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Experimental evaluation of microplastic consumption by using a size-fractionation approach in the planktonic communities

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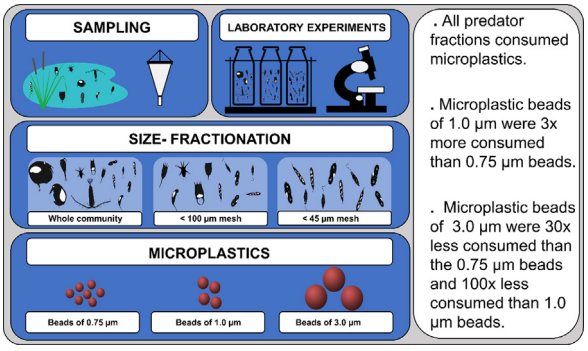
HIGHLIGHTS

- This study experimentally analyzed the effects of microplastic particles on different links of planktonic trophic webs.
- Impacts of different sizes of microplastic particles were estimated.
- The experimental approach identified that the highest particle consumption occurs mainly in the lower links of the trophic web.
- Smaller microplastic particles are significantly more consumed.
- Higher links do not consume microplastic particles directly, however they can absorb these compounds in alternative routes.

GRAPHICAL ABSTRACT



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ABSTRACT

The increasing amount of plastic particles introduced into continental aquatic environments has drawn the attention of researchers around the globe. These particles can be assimilated by a wide range of aquatic organisms, from microorganisms to fish, causing detrimental effects on trophic webs. Using an experimental approach, we investigated the effect of microplastic particles of different sizes on the planktonic trophic chain by sampling natural plankton communities from a lake located in the Upper Paraná River floodplain, Brazil. Zooplankton samples were collected at the beginning of the experiment and after 36 h of incubation. Microplastic particles (MP) samples were taken every 12 h. The effect of MP particle consumption from the control and treatment groups indicates significant effects by all plankton size fractions ($p < 0.05$). We demonstrated that the presence of MP particles can significantly affect the trophic web, furthermore, we detected the effect of higher consumption effect of smaller size MP particles. This study suggest that the largest MP consumption effects come from the lower trophic levels of the trophic chain, such as protists. The competitive effect of large predators is a crucial factor in controlling the abundance of populations, and

Abbreviations: MP, microplastic; NCR, net consumption rates; PSF, predator size fraction.

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although they did not directly consume MP particles, they ingest them indirectly through prey capable of absorbing these compounds in the environment. Our findings warn that MP particles enter the food webs of tropical regions when exposed to these pollutants, and that the presence of these particles should not be neglected when studying freshwater ecosystems.

1. Introduction

Plastic production increased considerably after the end of the 1940s (Carpenter and Smith, 1972). Changes in the modern lifestyle, with the influence of globalization and the growing demand for consumption, has encouraged the production and massive use of plastic to manufacture products (Alimi et al., 2018; Li et al., 2020). Recent estimates indicate that plastic production currently exceeds 350 million tons/year (Thompson et al., 2009; Plastics-the Facts, 2021). Of this amount, it is estimated that about 12.7 million tons are dumped every year into rivers, lakes, and oceans (Guzzetti et al., 2018; Jambeck et al., 2015; Lebreton et al., 2017; Meijer et al., 2021).

The excessive use of plastic has led to an environmental problem on a global scale, since currently half of all production is used to manufacture disposable and single-use products (Andrady, 1994; Barnes et al., 2009; Hopewell et al., 2009). Thus, plastic waste is dumped into aquatic ecosystems through illegal dumping directly into water bodies or through transport mediated by stormwater (Cordier and Uehara, 2019; Tessnow-von Wysocki and Le Billon, 2019). Besides modifying the landscape of rivers and lakes, the presence of plastic debris can lead to loss of biodiversity, affect the landscape, and also threaten the human health that uses these resources (Blettler et al., 2018; de Souza Machado et al., 2018; Al-Thawadi, 2020; Bertoldi et al., 2021).

Once inside aquatic ecosystems, debris is weathered by the environment and undergoes a slow process of fragmentation (Harrison et al., 2018), releasing chemicals (trace metals and organic pollutants) directly into the water bodies (Blettler et al., 2018). The problem is exacerbated because large plastic particles break up, forming smaller and smaller particles called microplastics (MP) (Guzzetti et al., 2018; Strungaru et al., 2019). The increasing amounts of plastic in continental aquatic environments are drawing the attention of researchers around the globe (Lebreton et al., 2017). Of the numerous studies on the topic, much of the literature is focused on the effects of microplastics in the oceans, while a smaller amount of effort is directed towards freshwater ecosystems (Wagner et al., 2014), where study efforts should be intensified, particularly in countries with rapid economic development and poor waste management (Blettler et al., 2018).

Microplastics can be assimilated by a wide range of aquatic organisms, such as fish and microorganisms, causing them direct detrimental effects, and can also cause indirect effects, such as reducing the abundance of prey and affecting the structure of the food web (de Souza Machado et al., 2018; Kokalj et al., 2021). Planktonic microorganisms are the base of these food webs, both via the herbivorous food chain and the microbial loop, and occupy different trophic niches. Most studies in freshwater that have addressed the impact or interaction of microplastics with the planktonic community have been carried out with zooplankton, followed by bacteria and algae (Blettler et al., 2018; Li et al., 2018; Yu et al., 2020).

The competition for food resources within planktonic communities leads these organisms to ingest microplastic particles (Jemec et al., 2016). Studies show that *Daphnia* (cladoceran) species have increased ingestion rates of microplastic granules under poor resource conditions (Hoffschröer et al., 2021). On the other hand, studies addressing the effect of microplastics on more than one component in planktonic communities are scarce (de Sá et al., 2018a; Jemec et al., 2016). Therefore, understanding how predation and body size influence the consumption of these pollutants in food webs becomes relevant (Quintana et al., 2014). In theory, microplastic particles are captured in the environment, and over a short period of time, they become allocated within organisms, and the transfer of these harmful substances in the trophic chains occurs when the pollutants accumulate at some trophic level through a process known as bioaccumulation (Setälä et al., 2014).

