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Bacterial consumption by nematodes is disturbed by the presence of polystyrene beads: The roles of food dilution and pharyngeal pumping

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ABSTRACT

Microplastics (MPs; <5 mm) released into freshwaters from anthropogenic sources accumulate in sediments, where they may pose an environmental threat to benthic organisms, such as nematodes. Several studies have examined the effects of nano- and microplastics on the nematode *Caenorhabditis elegans*, whereas reduced food availability was suggested as a possible explanation for the observed inhibitory effects. Therefore, this study should clarify whether micro-beads of different sizes (1.0 and 6.0 μm in diameter) and materials (polystyrene PS, silica) are able to interfere with the feeding of *C. elegans* on its bacterial diet (*Escherichia coli*), and, by this, lowering its consumption rate within 7 h of exposure. Moreover, it was examined whether an inhibited bacterial consumption was caused by a reduction of the nematode's pumping rate, as a primary indicator of food ingestion. Bacterial consumption by *C. elegans* was significantly decreased in the presence of 1.0- and 6.0-μm PS beads (49–67% lower bacterial consumption compared to control), whereas in the presence of 1.0-μm silica beads feeding was not impeded. Interestingly, the pumping rate was significantly lower in the presence of non-ingestible 6.0-μm PS beads with 161 ± 16 pumps min^{-1}, while it was largely unchanged for nematodes exposed to ingestible 1.0-μm PS beads with 205 ± 12 pumps min^{-1}, compared to control conditions with 210 ± 18 pumps min^{-1}, respectively. As reduced bacterial consumption leads to generally lower energy reserves in *C. elegans*, these results allow to link observed inhibitory effects of MPs on the nematodes to a lower food availability. Such indirect, food-web related, effects of MPs should raise concern of ecological consequences in natural habitats, where temporal food deficiencies can occur. Consequently, disturbances in food availability and feeding efficiency should be regarded as important parameters in environmental risk assessments focusing on MPs.

1. Introduction

Microplastic (MP; <5 mm) particles of diverse polymer types pose one of the largest environmental threats of our time (PlasticsEurope, 2019). Following the release of the MPs into aquatic environments, they undergo changes in their density due to biofouling/sedimentation processes (Cole et al., 2011; Galloway et al., 2017; Kaiser et al., 2017; Leiser et al., 2020) such that the majority come to rest in the sediments, as demonstrated in freshwater systems (Cera et al., 2020). Indeed, MP concentrations in riverine sediments were up to 600,000-fold higher than in the water phase (Scherer et al., 2020). The resulting risk posed by MPs to benthic fauna has been reported in several studies (Frei et al., 2019; Haegerbaeumer et al., 2019; Walkinshaw et al., 2020).

Nematodes account for up to 90% of meiobenthic organisms (Bergtold and Traunspurger, 2005; Majdi et al., 2017; Traunspurger, 2000; Traunspurger et al., 2020), reach high densities in a diverse range of habitats throughout the world (van den Hoogen et al., 2019), and perform key functions in benthic food webs (Majdi and Traunspurger, 2015). As such, nematodes are versatile bioindicators in environmental risk assessments of pollutant-contaminated aquatic and terrestrial ecosystems (Höss et al., 2011; Wilson and Kakouli-Duarte, 2009). For example, standardized tools for toxicity testing have been developed using the model organism *Caenorhabditis elegans* (Hägerbäumer et al., 2015; ISO,

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2020).

Nevertheless, there have been few studies on the interactions of meiofauna and especially nematodes with MPs (Haegerbaeumer et al., 2019). The ingestion of MPs by nematodes is generally dependent on the size of the particles in relation to that of the nematode's buccal cavity (Fueser et al., 2019). For example, *C. elegans* is able to ingest polystyrene (PS) beads <3.4 μm in size (Boyd et al., 2003; Fang-Yen et al., 2009; Kiyama et al., 2012). The effects of MPs on *C. elegans* have been examined using various toxicity endpoints, including survival, growth, reproduction, intestinal damage, and/or mobility (Hu et al., 2020; Lei et al., 2018; Mueller et al., 2020a, 2020b; Schöpfer et al., 2020; Zhao et al., 2017). In a recent study, the exposure of *C. elegans* to 0.3-μm PS beads at 10–100 mg l^{-1} resulted in decreased locomotion, increased reactive oxygen species production and the activation of the mitochondrial unfolded protein response, consistent with the direct toxicity of PS beads to nematodes (Li et al., 2020). In addition to direct effects, indirect effects of these artificial produced plastics on *C. elegans* can also occur, but these have seldom been considered so far (e.g., Hanna et al., 2018). Mueller et al. (2020b) showed clear dose-dependent inhibitory effects of PS beads (0.1–10.0 μm in diameter) on the reproduction of *C. elegans* and were able to relate toxicity to the ratio of the PS beads and the food (bacteria). This result suggested that a lower food availability rather than a direct toxicity of the PS beads was responsible for the observed effects. Also, Shang et al. (2020) explained effects of 1.0- and 5.0-μm spheres on the lifespan and gene expression of *C. elegans* with a reduced ingestion of bacterial cells. Similarly, in other aquatic organisms, food dilution also seemed to explain the negative effects of MPs (Aljaibachi et al., 2020; Besseling et al., 2013; Blarer and Burkhardt-Holm, 2016; Ogonowski et al., 2016). Although the phenomenon of nutrient dilution by non-nutritive debris was addressed in the late 1990s (McCauley and Bjorndal, 1999) and nematodes are well-suited for examining food ingestion mechanisms (Avery and You, 2012), to the best of our knowledge, the impact of MPs on nematode feeding behavior has yet to be investigated.

