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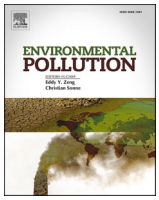

Abundance and distribution of microplastics in surface waters of the Kattegat/ Skagerrak (Denmark)☆

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ABSTRACT

Microplastics (MPs) are ubiquitous pollutants in the ocean, and there is a general concern about their persistence and potential effects on marine ecosystems. We still know little about the smaller size-fraction of marine MPs (MPs <300 μm), which are not collected with standard nets for MPs monitoring (e.g., Manta net). This study aims to determine the concentration, composition, and size distribution of MPs down to 10 μm in the Kattegat/Skagerrak area. Surface water samples were collected at fourteen stations using a plastic-free pump-filter device (UFO sampler) in October 2020. The samples were treated with an enzymatic-oxidative method and analyzed using FPA-μFTIR imaging. MPs concentrations ranged between 11 and 87 MP m^{-3}, with 88% of the MPs being smaller than 300 μm. The most abundant shape of MPs were fragments (56%), and polyester, polypropylene, and polyethylene were the dominant synthetic polymer types. The concentration of MPs shows a significant positive correlation to the seawater density. Furthermore, there was a tendency towards higher MPs concentrations in the Northern and the Southern parts of the study area. The concentration of MPs collected with the UFO sampler was several orders of magnitude higher than those commonly found in samples collected with the Manta net due to the dominance of MP smaller size fractions. Despite the multiple potential sources of MPs in the study area, the level of MPs pollution in the surface waters was low compared (<100 MP m^{-3}) to other regions. The concentrations of MPs found in the studied surface waters were six orders of magnitude lower than those causing negative effects on pelagic organisms based on laboratory exposure studies, thus is not expected to cause any impact on the pelagic food web.

1. Introduction

Microplastics (MPs) are found in the ocean from the surface to the seafloor (Choy et al., 2019; Lim, 2021). Their persistence and potential effects on marine ecosystems are a global concern (Andrady, 2011; UNEP, 2021); thus, it is crucial to determine the concentration (abundance), composition, and distributional patterns of MPs in marine ecosystems. Current knowledge of MPs abundance is mainly based on Manta trawls which collect particles larger than 300 μm (Cózar et al., 2014; Mai et al., 2018; De Lucia et al., 2018; Baini et al., 2018; Miller et al., 2021; Aigars et al., 2021; bib_citation_to_be_resolvedHänninen et al., 2021; Uurasjärvi et al., 2021). However, recent studies show that MPs smaller than 300 μm are significantly more abundant than larger ones (Enders et al., 2015; Rist et al., 2020). Kameda et al., (2021) emphasized the need for future studies, including "fine MPs'', which are those smaller than 300 μm. These smaller size fraction MPs are ecologically relevant because they overlap in size with plankton and can be ingested by planktivorous organisms (Sieburth et al., 1978; Andrady, 2011; Cole et al., 2011; GESAMP, 2016).

Sampling of small-size MPs fractions in marine waters is a challenging task. Although it is possible to collect MPs with nets of relatively small mesh size (Lindeque et al., 2020), the most promising methods are

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plankton pumps (Schönlau et al., 2020) and plastic-free pump filtration systems with small-mesh filters, either deployed off-board (Song et al., 2018; Kozak et al., 2021) or connected to the ship water intake (Enders et al., 2015). Manta trawl-based sample collection has established techniques for MPs identification, including microscopy and Attenuated Total Reflection Fourier Transform Infrared Spectroscopy (ATR – FTIR) (Gago et al., 2019). However, analyzing small-size MPs fractions in environmental samples is more difficult and laborious (Kameda et al., 2021; Vianello, 2020). The lack of an accepted standard method for identifying and quantifying MPs makes it difficult to compare MPs' data (Löder and Gerdts, 2015). There are different analytical methods used to identify/quantify and characterize the MPs such as optical and chemical analysis (Primpke et al., 2020a). Pyrolysis-gas chromatography (GC) in combination with mass spectrometry (MS), Raman spectroscopy, FTIR spectroscopy and microscopy are some examples of analysis for chemical identification of MPs (Löder and Gerdts, 2015; Primpke et al., 2020a). Several in-depth studies have used Focal Plane Array imaging micro FTIR (FPA-μFTIR-Imaging) to identify/quantify MPs that present in various compartments of the ecosystem and waste management systems (Lorenz et al., 2019; Vianello et al., 2019). Subsequently, there is a strong need to harmonize protocol to characterize the MPs, especially these small-size MPs fraction in the environmental matrices. Thereby, a proper analysis of these MPs requires laborious sample preparation with several steps of chemical and enzymatic treatments (Löder et al., 2017) and μFTIR techniques. Furthermore, prevention of contamination and strict quality control to estimate contamination during sampling and analyses of samples is particularly relevant for precisely determining the small-size MPs fractions (Wesch et al., 2017; Hermsen et al., 2018; Koelmans et al., 2019; Brander et al., 2020; Primpke et al., 2022).

Apart from methodological differences, the degree of MPs pollution and its magnitude also vary depending on geographical location, oceanographic features, and input sources, all variables affecting the potential MPs concentration in specific regions (Zhang et al., 2020). The present study focuses on the Danish marine waters, specifically on the Kattegat/Skagerrak region. Kattegat is a semi-enclosed sea that connects the brackish Baltic and the marine North Sea and one of the world's most ship trafficked areas (HELCOM, 2009; Moldanová et al., 2022). Furthermore, the severity of pollution entering into the Baltic Sea and the Kattegat area is influenced by approximately 90 million people living in the surrounding Baltic Sea countries (Elmgren et al., 2015). These anthropogenic pressure present multiple pathways for MPs to enter the Kattegat and the adjacent regions along three transit straits: Little Belt, Great Belt, and the Sound. A significant quantity of pollutants including MPs are also introduced in the Skagerrak from the North Sea (Anckar et al., 1998). Despite the anthropogenic-driven activities, seasonal variation, currents, and stratification in the region (Reckermann et al., 2022) also possibly impact the distribution and deposition of MPs.

