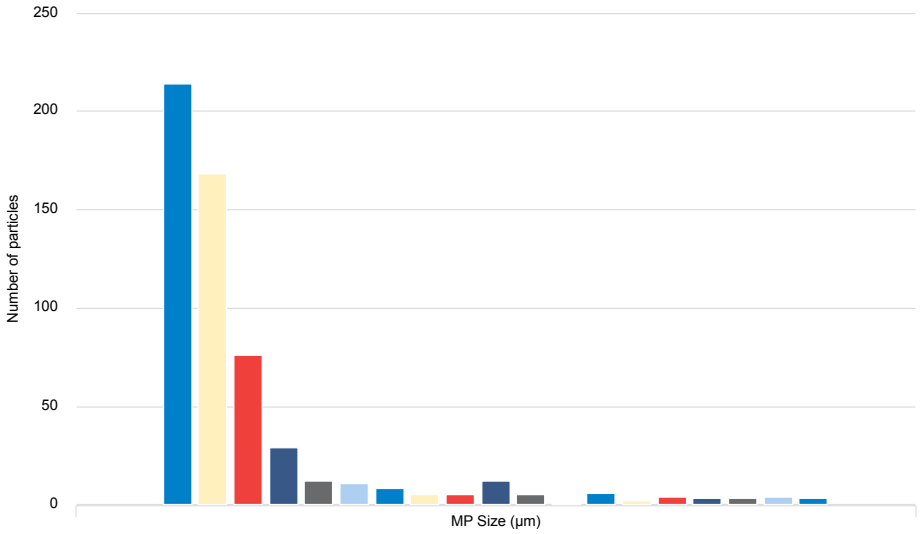

Figure 22: Size distribution of microplastics from sites above the LOD in *Mytilus* spp. from the Nordic environment. Microplastics below 1000 µm are included (n=571), while particles above 1000 µm up to 7386 µm (n=33) are excluded. The average size of microplastics was 158 µm. For the full data set, see appendix.

Table 9: Size of the microplastics (MPs) found in mussels at the sites exceeding the LOD.

				Longest dimension of MPs (µm)			
Site	**Country**	**Name**	**n-particles**	**Mean**	**St.dev**	**Min**	**Max**
M-11	Norway	Outer Trondheims-fjorden	5	1680	2290	138	5474
M-21	Denmark	Hvite Sande	18	1450	2042	161	7327
M-4		Hirtshals	22	899	1515	105	6952
M-9	Iceland	Bolungarvik harbour	27	892	1495	115	7386
M-10	Denmark	Øster Hurup	9	714	877	86	2743
M-16	Norway	Bodø harbour	59	309	841	30	6009
M-18		Singlekalven	4	271	320	80	749
M-15	Denmark	Hanstholm	34	168	90	61	496
M-14	Norway	Færder	158	164	243	42	2064
M-19		Akerhuskaia	208	135	233	34	1662
M-22	Stocholm	Outside Stockholm	46	99	68	35	416

Table 10: Results from a Tukey-Kramer HSD test on log transformed data of the microplastic sizes at the different sites. Sites not connected by same letters are significantly different. For example, sites M-21, M-11, M-4, M-10 and M-9 are significantly different from sites M-15, M-16, M-14, M-22 and M-19, but not from each other.

Sitesl	Country	Name				Mean µm (log)
M-21	Denmark	Hvite Sande	**A**			6.525505
M-11	Norway	Outer Trondheims-fjorden	**A**			6.506571
M-4	Denmark	Hirtshals	**A**			6.112987
M-10		Øster Hurup	**A**			6.073456
M-9	Iceland	Bolungarvik harbour	**A**			6.04526
M-18	Norway	Singlekalven	**A**	**B**	**C**	5.168594
M-15	Denmark	Hanstholm		**B**		5.011694
M-16	Norway	Bodø harbour		**B**		4.852382
M-14		Færder		**B**		4.795171
M-22	Sweden	Outside Stockholm		**B**	**C**	4.454091
M-19	Norway	Akerhuskaia			**C**	4.43451

3.1.4 Pyrolysis gas chromatography mass spectrometry (Py-GCMS)

Targeted approach

Since many methods are needed to find out what material microplastics are made of, mass estimations were also applied to some mussel samples. This showed us that for three of the sites where rubber particles were found, Mytilus spp. had measurable levels of two compound that are found in rubber. This increases the confidence that rubber is being detected in mussels from these sites. For other common plastics, no measurable levels were detected with this method.

Spectroscopic methods such as Fourier transform infrared (FT-IR) scanning of particles has its limitations, e.g. not providing mass information. Pyrolysis gas chromatography mass spectrometry (Py-GCMS) can provide such mass information (Löder & Gerdts 2015) and mass spectrometry methods can therefore be a good supplement, or addition, to FT-IR analysis. Mass spectrometry methods will also remove the need for manual hand-picking of particles, as well as the visual pre-identification step which can introduces human bias. However, the methods are not

yet fully developed for the heterogenous group of microplastics, and they tend to have quite high detection limits for many polymer types. One will also lack the morphological characteristics of microplastics that can be useful in a number of applications, e.g. a toxicological perspective or for source tracing. Black rubbery fragments are hard to analyse by FT-IR due to their high content of carbon black. Some selected *Mytilus* spp. samples were sent to Eurofins for Py-GCMS. The ten samples (ten GF/A filers representing one individual each) were chosen since they contained rubbery fragments and gave a good overall geographic distribution amongst the Nordic *Mytilus* spp. samples; M-19 (Akershuskaia; two individuals), Bodø harbour (M-16; two individuals), Færder (M-14; one individual), Bolungarvik harbour (M-9; one individual), Hanstholm (M-15; two individuals) and outside Stockholm (M-22; two individuals).

Isoprene and/or butadiene, substituents in rubber, were detected above the LOQ in mussels from three sites; Akershuskaia (isoprene and butadiene), Færder in the Oslofjord (butadiene) and Bolungarvik harbour at Iceland (butadiene) (Table 11). No other polymers above 100 µg/kg were detected with the current pyrolysis method. However, the results might have been interfered by organic material that in some samples had (based on Eurofins comments) strong peaks that could potentially mask plastic polymeric materials.

Table 11: Results from the targeted pyrolysis gas chromatography mass spectrometry (Py-GCMS) of ten mussels individuals for rubbers plus eight polymers; polyethylene (PE); polypropylene (PP); Polystyrene (PS); Polyvinylchloride (PVC); Polyethylene terephthalate (PET); Polyamide6/nylon (PA6); poly(methyl methacrylate) (PMMA); Polycarbonate (PC). The highlighted indicates that markers of rubbers were detected.

Site	Name	Ind no.	Parameter	Results (µg/kg)	LOQ
M-19	Akershuskaia; Norway	I	PE; PP; PS; PVC; PET; PA6; PMMA; PC	<100	100
			Indication of rubber; isoprene	0.45	ABOVE
		II	PE; PP; PS; PVC; PET; PA6; PMMA; PC	<100	100
			Indication of rubber; butadiene	1.2	ABOVE
			Indication of rubber; isoprene	0.86	ABOVE
M-16	Bodø harbour; Norway	I	PE; PP; PS; PVC; PET; PA6; PMMA; PC	<100	100
		II	PE; PP; PS; PVC; PET; PA6;	<100	100

			PMMA; PC		
M-14	Færder; Norway	I	PE; PP; PS; PVC; PET; PA6;P MMA; PC	<100	100
			Indication of rubber; butadiene	7.44	ABOVE
M-9	Bolungarvik harbour, Iceland	I	PE; PP; PS; PVC; PET; PA6; PMMA; PC	<100	100
			Indication of rubber; butadiene	5.58	ABOVE
M-15	Hanstholm, Danmark	I	PE; PP; PS; PVC; PET; PA6; PMMA; PC	<100	100
		II	PE; PP; PS; PVC; PET; PA6; PMMA; PC	<100	100
M-22	Baltic sea, Stockholm Sweden	I	PE; PP; PS; PVC; PET; PA6; PMMA; PC	<100	100
		II	PE; PP; PS; PVC; PET; PA6; PMMA; PC	<100	100

Non-target approach

When scanning broader for more type of plastic materials and not only the most common ones, four types of polyesters were detected in Mytilus spp. from ten urban sites. Since they are found across all sites though, there may be a common source to the marine environment. More research should be carried out to bridge this knowledge gap.

Four additional polymers were detected using the non-targeted approach when processing the pyrolysis data: polyhydroxybutyrate (PHB), polylactic acid (PLA), polycaprolactone (PCL) and polyethylene naphthalate (PEN) (Table 12). All of these are types of polyester. Polyester refers to a broad category of polymer types, of which PET is most common (used to produce plastic bottles and most polyester clothing). PHB, PLA and PCL were found in almost all samples except the second replicate of individual 7. PEN was found in seven of the individuals.

PHB is a type of biodegradable polyester which is used in both biodegradable/ compostable consumer products and some medical applications. PLA is another type of polyester and is often produced based on fermented plant starch such as from corn, cassava, sugarcane or sugar beet pulp. PLA has been used to produce a range of bio-based consumer products. PCL is also a biodegradable polyester and

was the earliest available synthetic polymer, while PEN polyester is similar to PET but with a higher temperature resistance. To our knowledge, these polymers have not been detected in environmental samples. Their specific source in this case is not known, but they must be abundant in environmental samples since they occur in all of the 'hot-spots' that were analysed in this study. However, results from the non-target sample analysis are relatively uncertain and only are an *indication* of the presence of PHB, PLA, PCL and PEN. Further confirmatory analyses are required to verify the occurrence of these polymer types in mussels from the Nordic environment.

Table 12: Non-targeted data treatment of pyrolysis gas chromatography mass spectrometry (Py-GCMS). +: ++: +++: means the levels of signal therefore indirectly the quantity of the plastics in the samples. Coloured field highlights polymers that were detected above the LOD.

		NORWAY											ICELAND		DENMARK			SWEDEN				
Polymers	Blank	M-19_1A	M-19_1B	M-19_2A	M-19_2B	M-16_1A	M-16_1B	M-16_2A	M-16_2B	M-14_1A	M-14_1B	M-9_1A	M-9_1B	M-15_1A	M-15_1B	M-15_2A	M-15_2B	M-22_1A	M-22_1B	M-22_2A	M-22_2
Polystyrene	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Butadiene	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Acrylonitrile	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Polyethylene terephthalate	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Polyglycolide	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Polylactic acid	+	++++	++++	++	++	+++	+++	+++	+++	+++	++++	++++	++++	++++	++++	++++	-	++++	++++	++++	++++	
Polycaprolactone	-	-	-	-	-	-	-	-	-	-	-	+++	+++	+++	+++	+++	-	++	++	-	-	
Polyhydroxybutyrate	-	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	+++	-	++++	++++	++++	++++	
Poly (ethylene adipate)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
POLY(3-HYDROXYBUTYRATE-CO-3-HYDROXYVALERATE)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Polytrimethylene terephthalate	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Polyethylene naphthalate	-	-	-	-	-	-	-	-	-	-	-	+++	+++	+++	+++	+++	-	++	+++	-	-	
Polyvinyl chloride	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Poly(methyl methacrylate)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4-Vinylcyclohexene	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Polycaprolactam	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Polypropylene	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Polycarbonate	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Polyethylene	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

3.1.5 Black rubbery fragments in the Nordic environment

Most of the identified particles found in mussels from this study were black rubbery fragments, most of which were found in the Oslofjord at Akershuskaia M-19 and Færder M-14 (Table 13), illustrated in Figure 25. The change in the composition of microplastics (more fragments than fibres which is a result of these black small rubbery fragments) found in mussels in current study compared to previously studies from the Norwegian environment, is discussed under section 3.1.3. The black rubbery fragments were detected at eight different sites in the Nordic coastal environment; namely, Norway (Oslofjord and Norwegian Sea; M-19, M-14 and M-16), Sweden (Baltic Sea; M-22), Denmark (Skagerrak and Kattegat; M-15, M-4 and M-10) and one site at Iceland (M-9). Mussels from less urbanised site did not contain these rubbery fragments, with exception of a harbour area in west Iceland. The black particles found at these sites resemble each other, as illustrated from Figure 26 to Figure 32 and is therefore likely to be from the same type of source or release pathway.

Table 13: Sites with *Mytilus* spp. containing black rubbery fragments in descending order of abundance.

Site code	Country	Name
M-19		Akerhuskaia
M-14	Norway	Færder
M-16		Bodø harbour
M-22	Sweden	Outside Stockholm
M-15		Hanstholm
M-4	Denmark	Hirtshals
M-10		Øster Hurup
M-9	Iceland	Bolungarvik harbour

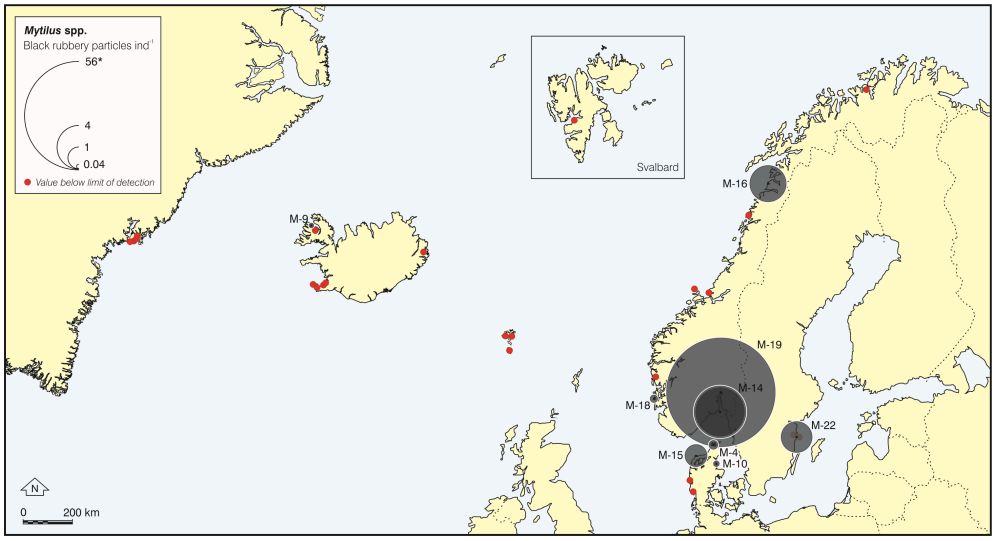

Figure 23: Occurrence of black rubbery fragments in *Mytilus* spp. from the Nordic environment. Asterisk * refers to upper quantification limit for particles at site M-19.

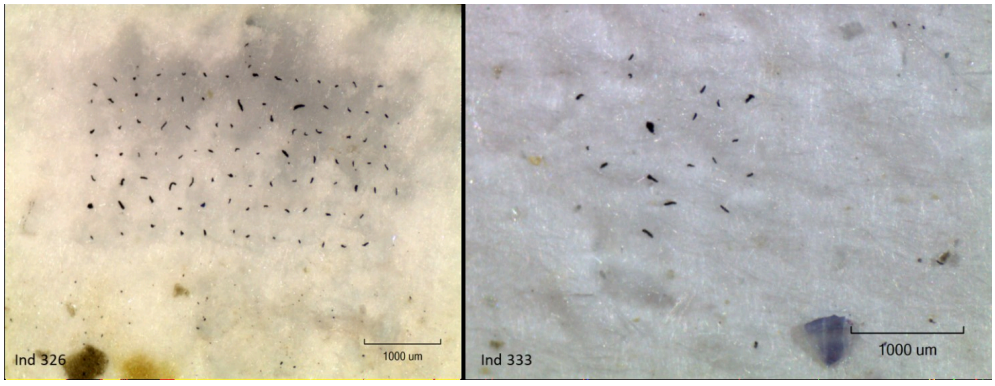

Figure 24: Examples of rubbery fragments found in two *Mytilus* spp. individuals from Akerhuskaia (M-19).

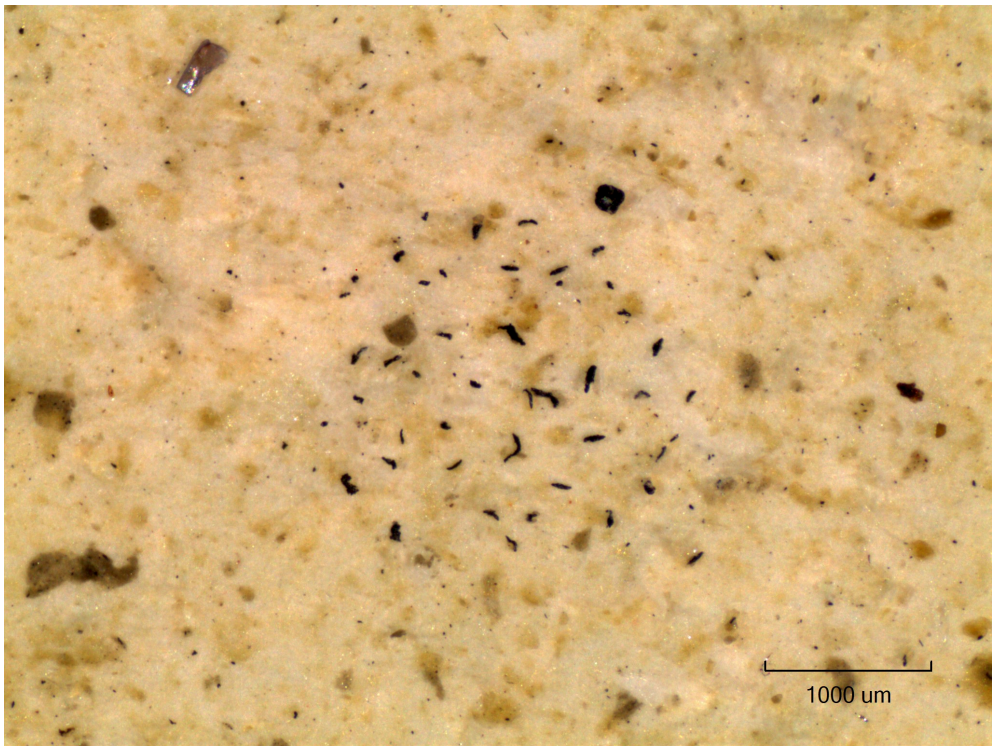

Figure 25: Examples of rubbery fragments found in one *Mytilus* spp. individual from 36A, Færder (M-14).

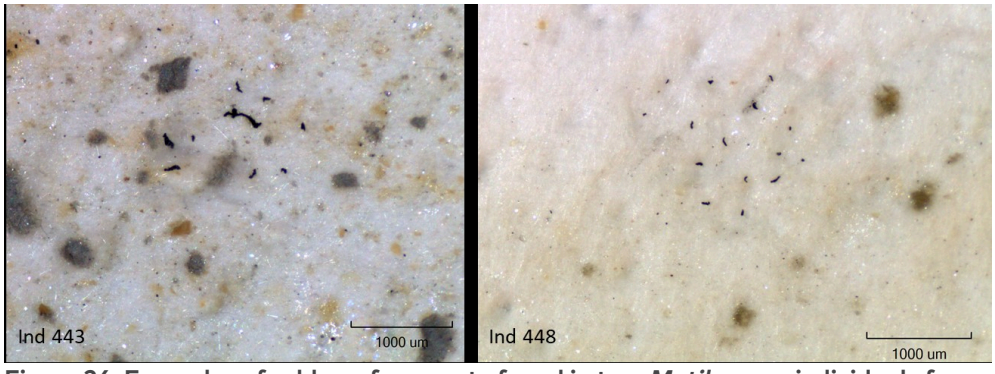

Figure 26: Examples of rubbery fragments found in two *Mytilus* spp. individuals from 97A2, Bodø harbour (M-16).

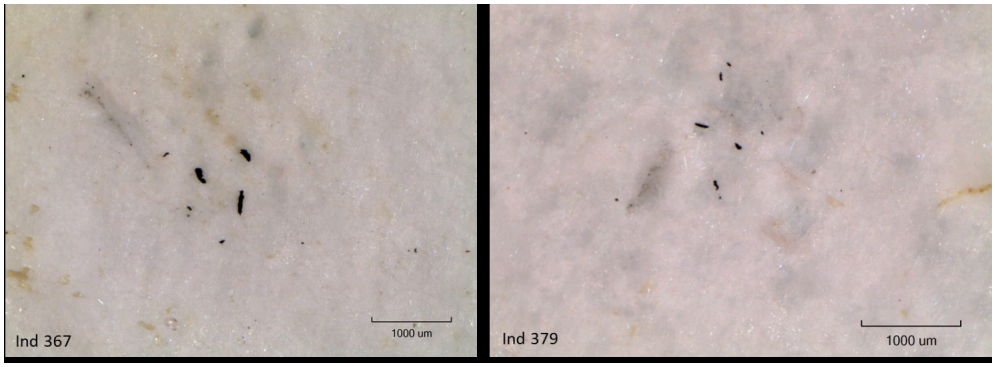

Figure 27: Examples of rubbery fragments found in two *Mytilus* spp. individuals from V4-ACES (M-22).

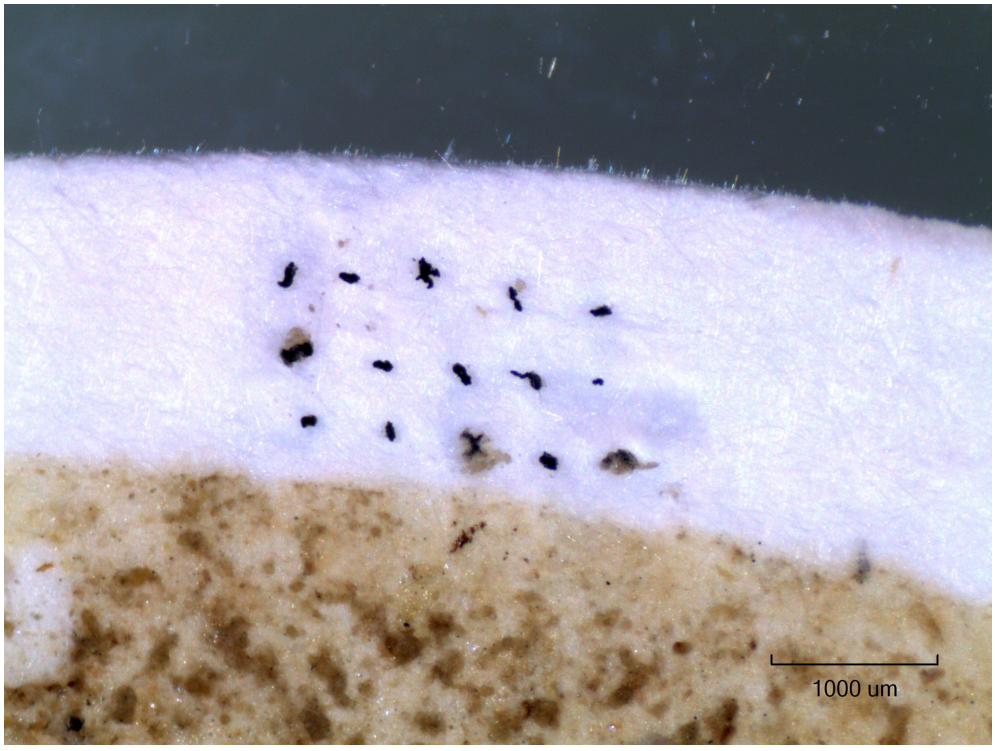

Figure 28: Examples of rubbery fragments found in one *Mytilus* spp. individual from DKW3, Hanstholm (M-15).

