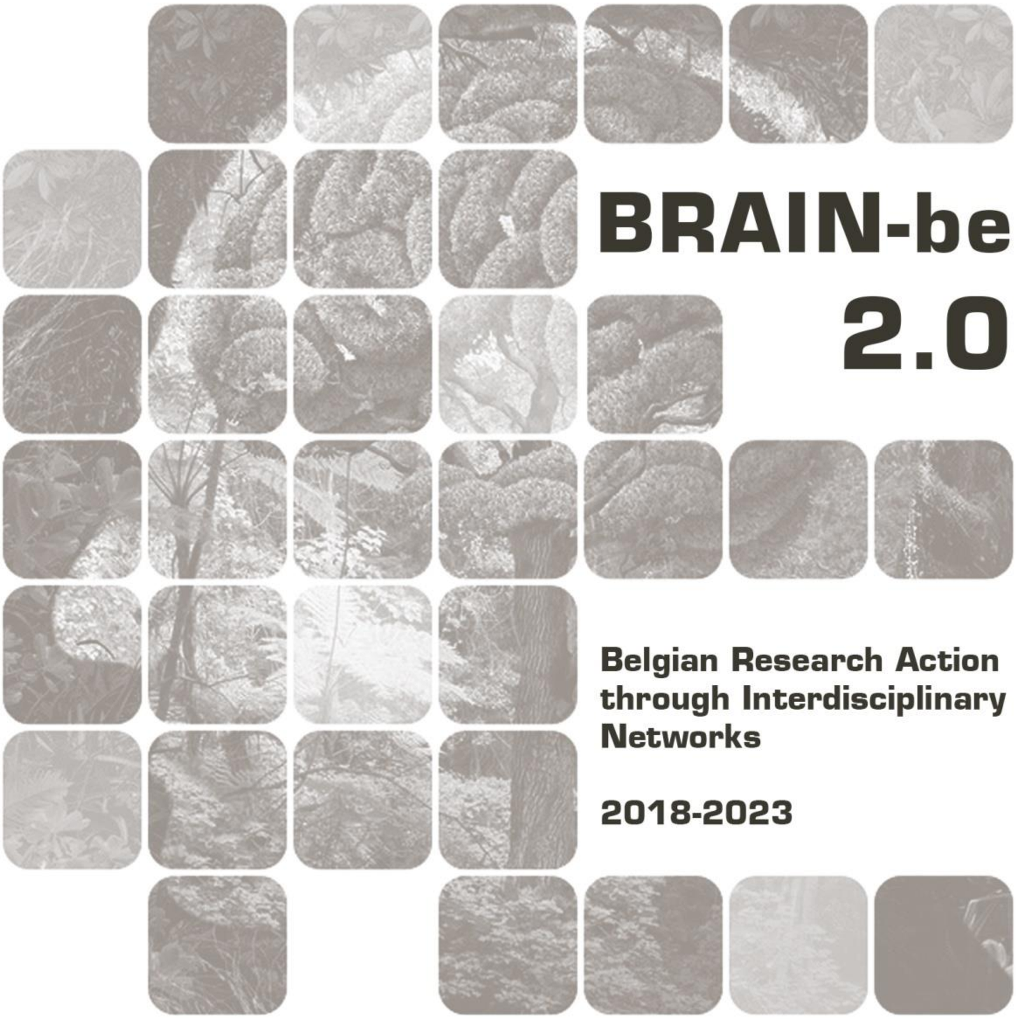

HOTMIC

Horizontal and vertical transport of microplastic particles

Gabriella Panto' (University of Ghent) – Carl van Colen (University of Ghent) – Ann Vanreusel (University of Ghent)

Pillar 1: Challenges and knowledge of the living and non-living world

belspo .be

BRAIN-be 2.0

Belgian Research Action through Interdisciplinary Networks

2018-2023

NETWORK PROJECT

HOTMIC

HORIZONTAL AND VERTICAL OCEANIC DISTRIBUTION, TRANSPORT, AND IMPACT OF MICROPLASTICS

Contract - B2/191/P1/HOTMIC

PROMOTORS: Ann Vanreusel (University of Ghent)
Carl Van Colen (University of Ghent)

AUTHORS: Gabriella Panto' (University of Ghent)

GHENT UNIVERSITY

belspo .be

Published in 2024 by the Belgian Science Policy Office
WTCIII
Simon Bolivarlaan 30 bus 7
Boulevard Simon Bolivar 30 bte 7
B-1000 Brussels
Belgium
Tel: +32 (0)2 238 34 11
http://www.belspo.be
http://www.belspo.be/brain-be

Contact person: Koen Lefever
Tel: +32 (0)2 238 35 51

Gabriella Panto’, HORIZONTAL AND VERTICAL OCEANIC DISTRIBUTION, TRANSPORT, AND IMPACT OF MICROPLASTICS (HOTMIC). Final Report. Brussels: Belgian Science Policy Office 2024 – 23 p. (BRAIN-be 2.0 - (Belgian Research Action through Interdisciplinary Networks))

TABLE OF CONTENTS

ABSTRACT

Context

The vast open ocean serves as a significant repository for plastics, acting as an expansive but unregulated reservoir for plastic waste from various global sources. Within this context, interactions between plastics and marine organisms likely play a pivotal role in processes such as fragmentation, aggregation, and vertical movement of plastic debris from surface waters to sedimentary layers. Consequently, these interactions may have adverse effects on the health of marine organisms. Despite the evident importance of plastic-biota interactions, our understanding of the scale and dynamics of plastic flux within marine ecosystems remains rudimentary. Critical questions persist regarding the rates of plastic migration from coastal areas to the open ocean and ultimately to deep-sea environments, as well as the ecological ramifications of these movements.

Objectives

To address these knowledge gaps, the HOTMIC initiative undertook a multifaceted approach encompassing fieldwork, open ocean research voyages, and experimental investigations. Within this framework, the role of the Marine Biology Research Group at UGent aimed at investigating MPs abundance, interactions and possible impact on benthos in marine sediments. The HOTMIC project was structured around four distinct technical work packages, each focused on a specific aspect of the plastic pollution issue: UGent was part of Work packages 1 and 3. Work package 1 dealt with investigating the abundance and polymer composition of microplastics particles along the Eastern Atlantic, while trying to unveil the complex dynamics involving plastics and ecologically important environmental variables and biological features. Work package 3 aimed at gaining a deeper understanding on MP-biota interactions. The Marine Biology research lab performed two different experiments in order to investigate the i) burial of MPs by means of bioturbation by macrobenthic organisms and ii) the ingestion of MPs and their possible influence on carbon assimilation in free-living marine nematodes, which constitute the most abundant biota in marine sediments worldwide.

