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Characterization of floating microplastic contamination in the bay of Marseille (French Mediterranean Sea) and its impact on zooplankton and mussels

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Abstract :

Microplastics (MPs) were sampled in three seasons from 2016 to 2018 in the Bay of Marseille, northwestern Mediterranean Sea, adjacent to a highly urbanized area. Six sites were selected according to their different characteristics (river mouth, treatment plants, protected marine area). Surface floating MPs were characterized (number, weight, typology and polymer) as was zooplankton. In addition, mussels were submerged and used to investigate ingestion. Finally, a hydrodynamic model was used to improve understanding of dispersion mechanisms. The annual averages of floating MPs values ranged from 39,217 to 514,817 items/km². The MPs collected were mainly fragments principally composed of polyethylene and polypropylene. The mean abundance ratio (MPs/zooplankton) was 0.09. On average 87% of mussel pools were contaminated and ingested 18.73 items/100 g of flesh. Two hydrodynamic patterns were identified: the first retaining the MPs in the harbor, and the second dispersing them outside.

Highlights

► The distribution and fate of microplastics were evaluated in the bay of Marseille (French Northwestern Mediterranean Sea). ► The floating MP reached more than 3.6 million items·km², mainly composed of polyethylene and polypropylene. ► 87% of mussels ingested microplastics, with concentrations at 18.73 items/100 g of tissues on the average. ► The ratio microplastics/zooplankton is a potential indicators of interactions between microparticles and zooplankton. ► Two hydrodynamic patterns were identified through modelling studies, including retention in harbor and dispersion offshore.

Keywords : Microplastics, Mediterranean Sea, Harbor, Impact, Zooplankton

1. Introduction

Plastic pollution has become a global issue causing major threats to the marine environment (UNEP, 2016b). The worldwide use and mass production of plastic in the last century has led to the accumulation of this type of debris in the global marine environment (Geyer et al., 2017). A study quantified that more than 12.7 million tons of plastic can enter the marine environment each year (Jambeck et al., 2015). Plastic properties such as lightweight, durability, and low cost explain the increase in production and have led to long-lasting contamination and accumulation in the marine environment (Andrady, 2011; Pirsahab et al., 2020). 75% of plastic pollution originates from terrestrial sources (fresh water input, residual and domestic activities, tourism and harbor) and from marine activities such as accidental loss of fishing gears and aquaculture (Thushari and Senevirathna, 2020). Plastic accumulation poses many threats to marine ecosystems by direct pollution (Sutherland et al., 2010), but it can also impact species inhabiting them

by causing, for example, strangulation and suffocation problems (Darmon et al., 2017; Fossi et al., 2018). In addition, smaller plastic particles can be ingested by marine organisms, resulting in various harmful effects (Giani et al., 2019; Kumar and Prasannamedha, 2021) such as chemical contamination, endocrine disruption and altered immune system responses. Indeed, plastic pollution is characterized by both macro plastic debris and smaller plastic particles known as Micro-Plastics (MPs).

MPs are defined as small plastic particles of sizes between 300 μm and 5 mm (Arthur et al., 2009). They are an important part of this pollution and are ubiquitous (Gago et al., 2018). They can be “primary”, coming directly from industrial production, the daily use of plastic products (e.g., cosmetics, scrubbing agents, pellets, etc.) and microfibers from clothing, or “secondary”, resulting from the fragmentation of MPs. Mesoplastics are sorted by size class between 5 and 20 mm. In addition, several studies have attempted to understand the different chemical properties and composition of polymer additives in order to

study the impact of pollutants associated with MPs (Guo and Wang, 2019; Vered et al., 2019). It has been shown that the polymers most present in the marine environment are mainly polyethylene (PE), polypropylene (PP), polystyrene (PS) and, in smaller amounts, other polymers such as polyurethane (PUR), polyethylene terephthalate (PET), ethylene-vinyl acetate (EVA), polyvinyl chloride (PVC), acrylonitrile butadiene styrene (ABS), fluorocarbon polymers, ethylene-propylene rubber and PEVA (De Haan et al., 2019; Kedzierski et al., 2019). The identification of microscopic particles involves two main steps: firstly, determining whether or not a particle is a synthetic polymer and, secondly, the identification of its chemical composition. The chemical identification of samples makes it possible to clearly attribute a sample to a certain polymeric origin. The study of the chemical nature of MPs allows both identifying sources and studying their degradation mechanisms.

These degradation phenomena are the result of mechanical forcing (wave), hydrological conditions (temperature, salinity) and UV radiations (Ter Halle et al., 2017). This contamination is omnipresent in all marine compartments (Bergmann et al., 2019), from the surface (De Haan et al., 2019) to benthic sediments (Harris, 2020), making MPs accessible to a wide range of biota occupying different habitats (Giani et al., 2019). Indeed, Coyle et al. (2020) listed a large number of studies indicating the presence of MPs at all levels of the marine environment, whether horizontal (surface), vertical (water column) or at the bottom in the sediments.

MPs are highly persistent in the marine environment (Alimba and Faggio, 2019) and this pollution is an emerging threat to human, animal and environmental health (Caruso, 2019; Landrigan et al., 2020). MPs also have many ecological impacts on marine fauna because, among other things, they affect the food web and release associated contaminants (Khalid et al., 2021). Through trophic transfer, MPs are found throughout the food chain (Wang et al., 2019). The impact of MPs on organisms depends on a combination of specific parameters, such as the position of these particles in the water column (Van Cauwenberghe et al., 2015), the polymers and additives composing them and organism ingestion systems. Many species are concerned such as zooplankton, mussels, oysters, corals, fish, turtles and even seabirds (Andrady, 2011; Wesch et al., 2016), some of which are consumed by humans, representing potential impacts on human health (Huang et al., 2020). Although several studies have already shown that MPs are ingested by different species, their mechanisms and effects are still poorly understood. Plastics decrease in size with weathering and fragmentation, making them accessible to a wide range of organisms. For example, MPs are similar in size to zooplankton (0.3 mm–5 mm), which can lead to confusion for predators regarding planktonic prey of this size. Therefore, they can be ingested and thus easily enter the marine food chain (Lusher et al., 2015; Van Cauwenberghe et al., 2015). It is therefore essential to improve our knowledge of the mechanisms involved, such as filter feeders and zooplankton.

