Journal of Hazardous Materials 500 (2025) 140551

Contents lists available at ScienceDirect

Journal of Hazardous Materials

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

Real-time visualization reveals copepod mediated microplastic flux

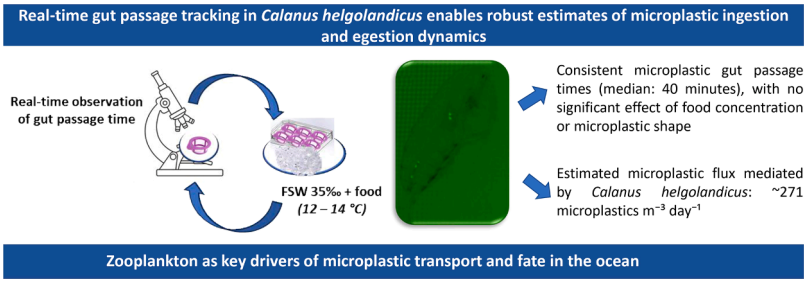
Valentina Fagiano [a,*], Matthew Cole [b], Rachel L. Coppock [b], Penelope K. Lindeque [b]

[a] Oceanographic Centre of the Balearic Islands, COB-IEO, CSIC, Moll de Ponent s/n, Palma, Balearic Islands 07015, Spain
[b] Marine Ecology and Society, Plymouth Marine Laboratory, Prospect Place, Plymouth PL1 3DH, UK

HIGHLIGHTS

- Real-time tracking shows median microplastic gut passage time of ~40 min in *Calanus helgolandicus*.
- Fragments, beads and fibers show similar microplastic gut passage times in *Calanus helgolandicus*.
- Microplastic gut passage time is consistent across food concentrations in *Calanus helgolandicus*.
- Estimated zooplankton-mediated microplastic flux: ~271 microplastics m^{-3} day^{-1}.
- Zooplankton are key drivers of microplastic transport and fate in the ocean.

GRAPHICAL ABSTRACT



ARTICLE INFO

Keywords:
Zooplankton
Calanus
Plastic
Pollution
Biological pump

ABSTRACT

Zooplankton are emerging as key actors in shaping the distribution and fate of microplastics in marine environments. Copepods are the predominant taxa in marine zooplankton communities, underpinning food webs and playing vital roles in biogeochemical cycling. Marine copepods have been identified as microplastic reservoirs that likely contribute to the biological transport of microplastics. Evaluating the extent to which copepods contribute to marine plastic cycles requires accurate measures of copepod-mediated microplastic fluxes. To address this critical research gap, real-time visualization is implemented to precisely measure microplastic gut passage time and ingestion intervals. The North Atlantic temperate copepod *Calanus helgolandicus* was exposed to fluorescent polystyrene beads, polyamide fibers and polyamide fragments under varying food concentrations. Copepods demonstrated consistent microplastic gut passage times (median: 40 min) with food concentration and microplastic shape having no significant effect. This study provides robust estimates of gut passage time and ingestion intervals, establishing a quantitative framework for assessing copepod-mediated microplastic fluxes. Estimated fluxes (~271 microplastics m^{-3} day^{-1}), based on the mean abundance of *C. helgolandicus* in the western English Channel (Northeast Atlantic), suggest that copepods may represent key drivers of vertical microplastic transport. These findings advance integration of copepod-driven processes into oceanographic models, reducing uncertainties in microplastic transport predictions and enhancing understanding of microplastics' ecological impacts on marine ecosystems and global biogeochemical cycles.

* Corresponding author.
E-mail address: valentina.fagiano@ieo.csic.es (V. Fagiano).

https://doi.org/10.1016/j.jhazmat.2025.140551
Received 7 September 2025; Received in revised form 17 November 2025; Accepted 19 November 2025
Available online 21 November 2025

1. Introduction

Microplastics are emerging as structural components of the global ocean, raising concerns about their potential impacts on ecosystem functioning [26,34]. Owing to their persistence and global abundance, marine microplastics are increasingly described as part of the plastic cycle [25,70]. Within marine ecosystems, microplastics move through ecological compartments or "reservoirs", interconnected by intertwined abiotic and biotic processes, both altering and being altered by marine environments and species [71]. While the effects of microplastics on marine biota have been well documented [3,5,16,41,45], the extent to which marine organisms can influence the distribution and fate of microplastics has received considerably less attention [10,25].

Copepods are predominant within zooplankton communities, underpinning marine food webs and contributing to biogeochemical cycling through the ingestion and egestion of auto- and heterotrophic phytoplankton. Marine copepods typically undergo diel vertical migration, whereby they feed in surface waters at night and migrate to depth during the day to avoid predation. The role of copepods as biotic conduits connecting microplastic reservoirs within the plastic cycle is gaining increasing attention [27,37]. Laboratory-based experiments and field studies have demonstrated copepods can ingest microplastic particulates and fibers [5,14,12,22,24,29]. Copepods exhibit relatively low microplastic ingestion rates compared with other marine organisms [2, 21,33,49,58,59,68,69], likely owing to copepods' capacity to discriminate between natural prey and microplastics [65]. However, their high population densities means copepod populations can still be considered as key biotic reservoirs for microplastics [1,19,22,24,29,53,58]. Indeed, retention values of up to 2700 microplastics/m^3 have been reported for calanoid copepods [6], and a recent meta-analysis estimates an average retention of approximately 8.7 microplastics per cubic meter across the global ocean [23]. However, these estimates remain static, capturing only the microplastics retained within a copepod population at a given time with limited understanding of their retention times. Such measures would help inform the transience of microplastics within zooplankton populations, in turn elucidating the susceptibility of predators - including commercially exploited organisms and charismatic megafauna - to consuming microplastics via trophic transfer.

Copepod fecal pellets are negatively buoyant organic aggregates that contribute to the biological pump through the vertical transport of carbon and nutrients to deeper waters and the seafloor [63]. Microplastics ingested by copepods are subsequently egested within these fecal pellets, facilitating their vertical flux within the water column [15, 17]. Conceptual models predict microplastic-laden feces will sink to the seafloor where benthic fauna can facilitate their decomposition and microplastic burial [48,18]. Zooplankton feces, together with marine snow, have been estimated to sequester between 230,000 and 660,000 tons of microplastics at the sea surface from 1980 to 2010 [36], representing one of the main biological pathways for the removal of microplastics from surface waters. However, the fate of microplastic-laden feces will ultimately depend on their remineralization rates, influenced by a variety of abiotic (e.g. temperature, salinity, pH) and biotic (e.g. microbial activity, consumption by coprophagous organisms) factors, and the properties of the fecal pellet, including their sinking velocity, structural integrity and size, and the depth at which they were released within the water column.

While the mechanisms through which copepod-microplastic interactions influence microplastic distribution have been outlined [28, 35,50,52,67], the quantitative contribution of copepods to microplastic fluxes remain uncertain [34,35,42]. To address this research gap, we aim to calculate the microplastic residence time and the frequency of ingestion events in an abundant marine copepod under the hypothesis that microplastics, similarly to natural prey, are rapidly processed and egested by copepods—within a few hours—and that this process is modulated by food concentration. In this study, we use real-time observations to assess microplastic gut passage time and ingestion event frequencies using a range of microplastics under differing feeding scenarios. Our copepod of choice is *Calanus helgolandicus,* a keystone species in European marine waters and the northeast Atlantic, where it can constitute up to 90 % of mesozooplankton biomass [14]. These measures will improve estimates of copepod-mediated microplastic fluxes, informing: 1. the transience of microplastics within copepod populations; 2. the depths at which copepods undergoing diel vertical migration may egest microplastics consumed in surface waters.

2. Material and methods

2.1. Copepod sampling and husbandry

Zooplankton samples were collected via vertical tows using a 735 μm mesh plankton net at the Western Channel Observatory (Station L4; 50°15′N, 4°13′W) in April and May 2024 aboard the RV Quest [54]. Samples were transported in 2 L of seawater within a cool box to the temperature-controlled facilities at Plymouth Marine Laboratory (Plymouth, UK) within three hours of collection. Upon arrival, adult female *Calanus helgolandicus* were identified by assessing their size, morphology and urosome under a dissecting microscope (Wild M5–49361; magnification ×20–×50). Specimens were carefully transferred using stork-billed forceps into aerated glass beakers containing 5 L of 0.2 μm filtered seawater (FSW; salinity 34.5–35 ‰, 24 h darkness). Copepods were maintained at a constant temperature of 11°C, reflecting the ambient sea surface temperature (SST) at the collection site and fed non-limiting concentrations of microalgae (Section 2.2). A preconditioning period of at least 72 h was implemented before initiating the experimental treatments [17].

2.2. Microalgae

The unicellular chlorophyte *Dunaliella tertiolecta (9 × 13 μm)* and dinoflagellate *Prorocentrum micans* (40 × 58 μm) are recognized as suitable microalgal prey for *Calanus* spp. and have been utilized for culturing *C. helgolandicus* and assessing gut passage times in calanoid copepods [4,17,60]. Microalgal cultures were obtained from the Culture Collection of Algae and Protozoa (*Dunaliella tertiolecta*, CCAP 19/6B) and Swansea University (*Prorocentrum micans*). Cultures were grown in Guillard's F/2 medium (Reefphyto Ltd, UK), prepared in sterilized FSW (salinity 34.5–35 ‰), and maintained at 20°C under a 16:8 light/dark cycle to ensure exponential growth. Weekly refreshing of cultures ensured optimal growth conditions. Algal populations were quantified using a Sedgewick Rafter counting chamber. Carbon biomass was estimated by applying a conversion factor, where 5 nL biovolume equates to approximately 1 μg C [31]. The biovolume was based on experimental estimations provided by Coppock et al. [17].

