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Species-specific effects of long-term microplastic exposure on the population growth of nematodes, with a focus on microplastic ingestion

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ABSTRACT

Microplastics (MPs; < 5 mm) released into freshwaters undergo changes in their density that make them highly bioavailable to the fauna in the sediments. In fine sediments, nematodes account for up to 90% of the meiobenthic organisms and have an important position in benthic food webs, by connecting lower (bacteria) and higher (e.g., macrofauna, fish) trophic levels. Due to their high ecological relevance, ubiquitous occurrence and very high individual densities, nematodes can serve as bioindicators of environmental pollution and especially MP pollution, since the ingested microscopic particles can be easily detected in the transparent bodies of these organisms. Single-species toxicity tests with *Caenorhabditis elegans* have revealed dose-dependent inhibitory effects on reproduction in nematodes exposed for 96 h to relatively high concentrations of polystyrene (PS) beads. Thus, in this study, we examined whether longer-term multigenerational tests of nematodes under continuous PS bead exposure are able to reveal more subtle impacts of MPs on population growth and whether the observed effects can be linked to the species-specific life history traits of the nematodes.

We therefore tested three bacterial-feeding nematode species (*C. elegans*, *Acrobeloides nanus*, *Plectus acuminatus*) by exposing them to 1.0-µm PS beads at an exposure concentration of 10^7 beads ml^{-1}. The experiment was conducted in semi-fluid medium for 21–49 days depending on the life-cycle characteristics of each nematode species. Ingested PS beads were quantified by fluorescence microscopy observations. Population growth rates, carrying capacities as well as doubling time and the time at which the maximum sustainable yield was reached were used to assess the effects of PS bead exposure on nematode population dynamics.

All three nematode species readily ingested 1.0-µm PS beads in semi-fluid medium, but the number of ingested PS beads varied between species. PS bead exposure significantly decreased the carrying capacity of *C. elegans*, whereas *A. nanus* populations grew significantly faster in the presence of the beads. Long-term multigeneration tests revealed more subtle impacts of MP on *C. elegans* than occurred following short-term exposure scenarios. Our results show that MP-induced changes in nematodes population dynamics can alter nematode communities, which in term may impact the benthic food web.

1. Introduction

Nematodes are used as bioindicators in assessments of anthropogenic pollution due to their high ecological relevance, ubiquitous occurrence and very high individual densities (Wilson and Kakouli-Duarte, 2009). The life-history strategies of nematodes are taxa-specific, allowing a trait-based bioindication by categorizing nematode families in classes of various strategies (colonizer-persister (cp) classes), ranging from fast-reproducing, more stress-tolerant colonizers (r-strategists) to well-adapted, more stress-sensitive persisters (K-strategists) (Bongers, 1990; Bongers and Ferris, 1999). For ecotoxicological experiments mainly typical colonizers (i.e. species belonging to cp-class 1 or 2) are used, as they are easy to culture, have a simple body plan and short generation time/life-cycle (Hägerbäumer et al., 2015). However, even within cp-classes, life-cycle parameters can vary considerably between various taxa (Muschiol and Traunspurger, 2007).

For nematodes, experiments involving full life-cycles were already used to assess the effects of chemical contaminants on ecologically relevant endpoints, such as population growth (Brinke et al., 2013; Vangheel et al., 2014; Fueser et al., 2018), and even in multigenerational set-ups (Vangheel et al., 2014). Population-level effects are a key endpoint in community ecology (Forbes and Calow, 1999; Hägerbäumer et al., 2015). To assess the impact of harmful substances at the population level, clearly interpretable measures, such as carrying

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capacity and population growth rate (Sibly and Hone, 2002), can be linked to the fitness of age-structured populations, such as by combining survival and fertility rates, as important life-cycle characteristics, into a single quantifiable factor (Charlesworth, 1994).

Microplastics (MPs) pollution affects all environmental compartments (water, soil, air) (Klein et al., 2015; Cai et al., 2017; Zhang and Liu, 2018) and has thus become a global environmental concern (Arthur et al., 2009; European Commission, 2013). Due to sedimentation and biofouling processes that increase the density of MP (Kaiser et al., 2017; Harrison et al., 2018; Kaiser et al., 2019), their concentrations in sediments may be up to 10,000-fold higher than at the water surface (Wendt-Potthoff et al., 2014), resulting in a higher risk to benthic compared to pelagic fauna (Frei et al., 2019; Haegerbaeumer et al., 2019; Walkinshaw et al., 2020). However, because sampling and analytical techniques suitable for MPs are still limited (Adam et al., 2019), estimates of the environmental concentrations of small MPs and nanoplastics (< 10 μm) are still based on extrapolations (Lenz et al., 2016). Indeed, a recent study suggested that the actual environmental concentrations of MPs may be much higher than currently recognized (Adam et al., 2019). Moreover, because the effects of nano- and MPs on freshwater organisms are for the most part unknown (Scherer et al., 2018), accurate assessments of the risk of these particles to freshwater organisms remain challenging. Scherer et al. (2017) showed that several freshwater invertebrates readily ingest MPs. Fueser et al. (2019) found that numerous species of nematodes are able to ingest polystyrene (PS) beads ranging in size from 0.5 to 10.0 μm in diameter and that the ingestion efficiencies were influenced by the feeding habit and buccal cavity size of the worms.

