Feeding of Marine Zooplankton on Microplastic Fibers

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Abstract
The goal of our study was to examine the effects of low abundances of nylon fibers on feeding rates of calanoid copepods (Crustacea, Copepoda) and doliolids (Tunicata, Thaliacea) in the presence of diatoms at near environmental concentration levels. In addition, we examined microscopically the fecal pellets produced by copepods and doliolids in the presence of fibers. Adult females of the calanoid *Eucalanus pileatus* and early gonozooids of *Doliolitta gegenbauri* (both of similar dry weight) cleared the diatom *Rhizosolenia alata* at similar rates. Nylon fibers were cleared at higher rates by *Doliolitta gegenbauri* compared to *Eucalanus pileatus*. Examination of fecal pellets revealed that copepods and doliolids could ingest the about 300 µm long fibers. The latter also ingested the occasionally occurring fibers of > 1 mm length. It appears that in seawater fiber abundances of about seven fibers ml⁻¹ did not have a negative effect on feeding of either *E. pileatus* or *D. gegenbauri*. As doliolids and copepods remove plastic fibers from seawater by packing them into their pellets, they might play a role in the reduction of microplastic pollution and the microplastic transfer from the water column to the seafloor. Calanoid copepods may limit ingesting fibers by not perceiving them, as compared to doliolids which do not seem to be able to avoid ingesting them.

The occurrence of microplastic particles in the ocean has been observed for decades (e.g., Andrady 2011). Initially, they were defined as smaller than 5 mm (Arthur et al. 2009). They occurred in the open ocean at high abundances in the range of 1–10 mm when collected with a 200 µm mesh net (Cozar et al. 2014). Those authors pointed out that our knowledge on particles < 1 mm is limited. Quantification of smaller sizes (> 0.7 µm) was achieved by Di Mauro et al. (2017) collecting with Niskin bottles and filtering on glass fiber filters. Their microscopic filter examination provided concentrations of microplastics of > 100 L⁻¹. Recently, Brandon et al. (2020) quantified < 333 µm microplastic particles in surface seawater samples and salp gut contents, using a new microscopic technique, and also those of > 333 µm length in the water column. Their results, from nearshore to offshore Pacific Ocean, revealed concentrations of microplastic particles from near 4000 to about 15,000 L⁻¹, mainly short fibers of an average of 60 µm length. Those small fibers are of a size which could be readily ingested by juveniles and adults of marine planktonic copepods.

There have been quite a few studies on the influence of microplastics on aquatic invertebrates, especially zooplankton (e.g., Cole et al. 2015; Coppock et al. 2019; Botterell et al. 2019). Among the zooplankton are the planktonic copepods which are the most abundant metazoans on our planet (Fryer 1986). Early it was shown that various species of planktonic copepods were feeding on polystyrene beads, but so did also doliolids (Tunicata, Thaliacea) and euphausiids (Cole et al. 2013). Later we learned that polystyrene beads led to a decrease of feeding on phytoplankton (Cole et al. 2015, Table 1). Such polystyrene beads would also negatively affect feeding, growth and oxygen consumption of doliolids (Paffenhöfer and Köster 2020, Table 1). Recently Coppock et al. (2019, Table 1) offered nylon fibers and fragments (10 µm diameter, 40 µm length) to the planktonic copepod *Calanus helgolandicus* resulting in a substantial ingestion decrease of chain-forming diatoms and also of unicellular microalgae. Cole et al. (2019) recorded a 40% reduction of phytoplankton ingestion by *C. finmarchicus* when fibers were present (Table 1). The concentration of beads or fibers ranged from 37 to 100 ml⁻¹ (Table 1).
### Table 1 The effects of polystyrene beads and fibers on the feeding of marine zooplankton

