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Environmental microplastics disrupt swimming activity in acute exposure in *Danio rerio* larvae and reduce growth and reproduction success in chronic exposure in *D. rerio* and *Oryzias melastigma*☆

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ABSTRACT

Microplastics (MPs), widely present in aquatic ecosystems, can be ingested by numerous organisms, but their toxicity remains poorly understood. Toxicity of environmental MPs from 2 beaches located on the Guadeloupe archipelago, Marie Galante (MG) and Petit-Bourg (PB) located near the North Atlantic gyre, was evaluated. A first experiment consisted in exposing early life stages of zebrafish (*Danio rerio*) to MPs at 1 or 10 mg/L. The exposure of early life stages to particles in water induced no toxic effects except a decrease in larval swimming activity for both MPs exposures (MG or PB). Then, a second experiment was performed as a chronic feeding exposure over 4 months, using a freshwater fish species, zebrafish, and a marine fish species, marine medaka (*Oryzias melastigma*). Fish were fed with food supplemented with environmentally relevant concentrations (1% wet weight of MPs in food) of environmental MPs from both sites. Chronic feeding exposure led to growth alterations in both species exposed to either MG or PB MPs but were more pronounced in marine medaka. Ethoxyresorufin-O-deethylase (EROD) and acetylcholinesterase (AChE) activities were only altered for marine medaka. Reproductive outputs were modified following PB exposure with a 70 and 42% decrease for zebrafish and marine medaka, respectively. Offspring of both species (F1 generation) were reared to evaluate toxicity following parental exposure on unexposed larvae. For zebrafish offspring, it revealed premature mortality after parental MG exposure and parental PB exposure produced behavioural disruptions with hyperactivity of F1 unexposed larvae. This was not observed in marine medaka offspring. This study highlights the ecotoxicological consequences of short and long-term exposures to environmental microplastics relevant to coastal marine areas, which represent essential habitats for a wide range of aquatic organisms.

1. Introduction

In the oceans, plastic debris are observed as large fragments (macroplastics; > 5 mm) or microplastics (MPs), defined as plastic particles between 5 mm and 1 μm in size (Jambeck et al., 2015). Primary MPs are produced for commercial purposes at the microscale size (Costa et al., 2010; de Sá et al., 2018; Ogata et al., 2009), while secondary MPs are produced from the breakdown of larger pieces (Anbumani & Kakkar, 2018; Li et al., 2018; Thompson et al., 2004). MPs are now considered as a potential threat for aquatic ecosystems (Castro-Castellon et al., 2022; Ma et al., 2020). Albeit fluxes estimations have been revised to much lower values than previously thought, the number of MPs floating at the ocean surface is currently estimated as tens to hundreds of thousand metric tons (Weiss et al., 2021). Plastic debris tend to accumulate in oceanic gyres (Gove et al., 2019) and in coastal areas (Eriksen et al., 2014; Gove et al., 2019; Law et al., 2014; Lebreton et al., 2018), as well

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as washing up on beaches (Andrady, 2011). As an example, the North Atlantic Gyre contribute significantly to the MPs found on beaches of the Caribbean Sea (Acosta-Coley et al., 2019; Acosta-Coley & Olivero-Verbel, 2015; Bosker et al., 2017).

Plastic production uses various additives such as plasticizers, flame-retardants, UV filters, dyes etc. to improve specific characteristics (Andrade et al., 2021; Hahladakis et al., 2018). It has been demonstrated that some of these additives can be toxic to aquatic organisms (Andrade et al., 2021). Besides additives, MPs particles have been shown to sorb a wide range of persistent organic pollutants and trace metals from the surrounding environment (Liu et al., 2021; Menéndez-Pedriza & Jaumot, 2020; Wang et al., 2020; Yu et al., 2019). Weathering processes, combined with fragmentation, can lead to an increase in the reactive specific surface area of particles, facilitating the sorption of pollutants (Liu et al., 2020) while decreasing the leakage of additives (Yan et al., 2021). Chemicals sorbed on MPs are mainly hydrophobic organic compounds, i.e. polychlorinated biphenyls (PCBs), polycyclic aromatic compounds (PAHs) or organochlorine pesticides (Derraik, 2002; Karapanagioti & Klontza, 2008; Wang et al., 2020). However, the sorption can also include trace metal elements (Catrouillet et al., 2021; El Hadri et al., 2020; Liu et al., 2021).

Desorption of chemicals from MPs to biota has been demonstrated (Cormier et al., 2021a; Cousin et al., 2020), but the importance of MPs contribution, compared to other routes, to chemical load of biota is disputed (Koelmans et al., 2016; Lee et al., 2021). In addition, micrometric size of MPs favor ingestion by organisms, and their transfers into food webs have been reported in laboratory studies (Cousin et al., 2020; Farrell & Nelson, 2013; Pannetier et al., 2020; Setälä et al., 2014). A wide range of organisms, including zooplankton, mollusks, fish or cetaceans are known to be able to ingest MPs (Cole et al., 2013; Desforges et al., 2015; Giani et al., 2019; Panti et al., 2019). In fish, the ingestion of MPs has been well-documented for numerous wild species and at all life stages (Alimba & Faggio, 2019; Gove et al., 2019; Hermsen et al., 2017; Rummel et al., 2016; Steer et al., 2017; Watts et al., 2015). After ingestion, MPs can cause a diversity of toxic effects to aquatic organisms, as physical and/or chemical damages. The latter may cause endocrine disruptions and hepatic stress including *cyp1A* induction, oxidative stress, changes in metabolic parameters, decrease in enzyme activities, and cellular necrosis (Browne et al., 2013; Law & Thompson, 2014; Mazurais et al., 2015; Oliveira et al., 2013; Rochman et al., 2013; Rochman et al., 2014a; Teuten et al., 2009). However, in most cases, short-term exposures coupled to high MPs concentration were reported while recent research reported the consequences of long-term exposure of fish to industrial MPs (Cormier et al., 2021b; Yin et al., 2018; Yu et al., 2019). In long-term exposures, the ingestion of MPs has been shown to compromise growth and organism development, reproduction dynamics and the survival of a population (Cormier et al., 2021b). However, to date, studies investigating the toxicity of MPs collected in the environment in teleost are still scarce and limited to short (3–5 days) or medium-term (30 days) exposures (Pannetier et al., 2019; Zitouni et al., 2021).

In 2017, a sampling campaign was carried out around the world in all oceans (Race for Water Odyssey, 2017–2021) by the Race for Water Foundation, to collect and quantify MPs from different aquatic compartments. Sampling sites were selected in the vicinity of the North Atlantic gyre, with a stopover in the Caribbean Islands, including two beaches in Basse-Terre and Marie-Galante located in the Guadeloupe archipelago. After a first evaluation of the toxicity of leachates of these MPs on diverse marine organisms (Cormier et al., 2021a), the purpose of the present study was to investigate the toxicity and the spectrum of effects of environmental MPs on two life stages and two model fish species. In a first experiment, acute zebrafish embryo toxicity test (OECD, 2013), supplemented with additional sublethal endpoints was carried out. In the second experiment, a chronic feeding exposure with two different fish species, a freshwater model fish, zebrafish (*Danio rerio*) and a marine model fish, marine medaka (*Oryzias melastigma*), was performed to precisely evaluate their chronic toxicity on different biological functions. The two fish species were exposed over four months through diet to environmental MPs. Studied endpoints included biochemical biomarkers, survival, growth, reproduction, and swimming behaviour of exposed fish (F0 generation). Toxicity on offspring (F1 generation) was also investigated with survival, growth, and swimming behaviour as endpoints.

2. Materials and methods

2.1. Composition and chemical characterization of environmental MPs

MPs were collected and characterized as described previously in Cormier et al. (2021a). MPs were sampled from the sand surface of two beaches: Capesterre in Marie-Galante Island (MG), and Petit Bourg (PB) in Guadeloupe Basse-Terre Island, in October 2017. MPs were sampled according to the standardized NOAA protocol (Lippiatt et al., 2013) with slight modification for millimetric plastic debris. In brief, polymer composition was determined using FTIR in ATR mode (Vertex 70 V Bruker spectrometer, MA, USA) and data were analyzed using OPUS software. Then, MPs were cryomilled (SPEX, 6770 Freezer-mill) and powder was sieved for 12 h (100 and 53 μm stain sieves). The particle-size distribution was measured using a Malvern laser diffraction particle size analyzer.

Regarding sample preparation prior to chemical analysis, more information can be found in Cormier et al. (2021a) and are summarized in supplementary materials.

2.2. Husbandry and egg production

Rearing and exposures were performed at the laboratory facilities at Ifremer Research Centre, in L'Houmeau (EEA 171901, France). The experiments were performed under authorization number APAFIS#10883 and followed the recommendations of Directive 2010/63/EU. Detailed information on husbandry and egg production for both species are available in supplementary materials.

2.3. Acute fish embryo toxicity test, using zebrafish

Zebrafish embryos were exposed from 3 hpf (hours post fertilization) to MPs added to the E3 medium until the end of the eleutheroembryo phase (96 hpf) in a climate chamber (Snijders Scientific, Tilburg, Netherlands) at 26 ± 1 C° under a 14 h:10 h light:dark cycle. Embryos (n = 20 per replicate, 3 replicates per treatment) were exposed to 1 or 10 mg/L of MPs; controls were reared in E3 medium supplemented with methylene blue only (antifungal agent), and positive control exposed to 4 mM of 3,4-dichloroaniline, for the test validation (OECD, 2013). Detailed information on methods used to evaluate acute toxicity of environmental MPs on zebrafish embryos are available in supplementary materials.

