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Organic contaminants sorbed to microplastics affect fish early life stages

Oryzias melastigma development

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ABSTRACT

The role of polyethylene microplastics 4-6 μm size (MPs) in the toxicity of environmental compounds to fish early life stages (ELS) was investigated. Marine medaka *Oryzias melastigma* embryos and larvae were exposed to suspended MPs spiked with three model contaminants: benzo(a)pyrene (MP-BaP), perfluorooctanesulfonic acid (MP-PFOS) and benzophenone-3 (MP-BP3) for 12 days. There was no evidence of MPs ingestion but MPs agglomerated on the surface of the chorion. Fish ELS exposed to virgin MPs did not show toxic effects. Exposure to MP-PFOS decreased embryonic survival and prevented hatching. Larvae exposed to MP-BaP or MP-BP3 exhibited reduced growth, increased developmental anomalies and abnormal behavior. Compared to equivalent waterborne concentrations, BaP and PFOS appeared to be more embryotoxic when spiked on MPs than when alone in seawater. These results suggest a relevant pollutant transfer by direct contact of MPs to fish ELS that should be included in the ecotoxicological risk assessment of MPs.

KEYWORDS : marine medaka, microplastics, developmental toxicity, benzo(a)pyrene, perfluorooctanesulfonic acid, benzophenone-3.

INTRODUCTION

Plastics are biodegraded very poorly, in some cases remaining present for hundreds of years in the marine environment. During the time they spend in aquatic systems, plastics undergo physicochemical weathering and degrade into smaller particles called microplastics (MPs). A MP is defined as a plastic particle between 1 and 5000 μm in size ¹. While there has been increasing interest in water contamination by MPs in the literature, their toxicity and potential effects on aquatic fauna remain poorly investigated ²⁻⁴.

In addition to polymers, plastic components include additives such as metals, flame-retardants, plasticizers, dyes, and UV-filters that some are known to be toxic. In addition, it has been shown that different organic pollutants are able to adsorb to the hydrophobic surface of plastics, and

thus reach even higher concentrations than measured in the water column⁵⁻⁹. However, the significance of MPs as a vector for environmental pollutants compared to other exposure routes remains a source of debate¹⁰⁻¹³.

In the present study, we investigated the toxicity of selected model contaminants adsorbed onto MPs to fish early life stages (ELS); and compare their toxicity to MP alone and these chemicals alone. These developmental stages were selected as a key stage in the population dynamic because of their high sensitivity to toxicants¹⁴⁻¹⁶. In non-feeding ELS, MPs exposure occurs mainly through direct contact with the egg chorion or the larval body surface. Transfer of MP-associated chemicals to aquatic organisms upon ingestion has been thoroughly investigated^{3,17-24}. However, exposure via contact with body surface and potential effects caused by desorption of the contaminant into water or the direct transfer from MP to organism remain largely unknown. Therefore, in this case, MPs can be considered as a potential chemical rather than a physical threat. For this reason, our study did not investigate the physical effects of MPs on digestive tracts, focusing instead on the chemical effects of MPs and their potential role as vectors of contaminants.

Marine medaka ELS were exposed to suspended MPs spiked with model contaminants to evaluate their bioavailability via induced toxic effects. Polyethylene was chosen as a virgin matrix for its high adsorption ability²⁵. Plastic concentrations in surface water were measured from 0.01 up to 0.1 mg plastic /L in North Pacific²⁶⁻²⁹ (> 300 µm size, 0.2 m depth). Taking into consideration that it is not technically possible to measure environmental concentrations of MP < 20 µm, MP exposure concentration was set at 10 mg MP/L, two order of magnitude higher than high environmental plastic concentrations measured. We selected three model aquatic pollutants commonly measured at the surface of MPs: a polycyclic aromatic hydrocarbon benzo(a)pyrene (BaP), a flame retardant perfluorooctanesulfonic acid (PFOS) and a UV-filter benzophenone-3 (BP3)^{7,30-32}. BaP was measured up to 0.18 µg/g MP^{7,30} in plastic debris, 0.1 ng/L in water surface³³⁻³⁵ and 0.7 µg/g in sediment³⁶, PFOS was measured up to 0.7 µg/L in surfacewater^{37,38} and 0.5 µg/g in sediment³⁸, and BP3 was

detected in plastic debris in qualitative studies^{31,39} and was reported up to 3.3 µg/L in seawater⁴⁰ and 0.03 µg/g in sediment⁴¹. Marine medaka *Oryzias melastigma* was selected as a model organism because it is widely used as a model fish species to study marine ecosystems⁴². In addition, it possess a long development time of 12 days, compared to 5 days for zebrafish *Danio rerio* for example, which allows to investigate an extended exposure time. Experiments were designed according to OECD test guidelines with additional sub-lethal endpoints, including CYP1A enzyme activity as a biomarker of AhR agonist and biotransformation activity, and behavior assessment as an indicator of neurotoxicity and physiological status of larvae^{14,16,43}.

MATERIAL AND METHODS

1. Synthetic microplastics

This work was carried out using pristine polyethylene (PE) microparticles to simplify the matrix of contaminant adsorption. PE was selected in this study both for its high occurrence in marine ecosystems and for its high sorbing capacity of hydrophobic compounds^{11,13}. Non-fluorescent polyethylene microplastics fragment powder of 4-6 µm size and 0.96 density MPP-635XF were purchased from Micro Powders Inc. (NY-USA). Polymer composition of the powder was confirmed to be PE only using a spectrophotometer Raman WITec Alpha 300 coupled with a 532 nm laser (Supp. Mat. S1).