| Food type | Food microplastic concentration | Volume of vials (L) | Feeders per bottle | Temperature (°C) | Duration (h) | Results | References |
|-----------|--------------------------------|---------------------|--------------------|------------------|--------------|---------|------------|
| *Calanus helgolandicus* | Polystyrene beads 20 µm diameter | 75 mL$^{-1}$ | 0.617 | 5 | 11 | 24 | Feeder *C. helgolandicus* ingested 11% fewer algal cells when exposed to beads and *T. weissflogii*, no effects on survival and egg production | Cole et al. (2015) |
| *Thalassiosira weissflogii* | | 250 µg C L$^{-1}$ | | | | | |
| *Dolioetta gegenbauri* | Polystyrene beads 20 µm diameter | 37 mL$^{-1}$ | 0.96–1.9 | 9–11 | 20 | 23 two time-series | Feeding rates on phytoplankton decreased as did rates on growth and oxygen consumption | Paffenhöfer and Köster (2020) |
| Mixture of *T. weissflogii* and *I. galbana* | Offering beads and phytoplankton together | 67 µg C L$^{-1}$ | | | | | |
| *Calanus helgolandicus* females | Nylon fibers 10 µm diameter, 40 µm length | 100 mL$^{-1}$ | 0.615 | 5 adult females | 11 | 24 | Reduction of feeding rates on phytoplankton in the presence of fibers | Coppock et al. (2019) |
| Mixture of *Dunaliella* sp., *Prorocentrum* sp., *Thalassiosira rotula* | | 120 µg C L$^{-1}$ | | | | | |
| *Calanus finmarchicus* copepodid V | Nylon fibers 10 µm diameter, 30 µm length | 47 mL$^{-1}$ | 1.15 | 10 | 8.7 | 4 days | 40% reduction of phytoplankton ingestion in the presence of fibers | Cole et al. (2019) |
| Mixture of *Dunaliella* sp., *T. rotula*, *S. trochoidea* | | 327 µg C L$^{-1}$ | | | | | |
the accompanying phytoplankton concentrations from 67 to 327 µg C L$^{-1}$ (Table 1). As to the effects of microplastic particles on marine zooplankton, a review by Botterell et al. (2019) indicated that negative effects were reported in 45% of the studies, while in 14% (three studies) no effects were found.

Environmental observations revealed that fibers often predominate as microplastics in the ocean (e.g., Cole et al. 2011; Desforges et al. 2014). This led us to our present study, partly renewed daily removing most of the accumulated fecal pellets, following cultivating details from Paffenhöfer and Gibson (1999). We hypothesize (i) that nylon fibers at their low numerical concentration would not affect feeding rates of calanoid copepods and doliolids at near average environmental diatom abundances and (ii) that fecal pellet content of calanoids and doliolids would differ due to differences of feeding behavior when fibers and diatoms are offered simultaneously.

**Material and Methods**

**Zooplankton Collection**

The zooplankton species *Eucalanus pileatus* and *Dolioletta gegenbauri* were collected on a cruise on 29 January 2020 near the 40 m isobath on the US southeastern shelf at temperatures of 18 °C. The zooplankton were collected with a net of 0.5 m mouth diameter, 200 µm mesh size and a 4-L codend, being towed at near 0.5 m s$^{-1}$ from near surface to near bottom to the surface. Directly after collection, doliolids and copepods were placed into 3.8 L glass jars which were then fastened to a plankton wheel moving at near 0.5 r.p.m. in an on-board laboratory adjusted to 20–21 °C. At the Skidaway Institute of Oceanography, those jars were placed on a plankton wheel in an environmental room at 20 °C and a light–dark cycle of 12 h:12 h. Both zooplankton species were offered concentrations of the flagellates *Isochrysis galbana*, *Rhodomonas* sp. and the diatoms *Thalassiosira weissflogii* and *Rhizosolenia alata* at average levels of near 40–60 µg C L$^{-1}$.

**Culture Conditions**

To obtain animals for our experiments copepods and doliolids had to be cultured under controlled conditions. Several females of *Eucalanus pileatus* were placed in 3.8 L jars on a rotating wheel being offered *T. weissflogii* and *R. alata* at an average level of about 50 µg C L$^{-1}$, and reproduced readily nauplii that were grown to adult females offering the above-mentioned mixture. To obtain gonozooids of *D. gegenbauri* to start our experiments, we cultured this species in our environmental laboratory at 20 °C on a plankton wheel through its life cycle (see Paffenhöfer and Gibson, 1999, for details), offering the previously mentioned phytoplankton species at total average concentrations of 40–60 µg C L$^{-1}$. Concentrations of the different food organisms were quantified daily using a Beckman Coulter Multisizer IV with a 140 µm orifice diameter tubing. The doliolids were cultured in seawater of 36.0‰ (parts per thousand) salinity; this water was partly renewed daily removing most of the accumulated fecal pellets, following cultivating details from Paffenhöfer and Gibson (1999).
Preparation of Microfibers

We selected nylon fibers for our experiments since they are among the most abundant microplastic particles in nature (Cole 2016). The fibers were prepared for these experiments following the protocol developed by Cole (2016). Transparent nylon fibers (polyamide 6,6) of 10 µm width (Goodfellow GmbH, Hamburg, Germany) were aligned, embedded in a water-soluble freezing solution (Neg 50™ Thermo Scientific, UK), and frozen at −70 °C for at least 1 h. The frozen gel block containing the aligned fibers had a length of 12 cm and was cut with a scalpel into gel blocks with a length of 1 cm. After eight (2 × 4) of those 1 cm long gel blocks were 90°-oriented to the surface of an aluminum holder and closely placed together to a large block, they were embedded in an additional freezing solution and frozen at −70 °C for 1 h. The frozen gel block containing the fibers was sliced with a stainless steel microtome knife (5° angle) into about 300 µm thick sections in a Bright cryostat microtome (Huntington/Cambridgeshire, UK) at −25 °C. Frozen sections were collected and thawed in ultrapure water heated at 60 °C to release the fibers. Then, the fibers were filtered on polycarbonate filters (pore size 5 µm, diameter 47 mm) and washed with ultrapure water. Fiber-loaded filters were stored at −20 °C until experimental use. We had decided on a nominal fiber length of 300 µm because Desforges et al. (2014), using as their smallest collection mesh 64 µm, mentioned that the most abundant size fraction of microplastic particles was that of 100–500 µm (fibers and plastic fragments).