The use of microplastic beads (MPB) as an artifice to assess natural prey consumption and feeding preference of mesoplankton, microzooplankton and protists has been reported in previous studies (Bern, 1990; Fernández et al., 2004; Nygaard et al., 1988; Paffenhöfer and Van Sant, 1985; Zánkai, 1991). On the other hand, it is not yet known which size of microplastic is preferentially ingested by the different compartments of the trophic web in subtropical regions. Therefore, our investigation assessed (1) whether bead consumption increases over time, (2) which fraction of the planktonic community is responsible for the highest bead consumption, and (3) which size of beads is preferentially ingested by the different components of the planktonic community. We hypothesized that (i) microplastic consumption would increase over time as the resources would be depleted, because competition would increase and organisms would consume whatever is in suspension; (ii) the whole planktonic community treatment would consume most of the microplastic due to the presence of organisms of different sizes; and (iii) the smallest sizes of microplastics would be the most consumed, as their size is easily ingested by all organisms.

2. Material and methods

2.1. Study area

The study was conducted by sampling the plankton in Garças Lagoon (22°43′27.18″S; 53°13′4.56″W), located in the Upper Paraná River floodplain, in Mato Grosso do Sul state, Brazil. This lagoon is shallow (average depth 2 m), with an area of 14.1 ha, and permanently connected to the Paraná River by a narrow channel (Fig. 1). The littoral zone is home to several species of aquatic macrophytes, such as *Pontederia azurea*, *Nymphaea amazonum*, *Polygonum ferrugineum*, *Polygonum stelligerum* and *Salvinia auriculata* (Souza et al., 2017; Thomaz et al., 2009). Transparency is generally less than 1 m, with total phosphorus ranging between 30 and 90 μg/L and total nitrogen between 150 and 300 μg/L (Rocha and Thomaz, 2004). We measured some physical and chemical water parameters on the day of the sampling: the water temperature was 26.3 °C, pH 6.32, turbidity 25.4 (NTU) and dissolved oxygen 6.32 mg/L (Segovia et al., 2018). The total depth at the sampling point was 1.2 m and the Secchi disk depth was 0.55 m. Water was collected from the subsurface in 20 L plastic gallons, transported to the laboratory under dark conditions, and maintained at in situ temperature.

2.2. Sampling design

Water samples (80 L) from the sub-surface of the pelagic region of the lagoon were obtained with a graduated bucket (20 L) during the morning period to collect the planktonic communities (Fig. 2A), and kept on ice until the laboratory to set up of the experiment at the laboratory, as described by Segovia et al. (2018).

2.3. Experimental design

The experiment was conducted in the laboratory for 36 h at in situ temperature (26 °C) and low light conditions to avoid overgrowth and competition by phytoplankton (Calbet and Landry, 1999). One-liter polyethylene bottles were filled with 800 mL of water, with a total of 3 replicates for each treatment (control, 3 treatments with different plankton sizes, and 3 treatments with different microplastic sizes, for a total of 36 experimental units).

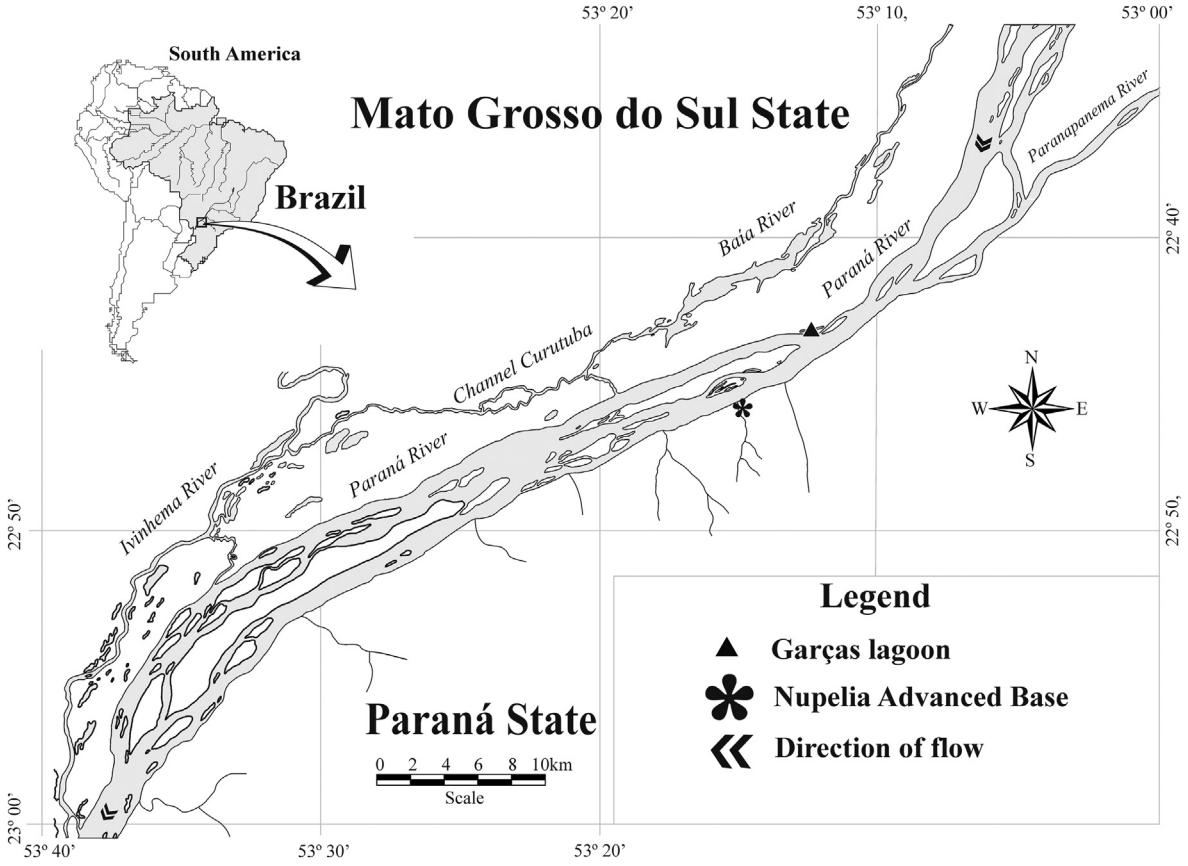

Fig. 1. The Upper Paraná River system, showing Garças Lagoon and Nupelia Advanced Base.

