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To cite this version:
Bettie Cormier, Chiara Gambardella, Tania Tato, Quentin Perdriat, Elisa Costa, et al.. Chemicals sorbed to environmental microplastics are toxic to early life stages of aquatic organisms. Ecotoxicology and Environmental Safety, Elsevier, 2021, 208, 10.1016/j.ecoenv.2020.111665. hal-03176074

HAL Id: hal-03176074
https://hal-univ-pau.archives-ouvertes.fr/hal-03176074
Submitted on 22 Mar 2021

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Research paper

Chemicals sorbed to environmental microplastics are toxic to early life stages of aquatic organisms

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\textbf{A R T I C L E  I N F O}

Edited by Professor Bing Yan

Keywords:
Environmental microplastics
Leachates
Early life stages
Aquatic organisms
Toxicity

\textbf{A B S T R A C T}

Microplastics are ubiquitous in aquatic ecosystems, but little information is currently available on the dangers and risks to living organisms. In order to assess the ecotoxicity of environmental microplastics (MPs), samples were collected from the beaches of two islands in the Guadeloupe archipelago, Petit-Bourg (PB) located on the main island of Guadeloupe and Marie-Galante (MG) on the second island of the archipelago. These samples have a similar polymer composition with mainly polyethylene (PE) and polypropylene (PP). However, these two samples are very dissimilar with regard to their contamination profile and their toxicity. MPs from MG contain more lead, cadmium and organochlorine compounds while those from PB have higher levels of copper, zinc and hydrocarbons. The leachates of these two samples of MPs induced sublethal effects on the growth of sea urchins and on the pulsation frequency of jellyfish ephyrae but not on the development of zebrafish embryos. The toxic effects are much more marked for samples from the PB site than those from the MG site. This work demonstrates that MPs can contain high levels of potentially bioavailable toxic substances that may represent a significant ecotoxicological risk, particularly for the early life stages of aquatic animals.

1. Introduction

Plastic have been a major concern for over 60 years, attracting interest from public, media and scientific community (Law and Thompson, 2014). One of the main reasons behind this interest is the abundance of plastics found in the aquatic environment. Mass production of plastics began in the 1950s, and global production has been rising continuously since. In 2017, it was estimated that around 348 million tons of plastic were produced worldwide (PlasticsEurope, 2018), with millions of tons of plastic waste being accidently or intentionally discharged into aquatic ecosystems (Geyer et al., 2017; Jambeck et al., 2015).

Plastics in the environment tend to degrade and fragment into smaller particles through chemical, physical or biological processes (Barnes et al., 2009), leading to debris with a size below 5 mm in
While plastic contamination in marine ecosystems is now widely documented (Law and Thompson, 2014); few studies have investigated the deleterious effects of MPs on aquatic organisms. However, recent studies have documented both physical and chemical damages induced by several types of MPs, mainly industrial ones. For instance, MPs can lead to blockage of the digestive tract (Cole et al., 2011; Pedá et al., 2016), false satiety (Rochman et al., 2013a, 2013b), growth retardation and death of fish embryos (Pannetier et al., 2020) and impairment of development and swimming activity of oyster larvae (Brieger et al., 2020; Sussarellu et al., 2016). Toxicological effects may also be induced by plastic additives, e.g. bisphenol A, phthalates, UV stabilizer, etc. (Koelmans et al., 2014, 2015; Rochman, 2015) or pollutants sorbed onto plastics (Pannetier et al., 2019a, 2019b; Le Bihanic et al., 2020; Coffin et al., 2020). Pollutants were shown to adsorb on plastic during the process of ageing while certain additives were readily released (Rochman et al., 2013a, 2014; Koelmans et al., 2014; Kedzierski et al., 2018). Chemicals sorbed on plastic are mostly hydrophobic organic compounds, including polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbon compounds (PAHs) and organochlorine pesticides (Derraik, 2002; Karapanagioti and Klontza, 2008; Rochman et al., 2020; Sussarellu et al., 2016). Toxic effects of artificially spiked MPs with pollutants have been reported, including endocrine perturbation, hepatic damage, oxidative stress induction and enzymatic activity modifications (Browne Mark et al., 2013; Pittura et al., 2018; Teuten et al., 2009).

