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1 **Microplastic contamination and pollutant levels in mussels and cockles**
2 **collected along the Channel coasts**

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24 **Abstract**

25 Nowadays, environmental pollution by microplastics (< 5mm; MP) is a major issue. MP are
26 contaminating marine organisms consumed by humans. This work studied MP contamination
27 in two bivalve species of commercial interest: blue mussel (*Mytilus edulis*) and common cockle
28 (*Cerastoderma edule*) sampled on the Channel coastlines (France). In parallel, 13 plastic
29 additives and 27 hydrophobic organic compounds (HOC) were quantified in bivalves flesh
30 using SBSE-TD-GS-MS/MS to explore a possible relationship between their concentrations
31 and MP contamination levels. MP were extracted using a 10% potassium hydroxide digestion
32 method then identified by μ -Raman spectroscopy. The proportion of contaminated bivalves by
33 MP ranged from 34 to 58%. Blue mussels and common cockles exhibited 0.76 ± 0.40 and 2.46
34 ± 1.16 MP/individual and between 0.15 ± 0.06 and 0.74 ± 0.35 MP/g of tissue wet weight. Some
35 HOC and plastic additives were detected in bivalves. However, no significant Pearson or
36 Spearman correlation was found between MP loads and plastic additives or HOC concentrations
37 in bivalve tissues for the two species.

38 **Keywords**

39 microplastic, mussel, cockle, plastic additive, hydrophobic organic compounds

40 **Capsule**

41 Microplastic contamination and pollutant levels of commercially important bivalves in France.

42

43 **1. Introduction**

44 Nowadays, plastic is a commonly used material with numerous benefits for everyday human
45 life (Thompson et al., 2009). To meet the growing demand, plastic production increased
46 exponentially since the 1950's from 2 million metric tons produced in 1950 to 381 million
47 metric tons in 2015 (Geyer et al., 2017). Simultaneously, plastics tend to accumulate in natural
48 environments due to their durability, resistance and trash mismanagement (Barnes, 2002;
49 Horton et al., 2017).

50 Small plastic particles, called microplastics (MP; <5 mm) (Arthur et al., 2009) are ubiquitous
51 in the marine environment (Li et al., 2016b) and are found in beach sediment (Claessens et al.,
52 2011; Dekiff et al., 2014) and in the water column (Collignon et al., 2012; Desforges et al.,
53 2014; Lattin et al., 2004). Coastal environments are also subjected to MP pollution. Indeed, as
54 these environments are interfaces between land and sea, pollution can either originate from
55 terrestrial or marine origins. Terrestrial activities are responsible on average for 80% of plastic
56 load in the Oceans (Andrady, 2011) but with high variability along the coasts (Filella, 2015).
57 Several studies found MP in coastal environments (Li et al., 2016b; Naidoo et al., 2015; Nel
58 and Froneman, 2015; Ng and Obbard, 2006) with the highest levels up to 16,272 MP/m³ in
59 coastal waters around Geoje Island, South Korea (Song et al., 2014) and up to 8,720 MP/kg
60 (dry weight) of beach sediment in Wanning, China (Qiu et al., 2015).

61 Numerous marine species are known to ingest MP (Lusher, 2015) including coastal species
62 harvested or cultivated for human consumption. Due to their commercial interest and the fact
63 that the whole animal is eaten by consumers, contaminations of bivalves are a major subject of
64 concern for food safety and human health. Their feeding mode directly exposes bivalves to
65 pollutants such as MP present in their surrounding environment. Consequently bivalves,
66 especially mussels (*Mytilus* spp.), are commonly used as a sentinel organism to monitor
67 anthropogenic pollution in marine coastal environments (Goldberg, 1975; Li et al., 2019).

68 Indeed, ingestion of MP has been demonstrated *in situ* in numerous bivalves species including
69 mussels (*Mytilus edulis* and *Mytilus galloprovincialis*), oysters (*Crassostrea gigas*) or clams
70 (*Venerupis philippinarum*) (Davidson and Dudas, 2016; Phuong et al., 2018; Van
71 Cauwenberghe and Janssen, 2014; Vandermeersch et al., 2015). For example Van
72 Cauwenberghe and Janssen (2014) reported up to 0.36 MP/g of tissue wet weight (ww) in
73 mussels collected on German coasts whereas Phuong et al., (2018) reported 0.23 MP/g of tissue
74 ww in mussels sampled on the French Atlantic coast. In laboratory experiments, uptake of MP
75 resulted in different side effects on bivalves physiology (Browne et al., 2008; Cole and
76 Galloway, 2015; Paul-Pont et al., 2016; Sussarellu et al., 2016; von Moos et al., 2012; Wegner
77 et al., 2012). For example, exposition of oysters (*C. gigas*) to polystyrene microspheres
78 modified their feeding capacity and affected reproductive outputs (Sussarellu et al., 2016).
79 Apart from the physical injuries caused by MP, their ingestion could also be associated with
80 the release of hydrophobic organic compounds (HOC) or plastic additives (Hermabessiere et
81 al., 2017). Some studies proposed the use of several chemicals as proxies of plastic ingestion
82 including polychlorinated biphenyls (PCB) (Teuten et al., 2009), di(2-ethylexyl) phthalate
83 (DEHP) (Fossi et al., 2014, 2012) or polybrominated diphenyl ethers (PBDE) (Tanaka et al.,
84 2013).

85 In the present study, two species of bivalves, the blue mussel (*M. edulis*) and the common
86 cockle (*Cerastoderma edule*) were chosen to study MP contamination. These species live in
87 different habitats and are both commonly found on French coasts. Cockles live at the interface
88 between sediment and water where higher contamination is expected (Besseling et al., 2014)
89 whereas mussels live in the water column on rocks or on lines and piling in aquaculture. In
90 addition, both species are commercially important seafood products. France is one of the top
91 producers of mussels (*Mytilus* spp.) in Europe with 47,394 tons produced in 2016 (FAO, 2018)
92 and 1,890 tons of cockle were produced in France in 2016 (FAO, 2018). Globally, in Europe,

93 mussels are one of the most consumed seafood products with an apparent consumption of 1.33
94 kg/capita in 2015 (European Commission, 2018).

95 The aims of this work were (i) to quantify MP content in two common commercial bivalves
96 species, the blue mussel and the common cockle, sampled along the Channel coast of France,
97 (ii) to quantify HOC and plastic additives in the bivalves tissues and (iii) to explore relationships
98 between MP loads in bivalves and HOC and plastic additive tissue concentrations.