The Predator Size Fraction (PSF) treatments with planktonic organisms were designed as follows: (1) Whole Community = unfiltered water, containing the mesoplankton (adult cladocerans and copepods and copepodites/young copepod forms) and the microplankton (nauplii/young copepod forms + rotifers + ciliates + flagellates); (2) < 100 μm = water filtered through a 100 μm mesh, containing all the microplankton smaller than 100 μm (nauplii + rotifers + ciliates + flagellates); (3) < 45 μm = water filtered through a 45 μm mesh, containing part of the microplankton smaller than 45 μm (ciliates + flagellates); and (4) < 1.2 μm = water filtered through a 1.2 μm mesh, this being the control treatment. For the control treatment, we filtered water samples through GF/C glass fiber filters (Whatman) that retain particles larger than 1.2 μm (Fig. 2).

In each of the community treatments, another treatment level of the experiment was established regarding Microplastic Size (MPs), where Fluoresbrite® carboxylate microspheres (fluorescent monodispersed polystyrene) were added. For each predator fraction size treatment, three different sizes of beads of 0.75 μm, 1.0 μm, and 3.0 μm diameter, were added separately, creating nine combinations of bead size x predator size fractions (Fig. 2).

The use of these particles represents an advantage due to their size gradient and stable fluorescence levels (Hammer et al., 2001; McManus and

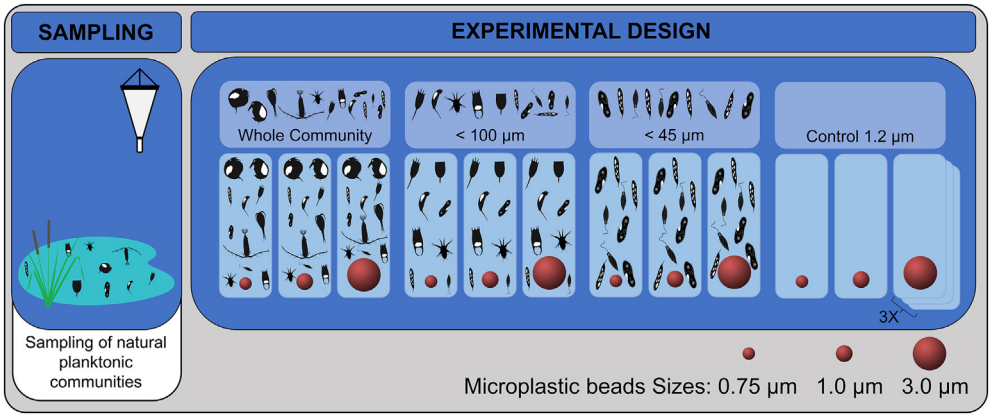

Fig. 2. Sampling and experimental design showing the treatments with the different predator size fractions and control. In the Whole Community treatment, adult microcrustaceans, nauplii, rotifers and protists were present. In the <100 μm treatment, adult microcrustaceans were removed and only nauplii, rotifers and protists were present. The <45 μm treatment was composed mainly of protists. The <1.2 μm treatment contained no predators (Control). MP beads of size 0.75 μm, 1.0 μm, and 3.0 μm were added separately for each predator size fraction. Three replicates were done for each treatment (3×).

Fuhrman, 1986). Another factor that represents an advantage for the use of these particles is that their behavior follows Stokes' law, where the sedimentation rate is translated by a relative velocity between the sphere (radius of the spheres) and the medium, with large particles settling in seconds and small particles (<10 μm) in hours (Scherer et al., 2017). The amount of beads used in the treatments simulated abundance values recorded in the seasonal dynamics and community structure of the naturally occurring bacterioplankton found in Garças lagoon (Chiaramonte et al., 2013; Lemke et al., 2009). We used microspheres (beads) as a surrogate for microplastics (MP) in the environment, and will use this terminology throughout the text when referring to the beads used in our experiment.

In the 0.75 μm MP treatment, an average of 2.74×10^{-2} (SE ± 1155) particles/L of spheres were added in the treatment with the entire planktonic community; 2.45×10^{-2} (SE ± 5840) particles/L in the <100 μm fraction treatment; 2.65×10^{-2} (SE ± 11,348) particles/L in the <45 μm treatment; and 2.71×10^{-2} (SE ± 9261) particles/L in the control treatment. In the 1.0 μm MP treatment, on average, 4.93×10^{-2} (SE ± 44,427) particles/L in the community-wide treatment; 5.77×10^{-2} (SE ± 68,521) particles/L in the <100 μm fraction treatment; 4.75×10^{-2} (SE ± 48,563) particles/L in the <45 μm treatment; and 5.89×10^{-2} (SE ± 82,002) particles/L in the control treatment. In the 3.0 μm MP treatment, on average, 1.59×10^{-1} (SE ± 395) particles/L in the Whole Community treatment; 1.54×10^{-1} (SE ± 351) particles/L in the <100 μm fraction treatment; 1.51×10^{-1} (SE ± 208) particles/L in the <45 μm treatment; and 1.51×10^{-1} (SE ± 433) particles/L in the control treatment.

The number of spheres used in each treatment was measured by epifluorescence (FACSCalibur model). We gently mixed all bottles every 2 h to keep the polyethylene beads in suspension, and to allow their effective filtration by the organisms.

2.4. Microplastic and plankton sampling

Water samples for microplastic counting were sampled from each treatment at the beginning of the experiment (0 h), and then at 12 h intervals after the start of the experiment (12 h, 24 h, and 36 h). These samples were immediately fixed with formalin buffered with borax (1% final concentration) and stored in liquid nitrogen until counting.

For the abundance estimates of predator size fractions, water samples were taken at the beginning (T0 h), and at the end of the experiment (T36 h). For flagellates, aliquots were taken from water and fixed with glutaraldehyde (1% final concentration). For ciliates and zooplankton, water samples were fixed with formalin buffered with borax (1% final concentration), Lugol, and thiosulfate.

To estimate the abundance of flagellates (cells/mL), 10 mL of sample were filtered through a 0.8 μm black polycarbonate filter and stained with 4′,6-diamidino-2-phenylindole (Porter and Feig, 1980), the epifluorescence microscopy method (Olympus BX51) was used at 1000× magnification. Ciliates (ind./L) were quantified under an inverted microscope (Olympus CK40) using Utermöhl cameras at 400× magnification. Zooplankton (ind./L) were quantified under a common optical microscope (Olympus CX31) using Sedgewick-Rafter counting chambers at 100× magnification.

