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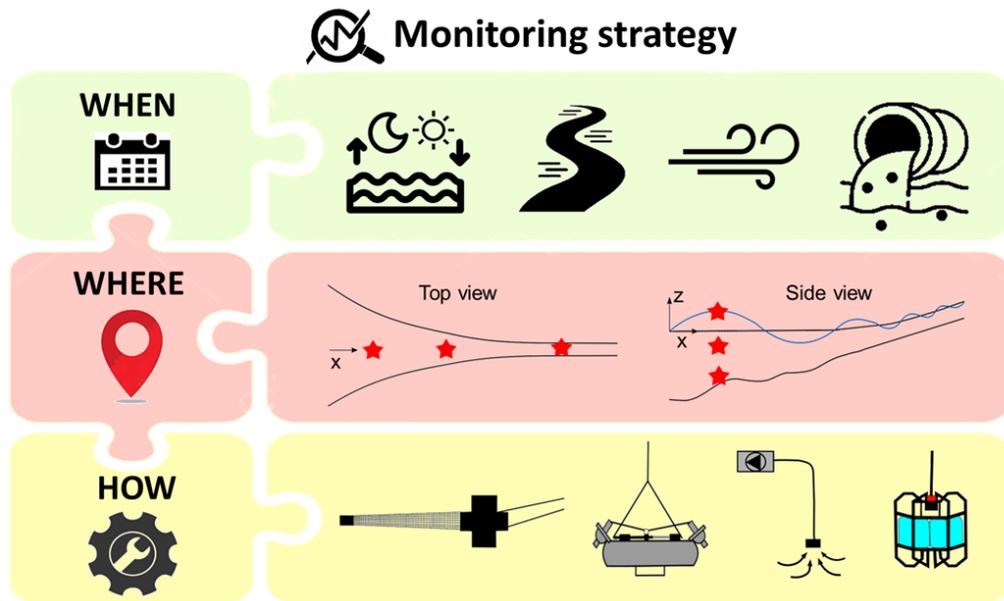


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1 Graphical Abstract

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

4 Sophie Defontaine ⁽¹⁾ and Isabel Jalón-Rojas ⁽¹⁾



5 Highlights

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

8 Sophie Defontaine ⁽¹⁾ and Isabel Jalón-Rojas ⁽¹⁾

- 9 • Strategies and methods for sampling microplastics in estuarine waters are reviewed
- 10 • The need of considering hydrodynamics when planning in-situ surveys are highlighted
- 11 • Recommendations on when, where and how sampling are discussed in relation to hydrody-
12 namics
- 13 • Recommendations on when, where and how sampling are discussed in relation to hydrody-
14 namics

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

17 Sophie Defontaine ⁽¹⁾ and Isabel Jalón-Rojas ⁽¹⁾

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19 **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.

20 *Keywords:* Microplastics, estuary, sampling, monitoring, physical processes, hydrodynamics

21 1. Introduction

22 Estuaries form the connection between marine and fluvial waters. They are highly productive
23 ecosystems that provide important environmental, social and economic services (Barbier et al.,
24 2011). Estuaries are, however, highly susceptible to both natural and human disturbances. Like
25 other aquatic systems around the world, estuaries are vulnerable to plastic pollution (Browne
26 et al., 2010). In particular, the abundance and risks associated with microplastic particles and
27 fibres (MPs), defined as plastics lower than 5 mm (Frias and Nash, 2019), are of high concern.
28 Their similar dimension to sediments and planktonic organisms make them easily ingestible by
29 the aquatic biota. The ingestion of MPs can be responsible for gut abrasion and blockage, as
30 well as intoxication by sorbed contaminants or toxic additives used in the compounding of plastics
31 (Andrady, 2011; Kazour et al., 2020). Living organisms may also develop on the MP surface in the
32 form of biofilms (Amaral-Zettler et al., 2020). MPs may thus act as dispersal vectors of pathogens
33 in moving estuarine waters (Forero-López et al., 2022).