MP were spiked at two environmental concentrations, one low and one high of BaP (0.01 and 16.64 µg BaP/g of MP, termed MP-BaP low and MP-BaP high); of PFOS (0.12 and 55.65 µg PFOS/g of MP, termed MP-PFOS low and MP-PFOS high); or of BP3 (0.14 and 24 ng BP3/g of MP, termed MP-BP3 low and MP-BP3 high). Spiking and MPs analysis were performed following published protocols^{4,17,44,45}. Briefly, for BaP and BP3 spiking 125 g/L of PE microplastic powder was transferred in double-deionized water in 400 mL Septa bottles (Thermo scientific) and agitated for 48h (20 rpm, 20 °C). BP3

and BaP final concentrations in the double-deionized water were 2500 and 20 µg/L. For PFOS spiking, 50 g/L of PE microplastic powder was transferred into 1L polypropylene bottles and agitated for 7 days (20 rpm, 20 °C). PFOS final concentration was 20 mg/L in double-deionized water. Detailed sorption and chemical analysis protocols are available in Cormier et al ⁴⁵.

2. Fish embryo-larval assay

Ethical requirement

The experimental work was conducted in compliance with both French and European animal experimentation policies. Marine medaka (*Oryzias melastigma*) broodstock was routinely maintained and bred in the Laboratoire Ressources Halieutiques, Ifremer (facility authorization A171901; project authorization APAFIS#10883)

Fish early life stages exposure

Oryzias melastigma embryos were obtained from the fish facility at Ifremer. At 1 day post fertilization (dpf), embryos (morula stage) were exposed under semi-static conditions to MPs suspended in seawater until the end of eleutheroembryo phase, the total resorption of the yolk sac at 13 dpf. The control condition consisted of embryos reared in 20 µm filtered-synthetic seawater only (Instant Ocean, 25 PSU, pH 7.4, 0.035 % methylene blue). The MP powder was directly added to the synthetic seawater after weighing; no dispersant was used to improve the homogenization of the suspension. For exposure, 55 mm diameter glass Petri dishes were used containing 20 mL of seawater and 30 embryos each. Exposure dishes were incubated in temperature-controlled chambers (Snijders Scientific, Tilburg, Netherlands) at 28±0.3 °C with a light:dark photoperiod 14:10 hours, 5000 lx white light, under 27 rpm rotation to synchronize hatching ⁴⁶. Half of the exposure medium was renewed daily. To re-suspend MPs before each renewal, MP exposure solutions were vigorously shaken and sampled at the middle volume of the glass bottle using a single-use 25 mL serological pipettes. After hatching at 10-11 dpf at 28 °C, larvae were transferred into glass beakers

containing 20 mL of exposure mediums until 13 dpf. Larvae were not fed during the experiment. Throughout the experiment, dissolved oxygen was checked daily using a fiber-optic oxygen mini-sensor Fibox 3 (PreSens Precision Sensor, Regensburg, Germany) and was above 85 %. For technical reasons exposures were divided in several experiments. Each experiment included a seawater control treatment (0(SW)), a pristine microplastic treatment (MP), and several spiked microplastic treatments (MP-). For each experiment, each treatment was replicated at least 3 times.

MPs particle concentrations in exposure solutions were measured with an acoustic focusing flow cytometer in triplicates in 500 μ L solution at 500 μ L/min speed (Attune, Applied Biosystems, CA-USA).

For the detection of MP locations, embryos were exposed to MPs stained with the fluorescent dye Nile Red (Sigma-Aldrich, US-MO) in a separate experiment with similar conditions to those described above. Virgin PE MPs were stained by incubation in a Nile Red solution at 50 mg/mL in acetone for 24 h at 150 rpm. Subsequently, stained MPs were dried for 24 h under a fume hood to allow acetone evaporation before embryos exposure. Embryos were observed with an Olympus epifluorescent microscope equipped with a monochrome CDD camera (Perceptive instruments, UK) using excitation and emission wavelengths of 480 and 510 nm respectively.

To investigate the impact of MPs on the pollutant toxicity, waterborne exposure of ELS to the three model aquatic pollutants was performed using the same procedure as described for MPs exposure. Exposure concentrations were selected to be equivalent to the total desorption of the chemicals from MPs at 10 mg MP/L spiked with the highest concentration. Therefore, nominal concentrations were calculated to be for BaP 166 ng/L, PFOS 556 ng/L, and BP3 0.24 ng/L.

Developmental toxicity

Dead embryos and larvae were recorded daily and immediately removed to avoid any alteration of the exposure medium. Fifteen randomly selected larvae from each replicate were individually

examined within the 24h following hatching than can occur between 10 to 13 dpf to record morphological measurement, head length and total body length. Once examined, larvae were placed in separate exposure units as and when hatching to continue exposure. Abnormalities and lesions were also recorded; specifically, edema (peritoneal and pericardial); body (scoliosis, lordosis and kyphosis), craniofacial (jaw and skull abnormalities), ocular (microphthalmia and anophthalmia), cardiovascular (anemia, hemorrhage, blood circulation) following previously published protocols ¹⁴. The percentage of abnormal larvae was the number of larvae presenting at least one abnormality compared to the total number of examined individuals. All measurements and records were conducted using a Leica MZ7.5 stereomicroscope equipped with a Leica DFP420C CDD camera using Leica Microsystems software v3.8 for image analyses (Leica, France).

