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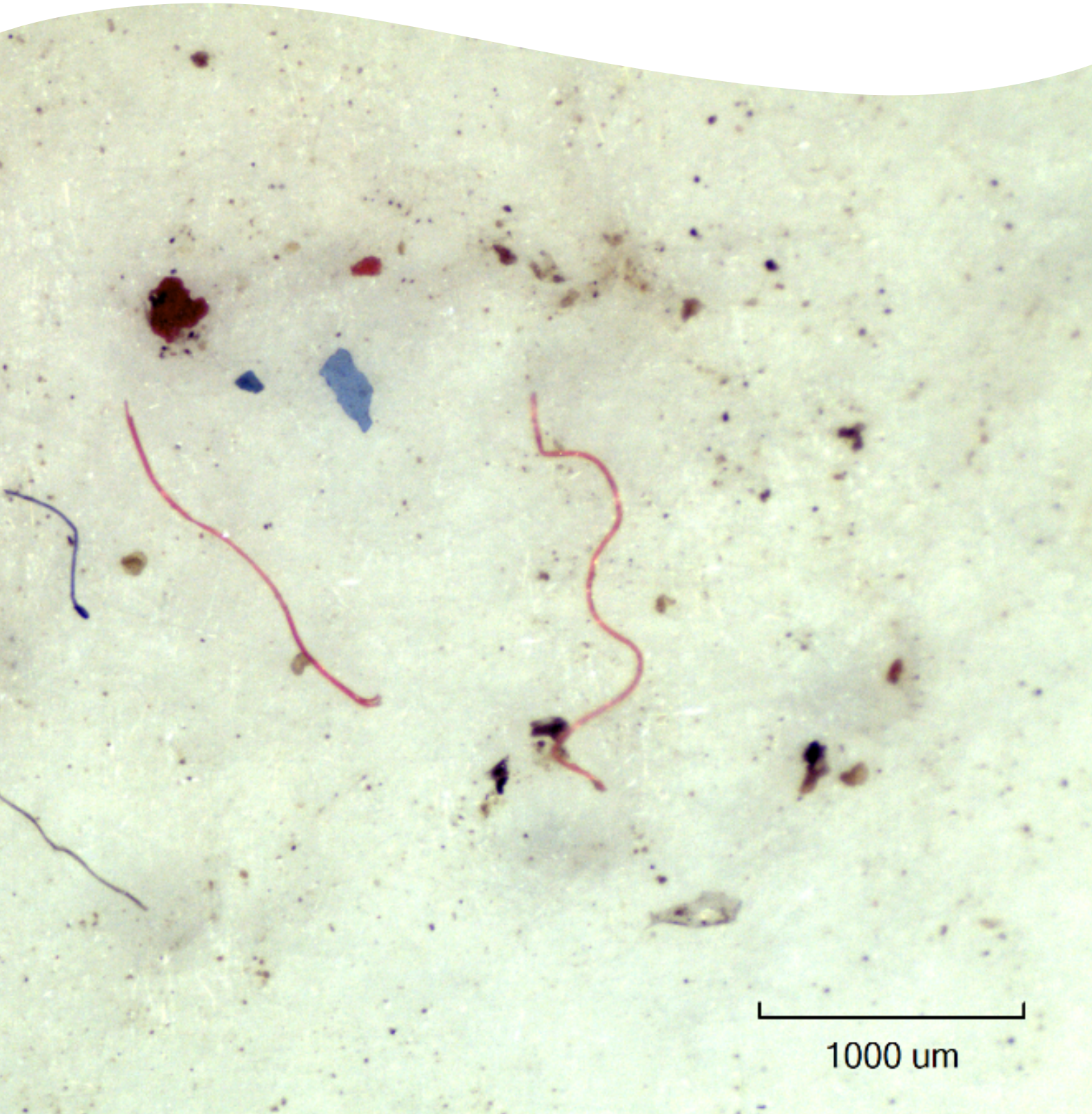
Monitoring of microplastics in Danish marine waters using the Oslo-Kiel ferry as a ship-of-opportunity



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Summary

The EU Marine Strategy Framework Directive require that EU Member States establish appropriate strategies and sampling programmes for microplastic in the marine environment. We report the results of a two-tiered pilot study consisting of a mini review focusing on sampling of microplastic in marine surface water and a test sampling of microplastic particles in the Inner Danish Waters using the FerryBox system on board the Oslo-Kiel ferry.

Four keywords	Fire emneord
1. Microplastics	1. Mikroplastik
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MSFD microplastic project

Monitoring of microplastics in Danish marine waters using the Oslo-Kiel ferry as a ship-of-opportunity

Client: Danish Environmental Protection Agency

Preface

Plastics and microplastics are regularly found in the marine environment around the world. Currently, the spatial and temporal dynamics of microplastics are poorly assessed and only limited long-term data is available on at-sea occurrence. Long-term data series are required to address changes in abundances of microplastics including variations in spatial and temporal distribution as well as to understand the influence of, for example, different seasons, changing weather or hydrological conditions.

To facilitate monitoring, harmonised and validated approaches are needed. One approach is to use ships of opportunity to collect data over replicated transects¬: these include research vessels as well as commercial vessels. Advances in technology enable assessment of micro-plastic abundance at large spatial scale using existing infrastructure in addition to the collection of oceanographic meta-data.

A microplastic sampling module was fitted to an existing marine monitoring system (Ferry Box) on a commercial ferry (M/S Color Line Fantasy) between Oslo and Kiel. It was used to acquire samples in the Danish part of the Skagerrak and Kattegat. In total thirteen samples were collected using a high-volume sampling and filters of mesh sizes 100 and 500 µm of which eleven were successfully processed. One of the challenges in processing the samples was the large variation in quantity of biological material simultaneously collected on the filters. Several samples did contain very little interfering material and could be directly processed, while other samples contained extreme amounts of biological material related to, for example algae blooms, and required several pre-processing steps before analysis.

Relatively small amounts of microplastics were found in the large volume samples (5340 L) ranging from 0 to 1.85 fragments or fibers per m3 (average 0.71 per m3). These levels agree with other studies in the same region. Most of the fragments and fibres consisted of polypropylene and polyester. Polyamide (nylon), polystyrene and rubber were also identified. Interestingly, no fragments of polyethylene were found which is one of the most commonly used consumer plastics. In addition to the synthetic fibers, large amounts of natural fibres (cellulose, wool) were present in the samples. They contributed up to 80% of the total particles.

Although a comparatively large number of samples were analysed over a period of 6 months, no clear temporal trend was found. The temporal resolution was most probably too small to explain the variation in the data.

Copenhagen, 26 August 2020

Jesper H. Andersen

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1 Introduction

This pilot study focuses on methods for monitoring of microplastics and also tests sampling of microplastic particles on a north-south transect through the Inner Danish Waters. Sampling was performed using a FerryBox system on the ferry M/S Color Line Fantasy between Oslo and Kiel. The pilot project was initiated and funded by the Danish Environmental Protection Agency and follows up on previous study of chlorophyll concentrations using this ferry (**Andersen et al. 2017**).

1.1 The need for monitoring of microplastics

Plastic is a ubiquitous environmental contaminant found around the globe which is threatening terrestrial, freshwater and marine ecosystems. This contamination includes microplastics (<5 mm) (**GESAMP 2019**, **Galgani el al. 2010**) which have been identified not only near populated areas but also in remote environments including polar regions, the deep sea and isolated mountain lakes. This widespread environmental presence highlights a need to understand sources and consequences of microplastics. Microplastic contamination is expected to increase in years to come, especially in view of increasing global plastic production and the subsequent degradation and fragmentation of larger plastics in nature.

International governing bodies now consider plastics to be an issue of emerging concern. The environmental monitoring of plastics is high on the agenda of countries and international organisations worldwide. UN Member States, Regional Sea Conventions and EU are in the process of ratifying monitoring frameworks and instruments, including stringent environmental assessment practices according to national and regional circumstances. An understanding of microplastic distribution and abundance at broad spatial scales is required to inform policy makers and governmental organisations on the fate of microplastics in the marine environment. Such assessment is therefore of legislative relevance within Europe's Marine Strategy Framework Directive (MSFD, 2008/56/EC) which requires the development of monitoring schemes for marine litter. Harmonised methods are required to enable regulatory compliance as well as to assess the effectiveness of environmental protection policies. To do so, robust and harmonised protocols and standards are required. There is an urgent need to reinforce existing initiatives and improve coordination between actors. Several working groups have now been established to compile recommendations for associated countries to begin to routinely monitor microplastics. Listed in Table 1 are some of the working groups and expert panels in the process of finalizing, or who have recently finalized their recommendations. These working groups are tasked with defining indicators, identifying baselines and recommending targets.

1.2 Status on monitoring of microplastics

Microplastics are introduced to the marine environment through various pathways, including direct input to coastal and marine systems, or transported from terrestrial and freshwater sources. Microplastics are generated from the breakdown and fragmentation of plastic items in the ocean or are introduced directly from land. Fragmentation in the ocean is facilitated through photodegradation, hydrolysis and biodegradation, as well as grazing and shredding by macro fauna. All of these processes affect the density, and buoyancy of plastics, which governs their presence in the water column (**Andrady 2011**). Once in the marine environment microplastics can move between compartments. Winds on the ocean surface cause the mixing of surface waters and the upper layers of the water column, affecting microplastic distribution in the water column. Surface currents and tides move microplastics between coastal and offshore areas, whereas gyres and other large oceanographic features create accumulation zones (reviewed in **van Sebille et al. 2020**).