Conclusions

In light of the increasing significance of understanding the dynamics of microplastic pollutants and their interactions with marine fauna, it becomes evident that the development of precise and reliable protocols for their analysis is imperative. Our preliminary investigations underscore the pressing need for such protocols, as they provide crucial insights into the intricate relationship between microplastics and benthic organisms. Through our initial experiments, we have uncovered compelling evidence of the intimate interaction between microplastics and benthic fauna, further confirmed by the analysis of field samples.

Of particular note is our observation that microplastics exhibit a propensity to be sequestered to deeper sediment layers, facilitated by the burial activity of macrofauna organisms. This phenomenon serves to reinforce the concept of marine sediments as a pivotal sink for microplastics, albeit one subject to a myriad of diverse and dynamic influences.

Building upon the insights gleaned from our subsequent experiment, it becomes evident that microplastics (MPs) exhibit a direct incorporation into nematodes through ingestion. This observation underscores the imperative to integrate microplastic studies into the broader framework of predator-prey dynamics within marine ecosystems.

Finally our study on field samples highlights the pervasive presence of microplastics in marine sediments across diverse temperate continental shelf areas. Our model results show that seabed resuspension, the presence of phytoplankton-plastic aggregates and their interaction with macrofauna likely play important roles in determining the spatial variability in polymer composition, including the vertical distribution at the local scale. The highly variable concentration and composition of microplastics across sediment layers confirms the notion that sampling of microplastics in marine sediments should not be confined to the surface 'fluffy layer,' as this may result in underestimation of microplastic densities and, notably, a misrepresentation of microplastic polymer composition. Our findings indicate that microplastics contribute to the large-scale spatial variability in macrofauna that was not predicted by other local water column or abiotic seabed properties, emphasizing its importance for benthic ecology.

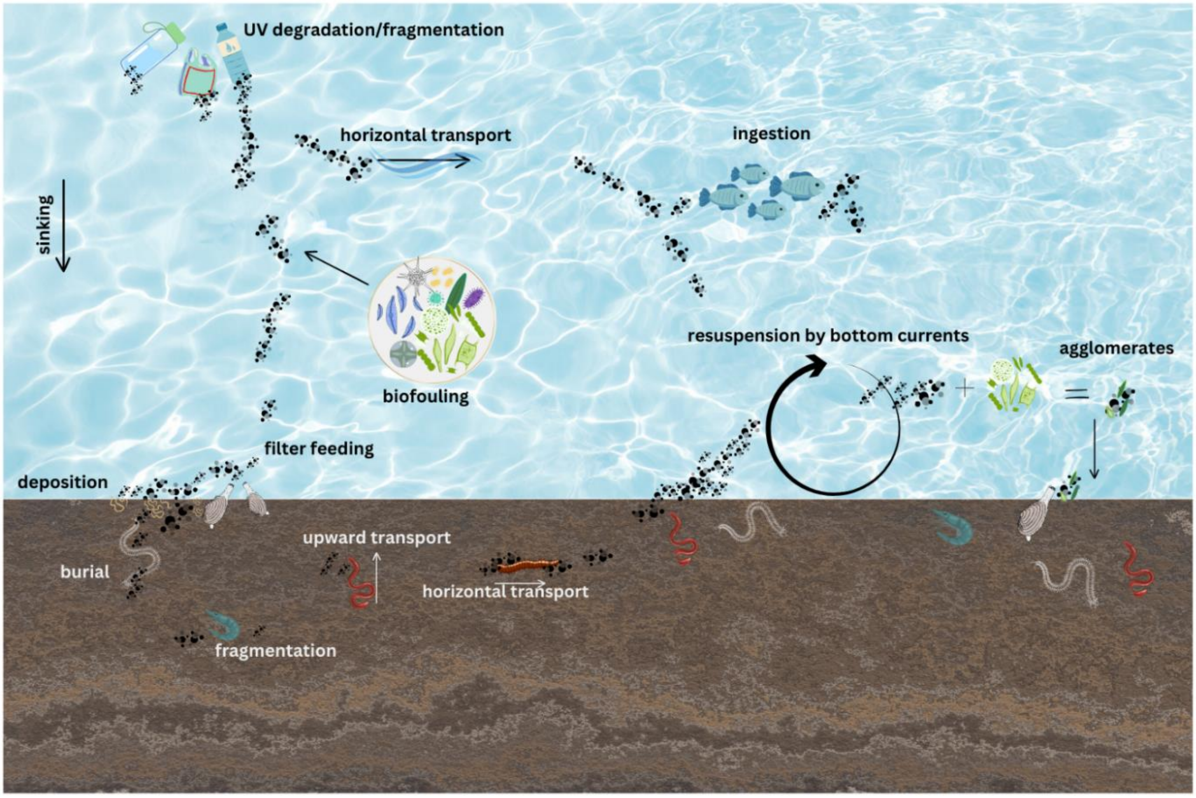

Figure 1: graphical abstract showing the possible transport and sequestration pathways of microplastics, from degradation of bigger plastic items to deposition, biofouling, agglomerates formation, burial and resuspension.

Keywords

Microplastics, Macrobenthos, Nematodes, Burial, Ingestion, Polymer, Marine Sediments, Benthos

1. INTRODUCTION

The presence of microplastics (MPs) in marine sediments has emerged as a pressing environmental concern, reflecting the pervasive nature of plastic pollution in aquatic ecosystems. Originating from diverse sources such as industrial discharge, urban runoff, and improper waste management, plastic particles have infiltrated marine environments worldwide, posing significant threats to ecosystem health and biodiversity (Carpenter and Smith, 1972; Thompson et al., 2004; Avio et al., 2017). As these non-biodegradable materials persist in the environment, understanding their interactions with benthic communities, particularly macrofauna and meiofauna, has become paramount in elucidating the ecological ramifications of plastic pollution in marine sediments. Recent research endeavours have shed light on the widespread distribution of MPs in marine sediments, challenging previous assumptions of certain areas as being relatively pristine. Even remote offshore seabeds, once considered untouched by human activities, are now recognized as repositories for microplastics, highlighting the extent of plastic pollution in marine ecosystems (Poulain et al., 2018; Courtene-Jones et al., 2020; Jorquera et al., 2022). However, the complex dynamics governing the fate and transport of MPs in marine sediments remain poorly understood, influenced by a myriad of factors including physical, chemical, and biological processes (Kaiser et al., 2017; Yao et al., 2019; Yuan et al., 2023).

