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Size dependent uptake and trophic transfer of polystyrene microplastics in unicellular freshwater eukaryotes

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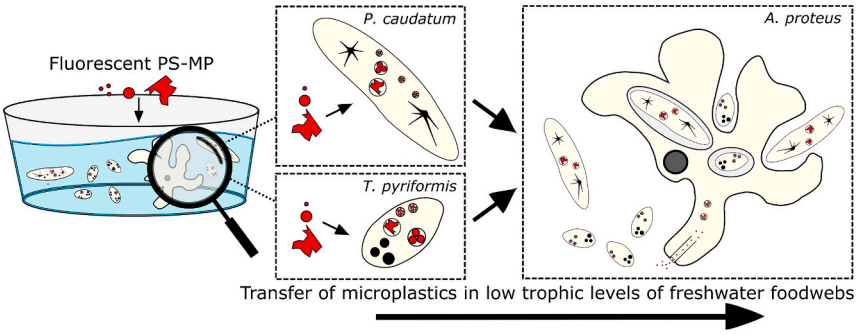
HIGHLIGHTS

- *P. caudatum* and *T. pyriformis* internalize large MP, relative to their cell size.
- *A. proteus* avoids ingesting MP larger than 6 μm suspended in the water column.
- *A. proteus* ingests larger microplastic particles when encountered during predation.
- Evidence of polystyrene MP transfer in lower trophic levels

GRAPHICAL ABSTRACT



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ABSTRACT

Microplastics (MP) have become a well-known and widely investigated environmental pollutant. Despite the huge amount of new studies investigating the potential threat posed by MP, the possible uptake and trophic transfer in lower trophic levels of freshwater ecosystems remains understudied. This study aims to investigate the internalization and potential trophic transfer of fluorescent polystyrene (PS) beads (0.5 μm, 3.6×10^8 particles/mL; 6 μm, 2.1×10^5 particles/mL) and fragments (<30 μm, 5×10^3 particles/mL) in three unicellular eukaryotes. This study focuses on the size-dependent uptake of MP by two freshwater Ciliophora, *Tetrahymena pyriformis, Paramecium caudatum* and one Amoebozoa, *Amoeba proteus,* serving also as predator for experiments on potential trophic transfer. Size-dependent uptake of MP in all three unicellular eukaryotes was shown. *P. caudatum* is able to take up MP fragments up to 27.7 μm, while *T. pyriformis* ingests particles up to 10 μm. In

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A. proteus, small MP ($PS_{0.5\mu m}$ and $PS_{6\mu m}$) were taken up via pinocytosis and were detected in the cytoplasm for up to 14 days after exposure. Large PS-MP ($PS_{<30\mu m}$) were detected in *A. proteus* only after predation on MP-fed Ciliophora. These results indicate that *A. proteus* ingests larger MP via predation on Ciliophora ($PS_{<30\mu m}$), which would not be taken up otherwise. This study shows trophic transfer of MP at the base of the aquatic food web and serves as basis to study the impact of MP in freshwater ecosystems.

1. Introduction

The extensive usage and careless handling of plastics since the second half of the 20th century led to an increasing accumulation of plastic waste in all environmental compartments (Ilyas et al., 2018; Li et al., 2016). Increasing pollution, not only with macro-, but also microplastics (MP, 100 nm - 5 mm, de Souza Machado et al., 2023), emerges as an ubiquitous hazard for the biota (Bellasi et al., 2020; Imhof et al., 2013; Kernchen et al., 2022). MP can be divided into primary (intentionally manufactured for specific applications) and secondary (fragments from larger plastic objects) plastic particles with variable shapes (spheres, fragments or fibers), sizes, and surface characteristics (Frias and Nash, 2019; Wieland et al., 2024). Due to their small size and ubiquitous presence, MP can be ingested by various organisms (Huang et al., 2021; Imhof et al., 2013; König Kardgar et al., 2023; Liu et al., 2022a; Magni et al., 2020). The impact of MP on aquatic ecosystems has been the subject of many investigations. MP research focused initially on marine compartments, and has nowadays extended to freshwater ecosystems (Li et al., 2023; Range et al., 2023), and the organisms that inhabit them (Triebskorn et al., 2019; Wagner et al., 2014). It has been shown that the ingestion of MP by aquatic organisms has the potential to induce different negative effects, such as impairment of food consumption, growth, and reproduction (Du et al., 2021). Different studies have been carried out on this issue, using both vertebrate and invertebrate organisms (Brehm et al., 2022; Brehm et al., 2023; Eltemsah and Bøhn, 2019; Imhof et al., 2017; Klein et al., 2021; Schwarzer et al., 2022; Xia et al., 2020; Xu et al., 2020). However, information regarding the potential impact of MP on organisms of lower trophic levels in freshwater ecosystems is still scarce. Nevertheless, continuous fragmentation and degradation of MP results in rising numbers of smaller MP particles in the aquatic environment, increasing their bioavailability for smaller biota (Al-Thawadi, 2020; González-Pleiter et al., 2019). For planktonic crustaceans, like Cladocera and Copepoda, it has been shown that many of them are prone to ingest MP, which can lead to various adverse effects like, e.g., increased oxidative stress, biomass reduction, fitness impairment, higher mortality rates, behavioral alterations, and impacts on their ability to feed and moult (Bosker et al., 2019; Cole et al., 2019; Cole et al., 2015; De Felice et al., 2019; Liu et al., 2022a; Schwarzer et al., 2022). Recent investigations also confirmed size-dependent uptake and toxicity in microzooplankton, like rotifers. For *Brachionus* sp., it was shown that MP ingestion has negative effects on their feeding activity, oxidative status, and gene expression (Drago et al., 2020; Jeong et al., 2016; Zheng et al., 2022). A recent study from Athey and co-authors (Athey et al., 2020) also showed that the marine tintinnid *Favella* spp. quickly ingests MP and that those particles are transferred via predation to estuarine fish larvae (*Menidia beryllina*). Unicellular eukaryotes hold an important role in aquatic ecosystems, as they generally feed on particulate matter, algae, bacteria or other, smaller unicellular eukaryotes (Mansano et al., 2014; Mansano et al., 2016). Furthermore, unicellular eukaryotic organisms also serve as prey for other protists and higher organisms. A decline in their population could, consequently, have an impact on both the bacterial communities that they prey upon, and on primary consumers, potentially altering the food web (Mansano et al., 2016). Nonetheless, only some studies employed these organisms for MP research to investigate, for instance, effects on photosymbiont productivity, food selectivity, population dynamics, and size dependent MP uptake (Bermúdez et al., 2021; Bulannga and Schmidt, 2022; Feng et al., 2021; Geng et al., 2021; Nałęcz-Jawecki et al., 2021; Nugroho and Fyda, 2020; Wang et al., 2024; Makin, 2023; Zhang et al., 2021).

Plenty of unicellular eukaryotes are non-selective feeders. Hence, food choice is dependent on availability (Nisbet, 2012). Given MP abundance in smaller size ranges (<50 μm) in virtually all freshwater compartments (Imhof et al., 2016), these particles are potentially available to a wide range of suspension feeders, including unicellular eukaryotes (Bulannga and Schmidt, 2022). If ingested, MP might be transferred to predators, as unicellular eukaryotes are usually subject to a high predation rate (Mansano et al., 2016). Ultimately, this could lead to a transfer of MP along the food web. Despite the constantly increasing effort spent by the scientific community on the investigation of the possible impact of MP exposure on different organisms, the uptake of MP by eukaryotic unicellular organisms of freshwater ecosystems as well as trophic transfer in these lower trophic levels are still scarcely researched.

In this study, two freshwater Ciliophora *Tetrahymena pyriformis* and *Paramecium caudatum*, that normally feed on suspended particulate matter, small bacteria and small eukaryotes, were employed as model organisms to investigate MP ingestion. Both organisms are likely to unintentionally ingest MP via phagocytosis, since they cannot differentiate between food (e.g., suspended particulate matter, bacteria and algae) and inorganic particulate matter (Scherer et al., 2018). We used *Amoeba proteus* as a generalist predator that feeds on other unicellular eukaryotes. Amoebae also feed on particulate matter via phagocytosis, but, unlike *T. pyriformis* and *P. caudatum,* they are known to be able to discern between organic and inorganic matter (Mast and Hahnert, 1935). Moreover, *A. proteus* shows a permanent, but minor pinocytosis-driven ingestion. As pinocytosis channels in *A. proteus* reach a diameter of up to 6 μm (Klein et al., 1988), they might be large enough for a passive uptake of MP smaller than this size.

Hence, the main objective of this study is to investigate if three distinct unicellular freshwater eukaryotes *P. caudatum*, *T. pyriformis*, and *A. proteus* are able to ingest MP particles of different sizes. To achieve this, we employed fluorescent polystyrene (PS) beads in two sizes (0.5 and 6 μm) as well as larger PS fragments (<30 μm). The selection of different size categories is essential for discerning potential size-dependent uptake patterns. We further analyzed in *A.proteus*, if the uptake of the different sized particles takes place via phagocytosis or pinocytosis. Finally, we investigated the potential for trophic transfer of MP by introducing *A. proteus* to its potential prey, *P. caudatum* and *T. pyriformis*, after these prey organisms have been exposed to MP.

2. Material and methods

2.1. Materials

If not otherwise indicated, materials and chemicals were obtained from Carl Roth GmbH (Karlsruhe, Germany) or Thermo Fisher Scientific (Schwerte, Germany). Fetal calf serum and bovine serum albumin (BSA) were obtained from Sigma-Aldrich (Taufkirchen, Germany), cell culture material from Greiner Bio-One (Frickenhausen, Germany). Dulbecco's phosphate-buffered saline (DPBS) was obtained from Lonza Group AG (Visp, Switzerland). Non-functionalized 0.5 and 6 μm polystyrene (PS) beads (fluorescent, Fluoresbrite® PC Red Microspheres, Cat. # 19507–5 and Cat. # 19111–2, Ex_{Max} 491 nm, Em_{Max} 565 nm, 3.64×10^{11} beads/mL ($PS_{0.5\mu m}$) and 2.10×10^{8} beads/mL ($PS_{6\mu m}$), size CV $\leq$ 5) were purchased from Polyscience (Polyscience Europe GmbH, Eppenheim, Germany). PS beads were delivered in a sterile aqueous suspension at a

concentration of 2.5 % (*w/v*). According to the supplier, all MP particles therein are plain beads with a slight residual anionic surface charge from sulphate ester. Larger PS fragments (Magic Pyramid, Frechen, Germany) were prepared in house by milling and sieving (detailed information in the SI, Figs. S1 and S2) to fractions of <30 μm (produced within the CRC 1357, department Macromolecular Chemistry I, University of Bayreuth; https://www.sfb-mikroplastik.uni-bayreuth.de/en/index.html, Fig. S1). The obtained fractions were then suspended in bi-distilled water, supplemented with 0.1 % (*w/v*) Tween20® to avoid aggregation. Afterwards, the suspension was centrifuged at 13,000 x*g* for 5 min at room temperature (RT), the supernatant was discarded, and the MP were resuspended in bi-distilled water ($PS_{<30\mu m}$, 5×10^5 fragments/mL). In the following, the respective MP particles will be referred to as $PS_{0.5\mu m}$, $PS_{6\mu m}$ (beads), and $PS_{<30\mu m}$ (fragments). The different particle sizes were chosen due to the different advantages of the respective size ranges. For $PS_{0.5\mu m}$ and $PS_{6\mu m}$, narrow size ranges with a well-defined characterization were possible due to the manufacturing (suspension polymerization). Larger fragments ($PS_{<30\mu m}$) are more comparable to environmental particles but were not available in narrow size ranges due to their manufacturing (milling and sieving of larger fractions). Before usage, the respective MP stock suspensions were diluted to the desired concentration in the suitable growth media.

2.2. Methods

2.2.1. Particle characterization

The ζ-potentials of the particle fractions were measured using the LiteSizer 500 (Anton Paar Germany GmbH, Ostfildern-Scharnhausen, Germany) and Omega cuvettes (Anton Paar Germany GmbH, Ostfildern-Scharnhausen, Germany). For the measurements, 2.5 μL of the particle suspension were directly diluted in the respective media (i.e., $Prescott_{ref}$ (pH 6.4), $Prescott_{NaCl}$ (pH 4.3), see below for compostion) and incubated overnight. Thereafter, the particles were collected by centrifugation (13,000 ×*g* for 10 min at room temperature (RT)) and resuspended in 1 mL of a 1 mM KCl (pH 6.0) solution for measurement. Three measurements with at least 100 runs each were performed at 21 °C with an adjusted voltage of 200 V. The ζ-potential was calculated using the Helmholtz-Smoluchowski equation (Smoluchowski, 1906).