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

48 In situ observations of microplastic concentrations in the abiotic compartment is a key approach
49 to address emergent research questions on plastic pollution at the land-sea continuum, e.g.: (1) the
50 sources and generation of MPs; (2) the pathways by which MPs reach estuarine, coastal and ocean
51 waters; (3) the abundance, distribution and fate of MPs through the continuum ecosystems; (4) the
52 transport mechanisms driving spatio-temporal variations and promoting the flushing of MPs to the
53 ocean; (5) MPs ageing, weathering and biofouling; and (6) ecosystems exposition and risks, among
54 others. Numerous protocols have been developed to study MP contamination in ocean surface
55 waters or beaches (Besley et al., 2017; Hanke et al., 2013; Masura et al., 2015; Miller et al., 2017,
56 2021). However, these protocols or strategies may not be suitable to evaluate MP contamination in
57 estuaries and transitional waters. Most of these protocols are based on the assumption that MPs
58 are floating on the surface or have been deposited on the bed depending on their density. The
59 complex three-dimensional hydrodynamics in estuaries can make this assumption, and therefore
60 these protocols, inappropriate. Table 1 compares sampling strategies previously implemented in
61 the water matrix of different estuaries around the world, highlighting that most of them focused on
62 surface water.

63 The specific hydrodynamics and properties of estuarine waters play a crucial role in the distri-

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

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

90 **2. When to monitor MP in estuaries?**

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

102 Various key time scales should be considered when planning samplings in estuaries, namely
103 tidal, fortnightly and seasonal time scales. The particular importance of a given time scale is
104 strongly related to the dominant forcing of the estuarine dynamics and is, therefore, site-specific
105 (Jay, 2010; Jalón-Rojas et al., 2017). In estuaries dominated by tides, MP concentration can vary

106 significantly between ebb and flood tides (Oo et al., 2021). This difference can be related to various
107 processes such as tidal asymmetry (the flood or ebb dominance of currents). For example, in a
108 flood-dominated estuary, sinking MPs could have a higher rate of resuspension and mixing during
109 flood tides. Microplastic concentration may also vary over the tidal cycle in stratified and salt-wedge
110 estuaries where tidal motion is weaker. This can be due to variations in currents and salinity driven
111 by tidal variations in vertical mixing and the along-channel density gradient. Defontaine et al.
112 (2020) show that the transport of MPs in suspension may be contained by the pycnocline due to
113 the turbulence damping induced by density stratification, whereas periods of intense mixing led to
114 homogeneous concentration along the water column. However, fewer studies sampled microplastics
115 at different tidal phases (Tab. 1). Given the importance of the tidal time scale, we recommend
116 sampling always at the same tidal phases to allow the inter-comparison of samples and, as far as
117 possible, sampling at different key tidal phases (e.g. flood, ebb, high water, low water).

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

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

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

143 The discussion of MP concentrations at the different key time scales should also consider the
144 variability of MP inputs from sources (Tab.2). Sources such as urban storm-water runoff or wastew-
145 ater effluents may have a variable discharge depending on the weather forecast (rainfall) or the peak
146 period of city occupation (e.g. tourism). Significant links between rainfall and MP concentration
147 have been found in the literature as it drains the land-based MP pollution through rivers to estu-
148 aries (Lima et al., 2015; Hitchcock, 2020). The wet season could therefore represent a period of a

149 strong abundance of MPs. However, the dry season may represent a period of denser population
150 and thus an increase of MP inputs from surrounding cities and shores. Collection during the wet
151 and dry seasons is therefore relevant to investigate potential seasonal differences in contamination.
152 In addition, wastewater effluent contamination may also vary between weekends and weekdays, and
153 between flood and ebb tides (e.g. WWTP discharge during ebb tide to favour flushing). Samplings
154 only on weekdays should be envisaged (Miller et al., 2021).

