

Intergenerational effects of environmentally-aged microplastics on the Crassostrea gigas

Arno Bringer, Jérôme Cachot, Emmanuel Dubillot, Grégoire Prunier, Valérie Huet, Christelle Clérandeau, Louise Evin, Hélène Thomas

▶ To cite this version:

Arno Bringer, Jérôme Cachot, Emmanuel Dubillot, Grégoire Prunier, Valérie Huet, et al.. Intergenerational effects of environmentally-aged microplastics on the Crassostrea gigas. Environmental Pollution, 2022, 294, pp.118600. 10.1016/j.envpol.2021.118600. hal-03482565

HAL Id: hal-03482565 https://hal.science/hal-03482565

Submitted on 8 Jan 2024

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers. L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NonCommercial 4.0 International License

1 Intergenerational effects of environmentally-aged microplastics on the 2 *Crassostrea gigas*.

- 3
- 4 <u>Arno Bringer¹⁺</u>, Jérôme Cachot², Emmanuel Dubillot¹, Grégoire Prunier¹, Valérie Huet¹, Christelle Clérandeau²,
- 5 Louise Evin¹, Hélène Thomas¹
- 6
- 7 ¹Littoral Environnement et Sociétés (LIENSs), UMR 7266, CNRS-Université de La Rochelle, 2 rue Olympe de
- 8 Gouges, F-17042 La Rochelle Cedex 01, France.
- 9 ² Université de Bordeaux, CNRS, EPOC, EPHE, UMR 5805, F-33600 Pessac, France.
- 10 *Corresponding author:* email: arno.bringer1@univ-lr.fr
- 11

12 Abstract

13 This study focused on the impacts of aged aquaculture microplastics (MPs) on oysters (Crassostrea 14 gigas). Adult oysters were exposed for two months to a cocktail of MPs representative of the 15 contamination of the Pertuis Charentais area (Bay of Biscay, France) and issuing from oyster framing 16 material. The MPs mixture included 28 % of polyethylene, 40 % of polypropylene and 32 % of PVC 17 (polyvinyl chloride). During the exposure, tissues were sampled for various analyzes (MP 18 quantification, toxicity biomarkers). Although no effect on the growth of adult oysters was noted, the 19 mortality rate of bivalves exposed to MPs (0.1 and 10 mg, $L^{-1}MP$) increased significantly (respectively 20 13.3 and 23.3 % of mortalities cumulative). On the one hand, the responses of biomarkers revealed 21 impacts on oxidative stress, lipid peroxidation and environmental stress. At 56 days of exposure, 22 significant increases were noted for Glutathione S-Transferase (GST, 10 mg. L⁻¹MP), Malondialdehyde 23 (MDA, 10 mg, L^{-1} MP) and Laccase (LAC, 0.1 and 10 mg, L^{-1} MP). No variations were observed for 24 Superoxyde Dismutase (SOD). Besides, ingestion of MPs in oyster tissues and the presence in 25 biodeposits was highlighted. In addition, in vitro fertilisations were performed to characterize MPs 26 effects on the offspring. Swimming behavior, development and growth of D-larvae were analyzed at 27 24-, 48- and 72-hours after fertilisation. D-larvae, from exposed parents, demonstrated reduced 28 locomotor activity. Developmental abnormalities and arrest as well as growth retardation were also

- 29 noted. This study highlighted direct and intergenerational effects of MPs from aged plastic materials on
- 30 Pacific oysters.
- 31

32 Graphical abstract



Plastic oyster equipment

33

Keywords Oyster, microplastics, reproduction, larval behaviour, development and growth,
 intergenerational effects, *Crassostrea gigas*

72 h

36

37 **1. Introduction**

38 The growing use of plastics dramatically increases their production in Europe (57.9 million tons,

- 39 PlasticsEurope, 2020) and in the World (368 million tons). Whether from land, atmosphere or rivers,
- 40 anthropogenic pollutants, including plastics, end up in the marine environment (Halpern *et al.*, 2008).
- 41 In recent years, microplastics (MPs) has drawn the public's attention. MPs are defined as plastic particles
- 42 between 1 µm and 5 mm (Cole *et al.*, 2011; Wagner *et al.*, 2014), while nanoplastics (NPs) are smaller

43 than 100 nm (Lambert & Wagner, 2016; Gigault et al., 2016). MPs are spread in all oceans (Barnes et 44 al., 2009), including in the Arctic Ocean (Zarfl & Matthies, 2010). The MP size range distribution in 45 the environment depends on hydrodynamic conditions, and degradation processes (Enders et al., 2015). 46 MPs occurrence in ocean is variable over time and space. Oceanic gyres and coastal areas as hot spots 47 of MP accumulation (Law et al., 2010). In Europe, 73 % of the plastic production resorts to 6 polymers 48 (PlasticsEurope, 2020): polypropylene (PP), high and low-density polyethylene (HDPE and LDPE), 49 polyvinyl chloride (PVC), polyurethane (PUR), polyethylene terephthalate (PET) and polystyrene (PS). 50 Once ingested, MPs cause abrasions, obstructions and alterations of physiological structures, affecting 51 the survival of individuals (Wright et al., 2013). MPs can also generate chemical damage, as they 52 contained a large variety of additives (Pérez-Albaladejo et al., 2020; Hermabessiere et al., 2017) and 53 can sorbed persistent organic pollutants and heavy metals (Rochman et al., 2013; Bakir et al., 2014; 54 Gorman et al., 2019; Naik et al., 2019). Recent studies have documented the potential effects of MPs 55 on marine organisms and in particular bivalves (Tallec et al., 2018; Sussarellu et al., 2016; Revel et al., 56 2020). However, most of the MPs studied were of commercial origin and very few studies focused on 57 the impacts of environmental MPs (Pannetier et al., 2020; Cormier et al., 2021; Gardon et al., 2020). 58 Bivalves are commonly used in studies to assess water quality and habitat. These filter feeders, in 59 particular Crassostrea gigas oysters, are sensitive to alterations in water quality, which makes them 60 good bioindicators and bioaccumulators for the study of pollutants (O'Connor, 2002), and in particular 61 of MPs (Ward et al., 2019; Bonanno & Orlando-Bonaca, 2018). In fact, ovsters (C. gigas) are considered 62 sentinel species of anthropized coastal ecosystems, due to their wide distribution, their sedentary 63 lifestyle, and their sensitivity to a large range of pollutants (Conger et al., 1978; His et al., 1999; Quiniou 64 et al., 2007). Found in the bivalve tissues, MPs appear as fibres, fragments or granules (Cho et al., 2019; 65 Jin-Feng et al., 2018). MP size and shape vary according to the tissue and species (Li et al., 2015). 66 Phuong et al. (2018a) highlighted that MP contamination fluctuated according to seasons, species and

the tissues of oysters living on the French Atlantic coasts (Van Cauwenbergh & Janssen, 2014; Phuong

their living conditions (wild or farmed). Several previous studies have shown the presence of MPs in

67

69 et al., 2018b). Sussarelu et al. (2016) demonstrated that the PS-MPs ingestion altered energy flows,

70 metabolism and homeostasis in oysters, resulting in a loss of energy dedicated to their reproductive

71 function. They also noted impacts on the gametogenesis, with a decrease in quantity and quality of the 72 gametes produced. Several studies underlined MPs effects on adult bivalves - including on their 73 gametogenesis mechanism, and early larval stages. Cole & Galloway (2015) demonstrated that oyster 74 larvae ingested PS-MPs, although their study differed in terms of larval stages and particle sizes. Tallec 75 et al. (2018) reported a significant increase in malformation and developmental arrest (early mortality) 76 frequencies in oyster larvae exposed to PS-NPs. Bringer et al. (2020b) studied the ingestion and effects 77 of fluorescent MPs (mixed polymers) on oyster larvae. They documented that 72h-old D-larvae ingested 78 MPs, which were detected in their digestive tract. This study also highlighted that MPs are able to stick 79 to the locomotor cilia of D-larvae, influencing their swimming behaviour at 24-h of development. In 80 addition, Bringer et al. (2020a) demonstrated that an exposure to 4-13 µm HDPE-MPs, affected the 81 oyster maximum swimming speed and trajectories of oyster larvae. A recent study focused on effects 82 environmental MPs on the early stages of the pearl oyster, Pinctada margaritifera (Gardon et al., 2020). 83 This work documented the impacts of aged plastics on the embryo-larval and larval stages, leading to 84 malformations and mortalities depending on the tested concentrations (Gardon et al., 2020).

Aquaculture professionals regularly use plastic tools (Lusher *et al.*, 2017). Following extreme hydroclimatic events (storms, winds or strong currents, etc.), professional materials become detached and drift towards beaches and coasts during high tides. Plastics, used in French oyster farming, could deteriorate, fragment and become toxic when broken down into particles of MPs/NPs. In addition, the additives released could potentially be more harmful to the marine environment than the polymer itself (phthalates, PAHs, metals ; Bringer *et al.*, 2021b). The French shellfish market is important. The sustainability of shellfish companies depends on the quality of the water.

This study aimed at assessing effects of environmentally-aged MPs (from shellfish farming) on adult oysters (*C. gigas*) and their offspring. The MPs used (138.6 \pm 2.3 µm) were a cocktail of three different aged-polymers: oyster crops (HDPE), collector cups (PP) and collector tubes (PVC). The data obtained included: (i) MP exposure of two-month-old adult oysters, with a follow-up on survival, biometrics, potential ingestion/excretion (tissues/biodeposits) and biomarker responses; (ii) larvae from exposed parents, with a 72-hour follow-up on swimming behaviour, development (malformations and developmental arrest) and length growth.