Estimation of microplastic beads abundance was performed using flow cytometry (FACSCalibur model), in which 200 μL aliquots of samples were stained with SYTO-13 (Molecular Probes; 2.5 μmol/L final concentration) and in the dark where microplastic beads were detected by plotting side scatter (SSC) versus FL3 (red fluorescence) (Fig. 3).

2.5. Data analysis

The counts of microplastic beads (MP) were calculated assuming no growth of this element in the treatments, according to the following expression:

$$\mathrm{MP} = (\mathrm{Count} \mid \mathrm{Singles}/\mathrm{Vol.Passed} * 1000)/\mathrm{Correction\ factor\ (fixation)}.$$

where Count|Singles are the MP beads, Vol. Passed (mL) is the amount of water filtered, and the correction factor is the Sample Volume (mL) / (Sample Volume (mL)) + (Fixation Volume (mL)).

The net consumption rates (NCR) of the microplastic beads by the plankton fractions were calculated by removing the potential natural loss of beads in the control treatments, at their respective times, as follow:

$$\mathrm{Microcrustaceans} + \mathrm{Rotifers} + \mathrm{Nauplii} + \mathrm{Protists}_{\mathrm{NCR}} = \left(\mathrm{MP}_{\mathrm{Whole\ Community}} - \mathrm{MP}_{\mathrm{control}}\right)$$

$$\mathrm{Rotifers} + \mathrm{Nauplii} + \mathrm{Protists}_{\mathrm{NCR}} = \left(\mathrm{MP}_{<\ 100\ \mu\mathrm{m}} - \mathrm{MP}_{\mathrm{control}}\right).$$

$$\mathrm{Protists}_{\mathrm{NCR}} = \left(\mathrm{MP}_{<\ 45\ \mu\mathrm{m}} - \mathrm{MP}_{\mathrm{control}}\right).$$

To investigate whether microplastic consumption increases over time (hypothesis 1), which predator size fraction consume most of the microplastic (hypothesis 2), and which particle sizes are potentially consumed the most (hypothesis 3), we evaluated net consumption rates (NCR) of microplastic over time (12 h, 24 h, 36 h) in the treatments with different predator size fractions (PSF) and different size of MP beads (0.75 μm, 1.0 μm, and 3.0 μm) using a three-way analysis of variance (Three-way ANOVA). We checked the assumptions for parametric models using the Shapiro Wilk test for normality and Levene's test for homogeneity of variances. Comparison between treatments was tested using Tukey's post hoc test.

The analyses were considered significant at $p < 0.05$ level and performed using the "vegan" package (Oksanen et al., 2018). The graphs were constructed using the "ggplot2" package (Wickham and Chang, 2007). All analyses were performed in R software (R Core Team, 2020).

3. Results

3.1. Planktonic community characterization

The abundance of flagellates was roughly similar across all treatments, decreasing slightly throughout the experiment compared to their initial abundances, and varying between an average of 161 cells/mL in the Whole Community treatment, 125 cells/mL in the <100 μm plankton fraction, and 140 cells/mL in the <45 μm plankton fraction (Fig. 4A).

Ciliate abundance increased considerably throughout the experiment to an average of 44,140 ind./L in the <45 μm treatment, and 67,430 ind./L in the <100 μm plankton fraction, whereas only a slight increase was found for the treatment with microcrustaceans (Whole Community), that averaged 8463 ind./L at the end of the experiment (Fig. 4B). The most abundant ciliate orders present in the experiment was Oligotrichida, followed by Prostomatida, Hymenostomatida, Gymnostomatida, Prostomatida, Suctoria, Peritrichia ande Hypotrichida.

Rotifers and nauplii were only present in the <100 μm and Whole Community treatments; both decreased slightly in abundance in the Whole Community treatment by the end of the experiment, but increased in abundance throughout the experiment in the <100 μm treatment. The average abundance of rotifers was 256 ind./L in the Whole Community treatment and 615 ind./L in the <100 μm treatment, with a dominance of the species *Polyarthra dolicoptera* (Fig. 4C). Nauplii had an average abundance of 104 ind./L in the Whole Community treatment and 231 ind./L in the <100 μm treatment, with a dominance of Cyclopoid nauplii (Fig. 4D).

Cladocerans and adult copepods were only present in the Whole Community treatment; both decreased slightly in abundance throughout the experiment. Cladocerans had an average abundance of 30 ind./L, with a dominance of the species *Bosmina hagmanni* (Fig. 4E). The average abundance of copepods was 64 ind./L, with a dominance of two Cyclopoid species: *Thermocyclops minutus* and *Thermocyclops decipiens*.