In experiments, the incubation medium consisted of FSW supplemented with microalgae at one of three distinct concentrations representing food availability in the western English Channel under specific scenarios [40]: "Low" (20 μg C L^{-1}), corresponding to food availability before a spring bloom; "High" (200 μg C L^{-1}), reflecting food availability during a spring bloom; and "Extreme" (800 μg C L^{-1}, representing the historical maximum food availability recorded. The incubation medium was subsequently added to incubation chambers and 6-well plates.

2.3. Microplastics

Fluorescent, additive-free polystyrene (PS) beads (20 μm; Spherotech) were prepared by repeatedly centrifuging source material in ultrapure water [13]. Polyamide fibers (10 × 30 μm were prepared by sectioning nylon 6,6 polyfilament (Goodfellow) using a cryotome [11]. Polyamide fragments (30 μm) were prepared by size fractionating nylon 6,6 powder (Goodfellow). Polyamide fibers and fragments were dyed using Nile Red [11]. Nile Red has been recommended as a stable and suitable dye for polyamide polymers that has been applied in numerous

studies [11,12,17].

Polystyrene, a critical component of rigid plastics, is ubiquitous in neustonic waters worldwide, while nylon comprises a substantial fraction of synthetic fibers present in diverse marine matrices [9,56]. The dimensions of the microplastics align with the size range commonly ingested by copepods in natural environments. In contrast, ingested fibers do not have reported dimensional constraints and are ingested across a broad size spectrum, including lengths comparable to the copepod's body length [23].

2.4. Experimental design

To determine the gut passage time of microplastics at varying food concentrations, two experimental protocols were conceived, with pilot experiments confirming suitable observation frequency. The protocols were designed to facilitate copepod-microplastic interactions and undertake real-time observation of the copepods with minimal handling stress.

2.4.1. Protocol 1

In the first protocol (Scheme 1), which served as a preliminary trial, individual adult female copepods (N = 16 per treatment) were exposed to FSW containing low, high and extreme food concentrations (Section 2.2; "incubation media") and 100 microplastics/mL of 20 μm PS beads in 60 mL Pyrex glass bottles, filled to capacity (75 mL total volume). The concentration of 100 MP/mL was chosen to maximize likelihood of microplastic ingestion events for visualization purposes, facilitating sufficient ingestion events while remaining within the range of spill scenarios or highly polluted waters under future modelling scenarios. Then, copepods were incubated overnight in the dark on a rotating plankton wheel (speed <5 rpm) at SST. After incubation, copepods were retrieved by gently filtering the bottle contents through a 500 μm mesh sieve, partially submerged in incubation media. Next, individual copepods were transferred into cell strainers (40 μm mesh) which were washed by gently submerging three times in FSW (S 35 ‰, T 11–12°C) to remove floating or adhered microplastics. Copepods were then transferred to a 6-well plate filled with incubation media and housed on crushed ice to maintain temperature.

To track the transit of fluorescent microplastics through the guts of the copepods, strainers were transferred to a glass Petri dish containing a thin layer of incubation media. Every 150 s copepods were examined under an Olympus SZX16 microscope with fluorescent light source (400–500 nm excitation, 450–550 nm emission). Observations included recording and photographing the number of microplastics in each gut segment (foregut, midgut, hindgut) and making notes on copepod physiology. In instances where multiple microplastics had been ingested, particle visualization became challenging owing to the rapid vertical movement of particles in the foregut and camera resolution. To address this, microplastics were quantified as exact counts (0–10 particles), or estimated (11–25 particles), or noted as "Saturated" when > 26 particles were observed. Groups of particles moving together from one gut segment to the next were further classified as aggregates (Fig. 1). Between observations, the strainers were returned to the 6-well plate.

2.4.2. Protocol 2

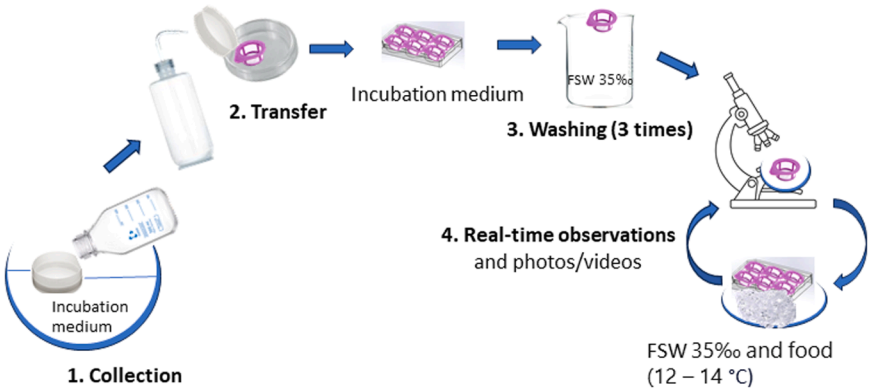
Protocol 2 (Scheme 2) was implemented based on the results of Protocol 1 to enable measurement of gut passage time from the moment of ingestion, thereby improving the precision of microplastic gut passage times, to assess the interval between exposure and ingestion, and to evaluate gut passage time under environmental-like conditions where typically no more than one MP is found in a copepod's gut [23].

Individual adult female copepods (N = 12 per treatment) were exposed to FSW containing low or high food concentrations (Section 2.2; "incubation media") and no microplastic, in experimental bottles as described in Protocol 1. Following incubations, copepods were transferred to single-cell strainers placed in a 6-well plate containing incubation media and either PS beads, PA fibers or PA fragments at concentrations of 100 microplastics/mL for 3 h in a multi-factorial design. To identify copepods that had ingested microplastics, strainers were transferred to a glass Petri dish containing a thin layer of exposure media and observed via microscopy. Yellow fluorescence (400–500 nm excitation, 450–550 nm emission) was used for PS beads, while red fluorescence (excitation 546/10 nm, and emission OG590) was used for PA fragments and fibers. Observations were repeated every two minutes until a copepod showed evidence of microplastic ingestion, returning strainers to the incubation media with microplastics in between. Copepods that ingested microplastics were washed and transferred to a 6-well plate containing incubation media and observed every 150 s as described for Protocol 1.

2.5. Data analysis

2.5.1. Protocol 1

In order to evaluate the gut passage time, the following variables were calculated for all ingested particles, including single particles and aggregates (Fig. 1): i) overall gut passage time, defined as the total time each particle spent within the copepod gut regardless of its location at T0; ii) gut passage time from the foregut, defined as the total time spent within the copepod gut by particles located in the foregut at T0; iii) gut passage time from the midgut, defined as the total time spent within the copepod gut by particles located in the midgut at T0; iv) frequency of ingestion, calculated as the proportion of copepods with microplastics in their gut relative to the total number of copepods analyzed, and v) the mean number of microplastics ingested per copepod, calculated across all individuals, including those that did not contain any microplastics at T_0. For copepods scored as "saturated", the number of ingested particles was estimated as 40, based on the median number of particles per aggregate, calculated using the best possible counts despite limitations



Scheme 1. Overview of the Protocol 1 workflow.

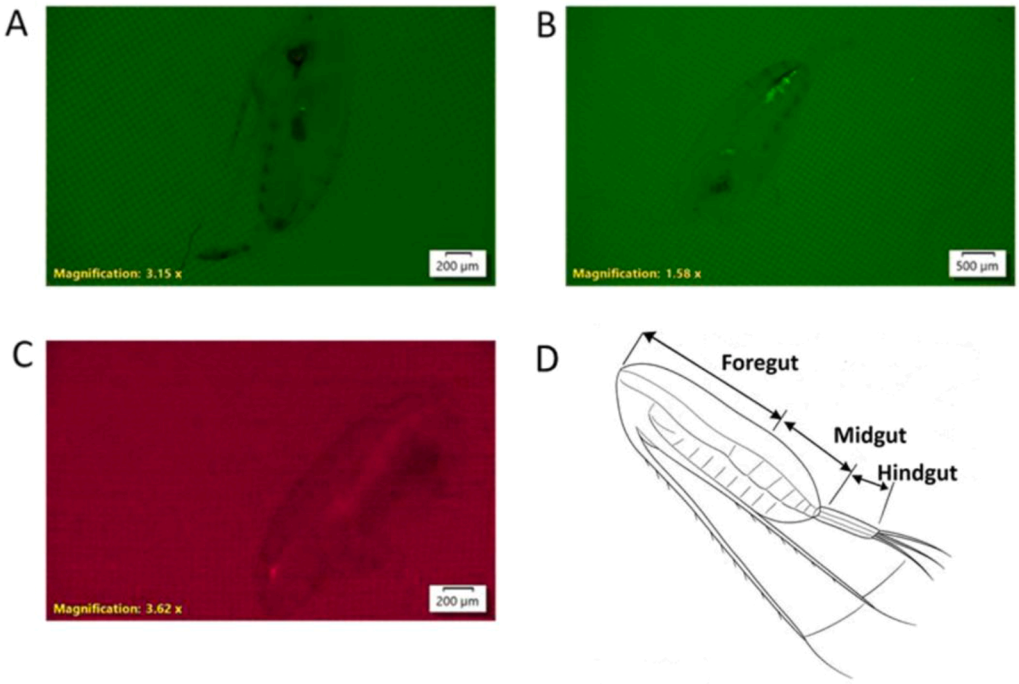
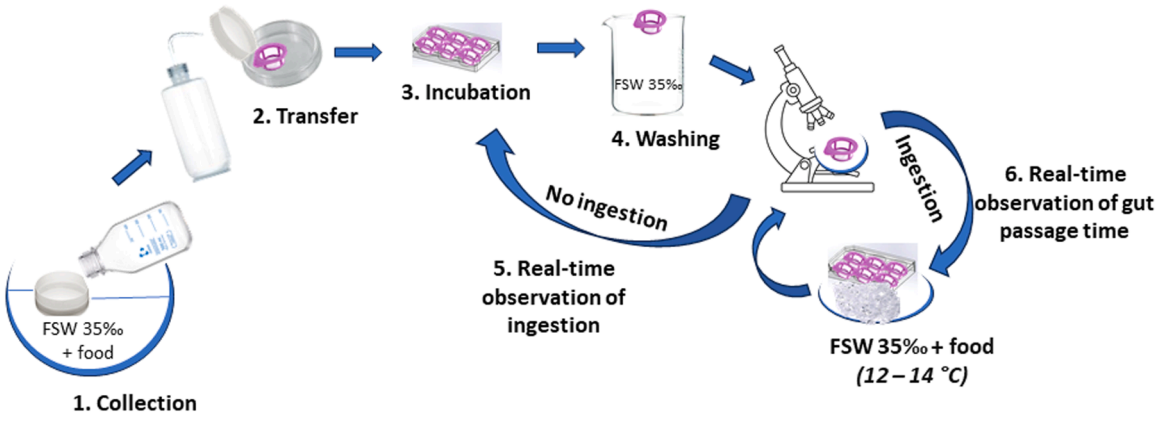


