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1 **High density polyethylene (HDPE) microplastics impair development and swimming**  
2 **activity of Pacific oyster D-larvae, *Crassostrea gigas*, depending on particle size**

3

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12

13 **Abstract**

14 Understanding the effects of plastic debris on marine ecosystems is essential in encouraging  
15 decision-makers to take action. The present study investigates the effect of a 24h  
16 experimental exposure to high density polyethylene (HDPE) microplastics (MPs) of different  
17 sizes (4-6, 11-13 and 20-25  $\mu\text{m}$ ) and at three concentrations (0.1, 1 and 10  $\text{mg MP.L}^{-1}$ ) on  
18 the development and locomotor activity of early stages of Pacific oyster, *Crassostrea gigas*.  
19 The bivalve embryo-larval assay (AFNOR, XP T90-382) was used in this study but with  
20 additional toxicity criteria: the developmental arrests, abnormal D-larvae, maximum speed  
21 and swimming trajectory. Copper (Cu), was used as a positive control. Our results show that  
22 smaller MPs (4-6 and 11-13  $\mu\text{m}$ ) induced higher rates of malformations and developmental  
23 arrests than the larger ones (20-25  $\mu\text{m}$ ). In addition, a dose-dependent decrease of  
24 maximum swimming speed was observed for larvae exposed to MPs of 4-6 and 11-13  $\mu\text{m}$ .  
25 On the other hand, there was no significant difference in swimming speed with the largest  
26 MPs size tested (20-25  $\mu\text{m}$ ). For all three sizes of MP, there was a decrease in straight-line  
27 swimming trajectories, and an increase in circular trajectories. This abnormal swimming  
28 behaviour could affect larvae survival as well as colonization of new habitats.

29

30 **Keywords:** Polyethylene microplastics; Pacific oyster; early life stage; development;  
31 swimming behaviour

32

33 **Capsule:** Polyethylene microplastics of different sizes induce differential deleterious effects  
34 on development and swimming activity of oyster D-larvae

35

## 36 **1. Introduction**

37 Plastic debris pollution is on the increase, and has been identified as major emerging global  
38 problem affecting marine organisms and humans alike (Sutherland *et al.*, 2010; Caruso,  
39 2015; Wang *et al.*, 2016).

40 In 2017, over 348 million tons of plastic were manufactured (PlasticsEurope, 2018). Eighty  
41 percent of plastic production in Europe is made up of 6 main polymers (PlasticsEurope,  
42 2016): polypropylene (PP), HDPE and LDPE (high and low-density polyethylene), polyvinyl  
43 chloride (PVC), PUR (polyurethane), polyethylene terephthalate (PET) and polystyrene (PS).  
44 Jambeck *et al.* (2015) estimated that between 2 and 5% of these plastics are discharged into  
45 oceans. In recent years, microplastics (MPs) have been increasingly in the public eye. MPs  
46 are defined as any forms of plastic particle between 1  $\mu\text{m}$  and 5 mm size (Cole *et al.*, 2011;  
47 Wagner *et al.*, 2014), while nanoplastics are defined as any plastic less than 100 nanometres  
48 in length (Lambert and Wagner, 2016; Gigault *et al.*, 2016). While micro and nanoplastics are  
49 often the result of fragmentation and degradation of larger volumes of plastic, they can also  
50 be released directly into the environment.

51 MPs are ubiquitous in marine water with reported concentrations ranging from 1.31 to  
52 102,000 particles per  $\text{m}^3$  (Van Cauwenberghe *et al.*, 2015; Auta *et al.*, 2017). PP and PE are  
53 the most common polymers in all environmental compartments (Isobe *et al.*, 2014; Enders *et*  
54 *al.*, 2015; Frere *et al.*, 2017). These plastics persist in the environment due to their resistance  
55 to biodegradation (Yoshida *et al.*, 2016) but also to chemical, photochemical and mechanical  
56 degradation (Cooper *et al.*, 2010).

57 During the 2009 International Coastal Cleanup, a number of different types of marine debris  
58 were collected from coastlines and waterways around the world: cigarettes, bags, food  
59 wrappers, caps, beverage bottles, cups, plates, spoons, etc. (Kershaw *et al.*, 2011). Recent  
60 publications have reported a high percentage (80%) of small MPs (25-50µm) in surface water  
61 or sediment (particles for which few data are available), when compared with larger particles  
62 (Enders *et al.*, 2015; Bergmann *et al.*, 2017). The estimated concentration of MPs in surface  
63 seawater along European coastlines is 2.505 mg.m<sup>-3</sup> for sizes between 10 and 1000 µm  
64 (Paul-Pont *et al.*, 2018).

65 Deleterious effects of MPs on feeding processes, behaviour, metabolism anomalies of  
66 holoplanktonic, meroplanktonic and benthic aquatic organisms have been reported for PS  
67 (Gerritsen and Porter, 1982; Holland *et al.*, 1986; Ward and Targett, 1989; Solow and  
68 Gallagher, 1990; Hart, 1991; Mayer, 1994; Ward, 1996; Baer *et al.*, 2008). Only a few studies  
69 have focused on PE effects, particularly on blue mussel (*Mytilus sp.*) with effects on tissues  
70 and cells (Détrée *et al.*, 2017; Von Moos *et al.*, 2012). Green, 2016 has shown that PE can  
71 influence the assembly of European oyster populations (*Ostrea edulis*). Au *et al.*, 2015  
72 observed that freshwater amphipods, *Hyalella azteca*, exposed to PE (fluorescent blue PE,  
73 10 to 27 µm) showed growth and reproduction alterations after 10 to 42 days of exposure.  
74 Beiras *et al.*, 2018 showed that exposure of zooplankton to PE (low-density PE, 1 to 500 µm)  
75 did not cause any significant toxic effects after 12 days of exposure. Cole *et al.*, 2015,  
76 observed the ingestion of MPs by the Pacific oyster larvae. In addition, a significant and  
77 linear increase in MPs uptake with increasing concentrations was observed at different larval  
78 stages of *Mytilus galloprovincialis* (Capolupo *et al.*, 2018).

79 The present study focuses on the potential effects of PE for their high occurrence in the  
80 oceans. Indeed, the vast majority of equipment used by aquatic farmers is made of polymers,  
81 particularly PE. The French shellfish industry produced 216,917 tons of marketable shellfish  
82 during the 2015-2016 year (CNC, 2016), giving it one of the largest outputs in the world. Of  
83 that figure, the Poitou-Charente region was the most productive, accounting for 23% of the

84 national total (CNC, 2016). It is therefore essential for better understanding the interaction of  
85 Pertuis Charentais' environment with oysters.

86 In addition to plastic pollution, coastal areas undergo several others anthropic pressures  
87 such as Copper (Cu) based fungicides widely used in viticulture to combat downy mildew in  
88 the Nouvelle-Aquitaine region (Singh, R.S., 2000), along with antifouling paint (Gatidou *et al.*,  
89 2007). In this study, copper will serve as a positive control.

90 The objective of this paper is to study the embryo-larval development and swimming  
91 behaviour of D-larvae of oysters (*C. gigas*) in response to direct acute exposure to HDPE  
92 micro-particles of three different sizes without ingestion. Indeed, oyster embryos and larvae  
93 of less than 24 hours old feed exclusively on their vitelline reserves and they cannot ingest  
94 particles.

95

## 96 **2. Material and methods**

### 97 **2.1 Animal collection**

98 Mature oysters (diploid male and female), *C. gigas* (Bayne *et al.*, 2019; Bayne *et al.*, 2017)  
99 came from a commercial hatchery specialized in production of mature oysters (France  
100 Naissain, France). Oysters were kept dry at 4°C for two days to get better spawning outside  
101 the natural breeding season and then. Oysters were acclimatized in 18 °C filtered seawater  
102 one hour before the beginning of experiments.

103

### 104 **2.2 Chemicals and seawater**

105 Seawater was collected from the Ile de Ré (SW France). After sampling, seawater was  
106 filtered using membrane filter of 20 µm, 5 µm, 1 µm and 0.2 µm to eliminate debris and  
107 microorganisms. Filtered seawater was stored at 4°C with continuous bubbling and was used  
108 within 7 days. A few hours before the experiment, filtered seawater was filtered again at 0.2  
109 µm. Water and sand samples were taken from the Atlantic coast.

110 Cu (CuSO<sub>4</sub>) and formaldehyde were purchased from Sigma-Aldrich Chemical (St. Quentin  
111 Fallavier, France). Three sizes of PE MPs (HDPE) were used (4-6 µm MPP-635 XF, 11-13

112  $\mu\text{m}$  MPP-635 G and 20-25  $\mu\text{m}$  MPP-1241, density 0.96, Micropowders Inc. USA). Stock  
113 solutions of  $100 \mu\text{g.L}^{-1}$  for Cu and  $100 \text{ mg MP.L}^{-1}$  for MPs were prepared in pure Milli-Q-  
114 water (Milipore) for Cu and in filtered seawater for MPs. Working solutions were obtained by  
115 diluting the stock solutions in filtered seawater and conserve at  $4^\circ\text{C}$  and in the dark for MPs.  
116 Before each exposure, working solutions were centrifuged at low speed for 2 min. Three  
117 concentrations of exposure were selected according to previous work for Cu: 0.1, 1 and 10  
118  $\mu\text{g.L}^{-1}$  and for MPs: 0.1, 1 and  $10 \text{ mg.L}^{-1}$  (Beiras *et al.*, 2018). The selected concentrations in  
119 MPs are higher than what can be found in the environment. For Cu, the range of  
120 experimental concentrations was chosen based on preliminary studies (Mai *et al.*, 2012) in  
121 order to get a complete dose-response curve.