Therefore, in the present study, bacterial (*Escherichia coli*) consumption by *C. elegans* was examined in the absence and presence of non-nutritious beads by comparing the bacterial densities in treatments with and without nematodes, considering the bead material (PS or silica) and the bead size (PS: 1.0 and 6.0 μm; silica: 1.0 μm). Moreover, we assessed pharyngeal pumping behavior, as a crucial process of food ingestion by *C. elegans* (e.g., Avery, 1993; Lee et al., 2017), in the absence and presence of beads. Following hypotheses were set up: (1) the dilution of *E. coli* suspensions with PS beads at effect-relevant dilution ratios causes a reduction of bacterial consumption; (2) given the lower toxicity of silica compared to PS beads (Mueller et al., 2020b), silica beads were also expected to have less impact on bacterial consumption; (3) pharyngeal pumping is affected by the presence of beads, as the pumping rate is dependent upon the availability of bacterial cells (Lee et al., 2017) via chemosensory perception (Bargmann, 2006).

2. Material and methods

2.1. Cultivation of the nematode Caenorhabditis elegans

Stock cultures of the nematode species *Caenorhabditis elegans* Maupas 1900 (N2 strain) were obtained from the Caenorhabditis Genetics Center (University of Minnesota, Minneapolis, MN, USA) and grown on nematode growth medium [17 g agar l^{-1}, 2.5 g peptone l^{-1}, 3 g NaCl l^{-1} supplemented with 1 ml 1M $CaCl_2$, 1 ml 1M $MgSO_4$, 25 ml 1M KH_2PO_4 buffer pH 6 (108.3 g KH_2PO_4 l^{-1}, 35.6 g K_2HPO_4 l^{-1}), with 1 ml of cholesterol solution (5 mg ml^{-1} in ethanol) added after autoclaving (Brenner, 1974)]. As food, agar plates were spiked with OP50, a uracil-requiring mutant of *Escherichia coli* that avoids overgrowth of the bacterial lawn (Brenner, 1974), following standard procedures (Stiernagle, 2006). Stock culture plates were stored at 20 °C in the dark.

2.2. Bacterial diet

Bacterial suspensions of *E. coli* were prepared according to the ISO guideline 10872 (ISO, 2020). An *E. coli* OP50 culture was grown overnight in Luria-Bertani medium (1% peptone, 0.5% yeast extract, 1% NaCl) at 37 °C for 17 h under constant mixing. After centrifugation of the culture for 20 min at 2000 g, the cells in the pellet were resuspended in K-medium (3.1 g NaCl l^{-1}, 2.4 g KCl l^{-1}) and then washed by a second centrifugation and resuspension procedure. The bacterial density was spectrophotometrically determined (Varian Cary 50 Bio UV–Visible) based on the optical density (OD_{600}) of three subsamples (1:20 dilution) and by using a calibration curve (Muschiol and Traunspurger, 2007).

2.3. Polystyrene and silica beads

Fluorescent polystyrene (PS) beads with diameters of 1.0 and 6.0 μm (Fluoresbrite® yellow-green microspheres, cat.# 17154 and cat.# 17156, respectively; excitation maxima: 441 nm; emission maxima: 485 nm) were purchased from Polysciences Europe GmbH (Hirschberg, Germany). PS beads were used because they have been frequently detected in river shore sediments and PS is a commonly used polymer (Klein et al., 2015; Scherer et al., 2020). Bead sizes of 1.0 and 6.0 μm were chosen because 1.0-μm but not 6.0-μm PS beads are readily ingested by *C. elegans* (Fueser et al., 2019).

A stock suspension of 1.0-μm silica (SiO_2) beads (cat. #SiO2-F-1.0) was purchased from microParticles GmbH (Berlin, Germany) as a non-plastic control to distinguish between mere particle effects and plastic-derived effects. Silica is naturally abundant in the environment and 1.0-μm silica beads have a similar size and shape as MP beads.

