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Microplastic analysis in the South Funen Archipelago, Baltic Sea, implementing manta trawling and bulk sampling

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ABSTRACT

Microplastic contamination in surface waters of the South Funen Archipelago in Denmark was assessed. Therefore, ten manta trawls were conducted in June 2015. Moreover, 31 low-volume bulk samples were taken to evaluate, whether consistent results in comparison to the net-based approach can be obtained. Microplastic contamination in the South Funen Archipelago (0.07 ± 0.02 particles/m^3) is slightly below values reported before. The sheltered position of the study area, low population pressure on adjacent islands and the absence of any major potential point sources were identified as major factors explaining the low concentration of microplastics. Within the Archipelago, harbors or marinas and the associated vessel traffic are the most probable sources of microplastics. The concentration of microplastics in low-volume bulk samples is not comparable to manta trawl results. This is mainly due to insufficient representativeness of the bulk sample volumes.

1. Introduction

The contamination of natural habitats with plastic litter has become an emerging topic for environmental scientists in recent decades (Galgani, 2015; Galgani et al., 2013; Wright et al., 2013; Thompson, 2004). The long-term durability of most plastic polymers, increasing production rates on a global scale, the unsustainable usage of plastics and inadequate waste management have led to the accumulation of plastics in ecosystems worldwide (PlasticsEurope, 2016; Barnes et al., 2009).

Microplastics in particular can pose various threats to ecosystems. Plastic particles may act as a transporting vector for toxic contaminants, when compounds such as Persistent Organic Pollutants (POPs) or heavy metals adsorb to the surface (GESAMP, 2015). Moreover, it was shown that various species can misinterpret microplastics for their actual food (Lusher et al., 2016, Battaglia et al., 2016). Besides potential negative impact on the respective metabolism (Lu et al., 2016; Watts et al., 2016), an accumulation of adsorbed contaminants into the food chain has to be considered, which includes potential threats to humans, as well (Galgani et al., 2015; Van Cauwenberghe et al., 2015; Cole et al., 2013). However, the impacts of plastic debris exceed solely ecological consequences. Economical (e.g. disadvantages for fishery and tourism) as well as social effects (e.g. reduced recreational value of natural landscapes) have to be taken into account and emphasize the need for further research (Newman et al., 2015). In general, the harmonization of sampling and analysis methods and the extension of the data base on microplastic abundances have largely been identified as important research topic (GESAMP, 2015; Löder and Gerdts, 2015).

Microplastics are commonly defined as plastic particles being smaller than 5 mm in their longitudinal orientation (GESAMP, 2015; Arthur et al., 2009). To the present, the lower size limit of about 10 µm is determined by analytical limitations (Enders et al., 2015; Lenz et al., 2015), but theoretically covers particles down to 1 µm in diameter (Magnusson et al., 2016).

A further distinction is made between primary and secondary microplastics (Cole et al., 2011; Fendall and Sewell, 2009). While the first enter the ecosystem directly, i.e. in the form of raw pellets or abrasive scrubs as an ingredient of cosmetics (Fendall and Sewell, 2009), the latter originate from the fragmentation of larger particles. UV(B)-oxidation and mechanical disintegration due to abrasion (e.g. in sand matrix), wave-action and turbulence are dominant processes in this regard (Cole et al., 2011; Barnes et al., 2009).

Microplastics are ubiquitous in the marine environment. To the present day microplastic contamination has been reported in surface waters (e.g. Setälä et al., 2016; Lusher et al., 2014; Song et al., 2014a), the water column (e.g. Enders et al., 2015; Reisser et al., 2015), embedded into sea ice (Obbard et al., 2014), seabed sediments (e.g. Zobkov and Esiukova, 2017; Woodall et al., 2014), coastal sediments (e.g. Graca et al., 2017; Stolte et al., 2015) and organisms such as fishes (e.g. Bellas et al., 2016) or annelids (e.g. Gusmão et al., 2016).

In situ littering from fishing or shipping (commercial and recreational) directly adds to microplastic pollution in the marine ecosystem.

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For terrestrial sources river based input, including waste water (WWTP effluents), stormwaters, lateral input via beaches and shore lines and atmospheric deposition have to be considered (Magnusson et al., 2016).

Major driving factors for the distribution of microplastics in surface waters and the water column are sea currents, waves as well as predominant wind patterns (Liubartseva et al., 2016; Gago et al., 2015; Reisser et al., 2015). The first is demonstrated by the concentration of microplastics in the large ocean gyres (Gago et al., 2015; Eriksen et al., 2013; Law et al., 2010). The latter can lead to a reduction of microplastic concentrations in the surface layer due to wind driven vertical mixing (Reisser et al., 2015; Kukulka et al., 2012).