The brackish signature of Baltic outflow water is detectable through the Kattegat and compensated by a deep-water inflow of North Sea origin (Edelvang et al., 2005; Rheinheimer, 1998). The Baltic Current (BC) flows towards the North and mixes with the Jutland Coastal Current (JCC), which flows east at the transition between the Kattegat and the Skagerrak (Alve and Murray, 1999). The Baltic current turns west in the outer Oslo Fjord as the Norwegian Coastal Current influences the influx and outflow of water from the Kattegat Sea (Alve and Murray, 1999). The Kattegat Sea density varies considerably with salinity and temperature changes (Rheinheimer, 1998; Edelvang et al., 2005), which can influence the distribution and abundance of MPs. The Kattegat is a two-layer system with lower saline surface water entering from the Baltic Sea and higher saline deeper water from Skagerrak, where the two water masses are typically separated by a strong pycnocline (Richardson, 1996; Matthews et al., 1999). A recent study in the Baltic has shown that the pycnocline is a potential accumulation zone for MPs in the water column (Uurasjärvi et al., 2021). However, little is known about the distribution of MPs in the Kattegat/Skagerrak area.

Thereby, the present study aims to investigate the abundance, distribution, composition, size, and mass of MPs (down to 10 μm) in the surface waters of the Kattegat/Skagerrak Sea on a basin scale. Additionally, we examined the influence of surface oceanographic parameters (Temperature and Salinity) on the observed abundance and distribution of MPs in the study area.

2. Materials and methods

2.1. Study area and sampling

The sampling was carried out onboard R/V DANA (DTU Aqua) from 20th October to November 1, 2020. Of the fourteen sampling locations, twelve were chosen in the Kattegat Sea and two located in the Skagerrak (Fig. 1 and Table S1). Stations were chosen to cover the different water masses. Profiles of temperature, salinity, and fluorescence (Chlorophyll *a*) were measured from the surface to a depth of 1 m above the seafloor using a Seabird CTD System (Sea-Bird SBE 9) equipped with a fluorometer. Water samples for chlorophyll *a* (Chl *a*) analyses were collected using 10 L Niskin bottles. *In situ* fluorescence depth profiles were calibrated against Chl *a* concentration in the water samples ($y = 144.68x - 2.0027$; $n = 14$, $r = 0.80$, $p < 0.01$). Phytoplankton dry weight was calculated from Chl *a* to carbon conversion factor of 42.6 (Juul-Pedersen et al., 2006) and carbon to dry weight (DW) conversion factor of 55% (Hansen et al., 1994).

MPs sampling was carried out from the ship's seawater intake. The seawater intake is located 3 m below the waterline on the forward port side of the vessel; the seawater received at the intake was considered as the first 3 m of the water column for this study due to the wave-induced vertical movements of the ship and the hydrodynamic turbulence from the ship bow (Enders et al., 2015). MPs samples >10 μm were collected using a plastic-free pump-filter device ("UFO"- Universal Filtering Objects system (Rist et al., 2020). In brief, the UFO system comprises three steel filtering devices interconnected, holding large diameter steel filters (Ø = 167 mm; 1 × 300 μm and 2 × 10 μm): the water is pre-filtered down to 300 μm in the first unit, then filtered in parallel down to 10 μm in the other units. The outflow is recombined, and the volume is measured with a mechanical flowmeter (Fig. S2). In the present study, the filtering device was directly connected to the ship's water intake through a short metal hose, and the water was filtered with no additional pumping (differently from Rist et al., 2020) since the flow provided by the intake was sufficient for effective filtration. The average filtered water volume was 1.04 ± 0.16 m^3 per station. Throughout the sampling period, three "air blanks" (field blanks) were taken by keeping petri dishes open while the filters were changed and transferred from the UFO system to the storage Petri dish.

2.2. Sample preparation

The enriched steel filters collected at each station by "UFO" (two of 10 μm and one of 300 μm each station (Fig. S2)) were processed according to the protocol slightly modified from Rist et al. (2020). In brief, the sample extraction consisted of five major treatment steps, as described in Fig. 2 (A). Initially, the filters were soaked in SDS (5% sodium dodecyl sulfate) for 24 h and filtered onto 10 μm steel filters (Ø = 47 mm). The samples were then incubated with enzymes, specifically: protease, for 48 h (Sigma, protease from *Bacillus* sp.), followed by cellulase (Sigma, cellulase enzyme blend) and Viscozyme® L (Sigma) for additional 48 h. The samples were then subjected to a Fenton oxidation (H_2O_2, NaOH, and $FeSO_4.H_2SO_4$) to remove any remaining organic matter (Simon et al., 2018). After oxidation, size fractionation was carried out with a 300 μm steel sieve to separate the particles above 300 μm. Finally, the density separation of the sample fraction between 10 and 300 μm was achieved by re-suspending the particulate with sodium polytungstate (SPT, ~1.7 g/cm^3) in a glass separatory funnel via air-mixing the solution for 15 min and then left to settle overnight. Then, the floating fraction was recovered, briefly sonicated, and rinsed with

