Science of the Total Environment 906 (2024) 167301

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Microcosm study of the effects of polyester microfibers on the indigenous marine amphipod (*Cyphocaris challengeri*) in the Strait of Georgia (BC, Canada)

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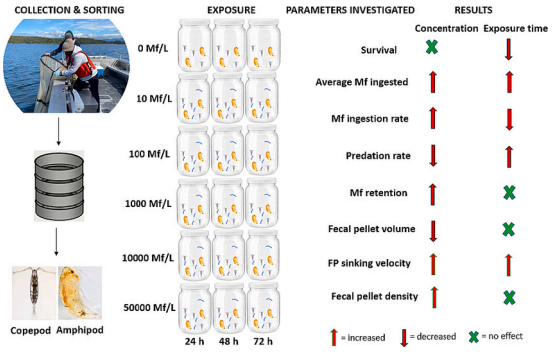
HIGHLIGHTS

- Amphipod ingested PET microfibers (Mf) (14.17 μm- diameter and 357.21 μm- length).
- Average number of ingested Mf and ingestion rate increased with Mf concentration.
- More than 50 % of exposed amphipods egested Mf after 48 h depuration.
- Predation rate of copepods by amphipods decreased with Mf concentration.
- Fecal pellet sinking velocities and densities increased with Mf concentration.

GRAPHICAL ABSTRACT



ARTICLE INFO

Editor: Yolanda Picó

Keywords:
Microfiber
Zooplankton
Ingestion
Retention
Predation
Fecal pellet

ABSTRACT

Microplastics (MP) remain contaminants of great concern in the ocean because of their abundance, prevalence, and threat to marine organisms. Still, there is a great need for studies on the impact of MP on marine zooplankton. Here, we investigated the effects of polyethylene terephthalate (PET) microfibers (Mf) on the survival, Mf ingestion and retention, predation, and fecal pellets (FP) of the marine amphipod (*Cyphocaris challengeri*) at environmentally relevant concentrations (0, 10, 100, 1000, 10,000 and 50,000 Mf·L^{-1}) and varied exposure time (24, 48 and 72 h). Our study demonstrated that exposure of *C. challengeri* to PET Mf did not affect their survival. The average number of ingested Mf and the Mf ingestion rate increased significantly with Mf concentrations. Nonetheless, the Mf ingestion rates by *C. challengeri* decreased significantly between 24 and 72 h in the two highest Mf treatments (10,000 and 50,000 Mf·L^{-1}), suggesting careful rejection of the Mf or reduced feeding activity. Indeed, PET Mf significantly reduced the copepod feeding rate of the amphipods at Mf concentrations ≥1000 Mf·L^{-1} after 24 and 48 h of exposure duration. Over time, prey intake reduction in amphipods due to Mf ingestion could affect their reproductive outcome, growth, development, and cellular and ecosystem function. The encapsulation of PET Mf into the FP of *C. challengeri* significantly increased the FP density and sinking velocities, ultimately doubling the transfer rate of the FP from the surface waters to the sediments in SoG. Conversely, ingesting PET microfibers and their incorporation in FP will potentially enhance the role of

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https://doi.org/10.1016/j.scitotenv.2023.167301
Received 5 June 2023; Received in revised form 21 August 2023; Accepted 21 September 2023
Available online 25 September 2023

C. challengeri in the biological C pump and sequestration in SoG. Our study showed that changes in Mf concentration had a more significant effect on *C. challengeri* Mf ingestion and ingestion rate, prey consumption, FP density and sinking velocity than the exposure time.

1. Introduction

Microplastics (MP) have emerged as one of the most severe environmental threats due to their abundance, widespread distribution and similarity to food items of many organisms. The presence of MP has cut across all aquatic habitats (He et al., 2021; Maynard et al., 2021; Aves et al., 2022; Yang et al., 2022), and their ingestion has been reported in many organisms, including corals (Rotjan et al., 2019), zooplankton (Botterell et al., 2022), echinoderms (Iwalaye et al., 2020), molluscs (Abidli et al., 2019), fish (Steer et al., 2017), seabirds (Nam et al., 2021) and cetaceans (Zhu et al., 2019). Various laboratory studies have examined MP ingestion by vertebrates and invertebrates (Wegner et al., 2012; Setala et al., 2014; Watts et al., 2014; Setala et al., 2016). But, the effects of MP remain largely unknown on zooplankton, despite their essential functions in aquatic ecosystems (Cole et al., 2013; Cole et al., 2016; Wieczorek et al., 2019; Fueser et al., 2020). Marine zooplankton could be especially prone to ingesting MP, given the resemblance between MP and their prey, and the high abundance of MP in surface waters, where zooplankton migrate nightly for feeding (He et al., 2021). Therefore, zooplankton may be affected by the ingestion of MP and/or serve as a vector of MP to organisms in upper trophic levels (Desforges et al., 2015; Steer et al., 2017; Botterell et al., 2019).

The Strait of Georgia (SoG) is a semi-enclosed and productive estuary in the NE Pacific Canadian coast, with an area of 6800 km^2, a volume of 1100 km^3, and a mean and maximum depth of 161 m and 420 m, respectively (Mackas et al., 2013; Perry et al., 2021). The SoG ecosystem houses a great diversity of phytoplankton and zooplankton, as well as fish and marine mammals (Perry et al., 2021). Most commercial fish species (i.e., salmon, herring and groundfish) in the SoG depend on zooplankton for growth and survival during their earlier stages, and variation in zooplankton population has been linked to poor salmon recruitment (Mackas et al., 2004; Li et al., 2013; Mackas et al., 2013; Mackas and Beaugrand, 2010). Amphipods, our model organisms, are the third largest (i.e., 14 %) contributors to SoG zooplankton biomass (Li et al., 2013; Perry et al., 2021), highlighting their importance in this ecosystem. Furthermore, field studies have shown that zooplankton from coastal British Columbia are ingesting microplastics (Desforges et al., 2015; Mahara et al., 2022), but to date, no study has investigated the effect of MP on SoG indigenous amphipods.

There are a few laboratory studies on MP ingestion in both freshwater and marine amphipods species (Chua et al., 2014; Blarer and Burkhardt-Holm, 2016; Carrasco et al., 2019; Mateos-Cardenas et al., 2020; Rani-Borges et al., 2022). But these studies have some shortcomings, including (i) the environmental irrelevance of MP polymers types (polyamide, polyethylene, polystyrene), shapes (fragments and microsphere), and concentrations used, given that PET fibers are the most abundant polymer in aquatic environments, and their concentrations in situ are much lower than those in these studies (Coppock et al., 2019; Mahara et al., 2022; Zhang et al., 2022; Zheng et al., 2020); (ii) amphipods were starved before and/or during the exposure experiments, conditions that are unlikely to occur in nature. Starvation could magnify MP ingestion and its effects on exposed organisms (Jemec et al., 2016).

To the best of our knowledge, there are no studies on the effects of PET microfiber on marine amphipods. This is the first study to investigate the effects of MP fibers on marine amphipods, using environmentally relevant MP concentrations, polymers and shapes, in the presence of their natural preferred prey. We specifically examined the effects of varied PET Mf concentrations and exposure durations on the SoG indigenous amphipod *C. challengeri*. We determined changes in *C. challengeri* survival, Mf ingestion rates, Mf retention, and copepods predation rates, as well as the volume, sinking velocity and density of their fecal pellets.

2. Materials and method

2.1. Phytoplankton culture

One liter of 0.22 μm filtered, natural seawater collected from the Pacific Science Enterprise (PSEC), autoclaved and enriched with nutrients (8 mL 0.04 M NO_3, 2 mL 0.01 M PO_4, 98 μL Fe stock, 1 mL Vitamins and 1 mL of Trace Metal stock), was inoculated with 10 mL of stationary phase phytoplankton 4–5 days before the start of feeding experiments. The phytoplankton cultures were kept at 20 °C with a light intensity of 200 μmol quanta m^{-2} s^{-1}, allowing phytoplankton biomass to peak during the amphipod experiment. Phytoplankton growth was determined daily by measuring in-situ chlorophyll fluorescence with a Turner Designs 10-AU fluorometer.

2.2. Seawater collection

Seawater used for the feeding experiments was collected in 20 L carboys from the Pacific Science Enterprise Centre (PSEC), West Vancouver, and stored in a fridge. Particle-free seawater (i.e., phytoplankton, bacteria, zooplankton, and MP) was obtained by gravity filtering using a 0.8/0.2 μm nitrocellulose capsule filter (AcroPak PN12941).

