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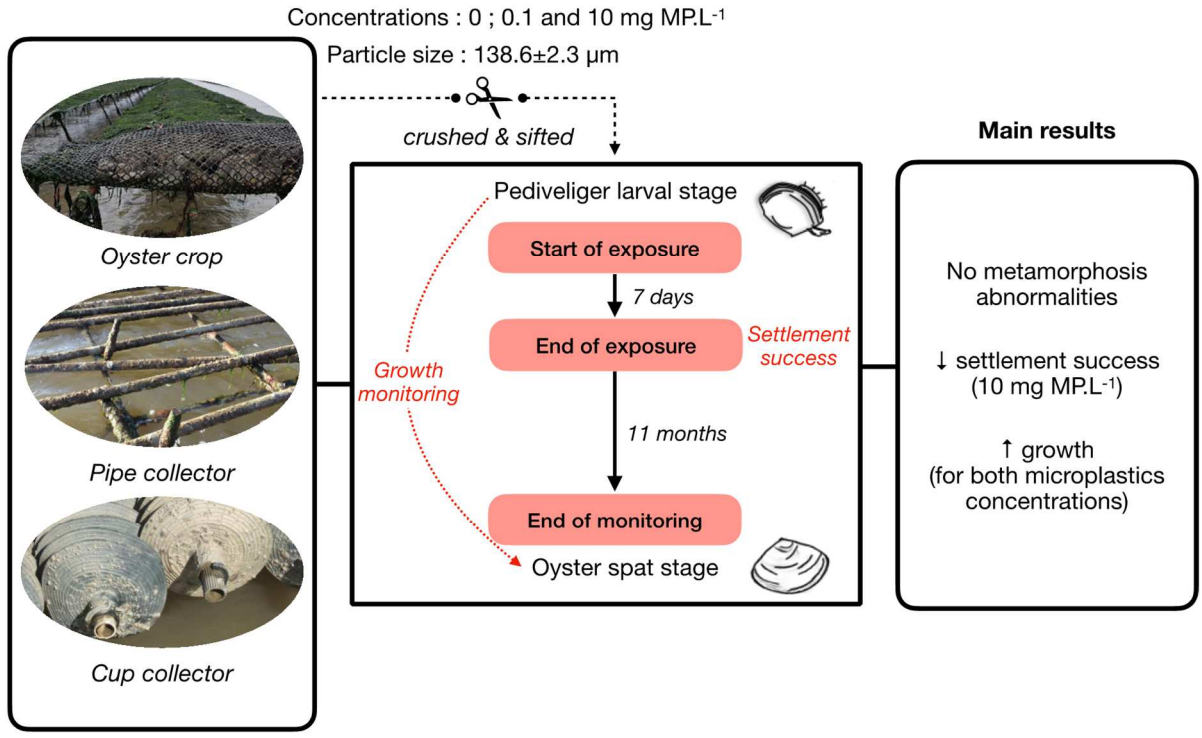
ABSTRACT

Plastic is currently used in aquaculture as a material for settlement and magnification of oyster spats. Plastic weathering and fragmentation under natural conditions can lead to the production of micro and nanoparticles and additive leakage, with potential toxic effects on marine life. This study investigates the effects of the exposure to microplastic (MPs) cocktail derived from aged aquaculture material on oyster pediveliger larvae (*Crassostrea gigas*). The cocktail was made of high-density polyethylene (HDPE), polypropylene (PP) and polyvinyl chloride (PVC). The concentrations tested were 0, 0.1, and 10 mg MP. L^{-1}. During the 7-day fixation phase, pediveliger larvae (17 days) were exposed to the MP cocktail in laboratory-controlled conditions. After exposure, the success of settlement was significantly lower for larvae exposed to 10 mg MP. L^{-1} (49±0.9 %) compared to control ones (61.8±1.6 %). No malformations or metamorphosis abnormalities were observed. Growth of pediveliger and spat stages was monitored up to 11 months. During the first twenty-eight days of development, spat growth was significantly lower for the two MPs exposure conditions (0.1 and 10 mg MP. L^{-1}; respectively -51.8 % and -44.4 %) compared to control groupe. Subsequently, the previously exposed oysters grew faster than the control condition, resulting in a significantly greater growth (0.1 and 10 mg MP. L^{-1}: +18.3 % and +19.7 %) than the control group at the end of follow-up. The nearly one-year follow-up highlighted the potential effects of MPs from aquaculture on larvae and spat of *C. gigas*.

GRAPHICAL ANSTRACT



KEYWORDS: Aged microplastics; Oyster; Pediveliger larvae; Spat; Growth; Settlement success.

1. INTRODUCTION

In 2019, plastic production reached 370 million tons worldwide, including almost 58 million tons in Europe (PlasticsEurope, 2020). Seen as revolutionary and versatile, plastic materials are part of one's daily life. Ideal for the current economic system, they incur low production costs, and are light, durable and malleable. The first studies revealing MP presence in marine habitats date back to the 1970s (Carpenter *et al.,* 1972). It is estimated that rivers carry between 70 % and 80 % of plastic waste, most of which ends up in oceans (Horton *et al.,* 2017). Each year, over 9.5 million tons of plastics are dumped into oceans (Boucher and Friot, 2020). In 2019 (PlasticsEurope, 2020), the plastics demand focused on Polypropylene (PP, 19.4 %), Low Density Polyethylene (LDPE, 17.4 %), High Density Polyethylene (HDPE, 12.4 %), and Polyvinyl-Chloride (PVC, 10 %). Plastic gear is necessary for the viability of many aquaculture and shellfish farming operations (Schoof and DeNike,

