Ingestion of microplastics by free-living marine nematodes, especially *Enoplolaimus* spp., in Mallipo Beach, South Korea

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**Abstract:** Many plastics cause pollution in the marine environment, with microplastics (0.1 µm–5 mm) representing a key research focus. The number of microplastics in sediments may increase rapidly, affecting organisms inhabiting marine sediments. The aim of this study was to determine how microplastics affect nematodes in intertidal sand. We assessed: (1) intake of microplastic particles (10 µm, 5 µm, 1 µm, or 0.5 µm) by *Enoplolaimus* spp. over 48 h; (2) microplastic intake by nematodes depending on feeding type (selective deposit feeders, non-selective deposit feeders, epistrate feeders, or predators/omnivores) over 48 h; and (3) microplastic egestion by *Enoplolaimus* spp. The proportion of *Enoplolaimus* spp. individuals containing microplastics was significantly less in the 10-µm microplastic treatment than in the treatments where *Enoplolaimus* spp. were exposed to microplastic particles of smaller sizes (5 µm, 1 µm, or 0.5 µm). The ingestion rates of microplastics by predators/omnivores, non-selective deposit feeders, and selective deposit feeders increased as the size of the microplastic decreased. After transferring *Enoplolaimus* spp. to filtered seawater following microplastic ingestion, the proportion of *Enoplolaimus* spp. individuals containing the smallest size microplastic (0.5 µm) decreased by 15% of the ingested amount in 3 days. In conclusion, there was a significant difference among microplastic-size treatments, but not among feeding types or in the interaction between microplastic size and feeding type. The size of microplastics, rather than feeding type of nematodes, impacted ingestion rates. It is possible that microplastics in the sediment are ingested by nematodes living in marine benthic ecosystems.

**Key words:** egestion, ingestion, meiofauna, microplastic, nematodes

**Introduction**

Plastic is an important and widely used material, from the production of basic goods (such as clothes and cosmetics) to complicated and high-tech products (such as aircraft and rocket nozzles). In the medical industry, plastic goods are crucial as aseptic and disposable materials (Hamid et al. 2018). Plastics are excellent packaging materials because of their low cost, excellent oxygen/moisture barrier properties, bio-inertness, and light weight (Andrady 2011). Annual global plastic production is continuously rising and reaching 348 million tons in 2017 (PlasticsEurope 2018), and, as of 2010, 5–13% of this annual plastic production ended up in the marine environment (Jambeck et al. 2015).

Additionally, approximately 18% of the plastic waste found in the ocean environment is associated with the fishing industry, with aquaculture also being a significant contributor to plastic debris in the oceans (Hinojosa & Thiel 2009).

Many plastics are transported to sediments in the marine environment (van Cauwenbergh et al. 2015b). Marine plastic debris represents one of the most serious environmental issues worldwide, with microplastics being the primary focus of many studies (Thompson et al. 2004, Eerkes-Medrano et al. 2015, Rochman et al. 2016, Galloway et al. 2017). Microplastics are generally defined as being 0.1 µm–5 mm in diameter (Thompson et al. 2004, Moore 2008) and in the marine environment, can be divided into two components: (1) primary microplastics that are manufactured directly for various consumer and industrial applications, and (2) secondary microplastics from the break-
down of larger plastic items through decomposition, such as weathering, oxidative processes, and biological degradation (Andrady 2011, Browne et al. 2007, Browne et al. 2011). In general, plastics (and microplastics) such as polystyrene, polyvinylchloride, and polyethylene terephthalate have higher specific gravity than seawater, resulting in a large proportion of plastic sinking to the sediment (Duis & Coors 2016, Auta et al. 2017, Kaiser et al. 2017, Harrison et al. 2018). Consequently, in marine environments, the concentrations of microplastics are higher and less variable in sediments than in seawater (Maes et al. 2017). In addition, the specific gravity of plastic particles increases as they become covered with biofilm (i.e., an aggregation of organic material that attaches to surfaces), resulting in their settling on sediments in the marine environment (Andrady 2011, Wright et al. 2013a, Galloway et al. 2017). Therefore, microplastics in sediments likely impact benthic fauna. The organisms that inhabit marine sediments might be continuously exposed to higher concentrations of microplastics than aquatic organisms in the marine environment (Haegerbaeumer et al. 2019). Karlsson et al. (2017) showed that microplastic concentrations in mussels are approximately one thousand-fold higher than those in sediment and surface water samples from the same location. Increasing quantities of microplastics in sediments increases the possibility of their ingestion by organisms inhabiting the sediment, such as marine worms (Wright et al. 2013b). Studies on the ingestion of microplastics by marine organisms have mainly been conducted on macrofauna. Microplastics, similar in size to plankton, are ingested by marine macrofauna by various feeding methods, such as by plankton-feeding organisms including mussels (filter feeders), lugworms (deposit feeders), and sea cucumbers (detritivores) (Browne et al. 2008, Graham & Thompson 2009, Bonanno & Orlando-bonaca 2018, Naji et al. 2018).

