Subchronic exposure to high-density polyethylene microplastics alone or in combination with chlortoluron significantly affected valve activity and daily growth of the Pacific oyster, *Crassostrea gigas*

Arno Bringer, Hlne Thomas, Emmanuel Dubillot, Stphane Le Floch,
Justine Receveur, Jrme Cachot, Damien Tran

► To cite this version:

Arno Bringer, Hlne Thomas, Emmanuel Dubillot, Stphane Le Floch, Justine Receveur, et al.. Sub-chronic exposure to high-density polyethylene microplastics alone or in combination with chlortoluron significantly affected valve activity and daily growth of the Pacific oyster, *Crassostrea gigas*. *Aquatic Toxicology*, 2021, 237, pp.105880. 10.1016/j.aquatox.2021.105880 . hal-03434008

HAL Id: hal-03434008

<https://hal.science/hal-03434008>

Submitted on 23 Nov 2021

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 destine au dpt et la diffusion de documents scientifiques de niveau recherche, publis ou non, manant des tablissements d'enseignement et de recherche franais ou trangers, des laboratoires publics ou privs.

Subchronic exposure to high-density polyethylene microplastics alone or in combination with chlortoluron significantly affected valve activity and daily growth of *Crassostrea gigas*.

Arno Bringer^{1*}, Hélène Thomas¹⁺, Emmanuel Dubillot¹, Stéphane Le Floch³, Justine Receveur³, Jérôme Cachot²⁺, Damien Tran²

¹ Littoral Environnement et Sociétés (LIENSs), UMR 7266, CNRS-Université de La Rochelle, 2 rue Olympe de Gouges, F-17042 La Rochelle Cedex 01, France.

² Université de Bordeaux, CNRS, EPOC, EPHE, UMR 5805, F-33600 Pessac, France.

³ Cedre, 715 Rue Alain Colas, 29218 Brest Cedex 2, France.

⁺: equal contributions

^{*}: Corresponding author: Arno Bringer: Tel: +33 645299739 (FR), Email: arno.bringer1@univ-lr.fr

Abstract

Nowadays, pesticides and microplastics (MPs) are commonly found in coastal waters worldwide. Due to their widespread use, their persistence and toxicity, they may induce adverse effects on physiology and behaviour of marine organisms such as the Pacific oyster (*Crassostrea gigas*). This study explored the growth and valve activity of juvenile oysters exposed for 24 days to two frequently detected pollutants in the Pertuis Charentais (South-West France): an herbicide (chlortoluron, 30 µg.L⁻¹) and high-density polyethylene microparticles (HDPE 20-25 µm, 10 µg MP.L⁻¹) alone or in combinaison. The characterisation of the valve activity of juvenile oysters, recorded by using a High Frequency and Non-Invasive valvometer (HFNI) depended on three parameters: the number of valve micro-closures (VMC), the Valve Opening Amplitude (VOA) and the Valve Opening Duration (VOD). Additionally, daily shell growth and the oyster daily rhythm were assessed. The exposure to MPs of oysters led to a significant, increase of VMC and a decrease of VOD and shell growth. The exposure to chlortoluron showed a significant, increase of VOA and a decrease of VMC.

In mixture with MPs, chlortoluron still increased VOA and decreased VMC but also reduced the shell growth. Chronobiological analysis did not reveal any effects on the daily rhythm of both contaminants. High experimental concentrations of MP and chlortoluron were tested. This work has underlined their selective impacts on the behavioral and physiological parameters studied.

Graphical abstract

Keywords Bivalve molluscs, Experimental biomonitoring, Microplastics, Pesticide, Valve behaviour, Growth.

1. Introduction

With a production of 135,000 tons of bivalves in 2017, including 81,000 tons of oysters, France, is the 2nd most important producer of bivalves in Europe (France Agrimer, 2020). Shellfish farming turnover was estimated at 774 million euros in 2019 (CNC, 2020). The Pertuis Charentais area (South-West, Atlantic coast, France) represents 23 % of the national oyster production (CNC, 2016). Since 2010, juvenile and adult oysters are facing excess mortality rates, reaching 70 to 80 % of oyster farms located in the Atlantic coast (Soletchnik *et al.*, 2018 ; Lucasson, 2018 and Girard & Agundez, 2014). The quality of the coastal waters, where bivalves are grown, is a source of concern today. The increased use of plastics (Phuong, 2018) in aquaculture generates MPs pollution, which could affect physiology of bivalves (*e.g.* growth, immune status, reproduction) (Sussarellu *et al.*, 2016; Paul-Pont *et al.*, 2018; Gardon *et al.*, 2020). Scientific studies are working to offer more and more toxicological tests on emerging contaminants such as MPs and pesticides, in order to better understand the modes of action of these pollutants (Wang *et al.*, 2020; Bhagat *et al.*, 2020).

Plastics are widely used polymers, which came as a revolution in the past decades (Xu *et al.*, 2017). In 2019, nearly 370 million tons of plastics were produced in the world and in Europe, plastics production almost reached 58 million tons (PlasticsEurope, 2020). In Europe, 80 % of the plastic production resorts to six main polymers (PlasticsEurope, 2016): polypropylene (PP), high and low-density polyethylene (HDPE and LDPE), polyvinyl chloride (PVC), polyurethane (PUR), polyethylene terephthalate (PET) and polystyrene (PS). It is estimated that rivers carry between 70 % and 80 % of plastic waste, most of which ends up in oceans (Horton *et al.*, 2017). Through wave activities, UV degradation and physical abrasion, larger plastic pieces in the marine environment are fragmented, resulting in producing MPs and nanoplastics (NPs) (Born & Schüttrumpf, 2019; Díaz-Mendoza *et al.*, 2020). MPs are plastic particles ranging from 1 µm to 5 mm (Cole *et al.*, 2011; Wagner *et al.*, 2014). MPs ingestion was observed in numerous aquatic species, notably in filter-feeding bivalves at different life stages (Rist *et al.*, 2019; Ward *et al.*, 2019; Betsill *et al.*, 2019; Bringer *et al.*, 2020b). It can cause several harmful effects at the physiological and tissue levels, affecting the growth and reproduction of bivalves (Lee *et al.*, 2013; Sussarellu *et al.*, 2016; Lo and Chan, 2018), as well as

physical injuries (Gall & Thompson, 2015) and reduced eating behavior (Cole *et al.*, 2015). At the individual level, this can lead to the depletion of energy reserves (Wright *et al.*, 2013 and Xu *et al.*, 2017), larval development leading to delays and malformations (Tallec *et al.*, 2018), and swimming activity (Bringer *et al.*, 2020a). Toxic effects are mainly observed using high laboratory exposures (and not *in situ*) compared to environmental concentrations reported in the literature (Paul-Pont *et al.*, 2018). The environmental concentrations mainly consider size classes of MP > 333 μm (Song *et al.*, 2015; Zhao *et al.*, 2014; Baldwin *et al.*, 2016) and therefore contain a significant bias, with respect to the small sizes used in the laboratory. Environmental concentrations ranging from 0.002 to 0.5 mg MP. L⁻¹ have been detected in surface water in different areas of the world (Eriksen *et al.*, 2013; Enders *et al.*, 2015).

Chlortoluron that belongs to substituted urea family is one of the most commonly used herbicides in wheat and barley crops. Chlortoluron inhibits photosynthesis and affects phytoplankton (Valiente Moro *et al.*, 2012). In 2017, a maximum value of 0.18 $\mu\text{g.L}^{-1}$ was detected at the mouth of a river (surface water) flowing into the Pertuis Charentais (Leonard, 2017). A concentration of 0.14 $\mu\text{g.L}^{-1}$ of chlortoluron was detected in 2018, at the mouth of the Charente (Pertuis Charentais), close to oyster farms (Action program, 2018). The herbicide family has already been studied on oysters, showing effects at the early stages of development on survival and development (Mottier *et al.*, 2013; Gamain *et al.*, 2017; Behrens *et al.*, 2016; Mai *et al.*, 2013), during gametogenesis with genotoxic effects (Akcha *et al.*, 2020), as well as by modulating molecular and biochemical parameters (Lee *et al.*, 2017).