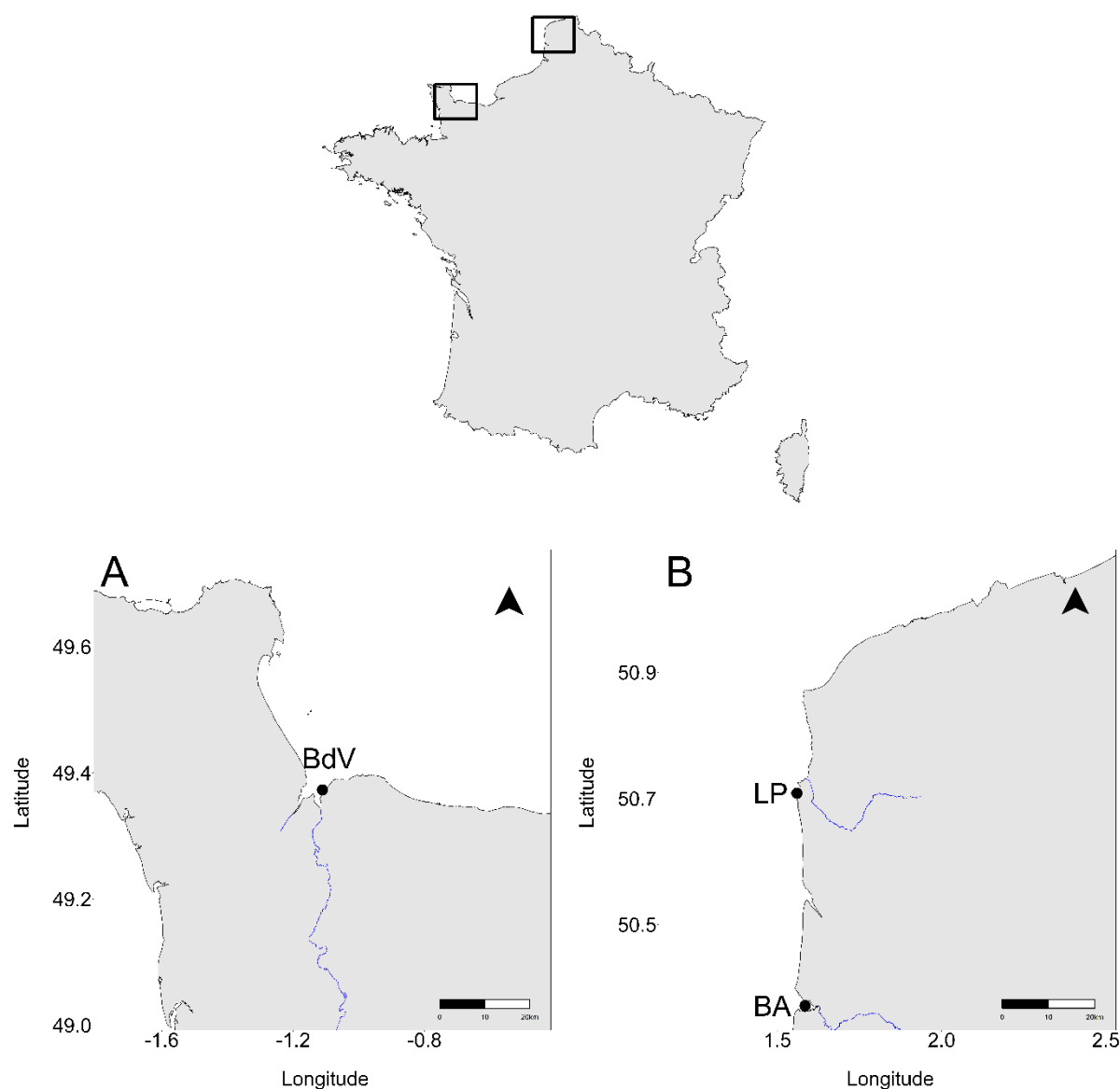
99 **2. Material and methods**

100 **2.1. Sampling**

101 Sampling sites were located along the Channel coasts (Fig. 1) which exhibit the most important
102 tide system in Europe associated with strong currents (Salomon and Breton, 1993). The Baie
103 des Veys (BdV) (Fig. 1A) is an estuarine bay under the influence of two rivers: the Taute and
104 the Vire with a total mean discharge of 53 m³/s. The intertidal part of the bay supports intensive
105 oyster farming with around 10,500 tons (Grangeré et al., 2009). The BdV also supports mussel
106 farming and professional fishing of *C. edule*. Moreover, this bay could also be influenced by
107 the Seine flow depending on meteorological conditions (Ellien et al., 2004). The Baie d'Authie
108 (BA) (Fig. 1B) is a small estuary influenced by the river Authie which has a mean flow of 9
109 m³/s mainly influenced by agricultural activities (Billon et al., 2001; Gillet et al., 2008). Finally,
110 Le Portel (LP) (Fig. 1B) beach is not influenced by any river but is located in an area under the
111 influence of 116,000 inhabitants in 2014 (INSEE, 2015). Overall, BdV and BA are small
112 estuaries with small influence of human activities whereas LP is located in a relatively high
113 populated area.

114 Mussels (n=50) were collected at LP (50°42'30.02"N, 1°33'34.43"E) on Oct 29th, 2015.
115 Mussels (n=50) and cockles (n=50) were then sampled at the BdV (49°22'23.4"N,
116 1°06'40.0"W) on Nov 1st, 2015. Finally, cockles (n=50) were sampled at the BA

117 (50°22'17.22"N, 1°35'4.8"E) on Nov 15th, 2015. In total, 100 mussels and 100 cockles were
118 collected. After field sampling, the shell was cleaned in the laboratory with filtered bidistilled
119 water and length was measured for all individuals. Then bivalves were shelled and their soft
120 tissue wet weights (ww) were recorded. Samples were wrapped in a piece of paper then in
121 aluminum foil and stored at -20°C before subsequent analysis. Atmospheric blank was
122 performed during opening and weighting (see 2.2.1).



123

124 **Fig 1: Blue mussel and common cockle sampling locations along the French coasts of the Channel in Normandy (A) and**
125 **in Hauts-de-France (B). BA: Baie d'Authie, BdV: Baie des Veys and LP: Le Portel.**

126 **2.2. Microplastics analyses**

127 **2.2.1. Prevention of procedural contamination**

128 To avoid overestimation of the MP concentration in bivalves due to airborne, container, and
129 tool contamination, preventative measures were applied. All used materials were made of glass.
130 All solutions: distilled water, 70% (v/v) ethanol, 10% potassium hydroxide (KOH) were filtered
131 through a 90 mm diameter GF/A 1.6 μm glass fiber filters (Whatman, Velizy-Villacoublay,
132 France) until no particle was found on the filter. Moreover, all glassware, tools and bench
133 surfaces were rinsed with filtered distilled water, filtered 70% ethanol then by filtered distilled
134 water before being used. Glass fiber filters were verified under a stereomicroscope to ensure
135 the absence of particle before being used.

136 Atmospheric blanks were performed at every step of the work: dissection, digestion and
137 filtration using glass petri dishes open to the environment during procedures. Furthermore, for
138 each digestion batch, a procedural blank made of only 10% KOH followed the same treatment
139 as the bivalve samples. Digestion and filtration were performed in a fume hood specifically
140 dedicated to MP analyses, with a switched-off aspiration system, to prevent contamination with
141 airborne particles from the ambient air. Finally, operators did not wear gloves and synthetic
142 clothing to limit contamination due to fixed airborne particles and they wore lab coats made of
143 cotton.

144 The used μ -Raman spectroscopy method did not allow the polymer identification of fibers
145 because these particles are too thin (Käppler et al., 2016). Thus, fibers were only counted. In
146 addition, in order to account for airborne contamination, whenever a fiber was found in a blank
147 (atmospheric or procedural), it was subtracted from the final result if a fiber of the same type
148 (*i.e.* color and shape) was found in the sample. Results for fiber counts without subtraction of
149 blank are available in Supplementary Table 1. Some particles were classified as pigment

150 containing particles as no confirmation of polymeric composition could be made. Together with
151 fibers, pigment containing particles were not considered in MP contamination results.

152 **2.2.2. Tissue digestion and filtration**

153 Digestion of mussels and cockles were performed according to Dehaut et al., (2016). After
154 thawing, individuals were placed in 300 mL Erlenmeyer flasks then 10% KOH was added. For
155 bivalves from LP and BA, 100-250 mL of 10% (w/w) KOH were added (ChimiePlus, Saint
156 Paul de Varax, France) and then samples were placed 24 h in an incubator (Binder BD 240,
157 Tuttlingen, Germany) set at $60 \pm 1^\circ\text{C}$ with agitation set at 200-300 rpm (IKA KS250, Staufen,
158 Germany or 2mag MIXDrive 6 HT, Munich, Germany). For bivalves sampled at BdV, a
159 solution of 10% KOH (w/v) was prepared using KOH pellets (Sigma-Aldrich, Saint-Quentin-
160 Fallavier, France) and distilled water. Bivalves were put in 250 mL of prepared 10% KOH then
161 placed on an agitation plate (IKA RT15, Staufen, Germany) set at 300 rpm and $60 \pm 1^\circ\text{C}$ for 24
162 h. After digestion, all samples were filtered on clean 90 mm diameter GF/A 1.6 μm glass fiber
163 filters (Whatman, Velizy-Villacoublay, France) using a vacuum system. Filters were then placed
164 in closed Petri dishes until subsequent analysis. Procedural blank was performed at the same
165 time as manipulating mussel and cockle soft tissue (see 2.2.1).

166 Except for mussels from LP due to a handling issue, two pools of 100 mL, of bivalve digestates
167 by species and location, obtained with 10 mL belonging to 10 different individuals were
168 prepared and conserved at -20°C until further analyses of HOC and plastic additives.

169 **2.2.3. Visual sorting and μ -Raman analysis**

170 Filters were observed under a Zeiss Stemi 508 stereomicroscope (Zeiss, Marly le Roi, France).
171 Particles resembling MP (MP-like) were counted and characterized according to their shape
172 (fragment, fiber, bead, foam or pellet) and color (Lusher et al., 2017). Colors were attributed
173 by a unique operator in order to allow comparisons. MP-like were then isolated for subsequent
174 μ -Raman spectroscopy analysis. Pictures of MP-like were taken and sizes were measured in

175 pixel using GIMP 2 software (2.8.16). The maximum length in μm of MP-like particles was
176 calculated using a scale bar. No particle smaller than 15 μm was observed on filters. Particles
177 were thus categorized depending on their maximum size according to the following class sizes:
178 15-50, 50-100, 100-500 and >500 μm .

