Land-Based Solutions for Plastics in the Sea

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D3.4. Report on MP and zooplankton indicator from analysed field samples

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Nº	Participant name	Acronym	Country	Type
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2	UNIVERSIDADE DA CORUÑA	UDC	SPAIN	HES
3	Bundesanstalt fuer Gewaesserkunde	BfG	GERMANY	RTO
4	LABORATORIO IBERICO INTERNACIONAL DE NANOTECNOLOGIA	INL	PORTUGAL	RTO
5	KATHOLIEKE UNIVERSITEIT LEUVEN	KUL	BELGIUM	HES
6	HELMHOLTZ ZENTRUM FUR OZEANFORSCHUNG KIEL	GEOMAR	GERMANY	RTO
7	NATIONAL OCEANOGRAPHY CENTRE	NOC	UNITED KINGDOM	RTO
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9	OPEN UNIVERSITEIT NEDERLAND	OUNL	NETHERLANDS	HES
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11	ASSOCIACAO PARA O DESENVOLVIMENTO DO ATLANTIC INTERNATIONAL RESEARCH CENTRE	AC	PORTUGAL	RTO
12	UNIVERSIDADE FEDERAL DO SAO PAULO	UNIFESP	BRAZIL	HES
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3.0	30/05/2024	Final version

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ABBREVIATIONS AND ACRONYMS

Abbreviation / Acronym	Description
UNEP	The United Nations Environment Programme
EU	European Union
MPs	Microplastics
MSFD	Marine Strategy Framework Directive
GES	Good Ecological Status
Ind.	Individuals
E	Elbe
T	Thames
St	Station
PZR	Plastic to zooplankton ratio

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1 Report on Microplastics (MPs) and Zooplankton indicator

1.1 Introduction

Protecting the marine environment has become one of the major concerns of our time, and a priority in Europe. Marine and coastal areas are home to a high level of human activity, which is exerting increasing pressure on the marine environment. The United Nations Environment Programme (UNEP) recognizes that we are facing a triple global crisis, characterized by anthropogenic climate change, biodiversity loss, and pollution. Human discharges of synthetic chemicals, including plastics, onto land and into the oceans have reached a critical threshold, with negative impacts on human health and the stability of the Earth system (Persson et al., 2022). Plastics are a ubiquitous source of pollution throughout their life cycle, including production, use, and waste management. The use of fossil fuels for their manufacture also contributes to climate change, as increased plastic production leads to a growing accumulation of marine debris, disrupting diversity and biogeochemical cycles and promoting the spread of invasive species, pathogens, and antibiotic-resistance genes (see Landrigan et al., 2023).

Plastic pollution is now omnipresent throughout the world, from our cities to the oceans, in the Arctic and Antarctic, via marine and atmospheric routes, and across layers of biological function (Morrison et al., 2022). The vast majority of aquatic plastic exists in the form of microplastics (MPs) often less than 5 mm in size. These micro-wastes comprise a very heterogeneous assemblage of parts that vary in size, shape, color, specific density, and chemical composition. More recent studies estimate that there are 24.4 trillion pieces of MPs in the world's upper oceans, which could total between 82,000 and 578,300 tons of these pollutants (Isobe et al., 2021).

Indeed, the fragmentation and disintegration of plastics promote the concentration of pollutants, which can be absorbed by filter-feeding organisms through biomagnification. This process occurs when small plastics are ingested by wildlife from lower trophic levels, such as zooplankton and small fish (Desforges et al., 2015, Provencher et al 2019), as well as large fishes and cetaceans (Fossi et al. 2012; Collard et al. 2019). It has been shown that plastic ingestion affects 65% of commercial fish species examined (Markic et al., 2020). Interactions between MPs and marine biota via direct or indirect pathways, such as ingestion and/or physical interaction (Wright et al., 2013). As a result, these particles contaminate the entire aquatic food web. Plastics can therefore easily enter the food chain and reach humans, without us really knowing the risks to our health.

Assessing the risk of plastic transfer along marine food webs and understanding the extent of this pollutant's impact on marine life, including humans (Savoca et al. 2021), is essential to developing conservation and mitigation strategies (Kershaw et al., 2019).

