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Facilitating microplastic ingestion in aquatic models: A verified protocol for daphnia magna as a trojan horse vector

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A R T I C L E I N F O

Method name:
Aquatic Microplastic Ingestion and Transfer Protocol (AMITP)

Keywords:
Daphnia magna
Microplastics
"Trojan Horse" vector
Pollution assessment
Food consumption

A B S T R A C T

Microplastic pollution poses a significant environmental threat due to its persistence, widespread distribution, and inherent toxic potential. Despite the increasing number of publications in this field, a standardized protocol for the laboratory intake of microplastics by *Daphnia magna* has yet to be established. In this study, we introduce a verified protocol designed to facilitate the ingestion of microplastic particles (MPs) by *D. magna*, ranging in size from 5–55 μm. This protocol can be further applied to evaluate the toxicity of MPs on *D. magna*, a crucial organism model in ecotoxicology. Furthermore, this protocol can be used to assess toxicity of MPs in other aquatic species, such as fish, by using daphnids as a vehicle for ensuring the ingestion of these particles. Consequently, this protocol can be applied to study also one of the most pressing concerns regarding exposure to MPs, the transfer of MPs through different trophic levels, which has a great potential for ecotoxicological studies.

- The influence of MPs concentration, duration and exposure dynamics and *D. magna* age/size in MPs intake were tested.
- We have determined the optimal conditions for promoting microplastic ingestion by *D. magna*.

Specifications table

Subject area:	Environmental Sciences
More specific subject area:	Microplastic toxicological assessment
Name of your method:	Aquatic Microplastic Ingestion and Transfer Protocol (AMITP)
Resource availability:	**EVOS M700 (FLUORESCENCE MICROSCOPY):** https://www.thermofisher.com/es/es/home/life-science/cell-analysis/cellular-imaging/evos-cell-imaging-systems/models/evos-m7000.html **LEICA E74 W (OPTICAL MICROSCOPY)** https://www.leica-microsystems.com/es/productos/microscopios-opticos/microscopios-estereoscopicos/p/leica-ez4-w/

(*continued on next page*)

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Microplastics	
https://www.cospheric.com/polymermicrospheres.htm	
Heidolph Reax 20 (DYNAMIC ROTATION)	
https://heidolph.com/emea/es/reax-20-para-8-botellas-1-16-rpm~p356	
Cospheric Microspheres:	
https://www.cospheric.com/	
Fluorescent Violet Polyethylene Microspheres: UVPMS-BV-1.00 20–27um-0.5 g	
Fluorescent Green Polyethylene Microspheres: UVPMS-BG-1.00 47–51um–1 g	
Colorless Poly(Methyl Methacrylate) PMMA Acrylic Microspheres: PMPMS-1.2 5–27um-10 g **and PMPMS-1.2 45–53um >95%-** 5 g	
Colorless Polystyrene Microspheres, Crosslinked Microspheres: PSMS-1.07 14–20um- 500 mg **and PSMS-1.07 38–48um- 500mg**	

Background

Plastic pollution is widely recognized as a significant environmental concern resulting from the substantial increase in global plastic production and the inadequate management of plastic waste. Special attention has focused on the appearance of small pieces of plastic, referred to as microplastics (MPs). In line with the National Oceanic and Atmospheric Administration (NOAA) definition, MPs are synthetic polymers of sizes between 5 mm and 1 μm [1], which can be categorized as primary or secondary MPs based on their source. Primary MPs are manufactured plastics for industrial or domestic use of microscopic scale. The main sources of this type of MPs can be the large amounts generated from the abrasion of car tires while driving or from the laundering of synthetic textiles [2]. These MPs can further fragment into smaller particles through degradation processes [3], which are more reactive and pose a greater risk to marine fauna and humans, leading to secondary MPs. These hazardous materials [4] impose relevant environmental threats on ecosystems, being classified into physical (i.e., entanglement), chemical (i.e., toxicity induced by residual monomers or incorporated compounds in their surface such as plasticizers, plastic additives and environmental pollutants) and biological (i.e., biofilms and microorganisms) [2]. The health impacts of MPs remain poorly understood due to the lack of comprehensive toxicological data. Consequently, MPs currently are central in ecotoxicology studies. Despite that, several challenges are still present when assessing the toxicity of MPs [5]. The lack of standardized analytical methodologies for collecting, processing and analyzing environmental samples potentially increases ambiguity, not allowing direct comparison between studies. Regarding MPs ingestion by aquatic species under laboratory conditions, there exists a large degree of uncertainty between the different feeding strategies used, highly affecting the quality of the toxicological results achieved [6].

Ingestion of MPs is generally classified into direct or indirect. Direct ingestion occurs when animals ingest them accidentally. In contrast, indirect ingestion is related to the trophic transfer being the result of the consumption of contaminated food. Direct ingestion is often conducted by spiking environmentally relevant concentration of MPs into the water [7]. However, these approaches may induce the intake of MPs not only by ingestion, but also by gills uptake. Recent evidence suggests that MPs can cross the gill wall and be translocated to other body tissues [8], enhancing the complexity of the toxicological evaluation. Alternatively, indirect ingestion can be achieved by feeding aquatic species with MPs-contaminated food (e.g., pellets [9] or living organisms [10]). These protocols have the capacity to reduce the variability across research studies, improving the toxicological evaluation of the ingestion of MPs by aquatic species, in addition to underline the MPs impact at several levels across the trophic chain through foodborne. In this work, an optimized protocol for the ingestion of MPs by *D. magna*, a keystone crustacean specie for aquatic ecotoxicity studies and one of the most commonly used model organisms in environmental risk assessment. They are highly sensitive to stressors like environmental pollutants, making them essential indicators for standardized chemical testing. Additionally, their short lifecycle and parthenogenetic reproduction make *D. magna* useful for assessing developmental toxicity and adaptation to stress [11]. In addition, this approach can be applied not only to conduct the toxicological threats of these particles on this primary consumer model, but also, as Trojan Horse vector, on other higher trophic aquatic species such as fish.

