Marine Pollution Bulletin
June 2023, Volume 191 Pages 114932 (8p.)
https://doi.org/10.1016/j.marpolbul.2023.114932
https://archimer.ifremer.fr/doc/00834/94597/

Physical processes matters! Recommendations for sampling microplastics in estuarine waters based on hydrodynamics

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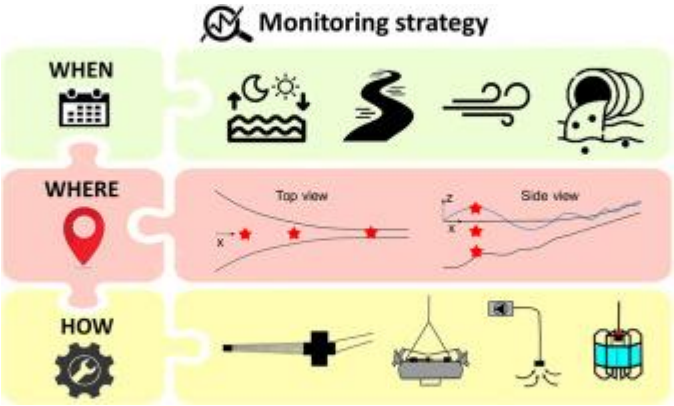
Abstract :

Monitoring the abundance and characteristics of microplastics in estuarine waters is crucial for understanding the fate of microplastics at the land-sea continuum, and for developing policies and legislation to mitigate associated risks. However, if protocols to monitor microplastic pollution in ocean waters or beach sediments are well established, they may not be adequate for estuarine environments, due to the complex 3D hydrodynamics. In this note, we review and discuss sampling methods and strategies in relation to the main environmental forcing, estuarine hydrodynamics, and their spatio-temporal scales of variability. We propose recommendations about when, where and how to sample microplastics to capture the most representative picture of microplastic pollution. This note opens discussions on the urgent need for standardized methods and protocols to routinely monitor microplastics in estuaries which should, at the same time, be easily adaptable to the different systems to ensure consistency and comparability of data across different studies.

Graphical abstract



Highlights

► Strategies and methods for sampling microplastics in estuarine waters are reviewed. ► The need of considering hydrodynamics when planning in-situ surveys is highlighted. ► Recommendations on when, where and how sampling are discussed in relation to hydrodynamics.

Keywords : Microplastics, Estuary, Sampling, Monitoring, Physical processes, Hydrodynamics

1. Introduction

Estuaries form the connection between marine and fluvial waters. They are highly productive ecosystems that provide important environmental, social and economic services (Barbier et al., 2011). Estuaries are, however, highly susceptible to both natural and human disturbances. Like other aquatic systems around the world, estuaries are vulnerable to plastic pollution (Browne et al., 2010). In particular, the abundance and risks associated with microplastic particles and fibres (MPs), defined as plastics lower than 5 mm (Frias and Nash, 2019), are of high concern. Their similar dimension to sediments and planktonic organisms make them easily ingestible by the aquatic biota. The ingestion of MPs can be responsible for gut abrasion and blockage, as well as intoxication by sorbed contaminants or toxic additives used in the compounding of plastics (Andrady, 2011; Kazour et al., 2020). Living organisms may also develop on the MP surface in the form of biofilms (Amaral-Zettler et al., 2020). MPs may thus act as dispersal vectors of pathogens in moving estuarine waters (Forero-López et al., 2022).

Estuaries are critical areas for plastic pollution due to their interface nature between the ocean and land. They are convergence areas of marine-based plastic pollution (Kuczenski et al., 2022), land-based plastic pollution from rivers (Lebreton et al., 2017), and also intrinsic estuarine-based plastic pollution from industries, cities, fishing, and port activities (Napper et al., 2022). In a similar way that estuaries are sinks for sediments, they can represent a temporary or permanent sink for MPs (Fok and Cheung, 2015; Nel et al., 2020; Simon-Sánchez et al., 2019). Estuarine hydrodynamic processes can form convergence zones of MPs or Estuarine MP Maxima (EMPM) within the estuary (Díez-Minguito et al., 2020; Bermúdez et al., 2021). Even after being trapped during long periods of time, MPs can still be flushed from estuaries by extreme events at time scales from annual to pluriannual (Tramoy et al., 2020). The high ecological values of estuaries together with their double role as an ocean source and a sink have motivated numerous research questions and attracted the recent attention of the interdisciplinary plastic research community (Gallagher et al., 2016; Gray et al., 2018; Hitchcock and Mitrovic, 2019; Sadri and Thompson, 2014; Zhao et al., 2014).