The Mediterranean is not immune to this pollution and is considered as one of the most polluted seas in Europe (Gerigny et al., 2019) mainly due to its semi-enclosed nature. This pollution is essentially due to its intense shipping activity, industrialization, strong tourist pressure, industrial development and significant fishing activity (UNEP, 2016a). Annual plastic inputs into the Mediterranean Sea have been estimated at 100000 tons per year (Cincinelli et al., 2019) and the degradation processes of large plastic items are thought to be slow and heterogeneous. Several studies have shown that all compartments of the Mediterranean Sea (water, sediment and biota) are polluted by MPs (De Haan et al., 2019; Giani et al., 2019) and that their distribution and composition are heterogeneous between two sub-basins (western and eastern basins) (Cincinelli et al., 2019). However, this distribution remains variable and highly dependent on surface currents and wind conditions. Despite the growing number of studies, including modelling works, knowledge of the spatial and temporal distribution of MPs, their

composition, forcing and behavior is still limited (Pabortsava and Lampitt, 2020). The same applies to the contamination of Mediterranean species. Indeed, the Mediterranean is known to be a biodiversity hotspot greatly threatened by plastic pollution (Compa et al., 2019) and the ingestion of plastic by fauna is fairly well documented with a census of over 76 species belonging to different taxonomic groups, including invertebrates, fish, sea turtles, sea birds and marine mammals (Fossi et al., 2020). The control and evaluation of plastic pollution in the Mediterranean basin is a priority for environmental policies at European level (Maes et al., 2019), such as the plastic strategy, the Barcelona Convention (UNEP, 2017) and the Marine Strategy Framework Directive (EU, 2008, MSFD). These policies point towards the need, among other things to enrich our knowledge and better assess pollution, including that generated by MPs, whether on the large scale of the Mediterranean Sea or on a more local scale. Better understanding of this pollution should help to fight it in the future.

This study was initiated to improve knowledge of MP contamination in the bay of Marseille, located in the French Mediterranean Sea, adjacent to a highly urbanized area with a major harbor, tourist activities and a national marine park. Its aim was to report on a wider analysis of MPs and their impact on biota like zooplankton and mussel and establish a plastic/zooplankton ratio. For each sample, MPs were characterized by number, weight, typology and polymer type. Their abundance was compared to the abundance of neustonic zooplankton in the size class of 0.3–5 mm considered as the optimal prey size ingested by filter feeders such as fish and their juveniles. The fauna associated to MPs was identified and mussel was used as a model in order to assess the potential for plastic ingestion by a filter feeder. Finally, a hydrodynamic model and a dispersion model were coupled to understand the global dynamics of MPs in the area and to identify, if possible, the main types of forcing.

2. Material and method.

2.1. Study area

Marseille is located in the South of France on the coast of the NW Mediterranean Sea. Samples were collected at 7 sites (Figure 1) in 2016 (November), 2017 (May) and 2018 (June). The “Cortiou”, “Huveaune” and “Estaque” sites were located near the outlets of treatment plants. In the “Cortiou” site, three consecutive transects, including stations (“Cortiou Z-1.1”, Cortiou Z-1.2” and “Cortiou Z-1.3”), were determined to evaluate the MP concentration gradient as a function of distance to the point of discharge. The “Les Goudes” site was located in the Calanques National Park and considered an area without a direct source of pollution. The “Aygade” site was located at the exit of the port of Marseille which also receives wastewater treatment plants. The “Planier” site was selected because of its considerable distance from the coast to study the coastal influence of the other sites. The “Rhône” site located far away from Marseille, was selected to determine the influence of the river mouth.

2.2. Hydrology and circulation in the study area

The dynamics of the Eastern part of the Gulf of Lion is rather complex as the area of Marseille is governed by strong interaction between intermittent wind forcing, various coast lines orientations, general circulation (The North Current) and inertial motion. The reader will find description of the processes previously observed and modelled in this area in previous studies (Frayse et al., 2014; Pairaud et al., 2011; Schaeffer et al., 2011). In brief, following the Ekman drift concept, in case of westerlies or north-westerlies “surface” water fluxes becomes progressively southwards or south-westwards leading to an upwelling and taking the Rhône plume away from the coast and the bay of Marseille. Conversely easterlies and south-easterlies lead to a downwelling

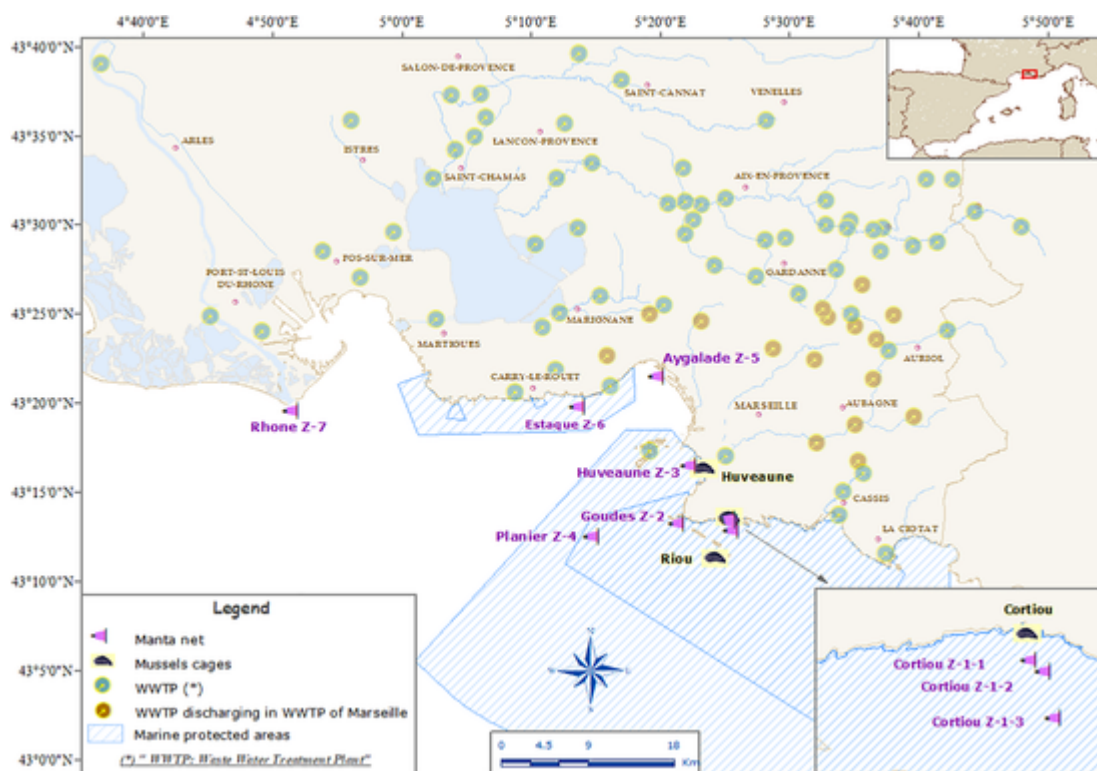


Fig. 1. Sampling sites for manta nets and mussel cages in the bay of Marseille (projection RGF93-Lambert 83).

pushing an eastward Rhone plume along the coast (see Fig. 4 in supplementary materials). Nevertheless, close to the surface the drift is closer to the wind direction (the classical drift at 45° to the right of the wind in the northern hemisphere). Moreover, wind direction is not constant and frequent wind shifts generates departures from this idealized stationary approach called relaxations or “transitory” dynamics. Additionally, an upwelling jet (a courant parallel to the front generated by the upwelling) progressively grows.