Fig. 1. *Calanus helgolandicus* with (A) single 20 µm polystyrene (PS) beads, (B) 20 µm PS aggregates, (C) 10–30 µm nylon fibers in the gut, and (D) schematic representation of the foregut, midgut, and hindgut.



Scheme 2. Overview of the experimental workflow for the ingestion/egestion experiments.

caused by the rapid vertical movement of particles in the gut and camera resolution.

Additionally, qualitative observations were made regarding the behavior of microplastics within each gut segment. These included taste discrimination (particles brought to the mouth and rejected without ingestion), regurgitation (particles visibly egested from the mouth after ingestion), and regurgitation-like events. The latter were defined as cases in which particles were present in the foregut at one observation time point and no longer visible at the next (2.3-minute interval), without any corresponding increase in the midgut, suggesting possible regurgitation.

To evaluate the variability in overall gut passage time, as well as in foregut and midgut passage times, a multivariate homogeneity of group dispersions analysis was performed using the betadisper function in the R package vegan [44]. The analysis was performed using 999 permutations, considering the distances to group centroids as a measure of dispersion.

2.5.2. *Protocol 2*

To evaluate gut passage time, the following variables were calculated: (i) gut passage time, defined as the total time each MP spent within the copepod gut; (ii) foregut retention time, defined as the time microplastics remained in the copepod foregut; (iii) midgut retention time, defined as the time microplastics remained in the copepod midgut; (iv) frequency of ingestion, calculated as the proportion of copepods that ingested microplastics within 3 h of exposure relative to the total number of copepods analyzed; (v) time to ingestion; and (vi) the mean number of microplastics ingested per copepod. Qualitative observations were recorded as described for Protocol 1.

Despite careful washing of copepods after initial ingestion, some microplastics occasionally remained adhered externally or within the medium, leading to secondary ingestion events. In such cases, all relevant parameters were recorded to assess intra-copepod variability in time to ingestion and gut passage time. However, these events were treated separately and excluded from statistical analyses.

Differences in gut passage time, foregut retention time, and midgut

retention time were evaluated using a nonparametric permutational multivariate analysis of variance (PERMANOVA), with food concentration, MP shape, and their interaction as factors. The analysis was performed with 999 permutations of the raw data. Additionally, for each MP shape, Kruskal-Wallis tests were used to separately assess differences in gut passage time, foregut retention time, and midgut retention time between low and high food concentrations. Moreover, to assess the overall variability in gut passage time and the impact of extreme values, a percentile-based approach was used to identify them, applying the 1st percentile for lower extremes and the 95th percentile for upper extremes. This method accounted for the asymmetric distribution of the data. The overall standard deviation (SD) was first calculated using all data points. A second SD calculation was then performed after removing the identified extreme values to evaluate their influence on variability estimates.

To assess the consistency between the two experimental protocols, gut passage times were compared using a Kruskal-Wallis test. For comparability, only midgut passage times were considered in both experiments. As such, for Protocol 1 data, only microplastics initially located in the foregut at T_0 were included, ensuring that their midgut passage time was accurately measured; foregut passage times were not considered, as the exact ingestion time of microplastics in protocol 1 was unknown.

Finally, to estimate the potential vertical flux of microplastics mediated by *Calanus helgolandicus*, we developed a simplified model based on ingestion and egestion dynamics observed during the experiment. The flux was calculated by integrating mean microplastic ingestion, gut passage time, and copepod abundance, according to the following formula:

$$\text{MPs flux } (\text{MPs}/\text{m}^3/\text{h}) = \frac{\text{Meaningestion } (\text{MPs}/\text{ind}) \times \text{Abundance } (\text{ind}/\text{m}^3)}{\min(\text{Time to ingestion } (\text{h}),\ \text{Gut passage time } (\text{h}))}$$

Where mean ingestion is expressed as microplastics per individual, abundance as individuals per cubic meter (based on data from the Western Channel Observatory temporal series), and time to ingestion and gut passage time as hours. The denominator accounts for the rate-limiting step between ingestion and egestion. This formula was applied under simplifying assumptions, including constant feeding rates, homogeneous exposure to microplastics, and the cumulative sinking of all fecal pellets through the full water column.

To express microplastic flux in units of MPs m^{-2} day^{-1} — consistent with values reported in the literature for vertical particle fluxes [50] — we multiplied the calculated flux per cubic meter by the height of the water column. This conversion reflects the cumulative sinking of fecal pellets through the entire water column, accumulating over a unit surface area at its base. The conversion was calculated as:

$$\text{Microplastic flux } (\text{MPs}/\text{m}^2/\text{day}) = \text{Flux}(\text{MPs}/\text{m}^3/\text{day}) \times \text{Water column depth}(\text{m})$$

3. Results

3.1. Protocol 1

Only two out of 65 individuals were found dead after the overnight incubation, confirming that, under the specific experimental conditions of this study, the microplastics utilized do not incite lethal toxicity over acute timescales. The behavior of PS beads within the gut of *Calanus helgolandicus* individuals was characterized by rapid oscillations in the foregut (video supplementary material), which ceased once the particles reached the midgut. In the midgut, beads were packed into fecal pellets until they passed into the hindgut. Retention time within the hindgut was in the order of seconds. The observed behavior of PS beads within the gut was consistent across the three tested food concentrations ("Low", "High", and "Extreme"). A single regurgitation event was observed and partially recorded; regurgitation occurred within a few seconds and involved a large number of particles being expelled from the foregut (Supplementary Material).

Supplementary material related to this article can be found online at doi:10.1016/j.jhazmat.2025.140551.

The proportion of copepods containing at least one microplastic particle was 87 %, 74 %, and 100 % at low, high, and extreme food concentrations respectively, while the mean ingestion values were 21.07 ± 15.40, 5.74 ± 7.26, and 32.27 ± 13.30 particles/individual, respectively (Supplementary Materials). No consistent trend in ingestion frequencies or mean ingestion values was observed with increasing food concentrations. At T_0, the majority of the particles were observed in the foregut (Table 1). Mean gut passage time ranged from 38.36 ± 17.67 min at low food concentrations to 47.09 ± 35.60 min at high food concentrations for particles located in the foregut at T_0. Comparatively, mean gut passage times ranged from 13.87 ± 17.67 min at high food concentrations to 22.30 ± 20.20 min at low food concentrations for particles located in the midgut at T_0 (Table 1). The slowest recorded gut passage time was 2.30 min from the start of the observation to complete evacuation. Variability in the overall gut passage time, and in midgut and foregut passage times, was consistent across food treatments (betadisper; $F = 0.128$, $p > 0.05$). However, the limited number of particles located in the midgut at T_0, particularly under high and extreme food treatments, limits the robustness of the comparison of midgut passage time across food treatments (Fig. 2).

When more than one microplastic was ingested, microplastics moved through the gut as aggregates, transitioning together from one segment to another prior to egestion. The majority of microplastics observed during the experiment were present as aggregates. Overall, particle aggregates exhibited higher gut passage times compared to individual particles (Fig. 2). However, due to an imbalance in the number of individuals in which aggregates versus single particles were observed — with aggregates recorded in a substantially greater number of copepods — statistical comparison between the two categories was not conducted.