Microfiber Counts and Sizes

Before experimental start, each fiber-containing filter was inspected for air contamination (e.g., fibers from other sources) and an even distribution of the fibers on the filter surface under a LEICA MZ 12 stereomicroscope (Leica Microsystems, Wetzlar, Germany). Filters that contained foreign fibers and/or revealed an uneven distribution of fibers were not used. For the determination of the number of 300 µm long fibers per filter, 20 microphotographs per filter were taken at 40 fold magnification with a digital 10 MP camera (ISH 1000; Tuscen Photonics, Fuzhou, China) connected to the stereomicroscope. The exact length of approximately 200 fibers was determined on each of five polycarbonate filters (diameter of 47 mm) using the software MikroCamLab II. The fiber length distribution of 1060 microtome-cut fibers (Fig. 1) shows that 42% of the fibers had a length of ≥ 300 to < 350 µm, 21% and 18% of fibers were in the size classes ≥ 250 to < 300 µm, and ≥ 350 to < 400 µm, respectively. The average length of the fibers was 336 µm (standard deviation 91). We observed that we had not always succeeded to produce fibers with the nominal length of 300 µm. Some fibers escaped sectioning and had double or triple length of the adjusted length. Fibers with multiple size (> 600 µm) contributed less than about 2% of the total number of cut fibers.

Experimental Design

All experiments were carried out at 20 °C on a plankton wheel running at 0.3 r.p.m. at a 12 h/12 h light–dark cycle. We offered the elongated diatom R. alata and nylon fibers together at similar particle concentrations each ranging on
average between 5.7 and 9.0 ml⁻¹. *R. alata* concentrations resembled those found in situ (Paffenhöfer, unpubl. results). The diatom cells were of 35–38 µm width and an average length of 250 µm. The fibers were of 10 µm width and on average 300 µm long, thus being of similar length as the diatoms. Our initial experiment with *E. pileatus* was run for 18 h, and the remaining five for 6.0 to 6.1 h. All experiments were run in 960 ml screw cap bottles with five young females, i.e., just molted adult females. All five experiments with *D. gegenbauri* were run for 6.0 to 6.1 h with five gonozooids of an average length of 4.5 to 4.9 mm. Those sizes were chosen as their average biomass carbon was 16–18 µg and similar to that of young females of *E. pileatus*. Controls were run in 960 ml jars to quantify the growth rate of and the feeding rates on *R. alata*. We decided not to offer the fibers by themselves as there are always living phytoplankton particles in the epipelagic ocean. Chlorophyll a levels in surface waters of the open ocean are always near or above 0.04 µg L⁻¹. Phytoplankton and fiber concentrations were quantified by inverted microscope counts. For each experiment, we counted fibers and *R. alata* cells in three settling chambers of 25 ml each at the start and end of each experiment. In parallel we ran control feeding experiments in 960 ml jars at same feeder and *R. alata* concentrations (five *E. pileatus* females, or five *D. gegenbauri* gonozooids of 4.5 to 4.9 mm length) over same feeding periods, but no fibers added!

Feeding/clearance/ingestion rates were calculated according to Frost (1972) who described all calculations in detail. The clearance rate is the amount of particles ingested by an individual zooplankter per hour or day; the ingestion rate is the amount of particles ingested by an individual zooplankter per hour or day. The lengths and stages of the experimental animals were determined at the beginning and end of each experiment. The length values of doliolid gonozooids were transformed to biomass carbon values using the equation weight (µg C) = 0.4643 length (mm)².³¹¹⁹ (Gibson and Paffenhöfer 2000). No copepods or doliolids died during our experiments. All escaped well at the start and end of each experiment.

**Pellet-Associated Microfibers**

Microphotographs of fecal pellets released by *E. pileatus* and *D. gegenbauri* were taken with a digital camera (ISH 1000, Tuscen Photonics, Fuzhou, China) connected to an inverted microscope DIAVERT (Ernst Leitz GmbH, Wetzlar, Germany). All pellets detected in settling chambers were documented at tenfold magnification. The number of fibers in pellets was determined on selected microphotographs. Length and width of fecal pellets were measured using an microscope calibration slide stage micrometer (0.01 mm = DIV). The software TCapture version 5.1 was used for photodocumentation.