Exposure to pristine MPs either has no effect or causes slight alterations in exposed organisms (Beiras et al., 2018; Cormier et al., 2019; Le Bihanic et al., 2020; Mazauris et al., 2015). However, toxicity assessment of MPs requires much more in-depth investigation, given that they are widespread, persistent and easily ingested by a wide variety of living organisms (Phillips, 2017). The jellyfish Aurelia sp., has been reported to be an consumer of zooplankton. Besides invertebrates, early life stages of three aquatic organisms, sea urchin (Danio rerio) and zebras (Danio rerio), using leachates.

2. Materials and methods

2.1. Sample collection

Environmental MPs were collected from Guadeloupe archipelago (France), located in the Caribbean (Fig. SM1), during the Race for Water Odyssey 2017–2021, in October 2017. This study focused on environmental MPs samples collected on two different sandy beaches, Capes-terre in Marie-Galante Island (MG), and Petit-Bourg (PB) in Guadeloupe Basse-Terre Island (Table SM1). This campaign took place just after the hurricane Irma that devastated the Caribbean in September 2017. These sites were sampled using the standardized NOAA protocol (Lippiatt et al., 2013) adapted for millimetric plastic debris. In short, debris between 1 and 5 mm were collected along a 100 m shoreline, at the surface of the sand. Sorting was based on a visual assessment. Samples were stored in aluminium tray with an aluminium lid at 4 °C.

2.2. MPs composition and chemical characterisation

MPs collected from each beach were counted, and then individually weighed. Plastic debris were monitored by a FTIR Vertex 70V Bruker spectrometer (Billerica, Massachusetts, USA) with a deuterated triglycine sulfate detector (DTGS), in ATR mode (attenuated total reflection), to identify polymer of each particles. Results are expressed in % mass. The absorption bands were recorded in the range of 400–4000 cm⁻¹ with 16 scans and a resolution of 2 cm⁻¹. The data were analyzed using OPUS software. Particles were put into a sample container containing a magnetic bar or impactor and both sides of the container were closed with two stainless steel caps. The container was inserted into a cryogenic mechanical miller (SPEX, 6770 Freezer-mill) filled with liquid nitrogen. Milling consisted in 2 min of pre-cooling followed by 2 cycles of milling. Each cycle lasted 8 min with a 1-min pause between the 2 cycles. The so-obtained powders of particles were then sieved during 12 h on a stainless sieve of 100 and 53 µm. The particle-size distribution was measured using a Malvern laser diffraction particle size analyzer.

Qualitative chemical analysis of organic micropolutants was conducted using gas chromatography (GC) hyphenated with Orbitrap mass spectrometry (Q Exactive GC Orbitrap, Thermo scientific, Bremen, Germany) with a non-target analysis (NTA) workflow. Liquid/solid extractions were performed using 0.3 g of mesoplastics in hexane (≥98%, Suprasolv; Merck, Darmstadt, DE); followed by 30 min of sonication and centrifugation at 8000 revolutions per minute (rpm) for 10 min. Extracts were reduced to 1 mL, using nitrogen flow (6.0) and filtrated through glass wool with sodium sulfate, and finally reduced to 5 µL prior injection. More information about the GC/MS parameters for the Q Exactive GC Orbitrap instrument can be found in supplementary materials (Table SM2).

