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# Polystyrene microplastics exposure modulated the content and the profile of fatty acids in the Cladoceran *Daphnia magna*

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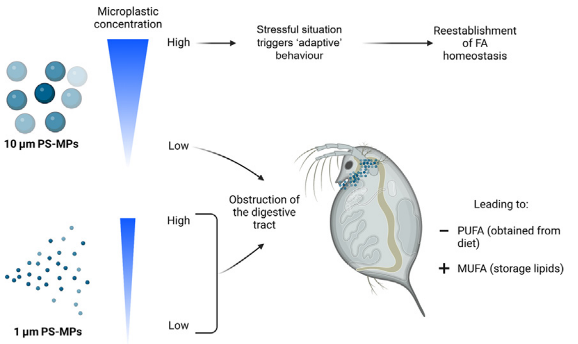
## HIGHLIGHTS

- Polystyrene microplastics (PS-MPs) affected *D. magna* fatty acid content and profile.
- Differently sized PS-MPs induced a decrease in fatty acids unsaturation.
- The biosynthetic ω3, ω6 and ω9 FAs pathways were modulated in the presence of PS-MPs.
- Differently sized PS-MPs affected fatty acids metabolism.
- The synthesis of EPA is promoted under low concentrations of PS-MPs.

## GRAPHICAL ABSTRACT



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## ABSTRACT

A growing number of studies has shown that the exposure to microplastics (MPs) of different polymeric compositions can induce diverse adverse effects towards several aquatic species. The vast majority of such studies has been focused on the effects induced by the administration of MPs made by polystyrene (PS; hereafter PS-MPs). However, despite the increase in the knowledge on the potential toxicity of PS-MPs, there is a dearth of information concerning their role in affecting energy resources and/or their allocation. The present study aimed at exploring the impact of 21-days exposure to three concentrations (0.125, 1.25 and 12.5 μg $mL^{-1}$) of PS-MPs of different sizes (1 and 10 μm) on fatty acids (FAs) profile of the freshwater Cladoceran *Daphnia magna*. The exposure to the highest tested concentration of PS-MPs induced an overall decrease in *D. magna* total FAs content, independently of the particle size. Moreover, a change in the accumulation of essential FAs by the diet was noted, with an enhanced synthesis of monounsaturated FAs-rich storage lipids. However, a sort of adaptation to counteract the adverse effects and to re-establish the FAs homeostasis was observed in individuals treated with high PS-MPs concentration, independently of their size. These results indicate that the exposure to PS-MPs could alter the allocation or induce changes in FAs composition in *D. magna*, with potential long-term consequences on life-history traits of this zooplanktonic species.

## 1. Introduction

Plastic contamination represents one of the main environmental issues that our society must tackle. Despite the undeniable benefits of plastics (Andrady and Neal, 2009), the improper disposal, and management, coupled with the persistence of plastic waste result in the accumulation of

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plastics in terrestrial and aquatic ecosystems. Once in the environment, plastics undergo weathering processes due to mechanical erosion, physical abrasion, solar radiation and biological degradation (De Sá et al., 2018) leading to their breakage and fragmentation in small-sized items. Microplastics (MPs) have been recently categorised as any plastic item in the 1 to <1000 μm size range (Hartmann et al., 2019). They are considered as a hot topic in environmental studies because of their presence in every ecosystem worldwide, as well as their potential toxicity towards organisms. A growing number of monitoring surveys has detected MPs in the atmosphere (Can-Güven, 2021), marine (Horton and Barnes, 2020), freshwater (Bellasi et al., 2020) and terrestrial (Rillig and Lehmann, 2020) ecosystems, as well as in the so-called remote areas, such as the deep sea, Arctic and Antarctica (Shahul Hamid et al., 2018; González-Pleiter et al., 2021), and high-mountain areas (Ambrosini et al., 2019; Bergmann et al., 2019; Parolini et al., 2021; Crosta et al., 2022).

Freshwaters ecosystems have been identified as the primary source of plastic contamination for seas and oceans (Wagner et al., 2014; Eerkes-Medrano et al., 2015). In recent years there has been growing interest in the assessment of MPs contamination in rivers and lakes, and their impact on freshwater organisms (Wagner and Lambert, 2018). The MPs contamination has been estimated as high as 0.001–0.1 items/$m^2$ in lake and 0.1–1 items/$m^2$ in river water, while 10–10,000 items/$m^2$ and 1–1000 items/$m^2$ range has been estimated for lake and river sediments, respectively (Dris et al., 2015). These estimates have been confirmed by recent field studies (Li et al., 2018, 2020). Depending on their physical and chemical features, a wide array of MPs with different environmental fate contributes to freshwater contamination. The pattern is dominated by polyethylene (PE), polypropylene (PP), polystyrene (PS) and polyethylene terephthalate (PET), according to the demand and use of plastics (PlasticsEurope, 2020).

