ÉCOLE DOCTORALE 548 Mer et Sciences

Equipe Laboratoire M.I.O

En partenariat international avec Universidade Federal Rural de Pernambuco (Brésil)

THÈSE présentée par :

Anne JUSTINO

soutenue le : **15 septembre 2022**

pour obtenir le grade de Docteur en Sciences de l'Univers

Spécialité : Géosciences de l'Environnement

From the estuary to the deep sea: microplastic contamination of fishes from Southwestern Tropical Atlantic

THÈSE dirigée par :

Mme. LUCENA-FRÉDOU Flávia	Full Professor, UFRPE	[Directrice de thèse]
Mme. LENOBLE Véronique	Maître de conférences, UTLN	[Directrice de thèse]

JURY :

M. LE LOC'H François	Directeur de Recherche, IRD	[Rapporteur]
M. TURRA Alexander	Full Professor, USP	[Rapporteur]
M. FRIAS João	Senior Researcher, GMIT	[Examinateur]
M. SEVERI William	Full Professor, UFRPE	[Examinatrice]
Mme. MONTAGNER Cassiana	Associate professor, UNICAMP	[Examinatrice]
Mme. PAUL PONT Ika	Associate professor, UBO	[Examinatrice]
Mme. LUCENA-FRÉDOU Flávia	Full Professor, UFRPE	[Directrice de thèse]
Mme. LENOBLE Véronique	Maître de conférences, UTLN	[Directrice de thèse]

Thesis to obtain the degree of doctor issued by the Université de Toulon – UTLN
and the Universidade Federal Rural de Pernambuco - UFRPE

UNIVERSIDADE FEDERAL RURAL DE PERNAMBUCO
PRO-RECTORATE OF RESEARCH AND GRADUATE STUDIES
GRADUATE PROGRAM IN FISHERIES RESOURCES AND AQUACULTURE

From the estuary to the deep sea: microplastic contamination of fishes from Southwestern Tropical Atlantic

ANNE K. S. JUSTINO

Thesis presented to the Doctoral School Sea and Science of the Université de Toulon and to the Graduate Program in Fishing Resources and Aquaculture of the Universidade Federal Rural de Pernambuco as a requirement to obtain the title of Doctor.

Flávia Lucena-Frédou (Supervisor)
Véronique Lenoble (Supervisor)

Recife,
15 September 2022

Thesis to obtain the degree of doctor issued by the Université de Toulon – UTLN
and the Universidade Federal Rural de Pernambuco - UFRPE

UNIVERSIDADE FEDERAL RURAL DE PERNAMBUCO
PRO-RECTORATE OF RESEARCH AND GRADUATE STUDIES
GRADUATE PROGRAM IN FISHERIES RESOURCES AND AQUACULTURE

From the estuary to the deep sea: microplastic contamination of fishes from Southwestern Tropical Atlantic

ANNE K. S. JUSTINO

Supervisors:

Dr. LUCENA-FRÉDOU Flávia	Full Professor, UFRPE	[Supervisor]
Dr. LENOBLE Véronique	Associate professor, UTLN	[Supervisor]

Juries:

Dr. LE LOC'H François	Director of Research, IRD	[Reviewer]
Dr. TURRA Alexander	Full Professor, USP	[Reviewer]
Dr. FRIAS João	Senior Researcher, GMIT	[Jury]
Dr. SEVERI William	Full Professor, UFRPE	[Jury]
Dr. MONTAGNER Cassiana	Associate professor, UNICAMP	[Jury]
Dr. PAUL PONT Ika	Associate professor, UBO	[Jury]
Dr. LUCENA-FRÉDOU Flávia	Full Professor, UFRPE	[Supervisor]
Dr. LENOBLE Véronique	Associate professor, UTLN	[Supervisor]

JUSTINO, A.K.S. **From the estuary to the deep sea: microplastic contamination of fishes from Southwestern Tropical Atlantic**

Cataloguing data for the Universidade Federal Rural de Pernambuco

Dados Internacionais de Catalogação na Publicação
Universidade Federal Rural de Pernambuco
Sistema Integrado de Bibliotecas
Gerada automaticamente, mediante os dados fornecidos pelo(a) autor(a)

J96f Justino, Anne K. S.
From the estuary to the deep sea: microplastic contamination of fishes from Southwestern Tropical Atlantic / Anne K. S. Justino. - 2022.
138 f. : il.

Orientadora: Flavia Lucena Lucena-Fredou.
Coorientadora: Veronique Lenoble.
Inclui referências.

Tese (Doutorado) - Universidade Federal Rural de Pernambuco, Programa de Pós-Graduação em Recursos Pesqueiros e Aquicultura, Recife, 2022.

1. Plastic pollution. 2. Trophic transfer. 3. Microplastic. 4. Fish ecology. 5. Atlantic Ocean. I. Lucena-Fredou, Flavia Lucena, orient. II. Lenoble, Veronique, coorient. III. Título

CDD 639.3

ACKNOWLEDGEMENTS

The development of this thesis was only possible thanks to the support of sponsors, whom I would like to thank immensely, and for the help of all the incredible people who have crossed my path during this insane yet astonishing journey.

The project on microplastic pollution was possible thanks to resources from the LMI-TAPIOCA project, which equipped the laboratory we use for the analyses and provided resources for missions to France through the support of IRD (Institute de Recherche pour le Développement). This research was also awarded with the FUNBIO (Fundo Brasileiro para a Biodiversidade) and Humanize Institute supports, which funded all laboratory materials and supplies necessary for the research development. The polymer analysis was only possible through the support of the MicroplastiX project funded by JPI Oceans and by FACEPE (Fundação de Amparo a Ciência e Tecnologia do Estado de Pernambuco). Thanks to the funding agencies CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior) and CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico), because without them it would be impossible to start. Also, thanks to PROTUNA and ABRAÇOS projects for providing the biological samples.

I wish to thank Flavia Lucena-Frédou for trusting my ideas, giving me subsidies to make them real throughout these five years, and sheltering me at such a necessary time. Furthermore, thank you, Flávia, for guiding me throughout my journey and being such an inspiring example of female leadership. I would also like to thank Véronique Lenoble for such warm hospitality and collaboration in all these years of cotutelle. It was a tremendous honour to have the support of two incredible women scientists. Special thanks to my co-supervisor in academic life, Guilherme Ferreira, for the collaboration in almost ten years working together and for exchanging ideas and shared experiences. Thanks to Vincent Fauvelle and Natascha Schmidt for their hospitality in Marseille and for rendering the project analyses possible. Thanks to Thierry Frédou, and all the Bioimpact members, the new and the old ones, we formed a great scientific team together.

Enormous thanks to the Várzea girl crew who supports my PhD dramas, and for all the exchange of ideas and ideals, Rafaela Passarone, Thaiza Barreto and my two favourite French girls in the world, Julie Salvetat and Latifa Pelage. Also, thanks to my UFRPE friends, Catarina Cardoso, Tatiana Beltrão and Karla Martins, for our conversations over the afternoon coffee. To my dear friends, Brenno Januário, Cibele Rodrigues and Raqueline Monteiro, who, even though distant, was present in my journey. My immense gratitude to my mother for all the support over three decades of existence; without her, nothing would be possible—finally, a special thanks to me for not giving up.

For all the women who came before,

for all the girls who will come after.

CONTENTS

LIST OF FIGURES

Page

Chapter 1
Article "Microplastic contamination in tropical fishes: An assessment of different feeding habits"

Chapter 2
Article "Microplastic contamination in coastal fishes from the Southwestern Tropical Atlantic"

Chapter 3
Article "The role of mesopelagic fishes as microplastics vectors across the deep-sea layers from the Southwestern Tropical Atlantic"

Chapter 4

Article "From prey to predators: evidence of microplastic trophic transfer in tuna and tuna-like species in the Southwestern Tropical Atlantic"

GENERAL CONCLUSIONS

LIST OF TABLES

Page

Chapter 1

Article "Microplastic contamination in tropical fishes: an assessment of different feeding habits"

Chapter 2

Article "Microplastic contamination in coastal fishes from the Southwestern Tropical Atlantic"

Chapter 3

Article "The role of mesopelagic fishes as microplastics vectors across the deep-sea layers from the Southwestern Tropical Atlantic"

Chapter 4

Article "From prey to predators: evidence of microplastic trophic transfer in tuna and tuna-like species in the Southwestern Tropical Atlantic"

ABSTRACT

Worldwide, microplastics (MPs; 5 < mm) have been identified in most marine ecosystems and organisms. The large availability of this contaminant has raised the concern of scientists about the impacts on biodiversity and ecosystems. However, despite being frequently reported, some regions still lack studies on the characterisation of MPs contamination. This is the case in the Southwestern Tropical Atlantic (SWTA) region. To fill this gap, this thesis aims to provide a general understanding of the distribution and patterns of MP contamination in the ichthyofauna inhabiting the SWTA region along the estuarine-oceanic gradient. Thus, a protocol for extraction of MPs for marine organisms was adapted to diminish the probability of airborne contamination in the samples, which would be practical and of low operational cost. The thesis is structured in four chapters presented as articles and a general conclusion. The first chapter explored an estuarine trophic chain, comprising a commercially important top predatory fish and two of its main prey. Both species have different feeding habits, which was also observed in the frequency of MP ingestion, which varied with feeding strategies. In the second chapter, four coastal demersal fish species were used to characterise the ingestion of MPs in reef fishes from the SWTA region. No differences were observed between the ingestion rate of MPs by coastal species; however, a wide range of polymers was identified. The ocean ecosystem is discussed in the last two chapters of the thesis, focusing on pelagic and mesopelagic oceanic species. The third chapter characterises the contamination of two abundant mesopelagic fish families in the SWTA and addresses the influence of these species on the transport of MPs in the deep ocean. In this chapter, MP contamination in deep-sea fishes in the region was identified for the first time and areas of possible accumulation of particles were discussed. Finally, in the fourth chapter, pelagic predators important for industrial and artisanal fisheries were evaluated for the presence of MPs. In addition to the characterisation of contamination, evidence of trophic transfer of MPs from prey to predators was observed. Furthermore, this chapter discusses the ingestion of larger particles (macroplastics > 5 mm) derived from the intense ocean fishing and tourism activity in the Fernando de Noronha Archipelago (FNA). Overall, it was possible through this thesis to confirm the contamination by MPs in fish from the SWTA region. The ecological patterns, such as feeding strategies and trophic level, increased MP contamination probability. Furthermore, the influence of the species’ behaviour in the water column in areas with an accumulation of MPs. In addition, it was also possible to observe that, in general, the most frequent shape of MPs ingested by fishes are fibres. However, large oceanic pelagic predators (tunas) tended to ingest pellets and foams, which was also observed in their primary prey, indicating trophic transfer. Moreover, a variety of polymers were identified, but the most frequent polymers in the coastal region were polyethylene (PE), Alkyd Varnish and styrene-butadiene rubber (SBR); for the mesopelagic fishes in the oceanic region were polyamide (PA), PE and polyethylene terephthalate (PET). However, the most frequent polymers for tuna and prey were SBR, PA, and PET. The availability of these polymers in the SWTA region indicates intense pressure from fisheries (*e.g.*, fishing nets and ship paint) and high tourist activity. The information available here can serve as a baseline to discuss and develop measures to mitigate the impacts caused by MPs contamination and plastic pollution in the SWTA region, thus contributing to the conservation of biodiversity and marine ecosystems.

Keywords: Plastic pollution, Trophic transfer, Atlantic Ocean, Fisheries resources, Fish ecology, Estuaries, Mesopelagic ecosystem, Tuna.

RESUMÉ

Les microplastiques (MPs ; 5 < mm) ont été identifiés dans la plupart des écosystèmes et organismes marins, ce qui suscite la préoccupation des scientifiques quant à leur impact sur la biodiversité et les écosystèmes. Cependant, certaines régions ne sont pas encore suffisamment documentées en ce qui concerne la caractérisation de la contamination par les MPs, comme la région de l’Atlantique tropical sud-ouest (ATSO). Afin de remplir ces lacunes, l’objectif général de cette thèse est de fournir une compréhension générale de la distribution et des modèles de contamination par les MP dans l'ichtyofaune de la région ATSO le long du gradient estuarien-océanique. Dans ce contexte, un protocole d’extraction rapide, efficace et d’un faible coût opérationnel, des MPs a été adapté aux organismes marins, tout en garantissant une probabilité minime de la contamination aéroportée dans les échantillons. La thèse est structurée en quatre chapitres sous forme d’articles, et une conclusion générale. Dans le premier chapitre, nous avons exploré une chaîne trophique estuarienne comprenant un poisson prédateur commercialement important et deux de ses principales proies. Ces espèces ont des stratégies alimentaires différentes, ce qui se retrouve dans la fréquence d’ingestion des MPs. Dans le deuxième chapitre, quatre espèces de poissons démersaux côtiers ont été étudiées pour caractériser l’ingestion de MPs chez les poissons de récif de la région ATSO. Aucune différence n’a été observée entre le taux d’ingestion de MPs par les espèces côtières, mais un large éventail de polymères a été identifié. L’écosystème océanique est abordé dans les deux derniers chapitres de la thèse, en se concentrant sur les espèces océaniques pélagiques et mésopélagiques. Le troisième chapitre caractérise la contamination de deux familles de poissons mésopélagiques abondantes dans l’ATSO, et aborde l’influence de ces espèces sur le transport des MPs dans l’océan profond. Ainsi, la contamination par les MPs des poissons d’eau profonde de la région a été identifiée pour la première fois et les zones d’accumulation probable de ces particules ont été discutées. Enfin, dans le quatrième chapitre, des prédateurs pélagiques importants pour la pêche industrielle et artisanale ont été étudiés. Outre la présence de MPs, le transfert trophique des MPs des proies aux prédateurs a été mis en exergue. Ce chapitre traite également de l’ingestion de macroplastiques (> 5 mm) provenant de l’intense activité de pêche et de tourisme dans l’Archipel de Fernando de Noronha. De façon générale, il a été possible, au travers de ce travail de thèse, de confirmer la contamination par les MPs des poissons de la région ATSO. Les comportements écologiques, tels que les stratégies d'alimentation et le niveau trophique, ont un impact sur la contamination. De plus, l'influence du comportement des espèces dans la colonne d'eau sur les zones d'accumulation des MPs a été mise en évidence. Il a également été possible d'observer qu'en général, la forme la plus fréquente de MP ingérés par les poissons sont les fibres. Cependant, les grands prédateurs pélagiques océaniques (thons) ont tendance à ingérer des pellets et des mousses, ce qui a également été observé chez leurs proies principales, indiquant un transfert trophique. En outre, les polymères les plus fréquemment retrouvés dépendent également des comportements écologiques des espèces ainsi que, dans les cas des thons, de la pression intense de la part des pêcheries (par exemple, filets de pêche et peinture des bateaux) et la forte activité touristique. Les informations disponibles ici peuvent servir de support pour discuter et développer des mesures visant à atténuer les impacts causés par la contamination par les MPs et la pollution plastique dans la région ATSO, contribuant ainsi à la conservation de la biodiversité et des écosystèmes marins.

Mots clés : Pollution plastique, Transfert trophique, Océan Atlantique, Ressources halieutiques, Écologie des poissons, Estuaires, Ecosystème mésopélagique, Thon.