Behavior assay

Changes in swimming behavior were investigated using the larval photomotor response test, which involved recording larval behavioral responses after a light stimuli challenge, based on the protocol detailed in Le Bihanic et al. (2014). The test was a sequence of 20 min acclimation in darkness followed by 5 min light on (LON1)+ 5 min dark (LOFF) + 5 min light on (LON2). For each replicate, the swimming activity of twelve randomly selected 13 dpf larvae was recorded at 28±0.5 °C using a Daniovision chamber (Noldus, Wageningen, The Netherlands). Larvae distance swam were calculated from videos using Ethovision software 12.0 (Noldus). Results were expressed regarding the control group as swimming alteration to normalize data within experiments.

CYP1A activity measurement

Activity of the enzyme CYP1A was quantified via measurement of *in vivo* EROD activity according previously published methods with modifications ⁴⁷⁻⁴⁹. Measurements were conducted on 13 dpf yolk-sac larvae. Five living larvae per single well were acclimated in a 48-well microplate containing 600 µL of 1 µM 7-ethoxyresorufin (7-ER), 3 replicate well per treatment. After 1h incubation in dark,

the 7-ER solution was replaced by freshly prepared 600 μ L 1 μ M 7-ER. Fluorescence of 100 μ L of the incubation media was measured at time 0 and after 4 h incubation in duplicate in white 96 well plate. Production of resorufin was measured with a BMG Labtech plate reader (Germany) using excitation and emission wavelengths of 560 and 590 nm respectively. Five larvae were exposed for 1h to 70 nM BaP serving as positive control.

Genotoxicity assay

Genotoxicity was investigated using the comet assay in 13 dpf yolk-sac larvae previously anesthetized in 0.1 mg/L of benzocaine. Larvae were crushed using a tissue grinder at 3000 rpm for 20 sec (G50, Coyote Bioscience, China) and then digested in 1.25 mg/mL dispase II (*Bacillus polymyxa*, Roche, France) at 37 °C, for 45 min and 150 rpm. After 10 min of centrifugation at 1000 rpm and room temperature, pellets were rinsed in 1 mL Minimum Essential Medium. Cell viability was determined in all samples using the Trypan blue exclusion assay and was constantly above 95 %. Comet assay was performed as previously described⁵⁰. For a better DNA migration and a longer comet tail, the duration of the alkaline bath and electrophoresis was increased to 30 min duration and 20 min, respectively. A hundred nuclei per slide were counted per slide and results are expressed as the percentage of DNA migrated in the comet tail.

3. Statistics

Data from all experiments were statistically analyzed as a single pool of data. Experiment-dependent data such as biometry and behavioral data were normalized and expressed based on their respective experiment seawater controls. Each set of exposure condition was repeated at least three times, and sea water control conditions were repeated 18 times. The normality of the data was verified using the Shapiro-Wilk test. In most cases, analysis of variance was performed followed by a Dunnet's post-hoc test compared to seawater control. For data that did not fulfill normal distribution, the Kruskal-

Wallis non-parametric test was performed. All statistical analyses were carried out at a 95 % level of significance and values are represented as mean±SD.

RESULTS

1. Microplastic exposure

Particles concentrations were measured in triplicates by flow cytometry. No dispersant was used in the exposure medium and particle aggregates up to 40 µm size were also included in the count. A concentration of 10 mg/L 4-6 µm size PE MP was equivalent to $16.5 \pm 2.5 \cdot 10^6$ particles/L (mean±SE, N=3), including aggregate particles. In the exposure units, MPs tend to float at the surface of the seawater and were found to accumulate as aggregates at the surface of the chorion stuck on the villi (Fig. 1). Therefore, embryos were in direct contact to MPs through their chorion. After hatching, no MP aggregation at the skin surface of larvae was detected.

2. No evidence of toxicity for virgin PE microplastic

The embryonic survival rate in seawater control was 88.8 ± 9.2 %, larval survival rate 99.8 ± 0.8 % and hatching rate 79.4 ± 9.9 % (Fig. 1). No significant acute toxicity of virgin MPs was observed after 12 days of exposure compared to seawater control (Fig. 1). Average head length of control larvae at hatching was 0.82 ± 0.04 mm, total length was 3.92 ± 0.20 mm, percentage of deformed individuals at hatching was 8.5 ± 6.4 % (Fig. 2) and percentage of tail DNA in comet assay was 16.9 ± 5.3 % (not shown). Similarly, no significant sub-lethal toxicity of virgin microplastic was observed compared to seawater control for endpoints monitored in this study (Fig. 2 and 3).

3. Toxicity of pollutants-spiked microplastic

Acute toxicity of spiked MP

Exposure to MP-BaP low did not induce lethality (Fig. 1). However, exposure to MP-PFOS high significantly reduced embryonic survival to 61.1 ± 10.7 % as well as hatching success to 50.0 ± 14.5 %. Surprisingly, hatching of individuals exposed to MP-BaP-high increased up to 96.7 % compared to lone MPs and seawater control. No impact of MP-BaP or MP-PFOS on the hatching time was noticed, irrespective of the concentrations tested. Exposure to MP-BP3 low significantly reduced embryonic survival to 41.0 ± 15.6 % and hatching rate to 29.9 ± 11.8 %⁴. Exposure to MP-BP3 high reduced hatching rate to 72.4 ± 2.4 % ($p < 0.05$) and induced premature hatching at 10.2 ± 0.1 days on average compared to 11.1 ± 0.2 days for the seawater control ($p < 0.05$)⁴.