The assessment of microplastic contamination in marine surface waters mostly relies on net-based (volume-reduced) sampling approaches (Eriksen et al., 2013; Hidalgo-Ruz et al., 2012). Manta trawl is a commonly used device, which was originally designed for the collection of plankton (Moore et al., 2002). Occasionally, neuston nets are used, which sample the air-water interface for microplastics (Morét-Ferguson et al., 2010). The lower detection size of both systems is defined by the specific net that usually (for sampling of aquatic environments) has a mesh size of 300 µm or 333 µm (Setälä et al., 2016; Mani et al., 2015; Eriksen et al., 2013). For sampling within the water column, bongo (Doyle et al., 2011) and other plankton nets have been applied (Hidalgo-Ruz et al., 2012). It has become evident that a majority of particles found in environmental samples is sized smaller than 300 µm (e.g. Enders et al., 2016). Since the application of finer nets could be hampered due to clogging effects, the need to implement bulk sampling techniques was identified (Setälä et al., 2016). Though, for this method sample volumes differ on a great scale. While Dubaish and Liebezeit (2013) took two replicates of 100 ml, Lusher et al. (2015) sampled 2000 l investigating sub-surface water. Several studies relied on sample volumes well below 100 l (Bagaev et al., 2017; Zhao et al., 2014; Dubaish and Liebezeit, 2013; Ng and Obbard, 2006).

To quantify microplastic in water samples it has to be distinguished from biogenic matter that can interfere the identification process (Song et al., 2015; Hidalgo-Ruz et al., 2012). To reduce the organic content of microplastic samples acidic (De Witte et al., 2014; Claessens et al., 2013), alkaline (Cole et al., 2014; Foekema et al., 2013), oxidizing (Avio et al., 2015; Collard et al., 2015; Tagg et al., 2015; Nuelle et al., 2014) or enzymatic digestion protocols have been applied (Löder and Gerdts, 2015; Cole et al., 2014).

Micro-Fourier transform infrared (FTIR) spectroscopy (Song et al., 2015; Tagg et al., 2015; Cole et al., 2014), micro-Raman-spectroscopy (Cole et al., 2013; Van Cauwenberghe et al., 2013) and Pyrolysis-gas-chromatography with mass spectrometry (Dekiff et al., 2014; Nuelle et al., 2014; Fries et al., 2013) are frequently applied to assess polymer abundance and composition (Ivleva et al., 2017; Löder and Gerdts, 2015).

Moreover, differential staining based on the fluorescent lipophilic dye Nile Red (9-diethylamino-5H-benzo[α]phenoxazine-5-one) has been implemented for microplastic quantification (Erni-Cassola et al., 2017; Maes et al., 2017; Fischer et al., 2016; Shim et al., 2016; Desforges et al., 2014; Shim et al., 2014; Song et al., 2014b; Andrady, 2010). The spectral characteristic of the fluorescence emission depends on the respective solvent as well as the polarity of the stained polymer (Maes et al., 2017; Shim et al., 2016). This approach allows a quick and inexpensive estimation of the microplastic load in a sample without giving detailed information on the chemical composition of the particles (Tamminga et al., 2017; Song et al., 2014b).

2. Material & methods

2.1. Study area

The South Funen Archipelago is situated in the transition zone between the Baltic Sea and the Kattegat in Denmark (Fig. 1). In total, it consists of 55 islands and is delimited by the islands of Lyø in the west, Ærø in the south, Langeland and Tåsinge in the east and Funen in the north. Svendborg (population 2016: 27,074) and Faaborg (population 2016: 7178), located at the southern coast of Funen are the largest cities in the region (Statistics Denmark, 2017).

Due to its remoteness and natural landscape, the region is a favorable destination for tourists. Especially water sports (e.g. sailing, angling or sea kayaking) are popular among both, the local population and visitors from abroad. Several small coastal villages such as Ærøskøbing or Søby on the island of Ærø are harboring marinas, which are frequented by smaller and larger vessels. Additionally, regular ferry connections between all major islands exist.

Complex hydrographic processes can be identified within the Skagerrak-Kattegat region. In general, a surface layer of low salinity (up to 1.5%) flows northwards through the Little and Great Belt straits (Omstedt et al., 2014). This is compensated by a denser, high-saline (up to 33%) and near-bottom stream of Skagerrak water entering the Baltic Sea. In the South Funen Archipelago the water column is mixed almost constantly without developing any considerable strata (Rask et al., 1999).

Mean wind conditions at Tåsinge Island (closest climate station with data available) are displayed in Fig. 2. Wind patterns are dominated by westerlies, in general. East to west wind flows develop as well, especially in winter. At this time increased frequencies of heavy winds lead to intensified mixing within in the water column (Rask et al., 1999).

2.2. Strategy for microplastic sampling in surface and subsurface water

All sampling was conducted between June 16th and 19th, 2015 aboard the sailing ship *Lovis* in the South Funen Archipelago, Denmark. A total of ten manta trawls were carried out to investigate microplastic contamination within the uppermost water layer. Additionally, 27 bulk-samples in open waters in three depths (0.5 m, 2.0 m and 5.0 m) and four bulk-samples in harbors (0.5 m depth in Faaborg, Ærøskøbing, Søby and Lyø) were taken throughout the area to test for the comparability of low-volume bulk-samples towards manta trawls. Table 1 provides an overview of manta sampling related data.