The valve activity on the oyster was measured using an HFNI (High Frequency Non-Invasive) valvometer biosensor (Andrade *et al.*, 2016). This biosensor has made possible to monitor behavioural responses of bivalves when exposed to stress events, such as, contaminants. Assessing the valve activity behaviour through an HFNI valvometer has already been used to study contaminants, including traces of metals (Tran *et al.*, 2004a, 2007), radionuclides (Tran *et al.*, 2004b, 2005) and harmful algae (Tran *et al.*, 2010, 2015; Haberkorn *et al.*, 2011). To our knowledge, no

valvometric behavioral study, under laboratory conditions, has been carried out to assess the consequences of exposure to pesticides and MPs. However, in recent years, several articles have underlined the relevance of modelling and analysing the behaviour of marine animals under experimental conditions mimicking environmental conditions (Mat *et al.*, 2014; Ahmed *et al.*, 2015). In the present study, under controlled conditions and to characterize the behaviour of the oyster valve, three parameters were recorded: VMC, VOA and VOD. In addition, a chronobiological analysis of the daily rhythm of the valve was carried out as well as a measurement of the daily growth. The aim of this study was to better understand the potentially disrupting effects of emerging contaminants (MPs and pesticides) alone or in cocktail on the daily growth and the valve activity of Pacific juveniles oysters (*C. gigas*).

2. Material and Methods

2.1. Individuals and acclimatization

Pacific oysters, *Crassostrea gigas* (Bayne *et al.*, 2019; Bayne *et al.*, 2017), were kindly provided by a hatchery (™France Naissain, France). This experiment was conducted on 12-month-old juvenile oysters (59.5 ± 1.1 mm, 22.6 ± 1 g total mass) during autumn season (after the period of gametogenesis and fertilization). Four homogenous groups of diploid oysters ($n = 8/\text{condition}$) were chosen. Each group was distributed in a 37.5 L tank. Each tank was continuously filled with oxygenated seawater coming from a large buffer tank. For the experiment, oysters were acclimated for 7 days in UV-treated running (78.3 L.h^{-1}) seawater before being exposed to pollutants for 24 days. Seawater was taken from the Pertuis Breton (South-West, France), which is directly connected to the experimental area, and filtered using a 50 μm and 10 μm membrane in order to eliminate debris. Filtered seawater (FSW) was used for experimental exposure tanks. The FSW temperature in tanks was of 16.8 ± 0.4 °C and the salinity of 35.7 ± 0.2 ‰. The temperature value and salinity were daily measured. Throughout the experiment, nitrates, nitrites, chlorine and pH (7.3 ± 1.0) were regularly

measured to ensure consistency. A continuous bubbling and a brewing system were set up. Throughout the duration of the experiment, a natural photoperiod was established. In addition, the oysters were nourished thanks to the natural presence of phytoplankton in the seawater used. During the acclimatization and experimental periods, no mortality events were recorded. Nevertheless, failures of the valvometric signal recording, due to an electrical leak at electrodes level during the study, reduced the number of oysters studied to respectively $n = 7$ in the control, $n = 6$ in the chlortoluron, $n = 8$ in the MP and $n = 5$ under cocktail conditions.

2.2 Experimental procedure

MPs were used ($^{\text{TM}}$ HDPE 20-25 μm MPP-1241, density 0.96, Micropowders Inc. USA) and chlortoluron ($^{\text{TM}}$ Pestanal, analytical standard, 45400-250MG-R reference) was purchased from Sigma-Aldrich Chemical (St. Quentin Fallavier, France). The solutions containing MPs and chlortoluron were prepared beforehand in test glass tubes. Stock solutions, prepared in pure Mili-Q-water, showed a MPs concentration of 10 mg MP.L^{-1} and a chlortoluron concentration of 3 mg.L^{-1} . They were kept cool in the dark in order to prevent bacterial growth. The stored solutions were then diluted in FSW to lower the concentrations to $10 \mu\text{g MP.L}^{-1}$ and $30 \mu\text{g.L}^{-1}$ respectively. After the 7-day acclimatization period, the pollutants are added to the experimental system, in the buffer tanks. Every two days, 30 % of the FSW in the buffer tank was renewed, for the well-health of the oysters. Upstream, FSW were stored in separate tanks, with the tested pollutants, at a temperature similar to that of tanks containing oysters. They were used to supply the buffer tanks during the renewal of FSW. Water stirrers have been installed in each buffer tanks in order to homogenize the plastic microparticles as much as possible in the seawater. In addition, the cytometric analyzes made it possible to determine a certain homogeneity in the exposure tanks. Polystyrene foams protected tanks and equipment to reduce vibrations from electrical equipment. In addition, capacitance chambers were installed to

reduce vibrations from the air pipes bringing oxygen into FSW, along with behavioral analyzes (Fig. S1).

2.3 HFNI valvometry analysis

To assess the pollutant impact (MPs and chlortoluron) on the oyster's valve behaviour, the valve activity was recorded using a High Frequency Non-Invasive (HFNI) valvometer. The oysters were equipped with light electromagnetic electrodes coated in resin (≈ 1 g), which were glued at the same spot on both shells. The electrodes were attached to free covered stranded copper wires (diameter: 0.98 mm, length: 120 cm), sending data to a device. The induced voltage, measured by biosensors, varied according to the distance between the electromagnetic electrodes. For each oyster, a measure was taken every 4.8 sec., *i.e.* 18 000 data/oyster/day. For more details, see Tran *et al.* (2015) and Andrade *et al.* (2016). Bivalve behaviour was monitored 24/24 hours and 7/7 days (Tran *et al.*, 2004a and Haberkorn *et al.*, 2011). The biosensor used consists of very sensitive electrodes, resulting in minimal experimental constraints. The valve activity was recorded non-stop, both for the 7-day acclimatisation and the 24-day experimental periods. Then, the data were analysed using the LabView 8.6 software (TMNational Instruments, Austin, TX, USA).

Three behavioural parameters were assessed: daily and hourly VMC, VOA and VOD. Micro-closures (VMC) designate partial and rapid closures followed by a re-opening of the valves. A VOA equal to 100 % and 0 % meant that the valves were opened at maximal and minimal amplitudes, respectively, during the studied duration. Finally, A VOD equal to 100 % and 0 % meant that the valves were opened or closed, respectively, during the studied duration. Mean daily VMC, VOA and VOD were the average results of the hourly values recorded for each oyster. The HFNI biosensor enabled to individually measure the daily growth of shells by calculating the minimal distance between the electrodes when the oyster valves were closed. For instance, in bivalve molluscs, calcification

appears at the shell internal surface. Thus, the cumulative daily shell length growth was calculated as indicated: minimal distance between electrodes (day (n) – day (1)) x 100 % (n/24), n representing the number of the day. To obtain the actual shell length growth, manual measurements (digital caliper, $\pm$ 0.1 mm) were performed at the start (D_0) and at the end (D_{24}) of the experiment. The difference in mm equals 100 % growth. Growth rates have been measured using the HFNI valvometer based on the fact that calcification in bivalves occurs in the mantle cavity, all over the shell's internal structure (Figure 4). When daily growth layers are deposited, the minimum distance between the electrodes glued to the shells increases providing a good proxy of growth (Schwartzmann *et al.*, 2011; Berge *et al.*, 2015).

To study the daily biological rhythm ($\tau = 24$ h) of the valve activity, a statistical chronobiological analysis was conducted using the software Time Series Analysis Sieriel Cosinor 6.3 (<http://www.euroestech.com>). Several steps were required to validate a significant biological rhythm (Gouthiere *et al.*, 2005). The first step included verifying the data quality of biological and physical phenomena. The absence of random distribution in data was assessed using an autocorrelation diagram, whereas the absence of a stationary phenomenon was calculated with a partial autocorrelation function (Box *et al.*, 2015). The second step involved searching significant periodicity ($p = 0.95$), using the Lomb and Scargle periodogram (Scargle, 1982). The third step added a modelling and statistical validation using the Cosinor model, which is a cosine function included in a regression model (Nelson *et al.*, 1979). Two tests validated the model and, thus, the existence of a biological rhythm. The elliptic test (Bingham *et al.*, 1982) had to be rejected, leading to a probability of null amplitude hypothesis of < 0.05 . When conditions were met, a chronobiometric parameter computed the Percent Rhythm (PR, %), which is the percentage of cyclic behaviour determined by the Cosinor model.

2.4 Contaminant analysis

Chlortoluron and MPs concentrations were measured twice: on day 5 (D_5 , 5 days after the start of the experiment) and day 24 (D_{24} , the last day of the experiment). Water samples ($V = 100$ mL/replicate with $n = 3$ replicates) and oyster tissues ($n = 7$ /condition) exposed to both chlortoluron and the cocktail solution were assayed using the QuEChERS method (Quick Easy Cheap Efficient Rugged and Safe, Anastassiades *et al.*, 2003) and the SBSE analysis (Stir Bar Sorptive Extraction, Baltussen *et al.*, 1999) (Pfannkoch *et al.*, 2010 and from the AOAC 2007.01 method of Lehotay, 2007).

