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Occurrence of microplastics in surface waters of the Gulf of Lion (NW Mediterranean Sea)

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Abstract Between 2014 and 2016 a total of 43 microplastic samples were collected at six sampling stations in the eastern section of the Gulf of Lion (located in the northwestern Mediterranean Sea), as well as upstream of the Rhône River. Microplastics were found in every sample with highly variable concentrations and masses. Concentrations ranged from $6 \times 10^3$ items km$^{-2}$ to $1 \times 10^6$ items km$^{-2}$ (with an average of $112 \times 10^3$ items km$^{-2}$), and mass ranged from $0.30$ g km$^{-2}$ to $1018$ g km$^{-2}$ DW (mean $61.92 \pm 178.03$ g km$^{-2}$). The samples with the highest and lowest microplastic count originate both from the Bay of Marseille. For the Bay of Marseille, it is estimated that the total microplastic load consist of $519 \times 10^3$ - $101 \times 10^6$ items weighing $0.07$ - $118$ kg. Estimations for daily microplastic transport by the Northern Current and the Rhône River, two important hydrologic features of the northwestern Mediterranean Sea, range from $0.18$ to $86.46$ t and from $0.20$ to $21.32$ kg, respectively. Particles $< 1$ mm$^2$ clearly dominated sampling stations in the Northern Current, the Rhône River and its plume ($52$, $53$ and $61 \%$, respectively), suggesting a long exposure time in the environment. Items between $1$ mm$^2$ and $5$ mm$^2$ in size were the most abundant microplastics in Marseille Bay ($55 \%$), which suggests coastal pollution sources or the removal of smaller particles from surface waters e.g. by ballasting owing to the presence of epibionts.
1. Introduction

Plastic and its chemical compounds have played an important role in the Anthropocene and might threaten human health (Kobrosly et al., 2014; Tranfo et al., 2012; Sathyanarayana 2008; Heudorf et al., 2007) and both terrestrial (Zhao et al., 2016; Lwanga et al., 2016; Oehlmann et al., 2009) and marine environments (Przybylinska & Wyszkowski, 2016; Van Franeker & Law, 2015; Sigler, 2014). In 2014, 311 million tons of plastic were produced worldwide, 15 % of which were consumed in Europe (PlasticsEurope, 2015). The degradation of large plastic items into microplastics (≤ 5 mm) in the ocean is a slow and heterogeneous process, varying with respect to the quality, shape and size of the plastic. This process is driven by mechanical forcing (e.g., waves), salt water, and UV radiation (Ter Halle et al., 2016). Because of its small size, micro debris can easily be ingested (e.g., Desforges et al., 2015; Neves et al., 2015). Approximately 270 $10^3$ tons of plastic are suspected to float in the world’s oceans (Eriksen et al., 2014). Estimates for floating microplastic loads range from 7 $10^3$ to 35 $10^3$ tons for global open-ocean surface waters (Cózar et al., 2014) or from 93 $10^3$ to 236 $10^3$ tons depending on the model used (Van Sebille et al., 2015). Plastic accounts for 60 to 80 % of all marine litter, followed in quantity by glass and metal (UNEP, 2009). About 370 $10^9$ plastic particles or 1,455 tons have been estimated to be floating on the surface of the Mediterranean Sea (Ruiz-Orejón et al., 2016). Other estimates range from 756 to 2,969 tons (Cózar et al., 2015) and from 874 to 2,576 tons (Suaria et al., 2016).