2.3. Fibers making

To mimic the weathering of microfibers during domestic laundry washing, 100 % blue virgin PET fleece was first fragmented with scissors and then blended in Milli-Q (MQ) water, using a hand-held Braun kitchen blender. Fibers were then poured through stacked sieves (1000, 500, 250, 125 and 64 μm), and the Mf retained on the 64 μm sieve were collected and resuspended in 1-liter MQ. After that, a subsample of Mf (mL) in triplicates was taken and examined under a light compound microscope to determine their abundance, length (357.21 ± 222.81 μm), and diameter (14.71 ± 4.05 μm).

2.4. Sample collection and size fractionation

Zooplankton were collected using a plankton net (75 cm mouth diameter, 250 μm mesh and 250 μm cod end) at 140–160 m depth from MacDonald inlet (49.299° N, 123.672° W) in SoG on the morning of December 16, 2021, and January 24, 2022, onboard the Kraken, our departmental small research boat. The zooplankton were resuspended in 20 L buckets filled with natural seawater, placed in a cooler containing ice packs (Botterell et al., 2020), and transported to the EOAS Department at UBC within the shortest possible time. The samples were immediately size-fractionated at UBC using stacked sieves of 2000, 1000, 500 and 250 μm. Zooplankton between 250 and 500 μm were mostly copepods and were kept in aerated seawater in 8 L buckets in the dark at 10 °C and fed 50 mL of phytoplankton culture (i.e., ~16.0 $\mu g \cdot L^{-1}$ of chlorophyll *a* concentration) daily. Approximately 50 amphipods from the >1000 μm fraction—which was dominated by amphipods with few euphausiids—were placed in 20 L of filtered seawater (FSW) and were fed with 25 copepods per amphipod, before they were left to acclimate in a temperature-controlled room (10 °C), in the dark for 24 h.

2.5. Exposure experiment

The experiment was comprised of six Mf concentrations – 0, 10, 100, 1000, 10,000 and 50,000 Mf·L^{-1} (i.e., 0, 0.013, 0.135, 1.341, 13.406 and 67.02 mg·L^{-1} respectively), which were carefully selected to reflect a) present environmentally-relevant Mf concentrations (i.e., no >10,000 L^{-1}; Bucci et al., 2020), and b) expected Mf concentrations in year 2100 (i.e., 50,000 Mf·L^{-1}; Everaert et al., 2018). The experimental temperature of 10 °C matched the in-situ seawater temperature (~ 7.4 °C in Dec. 2021; 7.7 °C in Jan. 2022). To determine whether prolonged accessibility to Mf will have a greater impact on the amphipods, each Mf treatment had 3 exposure durations (24, 48 and 72 h) in triplicates. Five amphipods were placed in each 1 L jar, containing FSW and the assigned Mf concentration, and were immediately positioned on orbital shakers set at 80 rpm in the dark. The shakers ensured homogenous mixing of PET Mf, given that the PET microfibers are denser (1.23–2.30 g.cm-^{3}) than seawater (1.02–1.03 g.cm-^{3}) (Uddin et al., 2020). Each amphipod was fed 25 copepods daily, given our preliminary findings on the food ration required to keep healthy amphipods in the laboratory. To avoid stressing the organisms via exposure to light and air, every 2 days, 500 mL of seawater from the jars were filtered through a 64 μm sieve and replaced with fresh FSW. The content on the sieve was carefully rinsed into the experimental jars. The amphipods' mortality in the experimental jars was monitored daily. If dead amphipods were identified (i.e., characterized by lack of appendages movement and swimming), they were carefully removed with a Pasteur pipette. At the end of each exposure duration (i.e., 24, 48 and 72 h), samples for each parameter were collected as described below.

2.6. Retention experiment

At the end of each exposure duration (i.e., 24, 48 and 72 h), three *C. challengeri* per jar were carefully collected, rinsed with FSW and transferred to clean jars filled with filtered seawater and no Mf. Each amphipod was fed every day with 25 copepods. The water in the jar was changed daily to prevent re-ingestion of egested Mf. The retention experiments lasted for 48 h.

2.7. Microfibers ingestion and retention

At the end of each exposure duration experiment (i.e., 24, 48 and 72 h), as well as of the retention experiment, two *C. challengeri* amphipods were collected from each of the Mf concentration triplicates, rinsed with FSW and placed in pre-labeled vials at −20 °C until further digestion and analysis (Chua et al., 2014; Beiras et al., 2018). After that, and before digestion, individual *C. challengeri* were rinsed thoroughly with MQ once more (Mateos-Cardenas et al., 2020; Botterell et al., 2022) before being placed in glass Petri dishes and broken into pieces with pre-cleaned forceps. Two milliliter of 18.5 % HCl were added to the samples and were left for 24 h at room temperature to digest (Blarer and Burkhardt-Holm, 2016). The digestate was filtered onto a 25 mm diameter GF filter (i.e., nominal 0.3 μm pore size) and placed in a pre-labeled, covered petri dish. Samples were then dried in a 60 °C oven for 24 h. Microfibers on the filters were counted and recorded using a Nikon Stereo microscope (SMZ18). Ingestion rate (IR) and retention rate (RR) were calculated as described by Iwalaye et al. (2021) with a slight modification:

$$IR\ \left(\text{ind}\ h^{-1}\right) = N_F / E_D$$

$$RR\ \left(\text{ind}\ h^{-1}\right) = N_F / (E_D + D_D)$$

where N_F = number of fibers found in individual, E_D = exposure duration, and D_D = depuration duration.

2.8. Fecal pellet sinking velocity

At the end of the exposure experiment (i.e., 24, 48 and 72 h), amphipods were removed, and the water in each jar was carefully filtered through a 64 μm sieve. The fecal pellets (FP) on the sieve were rinsed with FSW into the Petri dish and collected in 15 mL falcon tubes (Fig. 1), which were then placed in a cooler with 2–3 ice packs to reduce microbial activities that could cause fragmentation of the FP. The content of each tube was gently emptied into a petri dish and viewed under the microscope. The length and width of 3 fecal pellets were measured to the nearest micrometer. After capturing the FP images, the FP was picked with a Pasteur pipette, dropped gently in a 25 mL measuring glass cylinder, and filled with FSW (Bruland and Silver, 1981). Fecal pellets were made to sink to a certain distance before timing the sinking rate. Fecal pellet sinking speeds were measured and recorded to the nearest milliseconds. Fecal pellet lengths, widths, and sinking speeds were used to calculate the fecal pellet sinking velocity (v_{fp}), fecal pellet volume (V_{fp}) and density (ρ_{fp}, g.cm^{-3}) using the equations of Komar et al. (1981):

v_{fp} (m·day^{-1}) = d/t; where: d = distance travelled by fecal pellet (m), t = time elapsed (day)

$V_{fp} = \pi r^2 h$; where r = radius of fecal pellet, h = length of fecal pellet

$$\rho_{fp}\left(g{\cdot}cm^{-3}\right) = \frac{v_{fp}}{0.0790 \times (1/\mu SW)} \times \left(g \times h_{fp}{}^2\right) \times \left(h_{fp}/\phi_{fp}\right)^{-1.664} + \rho SW$$

where: μSW = seawater viscosity (g (cm·s)$^{-1}$), ρSW = seawater density (g·cm^{-3}), g = acceleration of gravity (981 cm·s^{-2}), v_{fp} = fecal pellet sinking speed (cm·s^{-1}) (i.e. settling velocity of fecal pellet), h_{fp} = length of fecal pellet (cm) and ϕ_{fp} = diameter of fecal pellet (cm). The salinity (PSU) of the 10 °C FSW was measured with a salinometer, and this was used to estimate μSW = 0.014 g (cm·s^{-1}) and ρSW = 1.024 g·cm^{-3}.

2.9. Predation rates

At the end of the exposure experiment (i.e., 24, 48 and 72 h), amphipods were removed and water in each jar was carefully filtered through a 64 μm sieve. The copepods on the sieve were rinsed with FSW into a Petri dish, collected in 15 mL falcon tubes, and stored at −20 °C until further analysis (Fig. 1). The samples were then thawed at room temperature, emptied, rinsed into a petri dish, and examined under a microscope for copepod counting. The change in the number of copepods over the predation time was used to calculate the predation rates (PR), following Botterell et al. (2020):

$$PR\ \left(\text{copepods}{\cdot}\text{amphipod}^{-1}\ h^{-1}\right) = (T_I - T_F) / (N_A \times P_T)$$

where: T_I = number of copepods added to the jar, T_F = number of copepods after predation, N_A = number of live amphipods in the jar, P_T = Predation time.