Particle size distributions were measured by laser diffraction (three lasers and two detectors) coupled with dynamic image analysis, using a Microtrac FlowSync Particle Size Analyzer (Microtrac Retsch GmbH, Haan, Germany, Fig. S1). Wet analysis was performed using deionized water as the mobile phase with 1 mg of particles added to 1 mL of 5 % (*w/v*) $Na_4P_2O_7$ and 2 % (*w/v*) Tween® 20 detergent to avoid aggregation. This mixture was poured into the water-filled measuring cell and diluted with 200 mL ultrapure water. A 180 s ultrasound treatment (40 kHz) was carried out to ensure complete suspension of the particles.

2.2.2. Cultivation of *Tetrahymena pyriformis, Paramecium caudatum and Amoeba proteus*

T. pyriformis (Icon biotech, Teltow, Germany) was cultivated at room temperature (20–25 °C) in 2 % (*w/w*) proteose-peptone broth. The freshwater ciliate *P. caudatum* originated from a well-established continuous in-house freshwater cultivation (Carolina Science GmbH, Berlin, Germany). The organisms were kept in 800 mL Erlenmeyer Duran flasks (DURAN group, Wertheim, Germany) filled with diluted Z medium ($Ca(NO_3)_2$ 16.66 mM, $NaNO_3$ 549.45 mM, K_2HPO_4 48.24 mM, $MgSO_4$ 10.14 mM, $NaHCO_3$ 199.98 mM, Na_2EDTA 199.98 mM, $FeSO_4$ 3.41 mM, H_3BO_3 0.23 mM, $MnSO_4$ 2.48 mM, $(NH_4)_6Mo_7O_{24}$ 0.02 mM, $ZnSO_4$ 0.07 mM, $Co(NO_3)_2$ 0.24 mM, $CuSO_4$ 0.01 mM; pH ~ 7.6) (Prusch and Minck, 1985). *P.caudatum* culture was maintained in static conditions in dark cabinets and fed ad libitum with an infusion of dried celeriac root (*Apium graveolens*) prepared in-house.

A. proteus (Carolina Science GmbH, Berlin, Germany) was cultivated as proposed by Prescott and James (1955) in the following referred to as $Prescott_{ref}$ method and medium. Briefly, amoebae were cultured in a 10 cm glass Petri dish (VWR, Germany) at room temperature in $Prescott_{ref}$ medium (KCl 12.4 mM, $CaHPO_4$ 2.9 mM, $MgSO_4$ 1.7 mM, pH 6.4) and were fed twice a week with approximately 10–20 *T. pyriformis* (Icon Biotech, Germany) per amoeba. The amount of *T. pyriformis* added was high enough to ensure a close vicinity between prey and predator. For transferring amoebae for experiments, individuals were pipetted with an Eppendorf pipette while observing them under the microscope (Zeiss Primovert trino, 0.5× Camera Adapter, Zeiss, Germany). For documentation purposes, the software Zen 3.0 blue edition 2019 (Carl Zeiss, Jena, Germany) was used.

2.2.3. Uptake of MP

A. proteus: Analogeous to experiments performed by Ahn and Jeon (1982) and Prusch and Minck (1985), initially, 50–100 amoebae per Petri dish were starved for 24 h by transferring them to a 5 mL Petri dish containing 2 mL $Prescott_{ref}$ medium without any food provided, to stimulate the need for food uptake. After 24 h, ~30 amoebae were transferred into single wells of a 12-well glass plate (iBL, Austria) for each treatment, containing 1 mL of $Prescott_{Ref}$ medium. The respective MP suspensions were added to the starved amoebae, corresponding to the following MP end concentrations in a total volume of 1 mL per well: $PS_{0.5\mu m} = 3.6 \times 10^8$ beads/mL (12.5 μg/mL); $PS_{6\mu m} = 2.1 \times 10^5$ beads/mL (12.5 μg/mL); fragments $PS_{<30\mu m} = 5 \times 10^3$ fragments/mL (5 μg/mL). Alongside the MP treatments, a control group was applied in an extra well, with $Prescott_{Ref}$ medium. This process was repeated three times in total, in which the number of particles, and the number of amoebae, containing MP, was recorded. The selection of these concentration ranges was deliberately chosen, aiming at maximizing the likelihood of interaction between particles and *A. proteus*. After the respective incubation time (0.5 h, 2 h, 4 h, 6 h and 24 h), the amoebae were washed by transferring them twice to fresh wells in 1 mL $Prescott_{Ref}$ medium to remove leftover MP. Thereafter, amoebae were observed under the fluorescent microscope and ingested particles were counted manually (CKX41, Olympus-Lifescience, Hamburg, Germany, magnification 400× and 100× for 0.5 μm beads and 6 μm beads / fragments, respectively).

P. caudatum: 200–300 Individuals were exposed to fluorescently labeled $PS_{0.5\mu m}$ (end concentration: 3.6×10^8 beads/mL, 12.5 μg/mL), $PS_{6\mu m}$ (end concentration: 2.1×10^5 beads/mL, 12.5 μg/mL) and $PS_{<30\mu m}$ (end concentration: 5×10^3 fragments/mL, 5 μg/mL) for 24 h in a 5 mL Petri dish containing 2 mL Z medium, respectively.

T. pyriformis were exposed to fluorescently labeled $PS_{0.5\mu m}$ (end concentration: 3.6×10^8 beads/mL), $PS_{6\mu m}$ (end concentration: 2.1×10^5 beads/mL) and $PS_{<30\mu m}$ (end concentration: 5×10^3 fragments/mL) for 24 h in a 5 mL Petri dish, containing 2 mL $Prescott_{Ref}$ medium, respectively.

Both Ciliophora species were not starved or washed before MP exposure. Ingestion of large PS beads ($PS_{6\mu m}$) and fragments ($PS_{<30\mu m}$) by Ciliophora was quantified microscopically for 15 randomly chosen individuals from each Petri dish at different time points (0.5 h, 2 h, 4 h, 6 h and 24 h). For observations, MP exposed Ciliophora were transferred onto a microscopy slide and covered with a cover slip. This way, the Ciliophora got gently squeezed while the water was evaporating, rendering them unable to move around freely, while they were still completely intact. For particle quantification, the samples (MP exposed Ciliophora) were sealed with nail polish (trend it up nail polish, dm-drogerie markt GmbH + Co. KG, Germany), which prevented the water from evaporating. The ingested particles were then quantified under a fluorescence microscope (Zeiss Imager.Z2m, 1× Camera Adapter, Carl Zeiss, Jena, Germany). For the smaller $PS_{0.5\mu m}$ beads ingested by the Ciliophora, an exact quantification of ingested particles was not possible. This difficulty arose from particle agglomeration inside the food vacuola. For that reason, individuals were divided into two groups ("particle uptake" or "no uptake") by estimating the presence of visible intracellular MP under the microscope.

Ciliophora length to particle ratio was measured and calculated

using ZEN 2 core v2.5 / ZEN 2 starter v2.5 (Carl Zeiss Microscopy GmbH, Jena, Germany).

2.2.4. Induction of pinocytosis in A. proteus

Induction of pinocytosis was conducted to analyze if MP particles are unintentionally internalized together with the medium. To induce pinocytosis, the pH of the $Prescott_{ref}$ medium was adjusted to pH 4.3 with HCl. In addition, the medium was supplemented with 125 mM NaCl (referred to as "$Prescott_{NaCl}$ medium"). For the experiments, the amoebae were incubated for 1 h in the $Prescott_{NaCl}$ medium and were then transferred back to the standard $Prescott_{ref}$ medium and observed for 14 days.

2.2.5. Induction of phagocytosis in A. proteus

To explore if phagocytosis of larger PS-MP is dependent on an eco-corona (Ramsperger et al., 2020) covering the MP surface, protein-coated PS-MP ($PS_{<30\mu m}$) were compared to non-coated ones. Particles were pre-coated with a protein corona as described by Jasinski et al. (2022), where also the characterization of the protein coating is described. Briefly, PS-MP ($PS_{<30\mu m}$) (1 μL of the 2.5 % (*w/v*) aqueous suspension) were incubated overnight at 37 °C in 1 mL of a 2 % (*w/v*) bovine serum albumin (BSA) solution or 1 mL of a 10 % fetal calf serum (FCS) solution in $Prescott_{ref}$ medium. Afterwards, the particles were centrifuged at 13,000 x*g* for 5 min at room temperature. The resulting pellet was once washed with bi-distilled water (13,000 x *g*, 5 min), resuspended in $Prescott_{ref}$ and used immediately for the experiments.

To evaluate whether the introduction of a known chemical trigger in the medium, such as chemoattractants produced by prey organisms, could stimulate the phagocytosis of larger PS-MP fragments ($PS_{<30\mu m}$), a second attempt to induce phagocytosis was undertaken by co-cultivating 100 individuals of *A. proteus* with *T. pyriformis* at a concentration of 5×10^4 Ciliophora/mL (prey: predator = 500:1), in the presence of $PS_{<30\mu m}$ particles at a concentration of 5×10^3 fragments/mL. This concentration was chosen to increase uptake probability of prey and particles, and to enable a possible enrichment of chemoattractants released by *T. pyriformis*. Subsequent analysis of the uptake of $PS_{<30\mu m}$ was conducted following the same methodology as previously described.

2.2.6. Trophic transfer of MP particles

T. pyriformis and *P. caudatum* were incubated with the respective amount of MP (end concentrations: $PS_{0.5\mu m} = 3.6 \times 10^8$ beads/mL; $PS_{6\mu m} = 2.1 \times 10^5$ beads/mL; $PS_{<30\mu m} = 5 \times 10^3$ fragments/mL) for 24 h. Afterwards, both Ciliophora were washed by centrifugation (400 x*g*, 5 min) to remove leftover MP, and resuspended in $Prescott_{ref}$ medium. The organisms were then offered to pre-starved amoebae in a prey: predator ratio of approximately 10:1. After the indicated incubation time (up to 24 h), the amoebae were washed (as described above for the uptake experiments), transferred to a microscopy slide and observed under a fluorescence microscope (Zeiss Imager.Z2m, 1× Camera Adapter, Carl Zeiss, Jena, Germany) to obtain images of amoeba feeding on MP burdened *T. pyriformis* and *P. caudatum*.

The remaining amoebae were observed for the respective time (up to 24 h) to record a possible excretion of particles.

2.2.7. Data analysis

Data analysis and visualization was performed using Origin software 2019b (Origin, Northampton, MA, USA). Figures obtained via fluorescence microscopy were prepared using ZEN 2 (blue edition) / ZEN 2 core v2.5 / ZEN 2 starter v2.5 (Carl Zeiss Microscopy GmbH, Jena, Germany) and Inkscape v1.1 (Open Source Scalable Vector Graphics Editor).

3. Results

3.1. Particle characterization

The $PS_{<30\mu m}$ used in this study were first characterized by their size (Figs. S1 and S2) and the ζ-potential of $PS_{0.5\mu m}$ and $PS_{6\mu m}$ was analyzed (Table 1). Since the zeta sizer is only applicable for particles of up to 10 μm in size, accurate ζ-potential measurements were only possible for the smaller particles ($PS_{0.5\mu m}$ and $PS_{6\mu m}$). The surface charge of the measured beads was always negative, but highly dependent on the surrounding matrix. While no major differences of the ζ-potential were detected after incubation in FCS compared to $Prescott_{ref}$, for $Prescott_{NaCl}$ and BSA incubated particles, a trend towards a decrease of the ζ-potential was detectable in beads of both sizes.

3.2. Microplastic uptake by unicellular eukaryotes

Our results show that the uptake of MP in Ciliphora is size-dependent. High uptake of $PS_{0.5\mu m}$ and $PS_{6\mu m}$ beads was observed in both organisms (>95 % of individuals) (Figs. 1, 2 and S2). *P. caudatum* took up larger particles in its vacuola ($PS_{<30\mu m}$, 75 % of the individuals, Figs. 1 and 2), with the largest particle found being 27.7 μm in diameter (Fig. S3). $PS_{0.5\mu m}$ beads were incorporated by both organisms, which can be identified by the accumulation of MP in food vacuoles (Fig. 2). An exact determination of the number of ingested $PS_{0.5\mu m}$ beads was not possible, due to the pronounced tendency for agglomeration inside the food vacuoles of both Ciliophora. Particle counting could not be improved by immobilizing or fixating the cells, as the high number of beads inside the vacuola and their small size did not allow an accurate quantification. Six μm beads were easier to identify, as they were individually found in *T. pyriformis* and, even if more than one was detected in the vacuola of *P. caudatum*, counting was still possible (on average 16.0 ± 13.8 MP per individual). Larger MP fragments ($PS_{<30\mu m}$) were detected in separate food vacuola in *P. caudatum,* so it was possible to perform an accurate count of the particles. In *T. pyriformis*, only a few of the observed cells, out of all the observed individuals were found to have ingested $PS_{<30\mu m}$ (Fig. 1). Particle size to cell length ratio showed a possible particle uptake of up to 20 % ($PS_{6\mu m}$, Fig. S4) of the total size of *T. pyriformis* (average size: 39.6 μm, Fig. S5) and up to 10 % ($PS_{<30\mu m}$, Fig. S3) of the total size of *P. caudatum* (average size: 195.5 μm, Fig. S6).