2.4. Long-term chronic trophic exposure to food supplemented with environmental MPs

The experimental design of the trophic exposure was performed according to Cormier et al. (2021b), as described in detail below.

2.4.1. Preparation of food supplemented with MPs for trophic exposure

Diets were prepared to obtain a homogenous distribution of MPs in the food at a final ratio of 1% wet weight (10 mg/g ww). Using glass tubes, MPs (53–100 μm) and pellets (Special Diet Services SDS, Dietex France, Argenteuil, France) were mixed with a 4 mm bottlebrush fixed on a drill for 3 min. Afterwards, to reinforce binding of MPs to pellets, flaxseed oil was added (5 μL per gram of food) and mixed for an additional time of 3 min. The control group consisted of fish fed with plain pellets (SDS, same as above) mixed only with flaxseed oil as described

above. Food sizes (200, 300 or 400 μm) were adapted as fish grew.

2.4.2. Experimental design

Sixty eggs were transferred to 10 cm diameter Petri dishes, and then incubated at 28 °C under a 14 h:10 h light:dark cycle. Dead eggs were daily removed and 20% of the medium was changed (E3 for zebrafish, and artificial seawater for marine medaka, Instant Ocean (Europrix, Lens, France). After hatching, 72 hpf and 8 days post fertilization (dpf) for zebrafish and marine medaka, respectively, chorion was removed from the medium. Then, hatched larvae were transferred and randomly assigned to each treatment including 3 replicates, to 1 L tanks for 7 days and then to 3 L cylinders tubes located in individual 10 L tanks in a dedicated flow-thought rearing system. 14 days later, tubes were removed from tanks, and larvae were poured into their 10 L tanks. Per treatment, each triplicate tank contained at least 20 to 30 fish. Embryos and larvae were monitored daily for mortality. At 30 dpf, marine medaka and zebrafish were exposed for 5 months (until 6 mpf, months post fertilization) to three different treatment diets: control, food supplemented with Marie-Galante MPs (MG, 1% wet weight) or supplemented with Petit-Bourg MPs (PB, 1% wet weight). Feeding regime was identical to that of brood stocks: two pellets meal per day at 3% of the biomass, and freshly hatched artemias once a day. At the end of the exposure, the offspring generation F1 was reared with no further exposure using the same protocol as mentioned above. During the exposure, fish were monitored for any possible signs of impaired health status (i.e., fish condition, external lesions, and modification of feeding behaviour). At the end of the exposure, fish from F0 generation were euthanized with 500 mg/L of benzocaine (Sigma Aldrich, Saint-Quentin Fallavier, France), stock solution in ethanol diluted 200-fold in water for euthanasia, for biomarkers analyses. Whole fish or dissected tissues were frozen in liquid nitrogen and stored at – 80 °C until analysis.

2.4.3. Biomarkers of toxicity in the F0 generation

Throughout the exposure, dead larvae or fish were monitored daily. Individual standard length (mm) and body weight (g) were measured at 2, 4 and 6 months of age according to previously published protocols (Vignet et al., 2014a) after a short anasthesia using 50 mg/L of benzocaine (Sigma Aldrich). At 6 months of age fish were euthanized with 500 mg/L of benzocaine (Sigma Aldrich). Depending on parameters, i.e., sex and time point, between 20 and 50 fish were monitored.

For all molecular biomarkers, three pools of 3 males or 3 females have been used to obtain 3 measures per sex and per treatment, for both species.

Liver, muscle and brain of individuals were sampled at the end of the exposure and homogenized in 0.1 M phosphate buffer (pH = 7.7; 0.1 M KCl) and then centrifuged at 9000 rpm for 20 min at 4 °C. The supernatant containing fraction S9 was then collected and stored at −80 °C before being used for all enzymatic activity measurements. The protein concentration in the S9 fraction was measured using Bradford's method (Bradford, 1976) with bovine serum albumin as standard. All spectrophotometric measurements were performed in a Biotek Synergy HT microplate reader (Biotek, VT, USA) and expressed as mg/mL. The Comet assay was performed on blood cells from marine medaka. Alkaline comet assay was performed following previously published protocols with minor modifications (Le Bihanic et al., 2014).

Detailed information on methods used to measure molecular biomarkers are available in supplementary materials.

Behaviour was monitored using the well-established procedure of the novel-tank diving test (Egan et al., 2009; Levin et al., 2007). For this purpose, fish were transferred in individual 1-L aquariums in a dedicated room the day before the test. On the day of the test, fish were gently introduced in the novel tank (trapezoid 1.5-L tank; Aquatic Habitats, Apopka, FL, USA; size in cm: height 15.2 × width 7.1 × length 27.9 at the top and 22.5 at the bottom) and their swimming behaviour was recorded for 5 min from the side using previously described camera set-up (Vignet et al., 2014a). A minimum of 12 fish (up to 21) were tested for each treatment. For space occupancy analysis, tanks were virtually separated into two areas (top and bottom halves each accounting for half of the volume) as previously described (Egan et al., 2009). EthoVision XT10 (Noldus, Wageningen, the Netherlands) was used for track extraction and analysis. The dependent variables measured were time spent in each area (s, top/bottom) and total distance travelled (cm) over the 5 min.

After 3 months of exposure, reproduction of both species was monitored for one month. Due to biological differences between species, monitoring was performed by pair-spawning or group-spawning for zebrafish and marine medaka, respectively.

For zebrafish, reproduction was evaluated according to Alfonso et al. (2019). To monitor reproduction, one female and one male were placed in a spawning box (AquaSchwarz, Göttingen, Germany) in early evening; five pairs were set up per attempt per treatment, to reach up to 60 attempts per treatment. Spawning boxes were inspected the following morning and eggs were collected and sorted to count the total number of eggs obtained per attempt and the fertilization rate was assessed. Eggs were kept and placed in Petri dishes filled with 30 mL of the isotonic mixture E3 supplemented with methylene blue (250 μL/L) at 26 ± 1 °C and their fate (hatching, survival, behaviour later monitored, see next section). Reproductive success was calculated according to the percentage of total number of spawning events obtained relative to the number of attempts (equal to the number of inspected rearing tanks per treatment).

For marine medaka, fish were kept in their rearing tanks and the day before eggs collection, tanks were siphoned to remove feces and eggs. On the next morning, tanks were siphoned, and eggs were collected, cleaned and placed in Petri dishes in artificial seawater at 26 ± 1 °C pending similar fate analyses as for zebrafish eggs.

2.4.4. Effects on the F1 generation

Larval behaviour was monitored at 5 dpf or 12 dpf, for zebrafish and marine medaka, respectively, using the larval photo-motor response test as described above, with minor modifications. Larvae were individually transferred into one well of a 24-well plate (Krystal 24, opaque wall, and clear bottom microplate, Dutscher, Bernolsheim, France) and acclimated for 10 min in the dark at 26 °C before the test. Larvae with tracking issues were removed resulting 50 to 60 larvae analyzed per treatment. At the end of the larval photo-motor response, larvae standard length was measured under anesthesia.

For the F1 generation, a survival experiment was performed using unfed offspring from exposed fish. The monitoring of F1 survival was performed by collecting 30 fertilized eggs from single spawns from zebrafish pairs or siphoned eggs in replicate tanks in marine medaka, kept in a Petri dish in 30 mL of E3 medium or artificial seawater at 25 at 26 ± 1 °C. There was a daily monitoring of F1 survival and hatching and a daily exchange of 20% of the medium. For both species, survival was monitored until 14 dpf.

2.5. Statistical analyses

Normality of the data was checked using the Shapiro-Wilk test, and the analysis of variance homogeneity was performed by Levene test. Variance analysis was performed followed by Fisher's test compared to control. ANOVA was used for normal data. For data that did not fulfill normal distribution, the Kruskal-Wallis non-parametric test was performed followed, if needed, by the Tukey HSD or multiple comparisons post-hoc tests to identify differences between treatments. Statistical analyses were carried out using the software Statistica (Tibco Software Inc., Palo Alto, CA, USA). For marine medaka, the kinetic of number of eggs produced was compared between treatments by using slope comparison methods (Zar (1984) in Prism software, Graphpad). Differences in survival were assessed using Log-rank (Mantel-Cox) test, and time at which 50% mortality is reached was calculated using four-parameter logistic regression. Akaike's information criterion was used for

comparison with control, both using Prism software (Graphpad). All statistical analyzes were carried out at a 95% level of significance and values are represented as means ± SD.

3. Results

3.1. Characterization of environmental samples

Microplastics were previously characterized, and results are detailed in (Cormier et al., 2021a). Briefly, the predominant shape was fragments (>98%) for both sites, composed of polyethylene (PE) and polypropylene (PP), with a ratio PE:PP of 78:21% and 75:25%, for MG and PB, respectively. Due to the small quantity of MPs for PB sample, only the particle-size of MG was analyzed, and the median particle size was 33.7 μm with a mean particle size of 87.1 μm.

Chemical load of both MPs was previously detailed in Cormier et al. (2021a). Briefly, non-target chemical analyses revealed some halogenated compounds in both samples, brominated flame retardant such as tribromophenol ($C_6H_3Br_3O$) and its metabolite tribromoanisole ($C_7H_5Br_3O$) and homologous series of pesticides from pentachlorobenzene (C_6HCl_5) and dichlorobenzene ($C_6H_4Cl_2$).

