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Mytilus spp. as sentinels for monitoring microplastic pollution in Norwegian coastal waters: A qualitative and quantitative study☆

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ABSTRACT

Microplastic (MP) contamination is ubiquitous in the environment and many species worldwide have been shown to contain MP. The ecological impact of MP pollution is still unknown, thus there is an urgent need for more knowledge. One key task is to identify species suitable as sentinels for monitoring in key eco-compartments, such as coastal waters. In Norway, mussels (*Mytilus* spp.) have been monitored for hazardous contaminants through OSPAR since 1981. Norway has the longest coastline in Europe and adding MP to the Norwegian Mussel Watch is therefore important in a European and global context. The present study reports MP data in mussels (332 specimens) collected from multiple sites (n = 15) spanning the whole Norwegian coastline. MPs were detected at all locations, except at one site on the west coast. Among the most surprising findings, mussels from the Barents Sea coastline in the Finnmark region, contained significantly more MPs than mussels from most of the southern part of the country, despite the latter sites being located much closer to major urban areas. Only mussels from a site located very close to Oslo, the capital, contained levels similar to those observed in the remote site in Finnmark. In total an average of 1.5 (±2.3) particles ind^{-1} and 0.97 (±2.61) particles w.w. g^{-1} was found. The most common MPs were <1 mm in size, and fibres accounted for 83% of particles identified, although there was inter-site variability. Thirteen different polymeric groups were identified; cellulosic being the most common and black rubbery particles being the second. This study suggests *Mytilus* spp. are suitable for semi-quantitative and qualitatively monitoring of MPs in coastal waters. However, some uncertainties remain including mussel size as a confounding factor that may influence ingestion, the role of depuration and other fate related processes, and this call for further research.

1. Introduction

Microscopic particles and fragments of plastics smaller than 5 mm (commonly called microplastics, MPs) are found in terrestrial and aquatic environments worldwide (Browne et al., 2007; Avio et al., 2017; Andrady, 2017). In marine biota, they have been detected in small organisms such as fish larvae (e.g. English Channel; Steer et al., 2017) to large marine mammals (e.g. Irish waters; Lusher et al., 2018). Efficient actions to combat MP pollution must be strictly knowledge-based. Monitoring therefore plays a key role in this respect to provide empirical evidence of, 1) the fate of various forms of plastic and MP; 2) spatial and temporal trends in different ecological compartments (seawater, filter feeder biota, etc.); and, 3) which species and ecosystem communities are potentially at risk for long-term impact. Coastal marine environments are among the key habitats for monitoring, as they act as recipients for plastics and MPs input sources, and they typically are more ecologically diverse and productive than oceanic systems (Costanza et al., 1997). The development of standardised biological

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monitoring (biomonitoring) of MPs in coastal waters requires the use of sentinel organisms that are common over large areas and that have biological traits which make them suitable for the purpose.

Mytilus spp. are seen by many as an optimal sentinels for such studies. The reasons for their suitability are many; mussels are common in temperate coastal seas all around the globe and they are sessile, which provides location-specific information. They are medium-sized (one individual provides suitable amount of tissue for analysis), they form (often large) mussel beds in shallow waters from where they easily can be collected, and they are hardy creatures that are easy to keep in culture, making them suitable for laboratory exposure studies as well as for translocation and caging exposure *in situ* (reviewed by Wesch et al., 2016 and Beyer et al., 2017). *Mytilus* spp. feed mainly on phytoplankton by filtering a large volume of water through their large ciliated gills, which may take in MPs. They are also popular as seafood, indicating a link to the human food chain. In Norway, mussels have been monitored for organic and inorganic hazardous contaminants as part of the national contribution to the Convention for the Protection of the Marine Environment of the North-East Atlantic (OSPAR) since 1981 (Green et al., 2017), and mussels are also proposed as a possible standard bioindicator for water-born MPs contamination in marine environments (OSPAR, 2015).

Along the Dutch coast, *Mytilus edulis* were reported to concentrate MP from ambient waters up to 1000 times (Karlsson et al., 2017), and a positive correlation has been found for the MP levels in water samples and corresponding mussel samples (Qu et al., 2018). This suggest that mussels act to integrate and amplify the MP contamination signal, potentially making monitoring easier and more reliable. A number of field studies on MPs in *Mytilus* spp. are reported worldwide (De Witte et al., 2014; Mathalon & Hill, 2014; Van Cauwenberghe et al., 2015; Li et al., 2016; Karlsson et al., 2017; Phuong et al., 2018; Qu et al., 2018), and although they are promising with regard to the relevance of *Mytilus* spp. as sentinels for MPs, they also indicate a need for standardisation of methods for MP identification and quantification to improve the inter-study comparability of MP data.

A key challenge for MP biomonitoring is the lack of standardisation associated with both the data that is provided (e.g., sometimes only MPs per ind^{-1} or per g^{-1}) and the different methodologies that are used (Supplementary information Table S1), such as the processing of samples that can affect the results (Lusher et al., 2017). Additionally, since the umbrella term *"microplastics"* covers such a heterogenous group of particles, there is also variation in which categories of anthropogenic particles are included within this term. MPs covers a wide range of shapes (e.g., fibres, fragments, films, beads) and polymeric materials (e.g., polyethylene and polypropylene). All plastics are made from polymers, but not all polymers are plastic, leading to the ongoing debate in the MP research field about which materials should be considered as *"plastic"*, since modified cellulose microfibres are sometimes included (e.g. Woodall et al., 2014; Obbard et al., 2014; Neves et al., 2015; Frias et al., 2016; Li et al., 2016). When converting natural cellulose into textile fibres several chemical modification steps are performed (Shen et al., 2010), which alter the properties of the cellulosic material, e.g., increasing the metal-binding capacity (O'Connell et al., 2008). It is currently unknown how these fibres might impact marine wildlife. Therefore, it can be argued that due to the modifications made to the cellulosic material to increase their durability, and since these particles are present together in the environment with petroleum-based MPs and in the same size range, then semi-synthetic polymers should be included as *microplastic-like* particles until the associated environmental risks have been established (Woodall et al., 2014). This discussion demonstrates the importance of reporting qualitative as well as quantitative results from MP monitoring. In this study, all anthropogenic particles, including cellulosic fibres, found are termed "microplastics" or "MPs".