Under the influence of the Earth's rotation, the freshwater of the Rh one flows globally westward, but can occasionally spread towards the Bay of Marseille in very exceptional hydrological and meteorological conditions (Gatti et al., 2006) and during the three period of observation the Rh one flow was in the range of 1000 m³/s (October 2016), of 1500 m³/s (may 2017) and of 2400 m³/s (June 2018). The mean flow rate is in the range of 1800 m³/s. Offshore, a permanent current called Northern Current (formerly Liguro-Proven al Current) flows westward over the continental slope (Millot, 1999). This current is unstable and may degenerate into eddies, meanders and occasionally transitory in-shelf intrusions towards the bay of Marseille (Casella et al., 2020).

2.3. Floating MPs and zooplankton: in situ sampling, extraction and processing

MPs and zooplankton were collected with a manta net (330 µm mesh size and 0.6 m mouth opening). The net was towed with winds under Beaufort scale 4, along a straight line behind the vessel and out of the wake, for 20 min at 2 knots. Once brought back on board, the net and collector were rinsed with filtered seawater (300 µm), and conditioned in one-liter of seawater (glass bottle) fixed with a buffered solution of 4% final formaldehyde (Galgani et al., 2013).

MPs were extracted by sedimentation of the biological material (carried out in graduated tubes of 1 l). The supernatant and MPs were collected. The bottoms of the graduated tubes with biological material, zooplankton and the remaining MPs (i.e. dense polymer such as PVC or MPs trapped in biological particles such as animal antennas) were

sorted manually to extract the MPs and zooplankton. Each matrix (MPs and zooplankton) was then analyzed separately.

The plastic particles were sieved and sorted by size class, with three classes for the MPs (300 µm-1 mm, 1-2 mm, 2-5 mm) and one class for the mesoplastic (size class > 5 mm). For each size class, MPs were counted, and their typology determined (fragment, pellet, filament/fiber, foam (mainly polystyrene) and film) under a binocular. For the typologies analyzed at RAMAN, fibers and filaments are differentiated. Filaments refer to fibers of long continuous lengths, their diameter is uniform. Fibers refer to staples fibers of shorter length, their diameter is irregular and varies from one part of fiber to another. Then, the MPs (more or less than 5 mm, all typologies combined) were weighed after drying (50 °C for 12 h) and data from each sample was normalized according to the surface sampled and expressed as items/km² (density) or g/km² (surface masse) unity.

In addition, plastic and plankton were enumerated by digital imaging using a ZoosCan scanner with a resolution of 2400 dpi (Gorsky et al., 2010). Image post-processing was performed with the Zooprocess and plankton Identifier software that gives a large set of morphological parameters for each object and the taxonomic identification. The ZoosCan calculated the bio-volumes occupied by both plastics and zooplankton using the geometric mean of the major and minor axis of the best-fit ellipse (Gorsky et al., 2010). The MPs were then analyzed under RAMAN (see part 2.7).

2.4. Statistical analyses

Variations of plastic quantity, mass, size class and typology between sites and years were analyzed using the Kruskal-Wallis test associated with Dunn's test for multiple comparisons. The choice of using nonparametric tests was made in view of the small number of samples and the non-normal distribution of the density data. The calculations were carried out with the software R^(C) (V4.0.2) and the “Kruskal.test” and “dunn.test” (V1.3.5) functions. The *p*-values were adjusted for multiple comparisons using the Bonferroni correction.

2.5. MPs ingested by mussels: in situ sampling, extraction and processing

Mussel bags were installed in three different sites in 2017 and 2018. The ‘‘Cortiou’’ and ‘‘Huveaune’’ sites were selected to cover the outflow of a Waste Water Treatment Plant (WWTP). The ‘‘Riou’’ site, located offshore, was selected as the reference site. For each site, two mussel bags were submerged under the surface and deeper (see Sup Materials - Table 2).

The MPs were extracted by chemical digestion, sedimentation and filtration according to the method of (Phuong et al., 2018). The samples were heated with KOH for 12 h at 40  C. Then, the solutions were sedimented in two steps, the first one without and then with potassium Iodide (IK) to increase the density of the solution. The supernatants of the two phases were filtered on a fiberglass filter. Each site sample was composed by a mean of five replicates (R1 to R5). MP data were normalized according to the quantity of mussel tissues and expressed as number of items/100 g of tissues. The filters were used directly for RAMAN analysis (see part 2.7). For each site, five replicate analyses were performed.

2.6. Chemical characterization of plastic

Micro-Raman analyses were conducted according to Fr ere et al. (2016). Particles were analyzed with a LabRam HR800 (HORIBA Scientific, Villeneuve d'Ascq, France) using the laser wavelength set at 785 nm (Laser diode, Oxixus, Lannion, France). Experimental conditions (integration time, accumulation, laser power) for  -Raman analyses were set to limit fluorescence and increase the spectral quality of the particles analyzed. Particle identifications were performed by comparing the spectra obtained to the home database. Home database spectra were obtained with reference polymers (PolyEthylene Low Density (PELD), PolyEthylene High Density (PEHD), PolyPropylene (PP), PolyStyrene (PS), unplasticized PolyVinyl Chloride (PVC), PolyEthylene Terephthalate (PET), PolyAmide-6 (PA-6), PolyAmide-12 (PA-12), PolyTetraFluoroEthylene (PTFE), PolyMethylMethAcrylate (PMMA), Acrylonitrile-Butadiene-Styrene (ABS), PolyURethane (PUR)) supplied by GoodFellow (Lille, France).

Then, downstream, chemometric analyses were carried out to obtain a better identification, based on the percentage of similarity (minimum value of 70%) between the particles and the reference spectra.

2.7. Hydrodynamic model and IBM

A two-step zoom of the Gulf of Lions embedded in the ‘‘MENOR’’ configuration of the hydrodynamic model MARS3D was performed to explore the circulation in the Bay of Marseilles during the sampling periods (Garnier et al., 2014). The MENOR configuration encompasses the whole circulation of the north western Mediterranean Sea with a horizontal resolution of 1.2 km (https://marc.ifremer.fr/resultats/temperature_et_salinite/modele_mars3d_mediterranee). This model, run routinely, has been largely used for environmental purposes (Garnier et al., 2014), and also recently for MPs in the NW Mediterranean Sea (Kane et al., 2020). In this study, a zoom with a horizontal resolution in the range of 400 m was built for a finer description of the Rhone's plume dynamics and a more precise simulation of the currents in the Marseille area. It extends from the Gulf of Saint Tropez (eastern limit) to Barcelona (western limit). Both models (parent and zoom) were forced by the high-resolution atmospheric forcing derived from the French met-office model ARPEGE-HR. The surface current computation considers wind forcing using a vertical mixing scheme and a near surface refined grid. This procedure allows realistic surface drifts (De Dominicis et al., 2016). The software Opendrift (Dagestad et al., 2018) was implemented to derive the Lagrangian drift of MPs (particle tracking).

3. Results

3.1. MP characterization

3.1.1. Densities and masses

For both MPs and mesoplastics (Table 1, Fig. 2), the mean density in summer 2018 (respectively 514,817.41 and 29,294.51 nb km⁻²) was higher than in spring 2017 (respectively 191,943.72 and 6074.93 nb km⁻²) and in winter 2016 (respectively 39,217.44 and 1104.40 nb km⁻²). For both types of particles, the standard deviations were relatively high compared to their respective averages; even well above the average for MPs in 2017. In total, 100% of manta nets were contaminated by MPs, but this was not always the case for mesoplastics since they were absent from four stations in winter 2016 and spring 2018.

