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RELAÇÃO ENTRE MICROPLÁSTICOS E ZOOPLÂNCTON NO ATLÂNTICO SUL TROPICAL

RECIFE,

Outubro de 2023

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Área de concentração: Oceanografia Biológica

Orientador: Profa. Dra. Sigrid Neumann-Leitão
Prof. Dr. Mauro de Melo Júnior

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RELAÇÃO ENTRE MICROPLÁSTICOS E ZOOPLÂNCTON NO ATLÂNTICO SUL TROPICAL

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RESUMO

Microplásticos são o tipo de lixo predominante nos ecossistemas marinhos. O presente trabalho tem o objetivo de estimar a abundância, distribuição, composição e tamanho de microplásticos (MPs) e zooplâncton no Atlântico Equatorial Ocidental (AEO). Para isso, avaliou-se duas regiões: 1) Uma Área de Proteção Ambiental (APA), com influência de pluma estuarina e 2) A quebra da plataforma continental no Nordeste do Brasil. Na APA, a amostragem bimestral foi possível no âmbito do Programa Ecológico de Longa Duração Tamandaré Sustentável (PELD TAMS/PE), em períodos com distintos regimes pluviométricos, através de arrastos com rede de plâncton (64 µm). Na quebra da plataforma, as amostras foram obtidas no âmbito do Cruzeiro Científico SOS MAR, através de arrastos com rede Bongo (64 e 300 µm) em 18 estações oceanográficas. Potenciais microplásticos foram isolados das amostras, a matéria orgânica foi digerida e espectroscopia no Infravermelho por Transformada de Fourier (FT-IR) foi utilizada para identificar os tipos de polímeros. Avaliamos a importância da identificação polimérica, visto que pouco mais de 40% dos plásticos suspeitos eram realmente plásticos. Os resultados demonstram que a abundância de microplástico varia espacialmente e é alta em períodos de chuvas intensas em ambientes costeiros (53,8 ± 89,6 partículas/m³), confirmando a importância dos rios como fonte de microplásticos no oceano. A forma predominante na coluna de água são as fibras e os polímeros polipropileno, poliamida e poliuretano representaram mais de 60% de todos os MPs em regiões costeiras. Enquanto poliésteres (28,16%), polietileno de alta densidade (20,41%), e polipropileno foram mais abundantes na região da quebra da plataforma. Os MPs se sobrepõem em tamanho zooplâncton e estes organismos, além de planctívoros, são mais vulneráveis à exposição em períodos de alta pluviosidade. Também registramos maior vulnerabilidade ao encontro com MP no setor com alta influência da Corrente do Brasil, quando há importante contribuição de polipropileno para a abundância de MPs. Nossos resultados auxiliam no entendimento das reais concentrações de MPs em ambientes marinhos costeiros e oceânicos e que potencialmente são uma ameaça para a teia trófica marinha.

Palavras-chave: Pluma estuarina, quebra da plataforma, plâncton, plástico, FT-IR.

ABSTRACT

Microplastics are the predominant type of marine litter in marine ecosystems. This present work goal is to estimate microplastics (MPs) and zooplankton abundance, distribution, composition and size in the Western Equatorial Atlantic (WEA) and verify the potential impact of MPs for marine food webs in this region. For this, two regions were evaluated: 1) An Environmental Protection Area (APA), with the influence of estuarine plumes and 2) The break of the continental shelf in Northeast Brazil. In APA, bimonthly sampling was possible within the scope of the Sustainable Tamandaré Long-Term Ecological Program (PELD TAMS/PE), in periods with different rainfall regimes, through trawls with a plankton net (64 µm). An area of shelf break, the samples were obtained as part of the SOS MAR Scientific Cruise, through trawls with a Bongo net (64 and 300 µm) at 18 oceanographic stations. Potential microplastics were isolated from the samples, organic matter was digested and Fourier Transform Infrared (FT-IR) spectroscopy was used to identify polymer types. We assessed the importance of polymeric identification, given that just over 40% of suspected plastics were plastics. The results demonstrate that the abundance of microplastic varies spatially and is high during periods of high rainfall in coastal environments (53.8 ± 89.6 particles/m^3), confirming the importance of rivers as a source of microplastics in the ocean. The predominant form in the water column is fibers. The polymers polypropylene, polyamide and polyurethane represented more than 60% of all MPs in coastal regions. While polyesters (28.16%), high-density polyethylene (20.41%), and polypropylene was more abundant in the shelf break area. Planktivorous species are more vulnerable to MP exposure in periods of high rainfall and more than 70% of MPs have a size fraction equivalent to zooplankton (200-2000 µm). The distribution, abundance and composition of suspended microplastics are not related to the boundary currents in the WEA during the low rainfall period. In more oceanic regions, meroplankton organisms have higher encounter rates with MPs when compared to holoplankton. The data generated in this thesis helps to understand the real concentrations of MPs in coastal and oceanic marine environments and which potentially pose a threat to the marine food web.

Key-words: Estuarine plumes, shelf break, plankton, plastics, FT-IR.

SUMÁRIO

1. INTRODUÇÃO GERAL

Apesar de ser uma invenção relativamente recente, o plástico tem se acumulado e se tornado o principal resíduo sólido presente em ecossistemas marinhos (Ivar do Sul et al., 2009; Wesch et al., 2016). Eles são reconhecidamente resistentes e de baixo custo, características que contribuíram fortemente para a ampla fabricação de diversos materiais com esse polímero em sua composição, desde embalagens até materiais utilizados na área médica. Porém, o que antes era uma descoberta extraordinária, tem se tornado um problema ambiental global (Frias e Nash, 2019). Em 2022, o Brasil gerou cerca de 13,7 milhões de toneladas de plástico e é o 4° maior gerador de lixo desse tipo do mundo (ABRELPE, 2022). Das cinco regiões brasileiras, o Nordeste é a segunda que mais gera resíduo sólido urbano (ABRELPE, 2022). No entanto, também é um dos países com a menor taxa de reciclagem, apenas 4%. Mais de 60% dos resíduos coletados têm destinação final em áreas inadequadas, como lixões (ABRELPE, 2022). Dessa forma, grande parte desses resíduos tem o oceano como destino.

Ecossistemas costeiros têm sido mais fortemente afetados por receberem um aporte considerável de lixo, proveniente principalmente de fontes terrestres (Zhang, 2017). Os rios, por exemplo, podem atuar como importantes transportadores de resíduos através das plumas (Araújo e Costa, 2007, Lebreton et al., 2017, Lima et al., 2023), onde o plástico é o material predominante (GESAMP, 2019). O problema da poluição por microplásticos é reconhecido globalmente no objetivo 14 (Vida na água), dentro dos Objetivos de Desenvolvimento Sustentável (ODS) da Organização das Nações Unidas (ONU). No âmbito desse objetivo, a meta 14.1, aborda a prevenção e redução significativa da poluição marinha de todos os tipos, em particular aquela proveniente de atividades terrestres, até 2025. Apesar de apenas um indicador dentro da ODS 14 mencionar o plástico como um problema, pelo menos 12 ODSs são afetadas pela poluição por (micro) plásticos direta ou indiretamente (Walker et al., 2021).

Quando chegam aos ecossistemas marinhos, a dinâmica ambiental oceânica (correntes, vento e a interação com os organismos) é responsável pela fragmentação de plásticos em pequenas partículas denominadas micro (1 µm - 5 mm) e nanoplásticos (<1 µm) (Frias e Nash, 2019), que se distribuem amplamente na coluna de água (Collignon et al., 2012; Frias et al., 2014; Wesch et al., 2016, Wright et al., 2013). Quando plásticos visíveis sofrem esse processo de fragmentação, resultando em partículas menores, os classificamos como microplásticos secundários. Porém, quando esses resíduos são fabricados e entram no oceano nessa escala de tamanho micro, os classificamos como primários (Wright et al., 2013).

Uma ampla gama de tipos de polímeros é registrada nos diversos compartimentos ambientais (água, ar e sedimento) (Trindade et al., 2023). A densidade dos polímeros é responsável pelo tempo em que ele permanece na coluna de água. Características como, leveza, resistência à corrosão e baixas taxas de degradação, permitirão que eles percorram grandes distâncias no oceano (Oluwoye et al., 2023). Vários tipos de MPs são mais densos que a água do mar (ρ > 1,02 g cm-3), e acredita-se que o destino final deles seja o fundo marinho (Woodall et al., 2014). Porém, os tipos mais fabricados no mundo são menos densos que a água do mar e consequentemente permanecem por mais tempo na coluna de água, como polietileno (PE) e polipropileno (PP). Ainda assim, esses plásticos podem afundar mais rapidamente quando incrustados por organismos (biofilme), por exemplo (Andrady, 2011). No entanto, em oceanos oligotróficos, é provável que a taxa de afundamento por formação de biofilme seja menor (Andrady, 2011).

No Brasil as pesquisas se concentram em investigar microplásticos em sedimentos (Castro et al., 2018; Gerolin et al., 2020; Martinelli Filho e Monteiro, 2019; Cannard et al., 2021), enquanto os estudos na coluna de água são mais escassos e têm sido realizados no mundo inteiro predominantemente nas camadas mais superficiais, e com redes de malha de 300 µm (Frias et al., 2020; Garcia et al., 2020; Ivar do Sul et al., 2014; Lima et al., 2014; Olivatto et al., 2019). Destaca-se que a maior parte dos MPs suspensos permanecem mais tempo na camada subsuperficial (Choy et al., 2019), e que a utilização de malhas menores nos fornece mais informações sobre o ambiente (Andrady, 2011; Figueiredo and Vianna, 2018; Lindeque et al., 2020).

A presença de microplásticos no oceano, o torna biodisponível para um grande espectro de organismos, particularmente o zooplâncton (Desforges et al., 2014). Este grupo inclui a grande maioria dos táxons marinhos e organismos de muitas ordens de tamanho, incluindo espécies comercialmente importantes; e desempenham papel fundamental no ecossistema marinho (Turner et al., 2004). Estudos em laboratório, verificaram transferência trófica entre organismos planctônicos de diferentes níveis (Setälä et al., 2014). Registros de ingestão de MPs por peixes pelágicos também evidenciaram transferência trófica, com presença de MPs em 70% das presas (Justino et al. 2023) e no sistema digestivo de mais da metade dos indivíduos que quando jovens tem como recurso alimentar, o zooplâncton (Ferreira et al., 2018). À medida que o plástico e plâncton ocorrem simultaneamente na coluna de água, há maior probabilidade de ingestão de plásticos por planctívoros, principalmente quando os organismos não forem capazes de discernir entre presas naturais e MPs (Bermúdez e Swarzenski, 2021; Frias e Nash, 2019). Esforços têm sido realizados para o aumento de dados com relação à abundância, tamanho e como esses polímeros ocorrem simultaneamente com o zooplâncton em ambientes reais (Collignon et al., 2014; Kang et al., 2015; Lima et al., 2023). Essas informações contribuem para que seja possível a identificação de fontes, percurso, destino e prováveis impactos desse poluente.

1.1. OBJETIVO GERAL

Quantificar a presença de microplásticos e zooplâncton que ocorrem simultaneamente nas águas subsuperficiais do Atlântico Equatorial Ocidental (AEO), para avaliar o risco potencial de encontro.