The objective of this study is to assess the suitability of *Mytilus* spp. as a sentinel species for water-borne MP contamination and to investigate, for the first time, the abundance of MPs (qualitatively and quantitatively) in mussels collected from multiple locations along the Norwegian coast.

2. Method

2.1. Sampling

Mussels from the Norwegian coast are dominated by *Mytilus edulis*, but some areas are also populated by the sub-species *M. trossulus*, *M. galloprovincialis* and hybrids of these (Brooks & Farmen, 2013). Therefore, the term *Mytilus* spp. is used in this study to cover all possible sub-species investigated. The mussel samples were provided as a part of the Norwegian nationwide long-term OSPAR coastal monitoring program *"Contaminants in the coastal waters of Norway"* (Green et al., 2017). *Mytilus* spp. were collected from August to November in 2016, with exception of mussels from sites S9 and S13 that were sampled both in 2016 (termed S9A and S13A), and in August 2017 (termed S9B and S13B) and site S10 that was only sampled in August 2017 (Table 1). One

Table 1
Description of site locations and collection method during the sampling of *Mytilus* spp. in 2016 and 2017. Year: year sampled, n: number of individual mussels analysed. Samples were collected between August to November.

Site code	Site name	Year	n	Position (depth in m)	Substrate	Sampling method
S1	Skallneset	2016	20	Shoreline, intertidal (0)	Rock	Hand selected
S2	Bodø harbour	2016	20	Subsurface (0–1)	Concrete pier	Hand selected
S3	Ørland	2016	20	Shoreline, intertidal (0)	Rock and sand	Hand selected
S4	Måløy	2016	20	Subsurface (0.2–1.2)	Pontoon	Hand selected
S5	Bergen	2016	20	Shoreline, intertidal (0)	Rock	Hand selected
S6	Lille Terøy	2016	20	Subsurface (0–0.5)	Pontoon	Hand selected
S7	Kvalnes	2016	20	Intertidal, subsurface (0–1)	Rock and sand	Snorkelling
S8	Byrkjenes	2016	20	Subsurface (0–1)	Attached to submerged branch	Snorkelling
S9A	Akerhuskaia	2016	20	Subsurface (0–1)	Quayside (cement with tyre fender)	Metal rake with net
S9B	Akerhuskaia	2017	20	Subsurface (0–1)	Quayside (cement with tyre fender)	Metal rake with net
S10	Lysaker	2017	20	Subsurface (0–1)	Concrete pier	Metal rake with net
S11	Gressholmen	2016	20	Subsurface (0–0.5)	Sandy/muddy bottom with some rocks	Hand selected
S12	Gåsøya	2016	20	Subsurface (0–1)	Rock	Snorkelling
S13A	Ramtonholmen	2016	12	Subsurface (1–2)	Rock and sand	Snorkelling
S13B	Ramtonholmen	2017	20	Subsurface (1–2)	Rock and sand	Snorkelling
S14	Solbergstrand	2016	20	Intertidal (0–1)	Sand and rock	Hand selected
S15	Singlekalven	2016	20	Subsurface (0.5–1.5)	Sandy bottom with some rocks	Snorkelling
Total			332			

additional site, S10, was not included in the monitoring program, but was included specifically for this study to expand the sites within the Oslofjord. Only living individuals with no visible signs of damage were collected. Individuals were frozen (- 20 °C) whole (in their shell) as soon as possible after collection.

2.2. Contamination precautions and LOD/LOQs

Strict contamination controls were carried out during collection, processing and analysis. Any presence of MPs in blank controls were accounted for in the results. Steps taken to avoid contamination included: use of pre-brushed cotton laboratory-coats, clean laboratory conditions in an enclosed room, filtered (0.22 μm) reverse osmosis (RO)-water for preparation of all solutions, and washing of all glassware including rinsing with filtered RO-water. In addition, mussels were visually inspected under a stereo microscope (NIKON, JAPAN) at 20× magnification for any visible surface contamination before they were dissolved in potassium hydroxide (KOH). Triplicates of negative controls were included per day of sample preparation, composed of 50 ml of 10% KOH with no mussels added. Also, if filter papers were exposed to the laboratory atmosphere during microscope work, an additional filter paper was left exposed for the same duration. Glassware was used for all steps of sample preparation. Limit of detection (LOD; mean + 3 x SD) and limit of quantification (LOQ; mean + 10 x SD) were calculated based on 24 blank samples for both fragment and fibre particle types.

2.3. Sample processing and method validation

The maximum length (mm) of individual mussels was measured using callipers. Excess water was discharged before the soft tissue was carefully dissected out with a scalpel. Byssus filaments and the foot were removed. Individual mussels were weighed (g w.w.), added to individual glass beakers and covered with aluminium foil.

To isolate MPs, biota needs to be processed by removing natural organic matter (NOM). Recently, 10% KOH has shown to be suitable for this purpose since it degrades NOM without degrading most polymers with exception of some alteration on cellulose acetate (CA) and polyamide (PA; brand name: Nylon)Foekema et al., 2013; Dehaut et al., 2016; Kühn et al., 2017). In addition, KOH has low health and safety risks, it is cost efficient and the procedure is not complicated, enabling a high throughput of samples. Therefore, the soft tissue of the mussels was degraded following the method from Dehaut et al. (2016) with small modifications; 10% KOH (10 times v/v) was added before the beakers were placed in an incubator (New Brunswick™Innova® 44/44R) at 60 °C and agitated at 140 rpm for 24 h. The digestate was filtered under vacuum onto glass microfibre filters (Whatman GF/D, pore size 2.7 μm). The filters were stored in sealed petri dishes at room temperature to dry prior to analysis. Recovery tests for several petroleum-based polymers, cellulosic material, wool and silk was also conducted (see supplementary Section 4).

2.4. Visual ID and μtransmission FT-IR

All samples were visually inspected for the presence of potential MPs. In total, 332 individual mussel samples were analysed from 15 sampling sites (Table 1) using a stereomicroscope with Infinity 1-3C camera and INFINITY ANALYZE and CAPTURE software to take pictures and to measure size (longest dimension) of all particles found above 70 μm. The size categories used were 0.70–0.99, 1.00–1.09, 1.10–1.19 and so on. Five main categories for shape were used; fibres, fragments, foams, films and beads (Lusher et al., 2017). All particles found were marked on the filter paper for easy identification prior to chemical characterisation. Visual identification of MPs, especially in the smaller size range (<1 mm), should always be supported by secondary analyses to confirm the polymeric material (Lusher et al., 2017). Therefore, representative sub-samples of those identified during visual ID were identified by μ-transmission FT-IR (ThermoScientific Nicolet iS50 FT-IR), accounting for 25% of the total particle count. Each microparticle was flattened and held in place using a diamond compression cell and the particle was exposed to infrared light (4000-400 cm^{-1}).

