NIVA Denmark

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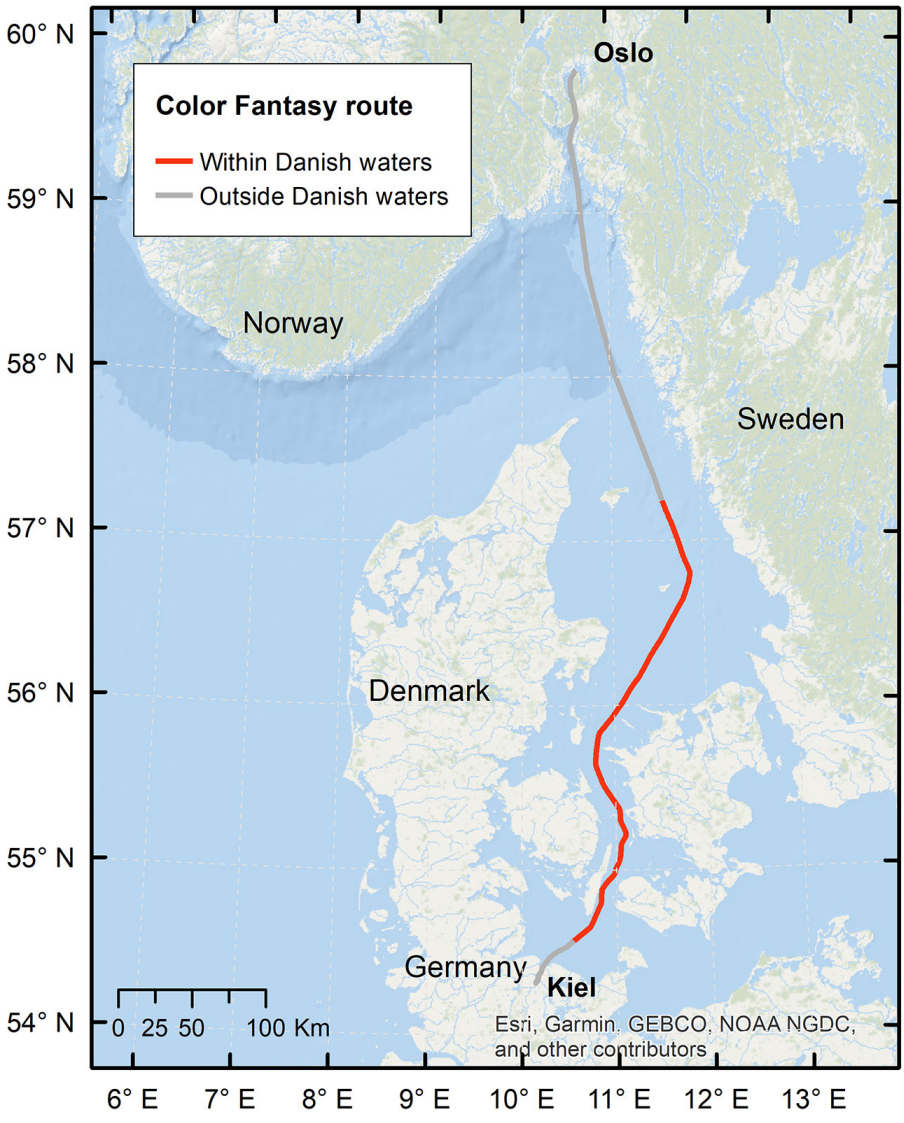
Frequent sampling of microplastic particles in surface waters in the open parts of the Kattegat and Great Belt, Denmark



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REPORT

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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 second study sampling microplastics in the Inner Danish Water using the Ferrybox system onboard the 'M/S Color Line Fantasy' ferry between Oslo and Kiel. This study aimed to supplement and extend a pilot project following recommendations to increase sample frequency and sample volume.

Four keywords	Fire emneord
1. Microplastic	1. Mikroplast
2. Monitoring	2. Overvågning
3. Marine Strategy Framework Directive (MSFD)	3. Havstrategidirektivet (HSD)
4. Inner Danish Waters (IDW)	4. Indre danske farvande (IDF)

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Ferrybox microplastic project, phase 2

Frequent sampling of microplastics in surface waters in the open parts of the Kattegat and Great Belt, Denmark

Client: Danish Environmental Protection Agency

Preface

NIVA has, on behalf of the Danish Environmental Protection Agency (Miljøstyrelsen), carried out a study of microplastics in the surface waters of the Kattegat and Great Belt, Denmark.

Water samples were collected by a microplastics sampling module connected to a Ferrybox on the "M/S Color Fantasy", a ferry operating between Oslo, Norway and Kiel, Germany while passing through the Inner Danish Waters.

Samples were collected by Louise Valestrand, Pierre F. Jaccard and Bert van Bavel. Cecilie Singdahl-Larsen performed the laboratory analyses, including sample processing, visual analysis and chemical analysis using FTIR. Amy Lusher and Cecilie Singdahl-Larsen were responsible for writing the report with input from the team. The report was controlled and edited by Bert van Bavel, Therese Harvey and Jesper H. Andersen (NIVA).

Copenhagen, 12 March 2021

Jesper H. Andersen
Chief scientist

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Summary

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 microplastic 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 (Ferrybox) 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. It is currently being tested and optimised for suitability in terms of sample volume and replicates.

In total seven samples were collected using the Ferrybox microplastics sampling module fitted with two filters with the mesh sizes of 300 and 500 µm. All samples collected could be processed with simplified methods only requiring filtering as the level of biological matter was limited during the autumn sampling period.

Relatively small amounts of microplastics were found in the large volume samples (average 5544 L) ranging from 0.39 to 1.85 particles per m^3 (average 0.91 per m^3). A total of 35 microplastics were included in the analysis, the majority of these were fibres (99 %). A substantial proportion of the microplastics were reported as viscose (46 %), polyester was the second most abundant polymer (27 %), followed by acrylic (8 %) and polypropylene (8 %) polymers.

The levels agree with other studies in the same region, including the former Ferrybox report. The temporal resolution consisted of daily sampling during the last week of October and resulted in a similar range and average as the sampling performed under a longer period (6 months) but less frequent (13 samples, van Bavel *et al.* 2020). Both pilot studies show a general background level of microplastics between 0- 1.85 microplastics per m^3, with most of the particles being classified as fibres. Interference of biological material is limited under certain periods (late autumn) to facilitate larger sample volumes and smaller filter mesh sizes. This will improve detection limits, the influence of blank samples and understanding of even smaller size microplastics (<100 µm) and optimise this method for repeated sampling of microplastics in the aquatic environment.

Sammenfatning

Titel: Hyppig prøvetagning af mikroplastpartikler i overfladevand i de åbne dele af Kattegat og Storebælt, Danmark
År: 2020
Forfattere: Bert van Bavel, Amy Lusher, Pierre Franqois Jaccard, Cecilie Singdahl-Larsen, Louise Valestrand, E. Therese Harvey & Jesper H. Andersen
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Vi rapporterer resultaterne af et pilot-projekt, der har fokuseret på intensive målinger af mikroplastik i overfladevandet i de indre danske farvande i oktober-november 2020. Formålet har dels været at følge op på et tidligere gennemført pilotprojekt med lavfrekevnt indsamling af prøver (månedlig), dels at tilvejebringe et bedre fagligt grundlagt for fastlæggelse af den fremtidige prøvetagningsfrekvens for den overvågning som skal finde sted i de åbne farvande i henhold til Havstrategidirektivet.

Indsamlingen af vandprøver er foretagtet med en såkaldt Ferrybox monteret på Oslo-Kiel-færgen ("M/S Color Fantasy"). De indsamlede prøver er oparbejdet på NIVA's laboratorier i Oslo. Resultaterne af de gennemførte analyser er følgende:

Der blev indsamlet i alt syv filterprøver fra Ferrybox'en, der var monteret med to filtre til indsamling af mikroplast: hhv. 300 and 500 µm. Alle prøver blev oparbejdet med simpel filtrering i det indholdet af organisk stof var lavt igennem hele indsamlingsperioden.

Relativt begrænsede mængder af mikroplast blev fundet på trods af at store mængder af vand blev filtreret (i gennemsnit 5544 L med koncentrationer) ranging fra 0,39 til 1,85 partikler per m^3, i snit 0,91 per m^3). I alt 35 mikroplastpartikler blev inkulderet i analyserne og hovedparten af disse var fibre (99 %). Den dominerede andel af mikroplasten var viskose (46 %), polyester var den næst mest forekommende polymer (27 %) efterfulgt af akryl (8 %) og polypropylen (8 %) polymerer.

De fundne niveauer stemmer overens med andre undersøgelser i samme region, herunder det for Miljøstyrelsen tidligere gennemførte pilotprojekt. Den tidslige opløsning bestod af daglige indsamlinger og gav stort set samme gennemsnit som i det første pilotprojekt, der fandt sted over 6 måneder men med en lavere indsamlingsfrekvens (13 prøver, van Bavel *et al.* 2020).

Begge pilotprojekter angiver et generelt baggrundsniveau for mikroplastpartikler på mellem 0 til 1.85 partikler per m^3, hvor hovedparten af partikler klassificeres som fibre. Interferens med biologisk materialer er begrænset i visse perioder (det sene efterår), hvilket muligør indsamling af store mængder af vand og også anvendelse af en mindre filterstørrelse. Dette vil forbedre detektionsgrænsen, betydningen af blanke vandprøver og forståelsen for betydningen af mindre størrelser af mikroplast (<100 µm) og således kunne optimere metoderne for gentagen prøvetagning i marine områder som de indre danske farvande.

Hvad angår en fremtidig overvågningsstrategi for plastpartikler i vandfasen, er der med de foreliggende resultater desværre endnu ikke det fornødne faglige grundlag for fastlæggelse af en frekvens for indsamling af vandprøver. Prøvetagningsfrekvensen bør indtil et sådant grundlag foreligger blive midlertidigt fastlagt eventuelt under hensyntagen til de økonomiske rammer.

1 Introduction

Long-term spatial and temporal sampling of microplastics is rare and often samples are taken occasionally without any consideration of time and place. Where continuous, or long-term sampling does exist, the information available is often incomparable between research teams as the methods applied are inconsistent. Furthermore, much of the long-term data sets collected using manta nets only focus on particles >300 µm (e.g. Cózar et al. 2017, Eriksen et al. 2014, Law et al. 2010, 2014, Suaria et al. 2020, Wilcox et al. 2020) thus, neglecting the smaller size, yet more abundant fraction of microplastics (e.g. Setälä et al. 2016, Rist et al. 2020, Ryan et al. 2020). The manta net has been identified as the best currently available method for sampling microplastics in surface waters (GESAMP et al. 2019, Michida et al. 2020). It has several advantages for use in coastal areas with high levels of contamination. However, particles <300 µm are underrepresented, as those particles are not floating in the surface water and fibres are often excluded because samples risk to be contaminated by airborne fibres during sampling including those generated from clothing during collection on deck. This is a major drawback as fibres are the most commonly identified particle in the marine environment (e.g. Gago et al. 2018). Another disadvantage of the use of manta trawl nets is that sampling volumes are difficult measure and often only rough estimates. This makes understanding the behaviour, distribution and source identification of microplastics nearly impossible. Therefore, other methods are explored to acquire more solid data for modelling purposes and risk assessment, which include uncertainty. Underway sampling using 'ships of opportunity' is a valuable resource for oceanographic data collection, and has been posited as an viable option for microplastic research (e.g. Cincinelli et al. 2017, Lusher et al. 2014, Kanhai et al. 2017, Jiang et al. 2020), but it is yet to be adopted and integrated for operational microplastic monitoring purposes. Pump methods have been developed for integration with ocean observing systems but still require to be tested and validated locally in terms of sample volumes, mesh seize, and collection frequency.