For *T. pyriformis*, a high initial rate of uptake (95 % of individuals with particles after 0.5 h of incubation) could be observed for $PS_{0.5\mu m}$ (Fig. 1A). The number of individuals without particles increased over time and was highest after 24 h at 75 % (Fig. 1A). For $PS_{6\mu m}$, no uptake was observable after 24 h (Fig. 1C). For $PS_{<30\mu m}$, low uptake rates were only observed at 2, 4, and 6 h (Fig. 1E).

In case of *P. caudatum*, $PS_{0.5\mu m}$ could be detected in all observed individuals over the whole observation time (0.5 h–24 h, Fig. 1B). Also, particle release could be observed for $PS_{0.5\mu m}$ (Fig. S7A/B). For $PS_{6\mu m}$, a time-dependent uptake could be observed. While the ingestion rate rose until 6 h (100 % of individuals with >5 particles), after 24 h, increased clearance rates decreased the number of ingested MP (Fig. 1D). Similar to $PS_{6\mu m}$, uptake decreased with increasing time for $PS_{<30\mu m}$, whereas only 25 % of individuals with ingested MP were detected after 24 h (Fig. 1F).

For *A. proteus* (500 μm in average, Marshall et al., 2012), a size-

Table 1
ζ-potential analysis and size distribution of the used MP.

	ζ-potential [mV]				Size [μm]
	$Prescott_{ref}$	$Prescott_{NaCl}$	BSA	FCS	
$PS_{0.5\mu m}$	−53.4 ± 0.2	−30.7 ± 0.2	−24.8 ± 0.4	−49.8 ± 0.3	0.5 ± 0.02
$PS_{6\mu m}$	−44.7 ± 0.5	−12.2 ± 1.2	−14.2 ± 1.1	−49.3 ± 5.7	6.0 ± 0.6
$PS_{<30\mu m}$	n.a.	n.a.	n.a.	n.a.	16.2 ± 7.4

n.a.: not applicable. BSA: bovine serum albumin. FCS: Fetal calf serum. $Prescott_{ref}$: standard Prescott medium, pH 6.4. $Prescott_{NaCl}$: standard Prescott medium supplemented with 125 mM NaCl, pH 4.3. Data represent mean values ± SD, ζ-potential measurement $n = 3$, for $PS_{<30\mu m}$ see Fig. S1.

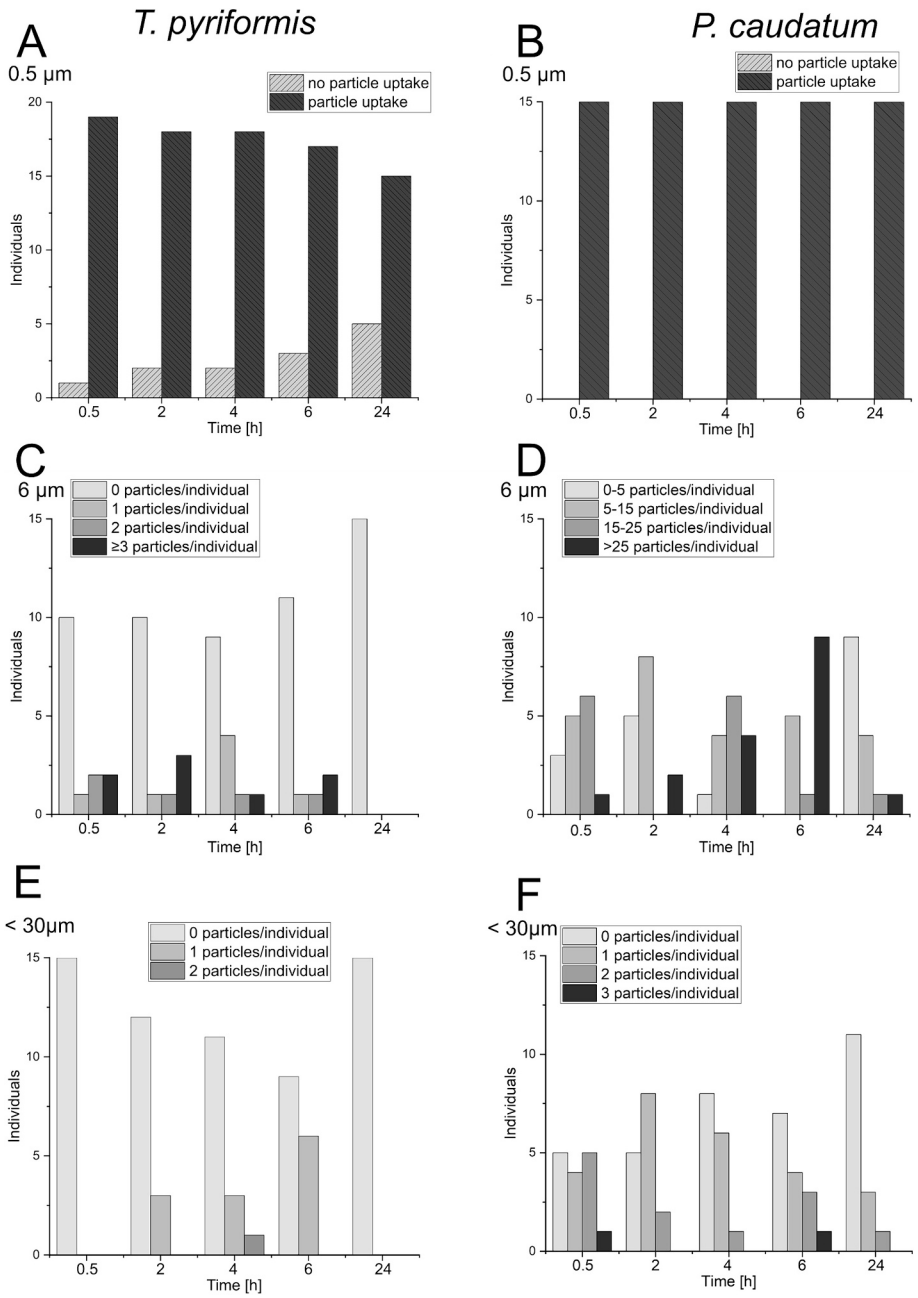

Fig. 1. Uptake of particles after ingestion in *T. pyriformis* (A, C, E) and *P. caudatum* (B, D, F). Both Ciliophora were exposed to $PS_{0.5\mu m}$, $PS_{6\mu m}$ and $PS_{<30\mu m}$. *T. pyriformis* exposed (A) to $PS_{0.5\mu m}$ (number of *T. pyriformis* = 20), (C) to $PS_{6\mu m}$ (number of *T. pyriformis* = 15) and (E) $PS_{<30\mu m}$ (number of *T. pyriformis* = 15). *P. caudatum* exposed to (B) $PS_{0.5\mu m}$ (number of *P. caudatum* = 15), (D) $PS_{6\mu m}$ (number of *P. caudatum* = 15) and (F) $PS_{<30\mu m}$ (number of *P. caudatum* = 15).

dependent uptake could be observed (Fig. 3). While beads with a size of ≤6 µm were taken up (Fig. 3A–F), larger fragments ($PS_{<30\mu m}$) were not observed inside any amoeba.

Quantification of the ingested beads per amoeba, by performing manual live-counting under the microscope, confirmed this size dependency (Fig. 3G). The number of MP, ingested by an individual *A. proteus*, exhibited substantial variation, ranging from as few as one to exceeding 50 beads for $PS_{0.5\mu m}$ (with an average of 18.8 ± 18.1 beads per amoeba). In contrast, *A. proteus* took up a maximum of 15 $PS_{6\mu m}$ particles with an average of 5.7 ± 3.4 beads per amoeba. For $PS_{<30\mu m}$, no uptake was observed in *A. proteus*.

3.3. Analysis of the MP uptake route in A. proteus

To unravel the size-dependent MP uptake route by *A. proteus*, pinocytosis and phagocytosis were investigated separately, by stimulating the respective uptake mechanism.

3.3.1. Induction of pinocytosis

Pinocytosis was successfully induced by using $Prescott_{NaCl}$ medium as shown by the high amount of pinocytosis channels (Fig. 4A/B) and the amount of $PS_{0.5\mu m}$ inside the amoebae (Fig. 4C).

Initially, amoebae ingested under pinocytic conditions about 3.8-fold more $PS_{0.5\mu m}$ beads ($Prescott_{NaCl}$ medium, 68.0 ± 44.0 beads per amoeba) than under non-inductive conditions ($Prescott_{ref}$ medium, average: 18.8 ± 18.1 beads per amoeba) (Fig. 5). Independently of the amount of $PS_{0.5\mu m}$ engulfed on day 0, no decrease of the number of particles per amoeba was detectable within the first three days of incubation. During this period, morphology and locomotion, representatives for cellular fitness, did not differ from the corresponding control

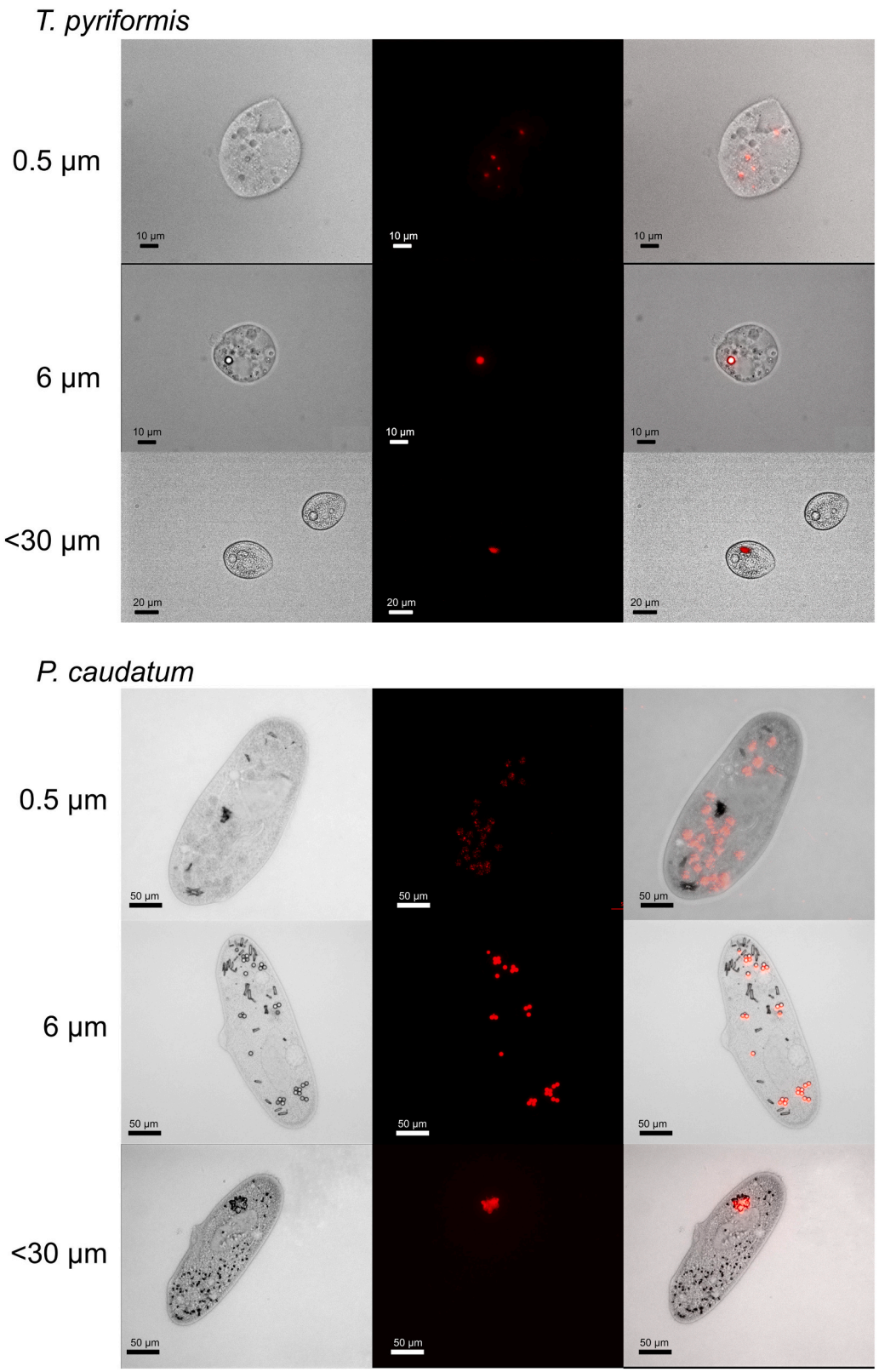

Fig. 2. Ingestion of $PS_{0.5\mu m}$ and $PS_{6\mu m}$ beads, and $PS_{<30\mu m}$ fragments by *T. pyriformis* and *P. caudatum* after 2 h of incubation. Fluorescently labeled MP beads and fragments taken up by Ciliophora, visualized by epifluorescence microscopy. Left column: bright field fluorescence, middle column: fluorescence microscopy, right column: merged pictures. Scale bar *T. pyriformis* $PS_{0.5\mu m}$ and $PS_{6\mu m} = 10$ µm (63× magnification); Scale bar *T. pyriformis* $PS_{<30\mu m} = 20$ µm (40× magnification). Scale bar *P. caudatum* = 50 µm (20× magnification).