3.2. Protocol 2

Within three hours of exposure, 75 % of adult female *C. helgolandicus* ingested microplastics (Table 2), with a mean ingestion of 1.7 microplastics per individual across treatments (Supplementary Materials). A higher ingestion frequency was observed for fibers (81 %) and beads (75 %), whereas only 50 % of individuals ingested fragments (Table 2). Time to ingestion varied among individuals, with a mean of 16.60 min (Table 2) and values ranging from 2 to 66 min (Supplementary Materials). Time to ingestion was consistent across treatments, with no significant effects of food concentration, microplastic type, or their interaction (PERMANOVA: $F = 0.56$, $p > 0.05$, $R^2 = 0.05$). Particles were tasted and rejected multiple times during observations, and three regurgitation-like events were recorded (Supplementary material). Microplastic transit behavior was similar to that observed in Protocol 1

Table 1
Summary of the overall gut passage time, defined as the total time each particle spent within the copepod gut regardless of its location at T0; the gut passage time from the foregut, referring to particles located in the foregut at T0; and the gut passage time from the midgut, referring to particles located in the midgut at T0. Data are presented for low, high, and extreme food concentrations.

Low food concentration			
Gut passage time from the foregut (min ± sec)			
Mean ± SD	**Min. value**	**Max. value**	**N. of observation**
38.36 ± 17.67	20.00	67.30	8
Gut passage time from the midgut (min ± sec)			
Mean ± SD	**Min. value**	**Max. value**	**N. of observation**
22.30± 20.20	2.30	42.30	3
Overall gut passage time (min ± sec)			
Mean ± SD	**Min. value**	**Max. value**	**N. of observation**
33.98 ± 18.84	2.30	67.30	11
High food concentration			
Gut passage time from the foregut (min ± sec)			
Mean ± SD	**Min. value**	**Max. value**	**N. of observation**
47.09 ± 35.60	15.00	110.00	7
Gut passage time from the midgut (min ± sec)			
Mean ± SD	**Min. value**	**Max. value**	**N. of observation**
13.87 ± 12.20	2.30	32.50	7
Overall gut passage time (min ± sec)			
Mean ± SD	**Min. value**	**Max. value**	**N. of observation**
30.48 ± 30.83	2.30	110.00	14
Extreme high food concentration			
Gut passage time from the foregut (min ± sec)			
Mean ± SD	**Min. value**	**Max. value**	**N. of observation**
44.53 ± 24.26	17.30	87.00	9
Gut passage time from the midgut (min ± sec)			
Mean ± SD	Min. value	Max. value	N. of observation
17.30 ± 00.00	17.30	17.30	2
Overall gut passage time (min ± sec)			
Mean ± SD	Min. value	Max. value	N. of observation
39.58 ± 24.33	17.30	87.00	11

experiments, with no notable differences between microplastic types. When more than one microplastic was ingested, microplastics moved through the gut as an aggregate, transitioning together from one segment to another prior to egestion.

After ingestion, *C. helgolandicus* readily egested microplastics, with an overall mean gut passage time of 46.41 min (Table 2). Values converged strongly around the median (40.00 min) (Fig. 3), reflecting overall homogeneity in gut passage times for fragments, beads, and fibers across food concentrations (PERMANOVA, $p > 0.05$; Table S1). Gut passage times remained consistent for each microplastic shape at both low and high food concentrations (Kruskal-Wallis test, $p > 0.05$). Retention times in the foregut and midgut were comparable for all microplastic shapes at both low and high food concentrations (Kruskal-Wallis test, $p > 0.05$; Table 2), except for fibers at high food concentrations, showing longer retention in the foregut compared to the midgut (Kruskal-Wallis test, $p < 0.05$).

The overall variability in gut passage time (SD = 31.34 min.) was primarily driven by a small proportion of particles transiting faster or slower than average. Specifically, 2 % of microplastics (1 out of 63) were egested in less than 2.5 min, while 6 % (4 out of 63) required more than 120 min. The highest extreme values were observed in individuals exposed to fibers and fed at high food concentrations, contributing to the slightly increased variability associated with this treatment (Table 2). Higher gut passage times were correlated with the absence of oscillations within the foregut. The fastest egestion was associated with regurgitation-like events. Removing these extreme values reduced the SD to 19.05 min, confirming the convergence of the majority of gut passage times around the median while maintaining a slight variability. Observations of second ingestion events revealed variability in the egestion process, with differences in gut passage times recorded within the same individual during exposure (Supplementary Materials). Finally, no significant differences were detected in gut passage time between the two experimental designs (Kruskal-Wallis test: $\chi^2 = 3.52$, $p < 0.05$).

4. Discussion

Microplastic-copepod interactions are emerging as critical pathways shaping the distribution of microplastics and their ecological impacts in marine environments. This study introduces a novel real-time visualization protocol that, for the first time, provides precise estimates of microplastic gut passage time and ingestion intervals in copepods,

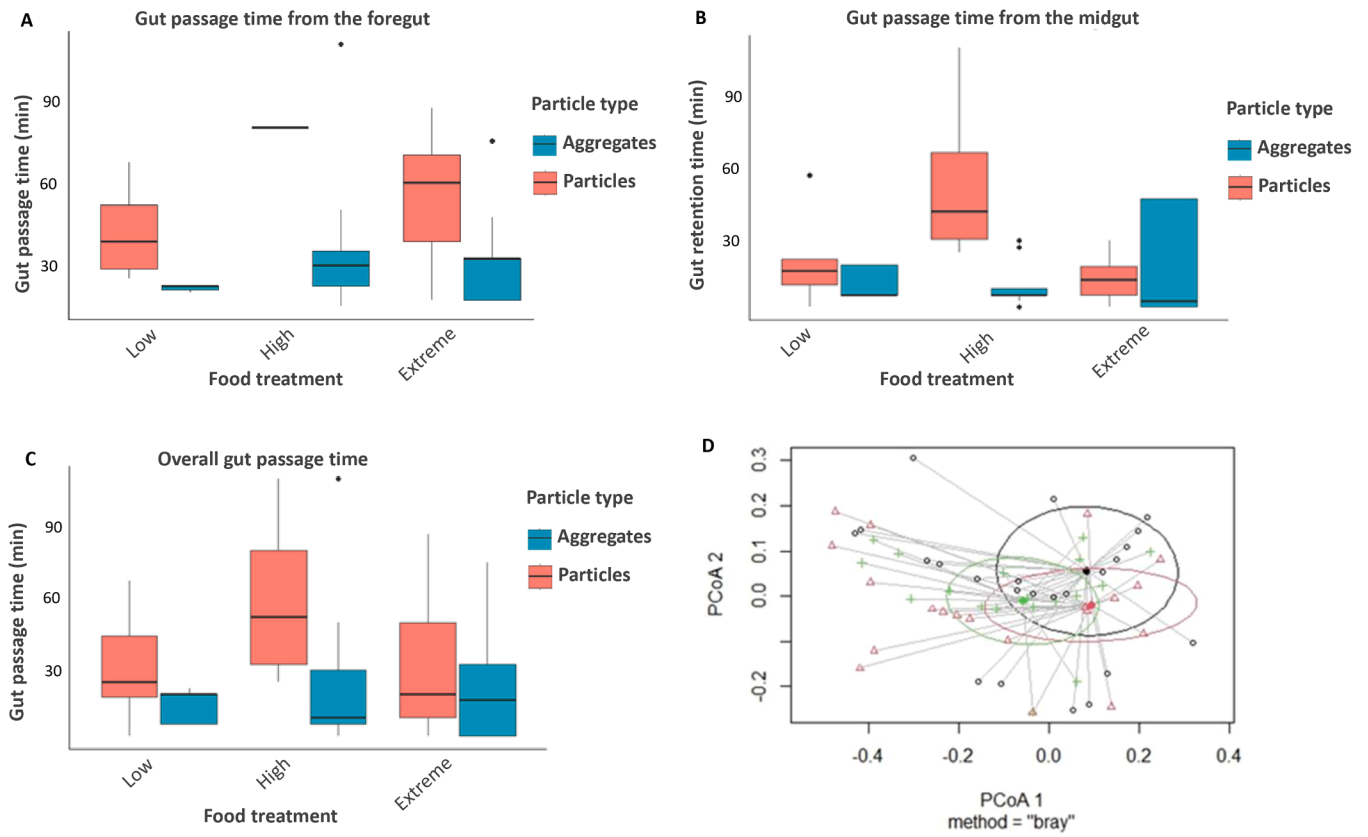


Fig. 2. Barplot showing (A) the overall gut passage time, defined as the total time each particle spent within the copepod gut regardless of its location at T0; (B) the gut passage time from the foregut, referring to particles located in the foregut at T0; and (C) the gut passage time from the midgut, referring to particles located in the midgut at T0, recorded in the pilot experiment for PS beads (particles and aggregates). (D) Schematic diagram of the multivariate homogeneity of group dispersion (variance) in gut passage time according to food concentrations. Grey lines represent the distance of each sample to its group centroid, ellipses represent the standard deviation, and PCoA1 and PCoA2 are the first and second principal coordinate axes in the variance analysis.

Table 2
Summarizes ingestion frequency, time to ingestion, foregut and midgut retention times, and gut passage time in *Calanus helgolandicus* exposed to different microplastic shapes (beads, fragments, fibers) under low and high food concentrations.