RESUMO

Globalmente, os microplásticos (MPs; 5 < mm) já foram identificados na maioria dos ecossistemas e organismos marinhos. A grande disponibilidade desse contaminante, vem crescendo a preocupação de cientistas sobre os impactos gerados na biodiversidade e nos ecossistemas. Entretanto, apesar de ser frequentemente reportado, algumas regiões ainda são carentes de estudos sobre a caracterização da contaminação por MPs. É o caso da região do Atlântico Sudoeste Tropical (AST). Visando preencher essas lacunas, esta tese tem o objetivo de fornecer uma compreensão geral da distribuição, e dos padrões de contaminação de MP na ictiofauna que habita a região AST, ao longo do gradiente estuarino-oceânico. Para isto, foi feita a adaptação de um protocolo de extração de MPs para organismos marinhos, com o objetivo de diminuir a probabilidade de contaminação cruzada nas amostras, que fosse um protocolo prático e de baixo custo operacional. A tese está estruturada em quatro capítulos no formato de artigo, e uma conclusão geral. O primeiro capítulo explorou uma cadeia trófica estuarina, compreendendo um peixe predador de topo de importância comercial, e duas de suas presas principais. Ambas as espécies têm diferentes hábitos alimentares e isso foi observado também na frequência de ingestão de MPs, que variou com a estratégia de alimentação. No segundo capítulo, quatro espécies de peixes costeiros demersais foram utilizadas para caracterizar a ingestão de MPs em peixes recifais da região do ATS. Não foram observadas diferenças entre a taxa de ingestão de MPs pelas espécies costeiras, mas uma grande variedade de polímeros foi identificada. O ecossistema oceânico é discutido nos dois últimos capítulos da tese, visando espécies oceânicas pelágicas e mesopelágicas. O terceiro capítulo caracteriza a contaminação de duas abundantes famílias de peixes mesopelágicos do AST, e aborda a influência dessas espécies no transporte de MPs no oceano profundo. Neste capítulo, foi identificada pela primeira vez a contaminação por MPs em peixes de profundidade na região, e áreas de provável acúmulo dessas partículas foram discutidas. Finalmente, no quarto capítulo, predadores pelágicos importantes para pescaria industrial e artesanal foram avaliados quanto a presença de MPs. Além da caracterização da contaminação, foram observadas evidências de transferência trófica de MPs das presas para os predadores. Ainda neste capítulo, é discutida a ingestão de partículas maiores (macroplásticos > 5 mm) oriundas da intensa atividade pesqueira oceânica e turismo no Arquipélago de Fernando de Noronha (AFN). De maneira geral, foi possível através dessa tese, confirmar a contaminação por MPs em peixes da região AST, identificar os formatos mais frequentes para cada ecossistema marinho avaliado e nível trófico da ictiofauna, além de caracterizar os principais polímeros encontrados. Os padrões ecológicos, como as estratégias de alimentação e o nível trófico, aumentaram a probabilidade de contaminação por MP. Além disso, a influência do comportamento das espécies na coluna d'água em áreas com acúmulo de MPs. Também foi possível observar que, em geral, o tipo de MP mais frequente ingerido pelos peixes são as fibras. Entretanto, grandes predadores pelágicos oceânicos (atuns) tenderam a ingerir pellets e espumas, o que também foi observado em suas presas principais, indicando uma possivel transferência trófica. Além disso, uma variedade de polímeros foi identificada, mas os polímeros mais frequentes na região costeira foram polietileno (PE), verniz alquídico e borracha de butadieno estireno (SBR); para os peixes mesopelágicos na região oceânica foram poliamida (PA), PE e polietileno tereftalato (PET). Entretanto, os polímeros mais frequentes para o atum e suas presas foram SBR, PA, e PET. A disponibilidade destes polímeros na região AST indica uma intensa pressão da pesca (por exemplo, redes de pesca e fragmentos da pintura das embarcações) e alta atividade turística. Ademais, foram feitas discussões importantes acerca dos principais fatores envolvidos na ingestão e disponibilidade de MPs, e as complexas interações tróficas entre as espécies e seus ecossistemas. As informações disponíveis aqui, podem servir como base para discutir e elaborar medidas que visam mitigar os impactos causados pela contaminação por MPs e a poluição plástica na região do AST, contribuindo assim para a conservação da biodiversidade e dos ecossistemas marinhos.

Palavras-chave: Poluição plástica, Transferência trófica, Oceano Atlântico, Recursos pesqueiros, Ecologia de peixes, Estuários, Ecossistema mesopelágico, Atuns.

GENERAL INTRODUCTION

Plastics are everywhere. Given the versatile, light, strong, flexible, low cost and relatively inert, plastics are handy for humans and are used in all industrial sectors (Zalasiewicz et al., 2016). Plastic resins are widely used in packaging (consumer sector), furniture and household (building and construction), and synthetic fibre production in the textile sector (Geyer, 2020). The constantly growing dependence of the industrial sector and consequently on society, associated with the current low rate of plastic recycling (approximately 9%) and improper waste management, turn it into a preoccupying environmental issue in the 21st century (Geyer, 2020). This concern increases in developing countries in the global south, which does not have adequate waste management strategy (Margallo et al., 2019). Hereafter, plastics could be recognised as stratigraphic markers because of their resistance and ubiquity and have been used to support the proposal of a new geological epoch called the Anthropocene (Zalasiewicz et al., 2016).

Once it is incorrectly disposed of in the environment, plastic can be distributed from the continental region through rivers to marine ecosystems (Koelmans et al., 2017; Meijer et al., 2021). In the marine environment, plastic waste undergoes weathering through physical (*e.g.*, solar radiation, hydrodynamics) and biological (*e.g.*, biofouling, interaction with biota) processes (Jambeck et al., 2015; Pinheiro et al., 2021; Thompson et al., 2004). The oceanographic and meteorological processes involved in the distribution of plastics in the environment make plastic pollution a transboundary problem (Krelling and Turra, 2019; Lima et al., 2020). In the environment, plastics are classified based on their size as macroplastics (> 5 mm) and microplastics (MPs; < 5 mm) (Arthur et al., 2009).

MPs are widely distributed in marine ecosystems, from coastal continental regions, polar regions, insular, and through the deep ocean, vertically and horizontally in the water column (Katija et al., 2017; Lins-Silva et al., 2021; Monteiro et al., 2020; Waller et al., 2017). As a consequence of their wide availability, small size and similarity (visual and olfactory) to aquatic organisms, MPs are highly susceptible to being accidentally swallowed by fish through the inadvertent ingestion or inhalation process of the species (Boerger et al., 2010; Li et al., 2021). Moreover, the ingestion of MPs is associated with various sublethal effects on organisms, including damage to the digestive system, decreased predation efficiency and induction of toxic effects when associated with pollutants adsorbed from the surrounded (Barboza et al., 2018; de Sá et al., 2015; Moore, 2008; Teuten et al., 2007). Besides the natural physical processes, marine biota seems to influence the transport of MPs from one pool to another (*e.g.*, epipelagic to mesopelagic layers) (Choy et al., 2019; Van Sebille et al., 2020). However, information on the transport of MPs associated with fishes in the source-to-sea continuum is scarce, and many questions remain open, mainly regarding the ecological factors involved in intake by fishes.

In South America, several studies have been conducted to investigate MPs contamination in fish; however, most were carried out to assess contamination of estuarine species (Dantas et al., 2020; Ferreira et al., 2019, 2018; Macieira et al., 2021; Neto et al., 2020; Pegado et al., 2018; Possatto et al., 2011; Ramos et al., 2012; Vendel et al., 2017). Nevertheless, only a few studies have considered the use of a digestion protocol to extract MPs from fish species (Arias et al., 2019; Calderon et al., 2019; Garcés-Ordóñez et al., 2020; Pozo et al., 2019; Ribeiro-Brasil et al., 2020), necessary to diminish the chance of cross-contamination (*e.g.*, airborne contamination) and misidentification or loss of particles during sample handling. Considering the preoccupying situation of plastic pollution in the marine ecosystem and the increasing need to quantify and comprehend the processes involved in MP contamination, it is crucial to use reliable and replicable research methods (Hermsen et al., 2018; Markic et al., 2020; Müller, 2021).

Located in the Southwestern Tropical Atlantic (SWTA), the northeast Brazilian coastal is recognised worldwide for its beaches, which receive tourists throughout the year (IBGE, 2011). In addition to tourism, the primary economic sources are located in or near the coastal region, represented by industries, agriculture and commercial and subsistence fishing (IBGE, 2011). Nonetheless, the unbridled increase in industrial and urban activities on the northeast Brazilian coast has been gradually modifying the marine ecosystems, contaminating and polluting, threatening biodiversity and the fisheries profitability in the region (Bruzaca et al., 2022; Lira et al., 2021; Pelage et al., 2019). This has been the case of MP pollution in this region, which is increasing in marine ecosystems, especially in fish species of economic importance (Ferreira et al., 2019; Savoca et al., 2021).

However, many questions imperative to advance into a mitigation process are still identified, not only at the regional level but also worldwide. Questions such as: how are particles moved and distributed from their continental sources to distant ocean areas?

Which regions are the most contaminated by MPs, from the estuaries to the deep sea (horizontally and vertically)? The level of contamination and the particles are differently related to the source-to-sea continuum? Can deep-sea fishes transport MP across ocean layers? Do ecological behaviours influence the contamination rate of species? Are predators more vulnerable to MPs contamination through trophic transfer? Comprehending such patterns is very important to establish local public policies and contribute to broader international policies for the sustainable use of marine ecosystems and resources. Moreover, due to the global concern for mitigating the effects of plastic pollution, the United Nations 2030 Agenda on the Sustainable Development Goals (SDGs) and the United Nations Decade of Ocean Science for Sustainable Development (2021-2030) have specific goals to achieve ocean sustainability, which is the case of Goal 14—life underwater.

Thus, to fill these gaps and contribute to the advance of oceanic science, this thesis is structured in four chapters and aims to characterise MPs contamination in fishes inhabiting the SWTA region and identify the ecological patterns involved in the contamination across the estuarine-oceanic gradient. The first chapter deals with the estuarine ecosystem by observing an estuarine trophic chain comprising a predator and two of its main prey in the region. The predator was chosen considering this species' economic and subsistence importance in the estuarine area of northeast Brazil. In addition, a reliable digestion protocol for extracting MPs in marine organisms is proposed, which will be adopted for the whole thesis (Chapter 1). The second chapter aims to fill a gap concerning the identification of microplastic contamination in coastal demersal reef fishes, some of the commercial importance for local fisheries.

The third and fourth chapters evaluate the oceanic ecosystem, the mesopelagic and pelagic regions, away from the continental sources. Chapter 3 deals with the characterisation of MPs contamination in four deep-sea fish species highly abundant in the SWTA region, also discussing the role of mesopelagic species in transporting MPs between deep ocean layers. Chapter 4, on the other hand, aims to answer the ecological question that links the estuarine ecosystem to distant oceanic regions, the contamination of an apex pelagic predator of significant socio-economic importance worldwide, the tuna. Moreover, it discusses the strong evidence of MPs trophic transfer from prey to predator.

Finally, we propose a general conclusion that synthesises the main findings of the thesis and integrates the characterisation of MP contamination in fishes of the SWTA region across the estuarine-oceanic gradient, describing the main ecological mechanisms involved in the MPs uptake by the species. Furthermore, we conclude with suggestions for future research and discussions of proposals that can be used by policy and decision makers, aiming at sustainable use of marine ecosystems, also highlighting the potential effects on the health of fish resources.

REFERENCES

Arias, A.H., Ronda, A.C., Oliva, A.L., Marcovecchio, J.E., 2019. Evidence of Microplastic Ingestion by Fish from the Bahía Blanca Estuary in Argentina, South America. Bull. Environ. Contam. Toxicol. 102, 750–756. https://doi.org/10.1007/s00128-019-02604-2

Arthur, C., Baker, J., Bamford, H., 2009. Workshop on the Occurrence , Effects , and Fate of Microplastic Marine Debris. Group 530.

Barboza, L.G.A., Vieira, L.R., Branco, V., Figueiredo, N., Carvalho, F., Carvalho, C., Guilhermino, L., 2018. Microplastics cause neurotoxicity, oxidative damage and energy-related changes and interact with the bioaccumulation of mercury in the European seabass, Dicentrarchus labrax (Linnaeus, 1758). Aquat. Toxicol. 195, 49–57. https://doi.org/10.1016/j.aquatox.2017.12.008

Boerger, C.M., Lattin, G.L., Moore, S.L., Moore, C.J., 2010. Plastic ingestion by planktivorous fishes in the North Pacific Central Gyre. Mar. Pollut. Bull. 60, 2275–2278. https://doi.org/10.1016/j.marpolbul.2010.08.007

Bruzaca, D.N.A., Justino, A.K.S., Mota, G.C.P., Costa, G.A., Lucena-Frédou, F., Gálvez, A.O., 2022. Occurrence of microplastics in bivalve molluscs Anomalocardia flexuosa captured in Pernambuco, Northeast Brazil. Mar. Pollut. Bull. 179, 113659. https://doi.org/10.1016/j.marpolbul.2022.113659

Calderon, E.A., Hansen, P., Rodríguez, A., Blettler, M.C.M., Syberg, K., Khan, F.R., 2019. Microplastics in the Digestive Tracts of Four Fish Species from the Ciénaga Grande de Santa Marta Estuary in Colombia. Water. Air. Soil Pollut. 230. https://doi.org/10.1007/s11270-019-4313-8

Choy, C.A., Robison, B.H., Gagne, T.O., Erwin, B., Firl, E., Halden, R.U., Hamilton, J.A., Katija, K., Lisin, S.E., Rolsky, C., S. Van Houtan, K., 2019. The vertical distribution and biological transport of marine microplastics across the epipelagic and mesopelagic water column. Sci. Rep. 9. https://doi.org/10.1038/s41598-019-44117-2

Dantas, N.C.F.M., Duarte, O.S., Ferreira, W.C., Ayala, A.P., Rezende, C.F., Feitosa, C. V., 2020. Plastic intake does not depend on fish eating habits: Identification of microplastics in the stomach contents of fish on an urban beach in Brazil. Mar. Pollut. Bull. 153, 110959. https://doi.org/10.1016/j.marpolbul.2020.110959

de Sá, L.C., Luís, L.G., Guilhermino, L., 2015. Effects of microplastics on juveniles of the common goby (Pomatoschistus microps): Confusion with prey, reduction of the predatory performance and efficiency, and possible influence of developmental conditions. Environ. Pollut. 196, 359–362. https://doi.org/10.1016/j.envpol.2014.10.026

Ferreira, G.V.B., Barletta, M., Lima, A.R.A., Morley, S.A., Costa, M.F., 2019. Dynamics of Marine Debris Ingestion by Profitable Fishes Along The Estuarine Ecocline. Sci. Rep. 9, 13514. https://doi.org/10.1038/s41598-019-49992-3

Ferreira, G.V.B., Barletta, M., Lima, A.R.A., Morley, S.A., Justino, A.K.S., Costa, M.F., 2018. High intake rates of microplastics in a Western Atlantic predatory fish, and insights of a direct fishery effect. Environ. Pollut. 236, 706–717. https://doi.org/10.1016/j.envpol.2018.01.095

Garcés-Ordóñez, O., Mejía-Esquivia, K.A., Sierra-Labastidas, T., Patiño, A., Blandón, L.M., Espinosa Díaz, L.F., 2020. Prevalence of microplastic contamination in the digestive tract of fishes from mangrove ecosystem in Cispata, Colombian Caribbean. Mar. Pollut. Bull. 154, 111085. https://doi.org/10.1016/j.marpolbul.2020.111085

Geyer, R., 2020. Production, use, and fate of synthetic polymers, Plastic Waste and Recycling. Elsevier Inc. https://doi.org/10.1016/b978-0-12-817880-5.00002-5

Hermsen, E., Mintenig, S.M., Besseling, E., Koelmans, A.A., 2018. Quality Criteria for the Analysis of Microplastic in Biota Samples: A Critical Review. Environ. Sci. Technol. 52, 10230–10240. https://doi.org/10.1021/acs.est.8b01611

IBGE, 2011. Atlas geográfico das zonas costeiras e oceânicas do Brasil. IBGE.