1.2. OBJETIVOS ESPECÍFICOS

1. Determinar a abundância de MPs suspensos presentes em pluma estuarina e na quebra da plataforma costeira do norte do AEO;
2. Determinar a distribuição e composição simultânea de MPs e zooplâncton espacial e temporalmente;
3. Determinar as faixas de tamanho dos microplásticos;
4. Quantificar os microplásticos nas amostras coletadas com redes de malhas com abertura diferente (64 e 300 m);
5. Estimar a ameaça potencial de MPs na teia trófica marinha;
6. Identificar a composição química dos microplásticos costeiros e oceânicos.

2. HIPÓTESES

H1 – A abundância de partículas de microplásticos em suspensão em áreas costeiras tropicais varia espacialmente e é mais alta próximo a plumas estuarinas (Capítulo I);

H2 - A maior abundância de microplásticos é observada no período de alta pluviosidade (Capítulo I);

H3 – Correntes de contorno influenciam na distribuição e abundância de microplásticos em suspensão (Capítulo II);

H4 – A probabilidade de encontro e ingestão de MP pelo zooplâncton é maior em regiões mais costeiras (Capítulo I e II).

H5 – Os microplásticos presentes na quebra da Plataforma Nordeste no período seco, tem fontes de MPs diferentes das regiões mais costeiras (Capítulo I e II).

3. METODOLOGIA GERAL SOBRE A ANÁLISE DE MICROPLÁSTICOS

O trabalho com microplásticos em laboratório envolve, basicamente, três etapas: i. Controle de contaminação e caracterização, ii. Digestão da matéria orgânica (MO) e iii. Análise polimérica.

Controle de contaminação e caracterização

Para evitar contaminação cruzada, todas as superfícies e materiais de campo e laboratório são limpos com água destilada ou etanol 70%, filtrados. No campo, os recipientes de armazenamento das amostras fixadas em formol (4%) são lavados com água destilada, e a rede de plâncton deve ser bem lavada externamente com água do mar, entre as estações, para evitar contaminação cruzada. Em laboratório, os recipientes de vidro utilizados são imersos em solução de ácido clorídrico (HCl) a 10%, por pelo menos 24 horas (Prata et al., 2021) e itens de metal (pinças e agulhas) são cuidadosamente lavados e inspecionados visualmente sob um estereomicroscópio de qualquer análise. Luvas nitrílicas, jaleco 100% algodão e touca são utilizados durante todo processo de extração de MPs. Para evitar a contaminação atmosférica a exposição das amostras é reduzida ao mínimo. A triagem é realizada em ambiente limpo e papel alumínio deve ser utilizado para cobrir as amostras sempre que necessário. Para levar em conta a possível contaminação atmosférica, um filtro (Papel de Filtro Qualitativo) ou água destilada filtrada são utilizados em uma placa de Petri de vidro aberta, como controle, próximo à amostra durante a análise. Imediatamente após essa etapa, os filtros são inspecionados visualmente sob um estereomicroscópio óptico. As partículas são, então, contadas e o número excluído da análise.

Amostras de plâncton são inspecionadas visualmente em estereomicroscópio óptico e as partículas podem ser categorizadas morfologicamente (Gago et al., 2019) em: (i) fragmento, (ii) fibra, (iii) filamento, (iv) filme e (v) outros tipos - partículas esponjosas e esferas. As partículas plásticas suspeitas foram armazenadas em tubos de vidro (5 ml) com Água Milli-Q.

É importante mencionar que a faixa de tamanho utilizada é >100 µm, devido a limitação visual e tamanho da rede da abertura da rede utilizada. Devido às características distintas das amostras utilizadas em cada sessão, o processo de digestão da matéria orgânica (MO) sobre as partículas suspeitas de MP foi realizado utilizando métodos distintos em cada seção.

Digestão da matéria orgânica

Para a seção I, as amostras foram digeridas seguindo o método adaptado de Lópes-Rosales et al. (2021) que consistem em: i. adição de solução surfactante SDS (dodecilsulfato de sódio) a 2% ao filtro; ii. filtração à vácuo após um período de 24h e adição de uma solução de H_2O_2 a 30% com incubação a 40°C; e iii. Filtração à vácuo após um período de 24h para finalizar o processo de digestão.

Para a seção II, as amostras foram digeridas seguindo uma adaptação de Enders et al. (2017): i. as partículas foram submetidas a solução de hidróxido de potássio (KOH 8%) e hipoclorito de sódio (NaClO). Todas as amostras foram expostas a 40°C por 5h ainda submersas no agente digestor, e em seguida filtradas à vácuo em filtros de microfibra de vidro (GF/F Whatman, 47 mm, profundidade 0,26 mm, tamanho de poro 1,6 µm).

Análise polimérica

Os métodos espectroscópicos vibracionais (FT-IR ou espectroscopia Raman) são os métodos mais comuns para identificação e quantificação de MPs. A espectroscopia infravermelha é baseada na interação da radiação com as vibrações moleculares, fornecendo uma imagem química não destrutiva e sem contato. Esse método possibilita a identificação precisa de partículas de polímero plástico com base nos espectros característicos de impressão digital de vibrações moleculares e caracterização com base na estrutura química polimérica, por comparação com espectros de referência conhecidos (Xu et al., 2019).

As partículas de MPs na Seção I foram analisadas em Espectrofotômetro Infravermelho por transformada de Fourier (FT-IR) convencional, modelo Shimadzu Prestige 21, com módulo de refletância difusa. As medições foram realizadas com uma faixa de número de onda de 400 a 4000 cm^{-1}, e realizando 24 varreduras por partícula. Cada espectro foi plotado usando o software Originlab e comparado com um banco de dados de referência de polímeros (Silverstein et al., 2007; Jung et al., 2018).

Para análise polimérica da Seção II, as amostras com polímeros foram analisadas pelo Centro Nacional de Pesquisa em Energia e Materiais (CNPEM), no Laboratório Nacional de Luz Síncrotron (LNLS). As partículas precisaram ser transferidas do filtro GF/F para substrato de ouro (Au). A identificação do polímero foi realizada em pelo menos 50% das partículas em cada filtro (n = 320, rede 64 µm; n = 201, rede 300 µm) sob um microscópio Agilent Technologies Cary 620 FT-IR usando uma fonte Globar, com um detector MCT (telureto de mercúrio e cádmio) detector. As medições foram realizadas no modo de refletância com uma faixa de número de onda de 400 a 4000 cm^{-1}, e realizando 256 varreduras por partícula e resolução espectral de 8 $cm-^{1}$. O tamanho do pixel no detector de matriz foi de 25 µm e um espectro de fundo foi adquirido, usando os mesmos parâmetros, antes da varredura de amostras individuais.

Para identificação dos polímeros foi utilizada uma ferramenta com espectros de referência de acesso aberto (OPEN SPECY). Nessa ferramenta é medido o grau de correlação entre o espectro de referência e o da amostra através do coeficiente de correlação de Pearson (r). Para melhor precisão dos resultados, só foram considerados coeficientes superiores a 0,6. Partículas com espectros com menos de 60% de correspondência foram classificadas como ‘naturais’ (por exemplo, quitina, celulose).

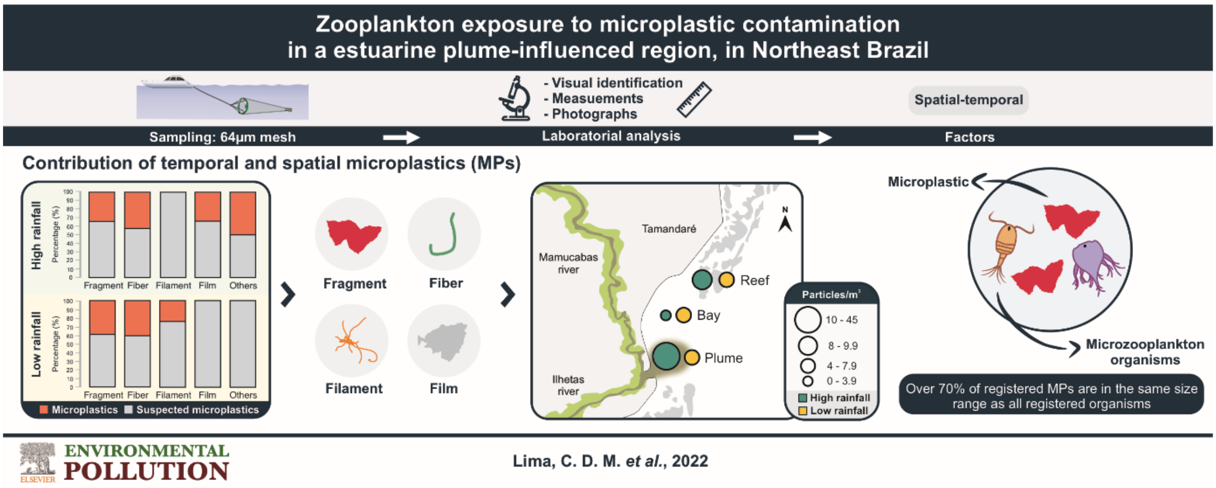
4. RESULTADOS

A tese está dividida em duas seções no formato de artigo científico. A seção I contém informações sobre microplásticos e zooplâncton em uma área costeira protegida (APA Costa dos Corais), influenciada por pluma estuarina. As amostras utilizadas nesta seção foram obtidas através do *Programa de Pesquisas Ecológicas de Longa Duração Tamandaré Sustentável (PELD/Tams)* com amostragens desde a desembocadura de dois rios (Mamucabas e Ilhetas) até os recifes de coral em uma Área de Proteção Ambiental (APA). Os resultados desta seção foram publicados na revista ‘Environmental Pollution’ no início do ano de 2023 (Lima et al., 2023).

A seção II contém informações sobre microplásticos e zooplâncton considerando uma amostragem espacial de mesoescala na costa leste do Brasil, desde Natal (RN) até Salvador (BA), no âmbito do projeto *SOS MAR*.

4.1. SEÇÃO I

ZOOPLANKTON EXPOSURE TO MICROPLASTIC CONTAMINATION IN A ESTUARINE PLUME-INFLUENCED REGION, IN NORTHEAST BRAZIL



REVISTA

Environmental Pollution

SITUAÇÃO

Publicado

Highlights

- Microplastic input in coastal is strongly influenced by the river plume.
- More than 40% of the suspected plastic identified as 'fibers' were validated.
- Not all brightly colored particles can be confirmed as plastic polymers.
- Over 70% of the MP are in the size fraction equivalent to the zooplankton spectra.
- Planktivorous species are more vulnerable to exposure by MP in the high rainfall.

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 2,079 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 2,000 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.

Keywords: Marine Protected Area, marine pollution, rivers, coral reef, zooplankton, Global South.

1. Introduction

Marine debris 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 however it 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). 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 (08º 45’36” and 08º 47' 20” S, 35º 03 '45 " and 35º 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-Lolaya 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 minutes at a speed of 1-2 knots. Samples were fixed in 4% neutral formalin for quantitative and qualitative analysis of microplastics and zooplankton.