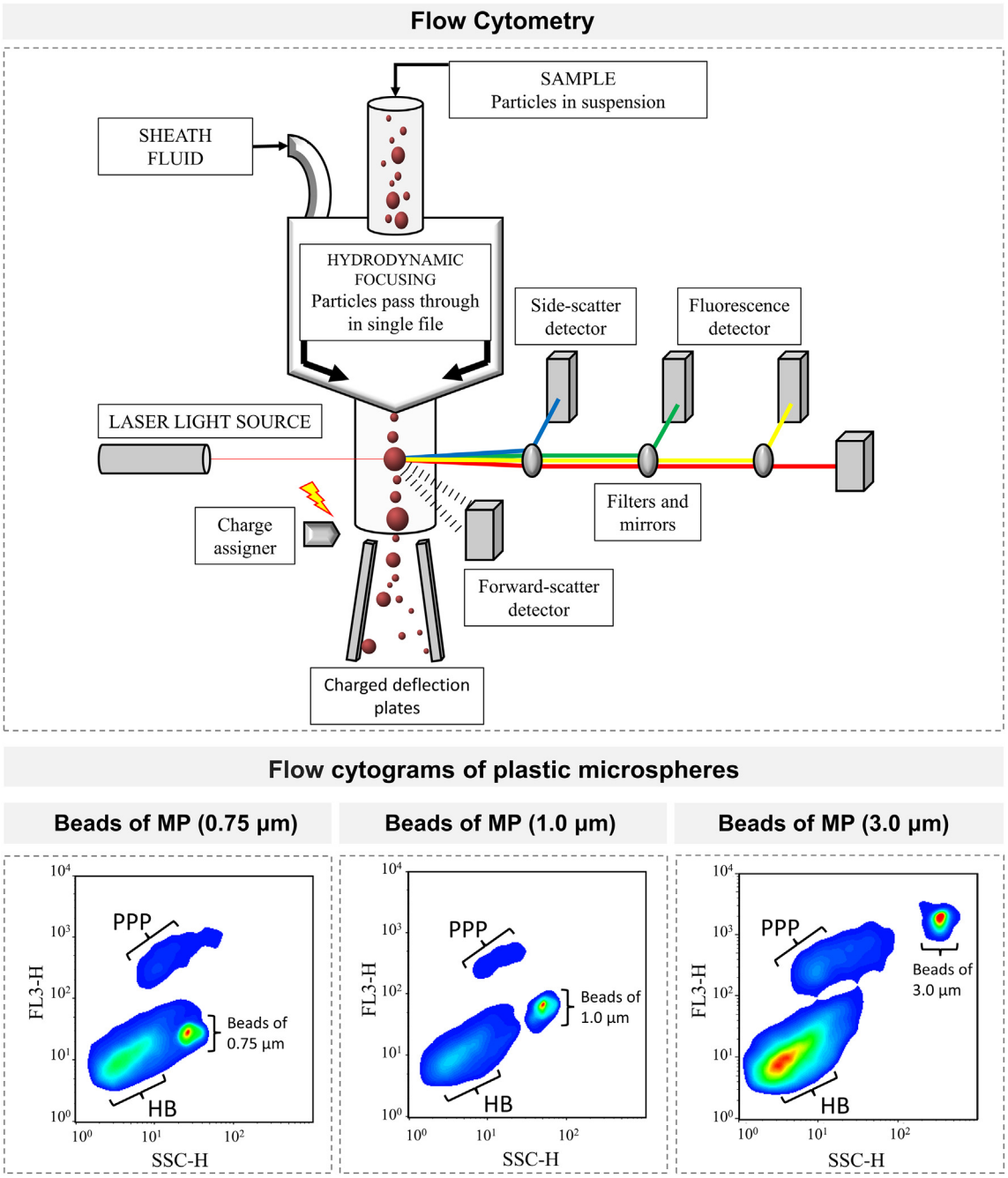

Fig. 3. Schematic representation of a flow cytometer and scatter plots displaying the SSC (X-axis) and FL3 (Y-axis). The FS3 scatter data provide information on the relative size of the particles, whereas the SSC data estimate the granularity. FL3-H, forward scatter area and SSC-H, side scatter area. For further details see Jahan-Tigh et al. (2012). Identification and distinction between heterotrophic bacteria (HB), picophytoplankton (PPP) and beads of microplastics (MP) of different size (0.75 µm, 1.0 µm, and 3.0 µm).

3.2. Evolution of microplastic consumption over time

To measure whether the consumption of microplastic particles by the treatments increases over time (12 h, 24 h, and 36 h), the NCR of the beads units (0.75 µm, 1.0 µm, and 3.0 µm) used in the PSF treatments were measured. Three-way ANOVA revealed that Time ($F = 6.658$; d.f. $= 2,72$; $p < 0.001$; Supplementary Material Table A.1) significantly influenced the amount of MP in the treatments. However, the post hoc test showed that particle consumption across sample Time had no significant differences (Supplementary Material Table A.1).

The combined effects of NCR treatments in relation to PSF and Time ($F = 1.149$; d.f. $= 6,72$; $p = 0.2083$; Supplementary Material Table A.1), revealed non-significant values for interaction of these factors on MP consumption over time. On the other hand, the interaction between experiment time and MP beads indicated significant effects caused by these factors ($F = 2.802$; d.f. $= 4,72$; $p < 0.05$; Supplementary Material Table A.1).

3.3. Microplastic consumption among predator size fractions

The results of treatments PSF in the three-way ANOVA showed significant differences ($F = 342$; d.f. $= 3,72$; $p < 0.05$; Supplementary Material Table A.1). Contrary to what we expected, we found no significant differences in MP consumption between the predator size fractions; this means that, for example, the <45 µm size predator treatment had similar rates of consumption than the treatment including all organisms (Whole Community). However, all treatments were different than the control