In situ observations of microplastic concentrations in the abiotic compartment is a key approach to address emergent research questions on plastic pollution at the land-sea continuum, e.g.: (1) the sources and generation of MPs; (2) the pathways by which MPs reach estuarine, coastal and ocean waters; (3) the abundance, distribution and fate of MPs through the continuum ecosystems; (4) the transport mechanisms driving spatio-temporal variations and promoting the flushing of MPs to the ocean; (5) MPs ageing, weathering and biofouling; and (6) ecosystems exposition and risks, among others. Numerous protocols have been developed to study MP contamination in ocean surface waters or beaches (Besley et al., 2017; Hanke et al., 2013; Masura et al., 2015; Miller et al., 2017, 2021). However, these protocols or strategies may not be suitable to evaluate MP contamination in estuaries and transitional waters. Most of these protocols are based on the assumption that MPs are floating on the surface or have been deposited on the bed depending on their density. The complex three-dimensional hydrodynamics in estuaries can make this assumption, and therefore these protocols, inappropriate. Table 1 compares sampling strategies previously implemented in the water matrix of different estuaries around the world, highlighting that most of them focused on surface water.

The specific hydrodynamics and properties of estuarine waters play a crucial role in the distribution, transport and trapping of MPs (Malli et al., 2022; Jalón-Rojas et al.). Estuarine waters are characterised by a changing density due to varying salinity and temperature that will largely impact the buoyancy of MPs (Defontaine et al., 2020). Estuarine waters are also rich in sediment and living organisms (blooms) that may favour MP flocculation. Andersen et al. (2021) suggested that the estuarine residence times of some MPs are long enough for sediment adhesion and possibly biofouling to occur. Flocculation and biofouling can modify the dynamical behaviour of particles (Andersen et al., 2021; Jalón-Rojas et al., 2022) and even induce the sinking of initially buoyant MPs (Kaiser et al., 2017; Laursen et al., 2022). The variety of forcing influencing estuarine hydrodynamics at different time scales, such as wind, tide, river discharge, and waves, also largely affects MP transport. However, only a few studies in the literature specify the tidal phase, current velocities, or water properties (e.g. salinity, turbidity) at the time of sampling (e.g. Defontaine et al. (2020); Gasperi and Cachot (2021), see Table 1). Compared to river or ocean transport, the three-dimensional estuarine circulation greatly increases the complexity of the dynamical behaviour of MPs and vertical transport plays a key role. Due to this complexity and the youth of this research field, the contamination, distribution and fluxes of MPs in estuaries are largely unknown.

In this manuscript, we sustain that to keep gaining knowledge on MP pollution in estuaries, monitoring protocols should be linked to estuarine hydrodynamics and their typical spatio-temporal scales. We also identify the lack of consistent and standardised methods and protocols for these systems (Table 1) as a limitation to inter-compare different systems and keep gaining understanding. The present work provides technical recommendations and suggestions for monitoring MPs in estuarine waters, focusing on sampling strategies; i.e. digestion, density separation, extraction, counting and chemical characterisation techniques being out of the scope of this note. Here, we present some of the most used techniques with their advantages and disadvantages depending on the type of estuary to be studied. In light of this, this note aims to answer the following questions : (i) when to monitor MPs in estuaries, (ii) where to monitor MPs in estuaries, (iii) and how to monitor MPs in estuaries. Good practices when sampling MPs are also discussed.

2. When to monitor MP in estuaries?

Two elements can affect the spatio-temporal variability of MPs in an estuary: flow patterns and MP sources. The environmental forcing (e.g., tides, river discharge, wind, waves) affecting the flow and the underlying physical processes driving the transport of suspended particulate matter (e.g., density stratification, exchange flow, tidal mixing, stokes drift, tidal pumping; Fig. 1) are well-known from decades of research in estuarine physics and sediment transport (Winterwerp and Van Kesteren, 2004; Scully and Friedrichs, 2007; Jay, 2010; Geyer and MacCready, 2014; Burchard et al., 2018). MP inputs from various sources (fluvial, marine and local) introduce additional temporal variability in MP abundance and distribution. This variability may depend on the environmental forcing (e.g. river and surface runoff inputs), or on human activities schedule (e.g. boat-based sources, sewage output). Advanced knowledge of the local hydrodynamics and the potential sources is therefore required to determine when to collect MPs.

Various key time scales should be considered when planning samplings in estuaries, namely tidal, fortnightly and seasonal time scales. The particular importance of a given time scale is strongly related to the dominant forcing of the estuarine dynamics and is, therefore, site-specific (Jay, 2010; Jalón-Rojas et al., 2017). In estuaries dominated by tides, MP concentration can vary significantly between ebb and flood tides (Oo et al., 2021). This difference can be related to various processes such as tidal asymmetry (the flood or ebb dominance of currents). For example, in a flood-dominated estuary, sinking MPs could have a higher rate of resuspension and mixing during flood tides. Microplastic concentration may also vary over the tidal cycle in stratified and salt-wadge estuaries where tidal motion is weaker. This can be due to variations in currents and salinity driven by tidal variations in vertical mixing and the along-channel density gradient. Defontaine et al. (2020) show that the transport of MPs in suspension may be contained by the pycnocline due to the turbulence damping induced by density stratification, whereas periods of intense mixing led to homogeneous concentration along the water column. However, fewer studies sampled microplastics at different tidal phases (Tab. 1). Given the importance of the tidal time scale, we recommend sampling always at the same tidal phases to allow the inter-comparison of samples and, as far as possible, sampling at different key tidal phases (e.g. flood, ebb, high water, low water).

