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Rapid ingestion and egestion of spherical microplastics by bacteria-feeding nematodes

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HIGHLIGHTS

- First fine-scale microplastic ingestion and egestion assay of nematodes.
- Rapid ingestion of ≤1.0-μm PS beads with first beads in the intestine within 5 min.
- Distinct nematode feeding behaviors influenced the PS bead body burdens.
- PS beads were completely egested within 20–40 min in presence of sufficient food.
- Spherical PS beads had very low bioconcentration factors in nematodes.

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ABSTRACT

Microplastics, anthropogenically released into freshwaters, settle in sediments, where they are directly ingested by benthic organisms. However, to the best of our knowledge, fine-scale studies of microplastic ingestion and egestion by nematodes, one of the most abundant meiofaunal taxa, are lacking.

We therefore conducted a time series of the ingestion and egestion by adult *Caenorhabditis elegans* and *Pristionchus pacificus* of 0.5- and 1.0-μm fluorescent polystyrene (PS) beads along with bacteria. The nematodes were exposed to 10^7 beads ml^{-1} in aqueous medium for 5 min–24 h and pumping rates of *C. elegans* were determined. In the egestion study, PS bead egestion was monitored in nematodes with high microplastic body burdens for 5 min–24 h in microplastic-free medium.

Ingested beads were detected already within 5 min and up to 203 ± 15 PS beads (1.0 μm; *C. elegans*) were found after 30 min. Overall, significantly more 1.0-μm than 0.5-μm PS beads were taken up. The distinct feeding behaviors of the two species influenced their PS bead body burdens. Ingested PS beads were almost completely egested within the first 20–40 min in the presence of sufficient food. In *C. elegans*, 1.0-μm beads were egested less rapidly than 0.5-μm PS beads.

Given the rapid ingestion and egestion of the beads, our study demonstrates that the actual amount of ingested and egested microplastics by nematodes in the environment may be several times higher than the microplastic body burdens may imply. However, spherical PS beads did not bioconcentrate in nematodes.

1. Introduction

The ubiquity of microplastics (polymer particles <5 mm; e.g., Arthur et al., 2009) in marine and freshwater ecosystems throughout the world is well-documented (Eerkes-Medrano et al., 2015; Peeken et al., 2018). Moreover, as their specific densities can be higher than that of water and biofouling enhances their sedimentation (e.g., Harrison et al., 2018; Kaiser et al., 2017; Kooi et al., 2017), microplastics released into riverine water bodies may end up in their sediments (van Cauwenberghe et al., 2015) at densities up to 10,000-fold (Wendt-Potthoff et al., 2014) or even 600,000-fold higher than in the water phase (Scherer et al., 2020). As a result, the risk of microplastic ingestion by benthic fauna is relatively high. The reported concentrations of microplastics in sediments vary and strongly depend on the considered particle size as well as the methods used in the assessment (e.g., Enders et al., 2015; Imhof et al., 2013; Ivleva et al., 2017). For microplastics <10.0 μm, technical limitations in their detection and identification

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impede reliable determinations of their sediment concentrations. Nonetheless, with decreasing particle size and increasing mobility, the ingestion risk for freshwater organisms increases (Triebskorn et al., 2019), since the smallest particles are presumably those that are readily ingested (Dris et al., 2015).

The uptake of microplastics by protozoans, freshwater invertebrates and vertebrates is an area of active research and several studies have shown that microplastics of various polymers, shapes and sizes are ingested by ciliates, flagellates, rotifers, annelids, crustaceans, mollusks, fishes (reviews by Adam et al., 2019; Scherer et al., 2018; Triebskorn et al., 2019) and field-sampled riverine macroinvertebrates (Windsor et al., 2019). The ingestion of microplastics by organisms across many trophic levels has been reported (Cole et al., 2013; Lusher et al., 2013) and multiple effects have been demonstrated, including in benthic invertebrates (Haegerbaeumer et al., 2019).

Among benthic invertebrates, nematodes are ubiquitous, occurring in very high densities in freshwater, marine and terrestrial environments (Heip et al., 1990; Traunspurger et al., 2019; van den Hoogen et al., 2019). They cover the entire food spectrum (Yeates et al., 1993; Traunspurger, 1997) and thus fill a key position in benthic food webs (Majdi and Traunspurger, 2015; Schmid-Araya and Schmid, 2000; Weber and Traunspurger, 2015). As such, nematodes may constitute a critical pathway for the entry of microplastics into higher trophic levels. These features, as well as the transparent body of nematodes and the ease of sample preparation, recommend the use of nematodes as test organisms in quantitative, microscopic determinations of microplastic ingestion (Fueser et al., 2019) by benthic invertebrates. In fact, numerous laboratory studies have investigated microplastic ingestion in the nematode *Caenorhabditis elegans* (e.g., Boyd et al., 2003; Fang-Yen et al., 2009; Fueser et al., 2019; Kiyama et al., 2012; Lei et al., 2018; Nika et al., 2016; Suzuki et al., 2019; Zhao et al., 2017). Polystyrene (PS) microspheres in the bacteria size range of 0.5- and 1.0-μm in diameter were shown to mainly enter the nematode's intestine (Fueser et al., 2019; Kiyama et al., 2012; Suzuki et al., 2019), with subsequent surface-related effects and the inhibition of reproduction due to reduced food availabilities (Mueller et al., 2020). In another study, the quantity of ingested PS beads was shown to depend on the nematode feeding type (Fueser et al., 2020), specifically, on the size and morphology of the buccal cavity, but also on the availability of the microplastics (Fueser et al., 2019) and nematode feeding behavior.

In rhabditids such as *C. elegans*, feeding consists of two processes: (i) coupled pumping by the corpus, anterior isthmus, and terminal bulb and (ii) peristalsis within the posterior isthmus (Avery and Horvitz, 1987). Food ingestion begins at the buccal cavity. Bacteria are sucked in through the contractions of pharyngeal muscle groups (pumping) followed by the posteriorly sweeping relaxation of muscles in the isthmus (peristalsis) (e.g., Avery and Horvitz, 1987; Avery and Shtonda, 2003). The ingested bacterial cells are then mechanically broken up in the grinder of the terminal bulb (e.g., Chiang et al., 2006; Riebesell and Sommer, 2017).

The diplogastrid *Pristionchus pacificus* differs substantially in its ecology and behavior from *C. elegans*. Although both species can be cultured on bacteria (e.g., Rae et al., 2008; Bumbarger et al., 2013), *P. pacificus* has no need for a pharyngeal grinder because it uses a predatory dorsal tooth to break open its prey (e.g., Bumbarger et al., 2013; Riebesell and Sommer, 2017). Thus, in *P. pacificus* and other diplogastrids, the terminal bulb is not engaged in pumping behavior but rather in peristalsis, which is coupled to that by the isthmus (Chiang et al., 2006).

An evaluation of the fate of microplastics in environments must include a determination of the residence time in the stomach or gut of resident biota (Rezania et al., 2018) and therefore quantifications of both the ingestion and egestion capabilities of those organisms (Frydkjær et al., 2017). In general, the bioconcentration potential of a contaminant is determined by its bioavailability and its rates of ingestion, metabolism, and elimination (Fent, 2007). Microplastics are highly bioavailable to nematodes (Fueser et al., 2019, 2020) but are indigestible, such that their ingestion and egestion rates alone determine their potential bioconcentration.

Studies dealing with the egestion of microplastics are underrepresented in the literature (e.g., Amphipoda: Au et al., 2015; Blarer and Burkhardt-Holm, 2016, Bivalvia: Chae and An, 2020; Rist et al., 2019, Cladocera: Elizalde-Velázquez et al., 2020; Frydkjær et al., 2017; Rist et al., 2017, Copepoda: Cole et al., 2013). For example, *Daphnia magna* almost immediately ingested 15-μm microplastics in large quantities especially as they were in the size range of natural food items (Aljaibachi et al., 2020). To the best of our knowledge, fine-scale ingestion and egestion studies for nematodes are missing.

For size fractions <63 μm, polystyrene (PS) beads are a common form and type of microplastics in riverine sediments (Klein et al., 2015; Scherer et al., 2020) and beads of 0.5 and 1.0 μm are readily ingested by both nematode species, without limitations of the buccal cavity (Fueser et al., 2019). Therefore, in this study, we evaluated the ingestion and egestion of fluorescent 0.5- and 1.0-μm PS beads by adult *C. elegans* and *P. pacificus* at finely resolved exposure-times. The body burdens resulting from the ingestion of fluorescent PS beads provided along with bacteria as food for 5 min−24 h were directly quantified by counting the PS beads in the nematode digestive system by fluorescence microscopy. In addition, as the pumping rate is the primary determinant of food ingestion by nematodes (e.g., Avery, 1993; Raizen et al., 2012) and pumping rates are a useful indicator of the responses to changes in food density (e.g., Chiang et al., 2006; Fischer et al., 2016; Nicholas et al., 1973), ingestion was also evaluated by visually determining the pumping rate of *C. elegans* exposed to PS beads provided in combination with an optimal food supply.

The specific aims of this study were to determine the proportion and speed at which the PS beads were ingested and egested by the two nematode species and the amount of time needed to reach a dynamic equilibrium of ingestion and egestion (saturation point). Bioconcentration factors were then calculated for both species and PS bead sizes. We hypothesized that, (1) nematodes rapidly ingest PS beads of 0.5 and 1.0 μm in size and (2) based on their different feeding behaviors, *C. elegans* with a pharyngeal pumping rate more than twice as high as that of *P. pacificus* (Kroetz et al., 2012) and a bacterial residence time in its intestine of <2 min (McGhee and Ghafouri, 2007), would more rapidly ingest and egest PS beads than *P. pacificus*. Moreover, according to McGhee and Ghafouri (2007), beads and other non-food objects may pass through the nematode intestine at a rate different than that of bacteria.