All measurements were performed in full scan mode with a resolution of 60 000 at m/z 200 between m/z 53.4 and m/z 800. All Q Exactive GC Orbitrap parameters are available in the SM. Plastic samples were analyzed using mass defect-plot. Halogenated compounds were detected using Cl-H/Br-H (mass scale = 34/33.9610 or 78/77.9105) (Jobst et al.,
3.8.2.2. Acute toxicity assay using zebrafish embryos
Fish husbandry conditions fully complied with OECD TG 236 (OECD, 2013; Westerfield, 2000) regarding maintenance of adult zebrafish (Danio rerio). Zebrafish, TU strain were bred in 10 L tanks at 27 ± 1 °C with a day/night cycle of 14 h/10 h (7.8 ± 0.2). 480 ± 130 µS, for pH and conductivity, respectively). Fish were fed twice-daily ad libitum
with Inicio + 0.5 mm (BioMar, France) and freshly hatched brine shrimp Artemia nauplii. Constant filtering or permanent flow-through conditions guaranteed that ammonia, nitrates, and nitrites were kept below detection limits (5, 1 and 140 mg/L, respectively). Zebrafish eggs were collected according to OECD TG 236 (OECD, 2013).

Leachate exposure started after hatching at 72 h post fertilization (hpf). Zebrafish embryos were exposed for 48 h to leachates in E3 medium until the end of the eleutheroembryo phase (96 hpf). Embryos were exposed to two equivalent concentrations of plastics 50,000 and 10,000 mg/L. Positive control was added, using 4 µM of 3,4-dichloroaniline (DCA) for the test validation (OECD, 2013). Glass petri dishes were exposed to two equivalent concentrations of plastics 50,000 and 10,000 mg/L. Positive control was added, using 4 µM of 3,4-dichloroaniline (DCA) for the test validation (OECD, 2013). Glass petri dishes containing 20 mL of E3 were used in semi-static conditions with a daily renewal of 15 mL of exposure medium. Twenty embryos were used for each condition, and each treatment condition was replicated 3 times. To maintain a temperature of 28 ± 1 °C, experiments were performed in temperature-controlled chambers (Snijders Scientific, Tilburg, Netherlands) with a photoperiod set up on 14:10, light:dark. Before hatching, embryos were kept in E3 medium, and then, transferred in glass beakers containing 20 mL of leachate. For the entire exposure period (from 72 to 96 hpf), dissolved oxygen was checked daily (>85%) with a fiber-optic oxygen mini-sensor Fibox 3 (PreSens Precision Sensor, Regensburg, Germany).

Individuals were examined following OECD 212 (OECD, 1998). Embryos were checked daily for survival and dead embryos were counted and immediately removed to avoid modification of the medium. Developmental anomalies and lesions were also recorded: edema; hemorrhages, skeletal axis (scoliosis, lordosis) and craniofacial deformations and cardiovascular anomalies (blood circulation) following previously published protocols for Japanese Medaka (Le Bihanic et al., 2014). Leica MZ7.5 stereomicroscope with Leica DFP420C CCD camera and Leica Microsystems software v3.8 were used to perform these measurements (Leica, France).

At 96 hpf, six to ten embryos per replicate were anaesthetized with 200 mg/L of benzocaine. Total length and head length, were measured using a Leica MZ7.5 stereomicroscope with Leica DFP420C CCD camera and Leica Microsystems software v3.8 (Leica, France), and the ratio of head/total length was calculated.

In vivo EROD activity was analysed according to published methods with modifications (Jonsson et al., 2002; Le Bihanic et al., 2013). Measurements were conducted on 96 hpf embryos. Five embryos per replicate were placed into individual wells of a 48-well microplate containing 1 µM of 7-ethoxyresorufin (7-ER) in 0.5 mL of E3. After 1 h of incubation in the dark, the 7-ER solution was removed and replaced by freshly prepared 1 µM 7-ER. Fluorescence of 100 µL of the incubation media was measured in duplicate in 96 white well plate at time 0 and after 4 h incubation. Resorufin formed was measured with a BMG Labtech fluorescent microplate reader (Germany) excitation and emission wavelengths of 560 and 590 nm respectively. Solution of 7-ER was added to the resorufin standard used, for a final concentration of 1 nM. A batch of five control embryos was exposed to benzocaine. Total length and head length, were measured using a Leica MZ7.5 stereomicroscope with Leica DFP420C CCD camera and Leica Microsystems software v3.8 (Leica, France), and the ratio of head/total length was calculated.