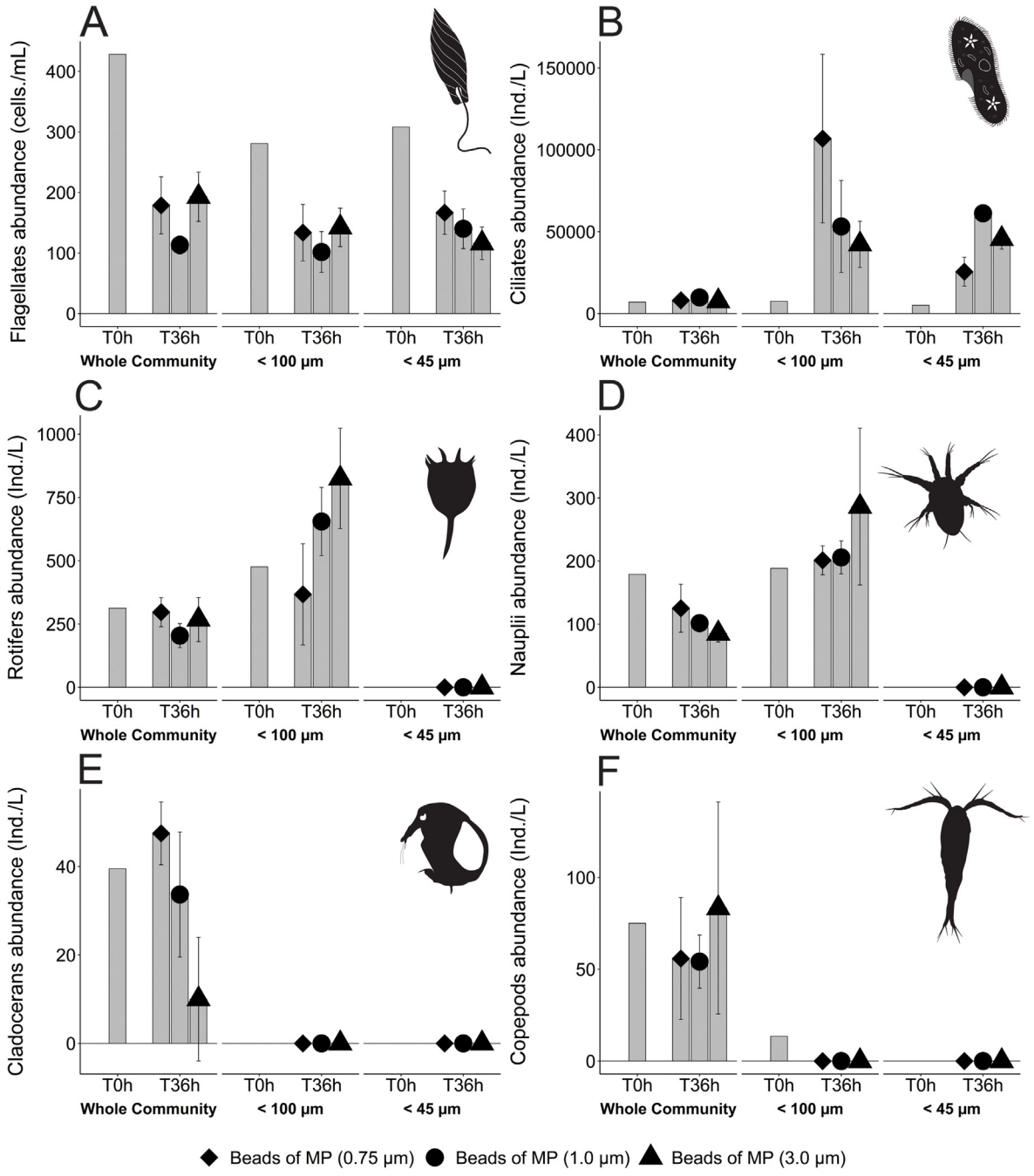

Fig. 4. Total predator abundance (ind./L) in the different predator size fraction treatments at the end of the experiment. Flagellates (A), ciliates (B), rotifers (C), nauplii (D), cladocerans (E) and copepods (F). The shapes diamond (0.75 μm), circle (1.0 μm) and triangle (3.0 μm) represent the microplastic size treatments. Shapes represent mean values and bars represent standard error (mean ± SE).

treatment, evidencing that all communities are potential consumers of microplastic in the environment. The effects of interaction in treatments PSF and MPs revealed significant differences ($F = 117$; d.f. = 3,72; $p < 0.001$; Table A.1) for these factors.

3.4. Most ingested particle sizes

A three-way ANOVA revealed that the consumption of different beads sizes ($F = 3.516$; d.f. = 2,72; $p < 0.05$; Table A.1) was significantly different between treatments. The post hoc test showed significant differences between the amounts of 1.0 μm and 0.75 μm beads (t-value = −3.586; $p < 0.001$) and 3.0 μm and 1.0 μm beads (t-value = 4.664; $p < 0.0001$).

The consumption of 0.75 μm and 1.0 μm MP particles was the highest across treatments (Fig. 5). On average, microplastic beads of 1.0 μm were 3× more consumed than 0.75 μm beads, whereas 3.0 μm beads were 30× and 100× less consumed than 0.75 μm and 1.0 μm beads, respectively, showing the strong preference of planktonic organisms for the smallest size of microplastic.

4. Discussion

By having treatments with different predator size fractions, and finding that the consumption of microplastic particles in the treatments was significantly higher for all fractions compared to the control, we show that all these predators are great consumers of microplastic in the environment.

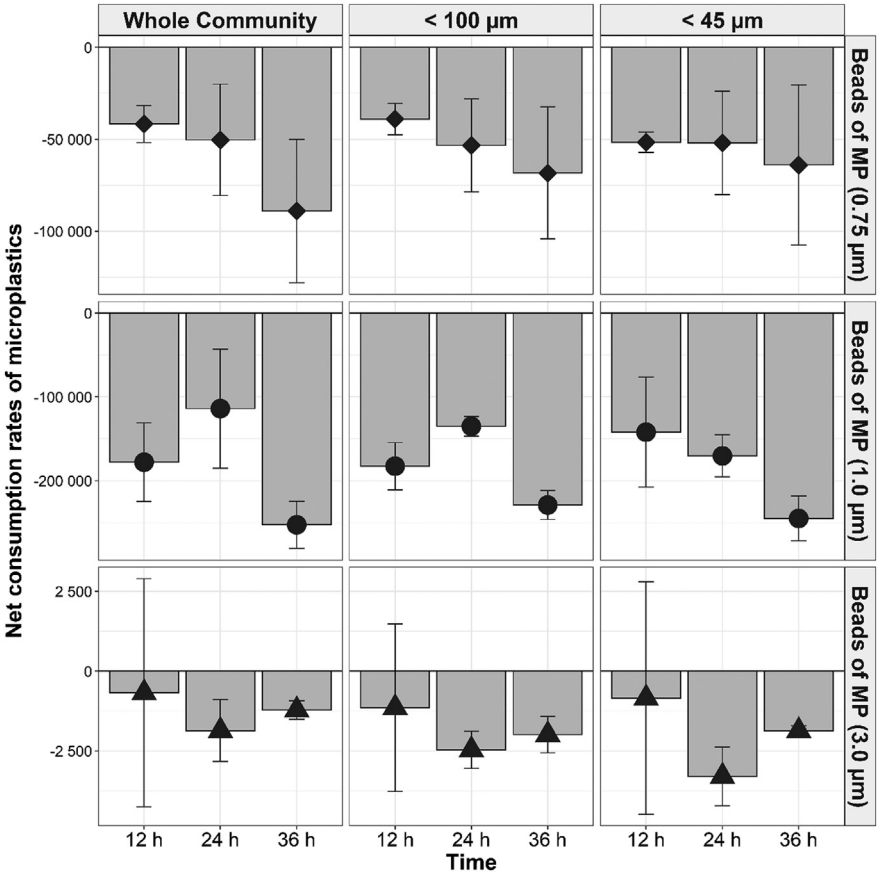

Fig. 5. Effects of the predators of different treatments (Whole Community, < 100 µm, and < 45 µm) on the net consumption rates of microplastics (MP) of different size (0.75 µm, 1.0 µm, and 3.0 µm), exerted over time. Dots represent mean values and bars represent standard error (mean ± SE). The shapes diamond (0.75 µm), circle (1.0 µm) and triangle (3.0 µm) represent the Microplastic size treatments.