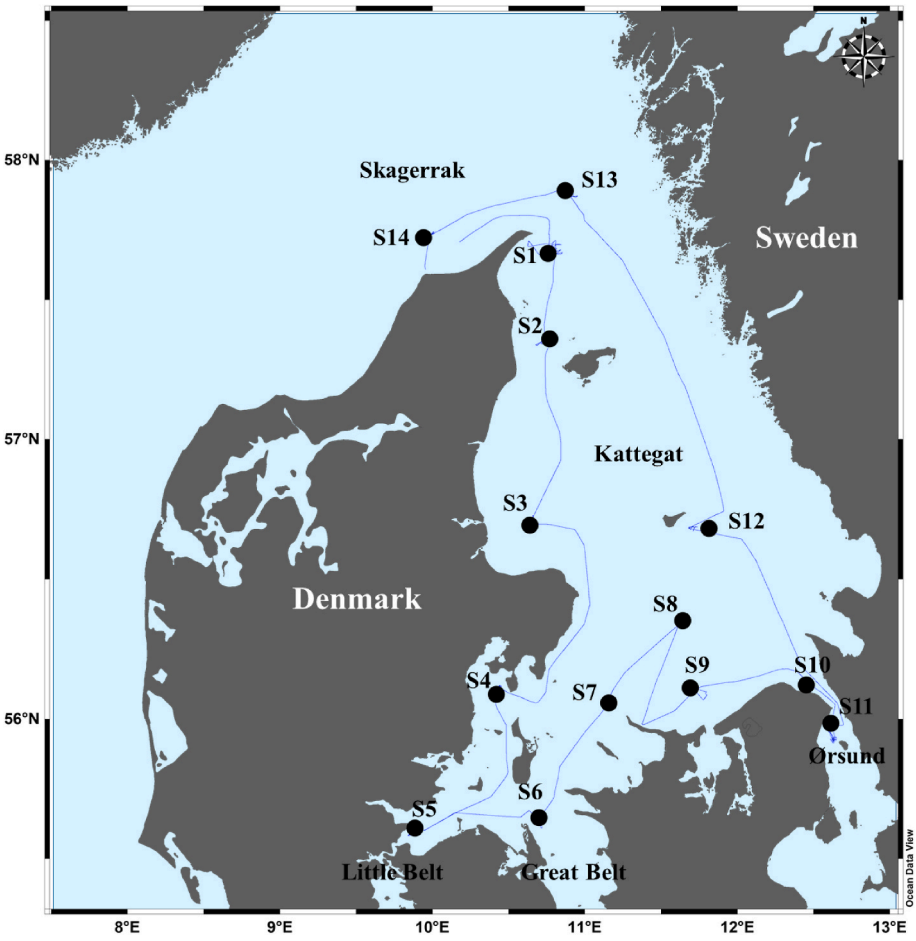

Fig. 1. The study sites along with the R/V DANA's track.

warm filtered water, and then 50% ultrapure ethanol. All liquid was sequentially transferred to 10 mL muffled glass vials and evaporated in a water bath at 50 °C using a stream of nitrogen (Biotage, TurboVap®). After evaporation, the sample's volume was fixed to 5 mL with 50% ultrapure ethanol.

2.3. MPs detection and data analysis

The extracted sample was homogenized with a vortex, and an aliquot corresponding to 50–60% of the sample was deposited onto multiple Zinc Selenide (ZnSe) infrared windows (13 mm Ø x 2 mm, Crystran, UK) in a compression cell (PIKE Technologies, Fitchburg, WI, USA) using a capillary glass micro-pipette (micro-classic, Brand GmbH, Germany). The deposited samples were covered with a muffled glass beaker and dried at 50 °C on a heating plate. Ultimately, the entire active surface of the ZnSe window (Ø = 11 mm) was analyzed by FPA-μFTIR-Imaging using an Agilent 620 FTIR microscope with a 128 × 128-pixel FPA detector combined with a Cary 670 FTIR spectrometer (Agilent Technologies, Santa Clara, CA, USA) in the spectral range of 3750–850 cm^{-1} at 8 cm^{-1} resolution applying 30 co-added scans in transmission mode (Fig. 2B). A background was collected by applying 120 co-added scans before each analysis to subtract the water vapor and CO_2 contribution.

The collected infrared maps were analyzed using the open access software siMPle (https://simple-plastics.eu/), which allows automated analysis of large FPA-μFTIR-Imaging datasets (Primpke et al., 2020b). The degree of spectral matching between the reference and sample spectra was assessed using Pearson correlation, which produced correlation coefficients for the raw spectra, first derivative, and second derivative (Liu et al., 2019; Vianello et al., 2019; Primpke et al., 2020b). Specific thresholds were set for different polymer clusters (see database in SI Table S2). Following that, the particle was counted as plastic if the correlation was greater than the set threshold and if all of the plastic reference peaks were present. Results were carefully inspected to remove the false positives which can arise from specific interferent materials. Simon et al. (2018) described an approach for the mass estimation of the particles. In brief, the length and width of the particles were designated from the major longest axis in the center of the particle and the longest axis running perpendicular to the major axis respectively. The thickness was calculated to be 67% of the width. Eventually, the volume of the particle, assuming an ellipsoid form, and the material's density (see database in SI Table S2)were used to compute the particle's mass.

2.4. Quality assurance and quality control of MPs sampling and sample preparation

Samples were processed in a laminar flow hood, and cotton laboratory coats were always worn. The use of plastic-containing equipment during sampling and sample preparation was avoided as much as possible. However, the plastic materials such as silicone and Teflon (PTFE) could not be avoided during sampling and sample preparation which were identified via FTIR and excluded from the MPs quantification. In addition, samples were taken from all the possible cross-contamination points, including the ship's paints, which are a relevant source of polymer-based materials (Leistenschneider et al., 2021). Eventually, seven matching DANA ship paint particles in the samples were excluded. All materials were muffled at 500 °C, if possible, rinsed with MilliQ water, and wrapped in aluminum foil until use. All chemical