1.2 Context and purpose of the study

The Marine Strategy Framework Directive (MSFD) is a European Union legislative framework aimed at protecting the marine environment across Europe. The MSFD aims to implement action and monitoring programs whose objective is to achieve a "good ecological status" (GES) of European marine waters focusing on methodological aspects and technical understanding including assessments of the current state of their marine waters. EU Member States have identified a set of characteristics that define GES to enhance more integrated management of human activities, based on a list of 11 qualitative descriptors. The MFSD-GES (2011) has recognized marine litter as one of the descriptors: properties and quantities of litter that do not cause damage to the coastal and marine environment (Galgani et al., 2011). To achieve the GES, we need to master

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indicator 10.1.3 Microplastics (trend from quantity, distribution, and, if possible, composition of microparticles). Although indicators are already in place to monitor the impacts of wastes on marine fauna- *i)* "macro-litter ingested by sea turtle (debris items >5 mm)", *ii)* "Marine wildlife entanglement in debris (all taxa)", *iii)* "micro-litter ingested by fish/ sea turtle (debris items <1mm)". It would be important to further develop other plastic indicators, especially those related to their biological impacts.

In this research, we investigate the potential risk of ingesting plastic particles mistaken for zooplankton. Plastic and zooplankton samples were taken from the North Sea, Elbe River, and Thames River to assess the proportion of zooplankton organisms in contact with microplastics in the surface layer (plastic: zooplankton ratio). This study aims to determine whether the quantities of plastic relative to zooplankton in the aquatic environment can serve as an environmental risk indicator and reflect the state of pollution in the sea.

The zooplankton community plays a crucial role in the marine biota by serving as a primary food source for larger filter-feeding animals such as larvae of many economically relevant species (e.g., crustaceans), fish, whales, and sharks, and by consuming plankton ten times smaller than their size. Zooplankton act as a link between lower and higher marine food webs, maintaining the balance of the greater ecosystem (Fenchel, 1998). Zooplankton potentially interact with microplastics (MPs) because MPs can confuse predators when they are similar in size to planktonic prey. The surface layer is a major accumulation zone for MPs that are the same size as zooplankton. Plastic fragments coexisting with zooplankton can threaten these marine organisms and the broader food chain (Ryan et al., 2009). The ingestion of plastic debris mistaken for zooplankton confuses predators, leading them to consume it as part of their normal diet. There is substantial evidence that available plastics can be mistaken for food (Botterell et al., 2019; Carrillo-Barragán et al., 2024).

The ratio of plastic to zooplankton (plastic: zooplankton ratio) could be used as an indicator to assess the risk of MPs entry into marine food webs, thus providing a valuable tool for assessing levels of micro-waste pollution. Predicting the impact of MPs requires a deeper understanding of various factors: the sources, abundance, distribution, and fate of MPs in aquatic environments, as well as their interactions with cohabiting organisms. These interactions are challenging to envisage without in-depth knowledge of the respective abundances involved.

This proposition is based on the following facts:

i) Plastics decrease in size as weathering and fragmentation occur, increasing the risk of ingestion by a wide range of organisms.

(ii) In the study area most of floating marine debris is composed of MPs, particles < 5mm in size.

(iii) These microplastics are similar in size to zooplankton, making them more likely to be mistaken for food by marine organisms

iv) Filter-feeding species, such as certain fish, and whales, could potentially ingest MPs

v) The potential ingestion of microplastics poses a risk of entering and transferring upwards the trophic chain, ultimately affecting humans (Wright et al., 2013).

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1.3 Implementation of the plastic: zooplankton indicator:

1.3.1 Sampling campaigns and laboratory analysis

Plastic and zooplankton for this study were collected along 18 stations in surface water samples from the Thames River, the Elbe River, and the North Sea during different season campaigns in 2022 and 2023. In the Thames River, UK, six sites (T1, T2, T3, T5, T6) were sampled during the winter and summer of 2022, and stations T5 and T6 were sampled in the winter, spring, and summer of 2023. For the seasonal sampling campaigns in the Elbe River, six sites (E13, E14, E15, E16, E17, E18) were sampled in the winter and summer of 2022. These sites spanned from the freshwater part (Dömitz, Dessau, and Wittenberg) to the tidal part (Hamburg and Geesthacht) and the estuary (Cuxhaven). In 2023, stations E13 and E17 were sampled in winter, spring, and summer. The North Sea was sampled at six stations (7, 8, 9, 10, 11, 12) along with intermediary ones during two campaigns in winter (February) and summer (June/July 2023). The summer campaign coincided with the North Sea cruise to maintain the connection between the Thames, Elbe, and the North Sea (Fig.1).