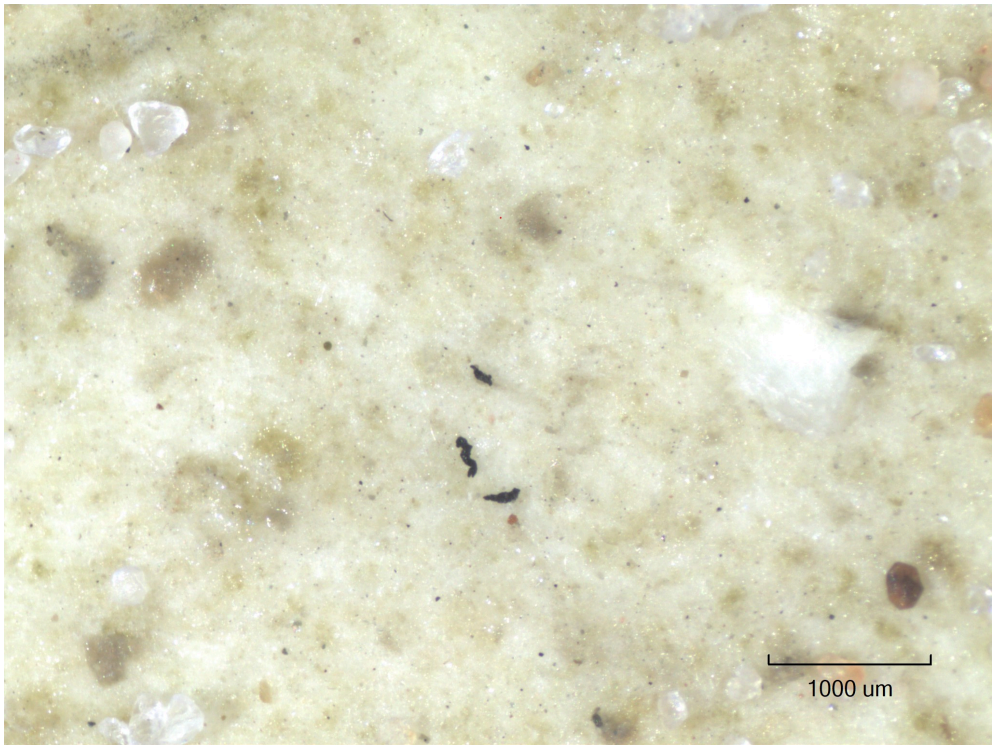

Figure 29: Examples of rubbery fragments found in one *Mytilus* spp. individual from DKW4 – Hirtshals (M-4).

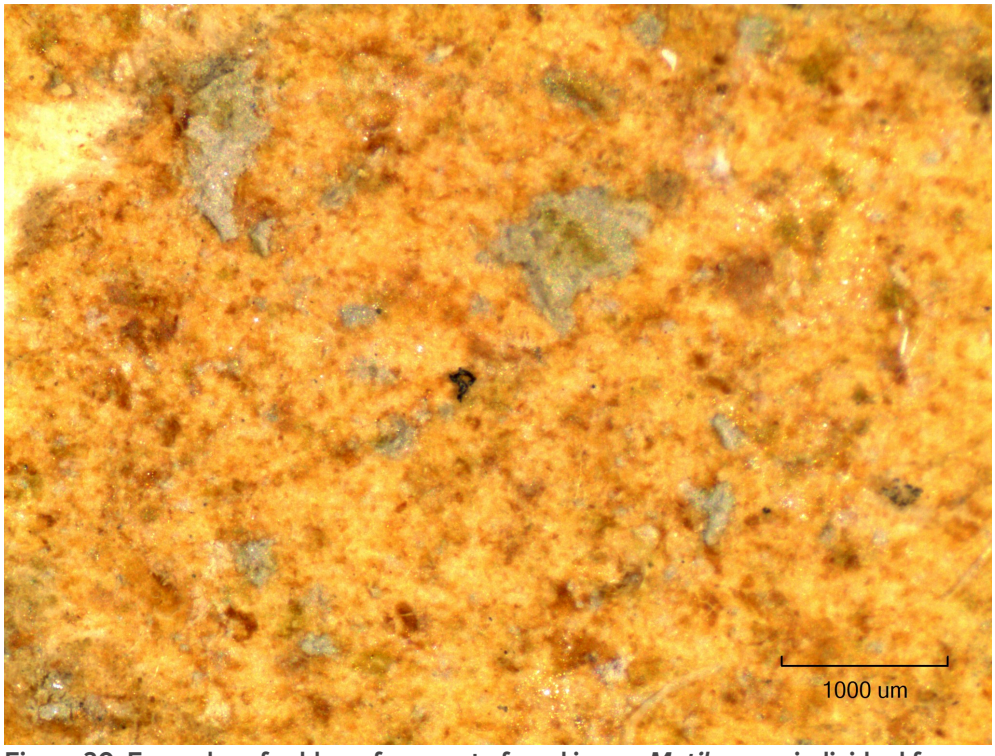

Figure 30: Examples of rubbery fragments found in one *Mytilus* spp. individual from DKE1, Øster Hurup novo strand (M-10).

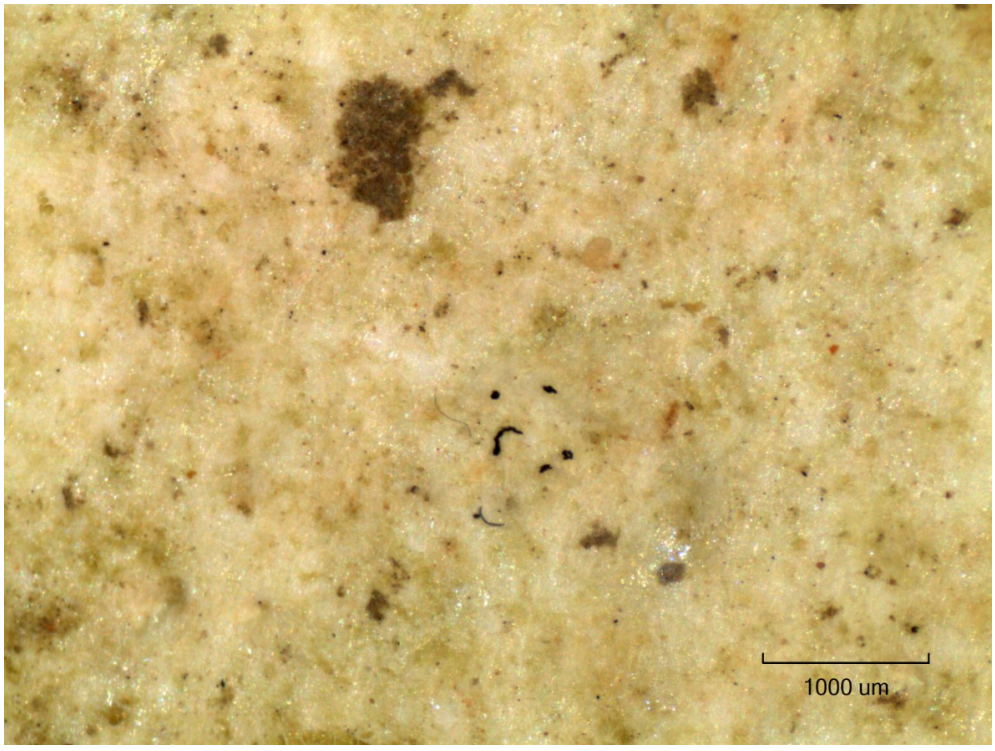

Figure 31: Examples of rubbery fragments found in one *Mytilus* spp. individual from St. IC7, Bolungarvik harbour, Iceland (M-9).

Small unidentifiable black particles were identified in water samples from the Nordic environment as early as 2009, they were considered to be of anthropogenic origin (Norén *et al.*, 2009). This was from a study conducted in Swedish waters (Baltic Sea and Kattegat) on behalf of the Swedish Environmental Protection Agency. The major component of anthropogenic particles found in the study were black rubbery fragments, illustrated in Figure 32, suggested to be from road and rubber wear. Similar particles were also detected in water samples from another study focusing on samples from Skagerrak, with a maximum load of 779 black particles per litre (Norén & Naustvoll, 2011). These studies illustrated that black rubbery fragments, of unknown origin and source, are abundant in water samples from at least some parts of the Nordic environment. Norén and Naustvoll (2011) also highlighted that the oil component in asphalt roads (bitumen) contains toxic hydrocarbons such as PAHs. This component can also be seen as having acute toxicity to the freshwater species *Daphnia magna* (Wik & Dave, 2006).

Additionally, in 2014, MEPEX published a report on behalf of the Norwegian Environment Agency that estimated that so-called road dust – microplastics derived from road associated activity – were the largest source of microplastics to the Norwegian environment (Sundt *et al.*, 2014). However, empirical data on black particles from the Norwegian environment is still lacking. Therefore, further analyses such as those performed in our study are required to fill knowledge gaps.

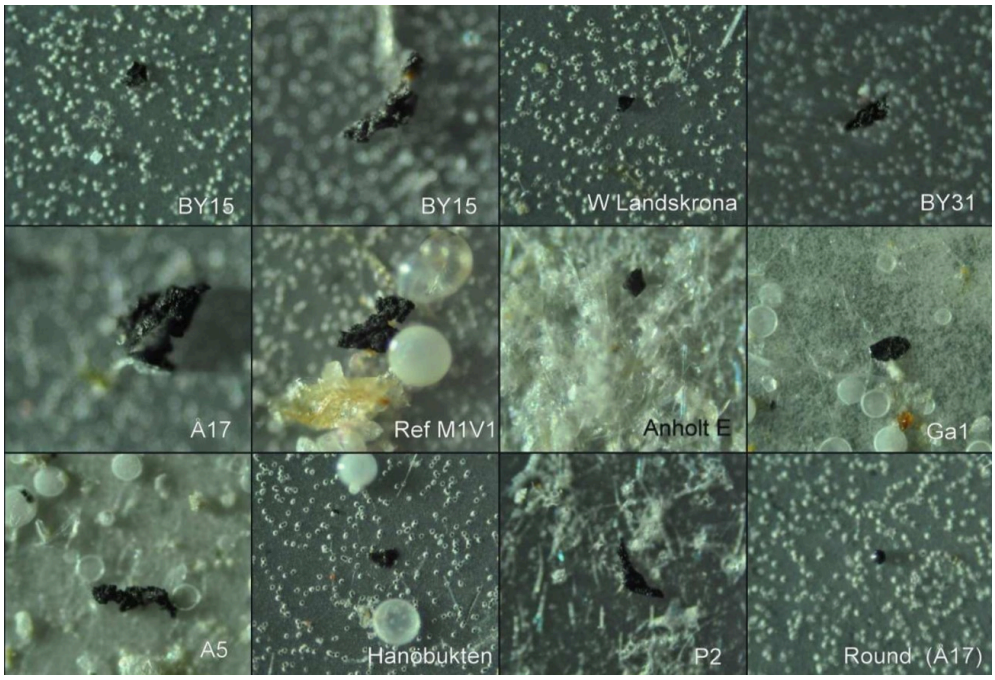

Figure 32: Picture of black particles in water samples from the Baltic Sea and Kattegat (from Noren *et al.*, 2009).

In 2019, a report on *Road dust-Associated Microplastic Particles* (RAMP) on behalf of the Norwegian Environment Agency was published (Vogelsang *et al.*, 2019). The main component of road dust was found to be rubber from tyre treads, polymers added to strengthen the bitumen used in road pavement and thermoplastic elastomers in road marking paints (Vogelsang *et al.*, 2019). However, very limited field studies are available on the characterization of these microplastics that are released from road-related activities. It is challenging to analyse rubbery fragments using the 'classical' methods available for microplastic research such as FT-IR analysis or similar, as discussed in more detail in section 2.4.1. Therefore, it has been suggested to use markers for such particles and several markers for tyre particles have so far been suggested. These markers can be used as a proxy to calculate the concentration of tyre particles in a sample. For example, extractable organic zinc, different benzothiazoles and SBR/IR content have been suggested (Wagner *et al.,* 2018; Vogelsang *et al.*, 2019). Isoprene and butadiene, as found in mussels from the Oslofjord and Iceland, can also be examples of such markers. Carbon black is also a potential marker since 90% of the carbon black on a global scale is used in the rubber industry as a reinforcing filler in a variety of products. Though, it is very challenging to distinguish between engineered and carbon black (e.g. from combustion sources) (Vogelsang *et al.*, 2019), and carbon black is also used as a reinforcing filler for certain other polymers.

Based on the limited literature on rubbery fragments, the main part of the tyre wear appears to be larger than 10 µm and up to 350 µm, with 85% ranging between 50 and 350 µm (Vogelsang *et al.*, 2019). When rubber treads are present in road dust, they are typically sausage-shaped conglomerates with rough surfaces (Vogelsang *et al.*, 2019). Many of the black particles found in this study were sausage- shaped. It would be beneficial to have a closer look at the shape of the particles found in this study with better magnification and resolution than a stereomicroscope, such as by

using scanning electron microscopy.

Klöckner *et al.,* (2019) highlights that the quantity and quality of particles emitted from road traffic is probably very variable depending on local circumstances such as traffic conditions, road material and surfaces; however, generally speaking, particles from road run-off will have a higher density than seawater (around 1 g/cm^3) and will therefore sink. Tyre rubber have an approximate density of 1.2 g/cm^3 (Degaffe & Turner 2011), and the particles collected from roads have been reported to range from 1.5 to 2.2 g/cm^3 (Kayhanian *et al.,* 2012) indicating that all types of particles emitted from road-runoff will sink towards the seafloor. Another potential pathway of road related particle, which is not mentioned a lot in the literature, is dumping of snow into the ocean. The M-19 site, Akershuskaia in the Oslofjord, has also previously been found to have the highest levels of microplastics in mussels dominating by similar rubbery fragments (Bråte *et al.*, 2018). This could be due to snow dumped from a snow melting cleaning facility near Akershuskaia.

Following the results obtained in this study and discussion above, it is not straight forward to determine the source of these rubbery fragments. However, based on an assembly of indications we hypothesize that the rubbery fragments in mussels from the eight urban sites in the Nordic area, could stem from either road runoff or harbour activity, or a combination.

This is based on the following indications:

- The visual assessment: black colour, rubber behaviour and often "sausage shaped" particles
- Carbon black indication from FT-IR analysis
- Py-GCMS analysis giving indications of rubbers for three sites (isoprene and/or butadiene)
- Size of the particles found in the mussels
- Previous findings of similar particles in water bodies and mussels from the Nordic environment

Lastly, since it was an apparent that there was a homogenic appearance of the particles across the Nordic sites, it indicates that the particles are from one 'type' of source or release pathway which must be common for the different sites. Road and/or harbour activity could be such a pathway. Which part of the harbour activity that might contribute to rubber release is not clear, but we cannot exclude that it is point sources in harbours that could result in this, such as boat traffic, dock protection, or as, mentioned above, snow dumping at specific sites.

Considerable effort is currently being put into solving these analytical issues, and this should help bridge the knowledge gap.

3.1.6 Other polymers

Rubbery fragments were by far the most dominant microplastic type observed in the mussels in this study; although other polymers were also found, including semi-synthetic biobased plastics and PE (Figure 33). For example, PE fragments were

dominant in one site from Denmark (M-21; North Sea) and two other sites in Denmark, M-4 and M-10 (Skagerrak and Kattegat) (from Figure 34 to Figure 36). PE was also found in mussels from Bolungarvik harbour at Iceland (M-9). Semi-synthetic biobased plastics were found in mussels from five sites, but they were not found to be dominant. This confirms earlier findings (Bråte *et al.*, 2018).

No individual mussels from Svalbard exceeded the LOD in regard to microplastic counts set in this study. However, when investigating the polymers found at this site, it is unlikely that the fragments found were a result of procedural contamination. In four out of fifteen *Mytilus* spp. individuals, epoxy fragments resembling paint fragments were present (Figure 37). If blank correction had performed for each morphology type (fibres, fragments), and not in the aggregated approach that was used in this study, these Svalbard results would have been eliminated. This is particularly the case when looking at all characteristics of the particles found in the blanks versus the samples (morphology, composition, etc.).

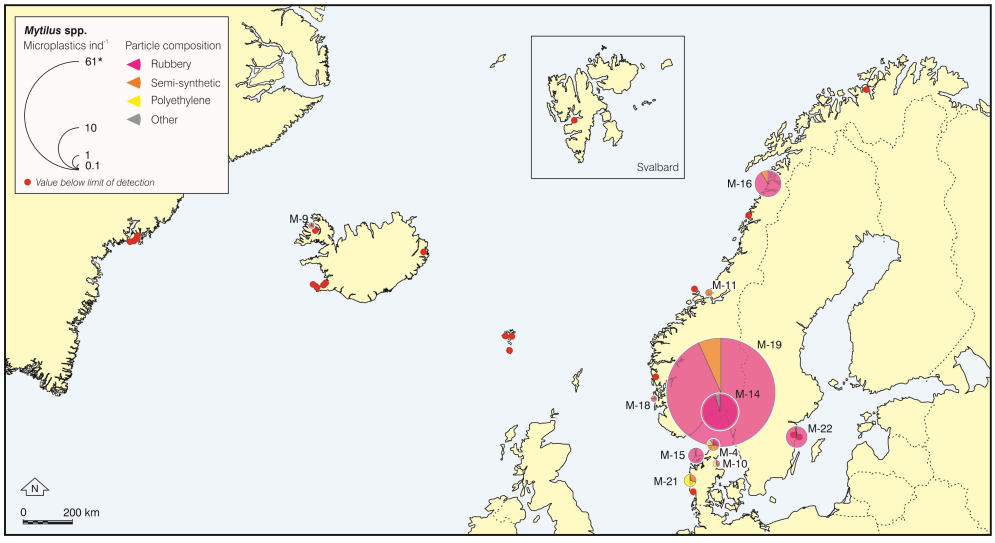

Figure 33: Polymeric composition of microplastics found in *Mytilus* spp. from the Nordic environment. Asterisk * refers to upper quantification limit for particles at site M-19. Only polymers representing >5% of the data for each site are shown, the rest are aggregated into 'other'.

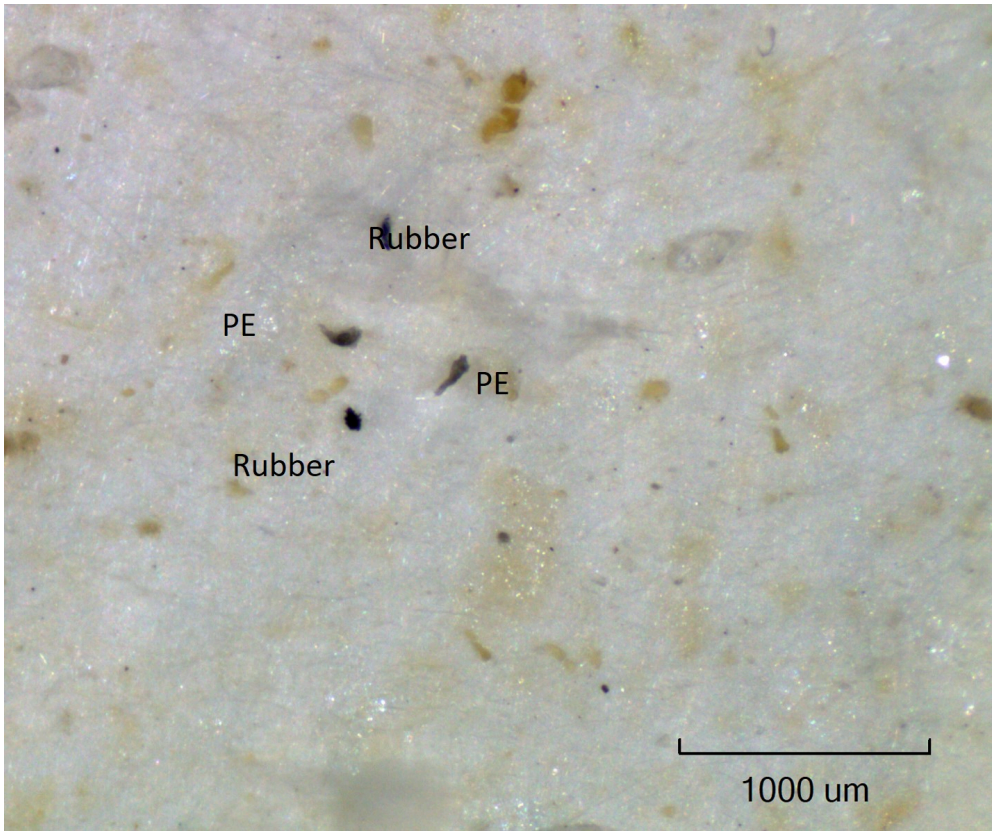

Figure 34: Microplastics in *Mytilus* spp. from DKW4, Hirtshals (M-4).

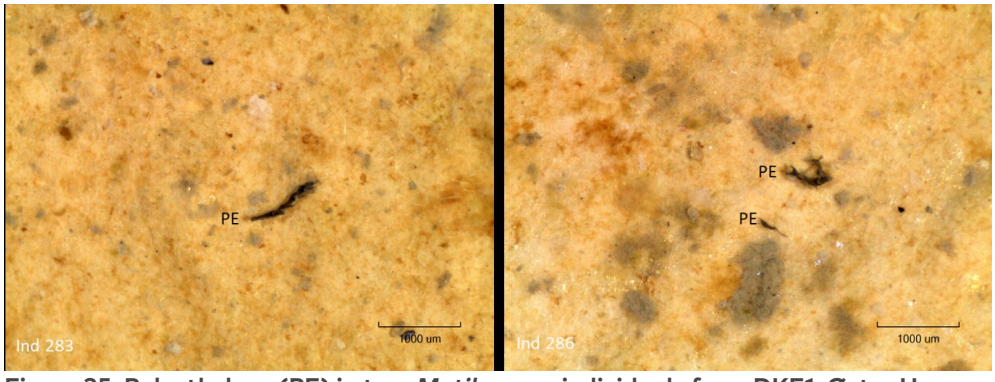

Figure 35: Polyethylene (PE) in two *Mytilus* spp. individuals from DKE1, Øster Hurup novo strand (M-10).