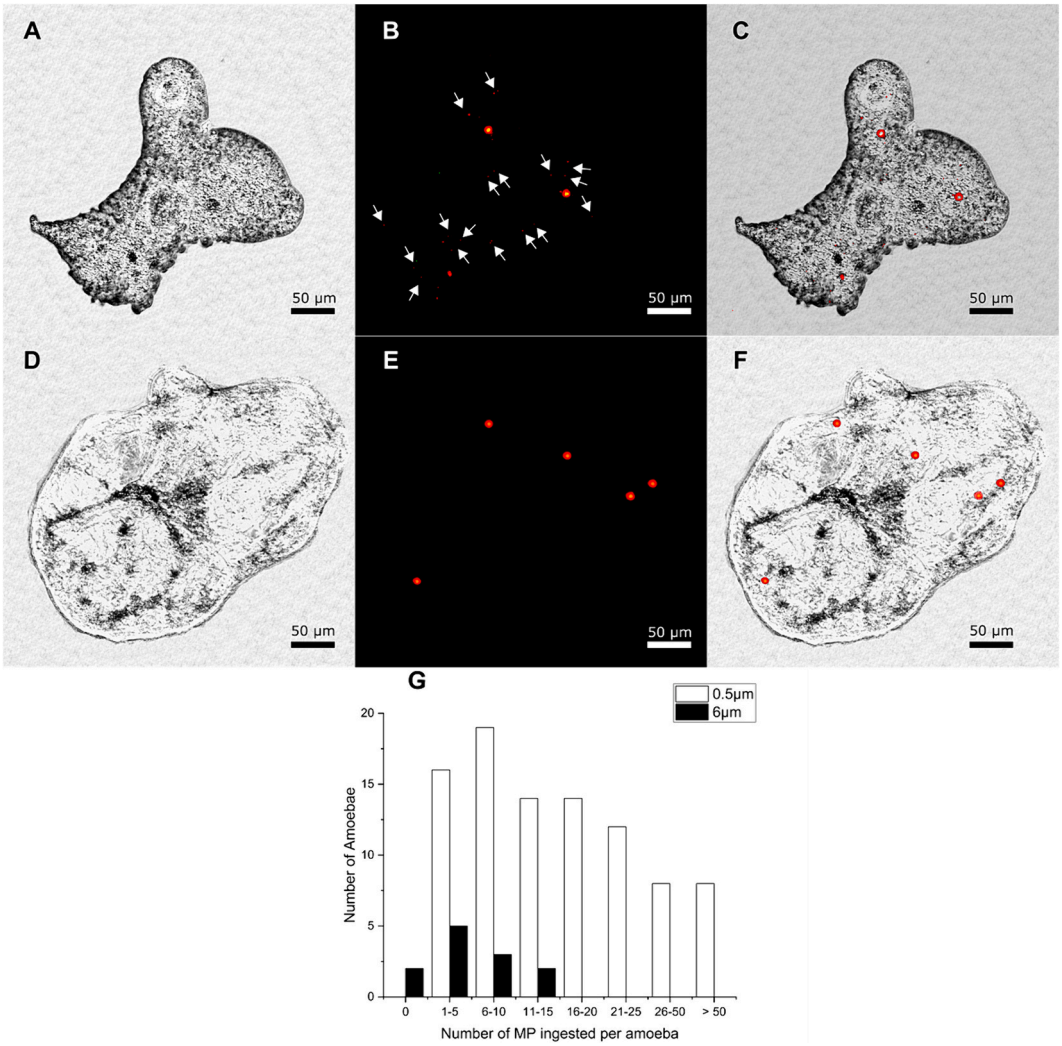

Fig. 3. Ingestion of $PS_{0.5\mu m}$ and $PS_{6\mu m}$ in *A. proteus* after 1 h of incubation. A–F: Fluorescently labeled MP beads engulfed by *A. proteus,* visualized by epifluorescence microscopy. A–C: *A. proteus* with $PS_{0.5\mu m}$ red fluorescent beads, white arrows indicate single beads, larger red dots represent agglomerations of various particles inside a vacuole. D–F: *A. proteus* with $PS_{6\mu m}$ red fluorescent beads. A, D: bright field; B, E: epifluorescence; C, F: merged photos. Scale bar = 50 μm, magnification 10×. G: Bar plot representing the number of amoebae that ingested $PS_{0.5\mu m}$ (white bars, number of *A. proteus* = 91) and $PS_{6\mu m}$ (black bars, number of *A. proteus* = 12).

group. After six days of cultivation, a 1.7-fold reduction of MP count was only recorded for amoebae cultured in $Prescott_{NaCl}$, indicating that some egestion of MP occurred. After 14 days, the remaining $PS_{0.5\mu m}$ per amoeba was low for both populations ($Prescott_{NaCl}$, $Prescott_{ref}$).

3.3.2. Induction of phagocytosis with pre-coated particles

In an attempt to trigger the receptor pathway for induced phagocytosis, $PS_{<30\mu m}$ particles were pre-coated with either BSA (a well-characterized single protein type) or FCS (a mixture of non-characterized proteins) to form a protein corona on their surfaces. However, despite this deliberate modification of the fragment surfaces, none of the coated microplastic particles were internalized by the organisms.

3.3.3. Induction of phagocytosis by co-incubation with prey organisms

To evaluate, whether the introduction of a known chemical trigger in the medium, such as chemoattractants produced by prey organisms, could stimulate the phagocytosis of larger PS-MP fragments, we conducted a co-incubation experiment involving a mixture of fragments ($PS_{<30\mu m}$) and *T. pyriformis* in the presence of *A. proteus*. However, here also, we observed no uptake of the larger MP fragments.

3.4. Trophic transfer of MP particles

To investigate the potential for trophic transfer of MP, we employed two specific prey organisms for *A. proteus*, namely, *T. pyriformis* and *P. caudatum*. These prey organisms were fed with $PS_{0.5\mu m}$ and $PS_{<30\mu m}$ particles for a period of 24 h. Subsequently, they were exposed to *A. proteus* in our experimental setup to verify the occurrence of trophic transfer from these to the latter. Independently of the species, shortly after co-incubation, the amoebae predated on the Ciliophora and took up the MP they carried (Figs. 6 and 7, S8 and S9). Especially in case of $PS_{0.5\mu m}$ beads, food vacuoles with clumped beads could still be observed 24 h post-ingestion inside the amoebae (Fig. S10).

24 h ($PS_{<30\mu m}$ fragments) and 72 h ($MP_{0.5\mu m}$ beads) post-ingestion of the prey, <10 % of amoebae still contained detectable amounts of MP (Fig. S9A–C). Our observations further showed that after digestion of the Ciliophora, a release of the remaining undigestible MP occurred (Fig. S9D–F).

4. Discussion

The uptake and transfer of MP in aquatic environments has been one of the major concerns of the ecological hazard and risk assessment in the

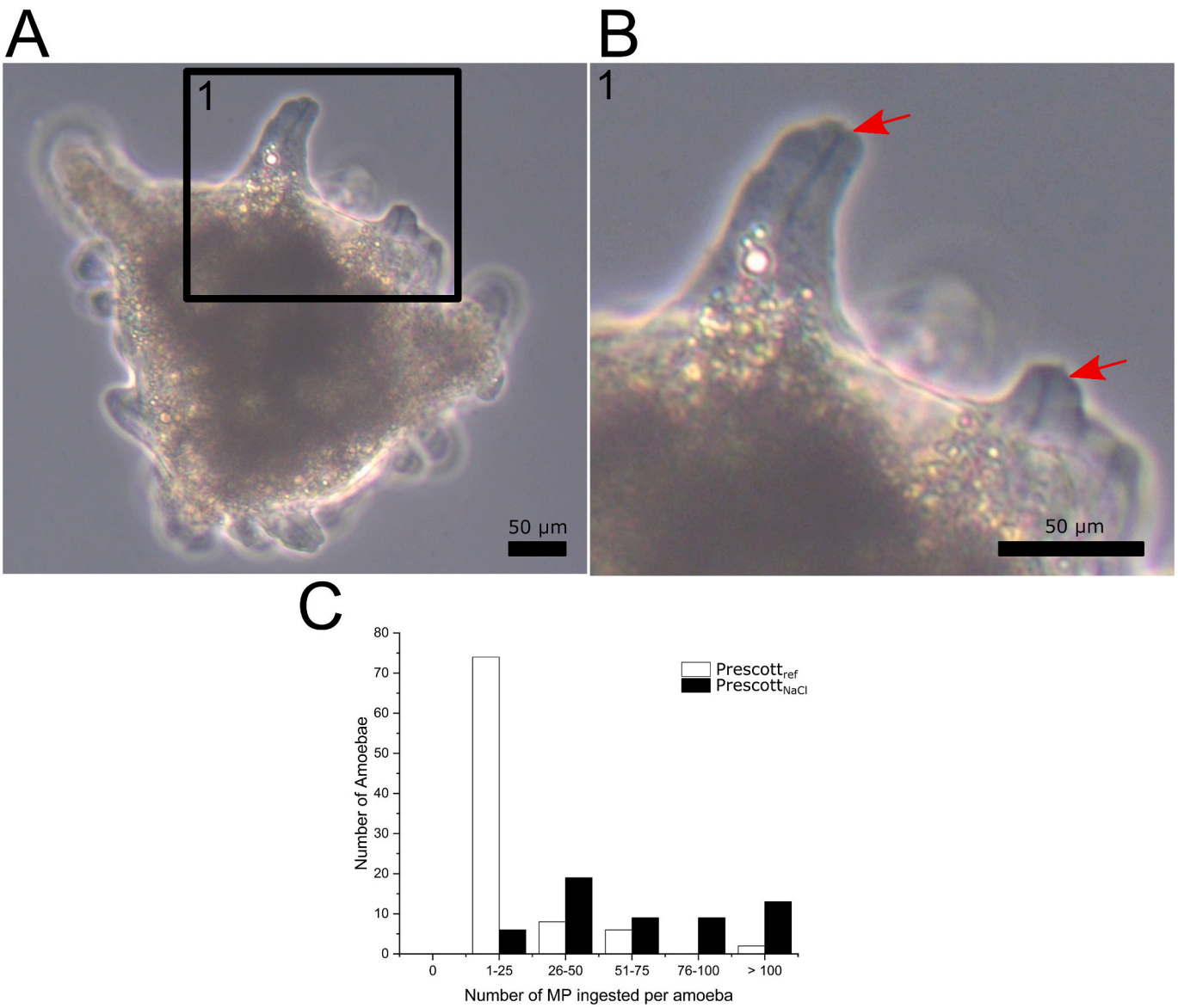

Fig. 4. A: Induction of the formation of pinocytosis channels in *A. proteus* after 20 min incubation in Prescott$_{NaCl}$ medium. B: Zoomed in section of the pinocytosis channels for better visibility. 10× magnification. Scale bar = 50 µm; C: Numerical distribution of PS$_{0.5µm}$ beads uptake in amoebae for Prescott$_{ref}$ and Prescott$_{NaCl}$ media. Quantitative analysis of the MP ingestion per amoeba after 60 min incubation with PS$_{0.5µm}$ beads in Prescott$_{ref}$ (white bars, number of *A. proteus* = 91) and Prescott$_{NaCl}$ (black bars number of *A. proteus* = 55) media.

last years (Koelmans et al., 2016). However, only a few studies exist which analyze MP transfer in the lower trophic levels, with them mainly focusing on the marine environment (Wagner et al., 2014). Regarding the uptake of MP in freshwater unicellular eukaryotes, which represent an important fraction of the lower trophic levels, only very little data is available. Therefore, this study analyzed the uptake of MP in a large size range (0.5 µm - 30 µm) in three freshwater unicellular eukaryotic organisms: *T. pyriformis, P. caudatum* and *A. proteus*. In addition, a possible trophic transfer of MP was examined.