100 2. Materials and Method

101 2.1 Preparation and analysis of environmentally-aged cocktail of MPs

102 Plastic macrodebris were recovered on the French Atlantic beach (South-West, France), more precisely 103 in the Angoulins-sur-Mer municipality (Bringer et al., 2021b). These were sorted to identify plastics 104 from the oyster farming industry. HDPE oyster culture crops, PP cup collectors and PVC tube collectors, 105 were selected. A GC-MS pyrolysis analysis (700 °C) of the three plastic materials selected (manual 106 cutting of 130-200 µg samples) allowed the identification of the main polymer. After a rough cut (3-4 107 cm), the plastics were structured using a stationary metal hammer, crushing the plastics several times. Then, the plastics were sees is sees in a more service out at the second s 108 109 room temperature so as *sephot* to influence the nature of the polymers materials. MPs cocktail of 138.6 110 $\pm 2.3 \,\mu$ m were recovered (three analytical replicates in laser granulometry, Malverne ®). MPs cocktail 111 was composed of 28 % HDPE, 40 % PP and 32 % PVC (Andrady, 2011). Each solution was prepared 112 in pure Milli-Q-water (Millipore ®) and kept in the dark at 4 °C (Figure 1). To carry out a quantitative analysis of MPs in experimental seawater, each cocktail solution (0.1, and 10 mg. L⁻¹ MP) was tested 113 114 through a flow cytometer (Attune Acoustic Focusing Cytometer ®) according to the protocole described 115 previously (Bringer et al., 2020a; Bringer et al., 2020b). Two-millilitre samples from each MP 116 exposition solution were vortexed (StarLab Vortex IR ®, 12,000 rpm for 20 sec) to homogenise the 117 solutions and then 300 μ L were taken for flow cytometry analysis. A calibration was carried out, thanks 118 to previous studies (Bringer et al., 2020a) with commercial MPs, to achieve an analysis rate of 500 119 μ L.min⁻¹ and a saturation of 10,000 particles maximum detected. From the control conditions (10 μ m 120 filtered seawater), control analytical sepsamples (n=12) were created. The blank obtained enabled to 121 remove background particles (natural present in seawater). Using seawater filtered at 0.2 µm, a first 122 calibration was conducted to select the < 200-time detected particles. MPs measurements in seawater 123 were assessed at T_1 (D₁₇), T_2 (D₃₅) and T_3 (D₅₆) for all exposure conditions.



Figure 1. Experimental design of adult oysters exposed to MPs cocktail obtained from different aged-plastic
materials used in French oyster farms.

129 2.2 Experimental exposure of adult oysters and larvae

130 In June 2019, adult oysters Crassostrea gigas (Bayne et al., 2019; Bayne et al., 2017) purchased from 131 a commercial hatchery (24 mo, 15.75 ± 1.5 g) were transferred to our laboratory. After a 7-day 132 acclimatisation period, the oysters were placed in 9 experimental tanks of 37.5 L (n=30 oysters per tank, 133 with three replicates tanks per condition) with 3 large buffer tanks (one buffer tank per condition). Tanks 134 were supplied with filtered (10 µm) UV-treated (78.3 L.h⁻¹) running seawater (filtered seawater, FSW) 135 at a temperature of 18.5 ± 0.1 °C, salinity of 35.3 ± 0.06 and natural photoperiod. During the experiment, 136 the tank temperature was gradually increased to faithfully recreate the environmental conditions of 137 spring on the French Atlantic coast (Zapata-Restrepo et al., 2019). Seawater came from the Atlantic 138 Ocean (South-West, France) in the Pertuis Breton and more precisely it was pumped in the Baie de 139 l'Aiguillon (La Rochelle, France). Once a day, oysters were fed with a mixed diet (T.Isochrysis galbana,

140 Pavlova, Tetraselmis, Thalassiosira and Nanno, Shelfish diet, VariconAqua). Tanks (n=3/condition) 141 containing the exposed oysters, were connected to a buffer tank, equipped with an air bubbling system 142 in order to aerate FSW, and carry out a continuous water renewal in a closed system. The buffer tanks 143 had the same physicochemical parameters as the exposure tanks. Every two days, water was renewed to 144 prevent bacterial growth and provide good conditions for oysters. Three conditions were set up: control 145 condition (without MPs), 0.1 mg. L⁻¹MP, and 10 mg. L⁻¹MP (Figure 2). In each buffer tank, a pumping 146 system was set up to homogenise MPs in water. After a two-month exposure, oysters were mating to 147 obtain embryos. Then, oyster larvae were grown in filtered seawater (0.2 µm) at 24 °C (optimal 148 temperature for development, Robinson, 1992) and potential effects of MPs on their swimming 149 behaviour and development were recorded at 24-, 48- and 72-hours (Figure 2).

150



152 Figure 2. Experimental design of adult oysters exposed to environmentally-aged MPs cocktail for two months.

153 After fertilisation, behaviour, development, and growth analyses were carried out on D-larvae.

154

155 2.3 Biometric analysis and survival

156 A length growth monitoring was performed on adult oysters exposed to MPs for two months. A follow-157 up was carried out at $D_0(T_0)$, $D_{17}(T_1)$, $D_{35}(T_2)$ and $D_{56}(T_3)$. Oysters (n=30/condition) were measured 158 (length, width and thickness) with a digital calliper and weighted (whole weight, wet flesh and empty 159 shell). A condition index was calculated in order to compare the growth of exposed oysters to control 160 ones. Bodoy et al. (1986) defined the index condition by the ratio of wet flesh to whole weight minus 161 empty shell. The delta condition index was the difference between the beginning and end of the exposure 162 period. A survival monitoring was carried out, removing dead oysters every two days and counting the 163 total dead oysters for each condition over the two-month period.

164

165 2.4 Microplastic analysis in adult oysters tissues and faeces

166 Methodological details have been placed in the section Supplementary data (S1).

167

168 2.5 Analyses of biochemical biomarkers in adult oysters

169 Individual sample processing

Adult oysters were removed from their experimental tank, dissected to extract shell tissue, and submerged in liquid nitrogen and stored at -80 °C for analysis. For each condition and each sampling date (T_1 , T_2 and T_3), digestive glands (n=5/conditions) were homogenised in ice-cold phosphate buffer (100 mM, pH 7.2, 1100 mOsm). The homogenates were then centrifuged for 15 min at 12,500 g at 4 °C and the Final Fractions (FF), corresponding to the supernatant, were used for biochemical assays. FF was stored at -80 °C for analyzes. Analytical protocols for each biomarker have been placed in the Supplementary data section (S2).

- 177
- 178

179 2.6 Reproduction of adult oysters after a two-month exposure

180 Mature oysters were cleaned to avoid external contaminations and remove stuck microorganisms. Male 181 and female oysters were induced to spawn by thermal stimulation (alternating immersion in FSW at 18 182 °C and 28 °C for 30 min) or by stripping the gonad (when thermal stimulation was ineffective) (Mai et 183 al., 2013; Gamain et al., 2017; Bringer et al., 2020a). Males and females were individually isolated in 184 beakers (500 mL of FSW) at their spawning temperatures (Parker et al., 2009) for 10 minutes, before 185 being removed. Eggs and sperm from two individuals were selected to create a single pairing. To 186 eliminate debris and faeces, both were sieved separately using 50 µm (sperm) and 100 µm (eggs) meshes 187 (Sefar Nitex ®). Sperm mobility was assessed and eggs counted under a microscope (Motic ®) at 100x 188 magnification. Eggs were fertilised with sperm at a ratio of 1:10 (egg:sperm). Fertilisation success was 189 studied under a microscope at 100x magnification, 1 hour after the start of the fertilisation. Embryos 190 were then counted and transferred to a 24-well microplate (Greiner Bio-One, Cellstar without detectable 191 DNase, RNase, human DNA and pyrogens (8) used in embryotoxicity according to previously published 192 protocols (His et al., 1999; Quiniou et al., 2005) and the AFNOR standard procedure (NF ISO 17244, 193 2015). The fertilized eggs (approximately 300-350 eggs) were placed in wells containing 2 mL of 194 filtered seawater (0.2 µm) and incubated in a dark climatic chamber at 24 °C for 24-, 48- and 72-hour.

195

196 2.7 Larval behaviour, development and growth

197 After a 24-, 48- and 72-hour incubation, the swimming behaviour was recorded under a microscope 198 (ZEISS Axio Observer Z1 ®) at 100x magnification in a naturally-lit air-conditioned room, set at 24 °C. 199 Two-minute videos were recorded. For the three replicates (corresponds to three different couples with 200 fertilisation success), results are expressed in mean \pm SEM, with 6 videos by couple, *i.e.*, n=18 201 videos/condition. A freeware application (VirtualDub, Windows ®) was used to subsample videos to 4 202 fps (frame per second) and convert them to AVI format (Gamain et al., 2019 and Bringer et al., 2020a,b). 203 ImageJ (1,52a software) was used to analyse the videos. These (AVI format) were first converted to 204 grayscale image, before being transformed into a stack of binary images. Swimming parameters of 205 numbered D-larvae, including their maximum speed (pixel/s) and trajectories, were calculated using the 206 wrMTrck plugin. Consequently, each larva was assigned an identification number reported in the result 207 file. Using the protocol published by Gamain et al. (2019) and improved by Bringer et al., 2020a, three 208 different types of larval path were noted: (1) rectilinear; (2) circular and (3) motionless. A graphic tablet 209 (Wacom Bamboo Pen&Touch ®) and image processing software (Photos, Windows 10) enabled to 210 quantify and characterise the swimming trajectories. Once processed with a plugin, the videos were 211 analysed to ensure each larva was only detected once. Then, 25 µL of 1 % buffered formaldehyde 212 (Sigma- Aldrich Chemical ®, St. Quentin Fallavier, France) were added to each well. The percentage 213 of abnormal D-larvae (developmental abnormalities) and developmental arrests were recorded (His et 214 al., 1999; Quiniou et al., 2005) according to the standardised AFNOR procedure (NF ISO 17244, 2015). 215 To validate the bioassays, the control condition should have contained less than 20 % of abnormal larvae 216 (NF ISO 17244, 2015). For the three replicates (three couples of oyster genitors), results are expressed 217 in mean ± SEM. In each condition, twelve wells of approximately 200 D-larvae were analysed, *i.e.*, 218 n=2400 D-larvae counted/condition.

A length growth analysis using the ImageJ software was carried out, studying larvae exposed for 24-, 48- and 72-hours (n=20/condition with three replicates, *i.e.*, a total of n=60/condition). Each larva were measured lengthwise (Talmage *et al.*, 2009; Helm *et al.*, 2006; Bringer *et al.*, 2021a). Exposed larvae were then visualised (ZEISS Axio Observer Z1, x20 objective) and photographed. Body length was determined from photographs. Results are expressed in mean \pm SEM.

224

225 *2.8 Statistics*

Data are expressed in mean \pm SEM. A logrank test, using the Kaplan-Meier method, was carried out to analyse the survival of adult oysters. In case of abnormal distribution, data were transformed using the following formula: P'=arcsin \sqrt{r} ; P corresponds to raw data (frequency of larvae abnormalities) specified in P values from 0 to 1 (Legendre & Legendre, 1998). Homogeneity of variance (Levene's test) and normality of distribution (Shapiro-Wilk) were assessed. In order to compare the various treatments, an analysis of variance (ANOVA) using Tukey's post-hoc test was performed. For data not entering in the scope of a parametric test, a statistical analysis was carried out through the Kruskal-Wallis Test.

