Microplastics accumulate to thin layers in the stratified Baltic Sea

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ABSTRACT

In the Baltic Sea, water is stratified due to differences in density and salinity. The stratification prevents water from mixing, which could affect sinking rates of microplastics in the sea. We studied the accumulation of microplastics to halocline and thermocline. We sampled water with a 100 μm plankton net from vertical transects between halo- and thermocline, and a 30 L water sampler from the end of halocline and the beginning of thermocline. Thereafter, microplastics in the whole sample volumes were analyzed with imaging Fourier transform infrared spectroscopy (FTIR). The plankton net results showed that water column between halo- and thermoclines contained on average 0.92 ± 0.61 MP m−2 (237 ± 277 ng/m3; mean ± SD), whereas the 30 L samples from the end of halocline and the beginning of thermocline contained 0.44 ± 0.52 MP L−1 (106 ± 209 ng L−1). Hence, microplastics are likely to accumulate to thin layers in the halocline and thermocline. The vast majority of the found microplastics were polyethylene, polypropylene and polyethylene terephthalate, which are common plastic types. We did not observe any trend between the density of microplastics and the sampling depth, probably because biofilm formation affected the sinking rates of the particles. Our results indicate the need to sample deeper water layers in addition to surface waters at least in the stratified water bodies to obtain a comprehensive overview of the abundance of microplastics in the aquatic environment.

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1. Introduction

Microplastics (MPs) are common pollutants in the marine environments worldwide (Auta et al., 2017). The definition of MPs is not consistent yet, but they are commonly suggested to be defined as plastic polymer particles between 1 μm–5 mm (Frias and Nash, 2019) or 1 μm–1 mm (Hartmann et al., 2019). MPs can be released to water bodies from manufacturing, use, disposal and fragmentation of large plastic items and products containing microplastics (Auta et al., 2017).

Though the presence of MPs in marine environments has been extensively studied, sampling has mostly been carried out in the surface waters (e.g. Eriksen et al., 2013; Setälä et al., 2016) and knowledge about vertical distribution of MPs in seawater is scarce (Gorokhova, 2015; Choy et al., 2019; Zobkov et al., 2019). In addition to water column, MPs have been detected from deep-sea sediments (Van Cauwenberghe et al., 2013; Woodall et al., 2014), where they presumably sink and accumulate. However, we do not know in detail what happens to MPs between the surface and deeper water column, where thermo- and haloclines form water layers with varying densities and temperatures (Kanhai et al., 2018).

One of the unique features of the Baltic Sea is the strong stratification of the water column. Baltic Sea is a brackish inland sea, which receives saline water only occasionally from the North Atlantic via the Danish Straits. These random saline water intrusions bring dense ocean water to the Southern Baltic Sea. Dense water enters the more northern basins near the bottom, while lighter and fresher waters originating from rivers and precipitation stay in the surface. This salinity and density difference between bottom and surface waters creates and maintains strong stratification i.e. halocline, which is in 60–80 m depth in the northern Baltic Sea. During summertime, surface waters warm up while the deeper waters remain cold. Stratification causes rapid vertical changes in the density of water, which largely prevents mixing of
the water column (Leppärenta and Myrberg, 2009). This could affect the sinking and accumulation of MPs.

The vertical distribution of MPs in the water column have been previously studied in the central Baltic Sea (Gorokhova, 2015; Zobkov et al., 2019). Zobkov et al. (2019) analyzed MPs from a sub-sample with Raman microspectroscopy, showing that MPs were stratified vertically, the count of particles being higher in thermohaline layers. Gorokhova (2015) also observed relatively high counts of MPs in separate layers in the water column by sampling with a 90 μm WP2 net. However, MPs were analyzed from old formalin-preserved zooplankton samples and were detected with light microscopy and melting test, which has lately been criticized to be highly inaccurate method to recognize small MPs (Silva et al., 2018).