Swimming behaviour was investigated following the protocol of Vignet et al. (2014) with a larval photomotor response test (LPMR) to record fish behavioural responses following a light stimulus. Embryos at 96 hpf were transferred into a 48-well plate filled with 500 µL of E3 medium without methylene blue and acclimated for 4 h in the dark prior to measurement. Then, embryos were moved to the DanioVision (Noldus, The Netherlands). Swimming behaviour of 10–12 embryos (96 hpf) per replicate was recorded at 28 ± 1 °C (DanioVision Temperature Control Unit, Noldus, The Netherlands). Swimming videos were analyzed with Ethovision software 12.0 (Noldus, The Netherlands).

2.5. Statistical analyses

Statistical analyses were conducted using IBM SPSS (Statistical Package for the Social Sciences) statistics software (v. 20 and 24). Normal distribution and homoscedasticity of the data was checked using Shapiro–Wilk’s and Levene’s tests respectively. ANOVA with Dunnett’s post hoc test was applied to determine the no observed effect concentration (NOEC) and the lowest observed effect concentration (LOEC). When data failed to meet the assumption of normality, nonparametric Kruskal Wallis test followed by Mann Whitney test were used to compare individual treatments. Effective median concentrations (EC50) and their 95% confidence limits were calculated using Probit and toxic units (TU) were calculated as TU = 1/EC50. For AFp, statistical analysis was performed using Frequency pulsation data (SPSS v.20). Data were considered significantly different when p < 0.05.

For zebrafish assay, three independent runs were performed, and data were tested for normality with the Shapiro-Wilk and Kolmogorov-Smirnov tests. If data were normally distributed, one-way analysis of variance (ANOVA) was run in combination with a post-hoc Dunnett’s test; otherwise, a non-parametric Kruskal-Wallis, Mann-Whitney U or Wilcoxon’s matched-pairs tests were used for statistical comparison. Data were first analyzed for differences between runs (biological replicates). Since there were no significant differences between independent runs, single data sets were merged for each laboratory, and tests on different exposure groups were performed. In the case of LPMR, a repeated-measure ANOVA was performed to take into account the three successive periods of the test. A p-value of 0.05 was considered statistically significant for all analyses. Graphical illustrations and statistical tests were performed with Sigma Plot 12.5 (Jandel-Systat, Erkrath, Germany).

3. Results

3.1. Characterisation of microplastics

The predominant shape of MPs collected was fragments (>98%) for both sites. Pellets were not found, and only a few particles were fibers, thin films and foams (<2%). Polymer composition was almost the same for the two beaches, with a majority of polyethylene (PE) and polypropylene (PP), few particles of polystyrene (PS) and 2 particles of polyvinyl acetate only at Petit-Bourg beach (Table 1).

Particle size was only measured on the MG site due to the small amount of particles available in PB site. Prior to granulometric analysis, MPs were sieved on 800 µm, and no particles were larger than 800 µm. The first decile (0.1) corresponded to a size of 6.2 µm, the median particle size was 3.7 µm, and the last decile (0.9) was 112.0 µm, while the mean size particles was 87.1 µm.

3.2. Pollutant fingerprint on MPs

3.2.1. Organic pollutants

Non-target chemical analyses were performed on samples from both sites. Chromatograms superposition shows that the two chromatograms contain peak similarities and dissimilarities (Fig. S2). For the chemical analysis, mass defect plot was used to detect chlorinated and brominated compounds. Some halogenated compounds were detected in both