The increasing relevance of MPs in toxicological assessment has led to several studies determining the potential threat of these ubiquitous particles to Daphnia magna species. However, a lack of a standardized protocol for conducting MPs exposure to these crustaceans has resulted in significant discrepancies across studies. This study aims to standardize the exposure of microspheres, the most studied type of MPs, by presenting a detailed step-by-step procedure, including previous optimizations conducted on the selection of MPs size, exposure conditions, and daphnids characteristics.

The increasing relevance of MPs in toxicological assessment has led to several studies determining the potential threat of these ubiquitous particles on *D. magna* species. However, a lack of a standardized protocol for conducting MPs exposure to these crustaceans, has resulted in significant discrepancies across studies. Therefore, this study aims to standardize the MPs exposure of microspheres, the most studied MPs type, to relevant aquatic species *D. magna* by presenting a detailed step-by-step procedure, including previous optimizations conducted on the selection of the MPs size, exposure conditions, and daphnids characteristics.

Method details

The goal of developing this method was to maximize the intake of microplastics in water flea (*D. magna*), with the intention of using them as a Trojan Horse vector to further expose fish to microplastics through indirect ingestion. This method also potentially evaluates the toxicological threats of MPs directly on *D. magna*. Optimization was performed based on three main factors that could

Table 1
Main specifications of the MPs used in the experiments.

Abbreviation	MPs type	Color	Excitation (λ_{ex}) and emission (λ_{em}) wavelength (nm)	Particle size (μm)	Density (g/cm^3)
MPs-50	Polyethylene (PE)	Fluorescent green	λ_{ex}: 414 λ_{em}: 515	47–51	1.00
MPs-20	Polyethylene (PE)	Fluorescent violet	λ_{ex}: 584 λ_{em}: 636	20–27	1.00

affect the MPs intake: MPs concentration, daphnids' age, and exposure approach (static/dynamic conditions). Below, we briefly describe the main optimization steps conducted, finally providing the standard operating procedure.

Microplastic characteristics

Fluorescent polyethylene microspheres with particle sizes of 20–27 μm (MPs-20) and 47–51 μm (MPs-50) were purchased from Cospheric (Somis, California, USA). Description of both microplastic types is summarized in Table 1. Herein, fluorescent particles were selected to better visualize MPs inside the crustaceans by fluorescence imaging. Also, to discriminate between both MPs size ranges, two different fluorescent colors were selected (green/violet).

Experimental animals and culture conditions

Adult *D. magna* (clone F) females were kept under standard culture conditions at a density of 10 animals per liter. Animals were maintained in ASTM hard synthetic water [12] under a 16 h light:8 h dark photoperiod cycle, and at 20 ± 1°C. Feeding occurred 3 times per week with 5×10^5 cells/mL of *Chlorella vulgaris* cultured under semi-axenic conditions. Cultures of juveniles were initiated with neonates (n=100–120), which were obtained from third to sixth brood reproductive females, cultured in 2 L of media and feed as described above. Cultures of juveniles were maintained to reach different ages previous to the microplastic exposures (a detailed culture schedule is provided in the summary on Section 6). Prior to MPs exposure, daphnids were placed in clean ASTM water for 24 h to facilitate the purging of the digestive system, and thus, the accumulation of microplastics.

MPs intake visualization

Two different image-based techniques were used to better characterize the optimal conditions for MPs intake by crustaceans. Optical images were acquired using a Leica EZ4W (Leica Microsystems, Germany). Alternatively, to maximize the visualization of fluorescent particles, an EVOS m7000 (Thermo Fisher Scientific, Germany) microscope was used. Fluorescence was conducted using two different light cubes: RFP (Color: Orange; Excitation wavelength: 542 nm; Emission wavelength: 593 nm) for visualizing violet particles and CFP/YFP (Color: Green; Excitation wavelength: 445 nm; Emission wavelength: 542 nm) for characterizing the green MPs. CFP/YFP was selected since the ability of CFP/YFP to visualize the MPs appeared superior to GFP (Color: Green; Excitation wavelength: 482 nm; Emission wavelength: 524 nm), leading to the selection of the first light cube as the optimal choice. Brightness, gain and exposure time parameters were adjusted for each experiment set to allow comparison between them. It is worth mentioning that the z-axis was adjusted in order to maximize the visualization of the digestive system of crustaceans. In Fig. 1, one image example of a daphnid for both techniques are presented. Fluorescence image presents both fluorescence light cubes individually and together with transmission light is presented.

Size and age correlation

In order to characterize the sizes of the *D. magna* F clone grown in our laboratory, we measured 5 juvenile and adult daphnia at different ages (3 of them included in Fig. 2). Mean sizes were as follows: at 0 days old they measured 0.9 ± 0.2 mm at 4 days old they measured 1.5 ± 0.2 mm; at 7 days old, 2.1 ± 0.2 mm; at 14 days old, 3.4 ± 0.3 mm; and at 30 days old, 3.5 ± 0.2 mm.