2.10. Sample collection and analysis

Due to insufficient amphipods collected from the SoG, the 50,000 Mf·L^{-1} treatment was not included in the calculations of Mf retention rate, as well as fecal pellet volume, density, and sinking velocity.

2.11. Precautionary measures to avoid Mf contamination

The Petri dishes and the glass apparatus were washed with 10 % Extran, rinsed thoroughly with tap water, followed thrice with MQ, and placed in a covered container to prevent air-borne contamination. A white laboratory coat was worn throughout the experiment, sample processing and analysis. Procedural blanks (in triplicate) containing the same volume of HCl used for digestion were added to glass beakers and processed as the samples. All digestion and filtration procedures were done in a fume hood.

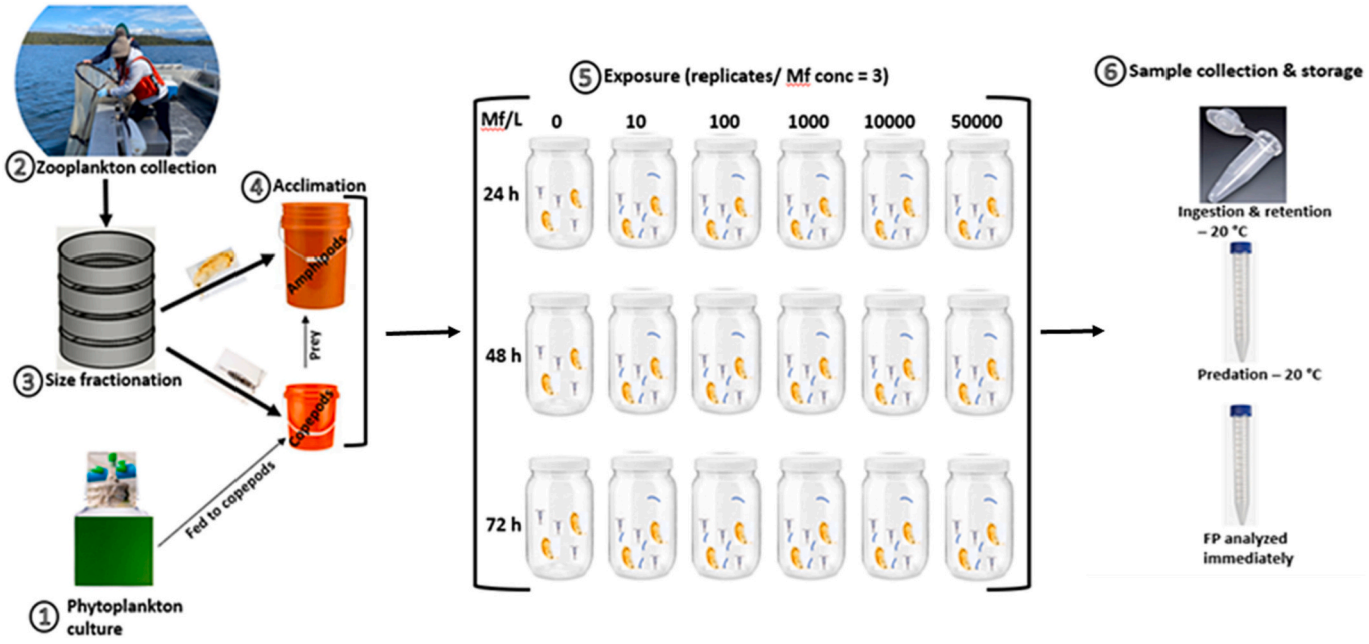

Fig. 1. Schematic image of the microcosm experiment.

2.12. Statistical analysis

Statistical analysis was conducted using IBM SPSS Statistics 27. Tests for normality and equal variance (homoscedasticity) were performed using Kolmogorov–Smirnov and Levene's tests, respectively. When these tests were passed ($p > 0.05$), the main effects and the interaction between independent variables were determined using General Linear Model (i.e., Univariate). When there was a significant difference, Scheffe Post hoc test was performed to determine where the differences existed.

Data were transformed by ranking when the normality or the equal variance tests failed ($p < 0.05$). After that, the main effects and interaction between independent variables were determined on ranks using General Linear Model. A Post hoc test using Scheffe determined where the significant difference existed. A significant difference was attributed at $p \leq 0.05$. Also, linear regression analysis was used to determine the relationship between two parameters.

3. Results

3.1. Survival

The overall survival of *C. challengeri* was not affected by the Mf concentrations, as the survival of *C. challengeri* in the control and the different Mf concentration treatments were not significantly different. However, the survival rate of *C. challengeri* was significantly lower ($p = 0.002$) after 72 h of exposure compared to the 24 h exposure time (Suppl. Table 1).

3.2. Average number of microfibers ingested per amphipod

Cyphocaris challengeri ingested PET microfibers (Suppl. Fig. 1 a-c). There was no significant interaction between Mf concentration and exposure duration on the average number of ingested Mf (Suppl. Table 2). The average number of Mf ingested by *C. challengeri* increased significantly ($p < 0.001$) with a) increasing Mf concentration regardless of exposure duration and b) between 24 and 72 h exposure duration in 100 and 50,000 Mf·L^{-1} (Table 1).

Table 1
Average number (± SD) of microfibers ingested per individual *C. challengeri* at varied microfiber concentrations (Mf·L^{-1}) and exposure duration (h).

Exposure duration (h)		24	48	72
Concentration (Mf/L)	10	$0.67 \pm 0.2^{+a}$	$1.0 \pm 0.3^{+a}$	$1.25 \pm 0.5^{+a}$
	100	$3.0 \pm 0.5^{+a}$	$5.0 \pm 0.8^{+*\ b}$	$6.5 \pm 1.0^{*ab}$
	1000	$7.33 \pm 1.3^{+b}$	$11.67 \pm 2.1^{+c}$	$12.50 \pm 1.8^{+bc}$
	10,000	$12.83 \pm 1.7^{+c}$	$19.17 \pm 2.6^{+d}$	$18.80 \pm 3.3^{+cd}$
	50,000	$16.83 \pm 1.0^{+c}$	$37.17 \pm 2.5^{*e}$	$32.33 \pm 4.9^{*d}$

2-way ANOVA on rank of means: within a Mf concentration, means with the same symbol are not significantly different from each other in average number of Mf ingested per *C. challengeri* ($p > 0.05$; $n = 6$). Within each time period, means with the same letter are not significantly different from each other ($p > 0.05$).

3.3. Microfibers ingestion rate by amphipods

There was no interactive effect between Mf concentration and exposure duration on Mf ingestion rates (Suppl. Table 2). Both Mf concentrations and exposure time significantly influenced the Mf ingestion rates by *C. challengeri* (Suppl. Table 2). Still, while the former had a positive effect (Fig. 2), the latter had a negative effect (Suppl. Fig. 2). For example, at each exposure time, the Mf ingestion rates increased steadily with Mf concentrations (Fig. 2). In contrast, exposure time had no effect on the Mf ingestion rates at the 3 lowest Mf concentration treatments, while for the 2 highest Mf concentrations (10,000 and 50,000 Mf·L^{-1}), the ingestion rate decreased significantly between the 24 and 72 h (Suppl. Fig. 2).

3.4. Predation rate

There was no significant interaction between exposure duration and Mf concentration on the copepods' predation rates by *C. challengeri* (Suppl. Table 2). Relative to the control and 10 Mf·L^{-1}, significant ($p < 0.001$) reductions in predation rates were recorded in ≥1000 Mf·L^{-1} at 24 h and at 48 h (Fig. 3). Interestingly, the predation rates were generally highest at 72 h for most Mf concentration treatments.