Table 1

<table>
<thead>
<tr>
<th>Polymer composition</th>
<th>Marie-Galante</th>
<th>Petit-Bourg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polyethylene</td>
<td>78.3</td>
<td>74.6</td>
</tr>
<tr>
<td>Polypropylene</td>
<td>21.2</td>
<td>24.8</td>
</tr>
<tr>
<td>Polystyrene</td>
<td>0.1</td>
<td>0.4</td>
</tr>
<tr>
<td>Polyvinyl acetate</td>
<td>0</td>
<td>0.2</td>
</tr>
</tbody>
</table>
samples. Brominated flame retardant as tribromophenol (C₈H₂Br₃O) and its metabolite tribromoanisole (C₇H₇BrO) were found in both samples. Tri- and di-polychlorinated biphenyl were detected as C₁₂H₇Cl₃ and C₁₂H₈Cl₂. Homologous series of pesticides from penta-chlorobenzene (C₅H₅Cl₅) and dichlorobenzene (C₆H₅Cl₂) were detected but only one chlorinated polycyclic aromatic compound (C₇H₇Cl₃) most probably chloroaenaphthylene. Analysis of PAHs was carried out using a mass defect plot with a PAHs scale, however no homologous series were detected for either MG or PB.

Finally, fold change comparisons of peak surfaces were made between MG and PB samples using TraceFinder, 26 features were selected. The results are presented in supplementary material (Table SM3). The concentrations of halogenated compounds as well as trichlorobenzene, bumerizole and octabenzene were higher at the MG site.

Phthalates were identified in MG sample as diisobutylphthalate, dibutyphthalate, diethylphthalate, ether di-(2-ethylhexyl) phthalate and di-n-octyl phthalate.

Using mass defect plot CH₂, more hydrocarbons were detected in the PB sample than from MG, qualitatively, these compounds are mainly C₇H₇O. This could be explained by the location of PB, close to a large industrial zone. Most PB-specific peaks identified, corresponded to alkanes. The three principal peaks identified were C₁₁H₈O₂ (Confidence level 4), C₁₀H₇O⁺ (Confidence level 4), seemed to correspond to a phenol C₆H₄O with an alkane chain and an isomer of octadecanoic acid C₁₈H₃₆O₂ (Schymanski et al., 2014).

3.2.2. Metallic contamination

The screening analysis of trace metals revealed the presence of various metals for both samples including aluminium (Al), chromium (Cr), manganese (Mn), cobalt (Co), nickel (Ni), gallium (Ga), arsenic (As), selenium (Se), strontium (Sr), silver (Ag), tin (Sn), antimony (Sb), caesium (Cs), cerium (Ce) and uranium (U). A quantitative analysis was then performed, and results are summarized in the Table 2. Experimental blank revealed absence of trace metal with a concentration of Al 3.0 μg/g, Zn 0.2 μg/g, Mn 0.2 μg/g, Fe 3.0 μg/g, Co 0.003 pmole/min/larvae). NOEC, LOEC and EC₅₀ were calculated for the different species (Table 3). Regarding jellyfish, results obtained after 48 h exposure to leachates were considered. A striking difference of sensitivity was observed between bioassays. Jellyfish ephyrae was the most sensitive species and frequency of pulsation was significantly altered from 0.33 g/ L equivalent MPs, after 48 h of exposure. Sea urchin larvae exhibited an intermediate sensitivity after 48 h of exposure while zebrafish embryos were clearly insensitive to MPs leachates whatever the considered endpoints at 72 or 96 hpf.

3.3. Leachate toxicity

3.3.1. Sea urchin

No toxicity was found in PE ‘virgin’ resin and in leachates of MG plastic sample up to a concentration of 3333 mg/L equivalent MPs. A moderate toxicity was noted for MG and a slight one for the reference PE for the highest tested concentration of leachate. However, the PB sample was much more toxic for P. lividus with a significant reduction in larval growth starting from 3333 mg/L equivalent MPs (Fig. 1).

3.3.2. Jellyfish

No effects (<1%) were reported in Aurelia sp. ephyrae exposed to PC, MG and PB leachates in terms of immobility after the two different exposure times (24 and 48 h) at all dilutions tested. Jellyfish behaviour was not affected after 24 h of exposure to PC and MG samples (Fig. 2A), while a significant effect higher than 20% was observed at 1/3 (333 mg MPs/L) and undiluted PB samples.