MPs shapes	MPs concentration	Food concentration	Number of individual	Frequency of Ingestion	Time to ingestion (min ± sec)	Foregut retention time (min ± sec)	Midgut retention time (min ± sec)	Gut passage time (min ± sec)	Number of observation
Beads	100 MPs/mL	Low	17	76 %	19.08 ± 21.21	23.45 ± 29.22	26.62 ± 22.74	51.06 ± 34.21	13
Beads	100 MPs/mL	High	15	73 %	12.00 ± 10.38	25.10 ± 11.42	23.53 ± 5.23	47.83 ± 13.61	11
Fibers	100 MPs/mL	Low	10	100 %	23.20 ± 18.30	16.71 ± 14.62	10.14 ± 6.38	28.90 ± 18.30	10
Fibers	100 MPs/mL	High	17	70 %	14.80 ± 13.03	40.38 ± 43.17	19.92 ± 13.04	63.68 ± 50.64	12
Fragments	100 MPs/mL	Low	12	50 %	12.20 ± 12.06	16.02 ± 12.60	16.32 ± 3.50	32.39 ± 14.86	7
Fragments	100 MPs/mL	High	12	58 %	16.86 ± 17.70	31.64 ± 24.91	21.26 ± 16.56	54.61 ± 17.63	9
TOTAL			**83**	**75 %**	**16.60 ± 15.99**	**25.97 ± 26.67**	**20.14 ± 14.08**	**46.41 ± 31.34**	**62**

helping to fill a missing piece of the puzzle in quantifying copepod-mediated microplastic fluxes.

In our study, microplastic gut passage time in *Calanus helgolandicus,* fed with a mix of algal monocultures *Prorocentrum micans* and *Dunaliella tertiolecta,* remained consistent across increasing food concentrations, ranging from pre-bloom-like natural prey concentrations to bloom conditions, including levels comparable to the highest algae concentrations observed at the sampling sites. Gut passage time is considered a key parameter for understanding feeding strategy adaptations and has been suggested to vary according to food type and concentration [60]. Jumars et al., [32] observed a decrease in natural prey gut passage time with increasing food abundance, along with a consequent increase in feeding rate, suggesting that copepods may benefit from extracting fewer nutrients per unit of food in favor of processing larger quantities under non-food-limited conditions. However, this correlation is not consistent among studies, with opposite patterns reported by other authors [4,39,46,62]. Additionally, no changes in gut passage time with increasing food concentration were observed by Head et al. [30] and

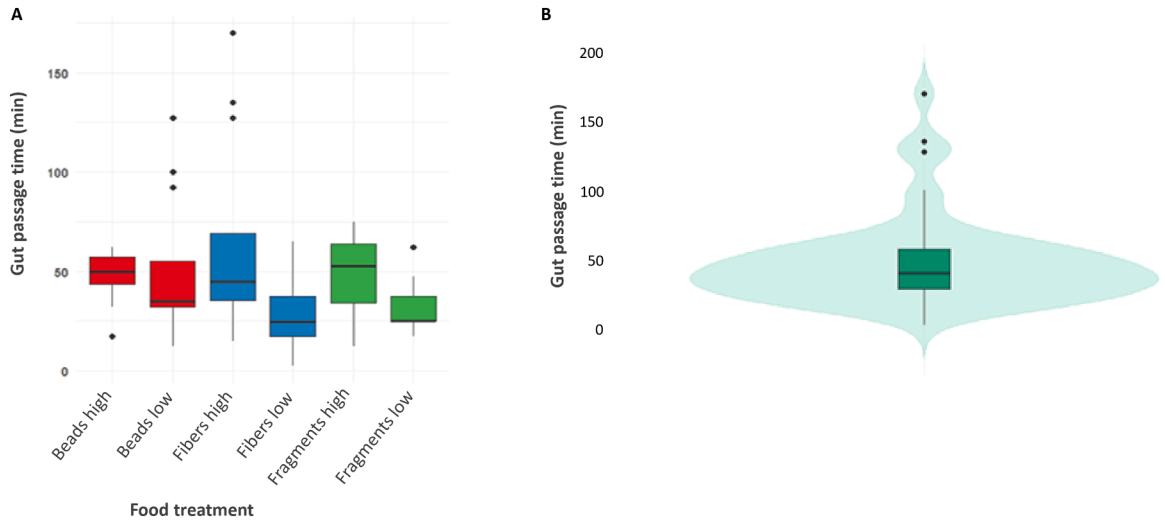


Fig. 3. (A) Barplot showing the gut passage time in *Calanus helgolandicus* according to the different microplastic shapes (beads, fibers, and fragments) under low and high food concentrations. (B) Violin plot illustrating the overall distribution of gut passage time for all microplastic shapes combined, with the width of the violins representing the density of data points. The dashed line represents the overall median gut passage time (40 min).

Tseitlin et al. [61]. Tirelli & Mayzaud [60], who found that the relationship between food concentration and gut passage time varied depending on the diet used to feed *Acartia clausii*, including dinoflagellates, detritus, and live diatoms; the study suggested that food quality, defined by protein and nutrient content, as well as food digestibility, are critical factors influencing gut transit time in copepods.

Overall, the mean microplastic gut passage times observed in this study ranged from 32 to 64 min across treatments. These values align with the range reported for the gut passage time of natural prey in different copepod species, *Neocalanus plumchrus* (35–85 min) and *Acartia clausii* (31–97 min) fed with *Thalassiosira weissflogii*, but are higher than those observed for *Calanus sinicus* (10–16 min) fed with the same algae [20,66,60]. For copepods fed with *P. micans*, the same studies report a range of 13–44 min for *C. sinicus* and approximately 106 min for *A. clausii*. *P. micans* has been proposed as a low-digestible food source despite its high nutrient content and has been associated with constant gut passage times, independent of food concentration [60]. In contrast, *T. weissflogii* has been considered as a high-quality food, and has been linked to gut passage times negatively correlated with food concentration [60,20]. While this would suggest copepod species and diet can influence gut passage times, it should be noted relationships between food type and gut passage time are not consistent across studies [66]. Our results also highlight individual variability in gut passage times confirmed by the observation of sequent ingestion events in the same individual.

Microplastic behavior observed within the gut of *C. helgolandicus* is in line with the digestion process previously described for natural prey, characterized by rapid oscillations in the foregut, ceasing upon reaching the midgut. This behavior, facilitating processes such as mixing and retention, aligns with the structures and functions described for the different copepod gut segments [57,7,43]. Natural prey, after ingestion, are initially processed in the foregut through agglutination with mucopolysaccharides and mixing with glycoproteins, facilitating their transit and subsequent digestion in the midgut [7]. The transfer from the foregut to the midgut, the segment primarily involved in enzymatic digestion and nutrient absorption, occurs via the action of the foregut muscles. Our results show that microplastic gut passage time in copepods closely aligns with the range observed for natural prey digestion [20,60,65], suggesting that microplastics follow similar transit dynamics as organic food. This further supports the hypothesis that copepods process microplastics in a manner analogous to their natural diet, reinforcing their role as efficient biological vectors in microplastic transport through marine ecosystems.

Microplastic gut passage time was unaffected by the number of microplastics ingested. No differences were detected between the two experimental protocols, one involving the simultaneous ingestion of up to 40 microplastics and the other predominantly involving the ingestion of a single microplastic per individual, with an overall mean of 1.7 microplastics per individual. Similarly, gut passage time showed no variation based on the shape of the microplastics tested—fibers, fragments, and beads. Previous studies have shown that the proportion of different types of microplastics ingested by copepods in marine environments exhibit temporal and spatial heterogeneity [1,2,22,24,49], but our results indicate gut passage time can be considered constant across varying environmental compositions of microplastics. Microplastic gut passage time converged strongly around the median of 40 min, with a few extreme values predominantly associated with fibers corresponding to the highest gut passage times. Real-time observation of microplastic behavior within the copepod gut revealed that fibers, which showed the slowest transit times, remained stable in the foregut without exhibiting rapid oscillations. It is unclear why fibers cause this response. This may be due to their shape increasing adhesion to the gut wall or limiting oscillation, thereby affecting their processing and hindering their transit. Fibers have been reported to induce significant biological reactivity in invertebrates due to mechanical disturbance and immune responses driven by their high aspect ratio [47] and to be more prone to causing physical damage than microplastic particles [12]. Thus, the response observed could be derived from fiber-induced localized inflammation.

Rapid egestion of microplastics by copepods has previously been highlighted by Uddin et al., [64], reporting *Pseudocalanus crossirostris* and *Acartia pacifica* egested 75–84 % and 61 % −71 % of microplastics within one hour following ingestion, with only 2 % of microplastics persisting in the gut for more than 2 h. Similarly, *P. annandalei* exposed to 2 μm and 10 μm microplastics demonstrated complete evacuation within 2 h of exposure [8]. These findings closely align with our observations, where 74 % of *C. helgolandicus* egested microplastics within 60 min, and 94 % egested microplastics within 2 h. Furthermore, our data shows that egestion begins as early as 12.5 ± 2.5 min post-ingestion (Experiment 2), with 30 % of microplastics egested within 12 min (Experiment 1). Field sampling studies have demonstrated copepod populations are substantial microplastic reservoirs [1,19,22,24,29,53,58], but given the time taken to haul in and preserve samples, existing data may be considered an underestimation. The

combination of copepod populations acting as microplastic reservoirs and rapid microplastic gut transit times reinforces the vital role copepods play in the short-term cycling of microplastics within marine environments. Rapid cycling and biotransformation of microplastics into microplastic-laden feces will enhance microplastic redistribution both longitudinally via currents and vertically via gravimetric-driven sinking, supporting the hypothesis that zooplankton-mediated transport is a key factor in determining the global fate of microplastics.