**Statistics**

Statistical analyses were made according to Zar (1974) applying the Kruskal–Wallis Test. This is a nonparametric single factor analysis of variance by ranks for *K* ≥ 2 independent samples (Conover 1980).

**Results**

**Particle Abundances and Feeding Rates**

As fibers are considered the dominant microplastics in subsurface waters of the northeast Pacific Ocean (Desforges et al. 2014), we chose them as microplastics representatives. While the average length of the *R. alata* cells at 250 µm was close to that of the fibers at 300 µm, the latter’s width at 10 µm was much lower than that of *R. alata* at 35–38 µm. The average volume of a *R. alata* cell was 0.250×10⁶ µm³ and that of a fiber 0.235×10⁵ µm³. The copepod *E. pileatus* readily consumed *R. alata* cells and fibers (Fig. 2A, B). Average experimental concentrations of *R. alata* were 7.71 ± 0.83 Standard Error SE cells ml⁻¹, corresponding to a biomass volume of 1.930×10⁶ µm³ ml⁻¹, or a biomass carbon of 28.9 µg C L⁻¹. Concentrations and volume of fibers amounted to 8.66 ± 0.39 SE fibers ml⁻¹ and a volume of 0.204×10⁶ µm³ ml⁻¹, respectively (Fig. 2 A). The average clearance rate on *R. alata* was at 21.4 ml copepod⁻¹ h⁻¹, (SE in Fig. 2 A as also for future clearance rates) significantly higher than that on fibers at 13.1 ml copepod⁻¹ h⁻¹ (Kruskall–Wallis test, *p* < 0.05). It was not possible to count the number of pellets produced by the copepods because they had attacked and damaged numerous pellets. The average clearance rate on *R. alata* in the presence of fibers (21.4 ml female⁻¹ h⁻¹) did not differ significantly from that of the control (no fibers, 21.5 ml copepod⁻¹ h⁻¹, Kruskal–Wallis test, *p* > 0.05, Fig. 2A).

Gonozooids of *Doloioletta gegenbauri* were feeding on *Rhizosolenia alata* at an average of 8.95 ± 0.91 (SE) cells ml⁻¹ (corresponding to 2.20×10⁶ µm³ ml⁻¹ or 33.6 µg C L⁻¹) and at 5.71 ± 1.65 fibers ml⁻¹ (corresponding to 0.134×10⁶ µm³ ml⁻¹; Fig. 2A). The clearance rates on fibers were at 27.2 ml gonozooid⁻¹ h⁻¹ significantly higher than on *R. alata* at 18.7 ml gonozooid⁻¹ h⁻¹ (Kruskall–Wallis test, *p* < 0.05). The average clearance rate of a gonozooid of *D. gegenbauri* on *R. alata* in the presence of fibers did not differ significantly from the clearance rate of the controls, i.e., when only *R. alata* was offered (Kruskall–Wallis test, *p* > 0.05, Fig. 2A). Ingestion rates were compared in relation to particle concentration (Fig. 2B). The different particle
volumes of fibers and diatom cells resulted in vastly different ingestion rates (Fig. 2B) which were about one order of magnitude higher on *R. alata* than on the fibers. While *D. gegenbauri* ingested about 10% more *R. alata* than *E. pileatus* (partly due to slightly higher *R. alata* abundance), the ingestion rate on fibers by numbers was 31% higher for *D. gegenbauri* than for *E. pileatus*. Enhanced fiber ingestion rates of *D. gegenbauri* occurred despite having a fiber concentration which was about one third lower than that for *E. pileatus* (Fig. 2B). No Kruskal–Wallis test was performed because volumetric fiber concentrations were vastly different.

The number of doliolid pellets produced per gonozooid and hour ranged from 4.2 to 6.2. Copepod pellets could not be counted because many of them had been damaged by having been captured by *E. pileatus*.

**Visible Fibers in Pellets**

Food particles enter the copepod’s gut after usually being broken in the esophagus while they are not broken entering the doliolid’s gut (Paffenhöfer and Köster 2005). We decided to document the contents of fecal pellets of both species. Pellets of *E. pileatus* adult females had an average length of 600 µm and 61 µm width (Fig. 3). The average number of fibers per pellet was 3.5 including some broken ones (Table 2). Any other structures could not been discerned. The copepods had attacked during these experiments many of their pellets and had broken them.