2. Material and methods

2.1. Nematode stock cultures

The nematode species used in this study, *Caenorhabditis elegans* and *Pristionchus pacificus*, are deposit-feeding nematodes that engulf whole prey in the bacterial size range. *Caenorhabditis elegans* (N2 strain) stock cultures were obtained from the Caenorhabditis Genetics Center (University of Minnesota, Minneapolis, MN, USA). *Pristionchus pacificus* was a wild isolate that has been maintained in our laboratory for over 10 years. Both species were grown on nematode growth medium agar plates (17 g agar l^{-1}, 2.5 g peptone l^{-1}, and 3 g NaCl l^{-1}, 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 cholesterol solution (5 mg ml^{-1} in ethanol) added after autoclaving (Brenner, 1974)). As food, the agar plates were spotted with OP50, a uracil-requiring mutant of *Escherichia coli* that avoids overgrowth of the bacterial lawn (Brenner, 1974), following standard procedures (Stiernagle, 2006). All stock culture plates were stored at 20 °C in the dark.

2.2. Bacterial suspensions

An *E. coli* OP50 culture grown overnight at 37 °C in Luria-Bertani (LB) medium (1% peptone, 0.5% yeast extract, 1% NaCl) was prepared as a suspension with a final density of 10^9 *E. coli* cells ml^{-1} and used in the ingestion and egestion assays to guarantee optimal feeding conditions (Schiemer, 1982) and PS bead ingestion by both nematode species (Fig. S1). The inoculated LB medium was centrifuged for 20 min at 2000 g and the bacterial cells were washed with K-medium (3.1 g NaCl l^{-1}, 2.4 g KCl l^{-1}). The bacterial density was spectrophotometrically determined in five subsamples (1:20 dilution) by measuring the optical density at 600 nm (Varian Cary 50 Bio UV–Visible). The cholesterol solution (5 mg ml^{-1} in ethanol) was then added to a concentration of 4 µl ml^{-1}.

2.3. Polystyrene bead suspensions

Microplastic suspensions were prepared by diluting stock suspensions of 0.5-µm (0.47 ± 0.01 µm) and 1.0-µm (0.91 ± 0.01 µm) PS Fluoresbrite® Yellow Green microspheres (excitation maximum: 441 nm, emission maximum: 485 nm; Polysciences Europe GmbH, Baden-Wuerttemberg, Germany) with K-medium to achieve final test concentrations of 10^7 PS beads ml^{-1} (0.5 µm: 0.69 mg l^{-1}; 1.0 µm: 5.49 mg l^{-1}). This amount ensured the ingestion and precise detection and quantification of the beads (Fig. S1, Fueser et al., 2019) but avoided toxicity to *C. elegans* (Mueller et al., 2020). Nominal PS bead concentrations were checked by fluorescence microscopy (Zeiss Axio Scope.A1, Jena, Germany) using a hemocytometer (Neubauer Improved; 0.02 mm chamber depth; Brand GmbH & Co KG, Wertheim, Germany) at 400× magnification. The test concentrations of the PS beads deviated from the nominal concentrations by ≤ 11.5% (0.5 µm) and 9.6% (1.0 µm). The surface charge of the PS beads was negative (zeta potential of fluorescent 1.0-µm PS bead: −82.2 ± 2.2 mV; measured in 1% M9-medium at 10^7 PS beads ml^{-1}; Zetasizer Nano ZS, Malvern Panalytical GmbH, Kassel, Germany). According to Hanna et al. (2018) and our own microscopy observations, PS beads do not heteroagglomerate with, e.g., negatively charged *E. coli* cells.

2.4. Experimental setup

Adult nematodes of *C. elegans* and *P. pacificus* retained, respectively, by a 10-µm or 20-µm mesh were used as test individuals for the assays (Fig. S2). In the ingestion assay, both species were exposed to 0.5- and 1.0-µm PS beads (10^7 PS beads ml^{-1}) in the presence of an optimal food supply (10^9 *E. coli* cells ml^{-1}), such that the microplastic to food ratio was 1:100. In the egestion assay, PS-bead-containing nematodes were placed in petri dishes (3.0 cm internal diameter) containing K-medium and again an optimal food supply was provided. The high concentration of *E. coli* cells ml^{-1} ruled out any competition for food between individuals. The ingestion and egestion of the PS beads was stopped at different time intervals by heat-killing the nematodes at 80 °C for 20 min. This procedure avoids further ingestion or egestion while maintaining the fluorescent properties and shape of the PS beads. The nematodes were then washed with K-medium to remove beads adhered to their cuticle and transferred onto a microscopic slide. PS bead body burdens, defined as the number of PS beads per individual at a given time point, were quantified at 400× magnification using a fluorescence microscope (Zeiss Axio Scope.A1). The potential of bioconcentration of PS beads is assessed by a bioconcentration factor ($BCF = C_N/C_M$), which is here expressed by the number of microplastics detected in nematodes (C_N) to the actual number of microplastics in the surrounding medium (C_M) at each sampling time interval. Since PS bead test concentrations of the PS beads may deviate from the nominal concentrations (Section 2.3), a BCF range was calculated (Tab. S1).

2.4.1. Ingestion assay

Thirty adult nematodes per PS bead size, sampling time and species (1200 nematodes in total) were starved for 30 min after which five adult nematodes per replicate (n = 6) were transferred to a 10-µl drop of K-medium in a petri dish (Fig. S2). One ml of K-medium containing 10^9 *E. coli* cells ml^{-1} and 10^7 PS beads ml^{-1} (microplastic to food ratio of 1:100) was then added to each dish, thus ensuring a synchronous start of PS bead ingestion. All petri dishes were sealed with Parafilm® and kept at 20 °C in the dark. After 5 min, 10 min, 20 min, 30 min, 1 h, 2 h, 4 h, 8 h, 16 h and 24 h, the nematodes were heat-killed to stop PS bead ingestion and washed with K-medium. The bead body burdens at each sampling time were measured as the number of ingested PS beads in the gut of 15 randomly chosen individuals from all replicates per PS bead size, sampling time and species (600 nematodes in total). Bead numbers were quantified by fluorescence microscopy.

2.4.2. Egestion assay

The assay required nematodes with a high PS bead body burden. Therefore, prior to the egestion assay, the nematodes were briefly (<1 min) centrifuged in a Falcon tube at 600 g and 300–500 nematodes were simultaneously removed using a pipette containing a minimum amount of K-medium. The nematodes were then exposed to PS beads at an exposure concentration of 10^7 PS beads ml^{-1} in K-medium containing 10^9 *E. coli* cells ml^{-1}. Based on the findings of the ingestion assay (Section 2.4.1), a maximum PS bead body burden was achieved in *C. elegans* and *P. pacificus* after an exposure time of 30 min and 210 min, respectively. Immediately thereafter, 30 nematodes per species and PS bead size were heat-killed and the ingested PS beads were quantified to determine the average number per nematode, as an initial value for the egestion assay. The remaining nematodes were divided into eight petri dishes, each containing 10^9 bacterial cells ml^{-1} in 2 ml of bead-free K-medium (Fig. S2). The petri dishes were sealed with Parafilm® and kept at 20 °C in the dark. After 5 min, 10 min, 20 min, 30 min, 1 h, 2 h, 4 h, 8 h, 16 h and 24 h, 30 nematodes were heat-killed, washed with K-medium and the number of remaining PS beads in the gut of 21–30 individuals per PS bead size, sampling time and species (934 nematodes in total) was quantified using fluorescence microscopy.

2.4.3. Pumping rate assay of C. elegans

A reliable estimate of the ingestion of PS beads or bacterial cells by nematodes requires a determination of the pumping rate. We therefore examined the average pumping rate in adult *C. elegans* individuals (Fig. S3). The nematodes were mounted on 3% Gelrite® pads fixed to microscope slides and containing a 10-µl droplet of one of the following: no food (K-medium only), food (10^9 *E. coli* cells ml^{-1}), PS beads without food (10^7 0.5-µm or 1.0-µm PS beads ml^{-1}) or PS beads with food (10^9 *E. coli* cells ml^{-1} with 10^7 0.5-µm or 1.0-µm PS beads ml^{-1}). Nematodes transferred from a culture plate onto a Gelrite® pad were allowed to acclimate to the new food supply conditions for 5–10 min. Average pharyngeal pumping rates were then measured at room temperature (20 °C) by counting

complete grinder backward movements in the terminal bulb in a single adult three times at 10-s intervals (adapted by Hobson et al., 2006) at 100× magnification using a microscope (Zeiss Axio Scope.A1). Pumping actions were scored only from nematodes actively pumping at the start of the recording period. The number of pumping actions per 10-s interval was then multiplied by six to obtain the average pumping rate per minute (ppm).