122

### 123 **2.3 Exposure solutions analysis**

124 For quantitative analysis of PE MPs at different concentrations (0.1, 1 and  $10 \text{ mg MP.L}^{-1}$ ),  
125 each solution was tested in a flow cytometer (Attune Acoustic Focusing Cytometer). Two-  
126 millilitres samples were prepared from the MP solution used for embryo-larval exposures  
127 ( $n=4/\text{condition}$ ). The samples were vortexed (StarLab Vortex IR, 12,000 rpm for 20 sec)  
128 before being transferred into the cytometer to homogenize the solution.  $300 \mu\text{L}$  were taken  
129 for flow cytometry analysis. Calibration was performed to obtain an analysis rate of  $500$   
130  $\mu\text{L.min}^{-1}$  and a saturation of 10 000 particles maximum detected. Control analytical samples  
131 ( $n = 12$ ) were made of filtered seawater (without the presence of MPs) to obtain a blank and  
132 remove background if necessary (particles naturally present in the water).

133 Chemical analyses of Cu were carried out in sandy sediments (surface and semi-depth 5 cm)  
134 and in coastal seawater close to shellfish areas. This gave us an indication of the  
135 environmental concentrations found. The water was acidified to 5% and then analyzed and  
136 diluted to one-third. For the sediment, 200 mg was weighed and then acidified with 6 mL of  
137 nitric acid and 2 mL of hydrochloric acid. The sediment was then mineralized by microwave  
138 (rise in temperature at  $120^\circ\text{C}$  and then plateau at  $120^\circ\text{C}$  for 30 minutes, then cooling to  
139 room temperature). The resulting mineralization was topped up to 50 mL with ultra-pure

140 water. The prepared samples were then assayed by ICPMS (Inductively Coupled Plasma  
141 Mass Spectrometry, Thermofisher, X, Model II). The limit of quantification was equivalent to  
142 0.02 µg.L<sup>-1</sup>.

143

#### 144 **2.4 Experimental design**

145 Mature oysters were cleaned to avoid any external contaminations and remove the  
146 microorganisms attached to them. Male and female oysters were induced to spawn by  
147 thermal stimulation (alternating immersion in filtered seawater of 18 °C and 28 °C for 30 min)  
148 or by stripping the gonad where thermal stimulation was ineffective (Mai *et al.*, 2013 and  
149 Gamain *et al.*, 2017). Spawning males and females were individually isolated in beakers (500  
150 mL of filtered seawater) at their respective spawning temperatures (LM Parker *et al.*, 2009).  
151 Individuals were left undisturbed for 10 min and then removed from beakers. Eggs and  
152 sperm from two individuals were selected to give a single pairing. Sperms and eggs were  
153 sieved separately through 50 µm and 100 µm meshes (Sefar Nitex), respectively to eliminate  
154 debris and faeces. Sperm mobility was verified and the number of eggs was counted under  
155 stereomicroscope (Nikon) at a magnification of 100.

156 Eggs were fertilized with sperm in ratio of 1:10 (egg:sperm). Fertilization success was  
157 verified under a microscope, and embryos were then counted and transferred to a 24-well  
158 microplate (Greiner Bio-One, Cellstar) for embryotoxicity assays following previously  
159 published protocols (His *et al.*, 1997; Quiniou *et al.*, 2005; NF ISO 17244, 2015). MP  
160 solutions were resuspended before injecting them into the microplates. Fertilized eggs  
161 (around 350 eggs/well) were exposed in wells containing 2 mL of toxicant solution and were  
162 incubated in the climatic chamber during 24h at 24 °C in the dark to obtain an optimized  
163 development (Robinson *et al.*, 1992 and AFNOR 2009).

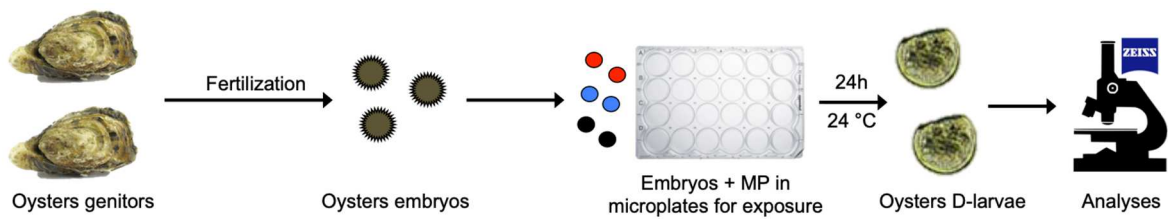
164

#### 165 **2.5 Swimming activity**

166 After 24h (e.g. 1 dpf, day post fertilization) of incubation, the swimming behaviour was  
167 recorded under a microscope (ZEISS Axio Observer Z1, UMR LIENSs, La Rochelle, France)

168 with a magnification of 100 in natural light condition and in air conditioned-room at 24 °C.  
169 Two-minute videos were recorded (2 or 3 videos per condition, and per replicate). In this  
170 experiment, a replicate corresponds to a pair of oysters (couple) having obtained fertilization  
171 success. Three different couples were used and four analytical replicates were performed for  
172 each condition. The experimental design presented above is shown schematically in Figure  
173 1.

174



175

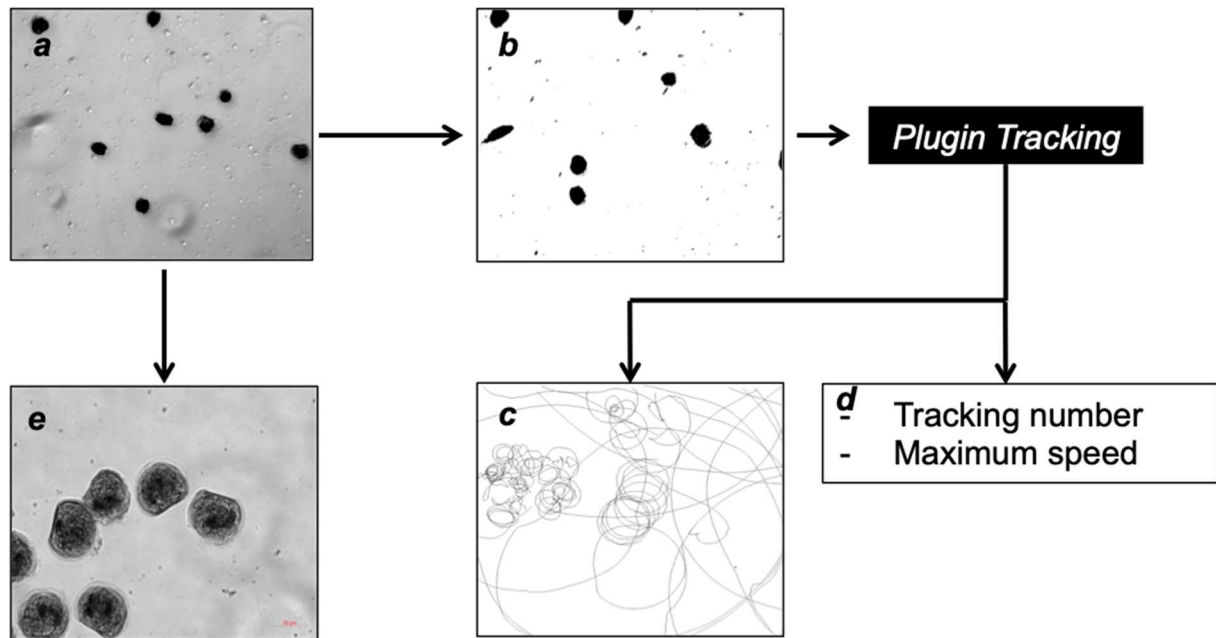
176 **Figure 1.** Experimental design. After a 24h-exposure to Cu or MPs, oyster D-larvae malformations  
177 and swimming behaviour were analysed. A 2 min-video was recorded for each batch of larvae and  
178 then abnormal D-larvae were quantified after formaldehyde fixation.

