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

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Abstract

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

Introduction

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

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

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

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

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

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

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

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

Material and Methods

Experimental design

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

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

Environmental parameters

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

Water flow data (m³/s) from the rivers Sèvre Niortaise, Charente and Seudre were extracted from the « Service de prévision des crues du Littoral Atlantique » (http://hydro.eaufrance.fr/selection.php?bassin_hydrographique=&btnValider=Ajouter%20Recherche&cmd=filtrer&code_station=&commune=&consulte=rechercher).

MPs analyses

In order to avoid background contamination of samples, specific measures were taken and followed most of the criteria and the procedures described in Hermesen *et al.*, (2018) and Woodall *et al.*, (2015). Handlings were made under an air flow cabinet preventing MPs from depositing on samples. All the equipment used were cleaned with alcohol, the glassware always rinsed three times with MilliQ water, covered when not used. The purity of MilliQ water was controlled for the presence of MPs after filtration on a 1.6 µm porosity GF/A filter. The chemical solutions used (H₂O₂, KOH and ZnCl₂) were filtered (using the same type of filter) three times before use. Workers were wearing cotton laboratory coats and nitrile gloves. One blank sample was included in each batch of studied samples in the laboratory. Those blank samples were processed using the same treatments as studied samples for tissue digestion and MPs extraction procedures in order to examine background MPs contamination.

MPs extraction from sea water. A motor pump onboard a research vessel was used to collect seawater samples in September and December 2019 at one meter depth. 1000 L of seawater was filtered using a 40 µm mesh size net, thoroughly rinsed and particles (> 40 µm) were collected in a pre-cleaned glass bottle. Microplastics were extracted using a density separation solution (ZnCl₂, density = 1.8 g/cm³) to recover plastics with a density below that of the solution. In a separating glass vial, 250 mL of seawater sample was mixed with 50 mL ZnCl₂ and let stand for 24h as described in Kazour *et al.*, (2019). The supernatant was then filtered on a GF/A filter (90 mm diameter, 1.6 µm porosity). Each filter was kept in a glass petri dish at 4°C and packed in parafilm to avoid desiccation until subsequent analyses.

MPs extractions from sediments. Each sample was thawed and dried in a drying chamber at 40°C for 24h. The organic matter was digested using hydrogen peroxide (H₂O₂, 30%) (Prata *et al.*, 2019). 10 g of sediment was mixed with 40 mL H₂O₂, then heated at 40°C on a heating magnetic stirrer for 24h, under a fume hood. Then, MPs were extracted using density separation as described above and the supernatant was then filtered and kept in a Petri dish.

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

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

counted, classified by type (fibre, fragment or bead) and by colour, and measured using Image J software (Table 1). The lowest limit size of a MP that could be assessed for these criteria was 4 μm .

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

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

Statistical analyses

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

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

Results

Environmental parameters

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

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

Evolution of growth parameters

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

Size of MPs

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

µm, min: 6 µm), oysters (mean: 27.0 ± 7.6 µm, min: 6 µm), sediments (mean: 35.1 ± 22.9 µm, min: 8 µm) and seawater (mean: 31.6 ± 25.7 µm, min: 4 µm) ($p > 0.05$). Contrarily to the fragments, the mean size of fibres was higher in the sediments (mean: 408.0 ± 407.1 µm, min: 57 µm) than in oysters (mean: 288.2 ± 463.5 µm, min: 12 µm) and the seawater (mean: 236.5 ± 293.5 µm, min: 23 µm) ($p < 0.05$). The size of fibres in oysters and seawater were similar to the size measured in mussels (mean: 287.1 ± 329.5 µm, min: 22 µm) ($p > 0.05$). No difference was observed between the size of fibres in mussels and in the sediments ($p > 0.05$).

Analyses of MPs polymers by spectroscopy

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

Abundance of MPs

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

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

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

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

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

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

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

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

Distribution of MPs colours

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

Discussion

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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Supplementary material.

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

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

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

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

Table S2. Water flow (m³/s) recorded for the Sèvre Niortaise, Charente and Seudre rivers. Data are from the « Service de prévision des crues du Littoral Atlantique » available online.

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Figure and Table Legend.

