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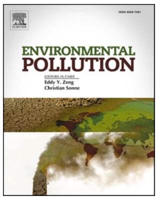

Zooplankton exposure to microplastic contamination in a estuarine plume-influenced region, in Northeast Brazil☆

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ABSTRACT

This work describes the spatio-temporal distribution of suspected plastic and microplastic (MP) particles in estuarine plumes and analyzes the microplastic/zooplankton ratio. Subsurface hauls with a conical-cylindrical net were deployed in the coastal area of Tamandare (Pernambuco, Brazil), covering the plume of two rivers and a bay adjacent to coral reefs. A total of 2079 suspected plastic particles were detected, mostly fibers and fragments (>60%). Organic matter digestion was made using a 30% hydrogen peroxide solution, of which approximately 50% of suspected particles were validated as MPs. The average MP abundance was significantly higher during the high rainfall season (53.8 ± 89.6 and 18.8 ± 32.3 particles/m^3, respectively), with higher values registered in the plume area (108.9 ± 158.5 and 44.6 ± 55.5 particles/m^3). Polymer identification using FT-IR confirmed that suspected particles were mainly polypropylene, polyamide, and polyurethane. These results confirm the hypothesis of a temporal transport variation of MPs from the river to the coastal environments, particularly since the plume influences debris input. Eleven animal phyla were identified, and the subclass Copepoda was predominant (90%), particularly the nauplius stage (70%). Over 70% of verified MPs range between 20 and 2000 μm, equivalent to the most common size of zooplanktonic organisms. Results support that coastal areas near estuarine plumes are exposed to microplastic contamination, affecting species dependent on zooplankton in marine coastal food webs.

1. Introduction

Marine litter comprises a wide range of materials such as processed wood, metal, glass and plastic, with the latter the most common (Iñiguez et al., 2016; Kroon et al., 2018; Purba et al., 2019). Plastic is persistent, durable (Thompson et al., 2009), and undergoes environmental degradation (Aliabad et al., 2019). Fragmentation into smaller particles known as microplastics [MP, 1 μm–5 mm (Frias and Nash, 2019)] occurs through physical, chemical, and biological processes (Aliabad et al., 2019). When inefficiently managed, plastics find their way into the environment where they remain for long periods of time (Hidalgo-Ruz et al., 2012) impacting organisms, mainly through ingestion (Cole et al., 2013; Sun et al., 2018; Amin et al., 2020). In 2020, Brazil produced about 226 tons per day of solid waste and approximately 40% of it, is disposed in the environment (ABRELPE 2021).

Zooplankton, the foundation of oceanic food webs, includes both ecologically important and socio-economic relevant animal groups (e.g. shrimps, crabs and fish larvae) (Amin et al., 2020). Zooplankton and microplastics share similar size ranges (Frias and Nash, 2019; Bermúdez and Swarzenski, 2021), however most studies do not include zooplankton analysis in monitoring approaches (Lima et al., 2014; Sun et al., 2018; Botterell et al., 2019). *In situ* studies assessing the relationship between zooplankton and MP, are essential to understand the socio-economic and ecological impacts on ecosystems (Sun et al., 2018).

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Evidence from field and laboratory studies have demonstrated negative impacts on zooplankton feeding behavior, growth, development, lifespan and reproduction (Botterell et al., 2019).

Marine Protected Areas (MPA) are thought to have high interaction rates between zooplankton and MP (Kang et al., 2015; Sun et al., 2018). Coastal ecosystems are not plastic free (Luna-Jorquera et al., 2019), having ecological implications, namely at community abundance and composition (Rochman et al., 2016). Approximately 80% of marine litter is derived from land-based sources, being transported and linked to several routes (Allsopp et al., 2006; Lebreton et al., 2017; Zhang, 2017). Rivers are carriers of sediments, nutrients, and plastic particles, which are dispersed into the ocean by plumes (Morris et al., 1995; Andrady, 2011; Giarrizo et al., 2019). Studies have shown that MP abundance transported by rivers is related to *a)* rainfall, *b)* local urban and industrial areas; and *c)* flow rates (Iñiguez et al., 2016; Lebreton et al., 2017).

This study aims to test the hypothesis that: (1) the concentration of suspended microplastic particles varies spatially in the coastal tropical area, with higher concentrations in the plume area; (2) the greater abundance of microplastic particles is observed in the period of high rainfall, and (3) there is a relation between the abundance and size spectra of suspended MP and zooplankton in MPA.

2. Material and methods

2.1. Study area

Samples were obtained within a Marine Protected Area (MPA) on the south coast of Pernambuco State, Brazil (08° 45′36″ and 08° 47′20″S, 35° 03′45″ and 35° 06′45″W). The Costa dos Corais Environmental Protection Area (EPA) is the largest Federal Marine Conservation Unit in the country, with 135 km in length. The sampling area includes three systems: (1) the plume of the rivers Ilhetas and Mamucabas, located south of the Tamandaré region, (2) a bay and the adjacent region of (3) coral reefs (Fig. 1). The bay area is a coastal embayment delimited by sandstone coral reefs that promote water trapping in the bay and can be influenced by the plume, especially during the high rainfall (Brito-Lolaia et al., 2020).

Pollution sources are mainly associated with agriculture (sugarcane monoculture), tourism and fishing, all important economic activities in the region (Moura and Passavante, 1994; Araújo and Costa, 2007). During the high rainfall, the study area can also be influenced by two other rivers, the Una (~10 km south) and the Formoso (~8 km north) (Barbosa et al., 2016). These rivers separate urban areas that have no basic sanitation. The region also includes slaughterhouses and mills (Magalhães and Araújo, 2012) that input the rivers.