Macrofauna, comprising a diverse array of organisms such as bivalves, polychaetes, crustaceans, and echinoderms, play crucial roles in sediment bioturbation, nutrient cycling, and ecosystem functioning (Mestdagh et al., 2018; De Borger et al., 2020; Gogina et al., 2020). These organisms interact with marine sediments at various trophic levels, influencing sediment stability, organic matter decomposition, and biogeochemical processes. However, the presence of microplastics in marine sediments can disrupt these ecological functions through mechanisms such as ingestion, entanglement, and burial. Ingestion of microplastics by macrofauna poses significant risks to individual organisms and can lead to adverse effects such as reduced feeding efficiency, physical harm, and internal injuries (Galloway et al., 2017; Coppock et al., 2021), microplastics ingested by benthic organisms can accumulate in their tissues over time, potentially biomagnifying through the food web and posing secondary threats to higher trophic levels. Additionally, microplastics can alter sediment characteristics by serving as substrates for microbial colonization, affecting sediment stability and biogeochemical cycling.

The interaction between microplastics and meiofauna, particularly nematodes, is still a challenging topic to investigate. Nematodes are among the most abundant and diverse organisms in marine sediments, playing vital roles in nutrient cycling, organic matter decomposition, and ecosystem resilience (Hope et al., 2021; Ladewig et al., 2023). These microscopic organisms inhabit the interstitial spaces between sediment particles, where they feed on bacteria, algae, and detritus, contributing to the processing and recycling of organic matter. Although some studies have showed that nematodes might be able to ingest microplastics, the extent of microplastic ingestion and its ecological implications remain poorly understood. Microplastics may alter the feeding behaviour, reproduction, and population dynamics of nematodes, ultimately influencing the structure and functioning of benthic communities.

Understanding the complex interactions between microplastics and subtidal benthic communities, encompassing macrofauna and meiofauna, is essential for elucidating the ecological consequences of plastic pollution in marine sediments.

2. STATE OF THE ART AND OBJECTIVES

While microplastics have garnered significant attention in recent years due to their pervasive presence in marine environments, the state of the art in understanding their abundance, composition in coastal sediments, and their interactions with macro- and meio-fauna presents a complex narrative shaped by multifaceted research efforts.

The occurrence, fate, and transport of microplastics (MPs) in aquatic environments, particularly in sediments, are influenced by various sources and environmental factors (Yu et al., 2018). Primary sources of MPs include intentional manufacturing of microscopic plastics and the release of microbeads from personal care products and plastic industries. Secondary sources involve the degradation of macro debris and wear and tear of synthetic materials such as clothing, tires, and fishing gear (Andrady, 2017). The transportation of MPs is facilitated by water currents, wind, and human activities, with rivers acting as significant pathways for transporting plastics from inland areas to coastal regions. The fate and distribution of MPs in water and sediments are influenced by their characteristics, including density, size, and shape. For instance, buoyant particles may travel long distances through surface currents, while high-density particles tend to settle in sediments. Additionally, factors such as biofouling and weathering can alter the density and behaviour of MPs in aquatic environments (Chubarenko et al., 2016; Corona et al., 2020).

The presence of microplastics (MPs) in marine sediments represents the ultimate sink for these particles due to dynamic coastal ecosystems and turbulent sediment mixing (Pagter et al., 2020). While the abundance of MPs decreases significantly with increased sediment depth, their longevity remains high in deeper layers due to low oxygen levels and limited degradation (Woodall et al., 2014).

The presence of MPs in benthic organisms is influenced by MP pollution levels in sediment and water. Studies have shown positive correlations between MP detection in benthic organisms and sediment, indicating that MP abundance in organisms is influenced by MP pollution levels in sediment(Porter et al., 2018; Van Colen et al., 2021). However, differences in MP occurrence and types between sediment and organisms suggest varying transport pathways, including trophic transfer. Fibers and fragments are the most common types of MPs detected in organisms, primarily in the digestive system. However, smaller particles may be transported to organs, indicating potential health risks for marine organisms.

The effects of microplastic (MP) exposure on aquatic organisms encompass various aspects of their health and well-being, including reproduction, digestion, toxicity, and development. Studies have consistently shown negative impacts on organisms exposed to MPs, with repercussions ranging from inflammation and intestinal damage to disruptions in metabolism, growth, reproduction, and survival(Athey et al., 2020; Porter et al., 2023). For instance, exposure to MPs can damage the digestive system of aquatic organisms, leading to inflammation and affecting food availability. Additionally, MPs can disturb the metabolism, growth, and reproduction of organisms, with significant decreases in energy and protein content observed in bivalves exposed to MP particles (Fabra et al., 2021). Larval stages of various species have also been negatively affected by MP exposure, with potential implications for body development and reproductive success (Urban-Malinga et al., 2021). Moreover, the presence of MPs in aquatic environments can impact the diversity and abundance of benthic fauna. High concentrations of MPs have been associated with reduced occurrence of benthic fauna, potentially disrupting entire ecosystems.

While microplastics are a significant area of research, methodologies and protocols for studying their interaction with marine environments are still in the experimental phase, making comparisons between studies challenging. One of the primary difficulties lies in ensuring the reliability of samples, as airborne contamination is often unavoidable and can be high. Additionally, the small size of microplastic particles poses limitations on investigating factors such as size, plastic shape (e.g., bead, fiber), and polymer type, although advanced technologies mitigate these challenges, particularly for nanoplastics (<1 µm). However, many laboratories lack access to these advanced technologies due to economic constraints, and analyzing particles remains time-consuming, especially for field samples.

With our study we aimed at filling gaps in the knowledge of MPs interaction with the benthic environment, both through experiments and analysis of field samples. Particularly:

- We quantified the different MP polymers present in the soft-sediment seabed along European shelf margins. We then analyzed the importance of water column and sediment abiotic properties in explaining the spatial variability of MPs and macrofauna communities at the sampled locations. Lastly, we analyzed whether and how much current levels of MPs pollution correlate with the spatial variability of macrofauna.
- We performed a mesocosm experiment with sediments sampled in the Belgian part of the North Sea to test the hypothesis that the residing macrobenthos community enhances the sequestration of surfaced MPs towards deeper depths, thus confirming marine sediments as a sink for MPs. In parallel, we examined the possible effects of MPs on the survival of macrobenthos after 30 days of exposure to the contaminant.
- We performed a mesocosm experiment with intertidal sediments from the Netherlands, isolating nematodes from the rest of the benthic community to test the hypothesis that a local nematode community is capable of ingesting microplastics. At the same time, we investigated weather the presence of microplastics in sediments and their ingestion have an impact on the carbon assimilation of free-living marine nematodes in presence of their natural food sources.

3. METHODOLOGY

Field samples

During research cruise AL 534/2, sediment samples were collected from 11 stations along the Mediterranean Sea and the Eastern Atlantic margins. Macrobenthic organisms were collected using a van Veen Grab deployed three times at each station, and sediment samples were collected using a Mini Corer for subsequent analysis. Plexi cores were used to collect intact sediment samples, with slices processed for various analyses.