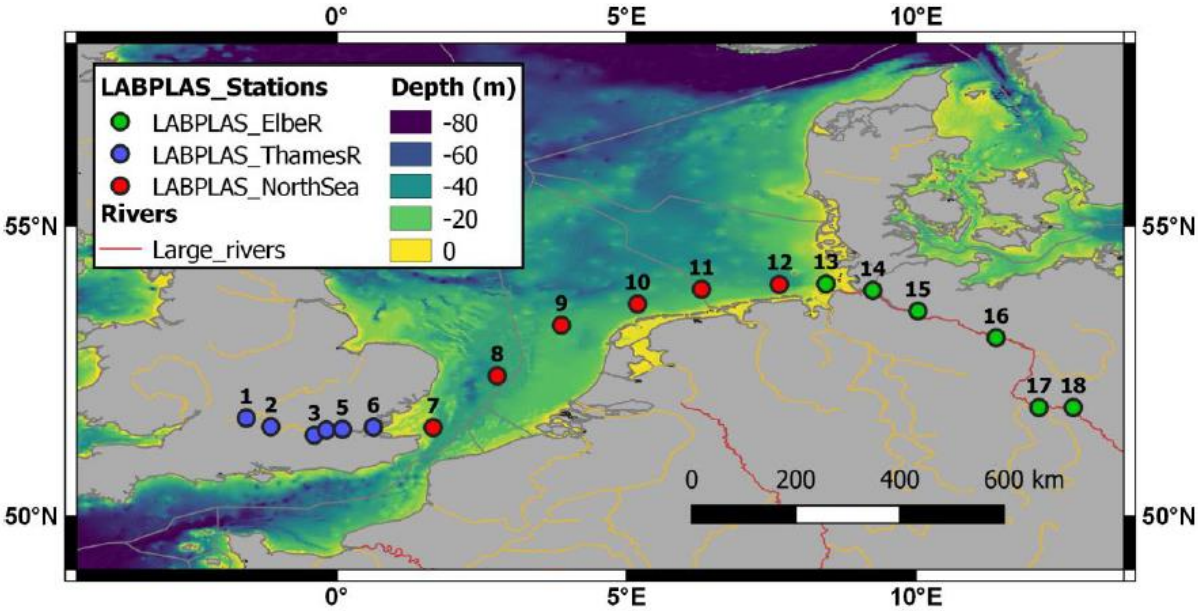

Figure 1. Sampling sites in 2022 and 2023 in Thames River (St1-St6), the North Sea (St7-St12), and the Elbe River (St13-St18).

In the Thames and Elbe rivers larger floating microplastics were collected with a Manta net (>335 µm) and in the North Sea, a catamaran was used. Samples were fixed immediately with 4% buffered formalin. In the laboratory, samples were gently transferred to a petri dish, and plastic particles were manually separated using a dissecting stereomicroscope from other components such as wood, zooplankton, and organic tissues. Each sample was examined twice to ensure the detection of most of the plastic particles. Plastics were counted and weighted grouped by size class: micro (<5 mm), meso (5-20 mm), and macro (> 20 mm). Zooplankton from the North Sea winter season were also weighted in 2 size classes (>5mm and < 5mm).

Plastics were digitally imaged using a ZooScan scanner system with a resolution of 2400 dpi (Gorsky et al., 2010). Plastic and zooplankton are automatically detected and their morphological attributes are extracted through post-processing with Zooprocess and Plankton Identifier software that provides a large set of morphological parameters for each object: surface area (mm^2), length (mm), equivalent spherical diameter, circularity, among others. Data was then exported to Ecotaxa (https://ecotaxa.obs-vlfr.fr) (Picheral et al., 2017).

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Plastics were semi-automatically classified into six shape categories (rigid fragments, films, foam, granules, rope filaments, and microfibers) through an AI-based machine-learning process. The polymeric composition of the plastic particles was sent for analysis in GEOMAR. The analysis of zooplankton has followed the same pattern with a semi-automatic taxonomic classification. At the end of the process, human validation corrects classification errors (Fig. 2). Analysis and quantification of plastic: zooplankton ratios derived from calculated zooplankton and plastics concentrations.