2017). More recently, questions have arisen regarding the potential contributions of aquaculture to marine debris and the formation of MPs particles. Most varieties of oyster culture around the world rely on equipment and gear to develop a specific product, respond to environmental conditions, or provide protection for bivalves (Pacific Coast Shellfish Growers Association, 2011; USACE, 2015). Plastic is primarily used in gear such as nets, crops, or attachment tubes/cups, designed to contain or protect farmed molluscs from predators or environmental conditions (Pacific Coast Shellfish Growers Association, 2011; USACE, 2015). In the seas, macroplastics first undergo physical degradation, by waves, salt, hydrolysis, temperature, and photodegradation from the sun's ultraviolet rays (Julienne *et al.,* 2019; Andrady, 2017; Alimi *et al.*, 2018; Chubarenko *et al.,* 2019; Hernandez *et al.,* 2019), before facing biological degradation, by bacterial processes (Dussud and Ghiglione, 2014), creating MPs (fragments <5 mm, Thevenon *et al.,* 2014). Plastics have invaded all marine habitats around the world (Kershaw and Rochman, 2015; Li *et al.,* 2016). Scientists have estimated that oceans concentrated more than 5 trillion pieces of plastic materials, gathering 1 to 102,000 particles/m^3 (Van Cauwenberghe *et al.,* 2015; Auta *et al.,* 2017). Plastic pollution affects marine ecosystems and human activities. When ingested by organisms, they cause abrasions, obstructions, and alterations in physiological structures, directly influencing the survival of individuals (Wright *et al.,* 2013). Recent studies have shown that commercial MPs can affect the early stages of bivalve development (Tallec *et al.,* 2018; Bringer *et al.,* 2020a). These studies have shown the effects of polystyrene (PS)-nanoplastics (NPs) (50 nm, 10 µg.mL^{-1}) and HDPE-MPs (4-13 µm, 0.1 mg.L^{-1}) on development (malformations and survival) and on swimming behaviour in oyster larvae, *C. gigas*. The early stages of development are the stages most sensitive to environmental hazards and to potential environmental pollution and contamination (Gamain *et al.,* 2020). Cole & Galloway (2015) demonstrated the ingestion of 70 nm to 20 µm PS-MPs/NPs particles in *C. gigas* larvae over the days 3 to 24 after fertilization. However, the study by Sussarellu *et al.* (2016) showed transgenerational effects of PS-MPs on larval growth following exposure of parents for two months. Nonetheless, information on environmentally-aged MPs is limited. Gardon *et al.* (2020) investigated the effects of both new and aged MPs on the early stages of pearl oyster development (*Pinctada margaritifera*). They concluded that a 48-hour exposure to MPs significantly increased the larval mortality rate. In addition, MPs with

a high surface/volume ratio tended to adsorb persistent organic pollutants while in marine environments (*e.g.* dichlorodiphenyltrichloroethane [DDT], polycyclic aromatic hydrocarbons and heavy metals; Beaumont *et al.,* 2019; Rochman *et al.,* 2013; Bakir *et al.,* 2014). During fragmentation or ageing in an aquatic environment, plastics can release additives, such as phthalates (Koumba, 2018), UV stabilizers, antioxidants (Rani *et al.,* 2017), biocides (Beiras *et al.,* 2021) or dyes. Thus, it is essential to assess toxicity of environmentally-aged MPs on marine fauna. In 1970, due to the economic development of modern societies, questions were raised internationally in relation to plastic waste (Rocher, 2008; Dupré, 2013). Few studies considered plastic materials from aquaculture and fishing activities. Nevertheless, materials and tools from aquaculture professionals are likely to interfere with bivalve organisms (production and capture phases). Fishing gear – abandoned, lost, or discarded – is the main source of plastic waste from the fishing and aquaculture sectors, although their implication in ocean pollution is overlooked by the public (Lusher *et al.,* 2017). The aquaculture and shellfish-farming sector has diversified and expanded massively with the importance of exports in France. Following extreme hydro-climatic events (*e.g.* storms, strong winds or currents, etc.), professional materials break off and drift to beaches and coasts during high tides. The French shellfish market is of importance. Each year, nearly 130,000 tons of oysters (*C. gigas*) are produced and marketed, while the activity generates more than 20,000 jobs (CNC, 2020). The sustainability of shellfish companies is water-quality dependent. Due to their filtration feeding activities, bivalves such as oysters absorb and accumulate MPs in marine environments (Li *et al.,* 2018; Phuong *et al.,* 2018; Teng *et al.,* 2019). In addition, oysters are key ecological and economic species in coastal ecosystems. They are also used as a model species in laboratory experiments for the assessment of MPs toxic effects (Teng *et al.,* 2021). Except for the work of Tallec *et al.* (2018) on commercial PS-NPs, no study has determined the toxicity of MPs or NPs on pediveliger larvae of oyster. For the experiment, pediveliger larvae were exposed for seven days to MPs from aquaculture (cocktail of HDPE crops, PP collection cups and PVC collection tubes) at two theoretical concentrations (*i.e.* 0.1 and 10 mg MP. L^{-1}). Study (i) determined larval settlement success after 7 days' exposure to different treatments, and (ii) monitored growth for nearly a year (11 months), until larvae reached the spat stage.

2. MATERIALS AND METHODS

2.1 Pediveliger larvae

Crassostrea gigas (Bayne *et al.,* 2019; Bayne *et al.,* 2017) 17-day-old pediveliger larvae were supplied by a commercial hatchery (France Naissain, France). Upon reception and before starting experiments, the mobility and good health status of larvae were assessed (Motic 1820, x20 objective).

