

Food bacteria and synthetic microparticles of similar size influence pharyngeal pumping of *Caenorhabditis elegans*

Hendrik Fueser^{a,*}, Marie-Theres Rauchschwalbe^a, Sebastian Höss^{a,b}, Walter Traunspurger^a

^a Bielefeld University, Animal Ecology, Konsequenz 45, 33615 Bielefeld, Germany

^b Ecosa, Giselastr. 6, 82319 Starnberg, Germany

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ABSTRACT

Toxicity tests using the model organism *Caenorhabditis elegans* have shown that exposure to small microplastics such as polystyrene (PS) beads lead to high body burdens and dietary restrictions that in turn inhibit reproduction. Pharyngeal pumping is the key mechanism of *C. elegans* for governing the uptake of food and other particles and can be easily monitored by determining the pumping rates. In this study, pharyngeal pumping of *C. elegans* was examined in response to increasing quantities of food bacteria (*E. coli*: 10^6 – 10^{10} cells ml^{-1}) and synthetic particles (10^7 – 10^9 beads ml^{-1}) of similar size (1 μm). While the average pumping rate of *C. elegans* exposed to *E. coli* depended on the density of the bacterial cells, this was not the case for the synthetic beads. At 10^7 items ml^{-1} , bacterial cells and synthetic beads triggered a basic stimulation of the pumping rate, independent of the nutritional value of the particle. At quantities $>10^7$ items ml^{-1} , however, the nutritional value was essential to maximize the pumping rate, as it was upregulated only by *E. coli* cells, which can be chemosensorially recognized by *C. elegans*. Given the unselective uptake of all particles in the size range of bacteria, restricting the pumping rates for particles with low nutritional value to a basic rate, prevents the nematodes from wasting energy by high-frequency pumping, but still allows a food-quality screening at low food levels.

1. Introduction

Nowadays, microplastics (<5 mm) can be found globally in all environmental compartments. In aquatic environments, their presence has been shown to increase significantly with decreasing size and with a decrease in the mesh size used in their detection, such that the concentrations of microplastics <10 μm have almost certainly been underestimated (Lindeque et al., 2020). Because of sedimentation processes, microplastic concentrations in riverine sediments can clearly exceed those in the water phase (Scherer et al., 2020), resulting in a greater risk of microplastic ingestion by benthic fauna (e.g., Frei et al., 2019; Haegerbaeumer et al., 2019).

As part of the benthic meiofauna, nematodes are a highly abundant (Traunspurger, 2000; Traunspurger et al., 2012) taxon all over the world (van den Hoogen et al., 2019). Their abundance is consistent with their major role in secondary production (Bergtold and Traunspurger, 2005; Majdi et al., 2017; Schmid-Araya et al., 2020) and benthic food webs (Majdi and Traunspurger, 2015). Moreover, as a simple model organism, the nematode *Caenorhabditis elegans* has been used to study a wide range of physiological processes, including the mechanisms underlying the

uptake and regulation of food (Avery, 1993; Avery and You, 2012; Song and Avery, 2013; Scholz et al., 2016; 2017) but also of non-food particles such as microplastics (e.g., Fang-Yen et al., 2009; Kiyama et al., 2012; Suzuki et al., 2019). Polystyrene (PS) beads of sizes in the range of bacteria (0.5- and 1.0- μm in diameter) were ingested by *C. elegans* without limitations due to the size of its buccal cavity, and their spatial distribution within the nematode's body was the same as that of *E. coli* cells, the nematode's preferred food (Fueser et al., 2019). Given these shared features, fluorescent PS beads have been used to study food uptake in nematodes (Fukushige et al., 1998; Ghafouri and McGhee, 2007; Raizen et al., 2012). Pharyngeal pumping and isthmus peristalsis are the fundamental processes of bacterial feeding by *C. elegans* (Avery and Horvitz, 1987) and also provide information about the food that is available, whereas pharyngeal pumping is the key mechanism relevant for directing food intake from the surrounding environmental medium (e.g., Scholz et al., 2016; Lee et al., 2017). Pumping takes place in the pharynx, a neuromuscular pump, generating pressure to force food into the pharyngeal lumen and with each pump a small amount of bacteria suspended in liquid is ingested (Avery and Shtonda, 2003; Scholz et al., 2017). Then, after expelling the liquid, concentration and mechanical

* Corresponding author.