The effects of MPs on freshwater meiobenthic organisms have scarcely been investigated hitherto and are also unclear (Haegerbaeumer et al., 2019), despite the ecological relevance of these organisms (Giere, 2019). The few studies evaluating the toxicity of MPs to meiobenthic organisms include those focusing on copepods (Cole et al., 2015; Jeong et al., 2017), rotifers (Jeong et al., 2016), and nematodes (Zhao et al., 2017; Lei et al., 2018; Mueller et al., 2020). Toxicity tests with the nematode *Caenorhabditis elegans* exposed to PS beads (0.1–10.0 μm in size) at relatively high concentrations (> 10^8 PS beads ml^{-1}) revealed dose-dependent inhibitory effects on reproduction, albeit more likely due to food dilution rather than to direct toxicity (Mueller et al., 2020). An alternative endpoint is population growth, which might be more sensitive than reproduction. For instance, the exposure of *Brachionus manjavacas* to 0.037-μm PS spheres at 0.3 μg ml^{-1} and 1.1 μg ml^{-1} resulted in reductions in population growth by 50% and 89%, respectively, whereas no effect on this endpoint occurred in response to PS spheres > 83 μm (Snell and Hicks, 2011).

Although *C. elegans* is considered to be representative of other freshwater nematodes in terms of their chemical sensitivity (Haegerbaeumer et al., 2018), species differing in their particle ingestion behavior (Fueser et al., 2019) and life-history strategies (Bongers, 1990) may respond differently to MPs. Moreover, as MPs are able to influence the availability of food for nematodes (Mueller et al., 2020), species-specific preferences in food densities (Schroeder et al., 2010) might lead to varying responses to MP. Therefore, in this study we investigated the effects of MPs on the population growth of three bacterial-feeding nematode species with low c-p-classes of 1 (*C. elegans*) and 2 (*Acrobeloides nanus* and *Plectus acuminatus*) but species differ in their life-cycle strategies and especially in generation times. The nematodes were exposed to 1.0-μm PS beads in semi-fluid medium at a concentration of 10^7 beads ml^{-1}, which is 100 times lower than the PS bead concentration causing a 50% decline in *C. elegans*' reproduction (Mueller et al., 2020), as we expected a higher sensitivity of population growth assessed via multigeneration exposure. Dietary ingestion of PS beads by the nematodes was quantified over the course of the experiment using fluorescence microscopy. The impact of the beads on population growth at several endpoints was determined as well. We hypothesized that (1) all three bacterial-feeding nematode species readily ingest 1.0-μm PS beads since their ingestion is not limited by their buccal cavities (Fueser et al., 2019), (2) that already a low PS bead concentration induce effects on the population dynamics of nematodes and (3) that the three nematode species respond differently to the PS beads, according to their reproductive strategies.

2. Material and methods

2.1. Nematode cultivation

Stock cultures of the nematode *Caenorhabditis elegans* Maupas, 1900 (wild-type, N2 strain) were obtained from the Caenorhabditis Genetics Center (University of Minnesota, Minneapolis, MN, USA). *Caenorhabditis elegans* and *Acrobeloides nanus* de Man, 1880 (wild isolate from young soils in Berzdorf, Germany) were 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 1 M $CaCl_2$, 1 ml 1 M $MgSO_4$, 25 ml 1 M KH_2PO_4 buffer pH 6 (108.3 g KH_2PO_4 l^{-1}, 35.6 g K_2HPO_4 l^{-1}) and 1 ml of cholesterol solution (5 mg ml^{-1} in ethanol), added after autoclaving (Brenner, 1974)]. *Plectus acuminatus* Bastian, 1865 (wild isolate from Lake Constance, Germany) was maintained on "pond water agar" plates (1.7% agar and filtered pond water without additional salts). All agar plates were seeded 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.

The three nematode species distinctly differed in their life-cycle strategies, especially in their generation times, and were therefore used in this study: *C. elegans* has a mean generation time of about 3.75 days (Muschiol et al., 2009), *A. nanus* of about 11.3 days (Sohlenius, 1973), and *P. acuminatus* of about 19.3 days (Kreuzinger-Janik et al., 2017). Following Bongers (1990), these nematode species can be ascribed colonizer-persister-classes based on their families: Rhabditidae = 1 (e.g., *C. elegans*), Cephalobidae = 2 (e.g., *A. nanus*) and Plectidae = 2 (e.g., *P. acuminatus*).