To analyse the oyster tissue and determine chlortoluron concentrations, 3 g of oyster tissue was spiked with an internal standard solution. Milli-Q water was added to the solution to reach 15 g. After being vortexed once, samples were added with 15 ml of acetonitrile (Pestnorm, VWR Chemicals), before being vortexed a second time (1 min, 160 rpm). Then, 6 g of magnesium sulphate (anhydrous, BioReagent, Sigma-Aldrich) and 1.5 g of sodium acetate (anhydrous, >99 %, Sigma-Aldrich) were added to the samples, which were then vortexed again (10 min, 250 rpm) and centrifuged (5 min, 4700 rpm, 5 °C). Two phases were formed and supernatant containing the organic phase with acetonitrile was sampled. Then, 10 mL of the supernatant were collected in the previous step and were taken and placed in a 125 mL flask, where 40 ml of sodium carbonate (0.1 M, ≥ 99.7 %, Sigma-Aldrich) was added. A Twister (magnetic stir bar coated with polydiméthylsiloxane) was inserted into the bottle. SBSE extraction was performed (16 h, 700 rpm), in the dark). After the completion of the extraction, the bars were rinsed off with Milli-Q water before being dried on a fabric.

To analyse the chlortoluron seawater exposure samples, 10 mL of methanol (Pestnorm, VWR Chemicals) was put in a bottle containing the internal standard solution. 100 mL of seawater and a Twister were then added. Protected from light, the solution was stirred for 16 hours at 700 rpm. Chlortoluron was quantified by gas chromatography (HP 7890N equipped with a Combipal MPS2 multifunction injection system, 300 °C, Gerstel, Switzerland) and mass spectrometry (GC/MS), coupled with a TDU (Thermal Desorption Unit, Gerstel: 50 °C (0.5 min) to 280 °C (6 min) at 15 °C/min)

and a temperature-programmed injector (Cooled Injection System, Gerstel: -10 °C (0.05 min) to 300 °C (10 min) at 12 °C/s). The temperature was programmed from 70 °C (5 min) to 150 °C increasing by 20 °C/min, then to 320 °C (5 min) increasing by 7 °C/min. The carrier gas, helium, was at constant flow rate (1 mL/min). The capillary column was a 5-ms RXi (Restek, Bellefonte, USA): 30m x 0.25mm ID x 0.25µm (film thickness) column. Through tandem mass spectrometry (Agilent 7000 Triple Quad), a chromatograph and a detector were coupled together. The quantitative analysis of chlortoluron was performed in MRM mode by internal calibration. Two transitions for each compound were set up: the first to quantify the molecules and the second to confirm their nature. The second step defined the nature by calculating the qualifier/quantifier ratio and comparing them to the reference values of pure compounds. The acquisition frequency of each fragment was of 2 cycles/s.

Due to the small sizes of particles (20-25 µm), the MPs analysis in oyster tissues was not performed. According to the protocol described in Bringer *et al.* (2020a & 2020b), flow cytometer (Attune Acoustic Focusing Cytometer) enabled a quantitative analysis of MPs in the 10 µg MP.L⁻¹ condition. 2 mL samples were prepared with the MPs solution (n=8/condition). The samples were vortexed (StarLab Vortex IR, 12,000 rpm for 20 s) before being transferred into the cytometer to homogenise the solution. 300 mL were taken to carry out a flow cytometry analysis. Calibration was conducted to reach an analysis rate of 500 mL.min⁻¹ and a saturation of maximum 10,000 particles detected. The blank obtained enabled to remove background particles (naturally present in seawater). Using seawater filtered at 0.2 µm, a first calibration step was conducted to select the < 200-time detected particles.

2.5 Statistics

All data were expressed as means ± standard error of the mean (SEM). Treatment differences were determined using a one-way analysis of variance (ANOVA), when both criteria of homogeneity of

variance (Levene's test) and normality of distribution (Shapiro-Wilk) were met. When these requirements were not met, a statistical analysis was performed using the non-parametric Kruskal-Wallis test, followed by the Nemenyi Post-hoc test for pair comparisons. Statistical comparisons were made on the means of each parameter according to the exposure conditions. Significant difference was accepted when p -value < 0.05. The statistical analysis was conducted using R.

3. Results

3.1 Pollutant exposure

It is noted, that no significant difference was observed concerning the assays carried out for chlortoluron (FSW and total tissues), nor for MP (FSW). Chlortoluron concentrations were determined in exposure waters (Table 1.a). Under control, water contamination increased over time. On D₂₄ (condition chlortoluron vs cocktail), an increase in the concentration of chlortoluron was noted. The concentration of chlortoluron in the cocktail condition was slightly higher than for the condition of chlortoluron alone (at D₅ and D₂₄). In both cases, the measured concentrations were well above the theoretical concentration of 30 µg.L⁻¹. The concentrations of chlortoluron measured on the soft body of the oyster are shown in Table 1.a. No trace of herbicide was detected in control individuals. While in exposed individuals, tissue concentrations of chlortoluron were measured at both sampling times D₅ and D₂₄.

The concentrations of MP in FSW (Table 1.b) were above the desired theoretical concentration of 10 µg of MP.L⁻¹. The approximate actual concentrations calculated in this study were determined using additional data from a previous study (Bringer *et al.*, 2020a), which used and tested the same commercial MP (HDPE, 20-25 µm), at different concentrations. The additional data allowed for calibration and validation of the current results. The concentrations of MP were stable over time (D₅

and D₂₄) and similar between the exposure conditions (MP alone vs cocktail). In addition, an analysis of the particles in the control FSW was performed and no MP particles were detected.

Table 1. (a) Theoretical and measured chlortoluron (herbicide) concentrations in oyster tissues (dry weight) and seawater for three conditions: control, chlortoluron and cocktail (MPs + chlortoluron). Values are mean $\pm$ SEM for n=7/condition for oyster tissues and n=3/condition for water samples. **(b)** Theoretical, measured MPs and approximate concentrations concentrations in three conditions: control (n=12), MPs (n=8) and cocktail (MPs + chlortoluron, n=8). Values are mean $\pm$ SEM.

a

Conditions	Samples time	Theoretical ($\mu\text{g.L}^{-1}$)	Oyster tissues (ng.g^{-1})	Water exposure ($\mu\text{g.L}^{-1}$)
control	Day 5	0	0	0.06 $\pm$ 0.0
	Day 24	0	0	1.5 $\pm$ 0.9
chlortoluron	Day 5	30	226.9 $\pm$ 69.5	58.3 $\pm$ 25.1
	Day 24	30	229.5 $\pm$ 92.0	85.5 $\pm$ 17.0
cocktail	Day 5	30	366.0 $\pm$ 132.7	74.6 $\pm$ 4.9
	Day 24	30	242.3 $\pm$ 199.6	96.6 $\pm$ 25.8

b

Conditions	Samples time	Theoretical ($\mu\text{g MP.L}^{-1}$)	Measured (MP.mL ⁻¹) 20-25 μm size	Approximate concentrations ($\mu\text{g MP.L}^{-1}$)
control	Day 5	0	0	0
	Day 24	0	0	0
MPs	Day 5	10	93.9 $\pm$ 31.2	47
	Day 24	10	111.7 $\pm$ 11.5	56
cocktail	Day 5	10	124.4 $\pm$ 30.3	60
	Day 24	10	107.6 $\pm$ 49.3	54

3.2 Valve activity of oysters

3.2.1 Valve Micro-Closures (VMC)

The number of VMC per day and per individual is shown on Fig. 1a. Compared to the other conditions, the daily VMC in MPs over the 24-day experiment highlighted a significant increase ($p < 0.001$). The mean VMC for MPs was 2.9 times higher than for the control condition (Fig. 1b). Fig. 1b clearly showed a significant decrease in the chlortoluron and cocktail VMC, compared to the control

condition VMC. The mean VMC for the conditions of exposure to chlortoluron and cocktail were respectively 40.3 % and 58 % lower compared to the control condition.

Fig. 1. (a) Daily VMC showing the 7-day acclimatisation and the 24-day exposure periods. **(b)** Histograms of mean VMC $\pm$ SEM for the 24-day exposure period. The different conditions are: control (white, $n = 7$), chlortoluron alone (green, $n = 6$), MPs alone (blue, $n = 8$) and cocktail (red, $n = 5$). Different letters indicate significant differences between concentrations ($p < 0.05$).

3.2.2 Valve Opening Amplitude (VOA)

In terms of VOA, the 24-day experiment underlined contrasting results between the MPs condition and the two other conditions containing herbicides (Fig. 2a, b, c). Although the VOA was similar for the MPs and control conditions (with no significant differences), it was significantly higher in chlortoluron and cocktail conditions compared to control (both $p < 0.001$). Over the entire experiment, the mean VOA displayed major differences between the four conditions ($p < 0.001$). Compared to the control condition, a significant increase in mean VOA of 18.2 % and 22.3 % were respectively noted for the chlortoluron alone and cocktail conditions (Fig. 2d).