155 **3. Where to monitor MP in estuaries?**

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

166 Intratidal and subtidal circulation may vary along the longitudinal and lateral axes as well as
167 water properties affecting the dispersion of suspended particles such as MPs (Lam et al., 2020).
168 Different locations along and across the estuarine channel should thus be considered (Tab.2). Even if
169 the choice of the locations should be based on the specific characteristics of the study site, a general
170 recommendation is to sample at different sites along the longitudinal density gradient, as physical
171 processes driving the transport of MPs and MP buoyancy vary over this gradient (Malli et al.,
172 2022; Jalón-Rojas et al.) (Fig. 1). For instance, density-driven circulation is a physical process
173 directly related to the density gradient. In estuaries characterized by decreasing salinity from the
174 ocean toward the upper estuary, light MPs might be transported as wash load to the ocean by a
175 surface seaward flow, while dense MPs might be transported as bed load by a landward flow near
176 the bottom (Defontaine et al., 2020). Wind stress can also promote a two-layer circulation (Li and
177 Li, 2011). Recent studies based on numerical and idealized models have suggested that estuarine
178 circulation may form hotspots of microplastics, also called Estuarine MicroPlastic Maxima (EMPM)
179 (Díez-Minguito et al., 2020; Bermúdez et al., 2021). In estuaries or estuarine regions dominated
180 by tides, tidal pumping can also generate longitudinal circulation and MP trapping (Stead et al.,
181 2020). In flood-dominated estuaries, the tidal wave may become increasingly asymmetric (higher
182 flood than ebb currents) along the estuarine channel depending on the competition between friction
183 and channel convergence. Non-buoyant MPs may be only resuspended and transported landward
184 during floods if the bed shear stress during ebbs does not exceed the critical shear stress. This
185 net landward transport of MPs may also form EMPM (Jalón-Rojas et al.). Readers can refer to
186 Burchard et al. (2018) for a whole explanation of estuarine trapping mechanisms. Longitudinal
187 gradients and hotspots of MPs can therefore be key estuarine features and should be considered
188 when planning monitoring campaigns.

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

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

199 Estuary cross-sections are generally composed of a navigation channel and shoals, where the
200 hydrodynamics may largely vary and induce differential advection of suspended matter (McSweeney
201 et al., 2016). Banks with vegetation can be sinks of MPs (Carmen et al., 2021; Stead et al., 2020). A
202 particular phenomenon of great importance for the accumulation and transport of MPs is estuarine
203 fronts (Largier, 1993). Fronts are related to strong convergence currents at surface waters that
204 form visible lines of foam and debris, promoting lateral gradients of MP concentration (Wang et al.,
205 2022). To adequately account for the lateral variation of contamination, sampling locations should
206 also be spread across the channel, at least two points to compare channel and shoals or the regions
207 inside and outside the front (Tab.2). It should be noted that trawling nets can be difficult to
208 operate in the front and can become rapidly clogged due to the high litter abundance (Green et al.,
209 2018). On the other hand, locations with morphological specificity (e.g. sills) or with distinctive
210 hydrodynamic features (e.g. bends) should be avoided unless studied on purpose, as they may
211 introduce site-specific phenomena (Tab.2).