Transmission spectral data were recorded using 32 scans, a resolution of 4, and an optical velocity of 1.8988. The spectra were automatically compared against commercial reference spectra libraries to obtain the chemical characterisation of the sample. Only spectra that matched more than 70% were accepted for polymer identification, with exception to rubbery particles which sometimes had a lower % match, but were interpreted and compared to reference spectrum manually.

2.5. Statistical analysis

Statistical analysis was performed using GraphPad Prism 5 (GraphPad Software, Inc., USA) and XLSTAT 2018.1 statistical software. ArcGIS was used to create maps. Normal distribution homogenous variance was tested (D'Agostino & Pearson omnibus normality test), and as data were non-normal, also after log-transformation, non-parametric ANOVA tests (Kruskal-Wallis) were performed to test for site effects, followed by multiple pairwise comparisons using Dunn's procedure. Since the means between the fifteen groups were compared against each other, and thereby to avoid Type 1 error, a Bonferroni correction of *p-value* was performed giving a Bonferroni corrected significance level of 0.0004. Spearman rank correlation test was performed to test any correlation between size of mussels and number of MPs.

3. Results and discussion

3.1. Size of mussels

There was significant variation in weight of mussel soft tissue among the local populations (Supplementary information Figure S1 and Table S2), complicating size standardisation of mussels collected across the sites. For all sites, the shell length ranged from 2.0 to 8.9 cm, with an average of 5.2 (SD ± 1.5) cm, and the wet weight varied between 0.14 and 16.30 g. It was not possible to compare same sized mussels between sites due to inherent local adaptions for the different populations. However, as normal procedure when monitoring other contaminants using mussels, the results were standardised towards weight to minimise the effects different sized mussels might have.

3.2. Blank corrections, LODs/LOQs and method validation

Even with the extensive contamination precautions carried out, fibres and fragments were found in the procedural blanks. The results presented here were therefore corrected by subtracting the daily mean value of the blank (for fibres and fragments separately) per sample, since the contamination varied between days (Supplementary information Table S3).

Quantifying uncertainties associated with obtained MP data is important. LOD and LOQ are widely used within analytical chemistry to give a measure of the uncertainties that come with the data (Arinbruster et al., 1994). Namely, LOD gives the lowest concentration where detection is feasible, while levels > LOQ are values that exhibit a greater probability to be a true quantitative value and not a random fluctuation of the blank (Arinbruster et al., 1994; Armbruster & Pry, 2008). Though, since LOD and LOQs mostly are

used for substances in solution, it is currently not known if this is the correct approach for MP analysis. Despite this, an attempt was made to give the uncertainties behind the data. Based on the 24 blanks, the fibre LOD was 7.64 and fibre LOQ 21.37, while fragment LOD was 2.36 and the LOQ 23.92 (Supplementary Table S4 and S5). The relatively high LOD and LOQs established in this study illustrates the uncertainties of quantitative data in MP studies. Although, site S9 (year 2017) in the Oslofjord, and site S1 in the Barents Sea, did both exceed LOD for fibres and for fragments. For a more in-depth discussion on the LOD and LOQ results, see supplementary section 3.

As found from the polymer degradation tests, oil-based polymers and cellulose-based material were not impacted by the KOH treatment (Supplementary Table S6), but both 100% silk and 100% wool were fully degraded. However, dye-leakage from the cellulosic material in the recovery test was noted, as also seen for some of the cellulosic fibres found following extraction from mussels (Fig. 5F and G). Colour of the particles are not presented here since 1) colour can be considered a subjective measure, 2) it was observed that KOH bleached some particles, and 3) weathering in the ocean can alter the colour of MPs (Duis & Coors, 2016). Transparent fibres had a low contrast to the white glass fibre filter, and this may have led to reduced detection. Additional analytical challenges include the obstacles researchers face when trying to chemically separate the different types of anthropogenic cellulose-based materials found in the environment (Comnea-Stancu et al., 2017). It is challenging to separate modified cellulose such as viscose from natural fibres such as cotton when using μtransmission FT-IR (Supplementary information section 4). This is in accordance with the findings of Comnea-Stancu et al. (2017). Unfortunately, due to this, the current study cannot distinguish semi-synthetics (i.e. Rayon and Viscous) from cotton.

Furthermore, we observed numerous pearls in the mussels (example in Fig. 5I). Up to 81 pearls were found in a single individual, with some pearls being very small in size (Fig. 5H). It is important to highlight this finding, given the possibility of misidentifying small pearls as polymeric materials such as microbeads.

3.3. Quantitative occurrence of MPs in Mytilus spp

Despite the analytical limitations discussed above, this study demonstrates that mussels can be used to monitor water-borne MPs, at least semi-quantitatively. Mussels from the Norwegian marine environment did contain MPs, with an overall average of 1.5 MPs per ind^{-1} (range: 0 to 6.9 MPs per ind^{-1}; Fig. 1) and correspondingly 0.97 MPs per g^{-1} w.w (range: 0 to 7.9 particles g^{-1} w.w.; Fig. 2) after correcting for average blank samples of 1.02 particles (Supplementary information Table S3).

Due to significant methodological variation between MP studies in mussels (Supplementary information Table S1), it is unfortunately not possible to quantitatively compare the MPs occurrence in Norwegian mussels with other studies. Nevertheless, within this study, MP concentrations can be compared between sites in at least a semi-quantitative manner.

MPs were observed in mussels from 16 out of 17 samples (including two replicated sites) along the Norwegian coast. Mussels from site S3, located at the west coast of Norway exposed to the Norwegian Sea, was the only site where no MPs were identified. Other spatial trends include: site S1 located in the Barents Sea, and site S9B, located inside the Oslofjord, which were identified as hot-spots of MPs ingestion by *Mytilus* spp. Site S1 had an average of 3.57 MPs per ind^{-1} and 7.9 MPs per g^{-1}, while site S9 (year 2017) had 6.9 microparticles ind-1 and 1.2 particles g^{-1} (Figs. 1 and 2). Both sites were significantly different from most of the other sites (Supplementary information Table S7 and S8).