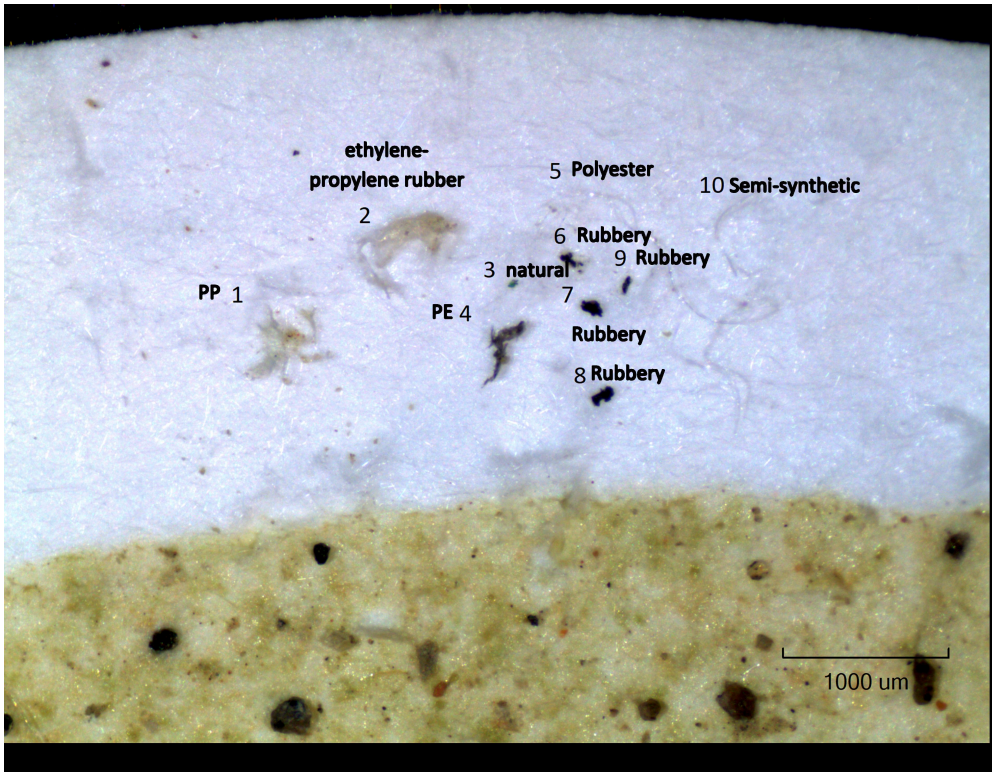

Figure 36: Microplastics in *Mytilus* spp. from St. IC7, Bolungarvik harbour (M-9).

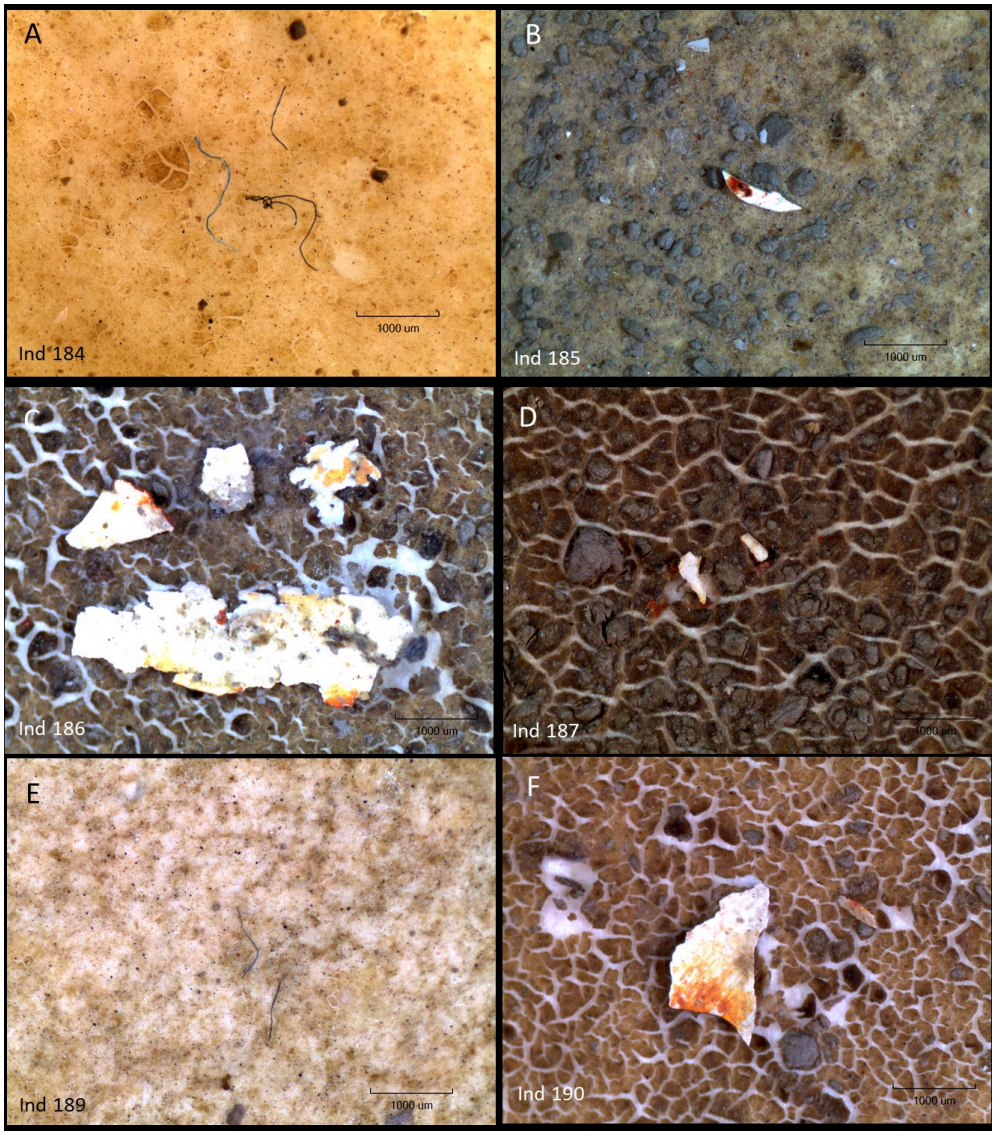

Figure 37: Microplastics in *Mytilus* spp. from Svalbard (M-1), values fell below LOD and were presumed not likely to be contamination. All from different *Mytilus* spp. individuals. A: Semi-synthetic biobased plastics and polyester (longest black) B-D: Epoxide polymer – a possible paint fragment(s) E: Semi-synthetic biobased plastics F: Epoxide polymer – a possible paint fragment.

3.2 Thyasira spp., Abra nitida, Limecola balthica and Hiatella arctica

Microplastics larger than 63 µm, which is the size of a sand grain, were also found in two other similar species, Abra nitida and the Baltic clam Limecola balthica living on soft bottom environment. Fewer microplastics were found in these species than in the Mytilus spp., but this was expected since they are typically a much smaller species that probably does not take in as many particles. Black rubbery fragments were the most common particles further strengthening the hypothesis of a large input of rubber particles into the Nordic marine environment.

3.2.1 Quantitative results and qualitative results, Fraction A (> 63µm)

Microplastics were found in Fraction A (above 63µm) in two out of four additional species studied, *Abra nitida* and *Limecola balthica* , Table 14, Figure 38, Figure 39 and Figure 40 (see also appendix). For *Abra nitida,* microplastics were only found in locations along the southern Norwegian coastline (North Sea and Skagerrak) and one site in Kattegat. The same applied for *Limecola balthica,* but microplastics were also found in two sites outside Stockholm and outside of Tromsø. It appears, as for mussels, that the most urbanized areas contain more microplastics (larger than 63 µm) in the two bivalve species. Based on this study, the Baltic clam *(Limecola balthica)* does filter microplastics larger than 63 µm and may be used to investigate microplastics in the Baltic Sea since mussels tend not to be very abundant in that region. Furthermore, *Abra nitida* also seem to contain microplastics of 'larger' size and might also be used as an addition to mussels and the Baltic clam to study microplastic abundance and composition. *Thyasira* spp. on the other hand, was not found to contain microplastics larger than 63 µm, despite studying many sites and individuals in this current study. No microplastics were found in *Hiatella arctica* or the three sites of small *Mytilus* spp. individuals, but these were only a few sites and few individuals so no firm conclusions can be drawn from this.

In Fraction A, all fibres were excluded from the results from *Abra nitida* and *Thyasira* spp. since they were stored in ethanol that was not pre-filtered. Fibres were sometimes observed in the ethanol solution. *Hiatella arctica* and the three-small sized *Mytilus* spp. sites included under Fraction A due to their small size, did not contain any fibres or any fragments. Fibres were also excluded for *Limecola balthica* samples that were stored in ethanol but included for the sites with frozen individuals. However, no fibres were found in the frozen *Limecola balthica*. Therefore, no fibres were included (or found for the appropriate samples) in any of the results of *Thyasira* spp., *Abra nitida*, *Limecola balthica* and *Hiatella arctica*. In the blanks (n=19), only fibres were found and therefore the LOD of fragments were set to 0.

The sizes of the particles detected in Fraction A for *Abra nitida* and *Limecola balthica* , ranged from 69 µm up to 210 µm in *Abra nitida*, and between 37µm and 329µm in *Limecola balthica* (see appendix for full table of size of microplastics). The microplastics found were smaller (in their longest dimension) than for the large mussels. This was expected due to the small size of these bivalves. For *Abra nitida*, a comparison of all means using Tukey-Kramer HSD, showed that the microplastic sizes did not vary significantly between sites, but for *Limecola balthica* it was found that microplastics in individuals from L-6 and L-14 were significantly larger than

microplastics in mussels from site L-3. Since the dataset is small, this data holds larger uncertainty.

For the polymeric composition, the same was found as for the mussels, with black rubbery fragments as the dominant fraction (Figure 40). Other polymers that were found were semi-synthetic biobased plastics and PVC. The latter polymer type was not identified in mussels.

For a further discussion of these results, see section 4.

Table 14: Overview of analysed samples for Fraction A, * indicates small mussels (not to be confused with mussels from section 3.1). Colour indicates the presence of microplastics (MPs).

Species	No of sites	Sites w/ MPs	No of ind	MPs (Y/N)	Total MPs	Samples w/MPs	Mean MPs	Max MPs	Min MPs
Abra nitida	31	6/31	589	Yes	20	9	0.29 ± 0.95	6	0
Limecola balthica	14	4/14	233	Yes	15	8	0.375 ± 0.86	4	0
Thyasira spp.	20	0/20	480	No	0	0	0	0	0
Hiatella arctica	3	0/3	17	No	0	0	0	0	0

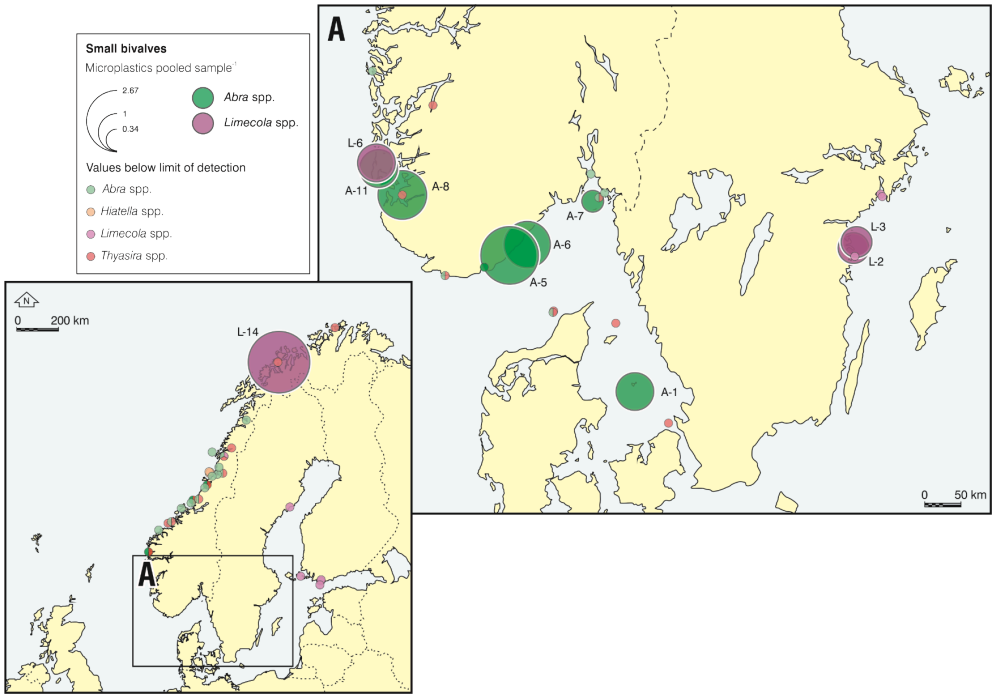

Figure 38: Microplastics in pooled samples of *Thyasira* spp., *Abra nitida* (A), *Limecola balthica* (L) and *Hiatella arctica*.

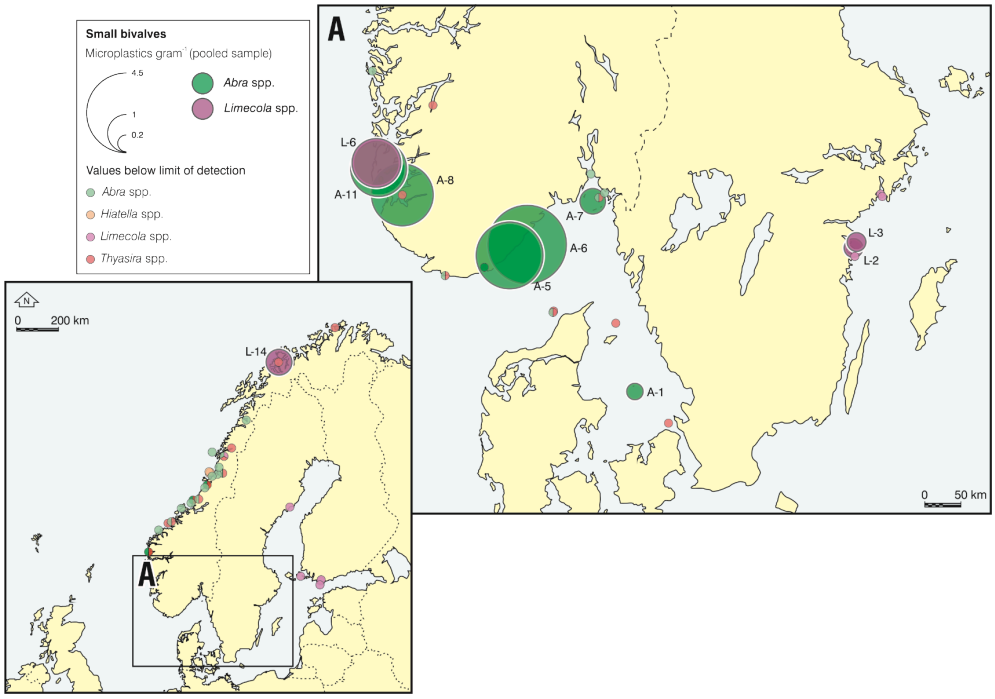

Figure 39: Microplastics in pooled samples and gram adjusted of *Thyasira* spp., *Abranita nitida*, *Limecola balthica* (T) and *Hiatella arctica* (H).

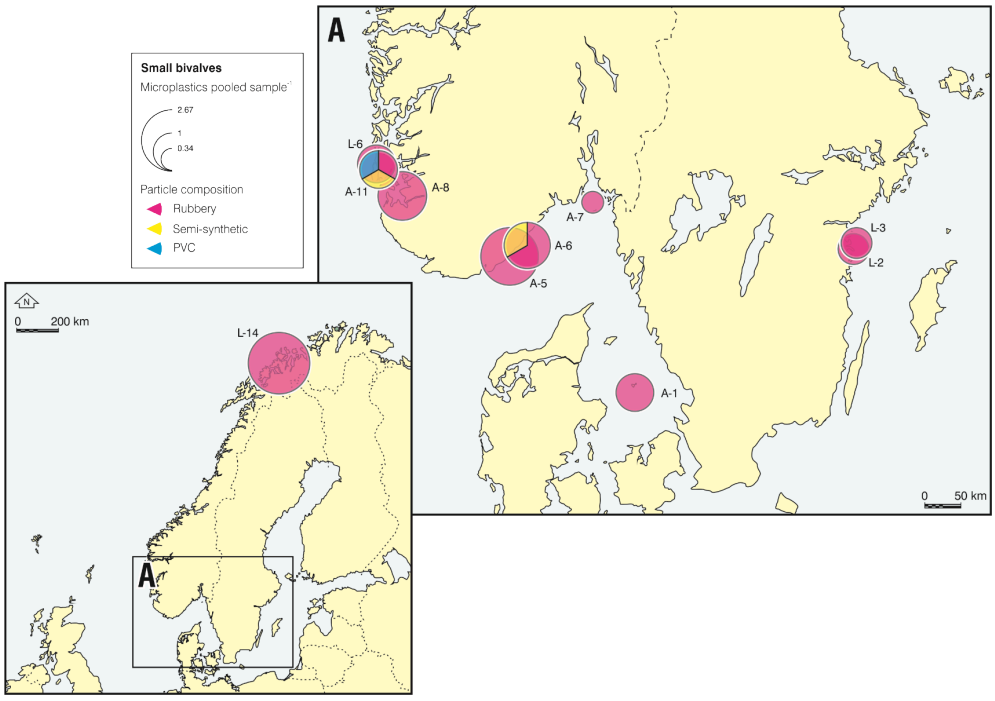

Figure 40: Polymeric composition of the microplastics found in *Abra nitida* (A) and *Limecola balthica* (L) based on pooled samples. Pink illustrates rubbery fragments, yellow illustrates semi-synthetic biobased plastics and blue indicates PVC.

3.2.2 Fraction B (<63µm)

Microplastics smaller than 63 µm were also studied for two species; Abra nitida and Thyasira spp., which are both found in soft bottom sediments. Since this fine-scale method is very time-consuming, only five sites were investigated. Microplastics were found in both species, and only one site did not contain plastics. Three different types of plastics, with different degrees of certainty, were identified: polyethylene, polyacrylate, and a form of silicone. More research is needed on these very small microplastics to understand where they come from and if they can cause harm for the marine ecosystem.

The results obtained for Fraction B (<63µm) for *Abra nitida* and *Thyasira* spp. is presented as presence/absence of plastic material and material composition (Figure 41). Please note that the analysis of Fraction B is only of 0.32% of the filter that represent particles in *Abra nitida* and *Thyasira* spp. that is smaller than 63 µm, and only performed for five sites. However, the sites chosen were sites that are likely to be heavily impacted by anthropogenic activity. Therefore, if polymers were present in this small area scanned, it is likely that the abundance of the polymeric material is quite high.

All of the different materials identified, including non-plastic components, are illustrated in the appendix. Most of the materials were, with different level of certainties, natural silicates. It is difficult to provide more detail on this using FT-IR, where one would need X-ray fluorescence (XRF image) to detect inorganic

constituents of silicate materials (Si, Al, Fe and so on).

Microplastics, or associated compounds, were found in both species studied (Table 15). Only one out of five study sites did not contain polymeric materials, *Thyasira* spp. from site T-6. Three suspected polymers were found in *Thyasira* spp. and *Abra nitida,* with different degree of certainty: polyacrylate, polydimethylsiloxane (silicone) and polyethylene (PE). Polyacrylate was found in many samples but with lower certainty, silicone with high degree of certainty but in few samples and PE with medium or low certainty in several samples.

Thyasira spp. contained two types of plastics: polyacrylate and PE. Calcium stearate was also found, which is possibly of anthropogenic origin. Calcium stearate is, for example, found in rubbers as an additive and in industrial lubricant for steel extrusion. Although, it is also often found in water because solid soaps are made with sodium stearate and the stearate anion precipitates as calcium stearate when it meets calcium from tap water. *Abra nitida* did contain two oil-based polymers, silicone and PE, in addition to the semi-synthetic material, rayon or similar, that are based on cellulosic material.

Microplastics were found in two out of three sites for *Thyasira* spp: T1 (all three replicates) and T2 (all three replicates) did contain plastics, but not in T6. For *Abra nitida*, microplastics were found in both stations; A10 (two of three replicates) and A12 (two of three replicates). The latter site was also the one where 37.5 – 50% of the Fraction A was analysed due to clogging of filters.

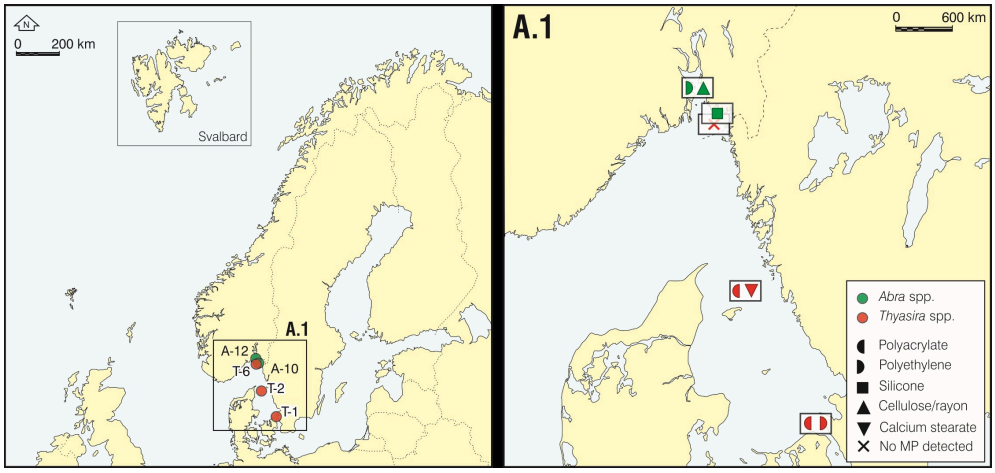

Figure 41: Presence/absence and material composition of microplastics of Fraction B for *Abra nitida* (A) and *Thyasira* spp. (T) from the Nordic environment.

Table 15: Overview of microplastic (MP) polymers and associated contaminants detected in *Thyasira* spp. and *Abra nitida*, Fraction B (below 63µm) with ATR scanning FT-IR.

Species	Site	Sample ID	Composition	MP	Certainty
Thyasira spp	T1	Rep1	Polyacrylate	yes	Medium
		Rep2	Polyacrylate	yes	Medium
		Rep3	Polyethylene (PE)	yes	Medium
	T2	Rep1	Calcium stearate	Additive?	High
			Polyacrylate	yes	Medium
		Rep2	Polyacrylate	yes	Medium
		Rep3	Polyacrylate	yes	Medium
	T6	Rep1,2,3	No microplastics		
Abra nitida	A10	Rep1	Polydimethyl-siloxane (Silicone)	yes	High
		Rep3	Polydimethyl-siloxane (Silicone)	yes	High
	A12	Rep 2	Cellulose/ Rayon	semi-synthetic	Low
			Polyethylene (PE)	Yes	Low
		Rep3	Polyethylene (PE)	Yes	Medium

4. Discussion

4.1 Combined microplastic results for all bivalves from the Nordic environment

This large-scale microplastic study is a significant contribution to address the lack of empirical data for the Nordic marine environment as highlighted by Bråte *et al.*, 2017. This study covers much of the sea areas in Nordic marine environment, ranging from Svalbard in the north, Greenland in the west, Baltic Sea in the east and the North Sea in the south. The amount of empirical data on marine bivalves from this area has dramatically increased with the 100 sites included in the survey. The results, especially the *Mytilus* spp., indicate that bivalves from urban sites in the Nordic marine environment are exposed to microplastics to a larger extent than less urbanised sites. This is not always the case for other microplastic studies that sometimes indicate, not local input, but rather less understood and studied sources/ pathways. A case in point, were high levels detected in mussels from the Barents Sea that might be explained by accumulation zones driven by currents (Bråte *et al.*, 2018).

In this current study, microplastics were identified at eleven sites for *Mytilus* spp., in six sites for Fraction A and two sites for Fraction B for *Abra nitida,* four sites of *Limecola balthica* from Fraction A, and two sites for *Thyasira* spp. from Fraction B (Figure 42).