Jambeck, J.R., Geyer, R., Wilcox, C., Siegler, T.R., Perryman, M., Andrady, A., Narayan, R., Law, K.L., 2015. Plastic waste inputs from land into the ocean. Science (80-.). 347, 768–771. https://doi.org/10.1126/science.1260352

Katija, K., Choy, C.A., Sherlock, R.E., Sherman, A.D., Robison, B.H., 2017. From the surface to the seafloor: How giant larvaceans transport microplastics into the deep sea. Sci. Adv. 3, e1700715. https://doi.org/10.1126/sciadv.1700715

Koelmans, A.A., Kooi, M., Law, K.L., Van Sebille, E., 2017. All is not lost: Deriving a top-down mass budget of plastic at sea. Environ. Res. Lett. 12. https://doi.org/10.1088/1748-9326/aa9500

Krelling, A.P., Turra, A., 2019. Influence of oceanographic and meteorological events on the quantity and quality of marine debris along an estuarine gradient. Mar. Pollut. Bull. 139, 282–298. https://doi.org/10.1016/j.marpolbul.2018.12.049

Li, B., Liang, W., Liu, Q.-X., Fu, S., Ma, C., Chen, Q., Su, L., Craig, N.J., Shi, H., 2021. Fish Ingest Microplastics Unintentionally. Environ. Sci. Technol. 55, 10471–10479. https://doi.org/10.1021/acs.est.1c01753

Lima, A.R.A., Silva, M.D., Possatto, F.E., Ferreira, G.V.B., Krelling, A.P., 2020. Plastic Contamination in Brazilian Freshwater and Coastal Environments: A Source-to-Sea Transboundary Approach, in: Handbook of Environmental Chemistry. pp. 427–460. https://doi.org/10.1007/698_2020_514

Lins-Silva, N., Marcolin, C.R., Kessler, F., Schwamborn, R., 2021. A fresh look at microplastics and other particles in the tropical coastal ecosystems of Tamandaré, Brazil. Mar. Environ. Res. 169, 105327. https://doi.org/10.1016/j.marenvres.2021.105327

Lira, A.S., Lucena-Frédou, F., Le Loc'h, F., 2021. How the fishing effort control and environmental changes affect the sustainability of a tropical shrimp small scale fishery. Fish. Res. 235, 105824. https://doi.org/10.1016/j.fishres.2020.105824

Macieira, R.M., Aparecida Silva Oliveira, L., Cardozo-Ferreira, G.C., Ribeiro Pimentel, C., Andrades, R., Luiz Gasparini, J., Sarti, F., Chelazzi, D., Cincinelli, A., Carvalho Gomes, L., Giarrizzo, T., 2021. Microplastic and artificial cellulose microfibers ingestion by reef fishes in the Guarapari Islands, southwestern Atlantic. Mar. Pollut. Bull. 167. https://doi.org/10.1016/j.marpolbul.2021.112371

Margallo, M., Ziegler-Rodriguez, K., Vázquez-Rowe, I., Aldaco, R., Irabien, Á., Kahhat, R., 2019. Enhancing waste management strategies in Latin America under a holistic environmental assessment perspective: A review for policy support. https://doi.org/10.1016/j.scitotenv.2019.06.393

Markic, A., Gaertner, J.-C., Gaertner-Mazouni, N., Koelmans, A.A., 2020. Plastic ingestion by marine fish in the wild. Crit. Rev. Environ. Sci. Technol. 50, 657–697. https://doi.org/10.1080/10643389.2019.1631990

Meijer, L.J.J., van Emmerik, T., van der Ent, R., Schmidt, C., Lebreton, L., 2021. More than 1000 rivers account for 80% of global riverine plastic emissions into the ocean. Sci. Adv. 7, eaaz5803. https://doi.org/10.1126/sciadv.aaz5803

Monteiro, R.C.P., Do Sul, J.A.I., Costa, M.F., 2020. Small microplastics on beaches of fernando de noronha island, tropical atlantic ocean. Ocean Coast. Res. 68, 1–10. https://doi.org/10.1590/S2675-28242020068235

Moore, C.J., 2008. Synthetic polymers in the marine environment: A rapidly increasing, long-term threat. Environ. Res. 108, 131–139. https://doi.org/10.1016/j.envres.2008.07.025

Müller, C., 2021. Not as Bad as It Seems? A Literature Review on the Case of Microplastic Uptake in Fish. Front. Mar. Sci. 8. https://doi.org/10.3389/fmars.2021.672768

Neto, J.G.B., Rodrigues, F.L., Ortega, I., Rodrigues, L. dos S., Lacerda, A.L. d. F., Coletto, J.L., Kessler, F., Cardoso, L.G., Madureira, L., Proietti, M.C., 2020. Ingestion of plastic debris by commercially important marine fish in southeast-south Brazil. Environ. Pollut. 267. https://doi.org/10.1016/J.ENVPOL.2020.115508

Pegado, T. de S. e. S., Schmid, K., Winemiller, K.O., Chelazzi, D., Cincinelli, A., Dei, L., Giarrizzo, T., 2018. First evidence of microplastic ingestion by fishes from the Amazon River estuary. Mar. Pollut. Bull. 133, 814–821. https://doi.org/10.1016/j.marpolbul.2018.06.035

Pelage, L., Domalain, G., Lira, A.S., Travassos, P., Frédou, T., 2019. Coastal Land Use in Northeast Brazil: Mangrove Coverage Evolution Over Three Decades. Trop. Conserv. Sci. 12. https://doi.org/10.1177/1940082918822411

Pinheiro, L.M., Agostini, V.O., Lima, A.R.A., Ward, R.D., Pinho, G.L.L., 2021. The fate of plastic litter within estuarine compartments: An overview of current knowledge for the transboundary issue to guide future assessments. Environ. Pollut. https://doi.org/10.1016/j.envpol.2021.116908

Possatto, F.E., Barletta, M., Costa, M.F., Ivar do Sul, J.A., Dantas, D. V., 2011. Plastic debris ingestion by marine catfish: An unexpected fisheries impact. Mar. Pollut. Bull. 62, 1098–1102. https://doi.org/10.1016/j.marpolbul.2011.01.036

Pozo, K., Gomez, V., Torres, M., Vera, L., Nuñez, D., Oyarzún, P., Mendoza, G., Clarke, B., Fossi, M.C., Baini, M., Přibylová, P., Klánová, J., 2019. Presence and characterization of microplastics in fish of commercial importance from the Biobío region in central Chile. Mar. Pollut. Bull. 140, 315–319. https://doi.org/10.1016/j.marpolbul.2019.01.025

Ramos, J.A.A., Barletta, M., Costa, M.F., 2012. Ingestion of nylon threads by gerreidae while using a tropical estuary as foraging grounds. Aquat. Biol. 17, 29–34. https://doi.org/10.3354/ab00461

Ribeiro-Brasil, D.R.G., Torres, N.R., Picanço, A.B., Sousa, D.S., Ribeiro, V.S., Brasil, L.S., Montag, L.F. de A., 2020. Contamination of stream fish by plastic waste in the Brazilian Amazon. Environ. Pollut. 266. https://doi.org/10.1016/j.envpol.2020.115241

Savoca, M.S., McInturf, A.G., Hazen, E.L., 2021. Plastic ingestion by marine fish is widespread and increasing. Glob. Chang. Biol. 27, 2188–2199. https://doi.org/10.1111/gcb.15533

Teuten, E.L., Rowland, S.J., Galloway, T.S., Thompson, R.C., 2007. Potential for plastics to transport hydrophobic contaminants. Environ. Sci. Technol. 41, 7759–7764. https://doi.org/10.1021/es071737s

Thompson, R.C., Olsen, Y., Mitchell, R.P., Davis, A., Rowland, S.J., John, A.W.G., McGonigle, D., Russell, A.E., 2004. Supporting material: Lost at sea: where is all the plastic? Science 304, 838.

Van Sebille, E., Aliani, S., Law, K.L., Maximenko, N., Alsina, J.M., Bagaev, A., Bergmann, M., Chapron, B., Chubarenko, I., Cózar, A., Delandmeter, P., Egger, M., Fox-Kemper, B., Garaba, S.P., Goddijn-Murphy, L., Hardesty, B.D., Hoffman, M.J., Isobe, A., Jongedijk, C.E., Kaandorp, M.L.A., Khatmullina, L., Koelmans, A.A., Kukulka, T., Laufkötter, C., Lebreton, L., Lobelle, D., Maes, C., Martinez-Vicente, V., Morales Maqueda, M.A., Poulain-Zarcos, M., Rodríguez, E., Ryan, P.G., Shanks, A.L., Shim, W.J., Suaria, G., Thiel, M., Van Den Bremer, T.S., Wichmann, D., 2020. The physical oceanography of the transport of floating marine debris. Environ. Res. Lett. https://doi.org/10.1088/1748-9326/ab6d7d

Vendel, A.L., Bessa, F., Alves, V.E.N., Amorim, A.L.A., Patrício, J., Palma, A.R.T., 2017. Widespread microplastic ingestion by fish assemblages in tropical estuaries subjected to anthropogenic pressures. https://doi.org/10.1016/j.marpolbul.2017.01.081

Waller, C.L., Griffiths, H.J., Waluda, C.M., Thorpe, S.E., Loaiza, I., Moreno, B., Pacherres, C.O., Hughes, K.A., 2017. Microplastics in the Antarctic marine system: An emerging area of research. Sci. Total Environ. https://doi.org/10.1016/j.scitotenv.2017.03.283

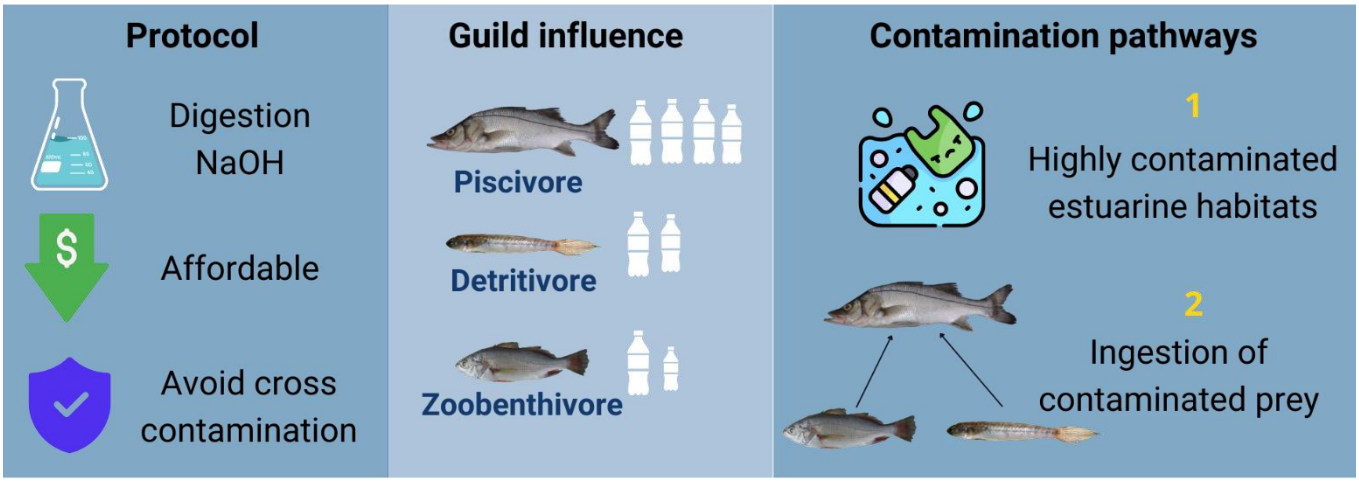
Zalasiewicz, J., Waters, C.N., Ivar do Sul, J.A., Corcoran, P.L., Barnosky, A.D., Cearreta, A., Edgeworth, M., Gałuszka, A., Jeandel, C., Leinfelder, R., McNeill, J.R., Steffen, W., Summerhayes, C., Wagreich, M., Williams, M., Wolfe, A.P., Yonan, Y., 2016. The geological cycle of plastics and their use as a stratigraphic indicator of the Anthropocene. Anthropocene 13, 4–17. https://doi.org/10.1016/j.ancene.2016.01.002

CHAPTER 1

Article "Microplastic contamination in tropical fishes: an assessment of different feeding habits"

Manuscript submitted on 27 December 2020 and published on 27 May 2021 in the Regional Studies in Marine Science.

https://doi.org/10.1016/j.rsma.2021.101857



Microplastic contamination in tropical fishes: an assessment of different feeding habits

Anne K. S. Justino [1,2], Véronique Lenoble [2], Latifa Pelage [1], Guilherme V. B Ferreira [1], Rafaela Passarone [1], Thierry Frédou [1], Flávia Lucena Frédou [1]

[1] Departamento de Pesca e Aquicultura da Universidade Federal Rural de Pernambuco – DEPAQ/UFRPE, Rua Dom Manuel de Medeiros, s/n, 52171-900, Recife, Brasil.

[2] Université de Toulon, Aix Marseille Univ., CNRS/INSU, IRD, MIO UM 110, Mediterranean Institute of Oceanography, CS 60584, 83041 – TOULON, France

Corresponding author: anne.karen@hotmail.com

Abstract

Marine ecosystems are reported to be contaminated by microplastics (MPs) (< 5 mm); however, the ecological mechanisms involved in the ingestion of debris by marine organisms are relatively unknown. By developing and optimising an appropriate protocol of gut digestion for fish species, this study explores a tropical estuarine environment to unriddle the processes responsible for the different ingestion rates of plastic debris. A total of 82 fishes with different feeding habits were analysed, *Centropomus undecimalis* (n = 30; Piscivore), *Bairdiella ronchus* (n = 21; Zoobenthivore) and *Gobionellus stomatus* (n = 31; Detritivore). The microplastic ingestion varied with the feeding strategy; *C. undecimalis*, the predator, was the most contaminated species. Overall, most MPs were fibres (47%), followed by pellets (40%) and fragments (13%), although these proportions varied among species. A high level of contamination was found in the Estuarine Complex of Santa Cruz Channel, Northeast of Brazil, with many potential input sources of MPs to the estuary, which likely accumulates in the sediment and water column, with unknown consequences for human health.

Keywords: Trophic transfer, Plastic extraction, Trophic guild, Plastic pollution, Tropical Atlantic

Introduction

Estuaries are well known for providing ecosystem services by supplying essential goods (raw materials and food) and offering an attractive environment for population growth and cultural activities (Atkins et al., 2011). In addition, they carry out important ecological functions since fishes use these environments for protection, feeding, reproduction, settlement, and nursery (Ferreira et al., 2019; Krumme et al., 2008; Lima and Barletta, 2016; Potter et al., 2013; Ramos et al., 2016). Worldwide, estuaries are usually surrounded by large metropolises, and the expansion of the urban population is directly associated with impacts on the coastal ecosystems (Freeman et al., 2019). Plastic pollution is one of the most significant environmental problems of the 21st century since large quantities of these materials are being mismanaged and/or illegally disposed of in marine ecosystems (Dauvergne, 2018; Ostle et al., 2019). Once in the environment, these plastic materials are weakened by natural processes (*e.g.*, hydrodynamic forces, solar radiation, and biological actions) (Jambeck et al., 2015; Thompson et al., 2004) and fragmented into smaller parts known as microplastics (< 5 mm).