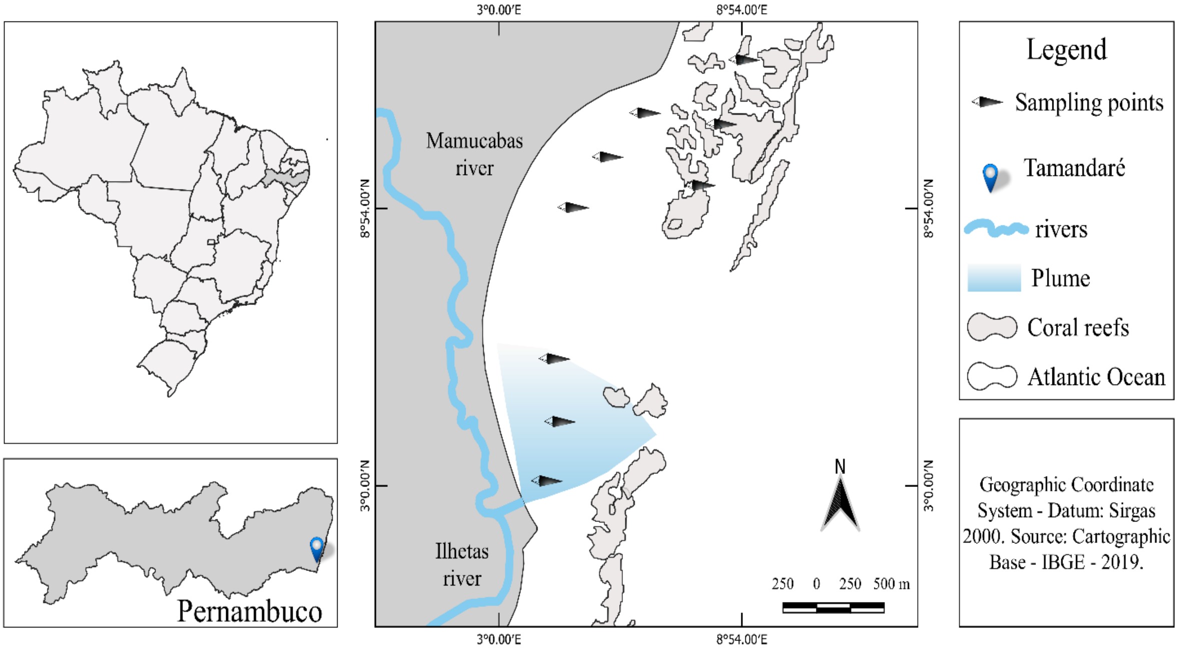


Fig.1: Study area and sampling stations in the coastal region of Tamandaré, Brazil. For each station, three plankton hauls were carried out (represented on the map by sampling stations).

2.1. 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 hours (Prata et al., 2021). 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 was 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 stereo microscope. 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 stereo microscope being the particles morphologically categorized, following Gago et al. (2019). The following 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 suspected MP particles was digested following a modified Lópes-Rosales et al. (2021) 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 24h, 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 hours, the samples incubated at 40ºC, 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.

2.2. FT-IR analysis

The particles were analyzed under a Shimadzu Prestige 21 Fourier Transform Infrared spectrophotometer, with a diffuse reflectance module. Measurements were carried out with a wavenumber 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'.

2.3. 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., Boltovoskoy, 1999). Taxon abundance was expressed as individuals/m³, 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).

2.4. Data analysis

All analyzes were conducted based on abundance, expressed in individuals or particles/m³. 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/m³) and zooplankton (ind./m³). 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 ºC in high rainfall and 29.2 ± 0.13 ºC 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 2,079 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; considerable contribution was from fibers common items in all areas (Table 1).

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).

3.3. Microplastics and Chemical composition

Validation of plastic particles varied with types (Fig.2). During high rainfall~~,~~ 42.4% of the fibers, 33.5% of the fragments, 33.3% of films and 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 as plastic (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 ± 3.5particles/m^3) (Table 1).

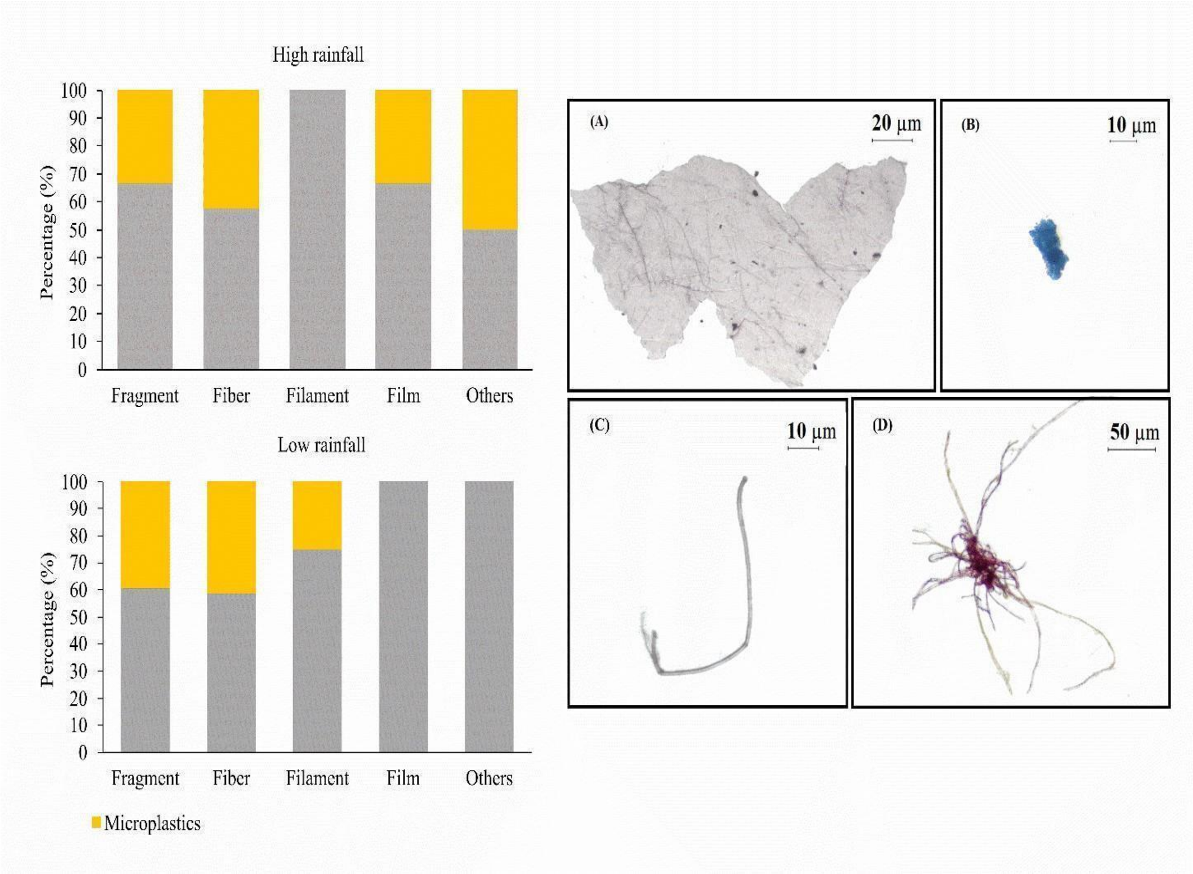


Fig.2: Percentage (%) of microplastics (identified in yellow in the plot) after peroxide digestion and examples of types and colors of microplastics registered in Tamandaré, Brazil. (A) Transparent fragment, (B) Blue fragment, (C) Transparent fiber, (D) Red filaments.

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 (PERMANOVA, 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.

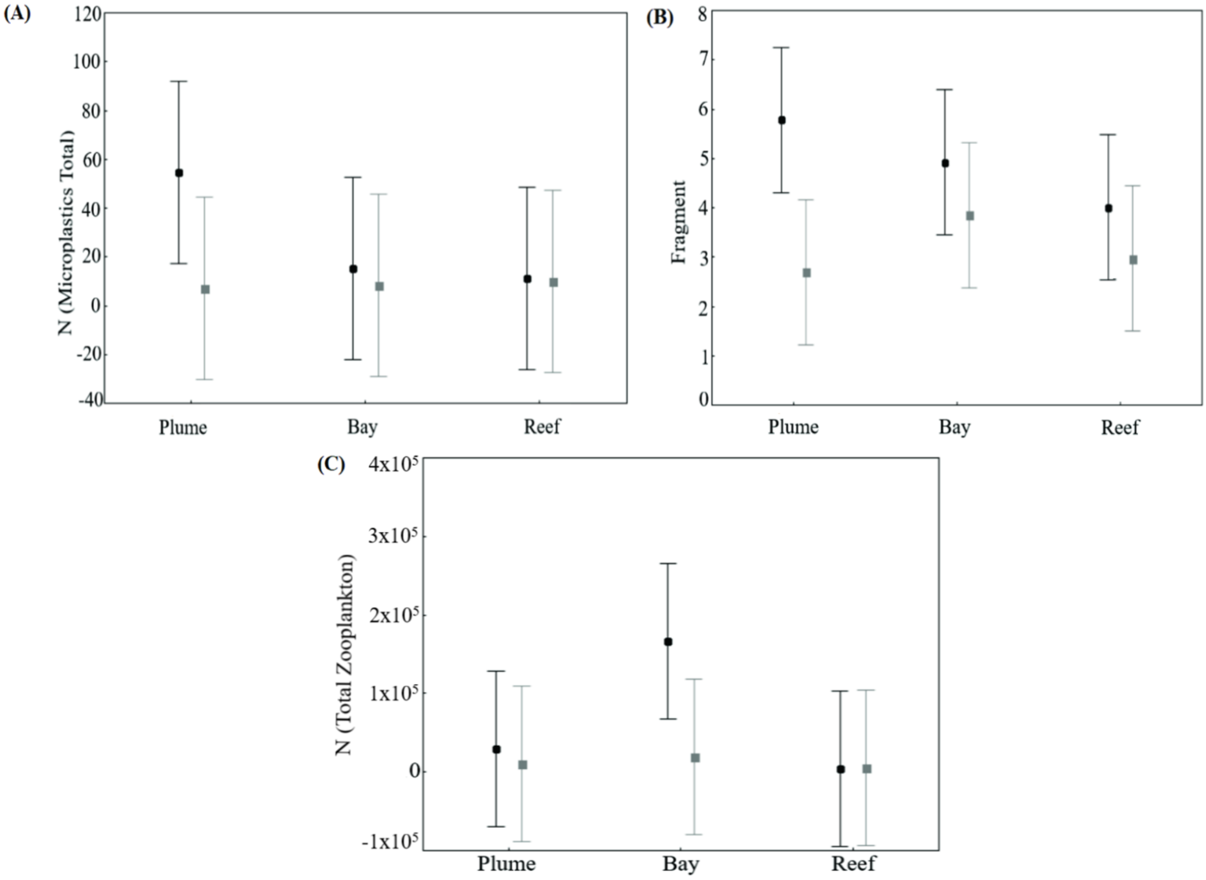


Fig.3. ANOVA results for total microplastics (A), fragment type (B) and microplastic: total zooplankton (C). Black points = high rainfall, gray points = low rainfall; error bars = standard deviation (A, B, C).

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./m³ and 10,919.6 ± 11,635.9 ind./m³, 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 average total abundance was 26,157.5 ± 10,2391.1 and 918.1 ± 1475 ind./m³ in high and low rainfall, respectively, with a greater contribution of Mollusc larvae and Foraminifera.

For the total zooplankton, PERMANOVA indicated a statistically 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).

