



**HAL**  
open science

## Spatio-temporal contamination of microplastics in shellfish farming regions: A case study

Adélaïde Lerebours, Marguerite Bathie, Maria Kazour, Rachid Amara, Valérie Huet, Hélène Thomas

### ► To cite this version:

Adélaïde Lerebours, Marguerite Bathie, Maria Kazour, Rachid Amara, Valérie Huet, et al.. Spatio-temporal contamination of microplastics in shellfish farming regions: A case study. *Marine Pollution Bulletin*, 2022, 181, pp.113842. 10.1016/j.marpolbul.2022.113842 . hal-03773030

**HAL Id: hal-03773030**

**<https://hal.science/hal-03773030>**

Submitted on 8 Sep 2022

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

1 **Spatio-temporal contamination of microplastics in shellfish farming regions: A case study.**

2

3 <sup>1</sup>Adélaïde Lerebours\*, <sup>1</sup>Marguerite Bathie, <sup>2</sup>Maria Kazour, <sup>2</sup>Rachid Amara, <sup>1</sup>Valérie Huet and <sup>1</sup>Hélène  
4 Thomas

5

6 <sup>1</sup>UMR CNRS LIENSs, Université de La Rochelle, Littoral Environnement et Sociétés, 2 rue Olympe  
7 de Gouges, La Rochelle, 17 000, France

8 <sup>2</sup>Université du Littoral Côte d'Opale, CNRS, Université de Lille, UMR 8187, LOG, Laboratoire  
9 d'Océanologie et de Géosciences, 32 Avenue Foch, 62930, Wimereux, France.

10

11

12

13

14

15

16

17

18

19

20 **Corresponding Author**

21 \*Adélaïde Lerebours, phone number: +33 (0) 5 46 50 76 23, e-mail: adelaiderebours@univ-lr.fr,

22 Address: UMR CNRS LIENSs, Université de La Rochelle, Littoral Environnement et Sociétés, La

23 Rochelle, 17 000, France

24

25 **Abstract**

26 The seasonal pattern of microplastics (MPs) contamination of the French littoral area of the Pertuis  
27 Charentais, one of the main French shellfish production regions, was assessed for the first time,  
28 between May 2019 and May 2020 at four different sites. The reference site was located at “Ile de Ré”  
29 and the other sites were located in the estuaries of the Sèvre Niortaise, Charente and Seudre rivers.  
30 Both blue mussels (*Mytilus edulis*) and Pacific oysters (*Magallana gigas*), that are considered sentinel  
31 species for the quality of the marine environment were analysed, along with sediment and seawater  
32 samples. MPs were extracted from each sample, counted, measured and sorted by colour and type.  
33 Micro-Raman spectroscopy was used to determine the proportion of confirmed MPs and the polymer  
34 types. The results showed that the contamination of mussels by fibres and fragments ( $1.9 \pm 2.1$  MPs/g  
35 ww) was significantly higher than for oysters ( $0.4 \pm 0.4$  MPs/g ww). Specifically, the contamination  
36 by fibres in both species was significantly greater than the contamination by fragments. Significant  
37 variations of MPs contamination were observed across the seasons and sites in bivalves, and depended  
38 on the species and the type of MPs (fibres or fragments). Mean concentrations of MPs measured in  
39 water and sediment were 0.007 MPs/L and 210 MPs/kg dw, respectively. Finally, blue was the  
40 dominant colour for fibres (79%) and fragments (81%). Blue fragments were mainly made of PS  
41 (70%) followed by PC (18%) and PP, PA or PLA (3%) whereas blue fibres were mainly made of PA  
42 (80%) followed by PET (13%) or PP (7%). This rare environmental case study of long-term chronic  
43 exposure of farming areas to MPs provides new knowledge on *in situ* variations of plastic fibres and  
44 fragments contamination throughout the seasons.

45

46

47

48

49

50

51

52

53

## 54 **Introduction**

55 Plastic pollution has drastically increased worldwide since the 1970's. 348 million tons of plastics  
56 were produced in the world in 2017, including 64 million tons in Europe (PlasticsEurope 2018).  
57 Among those, 19.3% were made of polypropylene (i.e. used for packaging and the automobile  
58 industry), 17.5% of low-density polyethylene, (i.e. a component of reusable bags and clingfilm),  
59 13.3% of high-density polyethylene (i.e. used in bottles and toys), 10.2% of polyvinyl chloride (i.e. a  
60 component of pipelines), 7.7% of polyurethane (i.e. used in mattress and insulating material), 7.4% of  
61 polyethylene terephthalate etc (PlasticsEurope 2018). It is estimated that between 4.8 and 12.7 Mt of  
62 macroplastics (Jambeck *et al.*, 2015) and 1.5 Mt of primary microplastics (Bouchet and Friot, 2017)  
63 enter the oceans annually and that 92% of the trillions of plastic fragments floating at sea would be  
64 microplastics (Eriksen *et al.*, 2014). Macro- and microplastic aquatic pollution from land is predicted  
65 to increase 2.6-fold between 2016 and 2040 under implemented remediation scenario (Lau *et al.*,  
66 2020). MPs called "primary MPs" enter the marine system directly, such as cosmetics, aerosols,  
67 microbeads and fibres. "Secondary MPs" result from degradation of bigger plastic pieces by  
68 mechanical (erosion, abrasion), (photo)chemical (ultra-violet, hydrolysis, oxidation) or biological  
69 processes (action of microorganisms) (Andrady, 2011). The degradation of MPs represents the major  
70 source of MPs contamination of the marine environment (Eriksen *et al.*, 2014).

71         MPs have been found in all the oceans and seas in the world, and in different compartments  
72 (water, glacier, sediments, biota) (Cole *et al.*, 2011) and a concentration gradient exists between the  
73 anthropized coasts and the open ocean (Enders *et al.*, 2015). Consequently, contamination by MPs was  
74 reported in several animal species representing different trophic levels: marine mammals (Hernandez  
75 *et al.*, 2018), seabirds (Amélineau *et al.*, 2016), fish (Avio *et al.*, 2017), crustaceans (Cau *et al.*, 2019)  
76 and bivalve molluscs (Beyer *et al.*, 2017). MPs contamination can exert a direct toxic effect (Alimba  
77 and Faggio, 2019; Wang *et al.*, 2020) or indirectly affect organisms through the release of chemicals  
78 like persistent organic pollutants (Hermabessiere *et al.*, 2017) and pesticides (Teuten *et al.*, 2017).

79         The blue mussel, *Mytilus edulis*, and the Pacific oyster, *Magallana gigas*, are the most  
80 common species in the French shellfish production, and have been used as sentinel species to monitor  
81 marine pollution (Beliaeff *et al.*, 1998; Beyer *et al.*, 2017; Lerebours *et al.*, 2021). Indeed, they are

82 both widespread and sessile so they provide information specific to their habitat. Moreover, they feed  
83 on plankton by filtering the water through their ciliated gills that can catch MPs leading to their  
84 ingestion (Bråte *et al.*, 2018). Therefore, as filter feeders, mussels and oysters are especially exposed  
85 to MPs. MPs can be caught by their ciliated gills and transported to the labial palps. The MPs can then  
86 go through the gut or be removed and trapped with the mucus to finally be excreted *via* faeces and  
87 pseudofaeces, respectively (Ward *et al.*, 2019). Capture, ingestion or rejection, are mediated by the  
88 particle size (Pouvreau *et al.*, 1999; Ward *et al.*, 2019). For instance, a recent study investigating  
89 different fraction sizes showed that most of the MPs accumulated in field deployed oysters were < 22  
90  $\mu\text{m}$  (Ribeiro *et al.*, 2021).

91 Recently, several studies proposed that bivalves could be used to assess MPs load in different  
92 environments (Ding *et al.*, 2021; Van Cauwenberghe, 2015; Gonçalves *et al.*, 2019; Kazour and  
93 Amara, 2020; Li *et al.*, 2019). Only a few studies reported long-term *in situ* variation of MPs  
94 concentrations in caged marine bivalves across the seasons. They were lasting 4 weeks maximum  
95 (Avio *et al.*, 2017; Catarino *et al.*, 2018; Kazour, 2019; Railo *et al.*, 2018), six months (Domogalla-  
96 Urbansky *et al.*, 2019) and one year (Catarino *et al.*, 2018).

97 Spatial and temporal factors such as population density, wastewater treatment plant effluent,  
98 rainfalls and water flow can influence the microplastics concentration in water bodies but studies need  
99 further investigations (for a review see Talbot and Chang, 2022). Species living in recipient bodies  
100 such as oysters and mussels, are thus prone to ingest and accumulate MPs. For instance, mussels  
101 collected in urbanized areas are more contaminated with MPs than mussels in non-urbanized areas  
102 (Cho *et al.*, 2021). The same goes for bivalves collected in industrialized and highly populated areas  
103 (Li *et al.*, 2016; 2018).

104 The Pertuis-Charentais littoral area, in south-west France, is a transition zone between the  
105 Atlantic Ocean and the estuaries of the rivers Sèvre Niortaise, Charente and Seudre. France is the  
106 second biggest bivalve producer in Europe and the Pertuis-Charentais region produces 22% of the  
107 French shellfish production. Therefore, a good quality of the surrounding waters is necessary to ensure  
108 the sustainability of bivalve farming. Questions about contamination levels of microplastics in these  
109 estuarine areas have been raised but have yet to be elucidated.

110 The objective of the study was to determine the contamination levels of MPs over a year in the  
111 marine ecosystem of the Pertuis Charentais, one of the main French shellfish production regions.  
112 Concentrations of MPs in blue mussels, Pacific oysters, sediments and seawater were determined in  
113 the estuaries of the rivers Sèvre Niortaise, Charente and Seudre and at a farther site in the South-West  
114 of the Ile de Ré. Characterization of MPs by size, colour, type and polymer was performed. Finally,  
115 the characteristics of water bodies were assessed to evaluate any potential site difference that could  
116 interfere with MPs contamination and/or bivalve physiology by analysing several environmental  
117 parameters (rivers' water flow, temperature, salinity, conductivity, turbidity, oxygen and chlorophyll  
118 *a*).

119

## 120 **Material and Methods**

### 121 **Experimental design**

122 All sites chosen in the study corresponded to shellfish farming areas. In March 2019, farmed mussels  
123 and oysters of 1.5 years were acclimatized for 1.5 month to the same environmental conditions at Le  
124 Martray site located in the South-West of Ile of Ré. In May 2019, oysters (average total length  $5.2 \pm$   
125  $0.7$  cm) and mussels (average total length  $4.0 \pm 0.7$  cm) were dispatched in cages at three exposed  
126 sites: off the Aiguillon Bay (near the Sèvre estuary), in the Charente and Seudre estuaries and one  
127 farther site from the estuaries and more exposed to marine waters, Le Martray site (Figure 1). Caged  
128 individuals with bivalves of identical genetic characteristics and life history were used to minimise  
129 confusion factors that may have an impact on contaminants' accumulation such as genetic variability,  
130 age and reproductive status (Kazour and Amara, 2020). At the start of the experiment, in May 2019,  
131 nine oysters, eight mussels and one surface sediment sample (at 5 cm depth and using a stainless-steel  
132 bowl of 10 cm diameter) were collected, then five mussels, six oysters and one surface sediment  
133 sample were collected every 4 months: in September 2019, January 2020 and May 2020. Sea water  
134 samples were collected in duplicates in September 2019 at the sites Les Faulx and Merignac, and, in  
135 December 2019 at the reference site and the Charente estuary (Figure 1). Other water samples were  
136 not collected due to technical reasons. After each sampling time, mussels, oysters, sediment and water

137 samples were frozen at -20°C until MPs analyses. The total length (cm), width (cm) and the wet  
138 weight (ww) (g) of the dissected flesh were measured for each organism.