Fig. 2. Valve Opening Amplitude (VOA) in percentage for each condition: **(a)** chlortoluron alone (green), **(b)** MPs alone (blue) and **(c)** cocktail (red), in comparison with the control condition (white). **(d)** Histograms of VOA (%) for the entire exposure and all conditions. Values are mean $\pm$ SEM for control ($n = 7$), chlortoluron ($n = 6$), MPs ($n = 8$) and cocktail ($n = 5$) conditions. Different letters indicate significant differences between concentrations ($p < 0.05$).

3.2.3 Valve Opening Duration (VOD)

Over the 24-day experiment (Fig. 3 a, b, c), the VOD for both the chlortoluron and cocktail conditions remained unchanged. In contrast, the VOD for the MPs condition significantly decreased compared to the control condition. Indeed, over the entire experiment, the mean VOD highlighted a significant decrease ($p < 0.001$) of 5.1 % for the MPs condition, compared to the control one (Fig. 3d).

Fig. 3. Valve Opening Duration (VOD) in percentage for each condition: **(a)** chlortoluron alone (green), **(b)** MPs alone (blue) and **(c)** cocktail (red), in comparison with the control condition (white). **(d)** Histograms of VOA (%) for the entire exposure and all conditions. Values are mean $\pm$ SEM for control ($n = 7$), chlortoluron ($n = 6$), MPs ($n = 8$) and cocktail ($n = 5$) conditions. Different letters indicate significant differences between concentrations ($p < 0.05$).

3.2.4 Chronobiological analysis

Through a statistical chronobiological approach, a daily rhythm analysis was performed using the Cosinor method. When comparing the three conditions to the control one, no significant disruption of VOA rhythm was observed. Figure S2 displays for each condition, the spectral analysis of the valve activity (Lomb and Scargle periodogram). It highlighted a significant periodicity in the valve behaviour. The daily mean VOA for all conditions were: $\tau = 24.0 \pm 0.1$ (control); 23.8 ± 0.1

(chlortoluron); 23.9 ± 0.1 (MPs); 23.9 ± 0.1 h (cocktail). Periodicities were observed using the Cosinor model, which validated the presence of a daily rhythm for all conditions ($p < 0.0001$ for all conditions). The percent rhythms (PR) between conditions were quite close, except for chlortoluron, which had a lower PR. PR were 46.4 % (control), 32.3 % (chlortoluron), 42.8 % (MPs) and 45 % (cocktail).

3.2.5 Shell Growth

The Fig. 4a displays the cumulative daily shell growth, monitored for 24 days. No significant difference was calculated between the control and chlortoluron conditions ($p = 0.07$) but this p-value is close to the statistical significance and, therefore express a trend. In contrast, over 24 days, oysters exposed to the MPs and cocktail conditions underlined a lower shell growth. Indeed, after the 24-day period, the mean shell length growth for the MPs and cocktail conditions respectively was reduced of 50.4 % ($p < 0.0001$) and 42.7 % ($p = 0.021$), in comparison with the control one (Fig. 4b). The oyster shell growth observed for the MPs and cocktail conditions were also lower than observed for the exposure to chlortoluron alone.

Fig. 4. (a) Daily shell length growth monitored for all conditions (mm). **(b)** After the completion of the 24-day experiment, histograms displaying the mean shell growth (mm) of the oysters equipped with electrodes. The

different conditions are: control (no pollutants, white), chlortoluron alone (green), MPs alone (blue) and cocktail (red). Values are mean $\pm$ SEM for control (n = 7), chlortoluron (n = 6), MPs (n = 8) and cocktail (n = 5) conditions. Different letters indicate significant differences between concentrations ($p < 0.05$).

3.2.6 Summary of the main results

The actual concentrations measured on D₂₄, in the FSW exposure (for MP and chlortoluron alone and cocktail), are presented in Table 2, as well as in the remainder of this present study. In our work, the analysis validated the existence of a daily rhythm for all tested conditions. No significant differences in chronobiological rhythms were noted between the four conditions tested. Regarding the behavioural parameters, our results suggest that, when exposed to chlortoluron, the oyster valve activity was significantly disrupted (Table 2). Indeed, in comparison with the control condition, a decrease in the number of VMC and an increase in VOA were noted. In terms of MPs exposure, a significant increase in VMC, and a decrease in VOD and growth were observed. Concerning the cocktail condition, a decrease in VMC and growth, and an increase in VOA were highlighted. Depending on the exposure conditions, the valve behaviour in oysters responded differently.

Table 2. Summary of the effects on the Pacific oyster valve activity induced after a 24-day exposure to chlortoluron alone (85 $\mu\text{g.L}^{-1}$), 20-25 μm HDPE MPs alone (112 MP. mL^{-1}) and a combination of both (cocktail, 97 $\mu\text{g.L}^{-1}$ of chlortoluron + 108 MP. mL^{-1}). Measured parameters are VMC (Valve Micro-Closures), VOA (Valve Opening Amplitude), VOD (Valve Opening Duration), daily rhythm (chronobiological analysis) and shell growth ($\uparrow$: significant increase compared to the control condition, $\downarrow$: significant decrease compared to the control condition and - : absence of significant effects compared to the control condition).

Conditions	VMC	VOA	VOD	Daily rhythm	Shell growth
Chlortoluron alone	↓	↑	-	-	-
MPs alone	↑	-	↓	-	↓
Cocktail	↓	↑	-	-	↓

4. Discussion

4.1 Experimental concentrations versus natural environment

In our FSW exposures, concentrations between 58 and 96 $\mu\text{g.L}^{-1}$ were measured. The study by Valiente Moro *et al.* (2012) reported an IC_{50} (half-maximum inhibitory concentration) of 50 $\mu\text{g.L}^{-1}$ on the freshwater phytoplankton *Ankistrodesmus fusiformis*. Although this concentration is higher than those commonly found in the Pertuis Charentais, it is quite close to the maximum concentrations detected in some European rivers. Indeed, concentrations of herbicide (chlortoluron in particular) of 20 $\mu\text{g.L}^{-1}$ and more (Beitz *et al.*, 1994) were determined. For herbicide exposure, control FSW highlighted a slight contamination to chlortoluron. Being volatile, it could easily evaporate in the atmosphere and contaminate the other tanks (Ineris, 2013). Nevertheless, the concentrations of chlortoluron measured in the control FSW samples were satisfactory, to validate the study. An increase of chlortoluron concentrations was observed between the start (D_5) and the end of the experiment (D_{24}). This increase could be explained by the initial presence of chlortoluron in natural seawater (0.14 $\mu\text{g.L}^{-1}$ in 2018, Action program, 2018) and regular water changes during the experiment. In this present study, the theoretical concentration of HDPE MPs (20-25 μm) was set at 10 $\mu\text{g MP.L}^{-1}$, where other studies have tested these same concentrations (Beiras *et al.*, 2018; Constant, 2018; Lei *et al.*, 2018 and Dris *et al.*, 2016). To assess MPs/NPs in water, flow cytometry was performed, as used in some studies (Long *et al.*, 2017; Caputo *et al.*, 2021; Kaile *et al.*, 2020). Instead of being 10 $\mu\text{g MP.L}^{-1}$, the average concentration for MPs-contained solutions was approximately of 50 $\mu\text{g MP.L}^{-1}$ at the two sampling periods. However, the assays by cytometry, we

confirm contents between 93.9 and 107.6 MP.mL⁻¹. The highest environmental concentration of MP detected in Artic sea ice cores is around 13.7 MP.mL⁻¹ (Peeken *et al.*, 2018). However, the size classes of MP detected in the natural environment are larger than MP tested in the laboratory (Paul-Pont *et al.*, 2018). Our present study could be defined as realistic in terms of size tested but not in terms of concentration. According to a study on the characterization of MP on the Atlantic coast, the majority of MP (64 %) were < 40 µm. In addition, about half (42 %) of them were PE (Enders *et al.*, 2015). These results justify the use of HDPE-MP (20-25 µm) in this study. In addition, some equipment used in aquaculture for bivalves consists mainly of PE (Lusher *et al.*, 2017 and Bringer *et al.*, 2021). The differences observed between the theoretical and measured MP concentrations could come from the aggregation of MP (Alimi *et al.*, 2018; Michels *et al.*, 2018) on the walls of the exposure tanks. Long *et al.* (2017) clearly showed that micro-PS may attach to glassware, form homo-aggregates and hetero-aggregates. In addition, regular water changes, for the welfare of oysters, could also have played a role in increasing theoretical concentrations.

