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**Microplastics enhance *Daphnia magna* sensitivity to the pyrethroid insecticide  
deltamethrin: effects on life history traits.**

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## Abstract

The aim of the study was to investigate the influence of microplastics (MPs) on the ecotoxicity of common contaminants of aquatic ecosystems. As a model contaminant, the hydrophobic pesticide deltamethrin (DM) was chosen, and its effects on life history traits of *Daphnia magna* were studied in the presence or absence of polyethylene MPs. Commercialized DM and MPs obtained as dry powder were used in the experiment. According the manufacturer (Cospheric, Santa Barbara, CA, USA) MPs were spherical (1-4  $\mu\text{m}$  in diameter), had a density of  $0.96\text{ g/cm}^3$  and were without any solvent. Three concentrations of polyethylene MPs were tested (0, 1, 10 mg/L) with two realistic concentrations of DM (0 and 40 ng/L) and a solvent control (acetone). During the 21 d experiment, *D. magna* neonates were individually exposed to the treatments, and the effects of MPs and DM alone and together were evaluated by assessing survival, number of cumulative molts, days to first brood, number of broods, number of neonates per surviving adult, and body length. Significant detrimental effects on survival were only observed for the two mixture treatments. DM alone (40 ng/L) delayed the days to first brood and reduced the number of neonates per surviving adult, whereas MPs alone (10 mg/L) induced significant reduction in the number of juveniles by surviving adults. The combined exposure to DM and MPs clearly had a synergistic effect on survival, brood number, and number of neonates per surviving female. For example, compared to exposure to 40 ng/L of DM alone, the addition of 1 mg/L of MPs resulted in a 51.1% reduction in number of neonates per surviving female and a 46% reduction in brood number. These results suggest the potential drastic effects of this kind of mixed exposure on daphnid populations, which are key components of freshwater food webs.

## Keywords:

Polyethylene microplastics, deltamethrin, *Daphnia magna*, survival, growth, reproduction, chronic ecotoxicity, interaction, mixture

## 1. Introduction

Plastic pollution is a major environmental concern worldwide. According to Geyer et al. (2017), 8300 million metric tons (Mt) of virgin plastics have been produced as of 2017, around 76% of which now are plastic wastes. Annual waste production is projected to rise to 3.4 billion Mt in the next 30 years (Silpa et al., 2018). As plastic is designed to be durable, it persists in the environment for long periods of time. Inappropriate dumping of disused or abandoned plastic wastes leads to contamination of the environment, affecting terrestrial, freshwater, and marine ecosystems (Rezania et al., 2017; Chae & An, 2018; Li et al., 2018).

Among plastics, macroplastics are defined as large item plastics that are > 5 mm in size, microplastics (MPs) are plastic particles ranging in size from 0.1  $\mu\text{m}$  to 5 mm, and nanoplastics are plastic particles ranging from 1 to 100 nm in size (Bråte et al., 2017). MPs are ubiquitous, and large quantities of MPs have been detected in freshwater, marine, and terrestrial ecosystems (in soil, sediment, water, ice, snow: Rezania et al., 2017; Chae & An, 2018; Wagner & Lambert, 2018; Bergmann et al., 2019), even in remote areas (i.e., from the Arctic to Antarctica; Waller et al., 2017; Bergmann et al., 2019). Their presence is related to inputs from other ecosystem compartments (e.g., terrestrial, rivers, sediment relocation from shorelines, wet and dry atmospheric deposition; Chubarenko et al., 2018; Bergmann et al., 2019; Klein & Fisher, 2019; Wetherbee et al. 2019). Concentrations of MPs vary from  $10^{-5}$  to  $10^5$  MPs/ $\text{m}^{-3}$  in surface water and from 40 to 400 MPs/L in sediments (reviewed in Li et al., 2018), and concentration in snow samples can be considerable even in remote areas (Bergmann et al., 2019: European sites  $154 \times 10^3$  MPs/L, Arctic snow :  $14.4 \times 10^3$  MPs/L). Among MPs found in natural environments, polyethylene (PE) MPs are generally predominant (Wagner & Lambert, 2018; Bergmann et al., 2019). To date, most ecotoxicological studies of MPs have focused on marine organisms, especially on marine fish, which highlights the need to acquire more information about freshwater organisms (de Sà et al., 2018; Wagner & Lambert, 2018).

Because MPs consist of numerous types, compositions, sizes, and shapes of plastics, which influence their bioavailability and then their ecotoxicity, results in the literature are highly

variable. The most consistent reported effect of MPs was the reduction in natural prey consumption in the presence of MPs, but negative effects on growth, reproduction, and survival were also reported for some taxa (Foley et al., 2018: meta-analysis). Accordingly, many studies reported that MPs are transferred into the aquatic food web (Setälä et al., 2014; Nelms et al., 2018). Ingestion of MPs (number of particles per individual, proportion of individuals ingesting plastic) was taxon- and life stage-specific, and depended on MP size, shape, and aging (Vroom et al., 2017). MPs ingested by organisms (feeding and/or ventilation processes) can have mechanical effects due to their attachment to external surfaces (e.g., reducing mobility and clogging the digestive tract) as well as chemical effects (e.g., inflammation, neurotoxicity, genotoxicity, hepatic stress, oxidative stress, disturbance of energy metabolism) (Setälä et al. 2016; de Sà et al., 2018; Wagner & Lambert, 2018; Prokić et al., 2019).

MPs also provide large surface areas that can adsorb, concentrate, and transport a range of pollutants in aquatic systems, especially persistent organic pollutants (POPs) and hydrophobic organic chemical (HOCs), and this process affects the mobility and bioavailability of pollutants for organisms (Wagner & Lambert, 2018; Wang et al., 2018). The interactive effect of MPs and other pollutants is problematic for the assessment of risks linked to MPs (Auta et al., 2017; Galloway et al., 2017; Wang et al., 2018). The sorbent capacity of MPs is influenced by the type of polymer and its size and state, stressing the need to conduct more studies on interactive effects, especially for freshwater organisms (de Sà et al., 2018, Wang et al., 2018; Prokić et al., 2019). To date, studies of the interactive effects of MPs and other pollutants have been focused mainly on pollutant accumulation or organism survival, whereas few studies have tested interactive effects on organism growth and reproduction, which are key parameters for population dynamics.

Pyrethroid insecticides are considered to be of low acute toxicity to mammals (Grosman & Diel, 2005), but they are highly ecotoxic to insects and non-target organisms (Saha & Kaviraj, 2008). They are widely used in households, for field pest control (agriculture, forestry), and for malaria control programs (Villarini et al., 1998; Ansari & Razdan, 2001). According to Mordor

Intelligence (2017), pyrethroid use has been increasing during the last two decades, and it accounts for more than 35% of the world market for insecticides. Deltamethrin (DM) is a hydrophobic and synthetic type II pyrethroid that can be found in diverse compartments, including air, water, and sediment, as well as in plants and animals (Pawlisz et al., 1998). DM concentrations in water were reported to range from 0.04 to 24 µg/L in Canadian agricultural areas (Pawlisz et al., 1998) and from 2 to 58.8 ng/L in the Ebro Delta in Spain (Feo et al., 2010). DM primarily acts on the nervous system, especially on the voltage-dependent ion channel in excitable membranes, but it also impacts various secondary targets involved in signal transduction (Narahashi, 1992) and in up and down regulation of the proteome (Toumi et al., 2014). DM is known to be toxic to diverse aquatic organisms (Fojut et al., 2012; US EPA, 2013), especially to daphnids, which are the crustacean zooplankton taxon most affected by exposure to DM (Crane et al., 2011; Toumi et al., 2013, 2015).

The cladoceran *Daphnia magna* is a standard organism in ecotoxicology studies and is routinely used as a model species to determine ecotoxicity of chemicals for risk assessment (Pereira et al., 2010) due to a suite of interesting features (Baird & Barata, 1998). In addition, *D. magna* was found to be sensitive to a large number of chemicals (Hanazato, 1998) such as pyrethroids (Ren et al., 2009; Toumi et al., 2013). Moreover, ingestion of MPs can have negative effects on *D. magna* (Rehse et al., 2016).

The aim of this study was to test the chronic ecotoxicity of DM in the presence or absence of MPs to test the potential interaction between both compounds and assess its outcome on *D. magna* life history traits. We first evaluated MP behavior in the water column of the experimental units, and then we analyzed the life history traits of *D. magna* (survival, number of cumulative molts, days to the first brood, number of broods, number of neonates per surviving adult, and body length). Lastly, we assessed the potential sensitivity modification (using a stress on stress study) in juveniles born under exposure to MPs or DM.