139

#### 140 **Environmental parameters**

141 Turbidity (NTU), conductivity (mS/cm), chlorophyll *a* (µg/L), salinity (PSU), temperature (°C),  
142 dissolved oxygen (mg/L) and pH were measured continuously by an immersed automated system in  
143 the Charente and Sèvre estuaries using multi-parameters probes (SAMBAT sea, nke instrumentation)  
144 from May 2019 until February 2020 (due to technical maintenance). In addition, those parameters  
145 (except for chlorophyll *a*) were recorded monthly at the four sites in surface water along the cages  
146 using portable multi-meter probe (HANNA HI 9829).

147 Water flow data (m<sup>3</sup>/s) from the rivers Sèvre Niortaise, Charente and Sèvre were extracted from the  
148 « Service de prévision des crues du Littoral Atlantique »  
149 ([http://hydro.eaufrance.fr/selection.php?bassin\\_hydrographique=&btnValider=Ajouter%20Recherche](http://hydro.eaufrance.fr/selection.php?bassin_hydrographique=&btnValider=Ajouter%20Recherche)  
150 [&cmd=filtrer&code\\_station=&commune=&consulte=rechercher](http://hydro.eaufrance.fr/selection.php?bassin_hydrographique=&btnValider=Ajouter%20Recherche&cmd=filtrer&code_station=&commune=&consulte=rechercher)).

151

#### 152 **MPs analyses**

153 In order to avoid background contamination of samples, specific measures were taken and followed  
154 most of the criteria and the procedures described in Hermsen *et al.*, (2018) and Woodall *et al.*, (2015).  
155 Handlings were made under an air flow cabinet preventing MPs from depositing on samples. All the  
156 equipment used were cleaned with alcohol, the glassware always rinsed three times with MilliQ water,  
157 covered when not used. The purity of MilliQ water was controlled for the presence of MPs after  
158 filtration on a 1.6 µm porosity GF/A filter. The chemical solutions used (H<sub>2</sub>O<sub>2</sub>, KOH and ZnCl<sub>2</sub>) were  
159 filtered (using the same type of filter) three times before use. Workers were wearing cotton laboratory  
160 coats and nitrile gloves. One blank sample was included in each batch of studied samples in the  
161 laboratory. Those blank samples were processed using the same treatments as studied samples for  
162 tissue digestion and MPs extraction procedures in order to examine background MPs contamination.

163

164 *MPs extraction from sea water.* A motor pump onboard a research vessel was used to collect seawater  
165 samples in September and December 2019 at one meter depth. 1000 L of seawater was filtered using a  
166 40 µm mesh size net, thoroughly rinsed and particles (> 40 µm) were collected in a pre-cleaned glass  
167 bottle. Microplastics were extracted using a density separation solution (ZnCl<sub>2</sub>, density = 1.8 g/cm<sup>3</sup>) to  
168 recover plastics with a density below that of the solution. In a separating glass vial, 250 mL of  
169 seawater sample was mixed with 50 mL ZnCl<sub>2</sub> and let stand for 24h as described in Kazour *et al.*,  
170 (2019). The supernatant was then filtered on a GF/A filter (90 mm diameter, 1.6 µm porosity). Each  
171 filter was kept in a glass petri dish at 4°C and packed in parafilm to avoid desiccation until subsequent  
172 analyses.

173

174 *MPs extractions from sediments.* Each sample was thawed and dried in a drying chamber at 40°C for  
175 24h. The organic matter was digested using hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>, 30%) (Prata *et al.*, 2019). 10 g  
176 of sediment was mixed with 40 mL H<sub>2</sub>O<sub>2</sub>, then heated at 40°C on a heating magnetic stirrer for 24h,  
177 under a fume hood. Then, MPs were extracted using density separation as described above and the  
178 supernatant was then filtered and kept in a Petri dish.

179

180 *MPs extraction from mussel and oyster tissues.* Each individual was thawed, measured (length, width  
181 and thickness), dissected, drained and weighted. Mussels and oyster were digested using a solution of  
182 potassium hydroxide (KOH) 10%, known to degrade organic matter without affecting MPs (Dehaut *et*  
183 *al.*, 2016). One oyster or one mussel and 250 mL of KOH 10% were added in each Erlenmeyer. Each  
184 mix was then heated at 60°C on a heating magnetic stirrer for 24h under a fume hood. After digestion,  
185 each digestat was filtered as described for water samples.

186

187 *MPs observation.* A total of 2,185 particles (1,433 in oysters, 523 in mussels, 143 in seawater and 86  
188 in sediments) were identified using a microscope (Leica) with a Leica camera at magnification x90  
189 according to the following criteria: no repetitive structure linking to a biological origin, an  
190 homogeneous colour, if fibrous, a constant thickness (Enders *et al.*, 2015). The potential MPs were



191 counted, classified by type (fibre, fragment or bead) and by colour, and measured using Image J  
192 software (Table 1). The lowest limit size of a MP that could be assessed for these criteria was 4  $\mu\text{m}$ .

193  
194 *MPs identification.* Polymer analysis of 176 randomly selected particles regarding their types  
195 (fragment, fibre or sphere) and colours, observed on fifteen and twelve filters from oysters and  
196 mussels tissue respectively, sampled at different times and sites during the biomonitoring and  
197 representative of the types and colours of MPs found in water and sediment samples, was performed  
198 using a microRaman spectrometer Xplora Plus (Horiba Scientific) equipped with 532 and 785 nm  
199 lasers. Each obtained spectrum is compared to a polymer database identification software (KnowItAll,  
200 Wiley<sup>®</sup>) and a personal library made with standard polymers obtained from Goodfellow (France). The  
201 identification was considered correct when the HQI (Hit Quality Index) was above 70%.

202 This technique was retained to identify MPs because it allows the analysis of the fragments, as small  
203 as 1  $\mu\text{m}$  (Phuong *et al.*, 2018b), and bivalves are more likely to catch small MPs (<100  $\mu\text{m}$ ) (Bringer  
204 *et al.*, 2020; Browne *et al.*, 2008; Kazour and Amara, 2020; Qu *et al.*, 2018). The total ratio between  
205 the confirmed MPs and the total number of particles observed using the microscope was calculated  
206 and then extrapolated to the results obtained from the previous observation performed under the  
207 microscope for mussels, oysters, water and sediments (Table 1).

208

## 209 **Statistical analyses**

210 Statistical analyses were performed using R Studio v4.0.1. After checking that the residuals were  
211 normally distributed using the Shapiro-Wilk test, the effect of the site (Ile de Ré, Sèvre Niortaise,  
212 Charente and Seudre estuaries) and time (May 19, Sept 19, Jan 20, May 20) on biometric parameters  
213 (length, weight, width and thickness), MPs concentrations in bivalves (number of MPs/ per gramme of  
214 tissue ww) and fragments or fibres concentrations were assessed using several one-way ANOVA tests.  
215 The difference between species was also tested using the same test. Potential size differences of MPs  
216 determined in water, sediment, oysters and mussels were checked. When significant, comparisons  
217 between sites or times were assessed in pairs by the LS-mean test (with adjusted  $p$  value according to

218 the Bonferroni method). When residuals were not normally distributed, the nonparametric test of  
219 Kruskal-Wallis was used followed, when significant, by a Wilcoxon pair-wise comparison test.

220

## 221 **Results**

### 222 **Environmental parameters**

223 *Seasonal variation.* The temperature and salinity slightly decreased during the winter (Table  
224 S1, Excel files S1 and S2) whereas the water flow rates increased to reach 29, 240 and 6.6 m/s for the  
225 Sèvre, Charente and the Seudre rivers, respectively (Table S2). Then, they decreased in spring 2020  
226 down to 9.5, 60 and 2.9 m/s, respectively.

227 *Variation across sites.* The DO, conductivity and pH data were comparable between the sites  
228 (Table S1, Excel files S1 and S2). The turbidity was the highest at the Charente estuary site during the  
229 autumn and winter periods and reached 41.6 FNU (Table S1, Excel file S1). The mean concentrations  
230 in chlorophyll *a* were comparable in the Charente and Seudre estuary, and were equal to 4 and 4.3  
231 µg/L, respectively (Excel files S1 and S2). Finally, the Charente River was characterised by the  
232 highest flow rate and the Seudre river, by the lowest during the year biomonitoring (Table S2).

233

### 234 **Evolution of growth parameters**

235 The oysters grew over the summer as revealed by the significant increase in length, width, thickness  
236 and weight between May 2019 and September 2019 ( $p < 0.05$ ) (Figure 2A, Figure S1A). Their growth  
237 was lower during the winter and spring period. Their mean maximal weight ( $13.3 \pm 1.0$  g) was reached  
238 after one year in May 2020 ( $p < 0.05$ ) (Figure 2A). The growth of mussels was not significant through  
239 the year ( $p > 0.05$ ) (Figure 2B, Figure S1B).

240

### 241 **Size of MPs**

242 The maximum length of microplastics was measured using Image J. A total of 25, 46, 175 and 319  
243 fragments were measured in sediments, seawater, mussels and oysters samples, respectively. In  
244 addition, 31, 77, 199 and 289 fibres were measured in sediments, seawater, mussels and oysters  
245 samples, respectively. The mean sizes of fragments were similar between mussels (mean:  $33.0 \pm 31.7$

246  $\mu\text{m}$ , min: 6  $\mu\text{m}$ ), oysters (mean:  $27.0 \pm 7.6 \mu\text{m}$ , min: 6  $\mu\text{m}$ ), sediments (mean:  $35.1 \pm 22.9 \mu\text{m}$ , min: 8  
247  $\mu\text{m}$ ) and seawater (mean:  $31.6 \pm 25.7 \mu\text{m}$ , min: 4  $\mu\text{m}$ ) ( $p > 0.05$ ). Contrarily to the fragments, the  
248 mean size of fibres was higher in the sediments (mean:  $408.0 \pm 407.1 \mu\text{m}$ , min: 57  $\mu\text{m}$ ) than in oysters  
249 (mean:  $288.2 \pm 463.5 \mu\text{m}$ , min: 12  $\mu\text{m}$ ) and the seawater (mean:  $236.5 \pm 293.5 \mu\text{m}$ , min: 23  $\mu\text{m}$ ) ( $p <$   
250  $0.05$ ). The size of fibres in oysters and seawater were similar to the size measured in mussels (mean:  
251  $287.1 \pm 329.5 \mu\text{m}$ , min: 22  $\mu\text{m}$ ) ( $p > 0.05$ ). No difference was observed between the size of fibres in  
252 mussels and in the sediments ( $p > 0.05$ ).