2.2 Seawater and preparation of microplastics from aquaculture

Plastic macrodebris were collected on the French Atlantic coast (South West, France), in Angoulins-sur-mer municipality beach area (Bringer *et al.,* 2021). They were sorted and classified, selecting only plastics aged in the marine environment, and from the oyster industry. Polypropylene cup collectors (PP), pipe collector (PVC) and high-density polyethylene oyster culture crops (HDPE) were chosen. A GC-MS pyrolysis analysis (700 °C) characterised the polymers of the selected materials (manual cutting to obtain samples of 130-200 µg). After a rough cut (3-4 cm), the plastics were crushed using a stationary metal hammer, and this was repeated several times. Then, the plastics were sieved using 5 mm, 1 mm and 100 µm sieves. The grinding was carried out at room temperature so as not to influence the nature of the material. The cocktail consisted of 28 % HDPE, 40 % PP, and 32 % PVC (Andrady, 2011). The size range of the cocktail of MPs was 138.6±2.3 µm (three analytical replicates in laser particle size distribution, Malverne). Seawater was sampled in the Atlantic (South West, France), before being filtered with 50 µm and 10 µm membranes to eliminate debris and microorganisms (*i.e.* filtered seawater, FSW).

2.3 Analysis of exposure solutions

To carry out a quantitative analysis of MPs in experimental seawater, each theoretical cocktail solution (0, 0.1, and 10 mg MP. L^{-1}) was analysed using a flow cytometer (Attune Acoustic Focusing Cytometer). Two-milliliter samples from each MP exposition solution (n=4/condition) were vortexed (StarLab Vortex IR, 12,000 rpm for 20 sec) to homogenise the solutions and then 300 µL was taken for flow cytometry analysis. A calibration was carried out, thanks to previous studies (Bringer *et al.,* 2020a) with commercial MPs, to achieve an analysis rate of 500 $\mu L.min^{-1}$ and a saturation of 10,000

particles maximum detected. From the control conditions (10 µm FSW), control analytical samples (n=12) were created. The blank obtained enabled removal of background particles (naturally present in seawater). Using seawater filtered at 0.2 µm, a first calibration was conducted to select the < 200-time detected particles. MPs measurements in seawater were assessed at D_0 (beginning of exposure) and D_7 (end of exposure) for all exposure conditions.

2.4 Experimental design

2.4.1 Exposure of pediveliger larvae in experimental microcosms

Pediveliger larvae (D_0) were acclimatised for a few hours in petri dishes at microcosm temperature (24±0.1 °C) for fixation (Figure S1). Microcosms have been defined as experimental systems simulating real-life conditions (Buffet *et al.,* 2014; Martinez-Sosa, 2019; Enya *et al.,* 2020). Fifteen thousand (15,000) larvae/condition with three replicates were added to experimental sieves, *i.e.* 45,000 larvae/condition. Then, larvae were exposed in the dark for 7 days (step 1: settlement phase, from D_0 to D_7, Figure 1). The exposure conditions were as follows: larvae were put in 195 µm sieves (three replicates per condition), at the surface of a 300-400 µm microbreak layer (Ovive industry, La Rochelle, France), and placed in 224 L tanks. Microbreaks correspond to very finely crushed oyster shells. Microbreaks was cleaned before use in microcosms. The sieves (n=3/conditions) were then placed on a table inside a tank where the FSW circulated. Sieves were all connected by an air-lift system creating a bottom-up movement of FSW (2.3 $L.h^{-1}$/sieve), allowing a continuous renewal of water in the sieves containing the pediveliger larvae. The temperature was 24.0±0.1 °C, and salinity 28.2±0.3 (Table 1). Continuous bubbling and brewing systems were set up. Brewers were installed in the microcosms in order to homogenize the dispersion of the MPs. The temperature value and salinity were measured daily. Throughout the experiment, nitrates, nitrites, chlorine, and pH (7.1±0.4) were regularly measured to ensure consistency. This experimental device was based on Glize's work (1992). The day before the start of the exposure, the cocktail of MPs was inserted into the tanks (224 L) according to three theoretical concentrations tested: 0 (control without MP); 0.1 and 10 mg MP. L^{-1}. To do this, precise quantities of MPs cocktail were weighed (n=7, *i.e.* 7 days of exposure with one renewal every day), before being inserted into the microcosms, for the two conditions of exposure to

MPs: 22.4 mg for 0.1 mg MP. L^{-1} and 2,240 mg for 10 mg MP. L^{-1}. The water was changed every day and the sieves were transferred to tanks prepared in advance, and the cocktail of MPs was added at each renewal according to the conditions. The pediveliger larvae were fed twice a day with algae paste (mixed diet of *T. Isochrysis galbana*, *Pavlova*, *Tetraselmis*, *Thalassiosira* and *Nanno*, Shelfish diet). At this stage of their development, the larvae are more sensitive to environmental risks (Connor, 1972; Martin *et al.,* 1981 and Quiniou *et al.,* 2005), hence the importance of respecting the protocols of renewal of water and daily food to avoid significant mortalities.

2.4.2 Experimental hatchery for long-term development

After a 7-day exposure period, pediveliger larvae were put in experimental hatchery devices (step 2, Figure 1). After D_7, fixed larvae were placed in a micronursery. In an open circuit, FSW (10 µm) circulated at a flow rate of 27 $L.h^{-1}$ (132 % renewal every day). The temperature was 21.3±0.5 °C, and salinity 25.1±0.3 (Table 1).

Table 1. Essential parameters (temperature, salinity, and light) for development of pediveliger larvae and oyster spat in various aquaculture devices.