## 2. Materials and Methods

### 2.1. Materials

#### 2.1.1. DM

DM used in this study was the technical active substance of diverse formulations commercialized by Bayer (DECIS EC25; Leverkusen, Germany). The chemical name of DM ( $C_{22}H_{19}Br_2NO_3$ ) is (s)-Cyano-(3-phenoxyphen) methyl (1R)-cis-3-(2,2-dibromovinyl)-2,2-dimethylcyclopropanecarboxylate). DM is a hydrophobic compound (log Kow = 6.2; Hansch et al., 1995).

#### 2.1.2. PE MPs

We used the same MPs as those used by Khan et al. (2015), Batel et al. (2016), and Rehse et al. (2016). They were PE MP particles, 1–4  $\mu m$  in diameter (clear PE microspheres 0.96 g/cm<sup>3</sup>), obtained as dry powder from Cospheric (Santa Barbara, CA, USA). As described by Rehse et al. (2016), i) these particles are well defined with a narrow size distribution and uniform spherical shape, ii) they consist of pure PE material with no additives such as pigments, iii) no solvents are used during manufacturing processes, iv) 1 mg of 1–4  $\mu m$  particles contains between  $3.1 \times 10^7$  and  $2.0 \times 10^9$  particles, and particles tend to v) attach to surfaces and vi) float close to the water surface in the *Daphnia* culture medium they used.

### 2.2. Organisms and breeding

The experiment was conducted using *D. magna* (Straus 1820) identified as clone A by the laboratory of Professor Calow (Sheffield University, UK). This biological material has been maintained for more than 40 years at the LIEC laboratory (University of Lorraine in Metz, France) in parthenogenetic cultures carried out in 1 L aquaria at 20 °C with LCV medium (a 20/80 mixture of Lefevre-Czarda (LC) medium (Ionescu et al., 2006) and French (Volvic) mineral water) under a 16-8 h light-dark photoperiod and at a density of 40 animals/L (Manar et al., 2009). The medium was supplemented with a mixture of vitamins (0.1 mL/L) containing

thiamine HCl (750 mg/L), vitamin B12 (10 mg/L), and biotin (7.5 mg/L), and was renewed three times a week. During breeding, the organisms were fed with a mixture of three algal species ( $5 \times 10^6$  *Raphidocelis subcapitata*,  $2.5 \times 10^6$  *Desmodesmus subspicatus*, and  $2.5 \times 10^6$  *Chlorella vulgaris* cells per *Daphnia* per day).

### **2.3. Preparation of stock and test solutions**

The medium of each treatment was renewed three times a week (every 2 d). During this step, for each treatment, new exposure media were prepared and placed in another set of tanks. Adult *D. magna* then were gently transferred from the old exposure medium to the renewed exposure medium specific to each replicate number of each exposure treatment.

#### **2.3.1. DM**

Before each experiment and medium renewal, stock solutions (80 ng DM/L) were prepared by dissolving the pesticide directly in acetone and afterwards in the *Daphnia* medium (LVC, see above). Briefly, 10 mg of DM were dissolved in 20 mL of pure acetone followed by dilution of 1/50, then 1/50, 1/5, 1/100, and 1/5 in LVC medium; thus, obtaining a solution of 80 ng DM/L. The acetone control (MP0-DM0ac) was prepared using the same procedure, but without DM. A last dilution of 1/2 was performed in LVC medium containing 20 mg MPs/L or 2 mg MPs/L to obtain MP10-DM40 or MP1-DM40 treatments (final acetone percentage:  $8 \times 10^{-7}$  %; v:v), whereas a 1/2 dilution was performed in LVC medium without MPs to obtain the MP0-DM40 treatment.

#### **2.3.2. MPs**

A bottle containing 200 mg of MPs was transferred to a 2 L-jauged flask. This bottle was rinsed five times with LCV medium. Next, LCV medium was gradually added under homogenization (500 mL by 500 mL). After each medium addition, the flask was ultrasonicated for 15 min to minimize aggregation of particles (cf. Rehse et al., 2016). This stock solution (100 mg MPs/L)



was used for the six medium renewals of the experiment. It was kept under agitation during the duration of the experiment (21 d) and ultrasonicated for 15 min every 2 d (before renewal of the medium).

All labware was rinsed with LCV medium. To prepare the exposure solutions at each renewal, a solution was prepared from the stock solution using the LCV medium in order to obtain 2 or 20 mg MPs/L. This solution was constantly homogenized during the preparation of MP-DM mixtures.

All MP-DM solutions (see Table 1) were prepared immediately before the beginning of the experiment and the medium renewals. The final volume of our experimental units was 50 mL. To prepare the treatment MP10-DM40 (10 mg MPs/L with 40 ng DM/L), we transferred 25 mL of the 20 mg MPs/L solution and, using the same 25 mL-jauged flask, we transferred 25 mL of culture medium containing 80 ng DM/L, algae, and daphnids. To prepare the treatment with 1 mg MPs/L, we transferred 2.5 mL of 20 mg MPs/L<sup>-1</sup> in a 25 mL-jauged flask and, using the same micropipette, we transferred 22.5 mL of daphnid culture medium. Then, we transferred the content in the experimental unit with 25 mL of daphnid culture medium containing 80 ng DM/L (MP1-DM40) or not (MP1-DM0).

## **2.4. DM and MP analysis**

### *2.4.1. DM analysis*

DM was extracted from exposure solutions with dichloromethane (CH<sub>2</sub>Cl<sub>2</sub>). After extraction, the solvent was concentrated to 1 mL in a rotary evaporator maintained at 45 °C and 800 mbar to evaporate the CH<sub>2</sub>Cl<sub>2</sub>. DM was analyzed using a gas chromatograph equipped with an ion trap mass spectrometer (FOCUS-ITQ 700, Thermo Scientific Inc.) in electronic impact mode. Each DM extract was injected (2 µL) in the splitless mode (1 min) on a Varian VF 5 capillary column (30 m × 0.25 mm × 0.25 µm film thickness), and detection was performed in MSMS mode (parent ion 181 m/z; daughter ion: 153 m/z). Detection and quantification limits were calculated for the extract as 0.2 and 0.5 µg/L respectively, with an uncertainty of 8%. After 24

and 48 h, nominal concentrations in treatments without MPs were decreased by 45% and 74%, respectively. To limit this concentration variation, and in accordance with the OECD (2012) guideline recommendations, media were renewed every 2 d.

#### *2.4.2. Measurement of MP concentration*

We filtered 770 mL of 20 mg MPs/L on glass microfibre filters (GF/F Whatmann filters; 47 mm diameter; porosity 0.7  $\mu$ m). The filters were weighed before and after filtration (Sartorius analytic A120S; accuracy: 0.1 mg; Goettingen, Germany). We detected a concentration of 19.58 mg MPs/L instead of a concentration of 20 mg MPs/L.

#### *2.4.3. Assessment of MP behavior*

Water was sampled using a micropipette at one centimeter from the surface of the liquid in units without daphnids and algae. We used a spectrophotometer to follow the MP distribution in the water column (Analytikjena Specord 205, Jena, Germany). The most discriminating optical density for following MPs was 900 nm. We measured the absorbance just after solution preparation and after 3 d in experimental units containing 0, 20, 40, 60, 80, and 100 mg MPs/L. There was a linear and significant relationship between MP concentration (x) and absorbance at 900 nm (y;  $y = 0.0022x$ ;  $R^2 = 0.9988$ ). However, after 3 d of rest, MP concentrations in the water column (1 cm from the surface) were 4.92 and 4.78 mg/L in 20 and 40 mg/L solutions, respectively, and 12.6, 10.06, and 10.62 mg/L in 60, 80, and 100 mg/L solutions, respectively. The increase of turbidity along the concentration gradient was less pronounced at the end of the test than at the beginning.

#### *2.4.4. MP distribution in the water column*

At the beginning and after 2 d in 60 mL test beakers (the same experimental units as used for daphnid exposure) in the absence of algae and daphnids, we measured the absorbance of solutions containing 10 and 20 mg MPs/L at the bottom of the units and at 1 cm from the surface

of the liquid (from 190 to 1100 nm; we focused our analyses on 900 nm). No differences were observed between the two sampling zones (10 mg MPs/L: 0.0134 vs. 0.0136; 20 mg MPs/L: 0.0236 vs. 0.0241). However, after 2 d of rest, we observed a loss of 50% of MPs in the water column. These MPs were floating on the surface of the water due to their low density. This effect was *de facto* limited because the exposure solutions were renewed every 2 d.