MPs exposure optimization

Exposure duration and selection of MPs water concentration

Organisms aged 15–18 days were employed to investigate the minimum exposure duration required to achieve significant microplastic (MPs) ingestion by *D. magna*, as well as to determine the optimal concentration of MPs. The daphnids were subjected to static conditions and exposed to MPs at concentrations of 1 and 10 mg/L for 2 and 24 hours. These concentrations and exposure times were chosen based on existing literature [13,14]. MP intake was evaluated using imaging microscopy with EVOS m7000 (section 4). All MPs concentration were conducted exposing daphnids to each MPs alone or to two different mixtures (1:1 and 1:2 MPs-20:MPs-50 (w/w)). In Fig. 3A, the results achieved for the green particles alone are presented as an example, achieving similar results for the other MP exposures conducted. The results indicated that a concentration of 10 mg/L was necessary for achieve the incorporation of MPs into the daphnia's digestive system. Concerning exposure duration, as shown in Fig. 3B, the tested durations proved to be

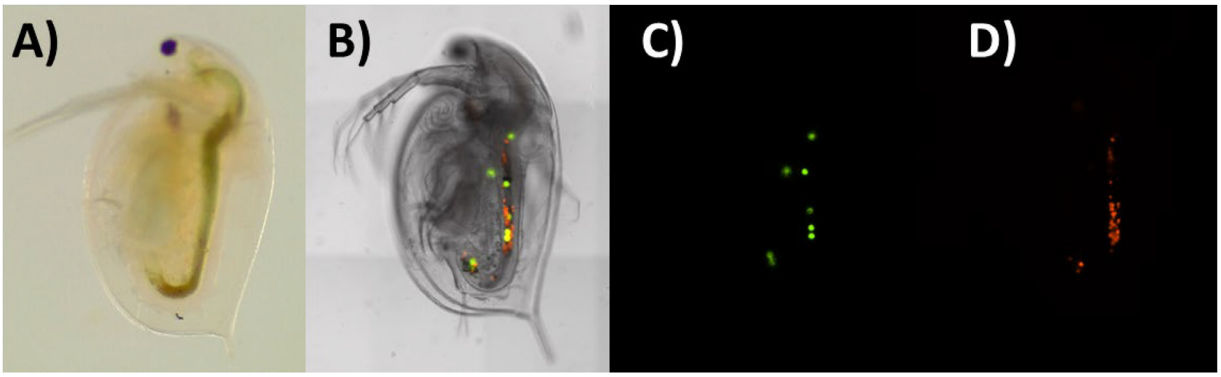

Fig. 1. *D. magna* images comparing daphnid visualization using A) optical image using the Leica EZ4W microscope (Leica Microsystems, Germany) working at 10X magnification, B) transmission light (monochrome camera) combined with RFP and CFP/YFP fluorescence, C) CFP/YFP fluorescence and D) RFP fluorescence using the EVOS m7000 Imaging system working at 10X magnification. The bright (31%), gamma (0.55) and saturation (140) were adjusted for the optical image acquired using the Leica microscope system without applying any further processing adjustment. The bright, coarse and fine parameters were adjusted in each image acquired by the EVOS system to achieve the best visualization of the MPs in the digestive system of daphnids.

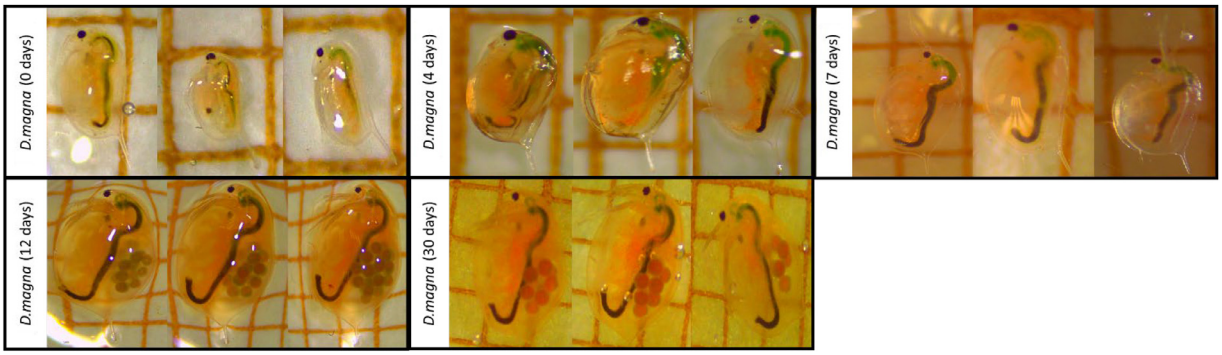

Fig. 2. Clone F *D. magna* images comparing their size from 0 to 30 days. Images were achieved using a Leica EZ4W microscope working at 30X magnification for *D.magna* at 0 days, 25X for *D.magna* at 4 days, 15 X at 7 days and 10X at 12 and 30 days. The bright (31%), gamma (0.55) and saturation (140) were adjusted for the optical images acquired using the Leica microscope system without applying any further processing adjustment.