Pellets of *D. gegenbauri* varied widely in dimensions and contents (Fig. 4): Many pellets were largely flat and pillow-like as the food particles are collected by the doliolid on the continuously-produced mucous net which is passed through the esophagus; this is followed by the digestion process and then pellets are released often in the form of a pillow. Of the morphologically diverse pellets produced by the rather small gonozooids of 4.5–4.9 mm length we are presenting a representative group (Table 3, Fig. 4). The number of fibers per pellet ranged from 3 to 16, while the number of *R. alata* cells ranged from 12 to 28 (all largely digested). Several pellets’ fibers included some far longer than the average length of about 300 µm, being as long as 1.45 mm (Fig. 4E), and also being curved (Fig. 4C–E, H). This reveals that the gonozooids feeding process can cover a wide range of particle lengths (without destruction) if the fiber width does not extend much beyond the about 60 µm wide esophagus.

**Discussion**

**Abundance of Microplastics in the Ocean and in Experiments**

In experimental laboratory studies on the effect of microplastics on marine zooplankton the microplastics concentration ranged from 111 to 314 µg dry weight L⁻¹ (Table 4). The number of particles ranged from 7 to 100 fibers ml⁻¹ and from 37 to 75 beads ml⁻¹ (Table 4). In the ocean
concentrations of microplastics were about two to three orders of magnitude lower by weight (Lenz et al. 2016, Table 4). These ocean observations were based on different methods of collection and analysis: Desforges et al. (2014) used 64 µm mesh to collect microplastics, and Di Mauro et al. (2017) filtered water collected with Niskin bottles on glass microfiber filters with a retention capacity of 0.7 µm followed by microscopic quantification. The most recent study used the most advanced microscopic technology (Brandon et al. 2020) resulting in the highest abundances of microplastic particles (5 ml⁻¹) found so far. We had calculated the dry weights of microplastics from these ocean studies from pictures (Di Mauro et al. 2017) and from two-dimensional values, provided in the papers. The numerical microplastic concentration applied in our present experimental study is numerically at seven fibers ml⁻¹ similar to the findings by Brandon et al. (2020) for the California Current at near five fibers ml⁻¹; however, the majority of the ocean fiber dimensions are far smaller resulting in dry weight amounts of two orders of magnitude lower.
Clearance Rates on Microplastic Particles and Phytoplankton

Whereas the first four papers of Table 4 revealed effects of microplastic on processes of zooplankton, as shown in Table 1, our recent study offering only about seven fibers ml\(^{-1}\) showed no effects of those fibers on clearance rates of calanoid copepods and doliolids at environmental abundances of phytoplankton (Fig. 2A). Botterell et al. (2019) in their review found that 45% of the papers showed negative effects of microplastics on zooplankton rates while only 14% showed no effects. Clearance rates of copepods and doliolids of similar weight feeding simultaneously on *Rhizosolenia alata* and fibers of similar length differed. While clearance rates by *E. pileatus* and *D. gegenbauri* on *R. alata* did not differ significantly (\(p > 0.05\), Kruskal–Wallis) *D. gegenbauri*’s rates on fibers were twice as high as those of *E. pileatus* (Fig. 2A). This will be discussed in the paragraph on Perception.

Our results from the experiments (*R. alata* in the presence of fibers) and controls (only *R. alata* offered) revealed that clearance rates of *E. pileatus* on *R. alata* did not differ significantly. The same was found for gonozoids of *D. gegenbauri* (Fig. 2A). These findings imply that the fibers at the experimental levels did not affect clearance rates of both zooplankton species.

Perception of Particles by Calanoid Copepods and Doliolids

Phytoplankton cells were perceived in the calanoid’s feeding current by chemoperception (e.g., Strickler 1982; Paffenhofer and Lewis 1990). When beads or fibers are offered alone to calanoids they are not or hardly ingested as they do not provide a chemical signal as phytoplankton cells do (Paffenhofer and van Sant 1985). However, beads were ingested when they arrived simultaneously with diatom cells at the copepod’s mouth (video observations, Paffenhofer and Van Sant 1985). As that co-occurrence does not happen often the feeding rates on beads were lower than on diatoms: At an abundance of 0.3 mm\(^3\) L\(^{-1}\) of both, *Thalassiosira weissflogii* of 12 µm width and beads of 20 µm diameter, being offered together, *Eucalanus pileatus* copepodid stage V (C V) ingested 2.7 × 10\(^6\) µm\(^3\) d\(^{-1}\) of beads and 50 × 10\(^6\) µm\(^3\) d\(^{-1}\) of the diatom. The ratio between ingestion of diatoms and beads was 18.5 to 1. These observations are being supported by Donaghay and Small (1979): when the diatom *T. fluviatilis* was offered together with 20 µm spheres very few spheres were eaten. Huntley et al. (1983) stated that bead consumption was low in all of the experiments when they offered polystyrene beads of 16.5 µm diameter to *Calanus pacificus* copepodid stage IV (C IV). While the diatom *T. weissflogii* was ingested at a clearance rate of 2.5 ml copepodid IV\(^{-1}\) h\(^{-1}\), the beads were eaten at a rate of only 0.3 ml copepodid IV\(^{-1}\) h\(^{-1}\). Nauplii of the copepod *Calanus pacificus* did not ingest polystyrene beads when offered together with phytoplankton (Fernandez 1979).