#### 2.4.5. Assessment of the effect of acetone on MPs

As acetone was used to solubilize DM (final acetone percentage:  $8 \times 10^{-7}$  %; v:v), we checked under a microscope (100x and 400x) for the possible alteration of MPs after 2 d in the experimental units (without food and daphnids) in the different treatments (i.e., MP10-DM0, MP10-DM40, MP1-DM0 and MP1-DM40). A drop of water was placed on a microscope slide. Particular attention was paid to homogenizing the water before analysis and to slightly pressing the slide to avoid bias due to thickness of items. Ten fields were chosen randomly at 100× magnification and then viewed at different magnifications from 100× to 400× to detect potential modifications in MP concentration (dissolution), shape (loss in sphericity), and color. No loss, deformation, or abnormality of MPs were observed in the MP10-DM40 and MP1-DM40 treatments compared to MP10-DM0 and MP1-DM0 treatments.

### 2.5. Chronic ecotoxicity

The experimental procedure was conducted in accordance with the OECD 211 (2012) guideline recommendations, except that we did not check the quantity of total organic carbon added due to the food (algae) supply, and we used LCV medium (see Section 2.2) instead of M4 or M7 medium.

Briefly, *D. magna* were exposed individually in 60 mL glass beakers containing 50 mL of test solution composed of LCV medium with algae and mixtures of DM and MPs at desired nominal concentrations. LCV medium was used because it is the usual culture medium for daphnids (and thus it did not induce stress), and it met the validity criteria for the test (OECD

211, 2012). During the test, daphnids were fed with a mixture of three algal species ( $5 \times 10^6$  *R. subcapitata*,  $2.5 \times 10^6$  *D. subspicatus*, and  $2.5 \times 10^6$  *C. vulgaris* cells per *Daphnia* per day). Temperature was controlled at 20 °C, photoperiod was maintained at 16-8 h light-dark, and the test solution (with food) was renewed every 2 days.

The assessment endpoints were survival, number of cumulative molts, days to first brood, number of broods, number of neonates per surviving adult, and body length (measured from the top of the head to the base of the tail spine using the image analysis software Motic Image Plus 2.0 (Motic China Group Ltd, Xiamen, China)).

To minimize DM adsorption, each exposure vessel was previously saturated for 24 h with the corresponding DM concentration before each test. Seven combinations of experimental conditions were studied (Table 1): a control (LCV medium only, MP0-DM0) and a solvent control (MP0-DM0ac; medium + acetone at  $8 \times 10^{-7}$  %), three MP concentrations (MP: 0, 1, and 10 mg/L) mixed with two DM concentrations (DM: 0 and 40 ng/L). At least ten replicates were carried out for each treatment.

In the present study, the DM concentrations used for the experiment were based on the IC<sub>20</sub><sub>21days</sub> value obtained in a previous study (Toumi et al., 2013) in which DM at 40 ng/L exerted no effect on survival, but had slight but significant effects on different sublethal parameters (number of cumulative molts, days to the first brood, and number of neonates per surviving adult). The MP concentrations (1 and 10 mg/L) were selected based on Rehse et al. (2016) in order to test a concentration high enough to cause death and a lower concentration without expected death. This experimental design allowed us to assess the interactive effects of the DM and MPs.

## **2.6. Sensitivity of juveniles born during the experiment: stress on stress experiment**

Acute tests with potassium dichromate were performed according to the International Organization for Standardization procedure 6341 (ISO, 1996). Four replicates of five neonates (< 24 h) born during the chronic test under different exposure conditions were placed in glass

test tubes containing 10 mL of each of the following test and control solutions: MP0-DM0; MP0-DM0ac; MP0-DM40; and MP1-DM0. The neonates were not fed during the test. The assessment endpoint was immobilization of *D. magna*. The juveniles that were able to swim were considered to be mobile, and those that still moved their antennae, but did not swim within 15 sec after gentle shaking were considered to be immobile. This experiment was not possible for the MP1-DM40 and MP10-DM40 treatments because too few juveniles were produced.

## 2.7. Statistical analyses

As very slight and not significant differences were reported between the two controls (MP0-DM0 and MP0-DM0ac: no acetone effect) regardless of the tested endpoint, these two groups were pooled before further analysis (Mann-Whitney test:  $p > 0.68$ ; Table 1). Data analyses were conducted within the R statistical environment (R Core Team, 2013) using the *drc* package for concentration-response modelling.  $P < 0.05$  was considered to be statistically significant.

### 2.7.1. Survival

Time-to-event data analysis (Hosmer et al., 2008) was used to investigate the effects of MP and DM concentrations on survival. This approach considers events occurring during the whole study period (not only at the final time point) and takes into account right-censored values (i.e., organisms for which death was not observed within the study period). Kaplan-Meier curves were used for time-to-event data representation. Log-rank tests were performed to compare survival curves between pairwise treatments (Peto & Peto, 1972). A Bonferroni correction was applied for multiple comparisons ( $n = 15$  comparisons). Interaction significance on survival was assessed using the two-way Scheirer-Ray-Hare test on *D. magna* longevity response (time alive). Because this test requires a perfectly balanced design, data were randomly selected to obtain the same number of replicates per treatment based on the lowest number of replicates among treatments (minimum of 13 replicates for longevity data).

### 2.7.2. Number of neonates per surviving female and body length

For these endpoints, the normality of data and homoscedasticity were checked, and subsequently parametric statistical methods were used. To compare the treatments, one-way analysis of variance (ANOVA) was performed followed by *post-hoc* Tukey tests for pairwise comparisons. Next, two-way ANOVA was performed to investigate the effect of the two factors under study (MP and DM) as well as their interaction. Because all organisms in the MP10-DM40 treatment died, data from the 10 mg MP/L treatments (MP10-DM0 and MP10-DM40) were discarded for the two-way analyses.

### 2.7.3. Cumulative number of molts, days to the first brood, and number of broods

For these endpoints, the normality of data and homoscedasticity were not checked, so non-parametric statistical methods were used. To compare the treatments, Kruskal-Wallis tests were performed followed by Mann-Whitney tests with Bonferroni correction for pairwise comparisons. Next, two-way Scheirer-Ray-Hare tests (used as a non-parametric equivalent of two-way ANOVA) were performed to investigate the effect of the two factors under study (MP and DM) as well as their interaction. Because these tests require a perfectly balanced design, data were randomly selected to obtain the same number of replicates per treatment based on the lowest number of replicates among treatments. Because all organisms in the MP10-DM40 treatment died, data from 10 mg MP/L treatments (MP10-DM0 and MP10-DM40) were discarded for the two-way analyses.

### 2.7.4. Stress on stress

Acute potassium dichromate LC<sub>50</sub>s (with 95% confidence interval) were calculated for neonates born during the chronic experiment (see Section 2.6) and further used in the acute toxicity test. For each time point (24 and 48 h) and for each pre-exposure treatment (MP0-DM0, MP1-DM0, MP10-DM0, MP0-DM40), two-parameter log-logistic models were fitted to survival data assuming a binomial error model.

325

### 326 3. Results

#### 327 3.1. *D. magna* survival

328 All *D. magna* survived in controls during the 21 d of the experiment (MP0-DM0; Figure 1).  
329 This result satisfies one validity criterion of the test ( $\leq 20\%$  mortality; OECD, 2012). Exposure  
330 to 40 ng DM/L alone (MP0-DM40) or 1 mg MP/L alone (MP1-DM0) for 21 d resulted in  
331 slightly lower survival (93.3%), but survival curves did not differ significantly from that of the  
332 control. When the two pollutants were combined (MP1-DM40), much lower survival was  
333 detected (20%), and the survival curve was significantly different from those of the three  
334 previously mentioned ones. Additionally, earlier first event of death (day 5 vs. day 15) was  
335 observed in the MP1-DM40 treatment. Survival of *D. magna* in the 10 mg MP/L alone (MP10-  
336 DM0) treatment was intermediate (69.2%), but the survival curve was similar to these of the  
337 control, MP1-DM0, and MP0-DM40 treatments. However, when 10 mg MP/L was combined  
338 with 40 ng DM/L (MP10-DM40), all daphnids were dead by day 9, and this survival curve was  
339 highly significantly different from those of the other five curves, again with earlier first event  
340 of death (day 3). Survival of *D. magna* was reduced by 78.6% in the MP1-DM40 treatment  
341 compared to unique exposure to DM (MP0-DM40) or MP (MP1-DM0). Finally, the combined  
342 effect of MP and DM was much greater than the sums of individual exposures (mortality of  
343 6.66% [MP1-DM0] and 6.66% [MP0-DM40] vs. 80% [MP1-DM40]; mortality of 31.8 %  
344 [MP10-DM0] and 6.66% [MP0-DM40] vs. 100% [MP10-DM40]), which clearly suggests  
345 synergy between MP and DM.