- 233 Differences in tested concentration means were then assessed using the Kruskal Nemenyi Post-hoc test
- with the PMCMR package (the Tukey test equivalent for non-parametric data).
- 235 Significance difference was accepted when *p*-value < 0.05. Statistical analysis was completed using R
- and graphs from Microsoft Excel ®.
- 237

238 **3. Results**

239 3.1 Analysis of MP concentrations

Conditions	Sampling time	Theoretical (mg. L⁻¹ MP)	Measured (MP. μL ⁻¹)	Measured (MP. mL⁻¹)
	T1	0.1	0.68 ± 0.067	677 ± 67.6
MPs cocktail	Т2	0.1	0.66 ± 0.05	655 ± 53.0
	Т3	0.1	0.94 ± 0.06	943 ± 60.7
	T1	10	3.30 ± 0.32	3,297 ± 319.0
MPs cocktail	T2	10	3.47 ± 0.14	3,466 ± 142.7
	Т3	10	3.21 ± 0.22	3,205 ± 221.3

240 **Table 1.** Theoretical and measured MP concentrations in FSW at different sampling times.

241

Through flow cytometry, the different concentrations of the MPs cocktail were measured in the experimental waters used for the exposure of adult oysters (Table 1) at different sampling times (T_1 , T_2 and T_3). At 0.1 mg. L⁻¹ MP, very little variation was noted between the first two sampling times (T_1 and T_2), ranging from 655 ± 53.0 to 677 ± 67.6 MP.mL⁻¹. At T_3 , the concentrations of MPs increased slightly, with 943 ± 60.7 MP.mL⁻¹. At 10 mg. L⁻¹ MP, values at the three sampling times (T_1 , T_2 and T_3) were stable. However, the difference in concentration factor between the two conditions of exposure to MPs seems to be lower than theory.

249

250 3.2 State of health and reproductive effort in adult oysters

To monitor growth and shape, a condition index was built from biometric measurements on adult oysters. However, there was no significant difference in the condition index between the control and exposed oysters (0.1 and 10 mg. L⁻¹ MP) with respectively 0.36 ± 0.03 ; 0.30 ± 0.02 and 0.31 ± 0.04 . Following a two-month exposure, oyster mortality rate differed significantly between the three exposure conditions (0, 0.1 and 10 mg. L⁻¹ MP). The control condition exhibited the lowest mortality rate of 2.2

% (n=90/condition). At 0.1 mg. L⁻¹ MP, a cumulative mortality of 13.3 % was quantified. At 10 mg. L⁻¹
 ¹ MP, the mortality rate reached 23.3 % (Figure 3.a). Over the exposure period, the cumulative mortality
 rate differed significantly between the conditions tested (Figure 3.b).





Figure 3. (a) Survival monitoring of adult oysters exposed to MPs for two months: control (without pollutants); 0.1 mg. L⁻¹MP and 10 mg. L⁻¹MP. (b) Histogram of cumulative mortalities for each exposure condition. Expressed in mean \pm SEM. Different letters indicate significant differences between concentrations (*p*<0.05, Kaplan-Meir Test, n=90/condition).

266 After two months exposure under controlled laboratory conditions, some adult ovsters reached sexual 267 maturity and developed mature gonads, allowing reproduction (n=10/conditions). Six oysters from the 268 control condition emitted gametes. Seven oysters from the 0.1 mg MP. L⁻¹ condition and 8 oysters from 269 the 10 mg. L⁻¹ MP condition were mature and ejected gametes through thermal shock (Table S3). In 270 terms of sex determination, oysters in the control condition were predominantly maturing females (4:2, 271 female: male) while oysters exposed to MPs were mostly males with mature gametes (2:5 for 0.1 mg. 272 L^{-1} MP and 3:5 for 10 mg. L^{-1} MP, Table S1). The quality of gametes (well-formed oocytes and motile 273 spermatozoa) and was observed under a microscope (Motic). In the control condition, 100 % of the 274 gametes ejected being of good quality (Figure S4). 57 % and 50 % of the gametes from oysters exposed 275 to 0.1 and 10 mg. L⁻¹MP were of good quality (Table S3). When bivalves expelled poor-quality gametes, 276 they were not selected to proceed to *in vitro* fertilisation. Three couples for each condition (*i.e.* three 277 replicates) were formed to obtain embryos, then becoming D-larvae.

279 3.3 MP analysis in adult oyster tissues and biodeposits

280 MPs were quantified in digested tissues and biodeposits from exposed adult oysters (Table 2).

281

Table 2. Concentrations of environmental MP cocktail (PE, PP and PVC) in oyster tissues (MPs.g⁻¹) and biodeposits (cumulate faeces and pseudo-faeces, MPs.mg⁻¹) at three sampling times (T_1 , T_2 and T_3). Data are expressed in mean ± SEM. Different letters indicate significant differences between concentrations (p < 0.05, ANOVA and Tukey post-hoc test).

286

Sampling time	T 1		T ₂		T ₃	
Conditions	Tissues	Biodeposits	Tissues	Biodeposits	Tissues	Biodeposits
Control	0	0	0	0	0	0
MP 0.1 mg. L ⁻¹	2.1 ± 0.8 ^a	0.084 ± 0.02 ^a	6.3 ± 2.8 ^a	0.78 ± 0.2 ^a	5.4 ± 2.5 ^a	0.93 ± 0.2 ^a
MP 10 mg. L ⁻¹	11.3 ± 3.6 ^b	6.4 ± 2.2 ^b	23.6 ± 9.2 ^b	7.6 ± 2.7 ^b	21.4 ± 7.3 ^b	7.3 ± 3.1 ^b

287

288 In the control oysters, there were no microplastics in the tissues for the three sampling times. In exposed 289 ovsters, MPs were detected in the tissues at all sampling times (T_1 , T_2 and T_3). At T_1 , a greater quantity 290 (increase factor of 5.4) of MPs is noted in the tissues of oysters exposed to 10 mg. L^{-1} MP compared to 291 the 0.1 mg. L⁻¹ MP condition. Same observation at T_2 and T_3 , with increase factors of 3.7 and 4 292 respectively. The two exposure conditions noted a decrease in the MPs present in the tissues at T_{3} , 293 compared to T₂. Under the control conditions, there were no MPs in the biodeposits. In exposed oysters, 294 MPs at T₁ were quantified as follows: 0.084 ± 0.02 at 0.1 mg of MP .L⁻¹ and 6.4 ± 2.2 MPs.mg⁻¹ at 10 295 mg. $L^{-1}MP$. At T₂ and T₃, the MPs in the biodeposits were markedly increased compared to T₁.

296

297 *3.4 Analysis of biochemical biomarkers in the adult oyster digestive gland*

298 Figure 4 presents biomarker results on adult oysters exposed to environmental MPs (cocktail of HDPE,

299 PVC and PP from oyster farming). SOD and GST activities are both induce under oxidative stress. The

300 MDA activity is defined as lipid peroxidation and the LAC activity as environmental stress.



Figure 4. Effects of environmental MPs on oxidative stress (Superoxide Dismutase (SOD) and Glutathione Stransferase (GST)), lipid peroxidation (Malondialdehyde (MDA) and environmental stress Laccase (LAC) in *C*. *gigas* adult digestive gland. The different sampling times correspond to: T_1 (D₁₇), T_2 (D₃₅) and T_3 (D₅₆). Three MP conditions were tested: 0 (control), 0.1 and 10 mg. L⁻¹ MP. The histogram data are expressed in mean ± SEM. Within series, the letters (a/b, A/B and α/β) above each bar indicate significant differences (p < 0.05, ANOVA and Tukey test, n=5/condition) between different sampling times for each condition.

302

310 Superoxide Dismutase (SOD) and Glutathione S-transferase (GST) assays: oxidative stress

The SOD activity in oysters from the three conditions tested (control, 0.1 and 10 mg. L⁻¹MP) decreased significantly at T₃ (respectively 34.3 ± 3.3 , 32.6 ± 2.5 and 30.0 ± 3.3 U/mg of protein), in comparison with T₁ (respectively 51.4 ± 5.4 , 60.9 ± 7.0 and 49.7 ± 8.3 U/mg of protein) and T₂ (respectively $60.5 \pm$ 13.4, 72.8 ± 7.2 and 73.5 ± 20.5 U/mg of protein; ANOVA, *p*<0.01). Both the control and exposed oysters followed similar trends. It cannot be concluded that there is a significant difference in SOD

activity at T3 for the exposure conditions because the control condition follows the same trend. The GST activity in control condition had no significant change at the three sampling times (ANOVA, p=0.9). The trend was similar to oysters exposed to 0.1 mg MP. L⁻¹, observing no significant difference between the three sampling times (ANOVA, p=0.5). In the 10 mg. L⁻¹ MP condition, the GST activity increased at T₃ (35.9 ± 5.4 U/mg of protein; ANOVA, p<0.05, Figure 4).

321

322 <u>Malondialdehyde (MDA) assay: lipid peroxidation</u>

In the control and 10 mg. L⁻¹ MP conditions, the MDA activity in oysters had no significant difference at the three sampling times (respectively, ANOVA, p=0.2 and p=0.9). In the 0.1 mg. L⁻¹ MP condition, the MDA activity increased between T₂ (1.3 ± 0.2 µM/mg of protein) and T₃ (1.9 ± 0.3 µM/mg of protein; ANOVA, p < 0.05) (Figure 4).

327

328 *Laccase (LAC) assay: environmental stress*

In control oysters, there were no significant differences of laccase activity between the three sampling times. The activity in oysters exposed to MPs (0.1 and 10 mg. L⁻¹ MP) increased significantly between T_2 (respectively 5.6 ± 0.6 and 4.1 ± 0.6 U/mg of protein) and T_3 (respectively 7.3 ± 0.6 and 6.7 ± 0.6 U/mg of protein; ANOVA, p < 0.05, Figure 4).