179

180 A freeware application (VirtualDub, Windows) for video conversion was used to subsample  
181 films to 4fps and convert them to AVI format (Gamain *et al.*, 2019). ImageJ (1,52a software)  
182 was used to analyse different videos. Videos (AVI format) were converted to grayscale image  
183 (Figure 2a), then converted into a binary stack of images (Figure 2b). Swimming parameters  
184 (Figure 2d) of each individual D-larvae numbered as maximum speed (pixel/s) and swimming  
185 trajectories were calculated using the wrMTrck plugin (Figure 2d). As a result, each tracked  
186 larva was assigned a number, with each number used to identify the tracked larvae in the  
187 results file (Figure 1). We identified three different types of larval path: (1) rectilinear; (2)  
188 circular and (3) motionless following protocol published by Gamain *et al.*, 2019 with  
189 improvements (Table 1). The use of a graphic tablet (Wacom Bamboo Pen&Touch) and  
190 image processing software (Photos, Windows 10) allowed swimming trajectories to be  
191 quantified and characterised. In this study, we used a single condition (control) without

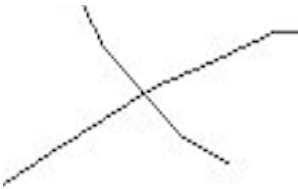
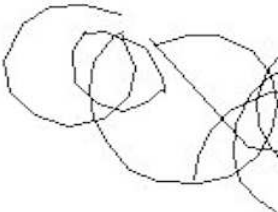



192 contaminant for trajectory analysis. Once the videos were processed with the plugin, we take  
 193 back video analysis to ensure that the same larvae were not detected more than once.  
 194



195  
 196 **Figure 2.** Procedure for swimming behaviour analysis of oyster D-larvae. (a) Original image after  
 197 conversion. (b) Binary image. (c) Traces of larval path. (d) Parameters studied. (e) Quantification of  
 198 abnormal D-larvae and developmental arrest after fixation with formaldehyde (1%).

199  
 200 **Table 1.** Paths analysis of oyster D-larvae with three categories of trajectories: rectilinear, circular and  
 201 motionless.

Rectilinear	Circular	Motionless
Straight or lightly curved lines + paths without any pattern	Large circles + paths with a repeating pattern or loops	Small circles + large points
		

## 212 **2.6 Developmental abnormalities**

213 After video recording, 25  $\mu$ L of 1% buffered formalin were added to each well, and the  
214 percentage of abnormal oyster larvae (Figure 2e) and developmental arrest were recorded  
215 (His *et al.*, 1999; Quiniou *et al.*, 2005). Abnormal larvae and developmental arrest were  
216 directly observed an inverted microscope (Nikon eclipse). An important prerequisite for this  
217 test is the presence, in control condition of less than 20% of abnormal larvae (Quiniou *et al.*,  
218 2005). Three different couples were used and four replicates were performed for each  
219 condition.

220

## 221 **2.7 Statistics**

222 All data are expressed as means  $\pm$  standard error of the mean (SEM). If data did not follow a  
223 normal distribution, it was transformed using the formula:  $P' = \arcsin \sqrt{r}$ ; P corresponds to raw  
224 data (frequency of abnormalities) specified in P values from 0 to 1 (Legendre and Legendre,  
225 1998). Homogeneity of variance (Levene's test) was verified and statistical analysis was  
226 performed by Kruskal-Wallis test. Differences between tested concentrations means were  
227 then performed using Kruskal Nemenyi Post-hoc test (equivalent to the Tukey test for non-  
228 parametric data). Significance difference was accepted when p-value < 0.05. Statistical  
229 analysis was conducted using R, and graphs prepared using Microsoft Excel.

230

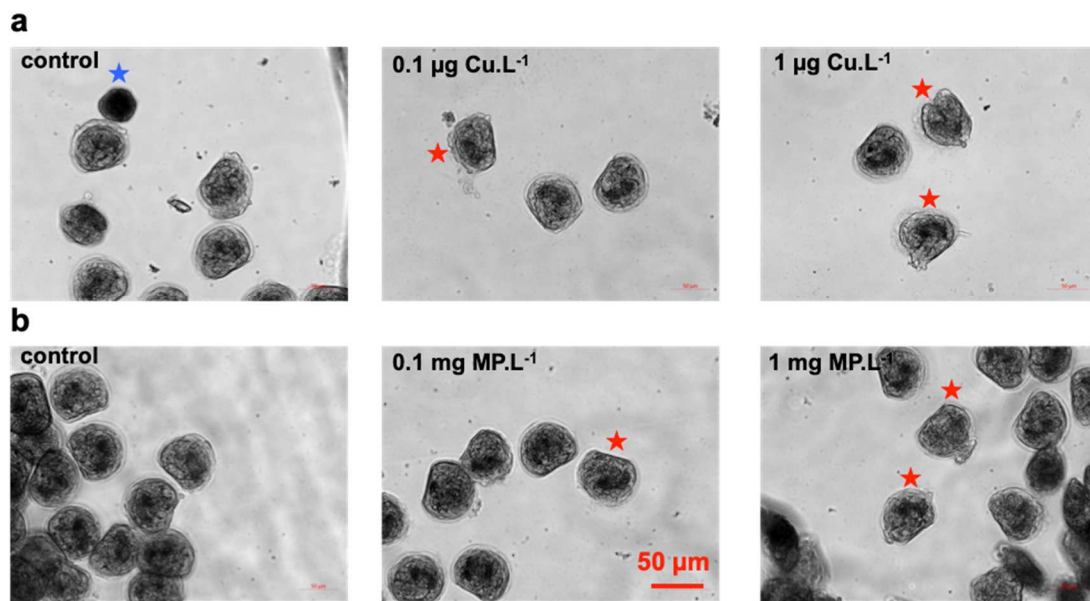
## 231 **3. RESULTS**

### 232 **3.1 Effects of different sizes of polyethylene microplastics on embryo-larval** 233 **development (abnormal D-larvae of *C. gigas*)**

234 Different abnormalities (abnormal shell, convex shell, scalloped shell, incomplete shell and  
235 mantle abnormality) observed in oyster D-larvae (Mottier *et al.*, 2013) were recorded after  
236 24h exposure to copper (Figure 3.a) and PE MPs (Figure 3.b and c). Abnormal larvae and  
237 developmental arrest average frequencies were respectively  $12.8 \pm 1.3\%$  and  $4.6 \pm 0.5\%$  for  
238 control conditions (Figure 4 and supplementary data, Table S1). The percentage of

239 developmental arrest was significant from 0.1  $\mu\text{g Cu.L}^{-1}$  ( $12.9\pm 2.5\%$ ) and increased to  
240  $37.8\pm 9.7\%$  (Figure 4.a and supplementary data Table S1). For the exposure to different sizes  
241 of PE MPs, the number of malformed oyster D-larvae increased significantly from the lowest  
242 PE MPs concentration (0.1  $\text{mg MP.L}^{-1}$ ) for the 4-6  $\mu\text{m}$  (Figure 4.b) and 11-13  $\mu\text{m}$  particle  
243 sizes (Figure 4.c). The percentage of developmental arrest showed a dose-dependent  
244 increase for the 11-13  $\mu\text{m}$  PE MPs (Figure 4.c) with a maximum effect at the highest tested  
245 concentration ( $17.7\pm 0.7\%$  at 10  $\text{mg MP.L}^{-1}$ ). For the larger MPs (20-25  $\mu\text{m}$ ), significantly  
246 different malformation rates were observed from the highest concentration at 10  $\text{mg MP.L}^{-1}$   
247 ( $16.3\pm 1.5\%$ , Figure 4.d). There are no significant differences in developmental arrest with  
248 embryo-larval exposure to MP 20-25  $\mu\text{m}$  (Figure 4.d).

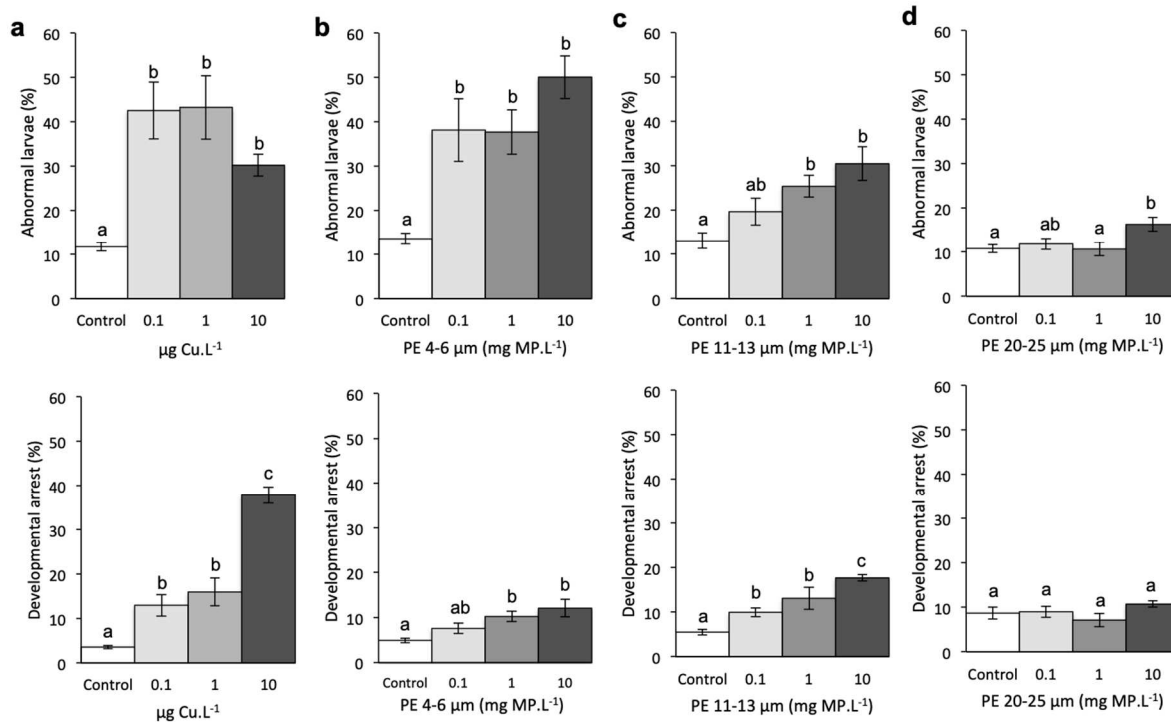
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250

251 **Figure 3.** Photographs of normal, abnormal D-larvae (red star) of *C. gigas* and developmental arrest  
252 (blue star) after 24h of exposure to 0.1 and 1  $\mu\text{g.L}^{-1}$  of Cu (**a**) or to 0.1 and 1  $\text{mg.L}^{-1}$  of PE MPs 4-6  $\mu\text{m}$   
253 (**b**). ZEISS Axio Observer Z1 photographs (x20).