4.2 Behavioural responses of juveniles' oysters (*C. gigas*) to chlortoluron exposure

Chlortoluron is a photosynthesis-disrupting herbicide, and more specifically as an inhibitor of photosystem II (Faggiano *et al.*, 2010). Phytoplankton communities have been shown to be very sensitive to herbicides (Solomon *et al.*, 1996). Indeed, effects on phytoplankton growth and development have been observed (El-Sheekh *et al.*, 1994), and could indirectly affect filter feeders such as oysters when they feed. Chlortoluron showed a significant decrease in VMC in number compared to condition control. An equivalent result has already been demonstrated for *Mytilus galloprovincialis* exposed to cypermethrin (insecticide) with a reduced the valve closure in a time-dependent manner (Ayad *et al.*, 2011). Higher VOA, at condition control, was also detected following exposure to chlortoluron. An apparent increase in the rate of opening of the shell under contamination with an herbicide (lenacil) was reported in an earlier study (Chmist *et al.*, 2019).

However, this parameter has been little studied in order to obtain relevant interpretations (Bae & Park, 2014). Chlortoluron may have a specific reducing effect on some distinctive phytoplankton species important for the physiology, development and behaviour of oysters (Stachowski-Haberkorn *et al.* 2008). Additional studies should be carried out in order to better understand the effect of this herbicide on phytoplankton populations and on oyster nutrition. Moreover, combined effect of VMC decrease and VOA increased may suggest that the herbicide lead to an apparent relaxed behaviour of valve activity. Next investigations should focus on putative neurotoxic effects of chlortoluron on oyster valve activity. Indeed, it would be of interest to study if the chlortoluron may block conduction of action potential at the voltage-gated sodium channels level, which plays a crucial role in membrane excitability in nerve cell, and lead to a decrease of neuro-muscular response. This neurotoxic effect has already been shown for many neurotoxins produced by animals or plants, such as saxitoxin and tetrodotoxin (Boulot *et al.*, 2017).

4.3 Behavioural responses of juveniles' oysters (*C. gigas*) to microplastics exposure

Oysters exposed to MP showed a higher number of VMC compared to the others conditions. VMC is defined as a marker of stress in oysters (Tran *et al.*, 2010). The presence of MP in the seawater, which could be detected by oysters, can induce mechanical stress (Sow *et al.*, 2011). The presence of harmful substances could activate chemoreceptors on the edge of the oyster mantle or directly affect the gills when the pollutants are in direct contact with the tissue of the pallial cavity, responsible for the rapid increase in the frequency of VMC. According to the work of Castrec *et al.* (2018), a similar hypothesis could be made, implying that the increase in the frequency of VMC is a protective behavioral response to accelerate interval water turnover and minimize potential contacts with MP, irritating the gills (Zhu *et al.*, 2020). A significant decrease in VOD was also measured. Likewise, a freshwater bivalve (*Corbicula fluminea*) had a behavioral closure reaction - as a protective strategy - when exposed to a metallic contaminant (Tran *et al.*, 2004a). The branchial cavities and the

labial palps (Ward *et al.*, 1998) determine the different modes of selection and sorting of particles, which could slow down the filtration of nutrients of exposed oysters, affect their feeding activity, their behaviour, and reduce their metabolism and their growth. Indeed, several studies have noted that toxins and foreign bodies can modify the nutritional activity of oysters through behavioral modifications (Tran *et al.*, 2010; Haberkorn *et al.*, 2010). Oysters exposed to MP grow more slowly than control oysters. Some previous work has shown non-effects of MP on oysters during subchronic exposure (Green, 2016; Revel *et al.*, 2020). However larger sizes of MP (103 μm and 300 μm) were tested contrary to this present study (20-25 μm). The study by Gardon *et al.* (2018) showed a decrease in the scope for growth from 25 $\mu\text{g MP.L}^{-1}$ of PS-MP in *P. margaritifera*. These results indicate that the exposed oysters must have drawn their energy from the reserves. In addition, the assimilation of microalgae was disturbed by the presence of micro-PS, and the energy provided by food intake was lower for exposed oysters (Gardon *et al.*, 2018). In the study conducted by Thomas *et al.* (2020), the condition index at the highest concentration (106 MP.L^{-1}) decreased significantly over time. The concentration tested is similar to our concentrations measured in MP exposure waters.

4.4 Effects of co-contamination by microplastics and chlortoluron on juveniles' oysters (*C. gigas*)

For oysters exposed to the cocktail, results similar to the chlortoluron condition were noted, on VMC and VOA. No effect on VOD was noted in contrast to its decrease for exposure to MP. Chlortoluron and MP may have antagonistic effects. A previous study demonstrated antagonistic effects between NP and glyphosate on the microalgae *Microcystis aeruginosa* (Zhang *et al.*, 2018). Combined exposure to diclofop-methyl and silver nanoparticles has been shown to antagonize the growth inhibition of *A. Thaliana* (Li *et al.*, 2018). In our study, growth decreased under the cocktail condition as for the MP condition. Studies have pointed out that MP can make hydrophobic organic chemicals

bioavailable by acting as an absorption vector (Horton *et al.*, 2018). However, our results do not show significantly different effects for the cocktail, compared to MP and therefore we cannot conclude on a potential vectorization of chlortoluron on MP. The results showed that the synergistic effect of chlortoluron and MPs is not significant, compared to the contamination conditions alone. Their common ecotoxic effects are affected by physical and chemical factors such as particle size, color, composition, function (Yu *et al.*, 2021). Due to their high filtration rate, marine bivalves, like oysters, are particularly sensitive to chemical contamination, which can be a major source of stress and behavioral alteration (Sokolova and Lannig, 2008; Islam and Tanaka, 2004).

5. Conclusions

On a larger scale, valvometric technology is a tool for monitoring water quality, using oysters as biosensors (Ahmed *et al.*, 2015). This study provided valuable data on the behavior of bivalves exposed to pollutants potentially present in the marine environment. Exposure to chlortoluron had little impact on their behavior and none on their growth. This study provided insight into the evidence that MP could potentially affect the physiology and behavior of *C. gigas*. MPs tended to act as stressors in bivalves, triggering urgent behavioral responses. In addition, MP could affect the filtration capacity of bivalves (nutrient system) and, indirectly, their growth. The combination of the two pollutants showed antagonistic responses to the conditions of contamination alone. Rarely used in studies, valvometric technology is an innovative, integrated and comprehensive marker that helps to understand the effects of environmental pollution. Future studies on the effects of MP on bivalves could use HFNI valvometry, mimicking environmental conditions (*e.g.* size range, concentrations, pollutant cocktail).

Acknowledgements

A. Bringer received a PhD grant (Comité Régional de la Conchyliculture de la Charente Maritime CRC17) as well as financial support from the Région Nouvelle Aquitaine and Comité Départemental de la Charente Maritime (CD17) to develop his research. The AQUAECOs project (**Amélioration de la QUALité Environnementale sur les zones COnchyliques des Pertuis Charentais**), funded by a partnership with CRC17, and the AFB (Agence Française de la Biodiversité, *Parc naturel marin de l'estuaire de la Gironde et de la mer des Pertuis*) contributed financially to this study. The funding was partly supported by the University of La Rochelle, the University of Bordeaux, the Centre National de la Recherche Scientifique (France). The authors thank the France Naissain hatchery for providing the oyster specimens as well as our collaborators for their work and help.

References

Action program, Coastal water quality in shellfish farming areas, Qualyse, CD17, 2018-2020 (accessed October 2020).

Ahmed, H., Ushirobira, R., Efimov, D., Tran, D., & Massabuau, J. C. (2015). Velocity estimation of valve movement in oysters for water quality surveillance. *IFAC-PapersOnLine*, 48(11), 333-338.

Akcha, F., Barranger, A., & Bachère, E. (2020). Genotoxic and epigenetic effects of diuron in the Pacific oyster: in vitro evidence of interaction between DNA damage and DNA methylation. *Environmental Science and Pollution Research*, 1-15.

Alimi, O. S., Farner Budarz, J., Hernandez, L. M., & Tufenkji, N. (2018). Microplastics and nanoplastics in aquatic environments: aggregation, deposition, and enhanced contaminant transport. *Environmental science & technology*, 52(4), 1704-1724.

Andrade, H., Massabuau, J. C., Cochrane, S., Ciret, P., Tran, D., Sow, M., & Camus, L. (2016). High frequency non-invasive (HFNI) bio-sensors as a potential tool for marine monitoring and assessments. *Frontiers in Marine Science*, 3, 187.