The aim of this study was to deepen the knowledge about the accumulation of MPs in the thermo- and/or halocline by collecting >50 and > 100 μm MP samples from the whole Gulf of Finland, in the northern Baltic Sea with two methods. The hypothesis of this study was that microplastics would be more abundant in halo- and thermoclines compared to the vertical water column between them. Moreover, we analyzed all the particles with imaging Fourier-transform infrared spectroscopy (FTIR) to get detailed data about particle counts, sizes and materials.

2. Materials and methods

2.1. Sampling

Samples were collected onboard R/V Aranda during a routine COMBINE3 monitoring cruise in the Gulf of Finland (Fig. 1) in late summer when both thermo- and haloclines are established. Stations were chosen to cover the whole basin as well as based on their stratification profiles. CTD (conductivity, temperature, depth) profiling was conducted with a SBE 911plus CTD system on every station prior to MP sampling and sampling depths were decided according to the vertical temperature and salinity profiles in the water column. MP samples were collected with two methods that enable sampling from the desired depth strata: a closing 100 μm WP2 net commonly used in mesozooplankton monitoring, which filters water from a vertical transect (filtered water volume varied between 8 and 67 m³ on different stations) and so-called Jussi sampler (large Limnos-type water sampler), which collects 30 L water from a specific depth.

WP2 net tows were taken vertically covering the water layer where temperature changed the most. The tow started from the deeper end of the thermocline or deeper, which often coincided in the Gulf of Finland with the halocline depth (Figs. 2 and 3). WP2 net was lowered with a winch to the chosen starting depth and raised up to the desired upper depth were the net was closed with a messenger. Jussi samples were always taken from the deepest depth of the WP2 tow in the halocline (60–70 m) and additionally from the depth where thermocline started (ca 20 m) and close to the bottom. Jussi-sampler was lowered with a winch to the chosen depth in the halocline and closed with a messenger. After samplers were closed, they were towed to the surface.

After towing with a WP2 net, the sample was rinsed from the net and cod end immediately at the deck to a glass beaker covered with a tin foil. Afterwards, samples were filtered in a fume hood through a 50 μm mesh to pre-cleaned glass bottles. Jussi sample waters were filtered through a 50 μm mesh and rinsed to glass bottles. Samples were stored in −20 °C for further processing in the laboratory before the FTIR analyses.

2.2. Sample pretreatment

Contamination was avoided with the following procedures: All
equipment were rinsed with ultrapure water, and reagents and solutions were filtered prior to use. Samples were always kept in closed containers and plastic items were avoided, except the sample storage bottle caps, which were made of polypropylene, and stir bars, which were made from polytetrafluoroethylene (Teflon). Sample processing was done in a fume hood and the operator wore cotton coat and nitrile gloves.

To prepare samples for FTIR imaging, they were thawed and non-plastic organic matter was digested with enzymatic purification method, modified from Löder et al. (2017). First, samples were filtered through 20 μm stainless steel filters (Manufacturer: G. BOPP + CO. AG). The filters were placed on beakers, 50–60 ml
sodium dodecyl sulphate (10% w/w, VWR) was added and samples were stirred in 50 °C for 24h. Second, samples were filtered to the same rinsed steel filters and 50 ml H₂O₂ (30%, Fischer) was added. They were stirred in 50 °C for 24h. Third, samples were again filtered to rinsed steel filters, 1 ml chitinase enzyme (ASA Special enzymes GmbH) and 30 ml pH 5 acetate buffer were added. Acetate buffer was prepared from sodium acetate and glacial acetic acid in the laboratory before treatments. Chitinase treatment was done in 37 °C for 5 days. After chitinase, the 50 ml H₂O₂ step was repeated, if sample still contained notable amount of solids.

Finally, samples were filtered to 0.8 μm gold-coated polycarbonate filters (Sterlitech Co). They were filtered to circular area

Fig. 3. Vertical profiles of temperature and salinity, and sampling and results from stations NAR2, 9F5, Arus and 6P in the eastern Gulf of Finland with Jussi sampler (light blue dotted line) and WP2 plankton net (red dotted line). Note the different scales. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)
with diameter 13 mm. Most of the samples were divided to two filters to ensure that the amount of solids would not be too high on one filter, because the treatment method did not dissolve solid matter completely. Filters were stored in closed glass Petri dishes. They were allowed to dry in room temperature and attached with two-sided tape to glass microscope slides.