Table 1. *Working Groups involved in the compilation and recommendations of Regional Action Plans (RAPs) and Guidelines for monitoring methods for assessing microplastics.*

Working Group	Level	Associated policy framework	Date	Outputs/reports	Focus
GESAMP WG 40	International (UN)	UN SDGs	2015-2019	Guidelines or the monitoring and assessment of plastic litter and microplastics in the ocean (GESAMP 2019)	Guidelines for plastics litter monitoring in the ocean
NOWPAP	International (UN)	UN SDGs	2008-	RAP launched 2008	Marine litter generation
MOEJ	International	G20	2017-	Guidelines v.1. Michida et al. 2019	Microplastics in surface waters
TSG-ML	European	MSFD, 2008/56/EC	2010-2016	Galgani et al. 2013 Gago et al. 2016	Monitoring marine litter in European seas Monitoring microplastics in seawater
ICES	European	MSFD, 2008/56/EC	2018-2020	In progress	Seafloor litter and microplastic monitoring
OSPAR	European	MSFD, 2008/56/EC	2014-2021	In progress	Beach litter, seabed litter, fulmar stomachs and new indicators
HELCOM	Baltic	MSFD, 2008/56/EC	2013-	RAP in progress	Marine litter
MAP	Mediterranean	MSFD, 2008/56/EC	2013	RAP launched 2013	Marine litter management
AMAP	Arctic Council	MSFD, 2008/56/EC	2019-	In progress	All arctic environments, all plastic sizes
PAME	Arctic Council	MSFD, 2008/56/EC	2017-	RAP in progress	All arctic environments, all plastic sizes

** AMAP (Arctic Monitoring and Assessment Plan); GESAMP (Joint Expert Working Group on Scientific Aspects of Marine Environmental Protection); HELCOM (Baltic Marine Environment Protection Commission); ICES (International Convention for Exploration of the Seas); MOEJ (Ministry of the Environment – Japan). MSFD (Marine Strategy Framework Directive); NOWPAP (Northwest Pacific Action Plan); PAME (Protection of the Arctic Marine Environment); RAP (Regional Action Plan); TSG-ML (European Union expert group on marine litter, the technical Subgroup on Marine Litter); UN SDGs (United Nations Sustainable Development Goals).*

The monitoring of microplastics (in the water column or on the seafloor) is not included as a global monitoring core parameter within Regional Action Plans (RAPs), or international policies, such as the SDG frameworks (**GESAMP 2019**). However, work is underway to develop such guidelines. Most surveys related to microplastics in the marine environment have therefore focused on method development or initial baseline surveys to assess the levels of contamination in one-off (not repeated) sampling. Such specific investigations in the marine environment have been performed in surface waters, the water column or in sediments (**Lusher 2015**). Currently, data on the presence of microplastics in the environment vary regarding quality, resolution and focus, which compromises of comparative assessments of contamination and limits confidence related to the impacts of plastic pollution.

Long-term spatial and temporal sampling of microplastics is rare and often samples are taken irregularly and not as part of a long-term sampling strategy. This hinders the understanding of behaviour, distribution and source identification of microplastics. Solid harmonised data for modelling the behaviour and source back-tracking of microplastics is missing and urgently needed. Long-term data series are required to address changes in abundances of microplastics including variations in spatial and temporal distribution (**Lusher et al. 2014**) and to understand the influence of, for example, different seasons, changing weather or hydrological conditions. Harmonised and validated approaches are required to facilitate monitoring. It is important to establish the origins, trajectory, and fate of microplastics in the environment in order to mitigate future effects. Once microplastics have been identified, and standardised sampling and analytical methods are developed, results can be fed into international monitoring strategies to map microplastic distribution worldwide (**Cutroneo et al. 2020**).

For clarification, we refer to the surface waters as the top 10m of the water column and distinguish between ***surface*** (at the air-water interface or directly below the interface) and ***sub-surface*** (below the surface but still within the upper mix-layers, affected by winds, surface currents and vessel movements).

1.3 Objective

The objective of this pilot project was to:

1) review current state of the art for monitoring microplastics in the marine environment and the tools available for monitoring surface waters of the marine environment; and

2) design and perform a monitoring study of microplastic in the Danish marine environment using NIVAs operating FerryBox system on the ferry between Oslo and Kiel Ferry sampling a route through Danish waters of the Skagerrak and the Kattegat from September to December 2019.

The aim of the project is to set a baseline for long-term spatial and temporal sampling and analysis of microplastics. In addition, metadata was collected during the sampling from the FerryBox system allowing interpretation of results using supplementary oceanographic observations.

2 Methods available for monitoring water bodies

Sampling strategies for monitoring microplastics in surface waters and the water column should be designed to relate to specific questions of the monitoring programme, reflecting requirements set by national and international policy. Choice of sampling location, frequency and method will all differ depending on the compartment of the water column (surface vs. sub-surface) as well as the size range of plastics being targeted (**GESAMP 2019**). Certainly, irrespective of the sampling method of choice, environmental metadata should be collected to support data interpretation, this includes bathymetry, water temperature, salinity, water currents, surface wind and weather conditions. Seasonal and temporal differences such as currents, tidal conditions, weather conditions (incl. short term wind and rain events or seasonal flooding), shipping routes and biological activity can all influence sampling conditions. Therefore, sampling design focusing on repeatability will help describe the variability within collected data. It is also important to establish baseline levels through an initial survey as this can provide a basis for monitoring future changes. Several techniques are currently used for sampling microplastics from the surface waters and the water column. Each method is described briefly in the following sections.

2.1 Surface and sub-surface sampling nets

Microplastics were first observed in water samples using surface sampling nets during research into planktonic communities as early as the 1970s (**Carpenter & Smith 1972**). These nets were originally used to sample plankton, although they have now been readily adopted for microplastic sampling. Plankton tows are one of the most common sampling devices for microplastics in surface water. Very little has changed in terms of net types and methodologies which has led to the establishment of some long-term data series (see Section 3). The main advantage of using a net is that a large volume of water can be quickly sampled (**Gago et al. 2016**). Nets can be towed horizontally, at the air-water interface, subsurface or at greater depths, as well as vertically from the bottom to the surface to sample the entire water column, or obliquely. Many surface trawls are limited to mesh sizes of around 300 µm although researchers have begun using equipment with mesh sizes targeting the lower µm range. A flow meter is normally attached to the mouth to allow measurement of the water volume passing through the net during sampling. Nets should be deployed to the side of vessels to avoid their wake as this disturbance may influence the particles floating in the surface water. Further, high sea states can also cause nets to jump"[1] on the ocean surface hindering sample collection and result in unreliable volume measurements. After samples are collected the nets are rinsed from the outside and the sample collected in the cod end can be volume reduced and processed directly (ideal for samples >1 mm) or preserved until later laboratory analysis. Nets can be used in singular or as replicates, such as the multinet set up deployed in the Baltic Sea **(Setala et al. 2019).** This special set up allowed sampling at different depths in the same water body.

Neuston nets are the typical plankton nets used to sample surface waters (see Figure 1). They generally have a rectangular or circular frame with a nylon mesh and cod end. Samples are collected by trawling surface waters for a pre-determined time period. Many nets are kept at the surface by floats or suspended beneath the water's surface. Neuston nets can capture the ocean surface layer even in wavy

[1] Jump- in rough weather, either wind or waves can cause the net to jump out of the water and not sample the sea surface. Therefore, under-sampling the desired surface area (Michida et al., 2019)

conditions, but it is difficult to estimate the volume of water filtered accurately because the net's immersion depth changes constantly (**Michida et al. 2019).**

Manta nets are one of the most common sampling devices for microplastics. Manta nets can sample a large volume of water in a quantitative fashion. They have been used in almost every regional sea including circumpolar and circum-global investigations (see Section 3). Manta nets are called as such due to their appearance resembling a manta ray. The wings provide lift, keeping the device close to the sea surface. The frame is generally rectangular in shape with a nylon mesh attached. Samples are washed down into the net as it sweeps the water surface and concentrates particulate matter in the cod end. Typically, manta nets have a mesh size of ~ 330 µm, therefore sampling the larger fraction of microplastics. Sampling using a manta net must be carried out in calm conditions, with a speed below 3-knots (**GESAMP 2019**). Manta nets are able to maintain a constant immersion depth under the sea surface and the filtered water volume can be estimated if there are no waves on the sea surface. If the wave height exceeds a certain level, the net tends to skip on the water surface (**Michida et al. 2019)**. Vessels should retain a constant direction and consider any water currents present in the area as this can influence the volume of water sampled. Flow meters can be used to help estimate water volume and results can be expressed as particles per m^3. Alternatively, the distance traveled can be recorded, and results expressed as particles per m^2 (**Michida et al. 2019).** Manta nets have also been modified to have smaller mesh sizes allowing the sampling of smaller particles, although this can be problematic in highly productive areas with high concentrations of organic matter (e.g. **Khalik et al. 2018, Lusher et al. 2015**). They have also been modified for high speeds (AVANI net**, Eriksen et al. 2018**).

A modification of plankton tows are **bongo nets**, which consist of two connected cylindrical-conical shaped frames, which are pulled horizontally through the water column. Bongo nets are often used for mid-water sampling (**Cai et al. 2018**) and can collect replicate samples.

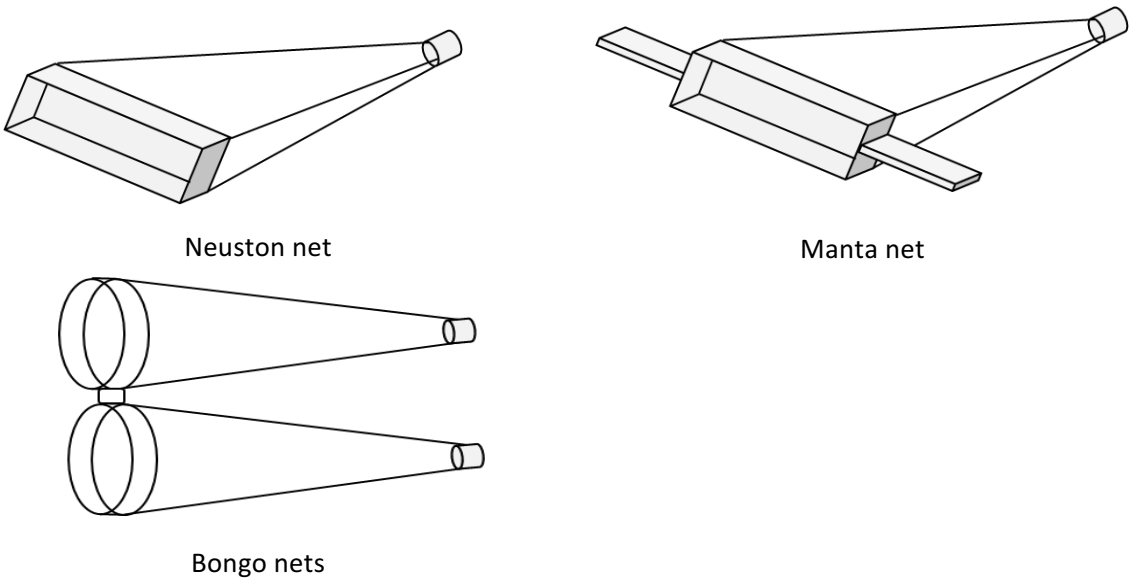


Figure 1. *Examples of surface and water column sampling nets.*

2.2 Continuous Plankton Recorders (CPRs)

Continuous Plankton Recorders (CPRs) can be used for microplastics sampling in subsurface waters. CPRs were originally introduced in 1931 as a plankton sampling instrument. They are a valuable tool to monitor plankton species composition, abundance and distribution through time. CPRs are designed to be towed from vessels. The CPR is towed at 10 m depth, whilst water slowly passes through the aperture containing a slow-moving band of silk, which traps planktonic material, including microplastics. The gathered sample is spooled into a storage tank containing formalin.