Among benthic fauna, meiofauna are small with short generation times and highly abundant, and their entire life cycle is spent within the sediment (McIntyre 1969, Higgins & Thiel 1988). In particular, free-living nematodes are major meiofauna taxa that have been used in impact assessment studies for many aquatic ecosystems (Mahmoudi et al. 2005, Gyedu-Ababio & Baird 2006). The characteristics of nematodes, such as short life cycle and high diversity, suggest that they could be potentially used in ecotoxicological monitoring (Hermi et al. 2009). However, few studies have investigated the effect of microplastics on marine free-living nematodes. Microplastic studies on nematodes have mainly focused on freshwater nematodes, such as Caenorhabditis elegans. (Lei et al. 2018, Kim et al. 2020, Mueller et al. 2020, Shang et al. 2020)

The aim of this study was to investigate how microplastics affect marine free-living nematodes in the intertidal sand zone at a site on the west coast of the Korean Peninsula. Eo et al. (2019) reported that the abundance of large (diameter 1–5 mm) and small microplastics (diameter <1 mm) are 0–2,088 n/m² and 1,400–62,800 n/m², respectively, in beaches of South Korea. Wang et al. (2019) reported that the abundance of microplastics (diameter 0.05–5 mm) is 560–4,205 n/kg dry weight in the surface sediments (the top 3 cm) of the South Yellow Sea, China. As nematodes are a food source for macrobenthic animals in the marine benthic ecosystem (Du et al. 2014), microplastics consumed by nematodes can be transferred to macrobenthic animals. Therefore, it is necessary to confirm the ingestion of microplastics by nematodes, which are low-level consumers in the food web. Additionally, differences in microplastic ingestion depending on the feeding type and egestion according to microplastic size were assessed. Our results are expected to provide baseline information on the transport of microplastics in the food web of the benthic ecosystem and the role of nematodes, an important biological resource.

Materials and Methods

Sediment sampling

The sediment and seawater used for the experiments were collected at low tide from Mallipo Beach (36°47′16.69″N, 126°83′4.97″E), Korea. Mallipo Beach is located on the west coast of the South Korean peninsula and is classified as an intertidal sand zone (Fig. 1). The first experiment was conducted in February 2016, the second was conducted in April 2017, and the third was conducted in October 2019. All samples containing meiofauna were collected from sandy sediments along the Mallipo Beach. Sediments collected from a depth of 5 cm were sampled using acryl cores of 10 cm² and transferred to clean bottles. The collected sediment samples and seawater were transported to a laboratory in an icebox containing ice packs. Nematodes were separated via a specific process using MgCl₂ anesthetic (See “Experimental Design, Experiment 1”). Seawater was filtered through a 0.2-µm filter to remove organic matter to prevent the growth of microorganisms and the deterioration of the experimental conditions. We also collected seawater from the site where the sediment samples were collected.