As many calanoids seem to have the ability to perceive food particles by chemosensory (e.g., Paffenhofer and Loyd 2000, showing chemosensory structures in setae of maxillipeds and second antennae) various particles in the ocean might develop biofilms over days and weeks (e.g., Phuong et al. 2016; Vroom et al. 2017). Polystyrene beads which had been kept in seawater for three weeks were ingested at a higher rate than fresh beads by the calanoid copepods *Calanus finmarchicus* and *Acartia longiremis* (Vroom et al. 2017). The authors assume that over that period a biofilm developed on those beads which provided

| Table 2 | Fecal pellets produced by *Eucalanus pileatus* females feeding on *Rhizosolenia alata* cells in the presence of nylon microfibers of near 10 µm width and about 300 µm length |
|---|---|---|---|---|
| Pellet No | Pellet length (mm) | Pellet width (mm) | Pellet volume (mm\(^3\)) | Number of fibers pellet\(^{-1}\) |
| A | 0.72 | 0.075 | 0.0032 | 6 |
| B | 0.63 | 0.067 | 0.0022 | 5 |
| C | 0.63 | 0.067 | 0.0022 | 4 |
| D | 0.43 | 0.050 | 0.0008 | 2 |
| E | 0.62 | 0.067 | 0.0022 | 3 |
| F | 0.66 | 0.057 | 0.0017 | 2 |
| G | 0.44 | 0.050 | 0.0009 | 2 |
| H | 0.63 | 0.058 | 0.0017 | 4 |
| Mean value | 0.60 | 0.061 | 0.0019 | 3.50 |
| S.D | 0.10 | 0.008 | 0.0007 | 1.41 |
| S.E | 0.03 | 0.003 | 0.0003 | 0.50 |
| Range | 0.44–0.72 | 0.050–0.075 | 0.0008–0.0032 | 2–6 |

Pellet numbers refer to microphotographs of pellets in Fig. 3. SD Standard Deviation, SE Standard Error
a chemical signal to those copepods. The possibility of chemical signals triggering the ingestion of microplastics has been supported by two studies: Procter et al. (2019) offered small nylon fibers (10 µm × 30 µm), which had been exposed to DMS (dimethyl sulfide) for six hours, to the calanoid Calanus helgolandicus. The copepods ingested significantly more of the DMS-treated-fibers than untreated ones. The results of a similar study by Botterell et al. (2020), also involving the feeding-current-creating C. helgolandicus, supported the findings of the previous study.

There are also results which appear not to agree with our earlier presented findings/interpretations: Coppock et al. (2019) offered microplastics at 100 ml⁻¹ (fibers or spheres) in 50 ml glass bottles to single C. helgolandicus females for 24 h. There was no mention of phytoplankton addition. All pellets revealed numerous microplastics (their Fig. 2) which would mean their ingestion occurred without phytoplankton

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**Fig. 4** Fecal pellets of gonozooids of Doliolettiga gegenbauri having ingested the diatom Rhizosolenia alata and fibers of 10 µm width and of about 0.3 to 1.45 mm length. The scale bar represents 100 µm.
being present. However, their Fig. 2c, when feeding on fibers, also shows several Prorocentrum micans cells in that pellet indicating that phytoplankton was offered with the plastics. This observation leads to the conclusion that phytoplankton could have contributed to the ingestion of fibers in those specific studies.

The feeding of doliolids does not seem to be affected by perception processes as long as the respective particles can enter their mouth (e.g., Deibel 1985, 1998; Köster and Paffenhöfer 2017). That appeared to be the case when we offered 300 µm long fibers and R. alata, similar in length to the fibers, simultaneously to gonozooids of D. gegenbauri. Yet fibers were cleared at a rate which was about 50% higher than that on diatoms (Fig. 2A). That may occur because the fibers have a smaller diameter than the R. alata cells and have no spikes at the end of each cell, making it easier to retain them on the mucous net than the diatom cells; and secondly, quite a few of the R. alata cells are dividing or in chains of two cells extending over 500 µm in length, and therefore are not that readily retained on the doliolid’s mucous net.