254



255

256

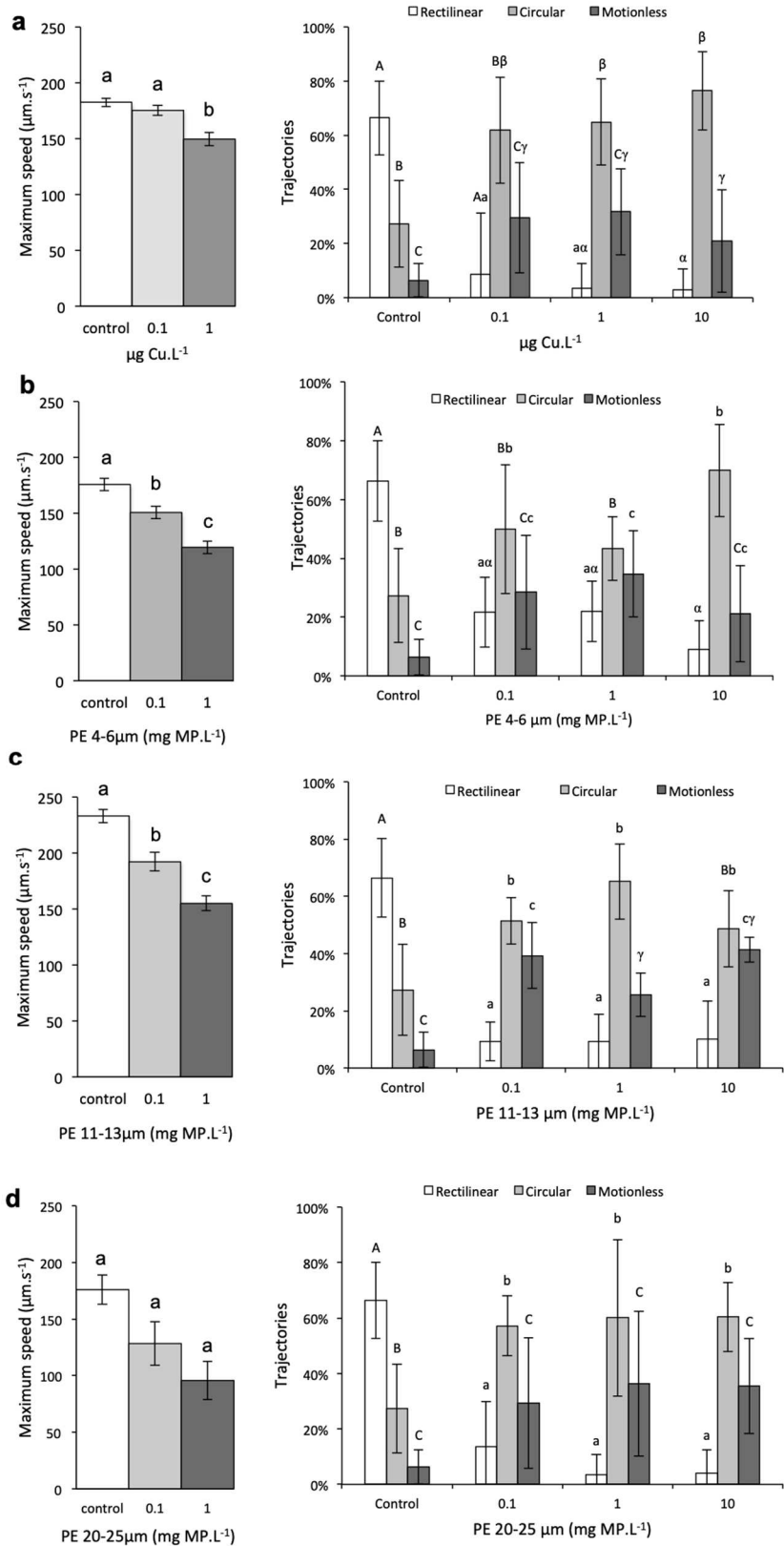
257 **Figure 4.** Abnormal larvae (%) vs. concentrations (Mean  $\pm$  SEM) of oyster D-larvae of *C. gigas*  
 258 (above) and developmental arrest (%) vs. concentrations (bottom) following 24h oyster embryonic  
 259 exposure to different concentrations of Cu (a) and PE MPs of 4-6  $\mu\text{m}$  (b), 11-13  $\mu\text{m}$  (c) and 20-25  $\mu\text{m}$   
 260 size (d). Control (without pollutants): 0.1, 1 and 10 correspond to concentrations of Cu ( $\mu\text{g Cu.L}^{-1}$ ) and  
 261 PE MPs using for exposures ( $\text{mg MP.L}^{-1}$ ). Values are mean  $\pm$  SEM for three replicates. Different  
 262 letters indicated significant differences between different concentrations ( $p < 0.05$ ).

263

### 264 **3.2 Effects of different sizes of polyethylene microplastics on D-larvae swimming** 265 **activities (maximum speed recorded and swimming trajectories) of *C. gigas***

266 In control condition (filtered seawater), the average speed of oyster D-larvae was  $109 \pm 3$   
 267  $\mu\text{m.s}^{-1}$  and the maximum speed was  $191 \pm 7$   $\mu\text{m.s}^{-1}$  (supplementary data, Table S2) while  
 268  $66.4 \pm 13.7\%$  of the D-larvae swam following a rectilinear trajectory and  $27.3 \pm 16.0\%$  shown  
 269 circular paths (Figure 6). Motionless larvae were rare within the control group with  $6.4 \pm 11.1\%$   
 270 (Figure 5). After  $1 \mu\text{g Cu.L}^{-1}$  exposure, the maximum speed significantly decreased  
 271 compared to control and the percentage of circular and motionless trajectories increased  
 272 significantly, whereas rectilinear trajectory declined (Figure 5.a). After exposure to MP 4-6

273  $\mu\text{m}$ , a decrease in maximum recorded swimming speeds was observed for the  
274 concentrations at 0.1 and 1 mg MP.L<sup>-1</sup> (with respectively 151±5 and 119±6  $\mu\text{m}\cdot\text{s}^{-1}$ , Figure  
275 5.b). We also observed a strong increase in circular trajectories for MP-exposed larvae, with  
276 49.9±22.0% at 0.1 mg MP.L<sup>-1</sup> and 69.8±15.6% at 10 mg MP.L<sup>-1</sup> condition (Figure 5.b). For  
277 11-13  $\mu\text{m}$  MPs, the same trend can be seen with a significant decrease in maximum  
278 swimming speed. Effectively, at 0.1 and 1 mg MP.L<sup>-1</sup> we find respectively 192±8 and 155±6  
279  $\mu\text{m}\cdot\text{s}^{-1}$  (Figure 5.c). At concentrations 0.1 and 1 mg MP.L<sup>-1</sup>, there is a significant increase in  
280 circular trajectories (with respectively 51.4±8.1 and 65.1±13.1%, Figure 5.c). While the  
281 straight paths decrease significantly for all 11-13  $\mu\text{m}$  MP concentrations. Motionless  
282 trajectories, on the other hand, increase with concentrations in MP. However, there were no  
283 significant differences in maximum speed recorded of D-larvae exposed to MP 20-25  $\mu\text{m}$   
284 (Figure 5.d). The trajectories analysed show an increase in "circular" and a reduction in  
285 straight-line trajectories (Figure 5.d).



286

287 **Figure 5.** Maximum speed and trajectories of D-larvae of *C. gigas* following 24h embryonic exposure  
 288 to different concentrations of Cu (**a**) and PE MPs of 4-6 µm (**b**), 11-13 µm (**c**) and 20-25 µm (**d**).  
 289 Control (without pollutants): 0.1, 1 and 10 correspond to concentrations of Cu (µg Cu.L<sup>-1</sup>) or PE MPs

290 (mg MP.L<sup>-1</sup>) used for exposures. Values are mean ± SEM for three replicates. Different letters  
291 indicated significant differences between different concentrations (p<0.05).

292

### 293 3.3 Exposure solution analysis

294 Concentrations of the different sizes of PE MPs were measured using flow cytometry in the  
295 filtered seawater working solutions, (Table 2). For 4-6 µm MPs we found dilution factors of  
296 8.7 and 9.3. These calculated dilution factors are consistent with theoretical concentrations.  
297 For 11-13 µm MPs, the first dilution factor was 3 for 0.1 mg MP.L<sup>-1</sup>, which is quite low  
298 compared to the theoretical dilution factor of 10. For 20-25 µm MPs, the dilution factors of 6  
299 and 6.8 are in adequacy with the theoretical concentrations of exposures.