Fig. 1. Location of the sampling site (A: specific location; B: general location).
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Microplastics

We experimented with fluorescent polystyrene microplastics to identify microplastics in the nematode body under a fluorescence microscope. Fluorescent polystyrene microplastics (FLUOR Polystyrene; MAGSPHERE, Pasadena, CA, USA), used in all experiments, are commercially available and were made with fluorescent dye (yellow-green color). Four diameters of microplastics were used: 10 μm (4.57×10^7 particles ml^-1), 5 μm (3.65×10^8 particles ml^-1), 1 μm (4.57×10^10 particles ml^-1), and 0.5 μm (3.65×10^11 particles mL^-1). The specific gravity of the microplastics used in this study was 1.05. Therefore, the microplastics used in the experiment sink by specific gravity, because the density of the microplastic is greater than that of seawater.

Experimental design

Experiment 1-Microplastic intake by Enoplolaimus spp.

In the first experiment, Enoplolaimus spp. were used to investigate how different sizes of microplastics affected ingestion rates. Before the experiment, we analyzed sediment in the intertidal zone of Mallipo Beach to identify the dominant nematode. Enoplolaimus was the dominant nematode genus at the sampling site and could be distinguished under a dissecting microscope owing to its large size. In addition, the feeding type of Enoplolaimus spp. is carnivorous/predator, and it has a large mouth through which 10 μm sized microplastics can enter.

Enoplolaimus spp. were separated from the collected sediments. We put the collected sediments containing living meiofauna in 500-ml bottles. To anesthetize living meiofauna, seawater containing 5% MgCl_2 was added to the bottles containing sediments. The bottles were then left for approximately 15 min to anesthetize the meiofauna. The bottle was stirred with a spoon so that the meiofauna would float to the surface, and the supernatant containing the meiofauna was poured into a 63 μm sieve. The nematodes in the sieve were transferred to a petri dish. Using a spray with seawater containing 5% MgCl_2, the nematodes were distinguished from other meiofauna under a dissecting microscope by using a hook or O-type loop, and they were confirmed to have been anesthetized (i.e., there was no movement).

Enoplolaimus spp. were then separated from other nematodes under a microscope by using a hook-or O-type loop. The selected Enoplolaimus spp. were transferred to a petri dish containing clean filtered seawater to ensure that they were alive and moving. As Enoplolaimus spp. emerged from anesthesia, they began to move. Twenty live Enoplolaimus spp. were placed on each of 16 petri dishes containing 5 mL of filtered seawater. One microliter of microplastic (10 μm, 5 μm, 1 μm, and 0.5 μm) was added to each of four petri dishes containing live Enoplolaimus spp. (i.e., there were four replicates for each microplastic size). The petri dishes were stored at 20°C in the dark. After 48 h, seawater containing Enoplolaimus spp. in a petri dish was placed in a sieve with a 38 μm mesh size. To remove the microplastics from the surface of Enoplolaimus spp., they were washed using a water spray containing fresh water. Then, the remaining Enoplolaimus spp. in the sieve were transferred to a petri dish and fixed with formalin. Enoplolaimus spp. were mounted on microscope slides to check and identify ingested microplastics by using fluorescence microscopy.

Experiment 2-Microplastic intake of nematodes, depending on feeding type

In the second experiment, the feeding type of nematodes was evaluated in relation to the microplastic ingestion rate. Twelve acrylic cores were collected for this analysis. All nematodes from one acrylic core were separated and transferred to a petri dish (i.e., there were 12 petri dishes each containing the nematodes from one sediment sample).

The seawater and living nematodes used in Experiment 2 were collected and extracted in the same way as in Experiment 1. The nematodes selected for Experiment 2 were transferred to filtered seawater to confirm movement. The nematodes were then transferred to a petri dish containing 5 mL of seawater. In each petri dish, approximately 100 nematodes sorted from one sediment sample were contained. One microliter of microplastic (10 μm, 5 μm, 1 μm, and 0.5 μm) was added to the petri dishes (three replicates for each microplastic size). The petri dishes were stored at 20°C in the dark.

After 48 h, all nematodes were removed using a fine pin under a stereoscopic microscope, transferred from formalin to 3% glycerin, and then mounted on stereoscopic microscope slides in anhydrous glycerine for identification. Nematodes were identified to the genus level by using the pictorial keys of Platt & Warwick (1983, 1988) and Warwick et al. (1998), with the aid of a microscope (Olympus BX51, Tokyo, Japan). The nematodes that had ingested microplastics were identified using fluorescence microscopy. The mounted nematodes were classified according to the original groupings of Wieser (1953) into four feeding groups: (1A) selective deposit feeders, (1B) non-selective deposit feeders, (2A) epistrate feeders, and (2B) predators/omnivores.