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

224 Local sources (e.g., sewage networks, industrial outflows, rainwater networks or marinas) should
225 be also considered when planning sampling locations. Recent studies have shown that MP pollution
226 is largely correlated to the proximity to urban areas (Lebreton et al., 2017; Rodrigues et al., 2019;
227 Yonkos et al., 2014). Unless otherwise desired, the sampling locations should be placed away
228 from such local sources to be as representative of the whole estuary pollution as possible (Tab.2).
229 However, the analysis of pollution pathways is particularly important for management and policy
230 regulations, as well as for developing numerical studies. In this sense, it is particularly interesting
231 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 μm - 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 μm - 500 μm	Subsurface (1-2 m)	235 m ³	Rodrigues et al. (2019)
Tamar Estuary, UK	flood and ebb tides	300 μm - 5 mm	Surface	270 m ³	Sadri and Thompson (2014)
Solent Estuary, UK	high tide	300 μm - 5 mm	Surface	-	Gallagher et al. (2016)
Ebro Estuary, Spain	-	5 μm - 5 mm	Surface	-	Simon-Sánchez et al. (2019)
Ría de Vigo, Spain	flood tide	300 μm - 5 mm	Surface	60 m ³	Díez-Minguito et al. (2020)
Guadalquivir, Spain	flood and ebb tides	1 mm - 5 mm	-	-	Bernúdez et al. (2021)
Chesapeake Bay, USA	-	330 μm - 5 mm	Surface	105 - 210 m ³	Yonkos et al. (2014), Bikker et al. (2020)
Delaware Bay, USA	low tide	200 μm - 5 mm	Surface	259 - 292 m ³	Cohen et al. (2019)
Tampa Bay, USA	-	300 μm - 5 mm 1.2 μm - 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 μm - 5 mm	Surface	-	Hitchcock and Mitrovic (2019)
Seine Estuary, France	ebb and flood tides	300 μm - 5 mm	Surface, Subsurface (50 cm), Bottom	-	Gasperi and Cachot (2021)
Chao Phraya Estuary, Thailand	flood and ebb tides	335 μm - 5 mm	Surface	\sim 60 m ³	Oo et al. (2021)
Raritan Hudson Estuary, USA	-	250 μm - 5 mm	Surface	\sim 40 m ³ /s	Bailey et al. (2021)
Pearl Estuary, China	-	333 μm - 5 mm	Surface	\sim 80 m ³ /s	Lam et al. (2020)
Pumping systems					
Yangtze Estuary, China	-	32 μm - 5 mm	Subsurface (1 m)	-	Zhao et al. (2014)
Changjiang Estuary, China	-	70 μm - 5 mm	Subsurface (50 cm)	100 L	Xu et al. (2018)
Minjiang Estuary, China	ebb tide	333 μm - 5 mm	Subsurface (30 cm)	20 L	Zhao et al. (2015)
Terengganu Estuary, Malaysia	-	20 μm - 5 mm	Subsurface (1-2 m)	1200 L	Taha et al. (2021)
Grab sampling					
Pearl Estuary, China	-	50 μm - 5 mm	Surface	5 L	Yan et al. (2019)
Tecolutla Estuary, Mexico	-	1.2 μm - 5 mm	Subsurface (30 cm)	5 L	Sánchez-Hernández et al. (2021)
Klang Estuary, Malaysia	-	300 μm - 5 mm	Surface	1 L	Zaki et al. (2021)
Juilong Estuary, China	ebb and flood	45 μm - 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

232 4. How to monitor MP in estuaries?

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

250 are presented.

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

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

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

282 **Pumping system.** Pumping systems generally consist in a high-capacity pump that pours
283 waters through a set of sieves (Defontaine et al., 2020; Xu et al., 2018; Zhao et al., 2015). In some
284 cases, water samples are collected in jars to be filtered at the laboratory. This method presents
285 different advantages. First, it allows us to precisely choose the finest MP size to be collected (e.g.
286 $70\ \mu\text{m}$, $50\ \mu\text{m}$ or $5\ \mu\text{m}$) as a function of the finest mesh size or filter pore. Second, this system
287 can easily collect MPs at different depths in the water column. A pressure sensor and a depressor
288 weight need to be fixed to the pump inlet to ensure a vertical fall and precisely estimate the depth
289 of measurement. Third, this method is relatively easy to set up on the field and duplicates can
290 readily be collected with a second pumping system working in parallel.

291 Nevertheless, the use of such technologies may introduce some contamination by the plastic com-
292 ponents of the pumping system and can trigger the fragmentation of MPs due to shear stress during

293 pumping (Enfrin et al., 2020; Skalska et al., 2020). Another major drawback is that, depending on
294 the pump capacity, the operation may take hours to collect some cubic meters of water (Defontaine
295 et al., 2020). During such a long operation, the estuarine hydrodynamics may have changed and
296 may not be representative of specific conditions. A compromise has to be found between a large
297 volume of water to gain statistically reliable data and a short operating time representative of one
298 hydrodynamic state.