The manta trawl was positioned at the windward side of the ship's hull to exclude any vessel-based contamination of the sample. The manta was recovered after 20 min of trawling. Special care was given to avoid any backflow during the recovery, which could have possibly flushed out parts of the sample at the cod-end. In previous sampling protocols, trawling times between one and three hours were suggested. However, Fischer et al. (2016) recommended trawling durations of < 60 min to prevent potential clogging effects of the net in case of high organic contents, which might lead to minor results. In order to avert these issues, trawling times were kept at 20 min in this study. Mean trawling speed was 4.2 km/h (maximum 5.95 km/h) and thereby well within the recommended range (5-Gyres Institute, 2014). The covered distance was tracked via D-GPS (Trimble Geo 7×) and ranged from 1146 m to 1746 m depending on the trawling speed. To calculate the sampled area and volume the recorded distance was multiplied with the width or the area of the manta opening, respectively.

Aboard the *Lovis,* the cod-end was detached and the sample volume was transferred into a stainless-steel bowl. Afterwards, the cod-end was inverted and thoroughly rinsed with purified water, until all sample material was recovered. It was then transferred into brown glass jars and treated with 10 ml of hydrochloric acid (HCl, 37%, Merck Emsure®) per jar to stop biological processes.

The bulk sampling was done by means of an Integrated Water Sampler (IWS, HYDRO-BIOS GmbH). After the entire sample volume (5 l) was automatically pumped into the plexiglass hull via a valve at the bottom of the IWS, the device was recovered on deck. Here, the sample was poured through a sieving cascade with mesh sizes of 5.0 mm, 1.0 mm and 0.3 mm. Subsequently, the sieve contents (5 mm– > 1 mm, 1 mm– > 0.3 mm) were transferred into brown glass vials via rinsing with little purified water. The sample

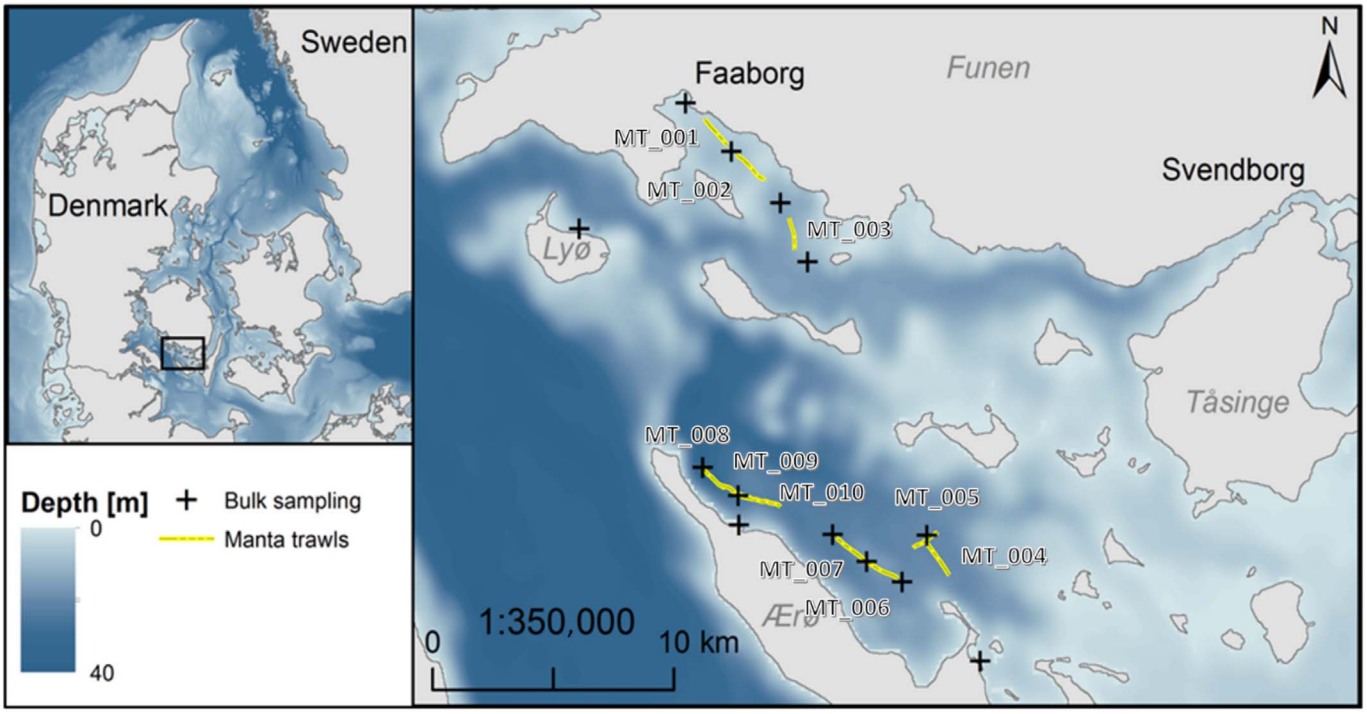

Fig. 1. The South Funen Archipelago and sampling sites, coordinate system: WGS_1984_UTM_Zone_32N, projection: Transverse Mercator, bathymetry: Baltic Sea Hydrographic Commission (BSHC) (2013).