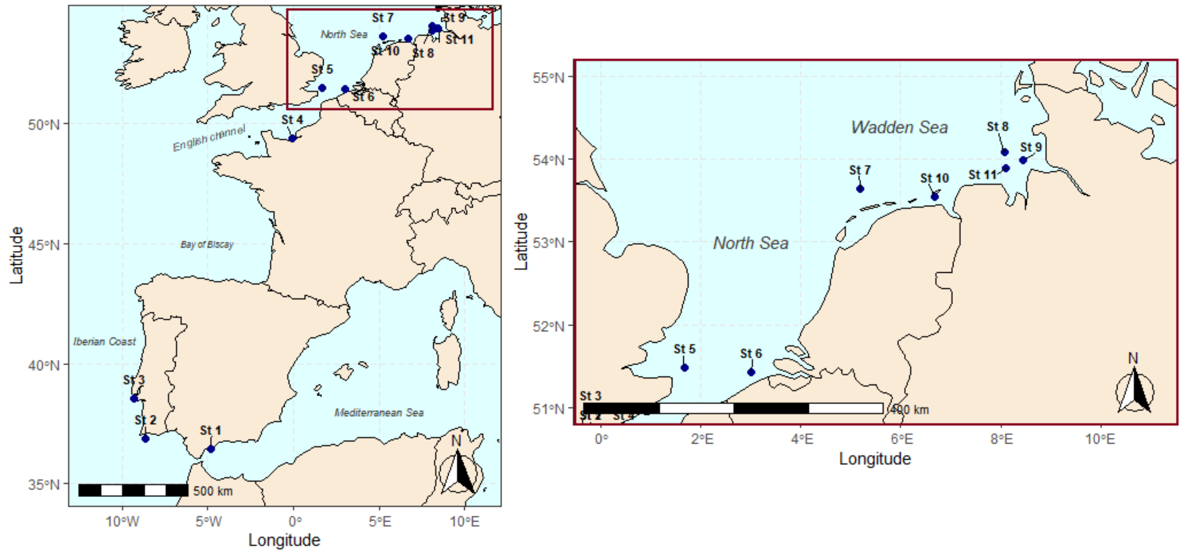

Figure 2: Map showing the sampling locations along the NE Atlantic and the Mediterranean sea (station 1). Stations 2 and 3 are located at the Iberian Coast, while stations 4 and 5 are in the English channel and 6 in the Belgian part of the North Sea; stations 7,8,9 and 11 are part of the Wadden Sea.

Environmental variables such as pigments, organic matter, and sediment grain size were analyzed from sediment samples. Pigments were extracted and analyzed using HPLC, while total organic matter content and sediment grain size distribution were determined. Microplastics were extracted from sediment samples using density separation, followed by filtration and spectroscopic analysis with a µFTIR spectroscope. Macrofauna stored in formaldehyde was washed, counted, and identified to the lowest possible taxonomic level, with relevant biological traits assigned.

Contamination checks were conducted throughout the sampling and analysis process to minimize potential sources of contamination. These included adherence to good field and laboratory practices, the use of metal and glass tools over plastic, and thorough rinsing of plastic containers used in the procedure. Blank samples were analyzed to assess possible airborne and equipment-related contamination, with minimal contamination detected.

Burial experiment - macrobenthos

In this study, sediment cores were collected from the Belgian part of the North Sea to investigate the impact of microplastics (MPs) on the macrobenthos community. The sampling station, located in the

Zeeland banks, is known for its rich benthic biodiversity. Sediment cores were collected using a Reineck box corer and transported to the laboratory for further analysis.

The experiment involved inoculating polyethylene (PE) fragments, representing realistic contaminants found in marine sediments, into sediment cores to simulate MPs pollution. Four treatments were considered: MP treatment with live fauna (MPF), MP treatment with dead fauna (MPD), procedural control with live fauna without MPs (MC), and field control with live fauna (FC). The MPD treatment involved freezing cores to kill fauna without disturbing the sediment structure.

The experiment lasted for one month, with two sampling times (T1 and T2). Samples were processed to extract both MPs and macrobenthos. MPs were extracted from sediment using density separation, followed by filtration and storage. Macroinvertebrates were sorted, identified, and assigned mobility and reworking scores.

Ingestion experiment – nematodes

In preparation for the experiment, fluorescent microplastics (MPs) of 1 µm and 6 µm, labeled algae, labeled glucose, and bacteria cultures were obtained. Five tanks with aerated filtered seawater were set up in a climate room. Thirty sediment cores, along with five backup cores, were collected from a muddier sediment area. The upper 3 cm of each core were sliced and sieved to exclude macrofauna. Cores were then acclimated in the tanks for a day.

Bacteria were cultured and labeled with glucose before injection into the samples. A mixture of labeled algae, bacteria, and fluorescent microplastics is injected into each sample, followed by a 1-day settlement period. The experiment ran for a week with intermediate sampling at 24 hours, 3 days, 5 days, and 7 days. After each incubation period, cores are sliced, with half processed for meiofauna extraction and MP ingestion assessment, and the other half processed for stable isotope analysis to assess carbon assimilation.

Control samples are used to compare carbon assimilation in the presence and absence of MPs, as well as to assess nematode mortality/survival. The experiment aims to quantify ingested particles, compare different MP sizes, identify feeding guilds, and assess carbon assimilation. Finally, remaining sediment is processed to assess the amount of non-ingested particles.

Digestion protocol for nematodes – analysis of ingested MPs in environmental samples

Currently, there is no knowledge on ingested particles by nematodes from environmental samples. One of the reasons for this knowledge gap, is the difficulties in i) digesting nematodes species – nematode's cuticules are particularly robust- and in ii) analyzing MPs particles < 10 µm. In an attempt to fill such knowledge gap, we tested a digestion protocol for nematodes using different enzymes and chemical solutions. We believe the resulting protocol will be useful in further research on MPs ingestion by nematodes (and possibly other meiofauna species, although such tests still need to occur). The protocol has been tested on samples from the 534/2 campaign and have been analyzed by the one the partner Universities within the HOTMIC project (Technical University of Munich). After providing a suspension of digested nematodes to our partners, they finally filtered it and analyzed it with µRaman spectroscopy. While preliminary results are discussed, we can currently share that the protocol seem to have been successful. However, further contamination checks are needed. This study will give, for the first time, information on future techniques to be employed for the detection and characterization of MPs particles in meiobenthic organisms.

4. SCIENTIFIC RESULTS AND RECOMMENDATIONS

Field samples

The environmental variables along the Atlantic margins exhibited distinct patterns across stations, with a clear separation observed through principal component analysis (PCA). Stations differed primarily along a north-south gradient, with varying characteristics such as salinity, sediment composition, and depth.