179 μ -Raman analyses were conducted according to Frère et al., (2016). Briefly, all MP-like were
180 analyzed with a LabRam HR800 (HORIBA Scientific, Villeneuve d'Ascq, France) using laser
181 wavelength set at 785 nm (Laser diode, Oxxius, Lannion, France) or 514 nm (Ar Laser, Melles
182 Griot, Bensheim, Germany). A laser wavelength of 785 nm was first attempted and if
183 identification was not conclusive, acquisition with laser wavelength set at 514 nm was carried
184 out. Experimental conditions for μ -Raman analyses - integration time, accumulation, laser
185 power and wavelength - were set to limit fluorescence and increase the spectral quality of the
186 analyzed particles. Particles identifications were performed by comparing acquired spectra to
187 reference spectra to home-made database including the following reference polymers: Low
188 Density Polyethylene (LDPE), High Density Polyethylene (HDPE), Polypropylene (PP),
189 Polystyrene (PS), Unplasticized Polyvinyl Chloride (uPVC), Polyethylene Terephthalate
190 (PET), Polyamide-6 (PA-6), Polyamide-12 (PA-12), Polytetrafluoroethylene (PTFE),
191 Polymethylmethacrylate (PMMA), Acrylonitrile-Butadiene-Styrene (ABS), Polyurethane
192 (PUR) acquired from GoodFellow (Lille, France). Then, downstream, chemometric analyses
193 were carried out in order to obtain a better identification for previously unidentified particles
194 (Batzan et al., 2018) (Supplementary Table 2). Identification was established based on the
195 similarity percentage (minimum value of 70%) between particles and reference spectra. In
196 addition, spectra with no identification in the home-made database were compared to spectra
197 described by Socrates (2004).

198 **2.3. Analyses of hydrophobic organic compounds and plastic additives**199 **2.3.1. Target chemicals**

200 Five groups of chemicals were analyzed including 15 polycyclic aromatic hydrocarbons (PAH),
 201 6 polychlorinated biphenyls (PCB), 6 organochloride pesticides (OCP), 6 phthalates and 7
 202 PBDE (Table 1).

203 **Table 1: List of the analyzed chemicals in digestates of cockles from Baie d'Authie (BA), cockles and mussels from Baie**
 204 **des Veys (BdV).**

Chemical family	Target chemical or congener	PubChem ID
PAH	Naphtalene	931
	Benzothiophene	7221
	Biphenyl	7095
	Acenaphtylene	9161
	Acenaphtene	6734
	Fluorene	6853
	Dibenzothiophene	3023
	Benzo(g,h,i)perylene	9117
	Phenanthrene	995
	Anthracene	8418
	Fluoranthene	9154
	Pyrene	31423
	Benzo(a)anthracene	5954
	Chrysene	9171
Benzo(b+k)fluoranthene	9158	
PCB	PCB-7	36399
	PCB-28	23448
	PCB-52	37248
	PCB-105	36188
	PCB-156	38019
	PCB-169	23448
OCP	Hexachlorobenzene	8370
	Aldrin	101611446
	Isodrin	10066
	Dieldrin	3048
	2,4-DDE ^a	246598
	Endrin	12358480
PBDE	BDE-28	154083
	BDE-47	95170
	BDE-99	36159
	BDE-100	154083
	BDE-153	155166
	BDE-154	15509898
	BDE-183	15509899

Phthalate	BBP ^b	2347
	DEHP ^c	8343
	DEP ^d	6781
	DMP ^e	8554
	DnBP ^f	3026
	DEHA ^g	7641
^a 2-(2-Chlorophenyl)-2-(4-chlorophenyl)-1,1-dichloroethene ^b butyl benzyl phthalate ^c di(2-ethylexyl) phthalate ^d diethyl phthalate ^e dimethyl phthalate ^f di-n-butyl phthalate ^g Diethylhexyl adipate		

205

206 2.3.2. Chemical analyses

207 Analytes were directly extracted then analyzed from the digestate pool using stir bar sorptive
208 extraction-thermal desorption-gas chromatography-tandem mass spectrometry (SBSE-TD-
209 GC-MS/MS) (Lacroix et al., 2014). Briefly, a polydimethylsiloxane stir bar (Twister 20
210 mm×0.5 mm, Gerstel, Mülheim an der Ruhr, Germany) was placed in the 100 mL digestate and
211 extractions were carried out on a magnetic laboratory agitator (MIX15, Munich, Germany) set
212 at 700 rpm for 16 h in the dark at room temperature. After the extraction step, stir-bars were
213 retrieved, rinsed with Evian® water and placed on a gas chromatography system Agilent 7890A
214 coupled with an Agilent 7000 triple quadrupole mass spectrometer (Agilent Technologies,
215 Little Falls, USA) and equipped with a Thermal Desorption Unit (TDU) combined with a
216 Cooled Injection System (CIS) (Gerstel, Mülheim an der Ruhr, Germany). Thermodesorption
217 was performed at 280°C for 6 min and samples were then cryofocused in the CIS at -10°C.
218 Injection in the GC-MS/MS system was carried out in splitless mode and the CIS was heated
219 to 300°C at 12°C/s. The GC temperature program was set as follows: 70°C for 0.5 min, then
220 increase to 150°C at 20°C/min and finally increase to 300°C at 7°C/min, maintained for 5 min.
221 A Rxi-5MS (30 m, 0.25 mm, 25 µm thickness) (Restek, Lisses, France) capillary column was
222 used. Helium was used as a carrier gas with a constant flow rate of 1 mL/min.

223 Limits of quantification (LOQ) were calculated according to Shrivastava and Gupta (2011)
224 using the calibrations curves method. Limits of detection (LOD) were calculated by dividing
225 the LOQ by 3.3. Calibration curves were drawn using mussel tissues digested in 10% KOH for
226 3 h at 80°C added with standard chemicals. Analytes were quantified relatively to deuterated
227 compounds using a calibration curve ranging from 0.01 ng to 10 ng. For PAH, PCB and OCP
228 quantification naphthalene d8, biphenyl d10, phenanthrene d10, pyrene d10, chrysene d12,
229 benzo(a)pyrene d12, benzo(g,h,i)perylene d12 were used as standards. For the plastic additives,
230 phthalates and PBDE, di (2-ethylhexyl) phthalate d4 and BDE 77 were respectively used as
231 standards. All standards were obtained from LGC Standard (Wesel, Germany) and Interchim
232 (Montluçon, France).

233 **2.4. Statistical analyses**

234 All statistical analyses were performed using R Statistical Software version 3.4.0 (R Core Team,
235 2015). As hypothesis of residuals normality, tested using Shapiro-Wilk test, and
236 homoscedasticity, tested on regression residues, were not verified, non-parametric Kruskal-
237 Wallis tests were performed instead of ANOVA. When significant differences were
238 highlighted, a post-hoc test using the Fisher's least significant difference (LSD) criterion and
239 Bonferroni correction was applied using the *agricolae* package (1.2-7) (De Mendiburu, 2014).
240 Microplastic color, sizes classes and polymer composition were compared using Chi-Square
241 test. To perform Chi-square test, data were summed to meet application requirements (Cochran,
242 1952). Relationships between MP or anthropogenic particle (AP) load and HOC or plastic
243 additive concentration in bivalves were assessed using correlation test (Pearson or Spearman)
244 with the *corrplot* package (0.84) (Wei et al., 2017). Differences were considered significant
245 when p-value was below 0.05.

246 Results are expressed as a mean \pm 2 standard error (S.E.), representing the 95% confidence
247 interval (95% CI). Contamination results were expressed as percentage of contaminated

248 individuals, mean particles/individuals and mean particles/g of tissue ww. Results were given
249 for contamination by MP, fiber, pigment containing particles (PCP) and all categories
250 (MP+fiber+PCP).

251 **3. Results and discussion**

252 **3.1. Biometric parameters**

253 Mussels from LP and BdV measured 47.3 ± 1.2 mm and 47.9 ± 1.5 mm and weighted 3.5 ± 0.5
254 g (soft tissue wet weight) and 5.7 ± 0.6 g, respectively. Cockles from BA and BdV measured
255 35.2 ± 0.4 mm and 27.3 ± 0.5 mm and weighted 3.2 ± 0.1 g and 3.0 ± 0.2 g, respectively. On
256 average, cockles and mussels measured legal market sizes: 27 mm and 30 mm for cockles for
257 professional and recreational fishing respectively and 40 mm for mussels for recreational and
258 professional fishing (LegiFrance, 2018; Préfecture de Haute Normandie, 2015a, 2015b;
259 Préfecture de Normandie, 2016).