2.3. Infrared imaging and data analysis

The entire circular 13 mm filtration area was analyzed with imaging FTIR (Agilent Cary 670/620 equipped with 128 × 128 FPA detector). Measurements were preformed from gold-coated filters in reflection mode, using 15X cassegrain objective, pixel size 5.5 μm, spectral resolution 8 cm⁻¹, spectral range 3800–750 cm⁻¹ and number of scans 8.

Spectral maps were analyzed with siMPle software (Primpke et al., 2020), which calculates Pearson’s correlation coefficients between sample and reference spectra and based on them automatically recognizes plastic types, particle sizes and calculates mass estimations from particles sizes and material densities (the algorithm for calculation of mass estimations is explained in detail in https://simple-plastics.eu/about.html). The reference spectral library was compiled from open source and in-house measured spectra. The library contained only the most common plastic types and natural protein and cellulose polymers, because increasing number of references would result in impractically long computing time. The following common polymer types were chosen for the analysis: polyamide (PA), polyethylene terephthalate (PET), polyethylene (PE), polypropylene (PP), polystyrene (PS), acrylonitrile butadiene styrene (ABS), polyurethane (PU), polyvinyl chloride (PVC), polyethylmethacrylate (PMMA) and polyacrylonitrile (PAN).

In siMPle, user can define the correlation thresholds for determining whether a particle is considered as plastic or not. The thresholds were set for this method according to the procedure reported by Liu et al. (2019). Shortly, the first correlation threshold was set to 60% and particles were manually examined to exclude false positives. If the correlation was >70% all plastic reference peaks were present and no extra peaks except minor carbonyl peak around 1700 cm⁻¹ (resulting from oxidation) were present, particle was counted as plastic. We used raw spectra, first derivative and second derivative with weights 1:1:1 for calculating correlations.

2.4. Quality control

Three replicates of blanks (negative controls) were prepared from ultrapure water similarly than samples, only stirring and heating times were shorter. Contrary to samples, 0.25 ml chinatase and 20 ml buffer was added to blanks. Control samples were analyzed similarly with imaging FTIR and siMPle.

Recovery of the pretreatment method was tested with two recovery test samples (positive controls). Polystyrene beads (NIST Traceable Size Standards, 90 μm, 58/57 pieces) were added to ultrapure water, and recovery samples were treated similarly than samples. However, chinatase and buffer were replaced with ultrapure water in same volumes and stirring and heating times were shorter. After the final filtrations to gold-coated filters, recovery samples were examined with a stereo microscope (Zeiss Stemi 508; 6.3–50× magnification; AxioCam ERC 55 camera) to count the beads. The average recovery rate was 75%.

Because the analysis method did not include sub-sampling, results are exact for the sampled volumes. The background resulting from plastic contamination during the sample treatment and analysis process was calculated as an average of the plastic particle counts or mass from three replicates. The background contamination was defined as 4.3 MPs/sample or 234 ng/sample, and it was used for all samples. Sample values, including particle counts and mass estimations, were corrected by subtracting the background, and resulting negative values were approximated to be zero. For comparison, the average raw values for samples before subtraction were 17.2 MPs/sample or 3218 ng/sample, which were multiple times higher than blank values.

The average particle size (major dimension) of MPs in blanks was 73 ± 45 μm (mean ± SD), largest was 154 μm and smallest 23 μm.

3. Results

3.1. Microplastic concentrations in water

The microplastic concentration range was 0–1.6 MP/m³ and 0–766 ng/m³ in plankton net (WP2) samples and 0.02–17 MP/L and 0–775 ng L⁻¹ in 30 L water sampler (Jussi). The highest particle counts were found at the open sea stations in the western Gulf of Finland at station LL7 from the halocline depth (80 m) and at station F62 in the depth where thermocline starts (21 m) (Table 1). Additionally, the highest estimated plastic mass was found from station F62 at the depth of 76 m, but the plankton net results were below limit of detection at that station. Both counts and masses were low at LL9 in both sampling depths (29 and 62 m), at 9F5 in plankton net sample and at NAR2 in 51 m.