The Mediterranean Sea is a semi-enclosed basin subject to significant anthropogenic pressures (e.g., The MerMex group, 2011; Blanfuné et al., 2016; Hassoun et al., 2015; Casale et al., 2015). Marine debris, including microplastics, are a particularly important concern in this region (Deudero & Alomar, 2015; Cózar et al., 2015; Ioakeimidis et al., 2014; Faure et al., 2015; Pedrotti et al., 2016). Concerns about marine litter in the Mediterranean Sea were first expressed in 1976 when the Barcelona Convention was signed with the goal of preventing and abating marine and coastal pollution (UNEP, 2009). In subsequent years, studies have been undertaken to better understand pollution sources and trajectories, through approaches as modeling the transport of floating marine debris (Mansui et al., 2014). However, knowledges on the spatial and temporal microplastic distribution remain limited (Ruiz-Orejón et al., 2016; Suaria et al., 2016; Cózar et al., 2015). Their contents are highly variable, although the sea surface circulation seems to be the main driver on the distribution of floating marine litter whatever their sizes. Currents affect time-dependent movements that remain difficult to predict, and cause several non-trivial Lagrangian mechanisms (Zambianchi et al., 2014). In semi-
enclosed seas, such as the Mediterranean Sea, aggregation patterns are not permanent and high variability is observed at a small scale (Suaria et al., 2016). Wind-induced effects on floating material and Stokes drift velocities require further investigation, such as refinement of regional models. Nevertheless, some available scenarios could be hypothesized with possible retention areas in the northwestern Mediterranean and the Tyrrhenian sub-basins (Poullain et al., 2012; Mansui et al., 2014). The Gulf of Lion is in the northwestern sector of the Mediterranean Sea, and its hydrodynamics are influenced by shallow water depths of the shelf, wind regimes (Mistral and Marin), the Northern Current (NC), and freshwater inputs from the Rhône River (Gatti et al., 2006; Fraysse et al., 2014). The NC has a high seasonal variability: while a decrease in intensity is observed in summer, it becomes faster, deeper and narrower in winter (Millot, 1991). Intrusion of the NC onto the shelf of the Gulf of Lion has been observed (Ross et al., 2016; Barrier et al., 2016 and references therein). This productive shelf is also highly exploited for commercial fishing (Bânaru et al., 2013) and the coastal area is strongly influenced by tourism activities. Given this areas great economic, touristic and environmental significance, monitoring threats, such as pollution sources, is essential. Therefore, the primary goal of this study was to provide insight into the temporal and spatial distribution of microplastics in the eastern sector of the Gulf of Lion. Furthermore, we wanted to examine relationships between microplastic size distributions and possible pollution sources and transportation routes.

2. Materials and Methods

Following the framework of the Particule-MERMEX and PLASTOX projects, microplastic debris were collected at different times between February 2014 and April 2016 (Table S1) in three distinct areas with specific hydrodynamic characteristics (Figure 1) within the eastern sector of the Gulf of Lion (northwestern Mediterranean Sea). The first area is located 40 km offshore at the eastern part (station #1, also called ‘Antares site’) and is within the direct influence of the Northern Current, which runs east to west along the shelf break over 2,475 m of water (Martini et al., 2016). The second area includes the Bay of Marseille (stations #2, 3 and 4), which is significantly influenced by a population of approximately 1 million inhabitants and by the daily volume of about 250 10^3 m^3 of waste waters released from the Marseille-Cortiou wastewater treatment plant (WWTP) (Savriama et al., 2015; Tedetti et al., 2012). To the west, the third study area is the downstream part of the Rhône River (station #6, Arles, 48 km from the river mouth) and within the dilution plume (station #5, about 2.5 km from the mouth) (Sempéré et al., 2000). Sampling dates, GPS coordinates, microplastic concentration,
mean wind speed and wind direction are provided in the supplementary data (Table S1) along with information on precipitation. Surface current speeds and directions were extracted from the Mars 3D model (http://marc.ifremer.fr).

Fig. 1.

The sampling stations situated in the eastern Gulf of Lion (A) including Antares site (station 1), Marseille Bay site (stations 2-4) and Rhône River site (stations 5-6). The Rhône River plume as observed during north/northwest wind conditions and the Northern Current (NC) are also indicated. Zoom of the Bay of Marseille (B) with the local WWTP (Cortiou). Map modified after Schlitzer, R., 2009.

Microplastic samples were collected using a Manta net (0.50 m x 0.15 m opening) mounted with a 780 µm mesh size and towed horizontally at the surface. Ten samples from March and April 2016 were collected (in Marseille at stations 3 and 4) with a 330 µm mesh size (Suppl. Table 1). Sampling was only conducted under low swell conditions (< 1 m). The net was towed for 20 minutes at an average speed of 2.5 knots approximately 50 m behind the research vessel. It was towed at a slight angle to avoid disturbances caused by the boat’s wake. Samples from the Rhône River (station #6) were collected from a fixed location on the dock of the river. Sampled superficials at this station were calculated by comparing the flow rate during sampling with reference flow rates and river speeds. Lower-limit river speeds were used for estimates, since river speeds tend to be slower near the dock.