In addition to surveys on the presence and the abundance of MPs in freshwaters, a growing number of investigations has focused on their impact on organisms. All the studies agreed that MPs of different size, shape and polymeric composition are efficiently ingested by many aquatic organisms at different trophic levels and stages of development (*e.g.*, Cole et al., 2013; Imhof and Laforsch, 2016; Scherer et al., 2017; Canniff and Hoang, 2018; Al-Jaibachi et al., 2018; Al-Jaibachi and Callaghan, 2018; An et al., 2021). However, the effects induced by the ingestion of diverse MPs by freshwater and marine invertebrates resulted as contrasting and depended on different factors, including the size, the shape, the polymer and the administered concentrations of MPs. For instance, some experimental studies has demonstrated that the exposure to MPs reduced food uptake and energy-supply related changes as a consequence of a false sense of satiation in the marine copepod *Calanus helgolandicus* (75 microplastics $mL^{-1}$ of 20 μm PS beads; Cole et al., 2015), in the shore crab *Carcinus maenas* ($9.4 \times 10^5$ microspheres $L^{-1}$ of 10 μm PS microspheres; Watts et al., 2014) and in the freshwater amphipod *Gammarus fossarum* (range 100–13,380 fibres $cm^{-2}$ base area of glass beakers of 500 × 20 μm polyamide fibres; Blarer and Burkhardt-Holm, 2016), the onset of oxidative stress in in larvae of the sea urchin *Pseudechinus huttoni* (range 10–10,000 microspheres $mL^{-1}$ of 1–5 μm of unknown polymer; Richardson et al., 2021) and the decrease in growth and reproduction rate in the oyster *Crassostrea gigas* (0.023 mg $L^{-1}$ of 2 and 6 μm PS microspheres; Sussarellu et al., 2016) and growth and development of veliger of the bivalve *Crepidula onyx* ($6 \times 10^4$ particles $mL^{-1}$ and $1.4 \times 10^5$ particles $mL^{-1}$ of 2.0–2.4 μm PS microspheres; Lo and Chan, 2018). In contrast, other investigations have reported slight or null effects on growth and survival of larvae of the sea urchin *Tripneustes gratilla* (1–300 spheres $mL^{-1}$ range of 10–45 μm of polyethylene (PE) microspheres; Kaposi et al., 2014), survival, morphological traits and reproductive parameters in the freshwater cladoceran *Daphnia magna* (290–580 particles $mL^{-1}$ of two mixtures of ~40 μm PA, polycarbonate, polyethylene terephthalate (PET) and polyvinylchloride and acrylonitrile-burtdiene-styrene terpolymer, plasticized polyvinyl chloride, polyoxymethylene homopolymer and styrene-acrylonitrile copolymer MPs; Imhof et al., 2017), and on the survival, development, metabolism and feeding activity of the freshwater amphipod *Gammarus pulex* (0.8–4000 particles $mL^{-1}$ of 0–150 μm PET items; Weber et al., 2018).

Zooplanktonic filter-feeder species indiscriminately ingest MPs during swimming and feeding activity (Gorokhova, 2015) resulted as particularly prone to the effects of these contaminants. Several studies have demonstrated that the presence of MPs in the digestive tract of different zooplanktonic species can result in a series of negative effects, including changes in physiological (*e.g.*, movement, growth, feeding, survival, gene expression), systemic (*i.e.*, digestive, reproductive and neuromodulation systems) and reproductive (*i.e.*, amount and size of offspring) endpoints (see He et al., 2021 and references therein). Focusing on freshwater zooplanktonic species, studies of *Daphnia magna* have demonstrated that the ingestion of MPs can negatively affect food availability (Al-Jaibachi and Callaghan, 2018), oxidative stress response (Liu et al., 2022), growth, swimming activity and reproduction (Jemec et al., 2016; Ogonowski et al., 2016), with different outcomes depending on the polymer composition, size and shape (Rosenkranz et al., 2009; Jemec et al., 2016; Frydkjær et al., 2017; Na et al., 2021; Song et al., 2021a, 2021b). However, our previous study has demonstrated that the 21-days exposure to increasing concentrations (0.125, 1.25 and 12.5 μg $mL^{-1}$) of polystyrene microplastics (PS-MPs) of different size (1 and 10 μm in diameter) induced 'putatively positive' effects (De Felice et al., 2019). Indeed, independently of MPs size, individuals exposed to the highest tested concentration grew more, swam longer distances and faster, and generated more offspring than conspecifics from the control group, suggesting that PS-MPs caused a modulation of the energy reserves of cladocerans (De Felice et al., 2019).

To check for this hypothesis, the present study aimed at replicating the experimental design used by De Felice et al. (2019) to explore if the 21-days exposure to three concentrations (0.125, 1.25 and 12.5 μg $mL^{-1}$) of differently sized PS-MPs (1 and 10 μm in diameter) could affect the energy reserves of *D. magna* individuals in terms of modulation of the fatty acids (FAs) profile and content. In fact, FAs are critical for the permeability and generation of the cell membrane, function as essential nutrients and energy reserves in the metabolic systems at all trophic levels (Lee et al., 2018; Neves et al., 2015) and are crucial for growth and reproduction (Arts and Kohler, 2009). Moreover, FAs are considered as markers of environmental stressors because when stressors emerge, FAs metabolism can be regulated through cellular physiological and biochemical responses (Gonçalves et al., 2016; Yang et al., 2021).

## 2. Materials and methods

### 2.1. Exposure to polystyrene microplastics (PS-MPs)

*Daphnia magna* individuals were reared in the facility located at the University of Milan, (Italy) according to the procedure described by Parolini et al. (2018) and De Felice et al. (2019). Individuals were reared in a commercial mineral water (San Benedetto®). Forty individuals/L were maintained in glass beakers at 20.0 ± 0.5 °C under a natural photoperiod (16 h light: 8 h dark) to ensure continuative parthenogenic reproduction. In the husbandry individuals were fed *ad libitum* with a suspension of the unicellular green alga *Raphidocelis subcapitata* ($8 \times 10^6$ cells $individuals^{-1}$ $day^{-1}$ until they were 8-days old, then $16 \times 10^6$ cells $individuals^{-1}$ $day^{-1}$ up to the end of the experiment) and the yeast *Saccharomyces cerevisiae* ($15 \times 10^6$ cells $mL^{-1}$). The culture medium was renewed every second day.