346 Significant differences in longevity were detected among the six tested treatments  
347 (Kruskall-Wallis ANOVA;  $H_{5,90} = 67.18$ ;  $P = 2.45 \times 10^{-13}$ ). *D. magna* exposed to control  
348 treatment lived for the entire 21 d of the experiment, which was similar to the longevity  
349 observed for the MP1-DM0, MP10-DM0, and MP0-DM40 treatments, but higher than that of  
350 the MP1-DM40 ( $11.60 \pm 5.62$  d) and MP10-DM40 ( $5.15 \pm 2.23$  d) treatments (Table 1; Figure  
351 2a). The two-way Scheirer-Ray-Hare test performed on the six treatments revealed that

longevity was significantly affected by DM exposure ( $H_{1,77} = 21.81$ ;  $P = 3 \times 10^{-6}$ ), MP exposure ( $H_{2,77} = 30.69$ ;  $P = 2.17 \times 10^{-7}$ ), and the interaction of MP and DM exposure ( $H_{1,77} = 11.08$ ;  $P = 0.0039$ ).

In subsequent sections, data for the MP10-DM40 treatment are not given, as all of the *D. magna* died early in the experiment.

### 3.2. Cumulative number of molts

*D. magna* exposed to control treatment during the 21 d experiment underwent eight molts, which was similar to the values for MP1-DM0 and MP10-DM0 treatments, but higher than those of the MP0-DM40 ( $7.86 \pm 0.36$ ) and MP1-DM40 ( $7.33 \pm 0.58$ ) treatments (Table 1; Figure 2b). Significant difference in cumulative number of molts was detected among the five test treatments (Kruskall-Wallis ANOVA;  $H_{4,60} = 21.37$ ;  $P = 0.0003$ ). The cumulative number of molts in the MP1-DM40 treatment was reduced by 6.67% and 8.33% compared to unique exposure to DM (MP0-DM40) or MP (MP1-DM0), respectively. The two-way Scheirer-Ray-Hare test performed on MP0-DM0, MP1-DM0, MP0-DM40, and MP1-DM40 treatments revealed that the cumulative number of molts was not affected by DM exposure ( $H_{1,11} = 2.2$ ;  $P = 0.14$ ), MP exposure ( $H_{1,11} = 2.2$ ;  $P = 0.14$ ), or the interaction of MP and DM exposure ( $H_{1,11} = 2.2$ ;  $P = 0.14$ ).

### 3.3. Days to the first brood

In the control treatment (MP0-DM0), the first brood of *D. magna* appeared close to day 11 (Table 1; Figure 2c). There was a significant difference in the days to the first brood among the five tested treatments (Kruskall-Wallis ANOVA;  $H_{4,67} = 46.42$ ;  $P = 2 \times 10^{-9}$ ). Mann-Whitney tests followed by Bonferroni correction revealed that compared to control and MP1-DM0 treatments, for which values were similar, the days to the first brood of *D. magna* were significantly delayed in MP0-DM40 and in MP1-DM40 treatments, whereas values for the MP10-DM0 treatment fell in the middle and were not significantly different from values in the



other treatment. Days to the first brood in the MP1-DM40 group were 14.13% and 26.51% higher compared to single exposures (i.e., MP0-DM40 and MP1-DM0), respectively. These results suggest synergy between MP and DM effects, as days to the first brood for the mixture MP1-DM40 was delayed by 3 d (26.51% longer compared to MP1-DM0 and the control), and the delay was much greater than that observed for MP0-DM40 (1.22 d). The absence of significant difference between days to the first brood in MP0-DM40 and MP1-DM40 treatments seems to be partly related to the low survival in the latter treatment (20%). The two-way Scheirer-Ray-Hare test performed on MP0-DM0, MP1-DM0, MP0-DM40, and MP1-DM40 treatments revealed that the endpoint of days to the first brood was significantly affected by DM exposure ( $H_{1,15} = 11.36$ ;  $P = 0.0007$ ), but not by MP exposure ( $H_{1,15} = 0.45$ ;  $P = 0.5$ ) or by interaction of MP and DM exposure ( $H_{1,15} = 1.82$ ;  $P = 0.18$ ).

### **3.4. Number of broods**

In the control treatment (MP0-DM0), *D. magna* produced four broods on average during the 21 d experiment (Table 1; Figure 2d). There was a significant difference in number of broods among the five tested treatments (Kruskall-Wallis ANOVA;  $H_{4,62} = 27.13$ ;  $P = 2 \times 10^{-5}$ ). Mann-Whitney tests followed by Bonferroni correction revealed that compared to control and MP1-DM0 treatments, for which values were similar, the number of broods of *D. magna* was significantly lower in the MP1-DM40 treatment. Although lower than the control group value, the number of broods in the MP10-DM0 and M0-DM40 treatments did not differ significantly from values in the other groups. The number of broods in the MP1-DM40 treatment was 46% and 50% lower compared to the single exposure groups (M0-DM40 and MP1-DM0), respectively. These results suggest synergy between MP and DM for the following reasons: i) the number of broods of daphnids exposed only to MP (MP1-DM0 and MP10-DM0) were 0% and 8.25% lower than that of the control, respectively; ii) the value for daphnids exposed to DM did not differ significantly from that of the control (M0-DM40: 7.25% lower than control); and iii) the number of broods of *D. magna* exposed to the mixture treatment MP1-DM40 was

50% lower than that of the control (2 vs 4 broods). The absence of significant difference between MP0-DM40 and MP1-DM40 seems to be partly related to the low survival in the latter treatment (20%). The two-way Scheirer-Ray-Hare test performed on MP0-DM0, MP1-DM0, MP0-DM40, and MP1-DM40 treatments revealed that the number of broods was significantly affected by DM exposure ( $H_{1,59} = 22$ ;  $P = 2.72 \times 10^{-6}$ ), MP exposure ( $H_{1,59} = 12.6$ ;  $P = 0.00039$ ), and the interaction of MP and DM exposure ( $H_{1,59} = 8.58$ ;  $P = 0.0034$ ).

### 3.5. Number of neonates per surviving adult

In the control treatment (MP0-DM0), the number of neonates produced by surviving females was  $93.90 \pm 3.84$  (Table 1; Figure 2e). This result satisfies one validity criteria of the test ( $\geq 60$  neonates produced by surviving female; OECD, 2012). There was a significant difference in the number of neonates produced among the five tested treatments (ANOVA;  $F_{4,60} = 37.16$ ;  $P = 2 \times 10^{-16}$ ). *Post-hoc* Tukey tests revealed that compared to the control and MP1-DM0 treatments, for which values were similar (difference of 0.64%), the number neonates per surviving adult was significantly lower in the MP10-DM0 (−15.75 %), MP0-DM40 (−19.36%), and MP1-DM40 treatments (−60.6 %), but no significant differences were detected among these three treatments. The number of neonates per surviving adult in the MP1-DM40 treatment was 51.13% and 60.85% lower than that in the single exposure (MP0-DM40 and MP1-DM0) treatments, respectively. Again, these results suggest synergy between MP and DM effects because the number of neonates for daphnids exposed to 1 mg MP/L (MP1-DM0) was similar to that of the control, whereas individuals exposed to the mixture MP1-DM40 was 60.85% lower than that of *D. magna* exposed to DM alone (MP0-DM40). The two-way ANOVA performed on MP0-DM0, MP1-DM0, MP0-DM40, and MP1-DM40 treatments revealed that the number of neonates per surviving adult was significantly affected by DM exposure ( $F_{1,47} = 50.91$ ;  $P < 0.0001$ ), MP exposure ( $H_{1,47} = 30.28$ ;  $P < 0.0001$ ), and the interaction of MP and DM exposure ( $H_{1,47} = 11.71$ ;  $P = 0.001294$ ).