2.2. Plankton sampling, hydrological and climate data

Samples were collected during four campaigns between March and October 2020. A total of 36 samples were collected from 3 stations, including the rivers' mouths (plume), bay and the adjacent coral reef area. At each station, three plankton trawls were carried out. Sampling was performed at spring tide during the diurnal ebb tide when there is a significant influence of the estuarine plume. Temperature and salinity were measured using a Multiparameter probe Horiba U-52, and rainfall data was obtained from the website of the Pernambuco Water and Climate Agency (APAC). Hauls were performed at the subsurface using a conical-cylindrical plankton net (30 cm ⌀), with a 64 μm mesh size, and a flowmeter (Hydrobios GmbH) that was fixed at the net mouth. At each station, the net was hauled for 3 min at a speed of 1–2 knots. Samples were fixed in 4% neutral formalin for quantitative and qualitative analysis of microplastics and zooplankton.

2.3. Quality control and MPs characterization

To avoid cross contamination, all surfaces and materials were thoroughly cleaned with Milli-Q water, distilled water or 70% ethanol, filtered by vacuum pump. Samples from distilled water, Milli-Q water and ethanol were visually inspected under stereomicroscope (Zeiss). In the field, the sample storage containers were washed with distilled water, and the net was washed thoroughly from the outside with seawater, between stations to avoid cross-contamination.

In the laboratory, glass containers used were immersed in a 10% hydrochloric acid solution (HCl) for at least 24 h (Prata et al., 2021).

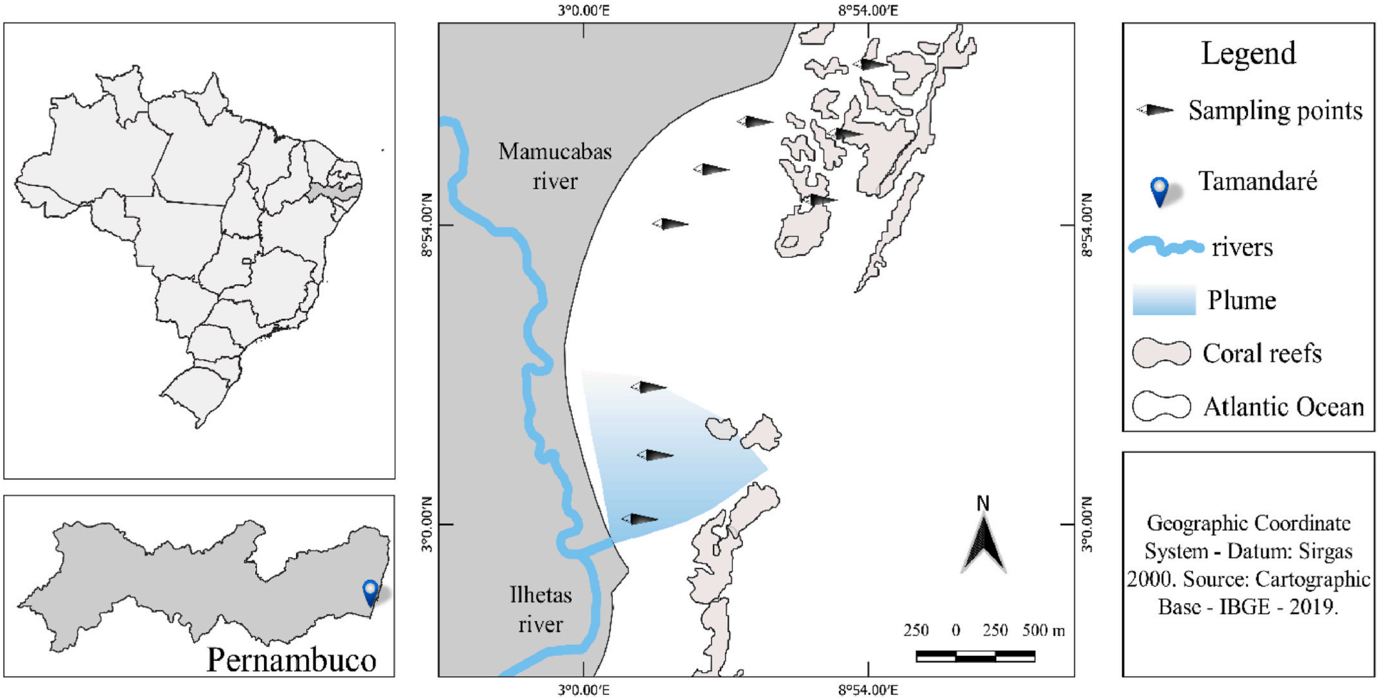

Fig. 1. Study area and sampling stations in the coastal region of Tamandaré, Brazil. For each station, three plankton hauls were carried out (represented on the map by sampling points).

Similarly, all glass containers and metal items (tweezers and needles) were thoroughly washed and visually inspected under an optical stereo microscope prior to any analysis.

Nitrile gloves, 100% cotton lab coat and a cap were used during these extraction processes. To avoid airborne contamination, exposure of samples were kept to a minimum, using pre-washed aluminum foil to cover them. To account for possible airborne contamination, one filter (Qualitative Filter Paper) was used in an open glass Petri dish as a control, close to the sample during analysis. Immediately after, filters were visually inspected under an optical stereomicroscope. Approximately 90% of the airborne contamination were fibers, mainly transparent and blue (72.4%). Similar fibers to the control were excluded from the analysis.

Plankton samples were visually inspected under an optical stereomicroscope being the particles morphologically categorized, following Gago et al. (2019). The MP types considered were (i) fragment, (ii) fiber, (iii) filament, (iv) film and (v) other types - spongy particles and spheres. For Color ID (i) blue, (ii) black, (iii) white, (iv) transparent, (v) red and (vi) other colors were considered. The suspected plastic particles were stored in glass tubes (5 ml) with Milli-Q water. Particle abundance was expressed as particles/m^3 (average value ± standard deviation).