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

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

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

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

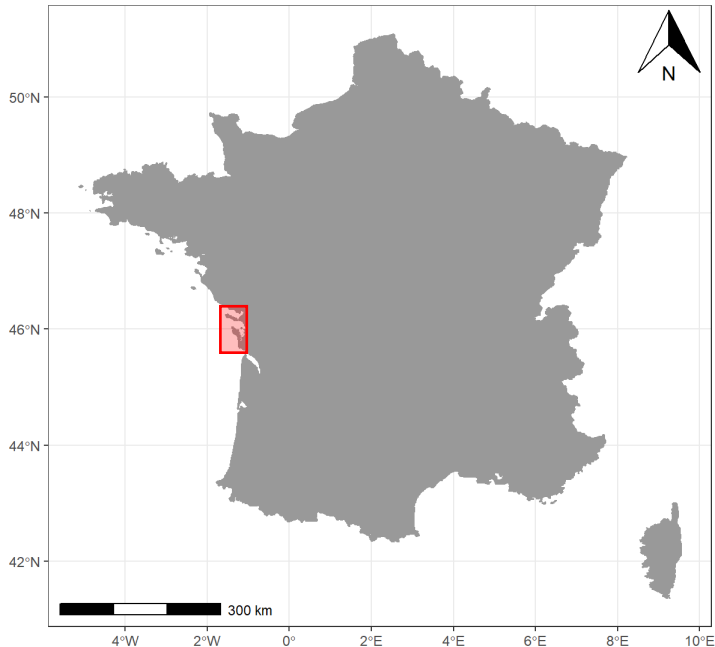
Figure 5. Length of fragments (A) and fibres (B) (μm) measured in oyster, mussel, water and sediment samples.

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

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

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

Figure 1.



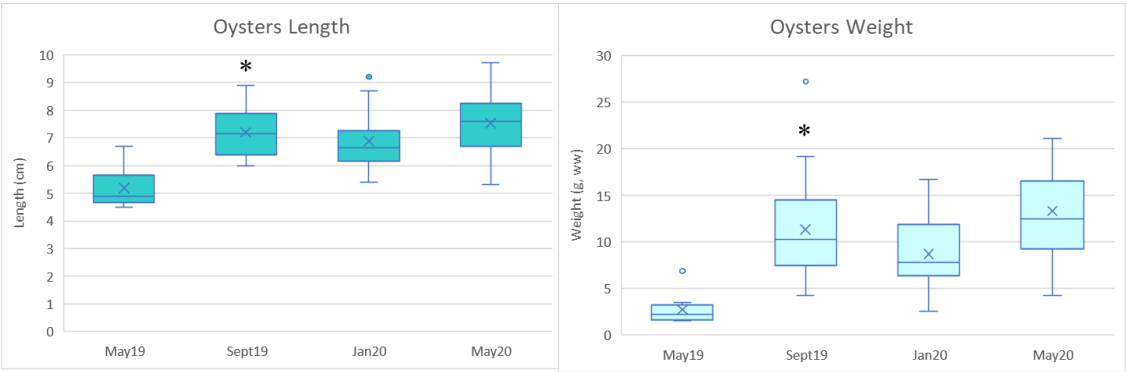
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829 **Figure 2.**

830 **A**



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832 **B**

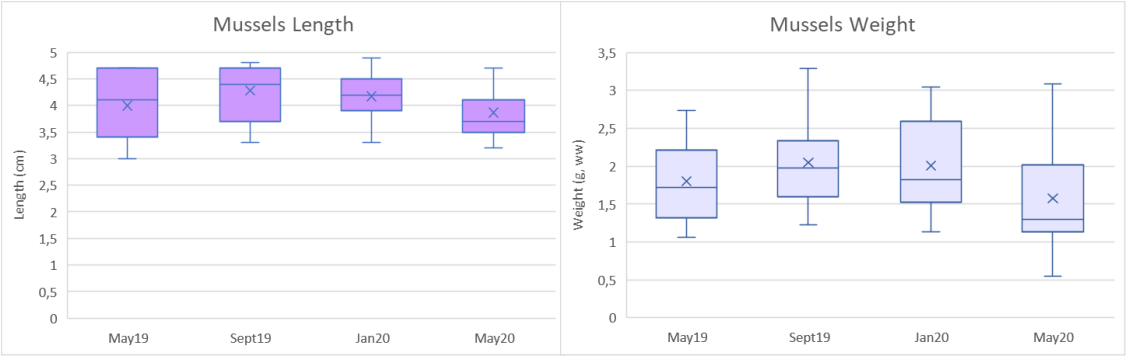


Figure 3.