Developmental toxicity of spiked MP

No developmental toxicity associated with MP-PFOS exposure was noted whatever the MPs or the PFOS concentrations (Fig. 2). Exposure to spiked MPs reduced larvae head length for MP-BP3 high, total length for MP-BaP low, and induced morphological abnormalities at hatching for MP-BaP low and high and MP-BP3 low. For MP-BaP and MP-BP3 exposures, the main deformities observed were yolk edemas and skeletal curvatures. This developmental toxicity of MP-BP3 and MP-BaP was observed from a microplastic concentration of 1 mg MP/L (Supp. Mat. S2).

a. CYP1A activity

No significant *in vivo* EROD activity induction was observed in larvae exposed to MP-BaP, MP-PFOS or MP-BP3 compared to seawater controls (Data not shown). EROD measurement was validated with BaP positive EROD control 100 times more concentrated than waterborne BaP exposure, which EROD activity was 5 times higher than EROD activity in seawater larvae.

b. Behavioral alteration

Exposure to either MP-PFOS-low or high did not alter the swimming activity of fish larvae (Fig. 3). Exposure to MP-BaP-high induced hypoactivity in larvae when exposed. Exposure to MP-BP3 also altered fish behavior with hyperactivity reported for MP-BP3 low. This hyperactivity was observed both after dark period and light period for MP-BP3 low particularly in the first minutes after the OFF or ON signal as illustrated in figure 3C. No significant disruption of swimming activity was observed at MP-BP3-high.

c. Genotoxicity

No significant DNA damage compare to the seawater control (data mentioned above section 2) was observed in fish exposed to MP-BaP, MP-PFOS or MP-BP3 using the comet assay (data not shown).

4. Comparison of toxicity between pollutant spiked on MPs and waterborne pollutants

To investigate the role of MPs in contaminant toxicity, the toxicity of spiked MP from data presented in Fig 1, 2 and 3 was compared to the toxicity of waterborne contaminants at equivalent high contaminant concentrations (Fig. 4). MP-BaP exposure induced abnormalities and altered swimming activity while the corresponding waterborne BaP concentration did not induce toxicity. MP-PFOS exposure induced more toxicity than PFOS alone, in particular, considering hatching success and total larvae length. Compared to waterborne BP3, the toxicity of MP-BP3 differed depending on endpoints. Interestingly, BP3 exposure did not follow a linear dose-response toxicity curve (Supp. Mat. S3).

DISCUSSION

This study aimed to investigate MP toxicity for marine fish ELS, as well as evaluating the role of MPs in the toxicity of organic contaminants sorbed onto their surface.

MP toxicity

In this study, no acute or sub-lethal toxicity of pristine polyethylene MPs was reported at the tested concentrations on marine medaka embryos and larvae. This was also noticed for other zooplanktonic organisms also exposed to different polymer types^{4,51,52}. *In natura* MPs can be composed of different polymers for which sorbing and desorbing properties may vary according to the polymer composition and additive content^{8,19,25}. For example, PAHs and PCBs were found to have better sorption on PE and polypropylene (PP) than polyethylene terephthalate (PET) and polyvinyl chloride (PVC)²⁵.

Passive internalization of MPs should not be possible at the embryonic stage, since MP size (4-6 µm) is about 10 times larger than chorion pore canals^{53,54}. In addition, no ingestion of MPs was observed by microscopy during exposure of marine medaka at the larval stage (2-3 days post-hatching).

MP-BaP, MP-PFOS and MP-BP3 toxicity

BaP, PFOS and BP3 induced different specific toxic effects to marine medaka ELS at physiological and organism levels when spiked to MPs. Observed toxicity increased as of organic contaminants concentrations went up. MP-PFOS and MP-BP3 (but not MP-BaP) induced acute toxicity.

Experiments were valid since in all experiments embryos in the control treatments did not exceed a steady hatching failure of 20 %. Hatching failure were mainly concomitant with a bacterial development at the surface of the embryos. An increase of the hatching success was observed on embryos exposed to MP-BaP-high. This could be explained by a bactericidal effect of BaP⁵⁵ that could improve the hatching conditions of the embryos. On the other hand BaP as a well-known Ah receptor inducer^{56,57}, triggered teratogenicity to marine medaka larvae expressed by blue sac

disease syndrome. This observed developmental toxicity was associated with the alteration of fish larvae swimming behavior. As an example, increase in Japanese medaka swimming activity has been shown after exposure to PAHs mixtures¹⁴.

This study also reveals acute toxicity in PFOS, even though no change was observed in the morphology of surviving exposed larvae. This acute toxicity could result from several organism dysfunctions, such as cardiac impairment and immunotoxicity, as previously reported in the literature on marine medaka embryos exposed for 10 days to 1 mg/L of PFOS^{58,59}.

Our study reported for the first time the developmental toxicity of BP3 and the disruption of fish larvae behavior at low exposure concentrations. These effects can be considered as an early warning of organism dysfunction at environmental chemical concentrations. Indeed, endocrine disruption has previously been reported after BP3 exposure of different fish species at transcriptional and protein levels⁶⁰⁻⁶². This raises concerns about the impact of BP3 on the survival and, in particular, the recruitment of fish species. Depending on the endpoints investigated, the lowest tested concentrations induced the highest toxic response. As a singular signature of endocrine disruptor compounds toxicity, the response of fish to BP3 was not monotonic and did not follow a linear dose-response curve. This could be due to several molecular target of the compound⁶¹⁻⁶³.