For MP surface masses, the mean value measured in spring 2017 (276.82 g km⁻²) was higher than in winter 2016 (3.66 g km⁻²) and in summer 2018 (116 g km⁻²). For mesoplastic surface masses, the mean value in summer 2018 (195.76 g km⁻²) was higher than in spring 2017 (149.31 g km⁻²) and in winter 2016 (1.30 g km⁻²). The correlation between the number of plastic particles by surface unity (density) and mass of plastic particles by surface unity was therefore tested, but no significant correlation appears between the two data sets.

The graphical analysis showed an overall increase in MP densities from year to year (or from season to season) for the Goude, Huveaune (particularly in 2018), Planier, Ayalades and Rhone sites (Fig. 2.a) whereas mesoplastic densities increased at the Cortiou 2 and 3, Huveaune, Planier, Ayalades and Estaque sites and stations (Fig. 2.c, e).

At Cortiou stations, MP densities decreased from near the outfall to the furthest offshore for the years 2016 and 2017, but this was not the case in 2018. On the other hand, this decrease from the coast towards the open sea was not observed for mesoplastics. The highest densities of MPs were sampled at the Ayalades site (2018 and 2017, Fig. 2 e), followed by Estaque (2017) and Huveaune (2018). For mesoplastics, the highest densities were sampled Ayalades (2017 and 2018) and Huveaune 2018. The largest masses of MPs were sampled in 2018 at the Ayalades site, and then in 2017 at the three stations of the Cortiou site and at the Goude site (Fig. 2.b). For mesoplastics, only the Ayalades site in 2018, and Cortiou stations 1 and 3 for the year 2017 accounted for larger masses when compared to the other sites (Fig. 2.d).

Overall, the statistical tests indicated that the data showed no spatial variability but that total densities (micro- and meso- plastics com-

Table 1

: Summary statistics of MPs and mesoplastics data. The calculations are based on the same 9 stations each year.

Year	Metric	Density (items/km ²)		Mass per unit area (g/km ²)	
		Microplastics	Mesoplastics	Microplastics	Mesoplastics
2016	Median	31,914.89	0.00	0.97	0.00
	Mean	39,217.44	1104.40	3.66	1.30
	Standard deviation	32,082.97	1495.25	5.17	2.35
	Min	4178.27	0.00	0.31	0.00
	Max	92,245.99	4172.46	15.90	7.21
2017	Median	64,602.96	4037.69	76.88	35.79
	Mean	191,943.72	6074.93	276.82	149.31
	Standard deviation	269,784.67	8997.98	292.96	256.51
	Min	40,322.58	0.00	0.00	0.00
	Max	768,777.61	26,509.57	632.19	608.33
2018	Median	70,769.23	4975.12	16.58	5.58
	Mean	514,817.41	29,294.51	116.00	195.76
	Standard deviation	1,167,273.47	60,633.10	262.49	538.16
	Min	27,881.04	1168.22	4.46	0.87
	Max	3,603,231.60	188,509.87	805.57	1629.44



Fig. 2. (a) Microplastic densities (item(s)/km²) and (b) surface weight (g/km²) by site or station in 2016 (winter), 2017 (spring) and 2018 (summer). (c) Mesoplastic densities (item(s)/km²) and (d) weight per area in 2016 (winter), 2017 (spring) and 2018 (summer). (e) Spatial distribution of microplastic densities (item(s)/km²) histograms per station and years.

bined) increased between 2016 and 2018 and that surface masses were higher in 2017 (*p* value < 0.05).

3.1.2. Size classes

In 2016 and 2017 (Supplementary Material – Fig. 1), the graphical analysis showed that the smallest size class (300–1000 μm) represented more than 70% of the samples. Over the 3 years, as the size class increased, the number of particles decreased. Concerning MPs however,

in 2018 the percentage of the size class 300–1000 μm was less than 50% (44.87%) and that of the size class 1–2 mm was almost equivalent (37.37%). Although their presence remained lower than that of the other size classes, particles in the 2–5 mm range and particles larger than 5 mm represented a larger part of the total particles in 2018 (respectively 12.43 and 5.32%).

Cortiou stations 1 and 2, and the Planier, Estaque and Rh ne sites had more than 60% of particles between 300 and 1000 μm. The propor-

tion of this size class for the other stations was lower, with a minimum of 41.25% for Cortiou station 3. Plastics larger than 1 mm had different particle size distributions depending on the year but not the sites, except at Cortiou station 3 which had more mesoplastics than the other sites.

For MPs and mesoplastics independently, and for both densities and surface masses, the Kruskal-Wallis tests did not identify significant differences between sites.

3.1.3. Types

For all years and all MP samples (<5 mm) combined (Supplementary Material - Fig. 2), fragments were the most common types, accounting for more than 80% of the particles collected, followed by films (between 3 and 7%) and foam (3–5%). The ratio pellets (0.78–2%)/fibers (0.62–4%) was not constant, with an alternating dominance of fibers in both 2016 and 2018, and pellets in 2017. Mesoplastic types were different from those of MPs, with a majority of fibers (83, 22 and 36% in 2016, 2017 and 2018 respectively), fragments (17, 42 and 28%) and films (0, 31 and 35%). Pellets were not observed in 2016 and 2018, and at less than 3% in 2017 whereas foam (mainly polystyrene), absent in 2016, remained under 3% in 2017 and under 1% in 2018.

For MPs, the Cortiou site showed the highest percentages of foam (9% at stations 1) and high percentages of films (8% and 13% for station 2 and 3 respectively). The Huveaune site had the highest proportion of films (15%). Concerning mesoplastics, there were very few pellets and little foam. The main types were fibers (between 20 and 100%), fragments (between 19 and 60%, absent from Cortiou stations 1 & 2 and from Planier) and films (between 9 and 38%, absent from Cortiou station 2 and Goudes).

As noted above, despite the absence of significant spatial differences, a significant time trend was observed for the fibers and films of the mesoplastic category and for foam MPs, with an increase between 2016 and 2018.

3.2. Polymers for MP marine water samples

Of all the MPs identified, the most abundant polymer was polyethylene ‘‘PE’’ (38–45%); followed by polypropylene ‘‘PP’’ (8–21%). Pigments such as blue phthalocyanine (PB15) and green phthalocyanine (PG7), titanium dioxide (TiO₂) which produces white coloring, and metal salts (Azoic: PR48) which produce red coloring, have been identified. These polymers have been identified alone or associated with polymers (for example PE + PB15).

There was a low percentage (≈ 1–10%) of natural compounds (shell, salt, sand, etc.) identified as MPs by optical techniques (Figure 3, OM). In 2016, this misidentification for all the samples combined was 9.4%, in 2017 and 2018, it was 5.87% and 1%. The maximum misidentification was therefore only 10% and is considered insignificant in view of the amount of particles analyzed under binocular.

Particles whose spectra did not match any polymer from the databases and particles that could not be identified (no signal or a saturated signal due to fluorescence) are represented by ‘‘NI’’ (unidentified spectra, 13–29%, Figure 3).