In the present study, the experimental design performed by De Felice et al. (2019) was faithfully replicated. Red PS-MPs with two different sizes (1 and 10 μm of diameter) were purchased from Sigma-Aldrich (product number 89904 and 72986 for particles of 1 and 10 μm of diameter, respectively; Milan, Italy). Chemical-physical properties of PS-MPs were provided by the supplier (1 μm nominal diameter − calibrated particle diameter = 1.07 ± 0.03 μm, density 1.51 g $cm^{-3}$; 10 μm nominal diameter − calibrated particle diameter = 9.86 ± 0.13 μm, density = 1.51 g $cm^{-3}$). The size and polymeric composition of MPs used in the experiments were confirmed through Scanning Electron Microscopy and Fourier Transformed Infrared Spectroscopy (FT-IR) analyses, respectively, according to De Felice et al. (2019). A stock solution for each MPs type was prepared through a 1:1000 (v/v) dilution of the commercial standard in the mineral water used to prepare the culture medium used. Briefly, *D. magna* individuals were independently exposed to three increasing, unrealistic concentrations of 1 μm and 10 μm PS-MPs (0.125, 1.25 and 12.5 μg mL$^{-1}$). The concentrations were the same tested in our previous study and in other experiments performed on marine and freshwater organisms (*e.g.*, Messinetti et al., 2018; De Felice et al., 2018). Three replicates per every single experimental condition were performed, for a total of 60 individuals per treatment. In each replicate, twenty daphnids (<24 h old) were exposed in 500 mL beakers filled with commercial mineral water (San Benedetto®) and maintained at 20.0 ± 0.5 °C under 16 h light:8 h dark photoperiod. Experiments were performed under semi-static conditions for 21 days, similarly to the *Daphnia magna* reproduction test (OECD test No. 211), with a daily renewal of the exposure medium (*i.e.*, water, feed and PS-MPs). During the duration of the experiments, the individuals were fed *ad libitum* as described for husbandry. At the end of the experiments, all live individuals were collected from each beaker, quickly frozen in liquid nitrogen and stored at −80 °C. *D. magna* individuals were then lyophilised in a freeze-dryer (Edwards Pirani 1001) for 36 h and stored in a desiccator until the FAs analysis. Three pools of twenty individuals each were collected per experimental group from both 1 and 10 μm PS-MPs experiments, including controls (without exposure to PS-MPs). Residual moisture was determined (Gibertini Eurotherm dry weight balance) to correct to dry weight (DW).

### 2.2. Fatty acids analysis

The fatty acids (FAs) composition of dried *D. magna* individuals was determined and expressed as FA methyl esters (FAMEs), as described by Nogueira et al. (2020). Briefly, FAs were converted to FAMEs by adding a mixture of ethyl acetate-methanol (1:19 v/v) to dry biomass that was then left at 80 °C for 1 h and then further extracted with heptane. FAMEs were analyzed by gas chromatography (Agilent HP 6890) equipped with a mass selective detector (Agilent 5973) and a capillary column DB-225 J&W (30 m × 0.25 mm inner diameter, 0.15 μm film thickness). The chromatographic conditions were as follows: initial temperature of the oven was 35 °C for 0.5 min; was increased by 25 °C min$^{-1}$ to 195 °C; followed by 3 °C min$^{-1}$ to 205 °C; and 8 °C min$^{-1}$ until reaching the final temperature of 230 °C for 3 min. The temperature of the injector was 250 °C, that of the transfer line, 280 °C; and the split ratio was 1:100. Helium was used as the carrier gas, with a flow rate of 2.6 mL min$^{-1}$. At least two replicates were performed for each gas chromatography analysis.

FAMEs were identified based on comparing their mass spectra with the equipment mass spectral library (Wiley-Nist) and comparing the retention times and mass spectra fragmentation to known standards (Supelco 37 component FAME Mix; Sigma-Aldrich CRM47885). Heneicosanoic acid (C21:0) was used as an internal standard. For quantitative analysis, GC–MS was calibrated with pure reference compounds (Supelco 37 component FAME Mix; Sigma-Aldrich CRM47885) relative to the internal standard. The respective response factors were calculated as an average of six GC–MS runs. The results presented are the mean values ± standard deviation (SD) of FAMEs expressed in the percentage of total FAs detected.

### 2.3. Statistical analysis

The effects of PS-MPs exposure on the levels of FAs in *D. magna* individuals were investigated by a one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparisons test. Normality and homogeneity of variance was previously checked by means of Shapir-Wilks and Bartlett's tests, respectively. Pearson's correlation analysis was performed to check for the relationships and changes in the relationships among different fatty acids after the exposure to increasing concentrations of differently shaped PS-MPs. *p*-Values < 0.05 were considered as statistically significant. Statistical analyses were performed using GraphPad Prism version 9.3.0 for Windows, GraphPad Software (San Diego, California, USA).

## 3. Results and discussion

A previous study performed under these same experimental conditions demonstrated that a 21-days exposure to 1 and 10 μm PS-MPs resulted in a quick PS-MPs ingestion and accumulation into the digestive tract of *D. magna* individuals without causing mortality (De Felice et al., 2019). Similarly, a quick and efficient ingestion (data not shown) and no mortality was observed over the whole duration of the present experiment, in agreement with the findings reported by other studies on *Daphnia magna* individuals exposed to different PE (17–34 μm; An et al., 2021) and PS (100 nm and 2 μm; Rist et al., 2017) microbeads in a similar range of concentrations.

### 3.1. Effect of PS-MPs exposure on the total FAs content

The 21-days exposure to three increasing concentrations of 1 and 10 μm PS-MPs induced the modulation of fatty acids (FAs) profile in *D. magna* individuals. The exposure to the highest tested concentration of PS-MPs induced an overall decrease in total FAs content independently of the particle size (Fig. 1). FAs can be used to monitor environmentally-induced stress (Filimonova et al., 2016; Gonçalves et al., 2016; Guschina et al., 2020; Yang et al., 2021), which can affect energy reserves (Lee et al., 2018; Neves et al., 2015). The significant decrease in the total content of FAs observed in *D. magna* exposed to 12.5 μg mL$^{-1}$ of both PS-MPs sizes suggests that treated individuals might suffer an high stressful situation, forcing them to activate biochemical or behavioral energy-demanding defense responses. Indeed, the exposure to the highest concentration of PS-MPs might induce a generic or oxidative stress condition in treated organisms, which could activate physiological or behavioral processes to prevent or to counteract negative consequences towards organism health status. Previous studies on aquatic organisms showed an increase in antioxidant defenses to tackle the overproduction of reactive oxygen species (ROS) due to PS-MPs (Zhang et al., 2019; Liang et al., 2021; Umamaheswari et al., 2021). For instance, a recent study by Tang et al. (2019), showed that the 10-days exposure to 1.25 μm PS-MPs (concentration range 2–8 mg L$^{-1}$) altered the expression of genes involved in oxidative stress defense, specifically the expression of thioredoxin reductase (TRxR), an enzyme involved in the thioredoxin system that plays a crucial role in cellular antioxidant defense eliminating the excess of ROS (Lushchak, 2011), with subsequent deregulation of genes related to energy production. Moreover, our previous study demonstrated that the exposure to 12.5 μg mL$^{-1}$ of PS-MPs induced an increase in swimming activity of *D. magna* individuals, suggesting an avoidance behavior fulfilled by the organism to swim away from a highly contaminated environment or an attempt made by the cladocerans to get rid of the particles on their body and appendixes (De Felice et al., 2019). Thus, the implementation of physiological or behavioral defense in a stressful situation requires a massive amount of extra energy (Tang et al., 2019). As PS-MPs retention in the digestive tract of *D. magna* can result in a false sense of satiation, reducing food ingestion and energy reserves (Wright et al., 2013), the decrease in