Clear patterns in MP concentrations were not found between sub-areas or depths sampled (Figs. 2 and 3). However, in the plankton net samples, MP concentrations by particle counts and masses per volume were nearly thousand times lower than in 30 L samples.

3.2. Plastic types and size distribution

By particle counts, polyethylene (PE) was the most common plastic polymer type in all the samples (Fig. 4). Additionally, Table 1 MP concentrations by particle counts and plastic masses at sampling stations. Note the different scale in MPs volume⁻¹ for different samples. All sizes columns are corrected values, from which contamination has been subtracted, and >100 μm are uncorrected numbers.

| Station | Depth (m) | Sampler | All sizes | >100 μm |
|---------|-----------|---------|-----------|---------|
| F62 | 75–20 WP2 | 0 | 0 | 0 |
| NAR2 | 50–31 WP2 | 1.2 | 222 | 0.3 |
| 9F5 | 44–45 WP2 | 0.41 | 38 | 0.2 |
| Arus | 25–18 WP2 | 1.6 | 766 | 1.5 |
| 6P | 21–10 WP2 | 1.3 | 157 | 0.5 |
| Mean ± SD | | 0.92 ± 0.61 | 237 ± 277 | 0.5 ± 0.5 |

| Station | Depth (m) | Sampler | All sizes | >100 μm |
|---------|-----------|---------|-----------|---------|
| F62 | 21 Jussi | 1.7 | 105 | 700 |
| F62 | 76 Jussi | 0.36 | 775 | 267 |
| UUS23 | 14 Jussi | 0.36 | 31 | 233 |
| UUS23 | 55 Jussi | 0.06 | 3.3 | 33 |
| LL9 | 29 Jussi | 0.02 | 0 | 33 |
| LL9 | 62 Jussi | 0.09 | 6.9 | 100 |
| L7 | 22 Jussi | 0.12 | 105 | 167 |
| L7 | 80 Jussi | 1.3 | 37 | 267 |
| L7 | 98 Jussi | 0.12 | 0.53 | 67 |
| NAR2 | 51 Jussi | 0.12 | 0 | 67 |
| 9F5 | 45 Jussi | 0.29 | 187 | 233 |
| Arus | 26 Jussi | 0.82 | 17 | 200 |
| Mean ± SD | | 0.44 ± 0.52 | 106 ± 209 | 197 ± 174 |
Polyethylene (PE) and polyethylene terephthalate (PET) were common, whereas only small numbers of polyamide (PA) and polystyrene (PS) were found. Other plastic types were absent. The majority of particles were fragments (97.6%) and minority fibers (2.4%). A particle was counted as a fiber, if the major dimension was at least ten times longer than the minor dimension. Otherwise, it was counted as a fragment.

Hypothetically, plastics less dense than water should float and denser should sink. In the calculation, density of seawater was estimated to be 1 g cm\(^{-3}\). PE and PP are less dense than seawater, whereas other polymers are denser. For Jussi samples, we calculated the percentage of floating plastics and plotted it against sampling depth, but we did not find any signal that denser plastics would be increasingly abundant towards the bottom, or less dense towards the surface (Fig. 5). However, the change of water density was not taken into account in this calculation, because the changes in water density are relatively small compared to the range of plastic densities.

The smallest detectable particle size was 20 μm because of the analysis method, and the largest was 724 μm in plankton net (WP2) samples and 8386 μm in 30 L (Jussi) samples. In 30 L samples <50 μm and in plankton net samples <100 μm particles are not representative, because they were mostly removed in sampling or right after it. However, MPs can fragment during storage, transportation and treatments, which could cause the observations of smaller particles. Moreover, smaller particles can attach to plankton net or filter with larger ones. The mean ± standard deviation of particle size was 126 ± 137 μm in WP2 and 96 ± 81 in Jussi. Particle sizes in all stations and sampled depths are presented in Fig. 6.