2.2. Microplastic suspension

A stock suspension of 1.0-μm Fluoresbrite® yellow-green fluorescent PS microspheres (cat.# 17152; excitation maxima: 441 nm; emission maxima: 485 nm; Polysciences Europe GmbH, Baden-Wuerttemberg, Germany) was prepared. The surface charge (zeta-potential) of the fluorescent 1.0-μm PS beads in 1% M9-medium (6 g Na_2HPO_4, 3 g KH_2PO_4, 5 g NaCl and 0.25 g $MgSO_4 \cdot 7H_2O$ l^{-1}) was -82.18 ± 2.16 mV and was measured with a Zetasizer Nano ZS (Malvern Panalytical GmbH, Kassel, Germany). The size distribution of the beads was determined (measurements of 225 beads) and the bead diameters corresponded well with those cited in the manufacturer's specifications (own measurements: 0.97 ± 0.003 μm; manufacturer's specifications: 1.0 ± 0.03 μm). PS beads were used because PS is a commonly used plastic and PS MPs have been frequently detected in river shore sediments (Klein et al., 2015). A bead size of 1.0 μm was chosen because beads of this size class were previously shown to be readily ingested by the three tested nematode species (Fueser et al., 2019).

PS beads in the appropriate concentrations were prepared in glass vials (A-Z Analytik-Zubehör GmbH, Langen, Germany) by diluting the stock suspensions with sterile deionized water to achieve a concentration ten times higher than the intended test concentration of 10^7 PS beads ml^{-1} (5.49 μg PS ml^{-1}), corresponding to 1% of the concentration that induced 50% inhibition on *C. elegans*' reproduction after 96 h exposure (EC50: $1.0 \pm 0.098 \times 10^9$ beads ml^{-1}; Mueller et al., 2020). A concentration of 10^7 PS beads ml^{-1} was used to simulate a 1% dilution of beads to bacteria (10^9 cells ml^{-1}). The nominal densities of the PS beads in the diluted stock suspension of 10^7 PS beads ml^{-1} were

checked using a hemocytometer (Neubauer improved; 0.02 mm chamber depth; Brand GmbH & Co. KG, Wertheim, Germany). Actual PS bead test concentrations deviated from the nominal concentrations by not more than > 10% (own measurements: $4.11 \times 10^{10} \pm 9.06 \times 10^{6}$ beads ml^{-1}; manufacturer's specifications: 4.55×10^{10} beads ml^{-1}).

2.3. Preparation of bacterial food

An *E. coli* OP50 culture was prepared in accordance with ISO guideline 10872 (ISO, 2010) by growing the bacteria overnight in Luria-Bertani (LB) medium (1% peptone, 0.5% yeast extract, 1% NaCl) at 37 °C for 17 h with continues shaking. The culture was centrifuged for 20 min at 2000*g*, the LB-supernatant was discarded, and the bacterial pellet was resuspended in K-medium (3.1 g NaCl l^{-1}, 2.4 g KCl l^{-1}) to wash the cells. After repeated centrifugation, the bacterial pellet was resuspended in K-medium. 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) using a calibration curve (Muschiol and Traunspurger, 2007). Defined bacterial pellets were prepared in centrifuge tubes to achieve a final density of 10^9 *E. coli* cells ml^{-1} when the appropriate volume of the test medium was added (Section 2.4), which is considered to be an optimal density for nematodes following standard conditions (ISO, 2010).

2.4. Test medium (component nematode growth gelrite)

Component nematode growth gelrite (CNGG), a semi-fluid medium with a consistency between standard laboratory liquid medium and sediment, was used in this study as it is recommended for nanoparticle testing on nematodes (Brinke et al., 2011). Particles added to CNGG can be evenly distributed and the nematodes can move freely in the medium (Brinke et al., 2011).

CNGG consists of (1) gellan gum solution (1.9 g l^{-1} Gelrite®, Merck & Co., Inc., Kelco Division), with 1.25 ml l^{-1} cholesterol solution (5 mg ml^{-1} in ethanol) added after autoclaving, and (2) salt solution (10 mM $MgSO_4$, 10 mM $CaCl_2$). The medium components were autoclaved and stored at 20 °C until needed. CNGG was then prepared in centrifuge tubes (including food bacteria) by mixing gelrite solution, salt solution and deionized water/PS bead suspension at a ratio of 8:1:1. Between steps, the contents of the centrifuge tubes were mixed thoroughly to ensure equal distribution of the three ingredients.

2.5. Experimental setup

The experimental setups were based on previous studies of the population growth dynamics of nematodes maintained in semi-fluid medium (Muschiol et al., 2009; Kreuzinger-Janik et al., 2017; Majdi et al., 2019).