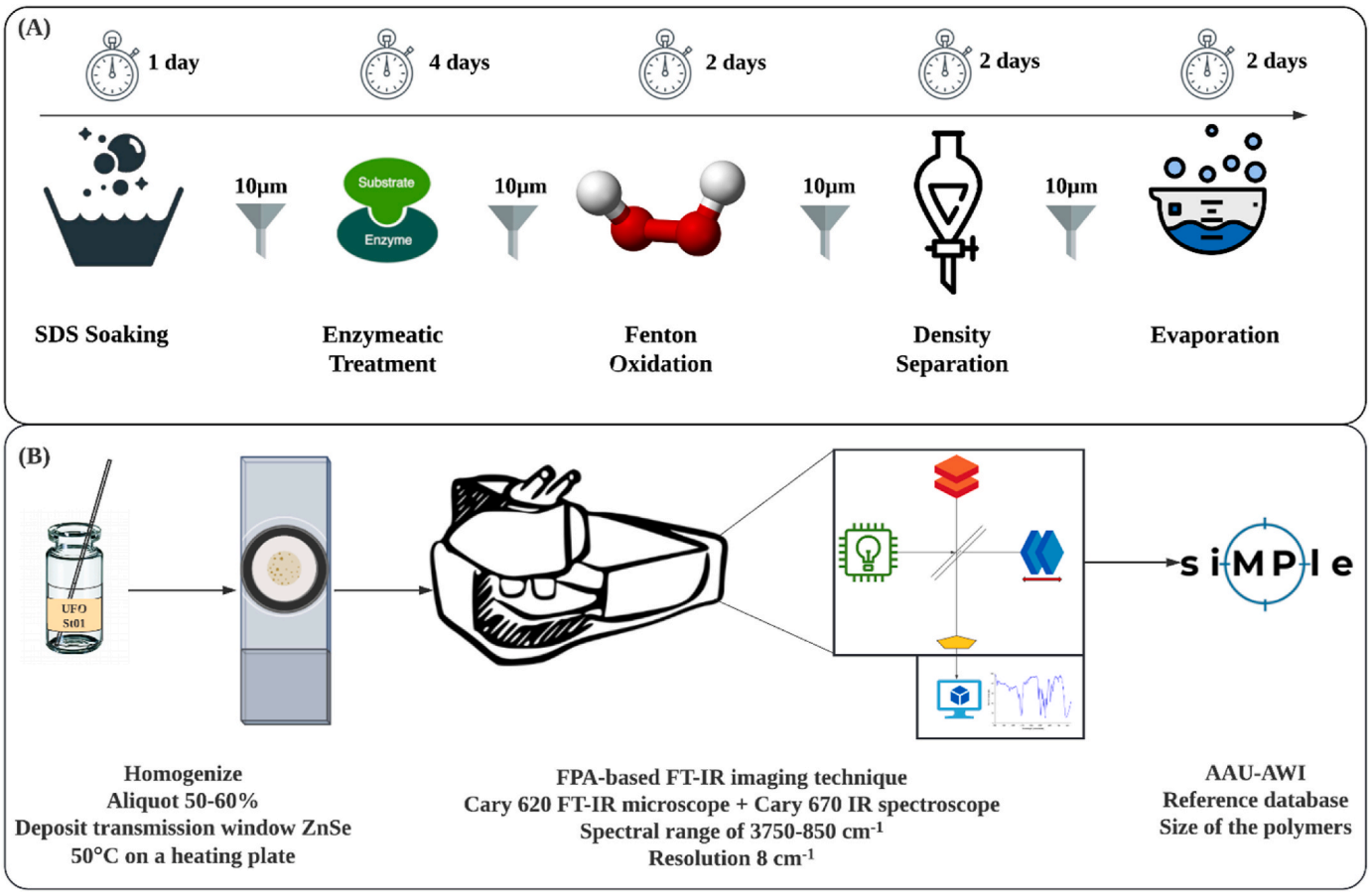

Fig. 2. Major steps of sample preparation (A) and detection/analysis of MPs >10 µm (B) used for surface water samples collected with the UFO pump-filter device.

solutions used for processing the samples were filtered over 0.7 µm GF/F filters. The "air blanks" were used to determine the contamination throughout the UFO sampling during the cruise. To estimate any possible air-borne contamination, the air blanks were collected during the sampling by opening a muffled Petri dish every time the UFO sample was transferred to a similar sized Petri dish (1min). The "air blanks" from the ship were rinsed with 50% ultrapure ethanol and evaporated. Furthermore, procedural blanks (all reagents and materials with no sample) from the laboratory underwent the same procedure as mentioned in section 2.2 to quantify MPs' field and procedural cross-contamination from sampling to the end of sample analysis (Rist et al., 2020). Fig. S1 illustrates the complete quality assurance and quality control measures at all stage of the project to minimize the sample contamination.

2.5. Data processing and statistical analysis

The number of MPs in each station was extrapolated to the respective samples based on the µFTIR-scanned fraction and corrected for cross-contamination recorded in procedural and field blanks. Descriptive statistics were applied to the MPs concentration, polymer type, size, and estimated mass. Polymer diversity among the stations was assessed by the Shannon-Wiener index (H' = $\sum$ [(*pi*) × ln (*pi*)]; *pi*: proportion of total polymers represented by polymer i), and richness (S = number of polymers, = polymer richness) was calculated based on the number of different polymers detected. Furthermore, Spearman's rank correlation was used to investigate the degree of association between the oceanographic parameters and characteristics of MPs (Total number of MPs and density of polymers (<1gcm^{-3} and > 1gcm^{-3})). Descriptive statistics, graphical illustrations and Spearman's rank correlation was conducted using R software (R 4.2.1). The maps of CTD data and plots were prepared using Ocean Data View (ODV) software version 5.4.0 (Schlitzer, R., 2021).

3. Results

3.1. Oceanographic parameter characteristics

The surface salinity in the study area clearly showed a two different water masses, the southern part of the Kattegat with salinities ranging from 18.9 to 21.6 Practical Salinity Unit (PSU) and the northern region ranging from 31.4 to 33.9 PSU (Fig. S3A). The temperature (T) of the surface waters showed less variation than the salinity, ranging from 12 to 14.5 °C (Fig. S3B). The T-S plot also confirmed the different surface water masses where the density of surface waters at stations 1, 13, and 14 highly deviated from the densities of other stations (Fig. S3C). Based on Chl *a* concentration, higher Chl *a* in the east and an area of lower Chl *a* in the west (Fig. S3D) with an average Chl a concentration of 0.66 ± 0.32 µg L^{-1}.

3.2. MPs abundance and distribution

MPs were found at all stations. The MPs concentrations varied between 11 and 87 MP m^{-3} (Fig. 3), with a median of 34 MP m^{-3} (average 39 ± 23 MP m^{-3}) (Table 1). The lowest MPs concentration was found at station 3 and the highest at station 1 (Fig. 3). The estimated total mass of MPs varied greatly among study sites and ranged from 0.2 to 193.1 µg m^{-3}, with a median of 6.1 µgm^{-3} (average 27.9 ± 52.2 µg m^{-3}) (Table 1). The distribution of total MPs shows a tendency toward higher concentrations in the southern and northern parts of the study area