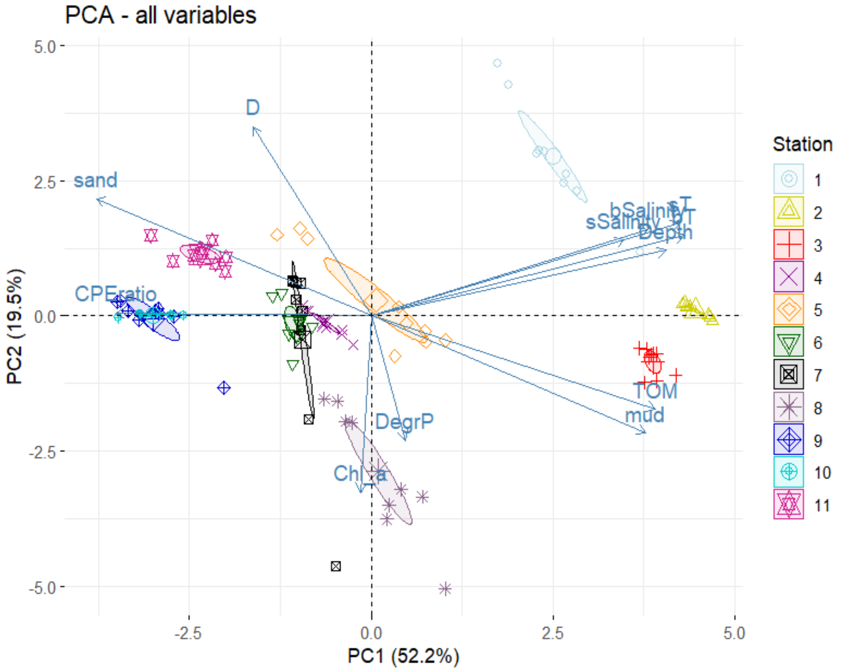

Figure 3: Principal Component Analysis (PCA) performed on the bulk data of all environmental variables. Each point represents a replicate, while ellipses delineate separate stations. Ellipses closer together are more similar compared to more isolated ones.

Microplastics were detected in all stations, with notable variation in concentration among sites. The Kruskal-Wallis test indicated non-significant differences in microplastic densities between stations. However, polymer composition varied significantly, with certain stations showing unique compositions.

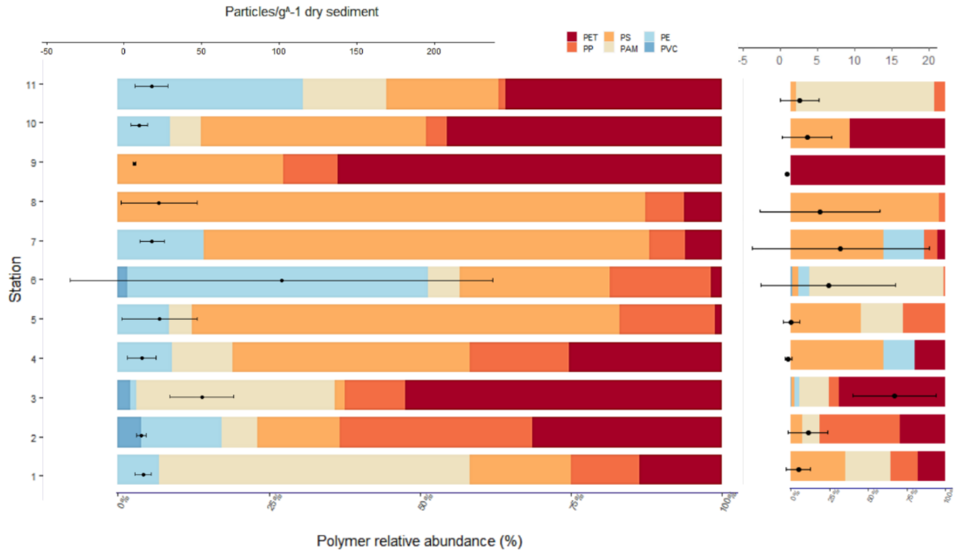

Figure 4: On the left graph, the relative abundance (%) of polymers per station for the bulk sample (0-3 cm). The dots represent the average MPs abundance ± sd at each station, calculated on three replicates and expressed in particles/g^{-1}. On the right, the same information is showed only for the 0-1 cm layer. Note the different scale of the upper x axis for the bulk fraction and the surface layer.

Further analysis revealed associations between environmental variables and polymer composition. For instance, sand content, bottom temperature, and total organic matter were significant predictors of polymer variation in the surface layer. Similarly, chlorophyll-a concentration played a significant role in polymer variation in the bulk sediment. The composition of the macrobenthic community also exhibited variability across stations, with distinct groupings observed through nonmetric multidimensional scaling (NMDS). Depth, total organic matter, and temperature emerged as significant factors influencing macrobenthic variation.

When considering the interaction between microplastics and macrobenthos, a model incorporating both environmental and polymer variables explained a substantial portion of macrobenthic variation. Depth, total organic matter, sediment temperature, polyacrylamide, and polyethylene were identified as significant predictors in this model. These findings highlight the complex relationships between environmental factors, microplastics, and macrobenthic communities, emphasizing the need for comprehensive assessments to understand ecosystem dynamics in marine environments.

Burial experiment - macrofauna

The study investigated the recovery and effects of microplastics (MPs) on benthic fauna, as well as the burial of MPs by macrobenthos. Approximately 75% of the MP fragments added to treatment cores were recovered in the MPF treatment, while recovery in the MPD treatment averaged 60%.