A sub-sample totaling just over 30% of the total samples was used for organic matter digestion and FT-IR polymer analysis. The organic matter (OM) in suspecteds MP particles was digested following a modified López-Rosales et al. (2021) protocol: Airborne contamination was avoided by vacuum filtering samples using a stainless steel filter (pore size, 26 μm) and rinsed with a Tween80 solution (0.1%). The filter was then placed in a glass beaker (250 ml), and a 2% SDS (Sodium Dodecyl Sulfate) surfactant solution was added to the beaker until the entire filter was covered. After 24 h, the sample was vacuum filtered and placed in a beaker (250 ml). A 30% H_2O_2 solution was then added gradually in 2 ml steps until the entire filter was covered. After a period of 24 h, the samples incubated at 40 °C, were again vacuum filtered to end the digestion process. Length measurements (μm)were used to categorize MP size ranges in the study area. A limitation associated with particles smaller than 100 μm was identified, as underestimated could be due to the mesh opening (64 μm) and the visual limit (100 μm). Nonetheless these were recorded and considered in the final count. Procedural blanks (n = 3) were used to quantify contamination of samples during processing. No contamination was registered in the procedural blanks.

2.4. FTIR analysis

The particles were analyzed under a Shimadzu Prestige 21 Fourier Transform Infrared spectrophotometer, with a diffuse reflectance module. Measurements were carried out with wave number range of 400–4000 cm^{-1}, and performing 24 scans per particle, to select the best signal/noise. Each spectrum was plotted using Origin Lab software and compared with a polymer reference database (Silverstein et al., 2007; Jung et al., 2018). The spectra are shown as acquired, without corrections, except for smoothing. Suspected particles that had matches <60% correspondence were considered ‘non-polymeric particles’.

2.5. Zooplankton analysis

Samples were diluted in a known volume, and three aliquots of 10 ml were subsampled until obtaining at least 100 individuals per aliquot. Counting and identification were performed under the light microscope (Leica), to the family level using specialized literature (e.g., Boltovskoy, 1999). Taxon abundance was expressed as individuals/m^3, using the filtered volume per tow. Plastics and zooplankton abundances were used to assess the microplastic:zooplankton ratio [(MPs particles/m^3)/(ind./m^3)]. For this ratio, only the most abundant groups were considered. The net used is not ideal for capturing organisms such as fish larvae and decapods, as there is avoidance of these fairly robust organisms (Gehrke, 1992; Kodama et al., 2022).

2.6. Data analysis

All analyzes were conducted based on abundance, expressed in individuals or particles/m^3. The original data were Box-Cox transformed to verify normality (Shapiro-Wilk test) and homogeneity of variances (Levene test). MP and zooplankton abundance were log x+1 transformed after considering its non-normal distribution. To assess MP and zooplankton spatial and temporal variations, a ANOVA test was applied (Fig. 3). The Bonferroni test ($p < 0.05$) was used to identify the sources of significant variations, with a statistical significance level of $p < 0.05$. All analyzes were conducted using Statistic 6.0 software. To evaluate how the composition of MPs and zooplankton differ spatially and temporally, the abundance matrices were transformed into the fourth root, and then a non-metric multidimensional scaling (nMDS) was performed using a Bray-Curtis matrix (Supplementary data - Fig. S1). A PERMANOVA was used to verify the effect of area and rainfall levels on the composition of microplastics and zooplankton using the PRIMER v.6.1 software package with the Permanova+ (Anderson, 2001). When differences were statistically significant, pairwise comparisons among levels were analyzed. Abundance was expressed as individuals/m3 (average value ± standard deviation). For each taxon standardizing the number of organisms for the sea surface volume filtered (same as in MP analyses). A Spearman correlation test was applied to test the correlation between the total abundance of MPs (particles/m^3) and zooplankton (ind./m^3). The numerical ratio of MPs to the most abundant taxonomic groups of zooplankton was proposed to express the MPs: Zooplankton ratio.

3. Results

3.1. Environmental variables

In 2020, rainfall data ranged from 170.3 to 320.4 mm during the high rainfall period (from March to August) and from 15.8 to 56.7 mm in the low rainfall period (from September to February). Temperature and salinity values were obtained only in the collection months. March/June represent the period with high rainfall, and September/October is the period with low rainfall. Average temperature in high rainfall was 28.5 ± 0.22 °C in high rainfall and 29.2 ± 0.13 °C in low rainfall. In general, salinity values presented a gradient from plume to the coral reef stations, with lower values in the plume and higher in the coral reefs, better visualized in the period with low rainfall (Supplementary data - Fig. S2).

3.2. Suspected plastic particles

A total of 2079 suspected plastic particles were registered with the most abundant types being fibers, fragments and filaments. The most abundant colors varied between types, with white and black representing more than 60% of fragments, blue fibers almost 50% and red and black filaments (70%). Transparent films had the highest abundance (87.7%). Blue plastics were identified across all areas (Table 1).

The average abundance of suspected plastic particles significantly differed between periods of high and low rainfall (ANOVA, p-value <0.05). The average abundance of suspected plastic particles was much higher in the plume (108.9 ± 158.5 particles/m^3) during the period with high rainfall (Fig. 3A; p-value <0.05), with a higher contribution of fragments in the plume (59.8 ± 89.4 particles/m^3) and bay (18.4 ± 7.1 particles/m^3), and fibers in the coral reef (10.7 ± 10.5 particles/m^3). In the period with low rainfall, the plume (14 ± 4.3 particles/m^3) had the lowest average abundance of suspected plastic particles. During this period, fibers were the most common item in all areas (Table 1).