Overall, MG MPs were characterized by higher concentrations of halogenated compounds, as well as trichlorobenzene, bumetrizole and octabenzone. The presence of phthalates was also investigated, and they were only identified in MG MPs as diisobutylphthalate, dibutylphthalate, diethylphthalate, ether di-(2-ethylhexyl) phthalate and di-n-octyl phthalate. However, PB MPs were contaminated with higher concentration of hydrocarbons than MG MPs. These compounds were mainly $C_xH_yO_z$. Peaks specific to PB have been identified and corresponded mainly to alkanes, corresponding to $C_{11}H_{18}O_3^+$, $C_{20}H_{34}O^+$ which seemed to correspond to a phenol C_7H_8O with an alkane chain and an isomer of octadecanoic acid $C_{18}H_{36}O_2^+$. All compounds listed have a confidence level of 4, corresponding to an unequivocal molecular formula (Schymanski et al., 2014). Both MPs samples contained different trace metals and quantitatively, MG MPs were characterized by higher concentrations of lead (102 μg/g), cadmium (222 μg/g) and chromium (47 μg/g), while PB contained more copper (85 μg/g) and zinc (292 μg/g).

3.2. Acute exposure in early life stages of zebrafish

Direct exposure of zebrafish embryos to MG and PB MPs at 1 or 10 mg/L did not induce any embryonic mortality (<5%) nor significant developmental anomalies compared to the negative control. Hatching time and hatching rate were also monitored and were unchanged for all tested conditions (Fig. SM1). Average head length of control larvae was 0.70 ± 0.05 mm with total length of 3.82 ± 0.05 mm. Neither lengths (total and head length) of individuals exposed to MPs nor ratio were significantly different from control larvae (Fig. SM2). No significant induction of *in vivo* Ethoxyresorufin-O-deethylase (EROD) activity was observed in larvae exposed to either MPs whatever the concentration when compared to negative control (0.067 ± 0.013 pmol/min/larvae) (Fig. SM3), while larvae exposed to 70 mM of BaP as positive control (PC) for 1 h showed a significant induction of EROD activity (0.12 ± 0.011 pmol/min/larvae). The larval photo-motor response was performed to monitor early behavioural disruption of larvae, and no significant modifications were observed during light-off (LOFF) period. However, during the first light-on (LON1), a decrease in the distance travelled was observed at the highest tested concentration of PB MPs as well as for LON2 with MG MPs at 10 mg/L and PB MPs at 1 mg/L (Fig. SM4).

3.3. Long-term chronic trophic exposure to food supplemented with environmental MPs: toxicity in the F0 generation

3.3.1. Survival and growth

Some sporadic mortalities were observed for both species, but there were no significant differences between conditions including control.

At 2 mpf, no significant differences between zebrafish exposed to MPs and controls were observed, neither on total length nor on weight (Fig. SM5, A–B). Total length of zebrafish at 4 mpf was identical across all treatments (Fig. SM5, C). However, significant lower weights were observed for female exposed to PB MPs compared to controls. Weight was also slightly lower for males exposed to MG MPs in comparison with the control (Fig. SM5, D).

At 6 mpf, significant decreases in length and weight were observed for both species and both treatments. In further detail, in marine medaka, body length was reduced after exposure to MG and PB MPs in both sex (female: ANOVA $F_{(2,133)} = 10.62$, $p < 0.0001$; male: ANOVA $F_{(2,129)} = 13.25$, $p < 0.0001$) (Fig. 1A–C).

Regarding body weight, it was significantly reduced in zebrafish females exposed to both MPs (ANOVA $F_{(2,73)} = 43.34$, $p < 0.001$) while it was unchanged in males (ANOVA $F_{(2,33)} = 0.34$, $p = 0.711$). For marine medaka, body weight decreased for both MPs, in females and males (ANOVA $F_{(2,133)} = 19.56$ and ANOVA $F_{(2,129)} = 14.05$ respectively; $p < 0.001$) (Fig. 1B–D).

3.3.2. Molecular and cellular biomarkers

EROD, acetylcholinesterase (AChE), and thiobarbituric acid reactive substance (TBARS) activities were analyzed in the liver, brain, and muscle, respectively. In zebrafish, there was no significant difference whatever the sex, or MPs treatment for all biomarkers (Table SM1). In zebrafish, the high variability of the comet assay results on blood cells prevented the use of this assay with confidence.

In marine medaka females exposed to PB, EROD activity was significantly reduced (Kruskal-Wallis, $p < 0.05$, Table SM2). In addition, both MG and PB MPs exposures led to a significant increase in AChE activity in the brain of marine medaka females (Kruskal-Wallis, $p < 0.05$, Table SM2). In marine medaka, the comet assay performed on blood cells revealed no evidence of DNA damage whatever sex or treatment (Table SM2).

3.3.3. Adults swimming and anxiety-like behaviour

Swimming activity and anxiety-like behaviour were monitored in adults. None of the treatments modified the activity or anxiety levels of exposed fish when compared to control fish whatever the species (Fig. SM6).

3.3.4. Reproduction

The reproductive success was evaluated for zebrafish. MG MPs exposure did not significantly modify the reproductive success ($p = 0.275$), while PB MPs exposure induced a significant decrease in reproductive success (−70%; $p < 0.0001$, Fig. 2). However, spawning characteristics were not modified whatever MPs origin, this included the total number of eggs per spawn (ANOVA, $F_{(2, 46)} = 1.13$; $p = 0.332$) and the fertilization rate (ANOVA, $F_{(2, 46)} = 1.91$; $p = 0.170$) (Fig. SM7).

In marine medaka, slopes of reproductive output (number of eggs) over time normalised to the number of females in each tank were obtained for each treatment (Fig. 3). No difference to control was observed for MG MPs exposed fish ($p = 0.740$), while PB MPs exposures induced a significant decrease in the slope (- 42%, $p = 0.0018$, Fig. 3). Fertilization rate was, however, unmodified for both MG and PB MPs exposures, in comparison with the control ($p = 0.412$, Fig. SM8).

3.4. Effects on the F1 generation

3.4.1. Larval survival and growth

Larval survival was monitored on unfed zebrafish larvae only.

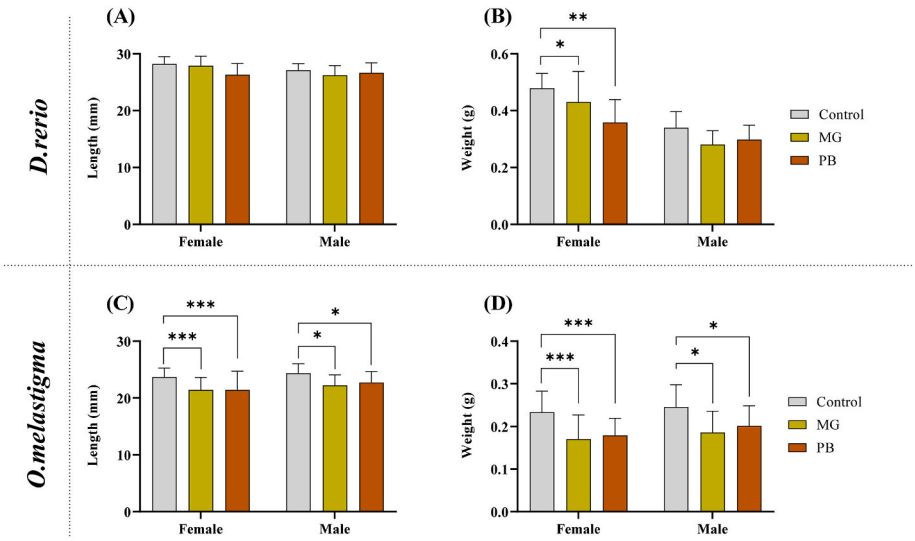

Fig. 1. Body length and weight of zebrafish (A and B) or marine medaka (C and D) at 6 mpf, after MG and PB MPs exposures. (Mean ± SD, Tukey HSD test, * indicates significant differences from respective control of same sex with * $p < 0.05$; **$p < 0.001$; ***$p < 0.0001$; n = 11 to 53 per sex and per treatment).

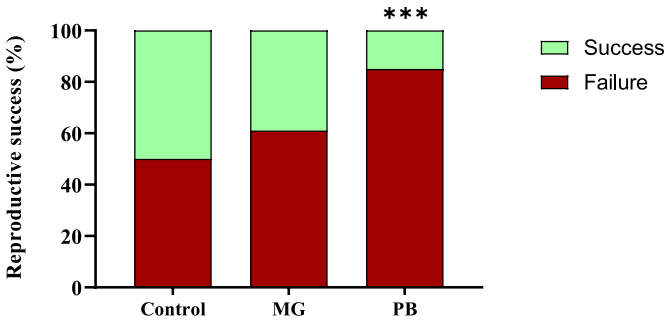

Fig. 2. Relative proportions of successful (white) and failed (black) attempts to obtain eggs from zebrafish pairs exposed to MG or PB MPs. (Fisher's exact test; ***: $p < 0.0001$. Attempts n = 56–66 from three replicates).

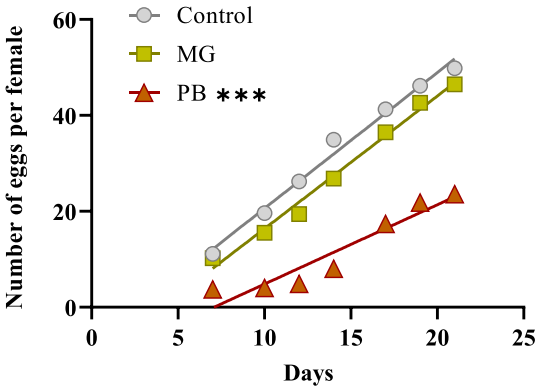

Fig. 3. Reproductive output of marine medaka exposed to MG or BP MPs. The number of eggs per day normalised according to the number of females present in the tank. R square of linear regressions are all above 0.9 (***: $p < 0.0001$; Attempts n = 18–38 from three replicates).