260 **3.2. Microplastics in mussels and cockles**

261 Overall, 1636 particles were visually isolated and sorted from the 200 sampled bivalves of BA,
262 LP and BdV. Among them, 324 were fibers varying from 2.4 to 50.2% of particles according
263 to sites and species. A total of 1312 particles (80%) were analyzed with μ -Raman spectroscopy
264 and identified as MP, PCP, natural particles, or unidentified (Table 2). The identified particles
265 correspond to MP (5 to 32.8%), PCP (0 to 2.5%), and natural particles (6.6 to 21.5%) (Table
266 2). Unidentified particles with μ -Raman account for 27 to 60.9% of analyzed particles (Table
267 2). Absence of identification was due to absence of peak in particles spectra, saturated signal
268 due to high fluorescence or mismatch with databases. Overall, natural particles (6.6 to 21.5%)
269 were mainly composed of minerals (exclusively quartz), organic and inorganic carbon
270 corresponding to sand or shell particles (Table 2). The majority of PCP contained a blue

271 pigment known as the phthalocyanine blue 15 (PB15). As this pigment is used in the plastic
 272 industry, it can be attributable to plastic (Lewis, 2005). Such PCP were also found in mussels
 273 sampled in Germany (Van Cauwenberghe and Janssen, 2014). Nevertheless, these could not be
 274 rigorously considered as MP since these PCP could also be paint particles, as demonstrated by
 275 Imhof et al. (2016).

276 **Table 2. Particles analyzed by μ -Raman spectroscopy and fibers visually isolated and sorted from mussels and cockles.**

Species	Sampling site ¹	Fiber ³	Unidentified particles ⁴	Identified ⁴			Total
				Natural particles ⁵	Microplastics	PCP ⁶	
Mussel	LP ²	77 <i>10.1%</i>	465 <i>60.9%</i>	164 <i>21.5%</i>	38 <i>5.0%</i>	19 <i>2.5%</i>	763 <i>100%</i>
	BdV ²	121 <i>50.2%</i>	65 <i>27.0%</i>	16 <i>6.6%</i>	39 <i>16.2%</i>	0 <i>0%</i>	241 <i>100%</i>
Cockle	BA ²	9 <i>2.4%</i>	169 <i>45.1%</i>	65 <i>17.3%</i>	123 <i>32.8%</i>	9 <i>2.4%</i>	375 <i>100%</i>
	BdV ²	117 <i>45.5%</i>	73 <i>28.4%</i>	39 <i>15.2%</i>	28 <i>10.9%</i>	0 <i>0%</i>	257 <i>100%</i>

¹ LP: Le Portel; BdV: Baie des Veys; BA: Baie d'Authie

² Results are expressed in terms of absolute number of particles and relative proportion of each items among the total count by line (in italic)

³ Not analyzed by μ -Raman

⁴ Analyzed by μ -Raman

⁵ Natural particle included minerals, organic and inorganic carbon.

⁶ Pigment containing particles

277

278 For mussels sampled at LP, MP were identified in 17 mussels (34%) whereas 23 mussels (46%)
 279 from BdV were contaminated by MP. Concerning cockles from BA, 29 (58%) individuals were
 280 contaminated by MP whereas 21 cockles (42%) from BdV were contaminated (Table 3). The
 281 presence of MP in cockles and mussels from the four sites varied from 0.56 ± 0.22
 282 MP/individual, namely 0.19 ± 0.08 MP/g of tissue wet weight (ww) for cockle from BdV to
 283 2.46 ± 1.16 MP/individual, namely 0.74 ± 0.35 MP/g of tissue ww for cockle from BA (Table
 284 3). MP contamination in mussels and cockles was significantly different according to location
 285 and species (Kruskal-Wallis, $p < 0.05$). Cockles sampled at BA were more contaminated by MP
 286 (Post-hoc after Kruskal-Wallis, $p < 0.05$) in comparison with mussels sampled at LP and cockles

287 sampled at BdV. These differences were both observed for results expressed as MP/individual
 288 and MP/g of tissue ww (Table 3). Higher contamination by MP in cockles sampled at BA could
 289 be due to their position in the water column. Indeed, as suggested by Besseling et al. (2014),
 290 MP concentration in sediment is expected to be higher in comparison with the water column.
 291 However, as cockles from BdV are less contaminated by MP in comparison with cockles from
 292 BA, plastic local sources may, in the present study, be a major source of contamination.
 293 However, to date, no study has been carried out to describe MP contamination at these sampling
 294 sites; consequently, it is difficult to clearly relate MP loads to the presence of MP in water or
 295 sediment of sites where bivalves were collected.

296 **Table 3. Microplastics (MP), fibers, pigmented particles and total (MP+fiber+pigment) contamination of mussels and**
 297 **cockles sampled at Le Portel (LP), Baie d'Authie (BA) and Baie des Veys (BdV).**

	Mussel		Cockle	
	LP	BdV	BA	BdV
% of contaminated individual by MP¹	34 %	46%	58 %	42 %
MP/individual^{1, 2, 3}	0.76 ± 0.40 ^a	0.78 ± 0.30 ^{a b}	2.46 ± 1.16 ^b	0.56 ± 0.22 ^a
MP/g of tissue ww^{1, 2, 3}	0.25 ± 0.16 ^a	0.15 ± 0.06 ^{a b}	0.74 ± 0.35 ^b	0.19 ± 0.08 ^a
% of contaminated individual by fiber	40 %	80 %	16 %	80 %
Fiber/individual^{2, 3}	1.54 ± 1.2 ^a	2.42 ± 0.55 ^b	0.18 ± 0.12 ^c	2.34 ± 0.77 ^b
Fiber/g of tissue ww^{2, 3}	0.49 ± 0.42 ^a	0.44 ± 0.1 ^b	0.06 ± 0.04 ^c	0.82 ± 0.28 ^b
% of contaminated individual by PCP^{1, 4}	24 %	0 %	8 %	0 %
PCP/individual^{1, 2, 3, 4}	0.38 ± 0.23 ^a	0 ^b	0.18 ± 0.2 ^b	0 ^b
PCP/g of tissue ww^{1, 2, 3, 4}	0.12 ± 0.07 ^a	0 ^b	0.06 ± 0.06 ^b	0 ^b
% of contaminated individual by all categories	68 %	86 %	72 %	86 %
Total/individual^{2, 3}	2.68 ± 1.33 ^a	3.20 ± 0.60 ^b	2.82 ± 1.14 ^{a b}	2.90 ± 0.77 ^{a b}
Total/g of tissue ww^{2, 3}	0.86 ± 0.47	0.59 ± 0.12	0.86 ± 0.34	1.02 ± 0.28

¹ Particles identification as MP and pigmented particles were obtained after μ -Raman spectroscopy.

² Results expressed as the mean \pm 2 S.E (95% confidence interval).

³ Superscript letters correspond to significant differences (per lines) after a Kruskal Wallis post-hoc test using the Fisher's least significant difference and Bonferroni correction ($p < 0.05$).

⁴ Pigment containing particles

298

299 In the present study, quantities of MP, *stricto sensu*, recorded for the bivalves along French
300 coasts of the Channel were in accordance with studies from other European coasts (Table 4)
301 where contamination in bivalve varied from 0.04 ± 0.09 MP/g of tissue ww in Mediterranean
302 blue mussels (*M. galloprovincialis*) sampled at Erbo Delta (Spain) to 4.44 MP/g of tissue ww
303 for blue mussels (*M. edulis*) sampled at Oban (Scotland) (Table 4). In this work and others
304 carried out in Europe, MP contamination appeared to be more influenced by location than by
305 species even though the number of investigated species is limited. However, MP contamination
306 in the two bivalves species is lower than contaminations recorded in bivalves sampled along
307 Chinese coasts (Li et al., 2016a; Li et al., 2015) (Table 4). Mussels along the Chinese coasts
308 were contaminated by 0.9 to 4.6 MP/g (Li et al., 2016a). These differences are likely due to MP
309 contamination level of the studied sites. Indeed, Chinese environments were reported to be
310 highly contaminated by MP and other plastic debris (Cai et al., 2018).