3.3. Microplastics and chemical composition

Validation of plastic particles varied with type (Fig. 2). During high rainfall, 42.4% of the fibers, 33.5% of the fragments, 33.3% of films and

Table 1
Average abundance of suspected plastic particles, microplastics, standard deviation (particles/m^3) and percentage of registered polymers in the environments between the periods of rainfall variation. *Not detected.

	Distribution (particles/m^3)					
	High rainfall			Low rainfall		
Area	Plume	Bay	Coral Reef	Plume	Bay	Coral Reef
Total average abundance (particles/m^3)	108.9 ± 158.5	30.4 ± 7.8	22.2 ± 9.9	14.0 ± 4.3	16.8 ± 5.0	20.0 ± 26.4
Fragment	59.8 ± 89.4	18.4 ± 7.1	8.1 ± 1.1	4.4 ± 3.5	6.7 ± 2.4	3.6 ± 1.5
Fiber	36.3 ± 52.2	11.0 ± 1.4	10.7 ± 10.5	9.4 ± 4.3	9.9 ± 3.9	15.6 ± 21.7
Filament	10.6 ± 15.7	0.15 ± 0.25	0.5 ± 0.5	0.07 ± 0.07	0.02 ± 0.04	0.1 ± 0.1
Film	1.6 ± 1.3	0.7 ± 0.5	1.2 ± 1.2	*	0.12 ± 0.2	0.5 ± 0.5
Others	0.7 ± 0.2	0.07 ± 0.12	3.2 ± 0.2	0.1 ± 0.1	*	0.1 ± 0.2
Total Microplastics (particles/m^3)	44.6 ± 55.5	3.7 ± 0.6	8.1 ± 11.4	4.0 ± 0.2	7.8 ± 3.5	4.3 ± 0.5
PP (%)	23,1	26,3	30,8	51,7	36,5	33,3
PE (%)	7,7	5,3	3,8	*	*	6,1
PS (%)	5,1	*	*	*	1,4	3,0
PA (%)	41,0	39,5	26,9	10,3	21,6	9,1
PU (%)	7,7	18,4	23,1	3,4	13,5	18,2
PC (%)	*	*	*	1,7	5,4	*
PVC (%)	2,6	*	*	5,2	*	9,1
PET (%)	2,6	*	*	*	*	*
PMMA (%)	5,1	2,6	*	6,9	6,8	3,0
EVA (%)	*	7,9	11,5	5,2	8,1	*
LATEX (%)	2,6	*	*	1,7	5,4	15,2
NITRILE (%)	2,6	*	*	1,7	*	*
ABS (%)	*	*	*	1,7	*	*
PTFE (%)	*	*	3,8	*	*	*
PU/PA (%)	*	*	*	10,3	1,4	*
PET/PP (%)	*	*	*	*	*	3,0

Polypropylene (PP), polyethylene (PE), polystyrene (PS), acrylonitrile butadiene styrene (ABS), polyethylene terephthalate (PET), polytetrafluoroethylene (PTFE) polyvinyl chloride (PVC), latex, nitrile, ethylene vinyl acetate (EVA), poly (methyl methacrylate) (PMMA), polycarbonate (PC), polyamide (PA) e polyurethane (PU).

50% of other types were validated as plastics. All filaments registered during this period were not plastic. In the period with low rainfall, fibers (41.2%), fragments (39.2%) and filaments (25%) were validated as plastics. During this period, films and 'other types'' were not identified as plastics (Fig. 3). Some blue and red suspected plastic particles were not plastic. The average total abundance of microplastics in the periods with high and low rainfall was 18.8 (±32.3) and 5.4 (±2.4) particles/m^3, respectively. In the plume area, a 10-fold increase in MP was registered in high rainfall (44.6 ± 55.5 particles/m^3) when compared to the low rainfall (4.0 ± 0.2 particles/m^3). In coral reefs, about 2-fold MPs was registered in the period with high rainfall (8.1 ± 11.4 particles/m^3). However, in the bay, 2-fold MPs concentration was registered in the low rainfall (7.8 ± 3.5 particles/m^3) (Table 1).

Although the composition of microplastic appears homogeneous, nMDS on a two-dimensional scale reveals a separation between periods (Supplementary data - Fig. S1). PERMANOVA supports these results indicating a significant statistical difference for fragments type between periods (p-value <0.05, Pseudo-F = 3.51) for plume ($t = 1.49$, p-value <0.05) and bay ($t = 1.43$, p-value <0.05).

Fourteen polymer types of floating MPs were registered in the study area: PP, PE, PS, ABS, PET, PTFE PVC, latex, EVA, PMMA, PC, PA and PU. The polymers PP, PA, and PU accounted for more than 60% of all MPs. In the high rainfall an unexpectedly large abundance of polyamide was registered and larger abundances of PP were identified in the low rainfall. On the reef, high abundances of PU were observed (Table 1). The present study detected that blue fibers were highly variable in polymer, as their composition determined 11 out of the 14 types of polymers identified in total.

3.4. Relation between microplastics and zooplankton

Taxonomic groups belonging to different classes of protists and animals were registered: Foraminifera, Dinoflagellata, Ciliophora, Ectoprocta, Cnidaria, Mollusca, Annelida, Arthropoda (Crustacea), Echinodermata, Chaetognatha and Chordata. Crustaceans (mainly, copepods) were predominant, with approximately 90% relative abundance and a high contribution of copepod nauplius (>70%) in both periods. The total average abundance of copepods was 40,932.5 ± 78,676.1 ind./m^3 and 10,919.6 ± 11,635.9 ind./m^3, in high and low rainfall, respectively. Copepods from the orders Calanoida (mainly, Paracalanidae), Canuelloida (mainly, Longipedidae) and Cyclopoida (mainly, Oithonidae) were present in larger abundances. For other zooplankton groups, the mean total abundance was 26,157.5 ± 10,2391.1 and 918.1 ± 1475 ind./m^3 in high and low rainfall, respectively, with a greater contribution of Mollusc larvae and Foraminifera.