333

334 *3.5 Larval behaviour, development and growth*

335 The recorded videos (ZEISS Axio Observer Z1) highlighted a swimming activity (maximum swimming 336 speed) increasing with the larvae age. The swimming speed was faster in 72-hour than for 24-hour larvae 337 (Figure 5). Compared to control larvae (153.3 \pm 12.2 μ m. s⁻¹), 24-hour D-larvae (Figure 5.a) from exposed adult oysters swam significantly slower (respectively 97.6 \pm 9.0 and 93.1 \pm 5.0 µm. s⁻¹ at 0.1 338 339 and 10 mg. L⁻¹ MP). 48-hour larvae (Figure 5.b) from parents exposed to 0.1 mg. L⁻¹ MP had a lower 340 swimming activity. 72-hour larvae (Figure 5.c) from parents exposed to the highest MP concentration 341 (10 mg. L^{-1} MP, 155.6 ± 26.4 µm. s⁻¹) swam slower than oyster larvae from other conditions (respectively 194.5 ± 12.2 and $158.5 \pm 16.0 \ \mu\text{m}$. s⁻¹ in the control and 0.1 mg. L⁻¹ MP conditions). In addition, over a 342

343 two-month period, the swimming trajectories of larvae from exposed parents were characterised (Figure 344 5). 24-hour D-larvae from unexposed parents (control condition) displayed rectilinear swimming 345 trajectories ($60.6 \pm 6.4 \%$, Figure 5.a). 48- and 72-hour larvae had predominant rectilinear swimming 346 paths (57.5 \pm 12.4 % and 54.3 \pm 5.8 % respectively, Figure 5.b & c). 24-hour D-larvae from parents 347 exposed to 0.1 and 10 mg. L⁻¹ MP mainly swam in circular trajectories, for 47.4 ± 8.1 and 65.0 ± 8.9 % 348 of the larvae, respectively (Figure 5.a). Forty-eight hour D-larvae from the 0.1 mg. L⁻¹ MP condition 349 showed predominant circular trajectories $(61.8 \pm 7.4 \%)$, in higher proportion than the control condition 350 $(32.5 \pm 10.4 \%)$. D-larvae from the 10 mg. L⁻¹ MP condition had motionless trajectories $(31.1 \pm 6.0\%)$, 351 in higher proportion than the control condition $(10.0 \pm 3.8\%)$, Figure 5.b). 72-hour larvae from parents 352 exposed to 0.1 and 10 mg. L^{-1} MP displayed fewer rectilinear trajectories (respectively, 28.6 ± 9.4 and 353 $23.8 \pm 6.5 \%$), in comparison with larvae from the control condition (54.3 ± 5.8 %, Figure 5.c).



Figure 5. Maximum speed and trajectories (rectilinear, circular or motionless) of 24- (a), 48- (b) and 72-hour (c)
D-larvae from adult oysters exposed for two months to 0 (control), 0.1 and 10 mg. L⁻¹MP. For the three replicates
(*i.e.* three couples), values are expressed in mean ± SEM. Different letters indicate significant differences between

359 concentrations (p < 0.05, Kruskal-Wallis and Nemenyi post-hoc test for maximum recorded speed and ANOVA

360 and Tukey post-hoc test for the trajectory statistical analysis, n=18 videos/condition).

361

362 After being fixed with formalin (1 %), 72-h D-larvae were classified according to their developmental 363 states (well-formed, malformed or in developmental arrest, Figure 6). D-larvae from unexposed adult 364 ovsters were well-formed (85.8 \pm 1.4 %) and statistically different (p<0.05) from ovsters exposed to 0.1 (p < 0.01) (81.1 ± 1.3 %) and 10 mg. L⁻¹ MP (p < 0.01) (74.2 ± 1.7 %). Malformed D-larvae from the 365 366 control and 0.1 mg. L^{-1} MP conditions were not statistically different, with respectively 8.5 ± 1.2 and 11.8 \pm 1.0 % (p=0.08). On the contrary, malformed larvae in the 10 mg. L⁻¹ MP condition were 367 368 significantly higher than in the control condition $(16.0 \pm 1.8 \% (p < 0.01))$. In addition, D-larvae with 369 developmental arrest had no significant difference between the first two conditions (control and 0.1 mg. 370 L^{-1} MP, with respectively 5.7 ± 0.7 and 7.1 ± 0.9 %, p=0.6). In comparison with the control condition 371 (p=0.03<0.05), the 10 mg. L⁻¹ MP condition differed significantly, with 9.8 \pm 1.2 %. Representative 372 photographs of malformed and developmental arrest larvae are presented in Figure S5.

373





Figure 6. Percentages of well-formed, abnormal and developmental arrest in 72-hour *C. gigas* larvae. Over a twomonth period, genitor oysters were exposed to three conditions: 0 (control), 0.1 and 10 mg. L⁻¹ MP. For the three replicates, values are expressed in mean \pm SEM. Different letters indicate significant differences between concentrations (p < 0.05, ANOVA and Tukey test, n=2400 larvae/condition).

Larval growth was slower in exposed than in control larvae (Figure 7). Significantly differences of size reductions of 10.6 % and 12.3 %, were observed in 72-hour larvae from parents exposed to 0.1 and 10 mg. L⁻¹MP. The total length of control D larvae was $63.0 \pm 1.5 \mu m$, and significantly greater than larvae of parents exposed to 0.1 mg. L⁻¹MP and up to 10 mg. L⁻¹MP measured $56.3 \pm 1.0 \mu m$ and 55.3 ± 0.8 µm.



385

Figure 7. Larva total length (μ m) of *C. gigas* after 24-, 48- and 72-h of development from parents exposed for 2 months to different concentrations of MPs cocktail : 0 (control), 0.1 and 10 mg. L⁻¹ MP. Values are means ± SEM. Different letters indicated significant differences between different concentrations and development time (p < 0.05, ANOVA and Tukey test, n=60/condition).

390

391 **4. Discussion**

392 The study aimed at evaluating the effects of environmentally-aged MPs (plastic materials from oyster

393 farms) on *C. gigas* oysters. Over a two-month period, adult oysters were exposed to aged MPs

394 obtained from a mixture of HDPE, PP and PVC. Their growth, survival, MP ingestion/excretion and

395 the response of biomarkers were monitored. Adult oysters were fertilised in laboratory conditions to

- 396 produce embryos. Then, the development and swimming activity of 72-hour oyster D-larvae (early
- 397 stages of development) were observed.
- 398

399 *4.1 Microplastics concentrations in experimental seawater*

400 MP concentrations in the exposure medium were assessed through flow cytometry. Except for the 0.1 401 mg. L⁻¹ MP condition at T₃, concentrations measured were constant. In the latter condition at T₃, the 402 increase in MP concentration over time might be due to particles binding on the tank walls while water 403 was renewed (Summers et al., 2018). Our present study focused on ecotoxicological test concentrations, 404 stronger than what is found in the marine environment (Lenz et al., 2016; Paul-Pont et al., 2018). The 405 previous study by Dubaish & Liebezeit (2013) determined concentrations in the North Sea of 1.7 MP.mL⁻¹ (MP > 80 μ m). In our work, the ranges of concentrations assayed reported means of 758 ± 60 406 407 and $3,322 \pm 228$ MP.mL⁻¹ for the two theoretical mass concentrations (0.1 and 10 mg. L⁻¹ MP). It is 408 therefore estimated that our study would have used concentrations 445 and 1,954 higher than the natural 409 environment. However, our concentrations are close to certain ecotoxicological studies that took into 410 account doses of 1,000 MP.mL⁻¹ (Sussarellu et al., 2016 and Paul-Pont et al., 2016). According to 411 literature, MP loads in seawater are within the $\mu g.L^{-1}$ range, and the highest MP loads reported in the 412 sea area round 0.08 to 0.3 mg.L⁻¹ (Lusher et al., 2014; Frias et al., 2014). Thereby, the theoretical 0.1 413 mg. L⁻¹ MP concentration correspond to the contamination peak of MPs in the marine environment 414 (Beiras et al., 2018). A recent study in the Bay of Biscay (South-West, Atlantic, France) near the study 415 area where our MPs were collected indicated MP concentrations between 0.03 and 0.06 MPs.L⁻¹ in 416 water, with a size distribution between $4-2,000 \,\mu\text{m}$, at the surface of the studied marine environment 417 (Lerebours et al., 2021). Wang et al. (2021), reported higher concentrations of 15-20 MPs.L⁻¹ in 418 seawater from the coastal areas in Zhuhai (China), with a size range of 101-500 µm for 45 % of the 419 detected MPs.

420

421 4.2 Microplastic in adult oysters tissues and faeces

MPs were not detected in tissues of control oysters. Like most animals, oysters lack enzymes and enzymatic pathways enabling to break down plastics (Aladaileh *et al.*, 2007; Wright *et al.*, 2013). After filtration-ingestion, MPs cannot be absorbed or used as energy, leading to their accumulation in the gills and digestive gland (Teng *et al.*, 2020). This work echoed previous results highlighting that bivalve accumulated MPs in both experimental and natural conditions (Browne *et al.*, 2008; Von Moos *et al.*, 2012; Magni *et al.*, 2018; Teng *et al.*, 2019; Birnstiel *et al.*, 2019 and Ward *et al.*, 2019). The presence 428 of MPs accumulated in the tissues at the end of exposure (T_3) could potentially affect reproductive 429 efforts and subsequently the quality of the gonads and embryo-larval development (Sussarellu et al., 430 2016). One must recognize that the concentrations tested and MP in tissues were much higher than those 431 in the environment (Keisling et al., 2020). Regarding the quantities of MPs contained in the biodeposits, 432 these present results are comparable to the results published by Revel et al. (2020). Oysters tended to 433 quickly excrete MPs (Graham et al., 2019). In a recent study (Thomas et al., 2020), MPs were detected 434 in tissues and biodeposits. Oysters could excrete MPs in faeces through cleaning processes, preventing 435 the gills from being clogged by particles (Jorgensen, 1981) and rejecting indigestible particles (Wegner 436 et al., 2012). Our results indicate low amounts of MPs in faeces. Although this suggests that oysters 437 might excrete MPs through feces, a small fraction of these microparticles might persist in the tissues or 438 be re-ingested by filtration, potentially leading to longer-term effects (Farrell & Nelson, 2013; Ribeiro 439 et al., 2017).