Plastic debris poses several risks to marine biota (Galloway et al., 2017). Ingestion can be hazardous, causing digestive injuries, decreasing predatory efficiency, or inducing toxic effects (Barboza et al., 2018; de Sá et al., 2015; Moore, 2008; Teuten et al., 2007). Microplastics can adsorb pollutants available in the water column, such as persistent organic pollutants (POPs) (Frias et al., 2010; Oehlmann et al., 2009; Rochman et al., 2013) or heavy metals (Ashton et al., 2010; Holmes et al., 2012), which may be further bioaccumulated and biomagnified in the food web (Batel et al., 2016; Teuten et al., 2009). Furthermore, microplastics may be transferred in the trophic chain by predating contaminated prey (Chagnon et al., 2018; Ferreira et al., 2016, 2019). The trophic transfer has already been pointed out as a relevant contamination mechanism for estuarine species (Athey et al., 2020).

Although the long-term effects of microplastic contamination are still unknown, the scientific community continually emphasises the importance of using reliable and replicable methods of investigations (Hermsen et al., 2018; Markic et al., 2020). A useful approach to obtain plastic in marine wildlife is based on chemical digestion protocols, which are efficient and low cost to work with (Karami et al., 2017; Kühn et al., 2017). Digestion protocols are a practical and secure way to extract and isolate microplastics in organisms, being widely used in the investigation of fish contamination by plastics (Bellas et al., 2016; Bessa et al., 2018; Foekema et al., 2013; Hermsen et al., 2017; Herrera et al., 2019; Pellini et al., 2018; Su et al., 2019; Tanaka and Takada, 2016). However, the lack of security procedures (*e.g.,* cleaned workroom and blanks procedures) during the implementation of digestion protocols may lead to the overestimation of contaminants since samples are more prone to airborne and cross-contamination (Hermsen et al., 2018, 2017; Torre et al., 2016). Moreover, the procedure must ensure data reliability through an effective and careful extraction of the microplastics and the implementation of a robust sample size with a minimum of 10 samples (Markic et al., 2020).

Several studies conducted in South Atlantic estuaries evaluated the ingestion of plastics by marine organisms, mammals (Attademo et al., 2015), mussels (Birnstiel et al., 2019; Santana et al., 2016), turtles (Guebert-Bartholo et al., 2011), and microplastics interactions with ichthyoplankton (Lima et al., 2016), and fish assemblages (Vendel et al., 2017). However, few studies investigated the ecological and biological dynamics associated with microplastic intakes on wild fishes (Amorim et al., 2020; Dantas et al., 2020; Ferreira et al., 2018, 2016). Furthermore, the bioaccumulation of microplastics in marine species is highly influenced by feeding strategies (Miller et al., 2020). However, the possible correlation between feeding habits and MPs ingestion is not yet well known.

Three estuarine species were chosen to test our hypothesis that microplastic ingestion varies according to the feeding strategies: (1) *Centropomus undecimalis* (Bloch, 1792); (2) *Bairdiella ronchus* (Cuvier, 1830); and (3) *Gobionellus stomatus* (Starks, 1913). *C. undecimalis* is an essential economic living resource for the commercial and subsistence fisheries in South America (Carpenter, 2002). While adults of *C. undecimalis* inhabit coastal areas and migrate towards the estuary, their juvenile stage uses the estuarine areas as a nursery ground (Ferreira et al., 2019). *C. undecimalis* is classified as opportunistic predator, feeding on a large variety of available preys in the environment, with a piscivorous tendency (Ferreira et al., 2019; Lira et al., 2017). *B. ronchus* is classified as zoobenthivore (Ferreira et al., 2019), which preys on invertebrates associated with the sediment (Elliott et al., 2007). *G. stomatus* is a detritivore fish (Ferreira et al., 2019) consuming detritus and microphytobenthos (Elliott et al., 2007). Although *B. ronchus* and *G. stomatus* were not of economic importance, they play a significant ecological role within the estuarine ecosystem and are the main energy source for the *C. undecimalis* (Gonzalez et al., 2019; Lira et al., 2018).

Understanding the role of microplastics as a component of anthropic pollution in this ecosystem is crucial to assess adverse impacts on regional biodiversity and the quality of fisheries resources that are being traded and consumed. Based on this information, the present study aims to (*i*) apply an adapted extraction protocol to assess MPs in the digestive tract of fishes, assuring the integrated quality control and appropriate sampling size, (*ii*) describe microplastics contamination in fishes in estuarine waters, and (*iii*) identify the main types of microplastics considering the different feeding strategies.

Materials and Methods

Study area

The Estuarine Complex of the Santa Cruz Channel (ECSC) (Fig.1) is located along the northeast Brazilian coast. The climate is classified as tropical, hot and humid, with an average of 26ºC (±2.8ºC) annual air temperature and two seasons defined according to the level of precipitation (rainy and dry seasons) (Medeiros et al., 2001).

The ECSC is a tidal channel that surrounds the Itamaracá Island, separating it from the mainland, with a total area of 22 km, a maximum width of 1.5 km and a depth between 4 and 5 m (Lira et al., 2017). The main tributaries are formed by the Arataca, Botafogo and Igarassu Rivers, and the predominant vegetation is the mangrove forest (Medeiros et al., 2001). The ECSC is surrounded by two cities (Itapissuma and Itamaracá), which have their economies mainly focused on the industrial and agricultural sectors (IBGE, 2011), whereas the local economy is primarily supported by artisanal fisheries, aquaculture, and tourism (de Moura et al., 2009).

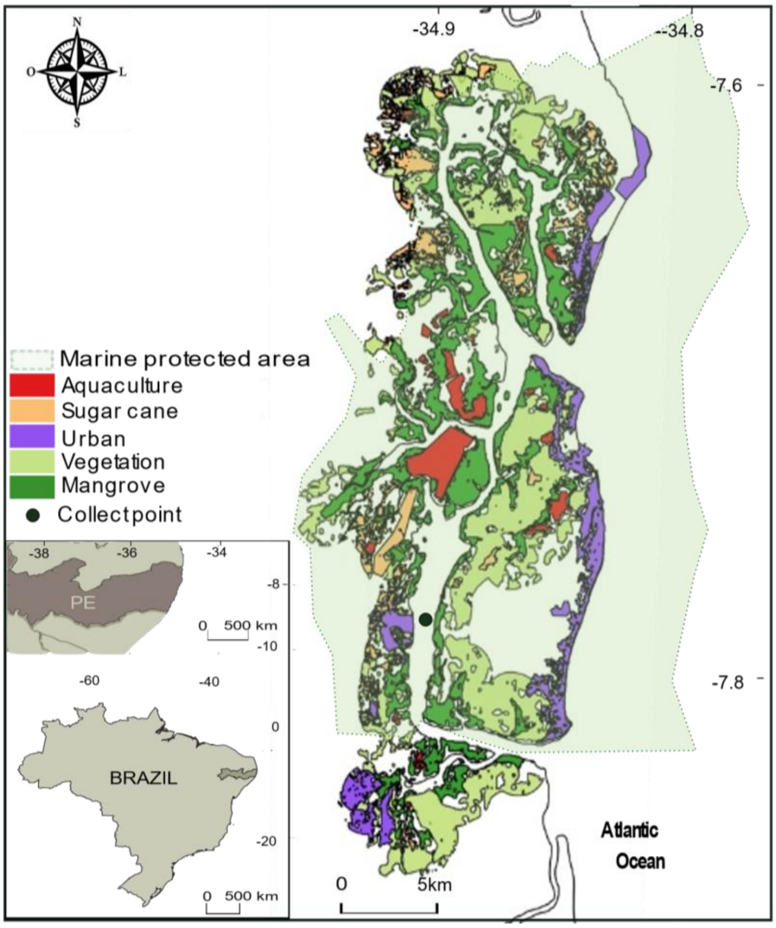


Figure 1. Map of the Estuarine Complex of the Santa Cruz Channel (ECSC) adapted from Pelage et al., 2019.

Sampling and Laboratory procedures

Three demersal species were selected for this study, given their commercial and or subsistence importance for riverine populations and their ecological interactions in the trophic chain (Lira et al., 2018, 2017): *Gobionellus stomatus*, *Bairdiella ronchus*, and *Centropomus undecimalis*, classified as Detritivores, Zoobenthivores and Piscivores respectively, according to Ferreira et al. (2019) for the area. For the common Snook *C. undecimalis*, individuals were collected in the juvenile stage (Total Length < 26.3 cm) (Ferreira et al., 2019), ensuring that the obtained specimens had not left the estuarine ecosystem. This procedure was carried out to ensure that contaminants' intake occurred within the estuarine boundary since adults of *C. undecimalis* perform migrations toward coastal areas. As *B. ronchus* and *G. stomatus* are not caught outside the estuary of ECSC (Ferreira et al., 2019), we did not fix these species' ontogeny, and juveniles and adults were analysed. A total of 82 individuals were obtained by local fishermen. After each sampling, the specimens were labelled, and the individuals were frozen. In the laboratory, individuals were identified (Menezes & Figueiredo, 2000), measured and weighted. Each individual had their organs (stomach and intestine) carefully removed, weighed and stored again for the digestion analysis. The minimum sample size for each species was 20 individuals to avoid the bias of a low sample size, which is twice the number suggested by Markic et al. (2020).

Quality control and Extraction protocol

Firstly, to guarantee quality control and avoid potential airborne contamination, several steps were implemented. The entire process of digestion, filtration, identification and storage of the microplastic samples was carried out in a cleaned and reserved room within the main laboratory, reserved only for microplastic analysis. The flow of people was limited; cotton lab coats and disposable latex gloves were worn during the entire process. Also, all used tools were previously cleaned with alcohol 70% and rinsed with filtered distilled water. The solutions utilised in the various procedures were filtered using a vacuum pump system (equipped with laboratory glassware) through a 47 mm GF/F 0.7 µm glass fibre filter (Whatman).

The digestive tracts were rinsed with distilled water before being placed in a beaker, submerged in NaOH (1 mol/L; PA 97%) solution, and covered by a glass lid (Fig. 2). The entire digestive tracts were submitted to NaOH without further dissection of those organs to avoid airborne contamination during handling. The proportion used was 1:100 w/v for 1 g of digestive tract weight, 100 ml of NaOH (1 mol/L) solution. The mixture was oven-dried at 60° C for 24 h, mixed from time to time with a glass stick. The samples digested in the previous step, and the procedural blanks were filtered using a vacuum pump system through a 47 mm glass fibre filter (GF/F 0.7 µm Whatman). After filtration, filters were carefully set in a Petri dish (47 mm diameter) and covered. These filters were oven-dried at 60° C for 24 h. The microplastics were identified using a stereomicroscope (Zeiss Stemi 508) with 6.3 – 50 times magnification with a detection limit of 20 µm, photographed (Axiocam 105 Color), measured (Zeiss Zen 3.2) from the filter and stored in covered Petri dishes (Fig. 2). They were then categorised by type: (*i*) fibres (filamentous shape), (*ii*) fragments (irregular shape) or (*iii*) pellets (spherical shape). The digestion protocol is a useful tool to separate the organic materials and facilitates visual identification, although it is not sufficient for identifying the polymers. Thereby, we also applied the method described by Ferreira et al. (2019) to confirm plastic debris by drying the samples in an oven to verify whether their physical characteristics changed or not.

Procedural blanks were made for each day of analysis before beginning the sample digestion. For blanks, a beaker was filled with 50 ml of NaOH (1 mol/L) solution and covered with a glass lid, and these blanks were exposed to the same protocol applied to the samples. A total of 10 blanks were made; among them, four blanks were observed with eight tiny particles

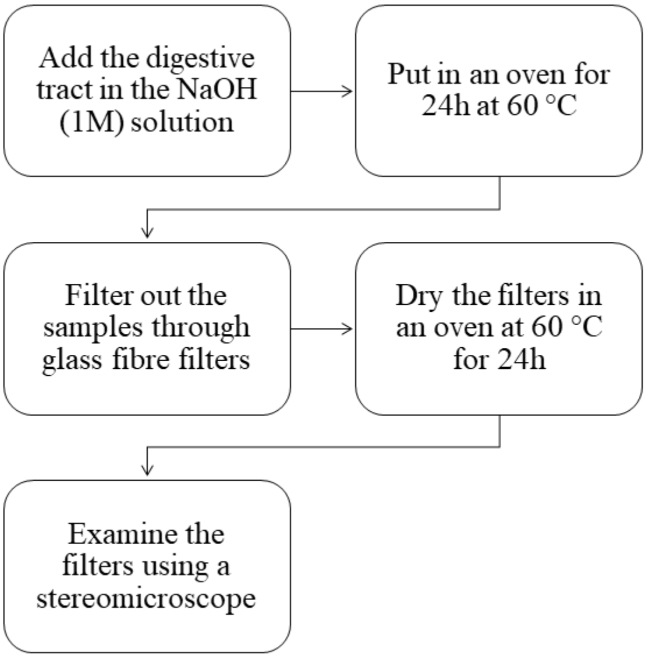


Figure 2. Flowchart with the stepwise of the extraction protocol.

(<100 µm) considered as paint fragments. Thereby, all particles further identified with any resemblance to those observed in the blanks were excluded from posterior analyses.

Data Analysis

Kruskal-Wallis tests were used to verify if ingested microplastics (total MPs and different types) presented significant differences in number and length in relation to species (*C. undecimalis*, *B. ronchus* and *G. stomatus*). When the Kruskal-Wallis showed significant differences, the *post hoc* pairwise comparisons Dunn's test was performed to investigate the sources of variance (Dunn, 1964). All statistical analyses were carried out with the software R version 3.6.3 (R Core Team, 2020) and were conducted considering a level of significance of 5%.

Results

A total of 30 individuals of *Centropomus undecimalis*, 31 *Gobionellus stomatus*, 21 *Bairdiella ronchus* were analysed. Microplastics were present in 77% of *C. undecimalis*, 74% of *G. stomatus*, and 67% of *B. ronchus* individuals. A total of 176 particles of MPs were recovered from 82 fishes (Table I).

According to the number of MPs, ingestion significantly differed between species (*p-value* ≤ 0.05), *C. undecimalis* being the most contaminated (3.3 ± 2.9 MPs fish^{-1}), followed by *G. stomatus* (1.7 ± 1.5 MPs fish^{-1}) and *B. ronchus* (1.2 ± 1.3 MPs fish^{-1}) (Fig. 3). Significant differences were recorded between *C. undecimalis* and *B. ronchus* (chi-squared = 7.873, df = 2, *p-value* ≤ 0.05). Concerning the length of ingested MPs, no significant differences among the same type of MPs were observed when comparing MPs' size between species.

Regarding the types of MPs ingested by fishes, most were fibres (47%), followed by pellets (40%) and fragments (13%), and proportions varied between the species. *C. undecimalis* registered 68% of pellets, 28% of fibres, and 4% of fragments, *B. ronchus* and *G. stomatus* registered 23% and 4% of pellets, 62% and 71% of fibres and 15% and 25% of fragments, respectively (Fig. 4 and 5).