### 3.6. Body length

In the control treatment (MP0-DM0), the length of daphnids after 21 d of exposure was  $3.74 \pm 0.06 \mu\text{m}$  (Table 1; Figure 2f). There was a significant difference in daphnid length among the five tested treatments (ANOVA;  $F_{4,60} = 10.81$ ;  $P = 0.0288$ ), but *post-hoc* Tukey's tests revealed no pairwise differences. Body length in the MP1-DM40 treatment was reduced by 1.14% and 2.54% compared to unique exposure (MP0-DM40 or MP1-DM0) treatments, respectively. Again, the lowest value was found in the MP1-DM40 treatment ( $3.65 \pm 0.04 \mu\text{m}$ ;  $-2.54\%$ ), whereas the mean length value was slightly higher in the MP1-DM0 treatment ( $+0.2\%$ ) compared to the control. The mean value was lower in the MP0-DM40 treatment ( $-1.2\%$ ) compared to the control. The two-way ANOVA performed on MP0-DM0, MP1-DM0, MP0-DM40, and MP1-DM40 treatments revealed that body length was significantly affected by DM exposure ( $F_{1,47} = 12.5$ ;  $P = 0.000944$ ), but not by MP exposure ( $H_{1,47} = 0.7$ ;  $P = 0.41$ ) or by the interaction of MP and DM exposure ( $H_{1,47} = 1.7$ ;  $P = 0.19$ ).

### 3.7. Sensitivity of juveniles born during the experimentation: stress on stress experiment

Due to the high mortality in mixture treatments and the low production of juveniles,  $LC_{50,24h}$  and  $LC_{50,48h}$  tests were only performed on the neonates ( $< 24$  h) from parents that survived to the former experiment with MP and DM (Table 2). No significant differences in potassium dichromate  $LC_{50}$  were detected among treatments, as indicated by the overlapping 95% confidence intervals. However, the potassium dichromate  $LC_{50,48h}$  for the MP10-DM0 and MP0-DM40 treatments were lower than that of the control (0.6 and 0.575 mg/L, respectively, compared to 0.647 for the control MP0-DM0:  $-7.3\%$  and  $-11.1\%$ , respectively).

## 4. Discussion

The interaction effects of PE MPs and the pyrethroid insecticide DM on *D. magna* were evaluated by i) analyzing the behavior of MPs, ii) analyzing the life history traits of *D. magna*

exposed to MPs, DM, and combinations of the two, and iii) assessing the acute sensitivity modification in juveniles born from parents previously exposed to MPs or DM.

#### **4.1. Behavior of DM and MPs**

DM nominal concentrations in treatments without MPs decreased over time (24h: -45%; 48 h: -74%). This loss in DM concentration was also reported in Toumi et al. (2013) under the same conditions. This loss was non-exclusively linked to DM degradation and uptake by daphnids, and it was partly counteracted by renewing the medium every 2 d.

No loss, deformation, or abnormality of MPs were reported due to use of acetone as the solubilizing solvent for DM concentration preparation. MP concentration assessment was conducted using filtration through glass microfiber filters and weighing, and there was a linear and significant relationship between MP concentration and absorbance. No differences in MP concentration were observed between measurement at the bottom of the units and at one centimeter from the surface of the liquid. The prepared MP concentration was very close to the expected value (i.e., 19.58 vs. 20 mg MP/L; difference of 2.1%). However, after 2 or 3 d of rest, MP concentration in the water column had decreased, and the turbidity along the concentration gradient was less pronounced than at the beginning of the test. This phenomenon is related to floating of MPs on the surface of the water due to MPs density. This trend was also observed by Rehse et al. (2016) using the same MPs. This effect was *de facto* limited because the exposure solutions were renewed every 2 d.

#### **4.2. Effects of DM**

Acute effects of DM on *D. magna* were previously described by several researchers who reported effective (lethal) concentration for 50% of organisms after 48h of exposure [E(L)C<sub>50</sub><sub>48h</sub>] values ranging from 0.031 to 5 µg/L (see Table 2 in Toumi et al., 2013), which is far above concentrations potentially found in freshwater ecosystems (see introduction: from 2 ng/L to 24 µg/L). Toumi et al. (2013), who used the same exposure conditions and the same *D.*

*magna* strain, reported a E(L)C50<sub>48h</sub> of 0.32 µg DM/L. This value is high compared with concentrations known to exert chronic effects. Toumi et al. (2013) also reported a 20 % inhibitory concentration after 21 d of exposure (IC20<sub>21d</sub>) of 22 ng/L for the number of neonates per surviving adult, 34 ng/L for the cumulative number of molts, 48 ng/L for the number of broods, 152 ng/L for the population growth rate, and 160 ng/L for the longevity. They reported the lowest 21 d No-Observed-Effect Concentration (NOEC) as 9 ng /L which was in the same range as values reported by McNamara (1991; 4–9 ng/L) and the NOEC reported by Hanson et al. (2007) for freshwater communities using mesocosms (9.6 ng/L). In the present study, significant chronic effects of DM (MP0-DM40) included reduced longevity, number of broods, number of neonates per surviving adult, and length as well as delayed days to first brood. These results were in accordance with Toumi et al. (2013), who reported the same significant responses for the same daphnid strain (strain 1) exposed to the same concentration. Finally, our results revealed that the choice of 40 ng DM/L as the test concentration was relevant, as it had no effect on survival (and a very slight effect on longevity), but slight and/or significant sublethal effects on other parameters (see Section 2.5).

#### **4.3. Effects of MPs**

Many authors have reported that toxicity of MPs differs due to their composition, shape, age, and size (Ma et al., 2016; de Sà et al., 2018), as well as the species or the clone considered (Imhof et al., 2016; Foley et al., 2018). In *D. magna*, ingestion of MPs is concentration- and exposure time-dependent (Scherer et al., 2017; Canniff & Hoang, 2018). According to Scherer et al. (2017), the presence of natural particles (including food, such as algae) reduced MP uptake. They also found that *D. magna* consumed 1 and 10 µm MPs depending of their life stage (larger daphnids ingested more and larger MPs), but not 90 µm MPs, and that *D. magna* ingested more MPs (up to 6180 particles/h) than other organisms due to their filtering feeding mode of food acquisition.

In the present study, the significant effects on *D. magna* induced by MPs alone were reduced longevity, number of broods, and number of neonates by surviving adults. The only difference found by pairwise comparisons of MP0, MP1, and MP10 treatments was a significant reduction in number of neonates by surviving adults, and this was only for the highest tested MP concentration (10 mg/L compared to the control; gap of -15.75%). No significant differences were observed between the 1 mg MP/L treatment compared to the control, whereas exposure to 10 mg MP/L led to a slight, but not significant decrease in survival (69.2% vs. 100%; gap of -30.8%), longevity ( $21 \pm 0$  vs.  $20.6 \pm 1.55$  d; gap of -1.9 %), number of broods ( $3.67 \pm 0.50$  vs.  $4 \pm 0$ ; gap of -8.25%), and length ( $3.706 \pm 0.052$  vs.  $3.736 \pm 0.061$   $\mu\text{m}$ ; gap of -0.8%) and to an increase in days to the first brood ( $11.69 \pm 0.85$  vs.  $11.05 \pm 0.22$ ; gap of + 5.79%). Reshe et al. (2016), who used the same MPs in a more acute test (4 vs. 21 d in our study), reported a  $E(L)C_{50-96h}$  of 57.43 mg/L for *D. magna*.

Some reports described detrimental effects related to organic pollutants added during plastic production (Bakir et al., 2014b; Wagner & Lampert, 2018). However, confounding effects due to additives were not an issue in our study because the manufacturer did not use them in the production of the MPs used in our experiments. Even at higher concentrations than the ones we tested, many researchers reported weak or absent effects of MPs on *D. magna* reproduction, growth, and survival; however, they often reported other confounding factors, such as aging, food presence, relative abundance, and organism age. For example, Ma et al. (2016) observed no effect (immobilization) on *D. magna* exposed for 48 h to polystyrene MPs at sizes of 5, 10, or 15  $\mu\text{m}$ , even at concentration up to 100 mg/L, but they found a strong effect of polystyrene nanoplastics on immobilization of *D. magna*. This result highlights the importance of MP size on toxicity. Rist et al. (2017) reported that exposure of *D. magna* to 1 mg/L of 1  $\mu\text{m}$  polystyrene MPs had no effect on feeding rate, reproduction (time to first offspring, number of broods, number of neonates per brood), growth (number of molts, size of the animals at the end of the test), and mortality. Although the gut of *D. magna* was filled with green fluorescent PE microbeads (size range: 63–75  $\mu\text{m}$ ; particle density: 0.99–1.01 g/cm<sup>3</sup>), no

effects of MPs on *D. magna* survival and reproduction were reported for concentration between 25 and 100 mg/L (Canniff & Hoang, 2018). Imhof et al. (2017) found that a mixture of four MP polymers had no effects on mortality, reproduction, and morphology (body length, body width, and tail spine length) of adult *D. magna*, but they had limited and significant effects on juvenile morphology (body length, body width, and tail spine length). After 48 h of exposure, adult *Daphnia* exhibited alterations in expression of genes related to stress responses (HSP60, HSP70, and GST) and body function and composition (sarco(endo)plasmic reticulum calcium ATPase) (Imhof et al., 2017).