253

#### 254 **Analyses of MPs polymers by spectroscopy**

255 The analyses of polymers using the microRaman technique revealed the polymer type of the  
256 confirmed MPs (fragments and fibres). Among the analysed blue fragments, 81% were identified as  
257 MPs and were mainly composed of: PS (70%), PC (18%), PP (3%), PA (3%) or PLA (3%). Only, 5%  
258 of the red fragments were confirmed MPs and made of PC (100%). The black, yellow, transparent,  
259 green and purple fragments observed under the microscope were of organic or mineral origin.  
260 Similarly, the blue fibres were mainly MPs (79%) but made of either PA (80%), PET (13%) or PP  
261 (7%). Approximately half of the black fibres were identified as MPs (42%) and made of PET (50%),  
262 PA (40%) or CA (10%). Also, around half of the pink-reddish fibres were identified as MPs (44%)  
263 made of PA (62.5 %), PET (25%) or PAN (12.5%). Finally, 33% of yellow and 25 % of transparent  
264 fibres were identified as MPs and made of PA. All the spheric particles observed under the microscope  
265 were of organic origin.

266

#### 267 **Abundance of MPs**

268  
269 MPs contamination of blank filters represented less than 5% of the abundance of microplastics  
270 detected  
271 in the samples and was considered negligible such as in recent studies (Pivokonsky *et al.*, 2018; Wang  
272 *et al.*, 2020).

273 *Concentrations of MPs in seawater.* The total concentrations of MPs measured in seawater at  
274 Merignac and Les Faulx sites ranged between 0.005 and 0.006 MPs/ L in September 2019. The

275 concentrations found in December 2019 appeared to be higher (statistical analyses were not performed  
276 as one replicate per site was chosen) and ranged between 0.008 and 0.01 MPs/ L (Table 2).

277 *Concentrations of MPs in sediments.* The MPs concentrations at the Ile de Ré site were  
278 characterized by low variations across the seasons, from 44 to 158 MPs/ kg of sediment, dry weight  
279 (dw). The MPs concentrations measured in sediments from other sites were influenced by the seasons.  
280 Indeed, the concentrations varied from 0 at the Sèvre, Charente and Seudre sites in autumn, to 247,  
281 652 and 934 MPs /kg dw, respectively during the winter. Then, during the spring 2020, the  
282 concentrations remained steady in at the Sèvre estuary (242 MPs/kg dw) or decreased at the Charente  
283 (84 MPs/kg dw) and Seudre (224 MPs/kg dw) estuaries (Table 3).

284 *Concentrations of MPs in mussels and oysters, and variation across the seasons and sites.*  
285 MPs were more concentrated in mussels ( $1.9 \pm 2.1$  MPs/g ww) than in oysters ( $0.4 \pm 0.4$  MPs/ g ww)  
286 on average ( $p < 0.05$ ). The MPs concentrations in mussels did not significantly vary over time and  
287 were similar between the sites ( $p > 0.05$ ) (Figure 3). Contrarily to mussels, the MPs concentrations in  
288 oysters decreased in September 2019 from  $0.7 \pm 0.4$  to  $0.3 \pm 0.3$  MPs/g ww ( $p < 0.05$ ), remained  
289 stable in January 2020 ( $p > 0.05$ ) and increased in May 2020 to reach  $0.6 \pm 0.5$  MPs/g ww ( $p < 0.05$ )  
290 (Figure 3). Specifically, the MPs concentrations increased between September 2019 and May 2020,  
291 from  $0.3 \pm 0.2$  to  $1.1 \pm 0.7$  MPs/g ww at the Seudre site ( $p < 0.05$ ) (Figure 3). During the same period,  
292 a subtle increase was observed at Le Martray site, from  $0.2 \pm 0.1$  to  $0.3 \pm 0.1$  MPs/g ww ( $p < 0.05$ )  
293 (Figure 3). In addition, the total MPs concentrations at the Sèvre and Charente sites did not vary  
294 significantly over this time laps ( $p > 0.05$ ) (Figure 3).

295 *Concentrations of fragments and fibres across the seasons and sites.* A species difference was  
296 found on the type of MPs accumulated: fragments or fibres, over a year and considering all sites. The  
297 fibres ( $1.2 \pm 1.4$  fibres/g ww) and fragments ( $0.7 \pm 1.3$  fragments/g ww) were more concentrated in  
298 mussels than in oysters ( $0.3 \pm 0.3$  fibres/g ww and  $0.1 \pm 0.2$  fragments/g ww) ( $p < 0.05$ ) (Figure 4A).  
299 In addition, the contamination by fibres was greater than the contamination by fragments for both  
300 species ( $p < 0.05$ ) (Figure 4A).

301 The fragment concentrations in mussels significantly varied between September 2019 and January  
302 2020 ( $p < 0.05$ ) (Figure 4A). Specifically, the concentrations dropped from  $0.4 \pm 0.3$  to  $0.1 \pm 0.1$

303 MPs/g ww in mussels at the Charente site ( $p < 0.05$ ) (Figure 4A). There was no significant variation of  
304 the fragment concentrations in mussels and oysters across the different sites ( $p > 0.05$ ) (Figure 4A).  
305 The fragment concentrations did not significantly vary across the year survey in oysters ( $p > 0.05$ )  
306 (Figure 4A).  
307 The fibres concentrations in mussels varied significantly over time ( $p < 0.05$ ) (Figure 4A).  
308 Specifically, the concentrations at Le Martray increased from  $0.4 \pm 0.2$  in January 2020 to  $1.5 \pm 1.2$   
309 MPs/g ww in May 2020 ( $p < 0.05$ ) (Figure 4A). The concentrations also increased at the Charente site,  
310 from  $0.4 \pm 0.4$  and  $0.6 \pm 0.5$  in September 2019 and January 2020 respectively, up to  $3.5 \pm 2.9$  MPs/g  
311 ww in May 2020 (Figure 4A). The fibres concentrations in oysters also varied across the seasons and  
312 the different sites ( $p < 0.05$ ) (Figure 4A). Specifically, the concentrations in oysters from the Seudre  
313 estuary increased from  $0.2 \pm 0.1$  in September 2019 to  $1.0 \pm 0.5$  MPs/g ww in May 2020 ( $p < 0.05$ )  
314 (Figure 4A).

315

#### 316 **Distribution of MPs colours**

317 The blue fragments were found in higher concentrations than red fragments on average in oysters  
318 ( $0.12$  blue and  $0.01$  red fragments/g) and mussels ( $0.70$  blue and  $0.02$  red fragments/g) ( $p < 0.05$ )  
319 (Figure 4B). The blue and black fibres were the most encountered in oysters with mean concentrations  
320 reaching  $0.13$  and  $0.10$  fibres/g respectively ( $p < 0.05$ ) (Figure 4B). Red and yellow fibres were found  
321 at a lower concentration;  $0.04$  and  $0.02$  fibres/g respectively ( $p < 0.05$ ) (Figure 4B). The pink fibres  
322 were the rarest with a mean of  $0.001$  fibres/g ( $p < 0.05$ ) (Figure 4B). Similarly to oysters, the blue and  
323 black fibres were the most encountered in mussels with mean concentrations reaching  $0.70$  and  $0.31$   
324 fibres/g respectively ( $p < 0.05$ ) (Figure 4B). The red and yellow fibres were also found at a lower  
325 concentration;  $0.11$  and  $0.03$  fibres/g respectively ( $p < 0.05$ ) (Figure 4B). Finally, the purple and  
326 transparent fibres in mussels were found at the lowest mean concentration of  $0.01$  fibres/g ( $p < 0.05$ )  
327 (Figure 4B).

328

#### 329 **Discussion**

330 The concentrations found in mussels ( $1.9 \pm 2.1$  MPs/g ww) and oysters ( $0.4 \pm 0.4$  MPs/g ww) in the  
331 present study were consistent with the concentration found in bivalves from other estuarine areas  
332 worldwide. Indeed, the present MPs concentrations found in oysters were comparable to the values  
333 measured in Pacific oysters collected from the French Atlantic coast ( $0.2 \pm 0.2$  MPs/g ww) (Phuong *et al.*,  
334 2018a), Brittany ( $0.47 \pm 0.16$  MPs/g ww) (Cauwenberghe and Janssen, 2014), the Oregon coast in  
335 U.S.A. ( $0.35 \pm 0.4$  MPs/g ww) (Baechler *et al.*, 2019) or the Jiaozhou Bay in China ( $0.3 - 3$  MPs/g  
336 ww) (Ding *et al.*, 2021). The present MPs concentrations found in mussels were comparable to those  
337 measured in Mediterranean mussels from the Jiaozhou Bay in China ( $1.6 - 2.6$  MPs/g ww) (Ding *et al.*,  
338 2021), in wild and farmed blue mussels along the Chinese coast ( $1.6 - 2.7$  MPs/g ww) (Li *et al.*,  
339 2016), in wild blue mussels from United Kingdom ( $0.7 - 2.9$  MPs/g ww) (Li *et al.*, 2018) or in caged  
340 mussels from the English Chanel coast ( $0.4 - 2.8$  MPs/g ww) (Kazour and Amara, 2020).

341 We also found that the concentrations in oysters measured in our study were five to ten times  
342 higher than the concentrations reported in Pacific oysters from Mexico bay (from  $0.04 \pm 0.02$  to  $0.08 \pm$   
343  $0.02$  MPs/g ww) (Lozano-Hernandez *et al.*, 2021). Our results in mussels were also above the  
344 concentrations of  $0.2$  MPs/g measured in mussels from the French Atlantic coast (Phuong *et al.*,  
345 2018a,b),  $0.15$  MPs/g in mussels from the French Channel coast (Hermabessiere *et al.*, 2019),  $0.28$   
346 MPs/g in wild mussels in Belgium (Van Cauwenberghe *et al.*, 2015) or  $0.36$  MPs/g in farmed mussels  
347 from Germany (Van Cauwenberghe *et al.*, 2014).

348 Our results showed that MPs were significantly more concentrated in mussels than in oysters.  
349 A few studies also found differences of MPs concentrations depending on bivalve species from the  
350 marine environment. For instance, Hermabessiere *et al.*, (2019), found significantly less MPs in blue  
351 mussels than in cockles *Cerastoderma edule* ( $0.15 \pm 0.06$  and  $0.74 \pm 0.35$  MP/g of tissue ww,  
352 respectively) from the French Channel coast (Hermabessiere *et al.*, 2019). Authors suggested that this  
353 difference may be explained by the position of the marine bivalves in the water column with mussels  
354 filtering particles present in the water column and cockle filtering particles closer to the sediments.  
355 However, this hypothesis does not explain the difference observed in our study as mussels and oysters  
356 cages were placed at the same hypsometric levels for all sites. Moreover, several physiological factors  
357 specific to the bivalve species may play a role in the accumulation of MPs such as filtration rate,

358 particle sorting ability, pseudofeces production or basal metabolism but this has yet to be  
359 demonstrated. Another study compared MPs accumulation in four bivalve species exposed at Jiaozhou  
360 Bay, in China, and showed that Pacific oysters and Mediterranean mussels (*Mytilus galloprovincialis*)  
361 accumulated less MPs (0.3 - 3 and 1.6 - 2.6 MPs/g of digestive gland ww, respectively) than the  
362 Japanese carpet shell (*Ruditapes philippinarum*) that contained MPs at concentrations that ranged from  
363 4.5 to 20.1 MPs/g of digestive gland (Ding *et al.*, 2021). However, as those bivalves were directly  
364 bought at the fishery market without information about their life history nor their specific *in situ*  
365 location on site and potential depuration step, it is difficult to interpret the species difference. In order  
366 to refine spatial and temporal variations of MPs contamination, environmental studies should therefore  
367 consider several bivalve species together with laboratory experiment to rigorously assess the influence  
368 of relevant physiological parameters as mentioned above, on MPs accumulation in different bivalve  
369 species.