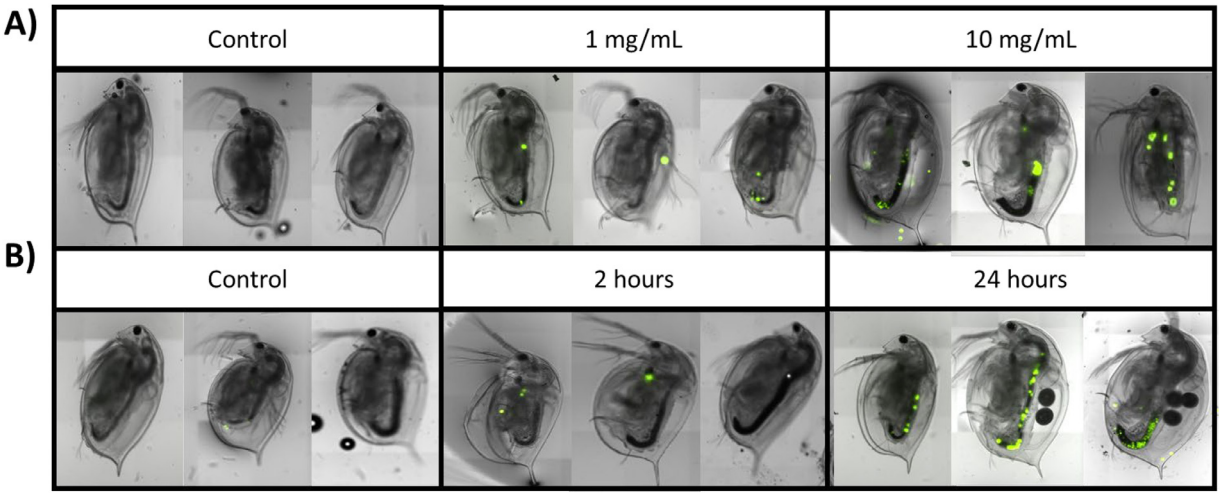

Fig. 3. *D. magna* adults exposed to A) 1–10 mg/L of the green particles for 2 h and B) 10 mg/L for 2 h and 24 h. All images were acquired using the EVOS m7000 Imaging system working at 10X magnification combining transmission light (monochrome camera) CFP/YFP fluorescence (green). The bright, coarse and fine parameters were adjusted in each image to achieve the best visualization of the MPs in the digestive system of daphnids.

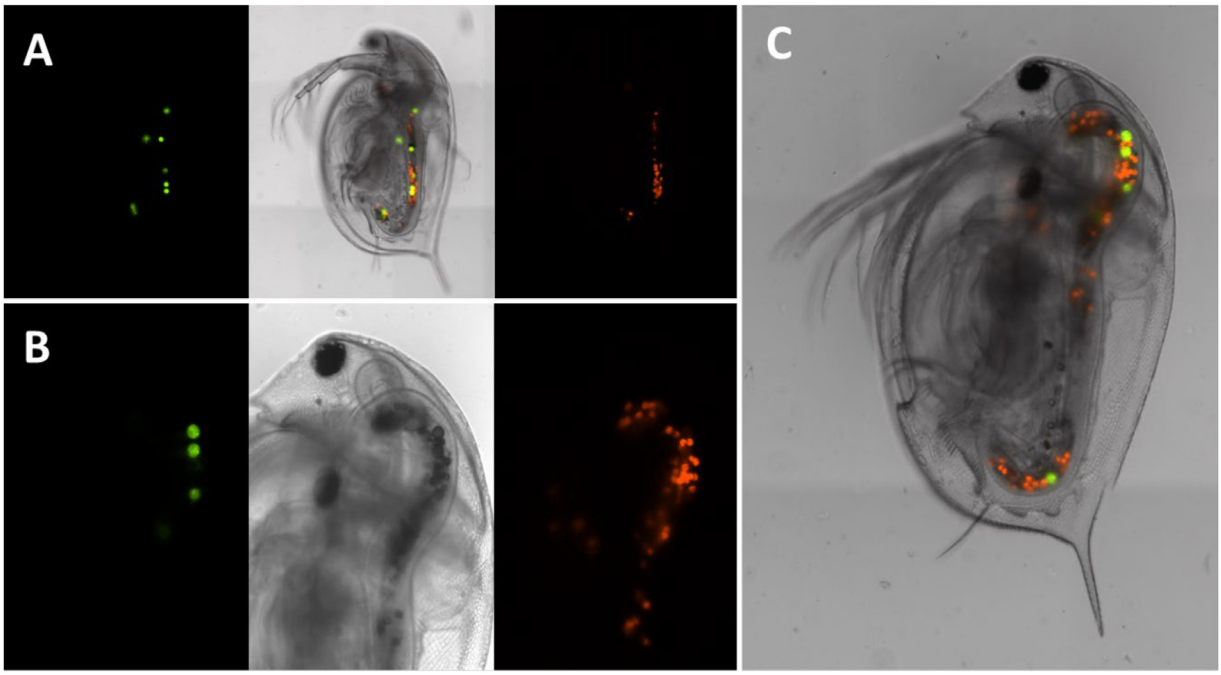

Fig. 4. Clone F *D. magna* at 8–10 days old (A) and 12–18 days old (B) exposed to green MPs alone and in combination of 1:2 MPs-20:MPs-50 microplastic mixture visualizing fluorescence signals individually and merged (C). All images were acquired working on an EVOS m7000 Imaging system at 10X magnification using fluorescence RFP (orange) and CFP/YFP (green) light cubes in combination with visible light (monochrome camera), and both fluorescence light cubes individually. The bright, coarse and fine parameters were adjusted in each image to achieve the best visualization of the MPs in the digestive system of daphnids.

essential for enabling MP intake. Nonetheless, the intake of MPs was enhanced after 24 hours compared to the shorter duration of 2 hours (which no significant uptake was achieved), prompting us to select the longer duration (24 hours) as optimal.

Also, preliminary results demonstrated that mixture of 1:2 MPs-20:MPs-50 outperforms individual exposures, being the improvement particularly relevant for green particles. It is worth mentioning that in this mixture, the number of particles of MPs-20 and MPs-50 are equalized, which may also explain the better results achieved compared to 1:1 MPs-20: MPs-50 mixture. In Fig. 4, some examples of daphnids at different ages exposed to the 1:2 MPs-20:MPs-50 mixture are presented to visualize MPs location on the digestive system of daphnids. As seen, despite being exposed to the same number of plastic particles, the smaller range tested (MPs-20) generally outperforms the MPs intake on the digestive system.