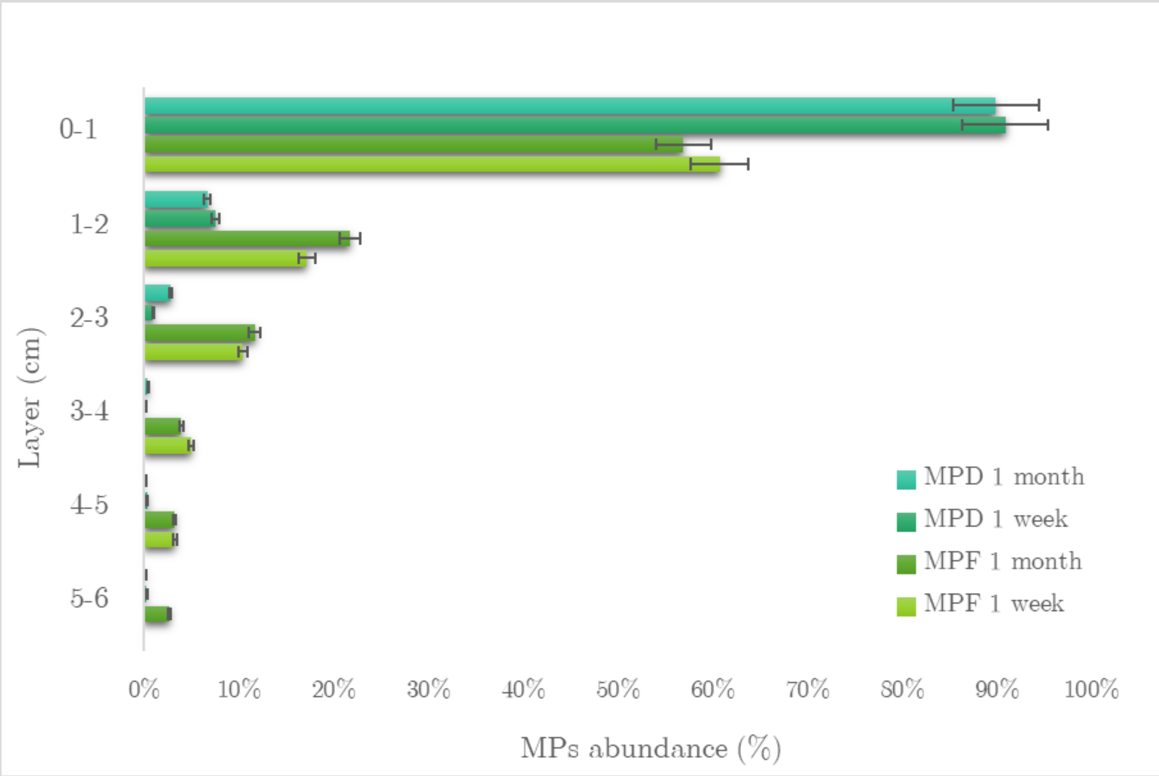

Figure 5: The graph shows the distribution of MPs (% of relative numbers) through sediment matrix (1 cm layers). Green bars represent cores with living fauna (MPF treatment), while blue bars indicate cores that were frozen to ensure the absence of living organisms (MPD treatment). The error bars represent standard deviation (SD).

The benthic community consisted mainly of surficial modifiers, with a significant negative effect of MPs observed on the density of the bivalves *A. alba, T. fabula, and M. bidentata*. However, the polychaete O. fusiformis was not affected by MPs, possibly due to its suspension feeding behaviour.

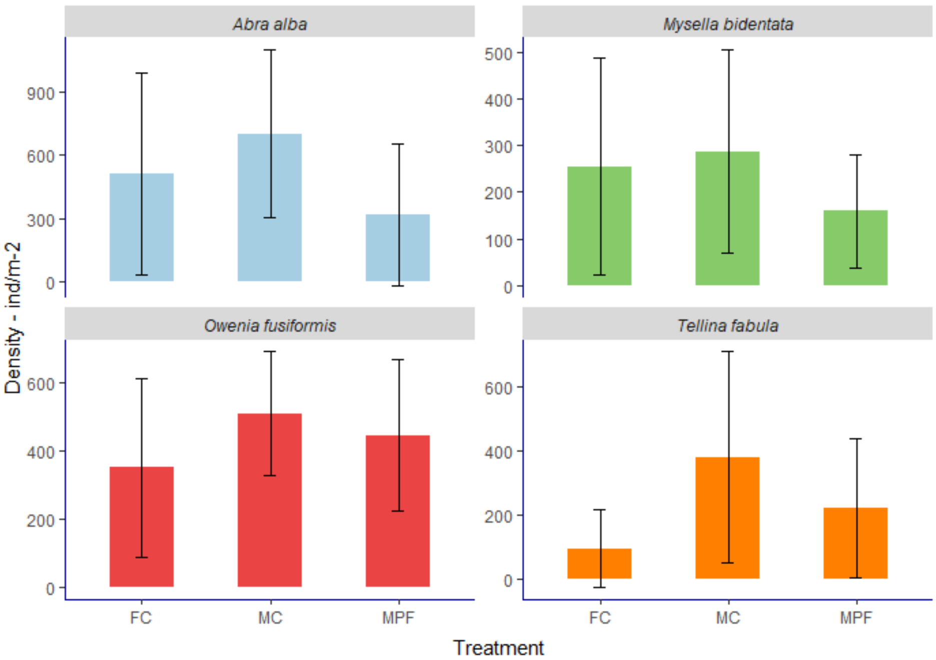

Figure 6: Average densities and standard deviation (sd) of the most dominant fauna in the macrobenthic community expressed in ind/m-2 ±sd (y axis, n=4). The x axis shows the three treatments considered (FC, MC and MPF). Note the different scale for each species.

Analysis revealed significant differences in MPs distribution through layers between treatments and within the same treatment over time. In the MPF treatment, more than 60% of MPs were found in the top cm layer after seven days, decreasing with depth over time. Conversely, in the MPD treatment, MPs remained predominantly in the top cm layer at both sampling times. The study highlighted the significant role of macrobenthos in the burial of natural low-density MPs, with up to 40% of surface MPs removed from the sediment surface after one week and transported to deeper layers. Benthic organisms' activities, such as burrowing and feeding, contributed to the downward transport of MPs. However, MPs-induced mortality of some species and experimental conditions may have limited burial over time. Overall, the findings underscored the complex interactions between MPs and benthic fauna, with potential implications for benthic ecosystem dynamics. While surface deposit feeders initially removed MPs from the sediment surface, continued exposure could lead to decreased removal efficiency due to species mortality.

Ingestion experiment – nematodes

Samples from the experiments have been processed but no final data are available. From the first half of the experiment, nematodes slides have been made and ingested particles have been observed under a stereomicroscope. Of the 2000 individuals chosen, fluorescent particles were only detected in two organisms (both 1 and 5 µm in size). Samples for stable isotope analysis have been processed and data are being currently analyzed. Samples will be used to assess the differences in carbon assimilation in presence and absence of MPs at different incubation times.

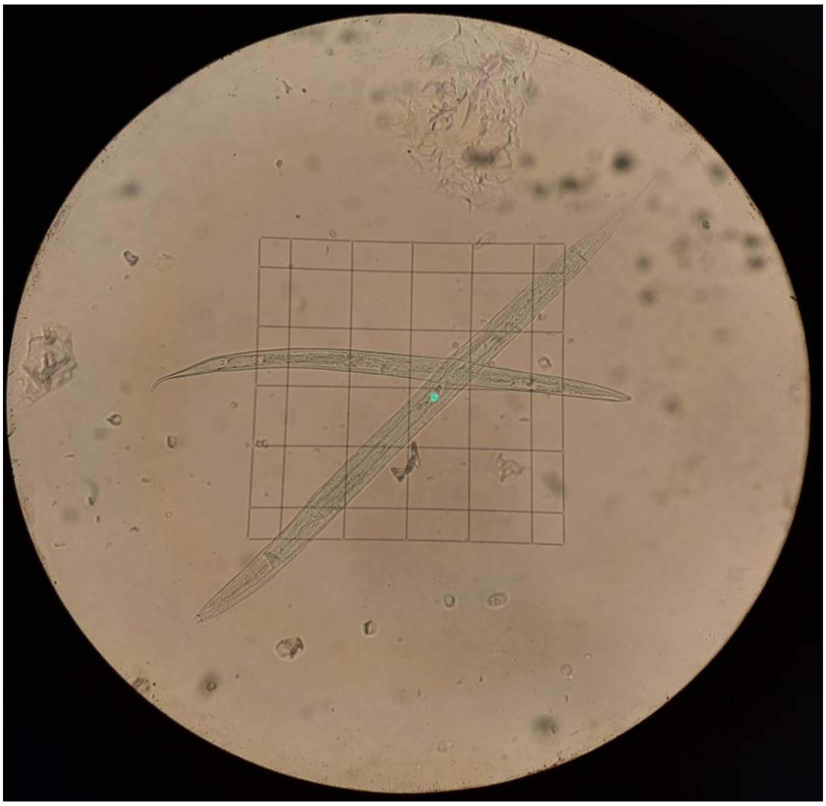

Figure 7: example of a 5 µm particle in the guts of a nematode after 7 days of incubation.