The results indicate that the North Sea, Skagerrak, Kattegat and the Baltic Sea are the most impacted areas. The three northern sites that with microplastics above LODs, were sites associated to harbours; M-9 (Bolungarvik harbour), L-14 (near Tromsø) and M-16 (Bodø harbour). Microplastics above LOD were not detected in bivalves from the east coast of Greenland, the Faroe Islands and Svalbard. No bivalves were studied from the north-east part of Varangerhalvøya (northern Norway). Despite not finding microplastics in mussels from the coast of Greenland, Svalbard and the Faroe Islands, there may still be some specific point sources in these areas. To address this, one could focus on possible point sources of microplastics such as wastewater treatment plants (WWTPs) that are known to have especially synthetic, semi-synthetic biobased plastics and natural fibres in their effluent (Magnusson & Norén 2014). Such an approach should take into consideration not only the population and industry the WWTP serves but also how developed the WWTP is.

One of the main objectives with this study was to identify the most likely sources of microplastics found in the bivalve species throughout the Nordic marine environment. Three different methods were applied and compiled: visual identification following point mode transmission Fourier transform infrared spectroscopy (μFT-IR), image scanning using automated attenuated total reflectance FT-IR (μATR-FT-IR) and, for ten *Mytilus* spp samples, pyrolysis gas chromatography mass spectrometry (Py-GCMS). Overall, black rubbery fragments were the dominate microplastic type for three species; *Mytilus* spp., *Abra nitida* and

Limecola balthica (Figure 43). This indicates that these rubbery fragments, possibly from road-runoff, are accumulating in bivalves from many sites in the Nordic marine environment, including North Sea, Skagerrak, Kattegat and the Baltic Sea. For a more in-dept discussion on these rubbery fragments, see section 3.1.5.

It was also evident from the three applied methods that these marine bivalves were exposed to a wide variety of polymeric materials, indicating numerous of sources or pathways. A total of 11 polymers were detected other than rubbery fragments:

Based on the visual ID and point µFT-IR (*Mytilus* spp., *Limecola balthica* and *Abra nitida*)

- Polyethylene (PE)
- Polypropylene (PP)
- Semi-synthetic biobased plastics (modified cellulose)
- Epoxy plastics (e.g. paint fragments)
- Polyvinyl chloride (PVC)

Based on scanning µFT-IR (*Abra nitida* and *Thyasira* spp.)

- Polyacrylate
- Polyethylene (PE)
- Polydimethylsiloxane (silicone)
- Calcium stearate (plastic additive)
- Semi-synthetics biobased plastics

Based Py-GCMS (*Mytilus* spp.)

- Polyhydroxybutyrate (PHB)
- Polylactic acid (PLA)
- Polycaprolactone (PCL)
- Polyethylene naphthalate (PEN)

On the whole, the composition of these polymers varied considerably from site to site and did not point strongly towards a specific source or pathway. The polymers reflect a wide range of densities such as the natural buoyant polymers like polyethylene (PE) and the denser polymers such as polyvinyl choride (PVC). The

polymers also represent a wide range of applications. Polyethylene is the most produced and used polymer worldwide (PlasticsEurope 2018), and hence, it is not possible to assign what is found in the bivalves to certain products or sources. Epoxy plastics, often used in paint, might be from boat paint but this can't be confirmed. Semi-synthetic biobased fibres might come from WWTPs or be a result of airborne deposition, but again, this is speculation. The polyester-like polymers found with the Py-GCMS method (PHB, PLA, PCL and PEN) are, to our knowledge, not commonly detected in environmental samples. Possible sources for these are, unfortunately, not possible to confirm within the scope of this study. However, since these are present at all sites studied with Py-GCMS, it indicates that the source(s) are common to urban sites in the Nordic environment. More comprehensive and targeted studies would help bridge this gap in knowledge.

One purpose of the sampling design was to observe whether the accumulation of microplastics differed among the target species. This related to the different habitats and feeding modes of the selected species. There were 18 sites at which two or more species were sampled. This overlap mostly corresponded with the species: *Abra nitida, Thyasira* spp., *Limecola balthica* and *Hiatella arctica*. For the majority of these sites, no values above the LOD were detected for any of the species. Three sites were characterised by only one of the species recording confirmed microplastics but, for two of these sites, this referred to the Fraction B of *Thyasira* spp. This complicates efforts to make comparisons, as the Fraction B of one species is not comparable to Fraction A of another. Hence, it is not possible to draw justified conclusions regarding the difference in exposure or ecology of the 5 bivalves tested. Further research is necessary to better understand these dynamics in regard to the ingestion of microplastic particles.

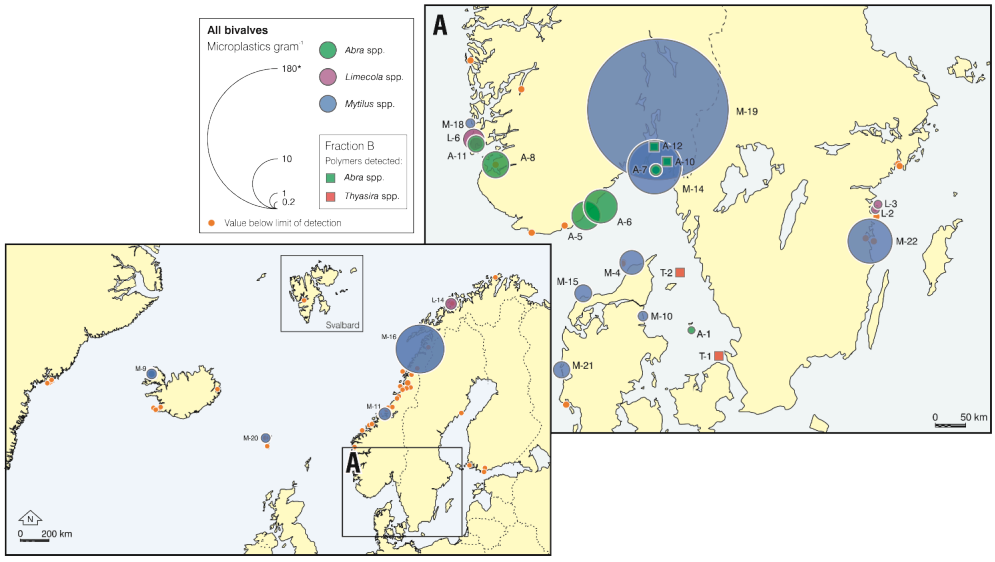

Figure 42: Combined results for all polymers found in *Mytilus* spp. (M), *Limecola balthica* (L), *Abra nitida* (A) and *Thyasira* spp. (T). Asterisk * refers to upper quantification limit for particles at site M-19.

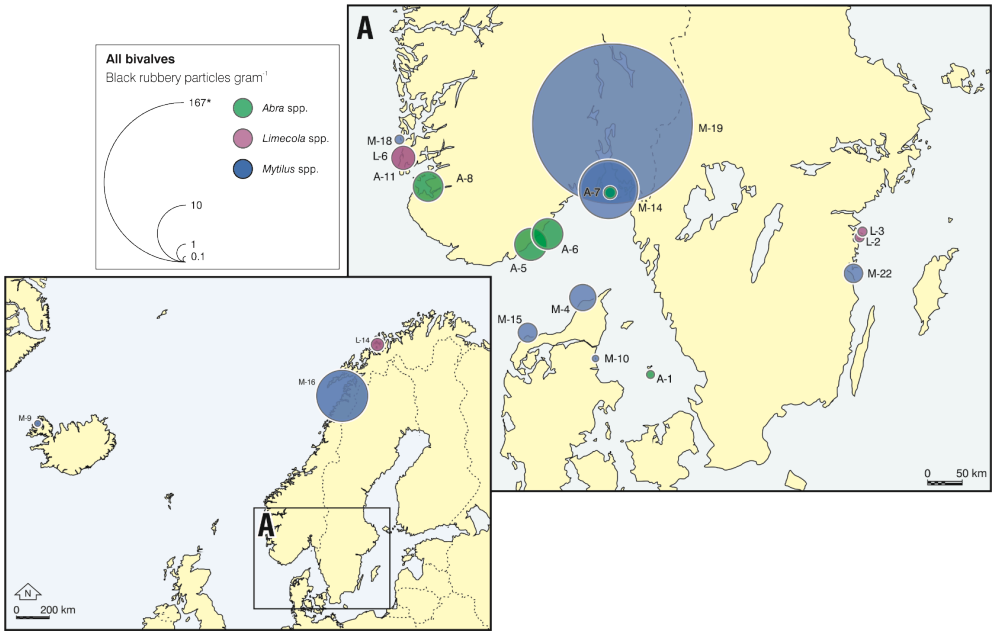

Figure 43: Combined results for the species containing black rubbery fragments from the Nordic environment; *Abra nitida* (A), *Limecola balthica* (L) and *Mytilus* spp. (M). Asterisk * refers to upper quantification limit for particles at site M-19.

4.2 Use of archived sampled for microplastic analysis

There are many factors to consider when collecting samples for microplastic analysis. For biota such as mussels, all containers for storage must be pre-rinsed with filtered (RO-)water before use to ensure there is no contamination. If the mussels are to be stored in a solution for preservation, the solution must also be pre-filtered to minimize any microplastic contamination. As atmospheric contamination cannot be accounted for except within lab facilities, fibres were excluded in samples stored in ethanol. Fibres, as large as 3000–8000 µm, were found in the pooled bivalve mussels. Also, fibres < 1000 µm long were detected in the several blanks from the NIVA lab. Fragments, however, can act as supporting material, if similarities between the data from the smaller mussels and the *Mytilus* spp. are observed such as the black rubbery fragments. Black rubbery fragments were never detected in the procedural blank samples. The *Mytilus* spp. were collected with the intent of analysing microplastics, and precautions were taken to minimize atmospheric and cross contamination from sampling areas to processing in the lab. They were also frozen and not fixed in ethanol, which is likely to be a better solution for microplastic studies. Due to the uncertainties of how the smaller mussels were collected, handled and stored, the data obtained from these samples should act as supporting material to the bivalve's data.

4.3 Some considerations regarding microplastics in Nordic marine bivalves

The presence of microplastics in bivalves, as with other marine species, raises a concern regarding the consequences. These include consequences to marine life, to seafood supply and to human health. Several international reports published in the last three years have address these issues. Most recently, VKM published a report in October 2019 to address concerns regarding the implications of microplastics to the environment and human health. Earlier reports addressing the same issue have come from EFSA Panel on Contaminants in Food (Alexander *et al.,* 2016), the Food and Agricultural organisation of the United Nations (Lusher *et al.,* 2017b) and Science Advice for Policy by European Academics consortium (SAPEA, 2019). On a general marine ecosystem scale, evidence shows that organisms can intake microplastics of different morphologies in varying concentrations. The VKM report shows that the amount of data available for environmental exposure and laboratories studies investigating effects, are disjointed. Therefore, we are currently not able to perform an assessment of environmental risk.

Importantly, the exposure of microplastics to individuals in the wild will depend on the concentrations in the specific environmental compartment. Microplastics in the water column are generally seen as transitory, they are either sinking or floating, due to changes in buoyancy and density. Ultimately, microplastics will sink to the sediment, which may suggest that benthic organisms are more exposed than pelagic species. There is ample information that suggests no matter which environmental compartment an organism inhabits, they could interact with microplastic. Understanding the impact on biota in the wild, requires assessment in the laboratory. When an organism ingests microplastics, the consequences of ingestion can be assessed on different levels of biological organisation: molecular, cellular, organ, individual, population and community. By far most of the information available focuses on the individual level (VKM 2019). Impacts on individuals can include impacts on feeding, growth, reproduction, behaviour, and ultimately mortality. However, many of the studies use exposure concentrations, and exposure material, which lack environmental relevance. Therefore, interpretation of any generated data in terms of the impact on the health of individuals must be made with caution, and especially at a higher level of organisation such as on a population level.

Shellfish farming has been highlighted as a potential source of microplastics to the environment (Lusher *et al.,* 2017b). There have been some studies which indicate mussels and clams collected from sites of aquaculture contained larger quantified of microplastics (Mathalon & Hill 2014; De witte *et al.*, 2014) whereas others have shown no difference or pattern in observed levels (Davidson & Dudas 2016). Further research is required to understand microplastics generated through fisheries and aquaculture and their consequences for food products. The types of pre-processing, such as depuration before reaching the point of sale, could reduce the levels of microplastics in seafood. There is evidence that when mussels are depurated, they contained fewer microplastics (e.g., Birnstiel *et al.*, 2019). This step is usually performed to prepare bivalves for human consumption.

There is sufficient data showing that many marine species that are consumed as food by humans does contain microplastics. However, further quantitative data is

still required to build a picture of the risk for consumption. Alexander *et al.*, (2016), Lusher *et al.*, (2017b) and SAPEA (2019) all state that there is sufficient published evidence that microplastics are present in seafood. This is affirmed by VKM, although they go on to state that an exposure assessment is not yet possible.

There is a general lack of information on the toxicity of micro and nano-sized plastics to human health. The consequences, in terms of a human health perspective, as outlined by three reports state that:

- There are few relevant studies for human hazard assessment (Alexander *et al.*, 2016)
- The toxicity of microplastics to humans is uncertain (SAPEA 2019)
- The available information does not provide sufficient basis to characterise potential toxicity to humans (VKM 2019)

5. Conclusions

In this large-scale survey of microplastics in five Nordic marine bivalves (*Mytilus* spp., *Limecola balthica*, *Abra nitida*, *Thyasira* spp and *Hiatella arctica)* from a total of 100 sites, three different methods were applied to assess the microplastic occurrence and composition. This study found that four out of five bivalve species contained microplastics. *Hiatella arctica* did not contain microplastics, however, there were only three sites, close together, with this species. Most of the sites with microplastics were mussels from highly urbanised areas, or harbour areas. This was more evident for *Mytilus* spp. and less so for *Abra nitida* or *Thryasira* spp. The results indicate that the North Sea, as well as Skagerrak and Kattegat, in addition to the area near Stockholm in the Baltic Sea, higher accumulation of most microplastics. Not all particles are simple to analyse, and this is especially true for rubbery fragments. However, nearly all microplastics found in this study were rubbery fragments. It is speculated that these are derived from road run-off or harbour activity based on the combination of methods applied in this study. In addition, to the rubber fragments, 11 other polymers were also detected, indicating many different sources and pathways contributing to the microplastic load in the Nordic environment.

Based on the empirical data now available, three bivalves living near, on, or in the sediment could be used to monitor microplastics in the range from 63 to 1000 µm in the Nordic environment. The three bivalves are: the hard-bottom dwelling blue mussel and closely related species (*Mytilus* spp.) for most of the coastal Nordic marine environment, and the two soft bottom dwelling Baltic clam (*Limecola balthica)* from the Baltic Sea and *Abra nitida* from the Norwegian coast and parts of the North Sea. There is an indication that with more effort *Thyasira* spp. and *Abra nitida* could be used for monitoring microplastics smaller than 63 µm as well.

How microplastics are impacting the marine ecosystem is still not understood, but by combining environmental concentration data, such as those from this study, with experimental data, one can better understand which areas are likely to be most impacted. Even so, there remain many unanswered questions with regards to the impact that microplastics have on the environment. To address these issues, further method development is needed to increase the resolution and understanding of microplastic occurrence and composition in biota. With this knowledge the challenging task of confirming the sources of the variety of microplastics that occur in the marine environment may be advanced.

6. References

Alexander J., Barregard L., Bignami M., Ceccatelli S., Cottrill B., Dinovi M., Edler L., GraslKraupp B., Hogstrand C., Hoogenboom L., Knutsen H.K., Nebbia C.S., Oswald I., Petersen A., Rogiers V.M., Rose M., Roudot A.C., Schwerdtle T., Vleminckx C., Vollmer G., Wallace H., Chain E.P.C.F., 2016. Presence of microplastics and nanoplastics in food, with particular focus on seafood. EFSA Journal 14:30. Available at: https://doi.org/10.2903/j.efsa.2016.4501

Andrady, A.L., 2017. The plastic in microplastics: A review. *Marine Pollution Bulletin*, *119*(1), pp. 12–22. Available at: https://doi.org/10.1016/j.marpolbul.2017.01.082

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, pp. 338–365. Available at: https://doi.org/10.1016/j.marenvres.2017.07.024

Birnstiel, S., Soares-Gomes, A. and da Gama, B.A., 2019. Depuration reduces microplastic content in wild and farmed mussels. *Marine Pollution Bulletin*, *140*, pp. 241–247. Available at: https://doi.org/10.1016/j.marpolbul.2019.01.044

Booth, A.M., Kubowicz, S., Beegle-Krause, C.J., Skancke, J., Nordam, T., Landsem, E., Throne-Holst, M. and Jahren, S., 2017. Microplastic in Global and Norwegian Marine Environments: Distributions, Degradation Mechanisms and Transport. Report for Miljødirektoratet M-918. 149p.

Bråte, I. L. B., Huwer, B., Thomas, K.V., Eidsvoll, D. P., Halsband, C., Almroth, B.C., Lusher, A., 2017. Micro-and macro-plastics in marine species from Nordic waters. Report for Nordic Council of Ministers, 101p. Available at: https://doi.org/10.6027/tn2017-549

Bråte, I.L.N., Hurley, R., Iversen, K., Beyer, J., Thomas, K.V., Steindal, C.C., Green, N.W., Olsen, M. and Lusher, A., 2018. Mytilus spp. as sentinels for monitoring microplastic pollution in Norwegian coastal waters: A qualitative and quantitative study. Environmental Pollution, 243, pp. 383–393. Available at: https://doi.org/10.1016/j.envpol.2018.08.077

Brooks, S.J. and Farmen, E., 2013. The distribution of the mussel *Mytilus* species along the Norwegian coast. Journal of Shellfish Research, 32(2), pp. 265–271.

Degaffe, F.S. and Turner, A., 2011. Leaching of zinc from tire wear particles under simulated estuarine conditions. Chemosphere, 85(5), pp. 738–743. Available at: https://doi.org/10.1016/j.chemosphere.2011.06.047

Dehaut, A., Cassone, A.L., Frère, L., Hermabessiere, L., Himber, C., Rinnert, E., Rivière, G., Lambert, C., Soudant, P., Huvet, A. and Duflos, G., 2016. Microplastics in seafood: benchmark protocol for their extraction and characterization. Environmental Pollution, 215, pp. 223–233. Available at: https://doi.org/10.1016/j.envpol.2016.05.018

Denisenko, S.G., Denisenko, N.V., Lehtonen, K.K., Andersin, A.B. and Laine, A.O., 2003. Macrozoobenthos of the Pechora Sea (SE Barents Sea): community structure and spatial distribution in relation to environmental conditions. Marine Ecology Progress Series, 258, pp. 109–123. Available at: https://doi.org/10.3354/

meps258109

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), pp. 146–155. Available at: https://doi.org/10.1016/j.marpolbul.2014.06.006

Davidson, K. and Dudas, S.E., 2016. Microplastic ingestion by wild and cultured Manila clams (Venerupis philippinarum) from Baynes Sound, British Columbia. *Archives of environmental contamination and toxicology*, *71*(2), pp. 147–156. Available at: https://doi.org/10.1007/s00244-016-0286-4

GESAMP 2019. Guidelines or the monitoring and assessment of plastic litter and microplastics in the ocean. Eds. Kershaw P.J., Turra A. and Galgani F. IMO/FAO/UNESCO-IOC/UNIDO/WMO/IAEA/UN/UNEP/UNDP/ISA Joint Group of Experts on the Scientific Aspects of Marine Environmental Protection. Rep. Stud. GESAMP No. 99, 130p. Available at: https://doi.org/10.1002/iroh.19840690208

Harbo, R.M., 2001. Shells and Shellfish of the Pacific Northwest, a field guide. Harbour Publishing, Canada, 270p.

Karami, A., Golieskardi, A., Choo, C.K., Romano, N., Ho, Y.B. and Salamatinia, B., 2017. A high-performance protocol for extraction of microplastics in fish. Science of the Total Environment, 578, pp. 485–494. Available at: https://doi.org/10.1016/j.scitotenv.2016.10.213

Kayhanian, M., McKenzie, E.R., Leatherbarrow, J.E. and Young, T.M., 2012. Characteristics of road sediment fractionated particles captured from paved surfaces, surface run-off and detention basins. Science of the Total Environment, 439, pp. 172–186. Available at: https://doi.org/10.1016/j.scitotenv.2012.08.077

Kiørboe, T., Mølenberg, F. and Nøhr, O., 1980. Feeding, particle selection and carbon absorption in Mytilus edulis in different mixtures of algae and resuspended bottom material. Ophelia, 19(2), pp. 193–205. Available at: https://doi.org/10.1080/00785326.1980.10425516

Klöckner, P., Reemtsma, T., Eisentraut, P., Braun, U., Ruhl, A.S. and Wagner, S., 2019. Tire and road wear particles in road environment–Quantification and assessment of particle dynamics by Zn determination after density separation. Chemosphere, 222, pp. 714–721. Available at: https://doi.org/10.1016/j.chemosphere.2019.01.176

Leopold, P., Renaud, P.E., Ambrose, W.G. and Berge, J., 2019. High Arctic *Mytilus* spp.: occurrence, distribution and history of dispersal. Polar Biology, 42(1), pp. 237–244. Available at: https://doi.org/10.1007/s00300-018-2415-1

Li, J., Lusher, A., Rotchell, J.M., Company, S.D., Turra, A., Bråte, I.L.N., Sun, C., Hossain, M.S., Li, Q., Kolandhasamy, P. and Shi, H., 2019. Using mussel as a global bioindicator of coastal microplastic pollution. Environmental Pollution, 244, 522–533. Available at: https://doi.org/10.1016/j.envpol.2018.10.032

Löder, M.G. and Gerdts, G., 2015. Methodology used for the detection and identification of microplastics—A critical appraisal. In M. Bergmann., L. Gutow., & M. Klages (Eds.), Marine Anthropogenic Litter (pp. 201–227). Berlin: Springer. Available at: https://doi.org/10.1007/978-3-319-16510-3_8

Lusher, A., 2015. Microplastics in the marine environment: distribution,

interactions and effects. In M. Bergmann., L. Gutow., & M. Klages (Eds.), Marine Anthropogenic Litter (pp. 245–308). Berlin: Springer. Available at: https://doi.org/10.1007/978-3-319-16510-3_10

Lusher, A., Bråte, I.L.N., Hurley, R., Iversen, K. and Olsen, M. 2017a. Testing of methodology for measuring microplastics in blue mussels (*Mytilus* spp) and sediments, and recommendations for future monitoring of microplastics (R & D-project). Report for Miljødirektoratet M-897. 90p.

Lusher A., Hollman P., Mendoza-Hill J. (2017b) Microplastics in fisheries and aquaculture, Food and Agriculture Organization of the United Nations, Rome.

Magnusson, K., Norén, F. 2014. Screening of microplastic particles in and down-stream a wastewater treatment plant. Report for the Swedish Environmental Protection Agency, 22p.

Marlin, 2019. The Marine Life Information Network, https://www.marlin.ac.uk/ [Accessed: 17 October 2019].

Mathalon, A. and Hill, P., 2014. Microplastic fibers in the intertidal ecosystem surrounding Halifax Harbor, Nova Scotia. *Marine Pollution Bulletin*, *81*(1), pp.69–79. Available at: https://doi.org/10.1016/j.marpolbul.2014.02.018

Mathiesen, S.S., Thyrring, J., Hemmer-Hansen, J., Berge, J., Sukhotin, A., Leopold, P., Bekaert, M., Sejr, M.K. and Nielsen, E.E., 2017. Genetic diversity and connectivity within Mytilus spp. in the subarctic and Arctic. Evolutionary Applications, 10(1), pp. 39–55. Available at: https://doi.org/10.1111/eva.12415

Norén, F. and Naustvoll, L.J., 2010. Survey of microscopic anthropogenic particles in Skagerrak. Report commissioned by Klima-og Forurensningsdirektoratet.