Water parameters	Microcosms	Micronursery	Nursery	Oyster farm
Temperature (°C)	24±0.1	21.3±0.5	19.4±0.6	15.9±2.5
Salinity	28.2±0.3	25.1±0.3	32.0+0.7	32.6±1.5
Light	dark	natural	natural	natural

At D_{28}, oyster spat were transferred to an outdoor nursery (seawater filtered at 30 µm). The flow rate was 2,117.6 $L.h^{-1}$ (7,260 % renewal every day), temperature 16.3±1.0 °C, and salinity 27.4±0.7 (Table 1). Once in the nursery, spat were exclusively fed with naturally-present phytoplankton. Larger mesh sieves were used (500, 1000 and 3000 µm) to allow larval magnification. Then, oyster spat were placed in 4000 µm mesh crops and placed in experimental beds (D_{190}, CNRS platform, La Rochelle, France). Figure 1 summarises the development cycle from pediveliger larvae to oyster spat. It includes the aquaculture devices used for oyster development, and indicates the beginning of the exposure

period (D_0), the settlement success (end of expsoure; D_7), and the growth monitoring analysis (D_0 to D_{338},).

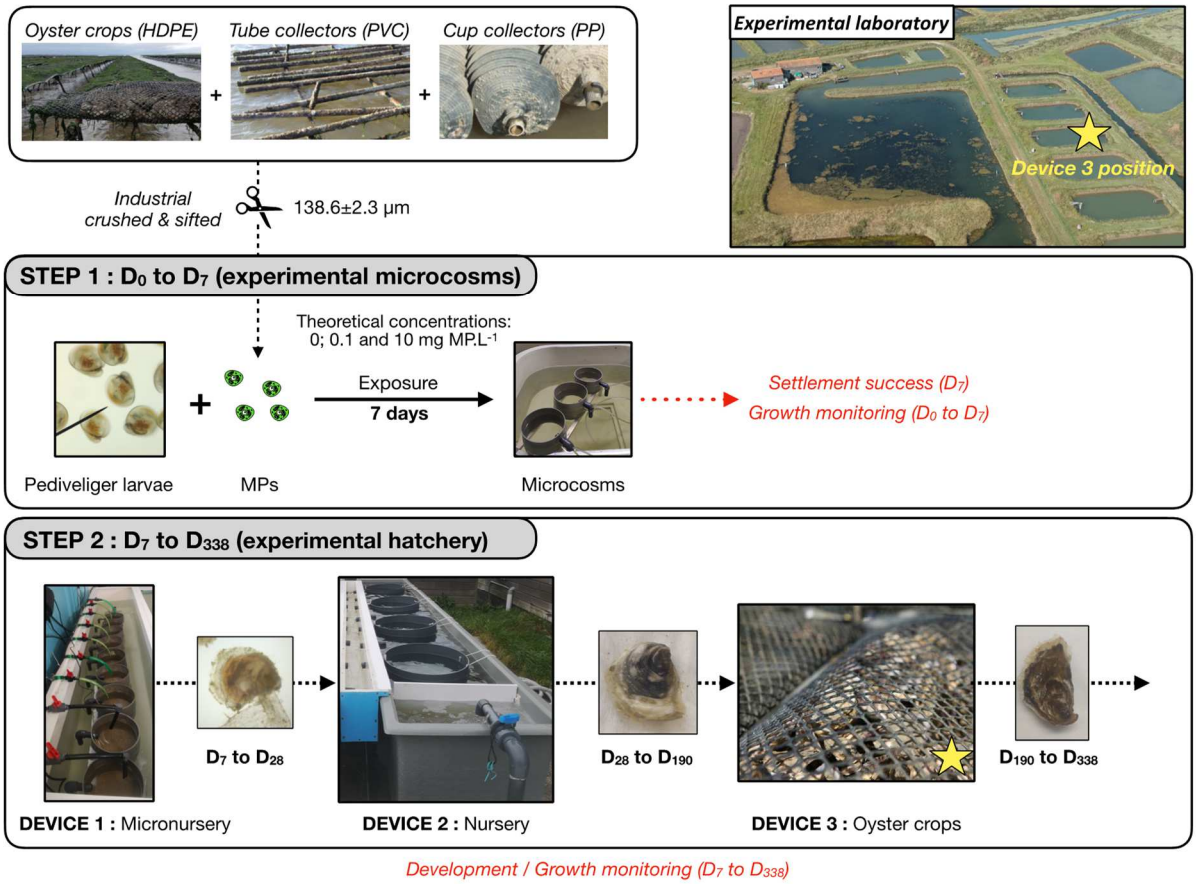


Figure 1. Experimental devices to develop pediveliger larvae into oyster spat *C. gigas*. Step 1: 7-day exposure (D_0 to D_7, settlement success) in experimental microcosms. Step 2: development (D_7 to D_{338}) in experimental hatchery. Length growth was monitored for the entire experiment (from D_0 to D_{338}, *i.e.* 11 months).

2.5 Settlement success and growth monitoring of pediveliger larvae

After a 7-day exposure period to MPs cocktail, at different concentrations, an analysis of the larval settlement ratio was performed, quantifying larvae that were fixed in each condition and replicate (n=3/condition). A ratio was calculated from the weight of samples taken (n=3/replicates, *i.e.* n=9/conditions) and the total weight corresponding to the total quantity of pediveliger larvae at the start of the experiment. After being weighed, the samples taken were analysed under a microscope (Motic 1820, 200x) and the number of larvae fixed was quantified. The oyster growth in length was