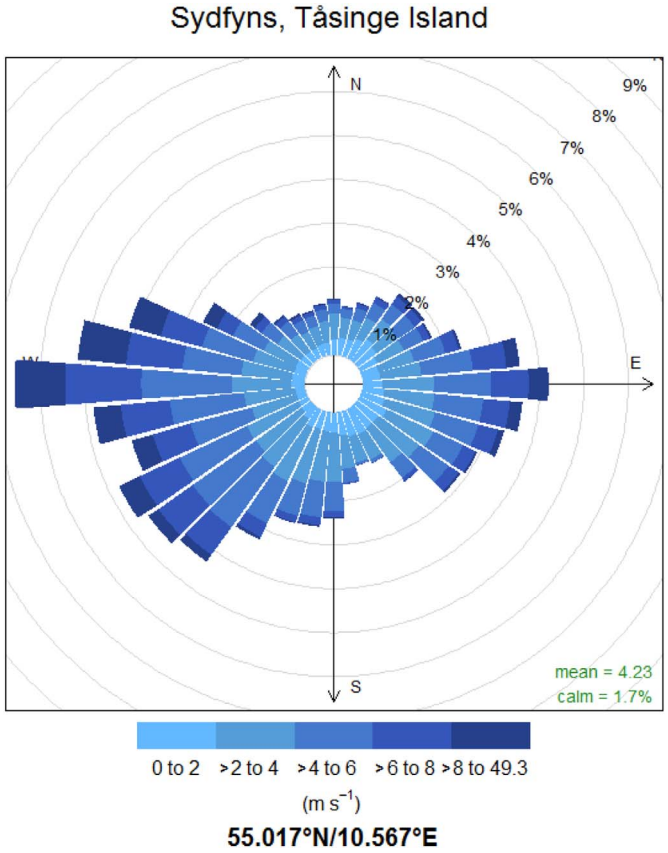

Fig. 2. Summary of the wind situation at Tåsinge Island between 1984 and 2014, wind direction as frequency (% of total), wind speed as the six hour mean, data: NOAA, 2016, visualization: Carslaw and Ropkins (2012).

volume ≤ 0.3 mm was collected in a beaker at the bottom of the cascade, vacuum filtrated (413, VWR International, particle retention 5–13 μm) and filters were stored in glass petri dishes. This filtration had to be limited to a volume of 2 l, since the utilized filter showed a tendency to clog, when larger volumes were applied.

To stop any biological processes, 1 ml of hydrochloric acid was added to each vial. All samples were stored in a cooling box until further processing in the laboratory of the Institute of Geography at the University of Hamburg.

2.3. Laboratory analysis

To allow a standardized processing of all manta samples, the contents of the brown glass jars were poured through a 0.063 mm sieve in order to remove the water volume from the sample. The sieve contents were then transferred into glass beakers via rinsing with little purified water.

The destruction of organic matter was conducted as follows:

At first, hydrogen peroxide (H_2O_2, 50%, AppliChem, 60 ml per 50 ml sample volume) was added to the sample (cf. Dris et al., 2015; Free et al., 2014). The beaker was covered with a watch glass and incubated for seven days at room temperature (168 h). Afterwards, the hydrogen peroxide volume was removed via sieving (0.063 mm) and rinsing with purified water. Secondly, after retransferring the sample into the beaker with little purified water, the volume was adjusted to 30 ml of purified water each. The sample was then treated with 10 ml sodium hypochlorite solution (NaClO, 9–14%, Merck Emplura) and allowed to rest for 24 h at room temperature to remove the remaining organic matter (Enders et al., 2016; Collard et al., 2015; Stojicic et al., 2010).

In the following, samples were size-fractioned by sieving (mesh sizes 1.0 mm, 0.63 mm, 0.3 mm) and relocated to a stainless-steel vacuum filtration system via rinsing with purified water (Satorius Stedim,

Table 1
Sampling data of conducted manta trawls and supplementary meteorological data.

Trawl no.	Transect	Date	Trawling parameters				Wind parameters		
			Duration (min)	Length (m)	Speed (km/h)	Direction	Speed (m/s)	Direction (°)	Beaufort number
MT_001	Faaborg - Ærøskøbing	15/06/16	20	1358	3.80	SE	1.4	302(NW)	1
MT_002	Faaborg - Ærøskøbing	15/06/16	20	1540	3.80	SE - E	4.8	300(NW)	3
MT_003	Faaborg - Ærøskøbing	15/06/16	20	1263	3.50	SE - S	5.8	282(W)	4
MT_004	Ærøskøbing - Søby	15/06/17	20	1624	4.80	NW	5	260(W)	3
MT_005	Ærøskøbing - Søby	15/06/17	20	1158	3.60	SW - WSW	6.7	250(SW)	4
MT_006	Ærøskøbing - Søby	15/06/17	20	1330	4.00	NW - NNW	3.5	265(W)	3
MT_007	Ærøskøbing - Søby	15/06/17	20	1509	4.50	NW	4.5	269(W)	3
MT_008	Søby - Lyø	15/06/18	20	1146	3.35	NW - NNW	4.5	281(W)	3
MT_009	Søby - Lyø	15/06/18	20	1460	4.45	SE	5	320(NW)	3
MT_010	Søby - Lyø	15/06/18	22	1746	5.95	ESE	4.5	315(NW)	3

500 ml funnel capacity). The sample material remaining on the filter was covered with a watch glass and dried for 48 h in glass petri dishes under a fume hood.