Experiment 3-Microplastic egestion of Enoplolaimus spp.

In Experiment 3, we investigated whether the microplastics ingested by Enoplolaimus spp. were egested. We allowed the Enoplolaimus spp. to ingest microplastics, then moved them to filtered seawater for incubation for 24 and 72 h. We then measured the amount of microplastics in the nematodes immediately after removal from the water containing microplastics and after 24 h and 72 h of incubation in the filtered seawater. A total of 24 petri dishes were prepared, and 10 Enoplolaimus spp. in each petri dish were placed in 5 mL of seawater containing microplastics of each size (10, 5, 1, and 0.5 μm). There were six petri dishes for each microplastic size. The petri dishes were stored at 20°C in the dark. After 48 h of microplastic ingestion,
the ingestion rate of *Enoplolaimus* spp. in two petri dishes from each microplastic size treatment was checked under a fluorescence microscope. Then, the *Enoplolaimus* spp. in the remaining petri dishes (four dishes for each microplastic size) were transferred to new petri dishes containing filtered seawater with no additional microplastics. The petri dishes were stored at 20°C in the dark. After a further incubation for 24 h, two petri dishes were removed and the amount of microplastics in the *Enoplolaimus* spp. was checked using a fluorescence microscope. After a further 72 h, the last two petri dishes were removed, and the amount of microplastics in the *Enoplolaimus* spp. was checked again.

**Statistical analysis**

The significance of differences in microplastic ingestion rates among microplastic-size treatments was determined using a one-way ANOVA. A two-way ANOVA was used to test the distribution of microplastic ingestion rate depending on feeding type and microplastic size. The ANOVA tests, followed by Scheffe’s post hoc analysis, were performed using SPSS statistics version 19. Statistical significance was set at *p* < 0.05.

**Results**

**Experiment 1-Microplastic intake of *Enoplolaimus* spp.**

Microplastics of each size were detected in the nematodes. Microplastics ingested by *Enoplolaimus* spp. were mainly observed in the buccal cavity and digestive system (Fig. 2). The proportion of *Enoplolaimus* spp. that contained microplastics increased as the size of the microplastics decreased. Overall, 27.5%, 60.9%, 67.9%, and 73.0% of *Enoplolaimus* spp. had 10, 5, 1, and 0.5 µm microplastics in the body, respectively (Fig. 3). The ingestion rate of 10 µm microplastics was significantly different from that of the other sizes (one-way ANOVA, Scheffe’s post hoc analysis, *p* < 0.05).

**Experiment 2-Microplastic intake by nematodes depending on feeding type**

A total of 11 genera were detected, with *Metachromodora* spp. and *Enoplolaimus* spp. being the most common (Table 1). The smaller the microplastic size, the greater the proportion of nematodes that contained microplastics (Table 1, Fig. 4). Overall, 9.6%, 28.9%, 92.3%, and 98.9% of nematodes had 10 µm, 5 µm, 1 µm, and 0.5 µm microplastics in the body, respectively. Most nematodes were predators/omnivores (2B), followed by non-selective deposit feeders (1B) and selective deposit feeders (1A). Nematodes of the epistrate feeder (2A) type appeared in very small numbers, therefore, we did not calculate the ingestion rate from the resulting values. Predators/omnivores (2B) had ingestion rates of 11.1%, 28.5%, 92.8%, and 98.9% for 10 µm, 5 µm, 1 µm, and 0.5 µm microplastics, respectively; non-selective deposit feeders (1B) had ingestion rates of 1.8%, 50%, 75%, and 100% for 10 µm, 5 µm, 1 µm, and 0.5 µm microplastics, respectively; and selective deposit feeders (1A) were not present in the 10 µm microplastic treatment and had 0%, 75%, and 100% ingestion rates for 5 µm, 1 µm, and 0.5 µm microplastics, respectively. Two-way ANOVA showed that there was a significant difference among the microplastic size treatments, but there was no significant difference among feeding types and in the interaction between microplastic size and feeding type (two-way ANOVA followed by Scheffe’s post hoc analysis *p* < 0.05).