**For more details on the sampling see: Deliverable D2.2 First sampling campaigns and sample preparation.*

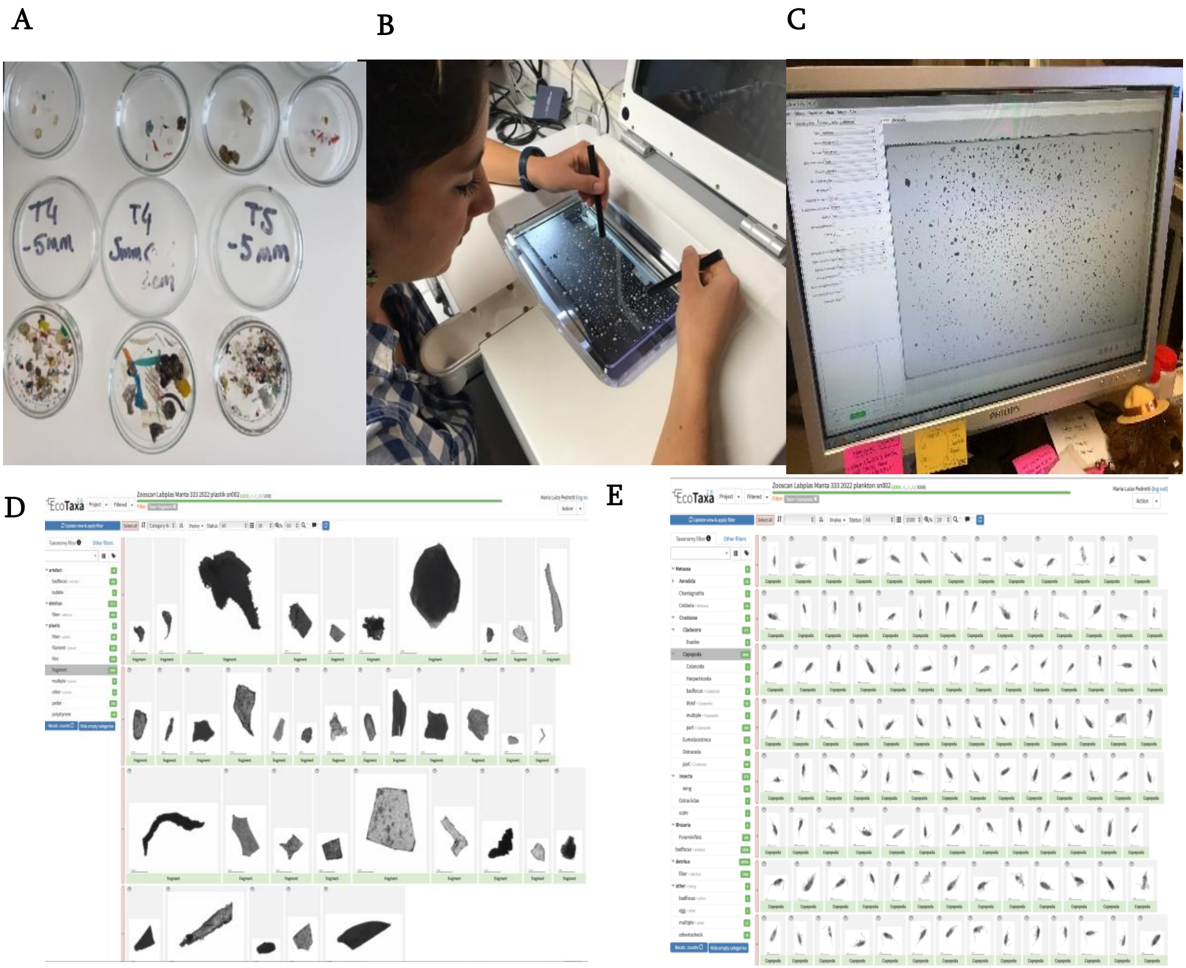

Figure 2. Pipeline for plastic analysis. A) Plastic debris were manually sorted from samples B-C) Plastics were scanned by ZooScan and image processed with Zooprocess to assess a large set of morphological parameters. D) Example of sorted plastic categories fragments and E) Copepods.

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1.4 Results

1.4.1 Plastic and zooplankton concentration

Plastic analyses revealed the presence of plastics at all sampling stations. Plastic concentration was highest in the Thames River, with an average of 10.10 plastics m^{-3}, followed by the Elbe (0.8 plastics m^{-3}) and the North Sea (0.38 plastics m^{-3}). In the Thames, average plastic concentrations were 2.53 plastics m^{-3} in winter and 2.44 plastics m^{-3} in summer 2022. However, in 2023, very high concentrations were observed at the two monitored stations closest to London: T5 (34.32 plastics m^{-3}) in winter and T6 (118.4 plastics m^{-3}) in spring.

In the Elbe, average plastic concentrations were 0.21 plastics m^{-3} in winter and 0.17 plastics m^{-3} in the summer of 2022. In 2023, higher concentrations were observed at upstream station E17 (average of 1.34 plastic m^{-3}) and station E13 closer to the sea (average of 3.65 plastics m^{-3}). The highest average plastic concentrations in both rivers were recorded in spring. In contrast, the North Sea showed similar plastic concentrations in winter (0.43 plastics m^{-3}) and summer (0.34 plastics m^{-3}) (Fig. 3).