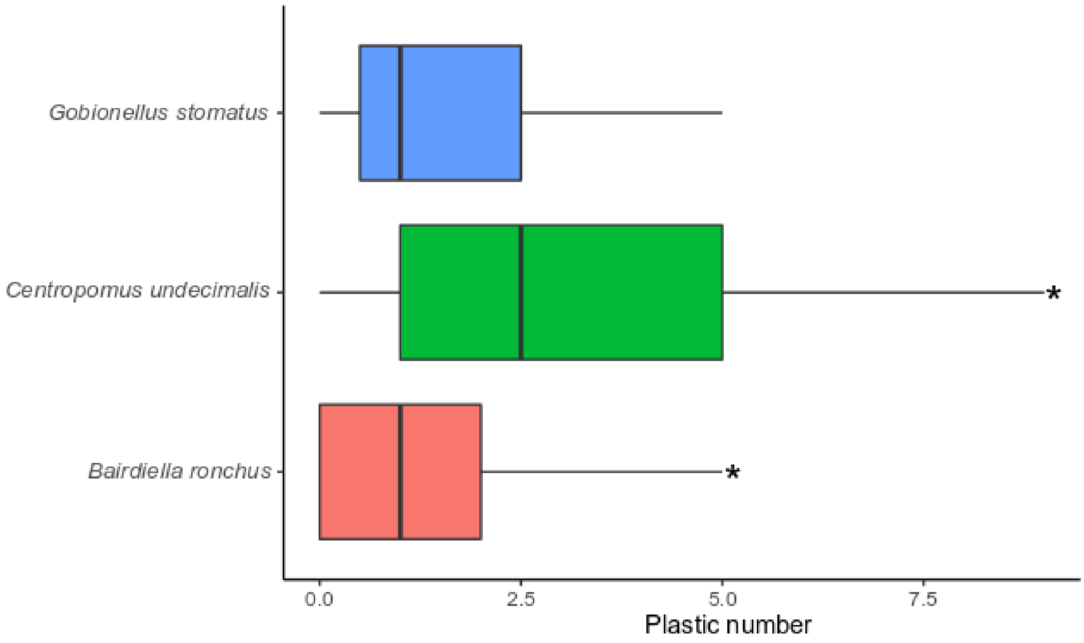


Figure 3. Mean number of microplastics ingested by fishes collected in the Estuarine Complex of the Santa Cruz Channel. The asterisks represent the statistical differences with a significance of 0.05.

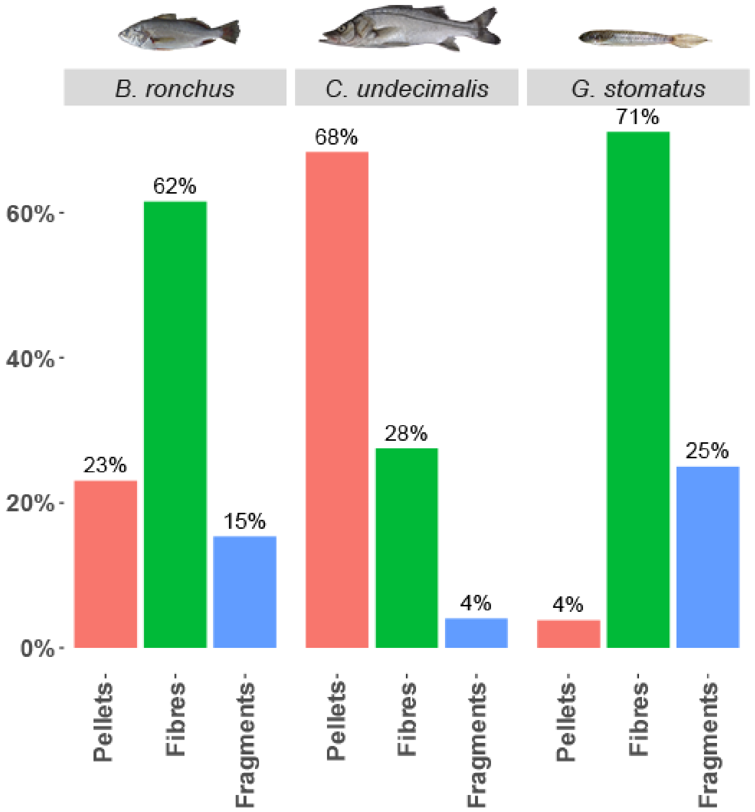


Figure 4. Different types of microplastics (fibres, fragments, and pellets) ingested by fish species, expressed as a percentage.

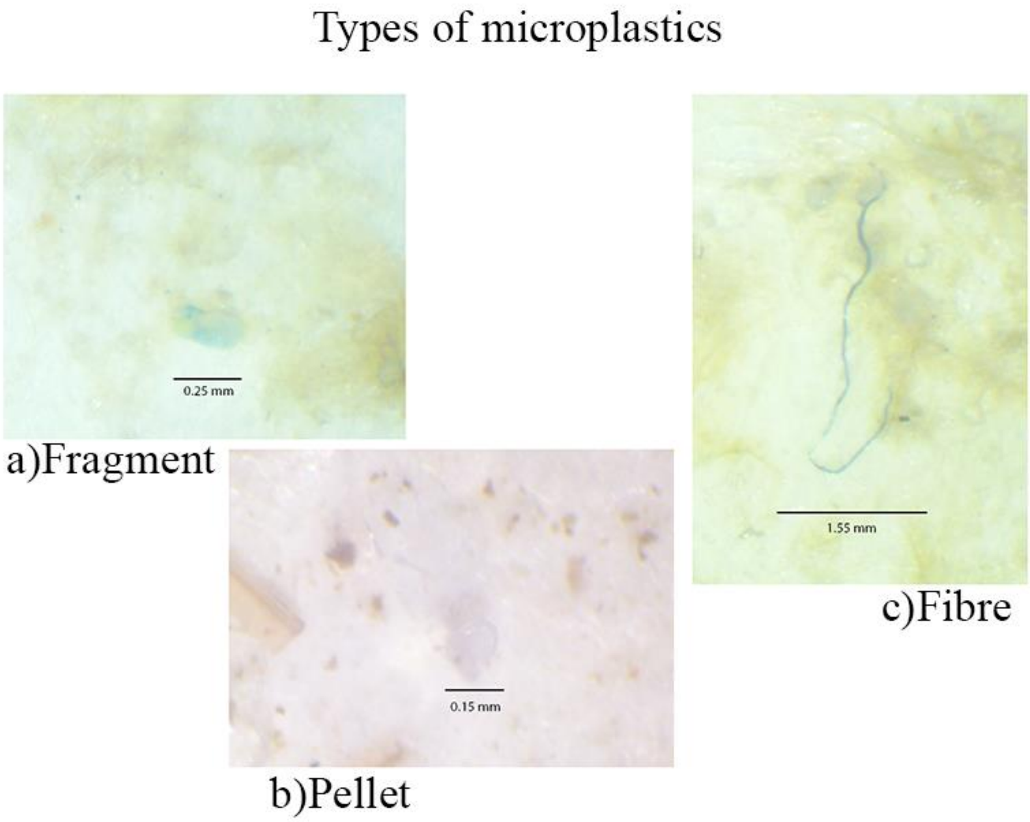


Figure 5. Types of microplastics ingested by fishes of the estuarine complex of Santa Cruz Channel. a) fragment (irregular shape); b) pellet (spherical shape); and c) fibre (filamentous shape).

Family/Species	Ecological parameters			Biometry			Microplastics occurrence		
	N	Habitat use	Feeding habit	TL (cm) Min-Max	SL (cm) Min-Max	TW (g) Min-Max	MPs	FO%	TL (mm) Min-Max
Gobiidae									
Gobionellus stomatus (Starks, 1913)	31	Demersal	DV	9.3 - 11.9	7.0 - 9.0	3.78 - 7.89	52	67	0.02 - 5.00
Sciaenidae									
Bairdiella ronchus (Cuvier, 1830)	21	Demersal	ZB	12.5 - 16.5	10.0 - 14.0	22.2 - 62.4	26	74	0.11 - 3.35
Centropomidae									
Centropomus undecimalis (Bloch, 1792)	30	Demersal	PV	8.9 - 14.0	7.3 - 11.0	5.8 - 16.78	98	77	0.06 - 3.79

Table I. The ecological parameters and biological aspects of the analysed species. Feeding habit: DV (Detritivores), ZB (Zoobenthivores) and PV (Piscivores) obtained for the area by Ferreira et al. (2019). TL (Total Length); SL (Standard Length); TW (Total Weight); FO% (Frequency of occurrence).

Discussion

Quality Assurance and Quality Control

In our study, we applied an analytical method to extract microplastics (MPs) in the digestive tract of fishes, involving a careful procedure of quality control, using sodium hydroxide (NaOH 1 M), and sample size with a minimum of 20 individuals for each species, following the recent recommendations of Markic et al. (2020) and Hermsen et al. (2018).

The application of digestion protocols to extract microplastics in marine biota has been growing worldwide (Lusher et al., 2017). Digestion protocol is a reliable method for isolating microplastics in the biota, facilitating the observation of non-organic materials. For fish, the most common method used is alkaline digestion. Indeed, for many authors, this is considered the most suitable method to remove organic material and isolate plastic debris (Karami et al., 2017; Kühn et al., 2017; Schirinzi et al., 2020). Although the efficiency of potassium hydroxide (KOH 10%) has been more often tested, the use of sodium hydroxide (NaOH 1M) has also been widely tested and demonstrated to be very useful (Baalkhuyur et al., 2018; Bellas et al., 2016; Budimir et al., 2018; Morgana et al., 2018; Su et al., 2019; Wieczorek et al., 2018), and ensures polymer integrity after chemical digestion (Budimir et al., 2018).

Although adaptations of digestion protocol have been carried out around the world (Karami et al., 2017; Kühn et al., 2017), so far, only three studies in estuaries of South America have used a chemical digestion protocol for microplastics extraction in the digestive tract of fishes, with the implementation of adequate quality assurance and quality control (QA/QC) (Arias et al., 2019; Garcés-Ordóñez et al., 2020; Ribeiro-Brasil et al., 2020). Implementing QA/QC procedures is essential to avoid cross-contamination of microplastic samples (Hermsen et al., 2017; Lusher et al., 2017; O'Connor et al., 2020). This treatment is necessary to minimise over/underestimation of microplastics due to airborne contamination and or loss of particles during sample handling. Moreover, the sample size is also an essential factor in the analysis. Among the studies in South America, only Arias et al. (2019) have chosen a sample size $n > 10$. Such bias is unlikely to occur in our study, where the sample size was the highest in South America. Therefore, our results are expected to adequately reflect the contamination in the Estuarine Complex of Santa Cruz Channel (ECSC), and the protocol here used can safely be replicated in other studies.

Microplastic ingestion by fishes

In our study, microplastic (MP) contamination rates were high (frequency of occurrence of 73%) for the three analysed demersal estuarine species. Overall, demersal estuarine species are frequently reported to have a high ingestion rate of MPs (Arias et al., 2019; Ferreira et al., 2019, 2016). Demersal species inhabit and feed on the fauna associated with the substrate (Elliott et al., 2007), which might be in direct contact with contaminated sediment. Indeed, the estuarine sediment is a significant accumulation zone for MPs (Zhang, 2017). As estuaries are a transitional ecosystem, often surrounded by urban areas and exposed to domestic sewage discharge, they receive contaminants of both riverine and tidal inputs (Lebreton et al., 2017). Thereby, these ecosystems are more prone to be contaminated by MPs, especially fibres (Bessa et al., 2018; Browne et al., 2011). In addition to the sewage discharge, fibres can also be originated by the use, maintenance, discarding and loss of fisheries gear (Lima et al., 2014). MPs fibres occurred the most in the estuarine bottom, possibly due to the rapid sinking of these types of MPs (Lima et al., 2014).

Overall, the most ingested MP type were fibres, as observed in most of the studies worldwide (Bellas et al., 2016; Bessa et al., 2018; Foekema et al., 2013; Herrera et al., 2019; Wright et al., 2013). Although all types of plastics were ingested by the species analysed in this study, ingestion rates varied. Fibres were the most frequent type in *G. stomatus* (representing 71% of the ingested MPs) and *B. ronchus* (68%). *G. stomatus* and *B. ronchus* are detritivore and zoobenthivore species, respectively, depending on the organisms associated with the substrate or the organic matter available. Consequently, they are more vulnerable to the MPs fibres contaminating estuarine sediments. However, pellets were the most frequent type in *C. undecimalis* (68%), differently from the results observed by Ferreira et al. (2019) for the same species, which registered mostly ingestion of filaments. Pellets also dominated the diet of fish along the coast of Salvador (Brazil) (Miranda and de Carvalho-Souza, 2016) and from the Amazon estuary (Pegado et al., 2018). Different from the other types of MP ingested, pellets are primary microplastics (Fendall and Sewell, 2009), which are manufactured as MPs mainly for the cosmetics industry (*e.g.*, microbeads). This type of MP can be accidentally discharged into the environment during the transport of this raw material (Ogata et al., 2009) or by the release of domestic sewage (Tanaka and Takada, 2016). The increase in urbanisation in the Santa Cruz Channel (Pelage et al., 2019) surely amplified the sewage discharge in this area.

Pellets can be found floating in the water column, and they can even have fish eggs attached to them (Ivar Do Sul and Costa, 2014). Predatory fishes such as *C. undecimalis* can ingest the pellets directly by confusing them with their natural prey. Moreover, when feeding, opportunistic predators ingest a large amount of prey, which might increase the momentary build-up of MPs particles prior to egestion.

Predator species are more vulnerable to microplastic contamination due to the trophic transference, which occurs when they ingest contaminated prey (Chagnon et al., 2018; Eriksson and Burton, 2003; Ferreira et al., 2019, 2016; Nelms et al., 2018). Consequently, as we observed among the three analysed demersal fish species, the predator, *C. undecimalis,* had the highest contamination rate (3.3 ± 2.9 MPs fish^{-1}) despite being in its early life stage (juvenile), followed by *G. stomatus* (1.7 ± 1.5 MPs fish^{-1}) and *B. ronchus* (1.2 ± 1.3 MPs fish^{-1}). Our study corroborated previous studies hypothesising that microplastic ingestion varies with the different feeding strategies (Ferreira et al., 2018, 2016; Mizraji et al., 2017). However, regardless of the diet preferences of the species, in our study area, there are several potential input sources of MPs contaminants, which probably accumulate in the sediment and water column, negatively affecting the life-strategies of fish species and mostly the juvenile stages which utilise estuaries as a nursery ground. Thus, the predators are more prone to be contaminated by microplastics through two main exposure routes: (1) the highly contaminated estuarine habitats and (2) the ingestion of contaminated prey.

Anthropogenic activities in the ECSC (urban areas, manufacturers, aquaculture plants, and sugarcane fields) are found surrounding the whole floodplain, and these activities might be an important source of microplastics and other contaminants (*e.g.*, pesticides and heavy metals). Indeed, the ECSC has registered many impacts such as habitat loss and mercury releases (Albuquerque et al., 2019; Araújo et al., 2019; Pelage et al., 2019), which likely affect the estuarine community. Besides, our study has identified high contamination by microplastics in fish species that are a relevant source of protein locally and regionally. Despite being a Marine Protected Area, which provides essential ecosystems services, there is a lack of awareness and public policies, highlighting the importance of monitoring and management policies to control and mitigate social and health problems.

Further studies regarding the microplastic impacts on marine fauna and whether they could transfer adsorbed pollutants such as persistent organic pollutants (POPs), heavy metals, and plastic additives to the food web are necessary, as microplastics particles can be transferred along the trophic chain, the chances to accumulate other pollutants in the food web increases. In fishery resources, this question is a public health matter because it is linked to human uptake of these pollutants. Our study also emphasises the importance of implementing protocols to extract microplastics in biological samples, which guarantee the quality of samples, avoid under or overestimation and airborne contamination, and which can be easily replicable.

Acknowledgement

This study was supported by the LMI TAPIOCA program CAPES/COFECUB (88881.142689/2017-01); FUNBIO and Humanize under the grant “Programa Bolsas Funbio - Conservando o Futuro 2019 (02/2019)”; FACEPE (Fundação de Amparo à Ciência e Tecnologia do Estado de Pernambuco); and MicroplastiX Project JPI-Oceans/CONFAP/FACEPE (n°APQ-0035-1.08/19). We thank the CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico), which provided research grants to Flávia Lucena Frédou (CNPq n° 308554/2019-1) and Thierry Frédou (CNPq 307422/2020-8); FACEPE for granting a scholarship to Guilherme V. B. Ferreira (BFP-0049-5.06/20) and CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior) for granting a scholarship at doctoral level for the first author. All authors thank the members of the laboratory BIOIMPACT for the support in the sampling procedures and the fishermen of Itapissuma for the sampling collect.