Estuarine physical processes may change from spring to neap tides or from equatorial to tropical tides (Defontaine et al., 2022; Valle-Levinson and Schettini, 2016). Microplastic concentration may therefore vary at the fortnightly time scale (Stead et al., 2020), analogously to suspended sediment concentrations (Jalón-Rojas et al., 2015). In addition, estuaries can be subject to the seasonal variability of forcing (e.g. river flow, wind) that may impact the stratification, residual currents, and therefore MP transport trends. In particular, the estuarine residual circulation (which includes several processes such as density-driven circulation, wind-driven circulation, internal asymmetry, non-linear tidal motion and the Stokes drift) can be subject to seasonal changes related to the annual river flow cycle and the precipitation/evaporation balance (Jay, 2010). To take into account all these variability time scales, it is highly recommended to monitor during full tidal cycles, under contrasting tidal ranges and river flow conditions.

Other forcing and processes such as wind gusts, sea breeze, harbour seiches and waves may also play critical roles in MP dispersion at different time scales. Browne et al. (2010) show that a prevailing wind direction can be responsible for MP accumulation at downwind sites of the Tamar estuary. Sánchez-Hernández et al. (2021) also showed that MP abundance was higher during periods of strong winds. In addition, wind and waves may also trigger vertical mixing of plastic debris (Kukulka et al., 2012).

In summary, contrasting conditions of prevailing forcing (e.g. low/high river flow, spring/neap tides, wind/unwind conditions, heavy swell/calm sea conditions) should be considered when planning monitoring campaigns. Table 2 summarizes key recommendations about when sampling microplastics depending on the main forcing and hydrodynamic processes of a given site. The post-processing of MP samples is expensive in terms of time and human resources, which can inevitably affect the final choice of the monitoring periods. When all the representative time scales of variability are not considered, results should be analysed keeping in mind that MP concentrations are only representative of the selected conditions.

The discussion of MP concentrations at the different key time scales should also consider the variability of MP inputs from sources (Tab.2). Sources such as urban storm-water runoff or wastewater effluents may have a variable discharge depending on the weather forecast (rainfall) or the peak period of city occupation (e.g. tourism). Significant links between rainfall and MP concentration have been found in the literature as it drains the land-based MP pollution through rivers to estuaries (Lima et al., 2015; Hitchcock, 2020). The wet season could therefore represent a period of a strong abundance of MPs. However, the dry season may represent a period of denser population and thus an increase of MP inputs from surrounding cities and shores. Collection during the wet and dry seasons is therefore relevant to investigate potential seasonal differences in contamination. In addition, wastewater effluent contamination may also vary between weekends and weekdays, and between flood and ebb tides (e.g. WWTP discharge during ebb tide to favour flushing). Samplings only on weekdays should be envisaged (Miller et al., 2021).

3. Where to monitor MP in estuaries?

As in many environmental pollution studies, the number of sampling sites and their location is of the foremost importance to ensure a truly representative study by probabilistic sampling. However, as explained above for the sampling frequency, the particularly expensive cost and time-consuming laboratory analyses that follow MP collection may be a strong limitation. Compared to lakes, rivers or the ocean, MP distribution in estuaries can strongly vary spatially in the three dimensions over small spatial and temporal scales (Malli et al., 2022; Jalón-Rojas et al.). A compromise should be found between spatial statistical coverage and time/financial costs. The site selection should then be restricted to a few sites as representative of the whole estuary as possible. Table2 also summarizes key recommendations on sampling locations depending on the major forcing and hydrodynamic processes.