370         The MPs concentrations in oysters in the present study varied across the seasons and sites.  
371 Indeed, they decreased between May 2019 and September 2019, from 0.7 to 0.3 MPs/g ww, and  
372 increased in May 2020 to reach 0.6 MPs/g ww, especially at the Seudre estuary site. Such seasonal and  
373 spatial variations were found in other coastal studies led worldwide (Baechler *et al.*, 2019; Lozano-  
374 Hernandez *et al.*, 2021). For instance, Lozano-Hernandez *et al.* (2021) found that MPs concentrations  
375 increased during the winter; and seasonal rainfalls were suspected to be one of the causal factors  
376 (Baechler *et al.*, 2019). Precipitations and river water flow are important environmental factors to  
377 consider when interpreting variations in MPs concentrations. However, MPs concentrations in water  
378 samples were not determined in that study to confirm this hypothesis. It is actually unclear if rainfall  
379 and subsequent catchment leaching increase, decrease or do not influence MPs concentrations in the  
380 water as the MPs input may be accompanied by important water input contributing to dilution.

381         In the present study, the MPs concentrations decreased in September in oysters and not in  
382 mussels. In parallel, the weight of oysters increased from to 2.7 to 11.3 g on average between May and  
383 September 2019 whereas the weight of mussels remained the same. This decrease observed in oysters  
384 may be due to the ponderal dilution linked to growth assuming that MPs stayed in the digestive tract

385 (and did not accumulate in the tissues). Further laboratory experiments including the study of the  
386 filtration and ingestion rates of MPs during oyster growth could help clarifying our hypothesis.

387 When looking at the fibre concentrations in mussels, they increased at the Charente estuary  
388 site, from 0.4 MPs/g in January 2020 to  $3.5 \pm 2.9$  MPs/g ww in May 2020. Interestingly, the MPs  
389 concentrations in oysters mainly composed of fibres increased between September 2019 and May  
390 2020, from  $0.3 \pm 0.2$  in to  $1.1 \pm 0.7$  MPs/g ww at the Seudre estuary. In parallel, the water flow of the  
391 Charente River decreased from 240 in January down to 60 m/s in May 2020 and from 6.6 in January  
392 down to 2.9 in May 2020. The increase of tourism combined with a decrease of the water flow may  
393 have contributed to the increase in MPs concentrations in mussels from the Charente estuary and in  
394 oysters from the Seudre estuary in spring 2020. The use of hydrodynamic models such as in Bernard *et*  
395 *al.*, (2010) or Zhang *et al.*, (2016) applied to MPs particles could allow a better understanding of the  
396 particle dispersal at those locations.

397 Interestingly, an outfall pipe of a WWTP nearby the site in the Seudre estuary was found. This pipe  
398 may have released more fibres when the touristic influence increased during the spring, contributing to  
399 increase fibres concentration in oysters. Washing clothes made of synthetic fabric mostly contributes  
400 to the release of synthetic microfibres in the aquatic system (Hernandez *et al.*, 2017) via WWTP  
401 effluents which represent important sources of MPs in the aquatic environment (Leslie *et al.*, 2017;  
402 Kazour *et al.*, 2019). Similarly to our results, other environmental studies found that fibres were the  
403 most encountered MPs in marine bivalves such as in oysters from Mexico bay (Lozano-Hernandez *et*  
404 *al.*, 2021), in mussels from Norwegian coastal waters (Bråte *et al.*, 2018), in Pacific oysters and  
405 Pacific razor clams from the Oregon coast in the United State of America (Baechler *et al.*, 2019) or in  
406 Mediterranean mussels and Pacific oysters from Jiaozhou Bay in China (Ding *et al.*, 2021). Moreover,  
407 previous studies found higher fibres concentrations in blue mussels (Leslie *et al.*, 2017; Kazour *et al.*,  
408 2019) and Pacific oysters (Leslie *et al.*, 2017) closer to WWTP effluents. However, a future study  
409 monitoring MPs seawater concentrations along the WWTP effluent in the Seudre estuary site will be  
410 necessary to confirm our present hypothesis.

411 As the size of fibres were similar between oysters (mean: 288  $\mu$ m, min: 12  $\mu$ m), mussels  
412 (mean: 288  $\mu$ m, min: 22  $\mu$ m) and seawater (mean: 237  $\mu$ m, min: 23  $\mu$ m), we assumed that both



413 species reflect the fibres contamination of the surrounding seawater. As such, mussels and oysters are  
414 good indicators of the quality of the estuarine environment regarding plastic microfibrils. The use of a  
415 40  $\mu\text{m}$  mesh size net in the present study may have refined those conclusions as most of past studies  
416 used 300  $\mu\text{m}$  mesh size nets initially dedicated to sample plankton. Indeed, the use of 300  $\mu\text{m}$  mesh  
417 size nets underestimates the concentrations of small particles. For instance, Lindeque *et al.*, (2020),  
418 found that concentrations of MPs when using 100  $\mu\text{m}$  mesh size nets were 2.5 and 10 times higher  
419 than the concentrations found when using 333 and 500  $\mu\text{m}$  mesh size nets, respectively. Interestingly,  
420 Gardon *et al.*, (2021), found that the large majority of small MPs (20-200  $\mu\text{m}$ ) were found in pearl  
421 oysters (95 %) and the water column (93.2 %). As this 40  $\mu\text{m}$  mesh size net allowed scientists to catch  
422 small particles in the water column and enabled them to make a coherent comparison with the MPs  
423 accumulated in oysters, they concluded, similarly to the present study, that the MPs contamination of  
424 marine bivalve reflected the seawater contamination. Furthermore, as the majority of small MPs are  
425 found in mussels (< 50  $\mu\text{m}$ ) (Phuong *et al.*, 2018a) and oysters (1 - 22  $\mu\text{m}$ ) (Ribeiro *et al.*, 2021), it is  
426 important to be able to identify and analyse such small sizes under the microscope and using  
427 appropriate analytical techniques. Finally, the use of a low net mesh size ( $\leq$  40  $\mu\text{m}$ ) for collecting the  
428 small fraction of seawater particles is required to ensure a reliable comparison of the contamination  
429 levels between biological and seawater matrices.

430         Regarding the MPs colours found in the present study, our results showed that the majority of  
431 MPs were blue. Other studies found a majority of blue MPs (Ding *et al.*, 2021; Kazour and Amara,  
432 2020). The fact that a majority of blue fibres made of PA was found may be the results of the net  
433 degradation used in fishing activities and mussel farming, or the fibre release of clothes made of  
434 synthetic fabric from the washing machine.

435         In terms of polymer distribution, we found that they depended on the type of MPs considered.  
436 Indeed, fragments were mainly made of PS (70%) followed by PC (18%) and PP, PA, or PLA (3%)  
437 whereas the fibres were mostly PA (80%) followed by PET (13%) and PP (7%). Our results are  
438 consistent with studies also finding a majority of MPs made of PA (Ding *et al.*, 2020; Lozano-  
439 Hernandez *et al.*, 2021) or PS (Cho *et al.*, 2019) but other studies found a majority of MPs made of PP  
440 (Abidli *et al.*, 2019), PE (Bour *et al.* 2018; Cho *et al.*, 2019; Hermabessière *et al.*, 2019) or polyester

441 (Li *et al.*, 2018). Therefore, the distribution of MPs types found in marine bivalves in the present study  
442 do not follow the same trend of the global plastic production (PlasticEurope, 2018). Instead, our  
443 results and those from the literature revealed that MPs types found in mollusks may be more specific  
444 to the coastal region studied.

445 Consumption of seafood is one of the main dietary routes of human exposure to MPs. A meta-  
446 analysis study that reviewed the most relevant studies on MPs contamination in seafood worldwide  
447 found that the MPs content varied as followed mollusks > crustaceans > fish > echinoderma  
448 (Danopoulos *et al.*, 2020a). Global human mollusk consumption was estimated to 2.65 kg/person per  
449 year, with a great variability across countries (FAO, 2020). The study estimated a corresponding  
450 human yearly consumption of 0-27,825 MPs based on a MPs content of 0 to 10.5 MPs/g in mollusks  
451 with the highest content reported in Asia (Danopoulos *et al.*, 2020a). In the present study, the  
452 consumption of mussels and oysters would be equivalent to a yearly human consumption of 6,095  
453 MPs which is much lower. However, as a depuration step for commercial bivalves before being sold to  
454 the consumers is a common procedure (Lee *et al.*, 2008), therefore, the key factor of the contamination  
455 of bivalves for human consumption is the level of MPs contamination of the water depuration system  
456 which allow either to depurate the bivalve of their MPs (Birnstiel *et al.*, 2019; Graham *et al.*, 2019) or  
457 further contaminate them. To our knowledge, no study in the literature highlighted an increase of MPs  
458 contamination after a depuration period. Therefore, if further studies assessing the MPs contamination  
459 in bivalves after the conventional depuration procedure would have been useful to conclude about  
460 human dietary exposure levels, the results from the present study suggest a low MPs exposure of  
461 humans *via* marine bivalve consumption. Moreover, the consumption of marine bivalves appeared as a  
462 minor contribution comparing to other sources of exposure such as drinking water or diet. Indeed, the  
463 yearly maximum human consumption of MPs from tap water was estimated to 458,000 MPs and  
464 3,569,000 MPs from bottled water, in Europe (Danopoulos *et al.*, 2020b). Moreover, Catarino *et al.*,  
465 (2020) estimated a yearly consumption of airborne fibres *via* the meals of 13,731 - 68,415 per person.

466 This rare environmental case study of long-term chronic exposure of farming areas to MPs  
467 provides new knowledge on *in situ* variations of plastic fibres and fragments contamination of two  
468 bivalve species throughout the seasons and sites. The present study encourages further water sampling

469 to confirm the influence of a WWTP on the fibres concentration in bivalves, the use of models to  
470 characterise MPs particles dynamic in the complex estuarine environment for a better understanding of  
471 the environmental factors influencing the MPs contamination levels and finally, future laboratory  
472 experiments allowing a robust assessment of the physiological factors involved in the species-specific  
473 differences observed for MPs accumulation.

474

#### 475 **Acknowledgements**

476 This research is part of the AQUAECOs project (Amélioration de la QUALité Environnementale en  
477 zones CONchylicoles des Pertuis Charentais), funded by the “Nouvelle Aquitaine Region”, the  
478 “Comité Régional de la Conchyliculture de la Charente-Maritime” (CRC17), the “Comité  
479 Départemental de la Charente-Maritime” (CD17) and the “Office Français de la Biodiversité” (OFB).  
480 Authors are grateful to the maritime school of La Rochelle, professionals and colleagues from the  
481 “Centre pour l’Aquaculture, la Pêche et l’Environnement de Nouvelle-Aquitaine » (CAPENA) for their  
482 technical and scientific support. Authors are also grateful to the captains P. Pineau and N. Lachaussée  
483 of the University of La Rochelle, for their help in sampling seawater onboard the research vessel.