We also found that protists are likely one of the major grazers of MP in the environment. As expected, the smallest size microplastic particles were the most consumed by all predators. Furthermore, no significant difference in microplastic consumption over time was detected.

4.1. Microplastic consumption was similar over time

Contrary to what we expected, the consumption of microplastic particles was similar between exposure times. Canniff and Hoang (2018) show that when it comes to the ingestion of MP particles, the amount of particles ingested increases with longer exposure time. In studies conducted by Rist et al. (2017) more particles were found in *D. magna* organisms after the 21-day period than at 5 days of exposure. In addition, filter-feeding organisms constantly capture particles to feed, which explains the increase in particle capture in the first hours of exposure. Increased ingestion rates of microplastic over time was also found in *Daphnia* when resources are scarce (Hoffschröer et al., 2021). In the presence of MPs, the energy transferred between trophic levels presents lower levels of energy return, especially when there are high concentrations of smaller size MPs, since they are diluted in the food particles and replace them in the gastrointestinal tract (Egbeocha et al., 2018). Considering that ours was a short-time experiment, it is possible that this difference in consumption over time was not captured during the time we measured the consumption, or that there were enough resources for the predators to consume along with the MP over that time period.

4.2. Microplastic consumption was similar among predator size fractions

Surprisingly, we found that all predator size fractions had similar MP consumption rates throughout the experiment, instead of the Whole Community being responsible for most of the consumption as we predicted. The fact that the <45 µm size predators, which included mostly protists, had similar rates of consumption than the Whole Community treatment, suggests that these organisms have a key role in the grazing of microplastics in the environment. In fact, ciliates and flagellates are considered the main grazers of planktonic prey of similar size to microplastics, such as bacteria and picophytoplankton, both in marine (Fenchel, 1982) and freshwater environments (Negreiros et al., 2017; Segovia et al., 2015).

In an ecologically point of view, it is important to highlight that the incorporation of the microplastics by protists, besides being harmful for them due to the poor nutrient quality, it also affects the entire ecosystem via trophic transfer, since higher trophic levels are contaminated by consuming prey that have previously ingested microplastic particles (Au et al., 2017).

4.3. Smallest microplastic size particles were consumed the most

The higher consumption of the smaller microplastic (0.75 µm and 1.0 µm) by most planktonic organisms, occurred probably occurred because these particles sediment slowly in the water column and are easily mistaken for food, since this is the size range of most bacteria and picophytoplankton in this environment (Meira et al., 2018).

In general, suspension- and filter-feeders are not as selective (Bermúdez et al., 2021; Colomer et al., 2019; Fenchel, 1980; Sun et al., 2019), but have some preference for food particles of a certain size; filter feeders usually prefer relatively smaller prey than predatory/raptorial feeders (Hansen et al., 1994). For example, Vadstein et al. (1993) showed similar results in their experiments, where particles smaller than <2 µm in diameter were mainly consumed by rotifers. Young forms of copepods exploit resources according to their body size, especially in terms of minimum particle size (Hansen et al., 1994). Microcrustaceans, particularly the small-size

cladocerans of the genus *Bosmina*, were also found to have a major impact on bacterial communities (Vaqué and Pace, 1992), suggesting that their potential as MP consumers is also considerable.

Filter-feeding protists, although using a lower energy investment, possess chemosensory and behavioral receptors to discriminate between different foods and particles (Thurman et al., 2010; Wootton et al., 2007). The prey size preference of protists generally falls within the size of the smallest MP sizes tested in our experiment. For instance, consumption of food particles by some bacterivorous ciliates were found to be most efficient on particles between 0.3 μm and 1 μm (Fenchel, 1980), whereas planktonic flagellates showed a preference for bacteria in the 0.8–1.2 μm size range (Chrzanowski and Šimek, 1990).

4.4. Implications of microplastic consumption for the organisms and the environment

In general, the microplastic particles are captured in the environment, and over a short period of time, they are allocated within the organisms, and then, through predation, these particles are transferred throughout the food web (Setälä et al., 2014) (Fig. 6).

The consumption of the microplastic depends, in part, on the strategy employed by the predator, so the presence of microplastics, especially the smaller particles, influences the structuring of the different trophic levels in the planktonic food chains. Ingestion rates also depend on the conditions and feeding strategies of the aquatic organisms evaluated (Wagner et al., 2014). Therefore, the accumulation of microplastic particles in the environment poses a serious risk to the maintenance of trophic relationships by affecting the physiological functions of organisms, and therefore ecosystem services, since different sizes of microplastic interact in different ways with aquatic organisms (Cole et al., 2015).

Studies have shown that microplastics have significant impacts on the feeding rates of marine copepods (Cole et al., 2013), in addition to the considerable reduction in survival and fecundity (Cole et al., 2015, 2019; Lee et al., 2013). Similarly, laboratory studies with nanoparticles confirm reduced fitness of filter-feeding organisms by blockage of the gastrointestinal tract, when consumption of this material occurs (Browne et al., 2008). In freshwater environments the size of prey available in the plankton can vary up to seven orders of magnitude (Kruk et al., 2010). This aspect is essential for the development and survival of copepods in this environment, since they use filtering and raptorial type strategies (Cole et al., 2015). Copepods are able to manipulate particles and have high food selectivity, however, filter feeders in this group may not distinguish small particles, whereas those employing the raptorial predation strategy may consume prey with microplastic particles allocated within their structures, and thus consume microplastic indirectly (Hansen et al., 1994).

Cladocerans usually filter particles in a size range from 1 μm to 50 μm (Colomer et al., 2019). Patterns of non-mortality are reported by Beiras et al. (2018), and no significant effect was observed on the survival and reproduction rates of *D. magna* in studies conducted by Canniff and Hoang (2018). On the other hand, microplastics increase in rates of mortality, reduced growth, total offspring, mobile juveniles, population growth rate and they produced immobile juveniles in studies that took into account consecutive generations of these organisms (Jaikumar et al., 2018; Martins and Guilhermino, 2018; Schür et al., 2020).