Nominal bead densities were verified by counting the beads in aliquots of defined dilutions of the stock suspensions using a hemocytometer (Neubauer improved; 0.02-mm chamber depth; Brand GmbH + Co KG, Wertheim, Germany). Actual test concentrations deviated from the nominal concentrations and thus from the manufacturer's specifications by not more than 4.4% for the 1.0-μm PS, 17.8% for the 6.0-μm PS, and 16.3% for the silica beads. Bead diameters corresponded well with the manufacturer's specifications and all PS and silica beads had a negative zeta potential between 60.78 ± 1.32 and 84.76 ± 1.78 (Mueller et al., 2020b). As a measure of the buoyancy of the different beads in water, the sedimentation velocities based on Stokes' law were calculated: 0.028 mm s^{-1} for the 1.0 μm PS beads, 0.464 mm s^{-1} for the 1.0 μm silica beads and 1.020 mm s^{-1} for the 6.0 μm PS beads, irrespective of depth.

2.4. Experimental designs

2.4.1. Feeding experiment

To examine whether the presence of PS or silica beads leads to a decreased food availability for *C. elegans*, which can at least partly explain inhibitory effects on reproduction, bacterial consumption by *C. elegans* was determined.

The bacteria were prepared in K-medium to achieve concentrations twice as high as the intended test concentration of 5×10^8 *E. coli* ml^{-1}. To inhibit the growth of *E. coli*, the antibiotic tetracycline hydrochloride ($C_{22}H_{25}Cl\ N_2O_8$; CAS Nr. 64-75-5; Carl Roth

GmbH + Co. KG, Karlsruhe, Germany) was added to the bacterial suspension to a final concentration of 2 mg l^{-1}. This concentration inhibits bacterial growth (Spann et al., 2015) but has no effect on nematodes (Vangheel et al., 2014). The bead suspensions were similarly prepared by diluting stock suspensions with K-medium to achieve concentrations twice as high as the intended test concentrations: 1.0-μm PS and silica beads at 5×10^8 beads ml^{-1}, respectively, and 6.0-μm PS beads at 5×10^7 beads ml^{-1}. PS bead concentrations were based on a 50% inhibition of *C. elegans* reproduction as determined in a previous study (Mueller et al., 2020b): a bead to bacteria ratio of 1:1 for the 1.0-μm PS beads and of 1:10 for the 6.0-μm PS beads. For silica beads, the same concentration as for 1.0 μm PS beads was chosen (bead to bacteria ratio of 1:1), at which only a 20% inhibition of nematode reproduction was observed (Mueller et al., 2020b).

All assays were carried out in standard polystyrene cell culture plates (cell growth area per well: 3.85 cm^2; VWR International GmbH, Darmstadt, Germany), with four replicates established for each of the following treatments: only *E. coli* (control), *E. coli* with nematodes, *E. coli* with nematodes and PS beads (1.0- or 6.0-μm) or silica beads. The effects of PS (experiment 1) and silica (experiment 2) beads on bacterial consumption were determined in two separate experiments. Each well, except the control treatment (only *E. coli*), contained 50 adult individual *C. elegans*, which were individually transferred into 1 ml of test medium (0.5 ml E. *coli* suspension +0.5 ml bead suspension or K-medium) using a glass pipette with an eyelash mounted at its tip to avoid additional dilution of the bacterial cells. In our test design, the water column in each test vial measured 2.6 mm. Based on their specific sedimentation velocities and assuming no perturbation, all types of beads reached the bottom of the well in less than 2 min. Even if considering a possible influence of living *E. coli* cells on the sedimentation of the beads (Singh et al., 2017), all types of beads were expected to be settled at the start of the experiment, when the nematodes were introduced.

After an exposure time of 7 h, the bacterial density of each replicate was determined by counting the number of *E. coli* cells in a 10-μl aliquot using a hemocytometer (Neubauer improved; 0.02×mm chamber depth; Brand GmbH + Co KG, Wertheim, Germany) and 400 × magnification (Zeiss Axio Scope.A1, Jena). The decrease of bacterial density in the test medium of the treatments containing nematodes was calculated by subtracting the number of bacteria in a respective treatment from the mean number of bacteria in the control without nematodes. It was assumed that the feeding of the nematodes was predominantly responsible for the removal of the bacteria from the medium, which was therefore defined as bacterial consumption. Thus, the consumption rate was expressed as consumed bacteria either per 50 nematodes in 7 h or per individual and minute (bacteria ind^{-1} min^{-1}).