Survival was first overall analyzed, then the age at which 50% of mortality was reached was calculated. In comparison with controls, MG MPs parental exposure induced significant premature mortality in F1 larvae (Chi^2 value = 5.823, p = 0.016). The age of 50% of mortality was significantly decreased by approximately 14 h and reached at 10.78 days [95% CI: 10.52–11.12 days] compared to the control larvae [11.37 days, 95% CI:11.35–11.39 days]. No difference in survival was observed for larvae from parents exposed to PB MPs (Chi^2 value = 0.966, p = 0.326).

F1 larvae were measured at similar developmental stage i.e. at 5 dpf in zebrafish and 12 dpf in marine medaka (Fig. 4). For both species, parental exposure to PB MPs modified the standard length of offspring. In the case of zebrafish, the analysis of individual standard length revealed a significant decrease for PB MPs exposure (ANOVA $F_{(2,31)} = 6.336$, p = 0.0049), while for marine medaka, PB MPs parental exposure induced a significant increase in standard length (ANOVA $F_{(2,153)} = 8.452$, p = 0.0003). In both cases, the differences were weak – 2% and +5%, for zebrafish and marine medaka respectively. No such difference was observed for F1 issued from MG MPs exposed parents.

3.4.2. Larval behaviour

The larval photo-motor response test was performed at 5 and 12 dpf, for zebrafish and marine medaka larvae, respectively, to monitor early behavioural disruptions. Independent of the treatment, the larval photo-motor response test showed a clear increase in the distance travelled during LOFF period compared to LON periods (zebrafish: ANOVA $F_{(2,663)} = 10.96$, $p < 0.001$; marine medaka: ANOVA $F_{(2,627)} = 148.6$, $p < 0.0001$). For offspring of zebrafish exposed to MG MPs, there was no difference in activity pattern when compared to control within each period. Parental exposure to PB MPs resulted in altered swimming behaviour of F1 zebrafish larvae (repeated–measure ANOVA, $F_{(2, 438)} =$

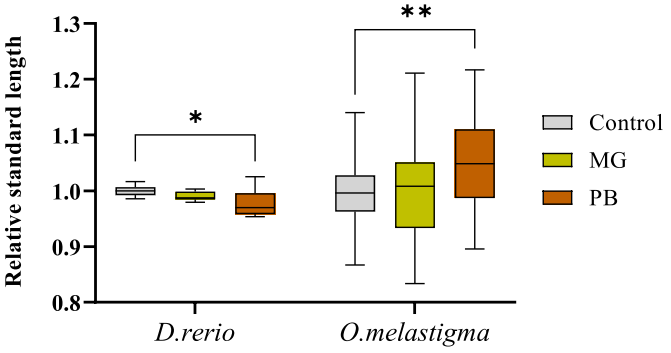

Fig. 4. Individual standard length of F1 larvae of adults zebrafish (left) and marine medaka (right) exposed to MG or PB MPs relative to control. (Mean ± SD; ANOVA; *: $p < 0.05$; **$p < 0.01$; zebrafish n = 9–15; marine medaka n = 44–60).

2.9; $p = 0.007$) by inducing hyperactivity during both LOFF ($p < 0.001$) and LON2 ($p < 0.05$) periods (Fig. 5A). However, for marine medaka, there were no significant differences between the different conditions (repeated–measure ANOVA $F_{(2,627)} = 0.69$, $p = 0.501$, Fig. 5B).

4. Discussion

MPs collected from beaches in Guadeloupe shared common characteristics with MPs collected from other beaches (Mazariegos-Ortíz et al., 2020; Pannetier et al., 2019; Rangel-Buitrago et al., 2021) with a majority of fragments (>98%) mainly composed of PE (75–80%) and PP (20–25%) and chemical analyses revealed the presence of sorbed pollutants. Previous studies reported that PE is the most abundant polymer found in plastic litter, followed by PP and polystyrene (Cheang et al., 2018; Fossi et al., 2017; Hidalgo-Ruz et al., 2012; Karthik et al., 2018), and are the predominant polymers of MPs collected on beaches over the world (Acosta-Coley et al., 2019; Frias et al., 2010; Mazariegos-Ortíz et al., 2020; Pannetier et al., 2019; Pérez-Alvelo et al., 2021). Organic compounds (brominated flame retardant, PCBs, chlorinated pesticides, and PAHs) were found on both sampling sites, but with higher concentrations in MG than in PB, along with the detection of phthalates. In contrast, a higher variety of hydrocarbons was detected in PB MPs and might be due to the proximity to the industrial area and harbor of Pointe-à-Pitre. Previous studies already demonstrated that PAHs, PCBs, and pesticides can sorbed to environmental MPs (Pannetier et al., 2019; Schönlau et al., 2019). For both MPs samples, selected trace metals were quantified in the μg/g range in agreement with previous studies (Acosta-Coley et al., 2019; Dobaradaran et al., 2018; Li et al., 2020; Vedolin et al., 2018). As for organic contaminants, metal composition differed between MG and PB MPs with MG MPs contaminated with higher concentrations of Pb, Cd and Cr while Cu and Zn were detected at higher concentrations in PB. Differences in chemicals concentrations between the two sites might be due to a combination of factors, including origin of MPs, residence time at sea leading to differences in weathering and surface erosion, photo-degradation stage, or biodegradation (Ashton et al., 2010; Holmes et al., 2012; Rochman et al., 2014b).

In the environment, MPs concentration in aquatic environments is still unknown due to technical limitations. However, fish are constantly exposed to MPs and particles are found in wild fish up to few particles/individuals (de Sá et al., 2018; Jovanović, 2017). For the 1st experiment using zebrafish larvae, concentrations were chosen according to previous studies in order to allow comparison with these studies (Beiras et al., 2018; Cormier et al., 2019; Le Bihanic et al., 2020). However, for the chronic exposure (2nd experiment), concentration of 1% ww was selected as the one used in a previous study (Cormier et al., 2021b). To represent an environmentally relevant concentration, we assumed a cubic particle of 330 μm (lower size collected with manta trawl) and calculated that for an adult fish fed at 3% of its body weight, it will correspond between 2 and 4 particles available per day and per individual. The amount of particles given per day is similar to the number of particles found in wild fish (de Sá et al., 2018; Jovanović, 2017).

Due to their high sensitivity to a wide range of chemical substances, fish early life stages are frequently used in ecotoxicology and as screening tests (Embry et al., 2010; Lammer et al., 2009). In the last years, fish early life stages were widely used for the evaluation of MPs toxicity (Cormier et al., 2019; Le Bihanic et al., 2020; Messinetti et al., 2018; Pannetier et al., 2019). As reported in previous studies (Beiras et al., 2018; Cormier et al., 2019), aquatic organisms directly exposed for a short duration to industrial MPs particles underwent weak toxic effects or no effects. This clearly suggests that this type of short-term acute exposure is not sensitive enough to evaluate toxicity of particulate MPs.

In agreement with many other studies, chronic exposure to environmental MPs from the two selected Guadeloupe beaches led to almost no modification of classical ecotoxicological biomarkers of toxicity (Batel et al., 2020; Beiras et al., 2018; Cormier et al., 2019; Le Bihanic et al., 2020). In female marine medaka only, PB MPs induced a slight decrease in EROD activity and increase in AChE activity. Modulation of EROD activity was already shown upon a 14 day-exposure of Japanese medaka larvae to MPs collected on one beach from Hawaii (Pannetier et al., 2020). However in this case, the authors revealed concurrent DNA damages which were not observed here. It is to note in larvae exposed to Hawaii collected MPs, EROD induction was much higher (more than a five-fold induction) than after exposure to PB MPs.

Chronic exposure to environmental MPs from the two selected Guadeloupe beaches led to significant reduction in growth in both zebrafish and marine medaka after 5 months of exposure. In zebrafish, effect was observed only in females while in marine medaka both sexes were similarly impacted. No difference in severity was observed between MG and PB MPs. In addition, in zebrafish, female body weight was already decreased after 3 months of exposure to PB MPs. In the absence of oxidative stress which was also observed in previous experiments with industrial (Cormier et al., 2021b) and environmental MPs (Pannetier et al., 2020), growth alterations may be explained by co-occurring processes either directly linked to chemical toxicity or indirectly due to particle burden leading to bioenergetic disruptions. In a previous experiment, we observed no difference in the severity of growth decrease depending on the presence of spiked chemicals or their type (Cormier et al., 2021b). The same result was obtained here since the load of chemicals from MG and PB MPs were quite different while the effects on growth were similar. This suggests, for this trait, that effect may not be attributable to chemicals but more likely to disruption of energy intake.