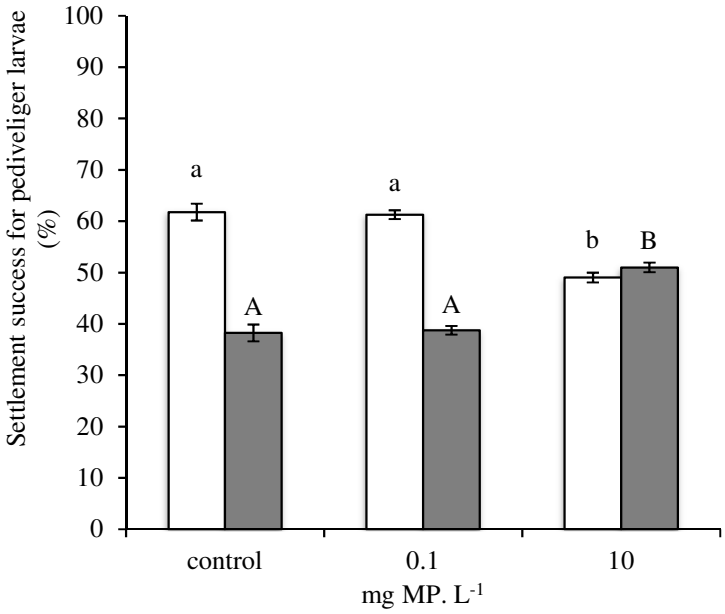
196 monitored at pediveliger and early spat stages, taking microphotographies under the Motic 1820
197 Binocular microscope (200x). Length of larvae was measured on photographs with Image J.
198 Pediveliger larvae were oriented lengthwise under the microscope (Motic 1820, x20 objective) and a
199 line was drawn with Image J (Talmage and Gobler, 2009; Helm *et al.,* 2004; Bringer *et al.,* 2020b). To
200 carry out the growth analysis, pediveliger larvae (n=30) in each replicate and condition
201 (n=90/conditions) were measured. Results are expressed in mean ± SEM. Then, oyster spat
202 (n=30/replicate, *i.e.* n=90/condition) were measured (length, width, and thickness) and weighed (total
203 weight) using a digital caliper (0.1 mm) and precision balance (0.1 g, Denver Instrument). Results are
204 expressed in mean ± SEM. During the exposure period (D_0 to D_7, microcosms, Figure 1), growth was
205 quantified every two days. During the experimental hatchery period (D_7 to D_{190}, device 1 and 2, Figure
206 1), growth was quantified every 4 days. Finally, during the period in oyster beds, growth was
207 quantified once a month (D_{190} to D_{338}, device 3, Figure 1).
208
209 ***2.6 Statistics***
210 A statistical analysis was conducted using R Studio and graphs on Microsoft Excel. Homogeneity of
211 variance (Levene's test) and normality of distribution (Shapiro-Wilk) were assessed. To compare the
212 settlement success in each treatment, an analysis of variance (ANOVA) was performed followed by a
213 Tukey's post-hoc test. A statistical analysis of growth biomonitoring data was carried out through the
214 Kruskal-Wallis test. Then, differences in mean concentrations were assessed using the Kruskal
215 Nemenyi Post-hoc test, coupled with the PMCMR package (equivalent to the Tukey's test for non-
216 parametric data). A significance was accepted for $p < 0.05$. Data are expressed in mean ± SEM.
217
218 **3. RESULTS AND DISCUSSION**
219 ***3.1 MP exposure concentrations***
220 The concentrations of MPs for the two exposure conditions (Table 2) varied between D_0 (start of
221 exposure) and D_7 (end of exposure). The results of this present study are comparable to the
222 concentrations assayed by flow cytometry in a previous study on commercial HDPE-MPs of 20-25 µm

223 (Bringer *et al.,* 2020a). In accordance with theoretical factors, the dilution factor of the two
224 concentrations tested should equate to 100. However, factors at D_0 and D_7 were of 8.88±0.62 and
225 9.17±0.37. The theoretical 10 mg MP. L^{-1} condition suggested that the real dilution factor was lower in
226 display tanks. The differences observed between the theoretical and measured concentrations of MPs
227 could come from aggregation of MPs togther (Alimi *et al.,* 2018; Michels *et al.,* 2018) and binding on
228 the walls of exposure microcosms. Despite the use of brewers, MPs might be heterogeneously
229 dispersed in the exposure medium. By taking into account the assays carried out at D_0 and D_7, we can
230 calculate the mean concentrations corresponding to the theoretical concentrations: 0.9±0.08 (0.1 mg
231 MP. L^{-1}) and 8.4±0.5 (10 mg MP. L^{-1}). No microparticles were found in the waters of the control
232 condition..
233
234 **Table. 2** Theoretical and measured MP concentrations (mean ± SEM) at the beginning (D_0) and at the end of the
235 pediveliger exposure (D_7).
236

Sampling times	Theoretical (mg MP. L^{-1})	Measured (MP. μL^{-1})	Concentration factor
D_0	0.1	0.88±0.07	-
D_7	0.1	0.97±0.09	-
D_0	10	7.8±0.65	8.88±0.12
D_7	10	8.9±0.27	9.17±0.54

237
238 Through flow cytometry, MP concentrations in exposure conditions were determined. Long *et al.*
239 (2017) assessed PS-MPs concentrations using flow cytometry. Recent studies have measured the
240 concentrations of nano and microparticles through flow cytometry (Caputo *et al.,* 2021; Kaile *et al.,*
241 2020). In this work, concentrations were higher than measured in the marine environment (Van
242 Cauwenberghe *et al.,* 2015; Auta *et al.,* 2017). A previous study measured 13.4±0.9 MP. L^{-1} in the
243 coastal Atlantic sector (Green *et al.,* 2018). In addition, the study published by Frere *et al.* (2017),
244 showed concentrations of 0.24±0.35 MP.m^{-3} in the Bay of Brest (France). These different results prove
245 to us that our experimental concentrations are stronger than the natural environment. However, the use
246 of aged aquaculture MPs in the marine environment has increased knowledge on the toxicity of plastic