### Ingestion Rates

Ingestion rates provide the actual amounts of food particles entering a feeder’s gut. Offering the fibers together with R. alata cells (~ 10 times the volume of fibers, Fig. 2B) resembles the relatively small numerical amount of fibers in situ, and reflects phytoplankton abundance in neritic intrusion waters (e.g., Yoder et al. 1985). The ingestion rates on fibers are less than 10% of those on R. alata, being determined by the size of the ingested particles (Fig. 2B). Not observing

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**Table 3** Fecal pellets produced by Dolioletta gegenbauri gonozooids (4.5 to 4.9 mm) feeding on Rhizosolenia alata cells in the presence of nylon microfibers of near 10 µm width and about 300 µm length

| Pellet No | Pellet area (mm²) | Number of fibers pellet⁻¹ | Number of R. alata cells pellet⁻¹ | Comments                        |
|----------|------------------|---------------------------|----------------------------------|---------------------------------|
| A        | 0.065            | 3                         | 12                               | Most R. alata cells aligned    |
| B        | 0.073            | 6                         | 14                               | Right pellet                   |
| C        | 0.136            | 4                         | 27                               | Elongated, curved fiber        |
| D        | 0.115            | 8                         | 12                               | Loose pellet, elongated fiber   |
| E        | 0.234            | 9                         | 28                               | Elongated fiber of 1.45 mm length |
| F        | 0.160            | 9                         | Counting not possible            | Cells out of focus             |
| G        | 0.085            | 5                         | 18                               | Most R. alata cells aligned    |
| H        | 0.105            | 16                        | Counting not possible            | Elongated fiber                |
| Mean     | 0.122            | 7.5                       | 18.5                             |                                 |
| S.D      | 0.052            | 3.8                       | 6.7                              |                                 |
| S.E      | 0.019            | 1.4                       | 2.9                              |                                 |
| Range    | 0.065–0.234      | 3–16                      | 12–28                            |                                 |

Pellet numbers refer to microphotographs of pellets in Fig. 4. SD Standard Deviation, SE Standard Error

**Table 4** Dry weight concentrations of microplastic particles in the ocean and in experiments (experiments from Table 1)

| Type of microplastics | Diameter (µm) or dimensions (µm × µm) | Concentration mL⁻¹ | Dry weight µg L⁻¹ | References                                      |
|-----------------------|---------------------------------------|--------------------|-------------------|------------------------------------------------|
| **Experiments**       |                                       |                    |                   |                                                |
| Polystyrene beads     | 20                                    | 75                 | 300               | Cole et al. (2015)                              |
| Polystyrene beads     | 20                                    | 37                 | 148               | Paffenhöfer and Köster (2020)                   |
| Fibers                | 10×40                                 | 100                | 314               | Coppock et al. (2019)                           |
| Fibers                | 10×30                                 | 47                 | 111               | Cole et al. (2019)                              |
| Nylon fibers          | 10×300                                | 5.7–9.0            | 165               | Köster and Paffenhöfer (this paper)             |
| **Ocean**             |                                       |                    |                   |                                                |
| Particles             | 10×606                                | max. 0.009         | 0.43              | Pacific                                        |
| Fibers                | n.d                                   | 0.090              | 0.24              | Gulf of Mexico                                  |
| Particles             | 10                                    | n.d                | 10⁻³–1           | Compilation of field data                       |
| Small fibers          | 2.7×60 (from their Fig. 2)            | 5                  | 1.7               | California Current                              |
| Large fibers          | 9.5×600                               | 0.01–0.1           | 0.43–4.3          | California Current                              |

References:

- Cole et al. (2015)
- Paffenhöfer and Köster (2020)
- Coppock et al. (2019)
- Cole et al. (2019)
- Desforges et al. (2015)
- Di Mauro et al. (2017)
- Lenz et al. (2016)
- Brandon et al. (2020)
any obvious fiber effects on feeding rates in our study might change when phytoplankton abundance and cell size is much lower as observed in oceanic waters like the North Atlantic Subtropical Gyre (NASG, e.g., Paffenhofer et al. 2003). Then, the volumetric concentration of fibers (Brandon et al. 2020) would be much closer to that of phytoplankton and heterotrophic nanoflagellates (e.g., Sherr and Sherr 2009).

Also the average size of small fibers in Brandon et al. (2020) of ~2.7 µm width and ~60 µm length represents a volume of 340 µm³ which is close to a dinoflagellate of 9 µm diameter, encountered in the NASG. Food perception by calanoids increases with decreasing food concentration (e.g., Paffenhofer and Lewis 1990). Thus, smaller food sizes of low abundance could still lead to relatively frequent perception, followed by ingestion, probably accompanied by co-ingestion of a similar-sized fiber. This assumption of significant ingestion of nanoplankton cells (2–20 µm diameter) was shown earlier for females of the calanoid Paracalanus aculeatus (Paffenhofer et al. 2003).