2.5. Data analysis

Data points 1.5× above or below the interquartile range were considered to be outliers and excluded from the data set. All data were assessed for normality (Shapiro-Wilk test) and homoscedasticity (Levene's test) but were not transformed to improve normality. A significance level of $p < 0.05$ was used for all comparisons. A one-factorial ANOVA (post hoc: Holm-Sidak method) was used to analyze pharyngeal pumping in the tested media, and a two-factorial ANOVA to detect the PS bead body burdens resulting from each bead size after a given time for each species separately (post-hoc: Holm-Sidak method). Mann-Whitney U-tests were performed to compare PS bead body burdens at different times between both species. The number of ingested PS beads is reported as the mean and standard error, and the PS bead body burden at dynamic equilibrium as the mean and standard deviation. Statistical analyses were carried out with SigmaPlot 12.0 (Systat Software Inc.) and graphic representations and curve fits were obtained using OriginPro, Version 2019b (OriginLab Corp., Northampton, MA, USA).

3. Results

3.1. Ingestion of polystyrene beads

Both, *P. pacificus* and *C. elegans*, readily ingested the 0.5- and 1.0-μm PS beads along with the bacterial diet. For *P. pacificus*, ingestion followed a sigmoidal logistic model as shown in Fig. 1.

Beads of either size were first observed in the *P. pacificus* intestine within 5 min and the rate of their ingestion increased continuously until a dynamic equilibrium was reached after about 200 min (0.5 μm) and 210 min (1.0 μm). The PS bead body burden (mean ± SEM) at this time was 28 ± 2 and 69 ± 4 PS beads, respectively. The ingestion rates were highest between 10 and 20 min (0.55 0.5-μm beads min^{-1}) and between 20 and 30 min (2.74 1.0-μm beads min^{-1}). Beginning at 30 min of ingestion, significantly more 1.0-μm than 0.5-μm PS beads were found in the nematodes' bodies ($F = 90.768$; $p < 0.001$).

By contrast, PS bead ingestion by *C. elegans* could be described only by a piecewise curve fit. The PS bead body burden resulting from beads of either size peaked at 30 min of ingestion, with 70 ± 18 (0.5 μm) and 203 ± 15 PS beads (1.0 μm). Due to different trends and for clarity, the curve fits before and after the peak were plotted separately (Fig. 2).

As in *P. pacificus*, in *C. elegans* 1.0-μm PS beads were found in significantly higher numbers than 0.5-μm PS beads ($F = 49.585$; $p < 0.001$). Maximal ingestion rates of 5.21 (0.5 μm) and 7.67 (1.0 μm) beads min^{-1} were reached within the first 5 min of the assay. Beginning at 30 min, the body burdens imposed by beads of either size decreased, following an exponential decay, until stabile body burdens of 29 ± 4 (0.5 μm) and 55 ± 5 PS beads (1.0 μm) per nematode were reached at 120 min and 60 min, respectively.

3.2. Egestion of polystyrene beads

Dynamic equilibrium, defined as the time at which egestion equaled ingestion, occurred in *C. elegans* after 30 min of ingestion and in *P. pacificus* after 210 min of ingestion. The mean PS bead body burden at the respective times was 203 ± 22 (0.5 μm) and 73 ± 12 (1.0 μm) PS beads in *C. elegans* and 17 ± 3 (0.5 μm) and 48 ± 6 (1.0 μm) PS beads in *P. pacificus* (Fig. 3).

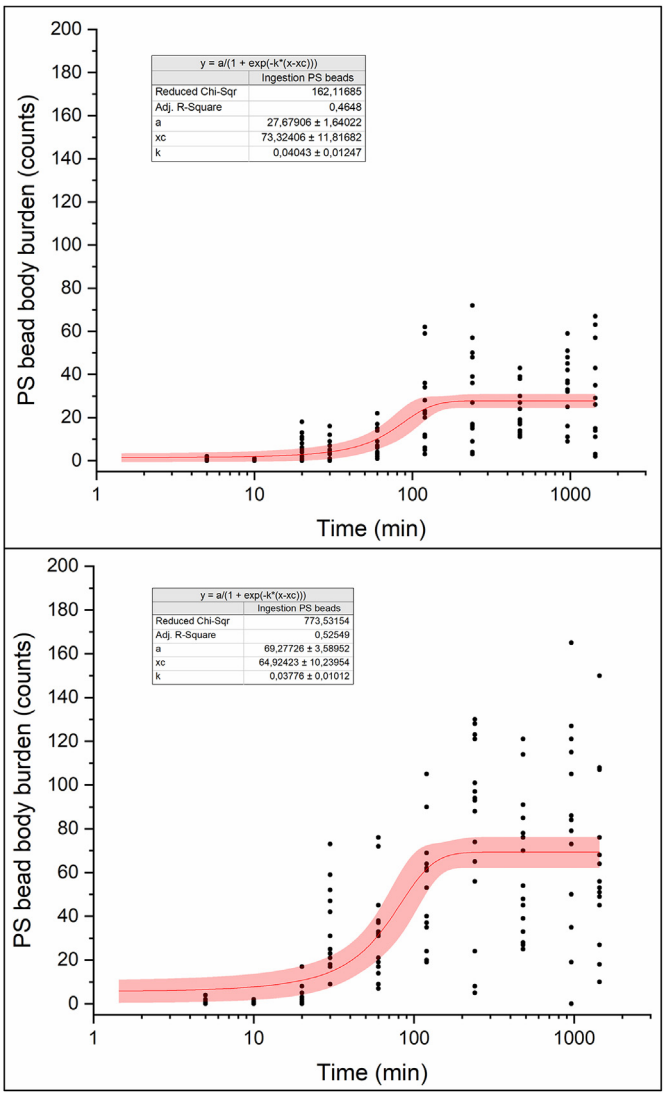

Fig. 1. Polystyrene (PS) bead body burdens of *Pristionchus pacificus* in the ingestion assay. *Pristionchus pacificus* was exposed to 0.5-μm (upper panel) and to 1.0-μm PS beads (lower panel) for 0–1440 min at 10^7 PS beads ml^{-1}. 95% confidence band (red). OriginPro V. 2019b (OriginLab Corporation, USA). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Regardless of species and PS bead size, PS beads were egested from nematodes placed in food-containing aqueous medium within 20–40 min (except 1.0-μm PS beads by *C. elegans*). Egestion followed an exponential decay, although the PS bead body burden at the start of the egestion time series was significantly higher in *C. elegans* than in *P. pacificus* individuals ($U = 28.500$; $p < 0.001$; Fig. 3).

Pristionchus pacificus readily egested 94% and 98% of the ingested 0.5 and 1.0-μm PS beads within 20 min. During that time, its mean body burden of 0.5-μm PS beads decreased from 17 ± 3 beads to 1 ± 0.2 PS beads at a rate of −0.85 beads min^{-1} while its mean body burden of 1.0-μm PS beads decreased from 48 ± 6 1.0-μm beads to 1 ± 0.6 PS beads at a rate of −2.35 beads min^{-1}. After 40 min of egestion, both egestion rates neared zero and the mean body burden reached a constant minimum of 0.5 ± 0.4 (mean ± SD;

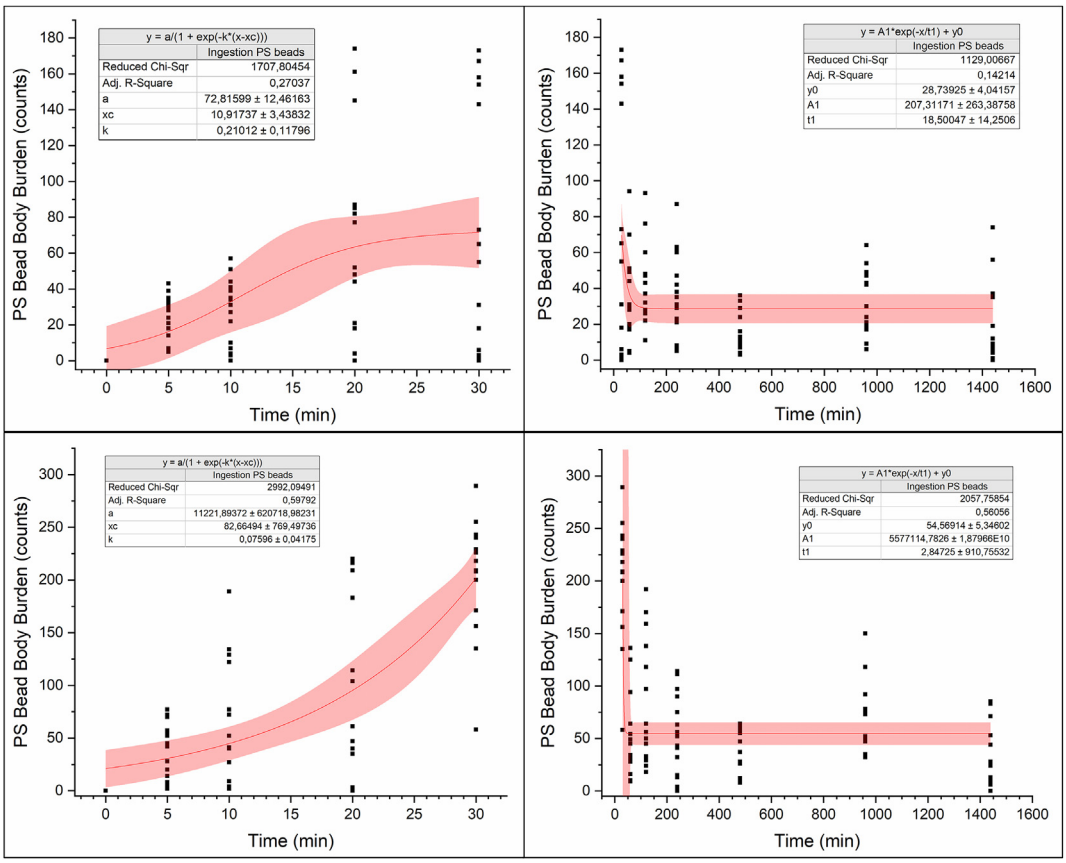

Fig. 2. Polystyrene (PS) bead body burdens of *Caenorhabditis elegans* in the ingestion assay. *Caenorhabditis elegans* was exposed to 0.5-μm (upper panels) and to 1.0-μm PS beads (lower panels) for 0–30 min (left panels) and for 30–1440 min (right panels) at 10^7 PS beads ml^{-1}. 95% confidence band (red). OriginPro V. 2019b (OriginLab Corporation, USA). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

0.5 μm) and 0.8 ± 1.0 (mean ± SD; 1.0 μm) PS beads per individual. A mean body burden of zero was never achieved, since 4–6 of the 30 individuals retained 1–2 PS beads in their intestines even after 24 h of egestion.