Dynamic versus static exposures

Based on the results from previous section (section 5.1 and section 5.2), from now on water flea exposures were set to 10 mg/L with a duration of 24 h. The next step on the methodological optimization was comparing the effect of conducting those exposures at static or under dynamic conditions. Thus, we tested two different approaches: 1) static and 2) dynamic rotation at 2 rpm using an orbital wheel Heidolph Reax 20 (Heidolph, Germany). To determine the optimal exposure approach, 1:2 MPs-20:MPs-50 mixture using daphnids from 12 to 18 days (section 5.3) were used. Results (Fig. 5) confirm that, rotation at 2 rpm improved MPs intake. The

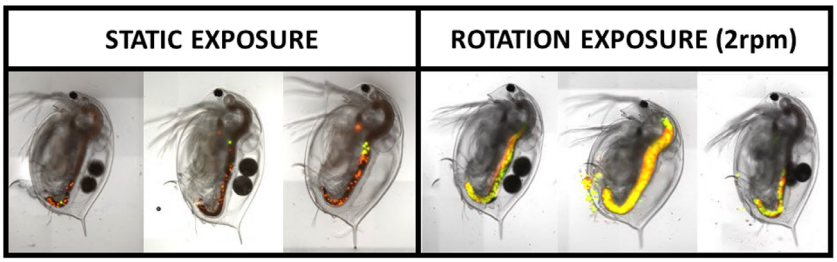

Fig. 5. Clone F *D. magna* at 12–18 days exposed to 1:2 MPs-20:MPs-50 microplastic by static and rotation (2 rpm) conditions. All images were acquired working on an EVOS m7000 Imaging system at 10X magnification using fluorescence RFP (orange) and CFP/YFP (green) light cubes in combination with visible light (monochrome camera). The bright, coarse and fine parameters were adjusted in each image to achieve the best visualization of the MPs in the digestive system of daphnids.

selected particle density of 1 g/cm3 may have affected the results. Under static conditions, MPs-20 and MPs-50 were only found in the upper phase, while dynamic conditions led to widespread distribution of MPs across the vial.

Influence of D. magna size and age in MPs intake

It has been previously demonstrated that the filtering area by *D. magna* varies depending on their size [15], which can be correlated with their age. Therefore, it is necessary to characterize the filtering capacity of water flea at different ages (meaning different sizes as shown in Fig. 2 to select the optimal age range in which ingestion of MPs is maximized. To test the capacity of filtration at different ages, exposures of both MP types individually and in combination (1:2 MPs-20:MPs-50 mixture) were tested working under rotation conditions (section 5.2). Our results (Fig. 6) underlined that juvenile (<8 days) and adult daphnids younger than 12 days had a limited capacity to incorporate MP particles in the range size used in this study. Particularly poor ingestion was seen for the higher size range (green particles). Therefore, results may lead to conclude that at younger ages, smaller MPs should be used in order to achieve significant MPs intake.

In contrast, when older daphnids were used (Fig. 7), a clear increase in the MP intake was observed regardless of their age, being particularly significant at the range age of 12 to 18 days. At the older age tested (28–30 days), MP we observed that the MPs-20 (violet, 20–27 μm) did not preserve the sphere shape and the digestive track seemed to be tinted with the violet fluorescence, hypothesizing that daphnids at this age might be capable of breaking the MP-20 when ingested. It must be noted that these crustaceans develop neck teeth, hypothesized to increase the predator resistance [16]. In concordance with the results achieved in Fig. 6A and Fig. 6B, at this older age stages *D. magna* seems to have the capacity to crush small MPs (violet). This break-down capacity for MPs have also been reported for similar organisms such as krill [17].

To better visualize the MP-20 present in the daphnids, we performed a peroxide digestion (32% H_2O_2 at 65°C for 24 hours). Our results (Fig. 8) confirmed the physical degradation of MPs by daphnids at the older age tested, since almost no entire MP-20