Surprisingly, mussels from the supposedly pristine site close to Varangerhalvøya National Park in the Barents Sea (site S1) was identified as a hot-spot. At this site, the mussels were collected from the shoreline in an intertidal area, and therefore these mussels may have been exposed to MPs present both in the water column as well as those in surface waters. The reason for the high microparticle abundance may be related to several factors including atmospheric deposition of airborne MPs, low tidal flow and amplitude, as well as limited circulation caused by the back-eddy present in the region. Furthermore, input of MPs from further afield, such as transport from the North Atlantic and entering the Barents Sea might also contribute with MPs. MPs might along with other plastic debris accumulate in this area, and the elevated levels in the mussels could be linked to the 6th gyre as proposed by van Sebille et al. (2012) and Cózar et al. (2017).

It must be noted, that mussels from S1 were the smallest-sized mussels of all analysed, with significantly smaller individuals than 10 of the other sites (Supplementary information Table S2). Smaller sized mussels may, for example, not be as efficient as larger mussels in egesting MPs. However, a positive significant correlation between mussel body weight and number of ingested particles was found ($p = < 0.0001$, Spearman $R = 0.2164$), showing that larger mussels, not surprisingly, are prone to contain more MPs than smaller sized mussels. The same thing was also observed by Catarino et al. (2018). Van Cauwenberghe et al. (2015) observed that 4 cm long mussels efficiently removed MPs, nevertheless no studies have investigated mussel size as parameter for MP depuration. Therefore, it is necessary to conduct laboratory studies to understand the effects of MP uptake and depuration in relation to body size.

There are other parameters in addition to body size that should be further investigated to find if these are impacting the MP levels found in wild mussels, and thereby might impair long-term monitoring; seasonal variation due to e.g. spawning cycle or life stage, or whether different sub-species are interacting differently with MPs. Despite trying to standardise these parameters as much as possible in the current study, it was not possible to avoid all variation within these parameters since it is environmental samples. Therefore, the present data should be considered as semi-quantitative and qualitative data, not as fully quantitative data until these parameters have been further investigated.

Mussels from the second hot-spot, S9, were located within the Oslofjord at a highly urbanised site. In addition to the MP levels quantified, numerous rubber-like black particles <70 μm were observed (>100 for some individuals), that resembled the larger rubbery particles in both years (Fig. 5E). Thus, the levels reported for both years may represent a large underestimation due to the studies detection limit. In addition to being located at one of the busiest sites for boat traffic in the Oslofjord, the site is also affected by freshwater input from two large rivers, Alna river and Akers river. Why the other sites within the urban Oslofjord did not have similarly high MPs levels to site S9, is currently not understood, but it might represent a local source of rubbery MPs. However, all the sites within the Oslofjord had at least 50% of the individuals containing MPs (Fig. 1).

4. Qualitative data

4.1. Size, shape and polymer composition

MPs found in this study had an average size of 770 μm (range: 70 μm–3870 μm, $R^2 = 0.91$), meaning that most of the MPs in Norwegian mussels were <1 mm (Fig. 3). Therefore, mussels appear to be appropriate to monitor “small microplastics” (<1 mm) while other marine species should be assessed to monitor “large