Similar results were obtained for *C. elegans* egesting 0.5-μm PS beads. The mean body burden of 203 ± 22 (mean ± SEM) PS beads had a high initial variance but decreased within 20 min to 4 ± 1 PS beads (98%) at a rate of −9.95 beads min^{-1}. After 40 min of egestion, the body burden declined to 1 ± 0.2 PS beads per individual, and after 60 min the egestion rate neared zero, with an absolute minimum of 0.9 ± 3 (mean ± SD) PS beads per individual. However, 1.0-μm PS beads were egested less rapidly by *C. elegans*. The mean body burden decreased by 14% within 20 min at a rate of −0.34 beads min^{-1}, from 49 ± 12 (mean ± SEM) PS beads to 42 ± 11 PS beads. A significant decrease in the mean body burden occurred between 20 and 40 min, when the number of PS beads in *C. elegans* decreased by an additional 83%, from 42 ± 11 to 7 ± 1 PS beads at a rate of −1.77 beads min^{-1} ($U = 133.500$; $p = 0.007$). The PS bead egestion rate neared zero after 120 min.

3.3. Pumping rates of C. elegans

The pumping rate of *C. elegans* on a Gelrite® pad containing a bacterial suspension of 10^9 *E. coli* cells ml^{-1} in bead-free K-medium was more than twice as fast than in pure K-medium (209 ± 22 ppm vs. 98 ± 13 ppm; Fig. 4). In all treatments that included a food supply, the pumping rate was significantly higher than in treatments without bacterial cells ($t = 13.229$; $p < 0.001$). Regardless of the food supply, however, the pumping rate of the 1.0-μm beads was significantly higher than that of the 0.5-μm PS beads ($p = 0.002$). The pumping rates in the food treatments with and without 1.0-μm PS beads did not significantly differ ($t = 0.922$; $p = 0.359$).

4. Discussion

Our results demonstrate the capacity of two model nematode species to ingest and egest large amounts of PS beads in the size range of their bacterial food within minutes. PS beads of 0.5- and 1.0-μm diameter were already detectable in *P. pacificus* and *C. elegans* within 5 min but their further accumulation by the two species occurred following distinct ingestion rates.

A steep increase in the body burden resulting from PS beads of either size was observed within the first 30 min only in *C. elegans* and might be explained by the 30-min starvation period prior to the start of the assay, since fasting affects refeeding by this species (You et al., 2008). You et al. (2008) quantified *C. elegans'* food intake during the 5 first min of refeeding by measuring the fluorescence intensity in the intestine of worms fed GFP-expressing bacteria and by counting ingested fluorescent PS beads mixed with food. Fasted worms consumed significantly more food than non-fasted worms (You et al., 2008). Moreover, when *C. elegans* is fed after a period of starvation, as in the ingestion assay, or when its eats highly preferred bacteria, it enters a phase of "satiety quiescence" after refeeding (You et al., 2008) in which both the pumping rate and food ingestion are drastically reduced. This termination of ingestion while the egestion of food and PS beads continues would explain the results of the ingestion assay, in which the PS bead body burden

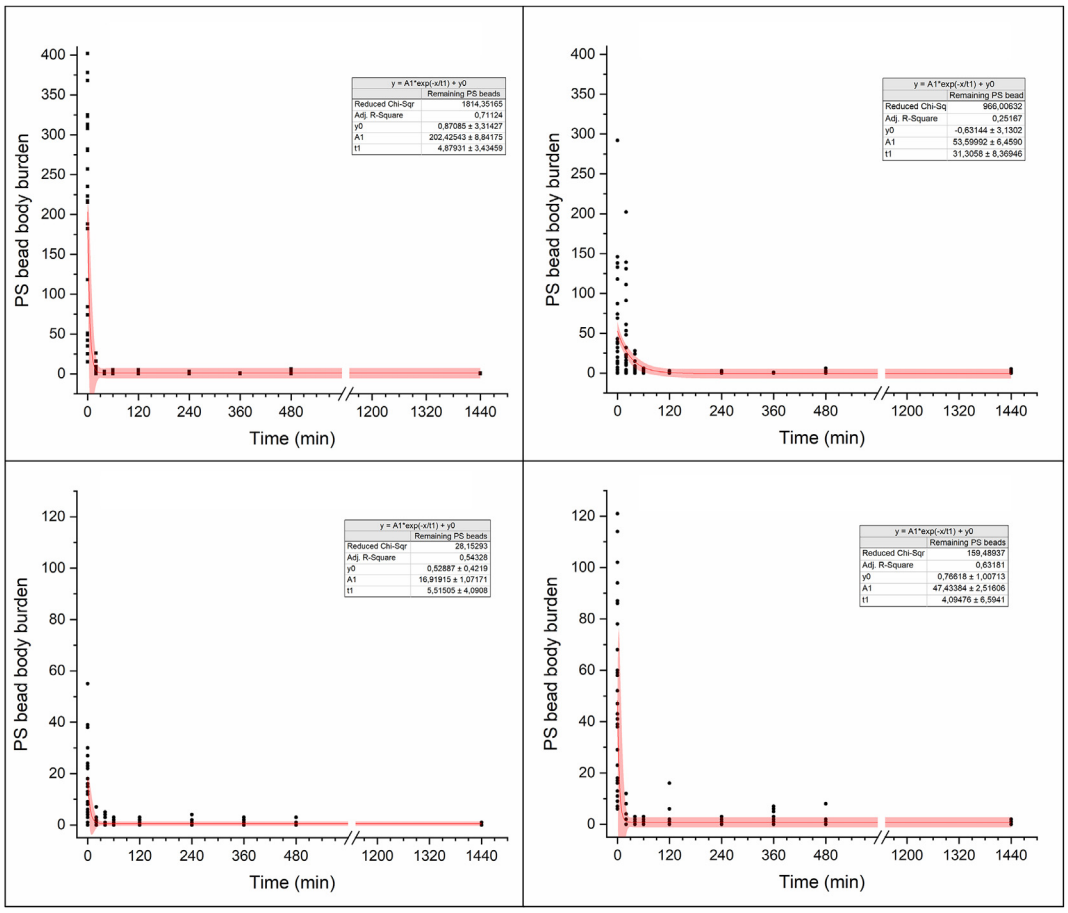

Fig. 3. Polystyrene (PS) bead body burdens of both species in the egestion assay. Nematodes of *Caenorhabditis elegans* (upper panels) and *Pristionchus pacificus* (lower panels) with high PS bead body burdens were allowed to egest PS beads of 0.5 μm (left panels) and 1.0 μm (right panels) into microplastic-free medium. 95% confidence band (red). OriginPro V. 2019b (OriginLab Corporation, USA). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

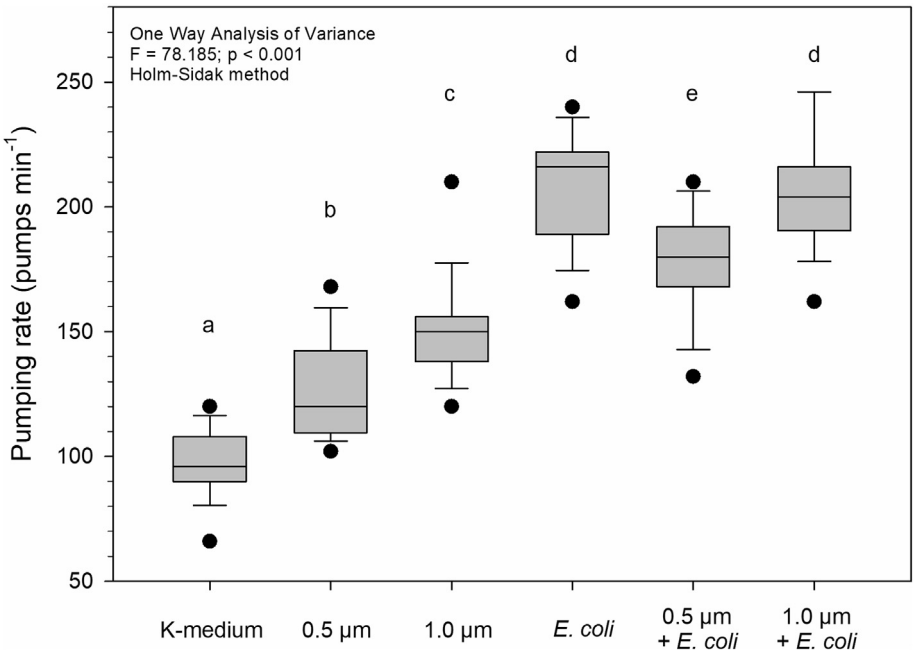

Fig. 4. *Caenorhabditis elegans*' average pumping rates in distinct media treatments. Pumps of *C. elegans* were counted in different treatments: no food (K-medium), food (bacterial suspension of *Escherichia coli* at 10^9 cells ml^{-1}), PS beads without food (0.5-μm PS beads at 10^7 beads ml^{-1}, 1.0-μm PS beads at 10^7 beads ml^{-1}) or PS beads with food (*E. coli* at 10^9 cells ml^{-1} with 0.5-μm PS beads at 10^7 beads ml^{-1}, *E. coli* at 10^9 cells ml^{-1} with 1.0-μm PS beads at 10^7 beads ml^{-1}). SigmaPlot 12 (Systat Software Inc., USA).

of *C. elegans* exponentially decayed after 30 min. By contrast, a similar sequence of events does not seem to occur in *P. pacificus,* and a similar response was not seen in our study.