2.4.2. Pumping rate experiment

The effect of food dilution by PS beads on the nematode pumping rate was determined in adult *C. elegans* under various food and/or PS bead conditions. Pharyngeal pumping rates were recorded at 20 °C by counting complete backward movements of the terminal bulb grinder (adapted from Chiang et al., 2006; Hobson et al., 2006), observed at 100 × magnification using a microscope (Zeiss Axio Scope.A1, Jena), at 10-s intervals. Single worms were transferred to a 70-μl 3% Gelrite® pad (0.5 g Gelrite® heated in 15 ml of deionized water and then cooled) placed on a microscopic slide together with a 10-μl droplet containing 5×10^8 *E. coli* ml^{-1}, 10^9 *E. coli* ml^{-1}, 10^9 *E. coli* ml^{-1} with 10^9 1.0-μm PS beads ml^{-1}, 5×10^8 1.0-μm PS beads ml^{-1}, 10^9 1.0-μm PS beads ml^{-1}, 10^8 6.0-μm PS beads ml^{-1}, or 10^8 6.0-μm PS beads ml^{-1} with 10^9 *E. coli* ml^{-1}.

The nematodes were allowed to acclimatize to the new conditions for 5–10 min, after which the pumping rates of five nematodes in each treatment were determined three times. Only nematodes actively pumping at the beginning of the recording time were scored. The number of pumps per 10 s was multiplied by six to achieve pumps per minute (pumps min^{-1}).

2.5. Data analysis

Data points 1.5 × the interquartile range of a boxplot were considered as outliers and excluded from the data set. The data were checked for normality (Shapiro-Wilk test) and for homoscedasticity (Levene's test) but were not transformed to improve normality. A significance level of $p < 0.05$ was set for all comparisons.

Since both the feeding and the pumping rate assay involved dependent data, significant differences between the bacterial densities after 7 h and between pharyngeal pumping rates in the different treatments (except bacteria start conditions at 0 h) were detected with a one-way repeated measures (RM) ANOVA (post-hoc Holm-Sidak method for bacterial densities; post-hoc Tukey test for pharyngeal pumping). As the feeding experiments with PS and silica beads were the same, but were performed at different times, they were separately analyzed, each with its own controls. Statistical analyses and figure generation were carried out using SigmaPlot 12.0 (Systat Software Inc.). The pumping rates of *C. elegans* and the bacterial densities are reported as the mean ± standard deviation.

3. Results

3.1. Feeding experiment

Despite the addition of tetracycline to the medium, the bacterial density increased slightly during the 7-h experiment. In the control treatments without nematodes, the bacterial density increased from 5.0 (±0.43) × 10^8 *E. coli* ml^{-1} at 0 h to 5.32 (±0.58) × 10^8 *E. coli* ml^{-1} after 7 h in experiment 1 (Fig. 1A), and from 5.09 (±0.61) × 10^8 *E. coli* ml^{-1} to 5.48 (±0.67) × 10^8 *E. coli* ml^{-1} in experiment 2 (Fig. 1B). A similar slight increase in the number of bacteria should be assumed in the treatments with nematodes. During the 7-h exposure, *C. elegans* significantly reduced bacterial density, by 19% and 14% in experiment 1 and 2, respectively (Fig. 1). Compared to the controls, bacterial densities were significantly lower in the presence of nematodes: 4.32 (±0.30) × 10^8 *E. coli* ml^{-1} ($t = 5.498$, $p < 0.001$) in experiment 1 and 4.71 (±0.39) × 10^8 *E. coli* ml^{-1} ($t = 4.365$, $p < 0.001$) in experiment 2. Hence, within 7 h 50 adult *C. elegans* consumed 1.0 × 10^8 and 0.77 × 10^8 *E. coli*, meaning individual consumption rates of 4762 and 3667 *E. coli* min^{-1} ind^{-1} for experiment 1 and 2, respectively.

Nematodes consumed significantly less bacteria with PS beads than in the treatments without PS beads. In the 1.0-μm PS bead treatment, the bacterial density was not significantly reduced compared to the control without nematodes ($t = 1.941$, $p = 0.113$; Fig. 1A), such that bacterial densities were significantly higher than in the treatments without PS beads but with nematodes (4.99 (±0.56) × 10^8 *E. coli* ml^{-1}; $t = 3.513$, $p = 0.005$; Fig. 1A). In the presence of 6.0-μm PS beads, *C. elegans* significantly reduced the bacterial density compared to the treatment without nematodes (4.81 (±0.58) × 10^8 *E. coli* ml^{-1}; $t = 2.850$, $p = 0.025$; Fig. 1A), albeit significantly less effectively than in treatments without PS beads but with nematodes ($t = 2.588$, $p < 0.037$; Fig. 1A). Thus, in the presence of 1.0-μm and 6.0-μm PS beads *C. elegans* consumed 0.33 × 10^8 and 0.51 × 10^8 *E. coli* within 7 h, which is 67% and 49% less than the control without PS beads (1.0 × 10^8 *E. coli* per 7 h).