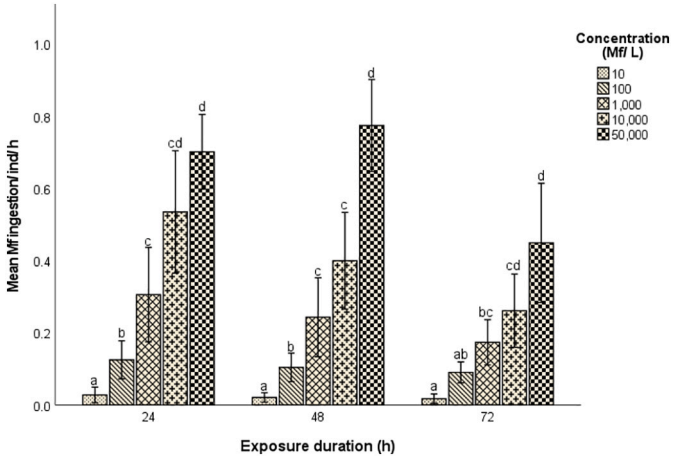

Fig. 2. Microfiber ingestion rate (Mf ingested $ind^{-1} \cdot h^{-1} \pm SD$) of *C. challengeri* exposed to varied Mf concentrations over different time periods. 2-Way ANOVA on rank of means: within a time period, means with the same letter are not significantly different from each other ($p > 0.05$).

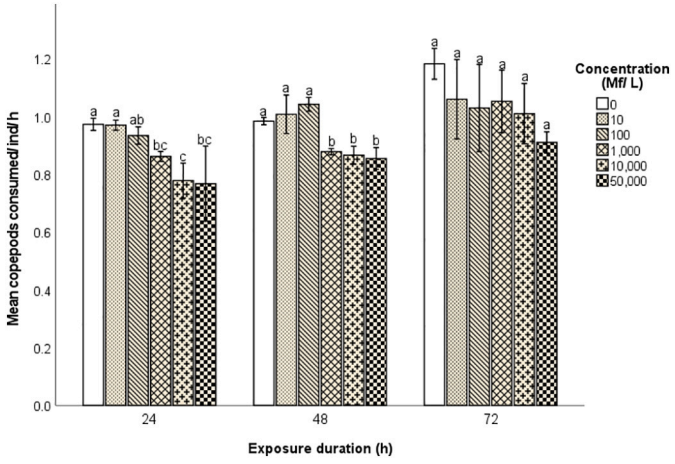

Fig. 3. Predation rates by *C. challengeri* (copepod $ind^{-1}\ h^{-1} \pm SD$) exposed to varied Mf concentrations over different time periods. 2-Way ANOVA on rank of means: within a time period, means sharing the same letter are not significantly different from each other ($p > 0.05$; $n = 3$).

However, this was significant in the 0, 1000 and 10,000 Mf·L^{-1} treatment (Suppl. Fig. 3).

3.5. Microfibers retention

The mean retention rate of ingested Mf after exposure to Mf for 24, 48 and 72 h, and depurated for 48 h was very low in all treatments (Suppl. Table 3), with the highest rates in the 10,000 Mf·L^{-1} treatment at 24 and 72 h (0.037 and 0.031 Mf $ind^{-1}\ h^{-1}$, respectively; Suppl. Table 3).

Regardless of exposure time, after 48 h of depuration, the percentage of *C. challengeri* with Mf and the average Mf retained increased positively with Mf concentration (Table 2). For example, after depuration, 50 % of the amphipods exposed to 10,000 Mf·L^{-1} still retained Mf, with an average of 2.17 ± 3.9 Mf per amphipod. In contrast, only 11 % of the amphipods initially exposed to 10 Mf·L^{-1} still retained Mf, at an average of 0.11 ± 0.3 Mf per amphipod (Table 2).

However, when the original Mf exposure time was longer (i.e., 72 h), a greater % of amphipods retained Mf (i.e., 38 % vs. 21 and 17 % of amphipods when the exposure time was 24 and 48 h, respectively). Still, the average number of Mf retained was similar between 24 and 72 h (Suppl. Table 4).

Table 2
Percentage of *C. challengeri* with microfibers and average number of microfibers per *C. challengeri* exposed to varied microfiber concentration (Mf·L^{-1}) for 24, 48 and 72 h, and depurated for 48 h ($n = 18$). Note: the variability (SD) is higher than the mean because the majority of the amphipods had zero microfiber after depuration.

Concentration (Mf/L)	*C. challengeri* with Mf (%)	Avg number of Mf retained per Amp ± SD (range)
10	11.1	0.11 ± 0.32 (0–1)
100	12.5	0.19 ± 0.54 (0–2)
1000	43.8	0.56 ± 0.73 (0–2)
10,000	50.0	2.17 ± 3.90 (0–14)

3.6. Fecal pellet volume

C. challengeri successfully incorporated the ingested Mf into their fecal pellets (Suppl. Fig. 4 a & b). Exposure duration did not significantly affect fecal pellet volume (Suppl. Table 2). On the other hand, the volume of the fecal pellets was significantly affected by the concentration of Mf in the treatments, but only at 24 h (Suppl. Table 2; Suppl. Fig. 5). In general, at the 24 h exposure time, the volume of the fecal pellets decreased as the concentrations of Mf in the treatments increased, and the fecal pellets produced by amphipods exposed to the 10,000 Mf·L^{-1} were significantly smaller than those produced by amphipods in the control and the 10 Mf·L^{-1} treatment (Suppl. Fig. 5).

3.7. Fecal pellet sinking velocity

A significant ($p = 0.023$) interaction exists between exposure duration and seawater Mf concentration on the fecal pellets' sinking velocity (Suppl. Table 2). Microfibers concentrations in seawater significantly affected FP sinking velocity at all exposure durations (Fig. 4). In general, sinking velocities were fastest at 72 h for all Mf concentrations, especially those produced in the highest Mf concentrations treatments (Suppl. Fig. 6). Regardless of exposure time, the significantly slowest FP sinking velocities were observed for those collected from the control treatments and the lowest Mf concentration (i.e., 10 Mf·L^{-1}) (Fig. 5).

3.8. Fecal pellet density

As the concentrations of microfiber increased in the treatments, the

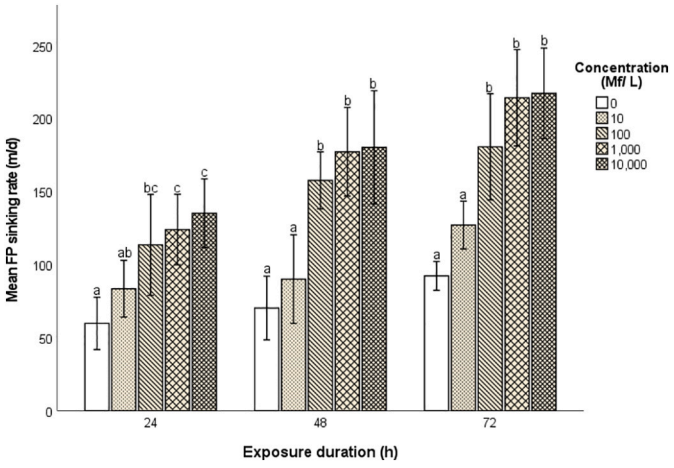

Fig. 4. *C. challengeri* fecal pellet sinking velocity (m $d^{-1} \pm SD$) when exposed to varied exposure durations and Mf concentrations. 2-Way ANOVA on unranked means: within a given time period, means with the same letter are not significantly different from each other ($p > 0.05$; $n = 9$).