**For more details on plastic results see "Deliverable D2.3 Preliminary report sampling 2nd year, sample preparation and results of 1st year" and "Deliverable 2.4 Sample preparation. Results of 2nd year and suggestions for sampling".*

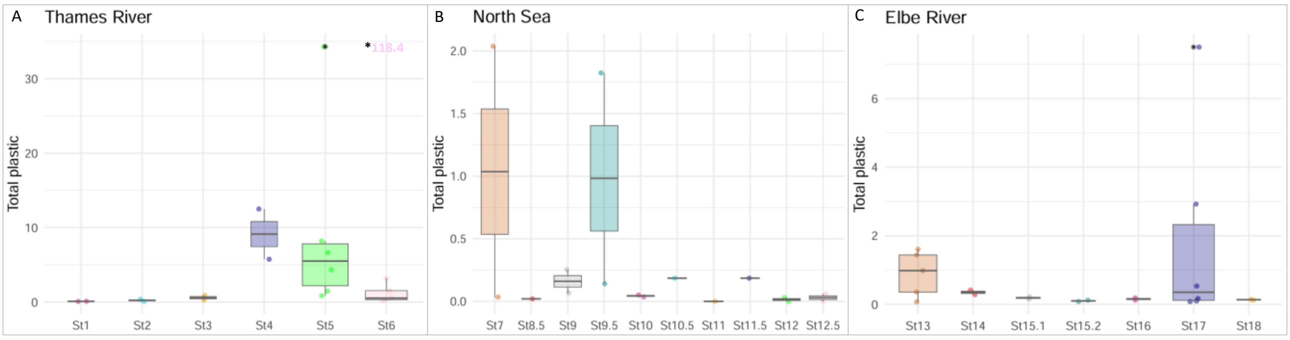

Figure 3. Average plastic concentration (m^{-3}) across the 18 stations sampled in 2022 and 2023. Thames River (A), North Sea (B), and Elbe River (C). Note that the y-axis is not at the same scale. When high outliers are present, they are written in the graph with an asterisk in their respective station: Thames River (118.4 plastics m^{-3}).

**For more details on plastic results see "Deliverable D2.3 Preliminary report sampling 2nd year, sample preparation and results of 1st year" and "Deliverable 2.4 Sample preparation. Results of 2nd year and suggestions for sampling".*

Analyses of zooplankton showed that they are present across the entire surface of the sampling stations, with the number of organisms and taxonomic composition varying among stations and periods of the year. The highest concentration was found in the North Sea during summer (St9; 10,772.3 ind. m^{-3}), while the lowest was in the estuarine part of the Thames River in winter 2023 (station T6; 0.07 ind. m^{-3}).

In the Thames River in 2022, zooplankton concentrations were almost similar between the two seasons (average 21.4 ind. m^{-3}), with higher values at stations T4 and T5. However, in 2023, zooplankton concentrations increased tenfold at the two studied stations, T5 and T6 (average 228.8 ind. m^{-3}). The North Sea exhibited a distinct seasonal pattern, with lower zooplankton concentrations in winter (22.35 ind. m^{-3}) and more than 100 times higher concentrations in summer (3,496.93 ind. m^{-3}), with no clear spatial pattern detected (Fig. 4).

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Overall, zooplankton concentrations in the North Sea (average 2,194.03 ind. m^{-3}) were higher than those in the Elbe River (471.5 ind. m^{-3}) and the Thames River (228.8 ind. m^{-3}).

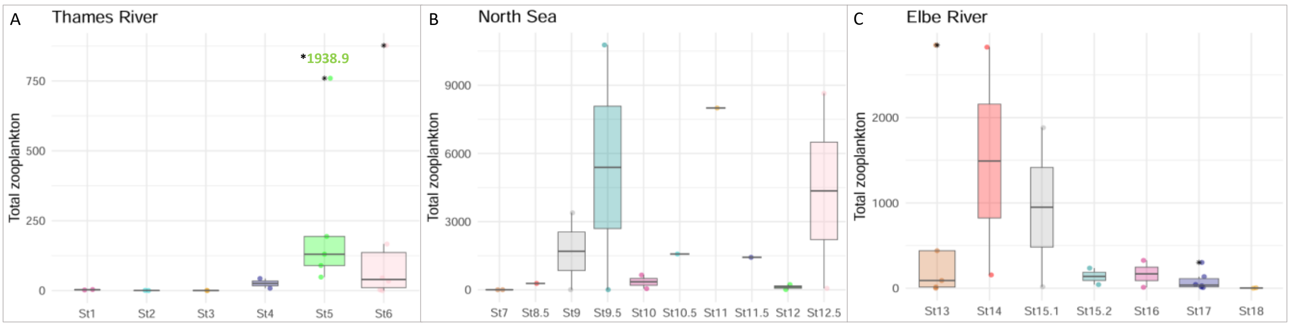

Figure 4. Average zooplankton concentration (m^{-3}) across the 18 stations sampled in 2022 and 2023. Thames River (A), North Sea (B) and Elbe River (C). Note that y axis is not at the same scale. When high outliers are present, they are written in the graph with an asterisk in its respective station: Thames River (1938.9 ind. m^{-3}).