Archived CPR samples have shown promise for microplastic sample collection and time-series analysis. Samples collected in the North Sea, dating back to the 1930s showed the emergence of microplastics in samples in the 1960s (**Thompson et al. 2004, Ostle et al. 2019**). The seminal publication by **Thompson et al. (2004)** used historical CPR records to show the change in abundance of microplastics over time. Even though it is the most cited microplastic publication, the method has failed to be adopted and employed widely. The method could be adapted for future investigations. The early reports of microplastics in CPR were taken further by **Sadri (2015)** who investigated additional silks from the Atlantic and North Sea, finding microplastics, mostly polyester fibres and lines, like those used in the fishing industry. CPR were introduced to the Southern Ocean in mid 2000s and initial data analysis found average microplastic concentration to range between 0.001 and 0.54 per m^3 (>300 µm, **Grover-Johnson 2018**).

2.3 Sample collection pump filter systems on board

Intakes of seawater which is used to cool the engine or to collect oceanographic measurements can be utilised, and water diverted to collect samples whilst vessels are travelling or performing other sampling operations (**Kanhai et al. 2018, Lusher et al. 2014** and **2015, Morganna et al. 2018).** Investigating how to routinely assess subsurface waters for microplastics, Lusher and colleagues set up a simple sampling stage to collect intake water into a sieve (250 µm) and filter the sample with a constant flow rate. The collected sample was then rinsed and filtered directly onto filter paper for analysis on return to land. Processing can be carried out around the clock and can collect a significant number of samples.

As an example, this method was employed on R/V Celtic Explorer, the ocean-going research vessel of Ireland's Marine Institute. The vessel was undertaking a variety of research cruises including marine mammal and fisheries surveys in the North Atlantic. Seawater was drawn from an intake valve at 3 m below the surface covering a cruise transect of 12,700 km. Samples of 2000 liters of seawater were collected while the ship was travelling at 10 knots. 94% of the 470 samples taken contained plastics between 0.25 mm and 5 mm (**Lusher et al. 2014**). Sampling ship intakes represents an easier and more reliable sampling technique than surface trawling, in terms of sample contamination and flow measurements. Another advantage is that metadata including sea, boat and weather conditions is collected. Modification to the method included reduced sampling during plankton blooms and large biological activity or the introduction of a second sieve to remove initial plankton bulk (1 mm). The heterogenous nature of plastic particles in the environment and the relatively small numbers of microplastics in the marine environment require sampling volumes >1000L to obtain statically relevant numbers.

Advances in technology will enable large spatial scale assessment of microplastic abundance using existing infrastructure in addition to the collection of oceanographic meta data (**Lusher et al. 2014**). It is further advantageous as it allows microplastic sampling whilst vessels are travelling to survey areas and thus long transects over several kilometres can be collected in connection with in-line analytical systems for other environmental metadata.

2.4 Sample collection pump filter systems (FerryBox)

Several marine monitoring systems utilise seawater intake on board of vessels and are specifically designed to continuously monitor environmental parameters. Several systems for the collection, analysis and presentation of water quality data combining information from sensors installed on board ships are available. These 'ships of opportunity' often travel along fixed routes collects data from the sensors that can be combined with other types of data, e.g. from environmental satellites or collected water samples for calibration and verification. These so called 'FerryBox' systems and other platforms for monitoring marine litter have been highlighted as an important move forward for large sample volumes, controlled sampling and broad spatial coverage (**Conchubhair et al. 2019**).

FerryBox sensors routinely measure temperature, salinity, oxygen, chlorophyll and particle content every minute at a depth of 2-4 meters below the surface along the fixed route of a vessel. Depending on the speed of the vessels, this results in one measurement every 500 meters. A fully equipped FerryBox system can measure and register more than 25 different parameters including weather and ocean conditions including sea state, oxygen, phytoplankton biomass and diversity, ocean surface stress, nutrients, zooplankton biomass and diversity, sea ice, inorganic carbon, sea surface height, transient tracers, sea surface temperature, particulate matter, subsurface temperature, nitrous oxide, seagrass cover, surface currents, subsurface currents, dissolved organic carbon, sea surface salinity and ocean colour. All data is transmitted in real time. In addition, FerryBox systems can activate sampling of predetermined locations, based on GPS coordinates or sampling can be remotely triggered. FerryBox system have been installed on more than 40 vessels in Europe and operated by several marine institutes including IFREMER, IMR, NIVA, SYKE, SMHI.

A microplastic sampling module has been tested and implemented for sampling on research vessels in the Siberian Arctic (**Yakushev et al. 2019**), cruise ships in the Southern Ocean and on commercial vessels in the European waters, Arctic and Antarctic waters (incl. Hurtigruten, Color Line).

2.5 Direct water sampling

Discrete sampling devices can be used to sample water from specific depths, either at the water surface or within the water column. Samples are usually collected this way with buckets, bottles or trays, and are processed directly on board or returned to laboratories for processing. Samples can be filtered through fine meshes or sieves. Nisken bottles, CTD rosettes and integrated water samplers are commonly used (**Bagaev et al. 2018, Dai et al. 2018, Tamminga et al. 2018**), along with buckets, bottles and steel samplers (**Dubaish & Liebezeit 2013, Khalik et al. 2018; Zhu et al. 2019**). Some methods can be as simple as a glass bottle of 1 litre of water. Citizen Science driven projects have used this approach with samplers collecting single bottles of 1 litre surface waters (**Barrows et al. 2018).** The small volume and transport of water samples is a disadvantage of direct water sampling, but the method can be used near sources including urban areas or for sampling nano particles.

2.6 Considerations for field sampling

Blank samples are for the analysis of microplastics. Blank samples allow researchers to see how clean their sampling and analysis process is. Blanks are used to account for procedural contamination from the sample vessel, the sampler or any processing performed of the sample before analysis. An example of a field blank is a sample collected in the field without being exposed to sea water. Procedural blanks with consistent contamination are used to either subtract average contamination from the samples,

or present data in terms of limits of detection and limits of quantification (**GESAMP 2019**). Samples of all equipment, clothing worn by samplers and vessel paints are sources of contamination.

Preservation methods for microplastic samples can include freezing of samples or the use of preservation solutions such as formalin and ethanol.

Metadata should be collected to support monitoring. Metadata, or ancillary data, to any sampling activity is vital. This data should be collected in a structured manner to allow reliable assessments to be made. Metadata parameters include location (start and end of sampling), date, equipment used, environmental variables (wind speed, wave height, currents, temperature, salinity etc), sampling variables (vessel speed, pitch, roll), record of procedural contamination as well as any non-conformance to protocols.

2.7 Brief summary of laboratory methods

It is still not possible to detect microplastics with a sensor or to prepare the samples for analysis onboard as the risk of procedural contamination is too high with current methods. For all the sampling methods described above, samples must be returned to laboratories for sample pre-treatment and assessment. The method chosen should be designed to lower the risk of contamination. Working in a clean environment is essential when investigating microplastics to ensure the samples are not contaminated, for example by airborne microplastic **(Wesch et al. 2017).**

Visual assessment to categorise particles by morphology, size and colour can be performed before categorisation based on polymeric composition. In some cases, if samples contain relatively large amounts of biological or organic material the samples must be pre-treated to allow efficient visual assessment (**Lusher et al. 2020**). Digestion approaches use peroxide, potassium hydroxide (KOH) or Fenton's reagent, whereas density approaches can utilise concentrated salt solutions to separate particles based on their density. These processes are reviewed in detail in **Lusher et al. (2020).**

Once samples are filtered, visual analysis can commence. Microplastic analysis of environmental samples are often based on visual identification of the particles using a light microscope. To avoid risk of visual misidentification, there is an increased interest in different spectroscopic techniques such as Near Infrared (NIR), Fourier Transform Infra-red (FTIR) and Raman, which can identify the polymeric or plastic component of particles. Polymer identification is important not only for quantification, but also holds information on possible sources. Currently no technology exists to analyse microplastic samples on-line or using flow through instrumentation.

2.8 Reporting requirements

To calculate reliable microplastics concentrations per unit volume, the total volume sampled must be accurately measured. Limitations of any method employed must be clearly described (Table 2).

Data management: currently there is no international data governance on microplastics as marine litter, although regional centres have been established. A coordinated solution for data management is envisaged to comply with the reporting requirements of SDG 14.1.1.

Table 2. *Advantages and limitations of methodological approaches used to sample microplastics.*

Sampling methods	Advantages	Limitations
Surface sampling nets (Neuston nets Manta nets)	Easy deployment from a range of vessel sizes. Easy to use, fast sampling of large volumes	Limited data available for <300 µm in long-term data sets Limited ability to prevent procedural contamination, especially fibres. Limited sample range Weather dependant Towing speed and time must be limited; vessel speed may be restricted
Vertically towed nets (Bongo nets)	Deployment at variable depths possible. Not weather dependant Paired sampling allows replicate sampling	Risk of sample contamination when handled on deck Under samples plastics smaller than net size (< 300 µm) Vessel speed may be resituated
Bulk water sample	Known sample volume No size discrimination	Volume reduction can lead to procedural contamination. Not suitable for larger plastics
Pumps systems (e.g. Ferry-Box)	Large volume of water over a trajectory Repeated sampling trajectory possible Remote operation possible	Constrained in terms of restricted vessel path, but replicable. Sensitive to large amounts of biological material (algae bloom)
Submersible pumps	Accurate sampling volumes at different depts	Vessel must be stationary Intakes are small and limit the upper size range
Continuous Plankton Recorder	Large range and trajectory Combined sampling	Restricted intake Difficult to employ

3 Relevant data/selected available data

3.1 Ongoing monitoring programmes for microplastics

There are currently no structured and large-scale monitoring programmes for microplastics in surface and subsurface waters. Currently the Ministry of the Environment, Japan are compiling and updating guidelines to support G7 and G20 initiatives (Michida et al. 2019) and Regional Seas are composing guidelines on a regional level (e.g. Mediterranean Action Plan and Arctic Monitoring and Assessment Programme). Further, several research projects targeting specific oceanic regions using different methodological approaches have been performed. Awaiting European harmonisation and standardisation we have summarised peer-reviewed data on microplastics using surface sampling nets and pump / filter sampling.