Rotifers are predators with filter-features, and in some cases, raptorial (Hansen et al., 1994). Sun et al. (2019) show that large microplastic particles do not significantly affect the life traits of these organisms, since rotifer ingestion of suspended particles is directly related to particle size. However, high concentrations of nano/microplastics are reported to decrease rotifer growth rates by 50% to 89% (Snell and Hicks, 2011). In addition, the presence of small microplastic particles decreases the reproduction rate, body size, lowered algae filtering capacity, prolongs the maturation time, reduces the body size, inhibition of energy metabolism, damages of cell membrane and oxidative stress of rotifers (Sun et al., 2019; Xue et al., 2021).

Studies addressing the influence of microplastics on protists are still scarce. Among the effects of MP presence on dinoflagellates, it was found that it interferes with their growth and ingestion rates of algal prey (Fulfer and Menden-Deuer, 2021). A substantial decrease in the abundance, body size, and biomass was also found in marine ciliates when exposed to microplastic (Zhang et al., 2021). The trophic transfer of MPs from protists to higher trophic levels has also been shown, with the tintinnid ciliate *Favella* spp. being consumed by the larval fish of inland silversides, which showed a high accumulation of particles in their gut (Athey et al., 2020).

4.5. Future directions

The lack of standardized bioassays and the number of studies conducted in freshwater and saltwater environments form a knowledge gap on the subject (de Sá et al., 2018b; Karami, 2017). On the other hand, due to the need to consider new models in experiments, future efforts should consider environmental conditions, size and shape of the microplastic particles, characteristics of the different groups, the place they inhabit, and the amount of particles present in the medium (Eerkes-Medrano et al., 2015).

We understand that the process of recognizing the risks associated with the presence of microplastic particles in the environment is moving towards being recognized as potential causes of ecological damage. Without causing

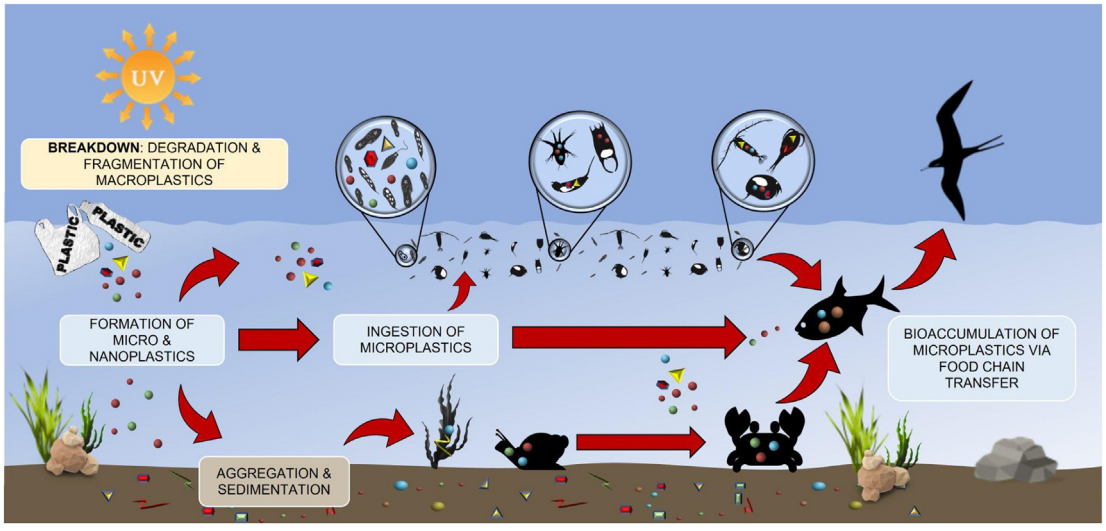

Fig. 6. Processes for the aggregation of microplastics under UV and heat irradiation, biodegradation and bioaccumulation in the aquatic environment. (Adapted from Picó and Barceló, 2019).

alarm, the preliminary considerations reported here demonstrate the ability of planktonic communities to consume microplastic, however we have not taken into consideration the ability of these microplastic particles to absorb compounds that, even at low concentrations (ng/L or μg/L), induce toxic effects, such as heavy metals, pharmaceutical products, and herbicide (Hernando et al., 2006). Understanding the prevalence, behavior, developing new techniques and adopting effective public policies correspond to the necessary measures to mitigate the impact of these contaminants.

5. Conclusion

Our results showed that different particle sizes and prey induced significant community responses. The results allowed for the detection of the effect of the consumption of smaller size microplastic particles consumed by protists compared to the treatments with all community links. The same effect occurred in the treatment with the presence of all zooplankton fractions. At the end of the experiment, the consumption of the smallest particles was preferred by all fractions of the trophic chain. Furthermore, the effect of competition from large predators is a crucial factor, which do not directly consume MP particles, but indirectly by consuming prey with high capacity to absorb these compounds in the environment.

The microplastics present in aquatic environments correspond to a fraction of all particles found in water and sediment. By using an experimental approach to assess the ability of aquatic communities to consume this material, we add knowledge of the specific risks and effects of these particles on these environmental components. We confirm that consumption of these particles is continuous, and that smaller particles are more easily assimilated.

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CRediT authorship contribution statement

João Vitor Fonseca da Silva: Formal analysis, Data curation, Writing – original draft, Writing – review & editing. **Fernando Miranda Lansac-Tôha:** Investigation, Formal analysis, Writing – original draft, Writing – review & editing. **Bianca Trevizan Segovia:** Investigation, Formal analysis, Writing – original draft, Writing – review & editing. **Felipe Emiliano Amadeo:** Investigation, Writing – review & editing. **Louizi de Souza Magalhães Braghin:** Writing – original draft, Writing – review & editing. **Luiz Felipe Machado Velho:** Methodology, Visualization, Investigation, Writing – review & editing. **Hugo Sarmento:** Methodology, Visualization, Investigation, Writing – review & editing. **Claudia Costa Bonecker:** Methodology, Visualization, Investigation, Writing – review & editing.

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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