Norén, F., Ekendahl, S. and Johansson, U., 2009. Mikroskopiska antropogena partiklar i svenska hav. 15p.

Oliver, P.G., Holmes, A.M., Killeen, I.J. and Turner, J.A., 2016. Marine bivalve shells of the British Isles (Mollusca: Bivalvia). Amgueddfa Cymru-National Museum Wales. Available online at http://naturalhistory. museumwales. ac. uk/britishbivalves. [Accessed: 20 October 2019].

Primpke, S., Wirth, M., Lorenz, C. and Gerdts, G., 2018. Reference database design for the automated analysis of microplastic samples based on Fourier transform infrared (FTIR) spectroscopy. Analytical and Bioanalytical Chemistry, 410(21), pp. 5131–5141. Available at: https://doi.org/10.1007/s00216-018-1156-x

Rees, H.L. and Dare, P.J., 1993. Sources of mortality and associated life-cycle traits of selected benthic species: a review. Fisheries Research Data Report No. 33, CEFAS Directorate of Fisheries Research, Lowestoft, UK, 36p.

Rexania, S., Park, J., Din, M.F.M., Taib, S.M., Talaiekhozani, A., Yadav, K.K., Kamyab, H., 2018. Microplastics pollution in different aquatic environments and biota: A review of recent studies. *Marine Pollution Bulletin*, *133*, pp. 191–208. Available at: https://doi.org/10.1016/j.marpolbul.2018.05.022

SAPEA; 2019. A Scientific Perspective on Microplastics in Nature and Society. The Science Advice for Policy by European Academies consortium (SAPEA), 2019.

Scott, N., Porter, A., Santillo, D., Simpson, H., Lloyd-Williams, S., Lewis, C., 2019. Particle characteristics of microplastics contaminating the mussel *Mytilus edulis* and their surrounding environments. *Marine Pollution Bulletin*, *146*, pp. 125–133.

Available at: https://doi.org/10.1016/j.marpolbul.2019.05.041

Strelkov, P., Nikula, R. and VÄINÖLÄ, R., 2007. *Macoma balthica* in the White and Barents Seas: properties of a widespread marine hybrid swarm (Mollusca: Bivalvia). Molecular Ecology, 16(19), pp. 4110–4127. Available at: https://doi.org/10.1111/j.1365-294x.2007.03463.x

Sundt, P., Schulze, P-E. and Syversen F. 2014. Sources of microplastic pollution to the marine environment. MEPEX. Report for Miljødirektoratet M-321. 108p.

Thiele, C.J., Hudson, M.D. and Russell, A.E., 2019. Evaluation of existing methods to extract microplastics from bivalve tissue: Adapted KOH digestion protocol improves filtration at single-digit pore size. Marine Pollution Bulletin, 142, pp. 384–393. Available at: https://doi.org/10.1016/j.marpolbul.2019.03.003

VKM, Utne Skåre, J., *et al.*, (2019). Microplastics; occurrence, levels and implications for environment and human health related to food. Scientific opinion of the Scientific Steering Committee of the Norwegian Scientific Committee for Food and Environment. VKM report 2019:16, ISBN: 978-82-8259-332-8, ISSN: 2535-4019. Norwegian Scientific Committee for Food and Environment (VKM), Oslo, Norway. Available at: https://doi.org/10.2903/sp.efsa.2018.en-1405

Vogelsang, C., Lusher, A. L., Dadkhah, M. E., Sundvor, I., Umar, M., Ranneklev, S. B., Eidsvoll, D. P., Meland, S., (2019). Microplastics in road dust – characteristics, pathways and measures. Report for Miljødirektoratet M-959. 174p.

Vollertsen, J., Hansen, A.A. 2017. Microplastic in Danish wastewater: Sources, occurrences and fate. Environmental Project 1906, Danish Environmental Protection Agency.

Wagner, S., Hüffer, T., Klöckner, P., Wehrhahn, M., Hofmann, T. and Reemtsma, T., 2018. Tire wear particles in the aquatic environment-a review on generation, analysis, occurrence, fate and effects. Water Research, 139, pp. 83–100. Available at: https://doi.org/10.1016/j.watres.2018.03.051

Wik, A. and Dave, G., 2006. Acute toxicity of leachates of tire wear material to Daphnia magna—variability and toxic components. Chemosphere, 64(10), pp. 1777–1784. Available at: https://doi.org/10.1016/j.chemosphere.2005.12.045

Wright, S.L., Thompson, R.C. and Galloway, T.S., 2013. The physical impacts of microplastics on marine organisms: a review. Environmental Pollution, 178, pp. 483–492. Available at: https://doi.org/10.1016/j.envpol.2013.02.031

Zanzerl, H., Salvo, F., Jones, S.W. and Dufour, S.C., 2019. Feeding strategies in symbiotic and asymbiotic thyasirid bivalves. Journal of Sea Research, 145, pp. 16–23. Available at: https://doi.org/10.1016/j.seares.2018.12.005

Zhao, S., Zhu, L., Gao, L. and Li, D., 2018. Limitations for microplastic quantification in the ocean and recommendations for improvement and standardization. In Microplastic Contamination in Aquatic Environments (pp. 27–49). Elsevier. Available at: https://doi.org/10.1016/b978-0-12-813747-5.00002-3

7. Appendix

7.1 Sample overview

Table 16: Sample overview. Location of sample through the site code was not known at the time to the those that did microplastic analyses. Method of sampling: VV = Van Veen 0.1m^2, HP = hand-picked.

Site-kode	Species	Latitude	Longitude	Country	Institute	Date of sampling	Method of sampling	Depth of sampling (m)	Comments	Preservation
A-1	*Abra nitida*	56.60000	11.60000	Denmark	DTU	2808_2018	VV	27		ethanol, clear
A-2	*Abra nitida*	57.40718	11.23662	Denmark	DTU	2909_2018	VV	38		ethanol, clear
A-30	*Abra nitida*	57.63436	9.61396	Denmark	DTU	2808_2018	VV	42		ethanol, clear
A-6	*Abra nitida*	58.40367	9.01667	Norway	NIVA	0000_2014	VV	=270	*Abra spp. Abra nitida*	ethanol, clear
A-7	*Abra nitida*	58.94637	10.63910	Norway	NIVA	0000_2015	VV	356	*Abra nitida*	ethanol, rose bengal
A-9	*Abra nitida*	59.04092	10.76027	Norway	NIVA	0000_2017	VV	456	*Abra nitida*	ethanol, rose bengal, large individuals
A-12	*Abra nitida*	59.35875	10.59050	Norway	NIVA	0000_2015	VV	305	*Abra nitida*	ethanol, clear
A-3	*Abra nitida*	58.01971	7.11888	Norway	NIVA	0000_2013	VV	89	*Abra nitida*	ethanol, rose bengal
A-4	*Abra nitida*	58.11847	8.03277	Norway	NIVA	0000_2014	VV	190	*Abra spp. Abra nitida*	ethanol, rose bengal
A-5	*Abra nitida*	58.32387	8.58826	Norway	NIVA	0000_2014	VV	33	*Abra longicallis, Abra nitida*	ethanol, clear, large individuals
A-8	*Abra nitida*	59.00783	5.97175	Norway	NIVA	0000_2016	VV	167	*Abra longicallis, r Abra nitida*	ethanol, rose bengal
A-10	*Abra nitida*	59.10532	10.97345	Norway	NIVA	0000_2015	VV	48	*Abra nitida*	ethanol, rose bengal
A-11	*Abra nitida*	59.35042	5.31080	Norway	NIVA	0000_2015	VV	51	*Abra nitida*	ethanol, rose bengal
A-13	*Abra nitida*	60.54113	4.97368	Norway	ApN	0000_2014	VV	120	*Abra nitida*	ethanol, clear
A-14	*Abra nitida*	61.36475	5.02635	Norway	ApN	0000_2015	VV	228	*Abra nitida*	ethanol, clear
A-15	*Abra nitida*	62.55235	6.15205	Norway	ApN	0000_2015	VV	122	*Abra nitida*	ethanol, clear

A-16	*Abra nitida*	62.95953	7.44777	Norway	ApN	0000_2014	VV	180	*Abra nitida*	ethanol, rose bengal
A-17	*Abra nitida*	63.61463	8.49222	Norway	ApN	0000_2014	VV	115	*Abra nitida*	ethanol, clear
A-18	*Abra nitida*	63.86981	9.66625	Norway	NIVA	0000_2017	VV	199	*Abra nitida*	ethanol, clear
A-19	*Abra nitida*	63.91047	9.82813	Norway	ApN	0000_2014	VV	88	*Abra nitida*	ethanol, clear
A-20	*Abra nitida*	63.93776	9.99133	Norway	NIVA	0000_2017	VV	60	*Abra longicallis, Abra nitida*	ethanol, clear
A-21	*Abra nitida*	64.55698	10.92478	Norway	ApN	0000_2015	VV	154	*Abra nitida*	ethanol, clear
A-22	*Abra nitida*	64.61025	11.26207	Norway	ApN	0000_2014	VV	264	*Abra nitida*	ethanol, clear
A-31	*Abra nitida*	65.10525	11.80293	Denmark	ApN	0000_2014	VV	198		ethanol, clear
A-23	*Abra nitida*	65.14492	12.32410	Norway	ApN	0000_2014	VV	426	*Abra nitida*	ethanol, clear
A-24	*Abra nitida*	65.25571	12.82145	Norway	ApN	0000_2015	VV	417	*Abra nitida*	ethanol, rose bengal
A-25	*Abra nitida*	65.45092	12.55332	Norway	ApN	0000_2015	VV	135	*Abra nitida*	ethanol, clear
A-26	*Abra nitida*	65.85710	13.17580	Norway	NIVA	0000_2015	VV	140	*Abra spp.*	ethanol, clear
A-27	*Abra nitida*	66.12422	11.87740	Norway	NIVA	0000_2014	VV	288	*Abra longicallis, Abra nitida*	ethanol, rose bengal
A-28	*Abra nitida*	67.46100	15.50698	Norway	ApN	0000_2015	VV	296	*Abra nitida*	ethanol, rose bengal
A-29	*Abra nitida*	70.89060	24.95362	Norway	ApN	0000_2015	VV	147	*Abra nitida*	ethanol, clear
H-3	*Hiatella arctica*	65.11095	11.79507	Norway	ApN	1411_2014	VV	149		ethanol, clear
H-2	*Hiatella arctica*	65.11152	11.79472	Norway	ApN	1411_2014	VV	158		ethanol, clear
H-1	*Hiatella arctica*	65.11182	11.79453	Norway	ApN	1411_2014	VV	167		ethanol, clear
L-1	*Macoma/ Limecola*	58.18800	16.91470	Baltic Sea	ACES	14-1501_2019	VV	19		frozen
L-2	*Macoma/ Limecola*	58.26630	16.91300	Baltic Sea	ACES	14-1501_2019	VV	25		frozen
L-3	*Macoma/ Limecola*	58.37160	16.96720	Baltic Sea	ACES	14-1501_2019	VV	16		frozen
L-4	*Macoma/ Limecola*	58.81860	17.61630	Baltic Sea	ACES	14-1501_2019	VV	22-60		frozen
L-5	*Macoma/*	58.83190	17.53940	Baltic Sea	ACES	14-1501_2019	VV	=30		frozen

	Limecola									
L-6	*Macoma/ Limecola*	59.36495	5.29001	Norway	NIVA	0000_2015	VV	22		ethanol, rose bengal, large individuals
L-7	*Macoma/ Limecola*	59.69050	23.25717	Baltic Sea	SYKE	0009_2018	VV	57		frozen
L-8	*Macoma/ Limecola*	59.85470	23.25500	Baltic Sea	ACES	14-1501_2019	VV	=25		frozen
L-9	*Macoma/ Limecola*	59.92690	23.34130	Baltic Sea	ACES	14-1501_2019	VV	=11		frozen
L-10	*Macoma/ Limecola*	60.05817	21.19833	Baltic Sea	SYKE	0009_2018	VV	89		frozen
L-11	*Macoma/ Limecola*	60.09480	6.53968	Norway	NIVA	0000_2015	VV	40	*Macoma calcarean*	ethanol, clear
L-12	*Macoma/ Limecola*	63.51850	19.80250	Baltic Sea	ACES	14-1501_2019	VV	=24		frozen
L-13	*Macoma/ Limecola*	65.85710	13.17580	Norway	NIVA	0000_2015	VV	140	*Macoma calcarean*	ethanol, clear
L-14	*Macoma/ Limecola*	69.65033	18.76352	Norway	ApN	0000_2016	VV	32	*Macoma calcarean*	ethanol, rose bengal, large individuals

Site-kode	Species	Latitude	Longitude	Country	Institute	Date of sampling	Method of sampling	Depth of sampling (m)	Comments	Preservation
M-1	Mytilus spp.	65.62818	-37.56950	Greenland	GINR	0009_2018	HP	*surface*		frozen
M-27	Mytilus spp.	64.02000	-22.15850	Iceland	UISRC	1308_2018	HP	surface	Substrate: rock and seaweed	frozen
M-28	Mytilus spp.	65.74296	-37.33328	Greenland	GINR	0009_2018	HP	surface		frozen
M-29	Mytilus spp.	65.79176	-37.34070	Greenland	GINR	0009_2018	HP	surface		frozen
M-30	Mytilus spp.	65.89867	-22.83342	Iceland	UISRC	2807_2018	HP	surface	Substrate: rock and seaweed	frozen
M-31	Mytilus spp. (tiny)	66.42107	13.01780	Norway	ApN	0000_2015	VV	61		ethanol, rose bengal
M-32	Mytilus spp. (tiny)	70.09077	22.75072	Norway	ApN	0000_2015	VV	50		ethanol, clear
M-2	Mytilus spp.	64.15767	-21.79158	Iceland	UISRC	1308_2018	HP	surface	Substrate: mud	Frozen
M-3	Mytilus spp.	62.11870	-6.75310	Faroe islands	FIEA	2710_2018	HP	surface		Frozen

M-4	Mytilus spp.	57.59190	9.99220	Denmark	NIVA-Denmark	2408_2018	HP	surface	Sandy sediment. Mussels found on the shore in the tidalzone. There were no mussels on the pier.	Frozen
M-5	Mytilus spp.	65.64260	-37.92260	Greenland	GINR	0009_2018	HP	surface		Frozen
M-6	Mytilus spp.	61.55519	-6.82818	Faroe islands	FIEA	2810_2018	HP	surface		Frozen
M-7	Mytilus spp.	65.19117	-14.01333	Iceland	UISRC	0909_2018	HP	surface	Substrate: rock and seaweed	Frozen
M-8	Mytilus spp.	55.48600	8.41220	Denmark	NIVA-Denmark	2308_2018	HP	surface	Collected on the northside of the habour. Collected on low water (tidalzone). Lots of shorebird. Sandy sediment with few medium-big sized stones.	Frozen
M-9	Mytilus spp.	66.06911	-23.11813	Iceland	De Vries	2508_2018	HP	surface		Frozen
M-10	Mytilus spp.	56.79530	10.27900	Denmark	NIVA-Denmark	2408_2018	HP	surface	Lots of blue mussels and other epifauna species. All the mussels were adhered to the seafloor with byssus.	Frozen
M-11	Mytilus spp.	63.65144	9.56386	Norway	NIVA	1110_2018	HP	surface		Frozen
M-12	Mytilus spp.	60.40077	5.30396	Norway	NIVA	1910_2018	HP	surface		Frozen
M-13	Mytilus spp.	63.94975	-22.64767	Iceland	UISRC	1108_2018	HP	surface	Substrate: rock and seaweed	Frozen
M-14	Mytilus spp.	59.02740	10.52500	Norway	NIVA	2509_2018	HP	surface	36A, Færder (need to know which year was used. A	Frozen

									nearby station (36A1 59,07357N 10,42522E) could have been used.)	
M-15	Mytilus spp.	57.12510	8.62250	Denmark	NIVA-Denmark	2408_2018	HP	surface	Collected just off the shore. Lots of the mussel with Fucus sp. There where big amounts of Ulva sp. The water were unclear. Sandy sediment.	Frozen
M-16	Mytilus spp.	67.41271	14.62193	Norway	NIVA	1011_2018	HP	surface		Frozen
M-17	Mytilus spp.	78.22927	15.60147	Norway	ApN	0000_2016	HP	surface		Frozen
M-18	Mytilus spp.	59.58711	5.15203	Norway	NIVA	2709_2018f	HP	surface		Frozen
M-19	Mytilus spp.	59.90533	10.73633	Norway	NIVA	0309_2018	HP	surface		Frozen
M-20	Mytilus spp.	62.14772	-7.17243	Faroe islands	FIEA	2510_2018	HP	surface		Frozen
M-21	Mytilus spp.	55.99770	8.11750	Denmark	NIVA-Denmark	2308_2018	HP	surface	Found under water in the tidalzone between rocks on pier.	frozen
M-22	Mytilus spp.	57.75683	16.64583	Sweden	ACES	0008_2018	HP	surface		frozen
M-23	Mytilus spp.	57.84633	16.48050	Sweden	ACES	0008_2018	HP	surface		frozen
M-24	Mytilus spp.	57.74583	16.76100	Sweden	ACES	0008_2018	HP	surface		frozen
M-25	Mytilus spp. (tiny)	63.84728	8.52760	Norway	ApN	0000_2014	VV	50		ethanol, clear
M-26	Mytilus spp.	64.00550	-22.96150	Iceland	UISRC	2807_2018	HP	surface	Substrate: rock and soft sediment	frozen
T-1	Thyasira spp.	56.12388	12.45895	Denmark	DTU	2808_2018	*VV*	27		ethanol, clear
T-2	Thyasira	57.40718	11.23662	Denmark	DTU	2909_2018	*VV*	38		ethanol,

	spp.									clear
T-19	Thyasira spp.	57.63583	9.61468	Denmark	DTU	2808_2018	*VV*	42		ethanol, clear
T-3	Thyasira spp.	57.63436	9.61396	Denmark	DTU	2808_2018	*VV*	42		ethanol, clear
T-6	Thyasira spp.	59.04092	10.76027	Norway	NIVA	0000_2017	*VV*	456	*Thyasira sarsii*	ethanol, rose bengal
T-18	Thyasira spp.	56.12388	12.45895	Denmark	DTU	2808_2018	*VV*	27		ethanol, clear
T-4	Thyasira spp.	58.01971	7.11888	Norway	NIVA	0000_2013	*VV*	88-90	*Thyasira obsulata, Thyrasira sp.*	ethanol, rose bengal
T-5	Thyasira spp.	59.00783	5.97175	Norway	NIVA	0000_2016	*VV*	167	*Thyasira obsulata, Thyrasira sarsii*	ethanol, clear
T-7	Thyasira spp.	60.09480	6.53968	Norway	NIVA	0000_2015	*VV*	40	*Thyasira sp., Thyrasira sarsii*	ethanol, rose bengal
T-8	Thyasira spp.	61.36475	5.02635	Norway	ApN	0000_2015	*VV*	228	*Thyasira equalis*	ethanol, clear
T-9	Thyasira spp.	62.44667	6.28944	Norway	NIVA	0000_2015	*VV*	40	*Thyasira sp.*	ethanol, rose bengal
T-10	Thyasira spp.	62.95953	7.44777	Norway	ApN	0000_2014	*VV*	180	*Thyasira equalis*	ethanol, rose bengal
T-11	Thyasira spp.	63.91047	9.82813	Norway	ApN	0000_2014	*VV*	111	*Thyasira equalis*	ethanol, clear
T-12	Thyasira spp.	63.93776	9.99133	Norway	NIVA	0000_2017	*VV*	60	*Thyasira sarsii*	ethanol, clear
T-20	Thyasira spp.	64.61025	11.26207	Norway	ApN	0000_2014	*VV*	264	*Thyasira equalis*	ethanol, clear
T-13	Thyasira spp.	65.25571	12.82145	Norway	ApN	0000_2015	*VV*	423	*Thyasira equalis,*	ethanol, clear
T-14	Thyasira spp.	65.85710	13.17580	Norway	NIVA	0000_2015	*VV*	140	*Thyasira sp.*	ethanol, rose bengal
T-15	Thyasira spp.	66.32500	14.12883	Norway	NIVA	0000_2015	*VV*	92-96	*Thyasira sp.*	ethanol, rose bengal
T-16	Thyasira spp.	69.65033	18.76352	Norway	ApN	0000_2016	*VV*	20	*Thyasira gouldi*	ethanol, clear
T-17	Thyasira spp.	70.89060	24.95362	Norway	ApN	0000_2016	*VV*	147	*Thyasira sarsii*	ethanol, rose Bengal

7.2 Reference material treated with KOH + acetic acid

Table 17: Recovery test of reference material in the presence and absence of biota tissue after incubation at 40 or 60 degrees for 24–72h with 10% KOH at 100 rpm shaking. The results represent average value (± SD) expressed in percentage of the number of initially spiked in polymers.

				Polymer recovery (%)			
Incubation time (h)	Temperature (oC)	Biota length (mm)	Biota w.w. (g)	Tyre frag	PET fibre	PVC frag	PET frag
24	60	50.1 ± 11.9	3.1 ± 1.8	86 ± 11	60 ± 37	78 ± 16	70 ± 19
48	40	57.7 ± 8.5	4.7 ± 1.7	75 ± 7	50 ± 0	70 ± 28	65 ± 21
	60	52.3 ± 8.2	3.2 ± 1.7	88 ± 8	6 ± 13	62 ± 13	58 ± 29
72	40	56.3 ± 8.8	3.6 ± 1.7	92 ± 13	60 ± 45	72 ± 19	76 ± 18
24	60	-	-	100 ± 0	15 ± 21	80 ± 0	75 ± 7
48	40	-	-	50	40	60	90
	60	-	-	82 ± 15	34 ± 11	78 ± 16	16 ± 22
72	40	-	-	76 ± 18	58 ± 20	88 ± 18	70 ± 28

Table 18 shows the quality score before after KOH + Acetic acid and Figure 44 represent FT-IR performed with Agilent Cary 360. Settings on the instrument were as following:

Sample Scans: 8
Background Scans:16
Resolution: 4
Range: 4000–650

Table 18: FT-IR before/after recovery test of the seven polymers tested for impact from KOH + acetic acid.