MPs can induce a reduction in natural prey consumption (Foley et al., 2018), but many studies reported the effect of food quantity or food/MP ratios on MP toxicity. For example, Aljaibachi and Callaghan (2018) tested polystyrene MPs and reported that daphnid mortality and reproduction were linked to availability of food rather than to MP concentration. Using the same MPs as those used in this study, Ogonowski et al. (2016) showed that at high concentration relative to the feeding suspension ( $10^5$  particles/mL contributing to 74% of suspended material), exposure of *D. magna* to secondary MPs led to high mortality, longer inter-brood period, and lower reproduction, whereas no effects were reported for the control treatment and primary MPs. Jemec et al. (2016) showed that ground PE terephthalate textile microfibers increased immobilization/mortality of *D. magna* after 48 h only when daphnids were not pre-fed with algae prior to the experiment (20–40% at exposure doses from 12.5 to 100 mg/L, with the maximum value for an exposure of 25 mg/L). If daphnids were fed before the experiments, no effect was reported.

In contrast, Canniff and Hoang (2018) observed that the algae *Raphidocelis subcapitata* grew more in the presence of MPs, suggesting that MPs could serve as substrates for *R. subcapitata* growth; subsequent intake of MPs by *R. subcapitata* could indirectly provide energy for survival and reproduction. Organisms serving as prey to larger predators (such as zooplankton) may be particularly susceptible to negative impacts of MPs, and they could have potential impacts throughout the food web (Foley et al., 2018) because MPs move up the food

chain from lower to higher trophic organisms (Hollman et al., 2013; Setälä et al., 2014; Nelms et al., 2018).

#### **4.4. Interactive effects of MPs and DM**

In the present study, a significant interaction (synergy) between MPs and DM was found for *D. magna* longevity. Significant differences in longevity between single exposures to MPs or DM and mixtures were observed: a reduction of 78.6% in the MP1-DM40 treatment was observed compared to MP0-DM40 or MP1-DM0 treatments, and 100% mortality within 9 d occurred in the MP10-DM40 treatment. In contrast, mortality was only 30.8% and 6.7% in the MP10-DM0 and MP0-DM40 treatments, respectively.

A synergistic effect was also reported for number of broods and number of neonates per surviving adult. No differences were observed among the control and the unique exposure to DM or MP treatments, except for number of neonates per surviving adult, for which a statistically significant 19.36% reduction was detected between control and MP0-DM40 treatments. Compared to the MP0-DM40 and MP1-DM0 treatments, exposure to the mixture MP1-DM40 resulted in a reduction up to 50% in number of neonates per surviving adult and a reduction of close to 50% in number of broods. Other tested endpoints showed the same trends, but effects were not detected through Sheirer-Ray-Hare tests and two-way ANOVA because the number of replicates was very low (3 to 13). Compared to unique exposure to DM or MP, exposure to the mixture MP1-DM40 resulted in the following: i) 14.13% and 26.51% increase in days to the first brood, respectively; ii) 6.67% and 8.33% reduction in cumulative number of molts; and iii) 1.14% and 2.54% reduction in body length. As is often the case, the number of neonates per surviving adult was the most sensitive tested endpoint.

Batel et al. (2016) used the same MPs as those used in our study and showed that food-borne MP-associated POPs (benzo[a]pyrene) can desorb in the fish intestine and then be transferred to the intestinal epithelium and liver. Bakir et al. (2014a) showed that polyvinylchloride and PE MPs can sorb and desorb C-14-DDT, C-14-phenanthrene (Phe), C-



14-perfluorooctanoic acid, and C-14-di-2-ethylhexyl phthalate. According to the authors, desorption rates were faster under simulated gut conditions compared to seawater (could be up to 30 times greater). Rochman et al. (2013a) showed that chemicals sorbed on MPs (marine fragments containing polycyclic aromatic hydrocarbons, polychlorinated biphenyls, and polybrominated diphenyl ethers) were ingested by the marine fish *Oryzias latipes* through vectorization by MP particles; they then bioaccumulated and induced pathological and oxidative stress as well as inflammation of the liver. When the fish exposed to PE MPs with low adsorbed chemicals (control) also showed signs of stress, they were less severe than in fish fed marine PE MPs.

Synergistic interaction effects of chemicals and MPs on aquatic organisms was reported in many studies. For example, Pacheco et al. (2018) showed that a mixture of high concentrations of 5 nm gold nanoparticles and 5 µm red microsphere MPs induced mortality that was higher than the sum of the lethal effects induced by gold nanoparticle and MPs alone. However, they also reported an antagonistic relationship on mortality when daphnids were exposed to a low concentration of the mixture as well as more pronounced effects than each type of particles when tested individually. Mixture treatments also damaged some reproduction endpoints (e.g., resulting in a higher number of aborted eggs), suggesting toxicological interactions. Toumi et al. (2018) also reported differences in type of interaction (antagonism, synergy) related to low or high exposure concentration. Kim et al. (2017) described the interactive effect of a mixture of polystyrene MPs and nickel on *D. magna* immobilization, but the type of interaction depended on the MPs used in the mixture: a slight synergistic effect was observed when using MPs coated with a carboxyl group, but a slight antagonistic effect was detected when using MPs without the carboxyl group coating. However, Horton et al. (2018) found no interactive effect when *D. magna* was acutely and conjointly exposed to polystyrene MPs and pesticides (dimethoate: 0.15–5 mg/L or DM: 0.016–10 µg/L). They concluded that polystyrene MPs are unlikely to act as a significant sink or as a vector for increased uptake of pesticides by aquatic organisms. There are several potential explanations for this difference from our results. For

example, Horton et al. (2018) used polystyrene MPs, which have lower affinity for hydrophobic chemical sorption than PE (Rochman et al., 2013b, Wang & Wang, 2018; Wagner & Lambert, 2018). Additionally, they conducted an acute test rather than a chronic one. Finally, they tested the interaction between polyamide MPs and bisphenol on immobilization of daphnids, Reshe et al. (2017) showed that chemical uptake through MPs is negligible compared to pollutant uptake through water for acute tests at high tested concentrations.

A growing body of studies illustrates the potential for plastic debris to sorb, concentrate, and transport POPs and metals (Bakir et al., 2014b; Wagner & Lambert, 2018; Prozić et al., 2019). Thus, MPs are considered to be a vector for hazardous HOCs (such as DM) in the aquatic environment, and therefore, as an important potential exposure route as well (Browne et al., 2013; Teuten et al., 2007). However, because MPs currently are less represented in the environment than other media that transfer HOC (e.g., colloids, phytoplankton, zooplankton), Koelmans et al. (2019) reported that the fraction sorbed by plastic remains low compared to the fraction held by other media. Nevertheless, MPs represent a new route of exposure for aquatic organisms that merits further study.

In the present study, PE microsphere MPs were used as a standardizable model to assess the interactive effect of MPs and DM on life history traits of *D. magna*. PE MPs are predominantly found in aquatic ecosystems, but more as fragments linked to secondary production than as microspheres (Wagner & Lambert, 2018). We tested higher MP concentrations than those found in the natural environment, but MP concentrations in the field are expected to increase in the near future due to atmospheric deposition, especially in lentic environments. Clear interactive effects of MPs and DM were found without any confounding effects linked to the food/MP ratio, as the ratios were comparable among treatment groups with or without exposure to DM. Although a recent WHO report (WHO, 2019) noted the absence of risk linked to the presence of MPs in drinking water, it is important to highlight that micropollutants in drinking water and water in natural ecosystems were not analyzed at the ng/L level and that a lot of the water is consumed by organisms throughout their life (i.e.,

chronic exposure). Therefore, caution is needed because the effects of micropollutant can be strongly enhanced in presence of MPs.

To better assess the risk linked to pollutant vectorization by MPs, further studies are needed. They should include more environmentally realistic MP concentrations, shapes/ages (fragments secondarily produced), and exposure conditions (e.g., mesocosms, in situ). Additionally, more pollutants, chemical and biological species, and effect of/on trophic routes should be considered.