No *in vivo* EROD activity was detected in larvae exposed to MP-BaP, -PFOS and -BP3 in comparison to control larvae both for waterborne and suspended MPs exposures. This means that no compound CYP450 1A induction was observed at the tested concentration and limit the discussion about the toxicity of the tested compounds. This might be explained by very low BaP exposure concentrations, and suggests either low sensitivity of the method or direct toxicity of parent compounds. Similarly, a recent study reported transfer, and therefore bioavailability, of BaP spiked on PE MPs to adult zebrafish after MP ingestion without evidence of EROD activity²¹.

Bioavailability of MP

The bioavailability of hydrophobic aquatic contaminants sorbed onto a matrix is a complex process that depends on several inter-dependent parameters^{9,19}. One of the focuses of the present work was on the role of MPs organic contaminants toxicity and MPs as a source of contamination for biota, which is an emergent issue. It raises the question of whether the contaminant is bioavailable via a direct transfer from the MPs in contact with the egg, via chemical desorption associated with the dissolution of the contaminant in the water, or via the surface water layer of the chorion between the villi. This layer presents specific physicochemical properties that evolve during embryonic development and differ from the rest of the exposure water media and, such as osmosis regulation^{64,65}. The investigation of the full mechanism of action undergoing contamination process was beyond the scope of this study. However, the results show that an increase in contaminant nominal concentrations, in particular for BaP and PFOS, leads to an increase of toxicity. All three tested compounds appeared to be directly bioavailable to fish embryos when spiked to MPs. One hypothesis would be that MPs aggregated on the surface of the fish egg in direct contact with the villi and the chorion that are sticky and may transfer contaminants and increase exposure concentration compared to exposure via the water column only. This transfer of contaminant has already been reported in zebrafish embryos exposed to polyethylene MP 1-5 μm ²⁰. Also, MPs may have abrasive or tension-active properties on to the chorion and thus facilitate passive transfer inside the egg leading to an increase of contaminant bioaccumulation. This could explain the higher toxicity of contaminants when spiked to MPs vs waterborne exposure observed in this study. Therefore, MPs exert organic contaminant toxicity and represent a chemical threat for fish early life stages.

Underestimation of the study

In addition, it is important to consider that ageing of polymers through mechanical erosion, bacterial degradation, biofouling, and photo-oxidation as well as modulation of temperature and salinity increases the sorption process of contaminants and their degradation^{9,19,66}. Therefore, this study may have underestimated the bioavailability, therefore the toxicity of contaminants sorbed onto

MPs. Indeed, aged plastic particles might be more toxic to fish ELS than pristine spiked ones ⁶⁷. Further research is required to investigate the effects of polymer aging on contaminant bioavailability and toxicity for aquatic fauna.

In conclusion, this study supports the relevance of the use of sub-lethal endpoints, such as biometry, developmental abnormalities and swimming behavior, to study the toxicity of chemicals to fish ELS. Our results underline the need for including such endpoints in the regulatory tests. All three contaminants sorbed onto MPs were directly bioavailable and toxic to fish early life stages without evidence of MPs ingestion. So direct contact to MPs and natural desorption of aquatic contaminants from MPs are a major concern in the evaluation of the risk of water contamination by MPs. These two sources of exposure to contaminants should be taken into consideration at least in non-feeding fish and invertebrates ELS.

Data availability

Data of this study are available on request to the corresponding author

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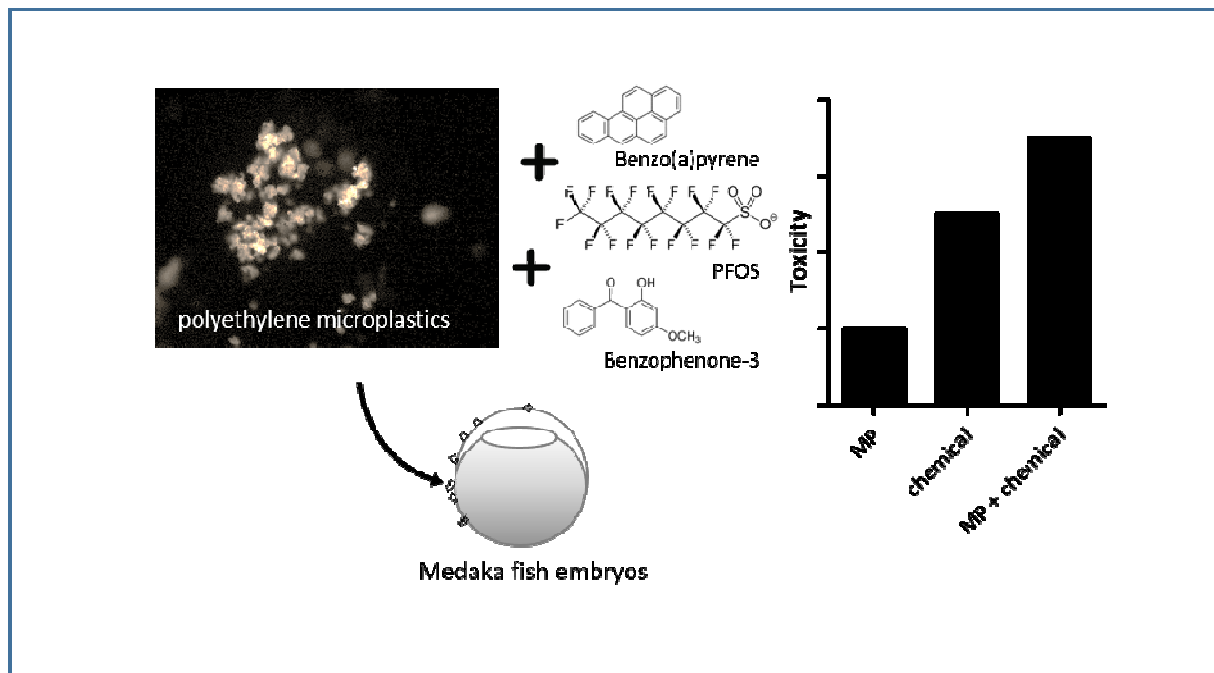
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Figures

For better quality figures, individual figure files can be downloaded on journal website.