E-mail address: h.fueser@uni-bielefeld.de (H. Fueser).

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disruption of the bacterial cells, the latter by the grinder, present in the nematode's terminal bulb (e.g., Avery and Shtonda, 2003; Chiang et al., 2006; Riebesell and Sommer, 2017), the transport to the intestine occurs via peristalsis (e.g., Seymour et al., 1983; Avery and You, 2012). Grinder activity is particularly coupled to pharyngeal pumping (Riebesell and Sommer, 2017) and therefore also correlates with food intake (e.g., Avery, 1993; Luedtke et al., 2010; Raizen et al., 2012; Scholz et al., 2017; Lee et al., 2017). Bacteria and particles smaller than the lumen diameter of the isthmus (~3.9 μm ; adult *C. elegans*) and procorpus (~4.9 μm ; adult *C. elegans*) will be forced to the center line by the geometry of the pharyngeal lumen, where they can be transported more efficiently (Avery and Shtonda, 2003). Among others, Scholz et al. (2016, 2017) and Lee et al. (2017) showed that pumping followed a burst-pause pattern with rapid pumping events interspersed by gaps of no pumping. Since feeding behaviours are costly in energy and can cause an internal exposure of toxicants, *C. elegans* needs to decide in certain intervals whether the benefits of feeding still exceed the costs and adjusts its feeding behavior, including the speed of pumping (e.g., Scholz et al., 2017). These feeding dynamics of *C. elegans* have been investigated in response to the quantity of bacterial cells in the surrounding medium (Scholz et al., 2017) but whether also the quality of food is important remains unknown. For nematodes, microplastic ingestion is closely linked to the mode of feeding (Fueser et al., 2020a) and, for *C. elegans*, especially to pharyngeal activity (Nika et al., 2016). Microplastics in the size range of bacterial cells are rapidly co-ingested by *C. elegans* in the presence of abundant bacteria, resulting in a high body burden (Fueser et al., 2020b). Furthermore, the ability of microplastics to reduce food consumption (Rauchschalbe et al., 2021) may alter responses related to the nematode energy budget, including reproduction. The latter effect is most likely of indirect nature, mediated by dietary interference (Mueller et al., 2020). Nematodes modulate the dynamics of feeding and therefore energy uptake is depending on the availability of food (e.g., Nicholas et al., 1973; Scholz et al., 2016; Lee et al., 2017), but whether the same responses are induced by the food-mimicking particles (e.g., microplastics and other synthetic particles) present in their environment is unclear and more studies that focus on particle uptake kinetics in biological systems are required (Barbosa et al., 2020). While the specific influence of food on nematode pharyngeal movements has been studied by direct observations (Mapes, 1965; Duncan et al., 1974; Woombs and Laybourn-Parry, 1984; Moens et al., 1996) or automatically in microfluidic devices (e.g., Scholz et al., 2016, 2017; Lee et al., 2017), the pharyngeal pumping behavior in response to quantities of microplastics, to the best of our knowledge, has only been addressed in two studies. Fueser et al. (2020b) discovered that PS beads could stimulate *C. elegans*' pumping rates most effectively when their diameter was similar to the size of *E. coli* cells (~1.0 μm). Rauchschalbe et al. (2021) showed that, PS beads of both ingestible (1.0 μm) and non-ingestible size (6.0 μm) for *C. elegans* reduced its bacterial consumption, whereas 6.0- μm beads also significantly lowered the pumping rate in the presence of bacteria. However, it is still not clear to which extent *C. elegans* can differentiate between quantities of bacteria and synthetic, non-nutritious particles and whether their ingestion can be regulated by adjusting pharyngeal pumping rates. In consideration of the theory by Scholz et al. (2017), we thus hypothesized that the average pumping rate of *C. elegans* is both quantitatively related to the presence of food and food-like items such as synthetic particles, and qualitatively related to the nutritional value of the presented items. Therefore, in this study we visually determined the average pumping rates of adult *C. elegans* during exposure to suspensions containing different quantities of *E. coli* (10^6 – 10^{10} cells ml^{-1}) or non-food PS and silica beads (10^7 – 10^9 beads ml^{-1}) of similar size.