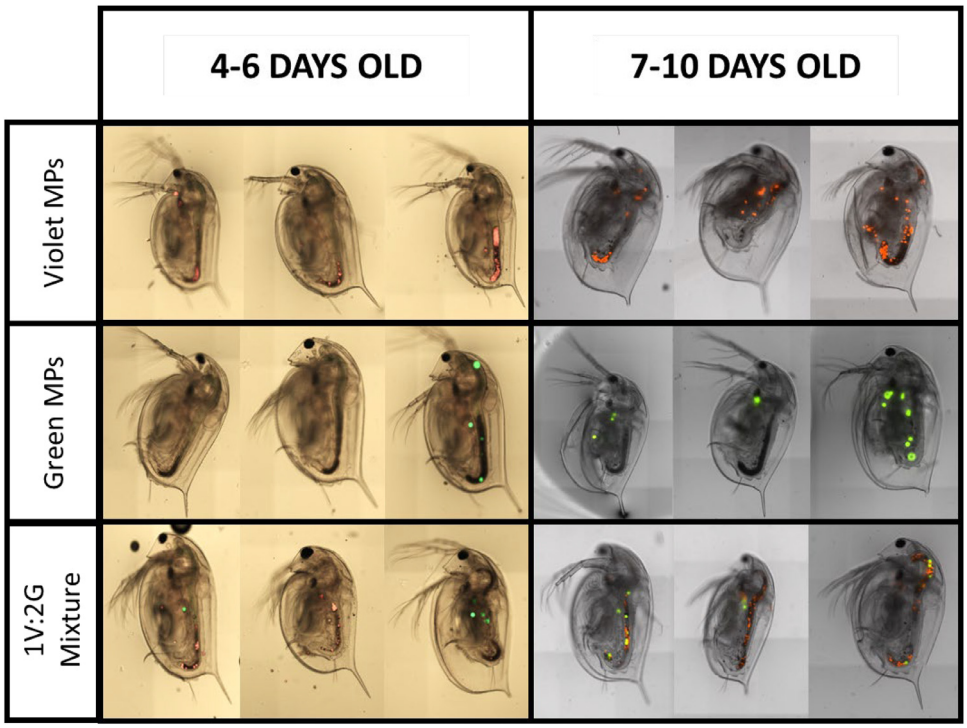

Fig. 6. Clone F *D. magna* juveniles at 4–6 days old (A) and 7–10 days old (B) exposed to violet MPs, green MPs alone and in combination of 1:2 violet: green (1V:2 G) microplastic mixture under rotation conditions. All images were acquired working on an EVOS m7000 Imaging system at 10X magnification using fluorescence RFP (orange) and CFP/YFP (green) light cubes in combination with visible light (4–6 Days old: color camera; 7–10 days: monochrome camera). The bright, coarse and fine parameters were adjusted in each image to achieve the best visualization of the MPs in the digestive system of daphnids.

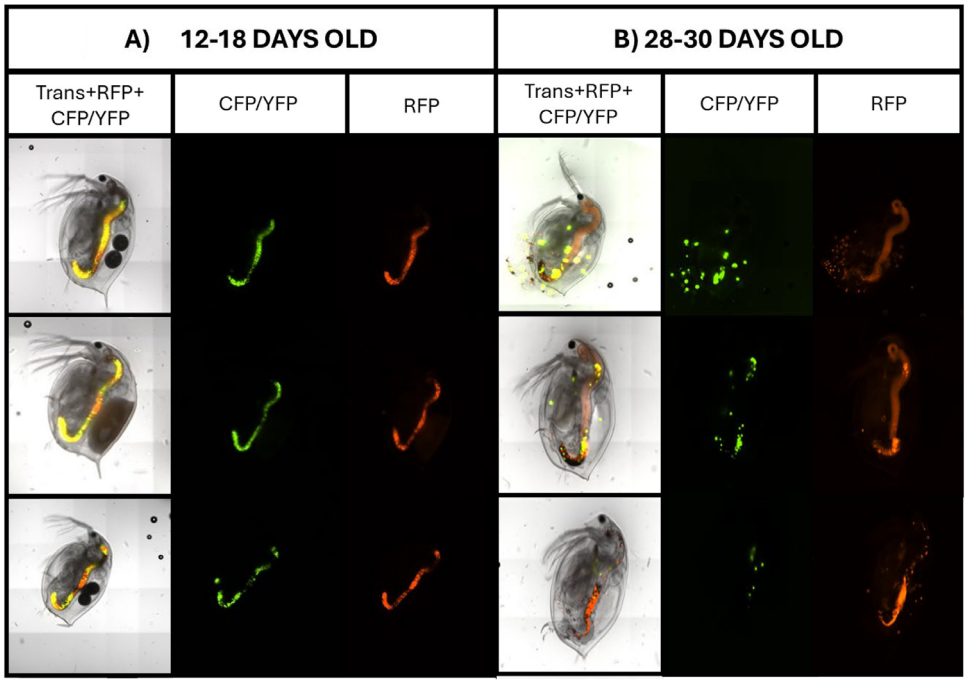

Fig. 7. Clone F *D. magna* at 12–18 days old (A) and 28–30 days old (B) exposed to green MPs alone and in combination of 1:2 MPs-20:MPs-50 microplastic mixture. All images were acquired working on an EVOS m7000 Imaging system at 10X magnification using fluorescence RFP (orange) and CFP/YFP (green) light cubes in combination with visible light (monochrome camera), and both fluorescence light cubes individually. The bright, coarse and fine parameters were adjusted in each image to achieve the best visualization of the MPs in the digestive system of daphnids.

were observed after digestion. All together allowed us to conclude that 12–18 days old is the optimal age for MP-20 and MP-50 incorporation.

Summary of the developed method

D. magna *maintenance*

1- In all cases, all individuals need to be maintained at 20 ± 1°C in ASTM hard water under a 16 h light:8 h dark photoperiod cycle.

Neonates are fed 2 times per week with 5 × 105 cells/mL of Chlorella vulgaris cultured under semi-axenic conditions. At the age of 7 days, feeding regime is changed and kept to 3 times per week.

2- After each reproductive cycle (by parthenogenesis of adult females), separate neonates from adults as soon as possible to prevent density-dependent food limitation, which could interfere with proper development, growth and energy storage.
3- Place 120 neonates in 2 L of ASTM water (density of 60 daphnids/L) (Fig. 9A).
4- Juvenile daphnids at 7 days are transferred to new 2L-pots with a maximum density of 30 daphnids/L.
5- Once daphnids are between 12–18 days, they are in the optimal conditions for MP intake.

MPs exposure

6- Prior MPs exposure, daphnids are fasted by placing them in a new 2 L pot with clean ASTM (Fig. 9B).
7- Using an analytical precision balance, the desired amount of MPs are weighted and placed inside 20 mL glass vials.
8- After 24 hours under food deprivation, 10 daphnids are placed in the 20-mL vials containing the MPs. ASTM water is added to reach the desired concentration of MPs (Fig. 9C).

Note: Be aware that reproducibility issues as well as cross-validation must be considered. Do not introduce the pipettes inside the vials to avoid MPs to attach to them.

9- Vials can be then placed in a rotary saker at 2 rpm for 24 h. In our case we placed the vials inside a plastic container that was deposited in a Reax 20 (Heidolph) (Fig. 9D).