3.2 Surface sampling nets

Several investigations have been conducted globally to assess the presence of microplastics in surface waters including two global studies (**Cozar et al. 2014, Eriksen et al. 2014**). All studies covering multiple sampling sites and at least a limited number of QA/QC measures are given in Table 3. Furthermore, these studies specifically reference the use of surface sampling nets for the development of monitoring programmes. For example, many studies have been carried out in the Mediterranean (reviewed in **Baini et al. 2018**) but few were directed towards monitoring. Investigations should consider that seasonality influences microplastic abundance (i.e. **Van der Hal et al. 2017, Bani et al. 2018**) as does locality and distance from urban locations (**Kwon et al. 2020**) or land. One of the best examples of long-term datasets, not collected for monitoring purposes, are those samples collected from the North Atlantic and the Pacific as part of SEA Semester (**Law et al. 2010, 2014**). These projects focused on visual identification of millimetre-sized plastics from 1972 to 2012. Surface sampling generally utilises larger mesh sizes, so there is an under-representation of smaller-sized microplastics.

3.3 Sample collection pump filter systems

Seawater intake systems have been used to investigate microplastics, although this method has been employed far less often than the net sampling methods. Use of seawater intake generally relies on research vessels which already monitor water characteristics, as has been employed in the Atlantic, Arctic, Antarctic and Pacific Oceans (Table 5). These studies can be compared because they use the same method of collection and data extrapolation. Studies utilising lower mesh sizes report the highest quantities of particles (**Desforges et al. 2014, Enders et al. 2015**).

Table 3. *Examples of studies with sampling nets, employed with the aim to kick-start monitoring programmes. Net types are reported with mesh sizes in parenthesis. N = number of samples, where depth is not at the ocean surface, the sampling depth is in parenthesis; MP ± SD per sample = Average microplastic concentrations with standard deviation where reported, units are displayed in parenthesis.*

Location	Approach	Net type	N	Processing steps	MP ± SD	Reference
Baltic Sea	Comparison of methods	Manta (330µm)	24	Visual sorting	0.04 (m^{-3})	Schonlau et al. 2020
Korea	8 different bays	Manta (330µm)	83	Digestion, visual sorting, FTIR	Urban: 2.85 (m^{-3}) Rural: 1.86 ((m^{-3}) *Range: 1.12-4.73*	Kwon et al. 2020
Tuscany, Mediterranean Sea	Seasonal sampling, repeated transects	Manta (330µm)	24 (surface)	Visual sorting, FTIR	0.26 ± 0.33 (m^{-3})	Baini et al. 2018
		Plankton net WP2 (200µm)	24 (100m)		0.16 ± 0.47 ((m^{-3})	
South China Sea	Comparison of methods	Bongo net (330µm)	19 (200m)	Digestion, visual sorting, FTIR	0.045 ± 0.093 (m^{-3})	Cai et al. 2018
South Funen Archipelago, Baltic Sea	Comparison of methods	Manta (330µm)	10	Digestion, Visual sorting	0.07 ±0.02 (m^{-3})	Tamminga et al. 2018
Stockholm Archipelago, Baltic Sea	Comparison of methods	Manta (330µm)	21	Digestion, Visual sorting, FTIR	0.19-7.73 (m^{-3})	Gewert et al. 2017
Gulf of Finland, Baltic Sea	Comparison of methods	Manta (330µm)	12	Visual sorting, combustion	0.3-2.1 (m^{-3})	Setala et al. 2016
Israel coast, Mediterranean Sea	17 sites, seasonal sampling	Manta (330µm)	108	Visual sorting	7.68 ±2.38 (m^{-3})	Van der Hal et al. 2017
Pacific Ocean	SEA Semester program 2001-2012	Plankton net (330µm)	2529	Visual sorting	33,090 (km^{-2})	Law et al. 2014
Global	Circumnavi. 2010-2011	Neuston net (200µm)	225	Visual sorting, Raman	0-2500 g ((m^{-3})	Cozar et al. 2014
Global	24 expeditions	Neuston net (330µm)	680	Visual sorting	1,000–100,000 (km^{-2})	Eriksen et al. 2014
North Atlantic and Caribbean Sea	SEA Semester program 1986-2008	Plankton net (330µm)	6136	Visual sorting	20,328 ± 2,324 (km^{-2})	Law et al., 2010

Table 4. *Examples of studies using sample collection pump filter systems. Lower size limits imposed by the sampling approach (mesh size) are presented mesh sizes in parenthesis. N= number of samples; MP ± SD per m3 = Average microplastic concentrations with standard deviation where reported, units are displayed in parenthesis.*

Location	Depth	Approach	N	Processing steps	MP ± SD	Reference
Norwegian Sea	6m	1000 L per sample, (80 µm)	7	Visual sorting, FTIR	2.4 ± 0.8 (m^{-3})	Morgana et al. 2018
Norwegian Sea	6m	2000 L per sample, sieve stack (250 µm)	75	Gravity separation, filtration, visual, FTIR	0.34 (m^{-3})	Lusher et al. 2015
North Atlantic	3m	2000 L per sample, sieve stack (250 µm)	470	Filtration, visual, FTIR	2.46 (m^{-3})	Lusher et al. 2014
North Atlantic	3m	Filter stack (10, 300 µm)	23	Visual, Raman	13–501 (m^{-3})	Enders et al. 2015
Atlantic Ocean	11m	2000 L per sample, sieve stack (250 µm)	76	Filtration, visual, FTIR	1.15 ± 1.45 (m^{-3})	Kanhai et al. 2017
North Pacific	4.5m	Sieve stack (62, 250 µm)	34	Acid digestion, visual sorting	2080 ± 2190 (m^{-3})	Desforges et al. 2014
South China Sea	0.5m	3000 L per sample, filter stack (44 µm)	22	Visual sorting, FTIR	2569 ± 1770 (m^{-3})	Cai et al. 2018
Ross Sea, Antarctica	5m	<2000 L per sample (1 µm)	15	Visual sorting, FTIR	0.17 ± 0.34 (m^{-3})	Cincinellu et al. 2017
Arctic Central Basin	8.5m	2000 L per sample, sieve stack (250 µm)	58	Visual sorting, FTIR	0 -7.5 (m^{-3})	Kanhai et al. 2018
Gulf of Finland, Baltic Sea		Submersible pump (330, 100µm)	12	Visual sorting, combustion	0-8.2 (m^{-3})	Setala et al. 2016
Baltic Sea		Submersible pump (50µm)	11	Visual sorting	0.10 (m^{-3})	Schonlau et al., 2020
Gullmar fjord, Sweden		Submersible pump (300µm)	6	Visual sorting, FTIR	0-0.4 (m^{-3})	Karlsson et al.
HAUSGARTEN, Arctic		Submersible pump attached to CTD (11µm)	18	Scanning FTIR	0-1287 (m^{-3})	Tekman et al. 2020
Baltic Sea		Self-priming pump, PLEX	19	Digestion, visual, FTIR	32.2 ± 50.4 (m^{-3})	Zobkov et al. 2019
Waters under ice flows in ACB		Manual surface pump (250µm)	22	Visual sorting, FTIR	0-18 (m^{-3})	Kanhai et al. 2020
East China Sea, coastal		Surface Teflon pump 20L 333 steel sieve	6	Digestion, visual, Raman	100-4100 (m^{-3})	Zhao et al. 2015
Yangtze Estuary		Surface Teflon pump 20L 333 steel sieve	7	Digestion, visual	4137 ± 2461 (m^{-3})	Zhao et al. 2014

4 Methods

This pilot study was performed using a newly designed FerryBox module for the sampling of microplastics. This module is based on the pump system used by **Schonlau et al. (2020)**. This system was the outcome of the EU project Clean Sea and the filter systems were made in close collaboration with KC Denmark, a partner in the project. This large volume microplastic filter system is easy to use, avoids contamination as much as possible and has the flexibility to use several filter setups in line using filters sizes from 100 µm to 500 µm. The most used set up contains a 300 µm and a 500 µm filter making data comparable with existing studies.

4.1 Sampling platform and study area

A FerryBox system has been installed on the M/S Color Fantasy since 2006. M/S Color Fantasy is a cruise ferry owned and operated by Color Line on the route between Oslo in Norway and Kiel (59.91°N-10.71°E to 54.33°N - 10.15°E, Figure 2). Sampling was carried out between September and December 2019 (Table 5). Two times a month samples were collected when entering Danish waters (latitude 57.066°N) for an 8-hour period until leaving the Danish EEZ (54.568°N) aiming at a sampling volume of around 5000 litres. An example of the read out of the exact position is given in Appendix A.

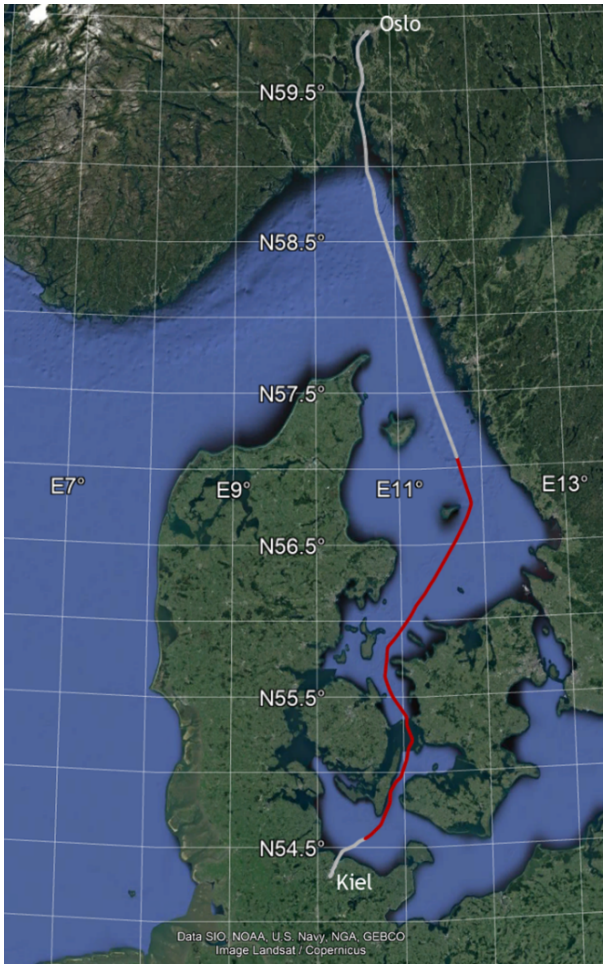


Figure 2. *Map of sampling trajectory in Danish marine waters. Red line indicates the Danish EEZ of the route by the Oslo-Kiel ferry 'Color Line'.*