311 **Table 4. Overview of microplastic contaminations in bivalves.**

Species	Individuals	MP/individual	Isolation procedure	Filter pore size (in μm)	Polymer chemical identification	Sampling location	Reference
<i>Mytilus galloprovincialis</i>	100	0.34 \pm 0.33 p/g	HNO ₃ :HClO ₄	13	Hot Needle	Tagus estuary, Portugal	[5]
<i>Mytilus galloprovincialis</i>	100	0.05 \pm 0.11 p/g	HNO ₃ :HClO ₄	13	Hot Needle	Po estuary, Italy	[5]
<i>Mytilus galloprovincialis</i>	25	0.15 \pm 0.33 p/g	HNO ₃ :HClO ₄	13	Hot Needle	Fangar Bay, Spain	[5]
<i>Mytilus galloprovincialis</i> ^a	25	0.25 \pm 0.26 p/g	HNO ₃ :HClO ₄	13	Hot Needle	Coro, Italy	[5]
<i>Mytilus galloprovincialis</i> ^a	100	0.04 \pm 0.09 p/g	HNO ₃ :HClO ₄	13	Hot Needle	Ebro delta, Spain	[5]
<i>Mytilus galloprovincialis</i> ^a	18	4.3 – 57.2 (2.1 – 10.5 p/g)	H ₂ O ₂	5	FTIR	Chinese coasts	[8]
<i>Mytilus galloprovincialis</i> ^b	80	1.9 \pm 0.2	H ₂ O ₂	1.2	FTIR	Ionian Sea, Greece	[14]
<i>Mytilus edulis</i>	25	0.06 \pm 0.13 p/g	HNO ₃ :HClO ₄	13	Hot Needle	Saint Brieuc bay, France	[5]
<i>Mytilus edulis</i>	25	0.32 \pm 0.22 p/g	HNO ₃ :HClO ₄	13	Hot Needle	Inschot, Netherlands	[5]
<i>Mytilus edulis</i>	36	0.36 \pm 0.07 p/g	HNO ₃	5	Raman	North sea, Germany	[3]
<i>Mytilus edulis</i>	-	0.2 \pm 0.3 p/g	HNO ₃	5	Raman ^c	North sea	[4]
<i>Mytilus edulis</i>	-	0.37 p/g	HNO ₃ :HClO ₄	13	Hot Needle	North sea, Belgium	[1]
<i>Mytilus edulis</i>	390	1.5 – 7.6 (0.9 – 4.6 p/g)	H ₂ O ₂	5	FTIR	Chinese coasts	[6]
<i>Mytilus edulis</i>	-	1.05 – 4.44 p/g	Enzyme	52	FTIR	Oban, Scotland	[9]
<i>Mytilus edulis</i>	120	0.60 \pm 0.56 (0.23 \pm 0.20 p/g)	KOH	12	FTIR	Atlantic coast, France	[10]
<i>Mytilus edulis</i>	162	1.1 – 6.4 (0.7 – 2.9 p/g)	H ₂ O ₂	5	FTIR	United Kingdom coast	[11]
<i>Mytilus trossulus</i>	450	0.04 \pm 0.19 (0.26 \pm 1.3 p/g)	Enzyme	20	FTIR	Baltic sea	[13]
<i>Crassostrea gigas</i>	11	0.47 \pm 0.16 p/g	HNO ₃	5	Raman	Atlantic ocean	[3]
<i>Crassostrea gigas</i> ^a	12	0.6 \pm 0.9 (2.10 \pm 1.71 p/g)	KOH	-	None	Pacific ocean	[2]
<i>Crassostrea gigas</i>	60	2.10 \pm 1.71 (0.18 \pm 0.16 p/g)	KOH	12	FTIR	Atlantic coast, France	[10]
<i>Saccostrea cucullata</i>	330	1.7 – 4.0 (1.5 – 7.2 p/g)	KOH	20	FTIR	Pearl River Estuary, China	[12]
<i>Venerupis philippinarum</i>	27	8.4 \pm 8.5 (0.9 \pm 0.9 p/g)	HNO ₃	1.2	None	British Columbia, Canada	[7]
<i>Venerupis philippinarum</i>	27	11.3 \pm 6.6 (1.7 \pm 1.2 p/g)	HNO ₃	1.2	None	British Columbia, Canada	[7]
<i>Venerupis philippinarum</i> ^a	24	4.3 – 57.2 (2.1 – 10.5 p/g)	H ₂ O ₂	5	FTIR	Chinese coasts	[8]
<i>Scapharca subcrenata</i> ^a	6	4.3 – 57.2 (2.1 – 10.5 p/g)	H ₂ O ₂	5	FTIR	Chinese coasts	[8]

<i>Tegillarca granosa</i> ^a	18	4.3 – 57.2 (2.1 – 10.5 p/g)	H ₂ O ₂	5	FTIR	Chinese coasts	[8]
<i>Patinopecten yessoensis</i> ^a	6	4.3 – 57.2 (2.1 – 10.5 p/g)	H ₂ O ₂	5	FTIR	Chinese coasts	[8]
<i>Alectryonella plicatula</i> ^a	18	4.3 – 57.2 (2.1 – 10.5 p/g)	H ₂ O ₂	5	FTIR	Chinese coasts	[8]
<i>Sinonovacula constricta</i> ^a	6	4.3 – 57.2 (2.1 – 10.5 p/g)	H ₂ O ₂	5	FTIR	Chinese coasts	[8]
<i>Meretrix lusoria</i>	18	4.3 – 57.2 (2.1 – 10.5 p/g)	H ₂ O ₂	5	FTIR	Chinese coasts	[8]
<i>Cyclina sinensis</i>	30	4.3 – 57.2 (2.1 – 10.5 p/g)	H ₂ O ₂	5	FTIR	Chinese coasts	[8]

^a Individuals sampled on local market; ^b Research of MP in gills and digestive gland; ^c Fibers were no considered as MP

“-“ : No data; p/g: Particle/g of tissue wet weight; FTIR: Fourier Transform Infra-Red

References : [1] De Witte et al. (2014); [2] Rochman et al. (2015); [3] Van Cauwenberghe and Janssen (2014); [4] Van Cauwenberghe et al. (2015); [5] Vandermeersch et al. (2015); [6] Li et al. (2016); [7] Davidson and Dudas (2016); [8] Li et al. (2015); [9] Courtene-Jones et al. (2017); [10] Phuong et al. (2018); [11] Li et al. (2018b); [12] Li et al. (2018a); [13] Railo et al. (2018); [14] Digka et al. (2018)

313 It is important to be aware that considering all recorded fibers as plastic particles could
314 overestimate the contamination. Indeed, fibers identification with Raman or FTIR spectroscopy
315 is an issue due to fibers being too thin (Cho et al., 2019; K  ppler et al., 2016). According to
316 Hermesen et al. (2017), studies with the lowest MP contamination levels in fish are those using
317 clean air conditions, high quality assurance criteria and polymer identification. Moreover, Dris
318 et al. (2016) demonstrated that fiber identification is important. Indeed, only 29% of analyzed
319 fibers could be considered as plastics while others fibers were mostly made of cotton (Dris et
320 al., 2016). Equivalent results were recently found in mussels sampled in the United Kingdom
321 with some fibers ($\approx 10\%$) identified as natural particles (Li et al., 2018b) and with 89% of fibers
322 being identified as natural particles or unidentified in bivalves sampled on fishery markets in
323 South Korea (Cho et al., 2019). More recently, Stanton et al. (2019) found that natural fibers
324 represent 93.8% of the total fibers found in freshwater and atmospheric fallout samples. All
325 these studies highlight that plastic fibers are not always dominating samples and as recently
326 recommended (K  ppler et al., 2018; Remy et al., 2015), fibers identification should be
327 performed to allow their inclusion in MP pollution counts, only if they are made of plastic.
328 However, to date, in some studies on MP contamination in bivalves, fibers were accurately
329 identified as plastic particles (Courtene-Jones et al., 2017; Murray and Cowie, 2011; Phuong et
330 al., 2018) but in other studies fibers or others particles were not verified using identification
331 techniques (Davidson and Dudas, 2016; De Witte et al., 2014; Santana et al., 2016) (Table 4)
332 possibly leading to an overestimation of MP contamination. Some studies (Avio et al., 2015;
333 Davison and Asch, 2011; Foekema et al., 2013; Ory et al., 2017; Santana et al., 2016; Van
334 Cauwenberghe et al., 2015) did not included fibers in their final MP *stricto sensu* counts, in
335 order to estimate accurate MP contamination levels in seafood products.

336 In addition to MP contamination variability according to locations, methodological approaches
337 can also be sources of heterogeneity and variability of the results found in the literature (Table

338 4). Indeed, to date, there is no harmonized protocol for the extraction and characterization of
339 MP from seafood products, despite the call from different institutions (Directive Strategy
340 Framework, 2017; OSPAR, 2016). Differences of methods used to determine MP
341 contamination in bivalves included different chemicals used to digest organism tissue which
342 may lead to substantial degradation of some polymers (Dehaut et al., 2016), different types of
343 filters (pore size and composition), identification of particles (visual vs chemical) which may
344 lead to false positive or false negative results (Lenz et al., 2015), inclusion of fibers in the results
345 and management of atmospheric contaminations. In this work, KOH 10% was used as its
346 suitability was shown for bivalve digestion without degrading multiple plastic polymers (Dehaut
347 et al., 2016; Kühn et al., 2017) whereas some acids (HNO_3 and HClO_4) discolored and degraded
348 some polymers (Dehaut et al., 2016) leading to possible underestimation of MP in organisms.
349 In addition, after chemical digestion, filtrations at 1.6 μm porosity were realized in order to
350 recover a broad size of MP even if the smallest particle found in the present work measured 15
351 μm . Then, particles resembling MP were chemically identified using μ -Raman spectroscopy.
352 Such chemical identification step is essential to accurately estimate MP contamination in the
353 environment and the biota (Dehaut et al., in press; Hermsen et al., 2018). Some guidelines were
354 recently suggested to improve and harmonize protocols used to study MP contamination in
355 seafood products (Dehaut et al., in press).