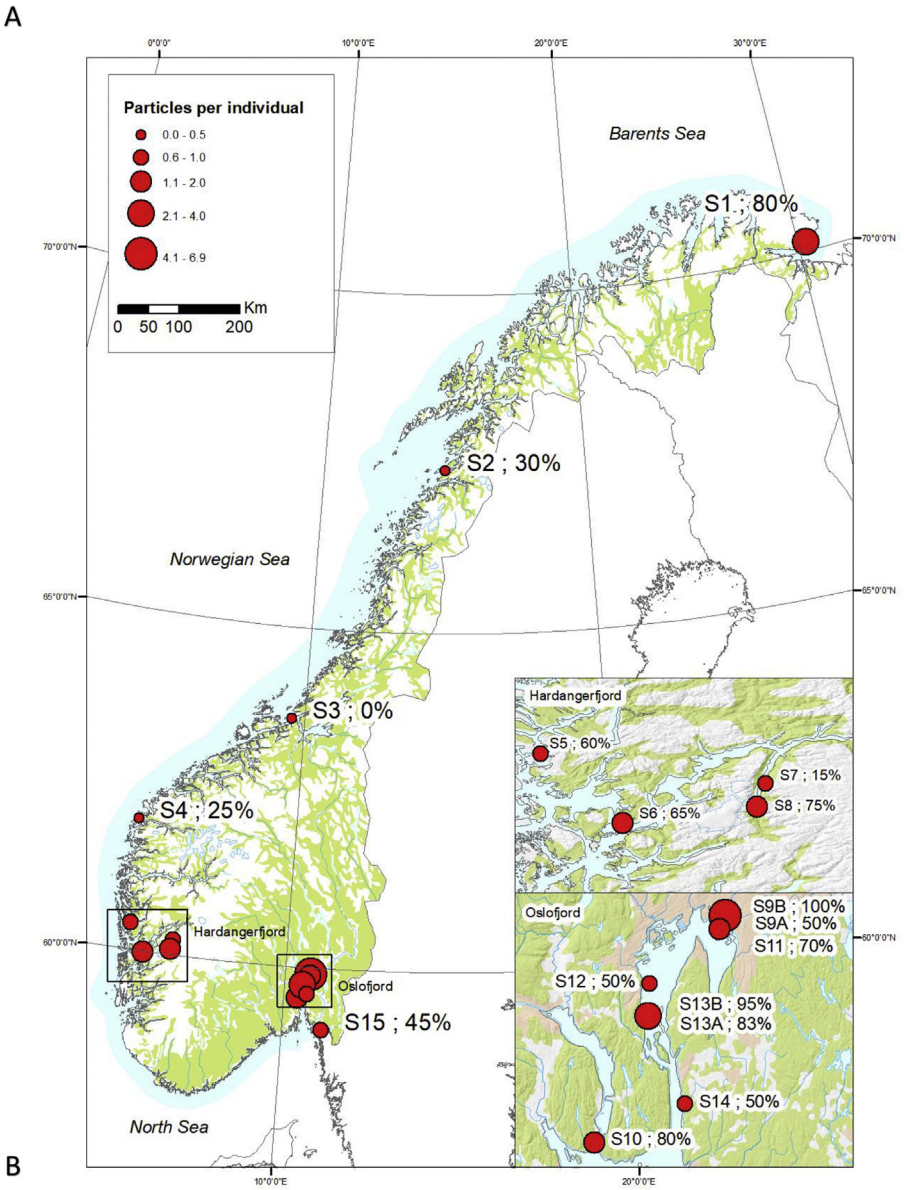


Fig. 1. A: **Map of sample sites in Norway investigated for microplastic presence in *Mytilus spp.*** with red circles giving the mean value of MPs per individual per site and the percentage (%) of individuals per site in which MPs were observed, **B: Box and whisker plots of log-transformed data of MPs per individua**l (n = 20 except for St. S13A with n = 12, see Table 1). +SB indicates sites with small black particles found below detection limit (<70 μm) resembling rubbery particles. Outliers are indicated as dots.

microplastics" (1-5 mm). Whether the relatively small sized MPs found in the mussels are due to mussels size preferences in regards to prey, or because the majority of MPs in the water column were typically in this size range, is currently unknown and should be further investigated.

Most of the MPs were fibres (81%), followed by fragments (12%) and "others" (3%; being films and foam) (Fig. 3). The high frequency of fibres are in accordance with other field studies (reviewed in e.g. Almroth et al., 2018). The only sites that did not show the same trend and were instead dominated by fragments, were mussels from S9 (the Oslofjord) and S7 (the Hardangerfjord).

Chemical analysis (μtransmission FT-IR) was performed on MPs found in mussels from 11 of the 15 sites, with a total of 224 out of the 894 MPs (25%) being analysed.

Of the particles tested on FTIR, 6.0% were the minerals analcime, travertine, mica or chitin and thereby excluded as MP particles. In total, 13 different groups of polymers were identified. Cellulosic fibres accounted for the highest proportions for all the sites, with exception of site S9, where 51% of the particles where "parking lot tar" and Ethylene-vinyl acetate (EVA) foam (Fig. 4). For all the polymeric particles from across all sites, cellulosic particles (identified as Cellophane; supplementary Figure S2) accounted for 63.9%, "parking lot tar" and EVA foam for 18.7%, polyethylene terephthalate (PET) for 9.9%, acrylic for 2.9%, polypropylene (PP) for 1.2%, polyethylene (PE) for 1% and <1% being PA, polybutadiene styrene rubber (SBR), epoxy resin (bisphenol A), *solprene plastomer*, styrene acrylonitrile (SAN), polyvinyl chloride (PVC) and polyacrylonitrile (PAN).

It is currently unknown why cellulosic-based articles were the most dominant in Norwegian mussels. Little information exists in the literature since many MP studies are lacking qualitative polymer composition data, or cellulosic-based particles may be excluded or included in the total MP counts without specifying this. However, similarly high levels of cellulosic polymers have been seen in other biota studies. Li et al. (2016) did find that cellulosic microfibers were the most abundant polymer type in *Mytilus edulis* from the Chinese coastal environment, accounting for over 40%, but this finding was not specifically discussed. Additionally, viscose was also found to be the largest component of MPs in an invertebrate community (Remy et al., 2015), >50% of the ingested particles found in fish from the English Channel were identified as Rayon (Lusher et al., 2013) and >49% of the polymers found in coastal fish from China were identified as Cellophane (Jabeen et al., 2017).

There are three likely sources of cellulosic fibres which may act in combination: fibres from atmospheric fall-out, fibres released with effluent from WWTPs or fibres from terrestrial application of sludge. The composition of microfibres from atmospheric fallout has not been extensively studied, but Dris et al. (2016) found that only 29% of the fibres from atmospheric fall-out were petroleum-based polymers, while the rest were cellulose-based particles (either cotton or semi-synthetics) in addition to wool. Cai et al. (2017) had similar findings when studying atmospheric microfibres, with 73% of the particles being cellulose-based particles.

Wastewater treatment plants (WWTPs) act as a source of MPs to the environment. Treatment processes are not 100% effective in capturing anthropogenic particles and low concentrations are released in the effluent (Murphy et al., 2016; Mason et al., 2016; Carr et al., 2016). Cellulosic fibres in effluent is rarely explicitly discussed; however, Michielssen et al. (2016) found that up to 88.9% of particles in final effluent were fibres, with cellulosic fibres included in this total. On the other hand, Magnusson (2014) found that natural fibres were a major proportion of the microfibres identified from a Norwegian WWTP in connection to the Oslofjord.

Many small anthropogenic particles that enter WWTPs are captured and much of this enters the sludge phase (Carr et al., 2016; Mahon et al., 2017). However, in many countries, sludge is used as a fertiliser for agricultural soils. This represents a potential release mechanism for very high concentrations of MPs through runoff into aquatic systems (Nizzetto et al., 2016). The composition of MPs found in sludge varies between plants; nevertheless, studies have observed high proportions of microfibres (e.g., Magnusson & Norén, 2014; Mahon et al., 2017). Although, the abundance of cellulosic fibres within this component has not yet been formally assessed. It is possible that some cellulosic fibres are degraded by wastewater and sludge treatment processes, but this is dependent on the degree of polymerisation and the crystalline structure of the material, which can vary (Park et al., 2004). Further work is required to effectively characterise this source of cellulosic microfibres to the environment.