The net was carefully rinsed and the content of the cod-end was poured into a 1 L glass bottle, preserved with a buffered seawater formalin solution (final concentration 5 %), and kept in cold and dark conditions until further analysis. Samples were then sieved (mesh size 125
µm), and rinsed with ultrapure water (ISO 3696). Plastic debris were picked out with tweezers under a dissecting microscope. Fibers were not taken into account due to the high risk of contamination. No Fourier Transform Infrared Spectroscopy (FTIR) Analysis was performed to verify the nature of the items, so despite all efforts to maximize result reliability, it cannot be excluded that some non-plastic items were estimated to be microplastics.

The number, size and shape of each item was identified using a ZooScan© (HYDROPTIC SARL). Each item was placed on the screen of the ZooScan without any water. Surface area measurements in pixels were obtained using the ImageJ software and then converted into mm² and the Equivalent Spherical Diameter (ESD). Plastic items ≤ 5 mm were considered. All microplastics from each sample were then weighed (Mettler AE 240, reliability ± 0.1 mg). Microplastic abundance (items km⁻²) and dry weight (g km⁻²) were calculated for each sample using the towing distance and the net opening surface. Analysis of variance (one-way and two-way ANOVA) with a 0.05 level of significance was performed to assess whether the microplastic abundance and size distribution varied with space (stations) and time. The Tukey test was used whenever significant differences were detected. All statistical analyses were performed using R version 3.3.2.

3. Results and Discussion

3.1. Microplastic abundance

Microplastic abundance ranged from 6 × 10³ to 1 × 10⁶ (mean 96 × 10³) items km⁻² in the Marseille Bay area, from 33 × 10³ to 400 × 10³ (mean 113 × 10³) items km⁻² in the Rhône River plume, from 7 × 10³ to 69 × 10³ (mean 34 × 10³) items km⁻² in the river itself and from 9 × 10³ to 916 × 10³ (mean 212 × 10³) items km⁻² off-shore (Fig. 2, top). The highest microplastic concentration (1 × 10⁶ items km⁻²) was observed at station #2 (Marseille Bay area). The day this sample was collected was characterized by calm conditions with no noteworthy surface currents near the station. In contrast, the other two stations on the coast of Marseille, stations #3 and #4, showed very low particle concentrations (averages 20 × 10³ and 10 × 10³ items km⁻², respectively). While a comparison between these both stations and the station #2 is difficult, because the samples were collected in different years (2016 vs. 2014), some assumptions can still be considered. Goldstein et al. (2013) reported that a high spatial heterogeneity for microplastic concentrations could be found not only at a large scale but also on a smaller scale for samples taken at distances of 10 km from one another. Heterogeneous spatial debris distribution can be the result of
currents, wave- and wind-driven turbulences, river inputs or hydrodynamic features such as upwelling, downwelling, gyres or fronts (e.g., Kukulka et al., 2012; Suaria and Aliani, 2014; Collignon et al., 2012). More generally, high concentrations of microplastics, especially small fragments, are found in coastal waters because of the proximity of densely populated areas, (Pedrotti et al., 2016) and continental inputs from the atmosphere or rivers (Collignon et al., 2012). Point pollutions could also play an important role in the Bay of Marseille, where the fierce northwestern Mistral wind can transport litter from city streets into coastal waters. Another possible source of microplastics in the Bay of Marseille is the local sewage facility (Cortiou) where treated wastewater enters the sea in the southeastern part of the city. On March 17, 2016 a slight surface current coming from Cortiou at a speed of approximately 0.5 m s\(^{-1}\) entered the area of stations #3 and 4. The microplastic concentrations observed that day were the highest ever found at station #4 (15 \(10^3\) items km\(^{-2}\)) and the second highest for station #3 (27 \(10^3\) items km\(^{-2}\)). Interestingly, microplastic abundance was always higher at station #3 compared to station #4, in spite of their geographical proximity (p < 0.05).