484

485

486

487

488

489

#### 490 **Supplementary material.**

491 **Figure S1.** Graphs representing the width and thickness of oysters (A) and mussels (B) across the  
492 seasons.

493 **Excel File S1.** Physicochemical data and graphs of fluorescence (chlorophyll *a* in µg/L), turbidity  
494 (NTU), salinity (PSU) and temperature (°C) measured using the immersed high throughput probe  
495 (SAMBAT sea, nke instrumentation) nearer the Charente estuary site.

496 **Excel File S2.** Physicochemical data and graphs of fluorescence (chlorophyll *a* in µg/L), turbidity  
497 (NTU), salinity (PSU) and temperature (°C) measured using the immersed high throughput probe  
498 (SAMBAT sea, nke instrumentation) nearer the Seudre estuary site.

499 **Table S1.** Physicochemical data: temperature (°C), Dissolved Oxygen (DO) (%), conductivity  
500 (mS/cm), pH, turbidity (FNU) and salinity (PSU) recorded using the multi-meter probe (HANNA HI  
501 9829) over a year at the four different sites.

502 **Table S2.** Water flow (m<sup>3</sup>/s) recorded for the Sèvre Niortaise, Charente and Seudre rivers. Data are  
503 from the « Service de prévision des crues du Littoral Atlantique » available online.

504

505

506

507

508

509

## 510 **References**

511 Abidli S, LahbibY, TriguiElMenifN. 2019. Microplastics in commercial molluscs from the lagoon of  
512 Bizerte (NorthernTunisia). *Marine Pollution Bulletin*, 142, 243–252.

513

514 Alimba, C. G., & Faggio, C. (2019). Microplastics in the marine environment : Current trends in  
515 environmental pollution and mechanisms of toxicological profile. *Environmental Toxicology and*  
516 *Pharmacology*, 68, 61- 74.

517

518 Amélineau, F., Bonnet, D., Heitz, O., Mortreux, V., Harding, A. M. A., Karnovsky, N., Walkusz, W.,  
519 Fort, J., & Grémillet, D. (2016). Microplastic pollution in the Greenland Sea : Background levels and  
520 selective contamination of planktivorous diving seabirds. *Environmental Pollution*, 219, 1131- 1139.

521

522 Andradý LA, (2011). Microplastics in the marine environment. *Marine Pollution Bulletin*, 62, 1596-  
523 1605.

524

525 Avio, C. G., Cardelli, L. R., Gorbi, S., Pellegrini, D., & Regoli, F. (2017). Microplastics pollution after  
526 the removal of the Costa Concordia wreck: First evidences from a biomonitoring case study.  
527 Environmental Pollution, 227, 207- 214.

528

529 Baechler BR, Granek EF, Hunter MV, Conn KE. (2020). Microplastic concentrations in two Oregon  
530 bivalve species: Spatial, temporal, and species variability. Limnology and Oceanography Letters 5,  
531 2020, 54–65.

532

533 Beliaeff B, O'Connor TP, Claisse D. (1998). Comparison of chemical concentrations in mussels and  
534 oysters from the United States and France. Environmental Monitoring and Assessment, 49, 87-95.

535

536 Bernard I, Le Moine O, Stanisière JY, Pouvreau S, Gouilletquer P and Dumas F. (2010). Site selection  
537 for stock replenishment using a hydrodynamic model: example of the Pacific oyster *Crassostrea gigas*  
538 culture in the Pertuis Charentais (France). ICES CM2010/A:18.

539

540 Beyer, J., Green, N. W., Brooks, S., Allan, I. J., Ruus, A., Gomes, T., Bråte, I. L. N., & Schøyen, M.  
541 (2017). Blue mussels (*Mytilus edulis* spp.) as sentinel organisms in coastal pollution monitoring: A  
542 review. Marine Environmental Research, 130, 338- 365.

543

544 Birnstiel S, Soares-Gomes A, da Gama BAP. (2019). Depuration reduces microplastic content in wild  
545 and farmed mussels. Marine Pollution Bulletin, 140, 241–247.

546

547 Boucher, J. and Friot D. (2017). Primary Microplastics in the Oceans: A Global Evaluation of Sources.  
548 Gland, Switzerland: IUCN. 43pp.

549

550 Bour A, Avio CG, Gorbi S, Regoli F, Hylland K. (2018). Presence of microplastics in benthic and  
551 epibenthic organisms: influence of habitat, feeding mode and trophic level. *Environmental Pollution*,  
552 243, 1217–1225.

553

554 Bråte, I. L. N., Hurley, R., Iversen, K., Beyer, J., Thomas, K. V., Steindal, C. C., Green, N. W., Olsen,  
555 M., & Lusher, A. (2018). *Mytilus* spp. as sentinels for monitoring microplastic pollution in Norwegian  
556 coastal waters: A qualitative and quantitative study. *Environmental Pollution*, 243, 383- 393.

557

558 Bringer, A., Thomas, H., Prunier, G., Dubillot, E., Bossut, N., Churlaud, C., Clérandeau, C., Le  
559 Bihanic, F., & Cachot, J. (2020). High density polyethylene (HDPE) microplastics impair  
560 development and swimming activity of Pacific oyster D-larvae, *Crassostrea gigas*, depending on  
561 particle size. *Environmental Pollution*, 260, 113978.

562

563 Browne, M. A., Dissanayake, A., Galloway, T. S., Lowe, D. M., & Thompson, R. C. (2008). Ingested  
564 Microscopic Plastic Translocates to the Circulatory System of the Mussel, *Mytilus edulis* (L.).  
565 *Environmental Science & Technology*, 42, 5026- 5031.

566

567 Canesi, L., Ciacci, C., Bergami, E., Monopoli, M. P., Dawson, K. A., Papa, S., Canonico, B., & Corsi,  
568 I. (2015). Evidence for immunomodulation and apoptotic processes induced by cationic polystyrene  
569 nanoparticles in the hemocytes of the marine bivalve *Mytilus*. *Marine Environmental Research*, 111,  
570 34- 40.

571

572 Catarino, A. I., Macchia, V., Sanderson, W. G., Thompson, R. C., & Henry, T. B. (2018). Low levels  
573 of microplastics (MP) in wild mussels indicate that MP ingestion by humans is minimal compared to  
574 exposure via household fibres fallout during a meal. *Environmental Pollution*, 237, 675- 684.

575

576 Cau, A., Avio, C. G., Dessì, C., Follesa, M. C., Moccia, D., Regoli, F., & Pusceddu, A. (2019).  
577 Microplastics in the crustaceans *Nephrops norvegicus* and *Aristeus antennatus*: Flagship species for  
578 deep-sea environments? *Environmental Pollution*, 255, 113107.  
579  
580 Cho Y, Shim WJ, Jang M, Han GM, Hong SH. (2019). Abundance and characteristics of microplastics  
581 in market bivalves from South Korea. *Environ Pollut* 245:1107–1116.  
582  
583 Clausen, I., & Riisgård, H. U. (1996). Growth, filtration and respiration in the mussel *Mytilus edulis*:  
584 No evidence for physiological regulation of the filter-pump to nutritional needs. *Marine Ecology-*  
585 *progress Series*, 141, 37- 45.  
586  
587 Danopoulos E, Jenner LC, Twiddy M and Rotchell JM. 2020a. Microplastic Contamination of  
588 Seafood Intended for Human Consumption: A Systematic Review and Meta-Analysis. *Environmental*  
589 *Health Perspectives*, 128, 1 - 32.  
590  
591 Danopoulos E, Twiddy M, Rotchell JM. 2020b. Microplastic contamination of drinking water: a  
592 systematic review. *PLoSOne*, 15, e0236838.  
593  
594 Dehaut, A., Cassone, A.-L., Frère, L., Hermabessiere, L., Himber, C., Rinnert, E., Rivière, G.,  
595 Lambert, C., Soudant, P., Huvet, A., Duflos, G., & Paul-Pont, I. (2016). Microplastics in seafood :  
596 Benchmark protocol for their extraction and characterization. *Environmental Pollution*, 215, 223- 233.  
597  
598 Ding JF, Li JX, Sun CJ, Jiang FH, He CF, Zhang M, et al. (2020). An examination of the occurrence  
599 and potential risks of microplastics across various shellfish. *Science of the Total Environment*, 739,  
600 139887.  
601

602 Ding J, Sun C, Hed C, Lic J, Juc P, Lia F. (2021). Microplastics in four bivalve species and basis for  
603 using bivalves as bioindicators of microplastic pollution. *Science of the Total Environment*, 782,  
604 146830.

605

606 Domogalla-Urbansky, J., Anger, P. M., Ferling, H., Rager, F., Wiesheu, A. C., Niessner, R., Ivleva, N.  
607 P., & Schwaiger, J. (2019). Raman microspectroscopic identification of microplastic particles in  
608 freshwater bivalves (*Unio pictorum*) exposed to sewage treatment plant effluents under different  
609 exposure scenarios. *Environmental Science and Pollution Research*, 26, 2007–2012.

610

611 Enders, K., Lenz, R., Stedmon, C. A., & Nielsen, T. G. (2015). Abundance, size and polymer  
612 composition of marine microplastics  $\geq 10 \mu\text{m}$  in the Atlantic Ocean and their modelled vertical  
613 distribution. *Marine Pollution Bulletin*, 100, 70- 81.

614

615 Eriksen M, Lebreton LCM, Carson HS, Thiel M, Moore CJ, Borerro JC, et al. (2014). Plastic pollution  
616 in the world's oceans: more than 5 trillion plastic pieces weighing over 250,000 tons afloat at sea.  
617 *PLoSOne*, 9, e111913.

618

619 FAO (Food and Agriculture Organization of the United Nations). 2020. Fishery statistical collections;  
620 consumption of fish and fishery products. Rome (Italy). [http://www.fao.org/fishery/statistics/global-](http://www.fao.org/fishery/statistics/global-consumption/en)  
621 [consumption/en](http://www.fao.org/fishery/statistics/global-consumption/en).

622

623 Lee, R., Lovatelli, A., Ababouch, L. (2008). Bivalve depuration: fundamental and practical aspects.  
624 FAO (Food and Agriculture Organization of the United Nations). Rome (Italy).

625

626 Frère, L., Paul-Pont, I., Rinnert, E., Petton, S., Jaffré, J., Bihannic, I., Soudant, P., Lambert, C., &  
627 Huvet, A. (2017). Influence of environmental and anthropogenic factors on the composition,  
628 concentration and spatial distribution of microplastics: A case study of the Bay of Brest (Brittany,  
629 France). *Environmental Pollution*, 225, 211- 222.



630

631 Gardon T, El Rakwe M, Paul-Pont I, Le Luyer J, Thomas L, Prado E, Boukerma K, Cassone AL,  
632 Quillien V, Soyez C, Costes L, Crusot M, Dreanno C, Le Moullac G, Huvet A. (2021). Microplastics  
633 contamination in pearl-farming lagoons of French Polynesia. *Journal of Hazardous Materials*, 419,  
634 126396.