Ayad, M. A., Fdil, M. A., & Mouabad, A. (2011). Effects of cypermethrin (pyrethroid insecticide) on the valve activity behavior, byssal thread formation, and survival in air of the marine mussel *Mytilus galloprovincialis*. *Archives of environmental contamination and toxicology*, 60(3), 462-470.

Bae, M. J., & Park, Y. S. (2014). Biological early warning system based on the responses of aquatic organisms to disturbances: a review. *Science of the Total Environment*, 466, 635-649.

Baldwin, A. K., Corsi, S. R., & Mason, S. A. (2016). Plastic debris in 29 Great Lakes tributaries: relations to watershed attributes and hydrology. *Environmental Science & Technology*, 50(19), 10377-10385.

Bayne, B.L., Ahrens, M., Allen, S.K., D'auriac, M.A., Backeljau, T., Beninger, P., Bohn, R., Boudry, P., Davis, J., Green, T., Guo, X., Hedgecock, D., Ibarra, A., Kingsley- Smith, P., Krause, M., Langdon, C., Lape_gue, S., Li, C., Manahan, D., Mann, R., Perez-Paralle, L., Powell, E.N., Rawson, P.D., Speiser, 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), 545e548.

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.

Behrens, D., Rouxel, J., Burgeot, T., & Akcha, F. (2016). Comparative embryotoxicity and genotoxicity of the herbicide diuron and its metabolites in early life stages of *Crassostrea gigas*: implication of reactive oxygen species production. *Aquatic Toxicology*, 175, 249-259.

Beiras, R., Bellas, J., Cachot, J., Cormier, B., Coussin, X., Engwall, M., Gambardella, C., Garaventa, F., Keiter, S., Le Bihanic, F., Lopez-Ibanez, S., Piazza, V., Rial, D., Tato, T., Vidal-Linan, L. (2018). Ingestion and contact with polyethylene microplastics does not cause acute toxicity on marine zooplankton. *Journal of Hazardous Materials*, 360, 452-460.

Beitz, H., Schmidt, H., & Herzel, F. (1994). Occurrence, toxicological and ecotoxicological significance of pesticides in groundwater and surface water. In *Pesticides in ground and surface water* (pp. 1-56). Springer, Berlin, Heidelberg.

Berge, J., Daase, M., Renaud, P. E., Ambrose, W. G. Jr., Darnis, G., Last, K. S., et al. (2015). Unexpected levels of biological activity during the polar night offer new perspectives on a warming arctic. *Curr. Biol.* 25, 2555–2561.

Betsill, M., Gonzalez, J., & Woods, A. (2019). The Impact of Microplastic Ingestion on the Bivalve Filtration Efficiency of the Hooked Mussel (*Ischadium recurvum*) from the Chesapeake Bay. Research Proposal for UH 1604: Honors Research Practices at Virginia Tech.

Bhagat, J., Nishimura, N., & Shimada, Y. (2020). Toxicological interactions of microplastics/nanoplastics and environmental contaminants: Current knowledge and future perspectives. *Journal of Hazardous Materials*, 123913.

Bingham, C., Arbogast, B., Cornélissen, G., Lee, J.-K., Halberg, F. (1982). Inferential statistical methods for estimating and comparing cosinor parameters. *Chronobiologia*, 9, 397–439.

Born, M. P., & Schüttrumpf, H. (2019, September). Microplastic in Coastal Areas-Impact of Waves, Sediments and Saltwater on the Degradation Behaviour. In *International Conference on Microplastic Pollution in the Mediterranean Sea* (pp. 158-163). Springer, Cham.

Boullot, F., Castrec, J., Bidault, A., Dantas, N., Payton, L., Perrigault, M., **Tran, D.**, Amzil, Z., Boudry, P., Soudant, P., Hégaret, H., Fabioux, C. (2017) Molecular Characterization of Voltage-Gated Sodium Channels and Their Relations with Paralytic Shellfish Toxin Bioaccumulation in the Pacific Oyster *Crassostrea gigas*. *Mar. Drugs*. 15(1): 21.

Box, G. E., Jenkins, G. M., Reinsel, G. C., & Ljung, G. M. (2015). *Time series analysis: forecasting and control*. John Wiley & Sons.

Bringer, A., Thomas, H., Prunier, G., Dubillot, E., Bossut, N., Churlaud, C., Clérandeau, 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*, 113978.

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.

606 Bringer, A., Le Floch, S., Kerstan, A., & Thomas, H. (2021). Coastal ecosystem inventory with
 607 characterization and identification of plastic contamination and additives from aquaculture
 608 materials. *Marine Pollution Bulletin*, 167, 112286.

609

610 Caputo, F., Vogel, R., Savage, J., Vella, G., Law, A., Della Camera, G., Hannon, G., Peacock, B., Mehn,
 611 D., Ponti, J., Geiss, O., Aubert, D., Prina-Mello, A., Calzolari, L. (2021). Measuring particle size
 612 distribution and mass concentration of nanoplastics and microplastics: addressing some analytical
 613 challenges in the sub-micron size range. *Journal of Colloid and Interface Science*, 588, 401-417.

614

615 Castrec, J., Soudant, P., Payton, L., Tran, D., Miner, P., Lambert, C., Goïc, N. Le, Huvet, A., Quillien, V.,
 616 Boullot, F., Amzil, Z., Hégaret, H., Fabioux, C., 2018. Bioactive extracellular compounds produced by
 617 the dinoflagellate *Alexandrium minutum* are highly detrimental for oysters. *Aquatic Toxicology*. 199,
 618 188–198.

619

620 Chmist, J., Szoszkiewicz, K., & Drożdżyński, D. (2019). Behavioural responses of *Unio tumidus*
 621 freshwater mussels to pesticide contamination. *Archives of environmental contamination and*
 622 *toxicology*, 77(3), 432-442.

623

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

626

627 CNC (Comité National de la Conchyliculture), 2020 ; Les statistiques. www.cnc-france.com (accessed
 628 October 2020).

629

630 Cole, M., Lindeque, P., Halsband, C., Galloway, T.S. (2011). Microplastics as contaminants in the
 631 marine environment: a review. *Marine Pollution Bulletin*, 62, 2588-2597.

632

633 Cole, M., Galloway, T.S. (2015). Ingestion of nanoplastics and microplastics by Pacific oyster larvae.
634 *Environmental Sciences Technology*, 49(24), 14625-14632.

635

636 Constant, M. (2018). Source, Transfert et Devenir des microplastiques (MP) en MerMediterranee
637 Nord-Occidentale. *PhD thesis. University of Perpignan-CEFREM*, pp277.

638

639 Díaz-Mendoza, C., Mouthon-Bello, J., Pérez-Herrera, N. L., & Escobar-Díaz, S. M. (2020). Plastics and
640 microplastics, effects on marine coastal areas: a review. *Environmental Science and Pollution*
641 *Research*, 1-10.

642

643 Dris, R., Lahens, L., Rocher, V., Gasperi, J., & Tassin, B. (2016). Premières investigations sur la
644 contamination en microplastiques d'une zone urbaine. Cas de l'agglomération parisienne, vol. 12.

645

646 El-Sheekh, M. M., Ktkat, H. M., & Hammouda, O. H. (1994). Effect of atrazine herbicide on growth,
647 photosynthesis, protein synthesis, and fatty acid composition in the unicellular green alga *Chlorella*
648 *kessleri*. *Ecotoxicology and environmental safety*, 29(3), 349-358.

649

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

653

654 Eriksen, M., Maximenko, N., Thiel, M., Cummins, A., Lattin, G., Wilson, S., Hafner, J., Zellers, A., &
655 Rifman, S. (2013). Plastic pollution in the South Pacific subtropical gyre. *Marine pollution*
656 *bulletin*, 68(1-2), 71-76.

657

Faggiano, L., de Zwart, D., García-Berthou, E., Lek, S., & Gevrey, M. (2010). Patterning ecological risk of pesticide contamination at the river basin scale. *Science of the Total Environment*, 408(11), 2319-2326.

France Agrimer, 2020. Chiffres clés des filières pêche et aquaculture en France, en 2020 (access in February).
<https://www.franceagrimer.fr/fam/content/download/64767/document/CC%20pêche%20aqua%20FR.pdf?version=22021>).

Gall, S. C., & Thompson, R. C. (2015). The impact of debris on marine life. *Marine pollution bulletin*, 92(1-2), 170-179.

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

Gardon, T., Reisser, C., Soyez, C., Quillien, V., & Le Moullac, G. (2018). Microplastics affect energy balance and gametogenesis in the pearl oyster *Pinctada margaritifera*. *Environmental science & technology*, 52(9), 5277-5286.

Gardon, T., Morvan, L., Huvet, A., Quillien, V., Soyez, C., Le Moullac, G., & Le Luyer, J. (2020). Microplastics induce dose-specific transcriptomic disruptions in energy metabolism and immunity of the pearl oyster *Pinctada margaritifera*. *Environmental Pollution*, 266, 115180.