The presence of 1.0-μm silica beads did not affect bacterial

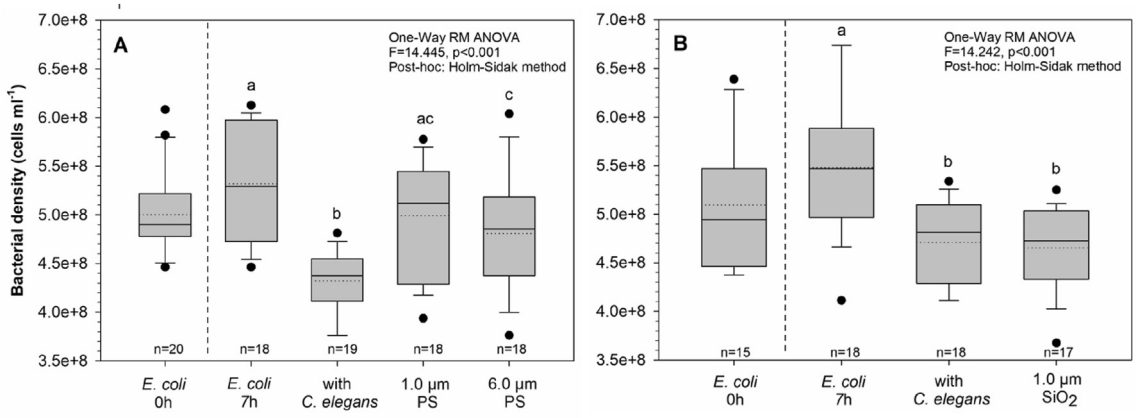

Fig. 1. Bacterial densities of *Escherichia coli*. The bacterial density (*E. coli* cells ml^{-1}) at the start (*E. coli* 0 h) and after *C. elegans* feeding for 7 h was counted for each treatment: only *E. coli* (*E. coli* 7 h), *E. coli* with *C. elegans* (with *C. elegans*), (A) *E. coli* + 1.0 μm PS beads with *C. elegans* (1.0 μm PS), *E. coli* + 6.0 μm PS beads with *C. elegans* (6.0 μm PS) and (B) *E. coli* + 1.0 μm (silica) SiO_2 beads with *C. elegans* (1.0 μm SiO_2). The dashed line separates the bacterial start conditions, which were not statistically analyzed. Different letters indicate significant differences (one-way RM ANOVA, post-hoc: Holm-Sidak method $p < 0.05$). Median (solid line), mean (dotted line); boxes represent 50% (interquartile range) and each whisker 25% of the data. SigmaPlot 12 (Systat Software Inc., USA).

consumption by *C. elegans*. After 7 h, similar densities of bacterial cells were found in treatments with nematodes, with or without silica beads (4.65 (±0.42) × 10^8 and 4.71 (±0.39) × 10^8 *E. coli* ml^{-1}, respectively; t = 0.577, $p = 0.568$; Fig. 1B). Slightly more bacteria were consumed during 7 h in the presence than in the absence of silica beads (0.83 × 10^8 vs. 0.77 × 10^8 *E. coli*, respectively).

3.2. Pumping rate experiment

In a subsequent experiment we examined whether lower pumping rates were responsible for the significant reductions in bacterial consumption by *C. elegans* in the presence of 1.0- and 6.0-μm PS beads.

The pumping rate of *C. elegans* decreased significantly, by 14%, when the bacterial density was reduced to 50% (from 210 ± 18 pumps min^{-1} at 10^9 *E. coli* ml^{-1} to 181 ± 14 pumps min^{-1} at 5 × 10^8 *E. coli* ml^{-1}; q = 6.097, $p < 0.001$; Fig. 2A). When bacterial cells were completely replaced by the same density of 1.0-μm PS beads (10^9 beads ml^{-1}), the pumping rate was considerably lower (23%) than at the corresponding bacterial density (161 ± 23 pumps min^{-1}; q = 10.248, $p < 0.001$) and slightly but not significantly higher than in the treatment with 5 × 10^8 1.0-μm PS beads ml^{-1} (145 ± 12 pumps min^{-1}; q = 3.804, $p = 0.071$; Fig. 2A). At the latter bead density, the pumping rate of *C. elegans* was 20% lower than in the presence of the same density of *E. coli* (q = 7.828, $p < 0.001$).