440

441 *4.3 Oysters state of health*

442 In the present study, MP exposure of adult oysters did not affect their growth, but their survival. The 443 highest dose of exposure (10 mg, $L^{-1}MP$) led to a significant increase of mortality rate. Sussarellu *et al.* 444 (2016) obtained similar results, showing that oysters exposed to PS-MPs had disrupted energy flows, 445 metabolism and homeostasis, increasing the mortality rate. Browne et al. (2013) reported in Arenicola 446 marina, accumulation of nonylphenol and triclosan from PVC that led to altered physiological stress, 447 immune system responses and increasing mortality. Stabilisation of mortality were noted at 35 and 44 448 days for oysters exposed to respectively 0.1 and 10 mg. L⁻¹ MP. The evolution in cumulative mortality 449 of exposed individuals may suggest an adaptation to MP exposure (Guo et al., 2015). However, it 450 remains complicated to conclude on the lethal effects of microplastics without setting up exclusion 451 analyzes for other factors (i.e. bacteria, nutrition, toxic algae, etc.). In addition, an impact on fertilisation 452 success was noted. The oysters exposed to MPs, having reached the stage of sexual maturation, had 453 more males than females, unlike the control oysters. In addition, the quality of female gametes was 454 poorer in adults exposed to MPs than in control. A previous study revealed that marine medaka (Oryzias 455 melastigma) exposed to PS-MPs faced sex-specific endocrine disruption. Wang et al. (2019) suggested 456 that PS-MPs had sex-specific reproductive disruption and decreased sex hormone levels in female 457 plasma by inhibiting their synthesis. Sussarellu *et al.* (2016) indicated that the quality of gametes 458 decreased following parental exposure to PS-MPs. This work reached similar conclusions on the quality 459 of gametes from exposed parents.

460

461 4.4 Biomarkers activities

462 The activities of biomarkers (SOD, GST, MDA and LAC) that can be modulated by environmental 463 pollutants were used as defense markers, providing information on the level of exposure and the 464 response capacities of ovsters exposed to cocktail of MPs. Oxidative stress biomarkers were chosen for 465 their early stress responses in bivalves (Valavanidis et al. 2006). Milinkovitch et al. (2015) confirmed 466 that SOD was an early indicator of oxidative stress. In their study, Revel et al. (2019) indicated that the 467 SOD activity in the digestive gland of blue mussels (Mytilus edulis) exposed for 10 days to a mixture of 468 MPs (PE, PP) was no affected. Our results show a decrease in SOD over time and with increasing 469 gonadal maturity in all conditions tested, including condition control. Similar effects have been 470 demonstrated in C. gigas with a decrease in SOD due to seasonal variations (Luna-Acosta et al., 2010b). 471 In contrast to this study, Ribeiro et al. (2017) revealed that the SOD activity in the digestive gland of 472 Scrobicularia plana exposed to PS-MPs increased at the end of the exposure period.

473 GST is involved both in the biotransformation of xenobiotics (Dos Santos et al., 2021) but also in the 474 recycling of reduced glutathione and therefore in the fight against oxidants (Fabioux et al., 2015). This 475 phenomenon could be a reaction to an increased xenobiotic biotransformation, as underlined by 476 Breitwieser et al. (2016). Excessive oxidative stress can lead to a significant increase in oxidative 477 damage (Araújo et al., 2016), potentially due to the depletion of organisms due to the activation of 478 defense mechanisms to fight against the polluted environment. Concerning the GST enzyme, also linked 479 to oxidative stress, an increase in this enzymatic activity was noted in freshwater clams Corbicula 480 fluminea exposed to PS-NP (Li et al., 2020). GSTs are key players in the removal of reactive oxygen 481 species (ROS), intervening indirectly by controlling the level of reduced glutathione.

482 If the balance is upset in favor of ROS, oxidative stress is generated due to environmental stress and483 xenobiotics could influence this balance by catalyzing the production of ROS, and altering the metabolic

balance (Donaghy *et al.*, 2015; Manduzio *et al.*, 2015). In addition, Wang *et al.* (2019) noted an
increased GST activity in the intestine and gills of the marine medaka (*Oryzias melastigma*) exposed to
20 and 200 μg PS-MP. L⁻¹, concentrations similar to this study.

The MDA content reflects the rate and intensity of lipid peroxidation (Qiao *et al.*, 2019a, b). In the 0.1 mg. L⁻¹ MP condition at T₃, the MDA content increased. The increase in MDA content, decreased the antioxidant protection and immune modulation (Pan *et al.*, 2009; Breitwieser *et al.*, 2016). Indeed, Sussarellu *et al.* (2016) and Palais *et al.* (2012) suggested that oysters exposed to particles can modify their energy flow and induced antioxidant and detoxification processes.

492 Laccase (LAC) is a marker of environmental stress (Luna-Acosta et al., 2010a). In the two exposure 493 conditions (0.1 and 10 mg, $L^{-1}MP$) at T₃, the LAC activity increased over time. The environmental study 494 conducted by Breitwieser et al. (2020) correlated an increase of the LAC activity in Mimachlamys varia 495 over time in presence of metals (Cu, Cr, Mn, Sn, and As). Bivalves are able to detect pollutants in their 496 environment and respond to stress by increasing their LAC activity (Luna-Acosta *et al.*, 2015). At T_3 – 497 the last sampling time - LAC and MDA biomarkers were significantly increased in comparison to 498 control group. Exposure to MPs should be investigated on a long-term basis to understand adaptive 499 responses or toxic effects on bivalves. Understanding mechanisms triggered by organisms to fight 500 against chronic exposure to MPs is key to conduct risk assessments. It is crucial as coastal areas are 501 constantly impacted by plastics (Santana et al., 2018). The main limitations of biomarker measurements 502 for assessing the quality of ecosystems are thus the difficulty in discriminating between "natural" 503 adaptive responses and responses to chemical stress, in extrapolating responses from one scale of 504 biological organization to another (cell-individual-population-community) and from one species to 505 another (Forbes et al., 2006). In addition, the use of biomarkers such as SOD and GST lead to increased 506 sensitivity in the results which could create bias in the observed effects.

507

508 4.5 Intergenerational effects on D-larvae

The present study also focused on the offspring obtained from adult oysters exposed to MPs over a twomonth period during gametogenesis (Enríquez-Díaz *et al.*, 2009). The larval growth was slower in the MPs-exposed groups in comparison to the control group. This result is consistent with those obtained 512 by Sussarellu et al. (2016) and Bringer et al. (2020b), suggesting that MPs exposure can delay C. gigas 513 larval growth. In this work, the exposure conditions revealed a significant increase in developmental 514 abnormalities, including malformations and developmental arrest. Thus, when parents were exposed to 515 high MP concentrations, developmental abnormalities were more frequent in their offspring. A lot 516 studies highlighted that micro-pollutants such as heavy metals and pesticides, caused developmental 517 defects in oysters (Mai et al., 2012; Gamain et al., 2016, 2017; Bringer et al., 2021b). Tallec et al. (2018) 518 reported greater toxicity of smaller plastic particles. In comparison with PS-MPs (2 µm), PS-NPs (500 519 and 50 nm) had greater impacts on the fertilisation success and embryo-larval development of oyster C. 520 gigas. Our results revealed a shift in the swimming behaviour of larvae from parents exposed to MPs. 521 Indeed, circular trajectories increased, in comparison to rectilinear trajectory. Gamain et al. (2019) 522 demonstrated that, under optimal developmental conditions, C. gigas D-larvae tended to have a 523 rectilinear swimming behaviour (2-D analysis). In a previous study, Bringer et al. (2020a) noted that 524 circular trajectories were significantly increased in larvae exposed to HDPE-MPs (4-13 µm). For both 525 MP concentrations, a decrease in maximum speed recorded was noted in 24- and 72-hour D-larvae. 526 These results are consistent with previous studies (Bringer et al., 2020a & b) in which D-larvae exposed 527 to MPs (HDPE and polymer cocktail) showed a decrease in maximum swimming speed. Yin et al. 528 (2019) indicated that black rockfish *Sebastes schlegelii* exposed to MPs (0.19 mg, L^{-1} PS-MP of 15 µm) 529 had a restricted swimming speed and range of motion, affecting their hunting behaviour and exploration 530 skill.

Altogether our data reinforced the hypothesis that MPs impacted both the physiology and behaviours of
oysters *C. gigas*. This study relied on the use of secondary MPs – aged in the marine environment – as
well as on plastic materials from oyster farming.

534

535 **5.** Conclusions and perspectives

To conclude, this experiment showed that the cocktail of MPs obtained from oyster material and aged in the marine environment can be toxic for adult oysters and the larval generation. The results indicated that exposure to MPs could affect survival, responses to defense biomarkers, and fertilization success, in adults (*C. gigas*) exposed for 2-months. D-larvae obtained from exposed oysters, having reached a 540 mature stage, exhibited alterations in swimming behavior, development and growth. Bad weather and 541 degradation of plastics from mariculture could therefore represent a possible threat to oyster farming 542 and surrounding marine life. In the future, it would be useful to study the life cycle of aquaculture 543 plastics and the potential risk of additives present on plastics on the *Crassostrea gigas* study model and 544 the health of marine ecosystems in general.

545

546 Acknowledgements

547 Arno Bringer received a PhD grant (Comité Régional de la Conchyliculture de la Charente-Maritime 548 CRC17) as well as financial support from the Région Nouvelle Aquitaine and Comité Départemental de 549 la Charente Maritime (CD17) to develop this research. The AQUAECOs project (Amélioration de la 550 QUAlité Environnementale sur les zones COnchylicoles des Pertuis Charentais), funded by a 551 partnership with CRC17 and OFB (French Biodiversity Office), contributed to this study. The funding 552 was partly supported by the University of La Rochelle, the University of Bordeaux, the Centre National 553 de la Recherche Scientifique (France). The authors thank the France Naissain hatchery for the oyster 554 specimens as well as Oriane Titeca for her proofreading services.