247 particles at early stages of development in *Crassostrea gigas*. Apart from this research, few studies
248 focused on aquaculture plastics and their impacts.
249
250 ***3.2 MP effects on the settlement success of pediveliger larvae***
251 After a 7-day exposure, pediveliger larvae were removed from the microcosm devices and were put in
252 the micronursery (device 1 of experimental hatchery, Figure 1). Fixed larvae were counted using a
253 microscope. In the control condition (without MPs), 9,266±246 (n=3 replicates) out of the 15,000
254 larvae at D_0 were fixed. In the 0.1 and 10 mg MP. L^{-1}conditions, 9,188±129 and 7,352±142 larvae
255 were fixed (Figure 2).
256



257

258 **Figure 2.** Settlement success of pediveliger larvae of *C. gigas* a*fter a 7-day exposure:* control (0 mg MP. L^{-1}),
259 0.1 and 10 mg MP. L^{-1}. Values are expressed as mean ± SEM (n=3 replicates/conditions, *i.e.* n=9/conditions).
260 Different letters at the top of the bars indicate significant differences between concentrations ($p < 0.05$, Anova
261 and Tukey test).
262
263 In the control condition, 61.8±1.6 % larvae were successfully fixed. In the 0.1 mg MP. L^{-1} condition,
264 the ratio indicated 61.3±0.9 %, which did not statistically differ from the control condition (p=0.95).
265 However, in the 10 mg MP. L^{-1} condition, the ratio was 49.0±0.9 %, differing significantly from the

other two conditions (p<0.001). The average rate of *C. gigas* larval settlement in remote microbreaks currently reaches 65 % (Bodoy, 1990). In this work, the control condition displayed a binding rate of approximately 62 %. The experimental system was thus considered valid. After a few days of development in the micronursery, fixed pediveliger larvae began to grow shells (Figure 3). In all conditions, no delay in shell development nor developmental abnormalities (malformations) were noted.

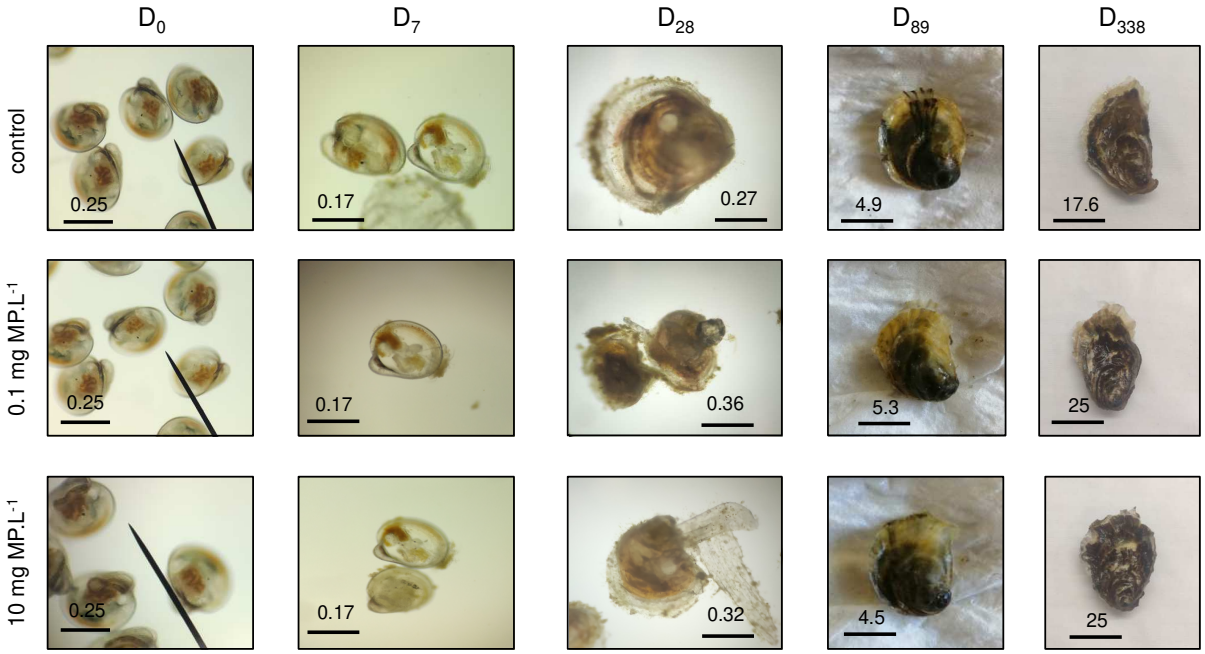


Figure 3. Microphotographs and pictures of pediveliger larvae from the beginning (D_0) to the end of the experiment (D_{338}) – including MP exposure (D_7 – step 1), spat development (D_{28} and D_{89} – step 2), and monitoring (D_{338}). Photos were taken in each condition: control (0 mg MP. L^{-1}); 0.1 and 10 mg MP. L^{-1}. Scales are expressed in mm. Observations and photographs were performed at 5X or 20X using a binocular microscope (Motic). "D_X" indicates the sampling times in days.

In an earlier study, pediveliger larvae (*C. gigas*) were exposed for a period of 24 hours, showing decreased metamorphosis rates with increasing drug concentrations tested, greater than what is found in the natural environment (Di Poi *et al.,* 2014). In the study conducted by Vignier *et al.* (2016), exposure of oyster pediveliger larvae (*C. virginica*) for 72 h to increasing doses of CEWAF (dispersed oil) induced settlement inhibition in a dose-dependent manner. The authors concluded that the settlement success was sensitive to environmental parameter variations and pollutant exposure