3.3. Plastic: zooplankton ratio and interactions with zooplankton

The abundance of MPs and zooplankton organisms in the 0.3 to 5 mm size class was calculated for each sample in Marseille Bay and their proportion was expressed as the MP/zooplankton ratio. This ratio was on average 0.09 ± 0.23 for the 3 years of the campaign. In the Bay of Marseille, the average ratio was 0.03 ± 0.04 with minimum values of 0.002 at the Planier site in 2016 and maximum values of 0.35 for the Ayalades site, situated in the middle of the bay, in summer 2018. At the site near the Rhone, the average ratio was higher (0.58) over the 3 years of the study with a steady increase over the years, reaching maximum values of 1.15 in 2018 (Figure 4).

The analysis of surface zooplankton allowed us to identify the fauna associated with MPs. If we focus on the mesozooplankton (0.3–5 mm), on average 37% of the fauna was composed of copepods, chaetognaths, mysids, cladocerans, larvae (zoea, megalopod crab) and eggs, with MPs representing 63% of the biovolume of samples (Supplementary material - Fig. 3). Macroplankton (> 5 mm) were mainly composed of gelati-

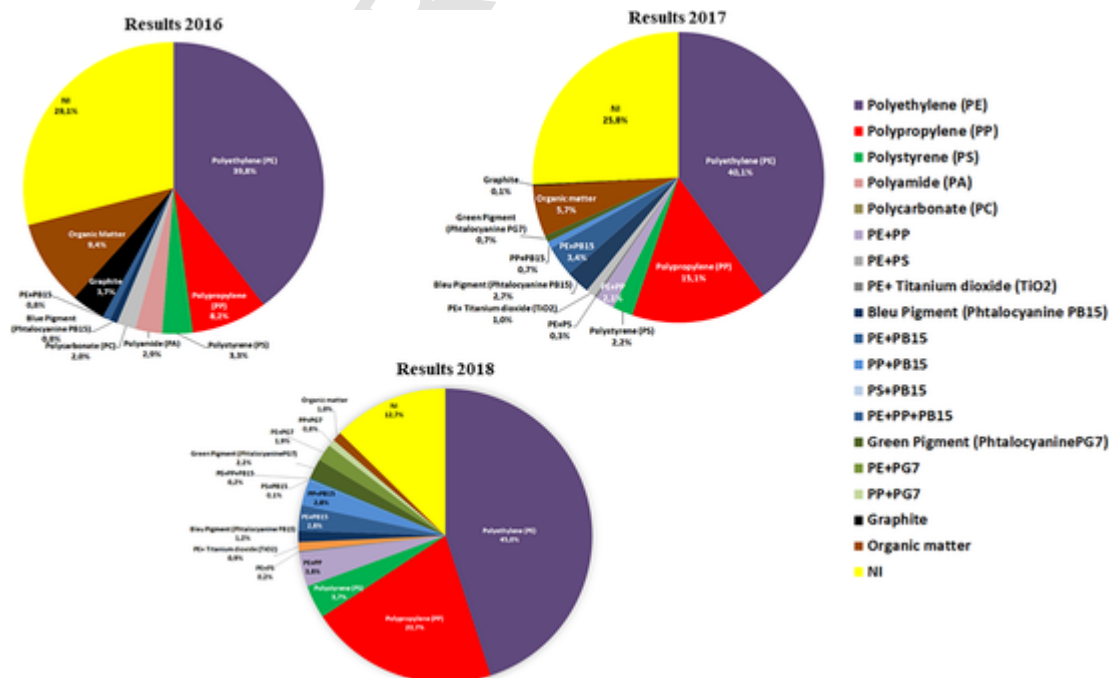


Fig. 3. Summary of particle types at all sampling points - 2016-2017-2018 campaigns. N.B. PB15, LePG7 and PR48 are pigments added to plastics.

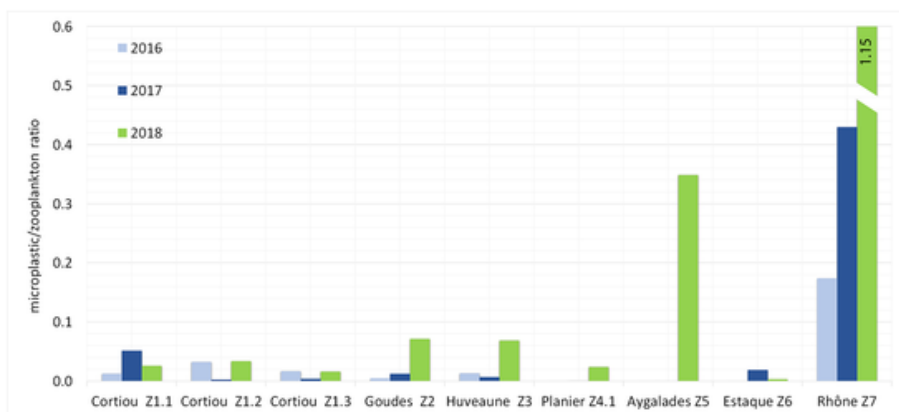


Fig. 4. Plastic: zooplankton ratio in the 0.3 to 5 mm size class at the 28 sites and stations of the Bay de Marseille during 2016 (winter), 2017 (spring) and 2018 (summer).

nous organisms such as Velelles, Doliolles, and several suborders of Siphonophores representing 87% of the total biovolume of the sample while the MPs represented 8%.

The biovolume of MPs at the site near the mouth of the Rhone River averaged 50% of the total biovolume in the samples for the 3 years of the study. The associated fauna was very different from the other stations in the bay, probably due to the influence of the river, and mainly composed of crustaceans, small grazers such as cladocerans, amphipods, isopods and cirripeds (Supplementary material - Fig. 3).

3.4. MPs in mussels

About 77% of the filters analyzed (all years, all sites) were contaminated with MPs. In 2017, 85% of filters (all replicates) were contaminated by MPs, compared to 76% in 2018. If both years are taken into ac-

count, an individual ingested an average of 1.18 items/mussel for 2017 and 1.30 items/mussel in 2018.

For all sites and depths combined (Figure 5.a), this gives an average of 18.73 ± 12.52 items/100 g of tissues (wet weight) with values ranging from $7.1 (\pm 12)$ for Riou, 2017/surface and ± 8 for Huveaune 2018/background). Statistical analysis was not performed due to limited data, through there was no apparent trend.