The digested solution has been processed and microplastic particles have been identified. Preliminary results are still being discussed as further contamination checks are needed at this stage.

RECOMMENDATIONS

The highly variable concentration and composition of microplastics across sediment layers confirm the necessity to expand sampling beyond the surface 'fluffy layer' in marine sediments. Focusing solely on surface sampling may lead to underestimation of microplastic densities and misrepresentation of polymer composition. Our findings also reveal that microplastics contribute to large-scale spatial variability in macrofauna, which cannot be fully explained by local water column or abiotic seabed properties alone. This underscores the importance of microplastics in shaping benthic ecology. Future research should delve into the effects of different polymer types on benthic communities' functionality and mortality, elucidating the multiple interaction pathways between microplastics and macrobenthos. Our results demonstrate the rapid transport of microplastics from the seabed to deeper layers facilitated by bioturbation of the macrobenthos community. Additionally, we observe increased mortality in *A. alba* due to microplastic exposure, highlighting potential cascading effects on biodiversity and ecosystem functioning. Given the crucial functional role of macrofauna in nutrient cycling and biotic interactions, understanding the implications of microplastics on these processes is paramount. Future investigations should focus on natural benthic communities under flow conditions that induce physical mixing and resuspension. Moreover, exploring potential ingestion-induced biological fragmentation and ecotoxicological effects of microplastics, such as oxidative stress, will enhance our comprehension of the intricate dynamics governing microplastic-macrofauna interactions in real-world scenarios. Finally, although final results are not yet available, our experiment on free-living marine nematodes confirms the notion that nematodes are in fact capable of ingesting microplastic particles even in presence of their natural food source, with unknown effects on the nematode community and in turn the higher levels of the food web they sustain. Future studies should focus on the possible effects of microplastics on marine nematodes with an interdisciplinary approach to test for bioaccumulation in the food web, in order to understand the real impact of such particles in benthic ecosystems.

5. DISSEMINATION AND VALORISATION

Throughout the HOTMIC project, all partners engaged in various dissemination and outreach endeavours. These efforts encompassed press releases and media articles, presentations at conferences and public forums, educational initiatives at primary and secondary schools (including hosting a summer school at GEOMAR), and the publication of cruise reports and scientific papers. Furthermore, IWC-TUM actively participated in committees addressing standardization issues related to (micro)plastics. A significant outcome of HOTMIC was a comprehensive literature review focusing on the design of experiments concerning microplastic ingestion and its effects. This review identified notable deficiencies in previous studies and formulated a blueprint to inform future experimental design.

Particularly, the Marine Biology research group at UGent participated to two conferences for dissemination:

MICRO conference 2022 – international conference on plastic pollution, from macro to nano.

IMCO conference 2022 – 18th international meiofauna conference

6. PUBLICATIONS

Review paper

In collaboration with the other partner universities of HOTMIC, a review paper on “the ideal MPs experiment” has been written. The paper has been published and can be found at the following link: https://doi.org/10.1016/j.scitotenv.2022.156610

Burial experiment – macrofauna

A paper with the title: “Fauna – microplastics interactions: empirical insights from benthos community exposure to beached litter” has been submitted for peer review in the “Marine Pollution Bulletin” scientific journal.

Field samples

A paper with the title: “Microplastics in European continental shelf sediments: investigating relationships with benthos and environmental variables for a holistic understanding” is in the finishing stages and will soon be submitted for peer review.

7. ACKNOWLEDGEMENTS

The authors wish to thank all the HOTMIC partners: GEOMAR Helmholtz Centre for Ocean Research Kiel (GEOMAR) GERMANY (Project coordinator), University of Southern Denmark, Department of Biology (SDU) DENMARK, University of Tartu, Estonian Marine Institute (UT) ESTONIA, Institute of Hydrochemistry, Technical University of Munich, Department of Chemistry (IWC/TUM) GERMANY, University of Pisa, Department of Chemistry and Industrial Chemistry (UNIPI) ITALY, Portuguese Institute for the Sea and the Atmosphere, Division of Oceanography and Marine Environment (IPMA) PORTUGAL, Marine and Environmental Sciences Centre (MARE) PORTUGAL.

Tom Moens and Ulrike Braeckman from the Marine Biology research group (MarBiol) are specifically thanked for the support during the experimental setup and analysis of the ingestion experiment with nematodes.

We also wish to thank the crew of the RV/Alkor during the 2020 campaign along the Atlantic coast (March 2nd – March 27th) and the one of the RV/Sonne during the 2020-21 campaign to the Atlantic garbage patch (December 2nd – January 3rd).

The authors also wish to thank all the laboratory assistants of MarBiol and the Laboratory of Environmental Toxicology and Aquatic Ecology (GhEnToxlab) at UGent, which helped with sampling and the technical analysis of field and experimental samples: Bart Beuselinck and Emmy Pequeur.

References

Andrady, A. L. (2017). The plastic in microplastics : A review. *Mar. Pollut. Bull.* 119, 12–22. doi:10.1016/j.marpolbul.2017.01.082.

Athey, S. N., Albotra, S. D., Gordon, C. A., Monteleone, B., Seaton, P., Andrady, A. L., et al. (2020). Trophic transfer of microplastics in an estuarine food chain and the effects of a sorbed legacy pollutant. *Limnol. Oceanogr. Lett.* 5, 154–162. doi:10.1002/lol2.10130.

Avio, C. G., Gorbi, S., and Regoli, F. (2017). Plastics and microplastics in the oceans : From emerging pollutants to emerged threat. *Mar. Environ. Res.* 128, 2–11. doi:10.1016/j.marenvres.2016.05.012.

Carpenter, E. J., and Smith, K. L. (1972). Plastics on the Sargasso Sea Surface. *Science (80-.).* 175, 1240–1241. doi:10.1126/science.175.4027.1240.