Intratidal and subtidal circulation may vary along the longitudinal and lateral axes as well as water properties affecting the dispersion of suspended particles such as MPs (Lam et al., 2020). Different locations along and across the estuarine channel should thus be considered (Tab.2). Even if the choice of the locations should be based on the specific characteristics of the study site, a general recommendation is to sample at different sites along the longitudinal density gradient, as physical processes driving the transport of MPs and MP buoyancy vary over this gradient (Malli et al., 2022; Jalón-Rojas et al.) (Fig. 1). For instance, density-driven circulation is a physical process directly related to the density gradient. In estuaries characterized by decreasing salinity from the ocean toward the upper estuary, light MPs might be transported as wash load to the ocean by a surface seaward flow, while dense MPs might be transported as bed load by a landward flow near the bottom (Defontaine et al., 2020). Wind stress can also promote a two-layer circulation (Li and Li, 2011). Recent studies based on numerical and idealized models have suggested that estuarine circulation may form hotspots of microplastics, also called Estuarine MicroPlastic Maxima (EMPM) (Díez-Minguito et al., 2020; Bermúdez et al., 2021). In estuaries or estuarine regions dominated by tides, tidal pumping can also generate longitudinal circulation and MP trapping (Stead et al., 2020). In flood-dominated estuaries, the tidal wave may become increasingly asymmetric (higher flood than ebb currents) along the estuarine channel depending on the competition between friction and channel convergence. Non-buoyant MPs may be only resuspended and transported landward during floods if the bed shear stress during ebbs does not exceed the critical shear stress. This net landward transport of MPs may also form EMPM (Jalón-Rojas et al.). Readers can refer to Burchard et al. (2018) for a whole explanation of estuarine trapping mechanisms. Longitudinal gradients and hotspots of MPs can therefore be key estuarine features and should be considered when planning monitoring campaigns.

The specific location of EMPMs may be difficult to predict as it may depend on the physical properties of the particles. In other words, different EMPM may happen for different types of

MPs (Jalón-Rojas et al.), but more research is needed to understand this phenomenon. In some estuaries, EMPM might correspond to estuarine turbidity maximum (Díez-Minguito et al., 2020). These potential zones of accumulation should be considered in the conception of field campaigns and during the data analysis. In particular, when using filtering processes, the huge quantity of sediments or living organisms contained in estuarine waters may lead to the clogging of filters or nets (Hanke et al., 2013). If such areas should not be avoided during sampling, the choice of larger mesh sizes for filtration may be an option. It must be noted that cleaning nets or sieves during field campaigns is very challenging.

Estuary cross-sections are generally composed of a navigation channel and shoals, where the hydrodynamics may largely vary and induce differential advection of suspended matter (McSweeney et al., 2016). Banks with vegetation can be sinks of MPs (Carmen et al., 2021; Stead et al., 2020). A particular phenomenon of great importance for the accumulation and transport of MPs is estuarine fronts (Largier, 1993). Fronts are related to strong convergence currents at surface waters that form visible lines of foam and debris, promoting lateral gradients of MP concentration (Wang et al., 2022). To adequately account for the lateral variation of contamination, sampling locations should also be spread across the channel, at least two points to compare channel and shoals or the regions inside and outside the front (Tab.2). It should be noted that trawling nets can be difficult to operate in the front and can become rapidly clogged due to the high litter abundance (Green et al., 2018). On the other hand, locations with morphological specificity (e.g. sills) or with distinctive hydrodynamic features (e.g. bends) should be avoided unless studied on purpose, as they may introduce site-specific phenomena (Tab.2).

All the physical processes mentioned above are characterized by strong three-dimensional components. Vertical transport became particularly important in shallow waters. For instance, vertical mixing is a key process in transferring suspended particulate matter from the bottom to the surface and for keeping small particles in suspension (Shamskhany and Karimpour, 2022). Surface sampling is certainly not representative of MP contamination, underestimating MP fluxes by avoiding suspended MPs inside the water column (Defontaine et al., 2020; Gasperi and Cachot, 2021). For example, Defontaine et al. (2020) have shown that vertical density stratification affected MP abundance and size distribution through the water column of the Adour estuary: MP concentration and size distribution was different (similar) in surface and bottom waters during stratification (well-mixed) periods. We recommend sampling three depths in the water column to compare surface, mid-column and bottom contamination, especially in highly-stratified estuaries, or at least two depths when time and human resources are limited (Tab.2).

Local sources (e.g., sewage networks, industrial outflows, rainwater networks or marinas) should be also considered when planning sampling locations. Recent studies have shown that MP pollution is largely correlated to the proximity to urban areas (Lebreton et al., 2017; Rodrigues et al., 2019; Yonkos et al., 2014). Unless otherwise desired, the sampling locations should be placed away from such local sources to be as representative of the whole estuary pollution as possible (Tab.2). However, the analysis of pollution pathways is particularly important for management and policy regulations, as well as for developing numerical studies. In this sense, it is particularly interesting to sample MP source outflows to estimate discharged loads to the estuary (Bailey et al., 2021).