Mussels ingested three types of MPs: fragments, including fibers and filaments (Figure 5. b and c). For all the sites combined and for the 2 years, the percentages of fragments (53%) on the surface were higher than the percentages of other types of MPs (fibers and filaments – 47%) ingested by the mussels. At depth, the distribution of types was inverted with a maximum of fibers and filaments representing 62% versus 38% of fragments. Only the blanks in the samples from Huveaune (2018, surface) and Cortiou (2018, surface) were contaminated, but by types of

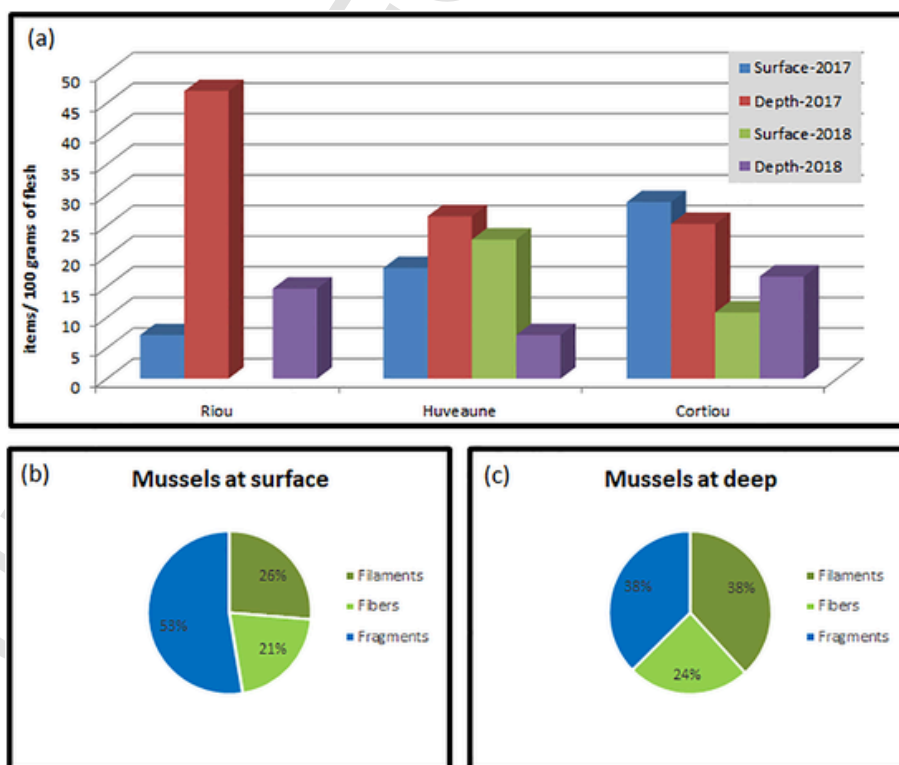


Fig. 5. (a) Average of the MPs ingested by the mussels at the three sampling sites (Riou, Huveaune and Cortiou) for 2017 and 2018, depending on mussel immersion depth. (b) MPs typologies submerged at surface (b) and at depth (c) for the 2 years 2017 and 2018 included.

particles not found in any of the samples, these particles were therefore not removed from the sample counts.

3.5. Hydrodynamism and MPs dispersion

Several sources of MPs were identified around the bay of Marseille in the course of the FUI project. Two important sources are outside the Bay: the Rhone River and the outlet of Marseille's water treatment plant at Cortiou. Three other potentially important sources exist inside the bay, named Estaque, Ayalade and Huvaune, respectively. Other small inputs probably exist all around the bay, but these five sources are considered as the most important ones.

To interpret the measurements, the MPs drifts coming from these main sources were estimated the week before the records were taken, using Opendrift software. A continuous release of particles was applied during this simulation period. MPs were advected in the thin surface layer under the action of the surface current forced by wind stress and local hydrodynamics. For the sake of clarity, tracks of MPs released from the inner bay sources (Estaque, Ayalade, Huvaune) and from the outer bay sources (Rhone, Cortiou) are presented separately (Figure 6).

During the week prior to the first recording (2016-10-13 to 2016-10-21), the Marseille area experienced such a wind shift. At the beginning of the period, strong southeast winds push the Rh one discharge westward along the coast. The relaxation just after the westerly wind shift allows the Rh one particles to be advected eastward towards the

bay of Marseilles before being exported south or southeastward under the effect of persistent west winds. The Cortiou discharges exhibited the same simulated export of MPs to the south or southeast, while the Inner Bay discharges remained confined and isolated from imports from the Rh one or Cortiou.

In May 2017, the meteorological situation was characterized by strong south-easterlies for 3 days (11–12–13 May). This resulted in an intrusion of the North Current into the shelf, generating a general westward circulation, but locally an anticyclonic (clockwise) gyre in the inner bay of Marseille that allowed the particles to escape. When the wind weakened and shifted westerly, the coastal water calmed and a south-westward coastal drift developed (a consequence of the relaxation), driving the particles released from Marseille towards La Ciotat. Particles from the Rh one also exhibited a southwestward trend at the end of the period. Loops observable in the trajectories reflect the inertial dynamics generated by this rather strong south-easterly gust.

For the latest records of MPs (June 2018), the situation is clearer and more classical. Five days of Mistral (from 14 to 18 June) caused the particles to advect southwards and southwestwards. For the three releases of the inner Bay, the upwelling induced pushed the particles offshore expelling the MPs from the bay from the south. At the end of the period, the wind abated and a north-westward coastal current drove it back into the bay.

The year 2016 was the year with the lowest MP concentrations in the bay. MP concentrations were moderate everywhere in 2016. Very