356 Fibers were found in all samples (Table 3). Mussels and cockles from BdV were significantly
357 more contaminated (Post-hoc after Kruskal-Wallis, $p < 0.05$) with respectively 2.42 ± 0.55
358 fiber/individual, namely 0.44 ± 0.10 fiber/g of tissue ww and 2.34 ± 0.77 fiber/individual,
359 namely 0.82 ± 0.28 fiber/g of tissue ww, in comparison with mussels and cockles sampled at
360 LP and BA (Table 3). Fibers and MP were found in bivalves from all sampling sites whereas
361 PCP were only found in bivalves sampled at LP and BA (Table 3). PCP were found in 12
362 mussels (24%) from LP with 0.38 ± 0.23 pigment/individual (0.12 ± 0.07 pigment/g of tissue

363 ww) and were found in 4 cockles (8%) from BA with 0.18 ± 0.2 pigment/individual ($0.06 \pm$
364 0.06 pigment/g of tissue ww). For mussels, all categories of particles, MP, fibers and PCP, were
365 found in 34 individuals (68%) from LP and 43 mussels (86%) from BdV (Table 3). In BdV, 43
366 cockles (86%) were contaminated while all types of particles were found in 36 cockles (72%)
367 from BA (Table 3). Levels of MP, fiber and PCP contamination per individual was significantly
368 higher in mussels sampled at BdV (3.20 ± 0.60) in comparison with mussels sampled at LP
369 (2.68 ± 1.33) (Post-hoc after Kruskal-Wallis, $p < 0.05$). Fibers were the dominant particles in
370 mussels and cockles sampled from BdV (Table 2). The Baie des Veys is influenced by two
371 rivers with a total mean discharge of $53 \text{ m}^3/\text{s}$ (Grangeré et al., 2009) and could also be
372 influenced by the Seine depending on meteorological conditions (Ellien et al., 2004). As
373 particles found in the Seine river are mainly in form of fibers (Dris et al., 2015), the Seine
374 discharge could contaminate mussels and cockles of the BdV with fibers. In the present study,
375 when PCP and fibers were included in MP counts, corresponding to all particles, contamination
376 levels for mussels and cockles were much higher (Table 3) but stayed between the observed
377 contamination levels found for bivalves sampled along the European coast (Table 4). Presenting
378 data with and without PCP and fibers allowed comparisons between all available studies
379 including those executed with and without chemically identified particles.

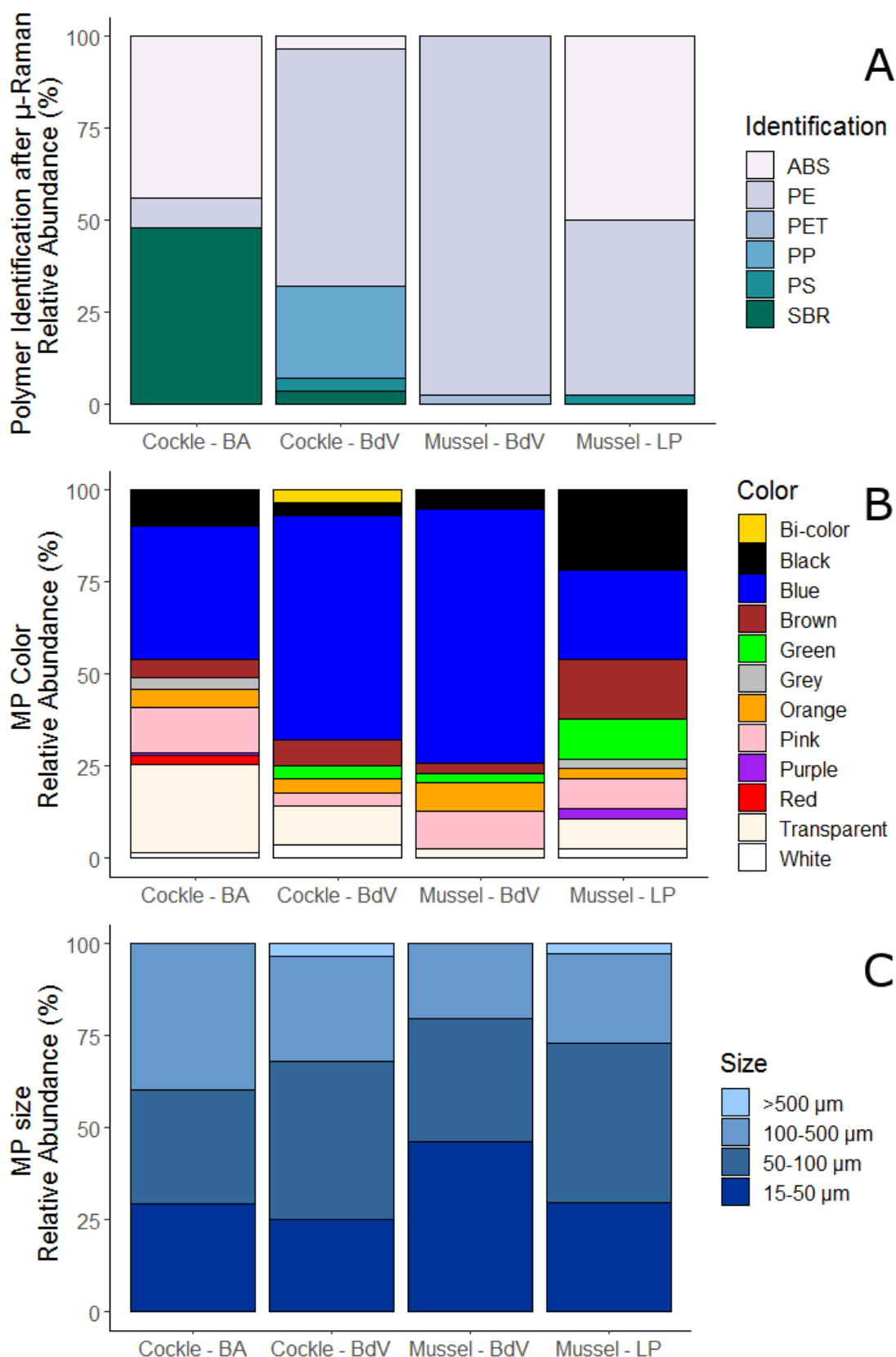
380 Among the 228 MP found in mussels and cockles, five different polymers and one copolymer
381 were identified using μ -Raman spectroscopy: PE, PP, PS, ABS, PET and styrene butadiene
382 rubber (SBR) copolymer. PE, ABS and SBR were the most common polymers found in the
383 cockles and mussels sampled with respectively 36.8%, 32.5% and 26.3% of all identified MP.
384 Each remaining polymer represented less than 5% of the MP found in the bivalves. Proportions
385 of polymers found in bivalves according to species and sampling sites were significantly
386 different (Chi-square, $p < 0.001$) (Fig. 2A). Indeed, PE was mainly found in mussels sampled at
387 BdV and LP and in cockles sampled at BdV whereas ABS and SBR were mainly found in

388 bivalves sampled in BA and LP. Moreover, PP was only found in cockles sampled at BdV (Fig.
389 2A). PE is common in samples which is in accordance with the available literature as PE is one
390 of the most common polymers found in the marine environment (Frère et al., 2017; Rezanian et
391 al., 2018) and it is the most abundant polymer product worldwide (Geyer et al., 2017;
392 PlasticsEurope, 2018). Polymers found in cockles sampled at BA were more contaminated with
393 SBR in comparison with other sites (Fig. 2A). SBR is mainly used to make tires (Hao et al.,
394 2001; Wagner et al., 2018) which could be a source of MP in marine environment (Rochman,
395 2018). Moreover, this plastic polymer is also used in road materials (Sundt et al., 2014). As a
396 highway is present above the Authie River, tire and road material could be the source of this
397 polymer found in cockles although particles identified as SBR were not all black. Additionally,
398 positions of common cockle and mussel in the water column could influenced MP exposure in
399 those species. Indeed, ABS density is greater than seawater (Tarrazó-Serrano et al., 2018) and
400 will tend to sink to the bottom explaining the large proportion of this polymer contaminating
401 cockles sampled at BA (Fig 2A). However, cockles from BdV (Fig 2A) are contaminated by
402 PE, PP and PS although their densities are lighter than seawater (Andrady and Rajapakse,
403 2017). Nevertheless, MP colonization by bacteria and MP incorporation into marine aggregates
404 may increase their sinking rate (Galloway et al., 2017) and explain PE, PP and PS contamination
405 of cockles in BdV. Furthermore, cockles live in intertidal sand flats where floating MP may be
406 deposited at low tide.