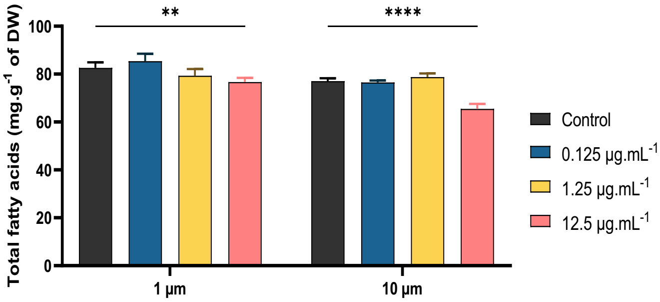

**Fig. 1.** Total fatty acids content of *Daphnia magna* after 21-days exposure to PS-MPs, with different sizes (1 and 10 μm) and concentrations (0.125, 1.25 and 12.5 μg mL$^{-1}$). Statistical significance: $^{**}p < 0.01$, $^{****}p < 0.0001$ after ANOVA followed by Dunnett's multiple comparisons test. Data are reported as mean ± SD (n = 3).

FAs content might be due to their use or re-allocation to support defense mechanisms of treated individuals. The allocation of food-derived resources towards growth and reproduction depends on their availability (Mariash et al., 2017; Nogueira et al., 2004). Previous studies have shown the importance of storage lipids for the fitness of *D. magna* individuals (Jordão et al., 2016; Mariash et al., 2017). The life-history traits of *D. magna* are strongly influenced by food concentration. Under food limitation, individuals mature later, present smaller body and clutch sizes compared to conspecifics experiencing high food availability (Klintworth and Von Elert, 2020). Although *D. magna* individuals were fed *ad libitum* during the whole duration of the experiments, the ingestion of PS-MPs and the filling of the digestive tract might cause a sort of fasting condition in treated organisms, reducing the uptake of food and forcing individuals to the mobilisation, and use of FAs as an energetic source to support organism functions. When organisms experience a stressful situation such as fasting, storage lipids can be metabolised, and FAs become available as a metabolic fuel (Tessier et al., 1983; Raclot, 2003). Under a potential limitation of food, due to the presence of PS-MPs in the digestive tract, individuals might consume internal lipid reserves to sustain the basic metabolic processes of the somatic body, causing the decrease of FAs content, as observed in both neonates and juveniles of *D. magna* experiencing short-term starvation (Yang et al., 2021).

The decrease of FAs content could be also related to the allocation of storage lipids from lipid droplets to eggs, a process that could explain the increase in offspring number observed in De Felice et al. (2019). The authors showed an increase in the reproductive effort of *D. magna* individuals exposed to the same high concentration of PS-MPs. This result was explained as an effort accomplished by cladocerans in a highly contaminated environment, where adults preferred to invest energy in their reproductive fitness rather than survival. The significant reduction of the total FAs content, especially after the exposure to 12.5 μg $mL^{-1}$ of PS-MPs of both sizes might promote the allocation of FAs to the eggs in order to provide offspring with sufficient energetic resources to tackle stressful situations caused by the exposure to a high concentration of MPs, as well as to outgrow the most vulnerable instars faster. Indeed, the allocation of FAs to offspring represents a part of life-history changes in response to environmental stressors, as demonstrated in *D. magna* adults exposed to predator-borne kairomones (Stibor and Navarra, 2000; Hahn et al., 2019; Klintworth and Von Elert, 2020). This hypothesis might be further confirmed through the comparison of the FAs content and profile of *D. magna* adults treated with PS-MPs and their offspring.

### 3.2. Size-related effect of PS-MPs on FAs profile

The analysis of the relative abundance of FAs allowed to elucidate changes in FAs accumulation and to identify potentially enhanced metabolic pathways (Figs. 2 and 3). In the presence of 1 μm PS-MPs, the relative abundance of monounsaturated fatty acids (MUFAs) was significantly increased, while polyunsaturated fatty acids (PUFAs) decreased according to the increase of the exposure concentrations. Although a similar trend was observed at the end of the exposure to 10 μm PS-MPs, it was only verified at the lower tested concentrations, as the exposure to the highest PS-MPs concentration led to a re-establishment of the homeostasis regarding to MUFAs/PUFAs abundance.

The analysis of FAs profile of *D. magna* exposed to 1 μm PS-MPs (Table 1) showed a significant increase in the abundance of MUFAs, except C18:1ω9 (oleic acid). A similar modulation of the FAs profile was also observed in a common and widespread algal species, *Chlorella sorokiniana*, exposed to PS-MPs (<70 μm in size), whereby the reduction in the content of essential FAs C18:2ω6 (linoleic acid) and C18:3ω3 (α-linolenic acid) was accompanied by an increase in C18:1ω9 content (Guschina et al., 2020).

The correlation analysis on the abundance of FAs (Fig. 4) allowed the identification of relationships occurring among different lipid species and to hypotesize biosynthetic events translating these variations. The synthesis of C14:1 and C16:1 was performed at the expense of saturated fatty acids (SFAs), while the increase of C16:1 and other MUFAs resulted as inversely correlated with C18:3 and C18:2 content (Fig. 4; correlations highlighted in box named with the letter A). Additionally, C18:1ω9 was also consumed to synthesize C14:1 and C16:1. Considering these findings, we might speculate that under the exposure to high concentrations of PS-MPs, treated individuals redirected the synthesis of FAs towards shorter and less unsaturated molecules, increasing C14 and C16 MUFAs. The variation of SFAs (Fig. 4; correlations highlighted in box named with the letter B) was also simultaneous, suggesting a whole regulation, as supported by the positive correlation indexes. As PUFAs are accumulated and transferred upwards in the food web, *D. magna* individuals accumulate these FAs according to their