For the total zooplankton, PERMANOVA indicated a statistically

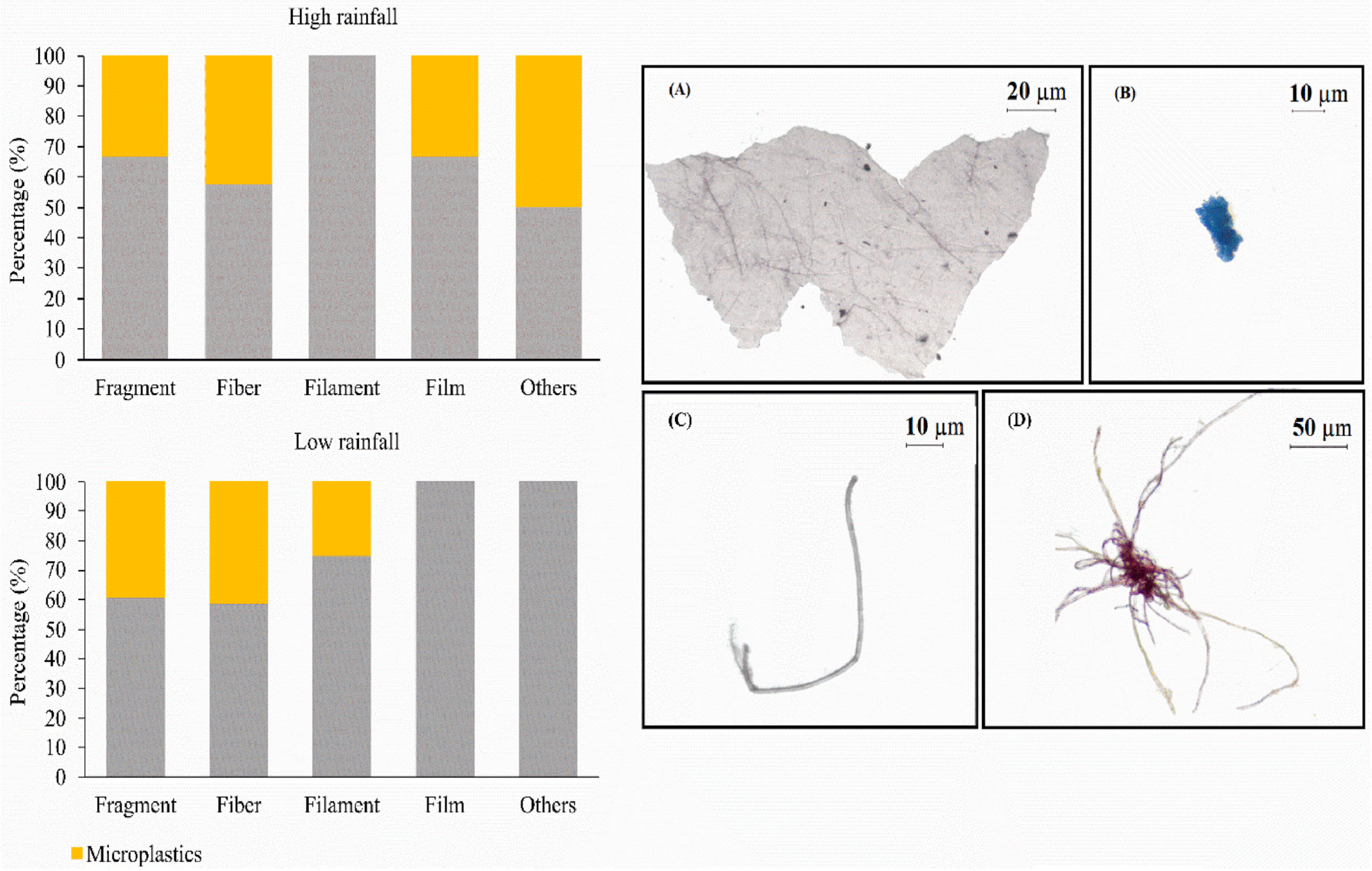

Fig. 2. Percentage (%) of microplastics (identified in yellow in the plot) after peroxide digestion and examples of types and colors of microplastics registered in Tamandaré, Brazil. (A) Transparent fragment, (B) Blue fragment, (C) Transparent fiber, (D) Red filaments. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

significant difference between the periods (PERMANOVA, p-value=<0.01, Pseudo-F = 3.03). This difference was observed for the bay (Fig. 3C; Kruskal-Wallis ANOVA, p-value=<0.05). Spatially, a significant difference was observed (PERMANOVA, p-value=<0.01, Pseudo-F = 2.51) between the plume and coral reef areas (t = 1.75, p-value=<0.05), in the period with high precipitation (Supplementary data - Fig. S1). The correlation between microplastics and zooplankton was not detected ($r^2 = 0.0013$).

Size classes were divised from Frias and Nash (2019). An adaptation suggested by Bérmudez and Swarzenski (2021) considers ranges for micro- (20–200 µm), meso- (200–2000 µm) and macroplastics (>2000 µm). In this study, micro-size includes fragments, being equivalent to dinoflagellates and tintinnids. This range is underestimated due to the limited mesh opening of the plankton net (64 µm) and visual identification (100 µm). The meso-size range accounts for 70% of MPs which are mainly fragments. This size range includes most marine zooplankton groups, including copepods (nauplius, juvenile and adult copepodites stage), and where most organisms were found. Macro-size (2000–5000 µm) includes most of the decapods (larvae), mysids, and euphausiids larvae (Fig. 4).