The species-specific progressions seen in the ingestion curve might also be explained by species-specific pumping rates and feeding behaviors. Pumping dynamics are not homogeneous in time (Lee et al., 2017) and under optimal bacterial conditions, the average pumping rate of *P. pacificus* is much lower (140 ppm; Chiang et al., 2006; Kroetz et al., 2012) than that of *C. elegans* (209 ± 22 ppm). Moreover, *P. pacificus* pumps faster in the absence than in the presence of food (Kroetz et al., 2012) and responds to a lack of food by decreasing its locomotion as well as its forward bending frequency, whereas in the absence of food *C. elegans* reduces pharyngeal pumping and increases locomotion (Kroetz et al., 2012; Rivard et al., 2010). *Pristionchus pacificus* would presumably have increased pharyngeal pumping during the 30-min food deprivation prior to the ingestion assay and decreased pumping at the start of the ingestion assay, when food was available again. The slow forward velocity of *P. pacificus* is because it increases its reversal frequency to enhance coverage of a local area rather than increasing its foraging range (Kroetz et al., 2012). Consequently, *P. pacificus* is less mobile than *C. elegans* and will encounter fewer PS beads within the same time, such that its ingestion rates will be lower. Those feeding behaviors of *P. pacificus* would have accounted for the gentler slope in the plot of its PS bead body burden vs. exposure time. Both species ingested more 1.0-μm than 0.5-μm PS beads, as the former is within the size range of their natural bacterial diet. In addition, for *C. elegans* this observation is also in accordance with its higher pumping rate following exposure to 1.0-μm PS beads.

The egestion assay started when PS bead body burdens were at a maximum. A rapid decrease in the bead body burdens occurred only when bacterial cells were available. Since PS beads are ingested passively along with the bacterial diet and the nematode's digestive system has no means of metabolizing, PS beads are instead rapidly egested intact. Due to the short retention times and the sole presence in the digestive system, PS beads of 0.5 and 1.0 μm do not translocate into nematode tissues. For both nematode species, actual mean body burdens never reached zero since in the egestion assay 1–2 PS beads persisted in the intestines of a few individuals even after 24 h. It is also possible that previously egested PS beads were re-ingested at late sampling times. However, it is unlikely because the encounter rates would have been extremely low given that the PS beads were egested into 3 ml of aqueous medium.

Several studies not focusing on nematodes demonstrated that the presence and abundance of food strongly influence ingestion, egestion and microplastic body burdens (e.g., Chae and An, 2020; Elizalde-Velázquez et al., 2020; Rist et al., 2017). However, in those studies both positive and negative correlations were reported. In the mussel *Mytilus galloprovincialis* exposed to microplastics, the microplastics were excreted rapidly when algal food was absent and more slowly when food was present (Chae and An, 2020). By contrast, Aljaibachi and Callaghan (2018) observed that when no food was offered to *Daphnia magna*, its microplastic body burden remained relatively stable but was significantly reduced over time when food was present. This can be attributed to the pressure imposed by newly ingested food in the gut system, which may be needed for to induce feces egestion (Ebert, 2005). In the copepod *Calanus helgolandicus*, microplastic-laden fecal pellets were egested within hours in the presence of food but remained in the intestinal tract for up to 7 days in the absence of food (Cole et al., 2013).

The transit times and egestion of PS beads are influenced by bead size and morphology. For example, in rotifers, 0.05-μm PS beads had longer retention times and exerted more negative effects than 0.5- and 6.0-μm PS beads (Jeong et al., 2016, 2017). *Daphnia magna* egested 2.0-μm more rapidly than 0.1-μm PS beads both in the absence and presence of food (Rist et al., 2017) while, in *Mytilus edulis*, egestion was independent of PS bead size (Rist et al., 2019). Our egestion data showed a rapid egestion for either 0.5- and 1.0-μm PS beads but, within the first 20 min, 98% of the 0.5-μm PS beads and only 14% of 1.0-μm PS beads were egested.

In this study, the body burden was examined, as it is the product of species-specific ingestion and egestion processes. PS bead ingestion started almost immediately whereas PS bead egestion was delayed, reflecting the time needed for passing the grinder (only in *C. elegans*), gut transit and aggregation of the beads in front of the rectum before their final egestion (Fig. 5).

Following this delay, egestion rates equaled ingestion rates and a constant net body burden of PS beads was observed.

According to literature reports, spherical microplastics at low concentrations have no major effect on organisms in terms of internal damage and gut transit times, as the egestion rates of the particles are similar to those of natural food items (Au et al., 2015; Cole et al., 2013). Thus, microplastics in size ranges close to those of natural prey were used in early studies to simulate predator-prey relationships (Huntley et al., 1983) or to model algal ingestion (Frost, 1977; Hart, 1991; Wilson, 1973). In *C. elegans*, the spatial distributions of ingested PS beads and *E. coli* are essentially identical such that PS beads have been used as a proxy for bacterial uptake in nematodes (McGhee and Ghafouri, 2007). The rapid egestion of regular-shaped microspheres, as also observed in the egestion assay of our study, generally implies a limited bioconcentration in the gastrointestinal tract (Grigorakis et al., 2017; Rosenkranz et al., 2009). At sampling times with the highest PS bead body burdens in the nematodes, very low BCF values of less than $7.66 \times 10^{-6} \pm 8.66 \times 10^{-7}$ (0.5 μm) and $2.24 \times 10^{-5} \pm 1.65 \times 10^{-6}$ (1.0 μm) were calculated for *C. elegans*

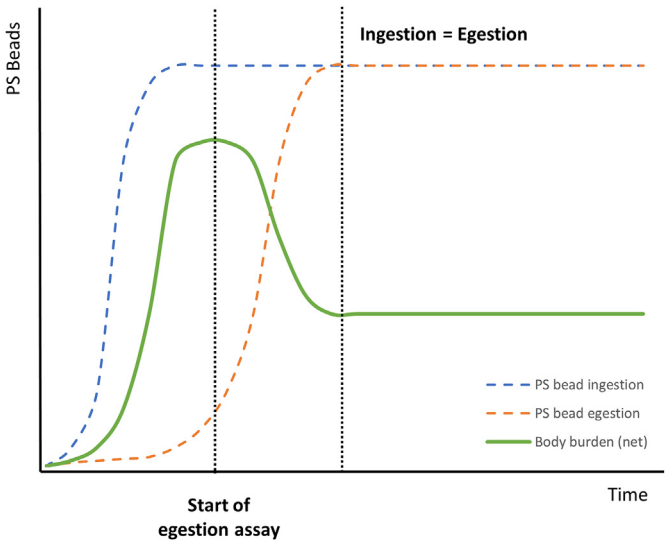

Fig. 5. Theoretical model of ingestion and egestion of polystyrene beads by *Caenorhabditis elegans*. The ingestion of PS beads (blue) immediately started at the beginning of the ingestion assay until reaching a saturation value. The egestion of PS beads (orange) started time-delayed and rates increased exponentially after a lag-phase until reaching the same saturation value. At the dynamic equilibrium, ingestion rates equaled egestion rates. The PS bead body burden curve (green) is a result of the ingestion and egestion rates. The egestion assay started when PS bead body burdens peaked right after the lag-phase of the egestion rate curve. Excel 2016 (Microsoft Corporation, USA). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

and $3.86 \times 10^{-6} \pm 4.49 \times 10^{-7}$ (0.5 μm) and $8.9 \times 10^{-6} \pm 1.15 \times 10^{-6}$ (1.0 μm) for *P. pacificus* (Tab. S1). According to the Regulation (EC) No. 1272/2008 of the European Parliament and the Council of the EU, substances with BCF >500 are considered to have a bioaccumulation potential.

However, PS beads have also been shown to decrease the feeding activity of many organisms (e.g., Besseling et al., 2013; Cole et al., 2011). Thus, over the long term, nutrient deficiencies (Connors et al., 2017) arise that (Andrassy, 1984) negatively impact (Schmid-Araya and Schmid, 2000) both the energy budget (Cole et al., 2015; Wright et al., 2013) and, by impeding reproductive output, fitness as well (Mueller et al., 2020).

In this study, only regular-shaped microspheres were tested but irregularly shaped microplastics (e.g., fragments, fibers) are found in abundance in the environment and they will differ in their sedimentation and aggregation behaviors as well as in their interaction with the intestinal tract, resulting in different ingestion and egestion rates (e.g., Au et al., 2015; Cole et al., 2013; Coppock et al., 2019; Ogonowski et al., 2016). For example, in amphipods, gut content egestion took two to four times longer when the organisms had ingested polypropylene microplastic fibers (Cole et al., 2013). *D. magna* rapidly ingested regular and irregular polyethylene microplastic particles but gut clearance and residence times were longer for the latter, since 94% of the test organisms fed with regular microplastics completely emptied their gut within 24 h compared to <1% of organisms fed irregular microplastics (Frydkjær et al., 2017). In *Hyalella azteca*, the egestion time for regular-shaped microplastics did not significantly differ from that of normal food but the egestion of microplastic fibers was significantly slower (Au et al., 2015), although microplastic fibers can be egested completely by amphipods (Wright et al., 2013). Furthermore, greater inhibition of growth and reproduction and greater toxicity were reported for irregular-shaped microplastics (Au et al., 2015; Frydkjær et al., 2017; Wright et al., 2013).