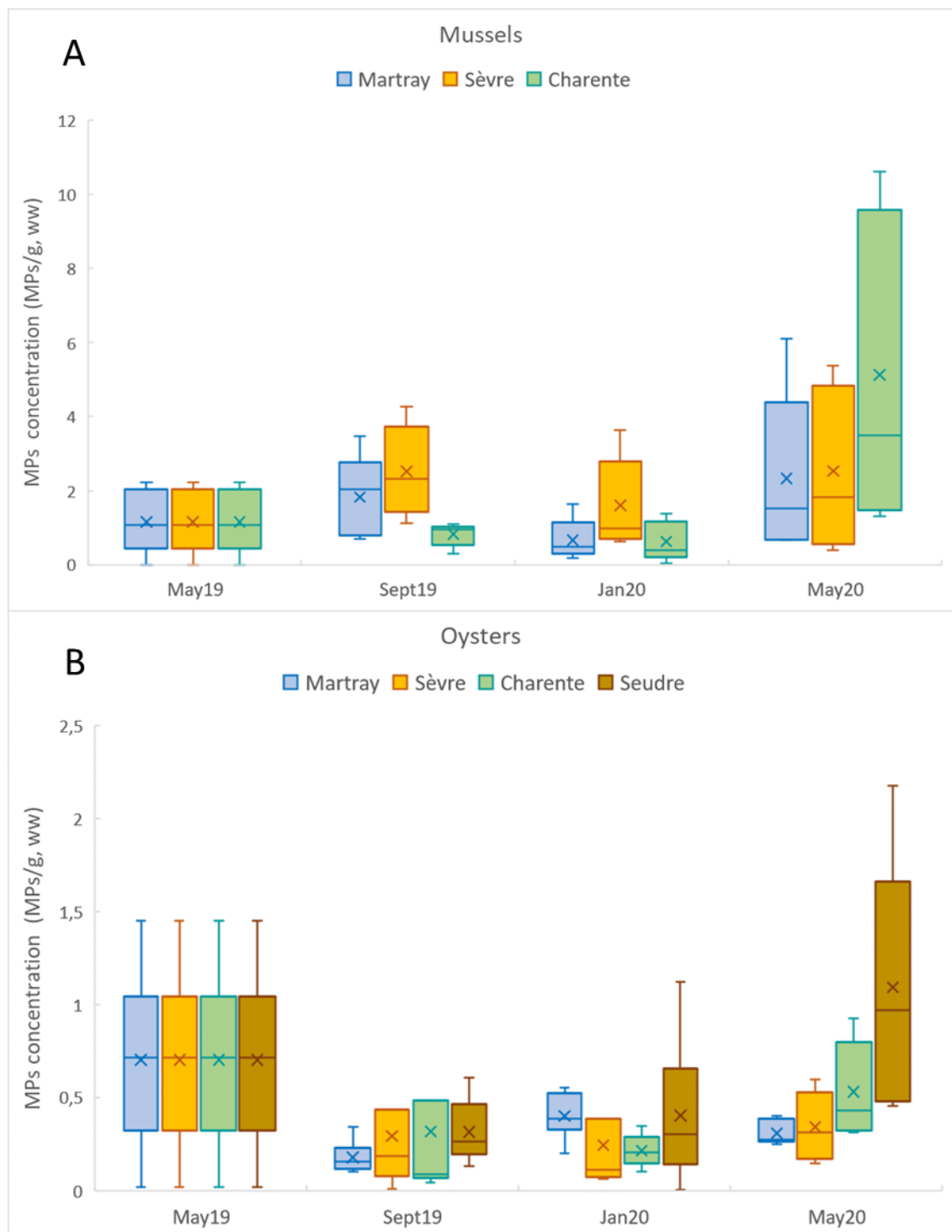


Figure 4.