Our median concentration (31 \(10^3\) items km\(^{-2}\)) was about one third of the mean value, highlighting potential surges in microplastic presence, possibly linked to climatic and hydrodynamic events. Hydrodynamic processes influencing microplastic distribution are e.g. vertical mixing or eddies and anticyclonic gyres. The latter of which are unsteady formations in the Mediterranean Sea (Pedrotti et al., 2016), but could lead to punctual increases in regional microplastic abundances. Additionally, in our study area, there is the Northern Current, which varies greatly in intensity, depth, and position (Millot, 1991). Data collected at station #1 showed temporal variability, with concentrations of microplastics being significantly higher on March 10, 2014 (p < 0.05) when the Northern Current was fast and narrow with maximum speeds of approximately 0.9 m s\(^{-1}\). However, triplicated trawls exhibited a range of microplastic abundances from 103 \(10^3\) to 916 \(10^3\) items km\(^{-2}\) at this sampling date, implying that a nine fold difference in abundances can be observed in the same sampling area within two hours. This further highlights the strong temporal variability observed for microplastic concentrations. Overall no seasonal differences were detected (p > 0.05), but the low number of observations limits the strength of any comparison. Goldstein et al. (2013) observed seasonal heterogeneity at much larger scale in the northeastern Pacific Ocean between summer 2009 and fall 2010.

Floating debris transported by the NC could be transported to the Balearic Islands, where models calculated high beaching probabilities (Mansui et al., 2014), or to the seafloor which is known to be a (micro-) plastic sink (Claessens et al., 2011; Ioakeimidis et al., 2014; Woodall et
Reasons of microplastic sedimentation can be the nature of the plastic material, (if its density is higher than the one of seawater, Tekman et al., 2017), the biofouling accumulation on microplastic surfaces (Woodall et al., 2014), the incorporation of free microplastics into marine aggregates or the incorporation of microplastics into fast-sinking faecal pellets after ingestion by zooplanktons and fishes (Cole et al., 2013).
Microplastic abundance (top; particles km\(^{-2}\)) and weight (bottom; g dry weight km\(^{-2}\)) for the six stations studied at the three sites. For samples collected the same day at the same station, points represent the averaged values and error bars the standard deviation. \(n\) = overall number of samples taken at this station. \textit{Note: the weight of one sample collected on 10/03/14 was not available and the data point hence only represents the weight of the two other samples collected this day at station #1.}

The overall average microplastic abundance for our samples was 112 \(10^3\) items km\(^{-2}\), which is in the same range as other areas in the northwestern basin, where mean densities have been estimated to 115 \(10^3\) items km\(^{-2}\) (Collignon et al., 2012), 130 \(10^3\) items km\(^{-2}\) (Faure et al., 2015) and 150 \(10^3\) items km\(^{-2}\) (De Lucia et al., 2014). Higher amounts were measured for the entire Mediterranean basin (243 \(10^3\) items km\(^{-2}\), Cózar et al., 2015), due to high concentrations in some Mediterranean areas. Densely populated areas as the semi-enclosed Adriatic Sea and the Levantine Basin were characterized by high densities of 1,050 \(10^3\) (max: 4,600 \(10^3\); Suaria et al., 2015) and 1,518 \(10^3\) (max: 65 \(10^6\); Van der Hal, 2017) items km\(^{-2}\), respectively. Our results are consistent with previous studies and indicate that the northwestern Mediterranean Sea contains similar mean microplastic concentrations as the Atlantic and Pacific Oceans (mean: 134 \(10^3\) items km\(^{-2}\) and 124 \(10^3\) items km\(^{-2}\), respectively, Eriksen et al., 2014). Hereby it needs to be considered that the Atlantic and Pacific Oceans are also known to be highly heterogeneous, with microplastic accumulation and non-accumulation zones. Examples for a heavily contaminated area are the East Asian Seas, where a mean microplastic abundance of 1,720 \(10^3\) items km\(^{-2}\) was measured (Isobe et al., 2015).

Microplastic abundances in the Rhône River at Arles (station #6; \(34 \ 10^3 \pm 19 \ 10^3\) items km\(^{-2}\); net size 0.50 m x 0.15 m, mesh size 780 \(\mu\)m) were relatively low, but were similar to values reported by De Alencastro (2014) upstream at Chancy (\(~ 52 \ 10^3\) items km\(^{-2}\); net size 0.60 m x 0.18 m, mesh size 300 \(\mu\)m). In comparison, a mean microplastic abundance of 893 \(10^3\) items km\(^{-2}\) was found in the Rhine River, a watercourse flowing through highly industrialized areas, such as North-Rhine Westphalia (Germany), where many plastic factories are located (Mani et al., 2015). Concentrations observed in the Rhône River plume (station #5, up to \(400 \ 10^3\) items km\(^{-2}\)) were higher than in the river itself, suggesting that the Rhône River – sea interface may generate a temporal accumulation zone for debris. In general, however, the
The area covered by our six sampling stations is not considered to be a retention area, but can better be described as a “transit area”. The size of the Mediterranean basin reduces the potential for formation of permanent gyres as in the Atlantic, Pacific and Indian Oceans, where plastic often concentrates (Cózar et al., 2015).