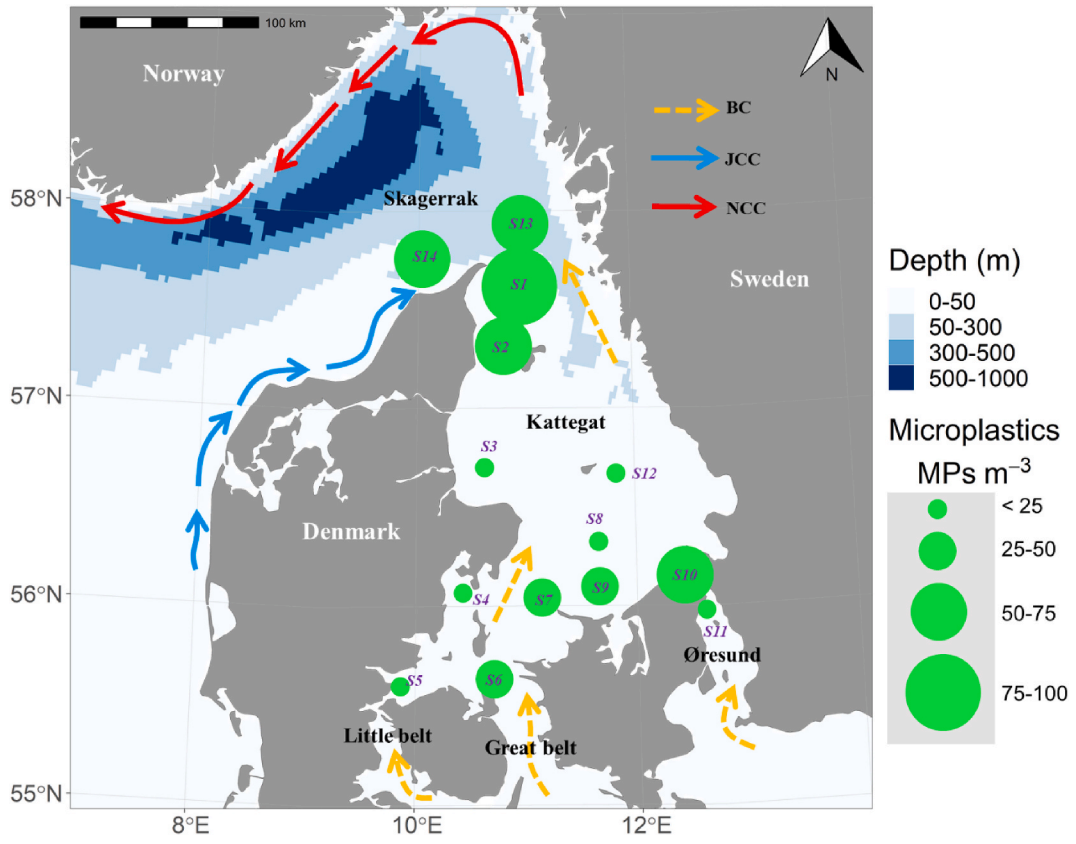

Fig. 3. General currents of Danish marine waters and horizontal distribution of MPs concentration (MPs m^{-3}).

Table 1
Summary of the data obtained from the UFO samples collected in surface waters of the Kattegat and ratio between phytoplankton and MP biomass. Volume = Filtered water volume per sample. Length = Average major dimension, Mass = total mass of MPs (μg m^{-3}) The phytoplankton biomass was calculated from the average *Chl* a at 5 m, a carbon/chl a conversion factor of 42.6 (Juul-Pedersen et al., 2006) and a carbon to dry weight (DW) conversion factor of 55% (Hansen et al., 1994).

Stations	Volume (m^3)	MPs m^{-3}	Length (μm)	Mass (μg m^{-3})	Phytoplankton biomass (DW, mg m^{-3})	Ratio Phytoplakton:MPs (x10^3 mass:mass)
St1	1.08	87	156	69.9	215	3.1
St2	1.25	59	191	32.6	32	1.0
St3	0.84	11	135	1.3	69	52.8
St4	1.06	22	138	1.9	86	45.4
St5	1.33	22	144	9.4	87	9.2
St6	1.18	39	235	193	102	0.5
St7	0.97	29	293	6.0	41	6.8
St8	1.17	20	164	4.7	161	34.2
St9	1.05	40	145	6.5	20	3.1
St10	1.01	57	66	2.3	17	7.2
St11	0.92	24	173	52.9	63	1.2
St12	0.90	16	50	0.2	20	99.4
St13	1.03	63	108	3.9	166	42.6
St14	0.73	62	109	6.2	254	41.0

(Fig. 3). Both the total number of MPs ($r = 0.57$, $p < 0.05$) and the low-density MPs (<1 gcm^{-3}) ($r = 0.65$, $p < 0.05$) were positively correlated with the density of the seawater in the studied area (Table S3).

3.3. Plastic polymer composition and shape

The polymer composition was dominated by polyester (46%), followed by polypropylene (16%), polyethylene (6%), antifouling paints (6%), cellulose ester (5%) and polyvinyl chloride (3%) (Fig. 4A). In addition, Fig. 4A also showed that there were other polymers including acrylic paint, polyamide, polyurethane, polystyrene, and modified cellulose, etc. Comprised up to 18% of the samples.

The particle type was classified based on the ratio between major dimensions (length) to minor dimensions (width). The MP particle was considered a fiber when the length was three times longer than the width; otherwise, it was counted as a fragment (Vianello et al., 2019). The majority of the plastic particles were fragments (56%), and fibers accounted for 44% (Fig. 4B). In both categories polyester and polypropylene were the dominant polymer group and most MPs were identified as polyester fibers (72%).

The polymer composition and proportion varied considerably among the study sites (Fig. 4C). For example at station 7, the percentage of polyester accounted for 93% and anti-fouling paints for 7%. On the other hand at station 1, the polymer composition was constituted of polyethylene (33%), polypropylene (30%), polyester (20%), and anti-fouling paints (9%). This polymer ("type") diversity was also examined by the