2. Material and methods

2.1. Test organism: *Caenorhabditis elegans*

The pumping rate assay was done with the N2 strain of the nematode *Caenorhabditis elegans*, which was obtained in stock cultures from the *Caenorhabditis* Genetics Center (University of Minnesota, Minneapolis, MN, USA). Following standard procedures (Stiernagle, 2006), *C. elegans* was further cultivated on agar plates with 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 CaCl₂, 1 ml 1 M MgSO₄ and 25 ml 1 M KH₂PO₄ buffer pH 6 (108.3 g KH₂PO₄ l^{-1} , 35.6 g K₂HPO₄ l^{-1}) and with 1 ml cholesterol solution (5 mg ml^{-1} in ethanol) added after autoclaving (Brenner, 1974)] and seeded with *E. coli* OP50. Cultures were stored at 20 °C in the dark.

2.2. Food organism: *E. coli*

According to Stiernagle (2006), Luria-Bertani (LB) medium (1% peptone, 0.5% yeast extract, 1% NaCl) was used as a growing medium for the *E. coli* OP50 culture, which was allowed to grow at 37 °C for 17 h under constant mixing. The *E. coli* cells were then washed three consecutive times by centrifuging for 20 min at 2000 g and resuspending the pellet in K-medium (deionized water with 3.1 g NaCl l^{-1} , 2.4 g KCl l^{-1}). Their density was spectrophotometrically determined (Varian Cary 50 Bio UV-Visible) based on the optical density (OD₆₀₀) of three subsamples (1:20 dilution) and by means of a calibration curve (Muschiol and Traunspurger, 2007) the OD was converted into bacterial density.

2.3. Synthetic particles: polystyrene and silica beads

As synthetic, non-nutritious particles with a diameter of 1.0 μm fluorescent PS beads (Fluoresbrite® yellow-green microspheres; excitation maxima: 441 nm; emission maxima: 485 nm) were purchased from Polysciences Europe GmbH (Hirschberg, Germany) and 1.0- μm silica beads from microParticles GmbH (Berlin, Germany). PS was chosen because it is a common plastic polymer type and beads are a common form of microplastics <200 μm , with high environmental quantities determined at microplastic "hot spot" sampling sites, including in riverine sediments (e.g., Scherer et al., 2020). PS beads with a diameter of 1.0 μm were previously shown to be readily ingested by *C. elegans* (e.g., Boyd et al., 2003; Fueser et al., 2019; Mueller et al., 2020) since their size is close to the size of *E. coli* cells ($1.13 \pm 0.18 \mu\text{m}$; Rauchschalbe et al., 2021). Avery and Shtonda (2003) already showed 0.8- μm latex beads being ingested, efficiently concentrated during pharyngeal pumping and readily passed through the intestine of rapid feeding *C. elegans* within 3–10 min. Since silica beads are similar in size and shape to PS beads and naturally abundant in the environment, they can be used as a non-plastic reference (Ogonowski et al., 2018) to differentiate mere particle effects and plastic-derived effects. The manufacturers' specifications of PS and silica beads were verified, and the measured diameters coincided with labeled specifications. Nominal bead densities were verified following Mueller et al. (2020), briefly, by using a hemocytometer (Neubauer improved; 0.02 mm chamber depth; Brand GmbH + Co KG, Wertheim, Germany) to count the beads in aliquots of defined dilutions of the stock suspensions and test densities differed from the nominal densities stated in the manufacturers' specifications by not more than 4% for 1.0- μm PS beads and 16% for 1.0- μm silica beads.