NIVA is operating a Ferrybox system on 'M/S Color Fantasy', a ferry travelling between Kiel in Germany to Oslo in Norway. This oceanographic sampling and measuring system have recently, in 2018, been equipped with a microplastic sampling module to enable long-term spatial and temporal sampling and analysis of microplastics. When fully operational, other oceanographic metadata during the sampling of the trajectories will be available from the Ferrybox system for further interpretation of trends in microplastic levels.

With the support of the Danish EPA, a test project was completed in Danish waters between September 2019 and February 2020 taking bi-monthly samples (van Bavel et al. 2020). The project showed that the microplastic concentration ranged from zero to 1.85 microplastics per m^3, over relative long periods between sampling occasions and different weather conditions. Although a large amount of hydrodynamics and other meta data was measured simultaneously in the test project, only a very small part of the meta data was used for evaluating different conditions during the time of sampling. It is hypothesized that weather and hydrodynamic conditions can play a major role in governing microplastics concentrations. In order to further study trends in microplastic levels in Danish waters NIVA recommended increasing the temporal resolution to ten samples over a two-week period to further establish baseline levels. Combining short-term variance (every other day) with the already established long term variance will result in a more reliable base line range of the levels of microplastic. Since the levels of microplastics and fibres during the test project were relatively low, the possibility of sampling larger volumes was investigated under a period with low biological activity. Hence, the present project aims to supplement and extend the foregoing sampling by following the recommendations to increase sample frequency and sample volume.

1.1 Ferrybox setup

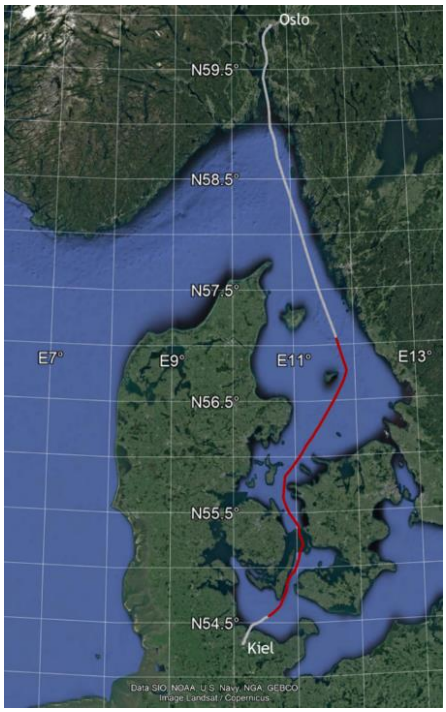
NIVA has developed a three-stage sampling tool which enables the sampling of relatively high numbers of microplastic particles (improving the limit of detection, LOD) and accurately measures of the volume of seawater (improving the accuracy of concentration reporting). The precision of the flow measurements is less than 0.2%. The system is incorporated as a module in NIVA's 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 100/50 µm filters. The design is such that it is possible to choose which combination of filters that are used, and to add other mesh sizes if required.

The Ferrybox system is set up to collect water from a seawater intake situated at 5 m depth on the starboard side of 'M/S Color Fantasy'. The system is remotely operated to start sampling and to stop again at designated positions along the vessels transect. The NIVA microplastic sampling module connected to the Ferrybox enables the sampling of relatively large volumes of sea water in the area of interest (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%).

1.2 Sampling platform and sampling location

Samples were collected using a Ferrybox system mounted on the 'M/S Color Fantasy' 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 1**). Samples were collected over a 10-day period at the end of October and the beginning of November 2020 on a trajectory through the Danish parts of the Kattegat, Great Belt and Mecklenburg Bay using a Ferrybox (**Figure 2**).

Panel A



Panel B

Figure 1: *Map of sampling trajectory in Danish marine waters. Red line indicates the Danish EEZ of the route by the Oslo-Kiel ferry (panel A). M/S Color Line Fantasy (panel B).*

Panel A

Panel B

Figure 2: *Ferrybox (panel A) and micro plastic (panel B) installations onboard M/S Color Fantasy.*

A total of 7 samples were collected. 10 samples had been anticipated but the global pandemic and closure of all vessel traffic prevented this. All but one sample represent a single direction trajectory (CF1-CF6), whilst CF7 was taken during both an outward and a return cruise, amounting to double sample volume.

Each sample was collected over roughly a 9-hour period during the crossing (**Table 1**) using the standard system set-up with two filters: 500 µm and 300 µm. These are stacked sequentially for size fractionation, however in one instance (CF3) the filters were installed in reversed order and, nevertheless, both filters were analysed in the laboratory. The volume of water filtered was measured by the built-in flow meter allowing all samples to be standardised to "per cubic metre filtered (m^3)". Following each sample period, the filters were removed from the Ferrybox and placed in sealed containers. These were stored in a fridge (6 °C) until processing under controlled laboratory conditions.

Table 1: *Overview of samples collected offshore in Danish parts of the Kattegat, Great Belt and Mecklenburg Bay via the Ferrybox on the Oslo-Kiel ferry.*

Sample ID	Date	Volume (litres)	Samples collected	Sample pre-treatment	Laboratory analysis
CF1	23 October	4958	300, 500 µm	Rinsed directly onto GF/A filters for analysis	Visual, FTIR confirmation
CF2	25 October	4865	300, 500 µm	Rinsed directly onto GF/A filters for analysis	Visual, FTIR confirmation
CF3	27 October	4820	500, 300 µm*	Rinsed directly onto GF/A filters for analysis	Visual, FTIR confirmation
CF4	29 October	4776	300, 500 µm	Rinsed directly onto GF/A filters for analysis	Visual, FTIR confirmation
CF5	30 October	5082	300, 500 µm	Rinsed directly onto GF/A filters for analysis	Visual, FTIR confirmation
CF6	31 October	4865	300, 500 µm	Rinsed directly onto GF/A filters for analysis	Visual, FTIR confirmation
CF7	31 October - 02 November	9444**	300, 500 µm	Rinsed directly onto GF/A filters for analysis	Visual, FTIR confirmation

** The filters were accidentally placed in the reverse order with 300µm on top of 500µm.*

*** Sample CF7 was taken during both an outward and a return cruise so the sample volume was doubled.*

1.3 Sample analysis

Filters were processed in the laboratory in controlled conditions as soon as possible after sampling to minimize the risk of contamination and to avoid any suspended particulate matter (SPM) and biota sticking to the mesh filter. Each sample size fraction was treated independently.

1.3.1 Basic filtering

All the samples contained low levels of SPM and organic matter, and a bottle with prefiltered (filtered through a filter with pore size 0.22 µm) reverse osmosis (RO) water was used to rinse the sample material directly from the mesh filters to Whatman glass microfiber filters (grade GF/A, pore size 1.6 µm, Ø 47 mm). Filtration was carried out in sterile conditions in a laminar airflow cabinet (with HEPA filter) using a Nalgene vacuum filtration system. In most cases, the material on the mesh filters were filtered on to 1-3 GF/A filters depending on the amount of SPM. After filtration, the filters with material were immediately transferred to a petri dish and covered prior to drying and analysis (**Figure 3**).

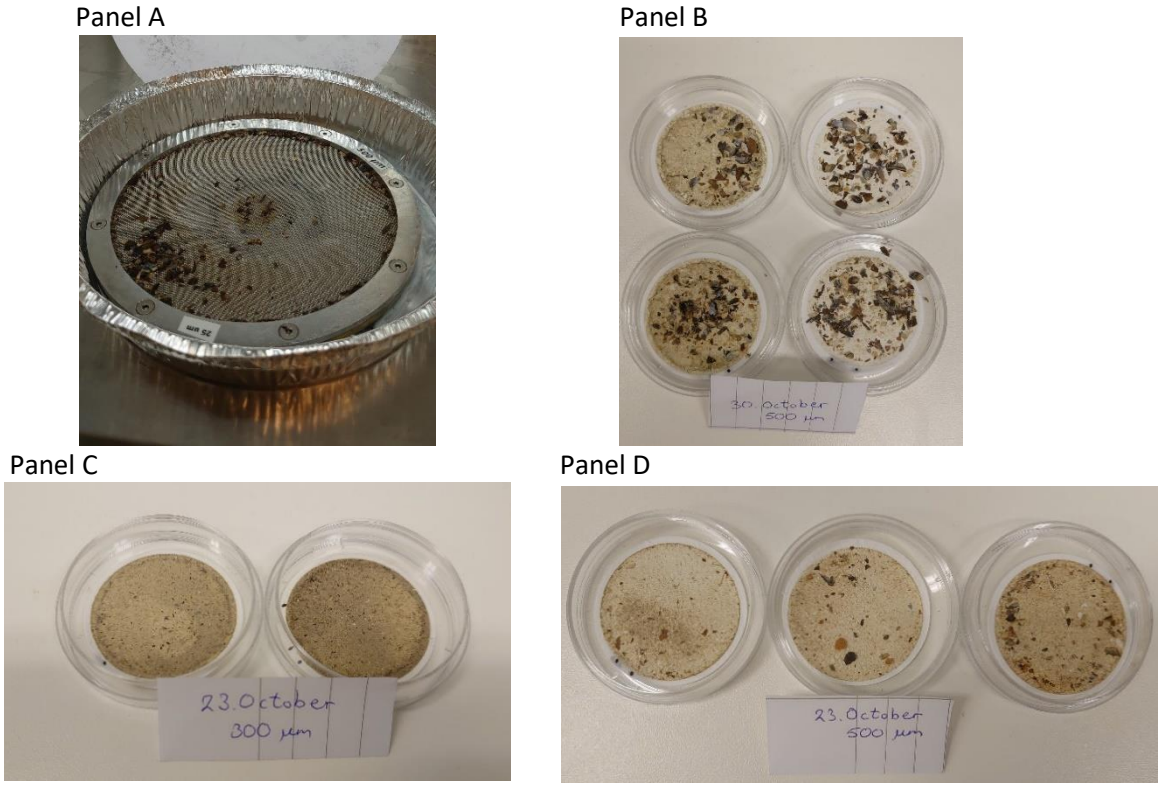


Figure 3: *Examples of samples collected during the sampling campaign 23/10-02/11 2020. Sample CF5 (500 µm) before filtration (A) and after filtration (B). This sample had the highest amount of organic material. Sample CF1 (300 µm) after filtration (C), and sample CF1 (500 µm) after filtration (D).*

1.3.1 Visual identification

After filtration, all samples were analysed by visual identification followed by chemical confirmation of the polymer material. A Nikon SMZ745T stereomicroscope (x20 magnification) with analysis software (Infinity Analyze v.6.5.6) was used to photograph and measure individual particles. The selection of particles was made following standard NIVA protocols which are mirrored in peer-reviewed literature (Lusher *et al*. 2020). Visual analysis followed where potential plastics were isolated, photographed, described in terms of morphology and colour, and measured along the longest length (µm). The following definitions were used for fragments, fibres and bead according to GESAM (2019). Fragments are defined as irregular

shaped hard particles having appearance of being broken down from a larger piece of litter, fibres as long fibrous material that has a length substantially longer than its width and beads as hard particle with spherical, smooth or granular shape.