An increasing MP/zooplankton ratio is observed in the plume area during the high rainfall period. In adjacent areas (bay and coral reef), the MP/zooplankton ratio fluctuates (Table 2).

4. Discussion

Results support the hypothesis that the concentration of suspected plastic and microplastic fragments varies spatially, with a significant difference in plume area during high rainfall. Although no relation was observed between suspended MPs and zooplankton in MPA, during high rainfall.

4.1. *Not everything is what it seems*

Fibers represent a significant portion of microplastics and depending on its color, identification can be challenging. Just over 40% of the suspected fibers were validated as plastic. According to Kroon et al. (2018), when subjected to digestion and/or spectrometry, most fibers are identified as having semi-synthetic or natural origin.

High recovery percentages are not necessarily a positive result. Studies found MP validation from visual identification, similar [e.g., 37% (Kanhai et al., 2017) and 36.4% (Lusher et al., 2014)] to the ones presented here (between 25 and 50%). Validation through spectrometric techniques are required to correctly identify microplastics (Hidalgo-Ruz et al., 2012). It is challenging to visually distinguish between organic and synthetic particles, particularly for yellowish/transparent colors (Lenz et al., 2015; Rodríguez-Seijo and Pereira, 2017).

Filaments and films can be mistaken for organic matter or natural debris. Hence the importance of not ignoring particles, as they are accidently or actively ingested by zooplankton (He et al., 2022). Color is an important factor in identifying plastics in plankton samples. However, results here demonstrate that even brightly colored particles, such as blue and red, need validation. Similarly, color particles similar to organic matter should not be ignored. These particles can be more easily ingested by zooplankton (He et al., 2022) or other organisms.

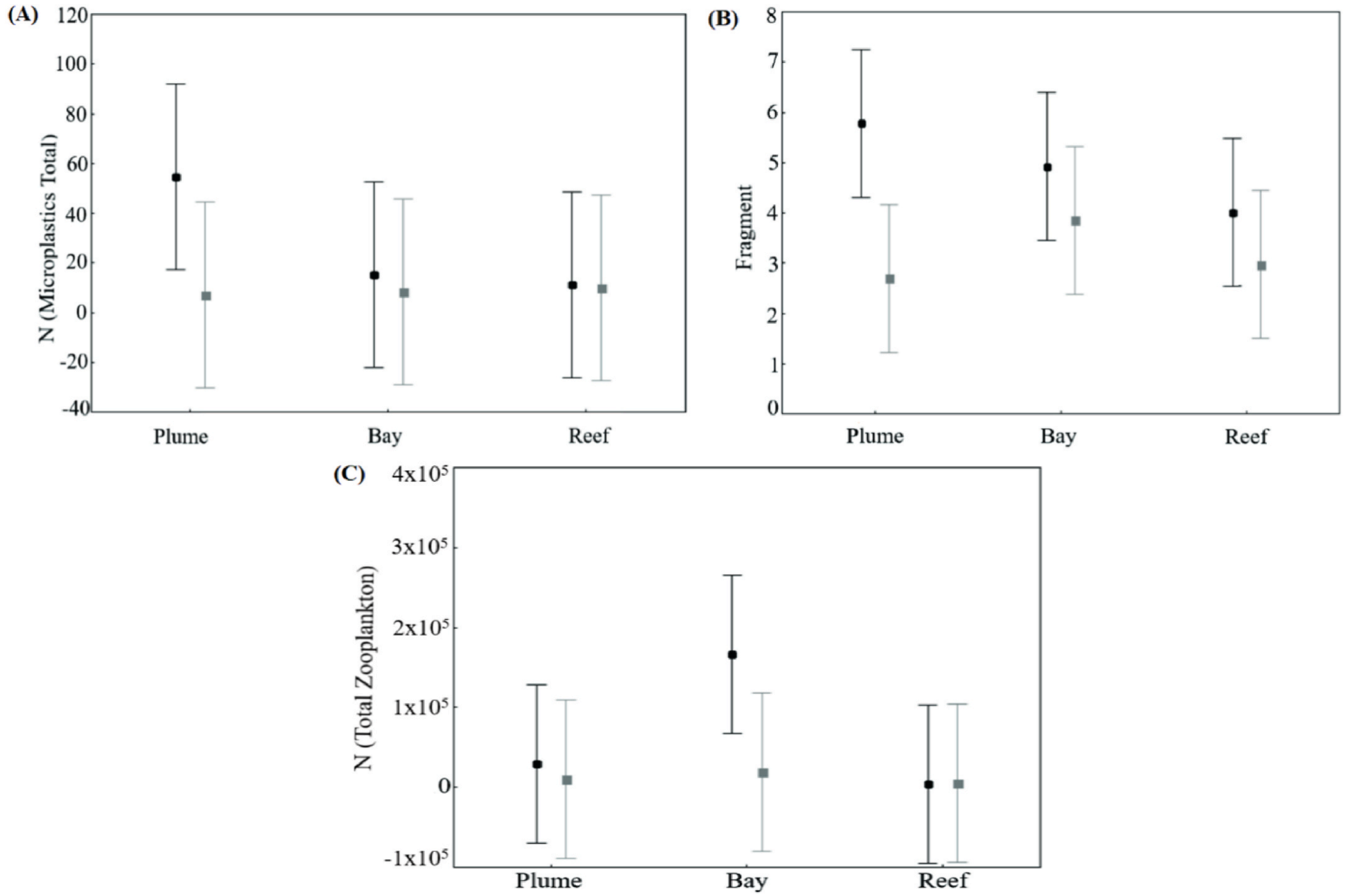

Fig. 3. ANOVA results for (A) Total microplastics, (B) Fragment type and (C) Microplastic:total zooplankton. Black points = high rainfall, gray points = low rainfall; error bars = standard deviation (A, B, C).