Abbreviation	Shape	FT-IR assessment
PP	Fragments	0.8570/0.9227
PA66	Fragments	0.8284/0.8493
LDPE	Fragment	0.8815/0.9595
PET	Fibres	0.9108/0.7243
PET	Fragment	0.7718/0.9526
PVC	Fragment	0.8787/0.8746
Rubber (Tyre)	Fragments	0.9805/0.8649

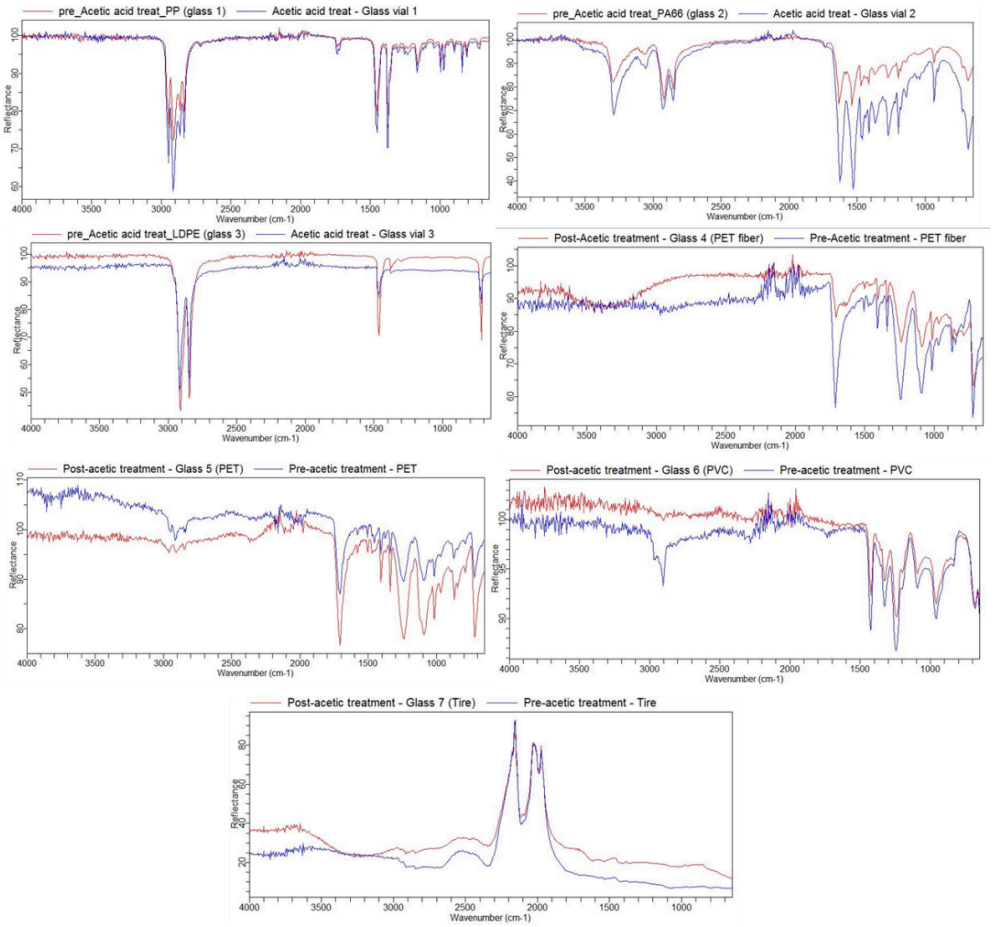

Figure 44: FT-IR spectra of the seven polymers tested for impact from KOH + acetic acid. See Table 18 for more information.

7.3 Weight and length of *Mytilus* spp.

Table 19: Wet weight and dry weight of analysed mussels.

Site	Rep	% d.w	% w.w	Mean
M-1	1	18.06	81.94	
M-1	2	17.16	82.84	
M-1	3	17.96	82.04	82.28
M-2	1	12.14	87.86	
M-2	2	13.95	86.05	
M-2	3	13.99	86.01	86.64
M-3	1	20.99	79.01	
M-3	2	18.34	81.66	
M-3	3	20.62	79.38	80.02
M-4	1	21.75	78.25	
M-4	2	16.52	83.48	80.87
M-6	1	11.38	88.62	
M-6	2	15.51	84.49	
M-6	3	18.43	81.57	84.89
M-7	1	9.62	90.38	
M-7	2	14.56	85.44	
M-7	3	11.09	88.91	88.25
M-9	1	15.50	84.50	
M-9	2	17.76	82.24	83.37
M-10	1	11.14	88.86	
M-10	2	11.08	88.92	88.89
M-11	1	19.76	80.24	
M-11	2	15.28	84.72	
M-11	3	17.29	82.71	82.56
M-12	1	10.79	89.21	
M-12	2	18.13	81.87	
M-12	3	13.04	86.96	
M-12	4	9.00	91.00	87.26
M-13	1	17.36	82.64	
M-13	2	17.01	82.99	
M-13	3	16.48	83.52	83.05
M-14	1	10.90	89.10	

M-14	2	9.77	90.23	
M-14	3	13.58	86.42	88.58
M-15	1	21.34	78.66	
M-15	2	20.59	79.41	79.04
M-16	1	11.33	88.67	
M-16	2	23.17	76.83	
M-16	3	12.68	87.32	84.27
M-18	1	20.10	79.90	
M-18	2	32.24	67.76	
M-18	3	NA	NA	73.83
M-19	1	13.44	86.56	
M-19	2	8.56	91.44	
M-19	3	12.36	87.64	88.55
M-20	1	18.88	81.12	
M-20	2	17.16	82.84	
M-20	3	88.32	NA	81.98
M-21	1	18.80	81.20	
M-21	2	20.18	79.82	
M-21	3	19.52	80.48	80.50
M-23	1	17.00	83.00	
M-23	2	11.40	88.60	
M-23	3	14.40	85.60	85.73
M-24	1	14.36	85.64	
M-24	2	15.32	84.68	
M-24	3	10.10	89.90	86.74
M-26	1	17.09	82.91	
M-26	2	15.49	84.51	
M-26	3	15.41	84.59	84.01
M-27	1	15.53	84.47	
M-27	2	11.89	88.11	
M-27	3	13.03	86.97	86.52
M-28	1	16.01	83.99	
M-28	2	10.21	89.79	
M-28	3	11.27	88.73	87.50
M-29	1	13.08	86.92	
M-29	2	13.22	86.78	
M-29	3	NA		86.85

M-30	1	14.58	85.42	
M-30	2	13.42	86.58	
M-30	3	13.62	86.38	86.13

Table 20: Estimated dry weight of *Mytilus edulis* based on Table 19.

ID	Length (mm)	Weight (g)	Mean w.w (%)	Mean d.w (%)	d.w. factor	Estimated d.w (g)
M-1	43.4	1.52	82.28	17.72	0.18	0.27
M-1	49.2	2.49	82.28	17.72	0.18	0.44
M-1	48.6	2.35	82.28	17.72	0.18	0.42
M-1	53.9	2.28	82.28	17.72	0.18	0.40
M-1	42.3	1.4	82.28	17.72	0.18	0.25
M-1	50.2	3.7	82.28	17.72	0.18	0.66
M-1	50.1	4.1	82.28	17.72	0.18	0.73
M-1	50.1	NA	82.28	17.72	0.18	NA
M-1	34.4	1.2	82.28	17.72	0.18	0.21
M-1	62.1	4.7	82.28	17.72	0.18	0.83
M-1	56.8	3.835	82.28	17.72	0.18	0.68
M-1	52.4	2.899	82.28	17.72	0.18	0.51
M-1	40	1.389	82.28	17.72	0.18	0.25
M-1	49.4	NA	82.28	17.72	0.18	NA
M-1	51.2	2.58	82.28	17.72	0.18	0.46
M-1	52.7	1.271	82.28	17.72	0.18	0.23
M-1	55.5	2.404	82.28	17.72	0.18	0.43
M-1	44.8	2.088	82.28	17.72	0.18	0.37
M-1	39.5	1.609	82.28	17.72	0.18	0.29
M-1	37.6	1.36	82.28	17.72	0.18	0.24
M-2	47.3	3.431	86.64	13.36	0.13	0.46
M-2	53.6	6.276	86.64	13.36	0.13	0.84
M-2	45.6	1.84	86.64	13.36	0.13	0.25
M-2	43.2	2.01	86.64	13.36	0.13	0.27
M-2	51.2	2.27	86.64	13.36	0.13	0.30
M-2	50.6	1.6	86.64	13.36	0.13	0.21
M-2	44.8	1.7	86.64	13.36	0.13	0.23
M-2	49.2	2.4	86.64	13.36	0.13	0.32
M-2	47.8	2.7	86.64	13.36	0.13	0.36

M-2	49.8	3.6	86.64	13.36	0.13	0.48
M-2	47.8	2.553	86.64	13.36	0.13	0.34
M-2	50	2.165	86.64	13.36	0.13	0.29
M-2	51	3.131	86.64	13.36	0.13	0.42
M-2	48	1.957	86.64	13.36	0.13	0.26
M-2	43.2	1.016	86.64	13.36	0.13	0.14
M-2	44.5	2.936	86.64	13.36	0.13	0.39
M-2	50.5	1.96	86.64	13.36	0.13	0.26
M-2	48.3	1.534	86.64	13.36	0.13	0.20
M-2	47.4	2.001	86.64	13.36	0.13	0.27
M-2	51.5	2.664	86.64	13.36	0.13	0.36
M-3	42.5	1.81	80.02	19.98	0.20	0.36
M-3	50.8	3.72	80.02	19.98	0.20	0.74
M-3	38	1.56	80.02	19.98	0.20	0.31
M-3	55.5	6.16	80.02	19.98	0.20	1.23
M-3	53.5	4.84	80.02	19.98	0.20	0.97
M-3	61.9	7.9	80.02	19.98	0.20	1.58
M-3	58.6	7.5	80.02	19.98	0.20	1.50
M-3	40.4	2	80.02	19.98	0.20	0.40
M-3	42.1	2.8	80.02	19.98	0.20	0.56
M-3	62	10.7	80.02	19.98	0.20	2.14
M-3	57	4.533	80.02	19.98	0.20	0.91
M-3	54	5.491	80.02	19.98	0.20	1.10
M-3	48.6	3.323	80.02	19.98	0.20	0.66
M-3	52	3.411	80.02	19.98	0.20	0.68
M-3	57.7	4.48	80.02	19.98	0.20	0.90
M-3	57.9	5.638	80.02	19.98	0.20	1.13
M-3	56.3	4.458	80.02	19.98	0.20	0.89
M-3	49.3	3.728	80.02	19.98	0.20	0.75
M-3	50.5	4.465	80.02	19.98	0.20	0.89
M-3	46.6	3.234	80.02	19.98	0.20	0.65
M-4	45.8	0.97	80.87	19.13	0.19	0.19
M-4	43.9	0.84	80.87	19.13	0.19	0.16
M-4	49	2.13	80.87	19.13	0.19	0.41
M-4	55.3	1.34	80.87	19.13	0.19	0.26
M-4	46.5	1	80.87	19.13	0.19	0.19
M-4	47.1	2.22	80.87	19.13	0.19	0.42

M-4	51.4	1.37	80.87	19.13	0.19	0.26
M-4	46.5	1.44	80.87	19.13	0.19	0.28
M-4	39.6	0.78	80.87	19.13	0.19	0.15
M-4	47.3	1.75	80.87	19.13	0.19	0.33
M-4	44.1	0.934	80.87	19.13	0.19	0.18
M-4	38.6	0.65	80.87	19.13	0.19	0.12
M-4	48.3	2.088	80.87	19.13	0.19	0.40
M-4	36.6	0.591	80.87	19.13	0.19	0.11
M-4	41.9	1.204	80.87	19.13	0.19	0.23
M-4	52.6	1.634	80.87	19.13	0.19	0.31
M-4	40.8	0.906	80.87	19.13	0.19	0.17
M-4	51.9	2.261	80.87	19.13	0.19	0.43
M-4	27.2	0.325	80.87	19.13	0.19	0.06
M-5	28.7	0.76	84.35	15.65	0.16	0.12
M-5	37.8	2	84.35	15.65	0.16	0.31
M-5	40.2	2.38	84.35	15.65	0.16	0.37
M-5	54.2	3.8	84.35	15.65	0.16	0.59
M-5	34.2	0.73	84.35	15.65	0.16	0.11
M-5	48.2	3.8	84.35	15.65	0.16	0.59
M-5	49.1	3.8	84.35	15.65	0.16	0.59
M-5	35.3	1.9	84.35	15.65	0.16	0.30
M-5	59.1	9.9	84.35	15.65	0.16	1.55
M-5	43.5	2.2	84.35	15.65	0.16	0.34
M-5	60.4	4.361	84.35	15.65	0.16	0.68
M-5	34.8	1.368	84.35	15.65	0.16	0.21
M-5	31.8	1.035	84.35	15.65	0.16	0.16
M-5	34.7	1.185	84.35	15.65	0.16	0.19

ID	Length (mm)	Weight (g)	Mean w.w (%)	Mean d.w (%)	d.w. factor	Estimated d.w (g)
M-6	58.4	6.31	84.89	15.11	0.15	0.95
M-6	50.2	2.73	84.89	15.11	0.15	0.41
M-6	64.2	3.68	84.89	15.11	0.15	0.56
M-6	52.1	4.68	84.89	15.11	0.15	0.71
M-6	44	2.05	84.89	15.11	0.15	0.31
M-6	48.11	3.01	84.89	15.11	0.15	0.45
M-6	40.05	1.4	84.89	15.11	0.15	0.21
M-6	50.05	2.67	84.89	15.11	0.15	0.40
M-6	57.1	6.37	84.89	15.11	0.15	0.96
M-6	52.2	3.01	84.89	15.11	0.15	0.45
M-6	65.8	5.987	84.89	15.11	0.15	0.90
M-6	52.3	3.868	84.89	15.11	0.15	0.58
M-6	56.3	3.411	84.89	15.11	0.15	0.52
M-6	48.2	1.612	84.89	15.11	0.15	0.24
M-6	48.1	3.207	84.89	15.11	0.15	0.48
M-6	55.8	3.189	84.89	15.11	0.15	0.48
M-6	51.7	3.839	84.89	15.11	0.15	0.58
M-6	50.1	1.631	84.89	15.11	0.15	0.25
M-6	50.6	3.174	84.89	15.11	0.15	0.48
M-6	49.6	3.505	84.89	15.11	0.15	0.53
M-7	51.8	1.19	88.25	11.75	0.12	0.14
M-7	52.5	1.64	88.25	11.75	0.12	0.19
M-7	52.6	2	88.25	11.75	0.12	0.24
M-7	55.2	1.92	88.25	11.75	0.12	0.23
M-7	53.1	1.86	88.25	11.75	0.12	0.22
M-7	54.2	2.45	88.25	11.75	0.12	0.29
M-7	47.8	1.66	88.25	11.75	0.12	0.20
M-7	43.4	1.43	88.25	11.75	0.12	0.17
M-7	52.1	2.57	88.25	11.75	0.12	0.30
M-7	44.9	0.76	88.25	11.75	0.12	0.09
M-7	50.2	2.793	88.25	11.75	0.12	0.33
M-7	50.5	1.849	88.25	11.75	0.12	0.22
M-7	57.5	2.122	88.25	11.75	0.12	0.25
M-7	49.9	1.647	88.25	11.75	0.12	0.19
M-7	45.9	1.625	88.25	11.75	0.12	0.19
M-7	45	1.01	88.25	11.75	0.12	0.12

M-7	52.5	1.67	88.25	11.75	0.12	0.20
M-7	47	1.492	88.25	11.75	0.12	0.18
M-7	44.8	0.999	88.25	11.75	0.12	0.12
M-7	53.6	2.021	88.25	11.75	0.12	0.24
M-8	59	3.59	84.35	15.65	0.16	0.56
M-8	42.9	0.94	84.35	15.65	0.16	0.15
M-8	32.4	0.89	84.35	15.65	0.16	0.14
M-8	36.1	0.78	84.35	15.65	0.16	0.12
M-8	40.6	0.79	84.35	15.65	0.16	0.12
M-8	56.1	3.54	84.35	15.65	0.16	0.55
M-8	36.2	1.01	84.35	15.65	0.16	0.16
M-8	51.8	1.14	84.35	15.65	0.16	0.18
M-8	33.9	0.8	84.35	15.65	0.16	0.13
M-8	36.8	0.83	84.35	15.65	0.16	0.13
M-8	49.5	2.262	84.35	15.65	0.16	0.35
M-8	31.8	0.709	84.35	15.65	0.16	0.11
M-8	53.1	2.471	84.35	15.65	0.16	0.39
M-8	32.4	0.763	84.35	15.65	0.16	0.12
M-8	30.5	0.723	84.35	15.65	0.16	0.11
M-8	33.1	0.905	84.35	15.65	0.16	0.14
M-8	50.3	2.4	84.35	15.65	0.16	0.38
M-8	40.1	1.653	84.35	15.65	0.16	0.26
M-8	36.1	1.234	84.35	15.65	0.16	0.19
M-8	47.1	1.1221	84.35	15.65	0.16	0.18
M-9	46.2	1.911	83.37	16.63	0.17	0.32
M-9	49	2.093	83.37	16.63	0.17	0.35
M-9	52.1	2.463	83.37	16.63	0.17	0.41
M-9	43	1.39	83.37	16.63	0.17	0.23
M-9	43.9	1.241	83.37	16.63	0.17	0.21
M-9	51.8	2.47	83.37	16.63	0.17	0.41
M-9	43.9	1.76	83.37	16.63	0.17	0.29
M-9	42.4	1.15	83.37	16.63	0.17	0.19
M-9	57.2	2.85	83.37	16.63	0.17	0.47
M-9	43.9	1.76	83.37	16.63	0.17	0.29
M-9	58.6	4.856	83.37	16.63	0.17	0.81
M-9	68.1	4.822	83.37	16.63	0.17	0.80
M-9	53.3	3.44	83.37	16.63	0.17	0.57

M-9	38.3	1.474	83.37	16.63	0.17	0.25
M-9	45.1	2.884	83.37	16.63	0.17	0.48
M-9	44.6	2.517	83.37	16.63	0.17	0.42
M-9	50.4	2.357	83.37	16.63	0.17	0.39
M-9	44.5	2.407	83.37	16.63	0.17	0.40
M-9	40.2	1.115	83.37	16.63	0.17	0.19
M-9	35.3	1.2	83.37	16.63	0.17	0.20
M-10	56.1	2.877	88.89	11.11	0.11	0.32
M-10	53.8	3.659	88.89	11.11	0.11	0.41
M-10	48.2	2.406	88.89	11.11	0.11	0.27
M-10	49.8	2.456	88.89	11.11	0.11	0.27
M-10	48.9	2.033	88.89	11.11	0.11	0.23
M-10	57.9	2.3	88.89	11.11	0.11	0.26
M-10	54.6	0.56	88.89	11.11	0.11	0.06
M-10	48	2.29	88.89	11.11	0.11	0.25
M-10	40.5	2.81	88.89	11.11	0.11	0.31
M-10	59.1	3.8	88.89	11.11	0.11	0.42
M-10	45	0.827	88.89	11.11	0.11	0.09
M-10	53.7	2.473	88.89	11.11	0.11	0.27
M-10	40.6	1.627	88.89	11.11	0.11	0.18
M-10	37.3	0.587	88.89	11.11	0.11	0.07
M-10	57	3.406	88.89	11.11	0.11	0.38
M-10	47.9	2.965	88.89	11.11	0.11	0.33
M-10	54.8	2.721	88.89	11.11	0.11	0.30
M-10	52	1.458	88.89	11.11	0.11	0.16
M-10	51	1.69	88.89	11.11	0.11	0.19
M-10	50.3	2.41	88.89	11.11	0.11	0.27

ID	Length (mm)	Weight (g)	Mean w.w (%)	Mean d.w (%)	d.w. factor	Estimated d.w (g)
M-11	46.9	2.181	82.56	17.44	0.17	0.38
M-11	53.4	3.39	82.56	17.44	0.17	0.59
M-11	50.2	3.436	82.56	17.44	0.17	0.60
M-11	58.2	2.762	82.56	17.44	0.17	<0.48
M-11	46.4	3.181	82.56	17.44	0.17	0.55
M-11	54.6	4.42	82.56	17.44	0.17	0.77
M-11	52.8	2.06	82.56	17.44	0.17	0.36
M-11	46.5	3.18	82.56	17.44	0.17	0.55
M-11	50.6	3.84	82.56	17.44	0.17	0.67
M-11	45	2.547	82.56	17.44	0.17	0.44
M-11	40.7	2.281	82.56	17.44	0.17	0.40
M-11	45.3	2.325	82.56	17.44	0.17	0.41
M-11	42.2	1.875	82.56	17.44	0.17	0.33
M-11	42	2.108	82.56	17.44	0.17	0.37
M-11	42.8	2.801	82.56	17.44	0.17	0.49
M-11	40.3	1.373	82.56	17.44	0.17	0.24
M-11	44.3	1.846	82.56	17.44	0.17	0.32
M-11	44.3	2.483	82.56	17.44	0.17	0.43
M-12	40.1	0.786	87.26	12.74	0.13	0.10
M-12	34.7	0.808	87.26	12.74	0.13	0.10
M-12	53.4	1.692	87.26	12.74	0.13	0.22
M-12	51.8	2.376	87.26	12.74	0.13	0.30
M-12	36.5	0.778	87.26	12.74	0.13	0.10
M-12	51.4	2.53	87.26	12.74	0.13	0.32
M-12	51.5	4.92	87.26	12.74	0.13	0.63
M-12	44.6	6.5	87.26	12.74	0.13	0.83
M-12	39.9	1.01	87.26	12.74	0.13	0.13
M-12	39.6	1.12	87.26	12.74	0.13	0.14
M-12	30.8	0.738	87.26	12.74	0.13	0.09
M-12	26.6	0.294	87.26	12.74	0.13	0.04
M-12	31.7	0.64	87.26	12.74	0.13	0.08
M-12	31.4	0.751	87.26	12.74	0.13	0.10
M-12	30.8	0.382	87.26	12.74	0.13	0.05
M-12	31.5	0.712	87.26	12.74	0.13	0.09
M-12	35.9	0.936	87.26	12.74	0.13	0.12
M-12	30.5	0.487	87.26	12.74	0.13	0.06

M-12	26	0.233	87.26	12.74	0.13	0.03
M-12	22	0.39	87.26	12.74	0.13	0.05
M-13	47.1	2.242	83.05	16.95	0.17	0.38
M-13	49.9	2.334	83.05	16.95	0.17	0.40
M-13	49.1	2.806	83.05	16.95	0.17	0.48
M-13	52.4	2.66	83.05	16.95	0.17	0.45
M-13	48.7	3.018	83.05	16.95	0.17	0.51
M-13	44.6	2.07	83.05	16.95	0.17	0.35
M-13	51.6	3.2	83.05	16.95	0.17	0.54
M-13	48.3	NA	83.05	16.95	0.17	NA
M-13	43.4	2.01	83.05	16.95	0.17	0.34
M-13	47.8	2.15	83.05	16.95	0.17	0.36
M-13	50.2	3.99	83.05	16.95	0.17	0.68
M-13	50.6	2.604	83.05	16.95	0.17	0.44
M-13	44.6	3.779	83.05	16.95	0.17	0.64
M-13	45.6	2.522	83.05	16.95	0.17	0.43
M-13	46.1	2.238	83.05	16.95	0.17	0.38
M-13	48.1	2.58	83.05	16.95	0.17	0.44
M-13	44.8	2.24	83.05	16.95	0.17	0.38
M-13	45.1	2.14	83.05	16.95	0.17	0.36
M-13	39.8	1.738	83.05	16.95	0.17	0.29
M-13	47.8	3.562	83.05	16.95	0.17	0.60
M-14	82.4	8.895	88.58	11.42	0.11	1.02
M-14	59.1	6.246	88.58	11.42	0.11	0.71
M-14	58	4.527	88.58	11.42	0.11	0.52
M-14	80.8	5.701	88.58	11.42	0.11	0.65
M-14	51	2.076	88.58	11.42	0.11	0.24
M-14	82.7	9.726	88.58	11.42	0.11	1.11
M-14	44.1	2.099	88.58	11.42	0.11	0.24
M-14	80.2	7.161	88.58	11.42	0.11	0.82
M-14	79.1	14.423	88.58	11.42	0.11	1.65
M-14	49	1.975	88.58	11.42	0.11	0.23
M-14	93.5	18.82	88.58	11.42	0.11	2.15
M-14	84.7	16.2	88.58	11.42	0.11	1.85
M-14	86.4	12.5	88.58	11.42	0.11	1.43
M-14	80.8	10.1	88.58	11.42	0.11	1.15
M-14	82.1	7.87	88.58	11.42	0.11	0.90