*Metachromodora* spp. were dominant and occupied more than 60% of the total nematode abundance in experiment 2. *Metachromodora* spp. were predators/omnivores (2B). On average, 0 of 37.0 *Metachromodora* spp. ingested 10 µm microplastics (a rate of 0%), 9.3 of 50.0 ingested 5 µm microplastics (a rate of 18.7%), 65.3 of 71.0 ingested 1 µm microplastics (92.0%), and 73.0 of 74.0 ingested 0.5 µm microplastics (98.6%) (Table 1). Information on the
Ingestion and egestion of MPs by marine nematodes

Ingestion and egestion of MPs by marine nematodes is shown in Table 1.

Experiment 3 - Excretion of microplastics by Enoplolaimus spp.

After transferring Enoplolaimus spp. that had ingested microplastics to filtered seawater, the proportion of individuals in which microplastics were found decreased (Fig. 5, Table 2). This decrease was attributed to the egestion of microplastics. The proportion of Enoplolaimus spp. with microplastics was 52.7% at 10 µm, 64.3% at 5 µm, 73.2% at 1 µm, and 88.2% at 0.5 µm after 48 h in a petri dish with microplastics. After transfer to and incubation in filtered seawater for 24 h, the proportion of Enoplolaimus spp. with microplastics was 43.7% at 10 µm, 50.0% at 5 µm, 74.1% at 1 µm, and 79.2% at 0.5 µm. After transfer to and incubation in filtered seawater for 72 h, the proportion of Enoplolaimus spp. with microplastics was 48.3% at 10 µm, 58.3% at 5 µm, 66.7% at 1 µm, and 75.0% at 0.5 µm. The proportion of individuals in which microplastics were found tended to decrease regardless of particle size. We also identified the location of microplastics in the body of Enoplolaimus spp. The microplastics were found close to the head of Enoplolaimus spp. after 48 h exposure to the microplastic environment; 72 h after being transferred to filtered seawater, the microplastics were closer to the tail (Fig. 6).

Discussion

In this study, we investigated the extent to which microplastics are ingested by nematodes (in particular Enoplolaimus spp.) collected from Mallipo Beach in South Korea. We showed that microplastics were ingested by free-living marine nematodes collected in the marine environment by using laboratory microcosm experiments. Few studies have demonstrated that free-living marine nematodes consume microplastics. However, there have been several other nematodes is shown in Table 1.

Table 1. The abundance of nematodes that ingested microplastics by genus in different microplastic-size treatments.

| Nematodes (spp.) | Feeding type | 10 µm | 5 µm | 1 µm | 0.5 µm |
|------------------|--------------|-------|------|------|--------|
|                  |              | Avg. Ind. | MP Ingest. | Avg. Ind. | MP Ingest. | Avg. Ind. | MP Ingest. | Avg. Ind. | MP Ingest. |
| Metachromodora   | 2B           | 37.0    | 0.0   | 50.0  | 9.3    | 71.0    | 65.3    | 74.0    | 73.0    |
| Enoplolaimus     | 2B           | 48.7    | 10.0  | 32.7  | 12.3   | 14.0    | 13.3    | 11.7    | 11.7    |
| Daptonema        | 1B           | 17.0    | 0.3   | 3.3   | 1.7    | 1.0     | 0.7     | 0.3     | 0.3     |
| Enopludis        | 2B           | 11.3    | 1.3   | 5.0   | 2.7    | 3.7     | 3.3     | 1.3     | 1.3     |
| Enoplus          | 2B           | 2.7     | 0.0   | 3.0   | 1.3    | 3.3     | 3.3     | 0.3     | 0.3     |
| Oncholaimus      | 2B           | 2.3     | 0.0   | 0.7   | 0.3    | 1.0     | 1.0     |         |         |
| Aearoaimus       | 1A           | 1.0     | 0.0   | 1.0   | 0.0    | 0.3     | 0.3     |         |         |
| Halalaimus       | 1A           | 0.7     | 0.0   | 0.7   | 0.0    |         |         |         |         |
| Anoplostoma      | 1B           | 0.7     | 0.0   | 0.7   | 0.0    |         |         |         |         |
| Theristus        | 1B           | 0.7     | 0.0   | 0.7   | 0.0    |         |         |         |         |
| Polysigma        | 2A           | 0.7     | 0.0   | 0.7   | 0.0    |         |         |         |         |
| Total            |              | 121.0   | 11.7  | 95.7  | 27.7   | 95.7    | 88.3    | 88.3    | 87.3    |
| Percentage (%)   |              | 9.6     | 28.9  | 92.3  | 98.9    |         |         |         |         |