The second most common polymer group found in Norwegian mussels were *"Parking lot tar"* and "*EVA foam*", accounting for 18.7% of the polymers identified. Based on a qualitative measure, S9 did, therefore, differ from the other sites in terms of polymeric composition (Fig. 4). Black particles can be challenging to measure using FT-IR since; 1) the IR beam can be fully absorbed by high concentrations of carbon black (22–40% carbon black is added as an UV-resistant (Kole et al., 2017), and 2) when "crushing" these particles with the crystal to flatten them prior to μtransmission FT-IR, they exhibit high elastic properties by "bouncing" back. Irrespective of these analytical challenges, the FT-IR spectra from these rubbery MPs matched with either EVA, "parking lot tar" or SBR rubber (Supplementary section 6). The origin of these rubbery MPs are unknown, but "wear and tear" of car ties or "road dust" is considered as the largest contributor of MPs into the Norwegian environment (Sundt et al., 2014). Until now, there has been no empirical data of their presence in biota, making our study a very important contribution to literature by illustrating that marine biota could be exposed to road derived MPs. Nevertheless, it is important to note that it is hard to fully determine the source of these particles. Similar particles have, however, been found in seawater from the Nordic area. A Swedish pilot study of coastal waters from the Baltic Sea, did find unidentified small black MPs resembling the ones found in Norwegian mussels (Norén, 2007). In passenger car tyres the most commonly used polymers are a mix of SBR, polybutadiene rubber and natural rubber (Vogelsang et al., 2018). It is, however, not only car tyres that are the source of road derived MPs. Road markings in Norway consist of different materials, including the polymers styrene-isoprene-styrene, EVA, PA and polyacrylate (Sundt et al., 2014). There is also a polymer composition within the asphalt itself, so-called polymer modified bitumen, that has been increasingly used on Norwegian roads since 2008 (Jørgensen et al., 2016), where the polymer component is often styrene-butadiene rubber (SBR) (Vogelsang et al., 2018). In addition to S9, mussels from S10 and S15 which are in connection to the Oslofjord, contained rubbery-like particles, specifically SBR rubber and *soloprene Plastomer* with the latter being a copolymer in SBR rubber. Mussels from S15 are from a protected national park, but the site is heavily impacted by recreational activities especially during the summer period, and fresh-water input from Norway biggest river, Glomma. Tyre rubber is known to contain toxic compounds such as PAHs, and exposure study with leachate from rubber particles on fish (*Oncorhynchus mykiss)* did find sub-lethal effects measured by e.g. increased EROD activity (Stephensen et al., 2003) which might make them of environmental concern.

In addition to road derived MPs being a possible source to these rubbery particles found in mussels from the Norwegian environment, rubber particles can also come from freshwater input due to granulate loss from artificial football turfs, which often consist of recycled car tyres. Lately, there has been a lot of attention towards this possible source of MP pollution into the Norwegian

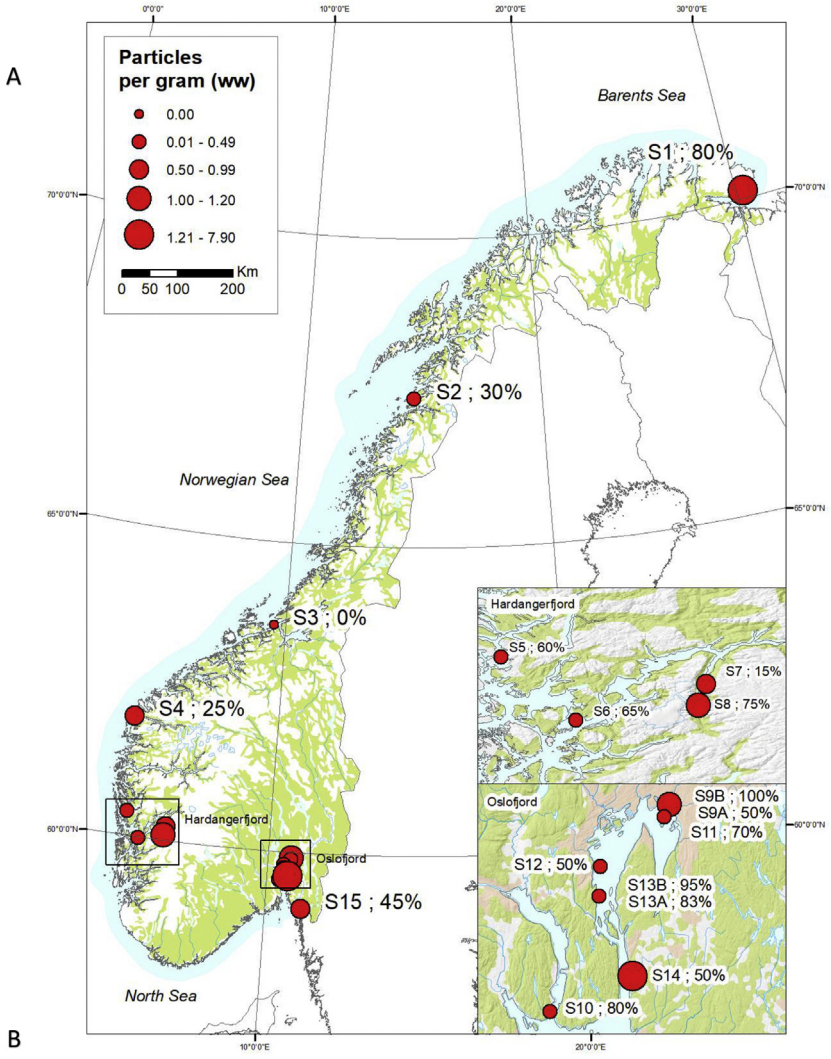
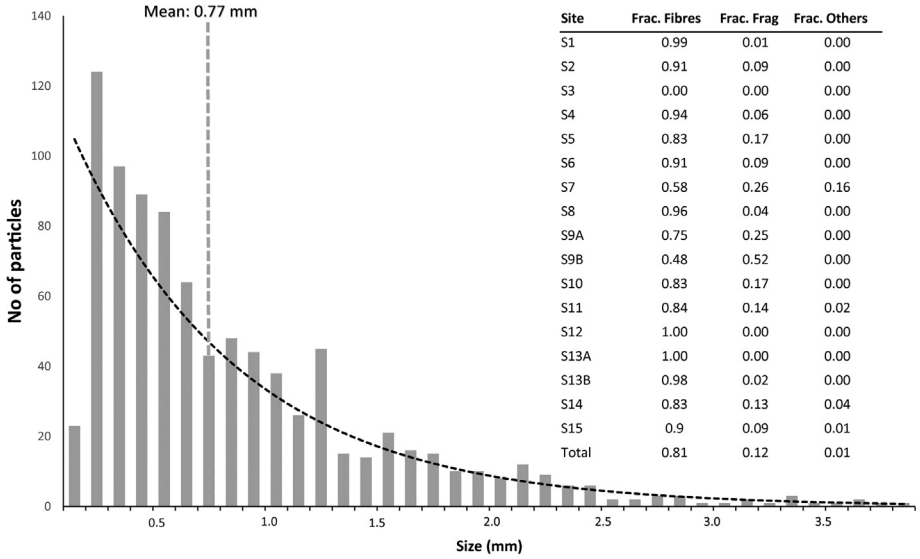


Fig. 2. A: **Map of sample sites in Norway investigated for microplastic presence in *Mytilus spp.*** with red circles giving the mean value of MPs per gram (w.w.) per site and the percentage (%) of individuals per site in which MPs were observed, **B: Box and whisker plots of log-transformed data of MPs per g w.w.** (n = 20 except for St. S13A with n = 12, see Table 1). +SB indicates sites with small black particles found below detection limit (<70 μm) resembling rubbery particles. Outliers are indicated as dots.