The slowest gut passage times observed, ranging 2.3–5 min, were associated with regurgitation or regurgitation-like events. This study represents the first video observation of microplastic regurgitation in copepods. In another study, high-speed videography has captured the copepod *Temora longicornis* rejecting microplastics prior to ingestion [65]. In *C. helgolandicus*, a significant reduction in grazing has been observed when copepods are exposed to microplastics [15,17], reaching a decline of 50 % when the ratio between microplastics and food reaches 0.5 [17]. We postulate the time and energy given to particle rejection and regurgitation, alongside gut blockages and food dilution, may contribute to such diminished feeding. Richon et al. [50] recently used the relationship between grazing and microplastic:food ratios [17] to project the impact of reduced grazing on the global carbon cycle. Based on field data and microplastic abundance projections, they estimate that this ratio could be reached within 10 years in highly contaminated regions, such as subtropical gyres and heavily impacted coastal areas. Over the next century, this trend could lead to a 20 % reduction in grazing even in areas currently experiencing lower microplastic contamination. Further studies are needed to assess the frequency of microplastic rejection and regurgitation in copepods to evaluate their potential effects on copepod grazing.

Microplastic gut passage time and ingestion interval metrics from this study provides a critical advance toward transitioning from static snapshots to dynamic flux assessments. The data represent a robust parameter for refining microplastic flux calculations in ocean systems and integration into model studies. However, it is important to acknowledge that this approximation was derived from experiments conducted at a fixed concentration of 100 microplastics/mL. Environmental abundances of the microplastic fraction ingested by copepods (up to 100 μm) remain largely unknown [38]. This fraction has been estimated to increment by one to two orders of magnitudes with respect to the 335 μm to 5 mm fractions normally considered in microplastic pollution studies [55]. Thus, it is possible that MP abundances used in this study could be higher than those actually found in the marine environment for this size fraction, even if potentially comparable to hotspot areas and proxime future scenarios. However, the comparability of the time to ingestion observed in accidentally second ingestion events, where only a few accidental particles were available in the exposure medium, and the other ingestion events suggest a limited influence of MP abundances on the time to ingestion. Thus, by assuming variability in mean microplastic ingestion, gut passage time, and ingestion intervals to be negligible and integrating these with in-field abundance data, it becomes possible to estimate copepod-mediated microplastic fluxes at both local and global scales.

Applying a mean ingestion of 0.05 microplastics/ind [23], a time to ingestion of 17 min, and a gut passage time of 40 min, the microplastics flux mediated by *C. helgolandicus* could be estimated at approximately 11 microplastics/m^3 /hour. This estimate is based on the mean *C. helgolandicus* abundance recorded at the sampling site in the Western Channel Observatory temporal series (64 ind/m^3; www.westernchannelobservatory.org.uk). This corresponds to 271 microplastics/m^3 per day. Under peak abundance conditions, considering the highest recorded density of *C. helgolandicus* in the Western Channel Observatory (1867 ind/m^3), the estimated microplastics flux would reach 329 microplastics/m^3 /hour, amounting to approximately 7907 microplastics/m^3 per day. Few studies have quantified the flux of microplastics in marine environments. Recently, Rowlands et al. [51] estimated a vertical flux of 306 microplastics/m^2 per day at a depth of 50 m. If we convert our copepod-mediated microplastics flux to the same units by assuming a cumulative sinking pathway, in which each meter of the water column integrates the microplastics incorporated within fecal pellets from the meters above, we obtain a mean flux of 5760 microplastics/m^2 per day at a depth of 50 m. These estimations are a first approximation, as they rely on simplifying assumptions regarding the sinking behavior of microplastics egested within fecal pellets, without considering the sinking dynamics of fecal pellets themselves, remineralization processes, or coprophagy, and simplified *C. helgolandicus* population dynamics. In reality, the properties of fecal pellets are also altered by microplastic incorporation [15,17], although this has not yet been evaluated under environmentally realistic scenarios of microplastic integration into fecal pellets. More accurate estimations of these alterations, as well as the parameterization and inclusion of the different factors governing both copepod population dynamics and fecal pellet sinking dynamics in copepod-mediated microplastic fluxes, are needed to provide more precise assessments. Nevertheless, this study provides preliminary quantitative evidence that copepods could act as major drivers of the vertical transport of microplastics, potentially surpassing the passive sinking of free microplastics, and highlights the urgency of advancing the estimation of these processes and their inclusion in analyses of the spatial distribution and fate of microplastics in marine environments.

5. Conclusion

Finally, for the first time, we provide precise estimates of microplastic gut passage times and ingestion intervals in copepods under environmentally realistic conditions, introducing a novel real-time visualization protocol. By establishing a quantitative framework for copepod-mediated fluxes, this study represents an important step toward integrating biological processes into large-scale oceanographic models to assess microplastic fate, exposure, and ecological risk. Our findings highlight the importance of including zooplankton-mediated fluxes in global assessments of microplastic transport and their long-term ecological impacts.

Environmental implications

The spatial distribution of microplastics is a key determinant of their ecological effects, and zooplankton is emerging as a major biological pathway for microplastics. This study establishes a quantitative framework to evaluate copepod-mediated fluxes by providing robust estimates of microplastics gut passage times and ingestion intervals. These advances represent an important step toward integrating biological processes into large-scale oceanographic models to assess microplastic fate, exposure, and ecological risk, with further implications for evaluating the effects of microplastics on the biological pump through alterations of fecal pellet dynamics.

CRediT authorship contribution statement

Lindeque Penelope: Writing – review & editing, Validation, Supervision, Resources, Project administration, Methodology, Conceptualization. **Coppock Rachel:** Writing – review & editing, Supervision, Methodology, Conceptualization. **Matthew Cole:** Writing – review & editing, Validation, Supervision, Methodology, Conceptualization. **Fagiano Valentina:** Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

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.

Acknowledgements

The authors would like to thank the Microplastics Group at the Plymouth Marine Laboratory for their support and training during this study. This work was supported by the Western Channel Observatory, funded by the UK Natural Environment Research Council through its National Capability Long-term Single Centre Science Programme, Atlantic Climate and Environment Strategic Science (AtlantiS), grant number NE/Y005589/1. Valentina Fagiano is the recipient of a Pre-doctoral Fellowship (FPI–CAIB), co-financed by the Government of the Balearic Islands (Govern de les Illes Balears) and the European Social Fund (Fons Social Europeu). She also gratefully acknowledges funding from the AJUTS A LA MOBILITAT INVESTIGADORA 2023–2025 (FPI–CAIB) fellowship, awarded by the Government of the Balearic Islands, which supported her international research stay. She gratefully acknowledges the continuous mentoring provided by Prof. Salud Deudero.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jhazmat.2025.140551.

Data availability

Data will be made available on request.

References

[1] Amin, R.M., Sohaimi, E.S., Anuar, S.T., Bachok, Z., 2020. Microplastic ingestion by zooplankton in Terengganu coastal waters, southern South China Sea. Mar Pollut Bull 150, 110616. https://doi.org/10.1016/j.marpolbul.2019.110616.

[2] Aytan, U., Esensoy, F.B., Senturk, Y., 2022. Microplastic Ingestion and Egestion by Copepods in the Black Sea. Sci Total Environ 806 (4), 150921. https://doi.org/10.1016/j.scitotenv.2021.150921.

[3] Bellas, J., Martínez-Armental, J., Martínez-Cámara, A., Besada, V., Martínez-Gómez, C., 2016. Ingestion of microplastics by Demersal Fish from the Spanish Atlantic and Mediterranean coasts. Mar Pollut Bull 109 (1), 55–60. https://doi.org/10.1016/j.marpolbul.2016.06.026.

[4] Besiktepe, S., Dam, H., 2002. Coupling of ingestion and defecation as a function of diet in the Calanoid Copepod Acartia tonsa. Mar Ecol Prog Ser 229, 151–164. https://doi.org/10.3354/meps229151.

[5] Botterell, Z.L.R., Beaumont, N., Dorrington, T., Steinke, M., Thompson, R.C., Lindeque, P.K., 2019. Bioavailability and effects of microplastics on Marine Zooplankton: a review. Environ Pollut 245, 98–110. https://doi.org/10.1016/j.envpol.2018.10.065.

[6] Buathong, D., Sriwisait, P., Pnengsakun, S., Chamchoy, C., Mue-Suae, O., Phoaduang, S., Thummasan, M., Sangiamdee, D., Yeemin, T., Sutthacheep, M., 2020. Accumulation of microplastics in zooplankton from Chonburi Province, the Upper Gulf of Thailand. Ramkhamhaeng Int J Sci Technol 3 (3).

[7] Calbet, A., Carlotti, F., Gaudy, R., 2007. The feeding ecology of the Copepod Centropages typicus (Kröyer). Prog Oceano 72 (2–3), 137–150. https://doi.org/10.1016/j.pocean.2007.01.003.

[8] Cheng, Y., Wang, J., Yi, X., Li, L., Liu, X., Ru, S., 2020. Low microalgae availability increases the ingestion rates and potential effects of microplastics on marine Copepod Pseudodiaptomus annandalei. Mar Pollut Bull 152, 110919. https://doi.org/10.1016/j.marpolbul.2020.110919.

[9] Cincinelli, A., Martellini, T., Guerranti, C., Scopetani, C., Chelazzi, D., Giarrizzo, T., 2019. A potpourri of microplastics in the sea surface and water column of the Mediterranean Sea. TrAC Trends Anal Chem 110, 321–326. https://doi.org/10.1016/j.trac.2018.10.026.

[10] Clark, J.R., Cole, M., Lindeque, P.K., Fileman, E., Blackford, J., Lewis, C., Lenton, T. M., Galloway, T.S., 2016. Marine microplastic debris: a targeted plan for understanding and quantifying interactions with marine life. Front Ecol Environ 14 (6), 317–324. https://doi.org/10.1002/fee.1297.