635

636 Gonçalves, C., Martins, M., Sobral, P., Costa, P.M., Costa, M.H., (2019). An assessment of the ability  
637 to ingest and excrete microplastics by filter-feeders: a case study with the Mediterranean mussel.  
638 *Environmental Pollution*, 245, 600–606

639

640 Graham P, Palazzo L, Andrea de Lucia G, Telfer TC, Baroli M, Carboni S. (2019). Microplastics  
641 uptake and egestion dynamics in Pacific oysters, *Magallana gigas* (Thunberg, 1793), under controlled  
642 conditions. *Environmental Pollution*, 252, 742-748.

643

644 Green, D. S. (2016). Effects of microplastics on European flat oysters, *Ostrea edulis* and their  
645 associated benthic communities. *Environmental Pollution*, 216, 95- 103.

646

647 Hermabessiere, L., Paul-Pont, I., Cassone, A., Himber, C., Receveur, J., Jezequel, R., El Rakwe, M.,  
648 Rinnert, E., Rivière, G., Lambert, C., Huvet, A., Dehaut, A., Duflos, G., Soudant, P., (2019).  
649 Microplastic contamination and pollutant levels in mussels and cockles collected along the channel  
650 coasts. *Environmental Pollution*, 250, 807-819.

651 Hermabessiere L, Dehaut A, Paul-Pont I, Lacroix C, Jezequel R, Soudant P, Duflos G (2017).  
652 Occurrence and effects of plastic additives on marine environments and organisms: A review.  
653 *Chemosphere*, 182: 781-793.

654

655 Hermsen E, Mintenig SM, Besseling E and Koelmans AA (2018). Quality Criteria for the Analysis of  
656 Microplastic in Biota Samples: A Critical Review. *Environmental Sciences and Technology*, 52,  
657 10230–10240.

658

659 Hernandez-Gonzalez, A., Saavedra, C., Gago, J., Covelo, P., Santos, M. B., & Pierce, G. J. (2018).  
660 Microplastics in the stomach contents of common dolphin (*Delphinus delphis*) stranded on the  
661 Galician coasts (NW Spain, 2005–2010). *Marine Pollution Bulletin*, 137, 526- 532.

662

663 Jambeck, J.R., Geyer, R., Wilcox, C., Siegler, T.R., Perryman, M., Andrady, A., Narayan, R., and  
664 Law, K.L. (2015). Plastic waste inputs from land into the ocean. *Science* 347, 768–771.

665

666 Kazour, M., Terki, S., Rabhi, K., Jemaa, S., Khalaf, G., & Amara, R. (2019). Sources of microplastics  
667 pollution in the marine environment: Importance of wastewater treatment plant and coastal landfill.  
668 *Marine Pollution Bulletin*, 146, 608-618.

669

670 Kazour, M., & Amara, R. (2020). Is blue mussel caging an efficient method for monitoring  
671 environmental microplastics pollution? *Science of The Total Environment*, 710, 135649.

672

673 Lau WWY, Shiran Y, Bailey RM, Cook E, Stuchtey MR, Koskella J, Velis CA, Godfrey L, Boucher J,  
674 Murphy MB, Thompson RC, Jankowska E, Castillo AC, Pilditch TD, Dixon B, Koerselman L, Kosior  
675 E, Favoino E, Gutberlet J, Baulch S, Atreya M, Fischer D, He KK, Petit MM, Sumaila UR, Neil E,  
676 Bernhofen MV, Lawrence K, Palardy JE (2020). Evaluating scenarios toward zero plastic pollution.  
677 *Science*, 369, 1455–1461.

678

679 Lerebours A, Bathie M, Receveur J, Jezequel R, Dubillot E, Brunello P, Barbier P, Le Floch S,  
680 Thomas H. (2021). Pesticides, nonylphenols and polybrominated diphenyl ethers in marine bivalves  
681 from France: A pilot study. *Marine Pollution Bulletin*, 172, 112956.

682

683 Li, J., Qu, X., Su, L., Zhang, W., Yang, D., Kolandhasamy, P., Li, D., Shi, H., (2016). Microplastics in  
684 mussels along the coastal waters of China. *Environmental Pollution*, 214, 177-184.

685

686 Li, J., Green, C., Reynolds, A., Shi, H., Rotchell, J.M., (2018). Microplastics in mussels sampled from  
687 coastal waters and supermarkets in the United Kingdom. *Environmental Pollution*. 241, 35-44.  
688

689 Li, J., Lusher, A.L., Rotchell, J.M., Deudero, S., Turra, A., Bråte, I.L.N., Sun, C., (...), Shi, H. (2019).  
690 Using mussel as a global bioindicator of coastal microplastic pollution. *Environmental Pollution*, 244:  
691 522-533.  
692

693 Lindeque PK, Cole M, Coppock RL, Lewis CN, Miller RZ, Watts AJR, Wilson-McNeal A, Wright S,  
694 Galloway TS (2020). Are we underestimating microplastic abundance in the marine environment? A  
695 comparison of microplastic capture with nets of different mesh-size. *Environmental Pollution*, 265,  
696 114721.  
697

698 Lozano-Hernández EA, Ramírez-Alvarez N, Rios Mendoza LM, Macías-Zamora JV, Sánchez-Osorio  
699 JL, Hernández-Guzmán FA. (2021). Microplastic concentrations in cultured oysters in two seasons  
700 from two bays of Baja California, Mexico. *Environmental Pollution* 290, 118031.  
701

702 McFarland K, Donaghy L and Volety AK. (2013). Effect of acute salinity changes on hemolymph  
703 osmolality and clearance rate of the non-native mussel, *Perna viridis*, and the native oyster,  
704 *Crassostrea virginica*, in Southwest Florida. *Aquatic Invasions* 8 (3): 299–310.  
705

706 Nelms, S. E., Galloway, T. S., Godley, B. J., Jarvis, D. S., & Lindeque, P. K. (2018). Investigating  
707 microplastic trophic transfer in marine top predators. *Environmental Pollution*, 238, 999- 1007.  
708

709 Phuong NN, Poirier L, Phamb QT, Lagarde F, Zalouk-Vergnouxa A. (2018a). Factors influencing the  
710 microplastic contamination of bivalves from the French Atlantic coast: Location, season and/or mode  
711 of life? *Marine Pollution Bulletin*, 129, 664-674.  
712

713 Phuong, N. N., Zalouk-Vergnoux, A., Kamari, A., Mouneyrac, C., Amiard, F., Poirier, L., & Lagarde,  
714 F. (2018b). Quantification and characterization of microplastics in blue mussels (*Mytilus edulis*):  
715 Protocol setup and preliminary data on the contamination of the French Atlantic coast. *Environmental*  
716 *Science and Pollution Research*, 25, 6135- 6144.

717

718 Pivokonsky M., Cermakova L., Novotna K., Peer P., Cajthaml T., Janda V. (2018). Occurrence of  
719 microplastics in raw and treated drinking water. *Science of the Total Environment* 643, 1644–1651.

720

721 PlasticsEurope. (2018). *Plastics-the Facts 2018 An analysis of European plastics production, demand*  
722 *and waste data*.

723

724 Pouvreau S, Jonquieres G, Buestel D. (1999). Filtration by the pearl oyster, *Pinctada margaritifera*,  
725 under conditions of low seston load and small particle size in a tropical lagoon habitat. *Aquaculture*,  
726 176, 295–314.

727

728 Prata JC, da Costa JP, Duarte AC, Rocha-Santos T. (2019). Methods for sampling and detection of  
729 microplastics in water and sediment: A critical review. *Trends in Analytical Chemistry*, 110, 150-159.

730

731 Qu, X., Su, L., Li, H., Liang, M., & Shi, H. (2018). Assessing the relationship between the abundance  
732 and properties of microplastics in water and in mussels. *Science of The Total Environment*, 621,  
733 679- 686.

734

735 Railo, S., Talvitie, J., Setälä, O., Koistinen, A., & Lehtiniemi, M. (2018). Application of an enzyme  
736 digestion method reveals microlitter in *Mytilus trossulus* at a wastewater discharge area. *Marine*  
737 *Pollution Bulletin*, 130, 206- 214.

738

739 Ribeiro F, Okoffo ED, O'Brien JW, O'Brien S, Harris MJ, Samanipour S, Kaserzon S, Mueller JF,  
740 Galloway T, Thomas KV. (2021). Out of sight but not out of mind: Size fractionation of plastics  
741 bioaccumulated by field deployed oysters. *Journal of Hazardous Materials Letters*, 2, 100021.  
742

743 Talbot R and Chang H, (2022). Microplastics in freshwater: A global review of factors affecting  
744 spatial and temporal variations. *Environmental Pollution*, 292, 118393.  
745

746 Teuten, E. L., Rowland, S. J., Galloway, T. S., & Thompson, R. C. (2007). Potential for Plastics to  
747 Transport Hydrophobic Contaminants. *Environmental Science & Technology*, 41(22), 7759- 7764.  
748

749 Thompson, R. C. (2004). Lost at Sea : Where Is All the Plastic? *Science*, 304(5672), 838- 838.  
750

751 Van Cauwenberghe, L., Claessens, M., Vandegehuchte, M.B., Janssen, C.R., 2015. Microplastics are  
752 taken up by mussels (*Mytilus edulis*) and lugworms (*Arenicola marina*) living in natural habitats.  
753 *Environmental Pollution*, 199, 10-17.  
754

755 Van Cauwenberghe, L., Janssen, C.R., 2014. Microplastics in bivalves cultured for human  
756 consumption. *Environmental Pollution*. 193, 65-70.  
757

758 Wang, W., Ge, J., Yu, X. (2020). Bioavailability and toxicity of microplastics to fish species: A  
759 review. *Ecotoxicology and Environmental Safety*, 189, 109913.  
760

761 Wang, Z., Lin T., Chen, W. (2020). Occurrence and removal of microplastics in an advanced drinking  
762 water treatment plant (ADWTP). *Science of the Total Environment*, 700, 134520.  
763

764 Ward JE, Rosa M, and Shumway SE. (2019). Capture, ingestion, and egestion of microplastics  
765 by suspension-feeding bivalves: a 40-year history. *Anthropocene Coasts* 2, 39–49.  
766

767 Woodall L. C., Gwinnett, C., Packer M., Thompson, R. C., Robinson, L. F., Paterson, G. L. J. (2015).  
768 Using a forensic science approach to minimize environmental contamination and to identify  
769 microfibrils in marine sediments. *Marine Pollution Bulletin*, 95, 40–46.

770

771 Zhang YJ, Yea F, Stanev EV, Grashorn S. (2016). Seamless cross-scale modeling with SCHISM.  
772 *Ocean Modelling*, 102, 64-81.

773

774

775

776

777

778

779

780

781

782

783

784

785

786 **Figure and Table Legend.**

787 **Figure 1.** The French littoral area of the Pertuis Charentais showing the sites where oysters, mussels,  
788 water and sediment samples were collected. The high-frequency probes were located in the estuaries  
789 of the Charente and Seudre rivers near the sampling sites.

790 **Figure 2.** Seasonal variations of the length and weight of oysters (A) and mussels (B).

791 **Figure 3.** Concentration of MPs (MPs numbers/g tissue wet weight) measured across the seasons  
792 (May 2019, September 2019, January 2020 and May 2020) and at the different sites (Le Martray,  
793 Sèvre, Charente and Seudre) for mussels (A) and oysters (B). Plots represent the median (line), mean  
794 (cross) and 25-75% percentile (box).

795 **Figure 4.** Distribution of fragments and fibres (A) and their colours (B) in mussels and oysters (mean  
796 fragment or fiber number/g of tissue wet weight).