407 MP colors varied significantly according to sampling sites and species (Chi-square, $p < 0.001$)
408 (Fig. 2B). For all sampling sites and species, the blue color dominated in all samples with 24%
409 and 69% of MP found in mussels sampled at LP and BdV and 36% and 61% in cockles sampled
410 at BA and BdV (Fig. 2B). Other colors representing important proportions were black,
411 transparent, pink, brown and green (Fig. 2B). In another study conducted on the Atlantic coast
412 of France, mussels and oysters were mainly contaminated by grey, black, green and red MP but

413 were less contaminated by blue MP (Phuong et al., 2018). Here differences in polymers and
414 particles colors found in bivalves could be related to differences in sources of plastic in the
415 studied sites that remain to be ascertain in environmental studies requiring further significant
416 analytical development.

417 No significant difference was found for MP size classes according to species and sampling sites
418 (Chi-square, $p=0.17$) (Fig. 2C). For all the 228 identified MP, size classes were represented as
419 follow: 31.7% 15-50 μm , 34.8% 50-100 μm , 32.6% 100-500 μm and 0.9% $>500 \mu\text{m}$ with the
420 smallest MP measuring 15 μm . A majority of the MP measured less than 100 μm (66.5%). It is
421 in agreement with a recent study using a slightly different protocol (10% KOH digestion step
422 followed by a density separation step; 5 μm pore size cellulose filters) and size cut-off (5-5000
423 μm), which demonstrated that 83% of MP found in mussels sampled on the French Atlantic
424 coast measured between 20 and 100 μm (Phuong et al., 2018). The presence of smaller MP in
425 both shellfish species reflects the fact that bivalves are filter feeders. Indeed, mussels ingest
426 preferentially particles measuring 7-35 μm (Strohmeier et al., 2012) and cockles ingest particles
427 measuring 7-11 μm (Iglesias et al., 1992). In addition, plastic particle numbers tend to increase
428 with decreasing particle sizes (Erni-Cassola et al., 2017).



429

430 **Fig 2: Relative abundance of microplastics (MP) according to polymer identification (A), color (B) size classes (C) found**
 431 **in mussels and cockles sampled at Le Portel (LP), Baie d’Authie (BA) and Baie des Veys (BdV). Microplastics were**
 432 **identified as polyethylene (PE), polypropylene (PP), polystyrene (PS), acrylonitrile butadiene styrene (ABS), styrene**
 433 **butadiene rubber (SBR) and polyethylene terephthalate (PET).**

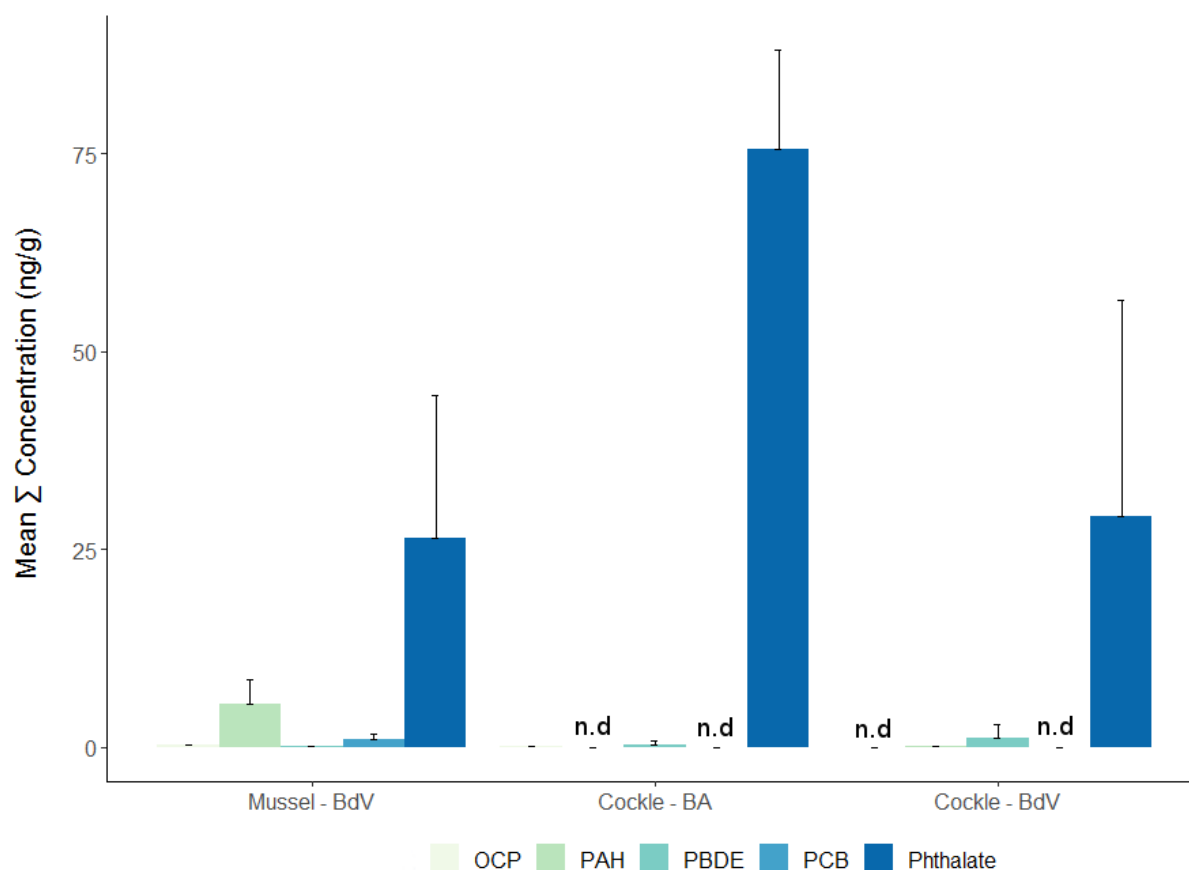
434 **3.3. Hydrophobic organic compounds and plastic additives concentrations in**
435 **mussels and cockles**

436 The second objective of this study was to combine particular and chemical analyses to test if
437 chemical pollutants can be a proxy of plastic contamination by comparing particles and
438 chemical analyses.

439 PAH were detected in mussels and cockles from BdV at 5.48 ± 3.09 ng/g and 0.06 ± 0.02 ng/g,
440 respectively (Fig. 3). The most abundant compound was phenanthrene (24-32% of Σ_{PAH}). PAH
441 concentrations are far below levels found in mussels from the Bay of Brest (France) (639 ± 73
442 ng/g and 492 ± 44 ng/g) and Barcelona (Spain) (273 – 405 $\mu\text{g/kg}$ dry weight) (Lacroix et al.,
443 2017; Porte et al., 2001). However, PAH concentrations are in the range of those found in
444 mussels and clams sampled in Milan market (Italy): not detected (n.d) - 13.95 ng/g and n.d -
445 4.35 ng/g (Chiesa et al., 2018).

446 PCB were only found in mussels sampled at BdV with 1.00 ± 0.59 ng/g, PCB 105 being the
447 most concentrated congener (Fig. 3). PCB concentration in mussels from BdV were below
448 concentrations found in mussels sampled at Milan market (Italy) (n.d – 49.2 ng/g) (Chiesa et
449 al., 2018) or sampled at Le Conquet (France) (10.46 ng/g dry weight) (Bodin et al., 2007).
450 Moreover, the PCB concentration found in mussels from the BdV, a non-polluted area, are far
451 below concentrations recorded in mussels (538.45 ng/g dry weight) sampled at Antifer, Bay of
452 Seine (France) which is a highly polluted estuary (Bodin et al., 2007).

453 Organochloride pesticides (OCP) were measured in mussels from BdV and cockles from BA at
454 concentrations of 0.23 ± 0.12 ng/g and 0.04 ± 0.08 ng/g respectively (Fig. 3). These levels are
455 below the concentrations found in mussels (max: 7.58 ng/g dry weight) from the Adriatic Sea
456 (Kožul et al., 2009).