2.4. Experimental design

Pharyngeal behavior in *C. elegans* can be measured by manually scoring the backward grinder movements in the terminal bulb (Duncan et al., 1974; Woombs and Laybourn-Parry, 1984; Moens et al., 1996). This is possible because there is a high visual contrast between the grinder and its surroundings and grinder movements are coupled to

pumping (e.g., Riebesell and Sommer, 2017). Thus, pharyngeal pumping rates were recorded at 20 °C following Fueser et al. (2020b) and Rauchschalbe et al. (2021). Briefly, single adult worms were always starved for 30 min and then transferred to a 10- μ l droplet of a test suspension on a flattened Gelrite®-pad (0.5 g Gelrite® heated in 15 ml deionized water) fixed to a microscopic slide. At 100 \times magnification using a microscope (Zeiss Axio Scope.A1), complete backward grinder movements were counted (adapted by Keane and Avery, 2003; Chiang et al., 2006; Hobson et al., 2006) only during burst phases of pharyngeal pumping. After acclimatization to the new conditions for 5–10 min, the temporal pattern of the bursts and pauses was more consistent and pumping rates of five nematodes in each treatment were determined for three 10-s intervals per nematode. Here, we applied 10- μ l droplets of a defined quantity of bacteria or synthetic beads suspended in K-medium (*E. coli*: K-medium, 10^6 , 10^7 , 10^8 , 5×10^8 , 10^9 and 10^{10} cells ml^{-1} ; 1.0- μ m synthetic beads: 10^7 , 5×10^8 and 10^9 beads ml^{-1}). Because a minute consisted of burst phases and pauses of pumping, counts of burst phases were divided by ten to obtain the average pumping rate within burst phases as the number of pump events per second. To minimize unintentionally bias, all pumping rates were counted at the same day and with randomized treatments.

2.5. Data analysis

The data were tested for normality (Shapiro-Wilk test) and for equal variance (Levene's test). Since single worms were measured three times, the study involved dependent data and significant differences between pumping rates were therefore detected using a one-way repeated measures (RM) ANOVA (post-hoc: Holm-Sidak; a significance level of $p < 0.05$ was set for all comparisons). Average pumping rates are reported as the mean \pm standard deviation. The relationship of the average pumping rate and the bacterial density was modeled with a logistic function (4 parameters, $y = y_0 + a/(1+(x/x_0)^b)$). The statistical analyses were carried out and the plots were created with SigmaPlot 12.0 (Systat Software Inc.).