Further, the hypothesis of a modification of the energetic budget due to a decrease in food consumption or assimilation due to the ingestion of the MPs particles or an increase in metabolic costs have been reported for numerous organisms upon exposures to MPs (Besseling et al., 2013; Rist et al., 2016; Sussarellu et al., 2016; Watts et al., 2015; Yin et al., 2018). In comparison with males, adult maturing female fish have higher metabolic costs as shown in e.g. northern pike, walleye, yellow

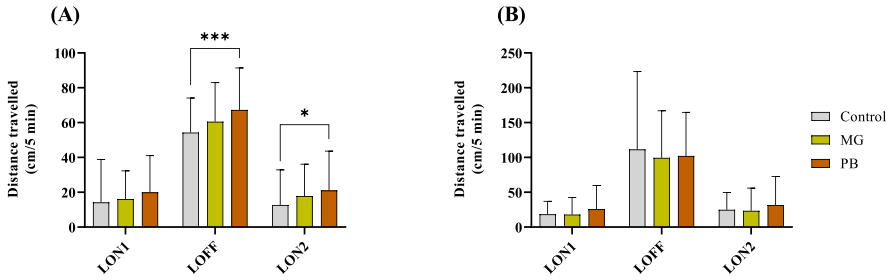

Fig. 5. Larval photomotor response in F1 generation, (A) 5 dpf zebrafish and (B) 14 dpf marine medaka after parental exposure to MG and PB MPs. Distance travelled over 5 min periods including two light-on periods (LON1, LON2) and one light off period (LOFF). (Mean ± SD repeated–measure ANOVA; differences between treatments within the same observation period as *$p < 0.05$; ***$p < 0.001$; $n = 50$ to 60 larvae per treatment).

perch, catfish or zebrafish (Beaudouin et al., 2015; Diana, 1983; Rennie et al., 2008; Xie et al., 1998). For that reason, a disruption in energetic budget would have greater consequences for females than for males, as we observed for both species in the present study.

Reproductive output was significantly reduced upon chronic exposure to PB MPs for both species while it was unchanged after exposure to MG MPs. In a previous experiment, we demonstrated that exposure to some industrial MPs resulted in similar disruptions (Cormier et al., 2021b). Since body weight of females was reduced upon exposure to either industrial PE or polyvinyl chloride (PVC) MPs and PB MPs, it is tempting to propose a link with reproduction defect, in relation with energy budget as proposed in *D. rerio* or *O. melastigma* (Cormier et al., 2021b) or the oyster *Crassostrea gigas* (Sussarellu et al., 2016). However, the absence of reproductive defects following exposure to MG MPs (this study) or to some PE or PVC MPs spiked or not (Cormier et al., 2021b) suggests a contribution of sorbed or intrinsic chemicals. Many chemicals can indeed interfere with reproduction success, some of them are known to be endocrine disruptors (Cao et al., 2019; Celino-Brady et al., 2021; Horri et al., 2018; Mills & Chichester, 2005; Muirhead et al., 2006; Vignet et al., 2016; Wang et al., 2019). This is, for example, the case of copper which was described as affecting gonadal development and disturbing steroid hormone levels in zebrafish (Cao et al., 2019).

Parental transfer of chemicals, e.g., heavy metals, pesticides, PAHs, PCBs, and additives to offspring has been documented in numerous species, including birds (Ackerman et al., 2016; Bargar et al., 2001), amphibians (Metts et al., 2013), and reptiles (Rauschenberger et al., 2004). In fish, different studies have demonstrated the maternal transfer of pollutants to eggs (Daouk et al., 2011; Miller, 1993; Niimi, 1983; Nyholm et al., 2008; Yu et al., 2011; Alfonso et al., 2019). In the present study, MG MPs exposure led to a significant premature mortality of offspring of exposed zebrafish. Exposures to different pollutants induced elevated mortality among unfed larvae progeny (Foekema et al., 2012; Foekema et al., 2014; Wang et al., 2019; Yu et al., 2011; Horri et al., 2018). Besides reduced larval survival, swimming activity was increased for the offspring of zebrafish exposed to PB MPs, whereas no changes in adult swimming and anxiety-like behaviour were observed for the F0 generation alike fish exposed to PE and PVC MPs (Cormier et al., 2021b). The MG MPs exposure induced embryotoxicity that can be linked to the presence of phthalates or flame retardants, while PB MPs parental exposure led to altered swimming activity of zebrafish larvae (hyperactivity). The presence of high hydrocarbon and PAHs contents in PB MPs may explain the hyperactivity of offspring larvae as PAHs are known to alter fish behaviour in response to light stimulation (Le Bihanic et al., 2014 & 2015; Vignet et al., 2014b) but also the presence of copper (Barjhoux et al., 2012; Weeks Santos et al., 2019).

5. Conclusion

This study highlighted adverse effects of environmental MPs from two beaches (MG and PB in Guadeloupe islands) on different life stages of two teleost fish. Short-term exposures of early life stages of zebrafish led only to weak toxic effects. However, the long-term trophic exposure revealed significant deleterious effects on adult zebrafish and marine medaka (growth and reproduction), as well as on their F1 offspring for both fish species (survival, growth, behaviour). The most prominent effects for both fish species were detected with PB MPs exposure and might be explained by chemicals. The polymer composition was quite similar between the two sampled sites; however, additives and adsorbed pollutants were different and might explain the greater toxicity of PB. The present study demonstrated that MPs are affecting different functions such as xenobiotic metabolization, growth and reproduction, and so, to evaluate the toxicity of MPs, scientific community should not only focus on some specific endpoints. Adverse effects were not only observed for exposed adults, but also on F1 offspring larvae that were unexposed. Deleterious effects observed on F1 offspring larvae for both fish species could be induced by either the parental transfer of chemicals desorbed from MPs (additives and/or pollutants) and/or as an indirect effect of the parental physiological status leading to poorer egg quality, showing the importance to investigate MPs toxicity using a multigenerational design study. This study raises the question of the environmental risk of MPs weathered at sea both on MPs directly exposed aquatic organisms but also on the progeny.

Authors statement

Bettie Cormier: Investigation; Formal analysis; Writing – original draft; Writing – review & editing, **Jérôme Cachot**: Funding acquisition; Conceptualization; Writing – review & editing, **Mélanie Blanc**: Investigation, **Mathieu Cabar**: Investigation, **Christelle Clérandeau**: Investigation; Formal analysis, **Florian Dubocq**: Investigation, **Florane Le Bihanic**: Investigation; Formal analysis, **Bénédicte Morin**: Investigation; Formal analysis; Writing – review & editing, **Sarah Zapata**: Investigation, **Marie-Laure Bégout**: Funding acquisition; Conceptualization; Investigation; Writing – review & editing, **Xavier Cousin**: Funding acquisition; Conceptualization; Investigation; Formal analysis; Writing – review & editing

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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.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2022.119721.

References

Ackerman, J.T., Eagles-Smith, C.A., Herzog, M.P., Hartman, C.A., 2016. Maternal transfer of contaminants in birds: mercury and selenium concentrations in parents and their eggs. Environ. Pollut. 210, 145–154.

Acosta-Coley, I., Mendez-Cuadro, D., Rodriguez-Cavallo, E., de la Rosa, J., Olivero-Verbel, J., 2019. Trace elements in microplastics in Cartagena: a hotspot for plastic pollution at the Caribbean. Mar. Pollut. Bull. 139, 402–411.

Acosta-Coley, I., Olivero-Verbel, J., 2015. Microplastic resin pellets on an urban tropical beach in Colombia. Environ. Monit. Assess. 187, 435.

Alfonso, S., Blanc, M., Joassard, L., Keiter, S.H., Munschy, C., Loizeau, V., Bégout, M.-L., Cousin, X., 2019. Examining multi- and transgenerational behavioral and molecular alterations resulting from parental exposure to an environmental PCB and PBDE mixture. Aquat. Toxicol. 208, 29–38.