Graphical abstract



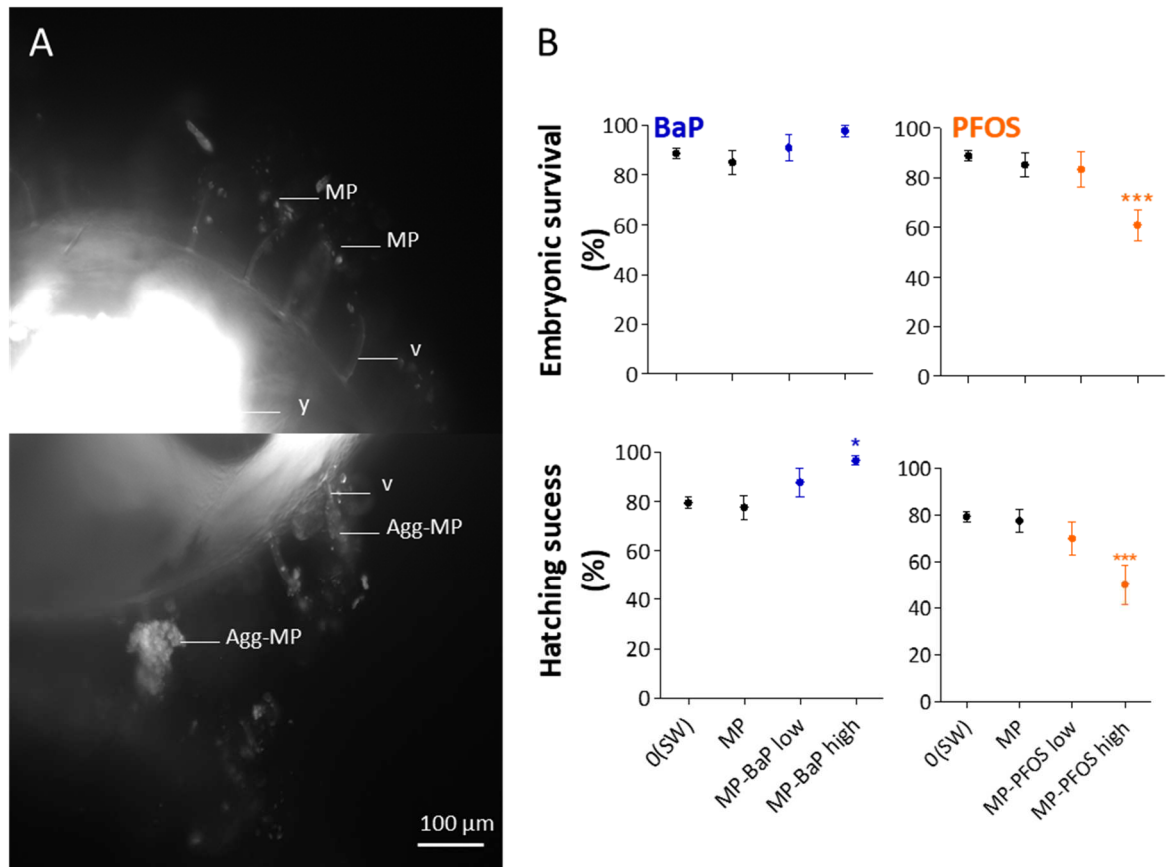


Fig 1. Spiked MPs induced acute toxicity in fish early life stage. A – Fluorescence microphotography of microplastics aggregated on the surface of embryonic chorion, embryos were exposed to 10 mg MP/L stained with Nile Red; v – villi, y – yolk (display an autofluorescence) MP – microplastic, Agg-MP – MP aggregates. B - Embryonic survival and hatching success after exposure to MP-BaP left panel, and MP-PFOS right panel. Mean \pm SD, N = 3-18 exposure replicates, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ compared to seawater controls (SW).