(Vignier *et al.,* 2016). Wang *et al.* demonstrated that the pediveliger larvae of *Pinctada maxima* are more prone to attach to natural collectors (palm rope collector) than to plastic collectors (Wang *et al.,* 2017). Larvae were considered metamorphosed if they displayed clear loss of velum and foot, shell growth and well-developed gills. Mottier *et al.* (2014) exposed pediveliger larvae for 24 h to glyphosate in commercial formulations and observed a reduction of the larval metamorphosis rate. Tallec *et al.* (2018) did not observe impacts on the metamorphosis of exposed *C. gigas* following 24 h of exposure. A variation in particle size (MPs or NPs) or concentration had no influence. In addition, after PS-MPs/NPs treatments, there were no developmental abnormalities in oysters (Tallec *et al.,* 2018). These results echoed our own observations, showing no impacts on larval metamorphosis and no developmental abnormalities during fixation. MPs had no impacts on the development of pediveliger larvae, most likely due to their protective shell (Hickman, 1999; Liebig and Vanderploeg, 1995; Schiaparelli *et al.,* 2004). Nonetheless, no studies reported MP impacts on fixation, *i.e.*, with lower fixation rate in larvae exposed to high concentrations. MPs could affect the energy dynamics of pediveliger larvae (Sussarellu *et al.,* 2016; Rico-Villa *et al.,* 2010). Larvae would lose their fixation capacities as they lack energy reserves, constantly fighting against MPs in their environment. Effects were noted on the energy reserves of sediment-dwelling bivalves. Indeed, the lipid content was lower in bivalves exposed to MPs (Auclair *et al.,* 2020; Bour *et al.,* 2018). The drifting and structure of marine organisms could be influenced by chronic MP exposure (Sendra *et al.,* 2021). In addition, recent studies have highlighted that MPs may affect immune enzyme activities and immune-related gene expression (Gardon *et al.,* 2020; Huang *et al.,* 2020). Authors hypothesised that the defence mechanisms at larval stages could be weakened and therefore individuals could be more sensitive to environmental factors such as pathogens or potential MPs (Thomas-Guyon *et al.,* 2009). Larvae of the Pacific oyster *C. gigas* appeared to acquire their own defence mechanisms during development. Potentially weakened by MPs, pediveliger larvae would have recourse to an adapted defense system to fight against plastic contamination, which can generate significant energy consumption, detrimental to growth for example.

3.3 MP effects on pediveliger larvae development and oyster spat growth

Length growth (in mm, Figure 4.a) was monitored at pediveliger larval stage from the beginning to the end of exposure (D_0 to D_7, Figure 3) and then until the spat stage, 11 months later (D_{338}, Figure 3). A 7-day exposure to MPs led to growth retardation of larvae up to 28 days (D_0 to D_{28}, Figure 4.c). Indeed, there was a significant difference of growth rate between the control condition (0.54±0.05 mm of growth for 28 days) and the 0.1 and 10 mg MP. L^{-1} conditions(0.26±0.02 and 0.30±0.02 mm of growth for 28 days). Oysters had similar growth rate between D_{28} and D_{89} (Figure 4.d), with no significant variation between the conditions tested. The larvae from the control had a growth of 9.5±0.9 mm, while the larvae from the 0.1 and 10 mg MP. L^{-1} conditions grew at 8.1±0.7 and 8.6±0.7 mm for 61 days (from 28 to 89 days). From D_{89} to D_{338} (end of growth monitoring), pre-exposed oysters exhibited high growth during their development (Figure 4.e). Control spat grew 32.1±2.0 mm while the spat from the 0.1 and 10 mg MP. L^{-1} conditions had similar growth of 41.5±2.5 mm and 41.3±2.1 mm, for 249 days (from 89 to 338 days, Figure 4.e).