Alimba, C.G., Faggio, C., 2019. Microplastics in the marine environment: current trends in environmental pollution and mechanisms of toxicological profile. Environ. Toxicol. Pharmacol. 68, 61–74.
Anbumani, S., Kakkar, P., 2018. Ecotoxicological effects of microplastics on biota: a review. Environ. Sci. Pollut. Control Ser. 25, 14373–14396.
Andrade, H., Glüge, J., Herzke, D., Ashta, N.M., Nayagar, S.M., Scheringer, M., 2021. Oceanic long-range transport of organic additives present in plastic products: an overview. Environ. Sci. Eur. 33, 85.
Andrady, A.L., 2011. Microplastics in the marine environment. Mar. Pollut. Bull. 62, 1596–1605.
Ashton, K., Holmes, L., Turner, A., 2010. Association of metals with plastic production pellets in the marine environment. Mar. Pollut. Bull. 60, 2050–2055.
Bargar, T.A., Scott, G.I., Cobb, G.P., 2001. Maternal transfer of contaminants: case study of the excretion of three polychlorinated biphenyl congeners and technical-grade endosulfan into eggs by white leghorn chickens (*Gallus domesticus*). Environ. Toxicol. Chem. 20, 61–67.
Barjhoux, I., Baudrimont, M., Morin, B., Landi, L., Gonzalez, P., Cachot, J., 2012. Effects of copper and cadmium spiked-sediments on embryonic development of Japanese medaka (*Oryzias latipes*). Ecotoxicol. Environ. Saf. 79, 272–282.
Batel, A., Baumann, L., Carteny, C.C., Cormier, B., Keiter, S.H., Braunbeck, T., 2020. Histological, enzymatic and chemical analyses of the potential effects of differently sized microplastic particles upon long-term ingestion in zebrafish (*Danio rerio*). Mar. Pollut. Bull. 153, 111022.
Beaudouin, R., Goussen, B., Piccini, B., Augustine, S., Devillers, J., Brion, F., Péry, A.R.R., 2015. An individual-based model of zebrafish population dynamics accounting for energy dynamics. PLoS One 10, 0125841.
Beiras, R., Bellas, J., Cachot, J., Cormier, B., Cousin, X., Engwall, M., Gambardella, C., Garaventa, F., Keiter, S., Le Bihanic, F., López-Ibáñez, S., Piazza, V., Rial, D., Tato, T., Vidal-Liñán, L., 2018. Ingestion and contact with polyethylene microplastics does not cause acute toxicity on marine zooplankton. J. Hazard Mater. 360, 452–460.
Besseling, E., Wegner, A., Foekema, E.M., Van Den Heuvel-Greve, M.J., Koelmans, A.A., 2013. Effects of microplastic on fitness and PCB bioaccumulation by the lugworm *Arenicola marina* (L.). Environ. Sci. Technol. 47, 593–600.
Bosker, T., Behrens, P., Vijver, M.G., 2017. Determining global distribution of microplastics by combining citizen science and in-depth case studies. Integrated Environ. Assess. Manag. 13, 536–541.
Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72, 248–254.
Browne, M.A., Niven, S.J., Galloway, T.S., Rowland, S.J., Thompson, R.C., 2013. Microplastic moves pollutants and additives to worms, reducing functions linked to health and biodiversity. Curr. Biol. 23, 2388–2392.
Cao, J., Wang, G., Wang, T., Chen, J., Wenjing, G., Wu, P., He, X., Xie, L., 2019. Copper caused reproductive endocrine disruption in zebrafish (*Danio rerio*). Aquat. Toxicol. 211, 124–136.
Castro-Castellon, A.T., Horton, A.A., Hughes, J.M.R., Rampley, C., Jeffers, E.S., Bussi, G., Whitehead, P., 2022. Ecotoxicity of microplastics to freshwater biota: considering exposure and hazard across trophic levels. Sci. Total Environ. 216, 151638.
Catrouillet, C., Davranche, M., Khatib, I., Fauny, C., Wahl, A., Gigault, J., 2021. Metals in microplastics: determining which are additive, adsorbed, and bioavailable. Environ. Sci. J. Integr. Environ. Res.: Process. Impacts 23, 553–558.
Celino-Brady, F.T., Lerner, D.T., Seale, A.P., 2021. Experimental approaches for characterizing the endocrine-disrupting effects of environmental chemicals in fish. Front. Endocrinol. 11, 619361.
Cheang, C.C., Ma, Y., Fok, L., 2018. Occurrence and composition of microplastics in the seabed sediments of the coral communities in proximity of a metropolitan area. Int. J. Environ. Res. Publ. Health 15, 2270.
Cole, M., Lindeque, P., Fileman, E., Halsband, C., Goodhead, R., Moger, J., Galloway, T. S., 2013. Microplastic ingestion by zooplankton. Environ. Sci. Technol. 47, 6646–6655.
Cormier, B., Batel, A., Cachot, J., Bégout, M.-L., Braunbeck, T., Cousin, X., Keiter, S.H., 2019. Multi-laboratory hazard assessment of contaminated microplastic particles by means of enhanced fish embryo test with the zebrafish (*Danio rerio*). Front. Mar. Sci. 7, 135.
Cormier, B., Gambardella, C., Tato, T., Perdriat, Q., Costa, E., Veclin, C., Le Bihanic, F., Grassl, B., Dubocq, F., Kärrman, A., Van Arkel, K., Lemoine, S., Lagarde, F., Morin, B., Garaventa, F., Faimali, M., Cousin, X., Bégout, M.L., Beiras, R., Cachot, J., 2021a. Chemicals sorbed to environmental microplastics are toxic to early life stages of aquatic organisms. Ecotoxicol. Environ. Saf. 208, 111665.
Cormier, B., Le Bihanic, F., Cabar, M., Crebassa, J.-C., Blanc, M., Larsson, M., Dubocq, F., Yeung, L., Clérandeau, C., Keiter, S.H., Cachot, J., Bégout, M.-L., Cousin, X., 2021b. Chronic feeding exposure to virgin and spiked microplastics disrupts essential biological functions in teleost fish. J. Hazard Mater. 415, 125626.
Costa, M.F., Ivar do Sul, J.A., Silva-Cavalcanti, J.S., Araújo, M.C.B., Spengler, Â., Tourinho, P.S., 2010. On the importance of size of plastic fragments and pellets on the strandline: a snapshot of a Brazilian beach. Environ. Monit. Assess. 168, 299–304.
Cousin, X., Batel, A., Bringer, A., Hess, S., Bégout, M.L., Braunbeck, T., 2020. Microplastics and sorbed contaminants – trophic exposure in fish sensitive early life stages. Mar. Environ. Res. 161, 105126.
Daouk, T., Larcher, T., Roupsard, F., Lyphout, L., Rigaud, C., Ledevin, M., Loizeau, V., Cousin, X., 2011. Long-term food-exposure of zebrafish to PCB mixtures mimicking some environmental situations induces ovary pathology and impairs reproduction ability. Aquat. Toxicol. 105, 270–278.
de Sá, L.C., Oliveira, M., Ribeiro, F., Rocha, T.L., Futter, M.N., 2018. Studies of the effects of microplastics on aquatic organisms: what do we know and where should we focus our efforts in the future? Sci. Total Environ. 645, 1029–1039.
Desforges, J.P.W., Galbraith, M., Ross, P.S., 2015. Ingestion of microplastics by zooplankton in the northeast Pacific Ocean. Arch. Environ. Contam. Toxicol. 69, 320–330.
Diana, J.S., 1983. An energy budget for northern pike (*Esox lucius*). Can. J. Zool. 61, 1968–1975.
Dobaradaran, S., Schmidt, T.C., Nabipour, I., Khajeahmadi, N., Tajbakhsh, S., Saeedi, R., Javad Mohammadi, M., Keshtkar, M., Khorsand, M., Faraji Ghasemi, F., 2018. Characterization of plastic debris and association of metals with microplastics in coastline sediment along the Persian Gulf. Waste Manag. 79, 649–658.
Egan, R.J., Bergner, C.L., Hart, P.C., Cachat, J.M., Canavello, P.R., Elegante, M.F., Elkhayat, S.I., Bartels, B.K., Tien, A.K., Tien, D.H., et al., 2009. Understanding behavioral and physiological phenotypes of stress and anxiety in zebrafish. Behav. Brain Res. 205, 38–44.
El Hadri, H., Gigault, J., Mounicou, S., Grassl, B., Reynaud, S., 2020. Trace element distribution in marine microplastics using laser ablation-ICP-MS. Mar. Pollut. Bull. 160, 111716.
Embry, M.R., Belanger, S.E., Braunbeck, T.A., Galay-Burgos, M., Halder, M., Hinton, D. E., Léonard, M.A., Lillicrap, A., Norberg-King, T., Whale, G., 2010. The fish embryo toxicity test as an animal alternative method in hazard and risk assessment and scientific research. Aquat. Toxicol. 97, 79–87.
Eriksen, M., Lebreton, L.C.M., Carson, H.S., Thiel, M., Moore, C.J., Borerro, J.C., Galgani, F., Ryan, P.G., Reisser, J., 2014. Plastic pollution in the world's Oceans: more than 5 trillion Plastic pieces weighing over 250,000 tons afloat at sea. PLoS One 9, 111913.
Farrell, P., Nelson, K., 2013. Trophic level transfer of microplastic: *Mytilus edulis* (L.) to *Carcinus maenas* (L.). Environ. Pollut. 177, 1–3.
Foekema, E.M., Fischer, A., Parron, M.L., Kwadijk, C., de Vries, P., Murk, A.J., 2012. Toxic concentrations in fish early life stages peak at a critical moment. Environ. Toxicol. Chem. 31, 1381–1390.
Foekema, E.M., Lopez Parron, M., Mergia, M.T., Carolus, E.R.M., vd Berg, J.H.J., Kwadijk, C., Dao, Q., Murk, A.J., 2014. Internal effect concentrations of organic substances for early life development of egg-exposed fish. Ecotoxicol. Environ. Saf. 101, 14–22.
Fossi, M.C., Romeo, T., Baini, M., Panti, C., Marsili, L., Campan, T., Canese, S., Galgani, F., Druon, J.N., Airoldi, S., Taddei, S., Fattorini, M., Brandini, C., Lapucci, C., 2017. Plastic debris occurrence, convergence areas and fin whales feeding ground in the Mediterranean marine protected area Pelagos Sanctuary: a modeling approach. Front. Mar. Sci. 4, 167.
Frias, J.P.G.L., Sobral, P., Ferreira, A.M., 2010. Organic pollutants in microplastics from two beaches of the Portuguese coast. Mar. Pollut. Bull. 60, 1988–1992.
Giani, D., Baini, M., Galli, M., Casini, S., Fossi, M.C., 2019. Microplastics occurrence in edible fish species (*Mullus barbatus* and *Merluccius merluccius*) collected in three different geographical sub-areas of the Mediterranean Sea. Mar. Pollut. Bull. 140, 129–137.
Gove, J.M., Whitney, J.L., McManus, M.A., Lecky, J., Carvalho, F.C., Lynch, J.M., Li, J., Neubauer, P., Smith, K.A., Phipps, J.E., Kobayashi, D.R., Balagso, K.B., Contreras, E. A., Manuel, M.E., Merrifield, M.A., Polovina, J.J., Asner, G.P., Maynard, J.A., Williams, G.J., 2019. Prey-size plastics are invading larval fish nurseries. Proc. Natl. Acad. Sci. USA 116, 24143–24149.
Hahladakis, J.N., Velis, C.A., Weber, R., Iacovidou, E., Purnell, P., 2018. An overview of chemical additives present in plastics: migration, release, fate and environmental impact during their use, disposal and recycling. J. Hazard Mater. 344, 179–199.
Hermsen, E., Pompe, R., Besseling, E., Koelmans, A.A., 2017. Detection of low numbers of microplastics in North Sea fish using strict quality assurance criteria. Mar. Pollut. Bull. 122, 253–258.
Hidalgo-Ruz, V., Gutow, L., Thompson, R.C., Thiel, M., 2012. Microplastics in the marine environment: a review of the methods used for identification and quantification. Environ. Sci. Technol. 46, 3060–3075.
Holmes, L.A., Turner, A., Thompson, R.C., 2012. Adsorption of trace metals to plastic resin pellets in the marine environment. Environ. Pollut. 160, 42–48.
Horri, K., Alfonso, S., Cousin, X., Munschy, C., Loizeau, V., Aroua, S., Bégout, M.-L., Ernande, B., 2018. Fish life-history traits are affected after chronic dietary exposure to an environmentally realistic marine mixture of PCBs and PBDEs. Sci. Total Environ. 610–611, 531–545.
Jambeck, J.R., Geyer, R., Wilcox, C., Siegler, T.R., Perryman, M., Andrady, A., Narayan, R., Law, K.L., 2015. Plastic waste inputs from land into the ocean. Science 347, 768.
Jovanović, B., 2017. Ingestion of microplastics by fish and its potential consequences from a physical perspective. Integrated Environ. Assess. Manag. 13, 510–515.
Karapanagioti, H.K., Klontza, I., 2008. Testing phenanthrene distribution properties of virgin plastic pellets and plastic eroded pellets found on Lesvos island beaches (Greece). Mar. Environ. Res. 65, 283–290.
Karthik, R., Robin, R.S., Purvaja, R., Ganguly, D., Anandavelu, I., Raghuraman, R., Hariharan, G., Ramakrishna, A., Ramesh, R., 2018. Microplastics along the beaches of southeast coast of India. Sci. Total Environ. 645, 1388–1399.
Koelmans, A.A., Bakir, A., Burton, G.A., Janssen, C.R., 2016. Microplastic as a vector for chemicals in the aquatic environment: critical review and model-supported reinterpretation of empirical studies. Environ. Sci. Technol. 50, 3315–3326.
Lammer, E., Carr, G.J., Wendler, K., Rawlings, J.M., Belanger, S.E., Braunbeck, T., 2009. Is the fish embryo toxicity test (FET) with the zebrafish (*Danio rerio*) a potential alternative for the fish acute toxicity test? Comp. Biochem. Physiol. C Toxicol. Pharmacol. 149, 196–209.