1.4.2 Calculation of total Plastic to zooplankton ratio (PZR) as a function of plastic and zooplankton concentration

Using total plastic and zooplankton abundance, we calculated the plastic-to-zooplankton ratio (PZR), defined as the number of plastic debris pieces per m^3 divided by the number of zooplankton organisms per m^3, across the entire study area. The spatial distribution of PZR varies by 5 orders of magnitude across the studied area with the lowest value (0.00002) in the North Sea during summer and the highest ratio found in the estuarine part of the Thames River in winter 2023 (23.3). Excluding this outlier, the average ratio is 0.29 (±1.451), with significant variations by season and location, as indicated by the large standard deviation. This suggests that filter feeders (fish and marine mammals), which consume zooplankton, have a 2.9 in 10 chance of ingesting plastic instead of plankton.

The Thames River had the highest PZR in 2022, with an average of 0.25 in winter and 0.14 in summer. Station T4, located near London, recorded the highest riverine ratio at 0.48. In 2023, the PZR decreased to 0.035. The North Sea exhibited a PZR ten times higher in winter (0.284) than in summer (0.002). The Elbe River showed a similar pattern, with a winter ratio of 1.48 and a summer ratio that decreased by 1000-fold to a very low value of 0.001 (Fig. 5). Overall, the PZR was higher in the two rivers than in the North Sea stations. We observed an elevated ratio in the two estuarine parts of the study area maybe due to the transition between zooplankton populations from freshwater to oceanic water. Further investigation is needed to determine whether changes in zooplankton composition, potentially due to varying tolerances to environmental parameters such as salinity, have occurred.

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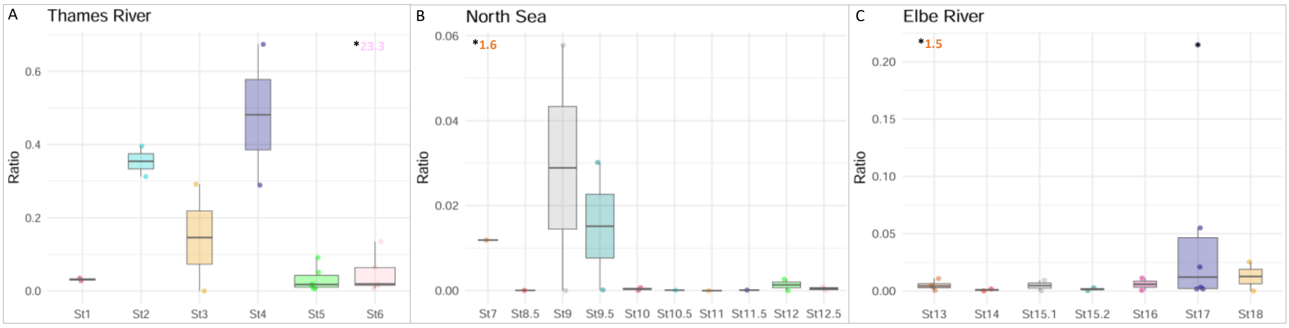

Figure 5. Plastic to zooplankton ratio (PZR) across the 18 stations sampled in 2022 and 2023. Thames River (A), North Sea (B), and Elbe River (C). Note that the y-axis is not at the same scale. When high outliers are present, they are written in the graph with an asterisk in their respective station: Thames River (23.3), North Sea (1.6), and Elbe River (1.5).

1.4.3 Calculation of microplastic to zooplankton ratio (PZR) as a function of zooplankton functional groups

Microplastics (<5mm) constituted an important proportion of the plastic debris found in the rivers (91%) and in the North Sea (80%) during the mentioned sample campaigns. Along with plastic debris, zooplankton organisms of the same size class (0.33—5.00 mm) were mainly composed of copepods, followed by small crustacean grazers such as mollusks and cladocerans. Copepods were the most abundant organisms in the surface layer, constituting 94%, 51%, and 88% of neustonic zooplankton in the Thames, North Sea, and Elbe, respectively. The highest concentrations of copepods were found in the North Sea during the summer of 2023, with 1783 ind. m^{-3}, while the lowest concentration was recorded in the winter of the same area, with 16.3 ind. m^{-3}. In the rivers, the highest numbers of organisms were found at the two stations near the estuary (Fig. 6). Small grazers, primarily Cladocera, were the second most abundant zooplankton group in the rivers, with 2.3 m^{-3} and 0.7 m^{-3} respectively, in the Thames River, and making up 16% of the zooplankton in the Elbe. In the North Sea, the two other abundant groups were gelatinous filter feeders (33%) and large crustaceans (12%). Other groups, such as mollusks, Chaetognatha, and Pteropoda, were also present mainly in the sea and estuaries.