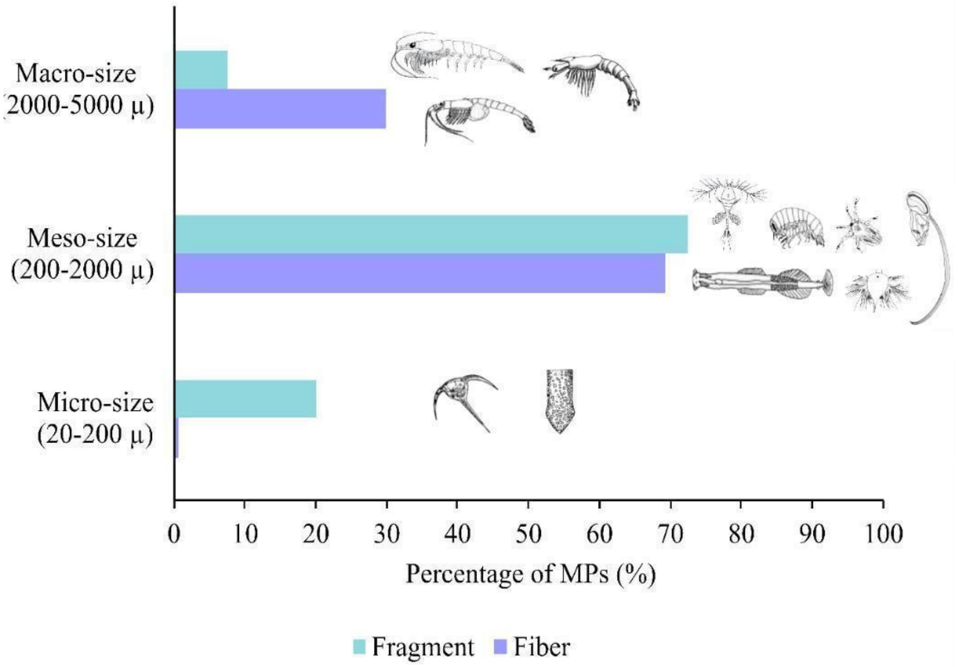


Fig.4: Percentage in size intervals of the most abundant suspended microplastics registered in the study area, with a representation of zooplankton organisms with an equivalent size.

Size classes were divided from Frias and Nash, 2019. An adaptation suggested by Bermúdez and Swarzenski (2021) considers ranges for micro- (20–200 µm), meso- (200–2,000 µm) and macroplastics (>2,000 µm). In this present 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 (2,000–5,000 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).

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

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 25-50%). Validation through spectrometric techniques is 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 accidentally or actively ingested by zooplankton (He et al., 2021). 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., 2021) or other organisms.

4.2. Suspected plastic particles and Microplastics

MPs were registered in 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-Lolaya 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 higher 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-Lolaya 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/m³, Ivar do Sul et al., 2014), Western Equatorial Atlantic (120 µm, 0.14 ± 0.11 particles/m³; 300µm, 0.02 ± 0.01 particles/m³; Garcia et al., 2019), Atlantic Ocean (250 µm,1.15 ± 1.45 particles/m³, Kanhai et al., 2017), Northeast Atlantic Ocean (250 µm, 2.46 ± 2.43 particles/m³, Lusher et al., 2014), Brazilian estuaries (300 µm, 0.26 particles/m³, Lima et al., 2014) and European coastal environments (300 µm, 0.45 ± 0.52 particles/m³, Rodrigues et al., 2020; 0.56 ± 0.33 particles/m³, 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 can 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 use size classes without do not consider size forming 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 us to make an 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 present study belonged to size ranges between 20 and 2,000 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 can 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). In this way, 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 Tanaka, 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; Fagianno 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 (Yu et al, 2021). In this way, 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, Boterell 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 it is necessary that the organisms be counted and measured and so that the MPs: Zooplankton ratio be performed using size ranges. Furthermore, investigating the impact of microplastics such microplastics on planktivory organism’s populations of 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 vulnerable to microplastic contamination when there is an increase in rainfall.

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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.

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Supplementary Data

S1. Material and Methods

2.6. Data analysis

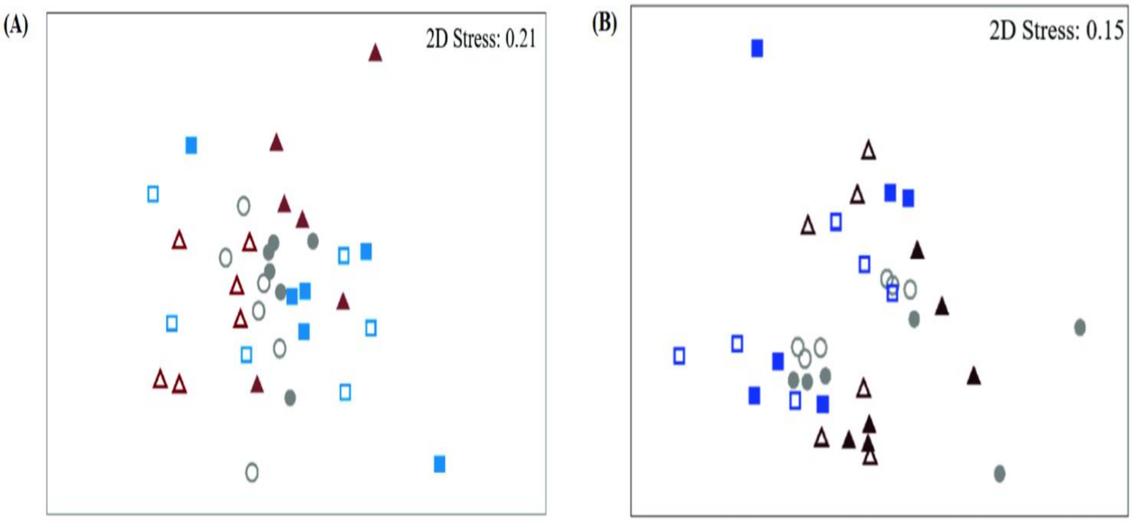

Fig. S1: Multidimensional scaling (nMDS) ordination based on Bray-Curtis dissimilarity matrix for the composition of microplastic registered in the study area (S = 0.21) and the zooplankton community registered in the study area (S = 0.15). Shapes: triangle =plume, circle = bay, square = coral reef. Full shapes = high rainfall, empty shapes = low rainfall (A, B).

S2. Results

3.1. Environmental variables

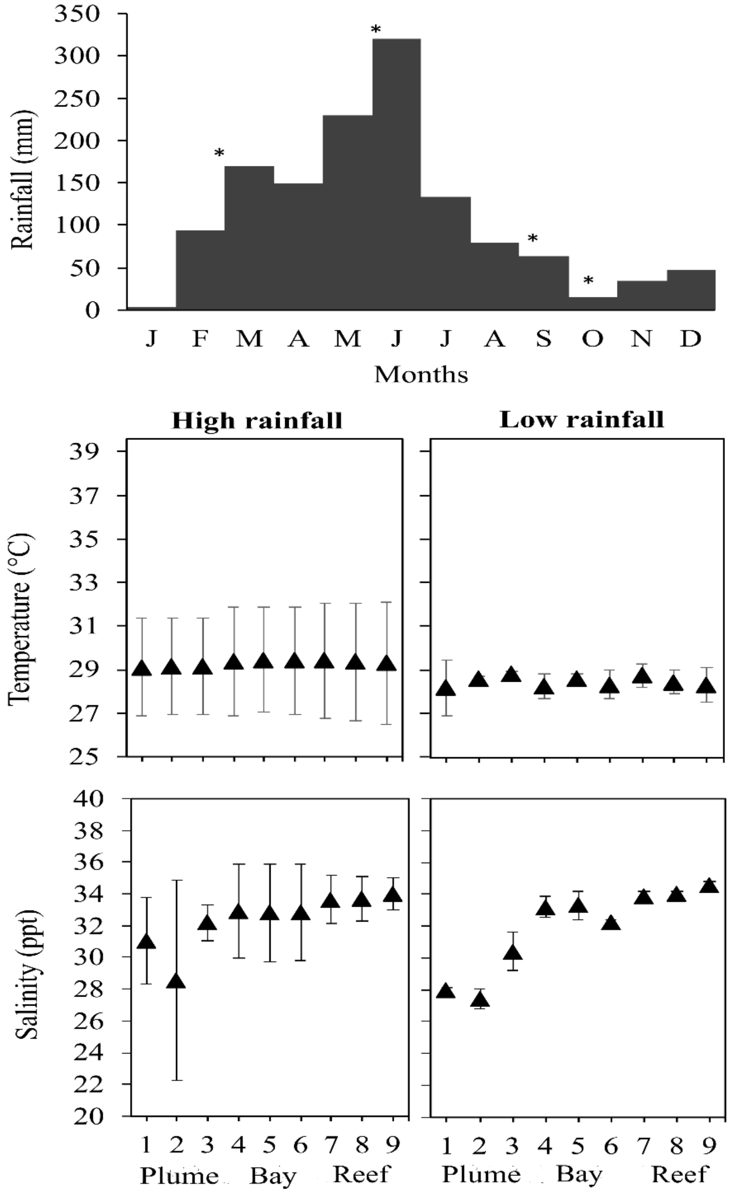

Fig.S2: Spatial and temporal variation of surface water temperature and salinity at Tamandaré region. (*) indicates the months in which the collections were performed, and error bars = standard deviation.

4.2. SEÇÃO II

ABUNDANT PLANKTON-SIZED MICROPLASTIC PARTICLES IN TO THE WESTERN EQUATORIAL ATLANTIC

Highlights

- There are hotspots of microplastics spatially and has no clear relationship with boundary currents.
- The majority of microplastics detected in the continental shelf break were fibers (95%).
- Polyester, high-density polyethylene and polypropylene dominate subsurface seawater in the Western Equatorial Atlantic.
- Zooplankton are more susceptible to the encounter with MP in sector with high influence of the Brazilian current.

Abstract

Floating microplastics are important pollutants in marine environments, mainly because they are similar in size to zooplankton and can enter food chains by trophic transfer or accidental ingestion. This study investigated the relation of the zooplankton and microplastic abundance, distribution, composition and size in the continental shelf break Western Equatorial Atlantic (WEA). Microplastics were sampled from sub-surface waters using Bongo net hauls (64 and 300µm mesh size). Potential microplastics were isolated from samples, the organic matter was digested and FT-IR spectroscopy was used to identify polymer types. We registered a total abundance average 2-fold higher in the samples with the 64µm net (5.05 ± 3.73 particles/m^3), when compared with the 300µm net (2.09 ± 2.77 particles/m^3). Of the particles analyzed, fibers (95%) are the dominant type of MPs. The majority of microplastics were identified as polyesters (28,16%), high density polyethylene (20.41%) and polypropylene (17.31%). Zooplankton are more susceptible to the encounter with MP in sector with high influence of the Brazilian current, when there is an important contribution of PP to the abundance of MPs.This study provides important information about the distribution of MPs in the Atlantic Ocean. It is also an important basis for future assessments of the potential changing trend of MP abundance over time in the oceans.

Key-words: Food web, zooplankton, marine Pollution, Global South.

1. Introduction

Global plastic production has increased exponentially and more than 400 million tons of plastic were production in 2022 (Plastics Europe, 2023), which equals 2 million individuals blue whales, the largest animal in the world. The problem of plastic pollution is recognized globally in objective 14 (Life in water), within the United Nations (UN) Sustainable Development Goals (SDGs) (Walker et al., 2021). Brazil is the 4th largest producer of plastic waste in the world, and one of the countries with the lowest recycling rate, just 4% (ABRELPE, 2022). More than 60% of waste collected in the country is disposed of in inappropriate areas, the ocean is the main destination for much of this waste (ABRELPE, 2022). Therefore, coastal ecosystems have been more strongly affected by litter coming mainly from terrestrial sources (Zhang, 2017). When arriving marine ecosystems, oceanic environmental dynamics, such as currents, wind and interaction with organisms; is responsible for the fragmentation of plastics into small particles called microplastics (1 µm - 5 mm) (Frias and Nash, 2019), which are widely distributed in the water column (Collignon et al., 2012; Frias et al., 2014; Wesch et al., 2016, Wright et al., 2013).