555

556 **References**

- Aladaileh, S., Nair, S. V., Birch, D., & Raftos, D. A. (2007). Sydney rock oyster (*Saccostrea glomerata*)
- hemocytes: morphology and function. *Journal of invertebrate pathology*, 96(1), 48-63.
- 559
- Andrady, A. L. (2011). Microplastics in the marine environment. *Marine pollution bulletin*, 62(8), 15961605.
- 562
- 563 Araújo, R.F.F. de, Martins, D.B.G., Borba, M.A.C.S.M., 2016. Oxidative stress and disease. In:
- 564 Morales-Gonzalez, J.A., Morales-Gonzalez, A., Madrigal-Santillan, E.O. (Eds.), A Master Regulator of
- 565 Oxidative Stress the Transcription Factor Nrf2. InTech.
- 566

- Bakir, A., Rowland, S. J., Thompson, R. C. (2014). Transport of persistent organic pollutants by
 microplastics in estuarine conditions. *Estuarine, Coastal and Shelf Science*, 140, 14-21.
- 569
- 570 Barnes, D.K.A., Galgani, F., Thompson, R.C., Barlaz, M. (2009). Accumulation and fragmentation of
- 571 plastic debris in global environments. *Philos. Trans. R. Soc.* B364, 1985–1998.
- 572
- 573 Bayne, B. L., Ahrens, M., Allen, S. K., D'auriac, M. A., Backeljau, T., Beninger, P., Bohn, R., Boudry,
- 574 P., Davis, J., Green, T., Guo, X., Hedgecock, D., Ibarra, A., Kingsley-Smith, P., Krause, M., Langdon,
- 575 C., Lapegue, S., Li, C., Manahan, D., Mann, R., Perez-Paralle, L., Powell, E. N., Rawson, P. D., Speiser,
- 576 D., Sanchez, J-L., Shumway, S., Wang, H. (2017). The proposed dropping of the genus *Crassostrea* for
- all pacific cupped oysters and its replacement by a new genus *Magallana*: a dissenting view. *J. Shellfish Res.* 36 (3), 545-548. [1]
- 579
- Bayne, B., D'Auriac, M. A., Backeljau, T., Beninger, P., Boudry, P., Carnegie, R., Langdon, C. (2019).
 A scientific name for Pacific oysters. *Aquaculture* 499, 373. [SEP]
- 582

583 Beiras, R., Bellas, J., Cachot, J., Cormier, B., Cousin, X., Engwall, M., Gambardella, C., Garaventa, F.,

Keiter, S., Le Bihanic, F., Lopez-Ibanez, S., Piazza, V., Rial, D., Tato, T., Vidal-Liñán, L. (2018).

- 585 Ingestion and contact with polyethylene microplastics does not cause acute toxicity on marine 586 zooplankton. *Journal of hazardous materials*, *360*, 452-460.
- 587
- Birnstiel, S., Soares-Gomes, A., & da Gama, B. A. (2019). Depuration reduces microplastic content in
 wild and farmed mussels. *Marine pollution bulletin*, *140*, 241-247.
- 590
- Bodoy, A., Prou, J., & Berthome, J. P. (1986). Etude comparative de différents indices de condition
 chez l'huître creuse (*Crassostrea gigas*). *Haliotis*, *15*, 173-182.
- 593

- Bonanno, G., & Orlando-Bonaca, M. (2018). Perspectives on using marine species as bioindicators of
 plastic pollution. *Marine pollution bulletin*, *137*, 209-221.
- 596
- 597 Breitwieser, M., Viricel, A., Graber, M., Murillo, L., Becquet, V., Churlaud, C., Fruitier-Arnaudin, I.,
- 598 Huet, V., Lacroix, C., Pante, E., Le Floch, S., Thomas-Guyon, H. (2016). Short-term and long-term
- biological effects of chronic chemical contamination on natural populations of a marine bivalve. *PloS one*, *11*(3), e0150184.
- 601
- 602 Breitwieser, M., Barbarin, M., Plumejeaud-Perreau, C., Dubillot, E., Guyot, T., Huet, V., Churlaud, C.,
- 603 Coulombier, T., Brenon, I., Fichet, D., Imbert, N., Thomas, H. (2020). Biomonitoring of *Mimachlamys*604 *varia* transplanted to areas impacted by human activities (La Rochelle Marina,
 605 France). *Chemosphere*, 243, 125199.
- 606
- Bringer, A., Thomas, H., Prunier, G., Dubillot, E., Bossut, N., Churlaud, C., Clerandeau, C., Le Bihanic,
 F., Cachot, J. (2020a). High density polyethylene (HDPE) microplastics impair development and
 swimming activity of Pacific oyster D-larvae, *Crassostrea gigas*, depending on particle size. *Environmental Pollution*, 260, 113978. EE
- 611
- Bringer, A., Cachot, J., Prunier, G., Dubillot, E., Clérandeau, C., & Thomas, H. (2020b). Experimental
 ingestion of fluorescent microplastics by pacific oysters, *Crassostrea gigas*, and their effects on the
 behaviour and development at early stages. *Chemosphere*, 126793. [1]
- 615
- 616 Bringer, A., Thomas, H., Prunier, G., Dubillot, E., Clérandeau, C., Pageaud, M., & Cachot, J. (2021a).
- 617 Toxicity and risk assessment of six widely used pesticides on embryo-larval development of the Pacific
- 618 oyster, Crassostrea gigas. Science of The Total Environment, 146343.
- 619

- Bringer, A., Le Floch, S., Kerstan, A., & Thomas, H. (2021b). Coastal ecosystem inventory with
 characterization and identification of plastic contamination and additives from aquaculture
 materials. *Marine Pollution Bulletin*, *167*, 112286.
- 623
- Browne, M. A., Dissanayake, A., Galloway, T. S., Lowe, D. M., & Thompson, R. C. (2008). Ingested
 microscopic plastic translocates to the circulatory system of the mussel, Mytilus edulis
 (L.). *Environmental science & technology*, 42(13), 5026-5031.
- 627
- Browne, M. A., Niven, S. J., Galloway, T. S., Rowland, S. J., & Thompson, R. C. (2013). Microplastic
 moves pollutants and additives to worms, reducing functions linked to health and biodiversity. *Current biology*, 23(23), 2388-2392.
- 631
- Cho, Y., Shim, W. J., Jang, M., Han, G. M., & Hong, S. H. (2019). Abundance and characteristics of
 microplastics in market bivalves from South Korea. *Environmental pollution*, 245, 1107-1116.
- 634
- Cole, M., Lindeque, P., Halsband, C., Galloway, T.S., 2011. Microplastics as contaminants in the marine
 environment: a review. *Mar. Pollut. Bull.* 62, 2588-2597.
- 637
- Cole, M., Galloway, T. S. (2015). Ingestion of nanoplastics and microplastics by pacific oyster larvae. *Environ. Sci. Technol.* 49 (24), 14625–14632. [SEP]
- 640
- 641 Conger, K. A., Swift, M. L., Reeves III, J. B., & Lakshmanan, S. (1978). Shell growth of unfed oysters
- 642 in the laboratory: a sublethal bioassay system for pollutants. *Life sciences*, 22(3), 245-254.
- 643
- 644 Cormier, B., Gambardella, C., Tato, T., Perdriat, Q., Costa, E., Veclin, C., Le Bihanic, F., Grassl, B.,
- 645 Dubocq, F., Karrman, A., Van Arkel, K., Lemoine, S., Lagarde, F., Morin, B., Garaventa, F., Faimali,
- 646 M., Cousin, X., Bégout, M.L., Cachot, J. (2021). Chemicals sorbed to environmental microplastics are
- 647 toxic to early life stages of aquatic organisms. *Ecotoxicology and Environmental Safety*, 208, 111665.

649	Donaghy, L., Hong, HK., Jauzein, C., Choi, KS., 2015. The known and unknown sources of reactive
650	oxygen and nitrogen species in haemocytes of marine bivalve molluscs. Fish Shellfish Immunol. 42, 91-
651	97.
652	
653	Dos Santos, R. N., Machado, B. R., Hefler, S. M., & Zanette, J. (2021). Glutathione S-transferase
654	activity in aquatic macrophytes and halophytes and biotransformation potential for biocides. Journal of
655	Plant Research, 1-8.
656	
657	Dubaish, F., & Liebezeit, G. (2013). Suspended microplastics and black carbon particles in the Jade
658	system, southern North Sea. Water, air, & soil pollution, 224(2), 1-8.
659	
660	Enders, K., Lenz, R., Stedmon, C.A., Nielsen, T.G. (2015). Abundance, size and polymer composition
661	of marine microplastics > 10 μ m m in the Atlantic Ocean and their modelled vertical distribution. <i>Mar</i> .
662	Pollut. Bull. 100 (1), 70-81. [SEP]
663	
664	Enríquez-Díaz, M., Pouvreau, S., Chávez-Villalba, J., & Le Pennec, M. (2009). Gametogenesis,
665	reproductive investment, and spawning behavior of the Pacific giant oyster Crassostrea gigas: evidence
666	of an environment-dependent strategy. Aquaculture International, 17(5), 491.
667	
668	Fabioux, C., Sulistiyani, Y., Haberkorn, H., Hégaret, H., Amzil, Z., & Soudant, P. (2015). Exposure to
669	toxic Alexandrium minutum activates the detoxifying and antioxidant systems in gills of the oyster
670	Crassostrea gigas. Harmful Algae, 48, 55-62.
671	
672	Farrell, P., & Nelson, K. (2013). Trophic level transfer of microplastic: Mytilus edulis (L.) to Carcinus
673	maenas (L.). Environmental pollution, 177, 1-3.
674	

- Forbes, V. E., Palmqvist, A., & Bach, L. (2006). The use and misuse of biomarkers in
 ecotoxicology. *Environmental Toxicology and Chemistry: An International Journal*, 25(1), 272-280.
- 677
- Frias, J. P. G. L., Otero, V., & Sobral, P. (2014). Evidence of microplastics in samples of zooplankton
 from Portuguese coastal waters. *Marine Environmental Research*, *95*, 89-95.
- 680
- Gagnaire, B., Thomas-Guyon, H., & Renault, T. (2004). In vitro effects of cadmium and mercury on
 Pacific oyster, *Crassostrea gigas* (Thunberg), haemocytes. *Fish & Shellfish Immunology*, *16*(4), 501512.
- 684
- 685 Gamain, P., Gonzalez, P., Cachot, J., Pardon, P., Tapie, N., Gourves, P.Y., Budzinski, H., Morin, B.,
- 686 2016. Combined effects of pollutants and salinity on embryo-larval development of the Pacific oyster,
- 687 Crassostrea gigas. Mar. Environ. Res. 113, 31-38.
- 688
- Gamain, P., Gonzalez, P., Cachot, J., Cle_randeau, C., Mazzella, N., Gourves, P.Y., Morin, B., 2017.
- 690 Combined effects of temperature and copper and S-metolachlor on embryo-larval development of the
- 691 Pacific oyster, Crassostrea gigas. Mar. Pollut. Bull. 115 (1-2), 201-210.
- 692
- 693 Gamain, P., Rome_ro-Ramirez, A., Gonzalez, P., Mazzella, N., Gourves, P.Y., Compan, C., Morin, B.,
- 694 Cachot, J., 2019. Assessment of swimming behavior of the Pacific oyster D-larvae (*Crassostrea gigas*)
- 695 following exposure to model pollutants. *Environ. Sci. Pollut. Res.* 1-11.
- 696
- 697 Gardon, T., Huvet, A., Paul-Pont, I., Cassone, A. L., Koua, M. S., Soyez, C., Jezequel, R., Receveur, J.,
 698 Le Moullac, G. (2020). Toxic effects of leachates from plastic pearl-farming gear on embryo-larval
- 699 development in the pearl oyster *Pinctada margaritifera*. Water Research, 115890.
- 700
- 701 Gerard-Monnier, D., Erdelmeier, I., Chaudiere, J., Yadan, J., 1998. Method of colorimetric analysis of
- 702 malonic dialdehyde and 4-hydroxy-2-enaldehydes as indexes of lipid per- oxidation, kits for carrying