For each nematode species and treatment (control, MP; Supplementary Material A1, Fig. A1), seven replicates were prepared in glass vials (to avoid additional plastic exposure by potential leachates of plastic test vessels) containing 4 ml of CNGG. For each species, 20 juveniles and 20 adult nematodes were transferred to the vials using a micropipette and a stereomicroscope (40× magnification), with 14 replicates per species and 42 replicates in total. The glass vials containing the nematodes were maintained under culture conditions, i.e., at 20 °C in the dark.

Depending on the growth rate of each nematode species, a 1-ml nematode-containing subsample was taken from the control and MP treatments after different exposure durations: *C. elegans*, after 0, 3, 6, 9, 12, 15, 18 and 21 days; *A. nanus*, after 0, 3, 6, 12, 18, 24, 27, 31, 35, 38, 42 and 45 days; *P. acuminatus*, after 0, 7, 14, 21, 28, 35 and 42 days. The subsamples were obtained by carefully mixing the contents of the vials by repeated pipetting to achieve a homogenous distribution of the nematodes in the medium (Fig. A2). To compensate for the medium removed during subsampling and to keep the food densities constant, 1 ml of freshly prepared CNGG including food bacteria was added to each vial after subsample collection.

To check for possible changes in food density due to the sub-samplings, the bacterial density was monitored every 3–7 days over the course of the experiment (replicate 7; see Fig. A1, Supplementary Material). The bacteria in three 10-µl aliquots of the subsamples were counted using a Neubauer improved hemocytometer (0.02-mm chamber depth; Brand GmbH + Co KG, Wertheim, Germany) to calculate the actual number of *E. coli* cells ml^{-1}.

Subsamples of replicates 1–6 (controls) and replicates 1–5 (MP) were liquefied and stained by the addition of 5 ml of an EDTA-Rose Bengal solution (0.93 g of 0.02 M EDTA, 0.15 g Rose Bengal in 500 ml of deionized water). EDTA breaks the bonds in the CNGG matrix (Muschiol and Traunspurger, 2007), while Rose Bengal stains the nematodes to facilitate counting. Nematodes were then counted under a stereomicroscope (40× magnification).

Subsamples of replicate 6 of the MP treatment were used to quantify the fluorescently labeled PS beads in the nematodes. The medium was therefore liquified by the addition of 5 ml of EDTA (0.93 g of 0.02 M EDTA in 500 ml of deionized water) without Rose Bengal, which avoided interactions with the fluorescent dye of the PS beads.

All nematodes in the subsamples were heat fixed for 20 min at 80 °C and stored at 4 °C in the dark until further investigations.

2.6. Population growth parameters

To determine the total number of nematodes in 4 ml of CNGG at each sampling date, individuals in the 1 ml-subsample were counted and the resulting number was multiplied by four.

Population density development over time can be described by a logistic growth function (Ricklefs and Miller, 2000):

$$N_t = \frac{K}{1 + \left(\frac{K - N_0}{N_0}\right) * e^{-rt}}$$

where N_t is the number of individuals in the population after t days, N_0 the size of the starting population, r the population growth rate per day and K the carrying capacity. Therefore, for each replicate, K and r were determined by logistic fitting using the package "growthcurver" (Sprouffske and Wagner, 2016) in the R environment (R Core Team, 2019). In addition, the time parameters T_{mid} (in days), defined as the time at which the maximum sustainable yield for a population is reached and which is equal to half of its carrying capacity ($T_{mid} = 0.5 \times K$), and T_{gen} (in hours), defined as the doubling time of the population at the largest decline of the curve ($T_{gen} = \ln 2/r$) were determined by logistic fitting (Sprouffske and Wagner, 2016). Since 25% of each sample replicate was removed per sampling day, leaving 75% for population growth, each value of r calculated by logistic fitting had to be corrected by the artificial mortality (m):

$$m = \frac{0.2n}{T_n - T_1}$$

where m is the artificial mortality per day, n the number of sampling times, T_1 the first sampling day and T_n the last sampling day.

2.7. Quantification of PS beads in nematodes

To quantify the ingestion of PS beads by the nematode species over the course of the population growth experiment, 10 adult nematodes were taken from the subsamples of replicate 6 (MP only treatment; Fig. A1, Supplementary Material) per sampling day, washed in test medium to remove PS beads adhered to the nematode cuticula and then transferred onto a microscope slide. A fluorescence microscope (Zeiss Axio Scope.A1, Jena, Germany) with a 400× magnification was used to count the ingested PS beads and to localize them in the buccal cavity,

Table 1
Ingestion of 1.0-µm polystyrene (PS) beads by three nematode species. Mean number ± standard error of PS beads ingested by *Caenorhabditis elegans* (n = 5–10), *Acrobeloides nanus* (n = 6–10) and *Plectus acuminatus* (n = 4–10) at the different sampling days and for all sampling days (overall mean).