The northeastern continental shelf is characterized as narrow, influenced by western boundary currents and by its oligotrophic waters (Ekau and Knoppers, 1999). The continental shelf-break is a marine ecotone, that is, an area of transition between coastal zones and the open ocean that is home to biological communities from these two environments (Olavo et al., 2011). As it is a transitional environment, the temporal and spatial distribution patterns of MPs in this environment can be associated with transport by hydrodynamic factors (e.g. wind, waves, currents) and terrestrial sources (e.g., tourism, sewage treatment plants, river discharge) (Araújo and Costa, 2007, Lebreton et al., 2017, Lima et al., 2023), marine (marine aquaculture, shipping, oil drilling) and by atmospheric dust (Zhang, 2017; GESAMP, 2019). Boundary currents along continental shelves are important in the transport and distribution of MPs from coastal areas to the open oceans, especially those that remain floating for longer in the water column (Thiel and Gutow, 2005).

Information about the abundance, distribution and composition of microplastics gives us insight into the extent of the plastic pollution problem in the global ocean. Because they are similar in size to zooplankton, trophic link between primary producers (phytoplankton) and higher trophic level organisms, microplastics have been extensively documented as a threat to marine ecosystems (Amin et al., 2020; Botterell et al., 2019; Lima et al., 2023). Despite the enormous importance, little effort has been dedicated to the study of plankton and MPs together.

The effects directly and indirectly caused by these residues have been studied for marine organisms in the laboratory (Cole et al., 2013; Costa et al., 2020). However, data on what environmentally relevant concentrations are necessary to understand real risks to which organisms are exposed. The present study aims to quantify how microplastics and zooplankton co-occur at larger spatial scales, in the shelf break in the Western Equatorial Atlantic (WEA). For this, we verified (1) the distribution, abundance and composition of microplastics in suspension in two sectors, with influence to the boundary currents in the WEA; (2) we estimated the probability of encountering and ingesting MP by zooplankton; and (3) we identified the types of plastic polymers present in the shelf break.

2. Methods and Materials

2.1. Study area

The ocean Western Equatorial Atlantic (WEA) is characterized by oligotrophic, nutrient-poor waters, and has width shelf that various along the coast, with average width of 50 km and the shelf break in depth from 40 to 80 m, (Leão and Domingues, 2000). The transatlantic South Equatorial Current (SEC) is a broad west-flowing current that extends from the surface to a depth of 100 m. Approximately 12 Sv from SEC flows northward to continue as the North Brazil Current (NBC), around 10° S. Only 4 Sv forms the southward flowing Brazil Current (BC) (Stramma et al., 1990, Ekau and Knoppers, 1999). The study area covers the continental shelf break to an average depth of approximately 52 m, off the eastern Brazilian coast between the parallels 5°S and 13°S. Therefore, we divided the sampling area into two sectors, according to the main eastern boundary currents of the South Atlantic: NBC (approximately 5°42 'S to -10°07' S) and BC (approximately 10° 24'S to 13°04' S). The shelf break is under the influence of the tropical water mass (TW) and by vertical mixing events between TW and the south Atlantic central water (SACW) (Castro and Miranda, 1998).

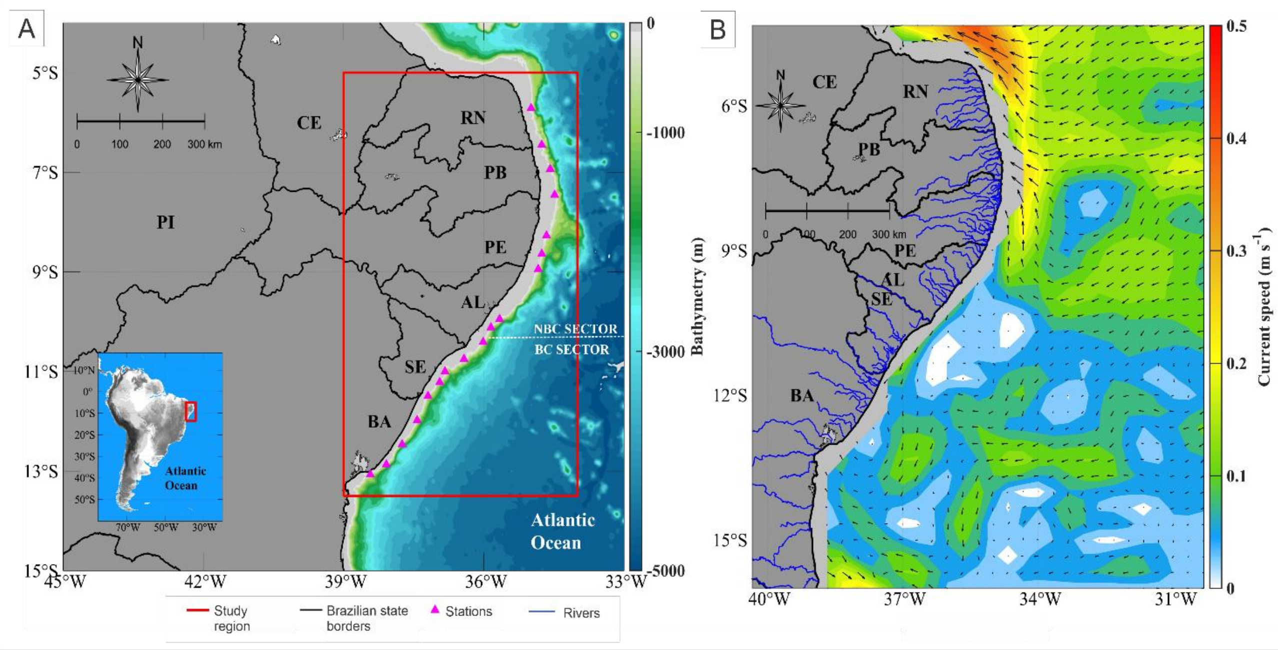

Fig. 1. Study area in the Western Equatorial Atlantic (WEA), with sampling stations in the latitudinal gradient (A) and average surface current registered in the sampling period (B).

2.2. Sampling and Datasets

The samples were obtained as part of the Scientific Cruise SOS MAR carried out by the Hydroceanographic Research Ship Vital de Oliveira (H-39), on the shelf-break of the Brazilian coast, from Rio Grande do Norte (34˚W, 5˚S) to Bahia (39˚W, 13.5˚S), during 2019 (November-December), in the period of low rainfall for this region. Over NE-Brazil, fall-winter rainfall occurs from March to August and the dry season is from September to February, during which the monthly rainfall is less than 100 mm. Simultaneous hauls were carried out with Bongo nets (64 and 300 µm) for 10 minutes (speed of 2 knots), along 18 sampling stations (9 for the BC sector and 9 for the BNC sector), totaling 36 samples. A Hydro-bios flowmeter coupled to the mouth of each net was used and the samples were preserved in 4% saline formalin. Throughout the Ship's route, measurements of physical variables were obtained using the conductivity-temperature-depth (CTD) and chlorophyll-a from 10 m depth to close to the sediment.

The Ocean Surface Currents Analyses Real-time (OSCAR) project computes near-surface ocean currents using quasi-linear and steady flow momentum equations. The horizontal velocity is derived from sea surface height, surface vector wind, and sea surface temperature data collected from satellites and in situ instruments. The model combines geostrophic, Ekman, and Stommel shear dynamics, along with a complementary term from the surface buoyancy gradient. OSCAR offers global 1/3-degree grid data with a 5-day resolution dating from 1992 to the present day. The project is generated by Earth Space Research (ESR) and more information can be found at their website https://www.esr.org/research/oscar/oscar-surface-currents/ (Bonjean and Lagerloef, 2002).

2.3. Quality control

During sampling, the net remained at a distance of more than 4 m from the ship; and after recovery at each sampling event, it was carefully rinsed with sea water from the outside. The sample storage Eppendorf was pre-washed and the samples were stored with distilled water. All distilled water used was previously vacuum filtered. In the laboratory, to avoid cross contamination, all surfaces and materials were thoroughly cleaned with distilled water or 70% ethanol, filtered by vacuum pump. Samples were visually inspected under stereomicroscope (Zeiss) and microscope when necessary. In the laboratory, the used glass containers were immersed in a 10% hydrochloric acid solution (HCl) for at least 24 h (Prata et al., 2021).

Similarly, all 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 was kept to a minimum, using pre-washed aluminum foil to cover them. To account for possible airborne contamination, distilled water filtered by vacuum pump was used in an open glass Petri dish as a control, close to the sample during analysis. Immediately after, Petri dishes were visually inspected under an optical stereo microscope. More than 90% of the airborne contamination were fibers, mainly transparent (69.6%). Blank controls (n = 3) were used to quantify contamination of samples during processing, with the average number of contaminants subtracted from all stations' final counts.

2.4. Laboratory Analysis

Plankton samples were visually inspected under an optical stereo microscope and classified according to Gago et al. (2019). The MP types considered were (i) fragment, (ii) fiber, (iii) other types - spongy particles and spheres. The suspected plastic particles were stored in Eppendorfs (5 ml) with distilled water. For zooplankton, the 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), using specialized literature (e.g., Boltovskoy, 1999).

Due to an excess of organic or inorganic particles that hampers MPs analysis, the suspecteds MP particles was digested following an adaptation of Enders et al. (2017) protocol: The suspected plastic particles were subjected to potassium hydroxide (KOH 8%) and sodium hypochlorite (NaClO) solution e maintained at 40°C for 5h. Then vacuum filtered in glass microfiber filters (GF/F Whatman, 47 mm, depth 0.26 mm, pore size 1.6 µm). Length and width/diameter measurements (µm) were used to categorize the MP size range (optical microscope Nikon Eclipse LV100ND) of all MPs registered in the study area. Particles <100 µm were underestimated due to the mesh opening and the visual limit, but were included in the analyses.

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. We considered ranges of: The size classes used here are adapted from Bermúdez and Swarzenski (2021), considering an ecological perspective between zooplankton and MPs (Table 1): 20–200 (Class 1), 200–2000 (Class 2) and 2000–5000 µm (Class 3).

2.5. µ-FTIR analysis

The microscope optic was used for particles transfer to gold (Au) substrate. Polymer identification was carried out on at least 50% of the particles in each filter (n=320, 64µm net; n=201, 300µm net) under a microscope Agilent Technologies Cary 620 FT-IR used the Globar source, with an MCT (mercury cadmium-telluride) detector. Was used in reflectance mode, using 256 scans per sample, with a wavenumber range of 4000–400 cm-^{1}, and spectral resolution of 8 cm-^{1}. The pixel size on the array detector was 25 µm and a background spectrum was acquired, using the same parameters, prior to scanning individual samples. Between 50% and 100% of the particles present in the filters were measured.

To identify the polymers, a tool with open access reference spectra (OPEN SPECY) was used. In this tool, the degree of correlation between the reference spectrum and that of the sample is measured using the Pearson correlation coefficient (r). For better precision of the results, matches with > 70% similarity were accepted while those with 55–69% were individually analyzed to observe whether there were corresponding peaks with standard polymers. Particles with spectra less than 60% and when analyzed individually had not matched were classified as ‘natural’ (e.g. chitin, cellulose or minerals). Open Specy’s data sharing and session log features ensure reproducible results.