797 **Figure 5.** Length of fragments (A) and fibres (B) ( $\mu\text{m}$ ) measured in oyster, mussel, water and  
798 sediment samples.

799 **Table 1.** Distribution of successfully analysed particles (classified by types and colours), MPs and  
800 polymers using the MicroRaman spectroscopy. The second column refers to the number of particles  
801 that were successfully analysed. The third column refers to the percentage of MPs identified among  
802 the particles analysed. The fourth column refers to the distribution of particles made of different  
803 polymers among the identified MPs.

804 **Table 2.** Concentration of fragments, fibres and total MPs in seawater (number per litre of seawater).

805 **Table 3.** Concentration of fragments, fibres and total MPs in sediment (number per kg of sediment,  
806 dry weight).

807

808

809

810

811

812

813 **Figure 1.**

814

815

816

817

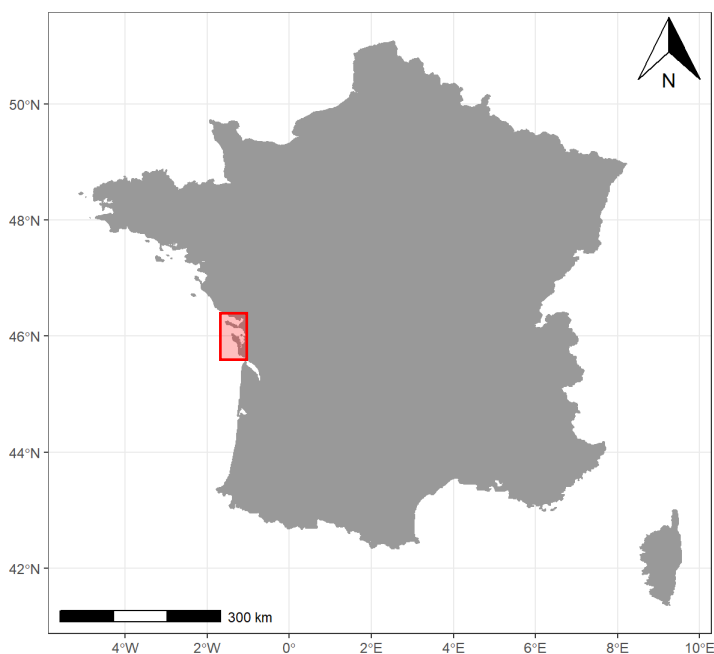
818

819

820

821

822



823

824



825

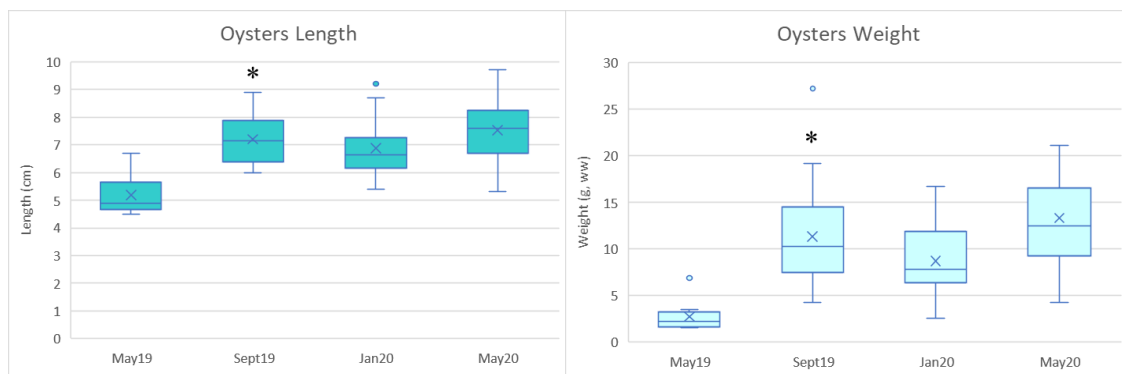
826

827

828

829 **Figure 2.**

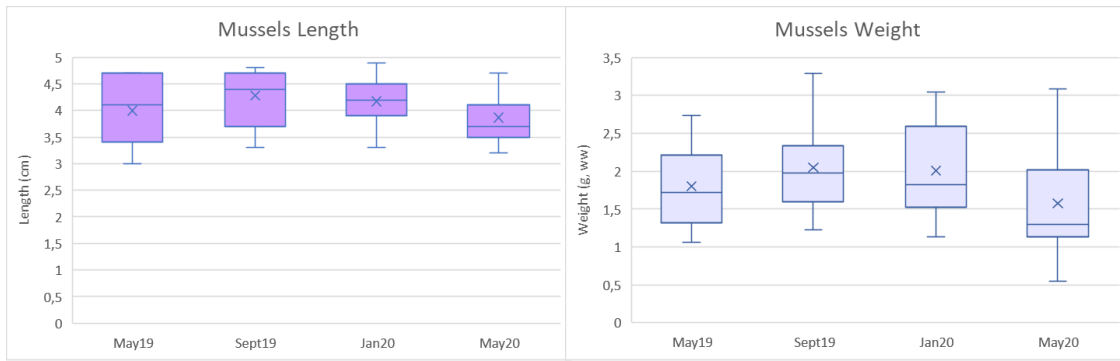
830 **A**



831

832 **B**





833

834

835

836

837

838

839

840

841

842

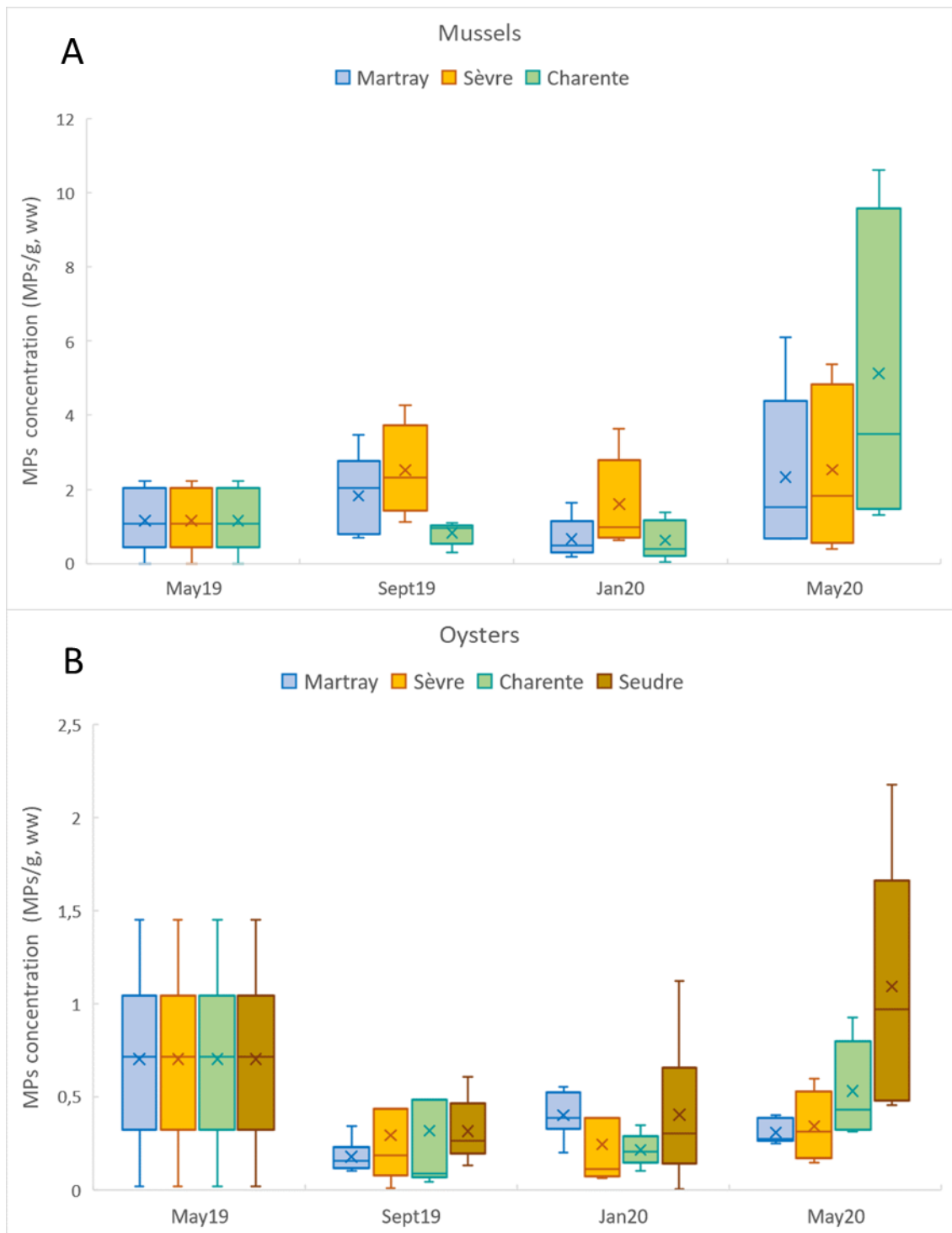
843

844

845

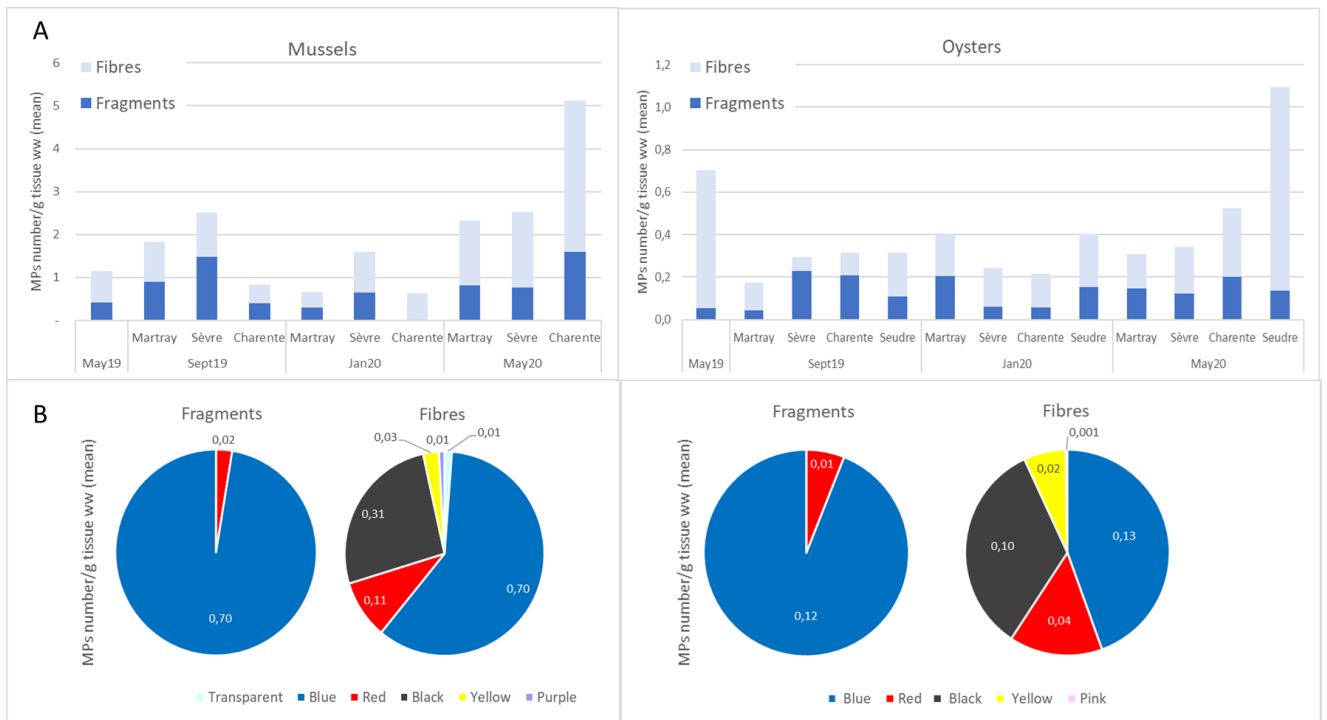
846

847 **Figure 3.**



848  
849  
850  
851  
852  
853  
854

**Figure 4.**



855

856

857

858

859

860

861

862

863

864

865

866

867

868

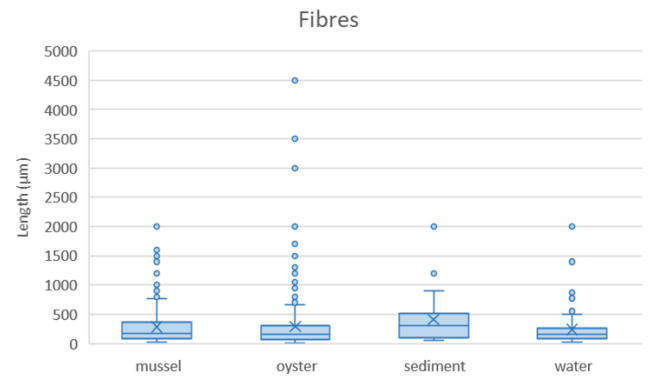
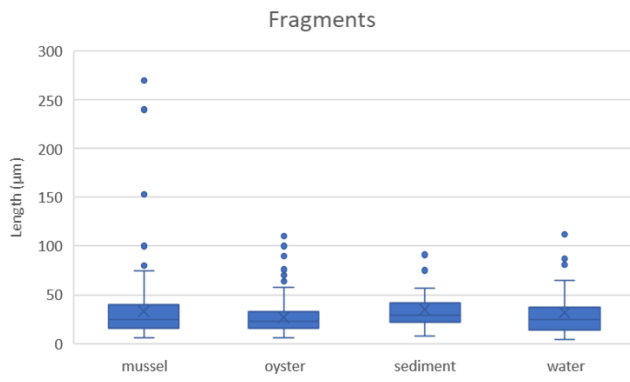
869

870

871

872 **Figure 5.**

873



874

875

876

877

878

879

880

881

882

883

884

885

886

887

888

889

890

891

892

893

894 **Table 1.**

Particle Type	Number identified/Total	MPs (%)	Type of polymer (%) and other origin
<b><i>Fragment</i></b>			
Blue	36/42	81%	MPs: 70% PS, 18% PC, 6% PP, 3% PA, 3% PLA, Other: mineral
Red	18/19	5%	MPs: 100% PC, Other: organic, mineral
Black	7/9	0%	organic
Yellow	4/4	0%	organic, mineral
Transparent	3/3	0%	mineral
Green	5/5	0%	mineral
Purple	1/1	0%	mineral
<b><i>Fibre</i></b>			
Blue	15/19	79%	MPs: 80% PA, 13% PET, 7% PP, Other: unknown
Black	16/24	42%	MPs: 50% PET, 40% PA, 10% CA, Other: organic
Transparent	8/8	25%	MPs: 100% PA, Other: organic, mineral
Yellow	3/3	33%	MPs: 100% PA, Other: unknown
Red-Pink	8/18	44%	MPs: 62.5% PA, 25% PET, 12.5% PAN, Other: unknown
<b><i>Sphere</i></b>			
Dark-Black	9/9	0%	organic
Transparent	7/7	0%	organic
Pink	4/4	0%	organic
Yellow	1/1	0%	organic
PS : Polystyrene, PA : Polyamide, PET: Polyethylene terephthalate, PC: Polycarbonate, PP: Polypropylene, PLA: Polylactic acid, PAN: Polyacrylonitrile, CA: Cellulose acetate			