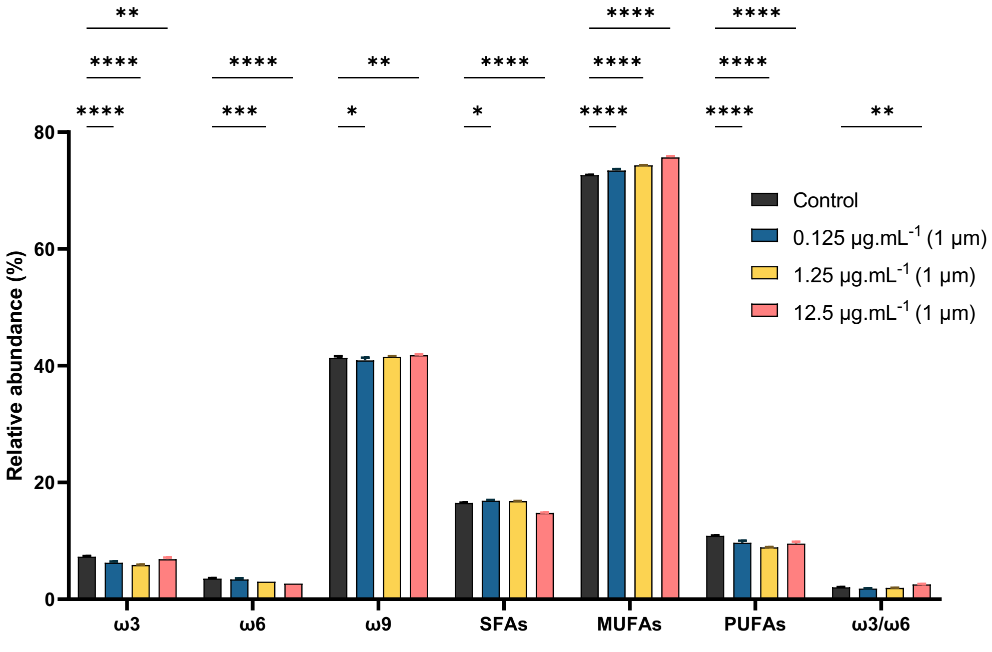

**Fig. 2.** Relative abundance of fatty acid groups from *Daphnia magna* grown 21-days in the presence of different concentrations (0.125, 1.25 and 12.5 μg $mL^{-1}$) of 1 μm PS-MPs. (Control: without exposure to PS-MPs; SFAs: saturated fatty acids, MUFAs: monounsaturated fatty acids, PUFAs: polyunsaturated fatty acids. Statistical significance: *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$ after ANOVA followed by Dunnett's multiple comparisons test. Data are reported as mean ± SD (n = 3)).

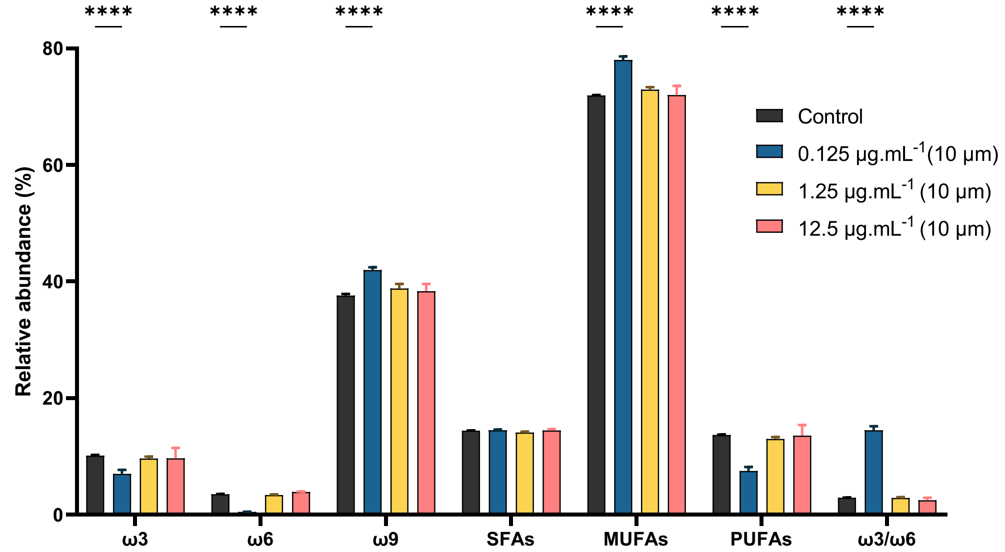

**Fig. 3.** Relative abundance of fatty acid groups from *Daphnia magna* grown 21-days in the presence of different concentrations (0.125, 1.25 and 12.5 μg mL$^{-1}$) of 10 μm PS-MPs concentrations. (Control: without exposure to PS-MPs; SFAs: saturated fatty acids, MUFAs: monounsaturated fatty acids, PUFAs: polyunsaturated fatty acids. Statistical significance: ****$p < 0.0001$ after ANOVA followed by Dunnett's multiple comparisons test. Data are reported as mean ± SD (n = 3)).

availability in the diet (Masclaux et al., 2012). Therefore, it is also possible that the decrease in the PUFAs/MUFAs ratio is related to less PUFAs being obtained.

Regarding the individuals grown in the presence of 10 μm PS-MPs, an increase in MUFAs, particularly C16:1, was observed (Table 1). However, while the C18:1ω9 content increased, a decrease in C18:3 occurred, suggesting an interconversion of these two FAs. In the 1 μm PS-MPs experiments, C18:1ω9 content increased, suggesting a different impact of this size of PS-MPs on FAs metabolism. All the effects caused by 10 μm PS-MPs were only observed at the lowest tested concentration, while homeostasis seems to be re-established after the exposure to the higher concentrations. These results might suggest i) a sort of adaptation in *D. magna* to the increasing concentrations of PS-MPs and ii) that also low concentrations of PS-MPs are sufficient to induce significant modulation of FAs metabolism and accumulation in this cladoceran species. Thus, smaller sized PS-MPs exposure should lead to a decrease in the accumulation of essential FAs by the diet, promoting the synthesis of monounsaturated FAs-rich storage lipids, as well as allocating resources towards their offspring. Larger MPs caused similar events on fatty acids management. However, at higher concentrations, the individuals look like to experience a sort of adaptation that counters the environmental stress and could promotes mobility, reproduction and food procurement, reestablishing fatty acids homeostasis and consuming storage lipids in the process.