2.5. Data analysis

All analyzes were conducted based on abundance, expressed in particles/m^3 to MPs and (ind/m^3) to zooplankton. The original data were Box-Cox transformed to verify normality (Kolmogorov-Smirnov test) and homogeneity of variances (Levene test). MP and zooplankton abundance were log (x+1) transformed after considering its non-normal distribution. PERMANOVA was used to compare MP size classes between sectors. When differences were statistically significant, pairwise comparisons were performed. All analyzes were conducted using Statistical software R v4.3.1 (R Core Team, 2021). To estimate the potential threat by microplastics for marine food webs, we calculated the MPs: Zooplankton ratio (Botterell et al., 2019), defined as the ratio between the number of MPs and the number of individuals per volume of water. The Spearman correlation test was applied to test the correlation between the total abundance of MPs and zooplankton and between MPs and environmental variables.

3. Results

3.1. Oceanographic conditions

The hydro oceanographic conditions are practically homogeneous between stations during the sampling period (low rainfall), with the presence of Tropical Water (AT) in the upper layers and Central Water of the South Atlantic (ACAS) permanently below. The temperature (°C) and salinity (PSU) is similar between NBC (average 26.9 and 36.8) and BC (average 26.3 and 37.1) sectors, respectively. The average to Chlorophyll-a (mg/m^3) and turbidity (NTU) were also similar between stations and NBC (average 0.12 e 0.23) and BC (average 0.14 e 0.24) sectors. We verified that there is no significant correlation between environmental variables (temperature, salinity, chlorophyll and turbidity) and Microplastics abundance.

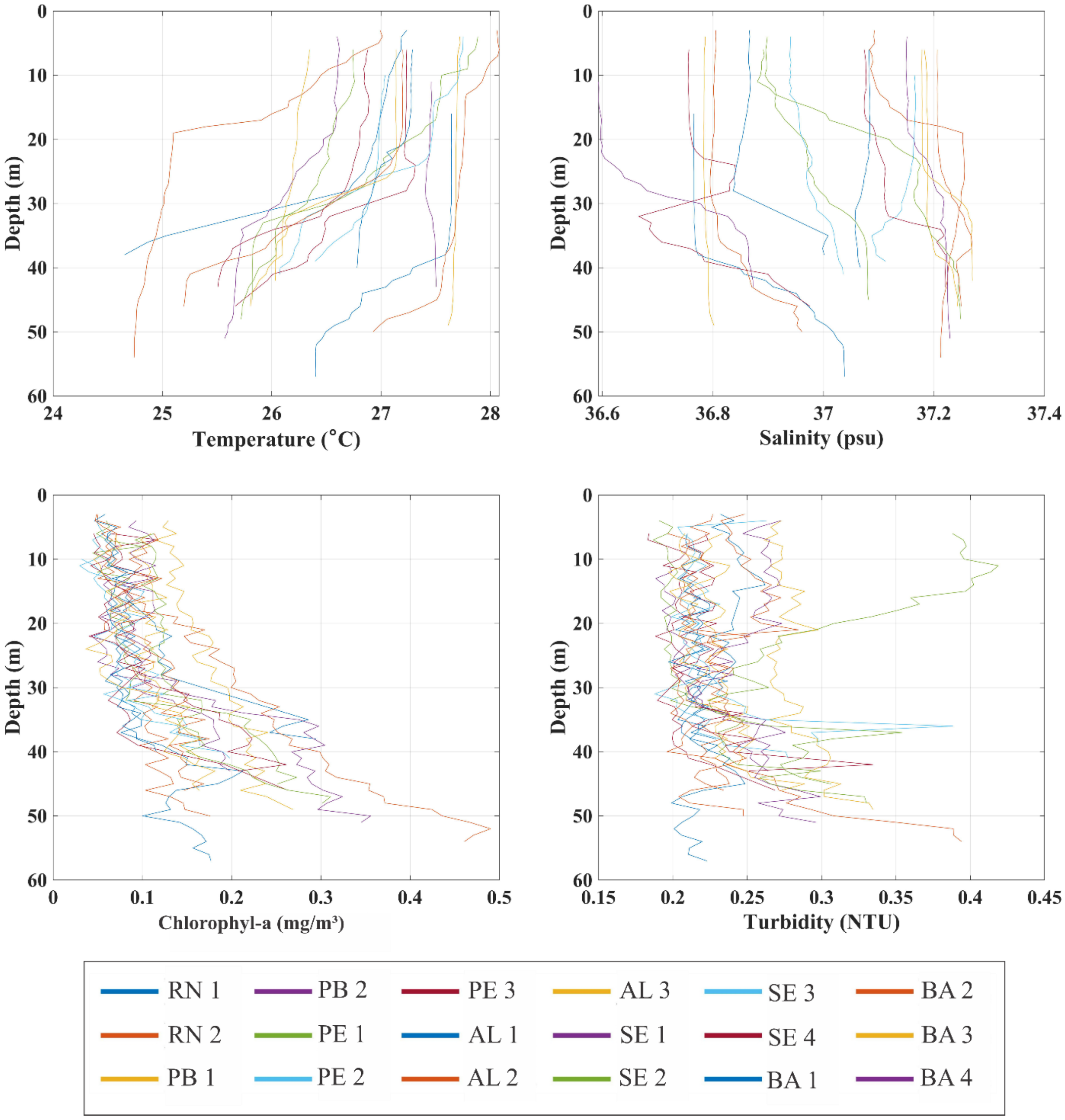

Fig.2: Environmental variables in the water (surface until 50 meters) in the Western Equatorial Atlantic (WEA). Rio Grande do Norte – RN, Paraíba – PB, Pernambuco – PE, Alagoas – AL, Sergipe – SE, Bahia – BA.

3.2. Microplastics in seawater

Were registered in all samples, post-digestion of organic matter, a total of 815 particles. The dominant type was fibers (95% of total particles). Plankton net pore size is a significant factor (Mann-Whitney; p-value < 0.05; Fig3C) for MPs abundance. Overall, was registered a total abundance average 2-fold higher in the samples with the 64 µm net (5.05 ± 3.73 particles/m^3), when we compare with the 300 µm size net (2.09 ± 2.77 particles/m^3) (Mann-Whitney - p-value: < 0.05; Fig3C). However, the abundance of MPs was much higher at most stations. At station 3, for example, they were registered 100-fold higher abundance with the 64 µm size net (Fig.3). For spatial distribution of microplastics, no trend nor significant difference in the abundance of MPs between sectors was recorded (Mann-Whitney, p-value: 0.05, Fig.3A, B). However, some MPs hotspots were registered, such in station 1, NBC sector (19.75 particles/m^3, Table 1).

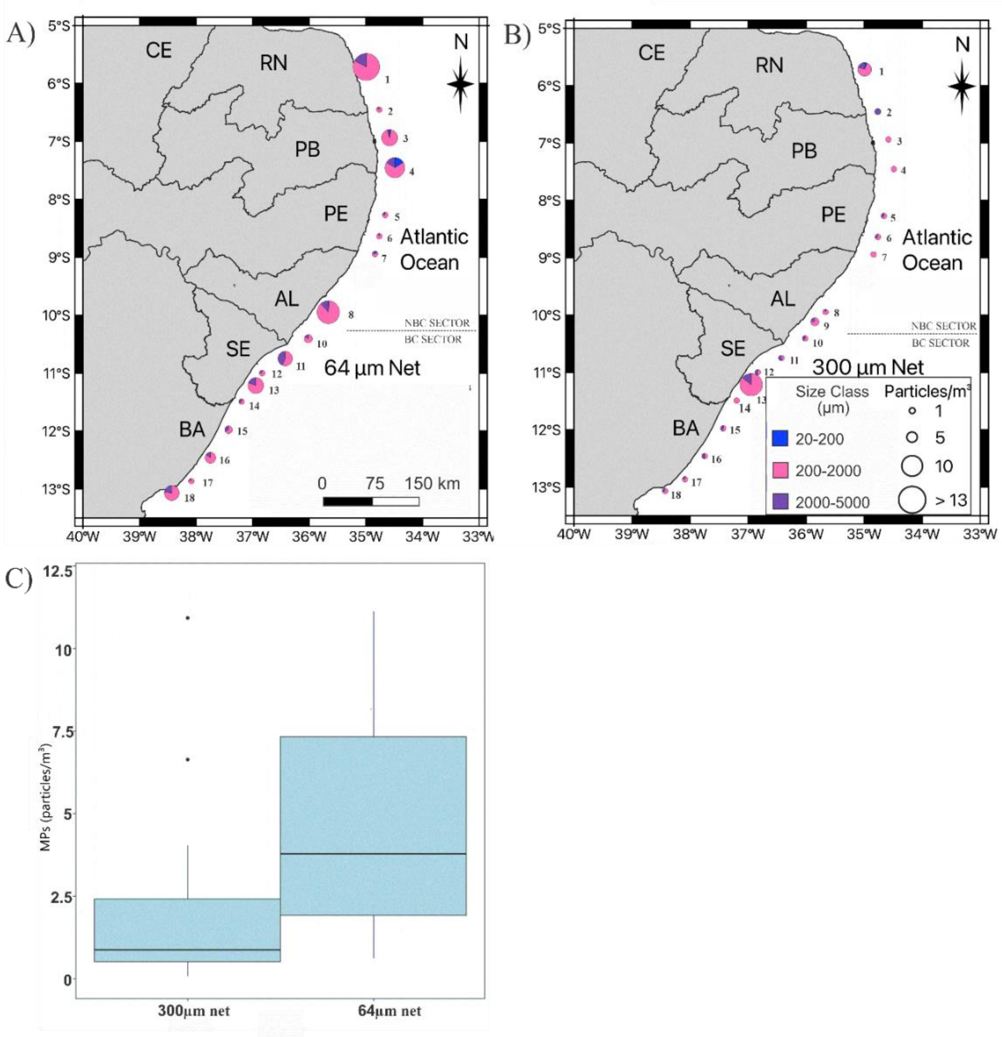

Fig.3: Microplastics abundance (diameter circle - particles/m³) of size classes (colors) in two mesh size nets (A, B) from Western Equatorial Atlantic (WEA). Boxplot graphic abundance in two mesh nets (C). **Note**: It is not possible to see station 9 (64 µm net, AL), as the previous station (8) overlaps it, due to the abundance of MPs (A).

Digestion protocols are needed to determine microplastics abundance and characteristics obtained in environmental matrices. We observed that the solution used in this study changed the color of the MPs and therefore we did not consider this information. Fourier transform infrared spectroscopy (FTIR) is a powerful tool in the field of material characterization and a non-destructive technique. Our small particles require the use of micro-FTIR (µ-FTIR) that allows simultaneous visualization, mapping, and collection of spectra. Filter mapping reduces FTIR operation time, but it is still a time-consuming technique. The scanning time for each particle lasted an average of 1 minute and 4 seconds. However, it was often necessary to perform more than two scans on each particle, due to irregular surfaces, especially on fibers.