5. Conclusion

A prerequisite for determining the bioconcentration, bioaccumulation and trophic transfer potential of microplastics by meiobenthic organisms is an understanding of the ingestion time, body burden, gut residence time and egestion time of these materials. Our findings demonstrated the ability of two species of nematodes to readily ingest 0.5- and 1.0-μm PS beads along with bacteria. Within minutes, large amounts of PS beads were ingested by both species but based on the smooth and spherical shape of the beads, with a size range close to that of bacteria, the very low BCF values and rapid egestion times in the absence of further exposure, the PS beads did not bioconcentrate. Due to these rapid ingestion and egestion times, the amount of microplastics ingested and egested by nematodes may be underestimated when samplings are limited to several days. While exposure to microplastics in natural habitats may be much lower than under laboratory experiments, where particulate matter is limited, our study was an experimental approach that was not intended to reflect microplastic concentrations in the environment. Moreover, in contrast to regular-shaped microspheres, irregular-shaped microplastics (e.g., fragments, fibers) will vary in their ingestion and egestion times and the potential for biomagnification is likely to be higher.

Credit author statement

H.F., M-T.M. and W.T. designed the ingestion and egestion assays. H.F. and M-T.M. guided the assays, analyzed the data and wrote the manuscript. W.T. supervised the work. All authors reviewed and approved the final manuscript

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

Supplementary data to this article can be found online at https://doi.org/10.1016/j.chemosphere.2020.128162.

References

Adam, V., Yang, T., Nowack, B., 2019. Toward an ecotoxicological risk assessment of microplastics: comparison of available hazard and exposure data in freshwaters. Environ. Toxicol. Chem. 38 (2), 436–447. https://doi.org/10.1002/etc.4323.

Aljaibachi, R., Callaghan, A., 2018. Impact of polystyrene microplastics on *Daphnia magna* mortality and reproduction in relation to food availability. PeerJ 6, e4601. https://doi.org/10.7717/peerj.4601.

Aljaibachi, R., Laird, W.B., Stevens, F., Callaghan, A., 2020. Impacts of polystyrene microplastics on *Daphnia magna*: a laboratory and a mesocosm study. Sci. Total Environ. 705, 135800. https://doi.org/10.1016/j.scitotenv.2019.135800.

Andrassy, I., 1984. Klasse Nematoda. Gustav Fischer Verlag, Stuttgart.

Arthur, C., Baker, J., Bamford, H., 2009. Proceedings of the International Research Workshop on the Occurrence, Effects and Fate of Microplastic Marine Debris. National Oceanic and Atmospheric Administration Technical Memorandum NOS-OR&R-30.

Au, S.Y., Bruce, T.F., Bridges, W.C., Klaine, S.J., 2015. Responses of *Hyalella azteca* to acute and chronic microplastic exposures. Environ. Toxicol. Chem. 34 (11), 2564–2572. https://doi.org/10.1002/etc.3093.

Avery, L., 1993. The genetics of feeding in *Caenorhabditis elegans*. Genetics 133 (4), 897–917.

Avery, L., Horvitz, H.R., 1987. A cell that dies during wild-type *C. elegans* development can function as a neuron in a ced-3 mutant. Cell 51 (6), 1071–1078. https://doi.org/10.1016/0092-8674(87)90593-9.

Avery, L., Shtonda, B.B., 2003. Food transport in the *C. elegans* pharynx. J. Exp. Biol. 206 (Pt 14), 2441–2457. https://doi.org/10.1242/jeb.00433.

Besseling, E., Wegner, A., Foekema, E.M., van den Heuvel-Greve, M.J., Koelmans, A.A., 2013. Effects of microplastic on fitness and PCB bioaccumulation by the lugworm *Arenicola marina* (L.). Environ. Sci. Technol. 47 (1), 593–600. https://doi.org/10.1021/es302763x.

Blarer, P., Burkhardt-Holm, P., 2016. Microplastics affect assimilation efficiency in the freshwater amphipod *Gammarus fossarum*. Environ. Sci. Pollut. Res. Int. 23 (23), 23522–23532. https://doi.org/10.1007/s11356-016-7584-2.

Boyd, W.A., Cole, R.D., Anderson, G.L., Williams, P.L., 2003. The effects of metals and food availability on the behavior of *Caenorhabditis elegans*. Environ. Toxicol. Chem. 22 (12), 3049–3055. https://doi.org/10.1897/02-565.

Brenner, S., 1974. The genetics of *Caenorhabditis elegans*. Genetics 77 (1), 71–94.

Bumbarger, D.J., Riebesell, M., Rödelsperger, C., Sommer, R.J., 2013. System-wide rewiring underlies behavioral differences in predatory and bacterial-feeding nematodes. Cell 152 (1), 109–119. https://doi.org/10.1016/j.cell.2012.12.013.

Chae, Y., An, Y.-J., 2020. Effects of food presence on microplastic ingestion and egestion in *Mytilus galloprovincialis*. Chemosphere 240, 124855. https://doi.org/10.1016/j.chemosphere.2019.124855.

Chiang, J.-T.A., Steciuk, M., Shtonda, B., Avery, L., 2006. Evolution of pharyngeal behaviors and neuronal functions in free-living soil nematodes. J. Exp. Biol. 209 (Pt 10), 1859–1873. https://doi.org/10.1242/jeb.02165.

Cole, M., Lindeque, P., Fileman, E., Halsband, C., Galloway, T.S., 2015. The impact of polystyrene microplastics on feeding, function and fecundity in the marine copepod *Calanus helgolandicus*. Environ. Sci. Technol. 49 (2), 1130–1137. https://doi.org/10.1021/es504525u.

Cole, M., Lindeque, P., Fileman, E., Halsband, C., Goodhead, R., Moger, J., Galloway, T.S., 2013. Microplastic ingestion by zooplankton. Environ. Sci. Technol. 47 (12), 6646–6655. https://doi.org/10.1021/es400663f.

Cole, M., Lindeque, P., Halsband, C., Galloway, T.S., 2011. Microplastics as contaminants in the marine environment: a review. Mar. Pollut. Bull. 62 (12), 2588–2597. https://doi.org/10.1016/j.marpolbul.2011.09.025.