300

301 **Table 2.** Theoretical and measured MP concentrations (mean±SEM) in the working solutions at the  
302 beginning of the embryotoxicity test.

303

Conditions	Theoretical (mg.L <sup>-1</sup> )	Measured (MP.µL <sup>-1</sup> )	Measured (MP.mL <sup>-1</sup> )	Dilution factor
MP 4-6 µm	0.1	0.3±0.0	300	-
	1	2.6±0.1	2600	8.7
	10	24.1±0.7	24100	9.3
MP 11-13 µm	0.1	0.3±0.0	300	-
	1	0.9±0.0	900	3
	10	16.1±0.1	16100	17.9
MP 20-25 µm	0.1	0.2±0.0	200	-
	1	1.2±0.0	1200	6
	10	8.2±0.2	8200	6.8

304

305

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#### 307 **4. Discussion**

308 The present study is the first to focus on swimming behavior of oyster larvae (*C. gigas*)  
309 exposed to PE MPs. It is an experimental integrative approach, designed to complement the  
310 previously published results on the effects of PS MPs on oyster embryos and larvae (Tallec  
311 *et al.*, 2018; Cole *et al.*, 2015; Sussarellu *et al.*, 2016).

312 Through this study, the main objective is to evaluate the impact of MP exposure (HDPE  
313 spherical particles) on the development and swimming activity of oyster embryos and larvae  
314 (early stages of development). It is important to understand that our swimming analyses  
315 consider two-dimensional rather than three-dimensional movement (Gamain *et al.*, 2019). A  
316 potential way of improving this in the future will be to capture behaviour in three dimensions,  
317 thus covering the whole water column.

318 A flow cytometry verification was set up to determine actual concentrations of MP particles in  
319 the exposure media. Long *et al.*, 2017, had also determined MP concentrations through flow  
320 cytometry. Apart from the concentration at 1 mg MP.L<sup>-1</sup> for PE 11-13 µm, the other  
321 concentrations seem consistent to the expected theoretical concentrations. The  
322 concentrations used in this study are high compared to what is currently found in the marine  
323 environment (Van Cauwenberghe *et al.*, 2015; Auta *et al.*, 2017).

324 In filtered seawater, oyster D-larvae adopted mainly a straight-line trajectory as already  
325 reported by Gamain *et al.* (2019) for the same species. Circular and motionless trajectories  
326 are considered as an abnormal swimming behaviour of D-larvae (His *et al.*, 1999; Gamain *et*  
327 *al.*, 2019). The average swimming speed recorded for this study was 100 µm.s<sup>-1</sup> lower than  
328 this recorded by Gamain *et al.*, (2019). These differences of behaviour could be explained by  
329 the fact that fertilization was carried out during the spawning period (June-August) whereas  
330 in our study, the fertilizations were realized during the October-November period. The  
331 temperature when recording videos plays also an important role and was maintained during  
332 our experimentation to 24 °C.

333 For all controls, abnormal D-larvae was below 20% and the mean rate was 12.8±1.3%.  
334 Exposure to Cu induced a significant increase of abnormal D-larvae from the first



335 concentration tested e.g.  $0.1 \mu\text{g Cu.L}^{-1}$  which is above the current water concentration of Cu  
336 ( $0.06 \mu\text{g Cu.L}^{-1}$ ) detected in the coastal waters of Pertuis Charentais in 2019. Regarding  
337 development arrests, a significant increase was observed from  $0.1 \mu\text{g Cu.L}^{-1}$ . These results  
338 are higher than in the paper of Gamain *et al.*, 2016 for the same copper concentrations but is  
339 in adequacy with Gamain *et al.*, 2019.

340 Greater toxicity of smaller plastic particles were reported by Tallec *et al.*, 2018 for PS  
341 nanoplastics (500 and 50 nm) compared to MP ( $2 \mu\text{m}$ ) on fertilization success and embryo-  
342 larval development of oyster *C. gigas*. With the same tested concentrations of PE particles,  
343 we observed a significant increase of developmental anomalies. From the results of our  
344 study, we can conclude that the smaller the PE MPs are, the more deformities and  
345 developmental arrests are visible on the oyster larvae. Different impacts on D-larvae have  
346 been reported following the exposure of spawners. A decrease in size and growth of oyster  
347 larvae were reported following exposure to PS MPs (Sussarellu *et al.*, 2016). Spherical  
348 particles are the most commonly used in laboratory studies, but fibers and fragments are the  
349 most common forms detected in wild organisms (de Sá *et al.*, 2018). Mesaric *et al.* (2015)  
350 investigated acute toxicity and performed swimming tests on *Artemia salina* larvae with MP  
351 concentrations between  $0.01$  and  $1 \text{ mg.L}^{-1}$ . They reported that nanomaterials can bind on  
352 external surfaces of *A. salina* larvae and affect their swimming activity. In addition to testing  
353 the effect of PE MPs on the early stages of *C. gigas*, copper exposures were performed in  
354 order to have the presence of additional, better referenced control (MacInnes *et al.*, 1979;  
355 Wang *et al.*, 2011; Mai *et al.*, 2012; Gamain *et al.*, 2017; Sussarellu *et al.*, 2018).

356 With respect to swimming behaviour (recording of maximum speed), larvae for control  
357 conditions (1 dpf) had a maximum swimming speed between  $160$  and  $182 \mu\text{m.s}^{-1}$ . Larvae  
358 exposed to the Pertuis Charentais environmental concentration had no altered swimming  
359 speed. A decrease in swimming speed was observed from  $1 \mu\text{g Cu.L}^{-1}$ . Contrary to this  
360 result, Gamain *et al.*, 2019 did not observe any effects on swimming behaviour at any of  
361 these Cu concentrations.

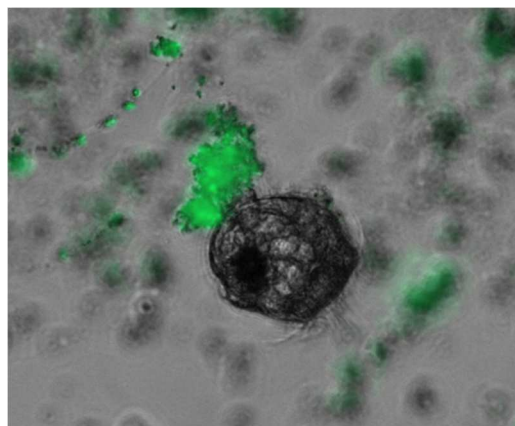
362 In our MP exposure conditions (24 °C, salinity of 33 usi, in the dark), the average speed and  
363 maximum swimming speed recorded for oyster larvae are respectively 109  $\mu\text{m}\cdot\text{s}^{-1}$  and 192  
364  $\mu\text{m}\cdot\text{s}^{-1}$  for control condition (without MP). According to Suquet *et al.*, 2012, the average  
365 speed recorded for oyster larvae without contamination was 105  $\mu\text{m}\cdot\text{s}^{-1}$ . The results would  
366 seem rather similar, despite the fact that they used a development temperature of 19 °C,  
367 whereas ours was 24 °C. In comparison with Gamain *et al.*, 2019, which found swimming  
368 values much higher at 144 and 297  $\mu\text{m}\cdot\text{s}^{-1}$  for average speed and maximum swimming  
369 speed, respectively. The differences in values could be explained by the quality of the  
370 parents but also the period of the year and the day when the videos are recorded. We also  
371 kept the optimal temperature of development during the video recording which may be the  
372 cause of the differences in swimming speed between these two studies.

373 For the small (4-6  $\mu\text{m}$ ) and medium size (11-13  $\mu\text{m}$ ) of PE particles, a dose-dependent  
374 decrease in swimming speed was observed, while for the larger ones (20-25  $\mu\text{m}$ ), no effect  
375 was reported. Lee *et al.* (2013) tested three different sizes of PS MPs (0.05, 0.5 and 6  $\mu\text{m}$  in  
376 diameter) on the marine copepod, *Tigriopus japonicus* in a two-generation chronic toxicity  
377 test. Their results highlighted that nanoplastics (0.05  $\mu\text{m}$  and 0.5  $\mu\text{m}$ ) but not microplastics (6  
378  $\mu\text{m}$ ) affect the survival of nauplii and copepodites in the F0 and F1 generations.

379 The analysis of the trajectories taken by the larvae during their displacements shows, for all  
380 the tested MPs, a decrease in straight-line trajectories and an increase in circular  
381 trajectories. This alteration of swimming behavior could lead to behavioral differences in  
382 larvae's ability to predate but especially in their ability to react to a predator. It seems that the  
383 smaller the plastics, the more the effects are noticeable on swimming behavior but also on  
384 developmental anomalies. This is comparable to the results of Tallec *et al.*, 2018, where  
385 nanoplastics had more effects on larval development than coarser MPs.