When 1.0-μm PS beads and *E. coli* cells were mixed at a ratio of 1:1 (10^9 *E. coli* ml^{-1} and 10^9 1.0 μm PS beads ml^{-1}), the pumping rate of 205 ± 12 pumps min^{-1} hardly differed from the 210 ± 18 pumps min^{-1} measured in treatments consisting only of bacterial cells at the same density (q = 0.780, $p = 0.981$; Fig. 2A). However, nematodes pumped significantly faster in the combined treatment than when provided with 5 × 10^8 *E. coli* ml^{-1} (q = 5.290, $p = 0.004$).

When 6.0-μm PS beads and *E. coli* cells were mixed at a ratio of 1:10 (10^9 *E. coli* ml^{-1} and 10^8 6.0-μm PS beads ml^{-1}), the pumping rate of *C. elegans* (161 ± 16 pumps min^{-1}) was significantly lower than that induced by the same concentration of bacteria (10^9 *E. coli* ml^{-1}; q = 10.054, $p < 0.001$; Fig. 2B). In the absence of bacteria, the pumping rate in response to 10^8 6.0-μm beads ml^{-1} (136 ± 15 pumps min^{-1}) was significantly lower than in either the mixed treatment (q = 4.494, $p = 0.011$) or the treatment containing only bacterial cells (q = 13.599, $p < 0.001$).

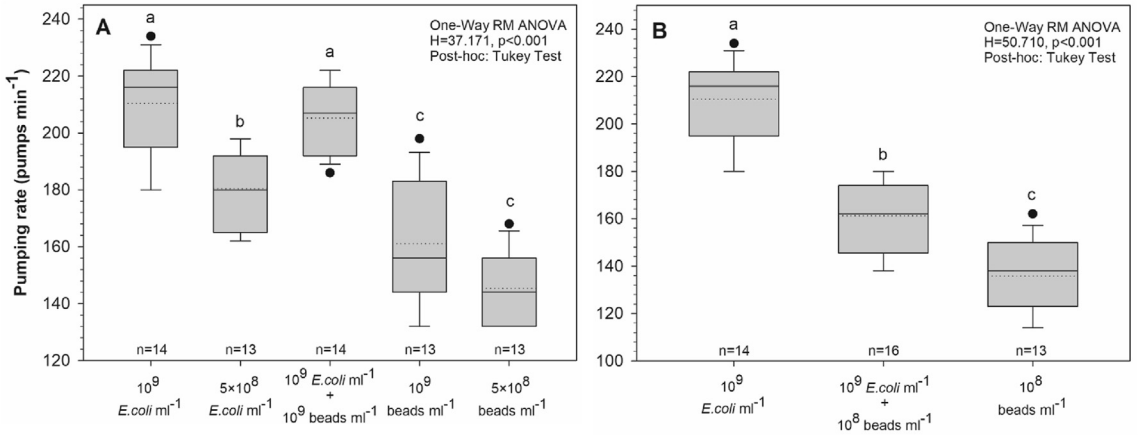

Fig. 2. Pumping rates of *Caenorhabditis elegans*. The pumping rate (pumps min^{-1}) of *C. elegans* under the following conditions: (A): 10^9 *E. coli* ml^{-1}, 5 × 10^8 *E. coli* ml^{-1}, 10^9 *E. coli* ml^{-1} with 10^9 1.0-μm PS beads ml^{-1}, 10^9 1.0-μm PS beads ml^{-1}, 5 × 10^8 1.0-μm PS beads ml^{-1}. (B): 10^9 *E. coli* ml^{-1}, 10^9 *E. coli* ml^{-1} with 10^8 6.0-μm PS beads ml^{-1}, 10^9 6.0-μm PS beads ml^{-1}. Different letters indicate significant differences (one-way RM ANOVA, post-hoc: Tukey test $p < 0.05$). Median (solid line), mean (dotted line); boxes represent 50% (interquartile range) and each whisker 25% of the data. SigmaPlot 12 (Systat Software Inc., USA).

4. Discussion

This study showed that, in accordance with hypothesis (1), the addition of PS beads to *E. coli* suspensions reduced the bacterial consumption by *C. elegans*. The inhibitory effects of PS beads on *C. elegans* reproduction, demonstrated by Mueller et al. (2020b), can thus be explained at least in part by a reduced food availability, measured as a reduced ingestion of bacterial cells. In the study of Mueller et al. (2020b), *C. elegans* reproduction was inhibited by 50% in the presence of a PS bead to bacteria ratio of 1:1 and 1:10 for 1.0-μm and 6.0-μm PS beads, respectively. In the present study, 50 nematodes consumed 1.0×10^8 *E. coli* during 7 h, which corresponds to individual consumption rates of 4762 *E. coli* min^{-1} ind^{-1}. Comparable consumption rates were found for the nematode *Plectus palustris* feeding on *Acinetobacter* sp. (5019 cells min^{-1} ind^{-1}; Duncan et al., 1974). In the presence of PS beads, however, the nematode consumed 49–67% less bacteria (Fig. 1A). While this effect was observed during a 7-h experiment, a considerable decrease in reproduction, caused by reduced energy inputs from food, can be expected during the 96-h exposure typically used in toxicity tests. Moreover, following their ingestion by *C. elegans*, 1.0-μm PS beads are transferred to the nematode's gut (Fueser et al., 2019), where they may interfere with digestion and nutrient assimilation and thus lower the energy input needed for reproduction and other physiological processes. This has been observed in the freshwater amphipod *Gammarus fossarum*, in which food assimilation efficiency decreased significantly following its exposure to polyamide fibers (Blarer and Burkhardt-Holm, 2016).