1.3.2 Chemical analysis

Visual identification of microplastics, especially in the smaller size range (< 300 µm) should always be supported by chemical analysis (Lusher et al. 2020) and FTIR confirmation was performed 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). As it is challenging to visually identify microplastics in environmental samples (Lusher et al. 2020, Isobe et al. 2019), and considering that this is a method development project, FTIR was performed on as many of the particles a possible within all samples, field and laboratory blanks.

All particles from each sample were subjected to further chemical characterisation using µFTIR microscope analysis. This was performed on a PerkinElmer Spotlight 400 µFTIR spectrometer. To improve the quality of the spectra generated, particles were prepared for analysis using a diamond compression cell (DCC) accessory. Particles were carefully transferred from glass microfiber filter papers to the DCC with use of extra fine micro-forceps. The DCC compresses the particles to a thin, homogenous thickness. The DCC was then loaded onto the µFTIR microscope stage for analysis. Measurements were obtained in transmission mode and at 4 cm^{-1} spectral resolution for the range 4000 to 600 cm^{-1}. Spectra were produced from a composite of 2 co-scans. Background measurements were taken before each batch of particles was analysed.

Library matching was performed in the Spectrum 10 software (v. 10.6.2). Each spectrum was compared to several different libraries available at NIVA: PerkinElmer ATR Polymers library, STJapan Polymers ATR library, BASEMAN library (Primpke et al. 2018), and several in-house libraries including reference polymers, different textile materials, and potential sources of laboratory contamination. All spectra were manually inspected to ensure that the library matches were acceptable. If the polymer type of a particle could not be confirmed (low intensity peaks, small particle size) but the spectra showed characteristic peaks of synthetic plastic it was included as 'other plastic'.

1.3.3 Contamination controls

To avoid contamination at all stages of the project, the following steps were taken:

- **Field blank** samples were performed on the vessel alongside sample collection. Field blanks consisted of the same filter material left exposed to air for the duration at which the filters were not housed in the Ferrybox (during changing and packaging of the samples). The field blanks were performed to monitor for potential contamination during the changing of the filters. The field blanks were processed in the same way as the samples to represent any contamination introduced during the sampling procedure. One field blank was performed per sample, apart from CF2 where no field blank was performed.

- Three **laboratory blanks** were included in each round of filtration (n=21) to test for laboratory contamination. Prefiltered RO-water was filtered at the start, in the middle and in the end of the filtration of the samples using the same set up as the samples. The purpose of this is to have control over the potential contamination during the whole filtration process with the blanks representing the levels of contamination that could have been introduced to the samples that were filtered the same day.

In the laboratory, standard NIVA practices were followed, which included that: 1) all equipment was cleaned with prefiltered RO-water and the use of plastic laboratory equipment was kept to a minimum, 2) filtration was performed in a laminar flow cabinet, and 3) all personnel wore cotton clothing and rinsed all equipment between samples.

1.3.4 Data corrections

Sample data was corrected based on the observed synthetic polymers in both the field and laboratory blanks. Particles observed in the samples which had the same morphology, colour and polymer combinations as those reported for the blanks were excluded from the final data set.

2 Results and discussion

2.1 Procedural controls

2.1.1 Field blanks

The field blanks presented varying levels of contamination during the sampling (**Figure 4**). Every field blank (n=6) contained some level of contamination. Visual analysis found a total of 63 particles (61 fibres and 2 fragments) across all of the field blanks, ranging from 6 to 20 particles (average 10). Only 18 of the particles were confirmed to be synthetic polymers (17 fibres, 1 fragment). The average number of synthetic polymers in the field blanks was 3 (range 1-6). All particles were further characterized by µFTIR, non-synthetic polymers were removed from the analysis.

Of the confirmed synthetic polymers, the smallest particle was 120 µm and the largest particle was 3400 µm (average 967 µm). The fibres were mostly blue and black in colour. Viscose (n=7) and polyester (n=6) were the most abundant polymers reported. Furthermore, acrylic fibres (n=2), a single polypropylene, and a polyamide were found in the field blank samples. The single fragment was a green unknown type of synthetic polymer (120 µm). No rubber particles were found in the field blanks.

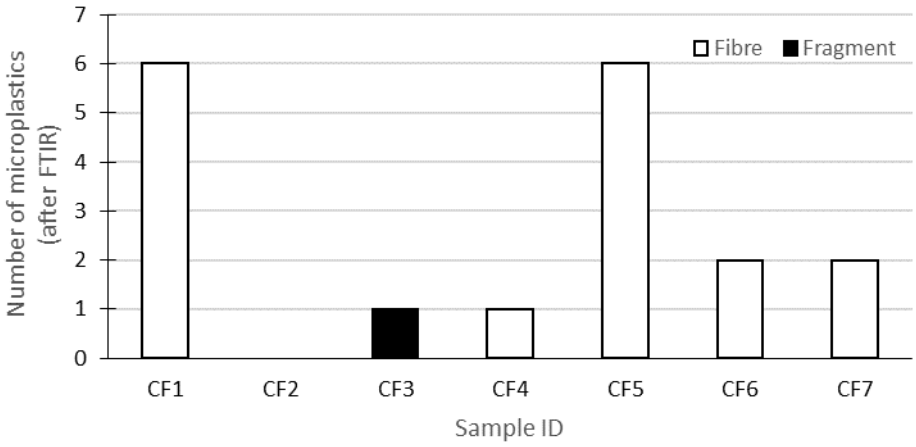


Figure 4: *Number of microplastics (confirmed by FTIR) present in the field blanks collected during the cruise. No field blank sample was collected together with sample CF2.*

2.1.2 Laboratory blanks

The laboratory blanks presented lower levels of procedural contamination than the field blanks (**Figure 5**). Three procedural blanks were taken for each sample (n=21), and the values were summed together for each sample to give an overall total. Only four out of seven procedural blanks contained particles (during visual identification), this number was reduced to two out of seven following FTIR confirmation. A total of nine particles (seven fibres and two fragments) where found in the laboratory blanks, but only two were confirmed to be synthetic polymers. These two synthetic polymers were a grey polyester fibre (1100 µm) and red rubber fragment (1500 µm), and they were found in two different blank samples (CF1 and CF6).

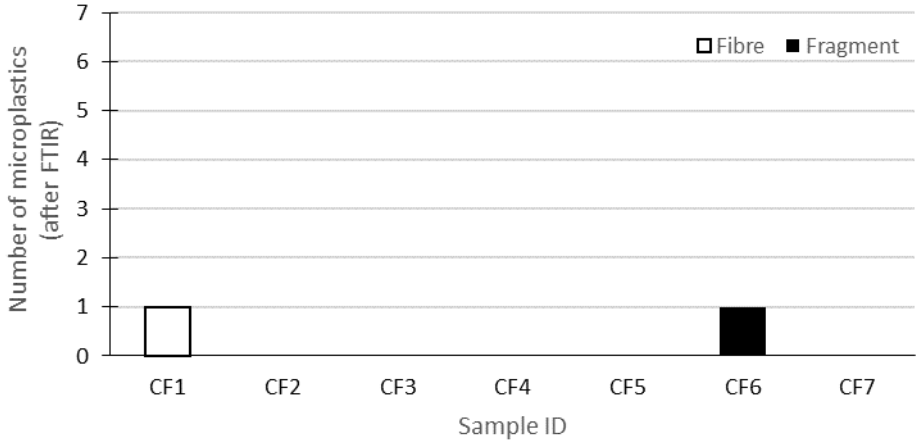


Figure 5: *Number of microplastics (confirmed by FTIR) present in the procedural controls performed during laboratory analysis.*

2.2 Visual analysis

A total of 149 particles were identified and they varied in size with a minimum length of 351 µm to a maximum length of 4700 µm. Most of the particles were classified as fibres (99%, range 13 – 29 per sample), whereas far fewer fragments (1%, range 0 – 1 per sample) were found. Beads were not observed in any of the samples. The results difference between samples are illustrated in **Figure 6**. For more fine details of the results, including the difference between both meshes is reported in the appendix.

It is often difficult to see the difference between natural and synthetic particles using visual analysis only, especially fibres (**Figure 7**). Therefore, no differentiation was made between natural and synthetic particles for the results from the visual analysis. Subsequent µFTIR analysis was used to distinguish these two categories.