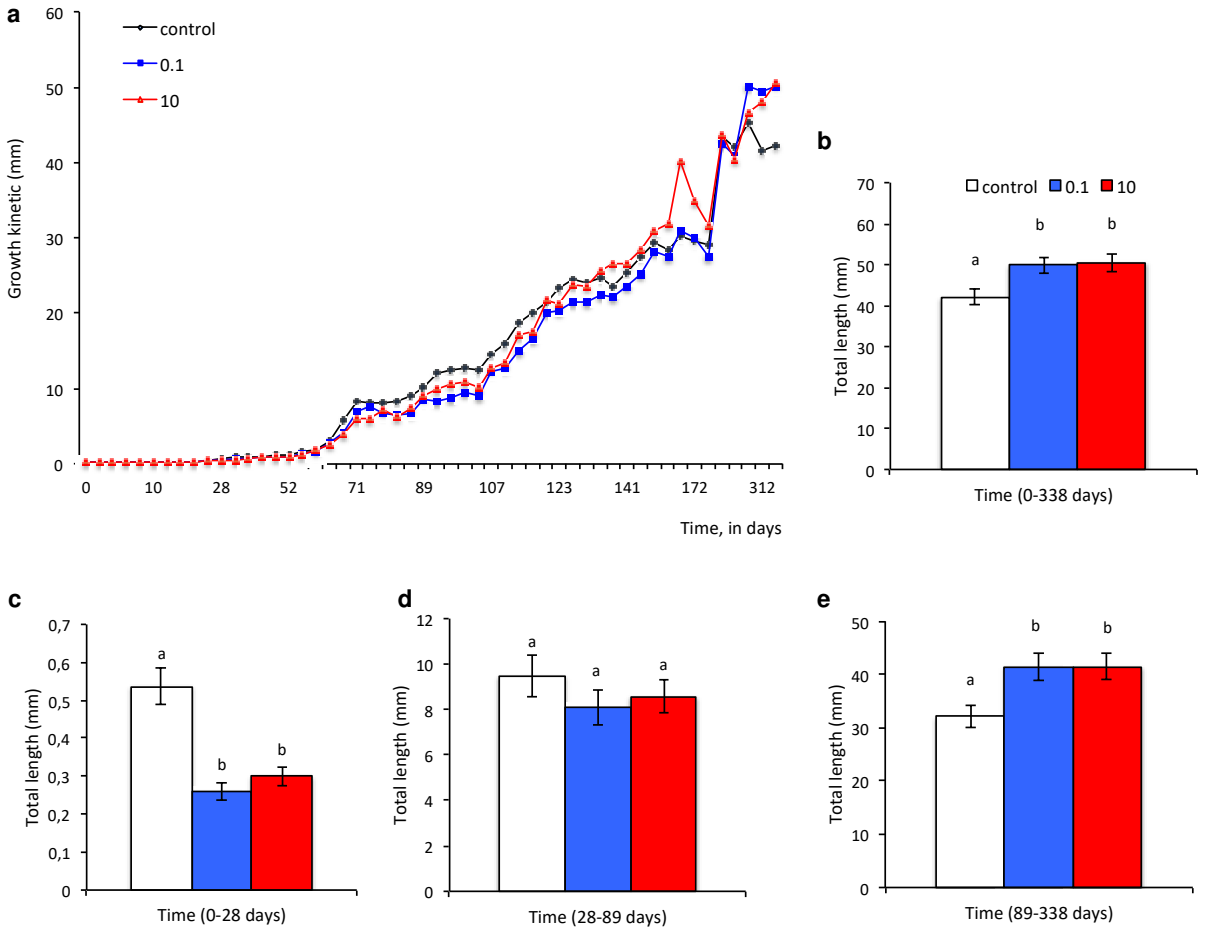


Figure 4. (a) Growth kinetic (D_0 to D_{338}) **(b)** and total length of *C. gigas* pediveliger larvae and oyster spat. **(c)** Graphical displays on total length from D_0 to D_{28}; **(d)** D_{28} to D_{89} and **(e)** D_{89} to D_{338} in various exposure conditions: control (0 mg MP. L^{-1}), 0.1 and 10 mg MP. L^{-1}. Values are expressed in mean ± SEM (n=30/replicates, *i.e.* n=90/conditions). Different letters at the top of the bars indicate significant differences between exposure conditions ($p < 0.05$, Kruskal-Wallis and Nemenyi test).

The total growth (D_0 to D_{338}) was significantly higher in MPs-exposed oysters in comparison to control individuals (Figure 4.b). Indeed, the control condition observed a total growth of 42.1±1.9 mm while the 0.1 and 10 mg MP. L^{-1} conditions displayed a total growth of 49.8±2.0 and 50.4±2.1 mm (Figure 4.b). Our results indicate that the oyster growth during the fixation phase was delayed by MPs exposure. Then, the growth at the end of experiment turned out to be higher than for oysters from the control condition. Few studies monitored the growth of exposed invertebrates on a long-term basis. In some cases, growth, respiration and filtration rates of oysters were not impacted by MPs, compared to other benthic study models that were weakened by exposures to MPs (Green, 2016). Revel *et al.* (2020) showed that PE and PP-MPs (<400 µm) had no significant toxicity on adult oysters after 10 days of exposure to 0-100 µg MP. L^{-1}. Cole and Galloway (2015) reported that PS-MPs of 1 and 10 µm restricted algal feeding of oyster larvae, but there were no consequences on the growth of oyster larvae exposed up to 100 MP. mL^{-1}. After a two-month exposure to low and high doses of PLA (polyactic acid) or HDPE-MPs, filtration and growth rates of adult *Ostrea edulis* were unaltered (Green, 2016). Because of the relatively low toxicity and long-term effects of MPs and additives, toxic effects analysis over a long periode of time is particulary relevant.. Analysis of metabolic rates and assimilation efficiency indicated a decrease in mean energy balance, when comparing adults from the control condition to those exposed for two months to 0.25 and 2.5 µg. L^{-1} micro-PS (Gardon *et al.,* 2018). The impacts on growth and energy dynamics are likely interconnected. Widdows and Johnson (1988) suggested that the energy available for growth should be a balance between the energy from food consumption and that used for respiration. Oyster larvae facing chronic MPs exposure could consume the energy dedicated to the fixation and development. Sussarellu *et al.* (2016) exposed adult oysters during gametogenesis for two-months to 23 µg. L^{-1} PS-microbeads (2-6 µm) and reported

delayed larval growth. Watts *et al.* (2015) fed crabs (*Carcinus maenas*) over a period of 4 weeks (fed 2-3 times per week) with different amounts of PP rope microfibers (0; 0.3; 0.6 and 1 % added to food). Those fed with plastics had a significant decrease in growth, mainly driven by a reduced food consumption over time. Larvae (*Crepidula onyx*) fed with higher concentrations (30 and 70 % of the algal concentration with final concentration of $6x10^4$ and $1.4x10^5$ particles.mL^{-1} respectively) of micro-PS (2-5 µm) than those found in the environment were smaller due to their reduced growth rate, in comparison to the control species (Lo and Chan, 2018).

5. CONCLUSIONS

This study observed the effects of a cocktail of MPs (138.6±2.3 µm) containing particles of HDPE, PP and PVC from aquaculture plastics aged in the marine environment. After an exposure period of 7 days, the settlement success of the pediveliger larvae was evaluated. Then, an 11-month follow-up was carried out to monitor the growth in length of the pediveliger larvae up to the spat stage. Larvae exposed to 10 mg MP. L^{-1} showed a lower binding rate to substrate. In addition, the larvae exposed to the two concentrations of MPs (0.1 and 10 mg MP. L^{-1}) exhibited growth retardation over 28 days of follow-up. At the end of the experiment (at 338 days), the spat produced from the larvae exposed to the MPs recovered their growth retardation, and over 11 months had a total growth greater than the control group. The energy used by larvae facing MPs exposure could affect the oysters' ability to grow. It may be relevant to conduct further studies on the potential effects of MPs on energy reserves and the immune system of oysters at early stages of development. This study provides new evidences on the environmental risks of realistic high concentrations of MPs on aquatic invertebrates.

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