Connors, K.A., Dyer, S.D., Belanger, S.E., 2017. Advancing the quality of

environmental microplastic research. Environ. Toxicol. Chem. 36 (7), 1697–1703. https://doi.org/10.1002/etc.3829.
Coppock, R.L., Galloway, T.S., Cole, M., Fileman, E.S., Queirós, A.M., Lindeque, P.K., 2019. Microplastics alter feeding selectivity and faecal density in the copepod, *Calanus helgolandicus*. Sci. Total Environ. 687, 780–789. https://doi.org/10.1016/j.scitotenv.2019.06.009.
Dris, R., Gasperi, J., Rocher, V., Saad, M., Renault, N., Tassin, B., 2015. Microplastic contamination in an urban area: a case study in Greater Paris. Environ. Chem. 12 (5), 592. https://doi.org/10.1071/EN14167.
Ebert, D., 2005. Ecology, Epidemiology, and Evolution of Parasitism in Daphnia. National Center for Biotechnology Information, Bethesda, Md.
Eerkes-Medrano, D., Thompson, R.C., Aldridge, D.C., 2015. Microplastics in freshwater systems: a review of the emerging threats, identification of knowledge gaps and prioritisation of research needs. Water Res. 75, 63–82. https://doi.org/10.1016/j.watres.2015.02.012.
Elizalde-Velázquez, A., Carcano, A.M., Crago, J., Green, M.J., Shah, S.A., Cañas-Carrell, J.E., 2020. Translocation, trophic transfer, accumulation and depuration of polystyrene microplastics in *Daphnia magna* and *Pimephales promelas*. Environ. Pollut. (Barking, Essex: 1987) 259, 113937. https://doi.org/10.1016/j.envpol.2020.113937.
Enders, K., Lenz, R., Stedmon, C.A., Nielsen, T.G., 2015. Abundance, size and polymer composition of marine microplastics ≥10μm in the Atlantic Ocean and their modelled vertical distribution. Mar. Pollut. Bull. 100 (1), 70–81. https://doi.org/10.1016/j.marpolbul.2015.09.027.
Fang-Yen, C., Avery, L., Samuel, A.D.T., 2009. Two size-selective mechanisms specifically trap bacteria-sized food particles in *Caenorhabditis elegans*. P. Natl. Acad. Sci. USA 106 (47), 20093–20096. https://doi.org/10.1073/pnas.0904036106.
Fent, K., 2007. Ökotoxikologie: Umweltchemie, Toxikologie, Ökologie ; 63 Tabellen, 3., überarb. und aktualisierte Aufl. ed. Thieme, Stuttgart, p. 338.
Fischer, F., Böhm, L., Höss, S., Möhlenkamp, C., Claus, E., Düring, R.-A., Schäfer, S., 2016. Passive dosing in chronic toxicity tests with the nematode *Caenorhabditis elegans*. Environ. Sci. Technol. 50 (17), 9708–9716. https://doi.org/10.1021/acs.est.6b02956.
Frost, B.W., 1977. Feeding behavior of *Calanus pacificus* in mixtures of food particles1. Limnol. Oceanogr. 22 (3), 472–491. https://doi.org/10.4319/lo.1977.22.3.0472.
Frydkjær, C.K., Iversen, N., Roslev, P., 2017. Ingestion and egestion of microplastics by the cladoceran *Daphnia magna*: effects of regular and irregular shaped plastic and sorbed phenanthrene. Bull. Environ. Contam. Toxicol. 99 (6), 655–661. https://doi.org/10.1007/s00128-017-2186-3.
Fueser, H., Mueller, M.-T., Weiss, L., Höss, S., Traunspurger, W., 2019. Ingestion of microplastics by nematodes depends on feeding strategy and buccal cavity size. Environ. Pollut. (Barking, Essex: 1987) 255 (Pt 2), 113227. https://doi.org/10.1016/j.envpol.2019.113227.
Fueser, H., Mueller, M.-T., Traunspurger, W., 2020. Ingestion of microplastics by meiobenthic communities in small-scale microcosm experiments. Sci. Total Environ. 746, 141276. https://doi.org/10.1016/j.scitotenv.2020.141276.
Grigorakis, S., Mason, S.A., Drouillard, K.G., 2017. Determination of the gut retention of plastic microbeads and microfibers in goldfish (*Carassius auratus*). Chemosphere 169, 233–238. https://doi.org/10.1016/j.chemosphere.2016.11.055.
Haegerbaeumer, A., Mueller, M.-T., Fueser, H., Traunspurger, W., 2019. Impacts of micro- and nano-sized plastic particles on benthic invertebrates: a literature review and gap analysis. Front. Environ. Sci. 7, 1596. https://doi.org/10.3389/fenvs.2019.00017.
Hanna, S.K., Montoro Bustos, A.R., Peterson, A.W., Reipa, V., Scanlan, L.D., Hosbas Coskun, S., Cho, T.J., Johnson, M.E., Hackley, V.A., Nelson, B.C., Winchester, M.R., Elliott, J.T., Petersen, E.J., 2018. Agglomeration of Escherichia coli with positively charged nanoparticles can lead to artifacts in a standard *Caenorhabditis elegans* toxicity assay. Environ. Sci. Technol. 52 (10), 5968–5978. https://doi.org/10.1021/acs.est.7b06099.
Harrison, J.P., Hoellein, T.J., Sapp, M., Tagg, A.S., Ju-Nam, Y., Ojeda, J.J., 2018. Microplastic-Associated biofilms: a comparison of freshwater and marine environments. In: Wagner, M., Lambert, S. (Eds.), Freshwater Microplastics. Emerging Environmental Contaminants?, vol. 58 Springer Open, Cham, Switzerland, pp. 181–201.
Hart, M.W., 1991. Particle captures and the method of suspension feeding by echinoderm larvae. Biol. Bull. 180 (1), 12–27. https://doi.org/10.2307/1542425.
Heip, C., Huys, R., Vincx, M., Vanreusel, A., Smol, N., Herman, R., Herman, P.M.J., 1990. Composition, distribution, biomass and production of North Sea meiofauna. Neth. J. Sea Res. 26 (2–4), 333–342. https://doi.org/10.1016/0077-7579(90)90095-X.
Hobson, R.J., Hapiak, V.M., Xiao, H., Buehrer, K.L., Komuniecki, P.R., Komuniecki, R.W., 2006. SER-7, a *Caenorhabditis elegans* 5-HT7-like receptor, is essential for the 5-HT stimulation of pharyngeal pumping and egg laying. Genetics 172 (1), 159–169. https://doi.org/10.1534/genetics.105.044495.
Huntley, M.E., Barthel, K.-G., Star, J.L., 1983. Particle rejection by *Calanus pacificus*: discrimination between similarly sized particles. Mar. Biol. 74 (2), 151–160. https://doi.org/10.1007/BF00413918.
Imhof, H.K., Ivleva, N.P., Schmid, J., Niessner, R., Laforsch, C., 2013. Contamination of beach sediments of a subalpine lake with microplastic particles. Curr. Biol.: CB 23 (19), R867–R868. https://doi.org/10.1016/j.cub.2013.09.001.
Ivleva, N.P., Wiesheu, A.C., Niessner, R., 2017. Microplastic in aquatic ecosystems. Angew. Chem. Int. Ed. 56 (7), 1720–1739. https://doi.org/10.1002/anie.201606957.
Jeong, C.-B., Kang, H.-M., Lee, M.-C., Kim, D.-H., Han, J., Hwang, D.-S., Souissi, S., Lee, S.-J., Shin, K.-H., Park, H.G., Lee, J.-S., 2017. Adverse effects of microplastics and oxidative stress-induced MAPK/Nrf2 pathway-mediated defense mechanisms in the marine copepod *Paracyclopina nana*. Sci. Rep-UK 7. https://doi.org/10.1038/srep41323.
Jeong, C.-B., Won, E.-J., Kang, H.-M., Lee, M.-C., Hwang, D.-S., Hwang, U.-K., Zhou, B., Souissi, S., Lee, S.-J., Lee, J.-S., 2016. Microplastic size-dependent toxicity, oxidative stress induction, and p-JNK and p-p38 activation in the monogonont rotifer (*Brachionus koreanus*). Environ. Sci. Technol. 50 (16), 8849–8857. https://doi.org/10.1021/acs.est.6b01441.
Kaiser, D., Kowalski, N., Waniek, J.J., 2017. Effects of biofouling on the sinking behavior of microplastics. Environ. Res. Lett. 12 (12), 124003. https://doi.org/10.1088/1748-9326/aa8e8b.
Kiyama, Y., Miyahara, K., Ohshima, Y., 2012. Active uptake of artificial particles in the nematode *Caenorhabditis elegans*. J. Exp. Biol. 215 (Pt 7), 1178–1183. https://doi.org/10.1242/jeb.067199.
Klein, S., Worch, E., Knepper, T.P., 2015. Occurrence and spatial distribution of microplastics in river shore sediments of the rhine-main area in Germany. Environ. Sci. Technol. 49 (10), 6070–6076. https://doi.org/10.1021/acs.est.5b00492.
Kooi, M., van Nes, E.H., Scheffer, M., Koelmans, A.A., 2017. Ups and downs in the ocean: effects of biofouling on vertical transport of microplastics. Environ. Sci. Technol. 51 (14), 7963–7971. https://doi.org/10.1021/acs.est.6b04702.
Kroetz, S.M., Srinivasan, J., Yaghoobian, J., Sternberg, P.W., Hong, R.L., 2012. The cGMP signaling pathway affects feeding behavior in the necromenic nematode *Pristionchus pacificus*. PloS One 7 (4), e34464. https://doi.org/10.1371/journal.pone.0034464.
Lei, L., Wu, S., Lu, S., Liu, M., Song, Y., Fu, Z., Shi, H., Raley-Susman, K.M., He, D., 2018. Microplastic particles cause intestinal damage and other adverse effects in zebrafish *Danio rerio* and nematode *Caenorhabditis elegans*. Sci. Total Environ. 619–620, 1–8. https://doi.org/10.1016/j.scitotenv.2017.11.103.
Lee, K.S., Iwanir, S., Kopito, R.B., Scholz, M., Calarco, J.A., Biron, D., Levine, E., 2017. Serotonin-dependent kinetics of feeding bursts underlie a graded response to food availability in *C. elegans*. Nat. Commun. 8, 14221.
Lusher, A.L., McHugh, M., Thompson, R.C., 2013. Occurrence of microplastics in the gastrointestinal tract of pelagic and demersal fish from the English Channel. Mar. Pollut. Bull. 67 (1–2), 94–99. https://doi.org/10.1016/j.marpolbul.2012.11.028.
Majdi, N., Traunspurger, W., 2015. Free-Living nematodes in the freshwater food web: a review. J. Nematol. 47 (1), 28–44.
McGhee, J., Ghafouri, S., 2007. Bacterial residence time in the intestine of *Caenorhabditis elegans*. Nematol 9 (1), 87–91. https://doi.org/10.1163/156854107779969718.
Mueller, M.-T., Fueser, H., Trac, L.N., Mayer, P., Traunspurger, W., Höss, S., 2020. Surface-related toxicity of polystyrene beads to nematodes and the role of food availability. Environ. Sci. Technol. 54 (3), 1790–1798. https://doi.org/10.1021/acs.est.9b06583.
Nicholas, W.L., Grassia, A., Viswanathan, S., 1973. The efficiency with which *Caenorhabditis briggsae* (rhabditidae) feeds on the bacterium, *Escherichia coli*. Nematol 19 (4), 411–420. https://doi.org/10.1163/187529273X00385.
Nika, L., Gibson, T., Konkus, R., Karp, X., 2016. Fluorescent beads are a versatile tool for staging *Caenorhabditis elegans* in different life histories. G3 (Bethesda, Md 6 (7), 1923–1933. https://doi.org/10.1534/g3.116.030163.
Ogonowski, M., Schür, C., Jarsén, Å., Gorokhova, E., 2016. The effects of natural and anthropogenic microparticles on individual fitness in *Daphnia magna*. PloS One 11 (5), e0155063. https://doi.org/10.1371/journal.pone.0155063.
Peeken, I., Primpke, S., Beyer, B., Gütermann, J., Katlein, C., Krumpen, T., Bergmann, M., Hehemann, L., Gerdts, G., 2018. Arctic sea ice is an important temporal sink and means of transport for microplastic. Nat. Commun. 9 (1), 1505. https://doi.org/10.1038/s41467-018-03825-5.
Rae, R., Riebesell, M., Dinkelacker, I., Wang, Q., Herrmann, M., Weller, A.M., Dieterich, C., Sommer, R.J., 2008. Isolation of naturally associated bacteria of necromenic Pristionchus nematodes and fitness consequences. J. Exp. Biol. 211 (Pt 12), 1927–1936. https://doi.org/10.1242/jeb.014944.
Raizen, D., Song, B.-M., Trojanowski, N., You, Y.-J., 2012. Methods for measuring pharyngeal behaviors. In: WormBook: The Online Review of C. elegans Biology, pp. 1–13.
Rezania, S., Park, J., Md Din, M.F., Mat Taib, S., Talaiekhozani, A., Kumar Yadav, K., Kamyab, H., 2018. Microplastics pollution in different aquatic environments and biota: a review of recent studies. Mar. Pollut. Bull. 133, 191–208. https://doi.org/10.1016/j.marpolbul.2018.05.022.
Riebesell, M., Sommer, R.J., 2017. Three-dimensional reconstruction of the pharyngeal gland cells in the predatory nematode *Pristionchus pacificus*. J. Morphol. 278 (12), 1656–1666. https://doi.org/10.1002/jmor.20739.
Rist, S., Baun, A., Hartmann, N.B., 2017. Ingestion of micro- and nanoplastics in *Daphnia magna* - quantification of body burdens and assessment of feeding rates and reproduction. Environ. Pollut. (Barking, Essex: 1987) 228, 398–407. https://doi.org/10.1016/j.envpol.2017.05.048.
Rist, S., Steensgaard, I.M., Guven, O., Nielsen, T.G., Jensen, L.H., Møller, L.F., Hartmann, N.B., 2019. The fate of microplastics during uptake and depuration phases in a blue mussel exposure system. Environ. Toxicol. Chem. 38 (1), 99–105. https://doi.org/10.1002/etc.4285.
Rivard, L., Srinivasan, J., Stone, A., Ochoa, S., Sternberg, P.W., Loer, C.M., 2010. A comparison of experience-dependent locomotory behaviors and biogenic amine neurons in nematode relatives of *Caenorhabditis elegans*. BMC Neurosci.