Chubarenko, I., Bagaev, A., Zobkov, M., and Esiukova, E. (2016). On some physical and dynamical properties of microplastic particles in marine environment. *Mar. Pollut. Bull.* 108, 105–112. doi:10.1016/j.marpolbul.2016.04.048.

Coppock, R. L., Lindeque, P. K., Cole, M., Galloway, T. S., Birgani, H., Richards, S., et al. (2021). Benthic fauna contribute to microplastic sequestration in coastal sediments. 415. doi:10.1016/j.jhazmat.2021.125583.

Corona, E., Martin, C., Marasco, R., and Duarte, C. M. (2020). Passive and Active Removal of Marine Microplastics by a Mushroom Coral (Danafungia scruposa). *Front. Mar. Sci.* 7, 1–9. doi:10.3389/fmars.2020.00128.

Courtene-Jones, W., Quinn, B., Ewins, C., Gary, S. F., and Narayanaswamy, B. E. (2020). Microplastic accumulation in deep-sea sediments from the Rockall Trough. *Mar. Pollut. Bull.* 154, 111092. doi:10.1016/j.marpolbul.2020.111092.

De Borger, E., Tiano, J., Braeckman, U., Ysebaert, T., and Soetaert, K. (2020). Biological and biogeochemical methods for estimating bioirrigation: A case study in the Oosterschelde estuary. *Biogeosciences* 17, 1701–1715. doi:10.5194/bg-17-1701-2020.

Fabra, M., Williams, L., Watts, J. E. M., Hale, M. S., Couceiro, F., and Preston, J. (2021). The plastic Trojan horse: Biofilms increase microplastic uptake in marine filter feeders impacting microbial transfer and organism health. *Sci. Total Environ.* 797, 149217. doi:10.1016/j.scitotenv.2021.149217.

Galloway, T. S., Cole, M., and Lewis, C. (2017). Interactions of microplastic debris throughout the marine ecosystem. *Nat. Ecol. Evol.* 1, 1–8. doi:10.1038/s41559-017-0116.

Gogina, M., Zettler, M. L., Vanaverbeke, J., Dannheim, J., Van Hoey, G., Desroy, N., et al. (2020). Interregional comparison of benthic ecosystem functioning: Community bioturbation potential in four regions along the NE Atlantic shelf. *Ecol. Indic.* 110, 105945. doi:10.1016/j.ecolind.2019.105945.

Hope, J. A., Coco, G., Ladewig, S. M., and Thrush, S. F. (2021). The distribution and ecological effects of microplastics in an estuarine ecosystem. *Environ. Pollut.* 288, 117731. doi:10.1016/j.envpol.2021.117731.

Jorquera, A., Castillo, C., Murillo, V., Araya, J., Pinochet, J., Narváez, D., et al. (2022). Physical and anthropogenic drivers shaping the spatial distribution of microplastics in the marine sediments of Chilean fjords. *Sci. Total Environ.* 814. doi:10.1016/j.scitotenv.2021.152506.

Kaiser, D., Kowalski, N., and Waniek, J. J. (2017). Effects of biofouling on the sinking behavior of microplastics. *Environ. Res. Lett.* 12. doi:10.1088/1748-9326/aa8e8b.

Ladewig, S. M., Coco, G., Hope, J. A., Vieillard, A. M., and Thrush, S. F. (2023). Real-world impacts of microplastic pollution on seafloor ecosystem function. *Sci. Total Environ.* 858, 160114. doi:10.1016/j.scitotenv.2022.160114.

Mestdagh, S., Bagaço, L., Braeckman, U., Ysebaert, T., Smet, B. De, and Moens, T. (2018). Functional trait responses to sediment deposition reduce macrofauna-mediated ecosystem functioning in an estuarine mudflat. 2587–2599.

Pagter, E., Frias, J., Kavanagh, F., and Nash, R. (2020). Varying levels of microplastics in benthic sediments within a shallow coastal embayment. *Estuar. Coast. Shelf Sci.* 243. doi:10.1016/j.ecss.2020.106915.

Porter, A., Barber, D., Hobbs, C., Love, J., Power, A. L., Bakir, A., et al. (2023). Uptake of microplastics by marine worms depends on feeding mode and particle shape but not exposure time. *Sci. Total Environ.* 857, 159287. doi:10.1016/j.scitotenv.2022.159287.

Porter, A., Lyons, B. P., Galloway, T. S., and Lewis, C. (2018). Role of Marine Snows in Microplastic Fate and Bioavailability. *Environ. Sci. Technol.* 52, 7111–7119. doi:10.1021/acs.est.8b01000.

Poulain, M., Mercier, M. J., Brach, L., Martignac, M., Perez, E., Desjean, M. C., et al. (2018). Small microplastics as a main contributor to plastic mass balance in the North Atlantic subtropical gyre ? doi:10.1021/acs.est.8b05458.

Thompson, R. C., Olsen, Y., Mitchell, R. P., Davis, A., Rowland, S. J., John, A. W. G., et al. (2004). Lost at Sea : Where Is All the Plastic ? 304, 2004.

Urban-Malinga, B., Jakubowska, M., and Białowąs, M. (2021). Response of sediment-dwelling bivalves to microplastics and its potential implications for benthic processes. *Sci. Total Environ.* 769. doi:10.1016/j.scitotenv.2020.144302.

Van Colen, C., Moereels, L., Vanhove, B., Vrielinck, H., and Moens, T. (2021). The biological plastic pump: Evidence from a local case study using blue mussel and infaunal benthic communities. *Environ. Pollut.* 274, 115825. doi:10.1016/j.envpol.2020.115825.

Woodall, L. C., Sanchez-Vidal, A., Canals, M., Paterson, G. L. J., Coppock, R., Sleight, V., et al. (2014). The deep sea is a major sink for microplastic debris. *R. Soc. Open Sci.* 1. doi:10.1098/rsos.140317.

Yao, P., Zhou, B., Lu, Y. H., Yin, Y., Zong, Y. Q., Chen, M. Te, et al. (2019). A review of microplastics in sediments: Spatial and temporal occurrences, biological effects, and analytic methods. *Quat. Int.* 519, 274–281. doi:10.1016/j.quaint.2019.03.028.

Yu, X., Ladewig, S., Bao, S., Toline, C. A., Whitmire, S., and Chow, A. T. (2018). Science of the Total Environment Occurrence and distribution of microplastics at selected coastal sites along the southeastern United States. *Sci. Total Environ.* 613–614, 298–305. doi:10.1016/j.scitotenv.2017.09.100.

Yuan, B., Gan, W., Sun, J., Lin, B., and Chen, Z. (2023). Depth profiles of microplastics in sediments from inland water to coast and their influential factors. *Sci. Total Environ.* 903, 166151. doi:10.1016/j.scitotenv.2023.166151.