After 24 hours, the digestive system of daphnids should be already filled with MPs.

Notes: Consider maintaining dark conditions whereas avoiding as much as possible static conditions when preparing daphnids for subsequent visualization. In case of working with many samples, we recommend working in different batches of 3 vials. This should improve reproducibility between samples.

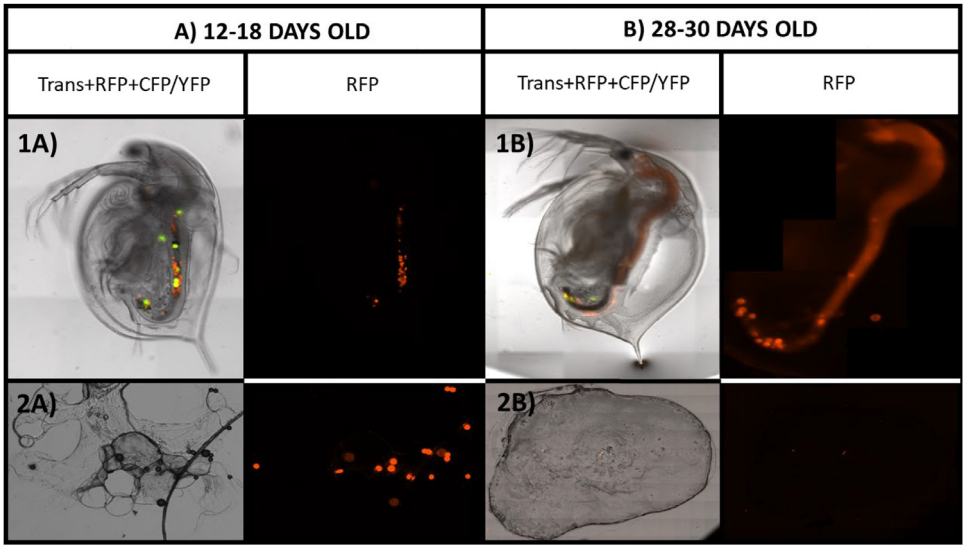

Fig. 8. Clone F *D. magna* at 12–18 days old (A) and 28–30 days old (B) exposed to 1:2 MPs-20:MPs-50 microplastic mixture 1) before and 2) after the peroxide digestion. All images were acquired working on an EVOS m7000 Imaging system at 10X magnification using fluorescence RFP (orange) light cube individually and in combination with visible light (monochrome camera). The bright, coarse and fine parameters were adjusted in each image to achieve the best visualization of the MPs in the digestive system of daphnids and the MPs on the peroxide digested tissue.

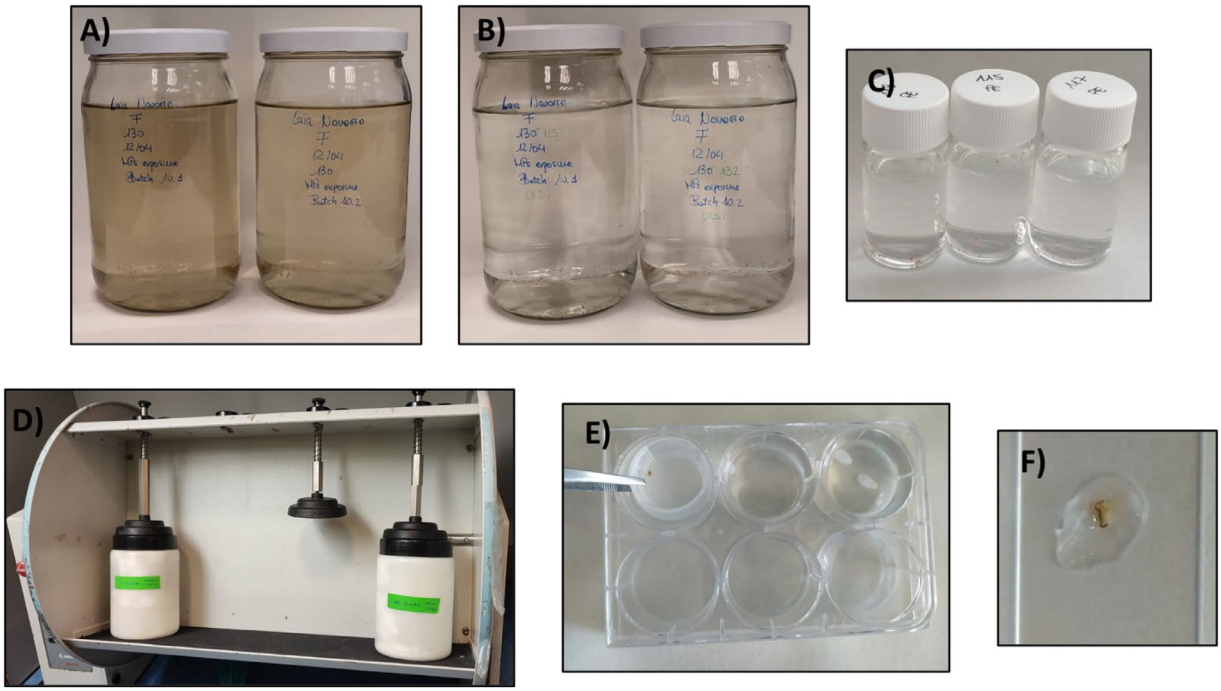

Fig. 9. Graphical illustration of the main steps of the experimental set-up: A) Growing daphnids until they are 12–18 days old; B) starving step in clean ASTM water; C) 10 daphnids are placed in 20 mL vials for the MPs exposure (10 mg/L); D) daphnids are exposed to MPs for 24 h under dynamic conditions (rotary shaker, 2 rpm); E) washing external MPS from daphnids previous to imaging acquisition; F) sample preparation for imaging visualization, embedding daphnids on 3% methylcellulose on regular glass slides.