The fact that 1.0-μm silica beads also added at a 1:1 bead to bacteria ratio had no effect on bacterial consumption by *C. elegans* supports the link between the inhibition of reproduction observed in the presence of beads and food availability (Mueller et al., 2020b). Due to their higher specific density (1.85 g ml^{-1}; manufacturer's specifications), silica beads are less buoyant than either PS beads (1.05 g ml^{-1}; manufacturer's specifications) or *E. coli* cells (1.07–1.11 g ml^{-1}; Baldwin et al., 1995, https://www.ncbi.nlm.nih.gov/pmc/articles/PMC176578/). According to Stokes' law, the 1.0-μm PS beads show in water a 16.5-fold lower sedimentation velocity than the silica beads of same size. Given that the bacteria and the beads had been slightly, but continuously resuspended by the nematode's movements, the PS beads were expected to be more buoyant together with the bacteria for a longer period than the silica beads. The weaker interference of silica beads with *C. elegans* bacterial feeding than observed with PS beads, also at a 1:1 bead to bacteria ratio (Fig. 1B), would explain their negligible effect on *C. elegans*' reproduction (<20% inhibition vs. 50% inhibition for 1.0-μm PS beads; Mueller et al., 2020b) and supports our second hypothesis.

Also for other organisms, negative effects of MP exposure were explained by decreases of energy uptake and nutrient assimilation caused by a dilution of their diet (Ogonowski et al., 2018; Scherer et al., 2018). Aljaibachi and Callaghan (2018) showed that the impact of 2.0-μm PS beads on the water flea *Daphnia magna* could be attributed to the density of food algae rather than to the concentration of the plastic particles. The pelagic copepod *Calanus helgolandicus* ingested 11% fewer algal cells within 24 h when exposed to 20-μm PS microspheres (Cole et al., 2015), whereas in the present study 6.0-μm and 1.0-μm PS beads reduced the bacterial consumption of nematodes by 49% and 67%, respectively. Watts et al. (2015) reported that the crab *Carcinus maenas* consumed significantly less food over time when fed with 1.0- to 5.0-mm polypropylene rope microfibers.

In the present study, both the 1.0-μm and the 6.0-μm PS beads caused a reduction in bacterial consumption. *Caenorhabditis elegans* non-selectively feeds on all particles small enough to pass the buccal opening. However, while the 1.0-μm PS beads are ingestible, the 6.0-μm PS beads are not, as they are too large for the buccal cavity of *C. elegans*, which has a maximum opening of 4.4 ± 0.5 μm (Fueser et al., 2019). The mechanisms leading to the reduced bacterial feeding triggered by the PS beads of different size therefore presumably differed based on their ability to enter the nematode's gut. Following their ingestion, the 1.0-μm PS beads suspended in liquid medium will be actively pumped into the intestinal system like bacterial cells (Avery and You, 2012). In a mixture of PS beads and bacteria, the proportion of ingested bacteria will be lower than in a pure bacterial suspension. Accordingly, for PS beads 1.0-μm in size a dilution mechanism most likely explained the observed effects. For the 6.0-μm PS beads, their disruption of foraging could explain the reduced bacterial consumption. Nematodes actively move in order to find areas with high food densities conducive to feeding (Boyd et al., 2003). Large particles might disturb their foraging activity and thus lead to a lower feeding efficiency. For particles of both sizes, their impact on pharyngeal pumping must also be taken into account. *Caenorhabditis elegans* is a deposit- and bacteria-feeder and the grinder within its terminal bulb mechanically crushes ingested bacteria (Fang-Yen et al., 2009) as the nematode moves through its aquatic particle-containing medium (Avery and Horvitz, 1990). Movements of the grinder are coupled to pharyngeal pumping (Riebesell and Sommer, 2017) so that the pumping rate is a primary determinant of food ingestion (Avery, 1993; Lee et al., 2017). Bacterial feeding is thus controlled by two processes, pharyngeal pumping and peristalsis (Avery and Horvitz, 1987). As the pumping rate of *C. elegans* depends on the food density (Lee et al., 2017), dilution of the food supply by artificial particles may slow pharyngeal pumping and thereby also decrease consumption.