After 48 h, a significant AFp was observed in ephyrae jellyfish exposed to all undiluted samples (PC, MG, PB) and to diluted MG and PB leachates (p < 0.05). Specifically, about 29–34% of AFp was observed in ephyrae exposed to MG leachates from undiluted leachate up to 0.1 g/ L (Fig. 2B); PB leachate induced a significant AFp at all dilutions.

3.3.3. Zebrafish

Zebrafish (72 hpf) were exposed for 48 h to MPs leachates. No larval mortality was recorded during the experiment except for the PC (40 ± 6%). No significant developmental anomalies (skeletal and cardiac deformities) were observed except for the PB (10 ± 2.8%) (Fig. SM3). Average length of negative control larvae was 2.45 ± 0.10 mm with head length of 0.49 ± 0.01 mm. Larval size for individuals exposed to MPs leachates from the two sites at both concentrations were not significantly different from negative control larvae (Fig. SM4). Fish exposed to PC were significantly smaller compared to negative control (total length 1.54 ± 0.2 mm; head length 0.23 ± 0.01 mm, Kruskal–Wallis, p < 0.05).

No significant induction of in vivo EROD activity was observed in larvae exposed to MPs leachates when compared to negative control (0.021 ± 0.003 pmole/min/larvae) (Fig. 3), while larvae exposed to 70 mM of BaP for 1 h showed a significant induction of EROD activity (0.039 ± 0.003 pmole/min/larvae).

The LPMR was performed at 4 dpf to monitor early behavioural disruption. There was no difference in mean velocity of larvae between treatments for a single set of light conditions (ANOVA, p > 0.05), (Fig. 4).

3.3.4. Sensitivity comparison between species

NOEC, LOEC and EC₅₀ were calculated for the different species (Table 3). Regarding jellyfish, results obtained after 48 h exposure to leachates were considered. A striking difference of sensitivity was observed between bioassays. Jellyfish ephyrae was the most sensitive species and frequency of pulsation was significantly altered from 0.33 g/ L equivalent MPs, after 48 h of exposure. Sea urchin larvae exhibited an intermediate sensitivity after 48 h of exposure while zebrafish embryos were clearly insensitive to MPs leachates whatever the considered endpoints at 72 or 96 hpf.

4. Discussion

4.1. Plastic characterization

In the present study, MPs (1–5 mm) collected from both study beaches were mostly fragments (>98%) composed of 75–80% PE and 20–25% PP. This is in agreement with previous studies reporting that PE is the most abundant polymer found in plastic litter (Cheang et al., 2018; Fossi et al., 2017; Hidalgo-Ruz et al., 2012; Karthik et al., 2018), followed by PP and PS. The density of plastic has an influence on the location of MPs in the water column. MPs with a density below one (e.g., PE, PP), tend to float (Hidalgo-Ruz et al., 2012) and to be brought back to the shore by the tide and waves. As a consequence, PE and to a lesser extent PP are the predominant polymers of MPs collected on beaches over the world (Frias et al., 2010; Pannetier et al., 2019b).

The chemical contamination profile of plastics collected on the two sites demonstrated similarities and dissimilarities. Halogenated compounds including brominated flame retardant, PCBs, chlorinated pesticides and chlorinated P AHs (chloroacaphthylene) were found on both sites, while unsubstituted PAHs were not detected. The major differences between the two sites consisted in higher concentrations of halogenated compounds in MG than in PB, along with the detection of phthalates (diisobutylphthalate, dibutylphthalate, diethylphthalate, ether di-(2-ethylhexyl) phthalate and di-n-octyl phthalate). MPs from PB site were characterized by a high variety of hydrocarbons. This result could be explained by the proximity of the industrial area and harbour of Pointe-à-Pitre. It was already shown that environmental samples of MPs often contained PAHs, PCBs and pesticides (Pannetier et al., 2019b;
The profile and concentrations of hydrophobic organic pollutants on plastics are linked to the polymer composition and to the chemical exposure of particles throughout their drift history (Rochman, 2015). In the present study, the sampling period was just after the hurricanes Irma and Maria that devastated the Caribbean in September 2017 and, at least some plastic debris collected on beaches came directly from inland, especially in PB due to its proximity with the industrial area and the harbour. In our study, selected metals (Pb, Cd, Cr, Cu and Zn) were quantified at µg/g range. These metals were commonly detected and quantified on environmental MPs at the same concentration range (Acosta-Coley et al., 2019; Dobaradaran et al., 2018; Li et al., 2020; Vedolin et al., 2018). The metals composition was different between the two MPs samples with higher concentrations of Pb, Cd and Cr in MG and higher concentrations of Cu and Zn in PB. Variations in metals concentrations might be due to a combination of factors, including residence time at sea leading to differences in weathering and surface erosion, photo-degradation stage but also to some extent of biofouling (Ashton et al., 2010; Holmes et al., 2012; Rochman et al., 2014).