- 703 out said method, substituted indoles for use in said method and their preparation. US Patent No.
- 704 US5726063. United States Patent and Trademark Office, Washington, DC.
- 705
- 706 Gigault, J., Pedrono, B., Maxit, B., Ter Halle, A., 2016. Marine plastic litter: the unanalyzed nano-
- 707 fraction. Environ. Sci.: Nano 3 (2), 346-350.
- 708
- 709 Gorman, D., Moreira, F. T., Turra, A., Fontenelle, F. R., Combi, T., Bícego, M. C., & de Castro Martins,
- 710 C. (2019). Organic contamination of beached plastic pellets in the South Atlantic: Risk assessments can
- benefit by considering spatial gradients. *Chemosphere*, 223, 608-615.
- 712 **SEP**
- 713 Graham, P., Palazzo, L., de Lucia, G. A., Telfer, T. C., Baroli, M., & Carboni, S. (2019). Microplastics
- vptake and egestion dynamics in Pacific oysters, Magallana gigas (Thunberg, 1793), under controlled
- 715 conditions. *Environmental pollution*, 252, 742-748.
- 716
- Guo, X., He, Y., Zhang, L., Lelong, C., & Jouaux, A. (2015). Immune and stress responses in oysters
 with insights on adaptation. *Fish & shellfish immunology*, 46(1), 107-119.
- 719
- Habig, W. H., Pabst, M. J., & Jakoby, W. B. (1974). Glutathione S-transferases: the first enzymatic step
 in mercapturic acid formation. *Journal of biological Chemistry*, 249(22), 7130-7139.
- 722
- Halpern, B.S., Walbridge, S., Selkoe, K.A., Kappel, C.V., Micheli, F., D'Agrosa, C., Bruno, J.F., Casey,
- K.S., Ebert, C., Fox, H.E., Fujita, R., Heinemann, D., Lenihan, H.S., Madin, E.M.P., Perry, M.T., Selig,
- E.R., Spalding, M., Steneck, R., Watson, R. (2008). A global map of human impact on marine
 ecosystems. *science*, *319*(5865), 948-952.
- 727
- Helm, M.M., Bourne, N., Lovatelli, A., 2006. Hatchery operation: culture of larvae basic methodology,
- feeding and nutrition, factors influencing growth and sur- vival, and settlement and metamorphosis.
- 730 Hatchery Culture of Bivalves: A Practical Manual. FAO Fisheries Technical Paper (471).

7	3	1
1	J	T

732	Hermabessiere, L., Dehaut, A., Paul-Pont, I., Lacroix, C., Jezequel, R., Soudant, P., & Duflos, G. (2017).
733	Occurrence and effects of plastic additives on marine environments and organisms: A
734	review. Chemosphere, 182, 781-793.
735	
736	His, E., Heyvang, I., Geffard, O., De Montaudouin, X., 1999. A comparison between oyster
737	(Crassostrea gigas) and sea urchin (Paracentrotus lividus) larval bioassays for toxicological studies.
738	Water Res. 33 (7), 1706-1718.
739	
740	Jin-Feng, D. I. N. G., Jing-Xi, L. I., Cheng-Jun, S. U. N., Chang-Fei, H. E., Jiang, F. H., Feng-Lei, G.
741	A. O., & Zheng, L. (2018). Separation and identification of microplastics in digestive system of
742	bivalves. Chinese Journal of Analytical Chemistry, 46(5), 690-697.
743	
744	Jørgensen, C. B. (1981). Feeding and cleaning mechanisms in the suspension feeding bivalve Mytilus
745	edulis. Marine biology, 65(2), 159-163.
746	
747	Karami, A., Golieskardi, A., Choo, C. K., Romano, N., Ho, Y. B., & Salamatinia, B. (2017). A high-
748	performance protocol for extraction of microplastics in fish. Science of the Total Environment, 578, 485-
749	494.
750	
751	Keisling, C., Harris, R. D., Blaze, J., Coffin, J., & Byers, J. E. (2020). Low concentrations and low
752	spatial variability of marine microplastics in oysters (Crassostrea virginica) in a rural Georgia
753	estuary. Marine Pollution Bulletin, 150, 110672.
754	
755	Lambert, S., Wagner, M., 2016. Characterisation of nanoplastics during the degradation of polystyrene.
756	Chemosphere 145, 265-268.

- 758 Law, K. L., Morét-Ferguson, S., Maximenko, N. A., Proskurowski, G., Peacock, E. E., Hafner, J., &
- Reddy, C. M. (2010). Plastic accumulation in the North Atlantic subtropical gyre. *Science*, *329*(5996),
 1185-1188.
- 761
- 762 Legendre, P., Legendre, L., 1998. Transformation:1-4. Numer. Ecol.
- 763
- Lenz, R., Enders, K., & Nielsen, T. G. (2016). Microplastic exposure studies should be environmentally
 realistic. *Proceedings of the National Academy of Sciences*, *113*(29), E4121-E4122.
- 766
- Lerebours, A. et al., 2021. Étude spatio-temporelle de la contamination en microplastiques chez les
 moules et les huîtres en mer des Pertuis : hétérogénéité ? Oral communication on Polymers & Oceans
 congress. [1]
- 770
- Li, J., Yang, D., Li, L., Jabeen, K., Shi, H. (2015). Microplastics in commercial bivalves from China. *Environ. Pollut.* 207, 190–195. [JP]
- 773
- Li, Z., Feng, C., Wu, Y., & Guo, X. (2020). Impacts of nanoplastics on bivalve: Fluorescence tracing of

organ accumulation, oxidative stress and damage. *Journal of Hazardous Materials*, 122418.

- Luna-Acosta, A., Rosenfeld, E., Amari, M., Fruitier-Arnaudin, I., Bustamante, P., Thomas-Guyon, H.,
- 2010a. First evidence of laccase activity in the Pacific oyster *Crassostrea gigas*. *Fish Shellfish Immunol*.
 28:719–726.
- 780
- 781 Luna-Acosta, A., Bustamante, P., Godefroy, J., Fruitier-Arnaudin, I., & Thomas-Guyon, H. (2010b).
- 782 Seasonal variation of pollution biomarkers to assess the impact on the health status of juvenile Pacific
- 783 oysters Crassostrea gigas exposed in situ. Environmental Science and Pollution Research, 17(4), 999-
- 784 1008.
- 785

- Luna-Acosta, A., Bustamante, P., Budzinski, H., Huet, V., & Thomas-Guyon, H. (2015). Persistent
 organic pollutants in a marine bivalve on the Marennes-Oléron Bay and the Gironde Estuary (French
 Atlantic Coast)-Part 2: Potential biological effects. *Science of The Total Environment*, *514*, 511-522.
- 789
- The The Terror T
- Atlantic Ocean: validated and opportunistic sampling. *Marine pollution bulletin*, 88(1-2), 325-333.
 792
- Lusher, A., Hollman, P., & Mendoza-Hill, J. (2017). *Microplastics in fisheries and aquaculture: status*of knowledge on their occurrence and implications for aquatic organisms and food safety. FAO.
- 795
- 796 Magni, S., Gagné, F., André, C., Della Torre, C., Auclair, J., Hanana, H., Parenti, C.C., Bonasoro, F.,
- Binelli, A. (2018). Evaluation of uptake and chronic toxicity of virgin polystyrene microbeads in
 freshwater zebra mussel *Dreissena polymorpha* (Mollusca: Bivalvia). *Science of the Total Environment*, *631*, 778-788.
- 800
- Mai, H., Cachot, J., Brune, J., Geffard, O., Belles, A., Budzinski, H., Morin, B., 2012. Embryotoxic and
 genotoxic effects of heavy metals and pesticides on early life stages of Pacific oyster (*Crassostrea gigas*). *Mar. Pollut. Bull.* 64 (12), 2663-2670.
- 804
- Mai, H., Morin, B., Pardon, P., Gonzalez, P., Budzinski, H., Cachot, J., 2013. Environmental
 concentrations of irgarol, diuron and S-metolachlor induce deleterious effects on gametes and embryos
 of the Pacific oyster, *Crassostrea gigas. Mar. Environ. Res.* 89, 1-8 [1]
- 808
- Manduzio, H., Rocher, B., Durand, F., Galap, C., Leboulenger, F., 2015. The point about oxidative
 stress in molluscs. *Invertebr. Surviv. J.* hal 32.
- 811
- 812 Mannervik, B., Helena-Danielson, U., Ketterer, B., 1988. Glutathione transferases-structure and
- 813 catalytic activity. *Crit. Rev. Biochem.* 23:283–337.

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

- 818
- 819 O'Connor, T. P. (2002). National distribution of chemical concentrations in mussels and oysters in the
 820 USA. *Marine Environmental Research*, *53*(2), 117-143.
- 821
- 822 Pannetier, P., Morin, B., Le Bihanic, F., Dubreil, L., Clérandeau, C., Chouvellon, F., Van Arkel, K.,
- 823 Danion, M., Cachot, J. (2020). Environmental samples of microplastics induce significant toxic effects
- 824 in fish larvae. *Environment international*, *134*, 105047.
- 825
- Paoletti, F., Aldinucci, D., Mocali, A., Caparrini, A., 1986. A sensitive spectrophotometric method for
 the determination of superoxide dismutase activity in tissue extracts. *Anal. Biochem.* 154:536–541.
- Parker, L.M., Ross, P.M., O'Connor, W.A., 2009. The effect of ocean acidification and temperature on
 the fertilization and embryonic development of the Sydney rock oyster *Saccostrea glomerata* (Gould
- 831 1850). *Glob. Chang. Biol.* 15 (9), 2123-2136.
- 832
- Paul-Pont, I., Lacroix, C., Fernández, C. G., Hégaret, H., Lambert, C., Le Goïc, N., Frere, L., Cassone,
 A.L., Sussarellu, R., Fabioux, C., Guyomarch, J., Albentosa, M., Huvet, A., Soudant, P. (2016).
 Exposure of marine mussels *Mytilus spp.* to polystyrene microplastics: toxicity and influence on
 fluoranthene bioaccumulation. *Environmental pollution*, *216*, 724-737.
- 837
- 838 Paul-Pont, I., Tallec, K., Gonzalez-Fernandez, C., Lambert, C., Vincent, D., Mazurais, D., Zambonino-
- 839 Infante, J.L., Brotons, G., Lagarde, F., Fabioux, C., Soudant, P., Huvet, A. (2018). Constraints and
- 840 priorities for conducting experimental exposures of marine organisms to microplastics. Frontiers in
- 841 Marine Science, 5, 252.