M-14	82.2	14.02	88.58	11.42	0.11	1.60
M-14	81.4	9.85	88.58	11.42	0.11	1.12
M-14	55.6	4.01	88.58	11.42	0.11	0.46
M-14	39.6	1.79	88.58	11.42	0.11	0.20
M-14	80.4	9.75	88.58	11.42	0.11	1.11
M-15	57.3	1.603	79.04	20.96	0.21	0.34
M-15	47.2	2.356	79.04	20.96	0.21	0.49
M-15	60.2	7.262	79.04	20.96	0.21	1.52
M-15	48	3.912	79.04	20.96	0.21	0.82
M-15	46.4	3.616	79.04	20.96	0.21	0.76
M-15	49.4	4.28	79.04	20.96	0.21	0.90
M-15	54.4	7.2	79.04	20.96	0.21	1.51
M-15	57.6	3.95	79.04	20.96	0.21	0.83
M-15	58.5	7.28	79.04	20.96	0.21	1.53
M-15	51.1	2.06	79.04	20.96	0.21	0.43
M-15	60.6	4.59	79.04	20.96	0.21	0.96
M-15	52.2	6.451	79.04	20.96	0.21	1.35
M-15	59.7	4.799	79.04	20.96	0.21	1.01
M-15	58.3	3.172	79.04	20.96	0.21	0.66
M-15	69.7	7.4312	79.04	20.96	0.21	1.56
M-15	54.5	4.312	79.04	20.96	0.21	0.90
M-15	64.6	6.406	79.04	20.96	0.21	1.34
M-15	60	5.573	79.04	20.96	0.21	1.17
M-15	59.1	4.054	79.04	20.96	0.21	0.85
M-15	50.8	2.532	79.04	20.96	0.21	0.53

ID	Length (mm)	Weight (g)	Mean w.w (%)	Mean d.w (%)	d.w. factor	Estimated d.w (g)
M-16	50.2	2.62	84.27	15.73	0.16	0.41
M-16	41.6	2.02	84.27	15.73	0.16	0.32
M-16	40.6	1.504	84.27	15.73	0.16	0.24
M-16	39.9	1.937	84.27	15.73	0.16	0.30
M-16	42.2	1.689	84.27	15.73	0.16	0.27
M-16	50	2.69	84.27	15.73	0.16	0.42
M-16	50.7	2.19	84.27	15.73	0.16	0.34
M-16	53.9	2.92	84.27	15.73	0.16	0.46
M-16	41.4	1.53	84.27	15.73	0.16	0.24
M-16	41.7	1.66	84.27	15.73	0.16	0.26
M-16	39.9	1.09	84.27	15.73	0.16	0.17
M-16	46.7	2.88	84.27	15.73	0.16	0.45
M-16	46.4	3	84.27	15.73	0.16	0.47
M-16	41	1.66	84.27	15.73	0.16	0.26
M-16	43.8	2.15	84.27	15.73	0.16	0.34
M-16	48.7	2.36	84.27	15.73	0.16	0.37
M-16	48	2.11	84.27	15.73	0.16	0.33
M-16	46.8	2.36	84.27	15.73	0.16	0.37
M-16	48.8	2.54	84.27	15.73	0.16	0.40
M-16	41.5	1.61	84.27	15.73	0.16	0.25
M-17	53.8	2.395	84.35	15.65	0.16	0.37
M-17	55.8	6.386	84.35	15.65	0.16	1.00
M-17	54.3	4.639	84.35	15.65	0.16	0.73
M-17	49.3	3.834	84.35	15.65	0.16	0.60
M-17	52.8	2.849	84.35	15.65	0.16	0.45
M-17	56.6	3.665	84.35	15.65	0.16	0.57
M-17	55.2	4.335	84.35	15.65	0.16	0.68
M-17	58.3	3.097	84.35	15.65	0.16	0.48
M-17	45.1	1.753	84.35	15.65	0.16	0.27
M-17	44.9	2.113	84.35	15.65	0.16	0.33
M-17	44.5	1.71	84.35	15.65	0.16	0.27
M-17	50.6	1.95	84.35	15.65	0.16	0.31
M-17	45.6	1.75	84.35	15.65	0.16	0.27
M-17	42.6	1.48	84.35	15.65	0.16	0.23
M-17	51.9	2.1	84.35	15.65	0.16	0.33
M-17	NA	NA	84.35	15.65	0.16	NA

M-17	NA	NA	84.35	15.65	0.16	NA
M-18	40.5	1.675	73.83	26.17	0.26	0.44
M-18	39.1	1.764	73.83	26.17	0.26	0.46
M-18	36.7	1.092	73.83	26.17	0.26	0.29
M-18	35.6	1.338	73.83	26.17	0.26	0.35
M-18	35.3	1.324	73.83	26.17	0.26	0.35
M-18	35.5	1.48	73.83	26.17	0.26	0.39
M-18	35.4	1.11	73.83	26.17	0.26	0.29
M-18	35.6	0.91	73.83	26.17	0.26	0.24
M-18	39.9	1.68	73.83	26.17	0.26	0.44
M-18	38.1	1.51	73.83	26.17	0.26	0.40
M-18	36.4	1.59	73.83	26.17	0.26	0.42
M-18	47.4	2.58	73.83	26.17	0.26	0.68
M-18	40.8	1.36	73.83	26.17	0.26	0.36
M-18	43.8	2.31	73.83	26.17	0.26	0.60
M-18	39.6	1.74	73.83	26.17	0.26	0.46
M-18	44.9	1.67	73.83	26.17	0.26	0.44
M-18	43.9	1.87	73.83	26.17	0.26	0.49
M-18	48.4	1.9	73.83	26.17	0.26	0.50
M-18	44.2	2	73.83	26.17	0.26	0.52
M-18	40.9	1.6	73.83	26.17	0.26	0.42
M-19	84.5	8.084	88.55	11.45	0.11	0.93
M-19	85.2	16.295	88.55	11.45	0.11	1.87
M-19	90	12.501	88.55	11.45	0.11	1.43
M-19	90.7	18.043	88.55	11.45	0.11	2.07
M-19	79.5	9.396	88.55	11.45	0.11	1.08
M-19	80	13.075	88.55	11.45	0.11	1.50
M-19	80.1	16.723	88.55	11.45	0.11	1.92
M-19	78.2	19.38	88.55	11.45	0.11	2.22
M-19	80.4	9.844	88.55	11.45	0.11	1.13
M-19	80.3	24.571	88.55	11.45	0.11	2.81
M-19	56.4	4.348	88.55	11.45	0.11	0.50
M-19	40.8	2.455	88.55	11.45	0.11	0.28
M-19	47.2	2.446	88.55	11.45	0.11	0.28
M-19	52.8	4.685	88.55	11.45	0.11	0.54
M-19	40.6	1.957	88.55	11.45	0.11	0.22
M-19	43.5	2.725	88.55	11.45	0.11	0.31

M-19	57.7	5.153	88.55	11.45	0.11	0.59
M-19	44.8	2.6	88.55	11.45	0.11	0.30
M-19	37	1.45	88.55	11.45	0.11	0.17
M-19	44.8	2.919	88.55	11.45	0.11	0.33
M-20	90.7	19.055	81.98	18.02	0.18	3.43
M-20	75.4	11.575	81.98	18.02	0.18	2.09
M-20	74.2	10.472	81.98	18.02	0.18	1.89
M-20	83.5	18.489	81.98	18.02	0.18	3.33
M-20	63.9	7.45	81.98	18.02	0.18	1.34
M-20	95.6	21.472	81.98	18.02	0.18	3.87
M-20	88	21.726	81.98	18.02	0.18	3.91
M-20	97.5	29.653	81.98	18.02	0.18	5.34
M-20	93.2	26.118	81.98	18.02	0.18	4.71
M-20	24.3	0.545	81.98	18.02	0.18	0.10
M-20	95.6	29.24	81.98	18.02	0.18	5.27
M-20	90.8	17.17	81.98	18.02	0.18	3.09
M-20	86	22.85	81.98	18.02	0.18	4.12
M-20	78.4	19.81	81.98	18.02	0.18	3.57
M-20	88.1	23.92	81.98	18.02	0.18	4.31
M-20	84.6	18.64	81.98	18.02	0.18	3.36
M-20	62.5	10.8	81.98	18.02	0.18	1.95
M-20	48.3	5.5	81.98	18.02	0.18	0.99
M-20	54.4	5.54	81.98	18.02	0.18	1.00
M-20	64.8	8.61	81.98	18.02	0.18	1.55

ID	Length (mm)	Weight (g)	Mean w.w (%)	Mean d.w (%)	d.w. factor	Estimated d.w (g)
M-21	47.2	1.99	80.50	19.50	0.20	0.39
M-21	51.6	3.06	80.50	19.50	0.20	0.60
M-21	49.1	2.18	80.50	19.50	0.20	0.43
M-21	56.6	3.84	80.50	19.50	0.20	0.75
M-21	59.1	5.14	80.50	19.50	0.20	1.00
M-21	56.9	3.41	80.50	19.50	0.20	0.67
M-21	51.9	2.47	80.50	19.50	0.20	0.48
M-21	50.1	2.47	80.50	19.50	0.20	0.48
M-21	48.9	2.71	80.50	19.50	0.20	0.53
M-21	49.1	2.5	80.50	19.50	0.20	0.49
M-21	52.9	2.72	80.50	19.50	0.20	0.53
M-21	62.5	4	80.50	19.50	0.20	0.78
M-21	62	4.88	80.50	19.50	0.20	0.95
M-21	59.6	4.47	80.50	19.50	0.20	0.87
M-21	45.7	1.45	80.50	19.50	0.20	0.28
M-21	51.5	2.64	80.50	19.50	0.20	0.51
M-21	46.1	2.06	80.50	19.50	0.20	0.40
M-22	27.9	0.7	84.35	15.65	0.16	0.11
M-22	24.8	0.63	84.35	15.65	0.16	0.10
M-22	30	1.59	84.35	15.65	0.16	0.25
M-22	27.5	0.72	84.35	15.65	0.16	0.11
M-22	29.2	0.86	84.35	15.65	0.16	0.13
M-22	29.9	1.39	84.35	15.65	0.16	0.22
M-22	26.6	1.3	84.35	15.65	0.16	0.20
M-22	21.5	0.88	84.35	15.65	0.16	0.14
M-22	25.5	0.74	84.35	15.65	0.16	0.12
M-22	24.6	0.8	84.35	15.65	0.16	0.13
M-22	28.8	1.26	84.35	15.65	0.16	0.20
M-22	21.6	0.27	84.35	15.65	0.16	0.04
M-22	28.6	0.77	84.35	15.65	0.16	0.12
M-22	22.8	0.55	84.35	15.65	0.16	0.09
M-22	28.2	1.49	84.35	15.65	0.16	0.23
M-22	9.7	0.03	84.35	15.65	0.16	0.00
M-23	27	0.84	85.73	14.27	0.14	0.12
M-23	30.9	0.96	85.73	14.27	0.14	0.14
M-23	26.4	1.14	85.73	14.27	0.14	0.16

M-23	25.7	0.45	85.73	14.27	0.14	0.06
M-23	22.4	0.58	85.73	14.27	0.14	0.08
M-23	25.8	0.67	85.73	14.27	0.14	0.10
M-23	15.8	0.42	85.73	14.27	0.14	0.06
M-23	25.3	0.8	85.73	14.27	0.14	0.11
M-23	24.5	0.86	85.73	14.27	0.14	0.12
M-23	26.9	0.55	85.73	14.27	0.14	0.08
M-23	23	0.32	85.73	14.27	0.14	0.05
M-23	17.4	0.65	85.73	14.27	0.14	0.09
M-23	24.8	0.72	85.73	14.27	0.14	0.10
M-23	26.2	0.79	85.73	14.27	0.14	0.11
M-23	11.6	0.12	85.73	14.27	0.14	0.02
M-23	26.8	0.55	85.73	14.27	0.14	0.08
M-23	23.2	0.5	85.73	14.27	0.14	0.07
M-23	20.5	0.26	85.73	14.27	0.14	0.04
M-23	13.8	0.18	85.73	14.27	0.14	0.03
M-23	25.8	0.79	85.73	14.27	0.14	0.11
M-24	13.6	0.14	86.74	13.26	0.13	0.02
M-24	16.4	0.17	86.74	13.26	0.13	0.02
M-24	13.1	0.12	86.74	13.26	0.13	0.02
M-24	19	0.26	86.74	13.26	0.13	0.03
M-24	36.1	0.77	86.74	13.26	0.13	0.10
M-24	13.9	0.11	86.74	13.26	0.13	0.01
M-24	15.3	0.15	86.74	13.26	0.13	0.02
M-24	13.1	0.13	86.74	13.26	0.13	0.02
M-24	16.3	0.18	86.74	13.26	0.13	0.02
M-24	13.5	0.12	86.74	13.26	0.13	0.02
M-24	14.7	0.12	86.74	13.26	0.13	0.02
M-24	22.3	0.36	86.74	13.26	0.13	0.05
M-24	14.4	0.18	86.74	13.26	0.13	0.02
M-24	14.9	0.19	86.74	13.26	0.13	0.03
M-24	14.6	0.17	86.74	13.26	0.13	0.02
M-24	10.8	0.08	86.74	13.26	0.13	0.01
M-24	18.7	0.25	86.74	13.26	0.13	0.03
M-24	14	0.18	86.74	13.26	0.13	0.02
M-24	12.5	0.1	86.74	13.26	0.13	0.01
M-24	14.4	0.24	86.74	13.26	0.13	0.03

ID	Length (mm)	Weight (g)	Mean w.w (%)	Mean d.w (%)	d.w. factor	Estimated d.w (g)
M-26	49.7	2.85	84.01	15.99	0.16	0.46
M-26	43.8	2.32	84.01	15.99	0.16	0.37
M-26	40.3	1.74	84.01	15.99	0.16	0.28
M-26	46.2	2	84.01	15.99	0.16	0.32
M-26	49.3	3.84	84.01	15.99	0.16	0.61
M-26	47.5	2.36	84.01	15.99	0.16	0.38
M-26	49.9	3.27	84.01	15.99	0.16	0.52
M-26	48.3	3.91	84.01	15.99	0.16	0.63
M-26	45	2.68	84.01	15.99	0.16	0.43
M-26	43.8	2.27	84.01	15.99	0.16	0.36
M-26	44	3.41	84.01	15.99	0.16	0.55
M-26	50.5	3.54	84.01	15.99	0.16	0.57
M-26	42.3	2.4	84.01	15.99	0.16	0.38
M-26	48.1	2.94	84.01	15.99	0.16	0.47
M-26	48.6	3.85	84.01	15.99	0.16	0.62
M-26	46.3	3.46	84.01	15.99	0.16	0.55
M-26	47.8	3.11	84.01	15.99	0.16	0.50
M-26	50.3	3.45	84.01	15.99	0.16	0.55
M-26	48	2.55	84.01	15.99	0.16	0.41
M-26	47	4.25	84.01	15.99	0.16	0.68
M-27	44.1	2.47	86.52	13.48	0.13	0.33
M-27	45.3	1.95	86.52	13.48	0.13	0.26
M-27	43.1	1.83	86.52	13.48	0.13	0.25
M-27	46.2	2.12	86.52	13.48	0.13	0.29
M-27	49.5	3.01	86.52	13.48	0.13	0.41
M-27	45.2	2.25	86.52	13.48	0.13	0.30
M-27	49.8	4.5	86.52	13.48	0.13	0.61
M-27	46.9	2.08	86.52	13.48	0.13	0.28
M-27	50.2	3.98	86.52	13.48	0.13	0.54
M-27	51.8	4.87	86.52	13.48	0.13	0.66
M-27	49	3.76	86.52	13.48	0.13	0.51
M-27	43.8	2.01	86.52	13.48	0.13	0.27
M-27	44.8	1.4	86.52	13.48	0.13	0.19
M-27	49.5	3.95	86.52	13.48	0.13	0.53
M-27	51.5	2.76	86.52	13.48	0.13	0.37

M-27	44.5	2.07	86.52	13.48	0.13	0.28
M-27	49.1	4.49	86.52	13.48	0.13	0.61
M-27	47.3	2.64	86.52	13.48	0.13	0.36
M-27	50.2	3.49	86.52	13.48	0.13	0.47
M-27	45.1	3.07	86.52	13.48	0.13	0.41
M-28	43	1.73	87.50	12.50	0.12	0.22
M-28	50.3	3.35	87.50	12.50	0.12	0.42
M-28	55.9	4.82	87.50	12.50	0.12	0.60
M-28	42.7	2.48	87.50	12.50	0.12	0.31
M-28	53.5	4.25	87.50	12.50	0.12	0.53
M-28	48.5	2.9	87.50	12.50	0.12	0.36
M-28	48.7	3.48	87.50	12.50	0.12	0.43
M-28	34.6	0.98	87.50	12.50	0.12	0.12
M-28	55.9	3.84	87.50	12.50	0.12	0.48
M-28	48.9	3.28	87.50	12.50	0.12	0.41
M-28	48.4	2.33	87.50	12.50	0.12	0.29
M-28	54.2	3.65	87.50	12.50	0.12	0.46
M-28	53.1	4.44	87.50	12.50	0.12	0.55
M-28	56.6	4.27	87.50	12.50	0.12	0.53
M-28	48.4	3.6	87.50	12.50	0.12	0.45
M-28	53.3	3.74	87.50	12.50	0.12	0.47
M-28	46.2	2.66	87.50	12.50	0.12	0.33
M-28	57	4.24	87.50	12.50	0.12	0.53
M-28	58.1	5.23	87.50	12.50	0.12	0.65
M-28	48.8	3.07	87.50	12.50	0.12	0.38
M-29	35.3	1.12	86.85	13.15	0.13	0.15
M-29	53.1	3.05	86.85	13.15	0.13	0.40
M-29	56.5	3.48	86.85	13.15	0.13	0.46
M-29	52.9	2.55	86.85	13.15	0.13	0.34
M-29	45.9	1.95	86.85	13.15	0.13	0.26
M-29	48.7	1.83	86.85	13.15	0.13	0.24
M-29	56.1	3.64	86.85	13.15	0.13	0.48
M-29	40.9	1.7	86.85	13.15	0.13	0.22
M-29	48.2	2.08	86.85	13.15	0.13	0.27
M-29	37.6	1.53	86.85	13.15	0.13	0.20
M-29	55.1	2.57	86.85	13.15	0.13	0.34
M-29	62.4	3.92	86.85	13.15	0.13	0.52

M-29	52.8	2.1	86.85	13.15	0.13	0.28
M-29	52.6	4.92	86.85	13.15	0.13	0.65
M-29	54.7	3.31	86.85	13.15	0.13	0.44
M-29	59	3.2	86.85	13.15	0.13	0.42
M-29	NA	NA	86.85	13.15	0.13	NA
M-29	49	1.85	86.85	13.15	0.13	0.24
M-29	46.8	2.09	86.85	13.15	0.13	0.27
M-29	53.6	3.51	86.85	13.15	0.13	0.46
M-30	47	2.81	86.13	13.87	0.14	0.39
M-30	42.9	2.55	86.13	13.87	0.14	0.35
M-30	44.6	2.26	86.13	13.87	0.14	0.31
M-30	44.4	2.46	86.13	13.87	0.14	0.34
M-30	47.2	3.4	86.13	13.87	0.14	0.47
M-30	43.5	1.94	86.13	13.87	0.14	0.27
M-30	39.1	1.72	86.13	13.87	0.14	0.24
M-30	43.3	3.12	86.13	13.87	0.14	0.43
M-30	43.4	2.88	86.13	13.87	0.14	0.40
M-30	49.7	2.22	86.13	13.87	0.14	0.31
M-30	43.5	1.9	86.13	13.87	0.14	0.26
M-30	44.4	1.49	86.13	13.87	0.14	0.21
M-30	44.1	1.71	86.13	13.87	0.14	0.24
M-30	49.3	2.25	86.13	13.87	0.14	0.31
M-30	47.2	2.2	86.13	13.87	0.14	0.31
M-30	41	1.77	86.13	13.87	0.14	0.25
M-30	42.2	2.05	86.13	13.87	0.14	0.28
M-30	48.5	3.78	86.13	13.87	0.14	0.52
M-30	40.2	1.57	86.13	13.87	0.14	0.22
M-30	46.4	1.78	86.13	13.87	0.14	0.25

7.4 Quantitative results *Mytilus* spp. per gram d.w

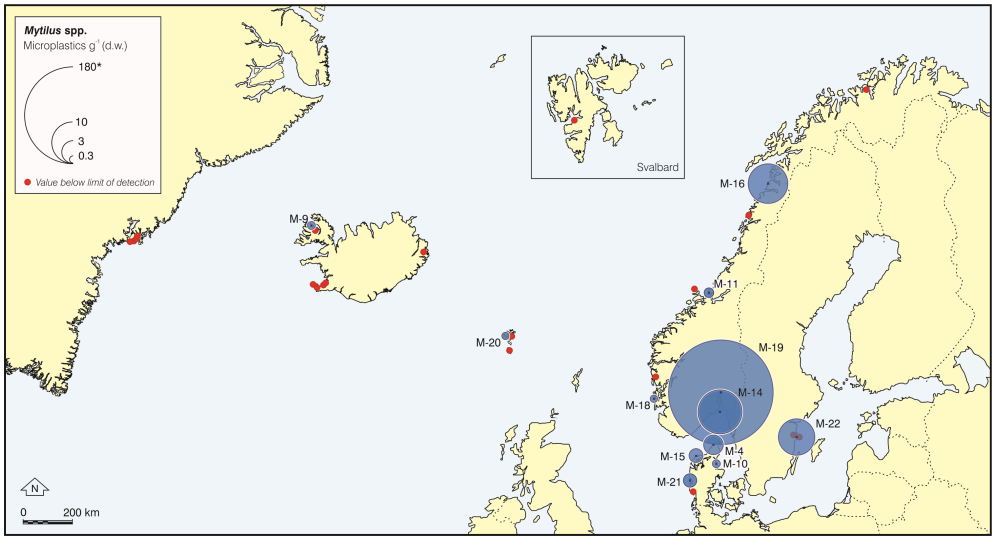

Figure 45: Microplastics (MPs) in *Mytilus* spp. (M) based on particles per gram d.w.