3. Results and discussion

3.1. Increase in average pumping rates with increasing bacterial densities

The quantitative relation of pharyngeal pumping to bacterial densities have already been shown in other studies (e.g., Scholz et al., 2016; Lee et al., 2017; Rauchschalbe et al., 2021). However, the systematic analysis of pumping rates over a large range of bacterial densities in this study allowed to describe the average pumping rate of *C. elegans* as a function of food (*E. coli* cells) availability (Fig. 1). With increasing bacterial densities, the average pumping rate increased until reaching an average maximal speed of 3.49 ± 0.29 pumps s^{-1} at 10^9 *E. coli* ml^{-1} (Fig. 1, 2). Average pumping rates were significantly related to the bacterial density but followed a non-linear relationship (Fig. 1; $r^2 = 0.9866$, $p = 0.0073$; logistic function: $y = y_0 + a/(1+(x/x_0)^b)$). When food was lacking (pure K-medium), the average basic pumping rate of *C. elegans* was 1.63 ± 0.15 pumps s^{-1} . Already in the presence of 10^6 *E. coli* ml^{-1} the pumping rate was significantly higher, 2.01 ± 0.28 pumps s^{-1} (1.2-fold over the basic pumping rate; $t = 2.791$; $p = 0.019$; Fig. 1), and it increased with increasing food concentrations until at 10^9 and 10^{10} *E. coli* ml^{-1} rates did not further increase (3.5 pumps s^{-1} ; >2-fold increase; $t > 13.957$; $p < 0.001$; Fig. 1). The pumping rates were in good agreement with those determined by Hobson et al. (2006) (3.2 – 4.8 pumps s^{-1}) and You et al. (2008) (3.3 pumps s^{-1}), who counted the pumping events of *C. elegans* on culture agar plates at unspecified bacterial densities and Scholz et al. (2016) (3.5 pumps s^{-1}) and Lee et al. (2017) (4.5 pumps s^{-1}) who automatically counted the pumping events of *C. elegans* in microfluidic devices. However, the pumping rates during burst phases observed in the present study did not reach the maximal values of 4–5 pumps s^{-1} that may only be observed with continuous food

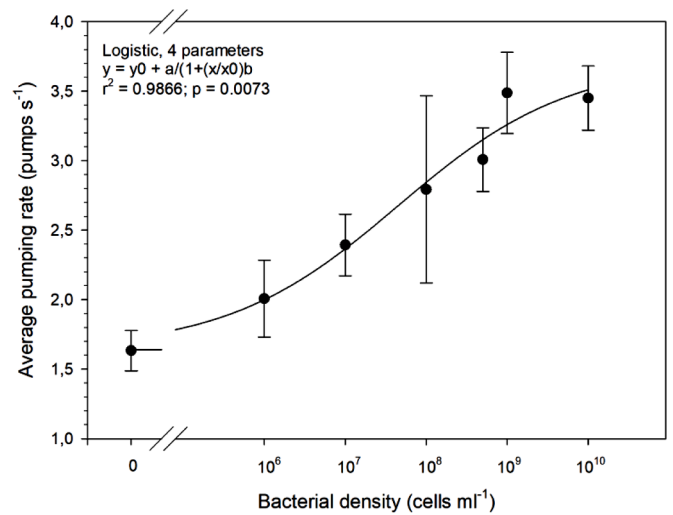


Fig. 1. Average pumping rates of *C. elegans* at fixed densities of *Escherichia coli*. The average pumping rates of the nematode were recorded on Gelrite®-pads containing *E. coli* densities of 0 (pure K-medium), 10^6 , 10^7 , 10^8 , 5×10^8 , 10^9 and 10^{10} cells ml^{-1} . The scatter plot: curve was fitted to the data with a logistic, 4-parametric curve ($r^2 = 0.9866$; $p = 0.0073$), mean \pm standard deviation, $n = 15$. SigmaPlot 12 (Systat Software Inc., USA).