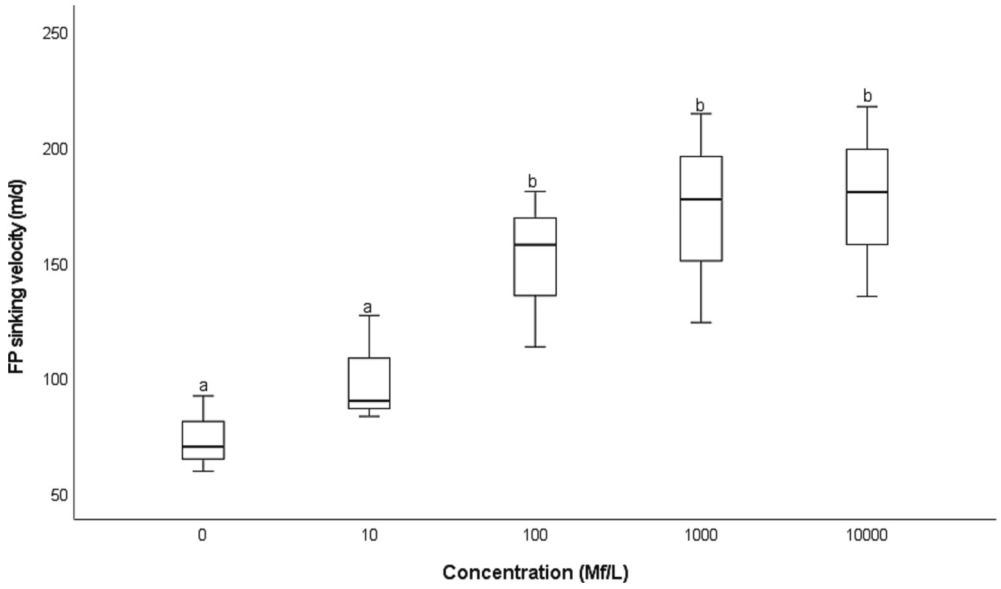

Fig. 5. *C. challengeri* mean fecal pellet sinking velocity (m d^{-1} ± SD) when exposed to varied Mf concentrations, regardless of exposure time. 2-Way ANOVA on unranked means: means with the same letter are not significantly different from each other ($p > 0.05$; n = 9).

higher the density of the FP produced by the amphipods (Figs. 6 and 7; Suppl. Table 2). Relative to the control (no Mf), the FP density significantly increased in the treatments with ≥100 Mf·L^{-1} concentration, regardless of exposure time (Fig. 6). Additionally, our study showed a positive significant correlation ($p \leq 0.001$) between FP density and sinking velocity (Suppl. Fig. 7).

4. Discussion

Our study is the first to explore the short-term impact of PET Mf on marine amphipods using environmentally relevant concentrations and conditions. The study confirms that *C. challengeri* inhabiting Mf-polluted environments can ingest microfibers. It also demonstrates that PET Mf did not impact *C. challengeri* survival within 72 h, but negatively affected their feeding. Furthermore, *C. challengeri* successfully ingested PET Mf (Suppl. Fig. 1), incorporated the ingested Mf into the fecal pellets (Suppl. Fig. 4) and efficiently cleared their gut within 48 h post-exposure to Mf. Incorporating Mf into FPs increased their density and sinking velocities and could ultimately affect the ocean's biological carbon pump. This study adds to the existing literature on the impact of microfiber exposure on zooplankton feeding and function.

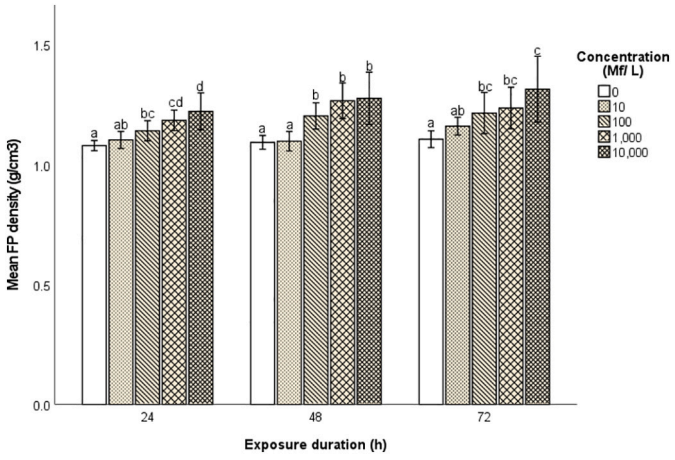

Fig. 6. *C. challengeri* fecal pellet density (g·cm^{-3} ± SD) when exposed to varied exposure durations and Mf concentrations. 2-Way ANOVA on rank of means: within a given time period, means with the same letters are not significantly different from each other ($p > 0.05$. n = 9).

4.1. Amphipods survival

Our study showed that exposure of amphipods to varied PET Mf concentrations did not affect their survival. But we observed an overall significant survival decrease at 72 h, most likely due to their confinement in 1 L jars (Suppl. Table 1). Other studies have also found that MP, at higher concentrations (75,000 PS beads·L^{-1}; 600,000 and 60,000,000 PE microsphere·L^{-1}; Suppl. Table 5) and exposure times (96 h and 9 days) than those used here (50,000 Mf·L^{-1}; 72 h), did not significantly affect zooplankton survival (Cole et al., 2015; Mateos-Cárdenas et al., 2019), while others reported MP effects on their survival (Besseling et al., 2014; Lee et al., 2013; Jemec et al., 2016). Starvation of organisms before or during exposure to MP could reduce their chance of survival, as demonstrated in a study with *Daphnia magna* (Jemec et al., 2016). The significant reduction in the amphipods' survival in our study could also be due to amphipods' stress due to the shaking of the experimental jars at 80 rpm (rpm). Based on the analysis of Beiras et al. (2018), the few mortalities recorded in our study could be due to the stress accumulated from the shaking of the experimental jars at 80 rpm (rpm). Beiras et al. (2018) recorded increased abnormal development of *M. galloprovincialis* larvae when exposed to MP and placed on the orbital shaker at 200 rpm compared to those placed on a rotatory shaker (Suppl. Table 5). Notably, the experimental jars in our study were shaken at considerably lower rpm (i.e., 80 vs. 200 rpm) than those in Beiras et al. (2018). For measuring the effects of MP exposure on organisms, some authors suggest that behavioral responses (i.e., more sensitive) might be more suitable endpoints than mortality (Beiras et al., 2018; Gambardella et al., 2019). For example, Bruck and Ford (2018) reported that the mortality of amphipods was related to molting, not MP exposure.

4.2. Microfibers ingestion versus copepods predation

The average number of ingested microfibers and Mf ingestion rate by *C. challengeri* depends on Mf concentration and exposure duration ($p < 0.001$, Suppl. Table 2) but was more affected by Mf concentration (PES = 0.893, 0.888) than exposure time (PES = 0.321, 0.249, Suppl. Table 2). Interestingly, throughout the experiment, exposure duration significantly and positively affected the average number of ingested Mf

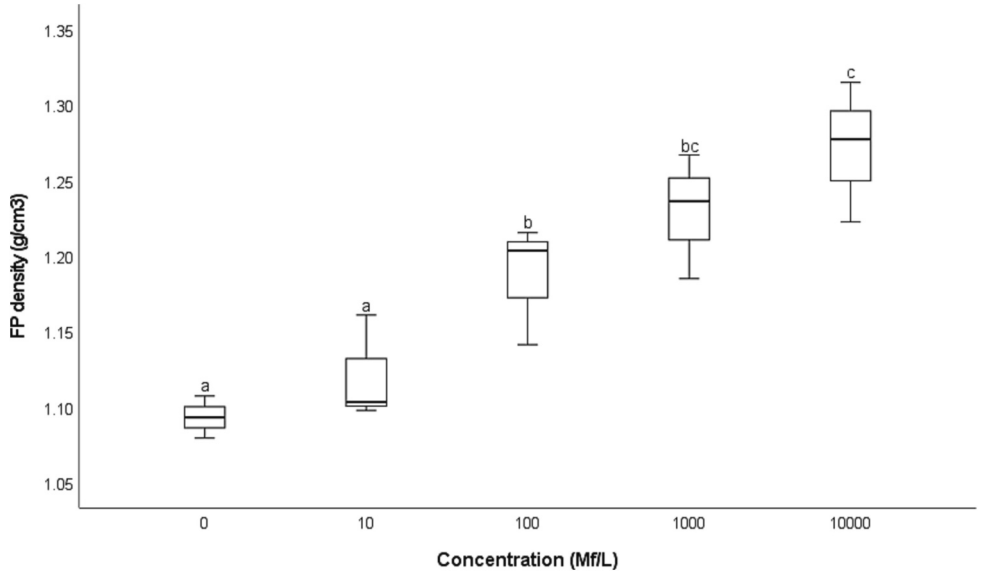

Fig. 7. *C. challengeri* mean fecal pellet density ($g \cdot cm^{-3} \pm SD$) when exposed to varied Mf concentrations. 2-Way ANOVA on ranked means: means with the same letter are not significantly different from each other ($p > 0.05$; n = 9).

by *C. challengeri* (Table 1), but only for 100 and the 50,000 $Mf \cdot L^{-1}$. In contrast, the exposure duration had a negative effect on the Mf ingestion rate, especially for the two highest Mf concentrations (Suppl. Fig. 2). Mateos-Cardenas et al. (2020) reported that MP concentration positively affected the occurrence of MP in the amphipod *Gammarus duebeni*, but in contrast to our results, exposure duration was positively correlated with MP in *Gammarus duebeni* (Suppl. Table 5).