The analysis of the correlation among FAs content measured in individuals exposed to 10 μm PS-MPs (Fig. 5), confirmed that C18 PUFAs were consumed when an increase in the abundance of C14, C16 and C18 MUFAs occurred (Fig. 5; correlations highlighted in box named with the letter A). Moreover, the positive correlation between all MUFAs (Fig. 5;

**Table 1**
Relative abundance (mean ± standard deviation) of fatty acids from *Daphnia magna* grown 21-days in the presence of different concentrations (0.125, 1.25 and 12.5 μg mL$^{-1}$) of 10 μm PS-MPs concentrations.

| Fatty acid (%) | Control | | 0.125 μg mL$^{-1}$ | | 1.25 μg mL$^{-1}$ | | 12.5 μg mL$^{-1}$ | |
|---|---|---|---|---|---|---|---|---|
| | 1 μm | 10 μm | 1 μm | 10 μm | 1 μm | 10 μm | 1 μm | 10 μm |
| C14:1 ω9c | 0.49 ± 0.01 | 0.47 ± 0.03 | 0.44 ± 0.02 | 0.60 ± 0.02 | 0.52 ± 0.02 | 0.55 ± 0.02 | 0.60 ± 0.03 | 0.50 ± 0.04 |
| C14:1 ω9t | 1.50 ± 0.02 | 1.69 ± 0.04 | 1.54 ± 0.03 | 1.97 ± 0.05 | 1.59 ± 0.02 | 1.90 ± 0.07 | 1.78 ± 0.04** | 1.78 ± 0.07 |
| C14:0 | 1.77 ± 0.03 | 1.48 ± 0.01 | 1.84 ± 0.03 | 1.55 ± 0.04 | 1.89 ± 0.03 | 1.46 ± 0.05 | 1.68 ± 0.05 | 1.46 ± 0.07 |
| C15:1 ω5c | 0.00 ± 0.00 | 0.16 ± 0.01 | 0.18 ± 0.02 | 0.00 ± 0.00 | 0.21 ± 0.01* | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| C15:0 | 1.26 ± 0.02 | 1.23 ± 0.02 | 1.33 ± 0.03 | 1.39 ± 0.04 | 1.39 ± 0.01 | 1.30 ± 0.05 | 1.32 ± 0.02 | 1.27 ± 0.03 |
| C16:1 ω9c | 8.30 ± 0.05 | 8.30 ± 0.06 | 8.12 ± 0.11 | 9.18 ± 0.14** | 8.32 ± 0.03 | 8.77 ± 0.67 | 9.24 ± 0.06**** | 9.02 ± 0.09* |
| C16:1 ω7c | 30.98 ± 0.26 | 33.91 ± 0.21 | 32.04 ± 0.48**** | 35.77 ± 0.40**** | 32.25 ± 0.11**** | 33.85 ± 0.39 | 33.70 ± 0.07**** | 33.36 ± 0.34 |
| C16:1 ω9t | 0.23 ± 0.01 | 0.22 ± 0.01 | 0.23 ± 0.01 | 0.27 ± 0.01 | 0.25 ± 0.01 | 0.00 ± 0.00 | 0.24 ± 0.01 | 0.25 ± 0.00 |
| C16:1 ω7t | 0.33 ± 0.01 | 0.25 ± 0.01 | 0.30 ± 0.02 | 0.27 ± 0.01 | 0.34 ± 0.01 | 0.24 ± 0.02 | 0.18 ± 0.01 | 0.27 ± 0.01 |
| C16:0 | 10.67 ± 0.08 | 9.22 ± 0.04 | 10.91 ± 0.14* | 9.01 ± 0.07 | 10.78 ± 0.03 | 8.79 ± 0.11 | 9.34 ± 0.00 | 9.07 ± 0.11 |
| C18:3 ω6c | 0.16 ± 0.02 | 0.16 ± 0.01 | 0.14 ± 0.01 | 0.18 ± 0.01 | 0.18 ± 0.00 | 0.17 ± 0.02 | 0.07 ± 0.00 | 0.19 ± 0.01 |
| C18:2 ω6c | 3.09 ± 0.02 | 3.09 ± 0.02 | 2.94 ± 0.15 | 0.00 ± 0.00**** | 2.82 ± 0.01** | 3.02 ± 0.14 | 2.46 ± 0.01**** | 3.26 ± 0.06 |
| C18:3 ω3c | 7.11 ± 0.11 | 9.98 ± 0.11 | 6.02 ± 0.20**** | 6.81 ± 0.67**** | 5.85 ± 0.05**** | 9.49 ± 0.35 | 6.67 ± 0.28**** | 9.55 ± 1.75 |
| C18:1 ω9c | 28.28 ± 0.30 | 24.13 ± 0.23 | 27.78 ± 0.51**** | 27.14 ± 0.49**** | 27.94 ± 0.19*** | 24.78 ± 1.45* | 27.55 ± 0.01**** | 23.70 ± 1.06 |
| C18:1 ω9t | 2.47 ± 0.02 | 2.73 ± 0.01 | 2.74 ± 0.01** | 2.74 ± 0.14 | 2.57 ± 0.02 | 2.73 ± 0.05 | 2.37 ± 0.01 | 3.04 ± 0.03 |
| C18:0 | 2.67 ± 0.02 | 2.39 ± 0.02 | 2.68 ± 0.03 | 2.42 ± 0.05 | 2.64 ± 0.01 | 2.44 ± 0.03 | 2.36 ± 0.00** | 2.54 ± 0.04 |
| C20:4 ω6c | 0.32 ± 0.01 | 0.26 ± 0.02 | 0.34 ± 0.03 | 0.30 ± 0.03 | 0.00 ± 0.00*** | 0.17 ± 0.02 | 0.16 ± 0.01 | 0.44 ± 0.01 |
| C20:5 ω3c | 0.19 ± 0.02 | 0.17 ± 0.02 | 0.25 ± 0.03 | 0.21 ± 0.01 | 0.03 ± 0.00 | 0.14 ± 0.02 | 0.18 ± 0.00 | 0.14 ± 0.00 |
| C20:1 ω9c | 0.07 ± 0.01 | 0.06 ± 0.01 | 0.07 ± 0.01* | 0.08 ± 0.01 | 0.31 ± 0.02 | 0.08 ± 0.01 | 0.00 ± 0.00 | 0.07 ± 0.01 |
| C22:0 | 0.08 ± 0.01 | 0.07 ± 0.01 | 0.07 ± 0.01 | 0.07 ± 0.00 | 0.07 ± 0.01 | 0.07 ± 0.01 | 0.05 ± 0.01 | 0.06 ± 0.01 |
| C24:0 | 0.05 ± 0.01 | 0.04 ± 0.00 | 0.05 ± 0.00 | 0.04 ± 0.01 | 0.05 ± 0.00 | 0.03 ± 0.01 | 0.03 ± 0.00 | 0.04 ± 0.01 |