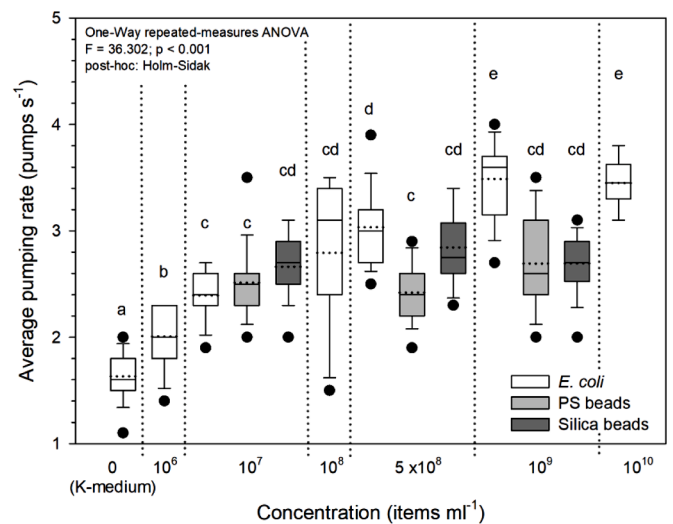


Fig. 2. Average pumping rates of *Caenorhabditis elegans* in the presence of fixed densities of bacterial cells and 1.0- μ m polystyrene (PS) and silica beads. Average pumping rates of *C. elegans* were recorded on Gelrite®-pads containing one of the following: 0 (pure K-medium), 10^6 (only *E. coli*), 10^7 , 10^8 (only *E. coli*), 5×10^8 , 10^9 and 10^{10} (only *E. coli*) items ml^{-1} . $n = 15$. Boxplots: different letters indicate significant differences (one-way repeated-measures ANOVA, post-hoc: Holm-Sidak method, $p < 0.05$). The median is shown by the solid line and the mean by the dotted line. Boxes represent 50% (interquartile range) and each whisker 25% of the data. Please see Fig. S3 for a dot density plot. SigmaPlot 12 (Systat Software Inc., USA).

flow (e.g., in microfluidic devices). Food availability seemed to reach an optimum at a bacterial density of 10^9 *E. coli* ml^{-1} since the pumping rate could not be enhanced further when higher bacterial densities were supplied (10^{10} vs 10^9 *E. coli* ml^{-1} : $t = 0.514$; $p = 0.608$) and the curve was close to saturation at 10^{10} *E. coli* ml^{-1} (Fig. 2). These findings are in accordance with those of Woombs and Laybourn-Parry (1984), who showed a constant pumping rate by the nematode *Rhaditis curvicaudata* at bacterial densities of $>10^9$ cells ml^{-1} , and with those of Schiemer (1982), who found only a weak increase in the production rates and respiration of *Caenorhabditis briggsae* in response to a surplus of food

(>10¹⁰ bacterial cells ml⁻¹). However, the feeding strategies of nematodes are species-specific: *R. curvicaudata* reduces its feeding activity as the bacterial density decreases, such that feeding eventually ceases entirely. In *Diplogasteritus nudicapitatus*, continuous pumping occurs at a more or less constant rate regardless of the food density (Woombs and Laybourn-Parry, 1984). For *C. briggsae*, Nicholas et al. (1973) mathematically modeled the removal of *E. coli* and showed that it was proportional to the bacterial density.

Feeding is essential for the survival of animals and it is regulated by a variety of signals generated in response to internal and external conditions (Takahashi and Takagi, 2017). *Caenorhabditis elegans* is able to detect food via olfactory, gustatory and mechanical cues (Bargmann and Mori, 1998; Sawin et al., 2000; Bargmann, 2006) perceived by ciliated sensory neurons localized in the head, the inner labia, and the tail (Bargmann, 2006). *Caenorhabditis elegans* is supposed to have a relatively direct mode of food sensing since pharyngeal secretory neurons (NSM) can detect food within seconds of ingestion (Rhoades et al., 2019). Through this constant sensing of the presence and quality of food in its environment (Avery and You, 2012) *C. elegans* modulates its pharyngeal pumping rate accordingly (Horvitz et al., 1982; Rogers et al., 2001). The effective ingestion of food is ensured only when an up-regulation of pharyngeal pumping is triggered (Calahorra et al., 2019) in which the rate matches the concentration of bacterial food in the nematode's environment (Scholz et al., 2016; Lee et al., 2017). In environments rich in bacteria, neither the duration of the individual pumps is affected nor is there a continuous tuning of the pumping rate; rather, the overall pumping pattern is modulated, such that the period of fast pumping is prolonged, and the duration of the long pauses is reduced (Lee et al., 2017). Conversely, following its removal from a food-rich environment (4–5-pumps s⁻¹; Song and Avery, 2012), *C. elegans* decreases its pumping rate by at least half (e.g., Avery and Horvitz, 1989; 1990) and its pumping dynamics are dominated by long pauses (Lee et al., 2017; Scholz et al., 2017). A previous study showed that the pharyngeal pumping rate can be reduced to a fifth (0.83 pumps s⁻¹) of the on-food rate during the first 120 min of food deprivation (Dallière et al., 2016), whereas we found that the off-food pumping rate was half the rate determined when the food supply was optimal. As compensation for the decrease in bacterial densities, *C. elegans* increases its locomotion, through body bends and head thrashes (personal observations). In other reports, *C. elegans* displayed a more exploratory behavior (e.g., Avery and Horvitz, 1990; Sawin et al., 2000), including sampling of the immediate environment for the presence of food (Dallière et al., 2016). Furthermore, starved individuals of *C. elegans* responded to lower densities of bacteria than well-fed individuals and slowly started to pump again after 4 h of food deprivation (Avery and Horvitz, 1990).