REFERENCES

Albuquerque, P.T.F., Frédou, T., Arruda, G.N., Filho, C.A.S., Nascimento, A.F., da Silva, M.J., De França, E.J., 2019. Tracking Hg historical inputs by Pb-210 geochronology for the Itapessoca Estuarine Complex, Pernambuco, Brazil. J. Radioanal. Nucl. Chem. 321, 875–883. https://doi.org/10.1007/s10967-019-06665-9

Amorim, A.L.A. de, Ramos, J.A.A., Nogueira Júnior, M., 2020. Ingestion of microplastic by ontogenetic phases of Stellifer brasiliensis (Perciformes, Sciaenidae) from the surf zone of tropical beaches. Mar. Pollut. Bull. 158, 111214. https://doi.org/10.1016/j.marpolbul.2020.111214

Araújo, P.R.M., Biondi, C.M., do Nascimento, C.W.A., da Silva, F.B.V., Alvarez, A.M., 2019. Bioavailability and sequential extraction of mercury in soils and organisms of a mangrove contaminated by a chlor-alkali plant. Ecotoxicol. Environ. Saf. 183, 109469. https://doi.org/10.1016/j.ecoenv.2019.109469

Arias, A.H., Ronda, A.C., Oliva, A.L., Marcovecchio, J.E., 2019. Evidence of Microplastic Ingestion by Fish from the Bahía Blanca Estuary in Argentina, South America. Bull. Environ. Contam. Toxicol. 102, 750–756. https://doi.org/10.1007/s00128-019-02604-2

Ashton, K., Holmes, L., Turner, A., 2010. Association of metals with plastic production pellets in the marine environment. Mar. Pollut. Bull. 60, 2050–2055. https://doi.org/10.1016/j.marpolbul.2010.07.014

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

Atkins, J.P., Burdon, D., Elliott, M., Gregory, A.J., 2011. Management of the marine environment: Integrating ecosystem services and societal benefits with the DPSIR framework in a systems approach. Mar. Pollut. Bull. 62, 215–226. https://doi.org/10.1016/j.marpolbul.2010.12.012

Attademo, F.L.N., Balensiefer, D.C., Freire, A.C. da B., de Sousa, G.P., da Cunha, F.A.G.C., Luna, F. de O., 2015. Debris ingestion by the Antillean Manatee (Trichechus manatus manatus). Mar. Pollut. Bull. 101, 284–287. https://doi.org/10.1016/j.marpolbul.2015.09.040

Baalkhuyur, F.M., Bin Dohaish, E.J.A., Elhalwagy, M.E.A., Alikunhi, N.M., AlSuwailem, A.M., Røstad, A., Coker, D.J., Berumen, M.L., Duarte, C.M., 2018. Microplastic in the gastrointestinal tract of fishes along the Saudi Arabian Red Sea coast. Mar. Pollut. Bull. 131, 407–415. https://doi.org/10.1016/j.marpolbul.2018.04.040

Barboza, L.G.A., Vieira, L.R., Guilhermino, L., 2018. Single and combined effects of microplastics and mercury on juveniles of the European seabass (Dicentrarchus labrax): Changes in behavioural responses and reduction of swimming velocity and resistance time. Environ. Pollut. 236, 1014–1019. https://doi.org/10.1016/j.envpol.2017.12.082

Batel, A., Linti, F., Scherer, M., Erdinger, L., Braunbeck, T., 2016. Transfer of benzo[*a*]pyrene from microplastics to *Artemia* nauplii and further to zebrafish via a trophic food web experiment: CYP1A induction and visual tracking of persistent organic pollutants. Environ. Toxicol. Chem. 35, 1656–1666. https://doi.org/10.1002/etc.3361

Bellas, J., Martínez-Armental, J., Martínez-Cámara, A., Besada, V., Martínez-Gómez, C., 2016. Ingestion of microplastics by demersal fish from the Spanish Atlantic and

Mediterranean coasts. Mar. Pollut. Bull. 109, 55–60. https://doi.org/10.1016/j.marpolbul.2016.06.026

Bessa, F., Barría, P., Neto, J.M., Frias, J.P.G.L., Otero, V., Sobral, P., Marques, J.C., 2018. Occurrence of microplastics in commercial fish from a natural estuarine environment. Mar. Pollut. Bull. 128, 575–584. https://doi.org/10.1016/j.marpolbul.2018.01.044

Birnstiel, S., Soares-Gomes, A., da Gama, B.A.P., 2019. Depuration reduces microplastic content in wild and farmed mussels. Mar. Pollut. Bull. 140, 241–247. https://doi.org/10.1016/j.marpolbul.2019.01.044

Browne, M.A., Crump, P., Niven, S.J., Teuten, E., Tonkin, A., Galloway, T., Thompson, R., 2011. Accumulation of microplastic on shorelines woldwide: Sources and sinks. Environ. Sci. Technol. 45, 9175–9179. https://doi.org/10.1021/es201811s

Budimir, S., Setälä, O., Lehtiniemi, M., 2018. Effective and easy to use extraction method shows low numbers of microplastics in offshore planktivorous fish from the northern Baltic Sea. Mar. Pollut. Bull. 127, 586–592. https://doi.org/10.1016/j.marpolbul.2017.12.054

Carpenter, K.E., 2002. The living marine resources of the Western Central Atlantic, FAO Fisheries Technical Paper.

Chagnon, C., Thiel, M., Antunes, J., Ferreira, J.L., Sobral, P., Ory, N.C., 2018. Plastic ingestion and trophic transfer between Easter Island flying fish (Cheilopogon rapanouiensis) and yellowfin tuna (Thunnus albacares) from Rapa Nui (Easter Island). Environ. Pollut. 243, 127–133. https://doi.org/10.1016/j.envpol.2018.08.042

Dantas, N.C.F.M., Duarte, O.S., Ferreira, W.C., Ayala, A.P., Rezende, C.F., Feitosa, C. V., 2020. Plastic intake does not depend on fish eating habits: Identification of microplastics in the stomach contents of fish on an urban beach in Brazil. Mar. Pollut. Bull. 153, 110959. https://doi.org/10.1016/j.marpolbul.2020.110959

Dauvergne, P., 2018. Why is the global governance of plastic failing the oceans? Glob. Environ. Chang. 51, 22–31. https://doi.org/10.1016/j.gloenvcha.2018.05.002

de Moura, A. R. L. U., Candeias, A. L. B., Limongi, C. M., 2009. A multi-temporal remote sensing and gis based inventory of the mangroves at itamaracá estuarine system, Northeastern Brazil. Tropical Oceanography, 37(1-2).

de Sá, L.C., Luís, L.G., Guilhermino, L., 2015. Effects of microplastics on juveniles of the common goby (Pomatoschistus microps): Confusion with prey, reduction of the predatory performance and efficiency, and possible influence of developmental conditions. Environ. Pollut. 196, 359–362. https://doi.org/10.1016/j.envpol.2014.10.026

Dunn, O.J., 1964. A Note on Multiple Comparisons Using Rank Sums. Technometrics 6, 241–252. https://doi.org/10.1080/00401706.1965.10490253

Elliott, M., Whitfield, A.K., Potter, I.C., Blaber, S.J.M., Cyrus, D.P., Nordlie, F.G., Harrison, T.D., 2007. The guild approach to categorizing estuarine fish assemblages: A global review. Fish Fish. https://doi.org/10.1111/j.1467-2679.2007.00253.x

Eriksson, C., Burton, H., 2003. Origins and Biological Accumulation of Small Plastic Particles in Fur Seals from Macquarie Island. AMBIO A J. Hum. Environ. 32, 380–384. https://doi.org/10.1579/0044-7447-32.6.380

Fendall, L.S., Sewell, M.A., 2009. Contributing to marine pollution by washing your face: Microplastics in facial cleansers. Mar. Pollut. Bull. 58, 1225–1228. https://doi.org/10.1016/j.marpolbul.2009.04.025

Ferreira, G.V.B., Barletta, M., Lima, A.R.A., Dantas, D. V., Justino, A.K.S., Costa, M.F., 2016. Plastic debris contamination in the life cycle of Acoupa weakfish (Cynoscion acoupa) in a tropical estuary . ICES J. Mar. Sci. J. du Cons. 73, 2695–2707. https://doi.org/10.1093/icesjms/fsw108

Ferreira, G.V.B., Barletta, M., Lima, A.R.A., Morley, S.A., Costa, M.F., 2019. Dynamics of Marine Debris Ingestion by Profitable Fishes Along The Estuarine Ecocline. Sci. Rep. 9. https://doi.org/10.1038/s41598-019-49992-3

Ferreira, V., Loc'h, L., Ménard, F., Frédou, T., & Frédou, F. L., 2019. Composition of the fish fauna in a tropical estuary: the ecological guild approach. Scientia Marina, 83(2), 133-142. doi: 10.3989/scimar.04855.25A

Ferreira, G.V.B., Barletta, M., Lima, A.R.A., Morley, S.A., Justino, A.K.S., Costa, M.F., 2018. High intake rates of microplastics in a Western Atlantic predatory fish, and insights of a direct fishery effect. Environ. Pollut. 236, 706–717. https://doi.org/10.1016/j.envpol.2018.01.095

Foekema, E.M., De Gruijter, C., Mergia, M.T., Van Franeker, J.A., Murk, A.J., Koelmans, A.A., 2013. Plastic in north sea fish. Environ. Sci. Technol. 47, 8818–8824. https://doi.org/10.1021/es400931b

Freeman, L.A., Corbett, D.R., Fitzgerald, A.M., Lemley, D.A., Quigg, A., Steppe, C.N., 2019. Impacts of Urbanization and Development on Estuarine Ecosystems and Water Quality. Estuaries and Coasts. https://doi.org/10.1007/s12237-019-00597-z

Frias, J.P.G.L., Sobral, P., Ferreira, A.M., 2010. Organic pollutants in microplastics from two beaches of the Portuguese coast. Mar. Pollut. Bull. 60, 1988–1992. https://doi.org/10.1016/j.marpolbul.2010.07.030

Galloway, T.S., Cole, M., Lewis, C., 2017. Interactions of microplastic debris throughout the marine ecosystem. Nat. Ecol. Evol. 1. https://doi.org/10.1038/s41559-017-0116

Garcés-Ordóñez, O., Mejía-Esquivia, K.A., Sierra-Labastidas, T., Patiño, A., Blandón, L.M., Espinosa Díaz, L.F., 2020. Prevalence of microplastic contamination in the digestive tract of fishes from mangrove ecosystem in Cispata, Colombian Caribbean. Mar. Pollut. Bull. 154, 111085. https://doi.org/10.1016/j.marpolbul.2020.111085

Gonzalez, J.G., Ménard, F., Le Loc’h, F., Andrade, H.A. de, Viana, A.P., Ferreira, V., Lucena Frédou, F., Lira, A.S., Munaron, J.M., Frédou, T., 2019. Trophic resource partitioning of two snook fish species (Centropomidae) in tropical estuaries in Brazil as evidenced by stable isotope analysis. Estuar. Coast. Shelf Sci. 226, 106287. https://doi.org/10.1016/j.ecss.2019.106287

Guebert-Bartholo, F.M., Barletta, M., Costa, M.F., Monteiro-Filho, E.L.A., 2011. Using gut contents to assess foraging patterns of juvenile green turtles Chelonia mydas in the Paranaguá Estuary, Brazil. Endanger. Species Res. 13, 131–143. https://doi.org/10.3354/esr00320

Hermsen, E., Mintenig, S.M., Besseling, E., Koelmans, A.A., 2018. Quality Criteria for the Analysis of Microplastic in Biota Samples: A Critical Review. Environ. Sci. Technol. 52, 10230–10240. https://doi.org/10.1021/acs.est.8b01611

Hermsen, E., Pompe, R., Besseling, E., Koelmans, A.A., 2017. Detection of low numbers of microplastics in North Sea fish using strict quality assurance criteria. Mar. Pollut. Bull. 122, 253–258. https://doi.org/10.1016/j.marpolbul.2017.06.051

Herrera, A., Ŝtindlová, A., Martínez, I., Rapp, J., Romero-Kutzner, V., Samper, M.D., Montoto, T., Aguiar-González, B., Packard, T., Gómez, M., 2019. Microplastic ingestion by Atlantic chub mackerel (Scomber colias) in the Canary Islands coast. Mar. Pollut. Bull. 139, 127–135. https://doi.org/10.1016/j.marpolbul.2018.12.022

Holmes, L.A., Turner, A., Thompson, R.C., 2012. Adsorption of trace metals to plastic resin pellets in the marine environment. Environ. Pollut. 160, 42–48. https://doi.org/10.1016/j.envpol.2011.08.052

Ivar Do Sul, J.A., Costa, M.F., 2014. The present and future of microplastic pollution in the marine environment. Environ. Pollut. https://doi.org/10.1016/j.envpol.2013.10.036

IBGE (Instituto Brasileiro de Geografia e Estatística) 2011. Estimativas da população nos municípios brasileiros em 1º de julho de 2011. Available in: http://www.ibge.gov.br/home/estatistica/populacao/estimativa2011/POP2011_DOU.pdf

Jambeck, J.R., Geyer, R., Wilcox, C., Siegler, T.R., Perryman, M., Andrady, A., Narayan, R., Law, K.L., 2015. Plastic waste inputs from land into the ocean. Science (80-.). 347, 768–771. https://doi.org/10.1126/science.1260352

Karami, A., Golieskardi, A., Choo, C.K., Romano, N., Ho, Y. Bin, Salamatinia, B., 2017. A high-performance protocol for extraction of microplastics in fish. Sci. Total Environ. 578, 485–494. https://doi.org/10.1016/j.scitotenv.2016.10.213

Krumme, U., Brenner, M., Saint-Paul, U., 2008. Spring-neap cycle as a major driver of temporal variations in feeding of intertidal fishes: Evidence from the sea catfish Sciades herzbergii (Ariidae) of equatorial west Atlantic mangrove creeks. J. Exp. Mar. Bio. Ecol. 367, 91–99. https://doi.org/10.1016/j.jembe.2008.08.020

Kühn, S., van Werven, B., van Oyen, A., Meijboom, A., Bravo Rebolledo, E.L., van Franeker, J.A., 2017. The use of potassium hydroxide (KOH) solution as a suitable approach to isolate plastics ingested by marine organisms. Mar. Pollut. Bull. 115, 86–90. https://doi.org/10.1016/j.marpolbul.2016.11.034

Lebreton, L.C.M., Van Der Zwet, J., Damsteeg, J.W., Slat, B., Andrady, A., Reisser, J., 2017. River plastic emissions to the world's oceans. Nat. Commun. 8, 1–10. https://doi.org/10.1038/ncomms15611

Lima, A.R.A., Barletta, M., 2016. Lunar influence on prey availability, diet shifts and niche overlap between Engraulidae larvae in tropical mangrove creeks. J. Fish Biol. 89, 2133–2152. https://doi.org/10.1111/jfb.13121

Lima, A.R.A., Barletta, M., Costa, M.F., Ramos, J.A.A., Dantas, D. V., Melo, P.A.M.C., Justino, A.K.S., Ferreira, G.V.B., 2016. Changes in the composition of ichthyoplankton assemblage and plastic debris in mangrove creeks relative to moon phases. J. Fish Biol. 89, 619–640. https://doi.org/10.1111/jfb.12838

Lima, A.R.A., Costa, M.F., Barletta, M., 2014. Distribution patterns of microplastics within the plankton of a tropical estuary. Environ. Res. 132, 146–155. https://doi.org/10.1016/j.envres.2014.03.031

Lira, A., Angelini, R., Le Loc'h, F., Ménard, F., Lacerda, C., Frédou, T., Lucena Frédou, F., 2018. Trophic flow structure of a neotropical estuary in northeastern Brazil and the comparison of ecosystem model indicators of estuaries. J. Mar. Syst. 182, 31–45. https://doi.org/10.1016/j.jmarsys.2018.02.007

Lira, A.S., Frédou, F.L., Viana, A.P., Eduardo, L.N., Frédou, T., 2017. Feeding ecology of Centropomus undecimalis (Bloch, 1792) and Centropomus parallelus (Poey, 1860) in two tropical estuaries in Northeastern Brazil. Panam. J. Aquat. Sci. 12, 123–135.