Avg. Ind.: Average number of individuals (average for three replicates). MP Ingest.: Average number of individuals that ingested microplastics. 1A, selective deposit feeders; 1B, non-selective deposit feeders; 2A, epistrate feeders; and 2B, predators/omnivores.

Fig. 4. Microplastic intake rate (%) of nematodes depending on microplastic size and feeding type. Error bars represent standard deviation.

Fig. 5. Proportion of Enoplolaimus spp. containing microplastic particles in different microplastic particle-size treatments and after transfer to filtered seawater.
recent studies investigating microplastic ingestion by one freshwater nematode species, *C. elegans* (Zhao et al. 2017, Lei et al. 2018, Fueser et al. 2019). *C. elegans* worms actively accumulate 0.5 and 1 µm fluorescent polystyrene microspheres in the presence and absence of bacterial food, whereas microspheres of <0.5 µm and >3 µm are rarely accumulated (Kiyama et al. 2012).

In the current study, Experiment 1 showed that smaller microplastics were more often detected in *Enoplolaimus* spp. bodies than larger microplastics. The reason for this result could be that given the smaller the size of the microplastics and the higher the number of microplastics particles added to the petri dishes, these small microplastics have a higher probability of entering the mouth of nematodes than large microplastics. Previous studies have reported that the size of microplastics influences the likelihood of ingestion. For example, a review paper summarizing microplastic studies conducted on various organisms showed that ingestion depends on the size of microplastic particles, among other factors (Phuong et al. 2016). Lehtiniemi et al. (2018) showed that the size of microplastics particles, rather than shape, strongly influences the amount of microplastics ingested in an experiment using fish and mysid shrimps. Few studies have evaluated the bioaccumulation of microplastics through food chains in the marine environment. However, lower trophic organisms, such as invertebrates, likely ingest and accumulate microplastics, introducing them to the food chains of the marine environment (Wright et al. 2013a).

In Experiment 2, we showed that the size of microplastics, rather than feeding type of nematodes, affected ingestion rates. The size and shape of microplastics, exposure concentration and time, morphological features of nematodes (e.g., buccal cavity size and intestine dimensions), and species-specific feeding habits probably constrain the uptake and ingestion of microplastics by nematodes (Gray & Weinstein 2017, Lehtiniemi et al. 2018, Fueser et al. 2019). Most nematodes collected in natural sediments for this experiment were predators/omnivores. Additionally, selective deposit feeders and non-selective deposit feeders appeared with low abundance. However, few nematodes were of the epistrate feeding type. For this reason, the results of non-predator/omnivore feeding-type nematodes may not have been reflected well. In contrast, the size of microplastics had a great influence on the intake rate. The small size of microplastics is an important factor affecting bioavailability, particularly for lower trophic organisms (Wright et al. 2013a). The ingestion of microplastics by nematodes is predominantly determined by their feeding habits and can be predicted by the morphology of the buccal cavity. Fueser et al. (2019) showed that feeding habit and the buccal cavity of nematodes have larger effects than other factors (such as species-specific feeding differences, exposure time, and exposure concentration) on the ingestion of microplastics and could be important for controlling the quantity of microplastic uptake.