Time (days)	*C. elegans*	*A. nanus*	*P. acuminatus*
3	93.5 ± 18.7		
6	40.0 ± 9.8		
7			31.0 ± 5.8
9	34.5 ± 11.4		
12	76.0 ± 15.3	18.6 ± 6.5	
14			85.3 ± 20.7
15	114.2 ± 19.3		
18	184.1 ± 45.7	13.1 ± 4.0	
21	100.8 ± 20.8		91.5 ± 17.4
24		18.0 ± 4.6	
27		33.6 ± 8.8	
28			60.0 ± 16.8
31		12.8 ± 7.9	
35		16.9 ± 3.9	74.6 ± 9.8
38		28.8 ± 5.6	
42		16.6 ± 4.2	152.0 ± 40.8
45		23.8 ± 9.8	
49			142.5 ± 26.2
Overall mean	91.9 ± 17.7	20.2 ± 2.2	91.0 ± 15.1

oesophagus, intestine and rectum of the nematodes.

2.8. Statistical analysis

All data were checked for normality (Shapiro-Wilk test) and homoscedasticity (Levene's test). Data that were not normally distributed (r, K, T_{gen}) were transformed logarithmically. A significance level of $p < 0.05$ was set for all comparisons. A two-way ANOVA (post-hoc: Holm-Sidak method) with species and treatment as independent factors was performed to analyze significant differences among nematode species for each population growth parameter (r, K, T_{mid}, T_{gen}) under control and PS bead exposure conditions.

3. Results and discussion

3.1. Ingestion of PS beads

Confirming hypothesis (1), all three investigated nematode species readily ingested 1.0-µm PS beads in CNGG (Table 1), but in varying amounts. These differences reflected species-specific differences in feeding behaviors, since fluorescent PS beads can be used as a proxy for bacterial cells (McGhee and Ghafouri, 2007). In the guts of *C. elegans* and *P. acuminatus*, a mean (± standard error [SE]) of 92 ± 18 (range: 35–184) and 91 ± 15 (range: 31–152) PS beads were detected, respectively (Table 1). This agrees well with the study of Fueser et al. (2019), in which 102 ± 20 (mean ± SE) and 69 ± 14 PS beads were detected in the guts of *C. elegans* and *P. acuminatus*, respectively, following exposure of these nematodes for 24 h to 1.0-µm PS beads in aqueous medium at a concentration of 10^7 ml^{-1}. *Acrobeloides nanus* ingested fewer PS beads compared to the other two species, with 20 ± 2 (range: 13–34) PS beads detected in its gut (Table 1). However, this was a much larger number than in the above-mentioned study of Fueser et al. (2019), in which *A. nanus* was exposed for 72 h to PS beads in aqueous medium (mean: < 1 PS bead). The discrepancy might be explained by (1) the much longer exposure time in the present study (12–45 vs. 3 days) and (2) the different test media, as in semi-fluid CNGG the PS beads are more evenly distributed than in water (Brinke et al., 2011).