**4. Discussion**

The results supported the hypothesis that MPs accumulate to thin layers in thermo- and haloclines to some extent, because 30 L samples (Jussi) contained thousand times more microplastics than plankton net (WP2). However, Jussi sampler collects only 30 L water, whereas WP2 filters tens of m\(^3\), and the sampled particle sizes were different — WP2 net collects only particles >100 μm whereas filtered Jussi samples represent MPs >50 μm. Because of the lower volumes, Jussi samples are less representative and possible background contamination affects them more. When only >100 μm MP are compared, Jussi samples contained on average 197 MP m\(^{-3}\) and WP2 samples 0.53 MP m\(^{-3}\). Therefore, if the smaller MPs in Jussi samples are omitted, they still contained nearly thousand times more MPs than WP2 samples. However, to minimize the effect of possible contamination and different volumes, we corrected the values expressed in results by subtracting the average contamination from raw numbers.

In previous studies from marine and freshwater environments, 20 μm filter has been found to collect about ten times higher counts of MPs than 100 μm (Hidalgo-Ruz et al., 2012; Railo et al., 2018; Uurasjärvi et al., 2020). Here the difference in MP counts between Jussi (50 μm) and WP2 (100 μm) samples was thousand times, which suggests that sampling methods and particle sizes do not explain it completely, but there is a real accumulation in MP concentrations in certain layers. As the MP monitoring efforts have concentrated to the surface water samplings worldwide (e.g. Andrady, 2011; Hidalgo-Ruz et al., 2012; Setälä et al., 2016), we lack proper understanding of the accumulation of MPs to different layers in the water column, driven by for example density stratification.

To compare MP counts of this study with previous studies from surface waters, the counts of >100 μm microplastics (WP2) in the stratified depth layers were the same or lower than previous results from the same study area (Setälä et al., 2016). Contradictory, concentrations in haloclines and the upper parts of thermoclines (Jussi) were hundred times higher than reported MP concentrations for the surface waters for small particle sizes (Railo et al., 2018). However, the results between different studies cannot be unambiguously compared because the sampling and analysis methods were different.

Considering the water column, our results covering the whole Gulf of Finland support the findings from the Central Baltic Sea (Gorokhova, 2015; Zobkov et al., 2019), which show higher concentrations of MPs in deeper water layers compared to the surface. Our WP2 results should be comparable to Gorokhova’s (2015) results as the same sampling equipment was used. However, our results are hundred times lower. The difference could be explained by contamination and identification procedures. Gorokhova (2015) analyzed MPs from old zooplankton samples, which were taken without taking care of plastic contamination as the purpose for sampling was different. MPs were identified with light microscopy with additional hot needle tests, which both could increase the false positives in the results.

Previous results have shown that MP concentrations of the surface waters are higher in the coastal areas compared to the offshore (Collignon et al., 2012; Railo et al., 2018; Setälä et al., 2016). However, our results from the depth layers did not reveal any trend that MPs would be more abundant closer to coastline than further offshore. Presumably, MPs are abundant in the coastal surface waters, because most of the sources of MPs are land-based (e.g. Andrady, 2011). After discharge, MPs enter the sea and they diffuse to the surface waters by wind and currents. Meanwhile, they start sinking towards the bottom. This could explain why we do not see the same trends in surface and deep waters. In the depths, MPs first accumulate to halocline, when present, and sink slowly towards the bottom. However, more studies are needed to examine how MPs drift, accumulate and sink in different water bodies and how physical phenomena such as waves, tides and upwelling affect them.

In this study, most of the MPs were PE and PP, which have been the most common MP types in other marine environments, too.
PE and PP are less dense than seawater and should float on the surface. The results did not provide any evidence that denser plastics would be more common in the deeper layers or low-density plastics would be more common towards the surface. In the marine environments, bacterial biofilms grow onto the surfaces of MPs, which can have notable impact on density, weight and surface characteristics of the particles, increasing or decreasing the sinking rates of them (Rummel et al., 2017; Zettler et al., 2013). Therefore, we suggest that the density of the virgin plastic does not play a major role in the sinking rate of microplastics in the marine environment. However, we did not examine whether the MPs found were covered by biofilms, because FTIR analysis required oxidative treatment, which decomposed biological materials.