Table 5. *Overview of samples collected between September and December 2019. (n.a. - not applicable samples could not be processed; OM- organic matter; KOH- potassium hydroxide).*

Sample ID	Date	Volume (litres)	Samples collected	Sample pretreatment	Laboratory analysis
DW-1	4-5 September	x[1]	*100, 300, 500 µm*	*Samples were not in a condition to process**	*n.a.*
DW-2	18 September	5338	100, 300, 500 µm	Clean filters, no sample pretreatment.	Visual Confirmation FTIR
DW-3	9 October	*4961*	100, 500 µm	Many shells present. Processed with KOH and acetic acid.	Visual Confirmation FTIR
DW-4	29 October	4873	100, 500 µm	Large amount of organic material. Repeated extraction with KOH	Visual Confirmation FTIR
DW-5	17 November	5076	100, 500 µm	Large amount of biological material, processed with KOH	Visual Confirmation FTIR
DW-6	22 November	4893	*100, 500 µm*	*Samples were not in a condition to process**	*n.a.*
DW-7	30 November	6796	100, 500 µm	Large amount of biological material, processed with KOH	Visual Confirmation FTIR
DW-8	12 December	6333	100, 500 µm	Large amount of biological material, processed with KOH	Visual Confirmation FTIR
DW-9	18 December	5143	100, 500 µm	Clean filters, rinsed directly onto GF/A filters for analysis	Visual Confirmation FTIR
DW-10	3-4 February	5051	100, 500 µm	100 µm processed with KOH, 500 µm rinsed directly onto GF/A filter	Visual Confirmation FTIR
DW-11	5-6 February	4950	100, 500 µm	100 µm processed with H2O2, 500 µm rinsed directly onto GF/A filter	Visual Confirmation FTIR
DW-12	18 February	5341 [2]	100, 500 µm	100 µm processed with KOH, 500 µm rinsed directly onto GF/A filter	Visual Confirmation FTIR
DW-13	26 February	5342 [2]	100, 500 µm	100 µm processed with KOH, 500 µm rinsed directly onto GF/A filter	Visual Confirmation FTIR

[1] *Flow stop, 100 µm blocked by biological material.* [2] *Estimated flow.* * *Large amount of biological material.*

4.2 FerryBox set up

The FerryBox system is set up to collect water from a seawater intake situated at 3 m depth on the starboard side of the M/S Color Line Fantasy. Although varying depending on the speed of the Fantasy, design calculations have shown that this represents mixed surface waters down to approximately 4m depth. The system is remotely operated to start sampling and to stop again at designated positions along the vessels transect. Water is passed over a series of metal sieves housed in a stainless-steel sieve holder (Figure 3). The NIVA three-stage microplastic sampling module connected to the FerryBox enables the sampling of relatively large volumes of sea water (5000-15000 L) thus improving the limit of detection (LOD numbers of microplastic particles/L). The system also accurately measures the volume of seawater improving the accuracy of the microplastic concentration (flow precision < 0.2%). The

system is incorporated as a module in NIVAs FerryBox systems and is designed with the option of running up to three different filter sizes simultaneously. The standard system is delivered with 500 µm, 300 µm and 50/100 µm filters. The combination of filters and stacked design is such that the user can change the filter sizes to other mesh sizes if required. For practical reasons two filters were used (500 µm and 100 µm) for most of the sampling with exception of the first two samples. Once sampling is complete and the vessel returned to dock the filters are removed and placed separately in aluminum boxes. There was no contact of mesh surface with box cover.

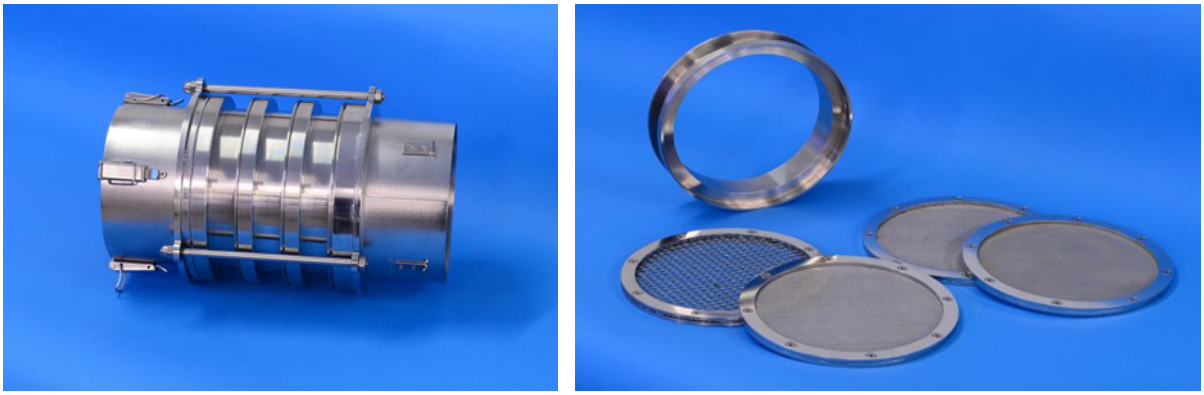


Figure 3. *FerryBox microplastic filter holder (left), with the option for three mesh size filters, 100 µm, 300 µm and 500 µm (right) (Clean Sea/KCDenmark).*

4.3 Sample analysis

Together with the microplastic collected on the mesh filters small particulate matter (SPM) and biota (mostly algae) could be present. Each filter is therefore processed as soon as possible after sampling at NIVA's laboratory in sterile conditions to minimize risk of contamination and to avoid SPM sticking to the mesh filter. Several different approaches can be used, depending on the content of SPM and biological material.

4.3.1 Basic filtering

When samples contained low levels of SPM and organic matter, they were directly rinsed from mesh filters using prefiltered water and filtered through GF/A filters or stainless-steel mesh filters (pore size <100 µm), Ø 47 mm. These filters were prechecked for microplastic content before the filtration. For samples with higher organic matter content, filtration was performed using stainless steel mesh filters before further processing. In most filter cases, 3 – 6 GF/A or stainless-steel filters were used to rinse all the material from the mesh filters, depending on volume of collected SPM. The filter with material was immediately transferred to a petri dish and covered prior to drying and analysis. Filtration was carried out in sterile conditions (laminar flow with HEPA filter).

4.3.2 KOH treatment

A digestion step using of 10% KOH followed by filtration was used to extract microplastics from samples which contained large amounts of organic matter:

- In the case of paper filters (GF/A), SPM from the GF/A was rinsed into a conical flask using 10% KOH and covered with aluminum foil. Conical flasks were put in an incubator for 24 hours at 40 degrees with 125 rpm. Once the sample was dissolved it was filtered through GF/A filters and immediately transferred to a petri dish and covered prior to drying and analysis. This processed doubled the number of filters requiring analysis.

- In the case where stainless-steel mesh filters (pore size <100 µm, Ø 47 mm) were used for first filtration step, these filters were placed directly in flasks with 10% KOH to improve the quality of analysis.

4.3.3 Acetic acid treatment

The acetic acid treatment was carried out for DW-3 from October 2019 and applied after the KOH treatment. 10% KOH was neutralized by the same volume of 10% acetic acid followed by adding of double amount of 5% acetic acid. Then the flasks containing this mixture were kept at 40 degrees and 125 rpm for 25 hrs. Once the sample was dissolved it was filtered through GF/A filters and was immediately transferred to a petri dish and covered prior to drying and analysis.

4.3.4 Visual identification

After preparation, all samples were analyzed by visual identification followed by chemical confirmation of the polymer material. Visual analysis followed standard NIVA protocols where potential plastics were isolated, photographed, described in terms of shape and colour, and measured along the longest and shorted length (mm). This was carried out using a stereomicroscope with an Infinity 1-3C mounted camera and INFINITY ANALYZE and CAPTURE software. All particles found were marked on the filter paper for easy identification prior to chemical characterization.

4.3.5 Chemical analysis

Visual identification of microplastics, especially in the smaller size range (> 300 µm) was performed using single-point FT-IR. NIVA conducted ATR-FT-IR on all extracted particles. This exceeds the recommendation for reporting under European Union's Marine Strategy Framework Directive (MSFD) where it is recommended that a proportion (5–10%) of all samples should be routinely checked to confirm the accuracy of visual examination (**Gago et al. 2016**). All FT-IR results, regardless of measurement techniques, were compared to an extensive library of polymers to identify the polymer type of each particle.

4.4 Contamination controls

The research team identified possible sources of procedural contamination prior to the start of the project and procedural blanks were taken throughout the project both in the field and in the laboratory. To avoid contamination at all stages of the project, thus ensuring comparable results the following steps were taken to avoid contamination.

- Field blank samples were performed on the vessel alongside sample collection.
- Procedural blanks were included in each batch under treatment to test for laboratory contamination.
- All equipment was cleaned with prefiltered water and the use of plastic laboratory equipment was kept to a minimum.
- Filtration and processing were performed in laminar flow cabinet.
- In addition, all personnel wore cotton clothing and rinsed all equipment between samples.

Any microplastic particles detected in the ship, procedural and laboratory controls were characterized and mostly contained cellulose fibres. On one occasion a single polyester fibre was found in the ship blank. As the levels of microplastics in all samples were relatively small, this could indicate that the amount reported in the sample (for polyester only) were very close to the LoD of the method based on this blank sample. Ship and laboratory background varied during the sampling period especially for the natural cellulose fibres.

5 Results

5.1 Sampling

Thirteen samples were collected between 4th of September 2019 and the 28th of February 2020. The samples varied largely in terms of the amount of biological material present on the filters. Samples were thus treated differently according to the amount of interfering material. We experienced problems with two of the samples due to sample storage and transport with sample DW 1 and extremely large amounts of organic matter for sample DW 6. These two samples were not processed further after initial sample preparation. For sample DW 2 three filters were used (100 µm, 300 µm, 500 µm) for the subsequent samples only the 100 µm and 500 µm filters were used. Figure 4 and 5 show some of the differences in sample composition throughout the sampling campaign. This complicated the analysis of samples and in some cases multiple processing steps were necessary, as indicated in Table 6.

5.2 Visual analysis

The results of the visual analysis are given in Table 6. Here the total number of particles collected on the two filters 100 µm and 500 µm are reported. The results are divided into fragment and fibres. Beads, which are also often reported, were not found, except for one bead-like structure which was of organic origin. Most of the particles found were classified as fibres (range 8 – 53 per sample) and a smaller number of fragments were found (range 0 – 6 per sample).