Lusher, A.L., Welden, N.A., Sobral, P., Cole, M., 2017. Sampling, isolating and identifying microplastics ingested by fish and invertebrates. Anal. Methods 9, 1346–1360. https://doi.org/10.1039/c6ay02415g

Markic, A., Gaertner, J.-C., Gaertner-Mazouni, N., Koelmans, A.A., 2020. Plastic ingestion by marine fish in the wild. Crit. Rev. Environ. Sci. Technol. 50, 657–697. https://doi.org/10.1080/10643389.2019.1631990

Menezes, N. A & J. L. Figueiredo., 2000. Manual de peixes marinhos do sudeste do Brasil. Teleostei (5). Museu de Zoologia, Universidade de São Paulo, p. 116.

Medeiros, C., Kjerfve, B., Araujo, M., Neumann-Leitão, S., 2001. The Itamaracá

Estuarine Ecosystem, Brazil, in: Coastal Marine Ecosystem of Latin America (Ecological Studies). Springer, Berlin, Heidelberg, pp. 71–81. https://doi.org/10.1007/978-3-662-04482-7_6

Miller, M.E., Hamann, M., Kroon, F.J., 2020. Bioaccumulation and biomagnification of microplastics in marine organisms: A review and meta-analysis of current data. PLoS One. https://doi.org/10.1371/journal.pone.0240792

Miranda, D. de A., de Carvalho-Souza, G.F., 2016. Are we eating plastic-ingesting fish? Mar. Pollut. Bull. 103, 109–114. https://doi.org/10.1016/j.marpolbul.2015.12.035

Mizraji, R., Ahrendt, C., Perez-Venegas, D., Vargas, J., Pulgar, J., Aldana, M., Patricio Ojeda, F., Duarte, C., Galbán-Malagón, C., 2017. Is the feeding type related with the content of microplastics in intertidal fish gut? Mar. Pollut. Bull. 116, 498–500. https://doi.org/10.1016/j.marpolbul.2017.01.008

Moore, C.J., 2008. Synthetic polymers in the marine environment: A rapidly increasing, long-term threat. Environ. Res. 108, 131–139. https://doi.org/10.1016/j.envres.2008.07.025

Morgana, S., Ghigliotti, L., Estévez-Calvar, N., Stifanese, R., Wieckzorek, A., Doyle, T., Christiansen, J.S., Faimali, M., Garaventa, F., 2018. Microplastics in the Arctic: A case study with sub-surface water and fish samples off Northeast Greenland. Environ. Pollut. 242, 1078–1086. https://doi.org/10.1016/j.envpol.2018.08.001

Nelms, S.E., Galloway, T.S., Godley, B.J., Jarvis, D.S., Lindeque, P.K., 2018. Investigating microplastic trophic transfer in marine top predators. Environ. Pollut. 238, 999–1007. https://doi.org/10.1016/j.envpol.2018.02.016

O’Connor, J.D., Mahon, A.M., Ramsperger, A.F.R.M., Trotter, B., Redondo-Hasselerharm, P.E., Koelmans, A.A., Lally, H.T., Murphy, S., 2020. Microplastics in Freshwater Biota: A Critical Review of Isolation, Characterization, and Assessment Methods. Glob. Challenges 4, 1800118. https://doi.org/10.1002/gch2.201800118

Oehlmann, J., Schulte-Oehlmann, U., Kloas, W., Jagnytsch, O., Lutz, I., Kusk, K.O., Wollenberger, L., Santos, E.M., Paull, G.C., VanLook, K.J.W., Tyler, C.R., 2009. A critical analysis of the biological impacts of plasticizers on wildlife. Philos. Trans. R. Soc. B Biol. Sci. 364, 2047–2062. https://doi.org/10.1098/rstb.2008.0242

Ogata, Y., Takada, H., Mizukawa, K., Hirai, H., Iwasa, S., Endo, S., Mato, Y., Saha, M., Okuda, K., Nakashima, A., Murakami, M., Zurcher, N., Booyatumanondo, R., Zakaria, M.P., Dung, L.Q., Gordon, M., Miguez, C., Suzuki, S., Moore, C., Karapanagioti, H.K., Weerts, S., McClurg, T., Burres, E., Smith, W., Velkenburg, M. Van, Lang, J.S., Lang, R.C., Laursen, D., Danner, B., Stewardson, N., Thompson, R.C., 2009. International Pellet Watch: Global monitoring of persistent organic pollutants (POPs) in coastal waters. 1. Initial phase data on PCBs, DDTs, and HCHs. Mar. Pollut. Bull. 58, 1437–1446. https://doi.org/10.1016/j.marpolbul.2009.06.014

Ostle, C., Thompson, R.C., Broughton, D., Gregory, L., Wootton, M., Johns, D.G., 2019. The rise in ocean plastics evidenced from a 60-year time series. Nat. Commun. 10, 8–13. https://doi.org/10.1038/s41467-019-09506-1

Pegado, T. de S. e. S., Schmid, K., Winemiller, K.O., Chelazzi, D., Cincinelli, A., Dei, L., Giarrizzo, T., 2018. First evidence of microplastic ingestion by fishes from the Amazon River estuary. Mar. Pollut. Bull. 133, 814–821. https://doi.org/10.1016/j.marpolbul.2018.06.035

Pelage, L., Domalain, G., Lira, A.S., Travassos, P., Frédou, T., 2019. Coastal Land Use in Northeast Brazil: Mangrove Coverage Evolution Over Three Decades. Trop. Conserv. Sci. 12. https://doi.org/10.1177/1940082918822411

Pellini, G., Gomiero, A., Fortibuoni, T., Ferrà, C., Grati, F., Tassetti, A.N., Polidori, P., Fabi, G., Scarcella, G., 2018. Characterization of microplastic litter in the gastrointestinal tract of Solea solea from the Adriatic Sea. Environ. Pollut. 234, 943–952. https://doi.org/10.1016/j.envpol.2017.12.038

Potter, I.C., Tweedley, J.R., Elliott, M., Whitfield, A.K., 2013. The ways in which fish use estuaries: a refinement and expansion of the guild approach. Fish Fish. 16, 230–239. https://doi.org/10.1111/faf.12050

R Core Team, 2020. R: A language and environment for statistical computing. R Foundation for Statistical Computing.

Ramos, J.A.A., Barletta, M., Dantas, D. V., Costa, M.F., 2016. Seasonal and spatial ontogenetic movements of Gerreidae in a Brazilian tropical estuarine ecocline and its application for nursery habitat conservation. J. Fish Biol. 89, 696–712. https://doi.org/10.1111/jfb.12872

Ribeiro-Brasil, D.R.G., Torres, N.R., Picanço, A.B., Sousa, D.S., Ribeiro, V.S., Brasil, L.S., Montag, L.F. de A., 2020. Contamination of stream fish by plastic waste in the Brazilian Amazon. Environ. Pollut. 266. https://doi.org/10.1016/j.envpol.2020.115241

Rochman, C.M., Hoh, E., Kurobe, T., Teh, S.J., 2013. Ingested plastic transfers hazardous chemicals to fish and induces hepatic stress. Sci. Rep. 3, 1–7. https://doi.org/10.1038/srep03263

Santana, M.F.M., Ascer, L.G., Custódio, M.R., Moreira, F.T., Turra, A., 2016. Microplastic contamination in natural mussel beds from a Brazilian urbanized coastal region: Rapid evaluation through bioassessment. Mar. Pollut. Bull. 106, 183–189. https://doi.org/10.1016/j.marpolbul.2016.02.074

Schirinzi, G.F., Pedà, C., Battaglia, P., Laface, F., Galli, M., Baini, M., Consoli, P., Scotti, G., Esposito, V., Faggio, C., Farré, M., Barceló, D., Fossi, M.C., Andaloro, F., Romeo, T., 2020. A new digestion approach for the extraction of microplastics from gastrointestinal tracts (GITs) of the common dolphinfish (Coryphaena hippurus) from the western Mediterranean Sea. J. Hazard. Mater. 397, 122794. https://doi.org/10.1016/j.jhazmat.2020.122794

Su, L., Nan, B., Hassell, K.L., Craig, N.J., Pettigrove, V., 2019. Microplastics biomonitoring in Australian urban wetlands using a common noxious fish (Gambusia holbrooki). Chemosphere 228, 65–74. https://doi.org/10.1016/j.chemosphere.2019.04.114

Tanaka, K., Takada, H., 2016. Microplastic fragments and microbeads in digestive tracts of planktivorous fish from urban coastal waters. Sci. Rep. 6, 1–8. https://doi.org/10.1038/srep34351

Teuten, E.L., Rowland, S.J., Galloway, T.S., Thompson, R.C., 2007. Potential for plastics to transport hydrophobic contaminants. Environ. Sci. Technol. 41, 7759–7764. https://doi.org/10.1021/es071737s

Teuten, E.L., Saquing, J.M., Knappe, D.R.U., Barlaz, M.A., Jonsson, S., Björn, A., Rowland, S.J., Thompson, R.C., Galloway, T.S., Yamashita, R., Ochi, D., Watanuki, Y., Moore, C., Viet, P.H., Tana, T.S., Prudente, M., Boonyatumanond, R., Zakaria, M.P., Akkhavong, K., Ogata, Y., Hirai, H., Iwasa, S., Mizukawa, K., Hagino, Y., Imamura, A., Saha, M., Takada, H., 2009. Transport and release of chemicals from plastics to the environment and to wildlife. Philos. Trans. R. Soc. B Biol. Sci. 364, 2027–2045. https://doi.org/10.1098/rstb.2008.0284

Thompson, R.C., Olson, Y., Mitchell, R.P., Davis, A., Rowland, S.J., John, A.W.G., McGonigle, D., Russell, A.E., 2004. Lost at Sea: Where Is All the Plastic? Science (80-.). 304, 838. https://doi.org/10.1126/science.1094559

Torre, M., Digka, N., Anastasopoulou, A., Tsangaris, C., Mytilineou, C., 2016.

Anthropogenic microfibres pollution in marine biota. A new and simple methodology to minimize airborne contamination. Mar. Pollut. Bull. 113, 55–61. https://doi.org/10.1016/j.marpolbul.2016.07.050

Vendel, A.L., Bessa, F., Alves, V.E.N., Amorim, A.L.A., Patrício, J., Palma, A.R.T., 2017. Widespread microplastic ingestion by fish assemblages in tropical estuaries subjected to anthropogenic pressures. Mar. Pollut. Bull. 117, 448–455. https://doi.org/10.1016/j.marpolbul.2017.01.081

Wieczorek, A.M., Morrison, L., Croot, P.L., Allcock, A.L., MacLoughlin, E., Savard, O., Brownlow, H., Doyle, T.K., 2018. Frequency of microplastics in mesopelagic fishes from the Northwest Atlantic. Front. Mar. Sci. 5, 1–9. https://doi.org/10.3389/fmars.2018.00039

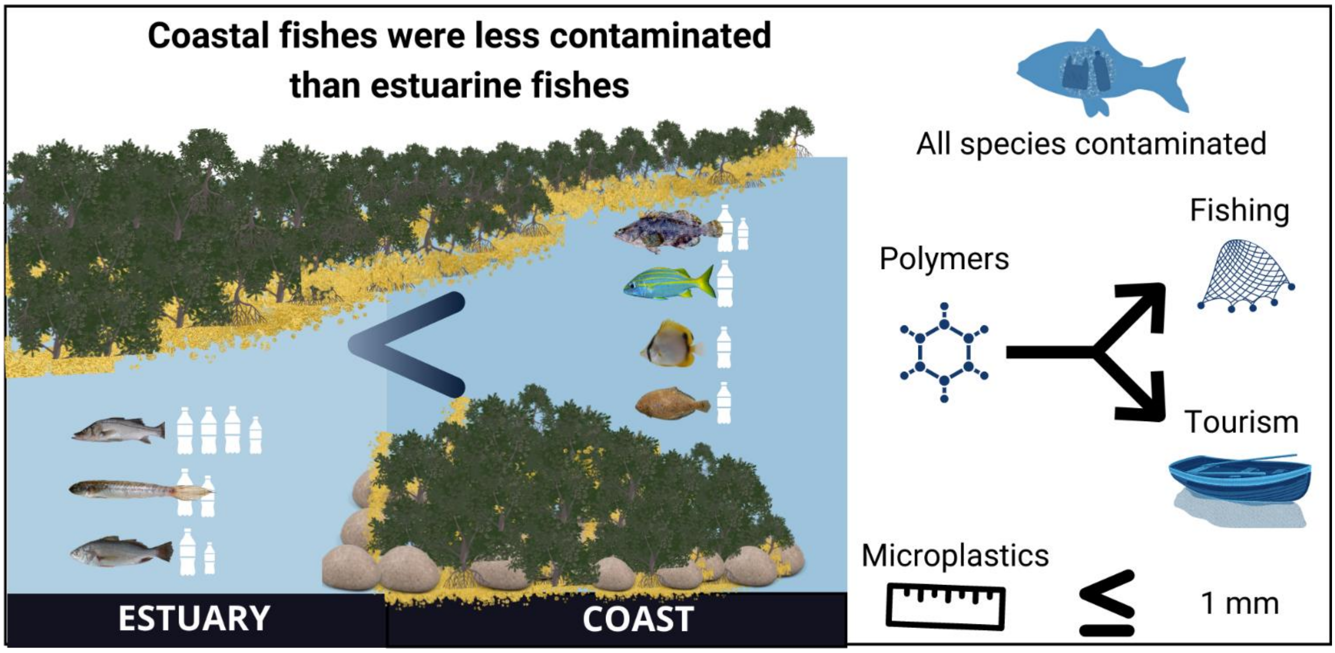
Wright, S.L., Thompson, R.C., Galloway, T.S., 2013. The physical impacts of microplastics on marine organisms: a review. Environ. Pollut. 178, 483–492. https://doi.org/10.1016/j.envpol.2013.02.031

Zhang, H., 2017. Transport of microplastics in coastal seas. Estuar. Coast. Shelf Sci. https://doi.org/10.1016/j.ecss.2017.09.032

CHAPTER 2

Article "Microplastic contamination in coastal fishes from the Southwestern Tropical Atlantic"

Manuscript to be submitted after thesis defence in the Marine Pollution journal as a baseline paper.



Microplastic contamination in coastal fishes from the Southwestern Tropical Atlantic

Anne K. S. Justino[1,2*], Guilherme V. B. Ferreira[1], Vincent Fauvelle[3], Natascha Schmidt[3], Véronique Lenoble[2], Latifa Pelage[1], Flávia Lucena-Frédou[1]

[1] Universidade Federal Rural de Pernambuco (UFRPE), Departamento de Pesca e Aquicultura (DEPAQ), Rua Dom Manuel de Medeiros, s/n, 52171-900, Recife, Brazil.
[2] Université de Toulon, Aix Marseille Univ., CNRS, IRD, MIO, Toulon, France.
[3] Aix Marseille Univ., Université de Toulon, CNRS, IRD, MIO, Marseille, France.