A **B** **C**

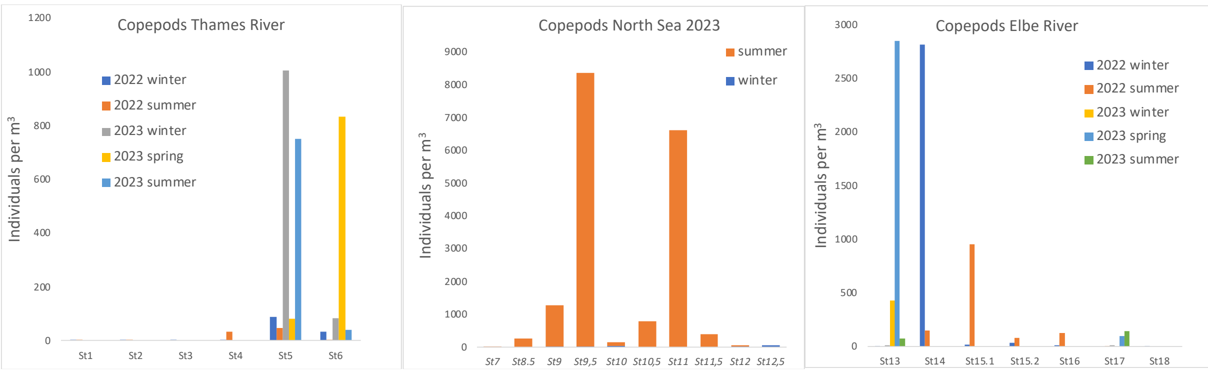

Figure 6. Copepod concentration (m^{-3}) in Thames River (A), North Sea (B) and Elbe River (C). Note that y axes are not at the same scale.

PZR using microplastics were calculated for copepods, small grazers, and filter-feeding gelatinous organisms, for different seasons and sites. We also calculated a ratio as a function of the zooplankton size class (not shown in the deliverable).

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Concerning copepods, the North Sea has the highest ratio (0.4) in winter 2023, an order of magnitude higher than in the summer of the same year. This ratio indicates that for every ten zooplankton organisms in a seawater sample, there were four plastic debris particles, an exceptionally high concentration for seawater (Table 1). We also observed high ratios in the two rivers: the Thames River had an average ratio of 0.23, and the Elbe River showed an average ratio of 0.09. Indeed, copepods may be very effective candidates for MP assessment. Copepods form an extremely diverse trophic group, highly abundant and they are present in almost all habitats. Their role in trophic ecosystems is becoming increasingly well-known (Romagnan et al., 2015). In particular, they are a preferred food for many fish. Overall, the potential for ingestion of MPs is estimated at 16% for organisms feeding on copepods. For filter-feeding gelatinous zooplankton, the overall ratio is very high, as it is for small grazers such as Cladocerans. But it needs to be considered with caution as these values are averages.

Microplastic and zooplankton concentrations have been measured in many oceanic locations, but only a few studies have simultaneously measured their concentrations, and there is a lack of information on freshwater systems. This gap makes it challenging to understand the impact of plastic pollution. The ratios from the literature are often based on calculations between the dry weight of both zooplankton and plastic particles and to a lesser extent their abundances. In the North Pacific Gyre, Golstein et al., (2013) obtained rations varying from 0.01 to 10. Along the California Coast Moore et al., (2002) obtained a ratio of 0.6, and Lattin et al., (2004) on the South California shore obtained a ratio of 0.3 for the size class plastics smaller than 4.75 mm. In the Mediterranean Sea, Collignon et al. (2012) obtained a total dry weight ratio of 0.5 and the PZR based on concentration averages was 0.1, ranging from 0.001 to 2.8 (Pedrotti et al., 2026; Geringy et al., 2022, Fabri-Ruiz et al., 2023). However, these ratios are difficult to compare due to the different approaches used to present the results, which incorporate different plastic sizes, and the contrasting trophic states of the areas studied. For instance, the California Current, influenced by nutrient upwelling, has higher biological productivity than the central gyre of the North Pacific. Our ratio values ranged from 23.3 to 0.00002, with an average of 0.29 (±1.451), which is intermediate compared to the rates reported in the literature. This range of values indicates that the potential impact of plastic pollution on marine filter-feeding organisms can vary widely. High ratio values suggest that small microplastic debris can be confused with neustonic zooplankton, such as copepods and cladocerans, and consequently be ingested by zooplankton predators. Conversely, low ratio values imply that fish or whales rarely encounter microplastics.