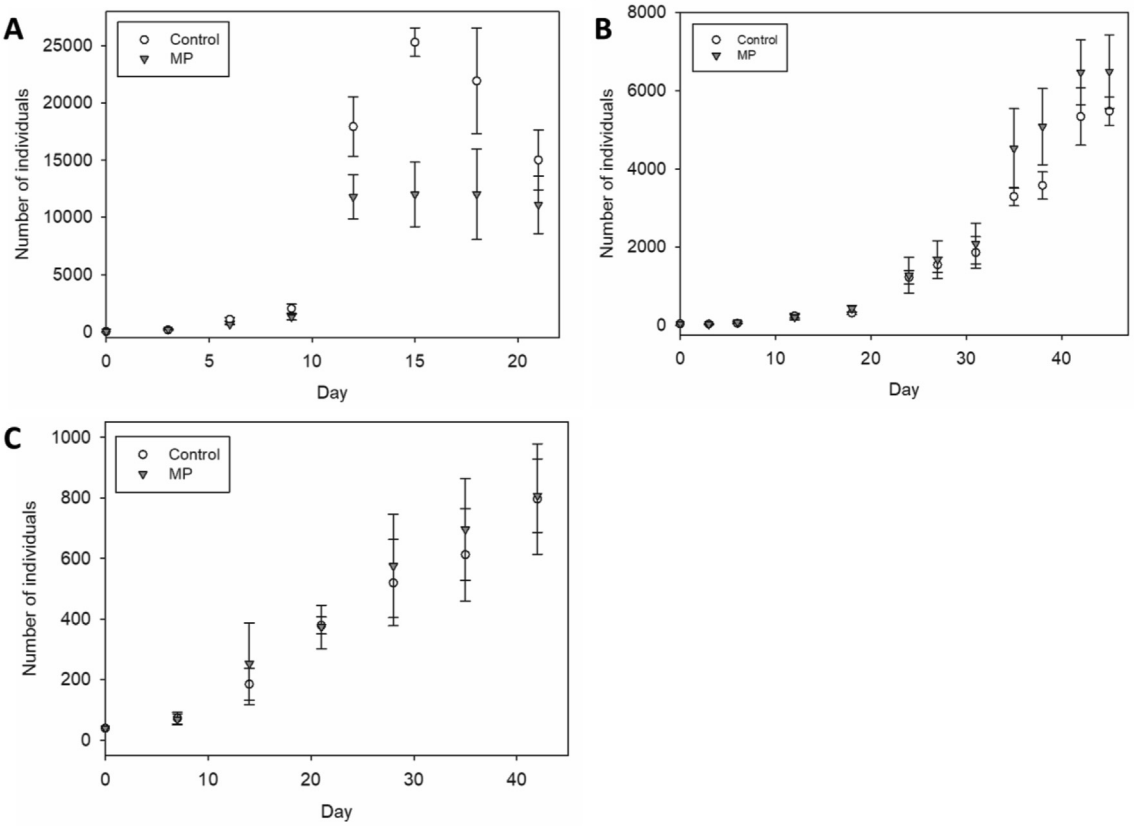

Fig. 1. Population growth curves of the three bacterial-feeding nematode species (A = *Caenorhabditis elegans*; B = *Acrobeloides nanus*; C = *Plectus acuminatus*). The number of individuals 4 ml^{-1} (mean ± SD; n = 5–6) in each treatment (Control; MP = 10^7 PS beads ml^{-1}) was plotted as a function of time using SigmaPlot 12.0 (Systat Software Inc.).

Table 2
Population growth parameters (r = population growth rate day^{-1}; K = carrying capacity [individuals 4 ml^{-1}]; T_{mid} [days] = time at which the maximum sustainable yield for a population is reached and which is equal to half of the carrying capacity; T_{gen} [hours] = doubling time of the population) of *Caenorhabditis elegans*, *Acrobeloides nanus* and *Plectus acuminatus*. Values are the mean ± standard deviation (n = 5–6) of each treatment (C = control; MP = microplastic). Two-way ANOVA (post-hoc: Holm-Sidak method; significant differences $p < 0.05$ are shown in bold).

Parameter	*C. elegans*		*A. nanus*		*P. acuminatus*	
	C	MP	C	MP	C	MP
r	1.59 ± 0.34	1.76 ± 0.58	0.21 ± 0.01	0.27 ± 0.04	0.18 ± 0.04	0.24 ± 0.12
	t = 1.023; p = 0.316		t = 1.315; p = 0.2		t = 1.045; p = 0.306	
K	21,266 ± 2,070	12,045 ± 2,865	7,829 ± 641	7,481 ± 742	834 ± 98	765 ± 118
	t = 6.212; **p < 0.001**		t = 0.5; p = 0.621		t = 1.027; p = 0.314	
T_{mid}	10.60 ± 0.48	10.26 ± 0.57	38.14 ± 0.95	34.12 ± 2.70	26.35 ± 2.49	24.24 ± 4.29
	t = 0.021; p = 0.836		t = 2.418; **p = 0.023**		t = 1.325; p = 0.197	
T_{gen}	11.21 ± 3.08	9.86 ± 3.53	118.99 ± 6.61	83.42 ± 14.95	134.16 ± 40.40	126.34 ± 82.52
	t = 0.587; p = 0.563		t = 1.424; p = 0.167		t = 0.918; p = 0.367	

3.2. Impacts of PS beads on nematode population growth

The measurements showed that the food supply was constant throughout the experiment for all nematode species, with bacterial densities ranging from 7.6 × 10^8 to 1.1 × 10^9, 8.6 × 10^8 to 1.1 × 10^9 and 8.6 × 10^8 to 9.8 × 10^8 *E. coli* cells ml^{-1} for *C. elegans*, *A. nanus* and *P. acuminatus*, respectively (Supplementary Material A3, Table A1). These minor fluctuations were not expected to affect population growth development of the nematodes (Schiemer, 1982a; Muschiol and Traunspurger, 2007), since population growth is restricted only at bacterial densities < 2.8 × 10^8 *E. coli* cells ml^{-1} (Schiemer, 1982b). Therefore, the food supply was assumed to be sufficient for nematode development, such that changes in population growth could be attributed to MPs exposure.