299 **Bottle sampling.** Grab sampling is a commonly used technique to estimate suspended sed-
300 iment concentration. It holds some advantages. For instance, it is simple to operate and allows
301 us to collect all types of MPs, including those difficult to capture with other techniques such as
302 fibres. Several studies have shown that fibres are more efficiently collected with bottle sampling
303 than with nets (Rebelein et al., 2021). The subsequent filtration at the laboratory with fine pore
304 filters can capture small MPs (down to $1\ \mu\text{m}$). Contamination is also lower compared to nylon net
305 and pumping system (Prata et al., 2019). Nevertheless, laboratory analyses are longer than with
306 other methods as no pre-sieving is realised in the field. The major drawback of this method is
307 the very small volume of water that is analysed, which leads to the need for replicates and thus
308 additional laboratory costs. Dubaish and Liebezeit (2013) have shown that replicates from bottle
309 samples displayed larger heterogeneity, especially for films and fragments. Although fragments and
310 films may be underestimated by grab sampling, the concentrations of fibres may yield between 3 and
311 4 orders of magnitude greater than those estimated by common zooplankton net methods (Green
312 et al., 2018). Therefore, bulk sampling may be envisaged to study fibres, but it is less recommended
313 when all types of MPs are investigated.

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

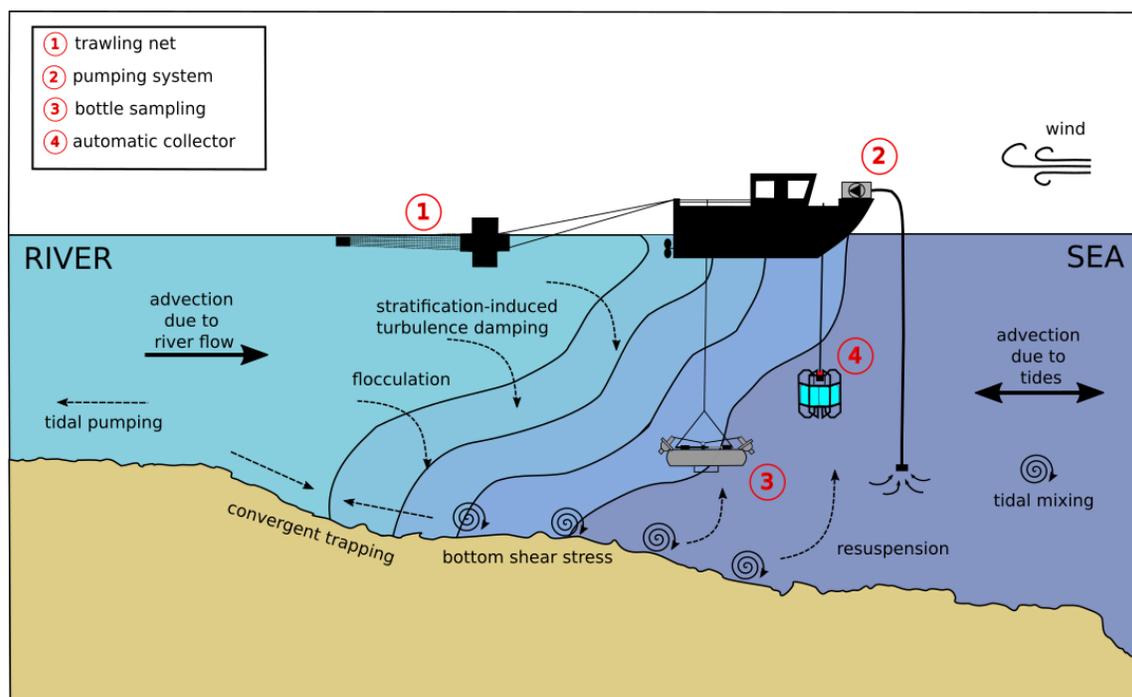


Figure 1: Key processes in regards to microplastic sampling strategies in estuaries

330 5. Best practice

331 When sampling microplastics, some good practices need to be applied to avoid sample contami-
 332 nation. It is important to ensure that samples are not affected by background contamination in the
 333 field or laboratory. Laboratory utensils should be made of glass or metal as far as possible, e.g. MPs
 334 should be stored in glass jars and petri-slides, and metallic sieves are preferred. They should be
 335 properly rinsed with pure water and covered with aluminium foil to avoid airborne contamination
 336 prior to any contact with the sample (Green et al., 2018). Synthetic clothing should be avoided
 337 when researchers recover and manipulate samples; cotton clothes should be favoured (Hanke et al.,
 338 2013).

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

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

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

357 **6. Research priorities**

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

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

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