Efforts are needed to establish a robust index for zooplankton encounter rates with various size ranges of microplastics in different marine pelagic environments. Such an index would enhance our ability to assess the ecological impact of plastic pollution more accurately and inform mitigation strategies.

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Table 1. Averages microplastic concentrations (MPs m^{-3}) and their respective ratios

Sites	Average MPs (m^{-3})	Ratio MPs:copepods	Ratio MPs:gelatinous feeders	Ratio MPs:small grazers	Ratio Plastic:zooplankton
Thames River					
winter 2022	2.31	0.38		1.6	0.25
summer 2022	2.22	0.32		1.2	0.14
St 5&6 2023	19.36	0.04		2.4	0.03
Two years	9.1	0.23		1.8	0.14
North Sea					
winter2022	0.34	0.4	2.45	8.33	0.28
summer2022	0.27	0.01	0.06	0.04	0.002
Elbe River					
winter 2022	0.19	0.24			1.48
summer 2022	0.15	0.003			0.0009
St 13&17 2023	2.27	0.02		0.21	
Two years	0.76	0.09	0.08	0.08	0.53

The presence of plastics along with this group of zooplankton indicates that, in addition to possible ingestion by fish, other interactions are possible (Table 2). Gelatinous organisms, transported by currents in the same way as plastics, are capable of filtering MPs. Larvae of benthic species and associated eggs can cling or adhere to plastic surfaces, completing their development and being carried away from recruitment sites by the currents.

Table 2. Ecological categories of plankton and ecological characteristics. Zooplankton functional groups studied.

Size	Plastic category	Zooplankton category	Zooplankton ecological Traits	Potential impacts
0.3-5mm	MPs	Copepods,Small grazers	Prey for larger zooplankton	Confusion in ingestion by filter feeders, colonization of plastics by their larvae
0.3-5mm	MPs	Gelatinous filters	Pelagic tunicates: wide range of prey from bacteria to zooplankton	Ingestion of plastics and plastic colonization by their larvae
>5mm	Meso-Macro plastics	Gelatinous carnivores	Planktonic predators in the adult stage, predation on crustaceans	Larval colonization, external contact

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1.5 Final remarks and further steps

This study examined the concentrations of plastics and zooplankton across a continuous freshwater and marine ecosystem, including often understudied rivers. It proposes a new tool for assessing the potential risk of plastic ingestion by marine fauna. The plastic:zooplankton ratio provides new insights into the environmental risk posed by this pollution on marine food webs. The initial findings on this ratio give an indication of the instantaneous encounter rate between plastic and zooplankton, highlighting the stress induced by plastic on marine ecosystems and the necessity for further research with larger spatial and temporal resolutions, including water column observations and rivers. Rivers with their higher flow rates compared to oceans, can wash away zooplankton, limiting their ability to establish stable populations and impacting the distribution and stability of these organisms (Wetzel, 2002). Future steps for the Labplas Project will include calculating the ratio based on the average surface area of microplastics and zooplankton, considering environmental drivers. This approach will help establish more accurate relationships between plastic and zooplankton abundances and environmental factors, aligning with the hydrology and current patterns of the studied area.

Currently, only surface data have been analyzed, while zooplankton inhabits the entire water column and exhibits diel vertical migration. Although our study collected some samples in the water column using WPII (200μm), the insufficient amount of plastic found prevented the detection of clear patterns. Changes in zooplankton composition, size, and density can impact higher trophic levels, such as the fitness of small pelagic fish. The next step for the Labplas Project will include analyses to assess the quality of zooplankton communities as a food supply for predators and to determine the pattern of zooplankton abundance in the North Sea, similar to the framework used in the Mediterranean Sea (Fabri-Ruiz et al., 2023).

Is also crucial to establish a threshold for this indicator and propose it as a baseline for future monitoring efforts. Identifying a plastic:zooplankton ratio that signifies a high risk of plastic ingestion is essential to assess and ensure the health of ocean ecosystems. In the Mediterranean Sea, an "extreme condition" threshold is considered higher than 0.1 (10% chance of encountering plastic), while a "high risk" threshold is estimated at >0.05 (more than 1 plastic piece for 20 potential prey) (Fabri-Ruiz et al., 2023). Differentiating these values for coastal, offshore areas, and rivers might be necessary to develop more targeted conservation strategies.

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