**Fig. 1.** Larvae of Paracentrotus lividus length increase with control corrected (ΔLc) in serial dilutions of leachates from microplastic samples. Virgin polyethylene resin obtained from Rotogal (PE Rotogal), Petit-Bourg (PB) and Marie-Galante (MG). Bars represent mean ± SD, N = 4. Asterisks refer to significant differences to the control treatment **p < 0.01 and ***p < 0.001.

**Fig. 2.** Percentage of alteration of frequency of pulsation (AFp), after 24 h (A) and 48 h (B) of exposure in rotary wheel to PC (white bars), MG (black bars) and PB (grey bars) samples from 0.033 to 1 g/L, using ephyrae of Aurelia sp. (*p < 0.05).
4.2. Toxicity assessment using early life stages

Embryos and larvae of fish and invertebrates play a fundamental role in the structure and functioning of marine ecosystems. The long-term sustainability of healthy populations depends on the good health and survival of larvae (Steer et al., 2017). In addition, early life stages are particularly sensitive to a wide range of pollutants (Emby et al., 2010; Lammer et al., 2009) including MPs (Bringer et al., 2020; Gambardella et al., 2017; Le Bihanec et al., 2020; Messinetti et al., 2018).

The lack of toxicity of virgin polymers compared to commercial plastics with chemical additives was previously reported. A comparison between leachates of pristine PVC and plastic toys made of PVC showed that the toys had the highest toxicity for _Paracentrotus lividus_ larvae (Oliviero et al., 2019). Leachate analyses have highlighted the presence of phthalates and metals used as plasticizers, stabilizers or colouring agents in plastics (Oliviero et al., 2019; Omolaoye et al., 2010). Plastic additives including metal stabilizers and heavy-metal pigments are not chemically bound to the polymers and thus can easily leak out of plastics (Guney and Zagury, 2012; Omolaoye et al., 2010).