Table 1: Comparison of sampling strategies in different estuaries around the world

Study area	Sampling time	Mesh size	Depth	Sampling volume	Reference
Trawling nets					
Adour Estuary, France	flood and ebb tides	$300\,\mu$m - 5 mm	Surface, subsurface (1 m), Bottom (1 m)	Net : 45 to 146 m^3, Pump : 2.8 to 5.1 m^3	Defontaine et al. (2020)
Douro Estuary, Portugal	ebb tide	$30\,\mu$m - $500\,\mu$m	Subsurface (1-2 m)	235 m^3	Rodrigues et al. (2019)
Tamar Estuary, UK	flood and ebb tides	$300\,\mu$m - 5 mm	Surface	270 m^3	Sadri and Thompson (2014)
Solent Estuary, UK	high tide	$300\,\mu$m - 5 mm	Surface	-	Gallagher et al. (2016)
Ebro Estuary, Spain	-	$5\,\mu$m - 5 mm	Surface	-	Simon-Sánchez et al. (2019)
Ría de Vigo, Spain	flood tide	$300\,\mu$m - 5 mm	Surface	60 m^3	Díez-Minguito et al. (2020)
Guadalquivir, Spain	flood and ebb tides	1 mm - 5 mm	-	-	Bermúdez et al. (2021)
Chesapeake Bay, USA	-	$330\,\mu$m - 5 mm	Surface	$105-210\,\mathrm{m}^3$	Yonkos et al. (2014), Bikker et al. (2020)
Delaware Bay, USA	low tide	$200\,\mu$m - 5 mm	Surface	$259-292\,\mathrm{m}^3$	Cohen et al. (2019)
Tampa Bay, USA	-	$300\,\mu$m - 5 mm $1.2\,\mu$m - 5 mm	Subsurface (1-2 m)	Net: 35 m^3, bottle : 1 L	McEachern et al. (2019)
Clyde, Bega and Hunter Estuaries, Australia	ebb tide	$45\,\mu$m - 5 mm	Surface	-	Hitchcock and Mitrovic (2019)
Seine Estuary, France	ebb and flood tides	$300\,\mu$m - 5 mm	Surface, Subsurface (50 cm), Bottom	-	Gasperi and Cachot (2021)
Chao Phraya Estuary, Thailand	flood and ebb tides	$335\,\mu$m - 5 mm	Surface	$\sim 60\,\mathrm{m}^3$	Oo et al. (2021)
Raritan Hudson Estuary, USA	-	$250\,\mu$m - 5 mm	Surface	$\sim 40m3/s$	Bailey et al. (2021)
Pearl Estuary, China	-	$333\,\mu$m - 5 mm	Surface	$\sim 80m3/s$	Lam et al. (2020)
Pumping systems					
Yangtze Estuary, China	-	$32\,\mu$m - 5 mm	Subsurface (1 m)	-	Zhao et al. (2014)
Changjiang Estuary, China	-	$70\,\mu$m - 5 mm	Subsurface (50 cm)	100 L	Xu et al. (2018)
Minjiang Estuary, China	ebb tide	$333\,\mu$m - 5 mm	Subsurface (30 cm)	20 L	Zhao et al. (2015)
Terengganu Estuary, Malaysia	-	$20\,\mu$m - 5 mm	Subsurface (1-2 m)	1200 L	Taha et al. (2021)
Grab sampling					
Pearl Estuary, China	-	$50\,\mu$m - 5 mm	Surface	5 L	Yan et al. (2019)
Tecolutla Estuary, Mexico	-	$1.2\,\mu$m - 5 mm	Subsurface (30 cm)	5 L	Sánchez-Hernández et al. (2021)
Klang Estuary, Malaysia	-	$300\,\mu$m - 5 mm	Surface	1 L	Zaki et al. (2021)
Juilong Estuary, China	ebb and flood	$45\,\mu$m - 5 mm	Surface	105 L	Wu et al. (2022)
Others					
Winyah Bay, USA	-	63 μm - 2 mm	Surface	4 L	Gray et al. (2018)
Itchen Estuary, UK	ebb and flood	0.45 μm - 5 mm	Surface	NA	Stead et al. (2020)

Table 2: Recommendations about when and where to sample MP depending on the main characteristics and hydrodynamic processes of an estuarine system

Estuarine characteristics	Hydrodynamic processes	When	Where
Tides	tidal pumping, tidal straining, tidal mixing, Stokes drift	ebb/flood/slacks (semi-diurne, diurne), neap/spring	along the tidal intrusion, surface/mid depth/bottom
Stratification	two-layer flow, gravitational circulation, stratification-induced turbulence damping	ebb/flood, spring/neap, wet/dry season	along the salinity intrusion, surface/mid depth/bottom
River	tide-river interaction, convergence area	wet/dry season	fluvial area, convergence areas, surface/mid depth/bottom
Fronts	shear fronts, tidal intrusion fronts	ebb and/or flood	inside/outside front, surface
Wind	wind mixing, wind driven-circulation	wind/unwind conditions	banks in the downwind direction, surface/subsurface
Waves	wave mixing, resuspension	swell/calm conditions	mouth of the estuary, banks at the entrance, surface/subsurface/bottom
Morphology	lateral circulation, differential advection	ebb/flood	thalweg/shoals, away from sills, bends ...
MP sources	-	ebb/flood, weekdays/weekends, tourist season wet/dry season	MP sources (WWTPs, sewage outflows, mouth/head of the estuary, industrial outflows ...), away from the sources