3.2. A yes/no-response to the stimulation of the average pumping rate by synthetic particles

Nematodes in their natural habitat are exposed to a broad range of nutritious and non-nutritious items of potentially ingestible size. The latter include synthetic beads, but it is still largely uninvestigated, whether their quantity in the surrounding medium is related to nematode feeding behavior, particularly to pharyngeal pumping. We found that, in the absence of food bacteria, ingestible PS and silica beads were able to stimulate pharyngeal pumping at 10⁷ items ml⁻¹ comparable to rates that were significantly higher than the baseline in pure K-medium (PS beads: $t > 6.901$, $p < 0.001$; silica beads: $t > 8.892$, $p < 0.001$; see also Fueser et al., 2020b). In contrast to bacterial cells, however, an increase of the bead quantities in the surrounding medium did not cause a further increase in the average pumping rate (Fig. 2; Fig. S1, S2). Thus, *C. elegans* may perceive the presence of items in the size range of its diet but its response does not include a stimulation of the average pumping rate in accordance with bead quantity. Moreover, the qualitative differences between the PS and silica beads (specific density of 1.05 g cm⁻³

and 1.85 g cm⁻³, respectively; manufacturers' specifications) did not result in any differences in the average pumping rate at any of the tested quantities (10⁷, 5 × 10⁸ and 10⁹ beads ml⁻¹; Fig. 2).

The average pumping rate of *C. elegans* in the presence of *E. coli* vs. synthetic beads (Fig. 2) at the relatively low density of 10⁷ items ml⁻¹ did not significantly differ (PS beads: $t = 0.941$, $p = 0.991$; silica beads: $t = 0.2451$, $p = 0.329$; Fig. 2), which suggests that the main trigger of pharyngeal pumping at this low concentration was the mere presence of appropriated shaped items in the medium. At a higher bead density (5 × 10⁸ items ml⁻¹), however, there was no further stimulation of pharyngeal pumping by the PS beads (see also Fig. S1) and the average pumping rate was 19% lower than that in response to the same high density of *E. coli* ($t = 4.810$, $p < 0.001$). At densities of 10⁹ items ml⁻¹ (the optimal food density), the average pumping rate triggered by the PS and the silica beads was 25% ($t = 6.371$; $p < 0.001$) and 28% ($t = 6.446$; $p < 0.001$) lower, respectively, than in response to bacterial cells and in both cases 39% ($t > 8.312$; $p < 0.001$) lower than in the control treatment without bacteria. Thus, at higher densities, the quality seems to become the relevant trigger of pharyngeal pumping. This suggests that *C. elegans* is able to detect *E. coli* via chemosensors (e.g., Bargmann, 2006) receiving signals to maximize pharyngeal pumping at high bacterial densities in the surrounding medium. By contrast, for other, non-odorant items, such as PS or silica beads, a further increase of pharyngeal pumping does not seem profitable.