In the present study, the toxicity of MPs leachates produced from two environmental samples of MPs has been tested on early life stages of sea urchin (_Paracentrotus lividus_), jellyfish (_Aurelia sp.)_ and fish (_Danio rerio_). Leachates of plastics collected in the two sites point out no toxicity in terms of survival (mortality, immobility) with the three bioassays. Sub-lethal effects were however observed in sea urchin embryo development and in jellyfish pulsatile behaviour, while no developmental anomalies, growth retardation and behavioural disruption were found in zebrafish larvae. Results of the present study are in agreement with previous results on sub-lethal effects of leachates of environmental MPs such as fishing nets, fishing cages and packaging collected at sea and commercial MPs, on the size and development of _P. lividus_ larvae (Oliviero et al., 2019). No data on plastic leachates toxicity in jellyfish and zebrafish are available so far for comparison. Here sub-lethal effects were only observed in sea urchin and jellyfish and not in zebrafish embryo-larval stages. It might suggest that early life stages of invertebrates and more particularly jellyfish ephyrae are particularly sensitive to MPs leachates. This hypothesis is supported by several studies on other emerging contaminants (i.e. MPs, nanoparticles, chlorpyrifos) and traditional ones (i.e. metals, pesticides), demonstrating the high sensitivity of ephyrae jellyfish compared to other zooplankton early life stages, such as crustaceans or mussels (Faimali et al., 2014; Costa et al., 2015, 2020; Gambardella et al., 2015). Regarding MPs, jellyfish ephyrae have been reported to be significantly affected in terms of immobility and frequency of pulsation at concentrations lower by 2–4 orders of magnitude
than those observed in other organisms (Beiras et al., 2018; Costa et al., 2020), thus being proposed as a good bioindicator species for plastic pollution (Macali and Bergami, 2020). Results obtained from sea urchin and jellyfish demonstrated that PB sample was more toxic than MG sample. In this regard, PB leachates affected sea urchin growth and jellyfish behaviour from a concentration of 3333 and 33 mg MPs/L, respectively. Although both samples were similar in polymer composition (>70% of PE and >20% of PP) the differences in toxicity could be due to the differences in chemical composition between both samples. For instance, MPs from PB sample were characterized by a much higher content of Zn and Cu in comparison with MG sample. Cu is known as one of the most toxic metal for a large range of invertebrate marine species with early life stage of the bivalve Crassostrea gigas (Mai et al., 2012), the sea urchin Paracentrotus lividus (Fernández and Beiras, 2001) and the polychaete Hydroides elegans (Gopalakrishnan et al., 2007). Cu and other metals could be, at least in part, involved in the toxicity of the MPs leachates. Further chemical analyses of leachates are necessary to ascertain this hypothesis. Also, in the same area as PB site, chlordecone and other metals could be, at least in part, involved in the toxicity of the MPs leachates. Previous studies have highlighted the chemical contamination at the surface of MPs with the presence of phthalates and metals (Oliviero et al., 2019; Omolaye et al., 2010). Another source of toxicity for environmental MPs are HOCs from the water column accumulated on the hydrophobic polymeric matrix. Gandara e Silva et al. (2016) found that beached pellets showed higher toxicity on bivalve larvae than virgin pellets. Similarly, Pannetier et al. (2019a) did not show toxicity of mixture of virgin MPs on medaka embryos but different degrees of toxicity of beached MPs.

5. Conclusion

This study documents the presence of toxic substances on the surface of MPs collected on two beaches in the Guadeloupe archipelago. Substances released by aqueous extraction are toxic to the early life stages of sea urchin and jellyfish. Results using behavioural endpoints are in agreement with previous reports on jellyfish exposure to MPs, chemicals, pesticides and nanoparticles, suggesting them as suitable endpoint to be further considered for the assessment of aquatic pollution by legacy and emergent pollutants, including MPs. This study provides also new evidence of the toxicity of chemicals sorbed to environmental plastics. It would be relevant to go further by characterizing the kinetics of sorption of pollutants and desorption of additives during the aging of plastics and the toxic kinetic and dynamic on different aquatic species.

Funding

This work was developed under RESPONSE (Towards a risk-based assessment of microplastic pollution in marine ecosystems) and EPHE-MARE projects (Ecotoxicological effects of microplastics in marine ecosystems) within the JPI Healthy and Productive Seas and Oceans (JPI Oceans program). In France, it was supported by the National Funding Agency (ANR-15-JOCE-0002-01). In Spain, it was funded by the Spanish Government (MINECO) (PCIN-2015-187-C02-03 and CTM2016-77945-C3) and Ricardo Beiras benefited from the Grant “Program of Consolidation and structuring of competitive research groups in the University system of Galicia” by the Galician Government (ED431C 2017/46). Italy also benefited of PRIN (Research Project of National Interest) EMME (Exploring the fate of Mediterranean microplastics: from distribution pathways to biological effects) project funded by the Italian Government (code: 2017WERYZP). Bettie Cormier was directly supported by a PhD grant from the University of Bordeaux (IdEx).

CRediT authorship contribution statement


Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The authors would like to thank James Emery for providing English proofreading services.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ecoenv.2020.111665.

References