More than 60% of the particles for both networks were analyzed in μ-FTIR. Fifteen polymer types of floating MPs were registered in the study area: Polypropylene (PP), high density polyethylene (HDPE), Low-density polyethylene (LDPE), polystyrene (PS), acrylonitrile butadiene styrene (ABS), polyethylene terephthalate (PET), polyester (PEST), polyethersulfone (PES) polyvinyl chloride (PVC), Polyvinyl chloride acetate (PVCA), ethylene-vinyl alcohol (EVOH), ethylene-vinyl acetate (EVA), polyamide (PA), epoxy resin and paint. Polymers identified as 'paint' probably come from the fragmentation of paint used on boats and are characterized by being a mixture of polymers. However, only five polymers represented more than 80% of the fibers in the study area: PEST (28, 16%) and HDPE (20.41%) are the most abundant types of plastic polluting this region, followed by PP (17.31%) and Paint (10.33%) (Fig.4). And two types represented over 60% of the fragments: HDPE (36.36%) and EVOH (27.27%). In relation to the stations, we observed a high abundance of PE and PEST in practically all of them. However, there is an important contribution of PP to the abundance of MPs in stations related to the BC sector.

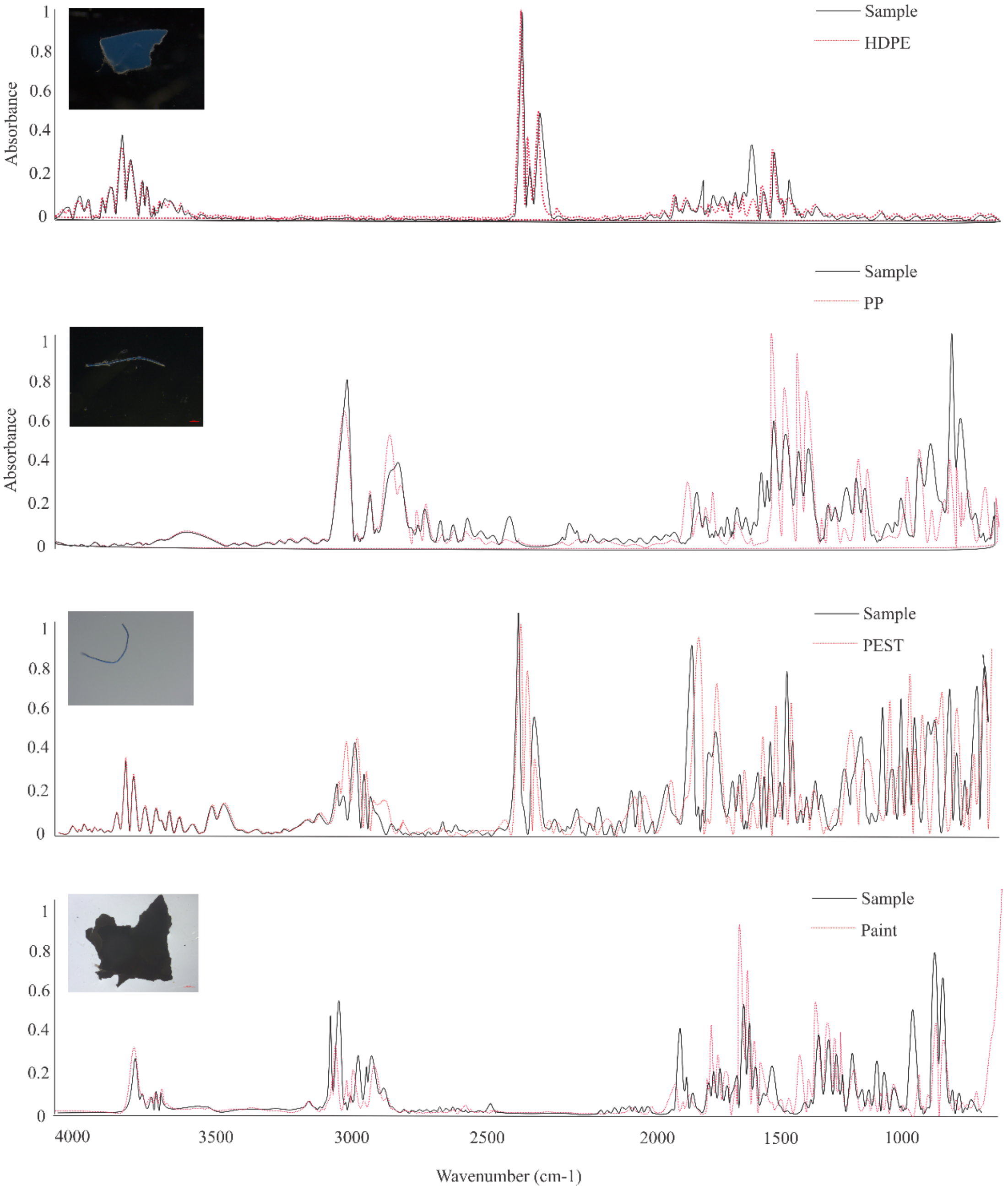

Fig.4: FTIR spectra of more abundant microplastics detected from seawater samples: high density polyethylene (HDPE), polypropylene (PP), polyester (PEST) e paint. Samples were measured with a MCT (mercury cadmium-telluride) detector in reflection mode from gold membrane filters.

The majority of microplastics (>70%) were up to 2 mm in length. The fibers ranged from 122.3 µm to almost 5 mm length (average, 1408.1 ± 1073.7 µm) and from 8.5 to 79.7 µm diameter (average, 24.2 ± 11.1 µm). The fibers recorded were not significantly larger in terms of length and diameter (Mann-Whitney, p-value > 0.05). The fragments, which represented only 5% of the recorded MPs, ranged from 51.4 µm to 1.4 mm (average, 201.5 ± 307.9 µm) in width.

No significant difference in the size distribution of MPs between sectors was registered (PERMANOVA, p-value > 0.05, Pseudo-F=1.04).

Table 1: Total Abundance (particles/m^3) and size classes abundance samples of MPs with two mesh nets (64 and 300 µm).

		Abundance (particles/m^3)							
		Microplastics		Size Classes					
Station	Sector	64µm	300µm	64µm			300µm		
				Class 1	Class 2	Class 3	Class 1	Class 2	Class 3
1	1	13.1	6.65	0,00	9.21	1.94	0.22	2.55	0.66
2	1	2.69	3.05	0,00	2.3	0.38	0,00	0,00	3.06
3	1	7.93	0.06	0.2	6.92	0.4	0,00	0.06	0,00
4	1	9.78	0.97	1.52	6.42	1.52	0,00	0.24	0,00
5	1	0.61	0.75	0,00	0.49	0.12	0,00	0.4	0.23
6	1	1.92	2.19	0,00	2.33	0.27	0,00	1.09	0.5
7	1	2.22	0.24	0.26	1.3	0.39	0,00	0.06	0,00
8	1	11.12	0.77	0.09	6.06	0.81	0.04	0.65	0.08
9	1	1.42	4.03	0,00	0.85	0.28	0,00	2.51	0.86
10	2	4.02	2.05	0,00	3.1	0.61	0,00	0.88	0.59
11	2	7.14	1.17	0,00	2.99	2.3	0,00	0.41	0.5
12	2	1.91	0.22	0,00	1.18	0.29	0,00	0.05	0.05
13	2	7.76	10.93	0,00	5.68	1.32	0,00	9.04	1.5
14	2	1.09	0.52	0,00	0.57	0.31	0,00	0.52	0,00
15	2	3.76	0.69	0,00	1.57	0.78	0,00	0.28	0.23
16	2	5.36	0.52	0,00	3.46	0.63	0,00	0.15	0.15
17	2	0.93	0.27	0,00	0.78	0.15	0,00	0.14	0.04
18	2	7.33	2.5	0,00	5.24	1.57	0,00	1.66	0.69

3.3. Microplastics: Zooplankton

The zooplankton organisms belonged primarily the clade Crustacea in both nets, representing more than 70% of the total zooplankton studied. In relation to the 64µm net were identified: Copepods (> 60%), Foraminifera (2.1%), Ciliophorans (0.5%), Radiolaria (0.5%), Cnidaria (0.4%), Annelida (1.1%), Mollusca (13.4%), Chaetognaths (1.4%) and Chordata (6.2%). For 300µm net were identified: Copepods (> 60%), Foraminifera (1%), Cnidaria (3.2%), Nematoda (0.03%), Annelida (0.15%), Mollusca (13.2%), Echinodermata (0.12%), Chaetognaths (5.6%) and Chordata (9.2%). It is possible to observe variation in the abundance of the total zooplankton among stations (Fig.5). However, no pattern was observed and no significant difference of the organism's abundance was observed between sectors and net meshes (Mann-Whitney, p-value >0.05, Supplementary data -Fig. S1). The highest total density of zooplankton individuals for 64µm net was recorded in the station 1 (34449 ind. m−3, BC sector) and the lowest total density was recorded in station 5 (612.71 ind. m−3; Fig.5). For 300µm net the highest total density was recorded in the station 16 (1558.8 ind. m−3, BC sector) and the lowest in station 9 (87.78 ind. m−3; Fig.5).

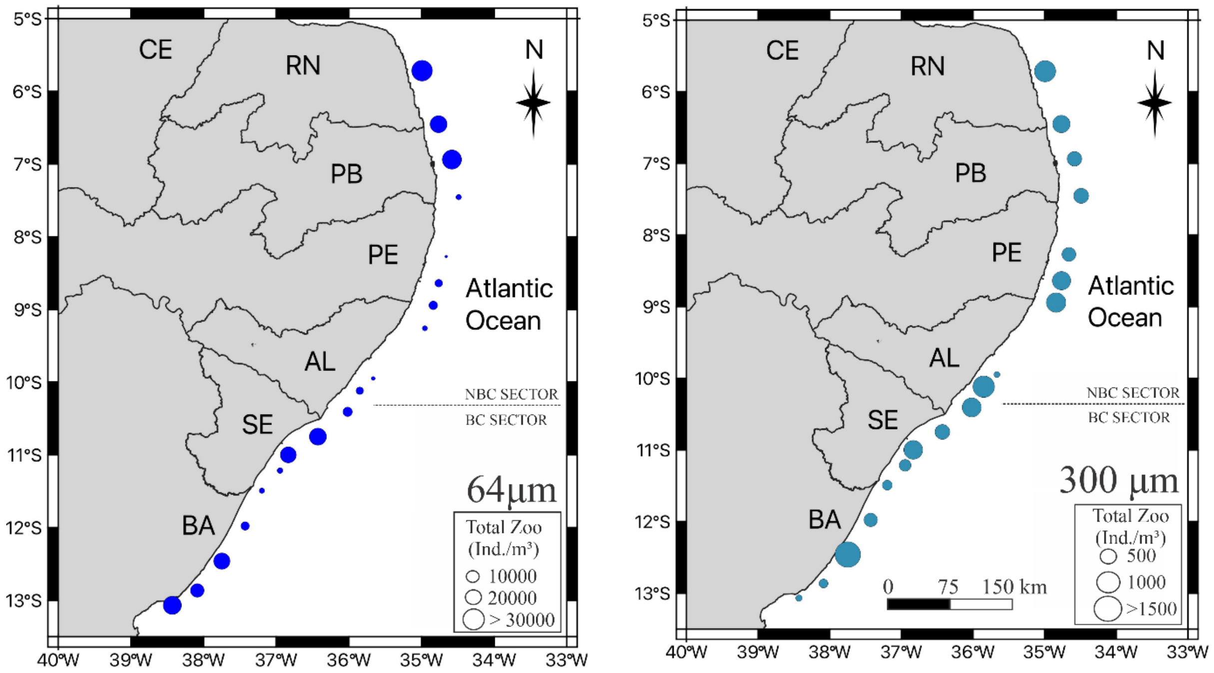

Fig.5: Total zooplankton (Total Zoo) abundance (ind./m³) registered in the two nets (64 e 300µm) in the Western Equatorial Atlantic (WEA).