386 There may also be effects on the survival, fitness, dispersion of larvae and colonization of  
387 new habitats (Gamain *et al.*, 2019). The observed effects of early embryo-larval exposure to  
388 MPs in oysters could have affected the following developmental stages (pediveliger, spat and  
389 adults). Indeed, a recent publication shows that blue mussels, *Mytilus edulis*, have difficulty

390 attaching to their substrate when in the presence of MPs (Green *et al.*, 2019). More recently,  
391 Yin *et al.*, 2019, have shown that black rockfish, *Sebastes schlegelii*, exposed to PS MPs of  
392 15  $\mu\text{m}$  to 0.19  $\text{mg}\cdot\text{L}^{-1}$ , had drastic reduction in their swimming speeds as well as reduction of  
393 range of movement, which may affect hunting behavior and exploration competence.  
394 The impact of PE MPs on swimming behaviour could be explained by interactions of these  
395 particles with larval cilia (Figure 6). Beiras *et al.* (2018) reported that PE particles (4-6  $\mu\text{m}$ )  
396 can stuck on villi of the chorion of *Oryzias melastigma* embryos. Agglomerates of MPs tend  
397 to settle and stick along the mantle of organisms, as with nanoplastics on brine shrimp *A.*  
398 *franciscana* on their antennules and abdomen (Bergami *et al.*, 2016). Nanosized latex  
399 particles (39.4 nm) have shown to be adsorbed on the fertilized egg surface of Japanese  
400 medaka, *Oryzias latipes* (Kashiwada, 2006). Like Medaka egg chorions, oyster D-larvae  
401 have cilia to move in the water column. This would explain the fact that D-larvae exposed to  
402 MPs exhibited altered swimming behaviour.  
403 Altogether, these results support the hypothesis that the smallest MPs at high concentrations  
404 can trigger deleterious effects on early life stage of Pacific oyster over short-term exposure.  
405 Future studies are needed to verify whether LDPE MPs have similar effects on D-larvae and  
406 investigate the impacts of ingestion of nanometer-sized PE particles on D-larvae. It would  
407 also be particularly relevant to evaluate the long-term effects of chronic exposure to  
408 environmental concentrations of MPs.  
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412 **Figure 6.** D-larva with MP stuck in her locomotor eyelashes. Screenshot from a video recorded with  
413 the ZEISS Axio Observer Z1 microscope (x20). In order to observe MP behaviour, we used  
414 fluorescent microbeads (1-5  $\mu\text{m}$ , Cospheric).

415

## 416 **5. Conclusions**

417 In conclusion, our experimental results highlight that small (4-6  $\mu\text{m}$ ) and medium sizes (11-13  
418  $\mu\text{m}$ ) HDPE particles at high concentrations are toxic for *C. gigas* early life stage. However, in  
419 comparison to the effects of Cu, the toxicity of HDPE MPs is much lower. Our data suggest  
420 that small HDPE particles (4-6  $\mu\text{m}$ ) are the most toxic to the embryo-larval development of  
421 oyster. PE of 20-25  $\mu\text{m}$  had very little effect on development anomalies and developmental  
422 arrest. With regard to swimming behaviour, D-larvae of *C. gigas* are more sensitive to PE  
423 particles < 15  $\mu\text{m}$ . Further research on early life stages of bivalves and other invertebrates, is  
424 crucial in strengthening the knowledge base required to establish recommendations and  
425 potential long-term effects of using plastics in coastal areas and estuaries.

426

## 427 **Supplementary data**

428 Supplementary data associated with this article can be found in the online version.

429

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442

#### 443 **References**

444 AFNOR, XP T90-382 Septembre 2009. Qualité de l'eau – Bio indicateur de la toxicité  
445 potentielle de milieux aqueux – Détermination de la toxicité potentielle d'échantillons aqueux  
446 sur le développement embryon-larvaire de bivalve – T90-382.

447 Andrady, A. L., & Neal, M. A., 2009. Applications and societal benefits of  
448 plastics. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 364 (1526),  
449 1977-1984.

450 Au, S. Y., Bruce, T. F., Bridges, W. C., & Klaine, S. J., 2015. Responses of *Hyalella azteca* to  
451 acute and chronic microplastic exposures. *Environmental Toxicology and Chemistry*, 34 (11),  
452 2564-2572.

453 Auta, H. S., Emenike, C. U., & Fauziah, S. H., 2017. Distribution and importance of  
454 microplastics in the marine environment: a review of the sources, fate, effects, and potential  
455 solutions. *Environment international*, 102, 165-176. Avio, C. G., Gorbi, S., & Regoli, F., 2017.  
456 Plastics and microplastics in the oceans: From emerging pollutants to emerged  
457 threat. *Marine Environmental Research*, 128, 2-11.

458 Baer, A., Langdon, C., Mills, S., Schulz, C., & Hamre, K., 2008. Particle size preference, gut  
459 filling and evacuation rates of the rotifer *Brachionus* "Cayman" using polystyrene latex  
460 beads. *Aquaculture*, 282 (1-4), 75-82.

461 Bayne, B.L., Ahrens, M., Allen, S.K., D'auriac, M.A., Backeljau, T., Beninger, P., Bohn, R.,  
462 Boudry, P., Davis, J., Green, T., Guo, X., Hedgecock, D., Ibarra, A., Kingsley- Smith, P.,  
463 Krause, M., Langdon, C., Lapègue, S., Li, C., Manahan, D., Mann, R., Perez-Paralle, L.,  
464 Powell, E.N., Rawson, P.D., Speiser, D., Sanchez, J.-L., Shumway, S., Wang, H., 2017. The  
465 proposed dropping of the genus *Crassostrea* for all pacific cupped oysters and its  
466 replacement by a new genus *Magallana*: a dissenting view. *Journal of Shellfish Research*, 36  
467 (3), 545-548.

468 Bayne, B., d'Auriac, M. A., Backeljau, T., Beninger, P., Boudry, P., Carnegie, R., & Langdon,  
469 C., 2019. A scientific name for Pacific oysters. *Aquaculture*, 499, 373.

470 Beiras, R., Bellas, J., Cachot, J., Cormier, B., Coussin, X., Engwall, M., Gambardella, C.,  
471 Garaventa, F., Keiter, S., Le Bihanic, F, Lopez-Ibanez, S., Piazza, V., Rial, D., Tato, T.,  
472 Vidal-Linan, L., 2018. Ingestion and contact with polyethylene micro- plastics does not cause  
473 acute toxicity on marine zooplankton. *Journal Hazardous Materials*, 360, 452e460.

474 Bergami, E., Bocci, E., Vannuccini, M. L., Monopoli, M., Salvati, A., Dawson, K. A., & Corsi,  
475 I., 2016. Nano-sized polystyrene affects feeding, behavior and physiology of brine shrimp  
476 *Artemia franciscana* larvae. *Ecotoxicology and Environmental Safety*, 123, 18-25.

477 Bergmann, M., Wirzberger, V., Krumpfen, T., Lorenz, C., Primpke, S., Tekman, M. B., &  
478 Gerdts, G., 2017. High quantities of microplastic in Arctic deep-sea sediments from the  
479 Hausgarten observatory. *Environmental Science & Technology*, 51 (19), 11000-11010.

480 Capolupo, M., Franzellitti, S., Valbonesi, P., Lanzas, C. S., & Fabbri, E., 2018. Uptake and  
481 transcriptional effects of polystyrene microplastics in larval stages of the Mediterranean  
482 mussel *Mytilus galloprovincialis*. *Environmental Pollution*, 241, 1038-1047.

483 Caruso, G., 2015. Plastic degrading microorganisms as a tool for bioremediation of plastic  
484 contamination in aquatic environments. *Pollution Effects and Control* 3, e112.

485 CNC (Comité National de la Conchyliculture), 2016. [http://www.cnc-france. com/La-](http://www.cnc-france.com/La-)  
486 [Production-francaise.aspx](http://www.cnc-france.com/La-Production-francaise.aspx) (accessed August 2019).

487 Cole, M., Lindeque, P., Halsband, C., Galloway, T.S., 2011. Microplastics as contaminants in  
488 the marine environment: A review. *Marine Pollution Bulletin*, 62, 2588-2597.

489 Cole, M., & Galloway, T. S., 2015. Ingestion of nanoplastics and microplastics by Pacific  
490 oyster larvae. *Environmental Science & Technology*, 49 (24), 14625-14632.

491 Détrée, C., & Gallardo-Escárate, C., 2017. Polyethylene microbeads induce transcriptional  
492 responses with tissue-dependent patterns in the mussel, *Mytilus galloprovincialis*. *Journal of*  
493 *Molluscan Studies*, 83 (2), 220-225.

494 Enders, K., Lenz, R., Stedmon, C. A., & Nielsen, T. G., 2015. Abundance, size and polymer  
495 composition of marine microplastics  $\geq 10 \mu\text{m}$  in the Atlantic Ocean and their modelled vertical  
496 distribution. *Marine pollution bulletin*, 100(1), 70-81.

497 Frere, L., Paul-Pont, I., Rinnert, E., Petton, S., Jaffre, J., Bihannic, I., Soudant, P., Lambert,  
498 C., Huvet, A., 2017. Influence of environmental and anthropogenic factors on the  
499 composition, concentration and spatial distribution of microplastics: a case study of the Bay  
500 of Brest (Brittany, France). *Environmental Pollution*, 225, 211-222.