## 5. Conclusions

MPs encompass a family composed of numerous types, compositions, sizes, and shapes of plastics, and all of these factors influence their bioavailability and ecotoxicity. Recent studies highlighted the capacity of MPs to vectorize toxicants due to their sorbing ability. The present study reports (for mortality, longevity, number of broods, and number of neonates per surviving adult) or suggests (for cumulative number of moult) a synergistic effect of DM and PE MPs on *D. magna*. Life history traits of *D. magna* were dramatically modified by exposure to a mixture of DM and MPs compared to exposure to MPs or DM alone. The detrimental effects of this synergy on daphnid survival, growth, and reproduction suggest potential drastic effects of these kinds of mixtures on daphnid populations, which are at the base of aquatic food chain. Literature suggests that MPs can reach all ecosystem on Earth through wet and dry atmospheric deposition. Thus, MP concentration in ecosystems with high residence time (such as lentic ecosystems) will increase over time, especially as plastic wastes will also increase over time. Hydrophobic micropollutants (defined as xenobiotic chemicals that have strong effects at low concentrations, such as pesticides and POPs) have a new route of exposure through ingestion or inhalation of MPs, which could pose a serious threat to organisms and ecosystems. In our study, sublethal effects of DM on daphnids were observed at ng/L concentrations. The same exposure in combination with MPs caused greater lethal effects. Therefore, caution is needed because the effects of micropollutants can be strongly enhanced by the presence of MPs.

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 858 distinct population segment, and delta smelt (*Hypomesus transpacificus*), and the federally  
 859 endangered California clapper rail (*Rallus longirostris obsoletus*), California freshwater  
 860 shrimp (*Syn caris pacificus*), California tiger salamander (*Ambystoma californiense*),  
 861 Sonoma county distinct population segment and Santa Barbara county distinct population  
 862 segment, San Francisco garter snake (*Thamnophis sirtalis tetrataenia*), and tidewater goby  
 863 (*Eucyclogobius newberryi*) available at <[https://www.epa.gov/espp/litstatus/effects/redleg-](https://www.epa.gov/espp/litstatus/effects/redleg-frog/2013/deltamethrin/analysis.pdf)  
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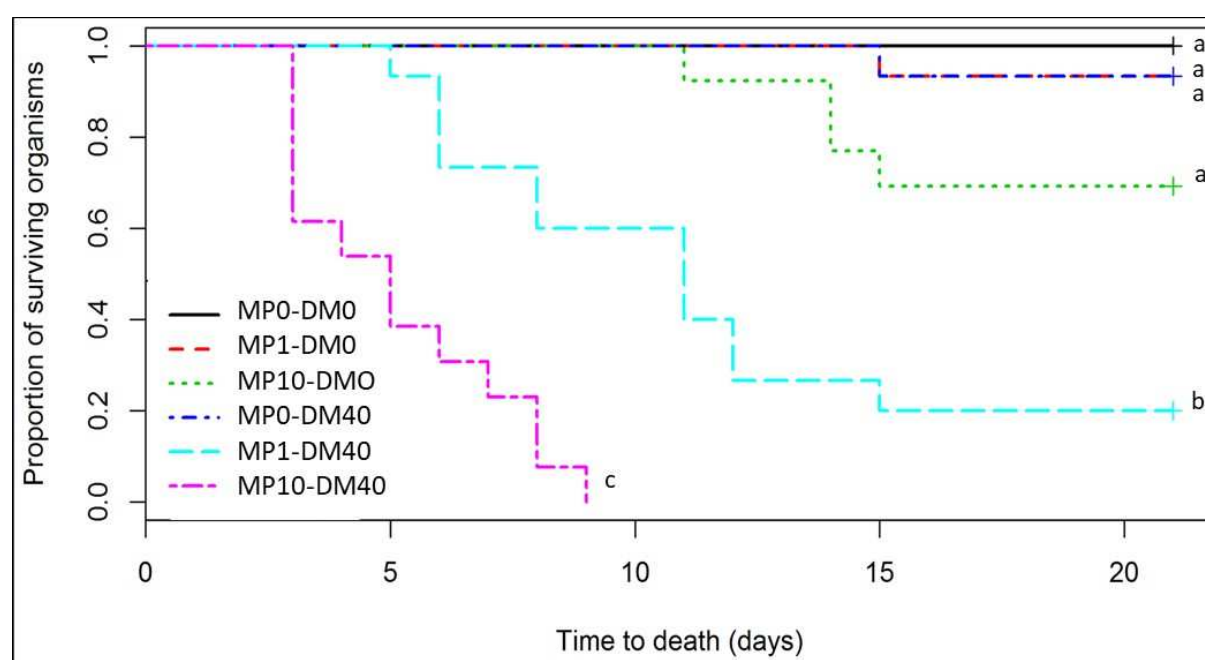
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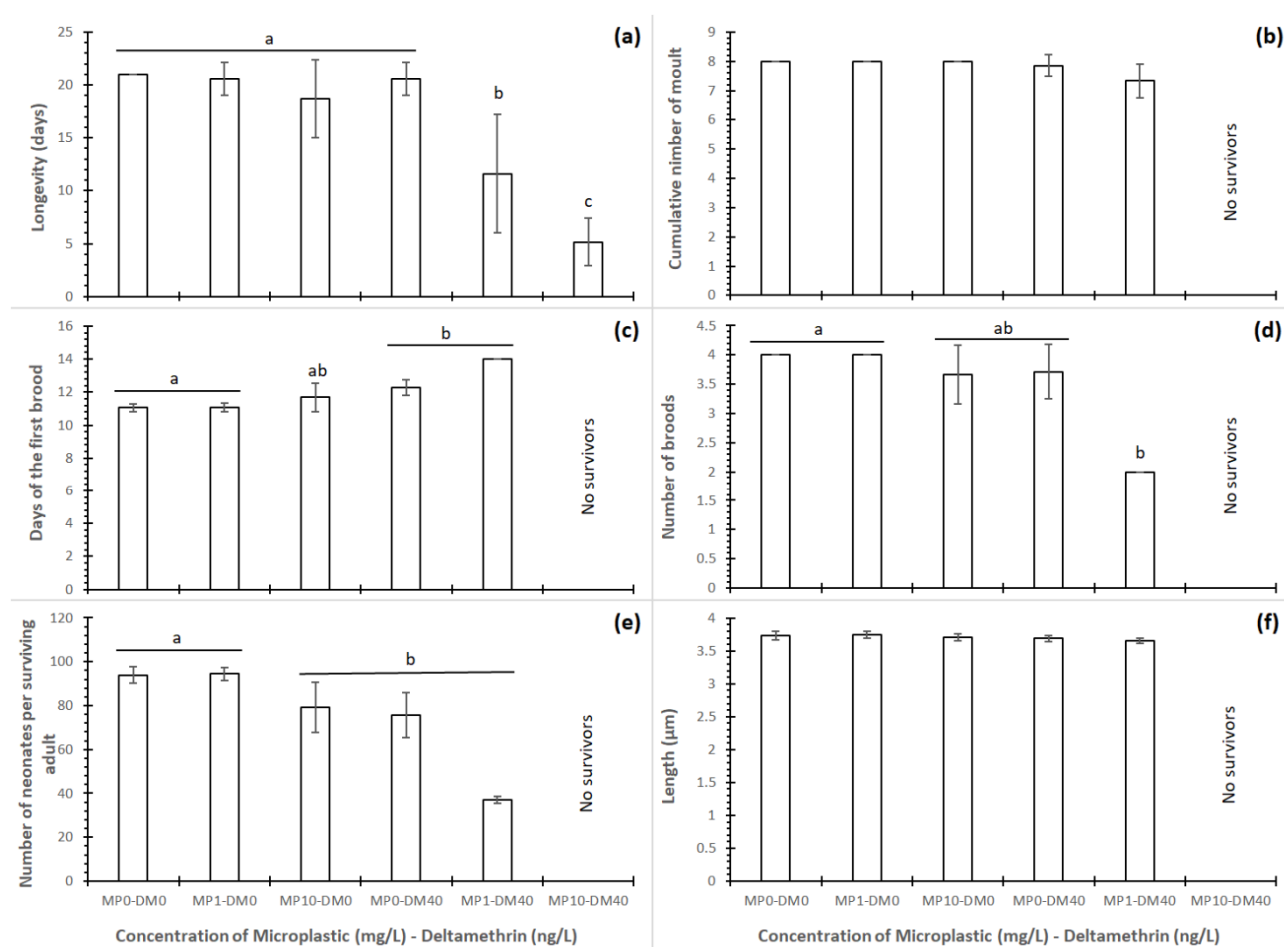
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**Figure 1:** Kaplan-Meier curves for the time-to-death of organisms exposed to 0, 1, or 10 mg MPs/L with 0 or 40 ng DM/L. The plus sign (+) at the end of curves represents censored data. Overall, out of 91 organisms, 60 were right-censored. The numbers after DM and MP in the combined treatment correspond to deltamethrin (DM) and microplastic (MP) concentrations, respectively (in ng/L and in mg/L, respectively). Different lowercase letters indicate significantly different survival curves after the Log-rank test with Bonferroni correction ( $n = 15$ ;  $p < 0.00333$ ).