Site	Frac. Fibres	Frac. Frag	Frac. Others
S1	0.99	0.01	0.00
S2	0.91	0.09	0.00
S3	0.00	0.00	0.00
S4	0.94	0.06	0.00
S5	0.83	0.17	0.00
S6	0.91	0.09	0.00
S7	0.58	0.26	0.16
S8	0.96	0.04	0.00
S9A	0.75	0.25	0.00
S9B	0.48	0.52	0.00
S10	0.83	0.17	0.00
S11	0.84	0.14	0.02
S12	1.00	0.00	0.00
S13A	1.00	0.00	0.00
S13B	0.98	0.02	0.00
S14	0.83	0.13	0.04
S15	0.9	0.09	0.01
Total	0.81	0.12	0.01

Fig. 3. Number of microplastics found in mussels in different size categories across all sites with black dotted exponential trend line (with equation and R^2) and dotted grey line giving total mean length (mm). Equation: y = 119.83e-0,134x and $R^2 = 0.9138$. **The morphology of the MPs found are given in the table for each site.**

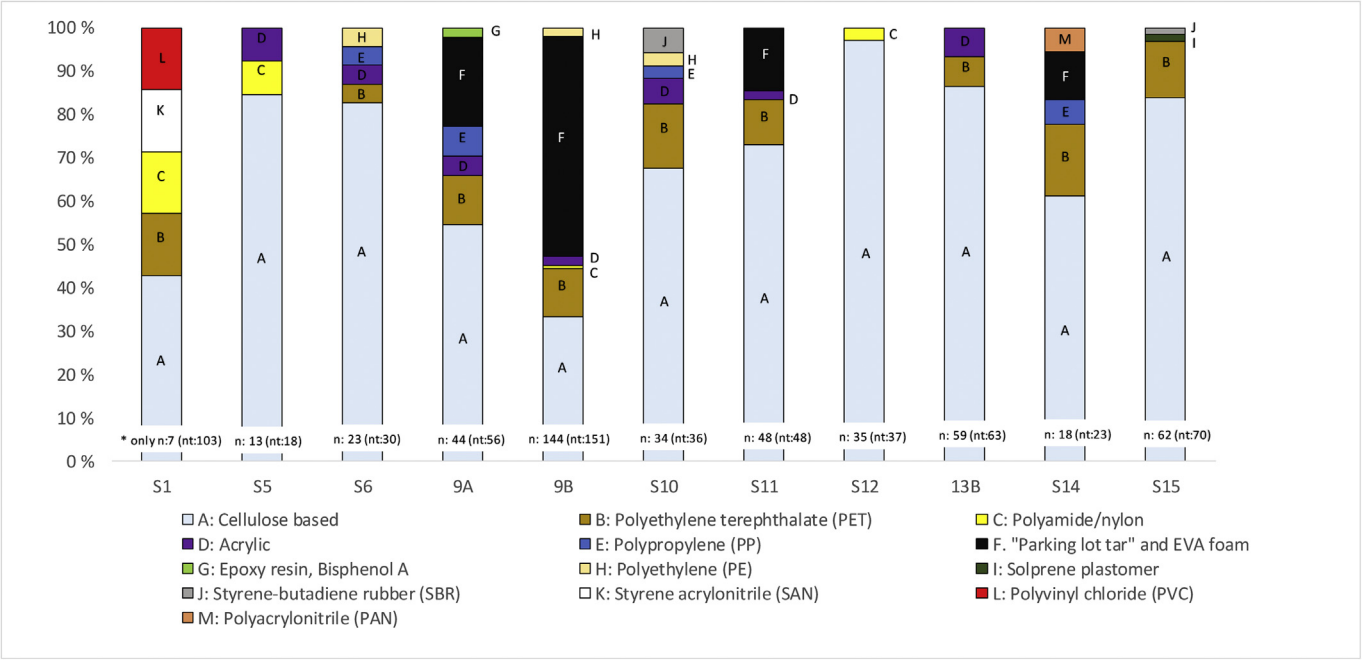


Fig. 4. Fraction of the different polymeric origion of the microplastics found in *Mytilus spp.* from 11 out of 15 sites in Norway based on μtrans FT-IR indicated by different colours and letters. For each of the sites "n" equals the total number of particles analysed by μtrans FT-IR and "nt" equals total number of particles found. * Only 7 out of 103 particles were analyse for site S1.

environment (Sundt et al., 2014) and preliminary results shows that granulates are found down-stream of Norwegian football fields (Beylich, B. A, *personal communication*). Yet, it is currently unknown whether these particles are entering the marine environment.

The third most commonly found polymer group in Norwegian mussels was polyester, specifically PET. PET was found as the largest polymer group in Atlantic cod (*Gadus morhua*) from the Norwegian environment (Bråte et al., 2016) and since synthetic clothing often is made from PET, this can be a possible source into the Norwegian environment. Furthermore, the next largest polymer group was acrylic, in the form of the brand name *Acrylic* and polyacrylonitrile (PAN). PA was also detected in Norwegian mussels despite Kühn et al. (2017) finding that nylon was affected by KOH treatment. However, based on our recovery test, no break-down was observed for *PA*. Following, the styrene monomer SAN resin was also found in Norwegian mussels. Not surprisingly, PP and PE were also found, being the most used and produced polymers worldwide (Plasticseurope, 2017) and being commonly found in marine biota (e.g., Rummel et al., 2016; Tanaka & Takada, 2016; Jabeen et al., 2017; Karami et al., 2017).