The positive relationship between exposed Mf concentration in seawater and either the average number of Mf ingested (Table 1) or the ingestion rate is due to increased numbers of Mf in the experimental jars with Mf concentration, which resulted in increased encounter rate between *C. challengeri* and Mf. These findings agree with those of Desforges et al. (2015) and Amin et al. (2020), who found a significant correlation between the number of fibers ingested by zooplankton (copepods, amphipods, shrimps, zoea, chaetognaths, and fish larva) and the concentration of fibers and MP in seawater, respectively. Our study reveals that higher Mf concentration increased its bioavailability to *C. challengeri* and the risk of ingestion by *C. challengeri*. Previous studies showed that MP ingestion by zooplankton increased with MP concentrations (Blarer and Burkhardt-Holm, 2016; Isinibilir et al., 2020; Botterell et al., 2022). Higher MP concentration has also been shown to increase the risk of MP ingestion in lugworm (*Arenicola marina;* Besseling et al., 2013), sea urchin (*Tripneustes gratilla;* Kaposi et al., 2014), freshwater amphipod (*G. duebeni;* Mateos-Cardenas et al., 2020) and sea cucumber (*Holothuria cinerascens*; Iwalaye et al., 2021). Prey dilution with MP in the natural environment could mislead predators to ingest more MP, while predators that can screen their prey will spend more time and energy feeding in the presence of MP or Mf.

Notably, the size (diameter: 14.91 μm and length: 357.21 μm) and shape (fibrous) of Mf in our study may have also contributed to its accessibility to the amphipod, resulting in increased average ingested Mf and Mf ingestion rate by *C. challengeri*. The microplastic size determines its capture efficiency and whether it falls within the range of organic/biological particles that are to be ingested (Desforges et al., 2015; Botterell et al., 2020; Mahara et al., 2022), while MP shape determines the prey resemblance, handling potential and ingestion capacity (Botterell et al., 2020). The study of Haro-Garay (2003) on the diet and functional morphology of two SoG amphipods showed that copepods accounted for 52 % and 33 % of prey found in the stomach of *P. pacifica* and *C. challengeri,* respectively. The length (357.21 μm) of the Mf used in our study was within the size range of copepods (i.e., 250–500 μm) fed to *C. challengeri*, which might have enhanced their availability, handling and ingestion.

The results obtained in our study show that *C. challengeri* cannot distinguish its prey from Mf. Other studies have also documented that amphipods could not distinguish their prey from microplastics (Iannilli et al., 2019; Mateos-Cardenas et al., 2022). Furthermore, the number of copepods added to the control and experimental treatment jars was identical (i.e., amphipods were not starved), yet, the average number of ingested Mf and ingestion rate increased with Mf concentration (Table 1 and Fig. 2). Thus, copepods cannot solely explain the increase in the average number of ingested Mf nor the Mf ingested rate as a function of Mf concentration. But, *C. challengeri* may have ingested Mf directly from the water or indirectly by consuming Mf-contaminated copepods. Other studies have confirmed that predators accumulate MP in their digestive tracts while ingesting MP-contaminated prey or plant biomass (Watts et al., 2014; Mateos-Cardenas et al., 2022; Xu et al., 2022).

Compared to previous investigations, the findings of this study suggest that *C. challengeri* could handle and ingest fibers more easily than fragments and microspheres by other amphipods. The average number of Mf (18.80 ± 3.3 Mf ingested/amp; Table 1) ingested by *C. challengeri* exposed to 10,000 $Mf \cdot L^{-1}$ concentration for 72 h is similar to the average number of MP particles (18.80 ± 3.5 particles ingested/amp; Suppl. Table 5) ingested by marine amphipod (*A. compressa*) exposed to 100 $mg \cdot L^{-1}$ irregularly shaped PE particles for 72 h (Chua et al., 2014). However, to enable a direct comparison between the present study and that of Chua et al. (2014), we converted 10,000 $Mf \cdot L^{-1}$ of PET to $mg \cdot L^{-1}$ (=13.4 $mg \cdot L^{-1}$) using the formula of Leusch and Ziajahromi (2021). After the conversion, the average number of Mf ingested by individual *C. challengeri* (i.e., 1.4 Mf per $mg \cdot L^{-1}$ of PET microfiber) was 7.5 times higher than the average number of MP particles ingested by individual *A. compressa* (i.e., 0.19 particles per $mg \cdot L^{-1}$ of PE fragments). Furthermore, to directly compare the average number of Mf ingested by *C. challengeri* at 50,000 $Mf \cdot L^{-1}$, to the average number of MP ingested by the amphipod *G. duebeni*, we divided their MP ingested per amphipod by 1000, given that their MP concentration was 1000-fold higher than ours (i.e., 53.4 ± 15.2 microsphere/amp; at 60,000,000 PE $microspheres \cdot L^{-1}$ & 96 h exposure, Mateos-Cardenas et al., 2020). After this normalization, the maximum and minimum average numbers of Mf ingested by *C. challengeri* (37 and 16 microfibers per amphipod at 50,000 $Mf \cdot L^{-1}$, respectively; Table 1) were far higher than that for *G. duebeni* (0.053 microspheres per amphipod). We recommend investigating the effect of

MP shapes on MP ingestion in amphipods.

Interestingly, the Mf ingestion rate by *C. challengeri* significantly decreased at 72 h in Mf concentrations ≥10,000 Mf·L^{-1} (Suppl. Fig. 2), in contrast to the PE microspheres accumulation in *G. duebeni*, which increased with exposure time (i.e., from 24 to 96 h, Mateos-Cardenas et al., 2020; Suppl. Table 5). Their increase in MP accumulated with time could be due to the reported fragmentation of PE microspheres within the *G. duebeni* gut. Our study's reduction in Mf ingestion rate at 10,000 Mf·L^{-1} and 50,000 Mf·L^{-1} (Suppl. Fig. 2) could suggest that *C. challengeri* had reached the optimum Mf ingestion capacity after 72 h of continuous feeding. The high Mf encounter rate in these two Mf concentrations could have made *C. challengeri* ingest more fibers, possibly resulting in a false sense of satiation, leading to a reduction in copepod feeding, as observed in the predation rate measurements at 24 and 48 h (Fig. 3). According to Iannilli et al. (2019) and Mateos-Cardenas et al. (2020), amphipods mistake microplastics for food. It could also be that amphipods exposed to these higher Mf concentrations reduced their feeding activities with time because the energy gained from feeding could not compensate for the energy used during feeding, given that Mf were 80 (10,000 Mf·L-1) and 400 (50,000 Mf·L-1) times more diluted than their prey (i.e., copepods). Nylon fibers have been documented to significantly alter prey selectivity in copepods (*Calanus finmarchicus*), resulting in reduced feeding (Cole et al., 2019).

The reduction in predation rate by *C. challengeri* exposed to ≥1000 Mf·L^{-1} concentrations at 24 and 48 h (Fig. 3) is likely due to the increased Mf ingestion rates at concentrations ≥100 Mf·L^{-1} (Fig. 2). The significant decrease in the number of copepods consumed by *C. challengeri* exposed to Mf concentrations ≥1000 Mf·L^{-1} (i.e., 88.9, 98.8 and 99.75 % of prey) for 24 and 48 h exposure durations (Fig. 3) agrees with the findings of Carrasco et al. (2019), who showed that amphipod – *Orchestoidea tuberculata* consumed significantly less food when 5 and 10 % of their prey were replaced by polystyrene microsphere (Suppl. Table 5). We acknowledge that the percentages of Mf to prey (88.9, 98.8, and 99.75 %) at which significant reductions in feeding were recorded in the present study were higher than those (5 and 10 %) used by Carrasco et al. (2019). It is possible that the consumption of microspheres gave the amphipods a quicker sense of false satiation than microfiber consumption. Other studies—conducted with a) lugworms (*A. marina)*, exposed to 0.22–150 mg of PS beads·L^{-1}, and b) crabs (*Carcinus maenas*), exposed to 0.6–2 mg of polypropylene Mf per 2 g of feed—also recorded a reduction in food consumption with increased MP concentration (Suppl. Table 5; Besseling et al., 2013; Watts et al., 2015).