Table 6. *Summary of the total number of particles collected on individual filters per sampling trajectory uncorrected for uFTR analysis. n.a. samples were not processed due to complications.*

	100 µm			500 µm			**Total 100 - 2000 µm**	
Sample ID	Fibres	Fragments	Total	Fibres	Fragments	Total	**Fibres**	**Fragments**
DW1	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	**n.a.**	**n.a.**
DW2	43	2	45	10	0	10	**53**	**2**
DW3	14	2	16	9	2	11	**23**	**4**
DW4	17	6	23	15	0	15	**32**	**6**
DW5	4	0	4	16	0	16	**20**	**0**
DW6	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	**n.a.**	**n.a.**
DW7	27	0	27	26	0	26	**53**	**0**
DW8	9	1	10	9	0	9	**18**	**1**
DW9	6	0	6	6	0	6	**12**	**0**
DW10	0	0	0	8	0	8	**8**	**0**
DW11	10	3	13	4	1	5	**14**	**4**
DW12	5	2	7	5	1	6	**10**	**3**
DW13	5	1	6	9	2	11	**14**	**3**

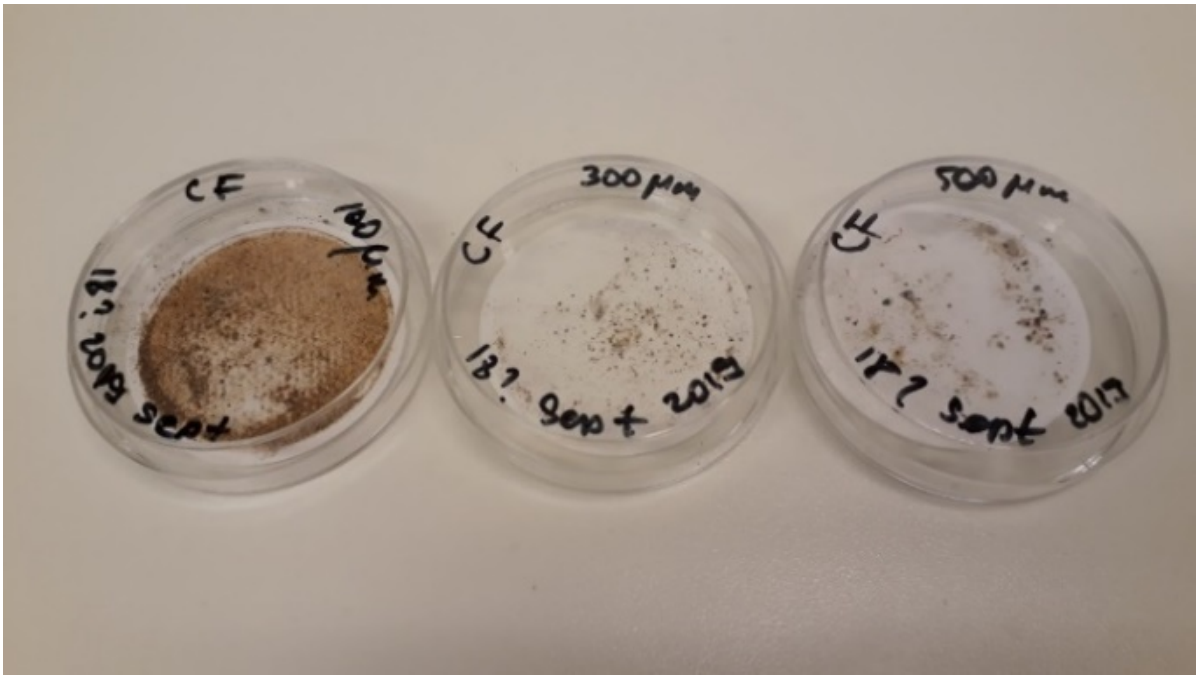


Figure 4. *Example of a relatively clean sample collected on GfA fiters from the 100 µm, 300 µm, 500 µm filters requiring no further pre-processing.*

Panel A **Panel B**

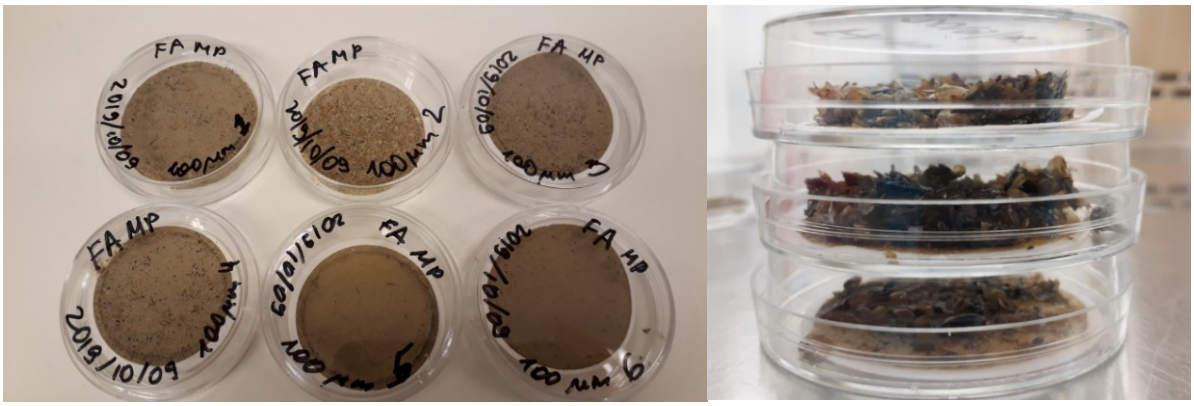


Figure 5. *Example of a sample containing large quantities of bivalves. Panel A: before sample processing; Panel B: following pre-treatment with acetic acid and filtration.*

A typical visual analysis is based on expert knowledge to determine if particles are of natural or synthetic origin. The determination of the origin of fibres is especially difficult because they can consist of synthetic fibres (polypropylene, polyamide, elastin or lycra), 'semi' synthetic fibres (rayon or viscose) from regenerated cellulose fibres or natural materials (cellulose and wool).

We have chosen to differentiate between the two classes of fibers where cellulose, wool and rayon fibres were classified as fibres of natural origin. The results of the visual analysis in Table 6 and displayed in Figure 6 are thus the sum of all fibres both natural and synthetic and subsequent µFTIR analysis is further used to distinguish these two categories.

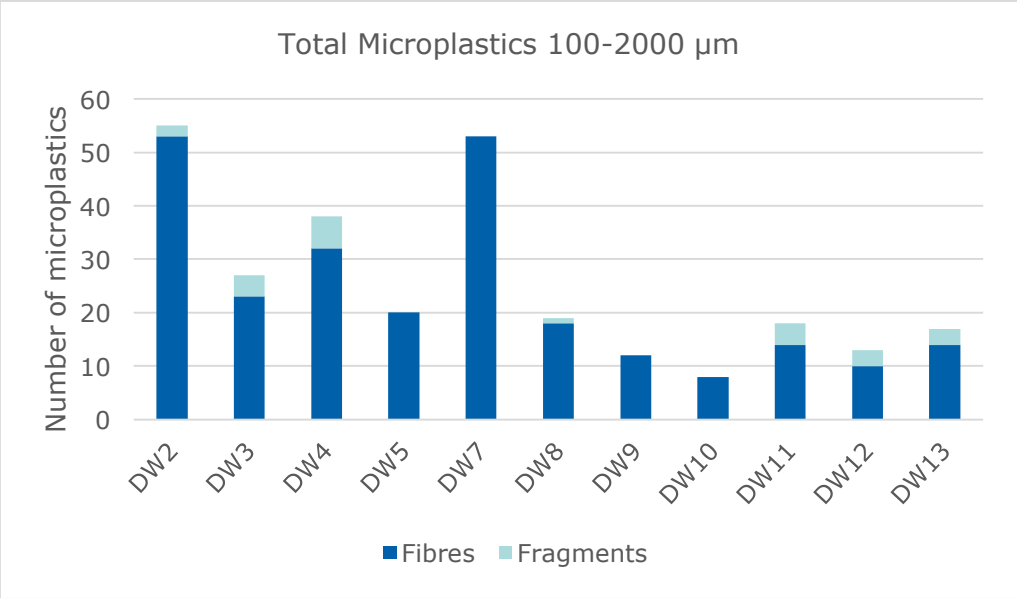


Figure 6. *Distribution of number of microplastics between fibres and fragments from sampling 100-2000 µm in a trajectory in Danish waters in the Skagerrak and Kattegat from September 2019 to February 2020.*

5.3 FT-IR confirmation

FT-IR confirmation analysis was performed on the fibres and fragments identified by the visual analysis, matching the FT-IR spectra against a large database. From 25% of fibres, the spectra produced were not of high enough quality to determining the chemical composition. This was due to the relatively small diameter of these fibres or because they were of biological origin. Examples of the identification are illustrated in Figure 7.

In Table 7 the identified polymers are given including polyethylene (PE), polypropylene (PP), polyester (PS), polyamide/nylon, acrylic fibres or synthetic rubber. The large majority of the fibres from the visual analysis were cellulose based, semi-synthetic or of biological origin (chitin). Of the total of 280 fragments (23) and fibres (257) found by visual analysis only 41 were confirmed as synthetic polymers or fibres. It is thus notable that only 20% of the total fibres and fragments were finally confirmed as synthetic.

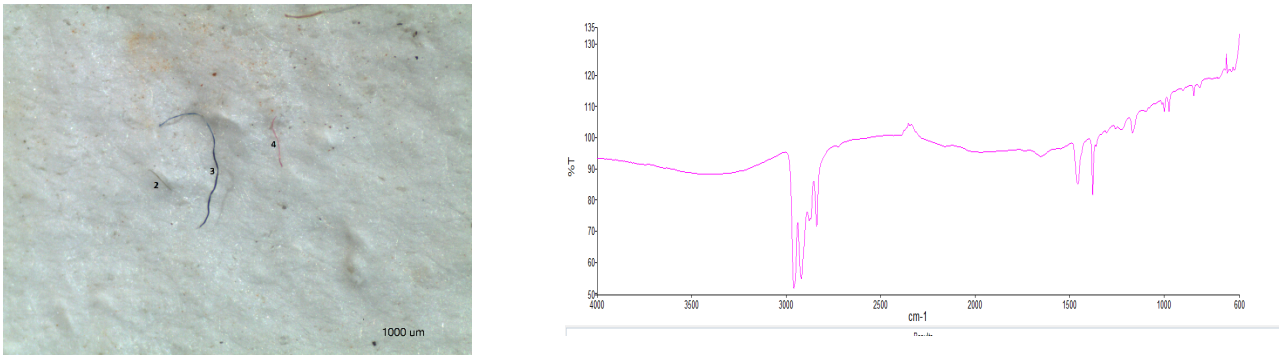
The polymer distribution in Figure 8 shows polyester and polypropylene fibres were present in most of the samples. Interestingly, rubber fragments were also found in all samples. No polyethene was found. This is surprising because it is one of the most widely used plastics and has a density (0.88–0.96 g/cm^3) lower than sea water (1.02- 1.03 g/cm^3) so would be expected to float and thus be present in especially beach samples and the marine environment.