- 843 Pérez-Albaladejo, E., Solé, M., & Porte, C. (2020). Plastics and plastic additives as inducers of oxidative
 844 stress. *Current Opinion in Toxicology*.
- 845
- 846 Phuong, N. N., Zalouk-Vergnoux, A., Kamari, A., Mouneyrac, C., Amiard, F., Poirier, L., & Lagarde,
- 847 F. (2018a). Quantification and characterization of microplastics in blue mussels (*Mytilus edulis*):
- 848 protocol setup and preliminary data on the contamination of the French Atlantic coast. *Environmental*
- 849 Science and Pollution Research, 25(7), 6135-6144.
- 850
- 851 Phuong, N. N., Poirier, L., Pham, Q. T., Lagarde, F., Zalouk-Vergnoux, A. (2018b). Factors influencing
- the microplastic contamination of bivalves from the French Atlantic coast: Location, season and/or
 mode of life? *Marine Pollution Bulletin* 129, 664-674. [see]
- 854
- PlasticsEurope, 2020. Plastics the Facts 2020. An Analysis of European Plastics. Production, Demand
 and Waste Data. https://www.plasticseurope.org/fr/resources/publications/4312-plastics-facts-2020
- Quiniou, F., His, E., Delesmont, R., Caisey, X., 2005. Bio-indicateur de la toxicité potentielle de milieux
 aqueux : bio-essai "développement embryo-larvaire de bivalve". In: Ifremer (Ed.), Méthodes d'analyse
 en milieu marin.
- 861

- 867 K., Decottignies, P., Cognie, B., Châtel, A., Mouneyrac, C. (2019). Tissue-specific biomarker responses
- 868 in the blue mussel Mytilus spp. exposed to a mixture of microplastics at environmentally relevant
- 869 concentrations. *Frontiers in Environmental Science*, 7, 33.

<sup>Quiniou, F., Damiens, G., Gnassia-Barelli, M., Geffard, A., Mouneyrac, C., Budzinski, H., & Roméo,
M. (2007). Marine water quality assessment using transplanted oyster larvae.</sup> *Environment international*, 33(1), 27-33.

⁸⁶⁶ Revel, M., Lagarde, F., Perrein-Ettajani, H., Bruneau, M., Akcha, F., Sussarellu, R., Rouxel, J., Costil,

871	Revel, M., Châtel, A., Perrein-Ettajani, H., Bruneau, M., Akcha, F., Sussarellu, R., Rouxel, J., Costil,
872	K., Decottignies, P., Cognie, B., Lagarde, F., Mouneyrac, C. (2020). Realistic environmental exposure
873	to microplastics does not induce biological effects in the Pacific oyster Crassostrea gigas. Marine
874	pollution bulletin, 150, 110627.
875	
876	Ribeiro, F., Garcia, A.R., Pereira, B.P., Fonseca, M., Mestre, N.C., Fonseca, T.G., Ilharco, L.M.,
877	Bebianno, M.J. (2017). Microplastics effects in Scrobicularia plana. Mar. Pollut. Bull. 122, 379–391.
878	
879	Robinson, A., 1992. Gonadal cycle of Crassostrea gigas kumamoto (Thunberg) in Yaquina Bay, Oregon
880	and optimum conditions for broodstock oysters and larval culture. Aquaculture 106 (1), 89-97.
881	
882	Rochman, C. M., Browne, M. A., Halpern, B. S., Hentschel, B. T., Hoh, E., Karapanagioti, H. K., Rios-
883	Mendoza, L. M., Takada, H., The, S., Thompson, R. C. (2013). Policy: classify plastic waste as
884	hazardous. <i>Nature</i> 494, 169–171.
885	
886	Santana, M. F., Moreira, F. T., Pereira, C. D., Abessa, D. M., & Turra, A. (2018). Continuous exposure
887	to microplastics does not cause physiological effects in the cultivated mussel Perna perna. Archives of
888	environmental contamination and toxicology, 74(4), 594-604.
889	
890	Smith, P.K., Krohn, R.I., Hermanson, G.T., Mallia, A.K., Gartner, F.H., Provenzano, M.D., Fujimoto,
891	E.K., Goeke, N.M., Olson, B.J., Klenk, D.C., 1985. Measurement of protein using bicinchoninic acid.
892	Anal. Biochem. 150:76–85.
893	
894	Summers, S., Henry, T., & Gutierrez, T. (2018). Agglomeration of nano-and microplastic particles in
895	seawater by autochthonous and de novo-produced sources of exopolymeric substances. Marine
896	pollution bulletin, 130, 258-267.
897	

898	Sussarellu, R., Suquet, M., Thomas, Y., Lambert, C., Fabioux, C., Pernet, M.E.J., Goïc, N.L., Quillien,
899	V., Mingant, C., Epelboin, Y., Charlotte, C., Julien, G., Johan, R., Ika, PP., Philippe, S., Arnaud, H.,
900	2016. Oyster reproduction is affected by exposure to polystyrene microplastics. Proc. Natl. Acad. Sci.
901	113 (9), 2430-2435.

- 902
- 903 Tallec K, Huvet A, Di Poi C, Gonzalez-Fernandez C, Lambert C, Petton B, Le Goic N, Berchel M,

Soudant P, Paul-Pont I (2018) Nanoplastics impaired oyster free living stages, gametes and embryos. *Environ. Pollut.* 242, 1226-1235. [J]

906

Talmage, S.C., Gobler, C.J., 2009. The effects of elevated carbon dioxide concentrations on the
metamorphosis, size, and survival of larval hard clams (*Mercenaria mercenaria*), bay scallops
(*Argopecten irradians*), and Eastern oysters (*Crassostrea virginica*). *Limnol. Oceanogr.* 54 (6), 20722080.

911

912 Teng, J., Wang, Q., Ran, W., Wu, D., Liu, Y., Sun, S., Liu, H., Cao, R., Zhao, J. (2019). Microplastic
913 in cultured oysters from different coastal areas of China. *Science of The Total Environment*, 653, 1282914 1292.

915

916 Teng, J., Zhao, J., Zhu, X., Shan, E., Zhang, C., Zhang, W., & Wang, Q. (2020). Toxic effects of
917 exposure to microplastics with environmentally relevant shapes and concentrations: Accumulation,
918 energy metabolism and tissue damage in oyster *Crassostrea gigas*. *Environmental Pollution*, 269,
919 116169.

920

921 Thiele, C. J., Hudson, M. D., & Russell, A. E. (2019). Evaluation of existing methods to extract
922 microplastics from bivalve tissue: Adapted KOH digestion protocol improves filtration at single-digit
923 pore size. *Marine pollution bulletin*, 142, 384-393.

- Thomas, M., Jon, B., Craig, S., Edward, R., Ruth, H., John, B., Dick, V.A., Heather, L.A., Matthew, S.
 (2020). The world is your oyster: low-dose, long-term microplastic exposure of juvenile oysters. *Heliyon*, 6(1), e03103.
- 928

929 Van Cauwenberghe L & Janssen CR (2014) Microplastics in bivalves cultured for human consumption.
930 *Environ. Pollut.* 193, 65–70 [see]

931

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

- 935
- 936 Wagner, M., Scherer, C., Alvarez-Munoz, D., Brennholt, N., Bourrain, X., Buchinger, S., Fries, E.,

937 Grosbois, C., Klasmeier, J., Marti, T., Rodriguez-Mozaz, S., Urbatzka, R., Vethaak, A., Winther-

Nielsen, M., Reifferscheid, G., 2014. Microplastics in freshwater ecosystems: what we know and what

939 we need to know. *Environ. Sci. Eur.* 26 (1), 12.

- 940
- Wang, J., Li, Y., Lu, L., Zheng, M., Zhang, X., Tian, H., Wang, W., Ru, S. (2019). Polystyrene
 microplastics cause tissue damages, sex-specific reproductive disruption and transgenerational effects
 in marine medaka (*Oryzias melastigma*). *Environmental Pollution*, 254, 113024.
- 944
- Wang, D., Su, L., Ruan, H. D., Chen, J., Lu, J., Lee, C. H., & Jiang, S. Y. (2021). Quantitative and
 qualitative determination of microplastics in oyster, seawater and sediment from the coastal areas in
 Zhuhai, China. *Mar. Pollut. Bull.*, *164*, 112000.
- 948
- 949 Ward, J. E., Zhao, S., Holohan, B. A., Mladinich, K. M., Griffin, T. W., Wozniak, J., & Shumway, S.
- 950 E. (2019). Selective ingestion and egestion of plastic particles by the blue mussel (*Mytilus edulis*) and
- 951 eastern oyster (Crassostrea virginica): implications for using bivalves as bioindicators of microplastic
- 952 pollution. *Environmental science & technology*, 53(15), 8776-8784.

- Wegner, A., Besseling, E., Foekema, E. M., Kamermans, P., & Koelmans, A. A. (2012). Effects of
 nanopolystyrene on the feeding behavior of the blue mussel (*Mytilus edulis* L.). *Environmental Toxicology and Chemistry*, *31*(11), 2490-2497.
- 957
- Wright SL, Thompson RC, Galloway TS (2013) The physical impacts of microplastics on marine
 organisms: A review. *Environ. Pollut.* 178, 483–492.
- 960
- 961 Yin, L., Liu, H., Cui, H., Chen, B., Li, L., Wu, F., 2019. Impacts of polystyrene microplastics on the
 962 behavior and metabolism in a marine demersal teleost, black rockfish (*Sebastes schlegelii*). *J. Hazard*963 *Mater.* 380, 120861.
- 964
- Zapata-Restrepo, L. M., Hauton, C., Williams, I. D., Jensen, A. C., & Hudson, M. D. (2019). Effects of
 the interaction between temperature and steroid hormones on gametogenesis and sex ratio in the
 European flat oyster (*Ostrea edulis*). *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, 236, 110523.
- 969
- 970 Zarfl C, Matthies M (2010) Are marine plastic particles transport vectors for organic pollutants to the
- 971 Arctic? Mar. Pollut. Bull. 60 (10), 1810–1814.