11, 22. https://doi.org/10.1186/1471-2202-11-22.
Rosenkranz, P., Chaudhry, Q., Stone, V., Fernandes, T.F., 2009. A comparison of nanoparticle and fine particle uptake by *Daphnia magna*. Environ. Toxicol. Chem. 28 (10), 2142–2149. https://doi.org/10.1897/08-559.1.
Scherer, C., Weber, A., Lambert, S., Wagner, M., 2018. Interactions of microplastics with freshwater biota. In: Wagner, M., Lambert, S. (Eds.), Freshwater Microplastics. Emerging Environmental Contaminants?, vol. 58 Springer Open, Cham, Switzerland, pp. 153–180.
Scherer, C., Weber, A., Stock, F., Vurusic, S., Egerci, H., Kochleus, C., Arendt, N., Foeldi, C., Dierkes, G., Wagner, M., Brennholt, N., Reifferscheid, G., 2020. Comparative assessment of microplastics in water and sediment of a large European river. Sci. Total Environ. 738, 139866. https://doi.org/10.1016/j.scitotenv.2020.139866.
Schiemer, F., 1982. Food dependence and energetics of freeliving nematodes: I. Respiration, growth and reproduction of *Caenorhabditis briggsae* (Nematoda) at different levels of food supply. Oecologia 54 (1), 108–121. https://doi.org/10.1007/BF00541117.
Schmid-Araya, J.M., Schmid, P.E., 2000. Trophic relationships: integrating meiofauna into a realistic benthic food web. Freshw. Biol. 44 (1), 149–163. https://doi.org/10.1046/j.1365-2427.2000.00594.x.
Stiernagle, T., 2006. Maintenance of *C. elegans*. The *C. elegans* Research Community. http://www.wormbook.org.
Suzuki, Y., Kikuchi, K., Tsuruta-Numayama, K., Ishikawa, T., 2019. Particle selectivity of filtering by *C. elegans*. Theor. Appl. Mech. Lett. 9 (2), 61–65. https://doi.org/10.1016/j.taml.2019.02.001.
Traunspurger, W., 1997. Bathymetric, seasonal and vertical distribution of feeding-types of nematodes in an oligotrophic lake. Vie Milieu 47 (1), 1–7.
Traunspurger, W., Wilden, B., Majdi, N., 2019. An overview of meiofaunal and nematode distribution patterns in lake ecosystems differing in their trophic state. Hydrobiologia 26, 32. https://doi.org/10.1007/s10750-019-04092-1.
Triebskorn, R., Braunbeck, T., Grummt, T., Hanslik, L., Huppertsberg, S., Jekel, M., Knepper, T.P., Krais, S., Müller, Y.K., Pittroff, M., Ruhl, A.S., Schmieg, H., Schür, C., Strobel, C., Wagner, M., Zumbülte, N., Köhler, H.-R., 2019. Relevance of nano- and microplastics for freshwater ecosystems: a critical review. Trac. Trends Anal. Chem. 110, 375–392. https://doi.org/10.1016/j.trac.2018.11.023.
van Cauwenberghe, L., Devriese, L., Galgani, F., Robbens, J., Janssen, C.R., 2015. Microplastics in sediments: a review of techniques, occurrence and effects. Mar. Environ. Res. 111, 5–17. https://doi.org/10.1016/j.marenvres.2015.06.007.
van den Hoogen, J., Geisen, S., Routh, D., Ferris, H., Traunspurger, W., Wardle, D.A., Goede, R.G.M. de, Adams, B.J., Ahmad, W., Andriuzzi, W.S., Bardgett, R.D., Bonkowski, M., Campos-Herrera, R., Cares, J.E., Caruso, T., Brito Caixeta, L. de, Chen, X., Costa, S.R., Creamer, R., Mauro da Cunha Castro, J., Dam, M., Djigal, D., Escuer, M., Griffiths, B.S., Gutiérrez, C., Hohberg, K., Kalinkina, D., Kardol, P., Kergunteuil, A., Korthals, G., Krashevska, V., Kudrin, A.A., Li, Q., Liang, W., Magilton, M., Marais, M., Martín, J.A.R., Matveeva, E., Mayad, E.H., Mulder, C., Mullin, P., Neilson, R., Nguyen, T.A.D., Nielsen, U.N., Okada, H., Rius, J.E.P., Pan, K., Peneva, V., Pellissier, L., Carlos Pereira da Silva, J., Pitteloud, C., Powers, T.O., Powers, K., Quist, C.W., Rasmann, S., Moreno, S.S., Scheu, S., Setälä, H., Sushchuk, A., Tiunov, A.V., Trap, J., van der Putten, W., Vestergård, M., Villenave, C., Waeyenberge, L., Wall, D.H., Wilschut, R., Wright, D.G., Yang, J.-I., Crowther, T.W., 2019. Soil nematode abundance and functional group composition at a global scale. Nature 572 (7768), 194–198. https://doi.org/10.1038/s41586-019-1418-6.
Weber, S., Traunspurger, W., 2015. The effects of predation by juvenile fish on the meiobenthic community structure in a natural pond. Freshw. Biol. 60 (11), 2392–2409. https://doi.org/10.1111/fwb.12665.
Wendt-Potthoff, K., Imhof, H., Wagner, M., Primpke, S., Fischer, D., Scholz-Böttcher, B., Laforsch, C., 2014. Mikroplastik in binnengewässern. In: Hupfer, M., Calmano, W., Fischer, H., Klapper, H. (Eds.), Handbuch Angewandte Limnologie. Grundlagen—Gewässerbelastung—Restaurierung—Aquatische Ökotoxikologie—Bewertung—Gewässerschutz, vol. 20. Wiley, pp. 1–35.
Wilson, D.S., 1973. Food size selection among copepods. Ecology 54 (4), 909–914. https://doi.org/10.2307/1935688.
Windsor, F.M., Tilley, R.M., Tyler, C.R., Ormerod, S.J., 2019. Microplastic ingestion by riverine macroinvertebrates. Sci. Total Environ. 646, 68–74. https://doi.org/10.1016/j.scitotenv.2018.07.271.
Wright, S.L., Rowe, D., Thompson, R.C., Galloway, T.S., 2013. Microplastic ingestion decreases energy reserves in marine worms. Curr. Biol.: CB 23 (23), R1031–R1033. https://doi.org/10.1016/j.cub.2013.10.068.
Yeates, G.W., Bongers, T., Goede, R.G.M. de, Freckman, D.W., Georgieva, S.S., 1993. Feeding habits in soil nematode families and genera—an outline for soil ecologists. J. Nematol. 25 (3), 315–331.
You, Y.-j., Kim, J., Raizen, D.M., Avery, L., 2008. Insulin, cGMP, and TGF-beta signals regulate food intake and quiescence in *C. elegans*: a model for satiety. Cell Metabol. 7 (3), 249–257. https://doi.org/10.1016/j.cmet.2008.01.005.
Zhao, L., Qu, M., Wong, G., Wang, D., 2017. Transgenerational toxicity of nanopolystyrene particles in the range of μg L^{-1} in the nematode *Caenorhabditis elegans*. Environ. Sci.: Nano 4 (12), 2356–2366. https://doi.org/10.1039/C7EN00707H.