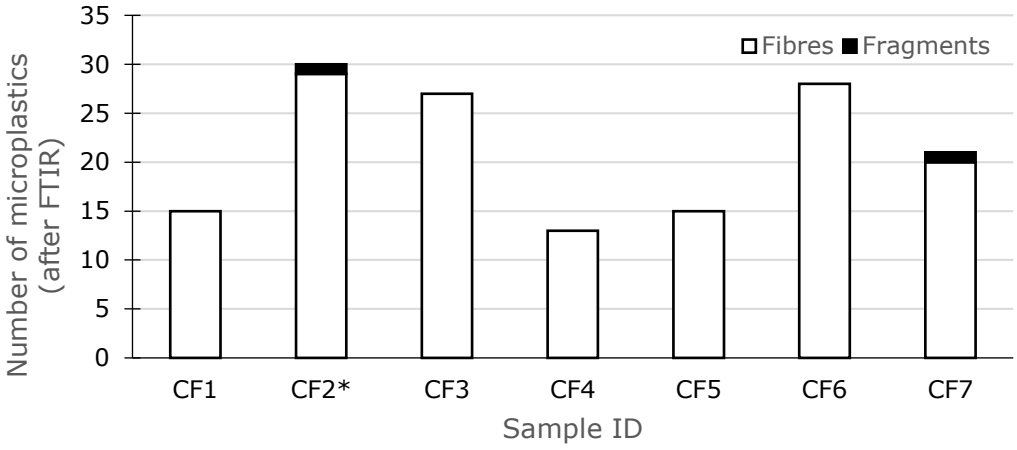


Figure 6: *Number of particles identified during visual analysis of the samples, displayed number of fibres and fragments. Data presented here is combined for both meshes (300 µm, 500 µm) and all samples collected 23/10-02/11 2020. *No field blank was taken together with CF2.*

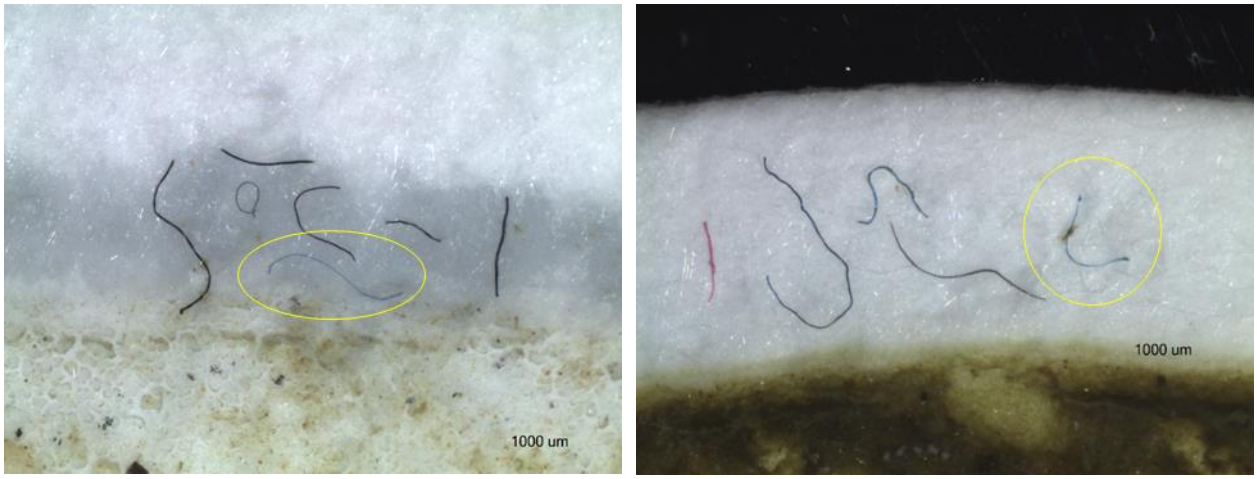


Figure 7: *The blue fibre (yellow circle) in the left picture was found in sample CF2 (500 µm) and it was confirmed with µFTIR to be a cellulose fibre, while the blue fibre in the right picture was found in sample CF6 (300 µm) and it was confirmed to be a polyester fibre.*

2.3 FTIR confirmation

A total of two particles which matched the morphology, colour and polymer combinations as the blank samples were removed. FTIR analysis was performed on all fibres and fragments identified by the visual analysis, and all FTIR spectra of the particles were matched against a large database to confirm the identity of the particles. Of the 149 particles originally identified through visual identification, 114 (77 %) particles from the visual analysis were found to be un-modified cellulose or of biological origin (chitin). These particles were excluded from the dataset as they do not fall under the definition of microplastics. The remaining 35 (23 %) particles were µFTIR confirmed to be synthetic polymers (**Table 2**). A substantial proportion of the confirmed particles were viscose (46 %), polyester was the second most abundant polymer (27 %), followed by acrylic (8%), polypropylene (8 %), and low amount of polyamide, rubber and other plastic types (**Figure 8**). No polyethylene (PE) or polystyrene (PS) particles were found.

The quality for 3% of the spectra were not good enough to determine the precise chemical composition, due to the relatively small size of the particle which reduced the intensity of the spectra. However, the spectra, although they did not pass the QA/QC criteria, showed peaks that are typical for synthetic polymers and were categorized and listed as “other plastic”.

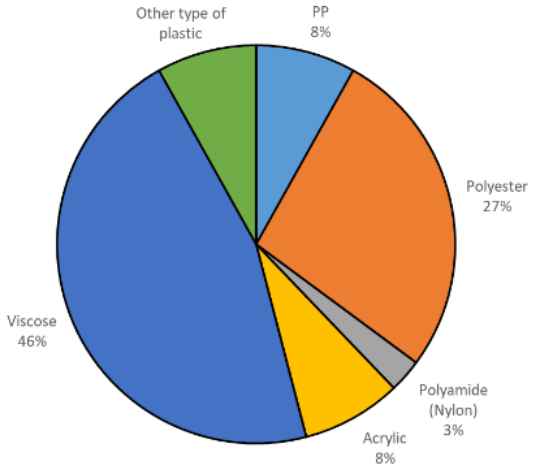


Figure 8: *Polymer composition of samples.*

Nine particles (1 from sample CF2, and 8 from the field blanks) were lost after the visual identification and before FTIR confirmation. The particle from CF2 was characterized as a black fibre, as no field blank was available for this sample this single fibre was excluded from the data set. The other 8 particles were from the field blanks corresponding to CF1 and CF5. None of the particles had similar morphology and colour characteristics to those in the samples and no further correction were made.

To correct for the procedural contamination, two fibres that matched those observed in the blanks were removed. These particles were a grey polyester fibre from CF1 (matched corresponding laboratory blank) and a grey polyester fibre from CF5 (matched corresponding field blank). The number of particles after µFTIR confirmation per sample ranged from 3 to 9 (**Figure 9**).

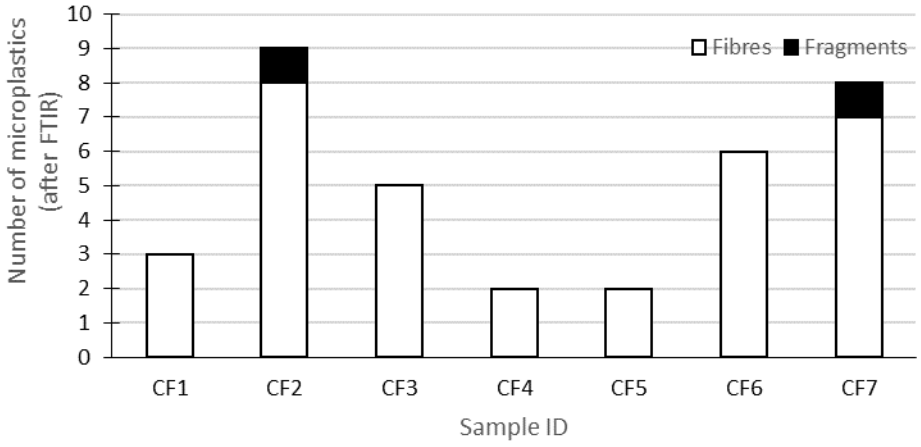


Figure 9: *Number of microplastics identified per sample, displayed number of fibres and fragments. Data presented here is combined for both meshes (300 µm, 500 µm) and all samples collected between 23/10-02/11 2020. Data corrected for procedural contamination except for sample CF2 where no field blank was available.*

2.4 Microplastic concentrations during transects

The FTIR corrected data was used to calculate the number of particles per m^3 and for comparision between the different samples. The total number of microplastics reported per sample ranged from 0.60 particles per m^3 to 1.85 particles per m^3 (**Table 2, Figure 10**). The average reported value was 0.91 particles per m^3 when fibres and fragments are summed together. There was a variation between the number of microplastics reported for each sample. Interestingly, sample CF7 with a double sample volume did not have a significantly low or high number of particles per m^3, and the sample with the highest number of particles per m^3 was CF2, and the second highest microplastic concentration was in CF6 with 1.23 particles per m^3. It should be noted that no field blank was taken together with sample CF2 and no blank correction was performed for this sample illustrating the challenges of operating just above the LoD of the method. Detailed results of the individual filters (300 µm, 500 µm) are given in Table A7 (visual analysis), Table A9 (FTIR confirmed) and Table A8 (seize > 500 µm) in the appendix.

Table 2: *Total number of fibres and fragments confirmed by µFTIR (n = number of particles), and the number of fibres and fragments normalised to sampling volume (n /m³ = number of particles m⁻³).*

	Fibres	Fragments	Total	Volume	Fibres	Fragments	Total
	n	n	n	m³	n/m³	n/m³	n/m³
CF1	3	0	3	4.96	0.60	0.00	0.60
CF2	8	1	9	4.87	1.64	0.21	1.85
CF3	5	0	5	4.82	1.04	0.00	1.04
CF4	2	0	2	4.78	0.42	0.00	0.42
CF5	2	0	2	5.08	0.39	0.00	0.39
CF6	6	0	6	4.87	1.23	0.00	1.23
CF7	7	1	8	9.44	0.74	0.11	0.85

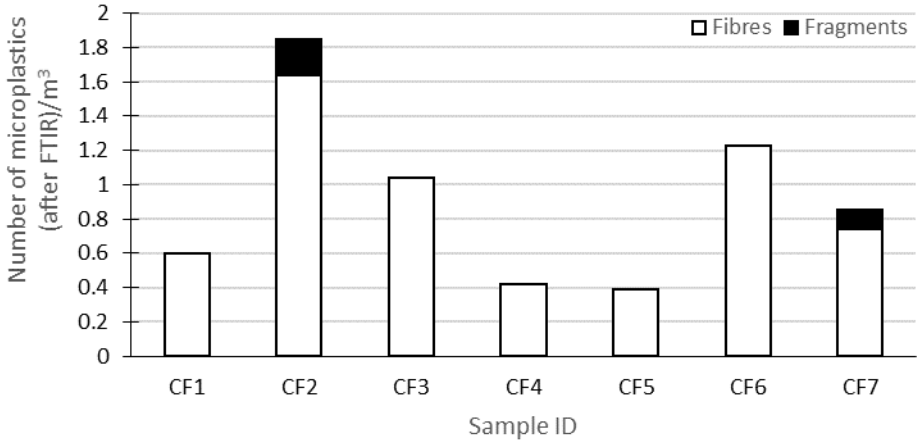


Figure 10: *Number of microplastics standardised per m³ and displayed number of fibres and fragments. Data presented here is combined for both meshes (300 µm, 500 µm) and all samples collected between 23/10-02/11 2020. Sample CF 2 is not corrected for field blanks.*

2.5 Logistical challenges

The number of samples collected was lower than originally planned. This was related to the COVID-19 situation with the cancellation of the ferry route. Because of this seven of the proposed ten samples were collected, including one test with a 'double' sample taken both from and to Oslo. The reduced number of samples affected the results and the validation of different options for further long-term monitoring, and it has limited the level of which the samples can be compared statistically. Future work should aim to collect a larger number of samples for the statistical analysis.

2.6 Procedural challenges

The number of particles in the procedural is challenging especially as the total number of microplastics in the sample are low (0.39-1.85 n/m³) and the results were corrected for the laboratory blank samples. A higher level of contamination was seen in the field blanks, which is not surprising as it is harder to control for the airborne particles in the open environment (**Figure 11**). To control for this, all particles that

matched both field blanks and samples were excluded which is a difficult and laborious process. All samples were blank corrected according to this procedure except for sample CF2 for which no field blank sample was available.