**Figure 2:** Mean ( $\pm$  standard deviation) of longevity (a), cumulative number of molts (b), days to the first brood (c), number of broods (d), number of neonates per surviving adult (e), and length (f) of *D. magna* exposed to MPs and DM for 21 days. The numbers after MP and DM in the combined treatment correspond to MP and DM concentrations, respectively (in mg/L and in ng/L, respectively). No significant differences were observed between the control (MP0-DM0) and solvent control (with only acetone MP0-DM0ac), so the data were pooled to constitute the control (MP0-DM0). Different lowercase letters indicate significantly different groups after *post-hoc* Tukey's tests ( $p < 0.05$ : for length, number of neonates) or the Mann-Whitney test with Bonferroni correction ( $n = 15$ ;  $p < 0.00333$ ; longevity, cumulative number of molts, days to the first brood, number of broods). No *D. magna* survived in the MP10-



DM40 treatment.

Felten *et al.*, Figure 2

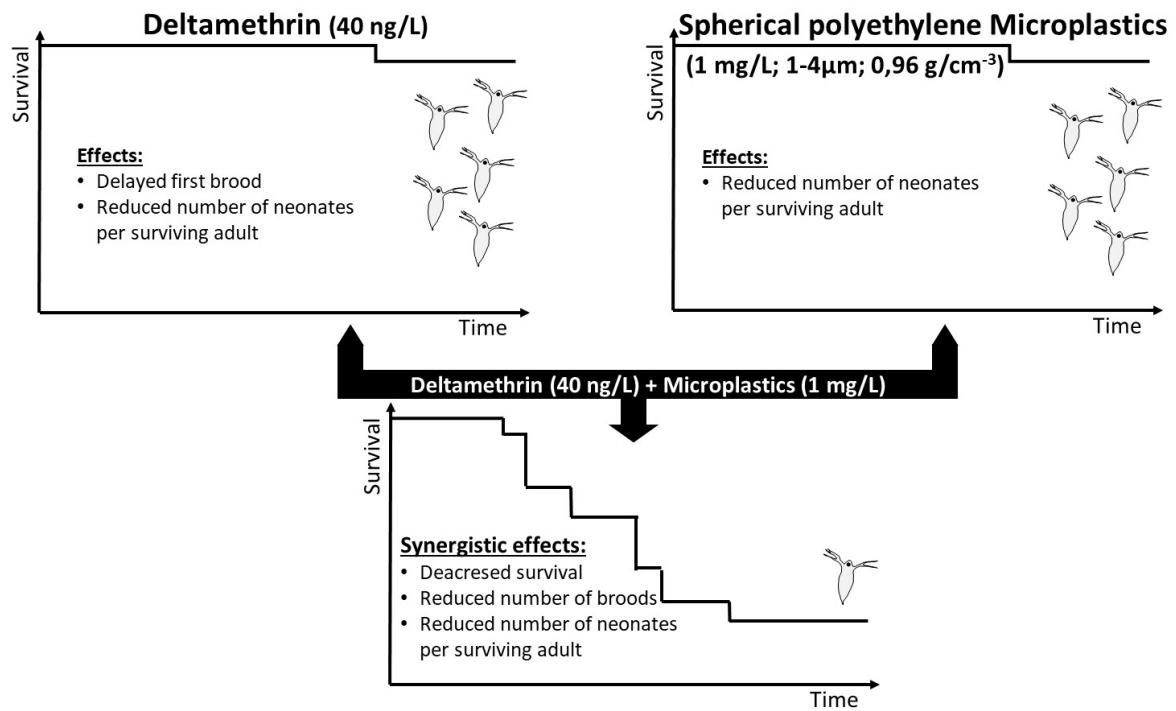
**Table 1:** Mean ( $\pm$  standard deviation) of longevity, cumulative number of molts, days to the first brood, number of broods, number of neonates per surviving adult, and length of *D. magna* exposed for 21 d to MPs and/or DM. The numbers after MP and DM in the combined treatment groups correspond to MP and DM concentrations, respectively (in mg/L and in ng/L, respectively). DM0ac is the solvent control treatment containing only acetone. Ns: no significant differences between the two controls (MP0-DM0 and MP0-DM0ac), which were pooled to constitute the control (MP0-DM0). For the considered endpoints, different lowercase letters indicate significantly different groups after *post-hoc* Tukey's tests ( $p < 0.05$ : for length, number of neonates per surviving adult) or the Mann-Whitney test with Bonferroni correction ( $n = 15$ ;  $p < 0.00333$ ; longevity, cumulative number of molts, days to the first brood, number of broods).

Labels	Microplastics (mg/L)	Deltamethrin (mg/L)	Survival (%)	Living daphnids after 21 days	Longevity (days)	Number of cumulative molts	Days to the first brood	Number of broods	Number of neonate per surviving adult	Length ( $\mu\text{m}$ )
<b><i>Controls</i></b>										
MP0-DM0	0	0	100	10	21.0 $\pm$ 0.00	8.00 $\pm$ 0.00	11.00 $\pm$ 0.00	4.00 $\pm$ 0.00	94.10 $\pm$ 4.25	3.733 $\pm$ 0.055
MP0-DM0ac	0	0	100	10	21.0 $\pm$ 0.00	8.00 $\pm$ 0.00	11.10 $\pm$ 0.32	4.00 $\pm$ 0.00	93.70 $\pm$ 3.59	3.738 $\pm$ 0.069
<b><i>Mean (MP0-DM0)</i></b>	0	0	100	20	21.0 $\pm$ 0.00	8.00 $\pm$ 0.00	11.05 $\pm$ 0.22	4.00 $\pm$ 0.00	93.90 $\pm$ 3.84	3.736 $\pm$ 0.061
<b><i>Treatments</i></b>										
MP1-DM0	1	0	93.3	14	20.6 $\pm$ 1.55	8.00 $\pm$ 0.00	11.07 $\pm$ 0.26	4.00 $\pm$ 0.00	94.50 $\pm$ 3.01	3.745 $\pm$ 0.051
MP10-DM0	10	0	69.2	9	18.7 $\pm$ 3.71	8.00 $\pm$ 0.00	11.69 $\pm$ 0.85	3.67 $\pm$ 0.5	79.11 $\pm$ 11.42	3.706 $\pm$ 0.052
MP0-DM40	0	40	93.3	14	20.6 $\pm$ 1.55	7.86 $\pm$ 0.36	12.27 $\pm$ 0.46	3.71 $\pm$ 0.47	75.71 $\pm$ 10.18	3.692 $\pm$ 0.049
MP1-DM40	1	40	20	3	11.6 $\pm$ 5.62	7.33 $\pm$ 0.58	14.00 $\pm$ 0.00	2.00 $\pm$ 0.00	37.00 $\pm$ 1.73	3.650 $\pm$ 0.040
MP10-DM40	10	40	0	0	5.15 $\pm$ 2.23	no survivor				

**Table 2:** LC<sub>50</sub> values and their 95% confidence intervals for neonates (whose parents were exposed to MPs or DM) after 24 h (LC50<sub>24h</sub>) and 48 h (LC50<sub>48h</sub>) of exposure to potassium dichromate. No mixture (MP + DM) effects were tested because mortality was too high under these conditions. The numbers after MP and DM in the combined treatment correspond to MP and DM concentrations, respectively (in mg/L and in ng/L, respectively). MP0-DM0ac is the solvent control treatment containing only acetone.

Treatment (Microplastic-Deltamethrin)		MP0-DM0	MP0-DM0ac	MP1-DM0	MP10-DM0	MP0-DM40
<b>LC50<sub>24h</sub></b>		<b>0.834</b>	<b>0.830</b>	<b>0.815</b>	<b>0.855</b>	<b>0.873</b>
95 % confidence interval	97.5%	0.881	0.883	0.877	0.906	0.921
	2.5%	0.788	0.777	0.751	0.804	0.825
<b>LC50<sub>48h</sub></b>		<b>0.647</b>	<b>0.684</b>	<b>0.641</b>	<b>0.600</b>	<b>0.575</b>
95 % confidence interval	97.5%	0.692	0.740	0.698	0.655	0.631
	2.5%	0.602	0.627	0.584	0.544	0.520





Felten *et al.*, Graphical abstract