4. How to monitor MP in estuaries?

The choice of the sampling method is not trivial when sampling MPs as it generally defines the lower MP size within the sample. If a consensus has been reached regarding the larger size limit of MPs to be 5 mm, it is not the case with the lower size limit. Some studies have defined a lower size limit of 1 μm, and plastic particles being smaller as nanoplastics (Thompson et al., 2009; Frias and Nash, 2019). However, the smaller size limit is defined operationally by the size of the finer mesh, sieve or filter pore used during sampling. Table 1 compares sampling methods employed in previous studies. These methods are diverse and imply different lowest-size limits. For example trawling nets classically only capture MPs greater than 300 μm (lowest mesh size), while the lowest size of MP collected with a pumping system or bottle sampling depends on the sieve size or filter pore, which can go down to 1 μm. Sampling techniques may be responsible for some sorting in MP collection. In particular, fibres are complex to collect due to the thin and elongated shape that allows them to pass through nets and sieves. Therefore, the different available sampling methods should be carefully evaluated during the study design. The two crucial characteristics to consider are the volume of analysed water and the finest mesh size, sieve or filter pore used. The possibility of sampling at different depths to capture the intrinsic vertical variability of estuaries, and during short periods to capture tidal variability, can also be important criteria to select a sampling method in estuaries. In this section, the advantages and disadvantages of the most commonly used techniques are presented.

Trawling nets. As classical protocols for sampling MPs were based on the hypothesis that most MPs are floating at surface waters, the most commonly used techniques are trawling nets such as plankton, neuston or manta nets (Gallagher et al., 2016; McEachern et al., 2019; Simon-Sánchez et al., 2019; Yonkos et al., 2014). Such nets are generally equipped with a rectangular opening and two floats to sample large volume in surface waters. On the other hand, bongo nets may be equipped with round openings and depressor weights to allow collection inside the water column. However, the sampling depth is hard to target precisely with a depressor weight. The volume of filtered water is estimated thanks to a flowmeter that is fixed on the opening frame (Hanke et al., 2013; Prata et al., 2019). Large volumes of water can be easily sampled ensuring solid statistical data and reducing the impact of background contamination. Paired bongo nets present the advantage to allow sampling duplicates. Trawling net seems to be the most common technique used in estuaries (Tab. 1), which permits comparison between sites.

The mesh size of the net is one of the most restrictive elements of trawling nets. A standard mesh of 300 μm is generally mounted on such systems that impede finer MPs to be collected, leading to an underestimation of the contamination (Green et al., 2018; Lindeque et al., 2020). However, some suppliers offer finer mesh sizes that may be considered. In the Seine River, a comparison between a plankton net equipped with an 80 μm mesh and a manta net equipped with a classical 330 μm mesh revealed that concentration may be largely underestimated (several orders of magnitude) with the larger mesh size (330 μm), even though a greater diversity of shape and types may be captured (Dris et al., 2015). Nevertheless, trawling nets with fine meshes are more susceptible to clogging, leading to a reduced volume of filtered water and to the collection of MPs finer than the mesh size (Dris et al., 2018). To avoid such drawbacks, it is recommended to deploy fine mesh trawling in relatively clear waters, which is not usual in (turbid) estuaries.

Trawling nets are towed at the rear of the vessel and have the benefit to be easy to deploy in coastal areas. However, they may be not appropriate for narrow channels or very busy shipping lanes. Such techniques may even be forbidden in some parts of the estuary to not disturb harbour activities and navigation. It is highly recommended to contact local authorities before deploying such equipment in estuaries. The European Commission recommends deploying trawling net out of the wake zone due to turbulence leading to unrepresentative sampling (Hanke et al., 2013). Michida et al. (2019) study recommends conducting trawling surveys in conditions where wave heights are under 0.5 meters as trawling nets can be relatively difficult to manipulate in rougher conditions.

Pumping system. Pumping systems generally consist in a high-capacity pump that pours waters through a set of sieves (Defontaine et al., 2020; Xu et al., 2018; Zhao et al., 2015). In some cases, water samples are collected in jars to be filtered at the laboratory. This method presents different advantages. First, it allows us to precisely choose the finest MP size to be collected (e.g. 70 μm, 50 μm or 5 μm) as a function of the finest mesh size or filter pore. Second, this system can easily collect MPs at different depths in the water column. A pressure sensor and a depressor weight need to be fixed to the pump inlet to ensure a vertical fall and precisely estimate the depth of measurement. Third, this method is relatively easy to set up on the field and duplicates can readily be collected with a second pumping system working in parallel.