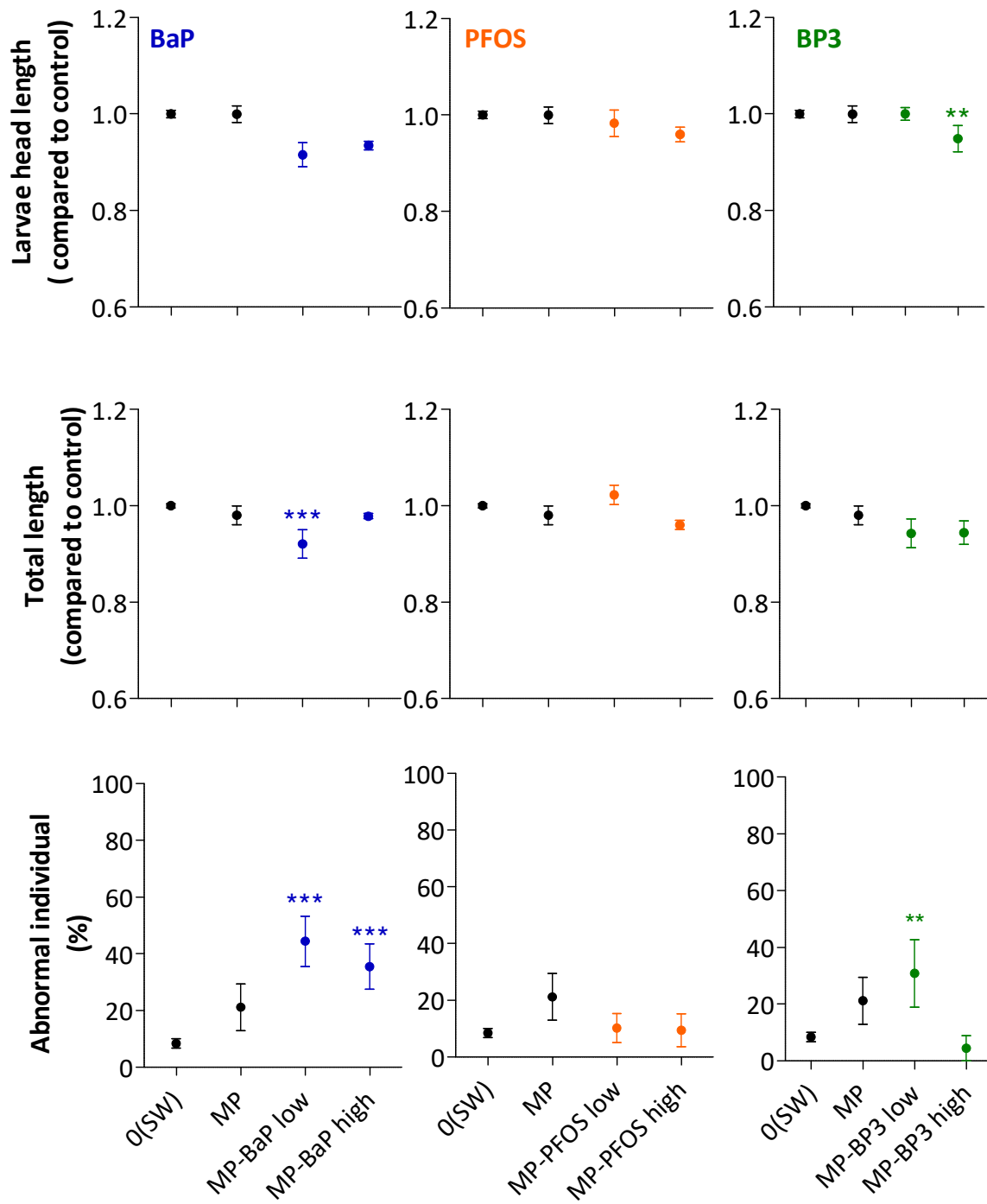


Fig 2. Spiked MPs induced developmental toxicity to fish early life stages. Sublethal endpoints include larval head and total length expressed relative to the seawater controls, and abnormal individuals (Mean \pm SD, N = 3-18 exposure replicates, * p < 0.05, ** p < 0.01, *** p < 0.001 compared to seawater controls (SW)).