All samples were analysed visually first and then confirmed by using µFTIR. During this process it is possible to lose some particles. This is most common for the fibres and often due to the weathering of the particles and fibres which results in fragile particles. The samples in this study were no exception and the number of particles that were lost (n=9) between visual analysis and µFTIR accounted for 6.1% of all particles observed. Most of the particles that were lost were from the field blanks (n=8). None of the particles that were lost were similar in morphology and colour to other particles observed in the sample field blank or corresponding samples.

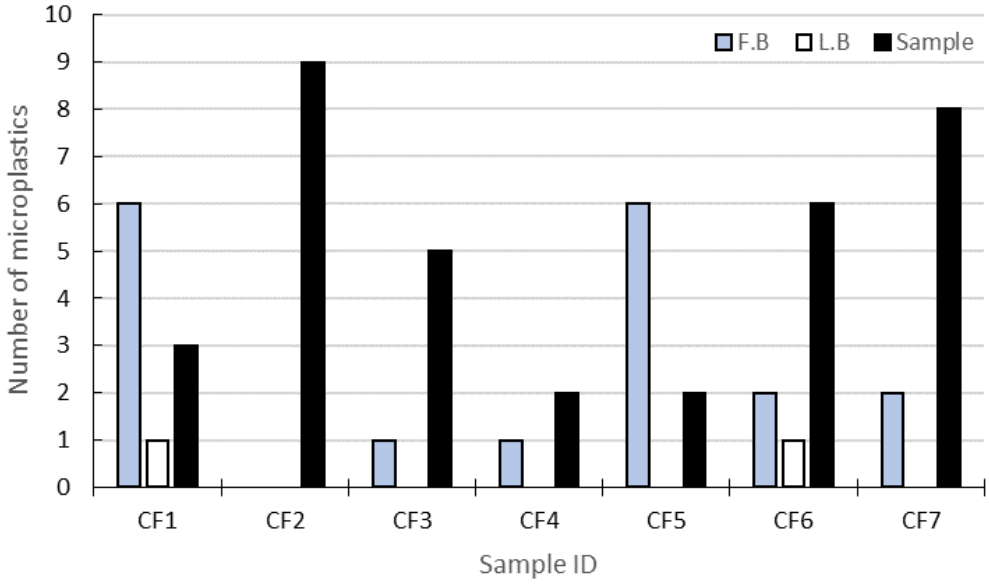


Figure 11. *Comparison of the observed number of microplastics in the samples to the controls sampled in the field (F.B) and laboratory (L.B). No field controls were collected for CF2.*

2.7 Microplastic results and meta data from the Ferrybox

The measurements of the Ferrybox during the sampling period are summarized in **Table 3** below and the corresponding sampling period is shown in **Figure A9** and **A10** in the appendix 5.5. An example of the graphical representation is given in **Figure 12**. The average variation between the different sampling days is relatively small for temperature, salinity and O_2 saturation (< 10%) but significantly larger for the chlorophyll A (29%) and turbidity (86%). The meta data on each day is given in detail in Appendix 5.5. During the sampling period no metrological data including wind speed, wind direction and wave height was logged by the Ferrybox on board due to a miscommunication when the M/S Color Fantasy was docked in harbor due to COVID-19 restrictions and electricity was cut off.

Table 3: *Selected Ferrybox data during the sampling period in Danish waters.*

	Temperature	Salinity	O2 Saturation	Chla-Fluorescence	Turbidity
	°C	g/kg	%	µg/L	FNU
mean	11.9	19.8	96.3	4.54	7.47
median	11.8	20.3	96.3	4.60	6.72
min	11.5	16.4	92.7	2.23	3.36
max	12.4	22.5	100.0	9.17	79.76
sdev	0.23	1.5	1.2	1.34	6.40

Figure 12: *Selected data from the Ferrybox meta data, temperature, salinity, O_2 saturation, chlorophyll-a and turbidity for two sample trajectories.*

However, if we look at the variation for temperature, salinity and O_2 saturation we see a large variation over the whole trajectory. The same is true for chlorophyll-a levels, which vary along the trajectory where the samples were taken. The turbidity measurements on the other hand are influenced by several extreme values (spikes) in relatively small areas. This illustrates the potential of difficulties to correlating microplastic levels with meta data. This is illustrated in **Figure 13** where the microplastic levels are plotted.

The Ferrybox microplastic module offer these opportunities for relating observations of microplastics with other environmental parameters including metrological data. Due to docking of the M/S Color Line Fantasy at the last sampling day and lock down of the ship this data could not be recovered for further evaluation. It should however be noted that for the project we only have looked at average meta data parameters during the 8-16-hour sampling periods in Danish waters. Further evaluation of the high resolution meta data falls out of the scope of the two pilot projects.

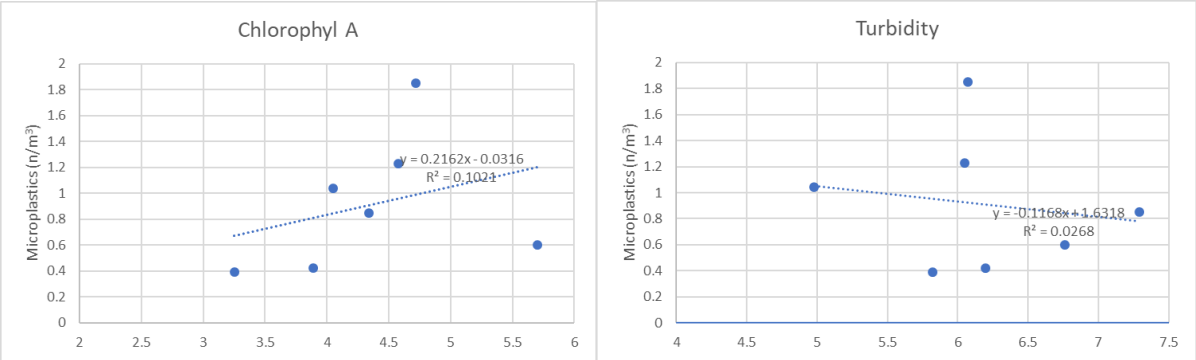


Figure 13: *Selected data from the Ferrybox meta data chlorophyll-a and turbidity plotted against the number of microplastics from the 7 measurements.*

2.8 Comparison to earlier sampling campaigns

The total number of microplastics reported per sample ranged from 0.39 particles per m^3 to 1.85 particles per m^3 (see **Table 2**). The average reported value was 0.91 particles per m^3 when fibres and fragments are summed together. These values were somewhat larger than those reported for the pilot study (van Bavel et al., 2020). In the former sampling campaign, the number of microplastics ranged from 0 to 1.85 microplastics per m^3 (average 0.71 per m^3). These values are within the range also reported for pump sampling in similar regions (**Table 4**).

There are many differences between the methods used in this survey and other similar sampling campaigns (for example, sampling method and mesh size), so for the purpose of comparison, only the pilot study (van Bavel *et al.* 2020) is compared further. Both campaigns in the Kattegat region utilised the Ferrybox system on the same vessel, covering the same trajectories, however different filter sizes were used. In the earlier report, 100 µm was the smallest size used, whereas this report used 300 µm as the smallest mesh size. There were complications related to the large amounts of biological material which was captured during periods of peak biological productivity in the first report. This was however not the case during the 10-day period from 23/10-02/11 2020 although the sampling periods were overlapping (4/9-26/2 and even 'double' sample of nearly 10 000 litres was taken without any problems with a 300 µm filter mesh. This shows the importance of monitoring other oceanographic parameters and eventually adjust sampling volumes and filter mesh size accordingly.

Table 4: *Reported microplastic concentrations in similar investigations.*

Location	Method (mesh size)	Average microplastics (m^3)	Reference
Norwegian Sea	Underway pump (80 µm)	2.50	Morgana et al. (2018)
North Atlantic	Underway pump (250 µm)	2.46	Lusher et al. (2014)
Norwegian Sea	Underway pump (250 µm)	2.68	Lusher et al. (2015)
North Atlantic	Underway pump (250 µm)	1.15	Kanhai et al. (2017)
Central Arctic Basin	Underway pump (250 µm)	Range 0-7.5	Kanhai et al. (2018)
Skagerrak/Kattegat, Baltic Sea and Gulf of Bothnia	Submersible pump (50, 300, 500 µm)	Range 0-10	Schonlau et al. (2020)
Baltic Sea	Submersible pump (100, 300 µm)	Range 0-8.2	Setälä et al. (2016)
Gullmar Fjord	Submersible pump (300 µm)	Range 0- 0.4	Karlsson et al. (2020)
Eurasian Arctic	Underway pump (1500, 100 µm)	0.8	Yakushev et al. (2021)
Skagerrak and Kattegat	Ferrybox (100, 500 µm)	0.71 (0-1.85)	van Bavel et al. (2020)
Skagerrak and Kattegat	Ferrybox (300, 500 µm)	1.16 (0.60-2.23)	This study.

3 Conclusions and recommendations

The data presented in this report is comparable to previous reports to different degrees. A range between 0.39 -1.85 microplastics per m^3 was established as background for the trajectory in Danish waters over a 10 days sampling period. This is in line with the earlier performed long term sampling where a range of 0-1.85 microplastics per m^3 was established. Both studies show that the levels on microplastics in open sea at the of the Kattegat, Great Belt and Mecklenburg Bay are relatively low in the size range 100 µm- 5000 µm or 300 µm-5000 µm (this study).

Considering the observed variability of other environmental parameters (e.g., temperature and chlorophyll a) in the meta data it is recommended to consider analysing smaller samples than used in this study. High intensity sampling across multiple transects has shown that it is possible to differentiate between water masses, distance from the coast, water temperatures and salinity (e.g. Lusher et al. 2014, Lusher et al. 2015, Kanhai et al. 2017). Therefore, recommended future actions include:

- The meta-data from the Ferrybox including weather and hydrodynamic data should be evaluated in relation to the varying microplastic concentration, but to do this a greater number of samples are needed with refined samples along a transect. For example, 3 samples could be taken per transect, but this would increase the demand on the microplastic Ferrybox system.

- Although not 'microplastics' most fibres that were rejected following µFTIR were of natural origin mostly unmodified cellulose, their occurrence in relation to synthetic fibres should be further investigated. Natural fibres including wool and cotton may originate from WWTPs with discharges and are a major but underestimated source of 'micro' litter.