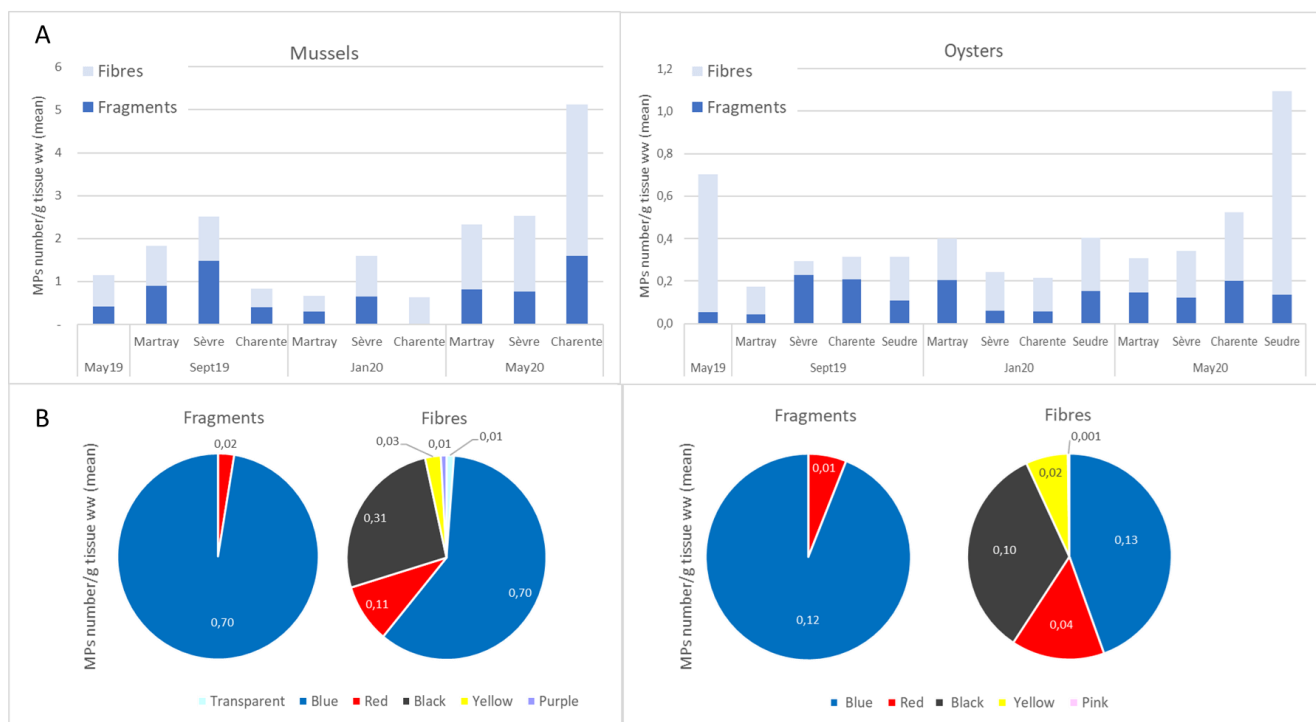
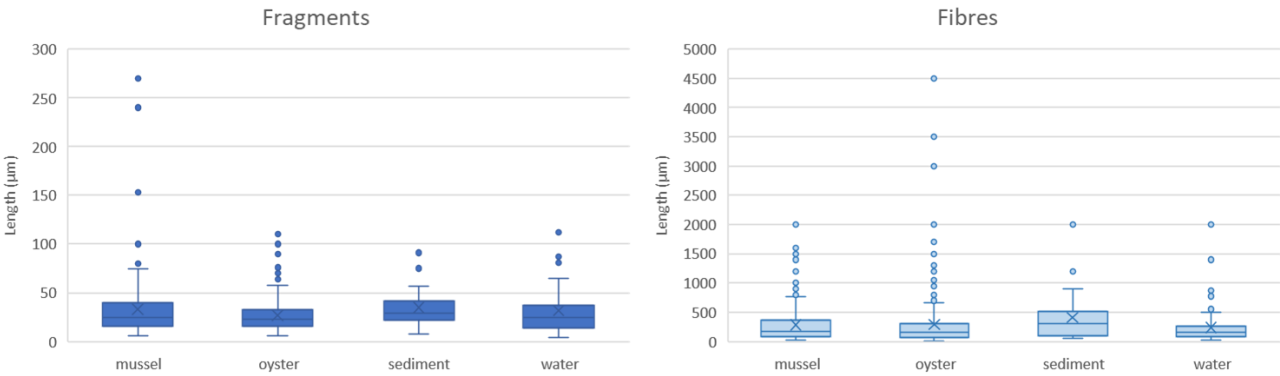


Figure 5.