[11] Cole, M., 2016. A novel method for preparing microplastic fibers. Sci Rep 6, 34519. https://doi.org/10.1038/srep34519.

[12] Cole, M., Coppock, R., Lindeque, P.K., Altin, D., Reed, S., Pond, D.W., Sørensen, L., Galloway, T.S., Booth, A.M., 2019. Effects of nylon microplastic on feeding, lipid accumulation, and moulting in a coldwater Copepod. Environ Sci Technol 53 (12), 7075–7082. https://doi.org/10.1021/acs.est.9b01853.

[13] Cole, M., Galloway, T.S., 2015. Ingestion of nanoplastics and microplastics by Pacific Oyster Larvae. Environ Sci Technol 49 (24), 14625–14632. https://doi.org/10.1021/acs.est.5b04099.

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

[15] Cole, M., Lindeque, P.K., Fileman, E., Clark, J., Lewis, C., Halsband, C., Galloway, T.S., 2016. Microplastics alter the properties and sinking rates of zooplankton Faecal Pellets. Environ Sci Technol 50 (6), 3239–3246. https://doi.org/10.1021/acs.est.5b05905.

[16] Compa, M., Capó, X., Alomar, C., Deudero, S., Sureda, A., 2024. A meta-analysis of potential biomarkers associated with microplastic ingestion in marine fish. Environ Toxicol Pharm 107, 104414. https://doi.org/10.1016/j.etap.2024.104414.

[17] Coppock, R.L., Galloway, T.S., Cole, M., Fileman, E.S., Queirós, A.M., Lindeque, P. K., 2019. Microplastics alter feeding selectivity and faecal density in the copepod, *Calanus helgolandicus*. Sci Total Environ 687, 780–789. https://doi.org/10.1016/j.scitotenv.2019.06.009.

[18] Coppock, R.L., Lindeque, P.K., Cole, M., Galloway, T.S., Näkki, P., Birgani, H., Queirós, A.M., 2021. Benthic fauna contribute to microplastic sequestration in coastal sediments. J Hazard Mater 415, 125583. https://doi.org/10.1016/j.jhazmat.2021.125583.

[19] Corradini, R., Menéndez, M.C., Gonella, F.M., Baleani, C.A., Texeira, V.G., Valdivia, A.E.L., Huillca, C.V., Oliva, A.L., Arias, A.H., 2025. Microplastic ingestion by zooplankton in surf zone waters of sandy beaches: are copepods potential reservoirs of these emerging pollutants? Environ Pollut, 126772. https://doi.org/10.1016/j.envpol.2025.126772.

[20] Dagg, M.J., Walser, W.E., 1987. Ingestion, gut passage, and egestion by the Copepod *Neocalanus plumchrus* in the laboratory and in the Subarctic Pacific Ocean. Limnol Oceano 32 (1), 178–188. https://doi.org/10.4319/lo.1987.32.1.0178.

[21] Desforges, J.-P.W., Galbraith, M., Ross, P.S., 2015. Ingestion of microplastics by zooplankton in the Northeast Pacific Ocean. Arch Environ Contam Toxicol 69 (3), 320–330. https://doi.org/10.1007/s00244-015-0172-5.

[22] Fagiano, V., Alomar, C., Ventero, A., de Puelles, M.L.F., Iglesias, M., Deudero, S., 2024. First Assessment of Anthropogenic Particle Ingestion in Pontellid Copepods: *Pontella mediterranea* as a Potential Microplastic Reservoir in the Neuston. Sci Total Environ 908, 168480. https://doi.org/10.1016/j.scitotenv.2023.168480.

[23] Fagiano, V., Compa, M., Alomar, C., Deudero, S., 2024. Global Meta-Analysis and Review of Microplastic in Marine Copepods. Environ Pollut 351, 124092. https://doi.org/10.1016/j.envpol.2024.124092.

[24] Fibbe, M.C., Carroll, D., Gowans, S., Siuda, A.N.S., 2023. Ingestion of Microplastics by Copepods in Tampa Bay Estuary, FL. Front Ecol Evol 11, 1143377. https://doi.org/10.3389/fevo.2023.1143377.

[25] Galgani, L., Goßmann, I., Scholz-Böttcher, B., Jiang, X., Liu, Z., Scheidemann, L., Schlundt, C., Engel, A., 2022. Hitchhiking into the Deep: How Microplastic Particles Are Exported through the Biological Carbon Pump in the North Atlantic Ocean. Environ Sci Technol 56 (22), 15638–15649. https://doi.org/10.1021/acs.est.2c04712.

[26] Galgani, L., Loiselle, S.A., 2021. Plastic Pollution Impacts on Marine Carbon Biogeochemistry. Environ Pollut 268, 115598. https://doi.org/10.1016/j.envpol.2020.115598.

[27] Galgani, L., Tzempelikou, E., Kalantzi, I., Tsiola, A., Tsapakis, M., Pitta, P., Esposito, C., Tsotskou, A., Magiopoulos, I., Benavides, R., Steinhoff, T., Loiselle, S. A., 2023. Marine Plastics Alter the Organic Matter Composition of the Air-Sea Boundary Layer, with Influences on CO_2 Exchange: A Large-Scale Analysis Method to Explore Future Ocean Scenarios. Sci Total Environ 857, 159624. https://doi.org/10.1016/j.scitotenv.2022.159624.

[28] Geng, X., Wang, J., Zhang, Y., Jiang, Y., 2021. How Do Microplastics Affect the Marine Microbial Loop? Predation of Microplastics by Microzooplankton. Sci Total Environ 758, 144030. https://doi.org/10.1016/j.scitotenv.2020.144030.

[29] Goswami, P., Vinithkumar, N.V., Dharani, G., 2020. First Evidence of Microplastics Bioaccumulation by Marine Organisms in the Port Blair Bay, Andaman Islands. Mar Pollut Bull 155, 111163. https://doi.org/10.1016/j.marpolbul.2020.111163.

[30] Head, E.J.H., 1986. Estimation of Arctic Copepod Grazing Rates *in Vivo* and Comparison with *in Vitro* Methods. Mar Biol 92 (3), 371–379. https://doi.org/10.1007/BF00392678.

[31] Jones, R., Flynn, K., Anderson, T., 2002. Effect of Food Quality on Carbon and Nitrogen Growth Efficiency in the Copepod *Acartia tonsa*. Mar Ecol Prog Ser 235, 147–156. https://doi.org/10.3354/meps235147.

[32] Jumars, P.A., Penry, D.L., Baross, J.A., Perry, M.J., Frost, B.W., 1989. Closing the Microbial Loop: Dissolved Carbon Pathway to Heterotrophic Bacteria from Incomplete Ingestion, Digestion and Absorption in Animals. Deep Sea Res Part A 36 (4), 483–495. https://doi.org/10.1016/0198-0149(89)90001-0.

[33] Kosore, C., Ojwang, L., Maghanga, J., Kamau, J., Kimeli, A., Omukoto, J., Ngisiag'e, N., Mwaluma, J., Ong'ada, H., Magori, C., Ndirui, E., 2018. Occurrence and Ingestion of Microplastics by Zooplankton in Kenya's Marine Environment: First Documented Evidence. Afr J Mar Sci 40 (3), 225–234. https://doi.org/10.2989/1814232X.2018.1492969.

[34] Kvale, K., 2022. Implications of Plastic Pollution on Global Marine Carbon Cycling and Climate. Emerg Top Life Sci 6 (4), 359–369. https://doi.org/10.1042/ETLS20220013.

[35] Kvale, K., Hunt, C., James, A., Koeve, W., 2023. Regionally Disparate Ecological Responses to Microplastic Slowing of Faecal Pellets Yields Coherent Carbon Cycle Response. Front Mar Sci 10, 1111838. https://doi.org/10.3389/fmars.2023.1111838.

[36] Kvale, K., Prowe, A.E., Oschlies, A., 2020. A Critical Examination of the Role of Marine Snow and Zooplankton Fecal Pellets in Removing Ocean Surface Microplastic. Front Mar Sci 6, 808. https://doi.org/10.3389/fmars.2019.00808.