Previous laboratory studies also showed that zooplankton are less likely to ingest the natural prey if they ingest MP (Cole et al., 2013; Coppock et al., 2019). This poses a great concern to zooplankton because feeding reduction due to MP ingestion has been reported to decrease metabolic rates and spawning time in copepods (*Calanus helgolandicus* at ~5,000,000 PS beads·L^{-1}, Isinibilir et al., 2020), fecundity in copepods (*Tigriopus japonicas* at 12.5 and 25 g of PS beads·L^{-1}, Lee et al., 2013), egg size in copepods (*Calanus helgolandicus* at 75,000 PS beads·L^{-1}, Cole et al., 2015), as well as causing premature molting in copepod (*Calanus finmarchicus*, exposed to PA nylon granule and fibers at concentration of 50,000·L^{-1}, Cole et al., 2019), stress-induced spawning in Arctic copepods (*Calanus finmarchicus*, *C. glacialis,* and *C. hyperboreus*; 200 and 20,000 PE beads·L^{-1}, Rodríguez-Torres et al., 2020), and behavioral abnormalities in *Daphnia* (*D. magna*; 12.5 and 200 mg of PE particles·L^{-1}, Rehse et al., 2016) (Suppl. Table 5). Thus, the implications of MP- or Mf-induced feeding reductions in various zooplankton will significantly impact the health and food availability of many upper trophic level organisms in marine food webs.

The increase in copepods consumption by *C. challengeri* exposed to 1000 and 10,000 Mf·L^{-1} for 72 h (Suppl. Fig. 3) coincided with a considerable decrease (not all are significant) in the ingestion of Mf at 72 h (Suppl. Fig. 2), which suggests that *C. challengeri* became more careful with their prey selection and/or very hungry. Nevertheless, this argument is weak because *C. challengeri* in the control (0 Mf·L^{-1}) also increased their predation rate at 72 h (Suppl. Fig. 3). However, further study is needed to investigate the possibility of prey selection in amphipods exposed to MP and Mf. So far, some studies have shown that, to avoid the consumption of plastic particles, copepods (*Calanus species*) shift their prey selection from microalgae (i.e., similar in size and shape to MP) to larger algae (Cole et al., 2019; Coppock et al., 2019) but no studies have investigated amphipod. The increased predation rate at 72 h in our study disagrees with the finding of Straub et al. (2017), who reported no difference in feeding rate with time in the amphipod *G. fossarum* when exposed to 100,000 MP particles·ind^{-1} (Suppl. Table 5).

4.3. Microfibers retention

Understanding amphipods' MP ingestion and egestion rates would help determine the potential of amphipods to transport MP to the deep ocean or cycle MP within the ocean interior (Jamieson et al., 2019). The low average Mf retention rate and number of Mf retained per amphipod in our study (Suppl. Table 3 and 4) suggest that *C. challengeri* is very efficient at evacuating ingested Mf within 48 h, even at high Mf concentrations (Suppl. Table 3; Table 2). During the depuration period, providing natural prey to *C. challengeri* enhanced the quick passage of ingested Mf through their digestive tract, hence the low Mf retained and percentage of *C. challengeri* with Mf. Two previous studies, Cole et al. (2015) and Bruck and Ford (2018), also reported that providing food to zooplankton reduced the resident time of MP in their guts. Murtaugh (1984) also documented that starved mysids crustaceans (*Neomysis mercedis*) significantly retained more digestible and indigestible materials in their digestive tract than those properly fed, and suggested that crustaceans can self-regulate the passage of material in their gut depending on food availability (i.e., reduce egestion rate during food scarcity).

The low Mf retention in the present study is consistent with findings from other MP studies that reported amphipods clearing their guts within a few hours after exposure (Suppl. Table 5). Chua et al. (2014) reported that 87 % of amphipods exposed to MP cleared their gut within 12 h and recorded <0.5 MP particles per amphipod at 32 h depuration. The study of Blarer and Burkhardt-Holm (2016) showed that *G. fossarum* evacuated ingested Mf within 16 h after exposure. Bruck and Ford (2018) also documented that all amphipods exposed to polystyrene microsphere eliminated the MP within 48 h after exposure. Despite that >50 % of *C. challengeri* (especially in ≤1000·Mf^{-1}) exposed to microfiber in our study were able to evacuate Mf ingested (Suppl. Fig. 4), it is worth noting that, after 48 h depuration, those exposed to higher Mf concentrations retained more Mf (i.e., as much as 20 times) than those in the lower Mf concentrations (Table 2), similarly to the findings for *G. duebeni* in Mateos-Cardenas et al. (2020). The increased Mf retention in high Mf concentration is because of the increased accessibility of *C. challengeri* to Mf, Mf encounter rate and ingestion rate at these concentrations.

The short residence time of Mf in the gut of *C. challengeri* (Suppl. Table 4) will arguably cause less toxicological effects on these organisms (Chua et al., 2014; and Kaposi et al., 2014), such as chemical desorption of persistent organic pollutants into their biological tissue or the chance of transferring Mf to their predators. In our study, *C. challengeri* was exposed to Mf for a few days (1–3 days) compared to the life span of males (i.e., 66–105 days) and females amphipods (i.e., 89–135 days) (Conradi and Depledge, 1998; Shahin et al., 2023a, 2023b). Thus, the continuous exposure of the amphipods to Mf through their entire life span may constitute a problem for their well-being and that of their predators. And, even though *C. challengeri* could clear their gut, Mf encapsulated in fecal pellets could be transferred to benthic microbes and organisms (Honjo and Roman, 1978; Cole et al., 2016). However, there is a need to investigate the resident time of other types of MP in amphipods and the possibility of nanoplastics (i.e., arising from further fragmentation of MP in the gut) translocation from the gut to their

tissue.

4.4. Fecal pellets

We observed a significant decrease in FP volume in the 10,000 Mf/L concentration treatment at 24 h (Suppl. Fig. 5). This could be due to FP fragmentation—weakened structural integrity due to the loss of organic or inorganic material—resulting from Mf incorporation in FP and/or the shaking effects of the experimental jars. The latter seems unlikely, as we did not observe any significant reduction in FP volume in any other treatment, even though *C. challengeri* was subject to the same experimental conditions (i.e., prey supply and orbital shaking). We also acknowledged that *C. challengeri* were collected from SoG using a net tow, followed by sorting with a stack of sieves (see methods). The collected *C. challengeri* (> 1000 μm) were placed in a holding bucket and randomly assigned to the experimental jars. Thus, it is unlikely that the *C. challengeri* specimens in the 10,000 Mf/L concentration treatment were specifically smaller and thus produced smaller fecal pellets. The decrease in FP volume after 24 h exposure agrees with the study of Wieczorek et al. (2019), who documented that salps exposed to polystyrene fragments egested smaller fecal pellets compared to those in the MP-free treatment (Suppl. Table 5). On the other hand, the insignificant reduction in FP size in our study (Suppl. Fig. 5) is consistent with the study of Cole et al. (2016) and Rodríguez-Torres et al. (2020), who reported that no significant size reduction in FP produced by copepods exposed to polystyrene and polyethylene (Suppl. Table 5).

Zooplankton fecal pellets are compactly packed organic matter waste covered by an organic membrane. Fecal pellets are a rich energy and carbon source for microbes and detritivore organisms and contribute to the recycling and vertical transport of particulate organic matter in the ocean (Honjo and Roman, 1978; Cole et al., 2016; Wieczorek et al., 2019). The contents of fecal pellets usually reflect the chemical composition of food consumed by zooplankton (Honjo and Roman, 1978; Small et al., 1979; Cole et al., 2016) and could influence (i.e., either by decreasing or increasing) the sinking velocity and density. The present study shows that FPs density and sinking velocity are a direct function of Mf concentrations in seawater (Figs. 4, 5, 6 and 7). This is unsurprising because the number of Mf incorporated in the FPs should be a function of the number of Mf ingested by *C. challengeri*. Although Mf within the FP were not enumerated, we expect the number of Mf encapsulated in FP to increase with an increasing number of ingested Mf. This could then account for the high density (Fig. 7) and faster sinking velocity (Fig. 5) of FP at high Mf concentrations (≥ 100 Mf·L^{-1}) given that PET Mf are denser (1.23–2.30 g.cm^{-3}) than *C. challengeri* FPs (< 1.2 g.cm^{-3}, Suppl. Fig. 7). The positive significant correlation between the FP density and sinking velocity (Suppl. Fig. 7) further strengthens our claim that the greater the number of PET Mf in the water, the greater the number of Mf in the FPs, and the higher the fecal pellet density and sinking velocity (Fig. 4 and 6).