**Feeding on Microplastic Particles in the Ocean**

Desforges et al. (2014, 2015) found on average 2080 plastic particles m⁻³ (majority ranged from 100–500 µm length) and 28 large calanoid copepods of Neocalanus cristatus m⁻³ in the northern Pacific Ocean. That copepod species (Copepodid stage V) cleared 290 ml copepod⁻¹ d⁻¹ of the diatom Thalassiosira weissflogii at 11 °C (Frost et al. 1983). Multiplying that rate with the number of N. cristatus m⁻³ results in 8120 ml cleared d⁻¹ m⁻³. In the Pacific Ocean 8120 ml contain on average 17 microplastic particles of ~600 µm average length (Desforges et al. 2014). Feeding at 290 ml copepod⁻¹ d⁻¹ ten copepods of N. cristatus would have encountered in situ 6.0 microplastic particles per day. Desforges et al. (2015) found a total of only 25 plastic particles in the guts of 960 N. cristatus, i.e., one particle in 38 copepods. Why had been so few ingested? In the Subarctic Pacific there is a scarcity of larger perceivable food particles, as compared to the US southeastern shelf. This limits the co-occurrence of plastics and food particles in N. cristatus’ feeding current which would be necessary for the ingestion of a plastic particle (e.g., Paffenhofer and Van Sant 1985). Therefore, effects of microplastic particles on feeding-current-producing calanoid copepods might be limited in those parts of the ocean where their ingestion depends on the simultaneous occurrence of perceived food particles like phytoplankton cells. While feeding-current producing copepods in the northeast Pacific Ocean do hardly appear to ingest microplastic particles, doliolids like Dolioioletta gegenbauri could ingest them readily as they are occurring intermittently in abundance on the west coast of the USA and Canada (e.g., Mackas et al. 1991). This would imply that a greater abundance of fibers might affect doliolids more than calanoid copepods.

It appears that fibers at the offered sizes and abundance do not affect the feeding of calanoids and larger doliolids, yet occur in different sizes and shapes of fecal pellets which again will be encountered as potential food particles by different zooplankton taxa.

**Fibers in Fecal Pellets**

Evaluating fecal pellets of Eucalanus pileatus and Dolioioletta gegenbauri, we observed major differences. Pellets of the copepod were compact and contained destroyed and digested diatom cells and fibers (Fig. 3). Doliolid pellets contained largely digested but physically undamaged diatom cells and fibers (Fig. 4). Those pellets can be compact, i.e., diatoms close together with few fibers (Fig. 4A, B, C, G), or of similar size but more fibers including long ones (Fig. 4D, E), and also pellets with numerous fibers and closely bunched diatoms (Fig. 4E, F H). While copepods like E. pileatus will find it difficult to ingest fibers of 1 mm or longer (pers. observation) doliolids appear to experience no difficulties in ingesting such larger fibers. Most of the fibers shown in Di Mauro et al. (2017, their Fig. 4) could be ingested by doliolids but most likely not by calanoid copepods. The doliolid pellets also contain on average more fibers per pellet than pellets produced by calanoids of similar size as doliolid zooids (Figs. 3 vs. 4). Doliolids, of similar weight as copepods, not only have more fibers in their pellets but also produce more pellets per hour (E. pileatus ~ 2 pellets h⁻¹, D. gegenbauri ~ 4 pellets h⁻¹). In summary, as doliolids remove far more fibers than copepods they also could be more affected by microplastics than copepods.

In comparison with the copepod pellets the doliolid pellets will sink slowly (Patonai et al. 2011). At a specific weight of very close to 1 g cm⁻³, the fibers should not affect the sinking rates of those pellets that much. These pellets can readily serve as food for other doliolids (Küster and Paffenhofer 2017) and also copepods as they contain organic matter with a high nutrient value. However, the food value of those doliolid pellets could be reduced in the presence of fibers and negatively affect the ingestion of those pellets.

We might obtain insights on the effects of such microplastic particles, as quantified by Brandon et al. (2020), by offering such in situ particulate matter over hours to days to juveniles and adult stages of the often abundant smaller calanoids like the genera Clausocalanus and Paracalanus. Particles of near 3 µm width and about 60 µm length, as found by Brandon et al. (2020), could be readily ingested by nauplii and older stages of such genera. That probably would occur only in the presence/co-occurrence of phytoplankton and heterotrophic cells. In the open ocean small phytoplankton (< 10 µm ESD) and heterotrophic cells (< 10 µm ESD)
dominate (Paffenhöfer et al. 2003). These food particles are readily perceived and ingested by early copepodid stages of small calanoids (e.g., Berggreen et al. 1988). Calanoid juveniles are more sensitive to food limitations than adults as shown by mortality rates (e.g., Paffenhöfer 1970). Such microplastics might have more likely an effect on those stages if the abundance of the accompanying living food particles is low as found in oceanic waters.

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Data Availability The data supporting the findings of this study are available within the article.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

Ethical Approval This article does not contain any studies with human participants or animals performed by any of the authors.

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