501 Gamain, P., Gonzalez, P., Cachot, J., Pardon, P., Tapie, N., Gourves, P.Y., Budzinski, H.,  
502 Morin, B., 2016. Combined effects of pollutants and salinity on embryo-larval development of  
503 the Pacific oyster, *Crassostrea gigas*. *Marine Environmental Research*, 113, 31-38.

504 Gamain, P., Gonzalez, P., Cachot, J., Clérandeau, C., Mazzella, N., Gourves, P. Y., & Morin,  
505 B., 2017. Combined effects of temperature and copper and S-metolachlor on embryo-larval  
506 development of the Pacific oyster, *Crassostrea gigas*. *Marine Pollution Bulletin*, 115 (1-2),  
507 201-210.

508 Gamain, P., Roméro-Ramirez, A., Gonzalez, P., Mazzella, N., Gourves, P. Y., Compan, C.,  
509 Morin, B., Cachot, J., 2019. Assessment of swimming behavior of the Pacific oyster D-larvae  
510 (*Crassostrea gigas*) following exposure to model pollutants. *Environmental Science and*  
511 *Pollution Research*, 1-11.

512 Gatidou, G., & Thomaidis, N. S., 2007. Evaluation of single and joint toxic effects of two  
513 antifouling biocides, their main metabolites and copper using phytoplankton  
514 bioassays. *Aquatic Toxicology*, 85 (3), 184-191.

515 Gerritsen, J., & Porter, K. G., 1982. The role of surface chemistry in filter feeding by  
516 zooplankton. *Science*, 216 (4551), 1225-1227.

517 Gigault, J., Pedrono, B., Maxit, B., & Ter Halle, A., 2016. Marine plastic litter: the unanalyzed  
518 nano-fraction. *Environmental Science: Nano*, 3 (2), 346-350.

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

521 Green, D. S., Colgan, T. J., Thompson, R. C., & Carolan, J. C., 2019. Exposure to  
522 microplastics reduces attachment strength and alters the haemolymph proteome of blue  
523 mussels (*Mytilus edulis*). *Environmental Pollution*, 246, 423-434.

524 Hart, M. W., 1991. Particle captures and the method of suspension feeding by echinoderm  
525 larvae. *The Biological Bulletin*, 180 (1), 12-27.

526 His, E., Seaman, M. N. L., & Beiras, R., 1997. A simplification the bivalve embryogenesis  
527 and larval development bioassay method for water quality assessment. *Water*  
528 *Research*, 31(2), 351-355.

529 His, E., Heyvang, I., Geffard, O., & De Montaudouin, X., 1999. A comparison between oyster  
530 (*Crassostrea gigas*) and sea urchin (*Paracentrotus lividus*) larval bioassays for toxicological  
531 studies. *Water research*, 33 (7), 1706-1718.

532 Holland, N. D., Strickler, J. R., & Leonard, A. B., 1986. Particle interception, transport and  
533 rejection by the feather star *Oligometra serripinna* (Echinodermata: Crinoidea), studied by  
534 frame analysis of videotapes. *Marine Biology*, 93 (1), 111-126.

535 Isobe, A., Kubo, K., Tamura, Y., Nakashima, E., & Fujii, N., 2014. Selective transport of  
536 microplastics and mesoplastics by drifting in coastal waters. *Marine pollution bulletin*, 89 (1-  
537 2), 324-330.

538 Jambeck, J., Geyer, R., Wilcox, C., Siegler, T.R., Perryman, M., Andrady, A.L., Narayan, R.,  
539 Law, K.L., 2015. Plastic waste inputs from land into the ocean. *Science* 347, 768-771.

540 Kashiwada, S., 2006. Distribution of nanoparticles in the see-through medaka (*Oryzias*  
541 *latipes*). *Environmental Health Perspectives*, 114 (11), 1697-1702.

542 Kershaw, P., Katsuhiko, S., Lee, S., & Woodring, D. (2011). Plastic debris in the ocean.  
543 United Nations Environment Programme.

544 Koelmans, A. A., Besseling, E., & Shim, W. J., 2015. Nanoplastics in the aquatic  
545 environment. Critical review. In *Marine anthropogenic litter* (pp. 325-340). Springer, Cham.

546 Lambert, S., & Wagner, M., 2016. Characterisation of nanoplastics during the degradation of  
547 polystyrene. *Chemosphere*, 145, 265-268.



548 Lee, K. W., Shim, W. J., Kwon, O. Y., & Kang, J. H. (2013). Size-dependent effects of micro  
549 polystyrene particles in the marine copepod *Tigriopus japonicus*. *Environmental Science &*  
550 *Technology*, 47(19), 11278-11283.

551 Long, M., Paul-Pont, I., Hegaret, H., Moriceau, B., Lambert, C., Huvet, A., & Soudant, P.  
552 (2017). Interactions between polystyrene microplastics and marine phytoplankton lead to  
553 species-specific hetero-aggregation. *Environmental Pollution*, 228, 454-463.

554 MacInnes, J. R., & Calabrese, A., 1979. Combined effects of salinity, temperature, and  
555 copper on embryos and early larvae of the American oyster, *Crassostrea virginica*. *Archives*  
556 *of Environmental Contamination and Toxicology*, 8(5), 553-562.

557 Mai, H., Cachot, J., Brune, J., Geffard, O., Belles, A., Budzinski, H., & Morin, B., 2012.  
558 Embryotoxic and genotoxic effects of heavy metals and pesticides on early life stages of  
559 Pacific oyster (*Crassostrea gigas*). *Marine Pollution Bulletin*, 64 (12), 2663-2670.

560 Mai, H., Morin, B., Pardon, P., Gonzalez, P., Budzinski, H., & Cachot, J., 2013.  
561 Environmental concentrations of irgarol, diuron and S-metolachlor induce deleterious effects  
562 on gametes and embryos of the Pacific oyster, *Crassostrea gigas*. *Marine Environmental*  
563 *Research*, 89, 1-8.

564 Mattsson, K., Ekvall, M. T., Hansson, L. A., Linse, S., Malmendal, A., & Cedervall, T., 2014.  
565 Altered behavior, physiology, and metabolism in fish exposed to polystyrene  
566 nanoparticles. *Environmental Science & Technology*, 49 (1), 553-561.

567 Mayer, S. (1994). Particle capture in the crown of the ciliary suspension feeding polychaete  
568 *Sabella penicillus*: videotape recordings and interpretations. *Marine Biology*, 119 (4), 571-  
569 582.

570 Mesaric, T., Gambardella, C., Milivojevic, T., Faimali, M., Drobne, D., Falugi, C., Makovec,  
571 D., Jemec, A., Sepcic, K., 2015. High surface adsorption properties of carbon-based  
572 nanomaterials are responsible for mortality, swimming inhibition, and biochemical responses  
573 in *Artemia salina* larvae. *Aquatic Toxicology*, 163, 121-129.

574 Mottier, A., Kientz-Bouchart, V., Serpentine, A., Lebel, J. M., Jha, A. N., & Costil, K., 2013.  
575 Effects of glyphosate-based herbicides on embryo-larval development and metamorphosis in  
576 the Pacific oyster, *Crassostrea gigas*. *Aquatic Toxicology*, 128, 67-78.

577 NF ISO 17244, 2015 - Qualité de l'eau. Détermination de la toxicité d'échantillons aqueux sur  
578 le développement embryo-larvaire de l'huître creuse (*Crassostrea gigas*) et de la moule  
579 (*Mytilus edulis* ou *Mytilus galloprovincialis*).

580 Parker, L. M., Ross, P. M., & O'Connor, W. A., 2009. The effect of ocean acidification and  
581 temperature on the fertilization and embryonic development of the Sydney rock oyster  
582 *Saccostrea glomerata* (Gould 1850). *Global Change Biology*, 15(9), 2123-2136.

583 Paul-Pont, I., Tallec, K., Gonzalez Fernandez, C., Lambert, C., Vincent, D., Mazurais, D.,  
584 Zambonino-Infante, J.-L., Brotons, G., Lagarde, F., Fabioux, F., Soudant, P., Huvet, A.,  
585 2018. Constraints and priorities for conducting experimental exposures of marine organisms  
586 to microplastics. *Frontiers in Marine Science*, 5 (252).

587 PlasticsEurope, 2018. Plastics – The Facts 2018. Brussels, Belgium: PlasticsEurope.  
588 <https://www.plasticseurope.org/fr/resources/publications/619-plastics-facts-2018>

589 Robinson, A., 1992. Gonadal cycle of *Crassostrea gigas* kumamoto (Thunberg) in Yaquina  
590 Bay, Oregon and optimum conditions for broodstock oysters and larval  
591 culture. *Aquaculture*, 106 (1), 89-97.

592 de Sá, L. C., Oliveira, M., Ribeiro, F., Rocha, T. L., & Futter, M. N., 2018. Studies of the  
593 effects of microplastics on aquatic organisms: what do we know and where should we focus  
594 our efforts in the future? *Science of the Total Environment*, 645, 1029-1039.

595 Solow, A. R., & Gallagher, S. M. (1990). Analysis of capture efficiency in suspension feeding:  
596 application of nonparametric binary regression. *Marine Biology*, 107(2), 341-344.