Further, polymers of special environmental concern were found in Norwegian mussels. Epoxy resin with bisphenol-A was detected

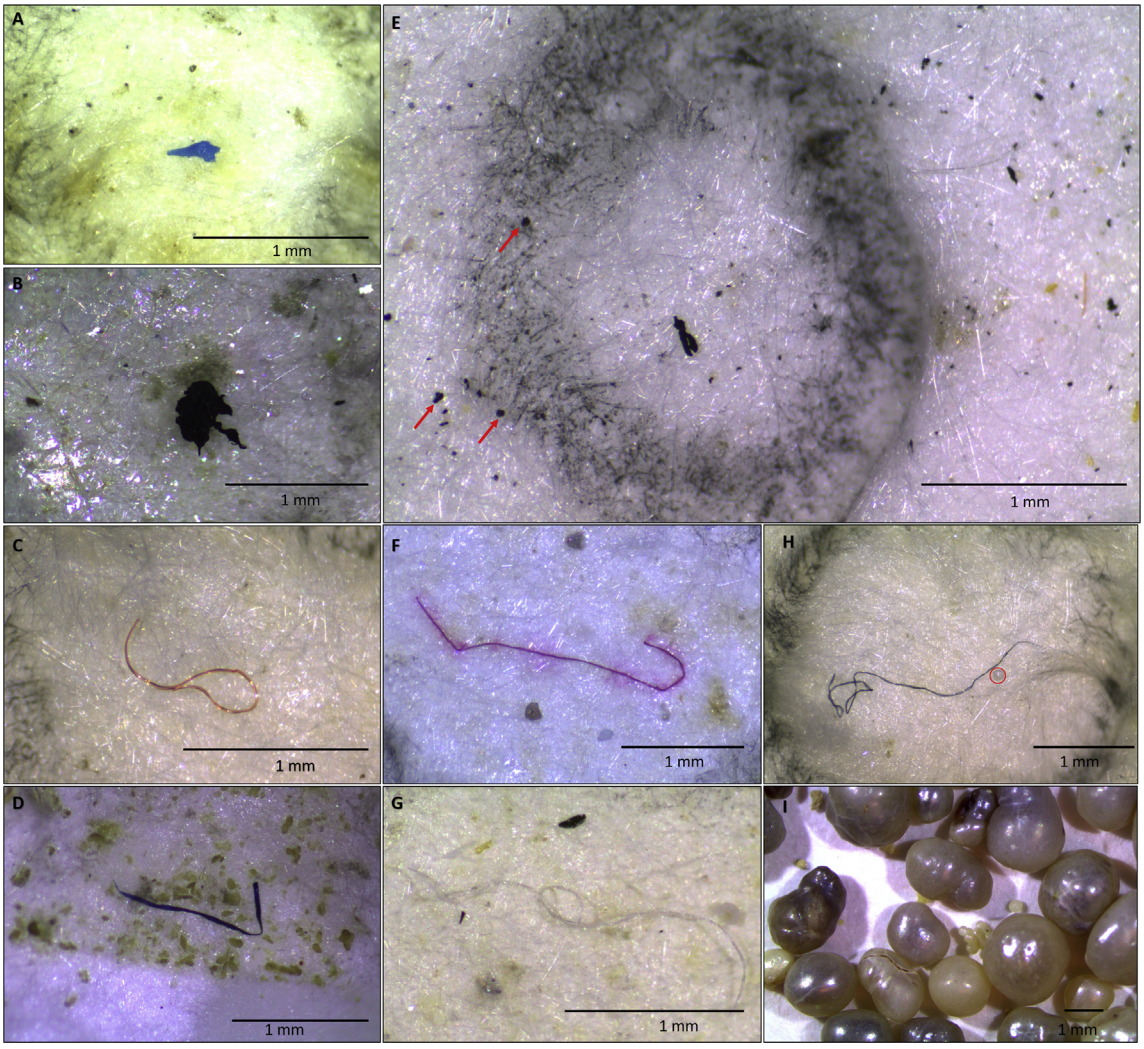


Fig. 5. Examples of microplastics isolated from *Mytilus spp.* in Norway A: Polyethylene fragment from site S9B, **B:** Polyethylene fragment from site S6, **C:** PET fibre from site S9B, **D:** Cellulose based fibre from site S12 **E:** Black fragment "Parking lot tar" and examples of small black particles below detection limit, **F:** Cellulose based fibre from site S15, with dye loss from KOH treatment **G:** Transparent cellulose based fibre from site S9B **H:** Cellulose based fibre from site S9B with a small pearl (red circle), **I:** Pearls from one individual from S9B, with 81 pearls for that specific individual.

in mussels from S9 (the Oslofjord). Expoxy resin with bisphenol A is used in for example boat paints and bisphenol A (a common component in epoxy resin) is of environmental concern being a well-known endocrine disruptor (Rubin, 2011). PVC was also identified in this study, more spesifically in mussels from the "pristine" Barents Sea, and PVC were also found in Atlantic cod (*Gadus morhua*) from the Norwegian environment (Bråte et al., 2016). PVC can have a high phthalate content, and phthalates are frequently found in the environment (reviewed in Net et al., 2015; Hermabessiere et al., 2017). Based on a laboratory study it has been found that mussels can be affected by phthalate exposure, such as by increased micronuclei formation post exposure (Barsiene et al., 2006). This is the second time PVC has been confirmed in marine mussels, being earlier found in marine mussels from the Chinese environment (Qu et al., 2018).

5. Conclusion

Marine mussels from the Norwegian environment are exposed to water-borne MPs with two hot-spots being identified from the Norwegian environment; mussels from the Barents Sea and mussels from the highly urbanised Oslofjord. Thirteen different polymers were identified, with cellulose-based polymers and small black rubbery particles possibly being from "road-dust", as the most abundant. Epoxy resin with bisphenol A and PVC were also found being polymers of special environmental concern. Based on this study, mussels are a promising sentinel species for small MPs (<1 mm) in the marine environment. To be a fully quantitative measure, some questions need to be answered and further methodological improvements should be considered. Future work should investigate the role of mussel size in relation to MP ingestion, accumulation and depuration, as well as the role of subspecies. A benefit of using mussels as a sampler for MPs in the marine environment and not directly measure MPs in water samples, is the knowledge obtained from the interaction between MPs and biota, that water samples cannot provide.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.envpol.2018.08.077.

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