Nevertheless, the use of such technologies may introduce some contamination by the plastic components of the pumping system and can trigger the fragmentation of MPs due to shear stress during pumping (Enfrin et al., 2020; Skalska et al., 2020). Another major drawback is that, depending on the pump capacity, the operation may take hours to collect some cubic meters of water (Defontaine et al., 2020). During such a long operation, the estuarine hydrodynamics may have changed and may not be representative of specific conditions. A compromise has to be found between a large volume of water to gain statistically reliable data and a short operating time representative of one hydrodynamic state.

Bottle sampling. Grab sampling is a commonly used technique to estimate suspended sediment concentration. It holds some advantages. For instance, it is simple to operate and allows us to collect all types of MPs, including those difficult to capture with other techniques such as fibres. Several studies have shown that fibres are more efficiently collected with bottle sampling than with nets (Rebelein et al., 2021). The subsequent filtration at the laboratory with fine pore filters can capture small MPs (down to 1 μm). Contamination is also lower compared to nylon net and pumping system (Prata et al., 2019). Nevertheless, laboratory analyses are longer than with other methods as no pre-sieving is realised in the field. The major drawback of this method is the very small volume of water that is analysed, which leads to the need for replicates and thus additional laboratory costs. Dubaish and Liebezeit (2013) have shown that replicates from bottle samples displayed larger heterogeneity, especially for films and fragments. Although fragments and films may be underestimated by grab sampling, the concentrations of fibres may yield between 3 and 4 orders of magnitude greater than those estimated by common zooplankton net methods (Green et al., 2018). Therefore, bulk sampling may be envisaged to study fibres, but it is less recommended when all types of MPs are investigated.

Automatic samplers, continuous plankton recorder and continuous-flow centrifuges. Recently, new technologies have been developed to investigate microplastic pollution in a more automatic way. Automatic rosette water samplers or even ROV have been used for MP sampling in ocean waters (La Daana et al., 2018; Dai et al., 2018; Choy et al., 2019). However, such massive equipment required a lift arm and is not adapted for shallow estuarine waters. Continuous flow centrifuges are able to separate particles denser than ambient waters from less dense particles and, when coupled with a filtration system, may be used for sampling MPs (Hildebrandt et al., 2019). However, it lasts several hours to process 100 L of water, so it may not be adapted to estuarine dynamics time scales. In-situ filtration devices consisting of a high-capacity pump associated with a filtration device (e.g. in-line steel filters, mesh bag) have shown promising results in sampling MPs (Li et al., 2020; Liu et al., 2019; Karlsson et al., 2020; Harrold et al., 2022). They can be equipped with a flowmeter and pressure sensor. However, such systems do not sample the surface microlayer, where light MPs may accumulate, to keep the pump inlet underwater during sampling (Karlsson et al., 2020). To our knowledge, there are no field studies in the literature using such new technologies in estuarine waters. However, it could be interesting to compare them with the most commonly used methods.