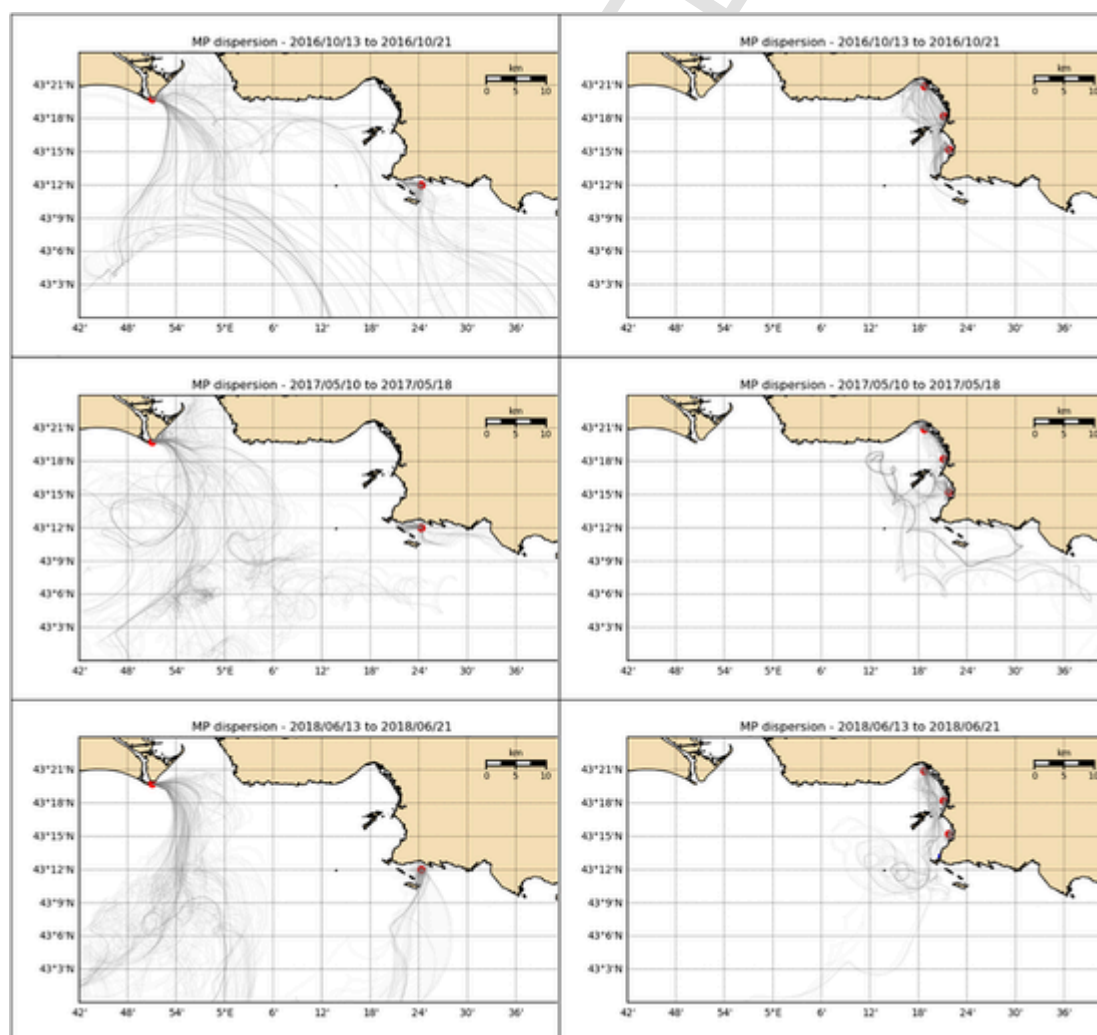


Fig. 6. Simulated surface trajectory of the microplastics released continuously during the week preceding the sampling by the sources of the outer bay - Rhone, Cortiou - (left column) and by the sources of the inner bay - Estaque, Ayalades, Huvaune - (right column).

high concentrations of MPs were detected at Aygalades and Estaque in 2017, and at Aygalades and Huveaune in 2018.

4. Discussion

The distribution of MPs is strongly dependent on hydrodynamic and meteorological drivers. It is therefore difficult to identify their source and area of accumulation. While many publications have dealt with the issue on a global scale, MP pollution needs more refinements at regional scale to understand the dynamics of particles, and support targeted reduction measures. The “FUI Microplastics” project attempted to improve knowledge of MP pollution in the bay of Marseille and better understand the harm it causes.

In the bay of Marseille, MP values varied between 4178 and 3,603,231 item·km⁻² with average values ranging between 39,217 and 514,817 items·km⁻². Compared to the literature on the Atlantic and areas of the Mediterranean Sea (Herrera et al., 2020), the Bay of Marseille appears to be highly contaminated. Compared to data collected in Marseille harbor between 2014 and 2016, indicating densities ranging from 6.10³ to 1.10⁶ item·km⁻² (Schmidt et al., 2018), the data presented here in this study covers a wider range of concentrations, with a lower minimum and a maximum that tripled. In another study, Schmidt et al. (2018), the wind was identified as driving the transport of MPs from the bay to offshore waters, and the main effluent of the city was shown to largely contribute to MP pollution with considerable temporal variability. The Rhône was also demonstrated as a main source of MPs pollution, as were the smaller rivers in the harbor, and large debris were mostly found close to the sources.

In this present work, the particles were mainly small-sized (class 300 µm-1 mm). Fragments, followed by films and foam (mainly polystyrene) were the most abundant shapes, as conventionally encountered in the literature (Zayen et al., 2020). Conversely, the mesoplastics were mainly fibers, followed by fragments and films. The fragments are considered to be secondary MPs, products of the degradation of a large plastic element, possibly at sea and not necessarily from wastewater treatment plants. Size distribution can be an indicator of the source of marine debris and its distance from the shore or source. While Pedrotti et al. (2016) observed small MPs in abundance in the first kilometer adjacent to the coast, Isobe et al. (2015) found larger particles generally in areas close to sources of pollution. It is therefore possible to attribute mesoplastic fibers to contributions from the city of Marseille. Generally, the surface of the area has limited contamination by pellets, as is often the case in the Mediterranean.

No spatial variability was found and it was not possible to clearly attribute the temporal variability to annual or seasonal variations. Lower values of MPs were observed distinctly in winter and these results appear consistent with the other literature results in other Mediterranean sites (Collignon et al., 2014). Moreover, while Schmidt et al. (2018) distinguished a difference between certain sites, notably between the Rhone and Cortiou, no spatial variability was noted in this study, an uncommon scheme, as MP concentrations are largely affected by waves, wind, riverine inputs and hydrodynamic characteristics, even at local scale (Goldstein et al., 2013). Limited sampling remains a possible explanation, as the focus of the study favored the nature and composition of microparticles (types, polymers), their interactions with zooplankton, and their transport. A future study could be carried out in the area to study in more detail the seasonal variability but with one spatial monitoring point following during 1 year.

As described above, the Rhône panache is very unlikely to reach this area of interest. But this does not mean that the surface current induced in strong mistral conditions cannot drive particles outside the dilution area. However, as the current is mainly directed offshore in Mistral conditions, the probability that particles originating from the Rhône reach the Bay of Marseille is quite low. In contrast, particles originating from Cortiou are very likely to reach the bay in easterly wind conditions or in

the case of intrusion of the Northern current. Overall, the sources of plastics simulated within the Marseille area tend to be trapped in the area. While those from the Rhône disperse more rapidly towards the open sea. Even if the hydrodynamic conditions in 2016 had a particle retention action in the Marseille area, the MP concentrations were the lowest. Conversely, the simulated MPs of 2017 and 2018 were less retained in the Marseille area than in 2016, but the concentrations were higher. The hypothesis would be that the concentrations of MPs observed in the Marseille area would be mainly to be attributed to the direct anthropogenic contributions which varies according to the season/year or human activities. The hydrodynamic conditions play at this scale a secondary role.

Polyethylene “PE” and polypropylene “PP” were the main polymers present in the samples studied. The dominance of these polymers in the bay is consistent with other surveys conducted in the Mediterranean Sea (De Haan et al., 2019; Kedzierski et al., 2019).

Synthetic polymers constitute the major part of floating marine debris. There are different types of polymer and mixtures of polymers in commercial production. PE and PP are frequently detected in the marine environment (Erni-Cassola et al., 2019) due to the common use of these materials (corresponding to 49% of world plastic production (PlasticsEurope, 2019) and their common use for the manufacture of short-life cycle products. PE and PP float because have lower densities (below 1 g/cm³) than seawater. As they are subject to chemical, biological, or photic degradation processes, they gradually degrade into smaller fragments until becoming MPs.

Many plastics are produced as a mixture of different polymers and various plasticizers, colorants, stabilizers and other additives. Several pigments used in the plastic industry were identified. They are standard pigments used in the packaging industry and in cosmetics, and can be found frequently in the marine environment (Frère et al., 2016; Lenz et al., 2015; Van Cauwenberghe et al., 2015). These pigments are used in colorful paintings and coatings is ubiquitous not only for plastics but also for products made of metal, wooden, fiberglass and carbon fibers (Imhof et al., 2016). The TiO₂ can be used in the manufacture of antifouling products on the hulls of boats (Szeto et al., 2020). The proximity of port activities in the harbor of Marseille, high anthropogenic activities and a large population could be one of the potential sources of these pigments and substances.