At the river mouth, microplastic concentrations were either significantly greater (p < 0.05) or similar to those observed upstream in the Rhône River. Concerning the river plume, we should highlight the similitude in zooplankton composition of two samples collected with the same Manta trawl, first, on the 10/03/14 at the station #1 (NC) and then (18/03/14), at the Rhône River Plume station (station #5). High abundances (> 1,000 individuals per sample) of *Velella velella*, a free-floating hydrozoan, were observed at both dates (Thibault D. pers. com.), implying a potential intrusion of water masses from the NC onto the shelf. Such intrusions have already been observed before (Barrier et al., 2016). Salinity data from the Mars 3D model support the hypothesis: while the Rhône River plume was extended in all directions on 10/03/14 and the following days, saltier surface waters pushed from the eastern direction into the area from 16/03/14 on and thus, reduced the extension area of the river plume. During the period examined, the velocity of the NC flowing through station #1 was about 0.4 m s$^{-1}$ (Suppl. Table 1), but currents leaving the main branch in northwestern directions flowed at reduced speeds of about 0.2 m s$^{-1}$. At this speed range (0.2-0.4 m s$^{-1}$), water masses could have travelled about 140-275 km in eight days, which is consistent with the straight line distance (120 km) between both stations. However, we would like to point out that these are only indications, since an accurate model would be needed to simulate the exact trajectory of the water masses and microplastics in question.

### 3.2. Microplastic weight

Microplastic dry weight showed a similar variability, ranging from 0.30 g DW km$^{-2}$ to 1018 g DW km$^{-2}$, with the maximum observed in Marseille Bay (Fig. 2, bottom). An average of 61.92 g DW km$^{-2}$ ($\pm$ 178.03 g DW km$^{-2}$) was found in the study area. This value is similar to averages of 60 and 63 g DW km$^{-2}$ reported for the western part of the northwestern Mediterranean Sea (Collignon et al., 2012) and the upstream part of the Rhône River (De Alencastro, 2014), respectively.

An estimated surface area of 87 km$^2$ of the Bay of Marseille would provide a total microplastic load of 0.07 to 118 kg (mean 9.94 kg), representing a range of concentrations from
0.5 $10^6$ to 101 $10^6$ (mean $8\ 10^6$) microplastic pieces in surface waters. For the Rhône River, the flow rate used for calculations varied between $1,150 \ \text{m}^3 \ \text{s}^{-1}$ and $1,600 \ \text{m}^3 \ \text{s}^{-1}$ during the sampling period. Using minimum and maximum concentration and weight values, we calculated a daily microplastic spill of 0.20 – 21.32 kg (dry weight), representing $10 \ 10^6$ - 40 $10^6$ items discharged by the Rhône River into the Mediterranean Sea. Similarly, microplastic loads for the Northern Current were calculated using volumetric transport rates of 0.7 Sv (Conan and Millot, 1995) and 2 Sv (Petrenko, 2003) and the minimum and maximum concentration and weight values. This method provided an estimate of daily transport ranging from 0.18 to 86.46 tons (dry weight) of microplastic, representing $4\ 10^9$ to $1\ 10^{12}$ items. These calculations give minimum ranges, since they are based on the assumption that microplastics concentrate within 15 cm under the surface. Turbulences, especially in rivers, may however transfer microplastics through several meters of the water column. As interesting as they are, these extrapolations should be considered with caution, since microplastic abundances show a high amount of variability and are difficult to predict.