895  
896  
897  
898  
899  
900  
901  
902  
903  
904  
905  
906

**Table 2.**

Sampling date	Site	Fragments/L	Fibres/L	Total/L
Sept 19	Mérignac	0.002	0.003	0.005
	Les Faulx	0.0002	0.006	0.006
Dec 19	Charente	0	0.008	0.008
	Ile de Ré	0.003	0.008	0.01

907

908

909

910

911

912

913

914

915

916

917

918

919

920

921

922

923

924

925

926

927

928

929 **Table 3.**

Sampling date	Site	Fragments/kg	Fibres/kg	Total MPs/kg
May 19	Seudre	5	0	5
Sept 19	Il de Ré	0	158	158
	Sèvre	0	0	0
	Charente	0	0	0
	Seudre	0	0	0
Jan 20	Il de Ré	0	44	44
	Sèvre	10	237	247
	Charente	175	477	652
	Seudre	850	84	934
May 20	Il de Ré	95	42	137
	Sèvre	0	242	242
	Charente	0	84	84
	Seudre	15	209	224

930

931

932

933

934

935

936

937

938

939

940

941

942

943

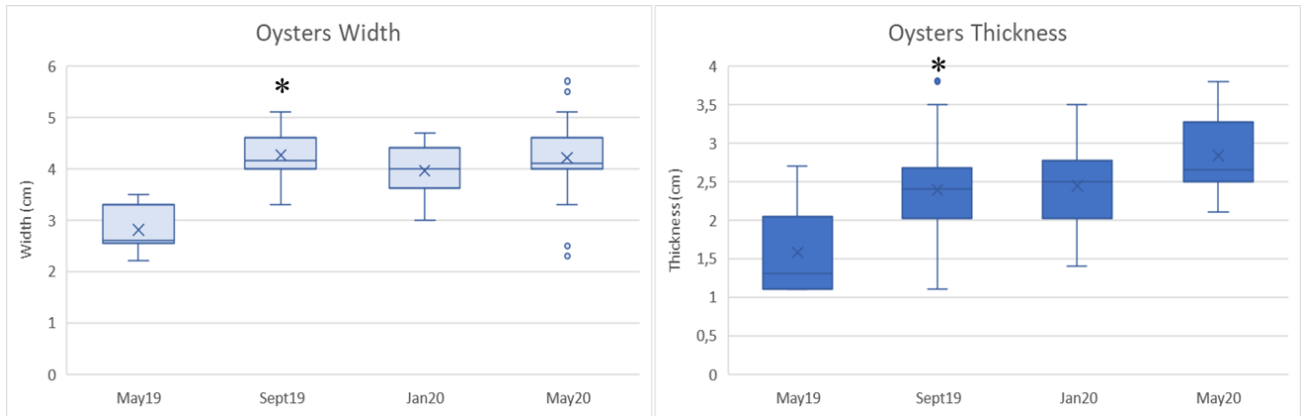
944

945

946 **Supplementary material**

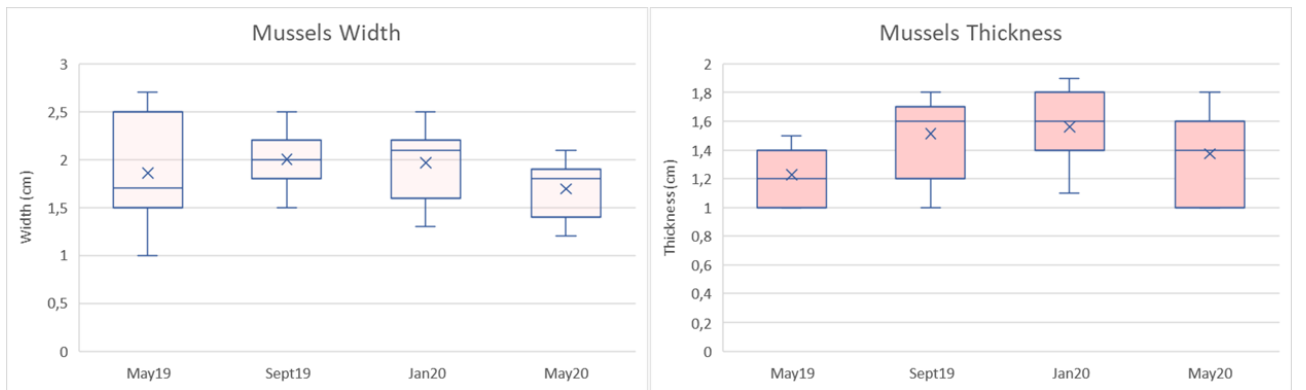
947 **Figure S1**

948 **A**



949

950 **B**



951

952

953

954

955

956

957

958

959

960

961

962

963

964

965 **Table S1.**



	2019						2020						
	May	June	July	Aug	Sept	Oct	Nov	Dec	Jan	Feb	March	April	May
<b>Ile of Ré</b>													
T (°C)	15	23		24	17	16	14	10	12	14	16	15	20
DO (%)	46	32			90	79	103	78	76	104	104	64	97
Cond (mS/cm)	58	57		59	49	47	45	43	44	43	44	46	46
pH	8,4	8,4		8,2	8,0	8,0	7,9	7,3	8,2	8,2	8,4	8,2	8,5
Turb (FNU)	56	52			9	20	61	75	11	30	83	180	63
Sal (psu)		38		39	32	31	29	27	28	29		30	30
<b>Charente</b>													
T (°C)	15			22	21		12	10	11			15	21
DO (%)	37				90	77	91	71	70	68	73	57	45
Cond (mS/cm)	46			59	49	32	22	22	35	15		43	43
pH	8,2			8,0	7,8	8,0	8,0	8,0	7,9	8,0	7,8	8,0	7,8
Turb (FNU)					500	1000	300	1000		1000		236	215
Sal (psu)	38			39	32							25	28
<b>Sèvre Niortaise</b>													
T (°C)			28		22	19	11		12	14	15	21	23
DO (%)			19		104	81	96		74	67	90	89	60
Cond (mS/cm)			62		51	39	36		33	18	43	42	43
pH			8,7		8,3	8,2	7,9		7,3	8,1	8,3	8,3	8,5
Turb (FNU)			50		123	76	238		140	170	47	51	339
Sal (psu)			41		34	33					28	27	28
<b>Seudre</b>													
T (°C)					19	17	11			11	13	16	18
DO (%)					93	70	90		74	83	83	48	43
Cond (mS/cm)					47	48	35		38	40	36	42	36
pH					7,8	7,9	7,9		8,0	8,0	8,0	7,9	7,8
Turb (FNU)					191	45	226		200	166	280	52	724
Sal (psu)					30	31				26		27	

966

967

968

969

970

971

972

973

974 **Table S2.**

---

	June 19 - Sept 19	Oct 19 - Jan 20	Feb 20 - May 20
Sèvre Niortaise	5 - 1.8	1.9 - 29	99 - 9.5
Charente	28 - 10	11 - 240	132 - 60
Seudre	0.6 - 0.05	0.1 - 6.6	4.9 - 2.9

---