In Experiment 3, we investigated whether the microplastic particles were retained in the bodies of *Enoplolaimus* spp. or egested. The excretion of microplastics by macroor-
organisms has been investigated by measuring the ingestion of microplastic particles and quantifying the microplastics in fecal debris. For example, Graham & Thompson (2009) quantified the excretion of microplastics by four species of sea cucumber. However, it is more difficult to quantify the excretion of microplastics by microorganisms such as marine nematodes. Therefore, in this study, we first determined the proportion of nematodes that ingested microplastics and then determined the proportion of nematodes that still contained microplastics after being transferred to and incubated in filtered seawater for 24 h or 72 h.

The proportion of Enoplolaimus spp. with 10 µm microplastics in the body decreased by 4.4 percentage points, that with 5 µm microplastics decreased by 6.0 percentage points, that with 1 µm microplastics decreased by 6.5 percentage points, and that with 0.5 µm microplastics decreased by 13.2 percentage points after 72 hours. When the size of the microplastic decreased, it was expected that microplastics would easily escape from the body along the digestive tract; however, this effect cannot be identified significantly differently due to the lack of sufficient samples to achieve relevant statistical power. Also, in Experiment 3, we transferred the nematodes to the filtered seawater and checked the discharge of microplastics for just three days. After three days of experiments in filtered sea water, we observed that the transferred nematodes slowed down and their health condition deteriorated, therefore, we no longer proceeded with the experiments. However, we expected that the egestion rates of microplastics would increase further if the nematode is provided conditions to maintain a healthy state in the petri dish for more than three days. Because the intestinal tract of nematodes is a simple, hollow, straight tube consisting of a single layer of epithelial cells, it is not difficult to move any sized microplastic from the mouth to the anus (Basyoni & Rizk 2016). Recently, Fueser et al. (2020) showed that the two nematode species, C. elegans and P. pacificus, rapidly ingest and egest PS beads (0.5 and 1.0 µm) along with bacteria. These two nematodes are not free-living marine nematodes, but the results are very important in relation to the bioconcentration of microplastics.

Finally, we checked the location of microplastics in the body of Enoplolaimus spp. In Experiment 1, the location of microplastics in the body of Enoplolaimus spp. was mostly close to the head. However, in Experiment 3, after transferring the Enoplolaimus spp. that ingested microplastics into filtered seawater, microplastics in the body of the Enoplolaimus spp. were mainly located close to the tail. As a result, the egestion of microplastics in the nematode body is expected to proceed slowly.

If organisms that ingest microplastics do not egest microplastics, then the plastics may accumulate in animals higher in the food chain. Conversely, if microorganisms egest microplastics, the adverse effects of plastics on animals higher in the food chain may be limited (Wright et al. 2013a). Some studies have been conducted on the egestion of microplastics by macroorganisms. For example, Graham et al. (2019) showed that Pacific oysters have efficient egestion rates of microplastics (84.6±2%) and Van Cauwenberghe et al. (2015a), who analyzed the fecal casts of Polychaeta, showed that they excrete some microplastic particles.

Nematodes are widely distributed globally, including in extreme environments (such as in polar, deep-sea, and hydrothermal areas). Microplastics are also widely distributed in the marine environment; thus, they are available to nematodes. Our study showed that sunk microplastics are ingested by nematodes living in marine benthic ecosystems. In these experiments, conducted on Enoplolaimus spp., which have a large mouth, the microplastics of the sizes used in this study could be sufficiently ingested. As a result, microplastics of all sizes were identified in the body of nematodes. In addition, since the number of nematodes used in Experiment 2 by feeding type is small, the difference in the ingestion rate of the nematode, depending on the feeding type is not clear. However, the intake rate can be expected to be more affected by the size of the microplastics rather than the difference in feeding types. In Experiment 3, contrary to expectations, the discharge rate of microplastics into the body of nematodes according to the size of microplastics could not be detected. After transferring Enoplolaimus spp. to filtered seawater, the proportion of nematodes containing the smallest size microplastic (0.5 µm) decreased by 15% of the ingested amount in 3 days. As microplastics sink through the water column when they become covered with biofilm, eventually settling onto sediments (Wright et al. 2013a), the microplastics ingested by nematodes will likely be transferred to animals that feed on them. Future studies should evaluate how microplastics are transmitted and how they accumulate within the benthic food web.

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