MPs visualization

10- Sample preparation for visualizing daphnids under image-based techniques can be carried out as follows:
   a. Daphnids are cleaned in a 6-mL well (3x) with clean ASTM water to remove plastic particles that superficial and not inside the organism (Fig. 9E).
   b. Daphnids are placed in a glass slide covered with a solution of methylcellulose at 3% at sagittal orientation (Fig. 9F).
   c. Fluorescence/ Optical image can be then obtained.

11- Two different image-based techniques can be used:
   a. Optical image (e.g., Leica EZ4W).
   b. Fluorescence microscopy (e.g., EVOS m7000 system).

12- Optional: After visualization, daphnids can be cleaned and recovered in ASTM water. However, the visualization step should be done as fast as possible. A maximum of 5 minutes has to be considered in maintaining the daphnids covered with 3% methylcellulose solution.

Some considerations:

- We strongly recommend using fluorescence microscopy when implementing the protocol in the laboratory to have a clear confirmation of MPs intake and location.
- Fluorescence images individually and combined with and without transmission light can be very helpful to better visualize the location of MPs. Consider also if working in greyscale (recommended) or color images.
- Fluorescence parameters (brightness, gain and exposure time) must be maintained during the experiment to allow comparison between images.
- MPs may be found outside the daphnia. Therefore, it is really important to focus on the area of interest (digestive system), to avoid misinterpretation of the images. Z-axis should be optimized for each daphnid individually.

Method validation

The method provided in this article has been optimized using fluorescent polyethylene particles which specifications are presented in Table 1. To validate the methodology, other plastic polymers (polysterene (PS) and polymethyl methacrylate (PMMA)) have also been tested. In Table 2, the specificacions of these additional MPs, also purchased from Cospheric, are presented.

Applying the same protocol already delineated in previous sections ([MPs]: 10 mg/mL of 1:2 (PS-15:PS-45 or or PMMA-10:PMMA-50) mixture; exposure approach: rotation at 2 rpm; Daphnia age range: 12–18 days), results (Fig. 10) demonstrated the MPs intake regardless of the MPs polymer type and its higher density. Interestingly, a slight different density and particle size seems not be significantly affect the potential application of the method.

Table 2
Main specifications given by Cospheric regarding the MPs used in the experiments.

Abbreviation	MPs type	Color	Particle size (μm)	Density (g/cm^3)
PS-15	Polystyrene (PS)	Colorless	14–20	1.07
PS-45	Polystyrene (PS)	Colorless	38–48	1.07
PMMA-10	Polymethyl methacrylate (PMMA)	Colorless	5–27	1.20
PMMA-50	Polymethyl methacrylate (PMMA)	Colorless	45–53	1.20

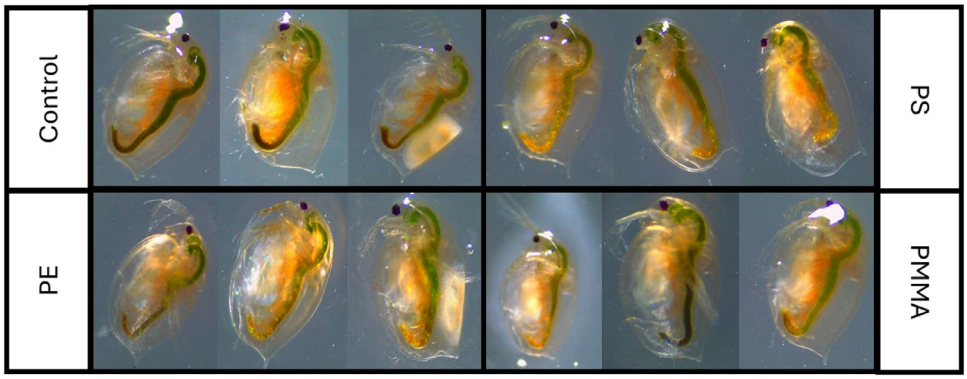

Fig. 10. *D. magna* individuals at 12–18 days old exposed to 3 different MP types (PE, PS and PMMA). All these optical images were acquired using a Leica EZ4W microscope system working at 10X magnification. The bright (31%), gamma (0.55) and saturation (140) were adjusted for the optical images acquired using the Leica microscope system without applying any further processing adjustment.

Limitations

Working with higher MP size (>55 μm) the method may not work. *D. magna* is capable of filtering particles with a maximum size of 50 μm. Also, working at lower MPs size (<5 μm) and nanoplastics (< 1 μm) has not been tested.

Used plastic particles have a density range between 1–1.2 g/cm^3. MPs may have lower/higher density. Therefore, if working in a different density range, a preliminary test should be conducted. Similarly, this approach should also be optimized for other types of MP morphology (e.g., fibers), as herein only spheric particles were used. Nevertheless, this developed protocol might serve as a first step to optimize and study other NPs and MPs intake by daphnids.

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.

CRediT authorship contribution statement

Albert Menéndez-Pedriza: Writing – original draft, Visualization, Methodology, Validation, Supervision. **Marta Gual:** Methodology, Validation, Visualization. **Lidia Molina-Millán:** Methodology, Writing – review & editing. **Ron M.A. Heeren:** Writing – review & editing. **Carlos Barata:** Writing – review & editing, Project administration, Supervision, Funding acquisition. **Laia Navarro-Martín:** Writing – original draft, Writing – review & editing, Project administration, Supervision, Funding acquisition, Conceptualization.

Data availability

Data will be made available on request.

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