Statistical significance: without* — no statistical significance; *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ****$p < 0.0001$ after ANOVA followed by Dunnett's multiple comparisons test. Data are reported as mean ± SD. (n = 3).

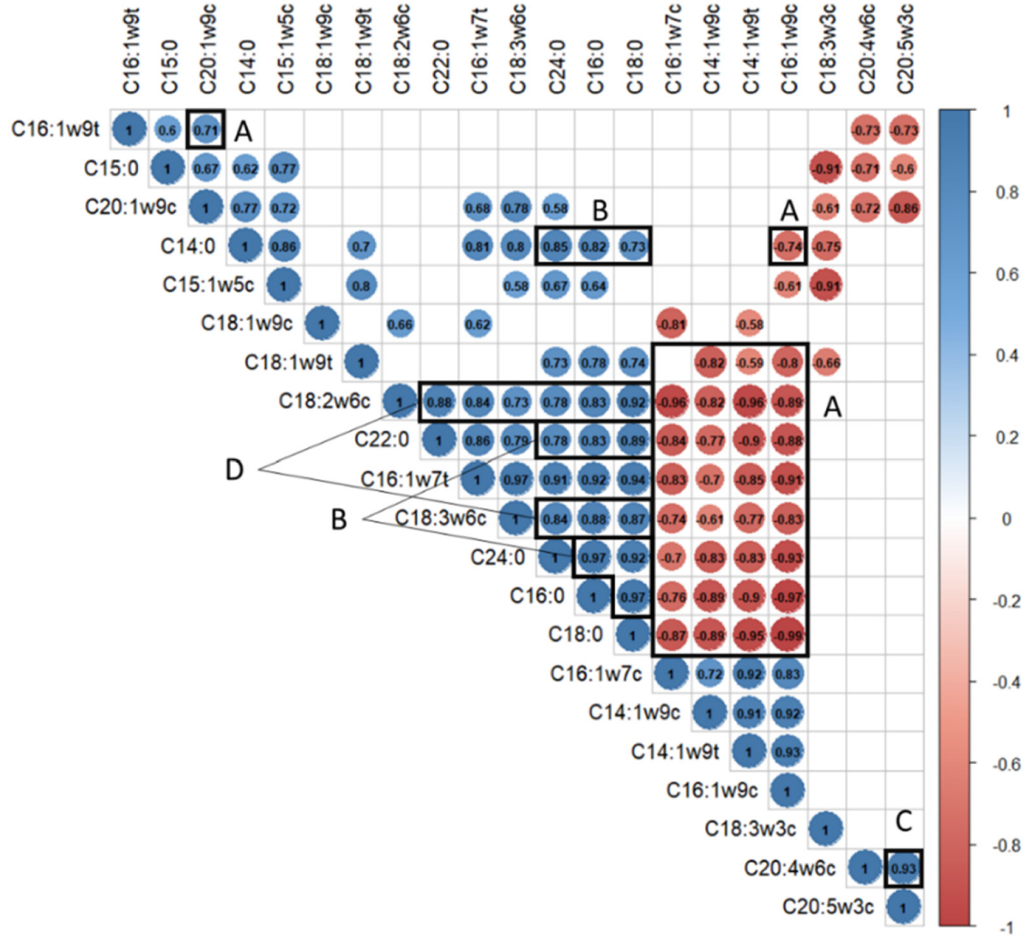

**Fig. 4.** Correlation matrix of fatty acids from *Daphnia magna* grown 21 days in the presence of different concentrations (0.125, 1.25 and 12.5 μg mL$^{-1}$) of 1 μm PS-MPs. Values are presented as linear correlation index between variables; the color gradient represent the strength of the correlations.

correlations highlighted in box named with the letter B) suggested a synchronism in this regulatory mechanism, as the synthesis of these FAs seems to be modulated collectively. Overall, these results showed that the presence of PS-MPs in the environment might force *D. magna* individuals to accumulate higher amounts of MUFAs rather than PUFAs. These changes might be induced by a limited food availability, leading to a rewiring of the FAs biosynthesis that promotes the conversion of longer and more unsaturated fatty acids into shorter and more saturated ones. The increase in MUFAs content could benefit individuals experiencing stressful conditions through the increase of the amount of storage lipids. Indeed, MUFAs and their derived fatty alcohols are important components of esters (Brett et al., 2009), the main form of storage lipid in daphnids (Lee et al., 2006).

### 3.3. Effect of PS-MPs exposure on the biosynthetic ω3, ω6 and ω9 pathways

A significant decrease in the abundance of ω3 and ω6 fatty acids, but an increase in ω9, was observed at the end of the exposure to 1 μm PS-MPs. Interestingly, at 10 μm PS-MPs exposure, such effects were observed only at the lowest tested concentrations (0.125 μg mL$^{-1}$), suggesting that the effect on ω3, ω6 and ω9 FAs are related to the mechanisms previously discussed regarding PUFAs/MUFAs abundance. For instance, the most abundant ω3 and ω6 FAs are C18:2 and C18:3, which were also hallmarks of the MUFAs decrease (Table 1).