The population growth parameters of the three species varied considerably in the MP-free controls and reflected their species-specific life-history strategies (Fig. 1, Table 2). *Caenorhabditis elegans* had a significant higher mean population growth rate with 1.59 day^{-1} than *A. nanus* (0.21 day^{-1}; t = 9.850; p < 0.001) and *P. acuminatus* (0.18 day^{-1}; t = 11.198; p < 0.001), both growing at the same rate (t = 0.910; p = 0.371). The highest carrying capacity was achieved by *C. elegans* with 21,266 individuals 4 ml^{-1} followed by *A. nanus* with 7,829 individuals 4 ml^{-1} (t = 10.551; p < 0.001) and *P. acuminatus* with 834 individuals 4 ml^{-1} (t = 35.795; p < 0.001). Moreover, the nematode species *A. nanus* produced significantly more individuals in terms of the carrying capacity than *P. acuminatus* (t = 24.775; p < 0.001). In addition to the highest carrying capacity, populations of *C. elegans* significantly grew faster by reaching T_{mid} about 16 days earlier than *P. acuminatus* (t = 9.891; p < 0.001) and about 27.5 days earlier than *A. nanus* (t = 16.560; p < 0.001; Table 2). *Acrobeloides nanus* significantly grew slower than *P. acuminatus* with a T_{mid} of 38.1 and 26.4 days, respectively (t = 7.406; p < 0.001). While *A. nanus* and *P. acuminatus* doubled their population sizes with same speed (t = 0.312; p = 0.758), *C. elegans* had the fastest doubling time (T_{gen}) of 11.2 h (p < 0.001; Table 2).

Generally, the energy requirements of fast-reproducing species (such as *C. elegans*) are assumed to be larger than those of slow-reproducing species (such as *P. acuminatus*). However, based on the ingestion of 1.0-µm PS beads as a proxy for the ingestion of bacteria, *P. acuminatus* was as effective as *C. elegans* in terms of food uptake (Table 1), although the abundance of PS beads in the gut is not necessarily indicative of the assimilation efficiency of bacterial nutrients. Nonetheless, *C. elegans* must be very efficient in nutrient uptake (assimilation efficiency = 1.0; Spann et al., 2015) to reach these high carrying capacities in a short time, as the residence time of bacteria in its intestine is less than two minutes (McGhee and Ghafouri, 2007). By contrast, since the carrying capacity of *P. acuminatus* was much lower than that of *C. elegans* with high PS bead ingestion rates at the same time, the food assimilation efficiency of *P. acuminatus* is apparently much lower.

Supporting our hypothesis (3), the three nematode species responded differently to long-term MP exposure (Fig. 1, Table 2). Although PS beads were ingested in large numbers by slowly growing *P. acuminatus* (Table 1), their effects on r, K, T_{mid} and T_{gen} were not significant when compared to the control values of these parameters (Table 2). By contrast, supporting our hypothesis (2), the population dynamics of *A. nanus* were affected by the MP treatment, but only after day 31 (Fig. 1). Beginning on day 31, *A. nanus* populations developed significantly faster in the MP than in the control treatment: the maximum sustainable yield (T_{mid}) was reached 4 days earlier (Table 2). Thus, at a certain nematode population density, PS beads may have a beneficial effect on certain species. But finally, after 49 days, MP and control treatments reached very similar K values (p = 0.314) and therefore similar numbers of *A. nanus* individuals were produced. Martinez et al. (2012) showed that *A. nanus* is stress tolerant since its individual abundances were not significantly affected by cadmium concentrations. When another bacteria-feeding nematode species was added, *A. nanus* tended to produce more offsprings with increasing cadmium concentrations (Martinez et al., 2012).

For *C. elegans*, despite the absence of a significant effect on the population growth rate (r), doubling time (T_{gen}) and time at which the maximum sustainable yield was reached (T_{mid}) following exposure of the nematode to the PS beads, the carrying capacity (K) was only 57% of that of the control (12,045 ± 2,865 individuals 4 ml^{-1} vs. 21,266 ± 2,070 individuals 4 ml^{-1}) and thus significantly lower (p < 0.001; Table 2), supporting our hypothesis (2). This result suggested a decrease in the digestion efficiency of this fast-reproducing species since PS beads are indigestible by nematodes and disturb their digestive processes.

Since this is the first study evaluating effects of MPs on population dynamics of nematodes, our findings cannot satisfactorily be compared with published nematode data. However, Jeong et al. (2016) examined the effects of 0.05-, 0.5- and 6.0-µm PS spheres on the population growth of the rotifer *B. koreanus* and used comparable exposure concentrations of PS spheres (0.1–20.0 µg ml^{-1}) to our study (5.49 µg ml^{-1}; 1.0 µm PS beads). In all cases where Jeong et al. (2016) discovered significant negative effects on the population growth of *B. koreanus* by PS spheres, PS spheres greatly outnumbered food items. Although Jeong et al. (2016) exposed the rotifers to comparable concentrations of beads on a mass basis (µg PS ml^{-1}), bead densities (beads ml^{-1}) of the various size classes of beads (0.05, 0.5, 6.0 µm) varied considerably. As the proportion of food items in relation to PS beads was very low (0.5-µm beads: 0.02–3.9%; 0.05-µm beads: 2.06 × 10^{-5}–4.1 × 10^{-3}%), the population growth of *B. koreanus* might have been restricted by the reduction of the food uptake efficiency. Also, significant effects of polyethylene microparticles of 5–10 µm in size on the population size of the copepod *Parvocalanus crassirostris* have been discovered at higher MP to food ratio of 1:1 (Heindler et al.,

2017) than in this study (PS beads to *E. coli* cells of 1:100).