Microplastics 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. There are currently several ongoing initiatives to identify the most appropriate monitoring strategy for microplastics in the environment. Water sampling is currently being considered for its inclusion in such campaigns and further refinement of methods will be necessary to help identify the most suitable methodologies. 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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5 Appendix

5.1 Sample trajectories

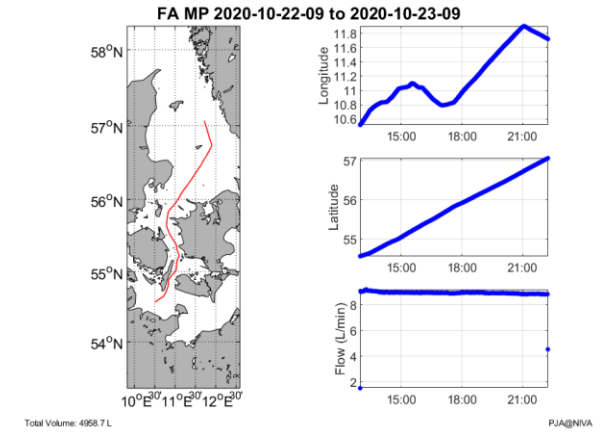

Figure A1: Map of sample collection for CF1.

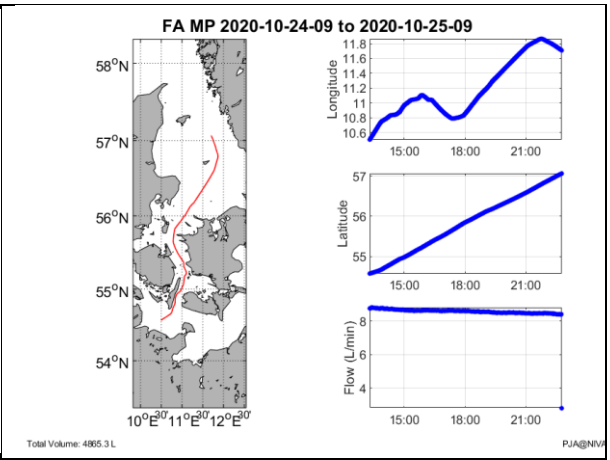

Figure A2: Map of sample collection for CF2.

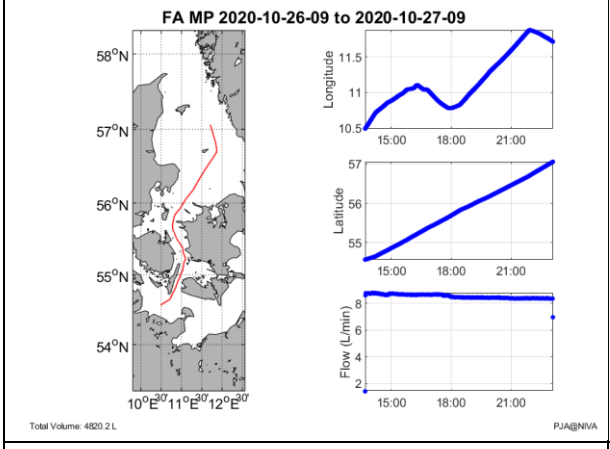

Figure A3: Map of sample collection for CF3.

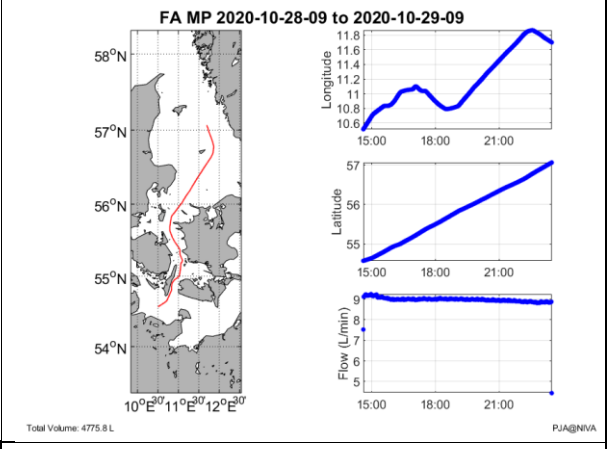

Figure A4: Map of sample collection for CF4.

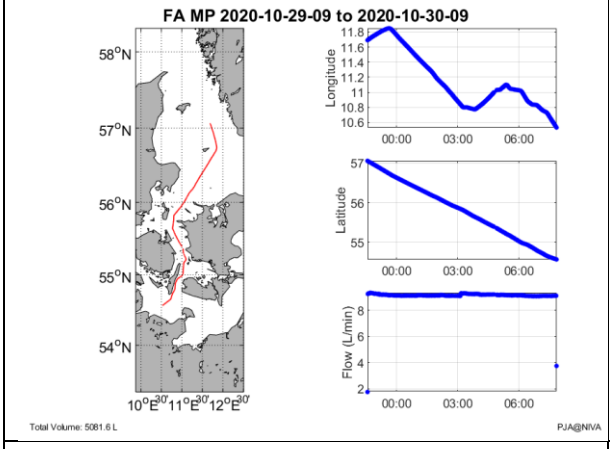

Figure A5: Map of sample collection for CF5.

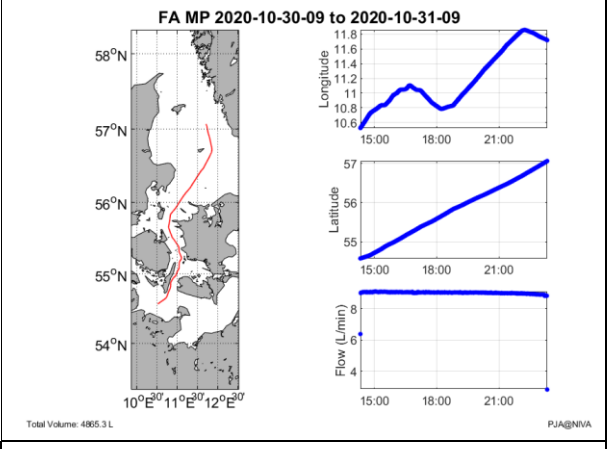

Figure A6: Map of sample collection for CF6.

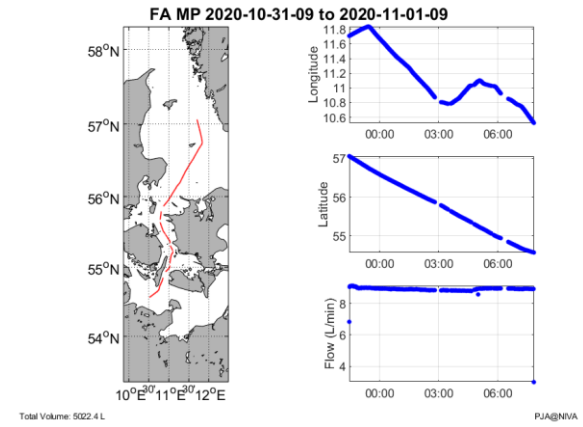

Figure A7: Map of sample collection for CF7 (outbound).

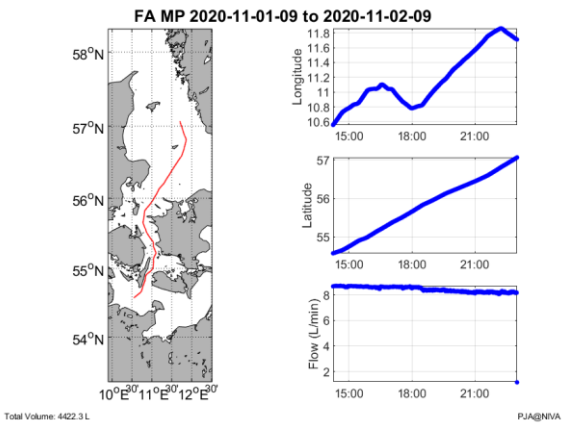

Figure A8: Map of sample collection for CF7 (return).

5.2 Field blanks

Table A1: Size distribution of particles identified in the field blanks (fibers and fragments)

	Visual	FTIR
100-500 µm	15	2
501-1000 µm	27	8
1001-1500 µm	13	5
1501-2000 µm	4	1
2001-2500 µm	0	0
2501-3000 µm	0	0
3001-3500 µm	4	2

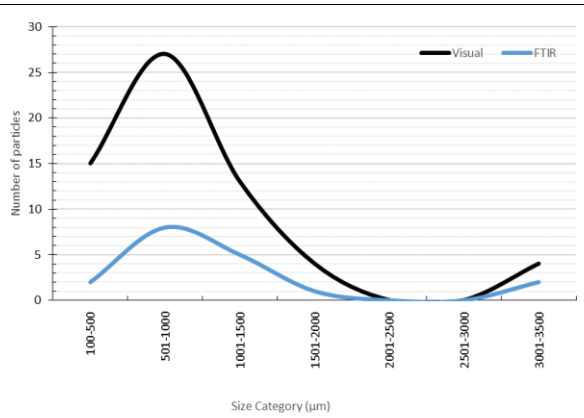

Table A2: Particles identified in the field blanks (fibers and fragments)

	Visual		Visual - Total	Confirmed with FTIR		FT-IR - Total
	Fibre	Fragment		Fiber	Fragment	
CF1 – field blank	11	0	**11**	6	0	**6**
CF2 – no field blank	-	-	**-**	-	-	**-**
CF3 – field blank	6	1	**7**	0	1	**1**
CF4 – field blank	10	1	**11**	1	0	**1**
CF5 – field blank	20	0	**20**	6	0	**6**
CF6 – field blank	8	0	**8**	2	0	**2**
CF7 – field blank	6	0	**6**	2	0	**2**
Total	***61***	***2***	***63***	***17***	***1***	***18***

Table A3: Polymers in the field blanks (fibers and fragments)

	PP	Polyester	Polyamide	Acrylic	Viscose	Other plastic	*Total*
CF1 – field blank	0	0	0	1	5	0	***6***
CF2 – no field blank	-	-	-	-	-	-	**-**
CF3 – field blank	0	0	0	0	0	1	***1***
CF4 – field blank	0	1	0	0	0	0	***1***
CF5 – field blank	1	4	0	0	1	0	***6***
CF6 – field blank	0	1	1	0	0	0	***2***
CF7 – field blank	0	0	0	1	1	0	***2***
Total	***1***	***6***	***1***	***2***	***7***	***1***	***18***

5.3 Laboratory controls

Table A4: Size distribution of particles identified in the laboratory blanks (fibers and fragments)

	Visual	FTIR
100-500 µm	4	0
501-1000 µm	2	0
1001-1500 µm	2	1
1501-2000 µm	1	1
2001-2500 µm	0	0
2501-3000 µm	0	0
3001-3500 µm	0	0

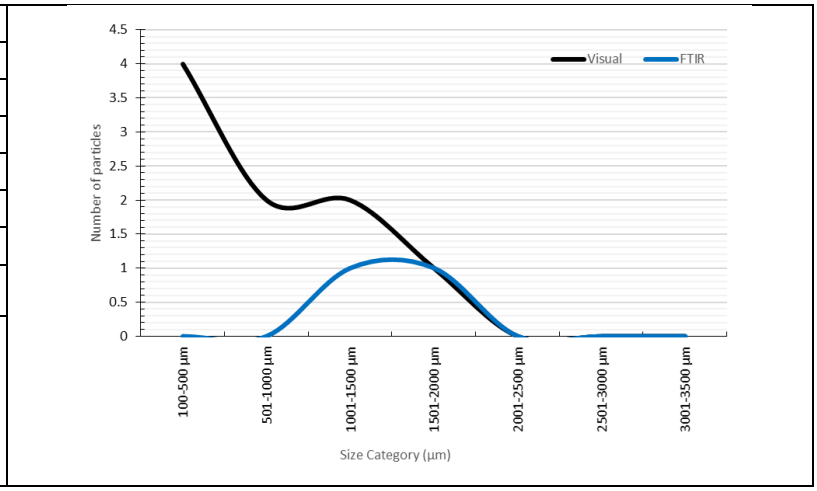

Table A5: Particles identified in laboratory blanks (fibers and fragments)