To conclude, our results from Jussi sampling showing on average 440 MP m$^{-3}$ in certain 1 m thick layers where the thermocline starts or close to the end of halocline reveal new hot spots for MP pollution. These stratification layers are restricting the vertical movement of biota (e.g. Lougee et al., 2002) as well as they restrict sinking of MPs through these barriers. Possibly, planktonic organisms and planktivorous fishes may feed in these layers (Schulz and Hirche, 2007), which could expose them to higher MP loads than in the surface waters.

Recently, most of the monitoring programs have targeted MPs in surface waters as required by the Marine Strategy Framework Directive (EU, 2008; European Commission, 2017). Moreover, most previous knowledge on MP abundances are also from the surface waters (Andrady, 2011; Hidalgo-Ruz et al., 2012). Our study, however, highlights the importance of sampling layers, where water density changes to get a better overview of the total abundance of MPs in the studied water bodies, as suggested by Gorokhova (2015). For sampling these thin horizontal layers common vertically towed plankton nets (e.g. WP2) are not good as they sample longer vertical transects than the possible thin layers, resulting in underestimations of the MP abundances per vertical area. However, for estimating MP abundances in the whole water column vertically towed plankton nets would work well. Large water samplers like Jussi sampler are good as they take water from a precise and desired depth but their disadvantages are still limited sample
volumes. The optimal sampling device for the thin layers would be horizontally towed net, that can be operated in a desired depth – for example MultiNet-type device, which samples large volumes per depth.

Considering the analytical methods, imaging FTIR and spectral library analysis of large data are relatively time-consuming, but the method recognizes microplastics, especially fragments, more accurately than light microscopy stand-alone (Löder et al., 2015). On the other hand, this method is not very good at identifying long plastic fibers, because they are usually not attached to the filter surface on the whole length and thus not in focus completely. Therefore, one long fiber can be identified as multiple smaller particles, which affects also the average particle size and mass estimation. To conclude, imaging FTIR likely underestimates the amount of fibers, whereas light microscopy overestimates it. Moreover, our samples were filtered through 50/100 μm nets, which probably reduced the amount of fibers. These aspects can explain why previous studies have reported higher proportions of fibers in the Baltic Sea (Tammenga et al., 2018; Zobkov et al., 2019).

5. Conclusion

The depth layers where the density of seawater changes rapidly contained higher concentrations of microplastics compared to the surrounding water column. However, different plastic types did not separate to different depth/density layers by the densities of the virgin plastic polymers. No areal hot spots were observed in the Gulf of Finland, though coastal areas have often been recognized as hot spots for microplastic pollution when surface waters have been studied. The current marine monitoring strategies aim to monitor the concentration and distribution of microplastics in the surface waters. Nevertheless, the results from stratified water bodies suggest that microplastics can accumulate to certain deeper depths, where they would be available for feeding organisms in higher amounts. Monitoring the changes in microplastic concentrations of surface waters reveals if the levels of microplastic pollution increases in certain areas, but in stratified water bodies, deeper water layers should also be monitored to get a comprehensive view.

Credit author statement

All authors have materially participated in the research and/or article preparation as following: Emilia Uurasjärvi: Methodology (sample and plastic analysis), Validation, Formal analysis, Investigation, Writing - original draft, Visualization, Minna Pääkkönen: Investigation, Formal analysis, Visualization, Outi Setälä: Conceptualization, Methodology (sampling), Investigation, Writing - original draft, Arto Koistinen: Writing - review & editing, Supervision, Project administration, Funding acquisition, Maiju Lehtiniemi: Conceptualization, Methodology (sampling), Investigation, Writing - original draft, Supervision, Project administration, 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.

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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.2020.115700.

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