Since the IWS samples were size-fractioned aboard the *Lovis* and the load of biogenic matter seemed negligible, they were filtered directly.

In order to identify microplastics on the filter, 1 ml of Nile Red solution (1 mg/ml) was added and left to stain for 48 h covered with a watch glass under a fume hood (Tamminga et al., 2017; Fischer et al., 2016). For manta samples chloroform was used to solve Nile Red, while IWS samples were treated with an acetone solution. Within our analysis it became evident that using chloroform, in contrast to acetone, a larger variety of polymers can be stained (see Tamminga et al., 2017). As IWS samples were processed beforehand acetone was used.

Stained samples were photographed (Pentax K-30, exposure time 2″, ISO 100, resolution 2420 × 2343) under UV-light (Omnilux UV 18 W G13, 365 nm) in a self-constructed photo box. The images were examined for stained particles using the counting tool in Adobe Photoshop CS3. An image catalogue of stained reference particles (various polymer types) was used as a verification measure for the quantification process (Tamminga et al., 2017). Two shape classes (fragments and fibers) were distinguished and recorded separately.

The data analysis was performed using the scripting language R (Version: 3.3.1; R Core Team, 2016) in a RStudio environment (Version: 1.0.136; RStudio Team, 2016). Geodata was processed and visualized with the software ArcGIS by ESRI (Version: 10.3).

2.4. Anti-contamination measures for microplastic analysis

The risk of background contamination is omnipresent in the field of microplastics analysis (Torre et al., 2016; Hidalgo-Ruz et al., 2012). Consequently, special care was given to avoid contamination during the whole analysis as far as possible: Only laboratory coats made of cotton were worn, surfaces were moistened before samples were handled in any way, the humidity within in the laboratory was increased (to avoid aerial contamination), all necessary analysis steps were carried out under a fume hood if applicable and samples were covered whenever possible (e.g. with watch glasses).

Furthermore, procedural laboratory blanks were run parallel with the field samples by undergoing the same processing steps starting with 50 ml of purified water. This was implemented for the laboratory analysis, only. A total of 4 blanks were conducted in terms of the manta samples and 10 blanks were used with regard to the IWS samples (ratio ~1:3). Since most processing steps of the IWS samples took place aboard the *Lovis*, the obtained blank results must be seen as an underestimation of the actual contamination, as only a part of the whole analysis process was covered. The average contamination per filter and shape (fragments and fibers) was subtracted from the microplastic counts (per sampling device).

Table 2
Mean number of microplastic contaminants per blank filter (± standard deviation).

Fraction (mm)	Manta		IWS	
	Fragments	Fibers	Fragments	Fibers
> 1.0–5.0	0	0.5 ± 1.0	–	–
> 0.63–1.0	0	0.7 ± 0.9	–	–
> 0.3–0.63	0	1.5 ± 0.6	–	–
> 0.013–0.3	–	–	0.3 ± 0.6	0.1 ± 0.3

3. Results

The results of blank samples are given in Table 2 and are characterized by the following abundances: For manta blanks, an average number of 0.9 (± 0.9) microplastic particles across all fractions were found. Blank contamination was composed of fibers only and showed distinct variations between the size fractions, with higher counts at smaller sizes. The IWS blanks were hardly affected at all.

Table 3 shows the concentration of microplastics in surface waters of the South Funen Archipelago. A total number of 137 microplastic particles (34 fragments; 103 fibers) was found in all trawl samples. This equals to an average of 0.07 particles/m^3 (± 0.02), including 0.02 fragments/m^3 (± 0.01) and 0.05 fibers/m^3 (± 0.02) or 12,897 particles/km^2 (± 3922), including 4057 fragments/km^2 (± 1388) and 8840 fibers/km^2 (± 3788).

Fig. 3 displays the mean microplastic concentration per size fraction by shape. In general, abundance increases rapidly towards smaller fractions. In particular, this shift is distinct between the fractions > 0.63–1.0 mm and > 0.3–0.63 mm. While the mean concentration of microplastics (both shapes) in surface waters is 0.006 particles/m^3 (± 0.008) for the largest fraction, this value increases to 0.045 particles/m^3 (± 0.025) for the smallest fraction. Additionally and in general, fibers are more abundant than fragments, except for the size range between > 0.63–1.0 mm, where both shapes show similar concentrations.

The spatial distribution of microplastic fragments within the South Funen Archipelago is shown in Fig. 4. The highest concentrations occur in proximity to the harbors of Faaborg and Søby in the western part of the area. Especially trawl no. 1 (MT_001) close to Faaborg revealed a microplastic concentration (0.040 particles/m^3) well above the other trawls (second highest concentration trawl no. 10: 0.026 particles/m^3).