7.5 Qualitative results *Mytilus* spp. – size of microplastics

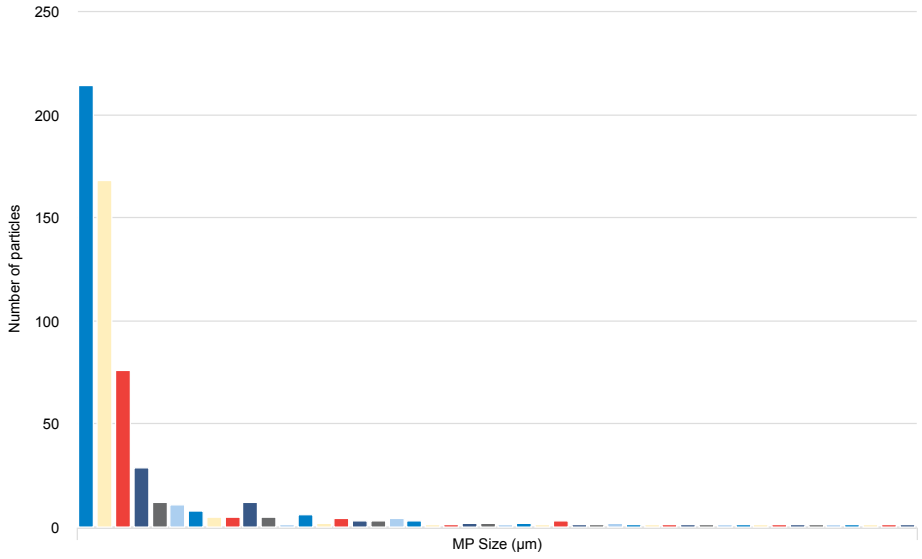

Figure 46: Size distribution of microplastics from sites above LOD in *Mytilus* spp. from the Nordic environment. All sizes included.

7.6 Fraction A – *Limecola balthica*, *Abra nitida*, *Thyasira* spp., *Hiatella arctica* and small *Mytilus* spp., processing and results

7.6.1 Bivalves processed and analysed for microplastics

Table 21: Number and weight of *Limecola* spp. and *Abra nitida* processed and analysed for microplastics.

Species	Site	Replicate	No of ind processed	w.w (g)	Average w.w. (g) per ind.
Limecola spp.	L-1	1	3	0.701	0.234
		2	3	0.635	0.212
		3	3	0.776	0.259
	L-2	1	10	2.265	0.227
		2	10	3.111	0.311
		3	10	2.685	0.269
	L-3	1	3	0.640	0.213
		2	3	0.708	0.236
		3	3	0.816	0.272
	L-4	1	3	0.854	0.285
		2	3	1.170	0.390
		3	3	1.001	0.334
	L-5	1	10	1.924	0.192
		2	10	1.917	0.192
		3	10	1.827	0.183
	L-6	1	3	0.858	0.286
		2	3	0.439	0.146
		3	3	0.502	0.167
	L-7	1	3	1.696	0.565
		2	3	1.708	0.569
		3	3	1.040	0.347
	L-8	1	10	4.044	0.404
		2	10	3.947	0.395
		3	10	4.537	0.454
	L-9	1	10	5.229	0.523
		2	10	4.794	0.479
		3	10	6.040	0.604

		1	3		2.182	0.727
	L-10	2	3		1.872	0.624
		3	3		1.590	0.530
	L-11	1	2		0.019	0.010
		1	3		1.052	0.351
	L-12	2	3		0.854	0.285
		3	3		0.953	0.318
		1	10		0.020	0.002
	L-13	2	10		0.036	0.004
		3	10		0.004	0.000
		1	6		5.479	0.913
	L-14	2	6		6.051	1.008
		3	6		5.587	0.931
	A-1	1		4	5.2816	1.320
	A-2	1		7	0.0489	0.007
		1	10		0.021	0.002
	A-3	2	10		0.0522	0.005
		3	10		0.0053	0.001
		1	10		0.2319	0.023
	A-4	2	10		0.2341	0.023
		3	10		0.2285	0.023
		1	10		0.8231	0.082
	A-5	2	10		0.7609	0.076
		3	10		0.6451	0.065
Abra nitida	**A-6**	1	7		0.352	0.050
		2	7		0.343	0.049
		1	10		0.8111	0.081
	A-7	2	10		0.6067	0.061
		3	10		0.7632	0.076
		1	5		0.45	0.090
	A-8	2	5		0.537	0.107
		3	5		0.537	0.107
		1	10		0.8539	0.085
	A-9	2	10		0.5943	0.059
		3	10		0.9961	0.100
	A-10	1	10		0.0658	0.007

	2	10	0.0852	0.009
	3	10	0.0845	0.008
A-11	1	10	0.5244	0.052
	2	10	0.5241	0.052
	3	10	0.3165	0.032
A-12	1	10	0.3189	0.032
	2	10	0.3918	0.039
	3	10	0.3256	0.033
A-13	1	10	0.122	0.012
	2	10	0.062	0.006
A-14	1	6	0.227	0.038
	2	6	0.232	0.039
	3	6	0.18	0.030
A-15	1	6	1.009	0.168
	2	7	0.014	0.002
	3	7	0.031	0.004
A-16	1	10	0.2111	0.021
	2	9	-0.0008	NA
A-17	1	6	0.08	0.013
	2	7	0.077	0.011
	3	7	0.067	0.010
A-18	1	10	0.0495	0.005
	2	10	0.0139	0.001
	3	10	0.037	0.004
A-19	1	10	0.0618	0.006
	2	8	0.0771	0.010
A-20	1	5	0.2491	0.050
A-21	1	10	-0.9875	NA
A-22	1	10	-0.0104	NA
	2	10	-0.2033	NA
A-23	1	10	0.0274	0.003
	2	10	0.0313	0.003
A-24	1	10	0.2241	0.022
	2	10	0.1637	0.016
A-25	1	9	-0.0078	NA
	2	5	0.0237	0.005

	A-26	1	5	0.0237	0.005
	A-27	1	7	-0.1148	NA
	A-28	1	9	0.189	0.021
		2	9	0.178	0.020
	A-29	1	10	0.0031	0.000
		2	10	0.0915	0.009
	A-30	1	5	0.0199	0.004
	A-31	1	10	0.0506	0.005
		2	10	-0.2558	NA

Table 22: Microplastics (MPs) in *Abra nitida* (pooled samples) from the Nordic marine environment.

	A-1	A-2	A-3	A-4	A-5	A-6	A-7	A-8	A-9	A-10	A-11	A-12	A-13	A-14	A-15	
Rep 1	1	0	0	0	0	3	1	2	0	0	3	0	0	0	0	
Rep 2			0	0	6	0	0	1	0	0	0	0	0	0	0	
Rep 3			0	0	1		0	2	0	0	0	0		0	0	
total no ind	**4**	**7**	**30**	**30**	**30**	**14**	**30**	**15**	**30**	**30**	**30**	**30**	**20**	**18**	**20**	
total no gram (w.w)	**5.282**	**0.049**	**0.079**	**0.069**	**2.229**	**0.695**	**2.181**	**1.863**	**2.444**	**0.236**	**1.365**	**1.036**	**0.184**	**0.639**	**1.054**	
Max MPs	**1**	**0**	**0**	**0**	**6**	**3**	**1**	**2**	**0**	**0**	**3**	**0**	**0**	**0**	**0**	
Min MPs	**1**	**0**	**0**	**0**	**0**	**0**	**0**	**1**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	
Mean MPs	**1.00**	**0.00**	**0.00**	**0.00**	**2.33**	**1.50**	**0.33**	**1.67**	**0.00**	**0.00**	**1.00**	**0.00**	**0.00**	**0.00**	**0.00**	
St.dev MPs	**NA**	**NA**	**0.00**	**0.00**	**3.21**	**2.12**	**0.58**	**0.58**	**0.00**	**0.00**	**1.73**	**0.00**	**0.00**	**0.00**	**0.00**	
	A-16	**A-17**	**A-18**	**A-19**	**A-20**	**A-21**	**A-22**	**A-23**	**A-24**	**A-25**	**A-26**	**A-27**	**A-28**	**A-29**	**A-30**	**A-31**
Rep 1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Rep 2	0	0	0	0			0	0	0	0			0	0		0
Rep 3		0	0													
total no ind	**19**	**20**	**30**	**18**	**5**	**10**	**20**	**20**	**20**	**14**	**5**	**7**	**18**	**20**	**5**	**20**
total no gram (w.w)	**0.210**	**0.224**	**0.100**	**0.139**	**0.249**	**NA**	**NA**	**0.059**	**0.388**	**0.016**	**0.024**	**NA**	**0.367**	**0.095**	**0.020**	**NA**
Max MPs	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
Min MPs	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
Mean MPs	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
St.dev MPs	**0.00**	**0.00**	**0.00**	**0.00**	**NA**	**NA**	**0.00**	**0.00**	**0.00**	**0.00**	**NA**	**NA**	**0.00**	**0.00**	**NA**	**0.00**

Table 23: Microplastics (MPs) in *Limecola balthica* (pooled samples) from the Nordic marine environment.

	L-1	L-2	L-3	L-4	L-5	L-6	L-7	L-8	L-9	L-10	L-11	L-12	L-13	L-14
Rep 1	0	0	0	0	0	1	0	0	0	0	0	0	0	2
Rep 2	0	1	2	0	0	2	0	0	0	0		0	0	2
Rep 3	0	1	0	0	0	0	0	0	0	0		0	0	4
total no ind	**9**	**30**	**9**	**9**	**30**	**9**	**9**	**30**	**30**	**9**	**2**	**9**	**30**	**18**
total no gram (w.w)	**2.11**	**8.06**	**2.16**	**3.02**	**5.5785**	**1.8**	**4.44**	**12.5**	**16.1**	**1.88**	**0.02**	**2.86**	**0.06**	**17.1**
Max MPs	**0**	**1**	**2**	**0**	**0**	**2**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**4**
Min MPs	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**2.00**
Mean MPs	**0.00**	**0.67**	**0.67**	**0.00**	**0.00**	**1.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**2.67**
St.dev MPs	**0**	**0.58**	**1.15**	**0**	**0**	**1**	**0**	**0**	**0**	**0**	**NA**	**0**	**0**	**1.15**

Table 24: *Thyasira* spp.. *Hiatella arctica* and tiny *Mytilus edulis* studied for microplastics (MPs) content.

Species	Site	No of ind	g w.w.	mg w.w.	Mean g w.w. per ind-1	Mean mg w.w. per ind-1	MPs (exl. fibres)
	T-1	10	0.050	50.00	0.005	5.00	0
	T-1	10	0.134	134.40	0.013	13.44	0
	T-1	10	0.105	104.60	0.010	10.46	0
	T-2	10	0.158	158.40	0.016	15.84	0
	T-2	10	0.059	58.50	0.006	5.85	0
	T-2	10	0.173	173.40	0.017	17.34	0
	T-3	10	0.291	291.00	0.029	29.10	0
	T-4	10	0.560	559.80	0.056	55.98	0
	T-4	10	NA	NA	NA	NA	0
	T-4	10	0.154	153.50	0.015	15.35	0
	T-5	10	NA	NA	NA	NA	0
	T-5	10	NA	NA	NA	NA	0
	T-5	10	NA	NA	NA	NA	0
	T-6	10	4.011	4011.20	0.401	401.12	0
	T-6	10	0.036	35.70	0.004	3.57	0
	T-6	10	0.104	103.60	0.010	10.36	0
Thyasira	T-7	10	0.130	129.60	0.013	12.96	0
	T-7	10	0.105	105.00	0.010	10.50	0
	T-7	10	0.120	120.00	0.012	12.00	0
	T-8	10	0.069	68.70	0.007	6.87	0
	T-8	10	0.068	67.50	0.007	6.75	0
	T-9	10	0.585	584.60	0.058	58.46	0
	T-9	10	0.816	815.80	0.082	81.58	0
	T-9	10	0.566	566.00	0.057	56.60	0
	T-10	10	0.175	175.00	0.018	17.50	0
	T-10	10	0.175	175.00	0.018	17.50	0
	T-11	10	0.119	119.00	0.012	11.90	0
	T-11	10	0.112	112.00	0.011	11.20	0
	T-12	10	0.088	87.50	0.009	8.75	0
	T-12	10	0.088	87.50	0.009	8.75	0
	T-12	10	0.081	81.40	0.008	8.14	0
	T-13	10	0.116	116.20	0.012	11.62	0
	T-13	10	0.092	92.10	0.009	9.21	0

	T-14	10	0.231	231.30	0.023	23.13	0
	T-14	10	0.237	236.90	0.024	23.69	0
	T-14	10	0.150	149.70	0.015	14.97	0
	T-15	10	0.048	47.70	0.005	4.77	0
	T-15	10	0.123	122.70	0.012	12.27	0
	T-15	10	0.049	49.00	0.005	4.90	0
	T-16	10	0.091	90.50	0.009	9.05	0
	T-16	10	0.096	95.80	0.010	9.58	0
	T-17	10	0.361	361.00	0.036	36.10	0
	T-17	10	0.289	289.00	0.029	28.90	0
	T-17	10	0.340	340.00	0.034	34.00	0
	T-18	10	0.421	420.50	0.042	42.05	0
	T-19	10	0.128	128.40	0.013	12.84	0
	T-20	10	0.049	49.00	0.005	4.90	0
	T-20	10	0.050	49.60	0.005	4.96	0
						0.00	
Hiatella	H-1	3	0.094	94.30	0.031	31.43	0
	H-2	4	0.018	17.50	0.004	4.38	0
	H-3	10	0.043	42.50	0.004	4.25	0
						0.00	
***Mytilus edulis* (tiny)**	M-25	10	0.062	62.00	0.006	6.20	0
	M-25	10	0.030	30.00	0.003	3.00	0
	M-31	10	0.070	70.00	0.007	7.00	0
	M-31	10	0.098	98.00	0.010	9.80	0
	M-32	9	0.373	373.00	0.041	41.44	0
	M-32	9	0.335	335.00	0.037	37.22	0

Table 25: Longest dimension of microplastics detected in *Abra nitida* and *Limecola balthica* Fraction A.

		Abra nitida					
		A-1	A-5	A-6	A-7	A-8	A-11
Longest dimension (µm)	**Part1**	68.7	84.4	76.5	147.9	104.3	114.3
	Part2	-	122.7	50.1	-	117.0	112.9
	Part3	-	108.2	177.8	-	123.2	81.4
	Part4	-	83.7	-	-	137.5	-
	Part5	-	78.7	-	-	103.4	-
	Part6	-	132.3	-	-	-	-
	Part7	-	210.5	-	-	-	-
	Mean size	**68.7**	**117.2**	**101.5**	**147.9**	**117.1**	**102.9**
	St.Dev	**NA**	**46.1**	**67.4**	**NA**	**14.2**	**18.6**

		Limecola balthica					
		L-2	L-3	L-6	L-14		
Longest dimension (µm)	**Part1**	111.9	40.9	147.6	207.8		
	Part2	117.0	36.6	147.3	185.6		
	Part3	-	-	328.9	187.1		
	Part4	-	-	-	131.9		
	Part5	-	-	-	73.0		
	Part6	-	-	-	104.4		
	Part7	-	-	-	90.2		
	Part8	-	-	-	80.2		
	Mean size	**114.5**	**38.8**	**207.9**	**132.5**		
	St.Dev	**3.6**	**3.0**	**104.8**	**53.9**		

7.7 Results Fraction B (*Abra nitida* and *Thyasira* spp.)

Table 26: Material composition of the different components identified with ATR image scanning followed by PCA analysis.

Species	Sample ID	PCA	Composition	Plastics	Level of Certainty
Thyasira spp.	T1_rep1	PCA 1	Polyacrylate	yes	Medium
Thyasira spp.	T1_rep1	PCA 2	Silicates	no	Low*
Thyasira spp.	T1_rep1	PCA 3	Kaolin	no	High
Thyasira spp.	T1_rep2	PCA 1	Polyacrylate	yes	Medium
Thyasira spp.	T1_rep2	PCA 2	Polyacrylate	yes	Medium
Thyasira spp.	T1_rep2	PCA 3	Proteins + carbohydrates; bacteria?	no	Medium
Thyasira spp.	T1_rep2	PCA 4.1	Polyethylene (PE) with biofouling	yes	Medium
Thyasira spp.	T1_rep2	PCA 4.2	Polyethylene (PE) with biofouling	yes	Medium
Thyasira spp.	T1_rep2	PCA 4.3	Polyethylene (PE) with biofouling	yes	Medium
Thyasira spp.	T1_rep3	PCA 1	Polyacrylate	yes	Medium
Thyasira spp.	T1_rep3	PCA 2	Proteins + carbohydrates; bacteria?	no	Medium
Thyasira spp.	T1_rep3	PCA 3	Polyacrylate	yes	Medium
Thyasira spp.	T1_rep3	PCA 4	Silicates same as in A10_A2	no	Low*
Thyasira spp.	T2_rep1	PCA 1	Silicates	no	Low*
Thyasira spp.	T2_rep1	PCA 2	Calcium stearate	maybe additive	High
Thyasira spp.	T2_rep1	PCA 3	Polyacrylate	yes	Medium
Thyasira spp.	T2_rep1	PCA 4	Silicates	no	Medium*
Thyasira spp.	T2_rep1	PCA 5	Proteins + carbohydrates +silicates	no	Low*
Thyasira spp.	T2_rep2	PCA 1	Polyacrylate	yes	Medium
Thyasira spp.	T2_rep2	PCA 2	Polyacrylate	yes	Medium

Thyasira spp.	T2_rep2	PCA 3	filter	no	High
Thyasira spp.	T2_rep2	PCA 4	Silicates	no	Low*
Thyasira spp.	T2_rep3	PCA 1	Polyacrylate	yes	Medium
Thyasira spp.	T2_rep3	PCA 2	Polyacrylate	yes	Medium
Thyasira spp.	T2_rep3	PCA 3	Quartz	no	High
Thyasira spp.	T6_rep1	PCA 1	Kaolin	no	High
Thyasira spp.	T6_rep1	PCA 2	Kaolin	no	High
Thyasira spp.	T6_rep1	PCA 3	Silicate	no	Low*
Thyasira spp.	T6_rep1	PCA 4	Silicate or phosphate	no	Low*
Thyasira spp.	T6_rep2	PCA 1	Silicate	no	Low*
Thyasira spp.	T6_rep2	PCA 2	Silicate	no	Low*
Thyasira spp.	T6_rep2	PCA 3	Silicate	no	Low*
Thyasira spp.	T6_rep2	PCA 4	Silicate+quartz	no	Low*
Thyasira spp.	T6_rep2	PCA 5	Kaolin	no	High
Thyasira spp.	T6_rep3	PCA 1	Silicate	no	Low*
Thyasira spp.	T6_rep3	PCA 2	Silicate	no	Low*
Thyasira spp.	T6_rep3	PCA 3	Silicate	no	Low*
Thyasira spp.	T6_rep3	PCA 4	Silicates same as in A10_A2	no	Low*
Thyasira spp.	T6_rep3	PCA 5	Kaolin	no	High
Abra nitida	A10_rep1	PCA 1	Silicates	no	Low*
Abra nitida	A10_rep1	PCA 2	Polydimethyl-siloxane (Silicone)	yes	High
Abra nitida	A10_rep1	PCA 3	Silicates	no	Low*
Abra nitida	A10_rep2	PCA 1	Silicates	no	Low*
Abra nitida	A10_rep2	PCA 2	Silicates	no	Low*
Abra nitida	A10_rep2	PCA 3	Silicates	no	Low*
Abra nitida	A10_rep2	PCA 4	Silicates but with a signal of aliphatic hydrocarbons	no	Low*
Abra nitida	A10_rep3	PCA 1	Silicates	no	Low*
Abra nitida	A10_rep3	PCA 2	Silicates same as in A10_A2	no	Low*
Abra nitida	A10_rep3	PCA 3	Silicates+oxide	no	Low*
Abra nitida	A10_rep3	PCA 4	Silicates+oxide	no	Low*
Abra nitida	A10_rep3	PCA 5	Polydimethyl-siloxane (Silicone)	yes	High

Abra nitida	A12_rep1 (*)	PCA 1	Silicate+quartz	no	Low*
Abra nitida	A12_rep1	PCA 2	Silicate+quartz	no	Low*
Abra nitida	A12_rep1	PCA 3	Protein	no	High
Abra nitida	A12_rep1	PCA 4	Silicates	no	Low*
Abra nitida	A12_rep1	PCA 5	Silicates	no	Low*
Abra nitida	A12_rep2 (**)	PCA 1	Silicates	no	Low*
Abra nitida	A12_rep2	PCA 2	Silicates	no	Low*
Abra nitida	A12_rep2	PCA 3	Silicate+quartz	no	Low*
Abra nitida	A12_rep2	PCA 4	Cellulose/ Rayon	Yes	Low
Abra nitida	A12_rep2	PCA 5	Polyethylene (PE) with biofouling	Yes	Low
Abra nitida	A12_rep3 (***)	PCA 1	Silicates same as in A10_A2	no	Low*
Abra nitida	A12_rep3	PCA 2	Polyethylene (PE) with biofouling	Yes	Medium
Abra nitida	A12_rep3	PCA 3	Silicate+quartz	no	Low*
	Blank 1	PCA 1	Cellulose nitrate filter	no	High
	Blank 1	PCA 2	Cellulose nitrate filter	no	High
	Blank 1	PCA 3	Cellulose nitrate filter	no	High
	Blank 2	PCA 1	Cellulose nitrate filter	no	High
	Blank 2	PCA 2	Cellulose nitrate filter	no	High
	Blank 2	PCA 3	Cellulose nitrate filter	no	High
	Blank 3	PCA 1	Cellulose nitrate filter	no	High
	Blank 3	PCA 2	Cellulose nitrate filter	no	High
	Blank 3	PCA 3	Cellulose nitrate filter	no	High
	Blank 4	PCA 1	Cellulose nitrate filter	no	High
	Blank 4	PCA 2	Cellulose nitrate filter	no	High
	Blank 4	PCA 3	Cellulose nitrate filter	no	High
	Blank 5	PCA 1	Cellulose nitrate filter	No	High

	Blank 5	PCA 2	Cellulose nitrate filter	No	High
	Blank 5	PCA 3	Cellulose nitrate filter	No	High

(*) 50% of fraction B analysed due to clogging. Subsample taken.
(**) 37.5% of fraction B analysed due to clogging. Subsample taken.
(***) 37.5% of fraction B analysed due to clogging. Subsample taken.

Three spectra per blank was obtained based on the PCA. All spectra were cellulose nitrate Figure 47, which is the filter material. All of these 15 spectra are representing the five blanks. Therefore, no particles smaller than 63µm were found in the blank detected by ATR image scanning. Polyacrylate was the most common polymer detected in *Thyasira* spp. and was found based on reference spectrum as illustrated in Figure 48.

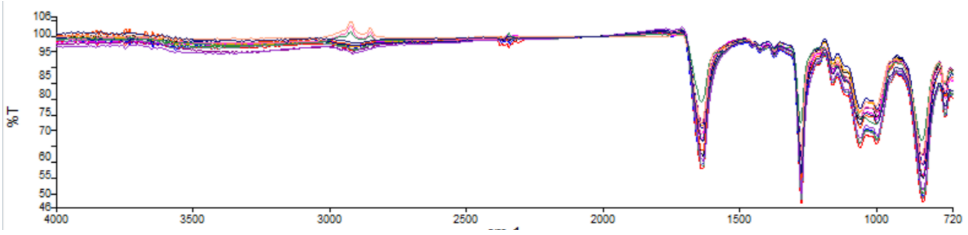

Figure 47: FT-IR Spectrum from all 15 spectra obtained from the blank.

Figure 48: FT-IR Spectrum where green is the reference spectrum of polyacrylate and the rest are spectra for suspected polyacrylate in the samples.

About this publication

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