The exposure of nematodes to toxicants has been shown to decrease mobility and feeding and, vice versa, the behavioral effects of toxicants on *C. elegans* can be affected by the availability of food (e.g., Boyd et al., 2003, 2007). There are contradictory reports on whether an accompanying reduction of pharyngeal pumping rates might act as a first-line defense to prevent the toxicants from being ingested (Boyd et al., 2003). While there are studies showing that *C. elegans* decreased feeding in a concentration-dependent manner after exposure to e.g., captan (Jones et al., 1996), Stringham and Candido (1994) found no effects of low toxicant exposure on pumping rates, suggesting that *C. elegans* were not able to avoid toxicant exposure by reducing pumping rates. Applying this, however, we found for the exposure of synthetic beads that nematodes increased the average pumping rate when PS and silica beads were added to pure K-medium but the stimulation could not be enhanced by raising the bead concentration. At quantities of 10⁷ beads ml⁻¹ beads were actually ingested at the same pumping rate as *E. coli* cells leading to a high body burden of synthetic beads (Fueser et al., 2019, 2020b). At higher synthetic bead quantities, the speed of pumping was slower than in *E. coli* suspensions with same densities. Restricting the pumping rates for particles with low nutritional value to a basic rate, prevents the nematodes from wasting energy by high-frequency pumping, but still allows a food-quality screening at low food levels. Our findings support the theory that the control of pumping rates helps *C. elegans* to gain information on the nutritional value of ambient particles (Scholz et al., 2017). For this purpose, an intermediate threshold pumping is apparently sufficient and no chemo-sensory stimulus is required.

At sufficiently high bacterial densities, however, even high quantities of 1-µm PS beads did not reduce the pumping rate of *C. elegans* (Rauchschwalbe et al., 2021), leading to a considerable co-ingestion of PS beads (Fueser et al., 2019). This, in turn, can lead to a lower feeding efficiency due to food dilution (Rauchschwalbe et al., 2021) and a consequently lower reproductive output (Mueller et al., 2020), which thwarts the feeding strategy to maximize food uptake at minimal energetic costs. However, here, only selective feeding could increase the nutritional content of the ingested particles, which *C. elegans* is not able to control by modulating the pumping rates.

4. Conclusion

Caenorhabditis elegans constantly assesses its environment for the presence and quality of food and modulates its pharyngeal pumping rate

accordingly. An increase in the average pumping rate with increasing bacterial densities ensures the effective ingestion of food. Also, non-nutritious items in the size spectrum of food bacteria (1.0- μm PS and silica beads) stimulated pharyngeal pumping similarly to bacteria. This stimulation is apparently triggered by the presence of any items mimicking bacterial cells by shape and size and allows a moderate uptake for the purpose of a quality screening. Only if ingested items are evaluated as food with nutritional value (e.g., bacteria), probably by chemosensory perception, the pumping rate can be increased with increasing particle abundance to optimize the exploitation of resources. In case of non-nutritional particles, it is probably not profitable to boost pumping. This feeding strategy seems to be beneficial in natural habitats of *C. elegans* (soils, sediment, compost), where small, non-nutritious particles (e.g., sand and clay particles) are abundant. However, at high food levels (e.g., high bacterial densities), the co-ingestion of non-nutritional particles (e.g., microplastics) cannot be avoided by regulating the pumping rate, which can lead to detrimental effects.

CRedit authorship contribution statement

Hendrik Fueser: Conceptualization, Methodology, Investigation, Data curation, Writing – original draft, Writing – review & editing. **Marie-Theres Rauchsvalbe:** Conceptualization, Methodology, Investigation, Data curation, Writing – original draft, Writing – review & editing. **Sebastian Höss:** Conceptualization, Supervision, Writing – review & editing. **Walter Traunspurger:** Conceptualization, Supervision, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.aquatox.2021.105827.

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