Law, K.L., Morét-Ferguson, S.E., Goodwin, D.S., Zettler, E.R., Deforce, E., Kukulka, T., Proskurowski, G., 2014. Distribution of surface plastic debris in the eastern Pacific Ocean from an 11-year data set. Environ. Sci. Technol. 48, 4732–4738.
Law, K.L., Thompson, R.C., 2014. Microplastics in the seas. Science 345, 144.
Le Bihanic, F., Clérandeau, C., Cormier, B., Crebassa, J.C., Keiter, S.H., Beiras, R., Morin, B., Bégout, M.L., Cousin, X., Cachot, J., 2020. Organic contaminants sorbed to microplastics affect marine medaka fish early life stages development. Mar. Pollut. Bull. 154, 111059.
Le Bihanic, F., Clérandeau, C., Le Menach, K., Morin, B., Budzinski, H., Cousin, X., Cachot, J., 2014. Developmental toxicity of PAH mixtures in fish early life stages. Part II: adverse effects in Japanese medaka. Environ. Sci. Pollut. Control Ser. 21, 13732–13743.
Lebreton, L., Slat, B., Ferrari, F., Sainte-Rose, B., Aitken, J., Marthouse, R., Hajbane, S., Cunsolo, S., Schwarz, A., Levivier, A., et al., 2018. Evidence that the great pacific garbage patch is rapidly accumulating plastic. Sci. Rep. 8, 4666.
Lee, Y., Yoon, D.-S., Lee, Y.H., Kwak, J.I., An, Y.-J., Lee, J.-S., Park, J.C., 2021. Combined exposure to microplastics and zinc produces sex-specific responses in the water flea *Daphnia magna*. J. Hazard Mater. 420, 126652.
Levin, E.D., Bencan, Z., Cerutti, D.T., 2007. Anxiolytic effects of nicotine in zebrafish. Physiol. Behav. 90, 54–58.
Li, J., Liu, H., Paul Chen, J., 2018. Microplastics in freshwater systems: a review on occurrence, environmental effects, and methods for microplastics detection. Water Res. 137, 362–374.
Li, W.H., Jian, M.F., Liu, S.L., Jiang, Y.M., Deng, Y.B., Zhu, L., 2020. Occurrence relationship between microplastics and heavy metals pollutants in the estuarine sediments of Poyang lake and the Yangtze river. Environ. Sci. J. Integr. Environ. Res. 41, 242–252.
Lippiatt, S., Opfer, S., Arthur, C., 2013. Marine Debris Monitoring and Assessment. NOAA Technical Memorandum.
Liu, G., Dave, P.H., Kwong, R.W.M., Wu, M., Zhong, H., 2021. Influence of microplastics on the mobility, bioavailability, and toxicity of heavy metals: a review. Bull. Environ. Contam. Toxicol. 107, 710–721.
Liu, P., Zhan, X., Wu, X., Li, J., Wang, H., Gao, S., 2020. Effect of weathering on environmental behavior of microplastics: properties, sorption and potential risks. Chemosphere 242, 125193.
Ma, H., Pu, S., Liu, S., Bai, Y., Mandal, S., Xing, B., 2020. Microplastics in aquatic environments: toxicity to trigger ecological consequences. Environ. Pollut. 261, 114089.
Mazariegos-Ortíz, C., de los Ángeles Rosales, M., Carrillo-Ovalle, L., Cardoso, R.P., Muniz, M.C., dos Anjos, R.M., 2020. First evidence of microplastic pollution in the El Quetzalito sand beach of the Guatemalan Caribbean. Mar. Pollut. Bull. 156, 111220.
Mazurais, D., Ernande, B., Quazuguel, P., Severe, A., Huelvan, C., Madec, L., Mouchel, O., Soudant, P., Robbens, J., Huvet, A., et al., 2015. Evaluation of the impact of polyethylene microbeads ingestion in European sea bass (*Dicentrarchus labrax*) larvae. Mar. Environ. Res. 112, 78–85.
Menéndez-Pedriza, A., Jaumot, J., 2020. Interaction of environmental pollutants with microplastics: a critical review of sorption factors, bioaccumulation and ecotoxicological effects. Toxics 8, 40.
Messinetti, S., Mercurio, S., Parolini, M., Sugni, M., Pennati, R., 2018. Effects of polystyrene microplastics on early stages of two marine invertebrates with different feeding strategies. Environ. Pollut. 237, 1080–1087.
Metts, B.S., Buhlmann, K.A., Tuberville, T.D., Scott, D.E., Hopkins, W.A., 2013. Maternal transfer of contaminants and reduced reproductive success of southern toads (*Bufo [Anaxyrus] terrestris*) exposed to coal combustion waste. Environ. Sci. Technol. 47, 2846–2853.
Miller, M.A., 1993. Maternal transfer of organochlorine compounds in salmonines to their eggs. Can. J. Fish. Aquat. Sci. 50, 1405–1413.
Mills, L.J., Chichester, C., 2005. Review of evidence: are endocrine-disrupting chemicals in the aquatic environment impacting fish populations? Sci. Total Environ. 343, 1–34.
Muirhead, E.K., Skillman, A.D., Hook, S.E., Schultz, I.R., 2006. Oral exposure of PBDE-47 in fish: toxicokinetics and reproductive effects in Japanese medaka (*Oryzias latipes*) and fathead minnows (*Pimephales promelas*). Environ. Sci. Technol. 40, 523–528.
Niimi, A.J., 1983. Biological and toxicologtcal effects of environmental contaminants in fish and their eggs. Can. J. Fish. Aquat. Sci. 40, 306–312.
Nyholm, J.R., Norman, A., Norrgren, L., Haglund, P., Andersson, P.L., 2008. Maternal transfer of brominated flame retardants in zebrafish (*Danio rerio*). Chemosphere 73, 203–208.
OECD, 2013. OECD guidelines for the testing of chemicals. In: Effects on Biotic Systems Test No 236: Fish Embryo Acute Toxicity Test. Organization for Economic Cooperation and Development.
Ogata, Y., Takada, H., Mizukawa, K., Hirai, H., Iwasa, S., Endo, S., Mato, Y., Saha, M., Okuda, K., Nakashima, A., et al., 2009. International pellet watch: global monitoring of persistent organic pollutants (POPs) in coastal waters. 1. Initial phase data on PCBs, DDTs, and HCHs. Mar. Pollut. Bull. 58, 1437–1446.
Oliveira, M., Ribeiro, A., Hylland, K., Guilhermino, L., 2013. Single and combined effects of microplastics and pyrene on juveniles (0+ group) of the common goby *Pomatoschistus microps* (Teleostei, Gobiidae). Ecol. Indicat. 34, 641–647.
Pannetier, P., Morin, B., Clérandeau, C., Laurent, J., Chapelle, C., Cachot, J., 2019. Toxicity assessment of pollutants sorbed on environmental microplastics collected on beaches: Part II-adverse effects on Japanese medaka early life stages. Environ. Pollut. 248, 1098–1107.
Pannetier, P., Morin, B., Le Bihanic, F., Dubreil, L., Clérandeau, C., Chouvellon, F., Van Arkel, K., Danion, M., Cachot, J., 2020. Environmental samples of microplastics induce significant toxic effects in fish larvae. Environ. Int. 134, 105047.
Panti, C., Baini, M., Lusher, A., Hernandez-Milan, G., Bravo Rebolledo, E.L., Unger, B., Syberg, K., Simmonds, M.P., Fossi, M.C., 2019. Marine litter: one of the major threats for marine mammals. Outcomes from the European Cetacean Society workshop. Environ. Pollut. 247, 72–79.
Pérez-Alvelo, K.M., Llegus, E.M., Forestier-Babilonia, J.M., Elías-Arroyo, C.V., Pagán-Malavé, K.N., Bird-Rivera, G.J., Rodríguez-Sierra, C.J., 2021. Microplastic pollution on sandy beaches of Puerto Rico. Mar. Pollut. Bull. 164, 112010.
Rangel-Buitrago, N., Arroyo-Olarte, H., Trilleras, J., Arana, V.A., Mantilla-Barbosa, E., Gracia, A., Mendoza, A.V., Neal, W.J., Williams, A.T., Micallef, A., 2021. Microplastics pollution on Colombian central caribbean beaches. Mar. Pollut. Bull. 170, 112685.
Rauschenberger, R.H., Sepúlveda, M.S., Wiebe, J.J., Szabo, N.J., Gross, T.S., 2004. Predicting maternal body burdens of organochlorine pesticides from eggs and evidence of maternal transfer in Alligator mississippiensis. Environ. Toxicol. Chem. 12, 2906–2915.
Rennie, M.D., Purchase, C.F., Lester, N., Collins, N.C., Shuter, B.J., Abrams, P.A., 2008. Lazy males? Bioenergetic differences in energy acquisition and metabolism help to explain sexual size dimorphism in percids. J. Anim. Ecol. 77, 916–926.
Rist, S.E., Assidqi, K., Zamani, N.P., Appel, D., Perschke, M., Huhn, M., Lenz, M., 2016. Suspended micro-sized PVC particles impair the performance and decrease survival in the Asian green mussel Perna viridis. Mar. Pollut. Bull. 111, 213–220.
Rochman, C.M., Hoh, E., Kurobe, T., Teh, S.J., 2013. Ingested plastic transfers hazardous chemicals to fish and induces hepatic stress. Sci. Rep. 3, 3263.
Rochman, C.M., Kurobe, T., Flores, I., Teh, S.J., 2014a. Early warning signs of endocrine disruption in adult fish from the ingestion of polyethylene with and without sorbed chemical pollutants from the marine environment. Sci. Total Environ. 493, 656–661.
Rochman, C.M., Hentschel, B.T., The, S.J., 2014b. Long-term sorption of metals is similar among plastic types: implications for plastic debris in aquatic environments. PLoS One 9, 85433.
Rummel, C.D., Löder, M.G.J., Fricke, N.F., Lang, T., Griebeler, E.M., Janke, M., Gerdts, G., 2016. Plastic ingestion by pelagic and demersal fish from the North sea and baltic sea. Mar. Pollut. Bull. 102, 134–141.
Schönlau, C., Larsson, M., Lam, M.M., Engwall, M., Giesy, J.P., Rochman, C., Kärrman, A., 2019. Aryl hydrocarbon receptor-mediated potencies in field-deployed plastics vary by type of polymer. Environ. Sci. Pollut. Control Ser. 26, 9079–9088.
Schymanski, E.L., Jeon, J., Gulde, R., Fenner, K., Ruff, M., Singer, H.P., Hollender, J., 2014. Identifying small molecules via high resolution mass spectrometry: communicating confidence. Environ. Sci. Technol. 48, 2097–2098.
Setälä, O., Fleming-Lehtinen, V., Lehtiniemi, M., 2014. Ingestion and transfer of microplastics in the planktonic food web. Environ. Pollut. 185, 77–83.
Steer, M., Cole, M., Thompson, R.C., Lindeque, P.K., 2017. Microplastic ingestion in fish larvae in the western English Channel. Environ. Pollut. 226, 250–259.
Sussarellu, R., Suquet, M., Thomas, Y., Lambert, C., Fabioux, C., Pernet, M.E.J., Le Goïc, N., Quillien, V., Mingant, C., Epelboin, Y., et al., 2016. Oyster reproduction is affected by exposure to polystyrene microplastics. Proc. Natl. Acad. Sci. USA 113, 2430.
Teuten, E.L., Saquing, J.M., Knappe, D.R.U., Barlaz, M.A., Jonsson, S., Björn, A., Rowland, S.J., Thompson, R.C., Galloway, T.S., Yamashita, R., et al., 2009. Transport and release of chemicals from plastics to the environment and to wildlife. Phil. Trans. Biol. Sci. 364, 2027–2045.
Thompson, R.C., Olsen, Y., Mitchell, R.P., Davis, A., Rowland, S.J., John, A.W.G., McGonigle, D., Russell, A.E., 2004. Lost at sea: where is all the plastic? Science 304, 838.
Vedolin, M.C., Teophilo, C.Y.S., Turra, A., Figueira, R.C.L., 2018. Spatial variability in the concentrations of metals in beached microplastics. Mar. Pollut. Bull. 129, 487–493.
Vignet, C., Larcher, T., Davail, B., Joassard, L., Le Menach, K., Guionnet, T., Lyphout, L., Ledevin, M., Goubeau, M., Budzinski, H., et al., 2016. Fish reproduction is disrupted upon lifelong exposure to environmental PAHs fractions revealing different modes of action. Toxics 4, 26.
Vignet, C., Le Menach, K., Mazurais, D., Lucas, J., Perrichon, P., Le Bihanic, F., Devier, M. H., Lyphout, L., Frère, L., Bégout, M.L., Zambonino-Infante, J.L., Budzinski, H., Cousin, X., 2014a. Chronic dietary exposure to pyrolytic and petrogenic mixtures of PAHs causes physiological disruption in zebrafish - part I: survival and growth. Environ. Sci. Pollut. Control Ser. 21, 13804–13817.
Vignet, C., Le Menach, K., Mazurais, D., Lucas, J., Perrichon, P., Le Bihanic, F., Devier, M.-H., Lyphout, L., Frère, L., Bégout, M.-L., et al., 2014b. Chronic dietary exposure to pyrolytic and petrogenic mixtures of PAHs causes physiological disruption in zebrafish - part I: survival and growth. Environ. Sci. Pollut. Control Ser. 21, 13804–13817.
Wang, J., Li, Y., Lu, L., Zheng, M., Zhang, X., Tian, H., Wang, W., Ru, S., 2019. Polystyrene microplastics cause tissue damages, sex-specific reproductive disruption and transgenerational effects in marine medaka (*Oryzias melastigma*). Environ. Pollut. 254, 113024.
Wang, T., Wang, L., Chen, Q., Kalogerakis, N., Ji, R., Ma, Y., 2020. Interactions between microplastics and organic pollutants: effects on toxicity, bioaccumulation, degradation, and transport. Sci. Total Environ. 748, 142427.
Watts, A.J.R., Urbina, M.A., Corr, S., Lewis, C., Galloway, T.S., 2015. Ingestion of plastic microfibers by the crab *Carcinus maenas* and its effect on food consumption and energy balance. Environ. Sci. Technol. 49, 14597–14604.
Weeks Santos, S., Gonzalez, P., Cormier, B., Mazzella, N., Bonnaud, B., Morin, S., Clérandeau, C., Morin, B., Cachot, J., 2019. A glyphosate-based herbicide induces sub-lethal effects in early life stages and liver cell line of rainbow trout, *Oncorhynchus mykiss*. Aquat. Toxicol. 216, 105291.