4.1. Size dependent uptake

The uptake of MP in all three unicellular eukaryotes proved to be size-dependent and varied between the organisms. *T. pyriformis* ingested 0.5 and 6 µm MP, which is in line with Lavin et al. (1990), who also found an uptake of 6.17 µm PS beads. Additionally, *T. pyriformis* was found to ingest PS fragments of up to 10 µm in size (Fig. S12). It is noteworthy, however, that the PS$_{<30µm}$ particles of this size (~10 µm) found inside *T. pyriformis* individuals possessed an elongated shape. Therefore, it can be assumed that these particles were taken up via the oral cavity in a lengthwise manner. Consequently, this uptake may not be possible with spheroid particles in the same size (~10 µm). *P. caudatum*, on the other hand, incorporated fragments up to 27.7 µm in size (Fig. S3). For *Paramecium* sp., past studies only showed an ingestion of particles (spherical) up to 10 µm in size (Bulannga and Schmidt, 2022; Nałęcz-Jawecki et al., 2021). Both unicellular organisms feed by creating a water current with their cilia that channels the potential food into their oral groove, where a food vacuole is then formed (Mast, 1947). Therefore, the variation in particle uptake between *T. pyriformis* and *P. caudatum* is likely attributed to the distinct sizes of these organisms (*T. pyriformis*: average size 39.6 µm, Fig. S5; *P. caudatum*: average size 194.5 µm, Fig. S6) and that of their oral grooves. However, since relatively small *T. pyriformis* cells were found with incorporated 6 µm MP, the particle-to-cell length ratio was higher for *T. pyriformis*. For *T. pyriformis* and *P. caudatum*, high initial uptake rates were observed (95 % and 75 %, respectively). Presumably driven by the organisms'

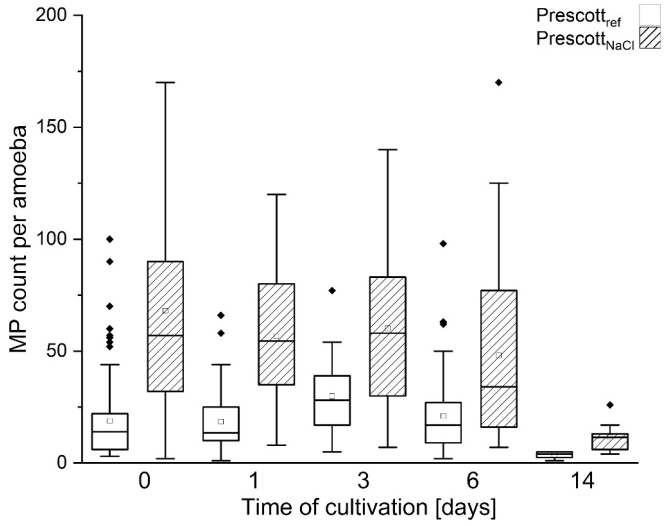

Fig. 5. $PS_{0.5\mu m}$ retention after pinocytic ingestion in *A. proteus*.
Amoebae were incubated with 3.6×10^8 $PS_{0.5\mu m}$ beads/mL in $Prescott_{ref}$ and $Prescott_{NaCl}$ media for 60 min and were thereafter placed into $Prescott_{ref}$ medium for the remaining cultivation time.

rapid doubling time (in this study approximately 2 h for *T. pyriformis* and 24 h for *P. caudatum*, in line with prior research (Ruehle et al., 2016; Takahashi, 2016)), the number of organisms harboring MP started to decline noticeably after 24 h, as illustrated in Fig. 1. In case of *P. caudatum*, a release of $PS_{0.5\mu m}$ could be observed (Fig. S7). Since only individuals with incorporated particles were found, released particles were probably re-ingested, probably due to the high concentrations of $PS_{0.5\mu m}$ particles provided. In *T. pyriformis*, particle release was also observed for $PS_{6\mu m}$ and $PS_{<30\mu m}$.

A. proteus (500 μm) directly took up beads up to 6 μm in size (Fig. 3), but none of the larger analyzed fragments ($PS_{<30\mu m}$). This is most likely associated with the uptake mechanism of the organism. *A. proteus* takes up extracellular matter either via pinocytosis or via phagocytosis. Both uptake pathways are initiated by the interaction between a solute/prey with membrane receptors on the surface of the amoeba. Depending on the targeted receptor, channels (pinocytosis) or pseudopods (phagocytosis) are formed with subsequent uptake of particulates (Prusch and Minck, 1985). For phagocytosis in *A. proteus*, an active food selection can be presumed, as it is also reported for, e.g., heterotrophic flagellates (Boenigk and Arndt, 2002; Xinyao et al., 2006). Hence, chemical and mechanical stimuli enable the amoeba to avoid ingesting inorganic matter through phagocytosis, such as MP. However, *A. proteus* generally also shows a permanent, but minor pinocytosis-driven ingestion in the uroid region (Wohlfarth-Bottermann and Stockem, 1966), with pinocytic channels reaching diameters of up to 6 μm (Klein et al., 1988), which might be large enough for a passive uptake of small MP (≤6 μm). The observed high increase of pinocytosis channels after incubation of Amoebae in $Prescott_{NaCl}$ is further accompanied by an increase in MP internalization for 0.5 μm particles (Fig. 4). It is, therefore, plausible to conclude that the passive pinocytotic pathway is responsible for the internalization of small MP in *A. proteus*. This increased uptake might further be correlated with the alteration of the surface charge of the particles in the different employed media ($Prescott_{Ref} = -53.4$ mV; $Prescott_{NaCl} = -30.7$ mV), that results in a pKa being closer to a typical organic one. Surprisingly, the ingested MP by pinocytosis remained inside the cytoplasm for up to 14 days (Fig. 5). As previously reported, organic (e.g., amino acids and proteins) and inorganic (e.g., cations) substances, that are taken up by the amoebae via pinocytosis, can then be excreted within minutes, to restore the standard concentration gradient of the medium to "normal" cultivation concentration (Klein and Stockem, 1995). However, it is important to consider that the MP utilized in our study may not disrupt the internal osmotic balance of the amoeba. In such a scenario, the amoeba may not recognize the unintentionally ingested particles, leading to the retention of these foreign matter particles within their cytoplasm.

While we could detect MP in the amoebae up to 14 days after the ingestion, it became evident that a decline in the number of retained MP particles started from day 3 onwards (Fig. 5). This phenomenon is most likely attributable to an asymmetrical cellular division process, which occurs at intervals ranging from 20 to 54 h (Ord, 1968; Prescott, 1955). This division process results in a gradual dilution of the MP content within the amoeba, rather than an active egestion of the particles. Due to the maximum diameter of *A. proteus'* pinocytic channels being limited to 6 μm, it becomes evident that larger particles can only be internalized through the process of phagocytosis.

In our study, phagocytosis could not be observed with fragments ($PS_{<30\mu m}$), despite the attempts of induction (particle coating with BSA and FCS proteins). In contrast, ingestion upon induction of phagocytosis was already recorded in other studies that observed the uptake of protein coated agarose beads (10–40 μm, coated with e.g., α-lactalbumin, RNase A) by *A. proteus* (Ahn and Jeon, 1982; Prusch and Britton, 1987; Prusch and Minck, 1985). Uptake efficiency was found to be highly dependent on the employed protein. The reason for a lack of phagocytosis of the protein-coated MP in our study can only be speculated upon and might be linked to a non-adequate surface charge. Since surface charge and size of MP was shown to be a critical parameter determining the ingestion of MP by ciliates as well as for the phagocytic ingestion by murine macrophages (Bulannga and Schmidt, 2022; Ramsperger et al., 2022; Rudolph et al., 2021), the same could be hypothesized for *A. proteus*.

Another important parameter in inducing phagocytosis might be the presence of an eco-corona or a biofilm for larger particles, which can alter the physicochemical characteristics of particles and make them more palatable for uptake (Liu et al., 2022b; Witzmann et al., 2021).

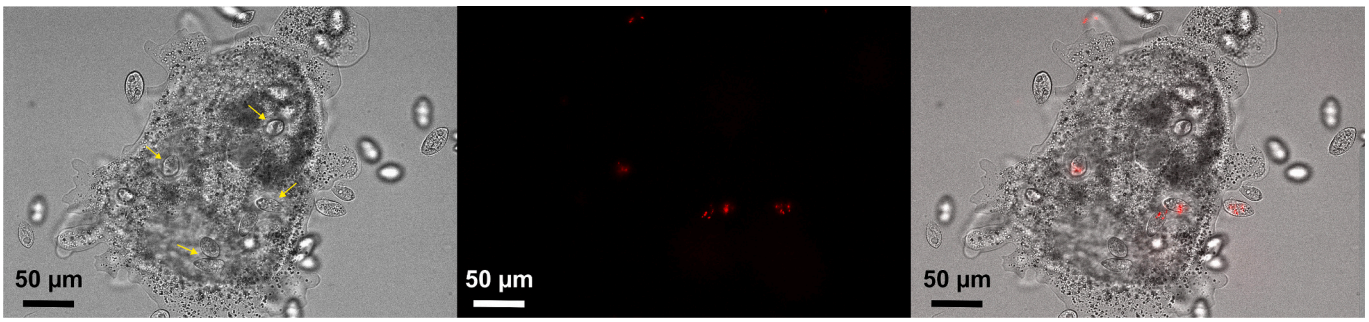

Fig. 6. *A. proteus* feeding on MP ($PS_{0.5\mu m}$) burdened *T. pyriformis*. At $t = 10$ min; multiple *T. pyriformis* have been taken up via phagocytosis, of which three cells contained MP. Left panel: bright field (arrows indicate *T. pyrifomis* inside *A. proteus*); middle panel: epifluorescence; right panel: merged (see Fig. S11 for larger image). 20× magnification. Scale bar = 50 μm.

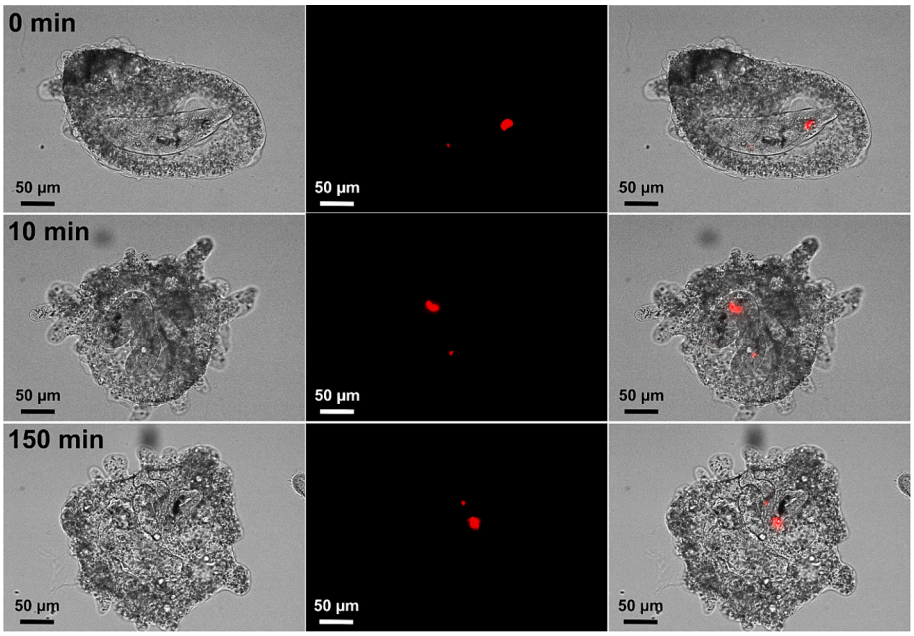

Fig. 7. Ingestion of $PS_{<30\mu m}$ loaded *P. caudatum* (two particles) by *A. proteus*. At $t = 0$ min one individual of *A. proteus* initiates the process of phagocytosis of one paramecium. The individuals are observed at t = 10 min in which *P. caudatum* is completely internalized, and at $t = 150$ min, when the digestion process made the *P. caudatum* cell almost unrecognizable. Left panel: bright field; middle panel: epifluorescence; right panel: merged. 20× magnification. Scale bar = 50 μm.

While a biofilm might not be relevant when considering small MP, the formation of an eco-corona is possible when particles are exposed to biological macromolecules which can adhere to their surface. As described in the pertinent literature, such natural MP coating significantly increases their uptake and, in some cases, biological effects in different organisms (Fabra et al., 2021; Ramsperger et al., 2022). In fact, while many studies report an increase in uptake and effects of coated MP (e.g. Fabra et al., 2021), contrasting findings have been recently reported (e.g. Amariei et al., 2022; Schür et al., 2021), which indicate a diminished influence (e.g., reduced uptake and toxicity) of coated MP.

In our experiments, a co-incubation of a prey organism (*T. pyriformis*) and MP fragments in a comparable size range ($PS_{<30\mu m}$) did not induce phagocytosis of MP in *A. proteus* either. *T. pyriformis* was chosen because it is preferably ingested by *A. proteus* via phagocytosis (Klein and Stockem, 1995) and secretes chemotactic substances, to which the amoebae are actively attracted (Jeon and Bell, 1962). It would also be possible that amoebae were exposed to fecal pellets potentially containing MP, released by the Ciliophora, as they are known to sometimes excrete inedible particles or pathogens this way (Denoncourt et al., 2017; Denoncourt et al., 2020). Nevertheless, the lack of particle ingestion despite the co-exposure with a known prey further confirms that amoebae can discriminate between prey and MP fragments and that soluble substances released by *T. pyriformis*, if any, are not sufficient to induce an inadvertent uptake of large MP fragments. In this study, not all the different employed particle sizes were coated or co-incubated with a known prey organisms. Therefore, even though a co-exposure with prey organisms did not alter the uptake in this study, it cannot be excluded that the size of the particles plays a role in this process. Further studies may consider this as an aspect to include.