[37] Kvale, K., Prowe, A.E.F., Chien, C.-T., Landolfi, A., Oschlies, A., 2020. The Global Biological Microplastic Particle Sink. Sci Rep 10 (1), 16670. https://doi.org/10.1038/s41598-020-72898-4.
[38] Lindeque, P.K., Cole, M., Coppock, R.L., Lewis, C.N., Miller, R.Z., Watts, A.J.R., Wilson-McNeal, A., Wright, S.L., Galloway, T.S., 2020. Are We Underestimating Microplastic Abundance in the Marine Environment? A Comparison of Microplastic Capture with Nets of Different Mesh Size. Environ Pollut 265 (A), 114721. https://doi.org/10.1016/j.envpol.2020.114721.
[39] Mayzaud, P., Tirelli, V., Bernard, J.M., Roche-Mayzaud, O., 1998. The Influence of Food Quality on the Nutritional Acclimation of the Copepod *Acartia clausi*. J Mar Syst 15 (1–4), 483–493. https://doi.org/10.1016/S0924-7963(97)00039-0.
[40] McEvoy, A.J., Atkinson, A., Airs, R.L., Brittain, R., Brown, I., Fileman, E.S., et al., 2023. The western channel observatory: a century of physical, chemical and biological data compiled from pelagic and benthic habitats in the Western English Channel. Earth Syst Sci Data 15 (12), 5701–5737. https://doi.org/10.5194/essd-15-5701-2023.
[41] Nelms, S.E., Duncan, E.M., Broderick, A.C., Galloway, T.S., Godfrey, M.H., Hamann, M., Lindeque, P.K., Godley, B.J., 2016. Plastic and marine turtles: a review and call for research. ICES J Mar Sci 73, 165–181. https://doi.org/10.1093/icesjms/fsv165.
[42] Noman, M.A., Adyel, T.M., Trevathan-Tackett, S., Macreadie, P.I., 2024. Plastic paradox in blue carbon ecosystems. Environ Sci Technol 58 (10), 4469–4475. https://doi.org/10.1021/acs.est.3c08717.
[43] Nott, J.A., Corner, E.D.S., Marvin, L.J., O'Hara, S.C.M., 1985. Cyclical Contributions of the Digestive Epithelium to Faecal Pellet Formation by the Copepod *Calanus helgolandicus*. Mar Biol 89 (3), 271–279. https://doi.org/10.1007/BF00393662.
[44] Oksanen, J.; Blanchet, F.G.; Friendly, M.; Kindt, R.; Legendre, P.; McGlinn, D.; Minchin, P.R.; O'Hara, R.B.; Simpson, G.L.; Solymos, P.; Stevens, M.H.H.; Szoecs, E.; Wagner, H. *vegan: Community Ecology Package, R package version 2.6-4*, 2022. ⟨https://CRAN.R-project.org/package=vegan⟩.
[45] Olmo-Gilabert, R., Fagiano, V., Alomar, C., Rios-Fuster, B., Compa, M., Deudero, S., 2024. Plastic webs, the new food: Dynamics of microplastics in a Mediterranean food web, key species as pollution sources and receptors. Sci Total Environ 918, 170719. https://doi.org/10.1016/j.scitotenv.2024.170719.
[46] Pasternak, A.F., 1994. Gut fluorescence in herbivorous copepods: An attempt to justify the method. In: Ferrari, F.D., Bradley, B.P. (Eds.), Ecology and Morphology of Copepods, 102. Developments in Hydrobiology, Springer: Dordrecht, pp. 241–248. https://doi.org/10.1007/BF00229947 (TO ADD PITTURA).
[47] Pittura, L., Nardi, A., Cocca, M., De Falco, F., d'Errico, G., Mazzoli, C., Regoli, F., 2022. Cellular disturbance and thermal stress response in mussels exposed to synthetic and natural microfibers. Front Mar Sci 9, 981365. https://doi.org/10.3389/fmars.2022.981365.
[48] Porter, A., Lyons, B.P., Galloway, T.S., Lewis, C., 2018. Role of Marine Snows in Microplastic Fate and Bioavailability. Environ Sci Technol 52 (12), 7111–7119. https://doi.org/10.1021/acs.est.8b01000.
[49] Rashid, C.P., Jyothibabu, R., Arunpandi, N., Santhikrishnan, S., Vidhya, V., Sarath, S., Arundhathy, M., Alok, K.T., 2022. Microplastics in copepods reflects the manmade flow restrictions in the Kochi backwaters, along the southwest coast of India. Mar Pollut Bull 177, 113529. https://doi.org/10.1016/j.marpolbul.2022.113529.
[50] Richon, C., Gorgues, T., Paul-Pont, I., Maes, C., 2022. Zooplankton exposure to microplastics at global scale: Influence of vertical distribution and seasonality. Front Mar Sci 9, 947309. https://doi.org/10.3389/fmars.2022.947309.
[51] Rowlands, E., et al., 2023. Vertical flux of microplastic: a case study in the Southern Ocean, South Georgia. Mar Pollut Bull 193, 115117. https://doi.org/10.1016/j.marpolbul.2023.115117.
[52] Shen, M., Ye, S., Zeng, G., Zhang, Y., Xing, L., Tang, W., Wen, X., Liu, S., 2020. Can microplastics pose a threat to ocean carbon sequestration? Mar Pollut Bull 150, 110712. https://doi.org/10.1016/j.marpolbul.2019.110712.
[53] Sipps, K., Arbuckle-Keil, G., Chant, R., Fahrenfeld, N., Garzio, L., Walsh, K., Saba, G., 2022. Pervasive occurrence of microplastics in Hudson-Raritan estuary zooplankton. Sci Total Environ 817, 152812. https://doi.org/10.1016/j.scitotenv.2021.152812.
[54] Smyth, T., Atkinson, A., Widdicombe, S., Frost, M., Allen, I., Fishwick, J., Queiros, A., Sims, D., Barange, M., 2015. The Western Channel Observatory. Prog Oceano 137, 335–341. https://doi.org/10.1016/j.pocean.2015.05.020.
[55] Song, Y.K., Hong, S.H., Jang, M., Kang, J.-H., Kwon, O.Y., Han, G.M., Shim, W.J., 2014. Large accumulation of micro-sized synthetic polymer particles in the sea surface microlayer. Environ Sci Technol 48 (16), 9014–9021. https://doi.org/10.1021/es501757s.
[56] Suaria, G., Achtypi, A., Perold, V., Lee, J.R., Pierucci, A., Bornman, T.G., Aliani, S., Ryan, P.G., 2020. Microfibers in oceanic surface waters: a global characterization. Sci Adv 6 (23), eaay8493. https://doi.org/10.1126/sciadv.aay8493.
[57] Sullivan, D.S., Bisalputra, T., 1980. The morphology of a harpacticoid copepod gut: a review and synthesis. J Morphol 164 (1), 89–105. https://doi.org/10.1002/jmor.1051640108.
[58] Sun, X., Li, Q., Zhu, M., Liang, J., Zheng, S., Zhao, Y., 2017. Ingestion of microplastics by natural zooplankton groups in the northern South China Sea. Mar Pollut Bull 115 (1–2), 217–224. https://doi.org/10.1016/j.marpolbul.2016.12.004.
[59] Taha, Z.D., Amin, R.M., Anuar, S.T., Nasser, A.A.A., Sohaimi, E.S., 2021. Microplastics in seawater and zooplankton: a case study from Terengganu estuary and offshore waters, Malaysia. Sci Total Environ 786, 147466. https://doi.org/10.1016/j.scitotenv.2021.147466.
[60] Tirelli, V., Mayzaud, P., 2005. Relationship between functional response and gut transit time in the calanoid copepod Acartia clausi: role of food quantity and quality. J Plankton Res 27 (6), 557–568. https://doi.org/10.1093/plankt/fbi031.
[61] Tseitlin, V.B., Pasternak, A.F., Drits, A.V., 1991. Does gut passage time in copepods depend on the food concentration? Oceanology 31 (1), 155–161.
[62] Tsuda, A., Nemoto, T., 1987. The effect of food concentration on the gut clearance time of *Pseudocalanus minutus* Kroyer (Calanoida: Copepoda). J Exp Mar Biol Ecol 107 (2), 121–130. https://doi.org/10.1016/0022-0981(87)90190-0.
[63] Turner, J.T., 2015. Zooplankton fecal pellets, marine snow, phytodetritus and the ocean's biological pump. Prog Oceano 130, 205–248. https://doi.org/10.1016/j.pocean.2014.08.005.
[64] Uddin, S., Behbehani, M., Habibi, N., Fowler, S.W., Al-Sarawi, H.A., Alonso-Hernandez, C., 2023. Microplastics residence time in marine copepods: an experimental study. Sustainability 15 (20), 14970. https://doi.org/10.3390/su152014970.
[65] Xu, J., Rodríguez-Torres, R., Rist, S., Nielsen, T.G., Hartmann, N.B., Brun, P., Li, D., Almeda, R., 2022. Unpalatable Plastic: Efficient Taste Discrimination of Microplastics in Planktonic Copepods. Environ Sci Technol 56 (13), 9344–9354. https://doi.org/10.1021/acs.est.2c00322.
[66] Xu, Y., Wang, W.-X., 2001. Individual responses of trace element assimilation and physiological turnover by the marine copepod *Calanus sinicus* to changes in food quantity. Mar Ecol Prog Ser 218, 227–238. https://doi.org/10.3354/meps218227.
[67] Zhao, S., Zettler, E.R., Amaral-Zettler, L.A., Mincer, T.J., 2021. Microbial carrying capacity and carbon biomass of plastic marine debris. ISME J 15 (1), 67–77. https://doi.org/10.1038/s41396-020-00756-2.
[68] Zheng, S., Zhao, Y., Liangwei, W., Liang, J., Liu, T., Zhu, M., Li, Q., Sun, X., 2020. Characteristics of microplastics ingested by zooplankton from the Bohai Sea, China. Sci Total Environ 713, 136357. https://doi.org/10.1016/j.scitotenv.2019.136357.
[69] Zheng, S., Zhao, Y., Liu, T., Liang, J., Zhu, M., Sun, X., 2021. Seasonal characteristics of microplastics ingested by copepods in Jiaozhou Bay, the Yellow Sea. Sci Total Environ 776, 145936. https://doi.org/10.1016/j.scitotenv.2021.145936.
[70] Zhu, X., 2021. The plastic cycle – An unknown branch of the carbon cycle. Front Mar Sci 7, 609243. https://doi.org/10.3389/fmars.2020.609243.
[71] Zhu, X., Rochman, C.M., Hardesty, B.D., Wilcox, C., 2024. Plastics in the deep sea – A global estimate of the ocean floor reservoir. Deep Sea Res Part I Oceanogr Res Pap 206, 104266. https://doi.org/10.1016/j.dsr.2024.104266.