Weiss, L., Ludwig, W., Heussner, S., Canals, M., Ghiglione, J.-F., Estournel, C., Constant, M., Kerhervé, P., 2021. The missing ocean plastic sink: gone with the rivers. Science 373, 107–111.

Xie, X., Long, T., Zhang, Y., Cao, Z., 1998. Reproductive investment in the *Silurus meridionalis*. J. Fish. Biol. 53, 259–271.

Yan, Y., Zhu, F., Zhu, C., Chen, Z., Liu, S., Wang, C., Gu, C., 2021. Dibutyl phthalate release from polyvinyl chloride microplastics: influence of plastic properties and environmental factors. Water Res. 204, 117597.

Yin, L., Chen, B., Xia, B., Shi, X., Qu, K., 2018. Polystyrene microplastics alter the behavior, energy reserve and nutritional composition of marine jacopever (*Sebastes schlegelii*). J. Hazard Mater. 360, 97–105.

Yu, F., Yang, C., Zhu, Z., Bai, X., Ma, J., 2019. Adsorption behavior of organic pollutants and metals on micro/nanoplastics in the aquatic environment. Sci. Total Environ. 694, 133643.

Yu, H., Huang, G.-H., An, C.-J., Wei, J., 2011. Combined effects of DOM extracted from site soil/compost and biosurfactant on the sorption and desorption of PAHs in a soil–water system. J. Hazard Mater. 190, 883–890.

Zitouni, N., Bousserrhine, N., Missawi, O., Boughattas, I., Chèvre, N., Santos, R., Belbekhouche, S., Alphonse, V., Tisserand, F., Balmassiere, L., Dos Santos, S.P., Mokni, M., Guerbej, H., Banni, M., 2021. Uptake, tissue distribution and toxicological effects of environmental microplastics in early juvenile fish *Dicentrarchus labrax*. J. Hazard Mater. 403, 124055.