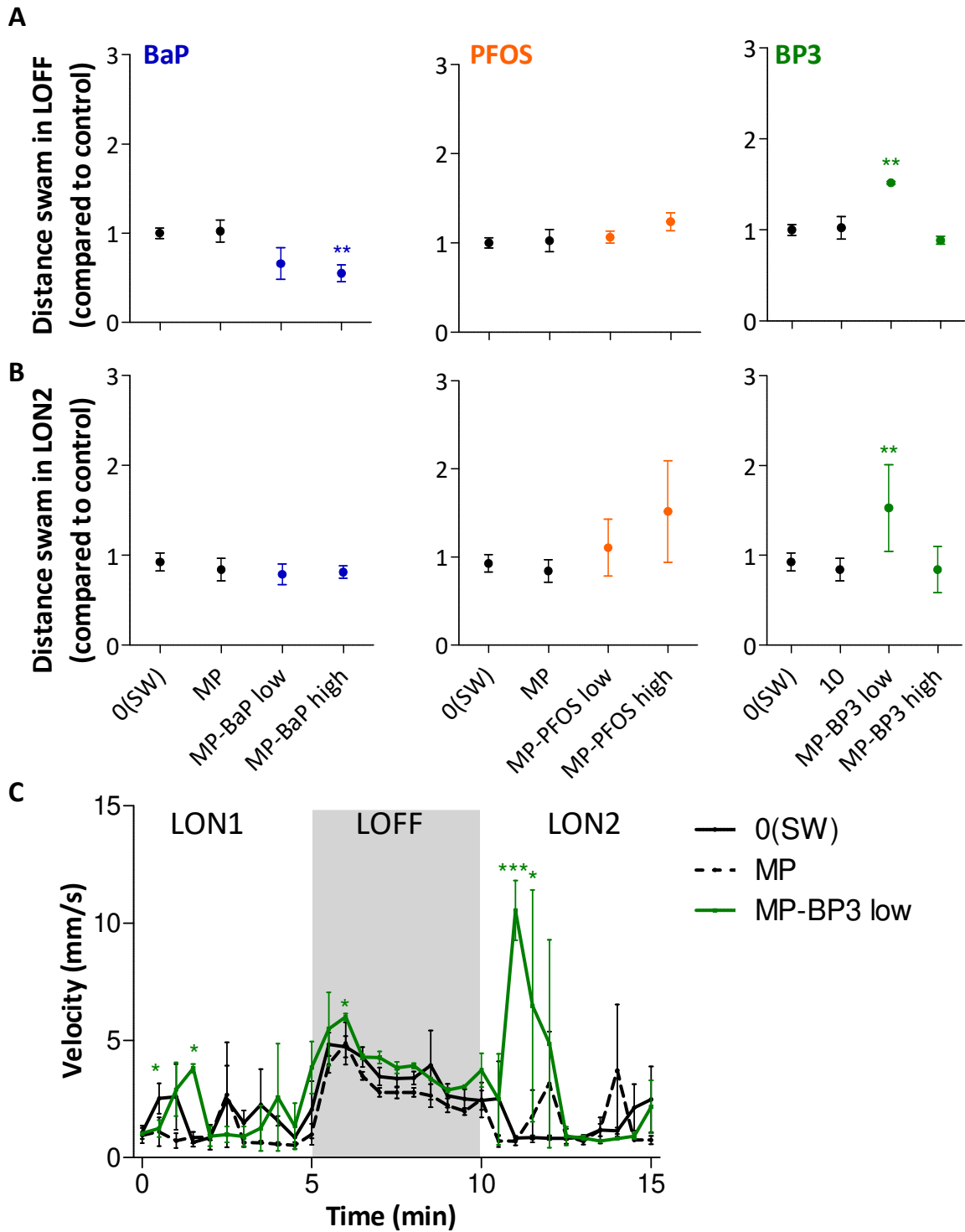


Fig 3. MP-BaP and MP-BP3 altered fish larval photomotor response. Alteration of the distance swam during the A- LOFF and B- LON2 period of the photomotor response test. Results are expressed

as distance swam relative to respective distance swam by seawater control larvae (Mean \pm SD, N = 3-18 exposure replicates, * p< 0.05, ** p<0.01, *** p<0.001 compared to seawater controls (SW). C-Larvae velocity exposed to seawater control, MP or MP-BP3 low (mean \pm SD, N = 3).

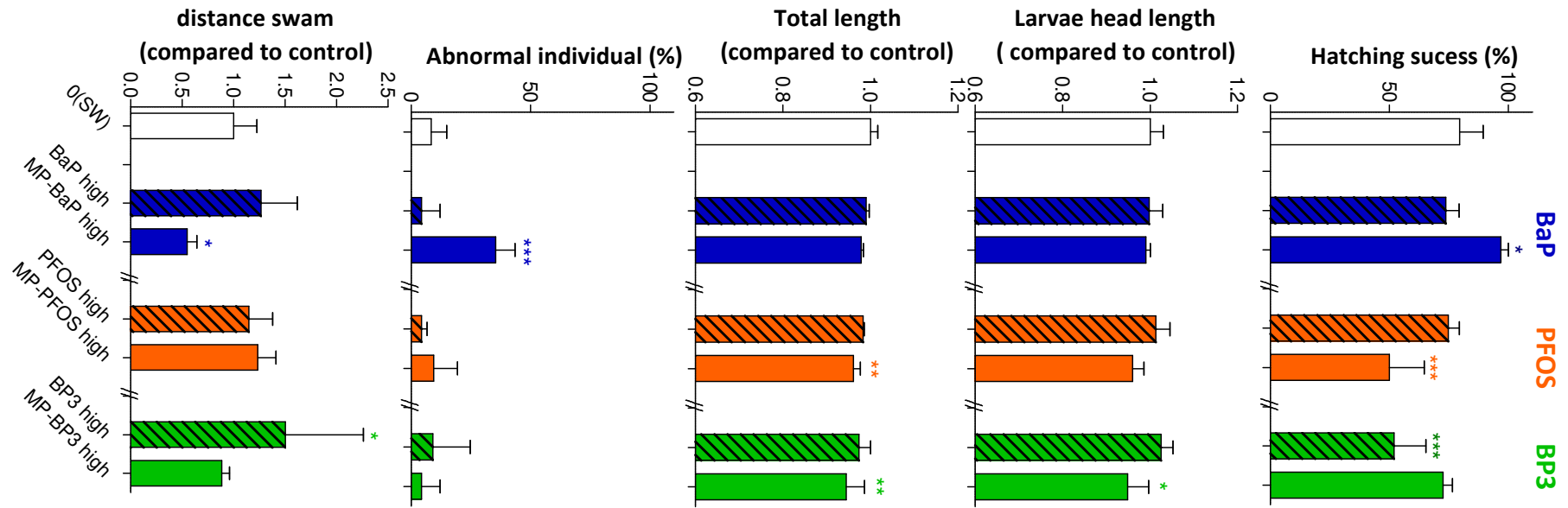


Fig 4. Waterborne contaminant toxicity compared to MP-contaminant toxicity. Fish embryos were exposed to seawater control; waterborne 166 ng/L BaP high, 556 ng/L PFOS high, 0.24 ng/L BP3 high nominal concentration equivalent to 100 % desorption of chemical from spiked MPs high (crosshatched bars); or spiked MP with BaP, PFOS or BP3 high (Mean \pm SD, N = 3-15 exposure replicates, * p< 0.05, ** p<0.01, *** p<0.001 compared to seawater controls (SW)).