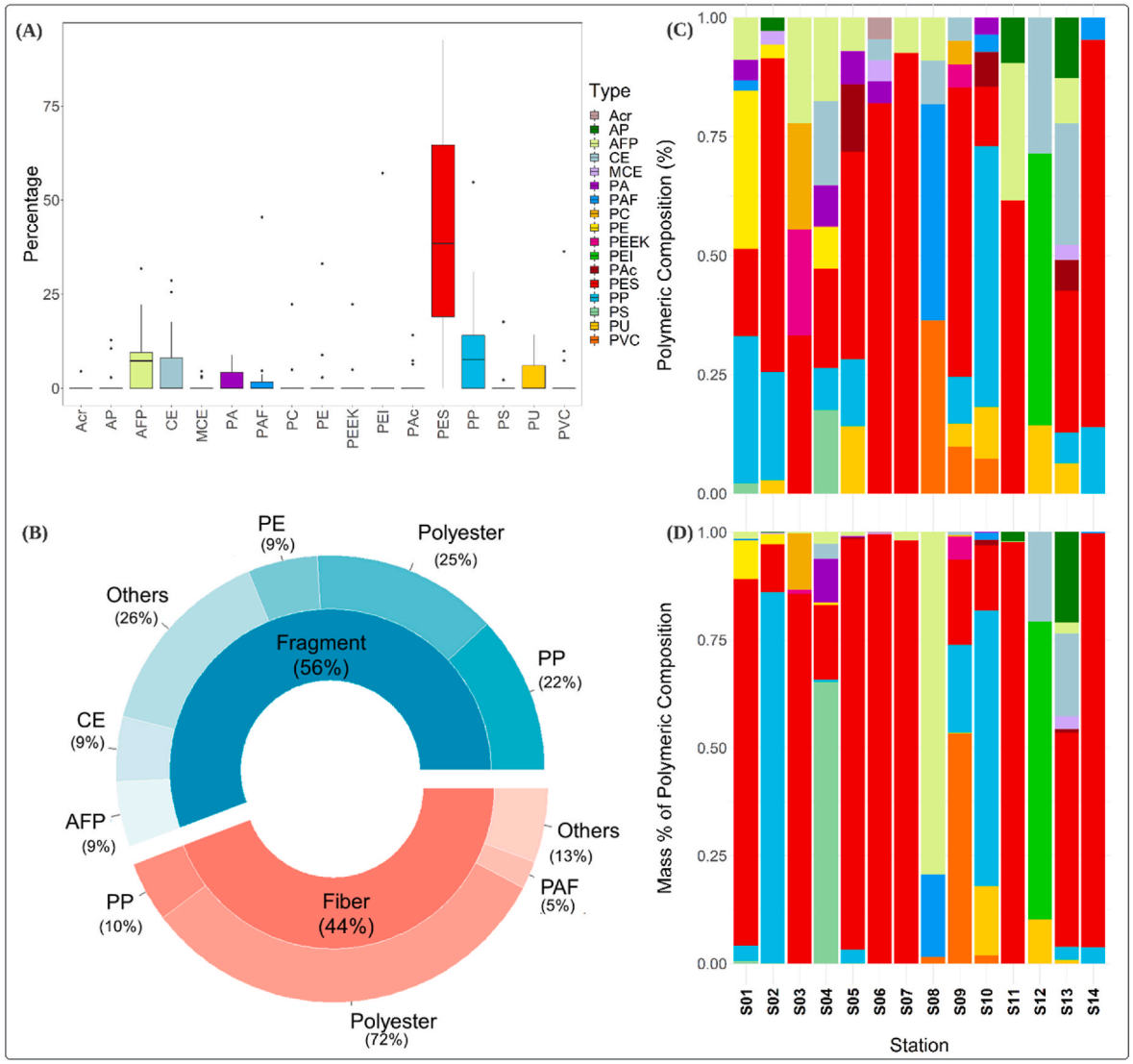

Fig. 4. (A) Average percentage of each polymer type (B) The distribution of particle type and major polymer composition of MPs (C) Spatial distribution of polymeric composition (%) (D) Spatial distribution of mass of polymeric composition (%) in the study sites. (Acr: Acrylic, AFP: Antifouling paint, AP: Acrylic paint, CE: Cellulose ester, MCE: Modified cellulose ester, PA: Polyamide, PAc: Polyacrylamide, PAF: Polyacrylonitrile fiber, PC: Polycarbonate, PE: Polyethylene, PEEK: Polyether ether ketone, PEI: Polyethylenimine, PES: Polyester/Polyethylene terephthalate, PP: Polypropylene, PS: Polystyrene, PU: Polyurethane, PVC: Polyvinylchloride).

Shannon-Wiener (H′) index, where a higher index was found at station 4 (H' = 1.88) and station 13 (H' = 1.83). At the same time, station 7 (H' = 0.26) and station 14 (H' = 0.40) exhibited the lowest indices. Further, the highest number of different polymers (richness = 8) were recorded at station 13, while only two type of polymers were detected in stations 7 and14 (richness = 2).

Concerning the mass of the polymers, polyester and polypropylene accounted for 94% of the total polymer composition while, other remaining polymers accounted for only 6% of the mass. The median of the MPs fiber mass was 0.24 μg (average: 0.79 ± 1.76 μg) while 0.05 μg (average: 0.31 ± 0.74 μg) was the median mass estimates of the MPs fragments. The total polyester mass was above 95% from St5, St6, St7, St11, and St14. Polypropylene was recorded at a higher percentage at St2 (86%) and St10 (64%). Further, antifouling paints were encompassed higher at St8 (79%). Overall, the mass of the different polymers considerably varied among the stations (Fig. 4D). Furthermore, the bioavailability of MPs in terms of the ratio between the mass of phytoplankton and MPs (mass of phytoplankton: mass of MPs) was highly variable between stations ranging from 528 to 99,400 times more phytoplankton than MPs (Table 1).

3.4. Size of MPs

With respect to the particle size range, 88% of the length and 98% of width of all particles was below 300 μm, (Fig. 5A). The median values of length and width for total MPs were 79.6 μm (average: 151.2 ± 196.5 μm) and 27.6 μm (average: 36.4 ± 37.2 μm) respectively. Among these MPs, fibers comprised 75% of the length and 99% of the width that were less than 300 μm (Fig. 5B). The median of length and width for fibers were 178.2 μm (average: 248.2 ± 246.2 μm) and 26.8 μm (average: 37.4 ± 41.0 μm) respectively. On the other hand, 98% of fragments were below 300 μm with a median length and width of 51.7 μm (average: 74.4 ± 83.8 μm), 27.6 μm (average: 35.5 ± 32.8 μm) respectively (Fig. 5C).

3.5. Quality control

A median of 1.16 MPs per sample was present in the three air blanks. On the other hand, a median of 4.7 MPs per procedural blank was discovered among the four procedural blanks. The polymer composition of the contaminating MPs in the blanks was 77% polyester, 9% polyamide, 5%, polyacrylonitrile fiber, 5% polyethylene, 2% of both