### 3.3. Microplastic size distribution

The mean size of microplastic was $1.48 \pm 0.88$ mm, however significant differences ($p < 0.01$) were observed between samples from the Bay of Marseille (stations # 2-4) and all other sampling stations. For better visualization of the size distribution of our samples, we calculated the equivalent spherical diameter (ESD) of each microplastic particle (Figure 3). A general exponential distribution curve was observed with the smallest items being the most important, except in the Bay of Marseille, where microplastics were more evenly distributed over the size range. The overall size distribution observed in this study closely resembles those observed for the Mediterranean Sea (Ruiz-Orejón et al., 2016; Cózar et al., 2015), open ocean waters (Cózar et al., 2014) and the Northeast Pacific Ocean (Goldstein et al., 2013). Manta nets are the most commonly used sampling device for microplastic sampling in aquatic ecosystems and were also used in this study. The mesh size of the net can influence the size distribution as well as the speed of the tow, as smaller particles avoiding the net can be forced aside from the net opening or large particles can squeeze out through the mesh. This study used mainly a 780 µm mesh sized net and a 330 µm mesh sized net only for ten sampling events at stations # 3 and #4 (Suppl. Table 1). We expected to collect more 0.0-0.4 mm items at both concerned stations (#3 and #4) by using the 330 µm mesh sized net, but microplastics of this size class were observed neither in samples from the 330 µm mesh size, nor in samples from the 780 µm mesh size. No
influence on the microplastic size distribution caused by the use of these different mesh sizes was hence observed. This was statistically confirmed by removing all samples collected with the 330 µm net from the dataset and repeating the one-way ANOVA with the following post-hoc test and obtaining the same significant results.

Size distribution can be an indicator of the source of marine debris and of its distance to the shoreline. While Pedrotti et al. (2016) observed that small sized microplastics were more abundant within the first kilometre adjacent to the coastline, Isobe et al. (2015) found that the percentage of larger plastic particles is typically greater in areas close to the pollution source.
Microplastic size distributions (ESD in mm) measured at six sampling stations with a ZooScan apparatus. Error bars represent the standard deviation.

The surface area distributions of stations #1 (NC) and 6 (Rhône River) clearly resemble each other. Both are dominated by small particles ($< 1 \text{ mm}^2$: 52 and 53 %, respectively, Figure 4). This size class only represented 27 % for stations in the Bay of Marseille, but represented 61 % of microplastic particles at the Rhône River plume. The second size class (1-5 mm$^2$) was
the most abundant in Marseille Bay (55%). The largest pieces (> 10 mm$^2$) were poorly represented (< 5%) at all stations. The size class distributions are likely related to the distance of the collected particles from pollution sources. In the case of station #1 (NC), it is likely that microplastics were transported by the Northern Current and may have originated in regions farther east, such as the Italian coast. At station #6 (Rhône River), the size distribution suggests that the collected microplastics were in the Rhône River watershed for some time and certainly originated from highly industrialized and/or populated regions higher upstream (e.g., Lyon with ~ 500,000 inhabitants). The position of the Rhône River plume varies based on wind and river flow; therefore, debris will be contributed from both the river itself and surrounding coastal areas in variable amounts. Since the smallest particles are most abundant here, it is probable that these microplastics, have also been transported by water masses for some time before collection. In the Bay of Marseille (stations #2-4) the dominance of larger particles (1-5 mm$^2$) suggests that the microplastics collected in this area were closer to their source and mainly originate from the urban area. A more efficient removal of the smallest floating particles in this region, via ballasting due to epiphytic growth, could also be a possible explanation (Ryan, 2015).
Spatial occurrence of microplastics and surface area distribution in the Northern Current (station #1), the Bay of Marseille (stations #2-4), the Rhône River plume (station #5) and the Rhône River (station #6). The size of the pie charts is hereby proportional to overall particle concentrations. Map modified after Schlitzer, R., 2009.

4. Conclusions

This study provides additional data on microplastic occurrence in the eastern Gulf of Lion. Our results revealed that surface water microplastic concentrations and size distributions in this area affected by anthropogenic impacts are consistent with those already published for
the western Mediterranean Sea. Significant temporal and spatial heterogeneity was observed for microplastic abundances. Our results confirm that the Rhône River, large cities, such as Marseille, and the Northern Current act as sources and/or transportation routes of microplastics collected in the northwestern basin of the Mediterranean Sea. As our microplastics are floating, it was shown that it can be pertinent to study the zooplankton composition of samples additionally to currentology data, in order to improve our knowledge on microplastic transport in the sea.
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