The effect of PS beads of both sizes (1.0- and 6.0-μm) on the pharyngeal pumping of *C. elegans* as a potential cause of the reduced food consumption was examined by determining the pumping rates in the absence and presence of PS beads. These experiments revealed that the pumping rate of *C. elegans* increased with increasing bacterial density, as reported in other studies (Lee et al., 2017). A 50% reduction in the bacterial density (from 10^9 to 5×10^8 *E. coli* ml^{-1}) lowered the pumping rate by 14% (Fig. 2A). Assuming that a 50% dilution of the bacteria by the 1.0-μm PS beads was perceived by the nematodes as a 50% decrease in bacterial density, a decrease of the pumping rate would have been expected (hypothesis 3). However, the results of the experiment showed that at a bacterial density of 10^9 *E. coli* ml^{-1} a 1:1 ratio of 1.0-μm PS beads to bacteria did not significantly reduced the pumping rate compared to the undiluted control. Moreover, even in the absence of bacteria, 1.0-μm PS beads slightly stimulated pharyngeal pumping (personal observation, Hendrik Fueser). Maybe 1.0-μm PS beads mimic nutritious *E. coli* cells to a certain extent, as their size and buoyancy are similar (Supplementary material, Fig. S1; Baldwin et al., 1995; Kiyama et al., 2012). However, because the 1.0-μm PS beads are unable to trigger a chemosensory response (Bargmann, 2006), they will be less effective than bacteria in stimulating pumping, as observed in this study (Fig. 2A). Given that the 1:1 mixture with ingestible 1.0-μm PS beads did not affect pharyngeal pumping, the reduced bacterial consumption cannot be explained by this mechanism (Fig. 1A). By contrast, a 1:10 ratio of 6.0-μm beads to bacteria significantly (by 23%) reduced the pumping rate (Fig. 2B), which is in accordance with hypothesis (3). Since, based on the results with 1.0-μm PS beads at a higher bead to bacteria ratio, a dilution effect does not seem to explain the decrease in the pumping rate of 6.0-μm PS beads, another mechanism must be involved. A previous study showed that gentle touch stimuli at the nematode tail tip inhibited pharyngeal pumping (Keane and Avery, 2003) such that *C. elegans* may have responded

similarly when moving through the suspension of 6.0-μm PS beads. Thus, a 23% slower pharyngeal pumping would explain a considerable part of the 49% reduction in bacterial consumption compared to the controls without 6.0-μm PS beads.

5. Conclusion

This study showed that high concentrations of 1.0- and 6.0-μm PS beads significantly limited bacterial consumption by the nematode *C. elegans*. This impact of the PS beads on food availability at least in part explains inhibitory effects of PS beads on nematode reproduction, shown to be strongly related to the ratio of beads to bacteria (Mueller et al., 2020b). This link between bead effects and food availability is supported by the findings for silica beads, which had no impact on food consumption, explaining the significantly lower inhibitory effects on *C. elegans* reproduction (Mueller et al., 2020b). The PS bead induced reduction in bacterial consumption could be attributed to different mechanisms, depending on the size of the PS beads. While in the presence of ingestible 1.0-μm PS beads food dilution is likely to explain the reduced bacterial consumption by *C. elegans*, the non-ingestible 6.0-μm PS beads lowered the pumping rate and may have disturbed foraging. To investigate the mechanisms accounting for the negative impacts of MPs on *C. elegans*, this study was carried out with unrealistically high MP concentrations and with food densities high enough to allow optimal laboratory conditions during toxicity testing but rarely found in natural habitats. In natural habitats, temporal food deficiencies may result in a negative impact of MPs on nematodes even at much lower concentrations. Moreover, we could show that even non-ingestible MPs can disturb feeding of nematodes, which enlarges the size range of potentially harmful MPs. By demonstrating the interference of MPs with food availability for benthic invertebrates, the results of this study contribute to the elucidation of the mechanisms underlying the effects of MPs in aquatic ecosystems and support prioritizing effect mechanisms in environmental risk assessments for MPs (Ruijter et al., 2020).

Author contributions

Marie-Theres Rauchschwalbe – Conceptualization, Methodology, Investigation, Data curation, Writing - original draft, Writing - review & editing. Hendrik Fueser – Methodology, Investigation, Data curation, Writing - original draft, Writing - review & editing. Walter Traunspurger – Supervision, Writing - review & editing. Sebastian Höss – Conceptualization, Supervision, 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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Appendix A. Supplementary data

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