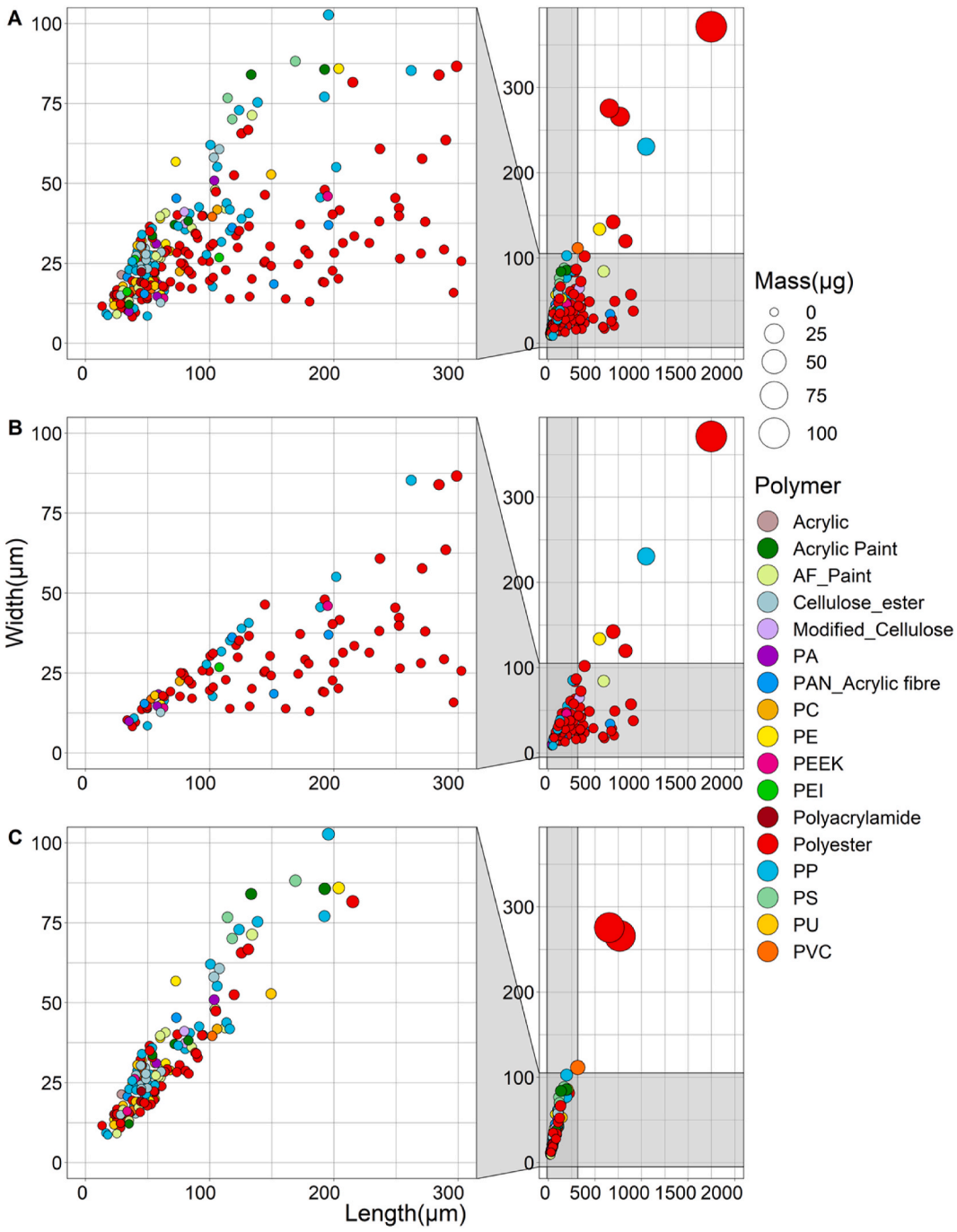

Fig. 5. The distribution of particle size (major dimension = length, minor dimension = width) and estimated mass of the sampled MPs in the Kattegat Sea (A) Total MPs (B) Fibers and (C) Fragments. AF_Paint: Antifouling paint, AP: Acrylic paint, PA: Polyamide, PC: Polycarbonate, PE: Polyethylene, PEEK: Polyether ether ketone, PEI: Polyethylenimine, PP: Polypropylene, PS: Polystyrene, PU: Polyurethane, PVC: Polyvinylchloride).

polypropylene and acrylic paint. For both air and procedure blanks, the polyesters had the medians of 1.25 MPs (particle numbers) and 0.03 μg (mass), whereas the others were zero. In order to correct contamination, the median contribution of each polymer discovered in the blank, which is polyester, was subtracted from the data (Table S4).

4. Discussion

4.1. Oceanographic parameters, concentration and distribution of MPs

Among oceanographic factors, the observed positive correlation between MPs and seawater density indicates that salinity, plays an important role in the distribution of MPs in surface seawater of the studied area. Further, the concentration of low-density polymers like polyethylene (0.85–0.92 gcm^{-3}) and polypropylene (0.89–0.98 gcm^{-3}) generally floating on the surface (Liu et al., 2022), also showed a positive correlation with seawater density ($r = 0.65$, $p < 0.05$). However, vertical mixing can bring the MPs into different layers (Uurasjärvi et al., 2021). Nevertheless, the morphology of coastal regions, sea surface temperature changes, ocean circulation patterns, coastal upwelling, and weather conditions also influence the distribution and aggregation of MPs hot spots (Welden and Lusher, 2017). According to Liu et al. (2022), current velocity strongly affects the accumulation, abundance, and distribution of MPs, in addition to the seawater temperature and

salinity, which affect the density and movement of water. Thereby, further studies accompanying sampling with other hydrological factors, including current velocity and seasonal sampling, could explain the abundance and distribution patterns of MPs in this study area are recommended.

On the other hand, knowledge of the abundance and distribution of MPs (<300 μm) is essential to determine the fate of plastic pollution in marine ecosystems. Although studies on these small size MPs below 300 μm are scarce, the concentrations of which small size MPs found in the studied area were approximately 2–10 times lower than in other ocean surface waters (Enders et al., 2015; Rist et al., 2020; Kameda et al., 2021) (Table S5). For example, using the same filter-pump system and analytical methods, the concentration of MPs in Greenland was, on average, three times higher than we found (Rist et al., 2020). These results are surprising since the Kattegat is a semi-enclosed rather shallow sea with multiple sources of plastic pollution, including the input of surface waters from the Baltic Sea. Northern and Southern Kattegat presented the highest concentrations of plastics likely because the sampling stations were close to harbor regions (in the North) or straits connecting with the Baltic Sea in the South. In addition, currents from the North Sea, mainly the Jutland Coastal Current, also play a crucial role to carrying the pollutants and nutrients to the Kattegat (Heilmann et al., 1994; Skogen et al., 1997).

The distribution of MPs in the ocean are patchy and high concentrations of MPs have been observed as aggregations of floating litter (Cózar et al., 2021; Adamopoulou et al., 2021). However, despite the very calm weather during the cruise, we did not observe litter aggregations in Kattegat. The seasonal changes, including weather conditions, also attribute MPs distribution differently in surface waters along with the hydrodynamic characteristics of seawater (Jiang et al., 2020). Meantime, the role of land sources seems unclear based on our data (e.g., St 3 shows the lowest concentration of MPs despite being close to potential land sources and river inputs). Semi-enclosed environments and stagnant waters may retain a comparatively high number of MPs due to limited water circulation (Frias et al., 2020). Simulations of hydrodynamic models demonstrating the transportation of MPs via water masses are needed to better evaluate the distribution and fate of MPs in the Kattegat/Skagerrak. Atmospheric deposition of airborne MPs can also be a potential source of marine MPs (Ferrero et al., 2022). However, the size limit and detection methods of these studies were different; thus, pretreatment, detection limit, and detection tools of the analysis of airborne MPs also influence the final outcomes of the study (Ferrero et al., 2022). Due to these uncertainties and the limited field data, further research is needed to assess the contribution of airborne MPs on plastic pollution in the surface waters of the Kattegat/Skagerrak.