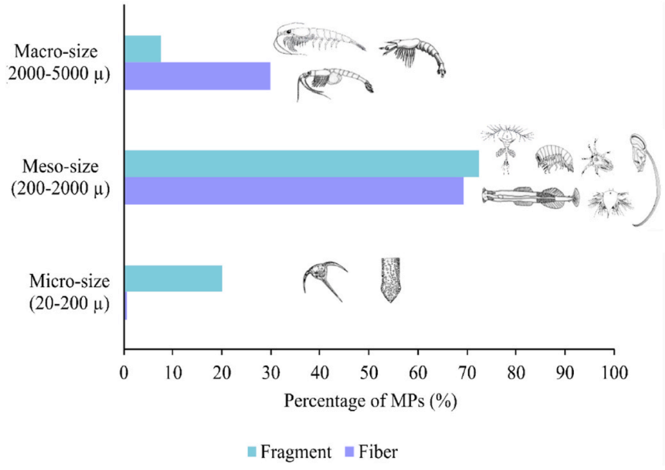

Fig. 4. Percentage in size intervals of the most abundant suspended microplastics registered in the study area, with a representation of zooplankton organisms with an equivalent size.

4.2. *Suspected plastic particles and microplastics*

MPs were registered in all samples (100%), indicating that these particles are ubiquitous in the subsurface layer of the study area, despite being located within a marine conservation unit, where fishing and touristic activities are reduced, monitored and/or prohibited since 1999. However, plastic marine litter from those sources can be found in MPA due to plume influence.

As reported in several studies, fibers and fragments are the most present types (see Lusher et al., 2014; Figueiredo and Vianna, 2018; Frias et al., 2020). The highest abundance of MP fragments was registered during the high rainfall, mainly in the plume, confirming the hypothesis of the effect of rain on plastic input in coastal environments. Brito-Lolaia et al. (2020) registered a high contribution of estuarine zooplankton species in the Tamandaré bay and coral reef area, confirming the important influence of rivers in coastal marine environments. A high abundance of fragments means that the MPs observed in the environment are aged, and potentially originate from distant sources (Metz et al., 2020). Only the bay area had a higher average abundance of MPs in the low rainfall period, probably due to water circulation rates being reduced. Coral reefs parallel to the bay limit water circulation (Brito-Lolaia et al., 2020) and in the period when there is less influence of the plume, the MPs can be retained for a longer time in this area (Barbosa et al., 2016).

Our results revealed that the average abundance of microplastics in periods of high and low rainfall (18.8 ± 32.3 and 5.4 ± 2.4 particles/m^3) is higher than in other coastal environments. The values registered for the two periods in the present study are higher than those registered for plankton samples from the tropical Atlantic Ocean (300 µm, 0.03 particles/m^3, Ivar do Sul et al., 2014), Western Equatorial Atlantic (120 µm, 0.14 ± 0.11 particles/m^3; 300 µm, 0.02 ± 0.01 particles/m^3; Garcia et al., 2020), Atlantic Ocean (250 µm, 1.15 ± 1.45 particles/m^3, Kanhai et al., 2017), Northeast Atlantic Ocean (250 µm, 2.46 ± 2.43 particles/m^3, Lusher et al., 2014), Brazilian estuaries (300 µm, 0.26 particles/m^3, Lima et al., 2014) and European coastal environments (300 µm, 0.45 ± 0.52 particles/m^3, Rodrigues et al., 2020; 0.56 ± 0.33

Table 2
Microplastic to zooplankton ratios between areas with high and low rainfall. *Not detected.

	High Rainfall			Low Rainfall		
Ratio MP/Zooplankton	Plume	Bay	Coral Reef	Plume	Bay	Coral Reef
Total Zooplankton	0.04	0.01	0.02	0.01	0.01	< 0.01
Paracalanidae	0.05	0.01	0.02	0.03	0.02	0.03
Acartidae	0.05	0.02	0.05	0.01	0.02	0.11
Pseudodiaptomidae	0.17	*	2.79	0.01	1.85	0.66
Longipedidae	0.02	< 0.01	0.01	< 0.01	< 0.01	0.01
Pontellidae	1.90	0.34	0.37	0.43	0.19	0.18
Temoridae	0.86	7.47	1.90	0.17	*	3.64
Oithonidae	0.01	< 0.01	0.01	< 0.01	< 0.01	0.01
Corycaeidae	7.77	*	1.42	1.10	1.33	0.77
Euterpinidae	0.22	0.07	0.12	0.16	0.01	0.03
Foraminifera	0.72	*	0.92	0.09	1.84	0.16
Mollusca	0.04	< 0.01	0.01	0.06	0.11	0.03
Cirripedia	0.37	0.93	0.07	0.10	2.97	0.05
Polychaeta	0.82	0.11	0.09	0.15	0.13	0.08

particles/m^3, Frias et al., 2020).

However, it is worth mentioning that studies that evaluate microplastics focus on the use of plankton nets with a mesh of 200 and 300 μm (Collignon et al., 2014; Frias et al., 2014; Ivar do Sul et al., 2014; Kang et al., 2015; Pasquier et al., 2022), the latter mainly with neuston net (surface drag). The lack of standardization of methods (Pasquier et al., 2022), makes comparisons difficult. In addition, some studies report that the abundance of MPs is significantly higher in samples collected with a 64 μm net (as used in the present study) (Figueiredo and Vianna, 2018; Bermúdez and Swarzenski, 2021) and that samplings with a neustonic net underestimate the MPs abundance present in the environment (Andrady, 2011). Samples of MPs performed with different size nets (100, 300, 500 μm) revealed abundances 2.5 to 10 times higher (Lindeque et al., 2020).