Furthermore, next to chemical stimuli, physical cues conveyed by the movement of the prey are also known to be important for the induction of phagocytosis (De la Fuente et al., 2019). Mast and Hahnert (1935) already showed that *A. proteus* mainly feeds on living organisms, indicating that the motility of the prey might be an important co-stimulus for ingestion. This could explain the absence of phagocytic uptake of MP. Overall, phagocytosis and the recognition of the prey is a complex process whose characteristics could not only vary for different prey organisms but for different amoeba strains as well (Shi et al., 2021). A phagocytic uptake of MP by amoeba in the natural environment, therefore, cannot be excluded.

Although we did not investigate whether any negative impact on the unicellular eukaryotes' survival occurred after the uptake of the offered MP, a low clearance rate within their cytoplasm could still pose a threat, as a leaching of plastic associated chemicals and residual monomers (Brehm et al., 2022; Setälä et al., 2014) into the organism could be possible. The release of plastic leachates in the environment potentially exposes the biota to a complex mixture of chemicals, of which only a fraction is known and regulated (Gunaalan et al., 2020). The released chemicals can include compounds that act as a potential threat to the ecosystem. However, even though evidence of the environmental risks of these chemicals already exists (e.g. Delaeter et al., 2022), research on the topic is still lacking.

However, even though release of MP was observable in *T. pyriformis* and *P. caudatum*, the MP particles stayed inside some cells for an, yet unknown, period of time. This uptake and retention could influence the metabolism and uptake rate of other nutritional particles, which is an important determining factor in ciliate physiology (Lavin et al., 1990; Sherr and Sherr, 1987). Additionally, increased metabolic costs, caused by the formation of food vacuoles and production and transport of digestive enzymes, could arise for MP-fed eukaryotes, as they try to digest the particles (Mueller et al., 1965). In this study, we used primary PS-MP, while in the environment, the organisms are exposed to a highly diverse composition of MP, differing substantially in their physico-chemical properties (Rochman et al., 2019). This could in turn cause diverse effects on these organisms in the environment, for which no data is available at present. Hence, our study on the uptake of different particle sizes may support research on the effects of this diverse array of contaminants on unicellular organisms. Moreover, a prolonged retention of MP inside the cytoplasm, while having no immediate negative influence on their survival, might increase the likelihood of a transfer of MP to higher trophic levels.

4.2. Trophic transfer of microplastic particles in a simplified food web

MP bioaccumulation in the lower trophic levels could be particularly concerning, as not only could it alter population dynamics, as it was

shown for marine ciliates (Zhang et al., 2021), but it could also cascade over time to higher trophic levels (Bulannga and Schmidt, 2024). Trophic transfer of MP was reported for different organisms, belonging to various genera in the marine (Carbery et al., 2018) and freshwater (Nair and Perumal, 2022) environment, e.g., from aquatic plants to gammarids (Iannilli et al., 2023), from copepods to jellyfish (Costa et al., 2020), from mussels to gastropods (Xu et al., 2022) and crabs (Farrell and Nelson, 2013), or from daphnids to fish (Mattsson et al., 2017). Additionally, biomagnification seems evident, as MP abundancies increase with an increase of the trophic level (Sarker et al., 2022). Given the ubiquitous presence of MP in the environment, trophic transfer in most ecosystems, therefore, seems inevitable.

When amoebae were offered MP-fed prey, we could observe trophic transfer of MP. *T. pyriformis* and *P. caudatum* cells, which had taken up $PS_{0.5\mu m}$ and $PS_{<30\mu m}$ were promptly ingested via phagocytosis by *A. proteus* (Figs. 6 and 7). This way, *A. proteus* ingested larger particles via prey, which would not have been taken up otherwise. Interestingly, MP, small and large alike taken up via prey organisms, were observed to be released by the amoebae within 24 h of observation (Fig. S9). On the contrary, 0.5 μm beads, that were taken up via pinocytosis, were detected in the cytoplasm up to 14 days after their internalization. It is known that inedible matter like glass, carbon beads, or indigestible remnants of prey organisms, such as the cuticle of small cladocerans, are excreted shortly after digestion (Mast and Hahnert, 1935). These observations could suggest that this process might take place also in the case of MP taken up via trophic transfer. Therefore, if this was the case, this process could reduce bioaccumulation, thanks to the shorter retention time of MP within the cytoplasm. A possible transfer through trophic levels depends hereby in part also on the retention time inside the organisms and the uptake of MP-fed prey by their predators. Further studies would be required to test this hypothesis. However, in favorable conditions, *A. proteus* can ingest up to 70 cells per day, which in turn are able to take up hundreds of MP of various sizes per cell. If the rate of ingestion exceeds the excretion rate, possible biomagnification of inedible, non-nutritive material at the low trophic levels can be proposed, posing a potential hazard to these organisms and their predators.

Unicellular predators are not the only organisms that could take up contaminated Ciliophora. For example, Stienbarger and coauthors showed that fish larvae of *Centropristis striata* ingest exponentially more MP while feeding on contaminated prey (*Favella* spp., Ciliophora) than when exposed to the particles alone (Stienbarger et al., 2021). Predators belonging to higher trophic levels could, furthermore, be impaired not only by feeding upon contaminated prey, but also by a decrease of their biomass. For example, a study conducted by Geng et al. (2021) showed a concentration dependent collapse of *Strombidium sulcatum's* population exposed to different sizes of PS fluorescent beads within 72 h from the beginning of the exposure, together with a reduction of their size (Geng et al., 2021). Hence, a subsequent death of *S. sulcatum* could directly affect the predators' population by the reduction of the number of prey and, indirectly, by the increase of MP available in the water. While we did not study population dynamics of the selected organisms exposed to PS-MP, we cannot exclude that a decrease of the population may happen over longer periods of time or with environmentally relevant MP.

5. Conclusion

This study suggests that an accumulation of MP in unicellular eukaryotes is likely and should be considered when assessing the hazard of MP contamination in aquatic environments. MP bioaccumulation may have detrimental effects, given the crucial role that these organisms have within the food web. Overall, while the potential impact on the survival of the exposed unicellular eukaryotes was not investigated, neither long term effects, nor the possibility of cascading effects over higher trophic levels can be excluded. In this context, further investigations are necessary to assess the potential effects of different MP on the life history parameters of unicellular organisms and their potential transfer along the food web. In future experimental designs, it would be appropriate to consider a more environmentally relevant scenario. In particular, it would be valuable to apply diverse protein or eco-corona coatings to all utilized particles and to investigate potential effects on the survival of the studied organisms depending on the physico-chemical properties of the particles.

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CRediT authorship contribution statement

Simona Mondellini: Writing – review & editing, Writing – original draft, Visualization, Validation, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Michael Schwarzer:** Writing – review & editing, Writing – original draft, Visualization, Validation, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Matthias Völkl:** Writing – review & editing, Writing – original draft, Visualization, Validation, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Julia Jasinski:** Investigation. **Valérie Jérôme:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Funding acquisition, Formal analysis, Conceptualization. **Thomas Scheibel:** Writing – review & editing, Resources, Project administration, Funding acquisition. **Christian Laforsch:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Funding acquisition, Conceptualization. **Ruth Freitag:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare no competing interest.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2024.172470.

References

Ahn, T.I., Jeon, K.W., 1982. Structural and biochemical characteristics of the plasmalemma and vacuole membranes in amoebae. In Exp. Cell Res. 137 (2) https://doi.org/10.1016/0014-4827(82)90026-x.

Al-Thawadi, Salwa, 2020. Microplastics and Nanoplastics in aquatic environments: challenges and threats to aquatic organisms. In Arab J Sci Eng 45 (6), 4419–4440. https://doi.org/10.1007/s13369-020-04402-z.

Amariei, G.; Rosal, R.; Fernàndez-Piñas, F.; Koelmans, A.A. (2022) Negative food dilution and positive biofilm carrier effects of microplastic ingestion by *D. magna* cause tipping points at the population level. In *Environmental Pollution* 294, pp. 118622. DOI: https://doi.org/10.1016/j.envpol.2021.118622.

Athey, Samantha N., Albotra, Samantha D., Gordon, Cessely A., Monteleone, Bonnie, Seaton, Pamela, Andrady, Anthony L., et al., 2020. Trophic transfer of microplastics in an estuarine food chain and the effects of a sorbed legacy pollutant. In *Limnol Oceanogr Letters* 5 (1), 154–162. https://doi.org/10.1002/lol2.10130.

Bellasi, Arianna, Binda, Gilberto, Pozzi, Andrea, Galafassi, Silvia, Volta, Pietro, Bettinetti, Roberta, 2020. Microplastic contamination in freshwater environments: a review, focusing on interactions with sediments and benthic organisms. In Environments 7 (4), 30. https://doi.org/10.3390/environments7040030.

Bermúdez, J.R., Metian, M., Oberhänsli, F., Taylor, A., Swarzenski, P.W., 2021. Preferential grazing and repackaging of small polyethylene microplastic particles (≤ 5 μm) by the ciliate Sterkiella sp. In Mar. Environ. Res. 166, 105260 https://doi.org/10.1016/j.marenvres.2021.105260.

Boenigk and Arndt, 2002. Bacterivory by heterotrophic flagellates: community structure and feeding strategies. Antonie Van Leeuwenhoek 81, 465–480. https://doi.org/10.1023/A:1020509305868.

Bosker, Thijs, Olthof, Gabriël, Vijver, Martina G., Baas, Jan, Barmentlo, S. Henrik, 2019. Significant decline of Daphnia magna population biomass due to microplastic exposure. In *Environmental Pollution* 250, 669–675. https://doi.org/10.1016/j.envpol.2019.04.067.

Brehm, Julian, Wilde, Magdalena V., Reiche, Lukas, Leitner, Lisa-Cathrin, Petran, Benedict, Meinhart, Marcel, et al., 2022. In-depth characterization revealed polymer type and chemical content specific effects of microplastic on Dreissena Bugensis. In. J. Hazard. Mater., 129351 https://doi.org/10.1016/j.jhazmat.2022.129351.

Brehm, J., Ritschar, S., Laforsch, C., Mair, M.M., 2023. The complexity of micro- and nanoplastic research in the genus *Daphnia* - a systematic review of study variability and a meta-analysis of immobilization rates. In. J. Hazard. Mater., 131839 https://doi.org/10.1016/j.jhazmat.2023.131839.

Bulannga, Rendani B., Schmidt, Stefan, 2022. Uptake and accumulation of microplastic particles by two freshwater ciliates isolated from a local river in South Africa. In *Environ. Res.* 204 (Pt B), 112123 https://doi.org/10.1016/j.envres.2021.112123.

Bulannga, Rendani B., Schmidt, Stefan, 2024. Micro- and nanoplastics in freshwater ecosystems—interaction with and impact upon bacterivorous ciliates. Front. Earth Sci. 12, 1349865 https://doi.org/10.3389/feart.2024.1349865.

Carbery, Maddison, O'Connor, Wayne, Palanisami, Thavamani, 2018. Trophic transfer of microplastics and mixed contaminants in the marine food web and implications for human health. In *Environment International* 115, 400–409. https://doi.org/10.1016/j.envint.2018.03.007.

Cole, Matthew, Lindeque, Pennie, Fileman, Elaine, Halsband, Claudia, Galloway, Tamara S., 2015. The impact of polystyrene microplastics on feeding, function and fecundity in the marine copepod Calanus helgolandicus. In *Environmental science & technology* 49 (2), 1130–1137. https://doi.org/10.1021/es504525u.

Cole, Matthew, Coppock, Rachel, Lindeque, Penelope K., Altin, Dag, Reed, Sarah, Pond, David W., et al., 2019. Effects of nylon microplastic on feeding, lipid accumulation, and Moulting in a Coldwater copepod. In Environmental science & technology 53 (12), 7075–7082. https://doi.org/10.1021/acs.est.9b01853.

Costa, Elisa; Piazza, Veronica; Lavorano, Silvia; Faimali, Marco; Garaventa, Francesca; Gambardella, Chiara (2020): Trophic Transfer of Microplastics From Copepods to Jellyfish in the Marine Environment. In *Front. Environ. Sci.* 8, Article 571732. DOI: https://doi.org/10.3389/fenvs.2020.571732.

De la Fuente, Ildefonso, M., Bringas, Carlos, Malaina, Iker, Fedetz, María, Carrasco-Pujante, Jose, Morales, Miguel, et al., 2019. Evidence of conditioned behavior in amoebae. In *Nat Commun* 10 (1), 1–12. https://doi.org/10.1038/s41467-019-11677-w.

de Souza Machado, Anderson Abel, Ghadernezhad, Nesar, Wolinska, Justyna, 2023. Potential for high toxicity of polystyrene nanoplastics to the European *Daphnia longispina*. In *Environmental Sciences Europe*. https://doi.org/10.1186/s12302-023-00763-y.