In toxicity tests in aqueous medium (Mueller et al., 2020), the first effects of the 1.0-µm PS beads on *C. elegans* reproduction occurred after 96 h of exposure to bead concentrations > 10^8 ml^{-1} (Mueller et al., 2020). By contrast, in this study, the prolonged multigenerational exposure to PS beads induced an effect (a significantly reduced carrying capacity) on *C. elegans* at a considerably lower concentration (10^7 PS beads ml^{-1}). Toxicity tests over several generations and thus with a more realistic exposure scenario may therefore reveal more subtle effects of MP on nematodes than is the case with similar tests based on relatively short exposure times (up to 96 h).

Crucial information on the ability of MPs to alter ecological structures (e.g., food web, competition) can be derived from experiments involving higher levels of organization (Browne et al., 2015). The observed species-specific effects of MP in the present study (i.e., the faster population development of one species and the reduced carrying capacity of another) may lead to shifts in the species composition and thus have consequences for the structure of benthic food webs, where nematodes occupy key positions (Majdi and Traunspurger, 2015). Bosker et al. (2019) showed that the presence of 1.0- to 5.0-µm PS beads had a significant effect on the total population of the water flea *Daphnia magna*, as exposure to 10^5 PS beads ml^{-1} resulted in a 21% reduction of total biomass compared to the control. Moreover, the impact of MPs on the population growth of nematodes may influence not only biomass but also secondary production, since the population growth characteristics of an organism are crucial to its role in secondary production (Faupel and Traunspurger, 2012). However, effects of MPs on nematode (and benthic) communities with respect to species composition, biomass, and secondary production are better investigated under more realistic exposure scenarios (e.g., in model ecosystems).

If PS beads have a stronger potential to induce effects in the nematodes than naturally occurring particles, which are abundant in the natural habitat of nematodes (sediments, soils) is a crucial question for interpreting the risk of nano- and MPs (Ogonowski et al., 2018; Triebskorn et al., 2019), however, cannot be answered within this study. Although, it could be shown that silica particles had a lower inhibitory effect on the reproduction of *C. elegans* than PS beads of the same size (Mueller et al., 2020), this might not be the case for other natural particles, such as clay or organic colloids. It could be shown that the reproduction of *C. elegans* was heavily influenced by clay particles in sediments (Höss et al., 1999) and by organic colloids in water (Höss et al., 2001).

4. Conclusion and future work

All three bacterial-feeding nematode species included in this study were able to ingest 1.0-µm PS beads in semi-fluid medium. The number of beads in the nematode gut was species-specific. As expected, nematode species with different life-history strategies responded differently to the presence of the beads, but the responses were not related to their gut concentrations. *Caenorhabditis elegans*, a fast-reproducing nematode with high nutrient demands, was more susceptible to MP exposure than the slowly reproducing *P. acuminatus*. Our results show that toxicity testing based on long-term multigenerational rather than short-term exposure is necessary to reveal the subtle impacts of MPs on *C. elegans*.

For instance, in the past European freshwaters were mainly sampled with cut-off sizes > 80 µm meaning that microplastics < 80 µm could not be detected (Adam et al., 2019) and therefore are underestimated (Lindeque et al., 2020). However, the estimated environmental concentration for 1.0 µm PS beads based on the extrapolation of Lenz et al. (2016) might reach 10^3 beads ml^{-1}, which is about 10,000 times lower than the actual concentration in this laboratory study. Since reliable data about the concentrations of small MP particles < 10 µm in field samples are lacking due to sampling and technical limitations (Adam et al., 2019), no clear conclusions can be made regarding the relevance of the MP concentration used in this study.

Population growth experiments are characterized by very stable conditions and allow assessments of ecologically relevant toxicity endpoints. Nematodes reach high densities in sediments and soils throughout the world (Traunspurger et al., 2020; van den Hoogen et al., 2019), which makes them to potentially good bioindicators of MP pollution. Moreover, their transparent bodies allow them to be easily screened for microscopic particles. Whether the species-specific effects of MP, revealed in this study by single-species population growth experiments, also translate into a shift in the species composition of nematode communities is a challenging question that has to be answered in future studies, using microcosm experiments with field-collected sediments.

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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Author contributions

M.-T.M., H.F., S.H. and W.T. designed the population growth experiment using the three nematode species. H.F. and M.-T.M. performed the experiment by counting the number of individuals in the subsamples and the number of PS beads ingested by the nematodes. M.-T.M. and H.F. wrote the manuscript. W.T. and S.H. supervised the work. All authors reviewed and approved the final version of the manuscript.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ecolind.2020.106698.

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