While Schmidt et al. (2018) recommended linking the long-term composition of zooplankton, MP concentrations and hydrodynamic conditions to obtain better insight, the present study considered a shorter period but focused on mechanisms with more elements analyzed, including plastic typology and polymer composition, in addition to zooplankton and hydrodynamic conditions.

In the Bay of Marseille, the MP/zooplankton ratio (0.3–5 mm) is similar to the ratio found by Pedrotti et al. (2016) for a coastal zone located less than 1 km from land in the NW Ligurian Sea (on average 0.03 ± 1.40) and higher by one order of magnitude than those found in the NW of the Sardinian Sea (0.006 ± 0.006) for sites beyond 10 km from the coast (Panti et al., 2015). The presence of MPs and zooplanktonic groups of the same size can lead to potential confusion for predators with respect to optimal size range of planktonic prey, therefore this ratio can be used to express the probability of ingesting MPs. For filter feeders such as fish, this means that adults and larvae have a 3% chance of confusing planktonic prey with MPs. This probability can increase to 35% in the center of the bay, while in the most polluted areas near the Rhone it can reach 50%. The higher ratio could be explained by a higher load of plastic from the river. This area should be considered for monitoring as food is a determining factor for the juvenile stages of fish, for example.

Another possible trophic relationship concerns macrozooplankton, studies have shown that MPs can be ingested by many taxa or even directly by their predators and thus contaminate the entire oceanic food web (Cole et al., 2013; Desforges et al., 2015). Gelatinous organisms

found in higher proportions in this study are transported by currents in the same way as plastics, and are thus likely to ingest MPs. A recent study showed that salps are capable of ingesting large quantities of mini-microplastics (5–333 μm) present in the marine environment at concentrations 5–7 times higher than MPs (Brandon et al., 2020). They are also able to filter MPs such as filaments and films (Arthur et al., 2009). Larvae and eggs associated with MPs may colonize or adhere to these surfaces to finish their development and be carried by currents away from recruitment sites. By studying the abundance, surface measurements and other morphological parameters of MPs and associated zooplankton, this study showed that the impact of MPs on the planktonic food web could be considerable.

In the context of the evaluation of methods for characterization of MPs, the identification of natural particles as a plastic particles represent a risk of overestimation evaluation methods (Cadiou et al., 2020). Indeed, the coupling of the different characterization methods used in this article (visual and RAMAN) showed that natural particles had been identified under binocular as plastic. However, the percentages of these overestimations, which vary between 1 and 10%, do not represent a significant portion of the samples and remain quite acceptable for analysis. In the context of several European directives and regional seas conventions (MSFD, plastic strategy, OSPAR and Barcelona), the monitoring and characterization of plastics has become a major issue. Monitoring programs therefore require reliable and affordable techniques. The results therefore indicate that counting under binoculars remains an efficient and inexpensive method for this monitoring perspective.

The ultimate goal of this research was to model the dispersion of MPs based on geographic sources and to predict areas of accumulation in order to assess their risk to biota and associated economic activities. This will encourage the implementation of a long-term monitoring program to provide reliable and effective assessments to generate awareness and support measures. In the context of DCSMM and the establishment of indicators of good ecosystem status, the quantities of plastics relative to zooplankton should be a good candidate as an indicator of the state of pollution in the marine environment.

Not all the mussel pools studied were contaminated by MPs, consequently this pollution was not omnipresent in all the filter-feeding marine organisms (mussels) on the site. The values of MPs ingested ranged from approximately 7 to 46 items/100 g of flesh, and the average was 18 items/mussel. Overall, the mussels ingested fragments and fibers or filaments. On the surface, the mussels ingested more fragments than fibers and filament, and vice versa at depth. Contrary to what could have been expected, the mussels at the Cortiou station, near the outfall, were not more contaminated than at the other sites. Conversely, those submerged on the Riou site (considered clean, due to the presence of the reserve) had the highest average. Currents could partly explain these results, with the presence of the Liguro-Provencal current oriented from east to west and favoring the accumulation of particles in the harbor of Marseille. In 2017, the particles released from the sources of contamination in the harbor of Marseille dispersed to the south, then to the west of Riou, where the mussels were positioned. The high MP concentrations of Riou could potentially be explained by this source. However, the dispersion study carried out over the few days does not allow this hypothesis to be robustly validated and could be the subject of a future study. Differences or the absence of differences of contamination in the bivalves between different sites (anthropized and virgin) have already been observed in the literature (Br ate et al., 2018; Marques et al., 2021), however it was difficult to clearly attribute this phenomena to marine currents, because these can mask the direct influence of a local anthropogenic source (Galgani et al., 2000; Yu et al., 2017). In addition, it appears that the concentrations of bivalve contamination by MPs also seem to be influenced by a mechanism that regulates mussels' ingestion/depuration rather than by the direct absorption response to the availability of MPs in the marine environment (Marques et al., 2021).

Initially, the choice of having two immersion depths was made in order to guarantee the data, because surface mussel pockets often disappear. However, this experiment showed that depending on the depth of immersion the ratio of the typologies of the MPs ingested was not the same and therefore, in parallel, the typology of surface MPs could potentially be different from those of MPs present in the water column. For instance, in the Northern Ionian Sea Digka et al. (2018) found an average of ingested MPs corresponding to 1.7 items/mussel with a dominance of fragments (77%) and fibers (22%). On Turkish coasts, Gedik and Erya sar (2020) found an average of ingested MPs corresponding to 0.69 items/mussel and 0.23 item/g fresh weight, with a maximum of 6.67 items/mussel and 2.78 items/g fresh weight with a fragment percentage of 67%. The ingested MP values of Marseille harbor are therefore much higher than those encountered in the literature.

5. Conclusion

The Bay of Marseille is largely affected by plastic pollution due to a highly populated area, a large harbor and associated industries, the inflows of the Rh one River, large volumes of effluents flowing directly into the sea, and hydrodynamic conditions that contribute to the retention of water in the area. In this study the amounts, the distribution, and more generally the fate of MPs were described in the bay. The results indicated the same pattern of contamination as many other areas in the Mediterranean Sea, however with higher amounts. The study confirmed the impacts of MPs on two components of biota, providing information on a potential indicator of interactions between microparticles and zooplankton, also showing the potential impact on the food chain. Overall, the results provided the scientific and technical basis for further monitoring, and better support for reduction measures.

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CRedit authorship contribution statement

O. G erigny: Conceptualization, chief of cruise, investigation, formal analysis, investigation, main writing (original draft, review & editing), visualization, supervision.

M-L Pedrotti: Conceptualization, investigation, writing.

M. El Rakwe: investigation, writing.

M. Brun: formal analysis, data curation.

M. Pavec: formal analysis, writing.

M. Henry: investigation.

F. Mazeas: investigation.

J. Maury: investigation.

P. Garreau: investigation, formal analysis, writing.

F. Galgani: Conceptualization, writing, supervision.

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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