Girard, S., & Agúndez, J. A. P. (2014). The effects of the oyster mortality crisis on the economics of the shellfish farming sector: Preliminary review and prospects from a case study in Marennes-Oleron Bay (France). *Marine Policy*, 48, 142-151.

Gouthiere, L., Mauvieux, B., Davenne, D., & Waterhouse, J. (2005). Complementary methodology in the analysis of rhythmic data, using examples from a complex situation, the rythmicity of temperature in night shift workers. *Biological Rhythm Research*, 36(3), 177-193.

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

Haberkorn, H., Lambert, C., Le Goïc, N., Moal, J., Suquet, M., Guéguen, M., Sunila, I & Soudant, P. (2010). Effects of *Alexandrium minutum* exposure on nutrition-related processes and reproductive output in oysters *Crassostrea gigas*. *Harmful algae*, 9(5), 427-439.

Haberkorn, H., Tran, D., Massabuau, J. C., Ciret, P., Savar, V., & Soudant, P. (2011). Relationship between valve activity, microalgae concentration in the water and toxin accumulation in the digestive gland of the Pacific oyster *Crassostrea gigas* exposed to *Alexandrium minutum*. *Marine pollution bulletin*, 62(6), 1191-1197.

Horton, A. A., Svendsen, C., Williams, R. J., Spurgeon, D. J., & Lahive, E. (2017). Large microplastic particles in sediments of tributaries of the River Thames, UK—Abundance, sources and methods for effective quantification. *Marine pollution bulletin*, 114(1), 218-226.

706 Horton, A.A., Vijver, M.G., Lahive, E., Spurgeon, D.J., Svendsen, C., Heutink, R., van Bodegom, P.M.,
 707 Baas, J., 2018. Acute toxicity of organic pesticides to *Daphnia magna* is unchanged by co-exposure to
 708 polystyrene microplastics. *Ecotoxicol. Environ. Saf.* 166, 26-34.
 709
 710 Ineris. (2013). Valeur guide environnementale: Chlortoluron (15545-48-9)
 711 <https://substances.ineris.fr/fr/substance/cas/15545-48-9/3>
 712
 713 Islam, M. S., & Tanaka, M. (2004). Impacts of pollution on coastal and marine ecosystems including
 714 coastal and marine fisheries and approach for management: a review and synthesis. *Marine pollution*
 715 *bulletin*, 48(7-8), 624-649.
 716
 717 Kaile, N., Lindivat, M., Elio, J., Thuestad, G., Crowley, Q. G., & Hoell, I. A. (2020). Preliminary results
 718 from detection of microplastics in liquid samples using flow cytometry. *Frontiers in Marine Science*, 7,
 719 856.
 720
 721 Lee, K.W., Shim, W.J., Kwon, O.Y., Kang, J.H., 2013. Size-dependent effects of micro polystyrene
 722 particles in the marine copepod *Tigriopus japonicus*. *Environmental Sciences Technology*, 47 (19),
 723 11278-11283.
 724
 725 Lee, D. H., Rhee, Y. J., Choi, K. S., Nam, S. E., Eom, H. J., & Rhee, J. S. (2017). Sublethal concentrations
 726 of atrazine promote molecular and biochemical changes in the digestive gland of the Pacific oyster
 727 *Crassostrea gigas*. *Toxicology and Environmental Health Sciences*, 9(1), 50-58.
 728
 729 Lehotay SJ (2007). Determination of pesticide residues in foods by acetonitrile extraction and
 730 partitioning with magnesium sulfate: collaborative study. *J AOAC Int* 90:485–520
 731

Lei, L., Wu, S., Lu, S., Liu, M., Song, Y., Fu, Z., ... & He, D. (2018). Microplastic particles cause intestinal damage and other adverse effects in zebrafish *Danio rerio* and nematode *Caenorhabditis elegans*. *Science of the Total Environment*, 619, 1-8.

Leonard, S. Dispersion des pesticides vers les eaux - Bassin de la Sèvre Niortaise et Marais Poitevin
Période 2004-2014, 2017 - http://www.sevre-niortaise.fr/wp-content/uploads/2017/04/etude_pesticides_SAGE_SNMP_31032017.pdf (accessed January 2020).

Li, X., Ke, M., Zhang, M., Peijnenburg, W. J. G. M., Fan, X., Xu, J., Zhang, Z., Lu, T., Fu, Z., & Qian, H. (2018). The interactive effects of diclofop-methyl and silver nanoparticles on *Arabidopsis thaliana*: growth, photosynthesis and antioxidant system. *Environmental pollution*, 232, 212-219.

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

Lucasson, A. (2018). Characterization and diversity of the mechanisms of the mortality syndrome affecting juveniles of *Crassostrea gigas*. *PhD thesis. University of Montpellier*, pp165.

Lusher, A., Hollman, P., & Mendoza-Hill, J. (2017). Microplastics in fisheries and aquaculture. *FAO Fisheries and Aquaculture Technical Paper (FAO) eng no. 615*.

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*. *Marine environmental research*, 89, 1-8.

758 Mat, A.M., Massabuau, J.C., Ciret, P., Tran, D. (2014) Looking for the clock mechanism responsible for
759 the circatidal behavior in the oyster *Crassostrea gigas*. *Mar. Biol.* 161: 89-99.

760

761 Michels, J., Stippkugel, A., Lenz, M., Wirtz, K., & Engel, A. (2018). Rapid aggregation of biofilm-
762 covered microplastics with marine biogenic particles. *Proceedings of the Royal Society B*, 285(1885),
763 20181203.

764

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

768

769 Nelson, W., Tong, Y.L., Lee, J.K., Halberg, F., 1979. Methods for cosinor-rhythmometry.
770 *Chronobiologia*, 6, 305–323.

771

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

776

777 Peeken, I., Primpke, S., Beyer, B., Gütermann, J., Katlein, C., Krumpen, T., Bergmann, M., Hehemann,
778 L., & Gerdt, G. (2018). Arctic sea ice is an important temporal sink and means of transport for
779 microplastic. *Nature communications*, 9(1), 1-12.

780

781 Pfannkoch, E. A., Stuff, J. R., Whitecavage, J. A., & Moran, J. H. (2010). High Throughput Method for
782 the Determination of PAHs in Seafood by QuEChERS-SBSE-GC-MS.

783

Phuong, N. N. (2018). Analytical developments for the characterization and quantification of microplastic contamination of sedimentary and biological matrices: application to shellfish farming areas in Pays de la Loire. *PhD thesis. University of Nantes*, pp232.

PlasticsEurope (2016) Plastics – the Facts 2016. URL <http://www.plasticseurope.de/Document/plastics—the-facts-2016-15787.aspx>

PlasticsEurope. (2020). Plastics - the facts 2020: an analysis of European plastics production, demand and waste data. PlasticsEurope Internal Report 4312 (64pp.). <https://www.plasticseurope.org/en/resources/publications/4312-plastics-facts-2020>.

Revel, M., Châtel, A., Perrein-Ettajani, H., Bruneau, M., Akcha, F., Sussarellu, R., Rouxel, J., Costil, K., Decottignies, P., Cognie, B., Lagarde, F., & Mouneyrac, C. (2020). Realistic environmental exposure to microplastics does not induce biological effects in the Pacific oyster *Crassostrea gigas*. *Marine pollution bulletin*, 150, 110627.

Rist, S., Baun, A., Almeda, R., & Hartmann, N. B. (2019). Ingestion and effects of micro-and nanoplastics in blue mussel (*Mytilus edulis*) larvae. *Marine pollution bulletin*, 140, 423-430.

Scargle, J. D. (1982). Studies in astronomical time series analysis. II-Statistical aspects of spectral analysis of unevenly spaced data. *The Astrophysical Journal*, 263, 835-853.

Schwartzmann, C., Durrieu, G., Sow, M., Ciret, P., Lazareth, C. E., and Massabuau, J.-C. (2011). *In situ* giant clam growth rate behavior in relation to temperature: a one-year coupled study of high-frequency noninvasive valvometry and sclerochronology. *Limnol. Oceanogr.* 56, 1940–1951.

810 Sokolova, I. M., & Lannig, G. (2008). Interactive effects of metal pollution and temperature on
811 metabolism in aquatic ectotherms: implications of global climate change. *Climate Research*, 37(2-3),
812 181-201.

813

814 Soletchnik, P., Bouquet, A. L., & Mille, D. (2018). Flash Info Maline 2008-2018, ten years already... of
815 communication on the mortalities of oysters (*Crassostrea gigas*) and mussels (*Mytilus edulis*) in the
816 Pertuis Charentais. *Ifremer report*, pp18.