597 Suquet, M., Le Mercier, A., Rimond, F., Mingant, C., Haffray, P., & Labbe, C., 2012. Setting  
598 tools for the early assessment of the quality of thawed Pacific oyster (*Crassostrea gigas*) D-  
599 larvae. *Theriogenology*, 78 (2), 462-467.

600 Sussarellu, R., Suquet, M., Thomas, Y., Lambert, C., Fabioux, C., Pernet, M.E.J., Goïc, N.L.,  
601 Quillien, V., Mingant, C., Epelboin, Y., Charlotte, C., Julien, G., Johan, R., Ika, P.-P.,

602 Philippe, S., Arnaud, H., 2016. Oyster reproduction is affected by exposure to polystyrene  
603 microplastics. *Proceedings of the National Academy of Sciences*, 113 (9), 2430-2435.

604 Sussarellu, R., Lebreton, M., Rouxel, J., Akcha, F., & Rivière, G., 2018. Copper induces  
605 expression and methylation changes of early development genes in *Crassostrea gigas*  
606 embryos. *Aquatic Toxicology*, 196, 70-78.

607 Sutherland, W.J. et al. (2016) A horizon scan of global conservation issues for 2016. *Trends*  
608 *in Ecology & Evolution*, 31(1), 44-53.

609 Tallec, K., Huvet, A., Di Poi, C., Gonzalez-Fernandez, C., Lambert, C., Petton, B., Le Goic,  
610 N., Berchel, M., Soudant, P., Paul-Pont, I., 2018. Nanoplastics impaired oyster free living  
611 stages, gametes and embryos. *Environmental Pollution*, 242, 1226-1235.

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

615 Von Moos, N., Burkhardt-Holm, P., & Köhler, A., 2012. Uptake and effects of microplastics  
616 on cells and tissue of the blue mussel *Mytilus edulis* L. after an experimental  
617 exposure. *Environmental Science & Technology*, 46 (20), 11327-11335.

618 Wagner, M., Scherer, C., Alvarez-Munoz, D., Brennholt, N., Bourrain, X., Buchinger, S.,  
619 Fries, E., Grosbois, C., Klasmeier, J., Marti, T., Rodriguez-Mozaz, S., Urbatzka, R., Vethaak,  
620 A., Winther-Nielsen, M., Reifferscheid, G., 2014. Microplastics in freshwater ecosystems:  
621 what we know and what we need to know. *Environmental Sciences Europe*, 26 (1), 12.

622 Wang, W. X., Yang, Y., Guo, X., He, M., Guo, F., & Ke, C., 2011. Copper and zinc  
623 contamination in oysters: subcellular distribution and detoxification. *Environmental*  
624 *Toxicology and Chemistry*, 30 (8), 1767-1774.

625 Wang, J., Tan, Z., Peng, J., Qiu, Q., & Li, M., 2016. The behaviors of microplastics in the  
626 marine environment. *Marine Environmental Research*, 113, 7-17.

627 Ward, J. E., & Targett, N. M., 1989. Influence of marine microalgal metabolites on the  
628 feeding behavior of the blue mussel *Mytilus edulis*. *Marine Biology*, 101 (3), 313-321.

629 Ward, J. E. (1996). Biodynamics of suspension-feeding in adult bivalve molluscs: particle  
630 capture, processing, and fate. *Invertebrate Biology*, Vol. 115 (3), 218-231.

631 Williams, L. G., 1978. Influence of Algal Cell Volume and Algal Culture Filtrates on  
632 Suspension Feeding Behavior of the Gastropod *Crepidula Fornicata* (Prosobranchiata:  
633 Calyptraeidae) (Doctoral dissertation, University of Delaware).

634 Yin, L., Liu, H., Cui, H., Chen, B., Li, L., & Wu, F., 2019. Impacts of polystyrene microplastics  
635 on the behavior and metabolism in a marine demersal teleost, black rockfish (*Sebastes*  
636 *schlegelii*). *Journal of Hazardous Materials*, 380, 120861.

637 Yoshida, S., Hiraga, K., Takehana, T., Taniguchi, I., Yamaji, H., Maeda, Y., Toyohara, K.,  
638 Miyamoto, K., Kimura, Y., and Oda, K., 2016. A bacterium that degrades and assimilates  
639 poly(ethylene terephthalate). *Science*, 351 (6278), 1196-1199.

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657 **SUPPLEMENTARY DATA**

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659 **Table S1.** Frequency of abnormal D-larvae (%) and developmental arrest (%) in D-larvae of  
 660 *C. gigas* exposed to Cu or PE MPs. Control (without pollutants): 0.1, 1 and 10 correspond to  
 661 concentrations of copper ( $\mu\text{g Cu.L}^{-1}$ ) or PE MP ( $\text{mg MP.L}^{-1}$ ) using for exposures. Means  
 662 values  $\pm$  SEM. Different letters indicated significant differences between different  
 663 concentrations ( $p < 0.05$ ).

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<b>Conditions</b>	<b>Abnormal larvae</b>	<b>Developmental arrest</b>
<b>Control</b>	12.8 $\pm$ 1.3 <sup>a</sup>	4.6 $\pm$ 0.5 <sup>a</sup>
<b>Cu 0,1</b>	42.5 $\pm$ 6.4 <sup>b</sup>	12.9 $\pm$ 2.5 <sup>b</sup>
<b>Cu 1</b>	43.2 $\pm$ 7.2 <sup>b</sup>	15.9 $\pm$ 3.2 <sup>b</sup>
<b>Cu 10</b>	30.2 $\pm$ 3.3 <sup>b</sup>	37.8 $\pm$ 9.7 <sup>c</sup>
<b>MP (4-6 <math>\mu\text{m}</math>) 0,1</b>	38.1 $\pm$ 7.1 <sup>b</sup>	7.6 $\pm$ 1.1 <sup>ab</sup>
<b>MP (4-6 <math>\mu\text{m}</math>) 1</b>	37.7 $\pm$ 5.0 <sup>b</sup>	10.2 $\pm$ 1.1 <sup>b</sup>
<b>MP (4-6 <math>\mu\text{m}</math>) 10</b>	50.1 $\pm$ 4.8 <sup>b</sup>	12.1 $\pm$ 1.9 <sup>b</sup>
<b>MP (11-13 <math>\mu\text{m}</math>) 0,1</b>	19.7 $\pm$ 3.0 <sup>ab</sup>	9.9 $\pm$ 1.0 <sup>b</sup>
<b>MP (11-13 <math>\mu\text{m}</math>) 1</b>	25.4 $\pm$ 2.4 <sup>b</sup>	13.1 $\pm$ 2.5 <sup>b</sup>
<b>MP (11-13 <math>\mu\text{m}</math>) 10</b>	30.5 $\pm$ 3.8 <sup>b</sup>	17.7 $\pm$ 0.7 <sup>c</sup>
<b>MP (20-25 <math>\mu\text{m}</math>) 0,1</b>	11.8 $\pm$ 1.0 <sup>ab</sup>	9.0 $\pm$ 1.3 <sup>a</sup>
<b>MP (20-25 <math>\mu\text{m}</math>) 1</b>	10.6 $\pm$ 1.5 <sup>a</sup>	7.1 $\pm$ 1.5 <sup>a</sup>
<b>MP (20-25 <math>\mu\text{m}</math>) 10</b>	16.3 $\pm$ 1.5 <sup>b</sup>	10.7 $\pm$ 0.7 <sup>a</sup>

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671 **Table S2.** Maximum and average speeds ( $\mu\text{m}\cdot\text{s}^{-1}$ ) recorded for D-larvae of *C. gigas* exposed  
 672 for 24h to different concentrations of Cu or PE MPs. Control (without pollutants): 0.1, 1 and  
 673 10 correspond to concentrations of copper ( $\mu\text{g Cu}\cdot\text{L}^{-1}$ ) or PE MP ( $\text{mg MP}\cdot\text{L}^{-1}$ ) used for  
 674 exposures. Means values  $\pm$  SEM. Different letters indicated significant differences between  
 675 different concentrations ( $p < 0.05$ ).  
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Conditions	Maximum speed	Average speed
<b>Control</b>	$192 \pm 7^a$	$109 \pm 3^a$
<b>Cu 0,1</b>	$175 \pm 4^a$	$120 \pm 3^b$
<b>Cu 1</b>	$150 \pm 6^b$	$97 \pm 4^c$
<b>MP (4-6 <math>\mu\text{m}</math>) 0,1</b>	$151 \pm 5^b$	$88 \pm 4^b$
<b>MP (4-6 <math>\mu\text{m}</math>) 1</b>	$119 \pm 6^c$	$66 \pm 4^c$
<b>MP (11-13 <math>\mu\text{m}</math>) 0,1</b>	$192 \pm 8^b$	$125 \pm 5^b$
<b>MP (11-13 <math>\mu\text{m}</math>) 1</b>	$155 \pm 6^c$	$100 \pm 4^c$
<b>MP (20-25 <math>\mu\text{m}</math>) 0,1</b>	$128 \pm 19^a$	$61 \pm 8^b$
<b>MP (20-25 <math>\mu\text{m}</math>) 1</b>	$96 \pm 17^a$	$49 \pm 9^c$

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**Graphical abstract**