4.2. Polymeric composition of marine microplastics

The present study shows that the dominant plastic polymer type detected was polyester. It has been noted that one of the top-produced and used polyester is polyethylene terephthalate, the most common thermoplastic polymeric resin (Geyer et al., 2017; Rist et al., 2020). Moreover, polyethylene (36%), polypropylene (21%), and polyvinyl chloride (12%) were also top-produced polymers globally (Geyer et al., 2017). Cellulose esters also have been accounted as one of the commercially important polymers for nearly a century around the globe (Edgar et al., 2001) and they are mainly used in cigarette filters in the form of cellulose acetate which are one of the most dominant forms of marine debris (Araújo and Costa, 2019). Antifouling paints are used to inhibit fouling organisms on boats and were found in significant amounts in the study area. It is worth mentioning that the identification of paint particles is not that straightforward, due to the large amount of different formulations and the risk of cross cross-contamination, as highlighted by Leistenschneider et al. (2021), they found that 45.5% of all recovered MPs in the Weddell Sea derived from vessel-induced cross cross-contamination. However, our study carried out a systematic quality control, including potential contamination from the vessel's paints, which were identified, included in the reference spectra library, and then excluded from the quantification of MPs. Furthermore, the recovery test indicated that the enzymatic digestion protocol possibly recovered 89–92% of the MPs particles from the samples which is collected from the accompanying study applying the same protocol (Liu et al. under review).

4.3. Sizes of marine microplastics (including methodological aspects)

MPs overlap in size with phytoplankton making them available to planktivorous organisms (e.g., Hansen et al., 1994; Rist et al., 2020; Rodríguez-Torres et al., 2020; Almeda et al., 2021) and potentially transferred up marine planktonic food webs. MPs sampling in surface waters has been commonly done with manta nets having a mesh size of around 330 μm, which capture small size MPs only in association with aggregates. Lindeque et al. (2020) reported that 100 μm surface trawls trap higher amounts of MPs than the 333 μm (2.5 fold) and 500 μm (10 fold) trawls. Also, plankton-sized MPs, equivalent to these small size MPs, down to 10 μm, have been quantified in Arctic and Atlantic waters (Enders et al., 2015; Rist et al., 2020). Rist et al., 2020, found that the concentration of MPs (>300 μm) collected with bongo net samples (median 0.12 MP m^{-3}) were 2–3 orders of magnitude lower than those collected by the UFO pump (<10 μm). Hänninen et al. (2021) also found similar low concentrations of MPs in the Arctic Ocean, the North Sea and the Baltic Sea when using the manta net (e.g., 0.06 MP m^{-3}). In general, the concentration of MPs in seawater collected with pumps using small mesh size filters (10–20 μm) is several orders of magnitude higher than those collected with a manta net. Although manta and other nets, have the advantage of filtering larger volumes and covering larger areas than the pump-filters, they underestimate the total amount of MPs since the particles below 300 μm MP are the dominant size fraction of MPs as observed here. Thus, the combined use of both sampling methods can provide a better estimation of the level of plastic pollution in surface waters.

4.4. Ecological implications

Potential bioavailability and uptake of small MPs raises concerns for ingestion and trophic transfer of MPs by many marine organisms (e.g., Cole et al., 2014). However, the concentration of MPs in The Kattegat surface water was found to be low, reducing the risk of encounter and ingestion of MPs by zooplankton. The *in situ* ratio between phytoplankton biomass to MPs in the studied surface waters (Table 1) was very high and consequently the risk of detrimental effects of MPs on zooplankton is minor. Also, the risk of ingestion of MPs by planktonic grazers is reduced by their selective feeding behavior, which allows copepods to discriminate MPs from similar sized phytoplankton cells (Xu et al., 2022).

Laboratory studies show that Effect Concentrations (ECs) of MPs on the main planktonic groups (phytoplankton, zooplankton, meroplankton and ichthyoplankton) are usually $>2.0 \times 10^8$ MP m^{-3} (>200 MP mL^{-1}) (e.g., Cole et al., 2013; Lee et al., 2013; Setälä et al., 2014; Vroom et al., 2017; Jeong et al., 2017; Choi et al., 2020; Rodrigues et al., 2021). Therefore, based on the ECs observed in laboratory studies, the concentrations of MPs found in surface waters of Danish marine waters ($<1.0 \times 10^2$ MP m^{-3}) six orders of magnitude lower are not expected to cause any impact on the planktonic food web. However, more research on the effects of different types of plastics (Bai et al., 2021) and their leached additives (Page et al., 2022) is required to better evaluate the potential impacts of microplastic pollution on the pelagic food web.

5. Conclusions

MPs were ubiquitously distributed in the surface waters of Kattegat and Skagerrak (Denmark) with polyester and polypropylene the

dominant polymers. MPs <300 μm constituted up to 88% of the total MPs in marine surface waters and their concentration in the study area was positively corelated to sea water salinity. The higher concentrations of MPs were found in the Northern and Southern stations, which connects towards the North Sea and the Baltic Sea region, respectively. Overall, the concentration of MPs in the Kattegat and Skagerrak in the study period was very low (<100 MP m^{-3}) compared to laboratory-effect concentrations on marine pelagic organisms (typically >200 MP mL^{-1}), suggesting a low impact on the pelagic food web.

Author statement

Kuddithamby Gunaalan: laboratory and data analysis methodological application, writing - original draft, graphical representations. **Rodrigo Almeda:** conceptualization, supervision, writing - review & editing, resources, funding acquisition. **Claudia Lorenz:** methodological application, writing - review & editing. **Alvise Vianello:** methodological application, writing - review & editing. **Lucian Iordachescu:** methodological application, review & editing. **Konstantinos Papacharalampos:** methodological application, review & editing. **Christian Mathias Rohde Kiær:** methodological application, review & editing. **Jes Vollertsen:** conceptualization, supervision, writing - review & editing, resources, funding acquisition. **Torkel Gissel Nielsen:** conceptualization, supervision, writing - review & editing, resources, funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2022.120853.

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