The ratio of microplastic to zooplankton for 64µm net was 0.0003 (i.e. 3 MPs for every 10,000 zooplankton organisms) in the Western Equatorial Atlantic region. When considering the ratio obtained between sectors, the result is not significant (Mann-Whitney, p-value >0.05), but higher in the NBC sector (0.0004) in relation to the BC sector (0.0003). For 300µm net, the ratio was 0.003 (i.e., 3 MPs for every 1,000 zooplankton organisms). When considering the ratio obtained between sectors, we also recorded higher values in the NBC sector (0.002) in relation to the BC sector (0.003).

4. Discussion

4.1. Microplastic in seawater

Aspects influenced by rivers, such as high turbidity and the presence of water with low salinity, were not detected at the sampling stations, even at stations close to larger rivers (e.g. Rio São Francisco). As the influence of rivers can be subject to seasonal variability, it is likely that sampling in the present study in periods with low rainfall is an important factor. Studies performed at WEA report that most materials from the coast are diluted and trapped near the coast, mainly larger plastics that travel shorter distances (Balzer and Knoppers 1996; Ekau and Knoppers 1999; Eo et al., 2021). However, some of the debris can still be transported across the shelf (Jennerjahn and Ittekkot, 1999).

Microplastics have a similar behavior to zooplankton, because they remain suspended in the water column and are transported in the direction of ocean currents and wind/waves (Cózar et al., 2014; Enders et al., 2015). However, unlike zooplankton, MPs act as passive particles. Field studies about the distribution, abundance and composition of microplastics in the ocean have important implications for evaluating the risks of the presence of these debris for several species of marine organisms (Rodrigues et al., 2021). Most studies with MPs *in situ* used plankton nets with a mesh higher than or equal 300µm (Conkle et al., 2018). Therefore, we sampled with 300 µm net so that our results could be comparable. However, how estimates of MP abundance is admittedly underestimated with use of this net (Lindeque et al., 2020) we also sampled with a 64 µm net, in order to obtain better estimates of MPs from the environment.

The results obtained demonstrate that there is great variability in the MPs spatially distribution. Fibers make up the highest percentage in the study area, as reported for other marine areas (Beer et al., 2018). It has mainly two sources in the marine environment: i. is the dominant type in the effluent of wastewater treatment plants (WWTPs) (Acarer, 2023) and ii. fishing equipment left into the ocean (Arat, 2024). Furthermore, this type of MP can remain floating longer and can reach greater distances, when compared to other types (Sambolino et al., 2022). When we evaluate the abundance and distribution patterns of MPs, not observed in the clear relationship with discharged rivers on the continent, same in stations close to rivers with higher volumes. Probably due to the low rainfall period in which the sampling was performed. It is likely that an important fraction of MPs sank before reaching the open ocean due to the smaller volume of water in rivers during this period and/or was retained by morphological structures (e.g., reefs) (Soares et al., 2023).

When we compare our results with other studies carried out in the water column (subsurface) Atlantic, we exceed the reported microplastic pollution in a North/South Atlantic transect, with average microplastic abundance records (1.15 ± 1.45 particles m− 3) (Kanhai et al., 2017, 11m depth), and obtained values similar to those carried out in the north eastern Atlantic Ocean (2.46 ± 2.43 particles m− 3, 3m depth) (Lusher et al., 2014), even when compared with the 300 µm net. Although there appears to be a decreasing trend in MP abundance as we move away from the coast (Steer et al., 2017), high abundances have been reported even in offshore areas (Queiroz et al., 2022). We note that there are some hot pots of MPs in this region, probably associated with hydrodynamic processes (Zhang, 2017). Boundary currents can be responsible for these hot spots, related to bodies of contaminated water (Goswami et al., 2023) or there can be nearby sources of MPs. It’s important to mention that MP abundance values in subsurface layers in the ocean can vary not only due to the pore size of the net used (both studies mentioned above used a 250 µm), but also due to the depth sampled. Plastic polymers are produced and also acquire different densities throughout the fragmentation process, and therefore, the abundance of polymer can be dependent on the depth at which it is sampled.

The particle dominance of class 2 (200-2000) can be mainly related to the method used here (Kang et al., 2015). Hauls 300µm net have been used for monitoring purposes (Frias et al., 2020) and although this study sampled with a 64µm net, the smaller fraction (20-200) is still underestimated. Further studies will be necessary to identify the abundance patterns of the smaller fraction. It is important to mention how marine environments are much more polluted than most studies can demonstrate. This is due to the difficulty of analysis and access to equipment capable of detecting smaller fractions. Identification of polymer types of MPs in seawater may be useful to predict potential sources. Polymer diversity was similar to that found in surface waters and closer to the coast (Lima et al., 2023). We recorded that the water column is mainly polluted by PEST, PP and PE (Erni-Cassola et al., 2019). These are the types of polymers most produced worldwide and commonly used in single-use products (Geyer et al., 2017). PP and PE are widely used in packaging, in addition to many other items used in daily life, such beverages (PE), shopping bags (PE) and textile products (PP) (Acarer et al., 2023). Similarly, PP is widely used in packaging, industrial plastic parts production, and textile products due to its superior properties. Although low density polymers such as those mentioned above are commonly registered in the water column, we registered mainly high-density polymers, such as HDPE and PEST, generally more abundant in deeper subsurface waters (Erni-Cassola et al., 2019). Ding et al. (2022) recorded rayon and PET as the main type of atmospheric MPs polluting the ocean. We do not register these types of MPs in high abundance. Therefore, it is probable that there are nearby sources of plastic in the shelf break or that there is resuspension in the water column.

4.2. Microplastics: zooplankton

The pelagic system dominates the food web of the Northeast shelf of Brazil. The Zooplankton is an important natural prey for several marine organisms and are primary consumers and can be directly linked to the entry of microplastics into the marine food web (Gunaalan et al., 2023). Most studies about the relationship between zooplankton and microplastics were conducted in the laboratory and report the negative effects of animals that ingest these particles (Cole et al., 2013; Yoo et al., 2021). Due to the ecological importance and trophic position of these organisms these effects can be extended along the food web. Increasing the ratio between MPs and zooplankton increases the probability of fish (Pereira et al., 2020, Hajisamae et al., 2022), birds (Susanti et al., 2020) and whales (Torres et al., 2023); ingest MP accidentally or by trophic transfer (contaminated zooplankton).

Modeling studies of MP accumulation along the food web indicated that these polymers spread rapidly along the web and reach higher trophic levels (Ma et al., 2021, Jian et al., 2023), including animals consumed by humans (Hara et al., 2020, Alava et al., 2020). In addition, even if the plastic is not bioavailable, that is, in a size compatible with the predator, the presence of these polymers in the water influences the predator-prey interactions and increases the concern of potential impacts derived from the presence of such particles. Fibers, type most abundant type registered, are reported in the literature as the microplastic most ingested by zooplanktonic and zooplanktivorous organisms (Ferreira et al., 2018; Hossain et al., 2019). There are records of copepods ingesting fibers more than 2 mm long (Yoo et al., 2021). Copepods are the most abundant plankton organisms, the ingestion of MPs by these organisms can lead to ecological risks for the entire pelagic community of organisms.

Many studies use the MPs: Zooplankton ratio to estimate the threat posed by microplastics to marine biota and indicate the probability of encountering and ingesting MPs by zooplankton (NGO et al., 2023). These values can be used as bioindicators in the study of protected areas (Soto-Navarro et al., 2021), or fishing areas (Colloca et al., 2017). There are no studies about ratio values as a parameter for more or less impacted areas. However, this number must be evaluated with caution. Our results make inferences about interactions in a specific space-time that they co-occur. Lima et al. (2023) found that estuarine plumes are very important in the input of MPs to the ocean, especially during periods of high rainfall. It is likely that the seasonality effect has a high impact on the MPs: zooplankton ratio, as the study area is subject to the influence of large rivers (eg. São Francisco river). Could they not be responsible for the increase in MPs in that area during these periods? Thus, it is necessary to have temporal studies to assess whether there are changes in the planktonic community related to the abundance of MPs and increased risk of contamination. Changes in the community of primary consumers and in the concentration of MPs can lead to structural modifications and threaten all trophic levels of the marine ecosystem. Still, we obtained results similar to those found in the Bay of Calvi (0.002, Collignon et al., 2014), Southern Sea of Corea (0.004- 0.086, Kang et al., 2015) and Australian estuaries (0.009, Hitchcock and Mitrovic, 2019). In oligotrophic environments, where there is low food supply, even if the water column is moderately contaminated, there is a threat to marine life due to high grazing activity (Richon et al., 2022).

5. Conclusions

Here we present the distribution and abundance pattern of microplastics floating over the WEA, with great variability and the presence of hotspots. We also reveal the potential sources of atmospheric microplastics based on the microplastic polymer type. The distribution and concentration of the microplastics were comparable to microplastics found previously in different regions worldwide. Although this study investigated the distribution and abundance of MPs subsurface in the water column, more sampling stations are needed to assess whether source MPs are more associated with the coast or open ocean and we also recommended to investigate the abundance of MPs temporally to better understand the level of MP contamination in the water column. Most of the polymers noted here were HDPE, PP, PEST and paint with types mainly of fibers. Overall, we can conclude that the abundance of MPs is higher than in other environments considered polluted in the world.

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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.

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5. CONSIDERAÇÕES FINAIS

O plástico como potencial ameaça para organismos é um tema de pesquisa relativamente recente, com relevante aumento no número de publicações científicas e ampla divulgação na mídia nos últimos 15 anos. Muitos esforços têm sido realizados pela comunidade científica para investigar a concentração real desses resíduos no ambiente. A abundância, distribuição e a caracterização dos plásticos presentes no oceano permitem que façamos estimativas do provável impacto desses resíduos nas teias alimentares e inclusive na saúde humana.

Os nossos resultados incluem uma área de proteção ambiental e a quebra da plataforma, áreas onde espera-se encontrar um volume menor de microplásticos. Porém, registramos valores semelhantes a áreas consideradas muito poluídas. O presente estudo revela a importância dos rios como fonte de microplásticos para ambientes costeiros marinhos, mesmo em unidades de conservação, principalmente em período de alta pluviosidade; e da predominância de fibras quando se fala em microplásticos suspensos em ecossistemas marinhos. Os valores encontrados da razão entre microplásticos e zooplâncton podem ser utilizados como bioindicadores, inclusive para região de Tamandaré (Capítulo I), que faz parte do Programa de Pesquisas Ecológicas de Longa Duração há 25 anos. Na área da quebra da plataforma, é necessário haver estudos temporais para avaliar se há alterações na comunidade planctônica relacionadas à abundância de MPs e aumento do risco de contaminação neste período.

O estudo sobre as concentrações de plástico e estimativas reais sobre a exposição de organismos ao plástico envolvem avaliações bastante complexas. Mas essas são questões necessárias para estabelecer formas de mitigar a contaminação plástica e compreender o caminho desses resíduos nos ecossistemas. Além disso, trabalhos como esse, acerca da poluição plástica nos oceanos precisam ser amplamente divulgados para a sociedade, e não apenas dentro da comunidade científica. A diminuição do uso e produção, principalmente de plásticos de uso único, dependem da sensibilização da comunidade fora da academia. Sem essas iniciativas, o volume de plásticos que produzimos e consumimos continuará alto.

6.. REFERÊNCIAS BIBLIOGRÁFICAS

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