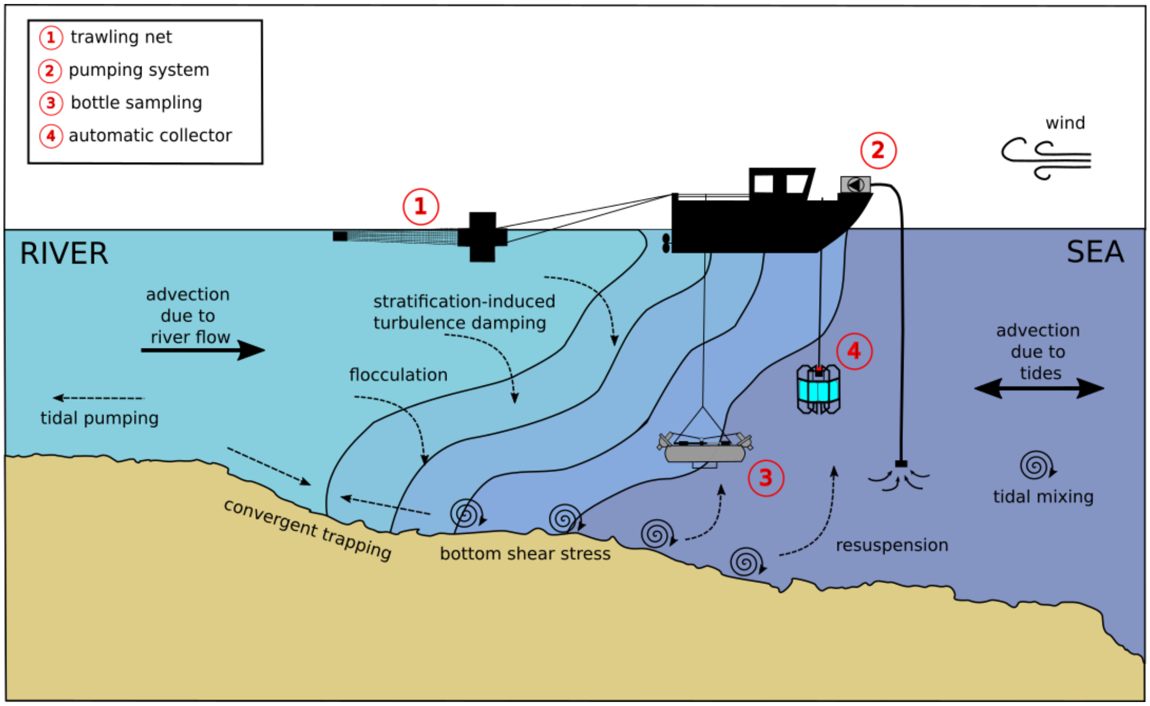


Figure 1: Key processes in regards to microplastic sampling strategies in estuaries

5. Best practice

When sampling microplastics, some good practices need to be applied to avoid sample contamination. It is important to ensure that samples are not affected by background contamination in the field or laboratory. Laboratory utensils should be made of glass or metal as far as possible, e.g. MPs should be stored in glass jars and petri-slides, and metallic sieves are preferred. They should be properly rinsed with pure water and covered with aluminium foil to avoid airborne contamination prior to any contact with the sample (Green et al., 2018). Synthetic clothing should be avoided when researchers recover and manipulate samples; cotton clothes should be favoured (Hanke et al., 2013).

When using trawling nets, they should be carefully rinsed between tows to reduce background contamination. Nets should be rinsed from the outside of the net and never through the net opening (Hanke et al., 2013). In order to investigate the potential background contamination, blank analyses should be carried out. It is recommended that laboratory analyses are carried out by a unique analyst to limit the analyst bias in the results (Green et al., 2018). During recovering and manipulation of samples on the field, the materials should be placed upwind to avoid additional contamination by the analyst. Trawling nets should be positioned by the side of the ship instead of at the rear when possible to avoid MP contamination from the ship and vertical mixing by wake and bow waves.

Replicates are standard practice to measure variability in sample collection and analysis. When small volumes of water are considered during sampling (e.g. grab sampling), replicate samples may be necessary to avoid heterogeneity in samples. Miller et al. (2021) recommend duplicating every ten samples or more if the study focuses on microfibers.

Another good but uncommon practice is to collect a wide range of physical parameters simultaneously to MP samples. MP abundance and distribution alone are very difficult to interpret and compare which may lead to biased conclusions. We highly recommend collecting additional data such as water levels, current intensity, water properties (e.g. salinity, turbidity, organic content) and weather forecast (e.g. rainfall, wind, waves).

6. Research priorities

Measuring the abundance and distribution of MPs in estuaries and identifying their sources is the primary step in gaining understanding of their dynamics and fate, evaluating environmental risks, and establishing future mitigation measures and management strategies. Nevertheless, as discussed in this work, sampling MPs in estuarine waters is not trivial. No established protocols have been developed so far to harmonise sampling methods in the water compartment. Given that different sampling methods and strategies can lead to concentration differences by orders of magnitude (Green et al., 2018), there is a critical need to establish standardized sampling protocols that ensure consistency and the inter-comparison of systems. However, it is also important for the protocols to be flexible, as estuaries are complex environments with a wide range of physical, chemical, and biological characteristics. Protocols should therefore take into account all the particularities of a specific estuarine environment (complex 3D hydrodynamics, ETM, variety of MP sources ..). Nevertheless, some elements may be harmonized such as the lower size limit of the collected MPs (e.g. mesh size, filter pores) and the minimum volume of sampled water. Although an optimal technique is highly dependent of the study site and the specific objectives, manual or automated high-capacity pumping system associated with any kind of filtering system presents a large number of advantages: easily available for deployment at different depths in complex areas (e.g. navigation, shoals with vegetation), collection of large volumes of water (statistically reliable data) in relative short period of time (representative of one hydrodynamic state). The monitoring protocol should cover the main time scales of variability (tidal cycles, spring/neap tidal cycles, contrasting conditions of river flow or wind) and the estuarine regions characterised by different hydrodynamic regimes. While these time scales and representative sampling locations strongly depend on the dominant forcing and may vary among systems, the hydro-meteorological conditions under which MPs have been collected should be clearly stated in the papers (tidal phase, current velocity, wind, waves, salinity etc...). Even if this study focuses on microplastic pollution, similar considerations should be taken into account for sampling other kinds of pollutants in estuarine environments.

Unlike classical sediment concentration analyses, MP concentration analyses have substantial financial and time costs that may not be neglected in the field campaign design. A relevant perspective is to test new methods of automatic sampling of MPs, reducing financial and time costs during laboratory analyses and field sampling.

Acknowledgments

This work was supported by the French National Agency for Research in the context of PLASTINEST project (grant N°ANR-22-CE01-0011-01).

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