Regarding polymeric composition, as plastics collected were environmentally degraded, adequate spectrometric matching is a challenge. Despite this, our results found a greater abundance of PP and PA, differing from most other studies, which found a greater abundance of PP and PE (Hidalgo-Ruz et al., 2012; Aliabad et al., 2019; Fagiano et al., 2022). Other plastic polymers were identified as PU and EVA were also more abundant than PE. This may result from the subsurface sampling and the smaller mesh used in this present study. Since most studies with MPs collect surface water samples (neuston net) and with larger mesh nets (Hidalgo-Ruz et al., 2012). PP and PE tend to be found at the surface, due to their positive buoyancy (Andrady, 2011). However, PA production has increased in recent years (Fernández-González et al., 2021) and PA particles have become important marine sources from fishing lines and nets (Castro et al., 2016). Nevertheless, its increase in the period of high rainfall can indicate that the greatest contribution of this polymer comes from land-based sources, such as household activities (mainly from domestic washing process). Other studies also found PA in great abundance, in a protected area (León et al., 2019) and in a polluted watershed (Yan et al., 2019). PP is one of the most produced types of plastic, widely used in packaging manufacturing. PP and PE represent for almost half of the MPs from Atlantic surface waters (Bergmann et al., 2017). We registered a high diversity of polymers. A study using the same mesh opening in Guanabara Bay identified only PP and PE (Figueiredo and Vianna, 2018). Ten polymers were registered on the west coast of Portugal (Rodrigues et al., 2020) and only 5 were registered on Chabahar Bay, Iran (Aliabad et al., 2019).

4.3. *Microplastics:Zooplankton*

Size, type, abundance and color of MP are relevant physical characteristics to understand the possible effects of these particles on the community of organisms (Rodríguez-Seijo and Pereira, 2017). Prey size is one of the main constraints determining zooplankton feeding (Figueiredo and Vianna, 2018). Studies assessing MP size and abundance generally do not consider size from an ecological perspective (Zhao et al., 2015; Gajšt et al., 2016). Considering the organism's size and defining the size classes of MPs allows the estimation of the MPs zooplankton ratio (Figueiredo and Vianna, 2018). Bermúdez and Swarzenski (2021) proposed ranges of size classes within the category 'microplastics', which can be ingested by certain groups of planktonic organisms, making it possible to investigate these interactions.

More than 70% of the MPs registered in this study belonged to size ranges between 20 and 2000 μm. This range is equivalent to the size of all registered organisms and mainly includes fragments. Microplastics are a potential hazard to marine organisms (Wright et al., 2013). A recent review comparing the effects of MP on different zooplankton groups showed that some groups are more sensitive (such as copepods) and that more tolerant groups may become more abundant in the environment to the detriment of others (Yu et al., 2020). With regard to the effects of MP barnacle larval development, Yu and Chan (2020a) did not identify impacts on barnacle larvae subjected to PS particles. However, when observing the intergenerational impacts of these larvae, there was a significant increase in the offspring larval mortality, among other effects (Yu and Chan, 2020b). Prolonged exposure to MP affects the sustainability of populations, and consequently, the zooplankton community in the long term Yu et al. (2020), Yu and Chan (2020b).

With plastic production increasing and inadequate disposal of plastics in the marine environment, the abundance of plastics could be higher than zooplankton in the future, having serious consequences in higher levels of the food web (e.g., Tanaka and Takada, 2016). Although no reference values for the MPs: Zooplankton ratios have been established yet, we consider ratios greater than or equal to 1 as high when compared to other studies (Frias et al., 2014; Fagiano et al., 2022). This means that zooplanktivorous organisms are more likely to find microplastics similar in size to zooplankton in a given period. High MP concentrations can also affect ingestion by zooplankton. Yu et al. (2021) demonstrated that the intestinal retention time in barnacle larvae is greater with decreasing MP size and that this time also differs according to the environment. Larvae that inhabit coral reefs are more susceptible to impacts per MP (Reichert et al., 2018; Huang et al., 2019; Yu et al., 2021). High ratios indicate higher marine biota vulnerability, mainly for those that inhabit sensitive environments, such as coral reefs.

Most studies that estimate the MPs:zooplankton ratio infer about the bioavailability of MPs in relation to zooplankton. Generally total MPs and zooplankton are considered to estimate the MP:Zooplankton ratio (Cole et al., 2013; Botterell et al., 2019; Lins-Silva et al., 2021). However, not all MP size ranges will be available to certain planktonic groups/species, as there is a size relationship between prey and predator. Therefore, to better understand the potential exposure level we recommend that the organisms be counted and measured so that the MPs:Zooplankton ratio be performed using size ranges. Furthermore, investigating the impact of microplastics on planktivorous organisms is fundamental.

5. Conclusion

The present study is one of the few studies that provides data on the abundance, composition and size of microplastics (MP) in a Marine Protection Area (MPA) influenced by an estuarine plume in the world. Results here confirm the important MP input through the plume in coastal marine environments, potentially affecting MPAs, where the human impact is reduced. We emphasize that food webs are more vulnerable to microplastic contamination when there is an increase in rainfall.

Credit author statement

C.D.M Lima: Conceptualisation, Methodology, Data curation, Formal analysis, Writing-review and editing. **M. Melo Júnior:** Conceptualisation, Writing-review and editing, Supervision. **S.H.L. Schwamborn:** Methodology, Writing-review and editing. **F. Kessler:** Writing-review and editing, Supervision. **L.A. Oliveira:** Writing-review and editing. **B.P. Ferreira:** Resources, Writing-review and editing. **G. Mugrabe:** Formal analysis, Writing-review and editing. **J. Frias:** Writing-review and editing. **S. Neumann-Leitão:** Conceptualisation, Resources, Writing-review and editing, Supervision.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2023.121072.

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