457

458 **Fig 3: Average concentration (ng/g) of Σ PAH, Σ OCP, Σ PBDE, Σ PCB and Σ Phthalate (+ confidence interval 95%) in**
 459 **mussels sampled at BdV (n=2) and cockles sampled at BA and BdV. n.d: values below limit of detection. BA: Baie**
 460 **d'Authie; BdV: Baie des Veys.**

461 Phthalates were the most concentrated pollutants in digestates for all samples (Fig. 3). Average
 462 $\Sigma_{\text{Phthalate}}$ were respectively 26.36 ± 18.16 ng/g, 75.53 ± 12.49 ng/g and 29.18 ± 27.23 ng/g for
 463 mussels sampled at BdV and cockles samples at BA and BdV with DEHA or DMP being the
 464 most concentrated phthalates for all samples (50-98% of $\Sigma_{\text{Phthalate}}$). These results are in
 465 accordance with studies on the contamination of mussels and oysters by phthalates at False
 466 creek, Vancouver, Canada. In the study by Mackintosh et al. (2004), mean $\Sigma_{\text{Phthalate}}$ (including
 467 DEP, diisobutyl phthalate (DiBP), DnBP, DEHP, di-n-octyl phthalate (DnOP) and
 468 dionylphthalate (DNP)) were 17.27 ng/g and 16.78 ng/g for mussels and oysters respectively.
 469 However, as phthalates studied by Mackintosh et al. (2004) are not the same, comparisons have
 470 to be made carefully. In another study, Blair et al. (2009) found 585 ng/g wet weight for the
 471 monobutyl phthalate (MnBP) in mussel tissue.

472 Finally, PBDE were detected in both species from all the sampling locations with 0.07 ± 0.05
473 ng/g, 0.23 ± 0.45 ng/g and 1.16 ± 1.71 ng/g for mussels from BdV and cockles from BA and
474 BdV, respectively (Fig. 3). These are below concentrations found in mussels from the coast of
475 Spain (0.229 ng/g) and France (2.71 – 9.88 ng/g) (Bellas et al., 2014; Johansson et al., 2006).
476 at relatively low levels compared with commonly reported levels in coastal and estuarine areas.
477 Overall, the contaminant levels found in soft tissue of bivalves are low and may not represent
478 a danger for seafood consumption. Indeed, for PCB, the regulatory threshold is fixed at 75 ng/g
479 in Europe (European Commission, 2011). For PAH, the regulatory threshold is fixed at 50 ng/g
480 for the sum of benzo(a)pyrene, benz(a)anthracene, benzo(b)fluoranthene and chrysene
481 (European Commission, 2014). No regulatory threshold exists in Europe for plastic additives
482 in seafood products.

483 Two hypotheses were tested by measuring HOC, plastic additives and MP contents in mussels
484 and cockles: (i) whether the HOC and plastic additive contamination could be used as indicators
485 of MP contamination (as suggested in Fossi et al. (2012)); (ii) whether highly contaminated
486 areas in terms of dissolved chemicals also correspond to MP hotspots. However, no correlation
487 was found between MP contamination and HOC or plastic additive concentrations possibly
488 because of the low number or concentrations of MP and chemicals found in the sampled
489 bivalves. Previous studies have demonstrated that HOC or plastic additives could be used as
490 indicators for MP contamination in marine mammals, birds or fish (Fossi et al., 2014, 2012;
491 Rochman et al., 2014; Tanaka et al., 2013). However, some other studies demonstrated that MP
492 are not a vector of HOC to marine organisms (Gouin et al., 2011; Kwon et al., 2017). Plastic
493 additives are not commonly studied despite the fact that plastics are sources of these chemicals
494 in the environment and for marine organisms (Hermabessiere et al., 2017). Indeed, Jang et al.
495 (2016) demonstrated that mussels (*Mytilus galloprovincialis*) living on expanded polystyrene
496 (ePS) buoys concentrated more hexabromocyclodecane (HBCD) than mussels living on other

497 substrates. As HBCD is a plastic additive present in ePS buoys, Jang et al. (2016) suggested
498 that transfer occurs when mussels ingest ePS particles. To date, this is the only reported
499 relationship between MP contamination and plastic additive concentration in bivalve.

500 **4. Conclusion**

501 This study is the first to describe MP contamination in commercially important bivalves from
502 the French Channel coast and the first to evaluate the microplastic contamination of the cockle
503 *Cerastoderma edule*. The present work contributes to the assessment of MP contamination in
504 bivalves used as seafood and highlights some important points. Blue mussels and common
505 cockles sampled from the French Channel coastlines exhibited between 0.76 ± 0.40 and $2.46 \pm$
506 1.16 MP/individual and between 0.15 ± 0.06 and 0.74 ± 0.35 MP/g of tissue wet weight. As
507 demonstrated in the present study, formal identification for MP studies is mandatory and has to
508 be performed for all studies on MP pollution to ensure correct estimations. Indeed, without
509 proper identification, MP contamination could be overestimated. Beyond the fact that formal
510 MP identification is mandatory to properly assess MP pollution, MP characteristics measured
511 by spectroscopy (shape, polymers) also provided some clues on MP sources and fates in the
512 environment. For instance, in the present study particles identification provided evidence that
513 plastic pollution in BA is different from a close site (LP) and that in a same site (BdV), bivalves
514 ingest different plastic polymer depending on their habitat meaning that plastic pollution is
515 different in the water column. In this work, no relationship between MP contamination of
516 bivalves and the concentration of HOC or plastic additive could be shown probably due to the
517 low number of MP and chemicals found in the bivalves soft tissues.

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527 **Declaration of interest**

528 None.

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Microplastic contamination and pollutant levels in mussels and cockles collected along the English Channel coasts

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Supplemental Table 1: Particles analyzed by μ -Raman spectroscopy and fibers (blank not subtracted) visually sorted from mussels and cockles.

Species	Sampling site ¹	Fiber ³	Unidentified particles ⁴	Identified ⁴			Total
				Natural particles ⁵	Microplastics	Pigments	
Mussel	LP ²	239	465	164	38	19	925
		<i>25.8%</i>	<i>50.3%</i>	<i>17.7%</i>	<i>4.1%</i>	<i>2.1%</i>	<i>100%</i>
Cockle	BdV ²	184	65	16	39	0	304
		<i>60.5%</i>	<i>21.4%</i>	<i>5.3%</i>	<i>12.8%</i>	<i>0%</i>	<i>100%</i>
Mussel	BA ²	188	169	65	123	9	554
		<i>33.9%</i>	<i>30.5%</i>	<i>11.7%</i>	<i>22.2%</i>	<i>1.6%</i>	<i>100%</i>
Cockle	BdV ²	156	73	39	28	0	296
		<i>52.7%</i>	<i>24.7%</i>	<i>13.2%</i>	<i>9.5%</i>	<i>0%</i>	<i>100%</i>

¹ LP: Le Portel; BdV: Baie des Veys; BA: Baie d'Authie

² Results are expressed in terms of absolute number of particles and relative proportion of each items among the total count by line (in italic)

³ Not analyzed by μ -Raman

⁴ Analyzed by μ -Raman

⁵ Natural particle included minerals, organic and inorganic carbon.

Supplemental Table 2: Particles analyzed by μ -Raman spectroscopy and fibers (blank not subtracted) visually sorted from mussels and cockles. Results are expressed before chemometrics treatment.

Species	Sampling site ¹	Fiber ³	Unidentified particles ⁴	Identified ⁴			Total
				Natural particles ⁵	Microplastics	Pigments	
Mussel	LP ²	239	513	140	2	31	925
		<i>25.8%</i>	<i>55.5%</i>	<i>15.1%</i>	<i>0.2%</i>	<i>3.4%</i>	<i>100%</i>
Cockle	BdV ²	184	84	15	0	21	304
		<i>60.5%</i>	<i>27.6%</i>	<i>4.9%</i>	<i>0.0%</i>	<i>6.9%</i>	<i>100%</i>
Mussel	BA ²	188	284	67	1	14	554
		<i>33.9%</i>	<i>51.3%</i>	<i>12.1%</i>	<i>0.2%</i>	<i>2.5%</i>	<i>100%</i>
Cockle	BdV ²	156	95	29	2	14	296
		<i>52.7%</i>	<i>32.1%</i>	<i>9.8%</i>	<i>0.7%</i>	<i>4.7%</i>	<i>100%</i>

¹ LP: Le Portel; BdV: Baie des Veys; BA: Baie d'Authie

² Results are expressed in terms of absolute number of particles and relative proportion of each items among the total count by line (in italic)

³ Not analyzed by μ -Raman

⁴ Analyzed by μ -Raman

⁵ Natural particle included minerals, organic and inorganic carbon.