5.4 Normalisation to sample volume

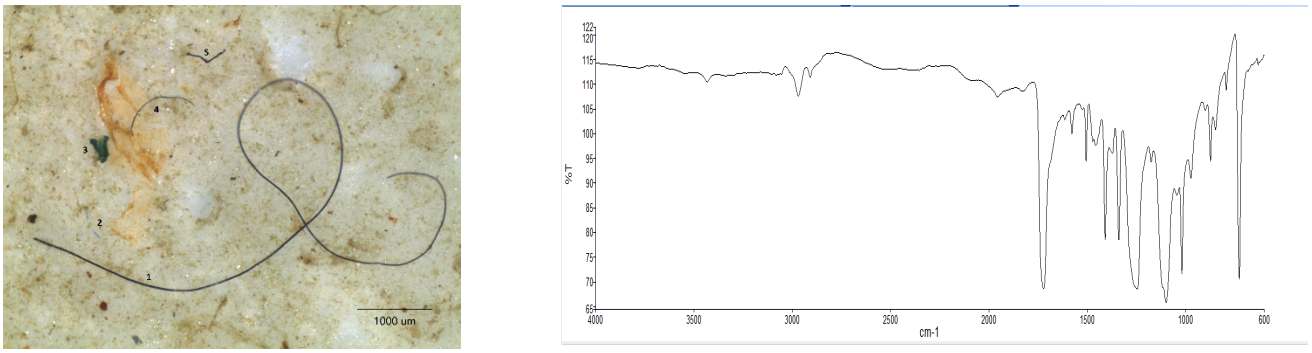
The number of fragments and fibres confirmed by FT-IR are given in Table 8, in addition to the number of microplastics normalised to the sampling volume. The true sampling volume of the last two samples DW 12 and DW 13 were not recorded due to data transfer problems while the Color Line Fantasy

operations were temporary discontinued. They were therefore estimated based on the average volume of the previous sampling volumes.

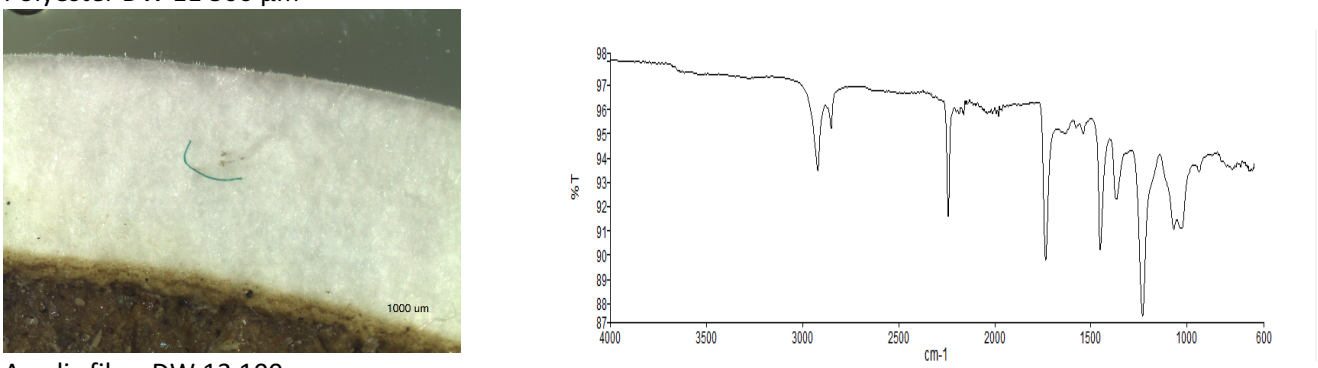
In total, 22 fibres and 19 fragments were collected during the sampling of the same route, starting and finishing the sampling at exactly the same location entering or leaving Danish waters. The volume normalised number of microplastics varied from 0.00 to 1.85 per m^3. The average concentration was 0.71 particles per m^3. In total more than 64,000 litres were sampled during the sampling campaign. In addition to the 41 microplastics, 239 other particles were analysed, most of these particles were fibres from natural (wool, cellulose) or semi-synthetic origin (viscose, 21 fibres).



Polypropylene DW2 500 µm



Polyester DW 11 500 µm



Acrylic fiber DW 13 100 µm

Figure 7. *Example of the FTIR spectra matching the different polymers and synthetic fibers. A polypropylene fiber from the 500 µm filter from sample DW 2, B polyester fiber from the 500 µm filter from sample DW 11 and C acrylic fiber from the 100 µm filter from sample DW 13.*

Table 7. *Confirmation analysis by FT-IR of the identified fibres and fragment from the visual analysis from the September 2019 to February 2020 sampling campaign. Polyethylene (PE), polypropylene (PP), polystyrene (PS).*

Sample	Mesh	*PE*	*PP*	*Polyester*	*Polyamide*	*Acrylic*	*PS*	*Other plastic*	*Rubber*	*Total plastic/rubber*
DW1	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	**n.a.**
DW2	100 µm	0	1	0	0	0	0	0	0	**1**
DW2	300 µm	0	2	0	0	0	0	1	0	**3**
DW2	500 µm	0	1	0	0	0	0	0	0	**1**
DW3	100 µm	0	1	1	0	0	0	0	1	**3**
DW3	500 µm	0	0	1	0	0	0	3	0	**4**
DW4	100 µm	0	4	1	0	0	0	0	1	**6**
DW4	500 µm	0	0	1	0	2	0	0	0	**3**
DW5	100 µm	0	0	0	0	0	0	0	0	**0**
DW5	500 µm	0	0	2	0	0	0	1	0	**3**
DW6	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	n.a	**n.a**
DW7	100 µm	0	0	1	1	0	0	0	0	**2**
DW7	500 µm	0	0	2	0	0	0	0	0	**2**
DW8	100 µm	0	0	0	0	0	0	0	1	**1**
DW8	500 µm	0	0	1	0	0	0	0	0	**1**
DW9	100 µm	0	0	0	0	0	0	0	0	**0**
DW9	500 µm	0	0	0	0	0	0	0	0	**0**
DW10	100 µm	0	0	0	0	0	0	0	0	**0**
DW10	500 µm	0	0	0	0	0	0	0	0	**0**
DW11	100 µm	0	0	2	0	0	1	0	0	**3**
DW11	500 µm	0	0	2	0	0	0	0	0	**2**
DW12	100 µm	0	0	0	0	0	1	0	0	**1**
DW12	500 µm	0	0	0	0	0	0	0	0	**0**
DW13	100 µm	0	1	1	0	1	0	0	0	**3**
DW13	500 µm	0	1	1	0	0	0	0	0	**2**
	Total	**0**	**11**	**16**	**1**	**3**	**2**	**5**	**3**	**41**

n.a. Not applicable samples could not be processed.

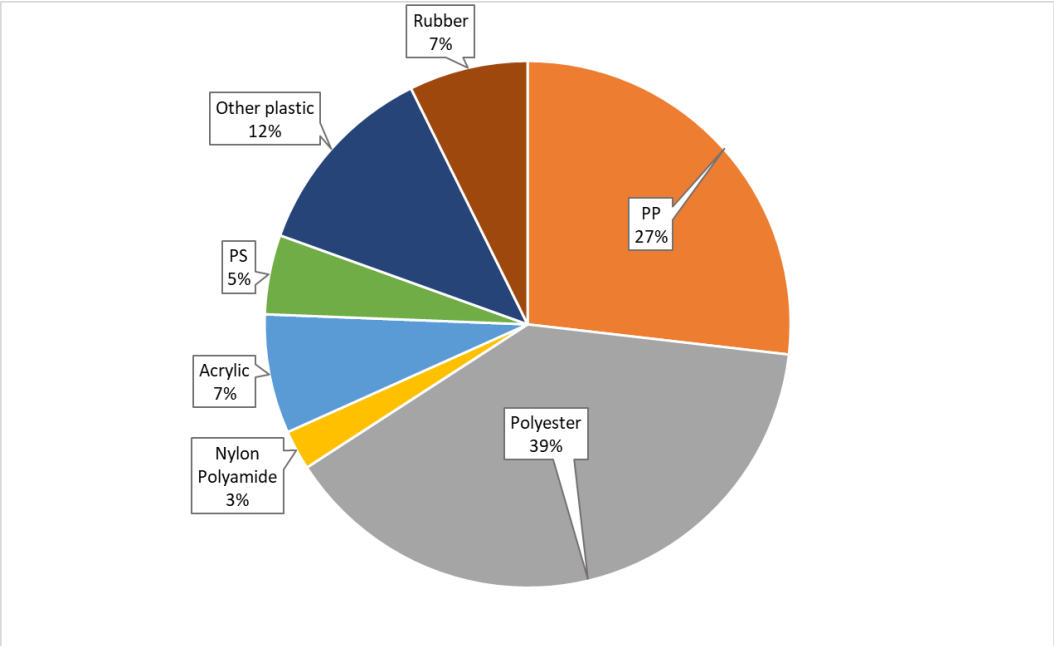


Figure 8. *Polymer distribution of synthetic polymers confirmed by µFTIR analysis of the complete data set of sampling of 11 trajectories corresponding of a total sampling volume of more than 64 000 liter. PP = Polypropylene, PS = Polystyrene.*

Table 8. *Total number of fibres and fragments (100 µm – 2000 µm) confirmed by µFTIR (n = number of particles), and the total number of fibres and fragments normalised to sampling volume (n /m^3 = number of particles per m^3).*

	Fibres	Fragments	Total	Volume	Fibres	Fragments	Total
	n	n	n	m^3	n/m^3	n/m^3	n/m^3
DW 1	n.a.	n.a	n.a	n.a	n.a.	n.a	n.a
DW 2	3	2	5	5.34	0.56	0.37	0.94
DW 3	3	4	7	4.96	0.60	0.81	1.41
DW 4	4	5	9	4.87	0.82	1.03	1.85
DW 5	3	0	3	5.08	0.59	0.0	0.59
DW 6	na	na	n.a.	4.89	na	na	n.a.
DW 7	4	0	4	6.80	0.59	0.0	0.59
DW 8	1	1	2	6.33	0.16	0.16	0.32
DW 9	0	0	0	5.14	0.0	0.0	0.0
DW 10	0	0	0	5.05	0.0	0.0	0.0
DW 11	1	4	5	4.95	0.20	0.81	1.01
DW 12	0	1	1	5.34*	0.0	0.19	0.19
DW 13	3	2	5	5.34*	0.56	0.37	0.94
Total	22	19	41	64.10			

* *Estimated sampling volume*

6 Discussion and conclusions

6.1 Discussion

The samples contained relatively small numbers of microplastics, ranging from 0 to 1.85 microplastics per m^3 (average 0.71 particles per m^3) compared to the large amount of natural fibres and semi-synthetic fibres (estimated average 4 'natural' fibres per m^3). This is in accordance with previously research sampling with similar methods and depths within the water column. **Moragana et al. (2018)** reported 2.5 per m^3 in the Norwegian Sea while **Lusher et al. (2014, 2015)** reported levels of 2.68 per m^3 in the Norwegian Sea and 2.46 per m^3 in the North Atlantic. Also, **Kahnai et al. (2017, 2018)** reported similar levels for the North Atlantic (1.15 per m^3) and the Artic Central Basin (0-7.5 m^{-3}).