Delaeter, Camille, Spilmont, Nicolas, Bouchet, Vincent M.P., Seuront, Laurent, 2022. Plastic leachates: bridging the gap between a conspicuous pollution and its pernicious effects on marine life. In Science of The Total Environment 826, 154091. https://doi.org/10.1016/j.scitotenv.2022.154091.

Denoncourt, A.M., Paquet, V.E., Charette, S.J., 2017. Packaging of *mycobacterium smegmatis* bacteria into fecal pellets by the ciliate *Tetrahymena pyriformis*. In Microbiology Letters 364 (23). https://doi.org/10.1093/femsle/fnx237.

Denoncourt, A.F., Denoncourt, A.M., Paquet, V.E., Charette, S.J., 2020. Evidence that Bacteria packaging by Tetrahymena is a widespread phenomenon. In Microorganisms 8 (10). https://doi.org/10.3390/microorganisms8101548.

Drago, Claudia; Pawlak, Julia; Weithoff, Guntram (2020): Biogenic Aggregation of Small Microplastics Alters Their Ingestion by a Common Freshwater Micro-Invertebrate. In *Front. Environ. Sci.* 8, Article 574274. DOI: https://doi.org/10.3389/fenvs.2020.574274.

Du, Sen, Zhu, Rongwen, Cai, Yujie, Xu, Ning, Yap, Pow-Seng, Zhang, Yunhai, et al., 2021. Environmental fate and impacts of microplastics in aquatic ecosystems: a review. In *RSC Adv.* 11 (26), 15762–15784. https://doi.org/10.1039/D1RA00880C.

Eltemsah, Yehia Sayed, Bøhn, Thomas, 2019. Acute and chronic effects of polystyrene microplastics on juvenile and adult Daphnia magna. In *Environmental Pollution* 254, 112919. https://doi.org/10.1016/j.envpol.2019.07.087.

Fabra, Monica, Williams, Luke, Watts, Joy E.M., Hale, Michelle S., Couceiro, Fay, Preston, Joanne, 2021. The plastic Trojan horse: biofilms increase microplastic uptake in marine filter feeders impacting microbial transfer and organism health. In Sci. Total Environ. 797, 149217 https://doi.org/10.1016/j.scitotenv.2021.149217.

Farrell, Paul, Nelson, Kathryn, 2013. Trophic level transfer of microplastic: Mytilus edulis (L.) to Carcinus maenas (L.). In *Environmental Pollution* 177, 1–3. https://doi.org/10.1016/j.envpol.2013.01.046.

De Felice, B., Sabatini, V., Antenucci, S., Gattoni, G., Santo, N., Bacchetta, R., Ortenzi, M. A., Parolini, M., 2019. Polystyrene microplastics ingestion induced behavioral effects to the cladoceran Daphnia magna. Chemosphere 231, 423–431. https://doi.org/10.1016/j.chemosphere.2019.05.115.

Feng, Jiaxin, Zhao, Hansen, Gong, Xiaoyun, Xia, Meng-Chan, Cai, Lesi, Yao, Huan, et al., 2021. In situ identification and spatial mapping of microplastic standards in paramecia by secondary-ion mass spectrometry imaging. In Analytical chemistry 93 (13), 5521–5528. https://doi.org/10.1021/acs.analchem.0c05383.

Frias, J.P.G.L., Nash, Roisin, 2019. Microplastics: finding a consensus on the definition. In Mar. Pollut. Bull. 138, 145–147. https://doi.org/10.1016/j.marpolbul.2018.11.022.

Geng, Xianhui, Wang, Jun, Zhang, Yan, Jiang, Yong, 2021. How do microplastics affect the marine microbial loop? Predation of microplastics by microzooplankton. In *Science of The Total Environment* 758, 144030. https://doi.org/10.1016/j.scitotenv.2020.144030.

González-Pleiter, Miguel, Tamayo-Belda, Miguel, Pulido-Reyes, Gerardo, Amariei, Georgiana, Leganés, Francisco, Rosal, Roberto, Fernández-Piñas, Francisca, 2019. Secondary nanoplastics released from a biodegradable microplastic severely impact freshwater environments. In *environ. Sci.* Nano 6 (5), 1382–1392. https://doi.org/10.1039/C8EN01427B.

Gunaalan, Kuddithamby, Fabbri, Elena, Capolupo, Marco, 2020. The hidden threat of plastic leachates: a critical review on their impacts on aquatic organisms. In Water Research 184, 116170. https://doi.org/10.1016/j.watres.2020.116170.

Huang, Zhuizui, Weng, You, Shen, Qichen, Zhao, Yao, Jin, Yuanxiang, 2021. Microplastic: a potential threat to human and animal health by interfering with the intestinal barrier function and changing the intestinal microenvironment. In Sci. Total Environ. 785, 147365 https://doi.org/10.1016/j.scitotenv.2021.147365.

Iannilli, Valentina; Passatore, Laura; Carloni, Serena; Lecce, Francesca; Sciacca, Giulia; Zacchini, Massimo; Pietrini, Fabrizio (2023): Microplastic Toxicity and Trophic Transfer in Freshwater Organisms: Ecotoxicological and Genotoxic Assessment in *Spirodela polyrhiza* (L.) Schleid. and Echinogammarus veneris (Heller, 1865) Treated with polyethylene microparticles. In *Water* 15 (5), p. 921. DOI: https://doi.org/10.3390/w15050921.

Ilyas, Muhammad, Ahmad, Waqas, Khan, Hizbullah, Yousaf, Saeeda, Khan, Kifayatullah, Nazir, Shah, 2018. Plastic waste as a significant threat to environment - a systematic literature review. In *Rev. Environ. Health* 33 (4), 383–406. https://doi.org/10.1515/reveh-2017-0035.

Imhof, H.K., Ivleva, N.P., Schmid, J., Niessner, R., Laforsch, C., 2013. Contamination of beach sediments of a subalpine lake with microplastic particles. In. Curr. Biol. 23 (19) https://doi.org/10.1016/j.cub.2013.09.001.

Imhof, Hannes K., Laforsch, Christian, Wiesheu, Alexandra C., Schmid, Johannes, Anger, Philipp M., Niessner, Reinhard, Ivleva, Natalia P., 2016. Pigments and plastic in limnetic ecosystems: a qualitative and quantitative study on microparticles of different size classes. In Water Research 98, 64–74. https://doi.org/10.1016/j.watres.2016.03.015.

Imhof, Hannes K., Rusek, Jakub, Thiel, Michaela, Wolinska, Justyna, Laforsch, Christian, 2017. Do microplastic particles affect Daphnia magna at the morphological, life history and molecular level? In *PloS one* 12 (11), e0187590. https://doi.org/10.1371/journal.pone.0187590.

Jasinski, J., Wilde, M.V., Völkl, M., Jérôme, V., Fröhlich, T., Freitag, R., Scheibel, T., 2022. Tailor-made protein Corona formation on polystyrene microparticles and its effect on epithelial cell uptake. ACS Appl. Mater. Interfaces 14, 47277–47287. https://doi.org/10.1021/acsami.2c13987.

Jeon, K.W., Bell, L.G.E., 1962. Pseudopod and foodcup formation in Amoeba proteus. In Exp. Cell Res. 27 (2), 350–352. https://doi.org/10.1016/0014-4827(62)90243-4.

Jeong, Chang-Bum, Won, Eun-Ji, Kang, Hye-Min, Lee, Min-Chul, Hwang, Dae-Sik, Hwang, Un-Ki, et al., 2016. Microplastic size-dependent toxicity, oxidative stress induction, and p-JNK and p-p38 activation in the Monogonont rotifer (Brachionus koreanus). In Environmental science & technology 50 (16), 8849–8857. https://doi.org/10.1021/acs.est.6b01441.

Kernchen, Sarmite, Löder, Martin G.J., Fischer, Franziska, Fischer, Dieter, Moses, Sonya R., Georgi, Christoph, et al., 2022. Airborne microplastic concentrations and deposition across the Weser River catchment. In Sci. Total Environ. 818, 151812 https://doi.org/10.1016/j.scitotenv.2021.151812.

Klein, Hans Peter, Stockem, Wilhelm, 1995. Nahrungsaufnahme und intrazelluläre Verdauung bei Amöben. In *Biologie in unserer Zeit* 25 (5), 293–299. https://doi.org/10.1002/biuz.19950250622.

Klein, H.P., Köster, B., Stockem, W., 1988. Pinocytosis and locomotion of *amoebae* XVII. Different Morphodynamic forms of endocytosis and microfilament Organization in *Amoeba proteus*. In: Tazawa, M. (Ed.), Cell Dynamics. Protoplasma, vol. 2. Springer, Vienna. https://doi.org/10.1007/978-3-7091-9011-1_9.

Klein, Kristina, Piana, Theresa, Lauschke, Tim, Schweyen, Peter, Dierkes, Georg, Ternes, Thomas, et al., 2021. Chemicals associated with biodegradable microplastic