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Table 1.

Particle Type	Number identified/Total	MPs (%)	Type of polymer (%) and other origin
Fragment			
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
Fibre			
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
Sphere			
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			

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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

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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

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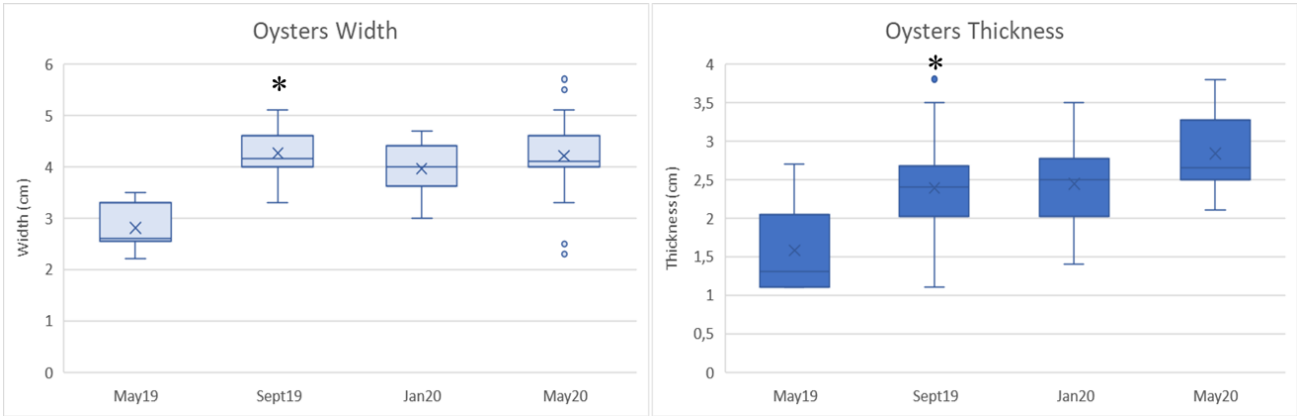
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946 **Supplementary material**

Figure S1

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B

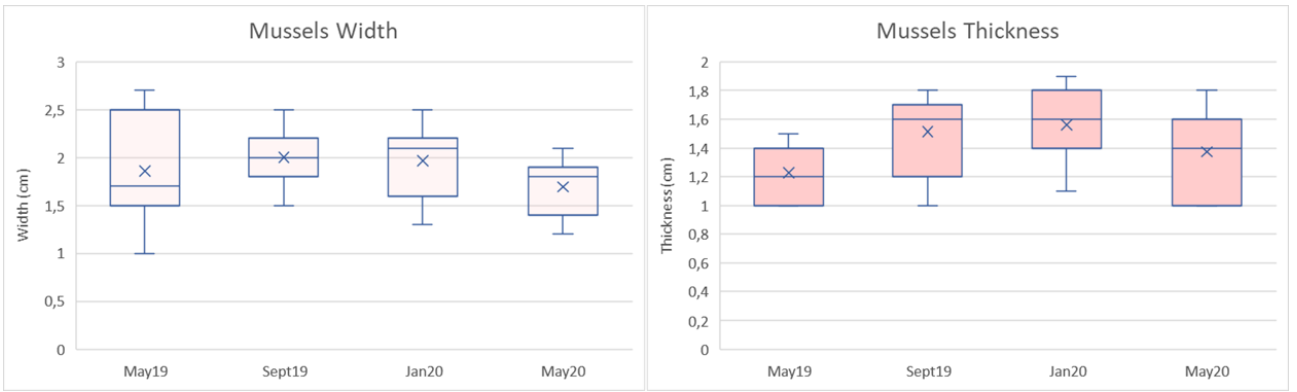


Table S1.

	2019							2020					
	May	June	July	Aug	Sept	Oct	Nov	Dec	Jan	Feb	March	April	May
Ile of Ré													
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
Charente													
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
Sèvre Niortaise													
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
Seudre													
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	

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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