For fibers, no obvious pattern was detected. Both, highest (e.g. MT_010: 0.068 particles/m^3) and lowest (e.g. MT_009: 0.019 particles/m^3) concentrations of microplastic fibers in the area were measured at adjacent sample tracks.

Considering the abundance of microplastic concerning the two different types of shape, the general dominance (share of fibers ≥ 50%) of fibers is obvious at nine out of ten locations sampled. Up to 84.3% of the total microplastic content from trawl no. 4 (MT_004) consisted of

Table 3
Microplastic concentration in surface waters of the South Funen Archipelago by sample location and shape.

Trawl no.	Transect	Trawling parameters				
		Length (m)	Volume (m^3)	Fragments (number/m^3)	Fibers (number/m^3)	Microplastics (number/m^3)
MP15_MT_001	Faaborg - Ærøskøbing	1358	150	0.04	0.04	0.08
MP15_MT_002	Faaborg - Ærøskøbing	1540	170	0.02	0.03	0.05
MP15_MT_003	Faaborg - Ærøskøbing	1263	140	0.02	0.07	0.09
MP15_MT_004	Ærøskøbing - Søby	1624	180	0.01	0.06	0.07
MP15_MT_005	Ærøskøbing - Søby	1158	128	0.02	0.03	0.05
MP15_MT_006	Ærøskøbing - Søby	1330	147	0.02	0.03	0.05
MP15_MT_007	Ærøskøbing - Søby	1509	167	0.02	0.07	0.09
MP15_MT_008	Søby - Lyø	1146	127	0.02	0.06	0.09
MP15_MT_009	Søby - Lyø	1460	162	0.02	0.02	0.04
MP15_MT_010	Søby - Lyø	1746	193	0.03	0.07	0.09

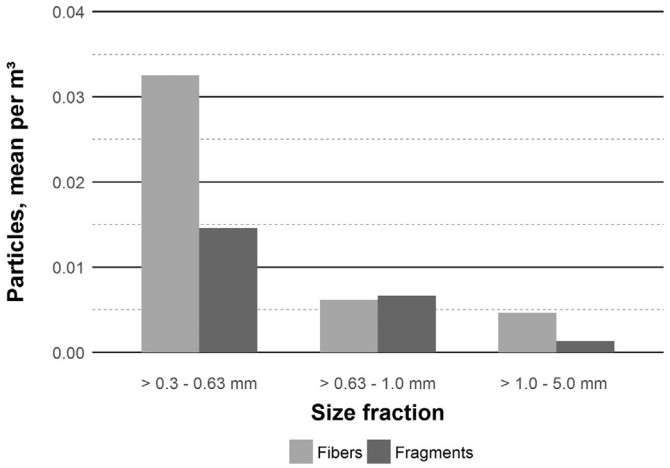

Fig. 3. Mean microplastic concentration in surface waters of the South Funen Archipelago by size fractions and shape.

fibers. The lowest share of fibers was registered at trawl no. 1 (MT_001) close to Faaborg.

A total number of 80 microplastic particles (34 fragments; 46 fibers) were found in all IWS samples (see also Supplementary Information for further details). Since the IWS samples are based on a water volume of maximum 5 l, concentrations refer to particles per liter. This leads to an average of 1.03 particles/l (± 0.80), including 0.35 fragments/l (± 0.38) and 0.68 fibers/l (± 0.62).

4. Discussion

Even though strict anti-contamination measures were taken in this study, it cannot be fully excluded that contaminating plastic particles accumulated on the filters, which strongly emphasizes the need to monitor contamination within the analytical process. These contaminants mainly had a fibrous shape, which is in good accordance with findings of previous studies (Torre et al., 2016; Setälä et al., 2016; Woodall et al., 2015; Nuelle et al., 2014; Fries et al., 2013). Nevertheless, it is advisable to carry out procedural blank samples during the whole sample treatment process, starting from sampling, in the future.

With 0.07 particles/m^3 the concentration of microplastics in surface waters of the study area is only slightly below values reported in the Baltic Sea earlier (Table 4). With the exception of sample locations situated close to large and/or industrial harbors (Norén et al., 2015; Norén, 2007), reported microplastic concentrations mostly are below 1 particle/m^3 (e.g. Setälä et al., 2016; Magnusson et al., 2016; Magnusson, 2014). Moreover, for the publications on studies of microplastic abundances in the Baltic Sea, extreme abundances often go along with lower detection limits due to the sampling equipment (Norén et al., 2014; Norén, 2007). Higher abundances in size fractions below the standard manta mesh size of 300 or 330 µm are indicated. In general, this distribution is also reflected in the results of this study with rising microplastic concentrations towards lower size fractions. However, it also emphasizes the uncertainty imposed by varying methods, which hampers the generation of compatible and comparable results or the discussion of fine-scale variations between different study areas. In contrast to previous findings fibers are more abundant in this study (Free et al., 2014; Eriksen et al., 2013). This is most likely due to agglomerations of synthetic fibers and organic debris (algae) that were observed during the sampling.