817

818 Solomon, K.R., Baker, D.B., Richards, R.P., Dixon, D.R., Klaine, S.J., LaPoint, T.W., 1996. Ecological risk
819 assessment of atrazine in North American surface waters. *Environmental Toxicology and Chemistry*
820 15 (1), 31-74.

821

822 Song, Y. K., Hong, S. H., Jang, M., Han, G. M., & Shim, W. J. (2015). Occurrence and distribution of
823 microplastics in the sea surface microlayer in Jinhae Bay, South Korea. *Archives of environmental*
824 *contamination and toxicology*, 69(3), 279-287.

825

826 Sow, M., Durrieu, G., Briollais, L., Ciret, P., & Massabuau, J. C. (2011). Water quality assessment by
827 means of HFNI valvometry and high-frequency data modeling. *Environmental monitoring and*
828 *assessment*, 182(1-4), 155-170.

829

830 Stachowski-Haberkorn, S., Quiniou, F., Nedelec, M., Robert, R., Limon, G., & de La Broise, D. (2008).
831 In-situ microcosms, a tool for assessment of pesticide impacts on oyster spat (*Crassostrea*
832 *gigas*). *Ecotoxicology*, 17(4), 235-245.

833

834 Sussarellu, R., Suquet, M., Thomas, Y., Lambert, C., Fabioux, C., Pernet, M.E.J., Goïc, N.L., Quillien, V.,
835 Mingant, C., Epelboin, Y., Charlotte, C., Julien, G., Johan, R., Ika, P.-P., Philippe, S., Arnaud, H., 2016.

836 Oyster reproduction is affected by exposure to polystyrene microplastics. *Proc. Natl. Acad. Sci. U. S.*
837 *A.* 113, 2430-2435.

838

839 Tallec, K., Huvet, A., Di Poi, C., González-Fernández, C., Lambert, C., Petton, B., Le Goic, N., Berchel,
840 M., Soudant, P., & Paul-Pont, I. (2018). Nanoplastics impaired oyster free living stages, gametes and
841 embryos. *Environmental pollution*, 242, 1226-1235.

842

843 Thomas, M., Jon, B., Craig, S., Edward, R., Ruth, H., John, B., Dick, V.A., Heather, L.A., & Matthew, S.
844 (2020). The world is your oyster: low-dose, long-term microplastic exposure of juvenile oysters.
845 *Heliyon*, 6(1), e03103.

846

847 Tran D., Fournier E., Durrieu G. and Massabuau J-C. (2004a) Copper detection in the asiatic clam
848 *Corbicula fluminea*: optimum valve closure response. *Aquatic Toxicology*, 66(3): 333-343.

849

850 Tran D., Massabuau J-C and Garnier-Laplace J. (2004b) The effect of carbon dioxide on uranium
851 bioaccumulation in the freshwater clam *Corbicula fluminea*. *Environmental Toxicology and Chemistry*,
852 23(3): 739-747.

853

854 Tran D., Bourdineaud J-P, Massabuau J-C and Garnier-Laplace J. (2005) Modulation of uranium
855 bioaccumulation by hypoxia in the freshwater clam *Corbicula fluminea*: induction of multixenobiotic
856 resistance protein and heat shock protein 60 in gill tissues. *Environmental Toxicology and Chemistry*,
857 24(9): 2278-2284

858

859 Tran D., Fournier E., Durrieu G. and Massabuau J-C. (2007) *Corbicula fluminea* valve closure response
860 as sensitivity threshold to inorganic mercury contamination. *Environmental Toxicology and*
861 *Chemistry*, 26(7): 1545-1551.

862

863 Tran, D., Haberkorn, H., Soudant, P., Ciret, P., & Massabuau, J. C. (2010). Behavioral responses of
864 *Crassostrea gigas* exposed to the harmful algae *Alexandrium minutum*. *Aquaculture*, 298(3-4), 338-
865 345.

866

867 Tran, D., Ciutat, A., Mat, A., Massabuau, J.C., Hégaret, H., Lambert, C., Le Goic, N., Soudant, P, (2015).
868 The toxic dinoflagellate *Alexandrium minutum* disrupts daily rhythmic activities at gene transcription,
869 physiological and behavioral levels in the oyster *Crassostrea gigas*. *Aquatic Toxicology*, 158, 41–49.

870

871 Valiente Moro, C., Bricheux, G., Portelli, C., & Bohatier, J. (2012). Comparative effects of the
872 herbicides chlortoluron and mesotrione on freshwater microalgae. *Environmental toxicology and*
873 *chemistry*, 31(4), 778-786.

874

875 Wagner, M., Scherer, C., Alvarez-Munoz, D., Brennholt, N., Bourrain, X., Buchinger, S., Fries, E.,
876 Grosbois, C., Klasmeier, J., Marti, T., Rodriguez-Mozaz, S., Urbatzka, R., Vethaak, A., Winther-Nielsen,
877 M., Reifferscheid, G., 2014. Microplastics in freshwater ecosystems: what we know and what we
878 need to know. *Environ. Sci. Eur.* 26 (1), 12.

879

880 Wang, F., Gao, J., Zhai, W., Liu, D., Zhou, Z., & Wang, P. (2020). The influence of polyethylene
881 microplastics on pesticide residue and degradation in the aquatic environment. *Journal of hazardous*
882 *materials*, 394, 122517.

883 Ward, J. E.; Levinton, J.; Shumway, S.; Cucci, T. Particle sorting in bivalves: *in vivo* determination of
884 the pallial organs of selection. *Mar. Biol.* 1998, 131 (2), 283–292.

885 Ward, J. E., Zhao, S., Holohan, B. A., Mladinich, K. M., Griffin, T. W., Wozniak, J., & Shumway, S. E.
886 (2019). Selective Ingestion and Egestion of Plastic Particles by the Blue Mussel (*Mytilus edulis*) and

887 Eastern Oyster (*Crassostrea virginica*): Implications for Using Bivalves as Bioindicators of Microplastic
888 Pollution. *Environmental science & technology*, 53(15), 8776-8784.

889

890 Wright, S.L., Rowe, D., Thompson, R.C., Galloway, T.S., 2013. Microplastic ingestion decreases energy
891 reserves in marine worms. *Current Biology* 23 (23), R1031–R1033.

892

893 Xu, X. Y., Lee, W. T., Chan, A. K. Y., Lo, H. S., Shin, P. K. S., & Cheung, S. G. (2017). Microplastic
894 ingestion reduces energy intake in the clam *Atactodea striata*. *Marine pollution bulletin*, 124(2), 798-
895 802.

896

897 Yu, H., Peng, J., Cao, X., Wang, Y., Zhang, Z., Xu, Y., & Qi, W. (2021). Effects of microplastics and
898 glyphosate on growth rate, morphological plasticity, photosynthesis, and oxidative stress in the
899 aquatic species *Salvinia cucullata*. *Environmental Pollution*, 279, 116900.

900

901 Zhang, Q., Qu, Q., Lu, T., Ke, M., Zhu, Y., Zhang, M., Zhang, Z., Du, B., Pan, X., Sun, L., & Qian, H.
902 (2018). The combined toxicity effect of nanoplastics and glyphosate on *Microcystis aeruginosa*
903 growth. *Environmental Pollution*, 243, 1106-1112.

904

905 Zhao, S., Zhu, L., Wang, T., & Li, D. (2014). Suspended microplastics in the surface water of the
906 Yangtze Estuary System, China: first observations on occurrence, distribution. *Marine pollution*
907 *bulletin*, 86(1-2), 562-568.

908

909 Zhu, X., Qiang, L., Shi, H., & Cheng, J. (2020). Bioaccumulation of microplastics and its in vivo
910 interactions with trace metals in edible oysters. *Marine Pollution Bulletin*, 154, 111079.

Supplementary data

Fig. S1. Experimental design located in an experimental laboratory (LIENSs La Rochelle – CNRS). The numbers correspond to: **(1)** experimental batch of exposed oysters equipped with valvometric electrodes (zoom picture), **(2)** second experimental batch of exposed oysters, **(3)** buffer tank, **(4)** reduced vibrations thanks to the capacitance chamber and **(5)** HFNI valvometer (High Frequency Non-Invasive) connected to the electrodes stuck on oysters.

Chronobiological analysis

Fig. S2. Rhythmic valve behaviour of *C. gigas*. For each condition, the daily periodicity (τ) was determined by spectral analysis (Lomb and Scargle periodogram). The X axis indicates the test duration in order to determine a significant period. The Y axis displays the power (arbitrary units) of the determined period. The red dotted line highlights a significant period for p -value = 0.95.