	Visual		Visual- Total	Confirmed with FTIR		FTIR-Total
	Fibre	Fragment		Fibre	Fragment	
CF1 – laboratory blank	3	0	3	1	0	1
CF2 – laboratory blank	0	0	0	0	0	0
CF3 – laboratory blank	1	0	1	0	0	0
CF4 – laboratory blank	0	0	0	0	0	0
CF5 – laboratory blank	3	0	3	0	0	0
CF6 – laboratory blank	0	2	2	0	1	1
CF7 – laboratory blank	0	0	0	0	0	0
Total	***7***	***2***	***9***	***1***	***1***	***2***

Table A6: Polymers in the laboratory blanks (fibers and fragments)

	Polyester	Rubber	*Total*
CF1 – laboratory blank	1	0	***1***
CF2 – laboratory blank	0	0	***0***
CF3 – laboratory blank	0	0	***0***
CF4 – laboratory blank	0	0	***0***
CF5 – laboratory blank	0	0	***0***
CF6 – laboratory blank	0	1	***1***
CF7 – laboratory blank	0	0	***0***
Total	***1***	***1***	***2***

5.4 Samples

Table A7: Summary of the total number of particles collected on the mesh filters per sampling trajectory (visual analysis only, not corrected with FTIR).

	300 µm			500 µm			**Combined**	
Sample ID	Fibres	Fragments	Total	Fibres	Fragments	Total	**Fibers**	**Fragments**
CF1	11	0	11	4	0	4	**15**	**0**
CF2	13	1	14	16	0	16	**29**	**1**
CF3	11	0	11	16	0	16	**27**	**0**
CF4	8	0	8	5	0	5	**13**	**0**
CF5	11	0	11	4	0	4	**15**	**0**
CF6	16	0	16	12	0	12	**28**	**0**
CF7	12	1	13	8	0	8	**20**	**1**

Table A8: Size distribution of particles identified in samples (fibers and fragments).

	Vis-ual	**FTIR**
100-500 µm	6	1
501-1000 µm	49	12
1001-1500 µm	43	10
1501-2000 µm	24	6
2001-2500 µm	12	3
2501-3000 µm	2	1
3001-3500 µm	7	1
3501-4000 µm	4	0
4001-4500 µm	1	0
4501-5000 µm	1	1

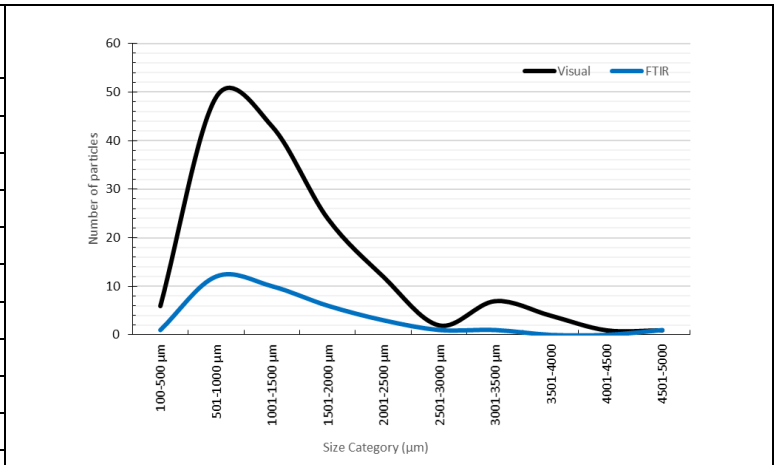

Table A9: Confirmation analysis by FTIR of the identified fibers and fragment from the visual analysis from 23/10-2/11 2020 sampling campaign. Data presented is corrected for procedural contamination.

Sample	**Mesh**	***PP***	***Polyester***	***Polyamide***	***Acrylic***	***Other plastic***	***Viscose***	***Total plastic/rubber***
CF1	300 µm	0	1	0	0	0	2	**3**
	500 µm	0	0	0	0	0	0	**0**
CF2	300 µm	0	2	0	0	1	1	**4**
	500 µm	1	1	1	0	0	2	**5**
CF3	300 µm	0	2	0	0	0	0	**2**
	500 µm	0	0	0	0	0	3	**3**
CF4	300 µm	0	0	0	1	0	0	**1**
	500 µm	0	0	0	1	0	0	**1**
CF5	300 µm	0	0	0	0	0	2	**2**
	500 µm	0	0	0	0	0	0	**0**
CF6	300 µm	0	2	0	1	0	1	**4**
	500 µm	1	0	0	0	0	1	**2**
CF7	300 µm	1	2	0	0	0	2	**5**
	500 µm	0	0	0	0	0	3	**3**
	Total	3	10	1	3	1	17	35

5.5 Meta data from the Ferrybox

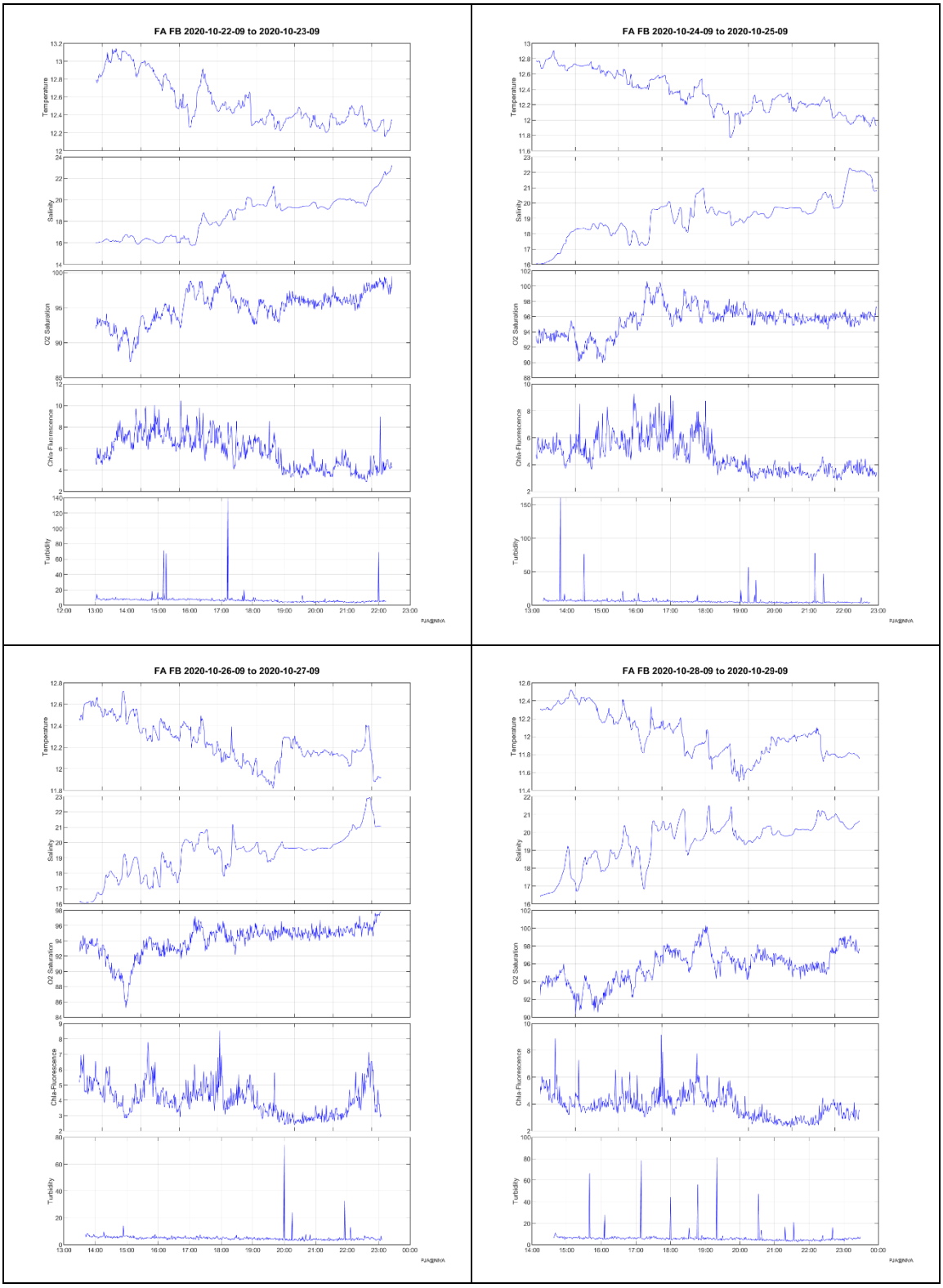

Figure A9: The following selected parameters for the Ferrybox meta data are given for every sampling period: temperature in °C, salinity in g/kg, oxygen saturation in %, uncalibrated Chl-a fluorescence in mg/L and turbidity in FNU.