The correlation analysis highlighted a collective variation in ω6 FAs, with positive correlations among FAs from this biosynthetic pathway (Fig. 4; correlations highlighted in box named with the letter D). Accordingly, the synthesis of the ω6 ARA, an essential precursor of signaling biomolecules and a key element in the reproduction in *D. magna* (Ginjupalli et al., 2015), is accumulated together with C18:3ω6 (Fig. 5; correlations highlighted in box named with the letter D).

These results also suggest that the ω9 pathway is regulated at the expense of C16:0, leading to the synthesis of C20:1ω9. When this synthesis is enhanced, there is an increment of C16, C18 and C20 MUFAs (Fig. 5; correlations highlighted in box named with the letter E). Overall, these results suggest the allocation of resources towards ω9 FAs, in addition to the MUFAs accumulation. Finally, a sharp increase in the ω3/ω6 ratio was observed in individuals that experienced the exposure to the lowest concentration of 10 μm and the highest one of 1 μm PS-MPs. This situation could likely occur as a consequence of a competition in the intake of essential ω3 FAs, which are not accumulated, as the synthesis of ω6 and ω9 storage lipids increases. These findings confirmed that FAs are involved in the response to PS-MPs exposure and their modulation might be considered as a valuable marker of MPs contamination in natural ecosystems, as daphnids seem to accumulate shorter FAs in the absence of ω3-rich food.

## 4. Conclusions

The present study showed that the exposure to increasing concentration of 1 and 10 μm PS-MPs, resulted in the modulation of fatty acids profile and content of *D. magna* individuals. Differently sized MPs affected fatty acids composition and profiles in distinct manners. In fact, higher concentrations of smaller MPs and lower concentrations of larger MPs exerted the most significant changes in the FAs profile. Although the concentrations of PS-MPs we tested in the present study can be considered as unrealistic, considering

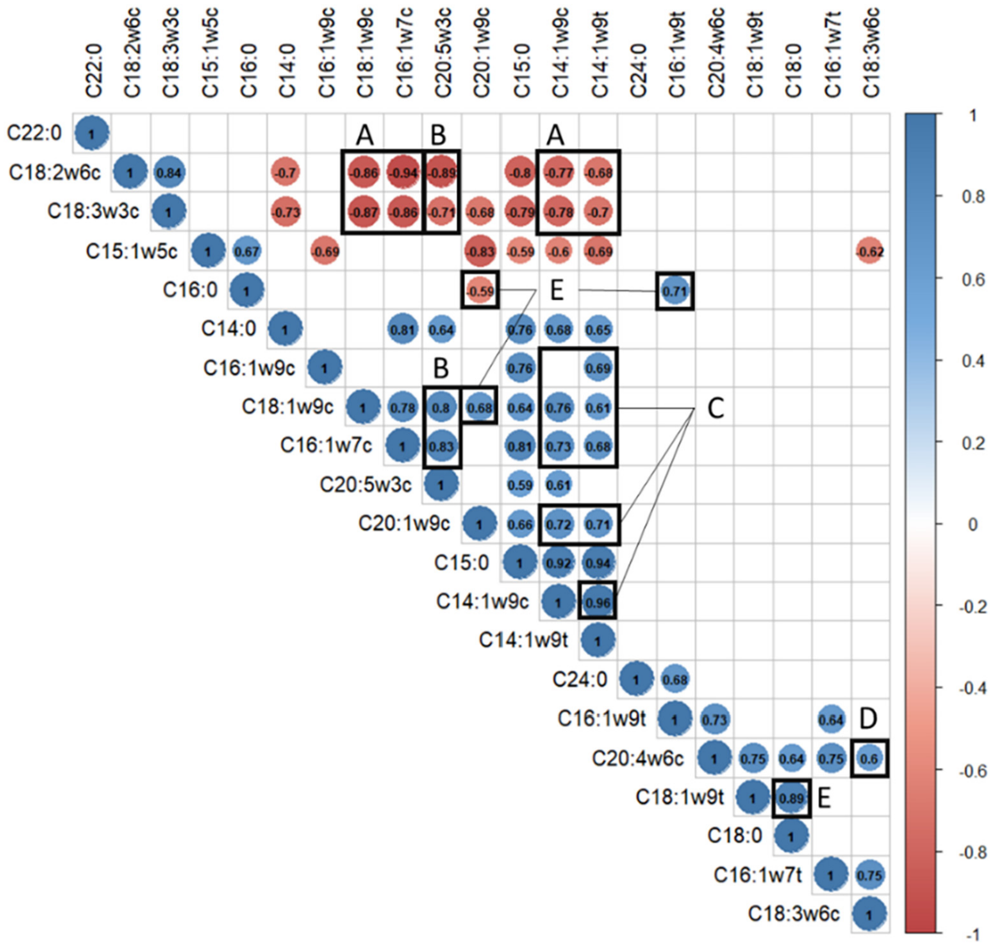

**Fig. 5.** Correlation matrix of fatty acids from *Daphnia magna* grown 21 days in presence of different concentrations (0.125, 1.25 and 12.5 μg mL$^{-1}$) of 10 μm PS-MPs concentrations. Values are presented as linear correlation index between variables; the color gradient represent the strength of the correlations.

the amount of large-sized plastics in the environment and their potential fragmentation, the levels of MPs could increase, returning effects similar to those observed in this experiments. These results enlarged the knowledge of the sub-lethal effects of MPs in zooplanktonic species. They returned helpful information to understand the mechanisms of action underlying the impact of these contaminants on different life-history traits, confirming that the ingestion of MPs can influence the energy budget and allocation, with potential consequences on growth, reproduction, and survival. In addition, further studies need to explore the adverse effects induced by mixture of MPs of different size, shape and polymeric composition towards aquatic organisms. This approach should increase the ecological realism of the experiments and allow to shed light on the effects induced by different interacting substances, returning useful, although not conclusive, information to assess the real risk of MPs exposure in natural ecosystems.

## CRediT authorship contribution statement

**Marco Parolini:** Conceptualization, Formal analysis, Investigation, Writing – original draft. **Beatrice De Felice:** Investigation. **André Gois:** Investigation, Writing – review & editing. **Marisa Faria:** Investigation. **Nereida Cordeiro:** Investigation, Methodology, Formal analysis, Writing – review & editing. **Natacha Nogueira:** Conceptualization, Investigation, Writing – review & editing.

## Data availability

Data will be made available on request.

## 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.

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