We understand that several factors, such as the quantity and density of MP ingested, the prey and other organic material incorporated into the FP, and a series of abiotic conditions (e.g., water- viscosity, salinity, temperature and turbulence) could all affect the sinking velocity and aggregation of FPs in the marine environment (Cole et al., 2016). In the present study, however, prey composition, density of Mf polymer and abiotic conditions were all the same for the treatments, negating their influence on the FP density and sinking velocity. The dose-response relationship between Mf concentration and both FP density and sinking velocity proves that Mf concentration is the main factor that altered the physical properties and behavior of FPs. Our result support Cole et al. hypothesis (Cole et al., 2016) that the encapsulation of MP in marine zooplankton FP can cause a significant alteration to the FP structural integrity, density and sinking rates.

According to the literature, alterations to the density and sinking velocity of the FP (either positive or negative) are dependent on MP density (Coppock et al., 2019). Our study demonstrated that encapsulation of PET Mf in *C. challengeri* increased their density and sinking velocity. Coppock et al. (2019) showed that the incorporation of high-density PE fibers in fecal pellets increased their sinking velocity (Suppl. Table 5), while Rodríguez-Torres et al. (2020) reported that PE spheres in the fecal pellets did not affect the sinking velocity (Suppl. Table 5). According to the study of Wieczorek et al. (2019), polystyrene and low-density polyethylene beads in fecal pellets decreased fecal pellet sinking velocity by 1.47-fold and 1.3-fold, respectively. The difference between these results could be due to the different plastic polymers used in each experiment. In the present study, we used PET Mf, which is denser (1.23–2.30 g.cm^{-3}) than PE (0.88–0.96 g.cm^{-3}), which was reported to be positively buoyant in seawater. The significant increase in FP density and sinking velocity in higher Mf concentration (Figs. 4 and 6) indicates that *C. challengeri* actively ingested Mf and effectively encapsulated the Mf in FPs (Suppl. Fig. 4 a & b), causing a change in the pellet density (Fig. 6) and sinking velocity (Fig. 4). The study of Cole et al. (2013) showed that polystyrene spheres ingested by copepods were aggregated in the anterior midgut and densely packed in the fecal pellets when egested.

Additionally, the sinking velocity of FP collected at the end of 72 h exposure was significantly higher than those collected after 24 h exposure in all treatments, including the control (Suppl. Fig. 6). This could be due to the increase in FP compactness and size with time, as shown by Small et al. (1979), where (i.e., within 2–3 h) lightweight and loosely packed FPs produced by copepods sank slowly, compared to compacted and heavily invested pellets with coccoliths, diatom silica frustules and sediment.

4.5. The implications of PET microfibers ingestion and encapsulation in fecal pellets on the SoG ecosystem

Mahara et al. (2022) confirmed that zooplankton in the Northeast Pacific Ocean are ingesting microplastics, but the impact on their health and their predators remains unknown Desforges et al. (2015). In the present study, exposure of *C. challengeri* to environmentally relevant Mf concentrations (i.e. 10 and 100 Mf·L^{-1}) similar to the microplastics concentrations found in the: NE Pacific Ocean (~10 MP·L^{-1}), Southeastern Black Sea (20 MP·L^{-1}), Yangtze River Delta (21.52 MP·L^{-1}), Southeast coast- India (23.7 MP·L^{-1}) and Charleston Habour and Winyah Bay (88 MP·L^{-1}) did not affect copepod consumption by *C. challengeri* (Desforges et al., 2014; Kabir et al., 2022; Elizalde-Velazquez and Gomez-Olivan, 2021; Sathish et al., 2020). However, their exposure to Mf concentration (≥ 1000 Mf·L^{-1}), lesser than the predicted seawater MP concentration by the year 2100, resulted in reduced copepod consumption by *C. challengeri* (i.e., for ≥1000 Mf·L-1 at 24 and 48 h, Fig. 3). Reduced feeding is a strong indicator that continuous exposure to and ingestion of Mf by *C. challengeri* will possibly affect their fitness, growth, development, energy reserves and reproduction resulting in a long-term population decline. Furthermore, insufficient nutrients and energy consumption, due to the ingestion of less prey and more Mf, could be detrimental to *C. challengeri* predators in the SoG ecosystem, especially juvenile salmon, herring and groundfish, which depend on amphipods for growth and survival if the seawater Mf concentration should increase than the concentration reported in the last decade.

Lastly, the average FPs travel time from surface waters to the ocean floor in the SoG was extrapolated using the data from FP sinking velocities (m/d) and average (161 m) and maximum (420 m) depths of SoG as reported in Mackas et al. (2013) and Perry et al. (2021). Our study shows a decrease in FP travel time with increasing Mf concentrations in seawater (Suppl. Fig. 8). Therefore, *C. challengeri* FP with no Mf will take 2.24 and 5.85 days to reach the average and maximum depth of SoG, respectively. However, FP with encapsulated PET Mf will travel the same distance in 1.07 and 2.90 days or less, depending on the Mf concentration. Therefore, ingestion of PET microfibers and their incorporation in FP will potentially increase the efficiency of *C. challengeri* in the

biological C pump and carbon sequestration from the surface to the deep ocean.

5. Conclusion

Our study showed that exposure of *C. challengeri* to environmentally relevant PET Mf concentrations (10 and 100 Mf·L^{-1}) did not significantly affect their survival, prey consumption and FP volume. While prey consumption by *C. challengeri* was not significantly affected by the presence of environmentally relevant MP, our study demonstrated that a further increase in the MP concentrations (predicted by Everaert et al., 2018) would reduce *C. challengeri* prey consumption. This can be detrimental to their health, physical activities, biological processes, and predators and could result in cascading effects on marine food webs. Poor salmon recruitment in the SoG was linked to variations in the zooplankton population (Mackas and Beaugrand, 2010). The dose responses of *C. challengeri* to Mf concentrations confirmed their capability of ingesting Mf that falls within the size of their prey. The direct positive relationship between Mf concentrations and Mf ingestion rates further supports that *C. challengeri* cannot differentiate natural prey from Mf.

This poses a great concern to the general well-being of zooplankton and other higher trophic-level organisms. The MP concentrations in marine environments have been predicted to increase over time regardless of the 4 Rs plastics' enforcement measures (refuse, reduce, reuse and recycle). The increase in the percentage of *C. challengeri* with Mf and the average number of Mf retained with Mf concentration after 48 h depuration proved their susceptibility to ingest and retain more Mf should the concentration of MP in their environment increase. More so, this study emphasized that Mf concentration and polymer density (compared to other studies) played a significant role in altering the density and sinking velocity of *C. challengeri* FP. The density and sinking velocity of their FP were significantly increased, even at Mf concentrations relevant to the environment, suggesting that the rate of transport of organic carbon and microplastics (i.e., high-density MP) from the surface water to the deep ocean by *C. challengeri* could be expected to double very soon. Lastly, it is important to mention that Mf concentrations significantly impacted the parameters investigated in this study more than the exposure time.

5.1. Limitation to study

In the current study, we tried to provide *C. challengeri* with conditions relative to what is obtainable in the natural environment regarding food and exposure to light. Notwithstanding, we cannot overlook the space limitation that might have hindered *C. challengeri* from performing their usual vertical migration to feed. Understandably, this might have stressed them during the experiment. Lastly, we could not simulate other factors, such as turbulence and currents similar to those in the natural environment.

Funding

This study was funded by the Increasing Knowledge on Plastic Pollution (IKPP) Initiative, Canada (Grant number: GCXE21S063).

CRediT authorship contribution statement

Maite T. Maldonado: Conceptualization, Funding acquisition, Review & editing. **Oladimeji A. Iwalaye:** Methodology, Experimentation, Data analysis and interpretation, Writing- original draft & 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.

Data availability

Data will be made available on request.

Acknowledgments

We want to thank Lori-Jon Waugh for making the microfibers used for this study and assisting with fecal pellet sample analysis. We also thank Kevin Landrini for assistance with zooplankton collection and fecal pellets sample analysis.

Appendix A. Supplementary figures and tables

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2023.167301.

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