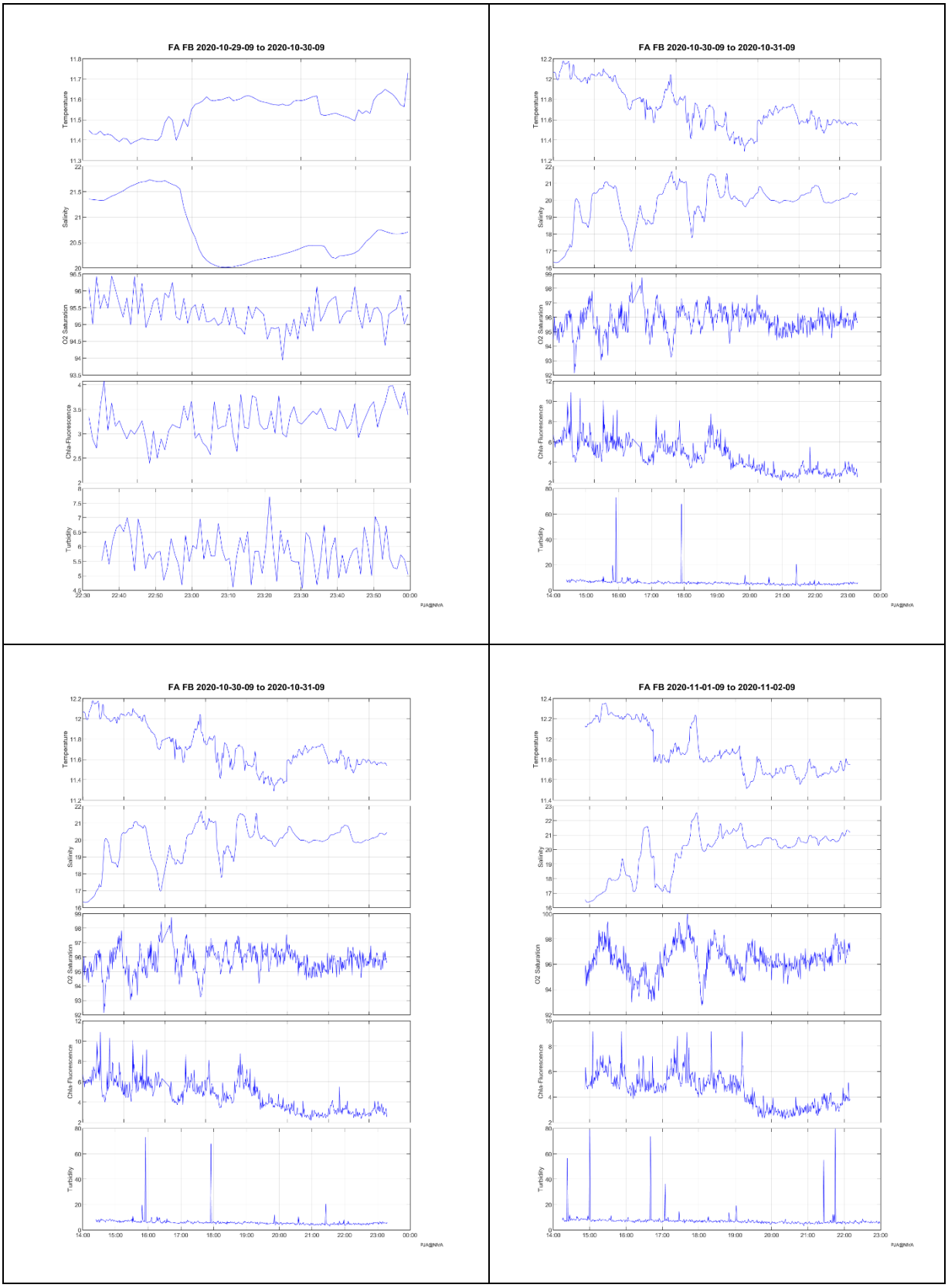

Figure A10: The statistics from selected Ferrybox parameters for each leg, where, red circles are mean values, blue x symbols median values. The vertical blue line is the extent of the standard deviation on each side of the mean values. The following parameters have been selected: temperature in °C, salinity in g/kg, oxygen saturation in %, uncorrected Chl-a fluorescence in mg/L and turbidity in FNU. Larger variation in turbidity measurements are reflected here as a higher standard deviation.

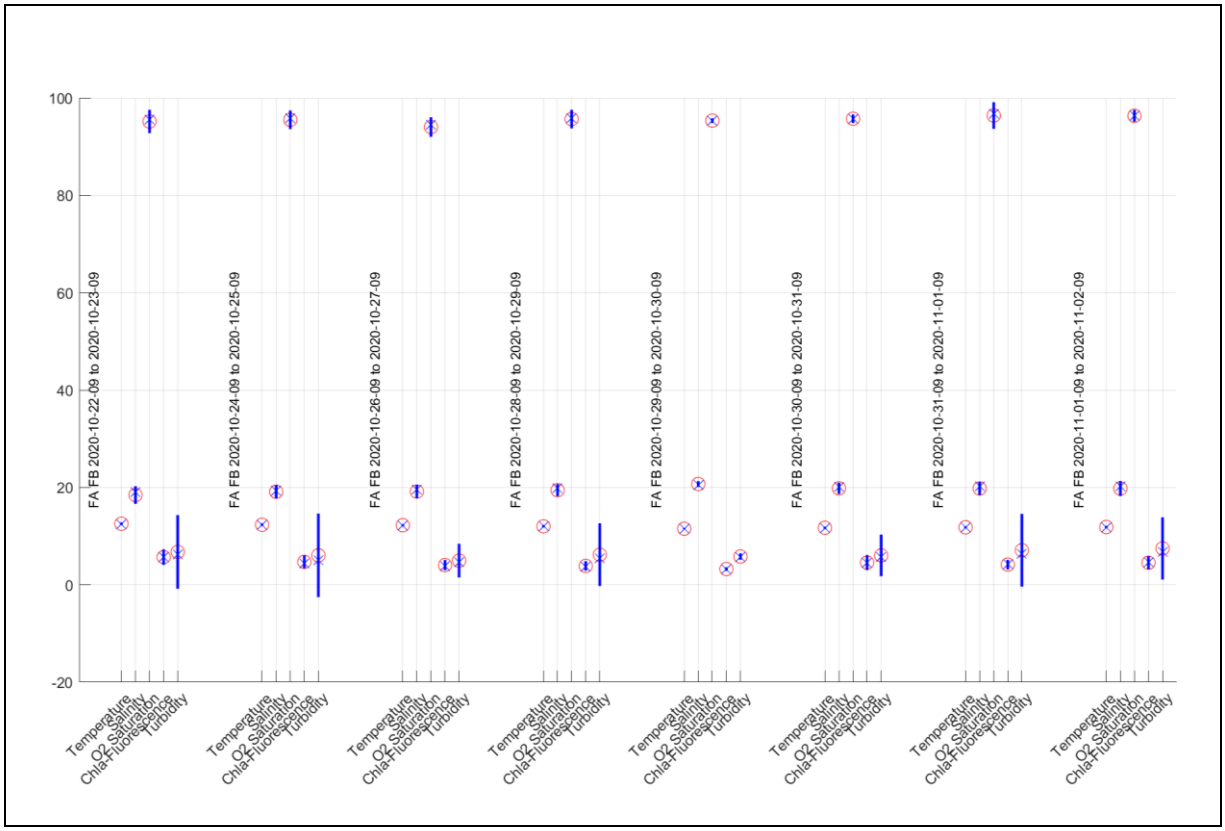

Figure A11: *Statistics from the Ferrybox parameters for each leg.*

Table A10: Ferrybox meta data.

CF1	mean	median	min	max	sdev	start	stop
Temperature [degC]	12.54	12.46	12.16	13.14	0.27	2020-10-22 12:59	2020-10-22 22:14
Salinity [g/kg]	18.44	19.11	15.77	23.25	1.82	2020-10-22 12:59	2020-10-22 22:14
O2 Saturation [%]	95.17	95.62	87.27	100.26	2.4	2020-10-22 12:59	2020-10-22 22:14
Chla-Fluorescence [µg/L]	5.7	5.7	2.91	10.44	1.6	2020-10-22 12:59	2020-10-22 22:14
Turbidity [FNU]	6.76	6.2	2.76	139.52	7.56	2020-10-22 12:59	2020-10-22 22:14
CF2							
Temperature [degC]	12.35	12.31	11.77	12.91	0.26	2020-10-24 13:18	2020-10-24 22:45
Salinity [g/kg]	19.12	19.29	16.03	22.28	1.38	2020-10-24 13:18	2020-10-24 22:45
O2 Saturation [%]	95.52	95.82	89.96	100.61	1.91	2020-10-24 13:18	2020-10-24 22:45
Chla-Fluorescence [µg/L]	4.72	4.43	2.76	9.29	1.33	2020-10-24 13:18	2020-10-24 22:45
Turbidity [FNU]	6.07	5.04	2.6	160.4	8.58	2020-10-24 13:18	2020-10-24 22:45
CF3							
Temperature [degC]	12.25	12.22	11.82	12.72	0.2	2020-10-26 13:41	2020-10-26 23:05
Salinity [g/kg]	19.16	19.53	16.07	22.94	1.42	2020-10-26 13:41	2020-10-26 23:05
O2 Saturation [%]	94.03	94.57	85.22	97.79	2.01	2020-10-26 13:41	2020-10-26 23:05
Chla-Fluorescence [µg/L]	4.05	3.91	2.4	8.54	1.05	2020-10-26 13:41	2020-10-26 23:05
Turbidity [FNU]	4.98	4.64	2.32	74.24	3.45	2020-10-26 13:41	2020-10-26 23:05
CF4							
Temperature [degC]	12.02	12	11.5	12.52	0.24	2020-10-28 14:37	2020-10-28 23:28
Salinity [g/kg]	19.44	19.82	16.41	21.51	1.24	2020-10-28 14:37	2020-10-28 23:28
O2 Saturation [%]	95.66	95.78	90.31	100.31	1.91	2020-10-28 14:37	2020-10-28 23:28
Chla-Fluorescence [µg/L]	3.89	3.78	2.26	9.17	0.98	2020-10-28 14:37	2020-10-28 23:28
Turbidity [FNU]	6.2	5.4	2.36	81.04	6.44	2020-10-28 14:37	2020-10-28 23:28

CF5	mean	median	min	max	sdev	start	stop
Temperature [degC]	11.53	11.56	11.38	11.73	0.08	2020-10-29 22:35	2020-10-29 23:59
Salinity [g/kg]	20.71	20.44	20.01	21.74	0.59	2020-10-29 22:35	2020-10-29 23:59
O2 Saturation [%]	95.35	95.3	93.94	96.44	0.47	2020-10-29 22:35	2020-10-29 23:59
Chla-Fluorescence [µg/L]	3.25	3.19	2.39	4.08	0.35	2020-10-29 22:35	2020-10-29 23:59
Turbidity [FNU]	5.82	5.76	4.56	7.72	0.66	2020-10-29 22:35	2020-10-29 23:59
CF6							
Temperature [degC]	11.72	11.7	11.29	12.18	0.21	2020-10-30 14:23	2020-10-30 23:18
Salinity [g/kg]	19.82	20.06	16.31	21.69	1.17	2020-10-30 14:23	2020-10-30 23:18
O2 Saturation [%]	95.74	95.76	92.13	98.75	0.88	2020-10-30 14:23	2020-10-30 23:18
Chla-Fluorescence [µg/L]	4.58	4.47	2.18	10.9	1.54	2020-10-30 14:23	2020-10-30 23:18
Turbidity [FNU]	6.05	5.64	2.96	73.16	4.27	2020-10-30 14:23	2020-10-30 23:18
CF7							
Temperature [degC]	11.85	11.795	11.45	12.285	0.22	2020-10-31 22:27	2020-11-01 22:59
Salinity [g/kg]	19.79	20.265	16.36	22.33	1.45	2020-10-31 22:27	2020-11-01 22:59
O2 Saturation [%]	96.37	96.535	91.22	100.89	1.97	2020-10-31 22:27	2020-11-01 22:59
Chla-Fluorescence [µg/L]	4.345	4.445	2.265	9	1.17	2020-10-31 22:27	2020-11-01 22:59
Turbidity [FNU]	7.285	6.54	3.72	106.18	6.94	2020-10-31 22:27	2020-11-01 22:59

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