drive the toxicity to the freshwater oligochaete Lumbriculus variegatus. In *Aquatic toxicology (Amsterdam, Netherlands)* 231, 105723. https://doi.org/10.1016/j.aquatox.2020.105723.
Koelmans, Albert A., Bakir, Adil, Burton, G., Allen; Janssen, Colin R., 2016. Microplastic as a vector for Chemicals in the Aquatic Environment: critical review and model-supported reinterpretation of empirical studies. In Environmental science & technology 50 (7), 3315–3326. https://doi.org/10.1021/acs.est.5b06069.
König Kardgar, A., Ghosh, D., Sturve, J., Agarwal, S., Carney Almroth, B., 2023. Chronic poly(L-lactide) (PLA)- microplastic ingestion affects social behavior of juvenile European perch (*Perca fluviatilis*). In *Sci. Total Environ.* 881, 163425 https://doi.org/10.1016/j.scitotenv.2023.163425.
Lavin, Daniel P., Hatzis, Christos, Srience, Friedrich, Fredrickson, A.G., 1990. Size effects on the uptake of particles by populations of Tetrahymena pyriformis cells. In The Journal of Protozoology 37 (3), 157–163. https://doi.org/10.1111/j.1550-7408.1990.tb01120.x.
Li, W.C., Tse, H.F., Fok, L., 2016. Plastic waste in the marine environment: a review of sources, occurrence and effects. In Sci. Total Environ. 566-567, 333–349. https://doi.org/10.1016/j.scitotenv.2016.05.084.
Li, W., Li, X., Tong, J., Xiong, W., Zhu, Z., Gao, X., Li, S., Jia, M., Yang, Z., Liang, J., 2023. Effects of environmental and anthropogenic factors on the distribution and abundance of microplastics in freshwater ecosystems. In *Sci. Total Environ.* 856, 159030 https://doi.org/10.1016/j.scitotenv.2022.159030.
Liu, Yang, Zhang, Jiale, Zhao, Haoyang, Cai, Ji, Sultan, Yousef, Fang, Haiyan, et al., 2022a. Effects of polyvinyl chloride microplastics on reproduction, oxidative stress and reproduction and detoxification-related genes in Daphnia magna. In *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology* 254, 109269. https://doi.org/10.1016/j.cbpc.2022.109269.
Liu, Shulin, Junaid, Muhammad, Liao, Hongping, Liu, Xinyu, Wu, Yan, Wang, Jun, 2022b. Eco-corona formation and associated ecotoxicological impacts of nanoplastics in the environment. In *Sci. Total Environ.* 836, 155703 https://doi.org/10.1016/j.scitotenv.2022.155703.
Magni, S., Bonasoro, F., Della Torre, C., Parenti, C.C., Maggioni, D., Binelli, A., 2020. Plastics and biodegradable plastics: Ecotoxicity comparison between polyvinylchloride and mater-bi® micro-debris in a freshwater biological model. In Sci. Total Environ. 720, 137602 https://doi.org/10.1016/j.scitotenv.2020.137602.
Makin, B., 2023. Microplastic contamination reduces productivity in a widespread freshwater photosymbiosis. In *Aquatic Biology* 32, 13–19. https://doi.org/10.3354/ab00758.
Mansano, A.S., Hisatugo, K.F., Hayashi, L.H., Regali-Seleghim, M.H., 2014. The importance of protozoan bacterivory in a subtropical environment (lobo-Broa reservoir, SP, Brazil). In Braz. J. Biol. 74, 569–578. https://doi.org/10.1590/bjb.2014.0081.
Mansano, Adrislaine S., Moreira, Raquel A., Pierozzi, Mayara, Oliveira, Thiessa M.A., Vieira, Eny M., Rocha, Odete, Regali-Seleghim, Mirna H., 2016. Effects of diuron and carbofuran pesticides in their pure and commercial forms on Paramecium caudatum: the use of protozoan in ecotoxicology. In Environmental Pollution 213, 160–172. https://doi.org/10.1016/j.envpol.2015.11.054.
Marshall, W.F., Young, K.D., Swaffer, M., Wood, E., Kimura, A., Frankler, J., Wallingford, J., Walbot, V., Qu, X., Roeder, A.H.K., 2012. What determines cell size? In *BCM Biology* 10 (101). https://doi.org/10.1186/1741-7007-10-101.
Mast, S.O., 1947. The food-vacuole in Paramecium. Biol. Bull. 92 https://doi.org/10.2307/1537967.
Mast, S.O., Hahnert, W.F., 1935. Feeding, digestion, and starvation in Amoeba proteus (Leidy). In Physiol. Zool. 8 (3), 255–272. https://doi.org/10.1086/physzool.8.3.30151248.
Mattsson, K., Johnson, E.V., Malmendal, A., Linse, S., Hansson, La, Cedervall, T., 2017. Brain damage and behavioural disorders in fish induced by plastic nanoparticles delivered through the food chain. In Scientific reports 7 (1). https://doi.org/10.1038/s41598-017-10813-0.
Mueller, M., Röhlich, P., Törö, I., 1965. Studies on feeding and digestion in Protozoa. VII. Ingestion of polystyrene latex particles and its early effect on acid phosphatase in Paramecium multimicronucleatum and Tetrahymena pyriformis. In *the*. J. Protozool. 12 (1), 27–34. https://doi.org/10.1111/j.1550-7408.1965.tb01807.x.
Nair, Haritha Thulaseedharan, Perumal, Siddhuraju, 2022. Trophic transfer and accumulation of microplastics in freshwater ecosystem: risk to food security and human health. In International Journal of Ecology 2022, 1–11. https://doi.org/10.1155/2022/1234078.
Nałęcz-Jawecki, Grzegorz, Chojnacka, Justyna, Wawryniuk, Milena, Drobniewska, Agata, 2021. Influence of Nano- and Small microplastics on ciliated protozoan Spirostomum ambiguum (Müller, 1786) Ehrenberg, 1835. In Water 13 (20), 2857. https://doi.org/10.3390/w13202857.
Nisbet, Brenda, 2012. Nutrition and Feeding Strategies in Protozoa. Springer Netherlands, Dordrecht, pp. 128–155. https://doi.org/10.1007/978-94-011-6555-6.
Nugroho, Failasuf Aulia, Fyda, Janusz, 2020. Uptake of plastic microbeads by ciliate Paramecium aurelia. In *Science, technology and Innovation* 9 (2). https://doi.org/10.5604/01.3001.0014.4173.
Ord, M.J., 1968. The synthesis of DNA through the cell cycle of Amoeba proteus. In Journal of cell science 3 (4), 483–491. https://doi.org/10.1242/jcs.3.4.483.
Prescott, D.M., 1955. Relations between cell growth and cell division. In Exp. Cell Res. 9 (2), 328–337. https://doi.org/10.1016/0014-4827(55)90106-3.
Prescott, D.M., James, T.W., 1955. Culturing of Amoeba proteus on Tetrahymena. In Exp. Cell Res. 8 (1), 256–258. https://doi.org/10.1016/0014-4827(55)90067-7.
Prusch, R.D., Britton, J.C., 1987. Peptide stimulation of phagocytosis in Amoeba proteus. In Cell Tissue Res. 250, 589–593. https://doi.org/10.1007/BF00218951.
Prusch, R.D., Minck, D.R., 1985. Chemical stimulation of phagocytosis in Amoeba proteus and the influence of external calcium. In Cell Tissue Res. 242, 557–564. https://doi.org/10.1007/BF00225421.
Ramsperger, A. F. R. M.; Narayana, V. K. B.; Gross, W., Mohanraj, J.; Thelakkat, M.; Greiner, A.; Schmalz, H.; Kress, H.; Laforsch, C. (2020):Environmental exposure enhances the internalization of microplastic particles into cells. In *Science Advances* 6 (50). DOI: https://doi.org/10.1126/sciadv.abd1211.
Ramsperger, A.F.R.M., Jasinski, J., Völkl, M., Witzmann, T., Meinhart, M., Jérôme, V., et al., 2022. Supposedly identical microplastic particles substantially differ in their material properties influencing particle-cell interactions and cellular responses. In J. Hazard. Mater. 425, 127961 https://doi.org/10.1016/j.jhazmat.2021.127961.
Range, D., Scherer, C., Stock, F., Ternes, T.A., Hoffmann, T., 2023. Hydro-geomorphic perspectives on microplastic distribution in freshwater river systems: a critical review. In Water Research 245, 120567. https://doi.org/10.1016/j.watres.2023.120567.
Rochman, Chelsea M., Brookson, Cole, Bikker, Jacqueline, Djuric, Natasha, Earn, Arielle, Bucci, Kennedy, et al., 2019. Rethinking microplastics as a diverse contaminant suite. In *Environmental toxicology and chemistry* 38 (4), 703–711. https://doi.org/10.1002/etc.4371.
Rudolph, Julia, Völkl, Matthias, Jérôme, Valérie, Scheibel, Thomas, Freitag, Ruth, 2021. Noxic effects of polystyrene microparticles on murine macrophages and epithelial cells. In *Sci. Rep.* 11 (1), 15702. https://doi.org/10.1038/s41598-021-95073-9.
Ruehle, Marisa D., Orias, Eduardo, Pearson, Chad G., 2016. Tetrahymena as a unicellular model eukaryote: genetic and genomic tools. In Genetics 203 (2), 649–665. https://doi.org/10.1534/genetics.114.169748.
Sarker, Subrata; Huda, A.N.M. Samiul; Niloy, Md. Nazmul Hasan; Chowdhury, Gawsia Wahidunnessa (2022): Trophic transfer of microplastics in the aquatic ecosystem of Sundarbans mangrove forest, Bangladesh. In *Science of The Total Environment* 838, p. 155896. DOI: https://doi.org/10.1016/j.scitotenv.2022.155896.
Scherer, Christian; Weber, Annkatrin; Lambert, Scott; Wagner, Martin (2018): Interactions of Microplastics with Freshwater Biota. In: Freshwater Microplastics: Springer, Cham, pp. 153–180. DOI: https://doi.org/10.1007/978-3-319-61615-5_8.
Schür, C., Weil, C., Baum, M., Wallraff, J., Schreirer, M., Oehlmann, J., Wagner, M., 2021. Incubation in wastewater reduces the multigenerational effects of microplastics in *Daphnia magna*. In Environmental Science & Technology 55, 2491–2499. https://doi.org/10.1021/acs.est.0c07911.
Schwarzer, Michael, Brehm, Julian, Vollmer, Martina, Jasinski, Julia, Xu, Chengzhang, Zainuddin, Shakir, et al., 2022. Shape, size, and polymer dependent effects of microplastics on *Daphnia magna*. In *J. Hazard. Mater.* 426, 128136 https://doi.org/10.1016/j.jhazmat.2021.128136.
Setälä, Outi, Fleming-Lehtinen, Vivi, Lehtiniemi, Maiju, 2014. Ingestion and transfer of microplastics in the planktonic food web. In *Environmental Pollution* 185, 77–83. https://doi.org/10.1016/j.envpol.2013.10.013.
Sherr, Evelyn B., Sherr, Barry F., 1987. High rates of consumption of bacteria by pelagic ciliates. In *Nature* 325 (6106), 710–711. https://doi.org/10.1038/325710a0.
Shi, Yijing, Queller, David C., Tian, Yuehui, Zhang, Siyi, Yan, Qingyun, He, Zhili, et al., 2021. The ecology and evolution of Amoeba-bacterium interactions. In Applied and environmental microbiology 87 (2). https://doi.org/10.1128/AEM.01866-20.
Smoluchowski, Marian von, 1906. Zur kinetischen Theorie der Brownschen Molekularbewegung und der Suspensionen. In *Annalen der Physik* 326 (14), 756–780. https://doi.org/10.1002/andp.19063261405.
Stienbarger, Cheyenne D., Joseph, Jincy, Athey, Samantha N., Monteleone, Bonnie, Andrady, Anthony L., Watanabe, Wade O., et al., 2021. Direct ingestion, trophic transfer, and physiological effects of microplastics in the early life stages of Centropristis striata, a commercially and recreationally valuable fishery species. In *Environmental Pollution* 285, 117653. https://doi.org/10.1016/j.envpol.2021.117653.
Takahashi, Toshiyuki, 2016. Simultaneous evaluation of life cycle dynamics between a host Paramecium and the endosymbionts of *Paramecium bursaria* using capillary flow cytometry. In Sci Rep 6 (1), 1–12. https://doi.org/10.1038/srep31638.
Triebskorn, Rita, Braunbeck, Thomas, Grummt, Tamara, Hanslik, Lisa, Huppertsberg, Sven, Jekel, Martin, et al., 2019. Relevance of nano- and microplastics for freshwater ecosystems: a critical review. In TrAC Trends in Analytical Chemistry 110, 375–392. https://doi.org/10.1016/j.trac.2018.11.023.
Wagner, Martin, Scherer, Christian, Alvarez-Muñoz, Diana, Brennholt, Nicole, Bourrain, Xavier, Buchinger, Sebastian, et al., 2014. Microplastics in freshwater ecosystems: what we know and what we need to know. In *Environ Sci Eur* 26 (1), 1–9. https://doi.org/10.1186/s12302-014-0012-7.
Wang, M., Huang, Z., Wu, C., Yan, S., Fang, H., Pan, W., Tan, Q., Pan, K., Ji, R., Yang, L., Pan, B., Wang, P., Miao, A., 2024. Stimulated Raman scattering microscopy reveals bioaccumulation of small microplastics in Protozoa from natural waters. In Environ. Sci. Technol. 58 (6), 2922–2930. https://doi.org/10.1021/acs.est.3c07486.
Wieland, S; Ramsperger, A. F. R. M.; Gross, W.; Lehmann, M.; Witzmann, T.; Caspari, A.; Obst, M.; Gekle, S.; Auernhammer, G. K.; Fery, A.; Laforsch, C.; Kress, H. (2024). Nominally identical microplastic model differ greatly in their particle-cell interactions. In *Nature Communications* 15, 922. DOI: https://doi.org/10.1038/s41467-024-45281-4.
Witzmann, Thomas, Ramsperger, Anja F.R.M., Wieland, Simon, Laforsch, Christian, Kress, Holger, Fery, Andreas, Auernhammer, Günter K., 2021. Repulsive interactions of eco-corona covered microplastic particles quantitatively follow modelling of polymer brushes. In Langmuir 38 (29), 8748–8756. https://doi.org/10.1021/acs.langmuir.1c03204.
Wohlfarth-Bottermann, K.E., Stockem, W., 1966. Pinocytose und Bewegung von Amöben. Z. Zellforsch. 73, 444–474. https://doi.org/10.1007/BF00329022.
Xia, Xiaohua; Sun, Menghan; Zhou, Miao; Chang, Zhongjie; Li, Li (2020): Polyvinyl chloride microplastics induce growth inhibition and oxidative stress in *Cyprinus*

carpio var. larvae. In *The Science of the total environment* 716, p. 136479. DOI: https://doi.org/10.1016/j.scitotenv.2019.136479.

Xinyao, L., Miao, S., Yonghong, L., Yin, G., Zhongkai, Z., Donghui, W., Weizhong, W., Chencai, A., 2006. Feeding characteristics of an Amoeba (*Lobosea: Naegleria*) grazing upon Cyanobacteria: food selection, ingestion and digestion Progress. Microb. Ecol. 51, 315–325. https://doi.org/10.1007/s00248-006-9031-2.

Xu, Elvis Genbo; Cheong, Rachel S.; Liu, Lan; Hernandez, Laura M.; Azimzada, Agil; Bayen, Stéphane; Tufenkji, Nathalie (2020): Primary and secondary plastic particles exhibit limited acute toxicity but chronic effects on Daphnia magna. In *Environmental science & technology* 54 (11), pp. 6859–6868. DOI: https://doi.org/10.1021/acs.est.0c00245.

Xu, Xiaoyu; Fang, James Kar-Hei; Wong, Chun-Yuen; Cheung, Siu-Gin (2022): The significance of trophic transfer in the uptake of microplastics by carnivorous gastropod Reishia clavigera. In *Environmental Pollution* 298, p. 118862. DOI: https://doi.org/10.1016/j.envpol.2022.118862.

Zhang, Yan, Wang, Jun, Geng, Xianhui, Jiang, Yong, 2021. Does microplastic ingestion dramatically decrease the biomass of protozoa grazers? A case study on the marine ciliate Uronema marinum. In *Chemosphere* 267, 129308. https://doi.org/10.1016/j.chemosphere.2020.129308.

Zheng, Jia-Lang, Wang, Dan, Chen, Xiao, Song, Hong-Zi, Xiang, Li-Ping, Yu, Han-Xiu, et al., 2022. Nutritional-status dependent effects of microplastics on activity and expression of alkaline phosphatase and alpha-amylase in Brachionus rotundiformis. In *Science of The Total Environment* 806, 150213. https://doi.org/10.1016/j.scitotenv.2021.150213.