Using a filter set-up similar to the Color Fantasy FerryBox system, Schonlau **et al.** (**2020)** used a submersible pump to sample the Baltic sea using a 50 µm filter instead of the 100 µm filter and reported 0-10 microplastics per m^3. Also, **Setala et al. (2016)** reported similar levels in for the Baltic sea and the Gulf of Finland (0-8.2 per m^3). **Karlsson et al. (2020)** recently published data from the same area (Gullmar Fjord) and reported 0- 0.4 particles per m^3.

Figure 9 shows the temporal distribution of the samples in the period from September 2019 to February 2020. No clear trend could be established from the limited sampling points, the temporal resolution is probably too small. However further evaluation of the meta data from the FerryBox including weather conditions and biological growth has not yet been performed.

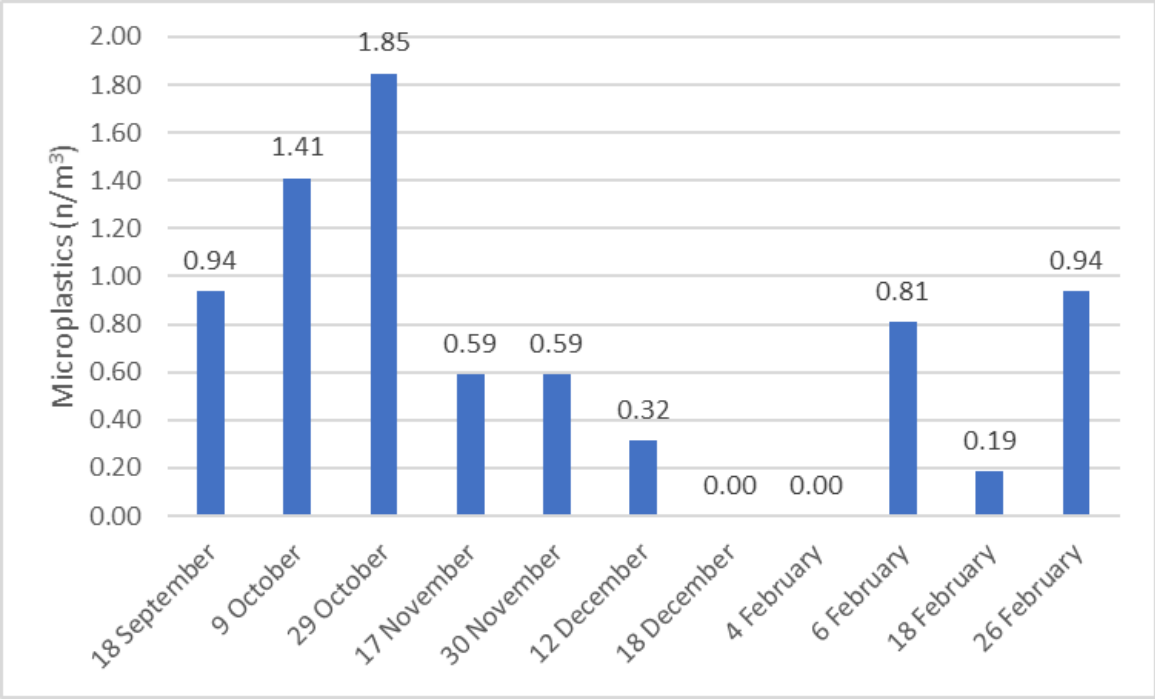


Figure 9. *Temporal variation of the total amount of microplastic in the Danish Skagerrak and Kattegat from September 2019 to February 2020. Microplastics in number of particles per m^3.*

6.2 Conclusion and recommendations

Based on the two tiers of this pilot project, i.e. 1) the mini review focusing on methods and 2) the test sampling in the Inner Danish waters, we conclude the following:

- Sampling of microplastic using existing marine monitoring infrastructure on so called 'ships of opportunity' is shown to be a good method for acquiring multiple samples.
- The samples varied considerably at different periods with regard to the amount of biological material they contained and therefore they required different pre-treatment steps before analysis
- The amounts of microplastic in Danish waters from Skagerrak and Kattegat were relatively small and varied from 0 to 1.85 particles per m^3. This agrees with several other studies in the same region.
- In addition to microplastics, large amounts of natural fibres were found, mainly consisting of cellulose and wool-based fibres which made up more than 75% of all particles and fragments collected.
- Although a significant number of large volume (average 5340 L) samples were taken over a long route, no temporal or spatial trends were found.

Further, we recommend the following:

- In order to study the temporal trends in the area it is recommended to increase the temporal resolution to every other day for a one-month period to establish a baseline.
- After this intensive sampling period, samples can be taken less frequently.
- The meta-data from the FerryBox including weather and hydrodynamic data was not evaluated in relation to the varying microplastic concentrations (0- 1.85 particles per m^3). Further exploration of this data might explain these concentrations.
- Limited attention has been focused on natural fibres from anthropogenic sources including wool and cellulose-based fibres. Their occurrence in relation to synthetic fibres should be further investigated.

Microplastic particles are an emerging threat in the marine environment, nationally, regionally and globally. More knowledge is required not only to monitor and assess the levels and trends of microplastic in marine systems, but also to implement appropriate actions to reduce inputs and mitigate negative effects. In a Danish context, the EU will be an important driver, especially when targeted monitoring of microplastic is implemented on a national scale. We believe our recommendation above will provide added value to the existing NOVANA monitoring program and will also enable future trend assessments relevant for the Inner Danish Waters.

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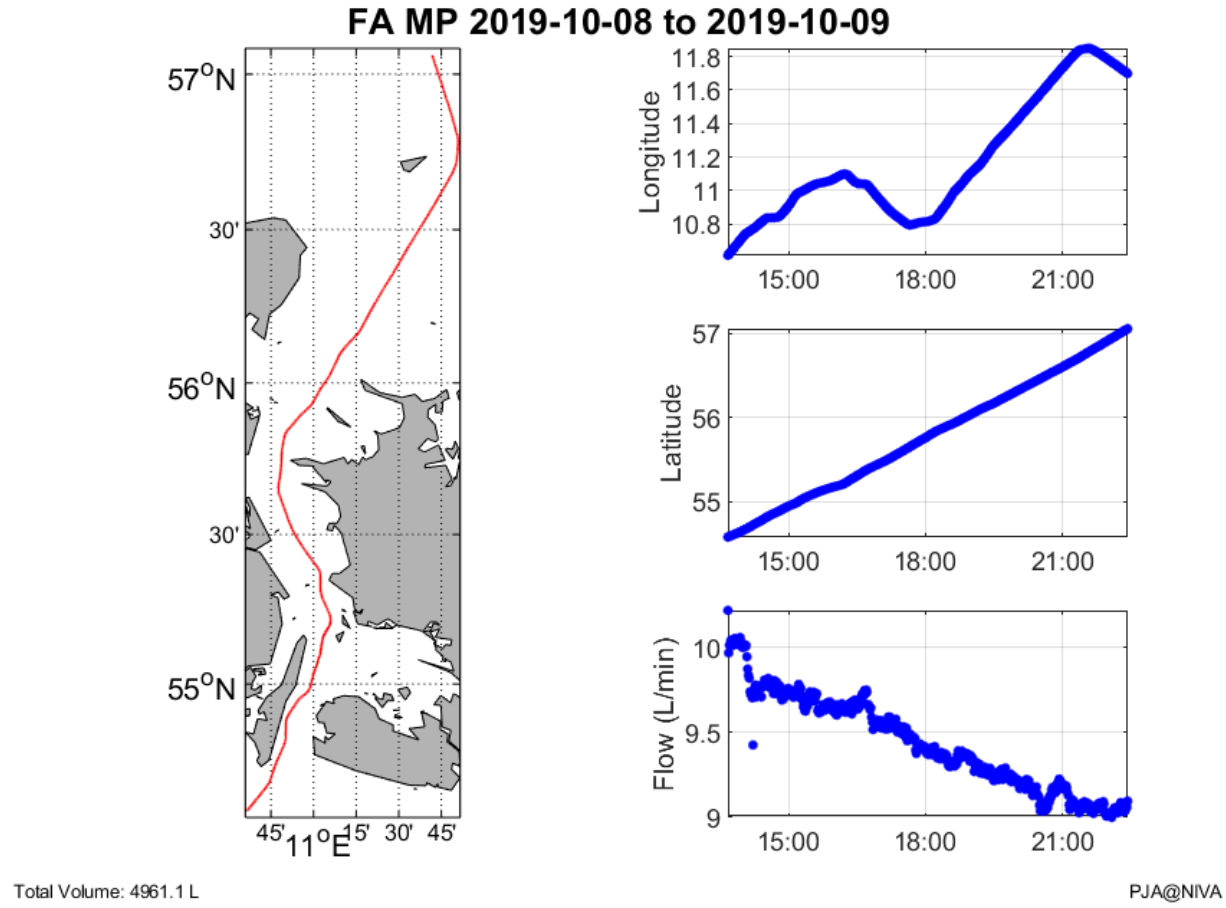
Appendix A.



Data extract from FerryBox system for sample DW-3 collected 8-9th October 2019.

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