The sheltered position of the South Funen Archipelago may prevent higher microplastic loads in the area. The general northward flow of low-saline water towards the Skagerrak containing contaminants from the eastern part of the Baltic Sea passes the investigated area only peripherally (Omstedt et al., 2014). In addition, the absence of large-scale industries and the low population density on the adjacent islands reduces the influence of terrestrial sources in the area. WWTPs, which have been identified as input pathways for plastic debris before, are either small (largest WWTP in the area: Faaborg Renseanlæg, capacity of 70,000 population equivalent) or do not discharge into the South Funen Archipelago (EEA, 2017). No data concerning sea based tourism and fishery is available for the study area, hence potential inputs cannot be quantified. Though, both activities must be seen as potential sources of microplastic pollution (Magnusson et al., 2016).

Looking at the abundance of microplastics within the study area, significantly different distribution patterns of fragments and fibers become apparent. For fragments, the highest concentrations occur in the western part of the archipelago (see Fig. 4). A share of the microplastic fragments might enter the South Funen archipelago through its largest “opening” in the west. Additionally, at least a part of the pollution might be caused in situ as littering in or around the harbors and ferry routes. This possibility is supported by the fact that the highest concentrations were recorded in proximity to the harbors of Faaborg and Søby (no trawl was carried out close to Ærøskøbing) and by the ship density derived from AIS signals (DMA, 2017). Both harbors are frequented by various kinds of vessels and are especially targeted by recreational boats (i.e. for fishing, sailing). As the latter are often made of or coated with synthetic polymers, they might act as source of microplastics (Lassen et al., 2015; Pichel et al., 2012). Abrasive particles from airblast cleaning of vessels and equipment associated with harbour activities may contribute to this, as well. For fibers, no distinct pattern is observable, but it has to be taken into account that these results are biased by the specific sample processing protocol, as stated above.

The distinct difference of microplastic abundances (even when assessing comparable size fractions only) between volume-reduced manta and IWS bulk samples in this study can be attributed to the lack of representativeness of the bulk samples, due to small sample volumes. It seems advisable to set a minimum to ensure that results are not biased by insufficient sample volumes, especially when low concentrations are to be expected. As microplastic concentrations in water are most

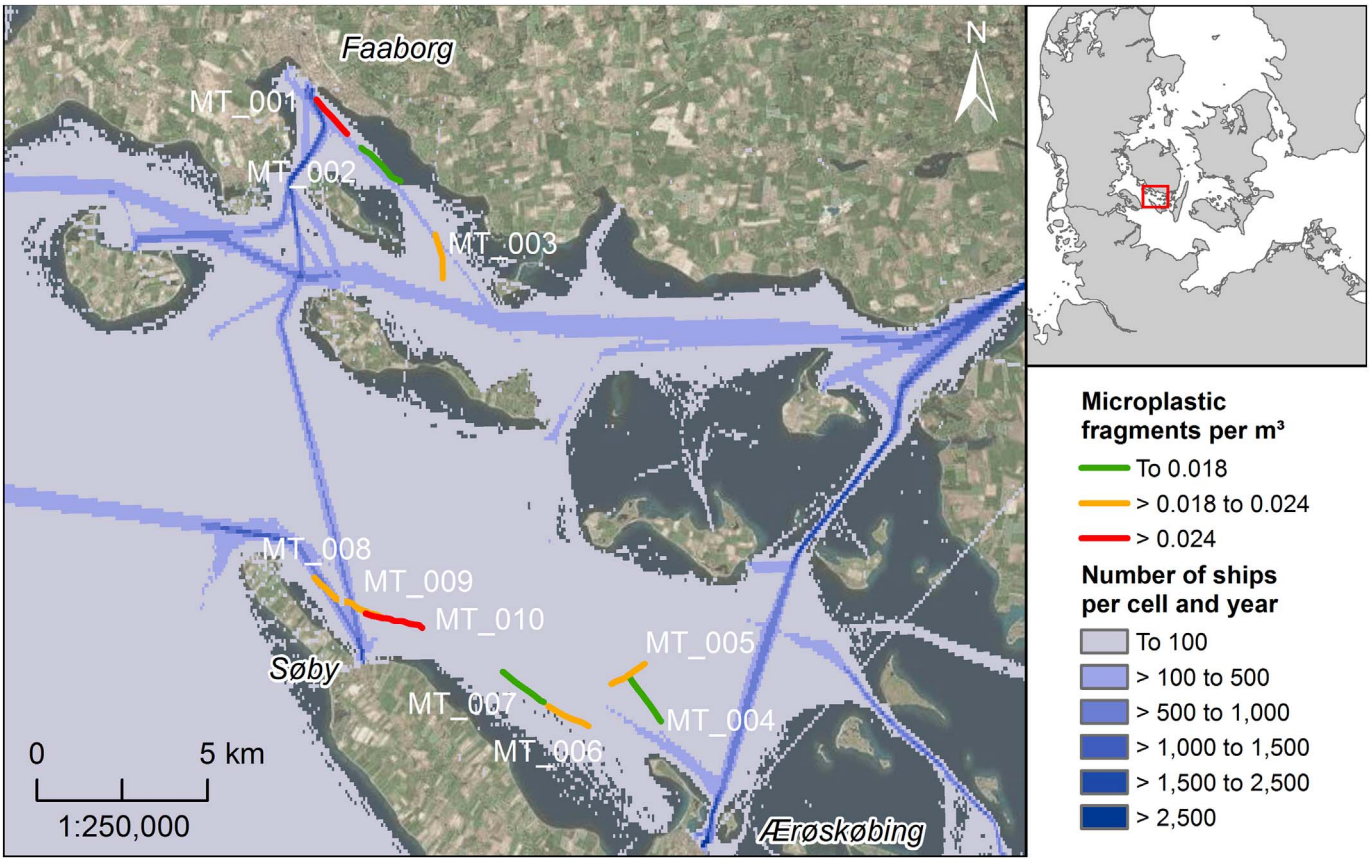

Fig. 4. Concentration of microplastic fragments by trawl location, ship density map: AIS-data (A and B transponders) for Danish waters 2016 (DMA, 2017), coordinate system: WGS_1984_UTM_Zone_32N, projection: Transverse Mercator, background: ESRI world imagery (2016).

commonly displayed as per cubic meter (e.g. Horton et al., 2017; Hidalgo-Ruz et al., 2012), a minimum sample volume of one cubic meter or multiples of it seems appropriate. Still, this threshold needs to be determined in field.

Additionally, sampling and processing induced variations play a role and are well reported and discussed within the scientific community (Setälä et al., 2016; Song et al., 2014a). Most obvious is the detection limitation of net based sampling methods (Dris et al., 2015) and the largely underestimating fiber concentrations when using nets, since fibrous structures tend to easily pass the net (Setälä et al., 2016; Faure et al., 2015), i.e. by aligning themselves in flow direction. The calculation of volume related concentrations via net sampling relies on the precise estimation of the sampled volumes. Otherwise, the sampled water volume will most likely be overestimated, as waves may lead to the partial submersion of the manta trawl (Free et al., 2014).

In our study, a difference in staining for IWS and manta samples lead to another bias in microplastic identification between bulk and net-based sampling.

5. Conclusion

Microplastic pollution in the surface water of the South Funen

Table 4
Abundance of microplastic contaminants as reported in former studies by location and sampling technique related lower detection limit.

Country	Specific site	Abundance (per m^3)	Lower detection limit	Publication	Additional Information
Denmark	North Sea	0.39	> 100 µm	Mintenig, 2014	No fibers included
Denmark	Kattegat	3.54	> 100 µm	Mintenig, 2014	No fibers included
Denmark	The Belt Sea	1.44	> 100 µm	Mintenig, 2014	No fibers included
Denmark	South Funen Archipelago	0.05–0.09	≥ 300 µm	This study	
Finland	Archipelago Sea	0.25	≥ 300 µm	Magnusson, 2014	
Finland	Gulf of Finland	0.62	≥ 333 µm	Setälä et al., 2016	
Sweden	Stockholm Archipelago	0.19–7.73	> 335 µm	Gewert et al., 2017	
Sweden	Göteborg harbour	0.9–2.9	≥ 330 µm	Magnusson et al., 2016	WWTP adjacent
Sweden	Gullmarfjord	0.41	≥ 330 µm	Magnusson et al., 2016	
Sweden	Kattegat	1.08	≥ 300 µm	Norén and Magnusson, 2011	
Sweden	Stenungsund, Industrial harbour	~102,550	> 80 µm	Norén, 2007	
Sweden	Skagerrak	7000–13,000	> 10 µm	Norén et al., 2014	No fibers included
Sweden	Malmö, Industrial harbour	43.01	≥ 300 µm	Norén et al., 2015	
Sweden	Ystad, inner harbour mouth	0.08	≥ 300 µm	Norén et al., 2015	
Great Britain	Western English channel	0.27	≥ 500 µm	Cole et al., 2014	
Great Britain	Northeast Atlantic	2.46	≥ 250 µm	Lusher et al., 2014	

Archipelago is slightly below values reported by previous studies. While a certain portion of microplastics might enter the area through its 'opening' in the west following wind driven surface currents, local harbors and marinas as well as the associated vessel traffic were identified as the most probable sources of microplastic pollution in the area. Moreover, the shape of the particles plays an important role for their behavior. This is reflected in the different distribution of fragments and fibers.

The methodical assessment of fibers was shown to be especially challenging (e.g. overestimation of fibers in small size fractions of manta samples due to agglomerations including organic debris). In this regard the implementation of blank samples within the analytical procedure was proven to be useful and should be extended to the complete sample processing (including sampling) in future investigations. The severely different results for manta trawls and IWS bulk samples underline the need for methodical harmonization and the implementation of sampling devices that are capable of gathering particles smaller than the mesh size of most volume-reduced approaches. To secure a quality standard regarding bulk sampling, the introduction of a minimum sample volume seems necessary.

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