* Corresponding author: anne.justino@ufrpe.br

Baseline paper

Abstract

Microplastics (MPs) are ubiquitous in all marine compartments, and their transboundary distribution favours the dispersion and accumulation of particles in the ecosystems. This study aimed to describe MP contamination in coastal fish from Northeast Brazil. An alkaline digestion protocol was applied in the digestive tract of four demersal fish species (*Haemulon squamipinna*, *Chaetodon ocellatus*, *Syacium micrurum* and *Alphestes afer*) to extract MPs. A laser direct infrared (LDIR) was used to identify the main polymers. All the coastal fish species analysed in this study were contaminated with MPs (frequency of occurrence FO= 70%). *Alphestes afer* was the most contaminated species (1.45 ±SD 1.09 MPs ind.$^{-1}$; FO= 80%), followed by *H. squamipinna* (1 ±SD 0.81 MPs ind.$^{-1}$; FO= 71%). However, no significant differences were observed between the mean number and mean size of particles found. The main shapes of MPs detected were fibres and films, and a wide variety of polymers were identified; the most abundant were polyethylene, polyamide, polyvinyl alcohol, polycarbonate, polypropylene, alkyd varnish and styrene-butadiene rubber. Overall, our study provides important baseline data on MP contamination of coastal fish species which inhabit complex habitat areas relevant for conserving marine biodiversity.

Keywords: Plastic pollution, Tropical Atlantic, Reef fish, Demersal fish.

Introduction

Plastic is a versatile, resistant and cheap material, and society is increasingly becoming more dependent on this synthetic material. However, the incorrect disposal of plastic waste makes its presence in ecosystems progressively hazardous. About 80% of plastics from continental sources enter the oceans, mainly through riverine discharges (Andrady, 2011; Meijer et al., 2021). In the marine environment, these anthropogenic materials are easily distributed through ocean currents and weathered by natural processes such as solar radiation and interaction with marine organisms (Jambeck et al., 2015; Thompson et al., 2004). The breakdown of larger plastics into particles smaller than 5 mm is called microplastics (MPs; Arthur et al., 2009).

MPs are widely available in marine ecosystems and can be mistaken as natural prey of marine species or swallowed by accident while breathing (Boerger et al., 2010; Li et al., 2021). Microplastics can pose various threats to marine biota (Galloway et al., 2017); their intake is associated with damage to the digestive system and decreased predation efficiency (de Sá et al., 2015; Moore, 2008). In addition, MPs can release additives burden into the environment and adsorb other available pollutants (Fauvelle et al., 2021; Rochman et al., 2013; Teuten et al., 2007). Furthermore, MPs can serve as habitats for microorganisms such as viruses and bacteria (Pinheiro et al., 2021), and transporting these organisms between areas can be potentially hazardous to ecosystems.

MPs have been detected in estuaries, coastal subsurface water, and oceanic islands in the western equatorial Atlantic region (Lima et al., 2016; Lins-Silva et al., 2021; Monteiro et al., 2020). Ingestion of MPs by marine species is widely documented (Savoca et al., 2021) and has also been reported for species in the equatorial Atlantic (Bruzaca et al., 2022; Ferreira et al., 2016; Morais et al., 2020; Vendel et al., 2017). However, globally, little information regarding the ingestion of MP by reef fish species is available (Baalkhuyur et al., 2018; Garnier et al., 2019; Macieira et al., 2021; Nie et al., 2019).

Along the Southwestern Tropical Atlantic (SWTA), the coastal region of northeast Brazil is recognised worldwide for its beaches, which receive tourists throughout the year. In addition to tourism, the primary economic sources are located in or near the coastal region, represented by industries, agriculture and commercial and subsistence fisheries (IBGE, 2011). The artisanal fishing in the area involves more than 200 thousand people and is responsible for the highest volume landed in the country (Nóbrega et al., 2009).

This large volume of fishing landings is due to the high fish diversity found on the continental shelf of northeast Brazil (MMA, 2006), a region included in an Ecologically or Biologically Significant Marine Areas (EBSA) (CBD, 2014). The incredible biodiversity, as a consequence, is explained by the presence of complex habitats in this region, such as reefs (Eduardo et al., 2018).

Given the relevance of this ecosystem, both in terms of biodiversity and fisheries importance and considering the gap of information in MP in reef areas of Northeast Brazil, our study aims to identify and characterise MP contamination in coastal demersal fish species, along with verifying the differences in MP ingestion rates among species.

Materials and Methods

Study area

The studied coastal areas are located on the continental shelf along the Northeast Brazilian coast in the Southwestern Tropical Atlantic and comprise the states of Rio Grande do Norte, Paraíba, and Pernambuco (Fig. 1). The climate is tropical, with an average air temperature of 20 to 25ºC throughout the year (IBGE, 2011) with a seasonality defined according to the rainfall patterns, with a dry and a rainy season (Macêdo et al., 2004). Within this area, several Marine Protected Areas have been established (*e.g.*, “APA dos Corais”, “APA Costa dos Corais”, “APA Guadalupe”, “APA Santa Cruz”, “APA Barra de Mamanguape”) (Ferreira and Maida, 2007; Prates et al., 2007).

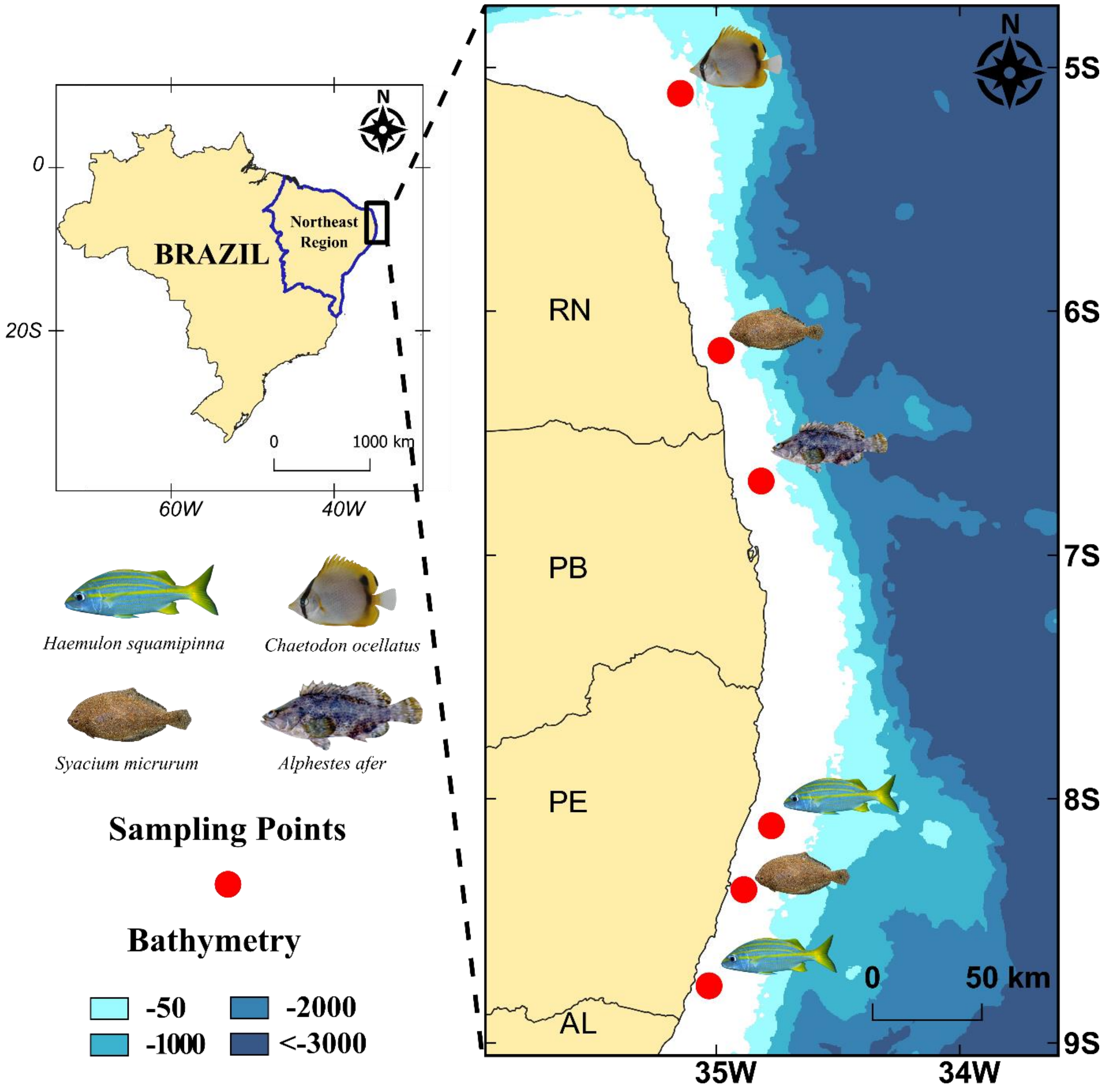


Figure 1. Map of the continental shelf on Northeast Brazil in the Southwestern Tropical Atlantic. The red dots represent the stations where the ABRACOS project sampled fishes.

Sample collection and laboratory procedures

Two scientific expeditions were performed on the oceanographic cruise R/V ANTEA in the years 2015 (August and September) and 2017 (April and May), where 52 stations were established along the continental shelf through the Acoustics Along Brazilian Coast (ABRACOS) campaign (Eduardo et al., 2018). The coastal fish samples were collected using a bottom trawl (mesh: 40 mm; bag mesh: 25 mm; mouth dimension: 28 × 10 m), between 15 and 65 m depth, and the haul was carried out for about 5 min at 3.2 kt. The SCANMAR system estimated the net’s height, depth, and width. Bobbins were added to the ground rope on the second cruise to reduce the impact on the benthic habitat caused by trawling and avoid damage to the net.

Four species were selected in this study due to their ecological and economic importance in the region. The coastal demersal species collected were: *Haemulon squamipinna* Rocha & Rosa, 1999; *Chaetodon ocellatus* Bloch, 1787; *Syacium micrurum* Ranzani, 1842 and *Alphestes afer* (Bloch, 1793). Individuals were frozen and measured at the laboratory (standard length and total length in cm), weighed (total weight in g) and separated for the microplastics analysis.

Contamination control and Microplastic identification

Several steps were carried out to ensure quality assurance and quality control to avoid cross-contamination, following the protocol proposed by Justino et al. (2021). The protocols include the use of cotton lab coats, and the filtration of all the solutions utilised through a glass fibre filter (47 mm GF/F 0.7 µm pore size, © Whatman) mounted on a vacuum pump system equipped with laboratory glassware. Blank procedures were done for each set of 10 samples and received the same treatment as samples. Particles found in the samples with similarities with the particles in the blank samples were excluded from the study. Only two black fragments were found in the blank samples and were identified as chitin. Additionally, the particles classified as biopolymers were excluded from further analysis.

To extract microplastics from the digestive tract (stomach and intestine) of fishes, we used an alkaline digestion protocol with sodium hydroxide (NaOH, 1 mol L^{-1}; PA 97%), which removed the organic matter around the particles (Justino et al., 2021). The digestive tracts were placed in a beaker and submerged in the NaOH solution, covered by a glass lid and oven-dried at 60 °C for 24 h. Later, samples were filtered through a 47 mm GF/F 0.7 µm pore size glass fibre filter (© Whatman) using a vacuum pump system. After filtration, samples were oven-dried again at 60 °C for 24 h and proceeded to visualise microplastics using a stereomicroscope (© Zeiss Stemi 508, with a size detection limit of 0.04–5 mm).

All the particles suspected to be microplastics were counted, photographed (© Zeiss Zen 3.2; Axiocam 105 Color), and measured in length (mm). Particles were first categorised according to their shape as fibres (filamentous shape), fragments (irregular shape), films (flat shape), foams (soft with an irregular shape), or pellets (spherical shape) (Justino et al., 2021). Thereafter, a subset of detected MP samples (52% of MPs detected) was sent in collaboration to the Mediterranean Institute of Oceanography (MIO) in

Marseille, France. The polymers were identified from the samples using the Laser Direct Infrared (LDIR) analyser Agilent 8700 Chemical Imaging System with the Microplastic Starter 1.0 library. The LDIR analyser operates by scanning the particles (size range 20–5000 µm) to obtain a spectral curve using a wavelength range of 1800–975 cm^{-1}. The information is collected with the Clarity image software (© Agilent version 1.3.9) and compared with the polymer spectrum library (~400 references spectra). The polymer nature was confirmed when the identification match was >70% (Ferreira et al., 2022; Justino et al., 2022).

Data analysis

The frequency of occurrence (percentage of individuals in a given species in which MPs were recorded; FO%) was calculated to assess the general contamination status of the species. Since the data did not meet the parametric premises, the Kruskal-Wallis test was used to verify if the MP detected in the digestive tract presented significant differences among the species according to the mean number and size. Additionally, a Spearman's correlation test was used to evaluate the relationship between microplastics and the biometry of fishes. All statistical analyses were performed with the software R version 3.6.3 (R Core Team, 2020) and were conducted considering a significance level of 5%.

Results

From the four coastal fish species analysed in this work, 52 microplastic particles were recovered (FO= 70%). However, the mean number and size of MPs ingested did not vary between the species ($p > 0.05$). The most contaminated species (number) were *A. afer* (1.45 ± 1.09 part. $ind.^{-1}$; FO= 80%), followed by *H. squamipinna* (1 ± 0.81 part. $ind.^{-1}$; FO= 71%), *S. micrurum* (0.90 ± 0.73 part. $ind.^{-1}$; FO= 70%), and *C. ocellatus* (0.70 ± 0.82 part. $ind.^{-1}$; FO= 50%) (Table 1, Fig. 2a and 2b). *Alphestes afer* showed the longest particle size ingested (0.63 ± 0.92 mm $ind.^{-1}$), followed by *C. ocellatus* (0.28 ± 0.72 mm $ind.^{-1}$), *S. micrurum* (0.24 ± 0.45 mm $ind.^{-1}$), and *H. squamipinna* (0.07 ± 0.10 mm $ind.^{-1}$). There is no relationship between the number and size of microplastics detected and the biological parameters of fishes (Spearman's rank correlation, $p > 0.05$).

Regarding the shapes of MPs ingested by fish species, *A. afer* registered 41% of fibres, 31% films, 37% fragments, and 10% of pellets, *S. micrurum* registered 22% fibres, 44% fragments, 22% films and 11% of pellets, while *C. ocellatus* and *H. squamipinna* reported 57% and 28% fibres, 42% and 71% of films, respectively (Fig. 2c). The colours blue, black, and white were predominant. Overall, plastic polymers were successfully identified in 33% of particles from the subset of samples analysed by the LDIR (Fig. 2d). However, MPs were considered partially identified when reaching 60 - 69.9% similarity to the reference spectrum and comprised 45% of the samples. The most common polymers were identified as polyethylene (PE) at 16% abundance, Polyamide (PA), Polyvinyl alcohol (PVA), Polycarbonate (PC), Polypropylene (PP) at 11% abundance, and Alkyd Varnish and Styrene-Butadiene Rubber (SBR) with a similar abundance at 10%. The other polymers such as Polyethylene Chlorinated (CPE), Polyurethane (PU), Ethylene Vinyl Acetate (EVA) and Polyvinyl chloride (PVC) contribute to a similar abundance of 5%. Biopolymers identified as cellulose, natural polyamide, and polylactic acid were observed in 22% of all particles (Fig. 2d and Fig.3).

Table 1. Summary of results regarding the mean (±standard deviation) number (particles individual^{-1}), size (mm), and FO% (frequency of occurrence) of microplastics detected in fish species, according to shape and colours. SL = Standard length; TW = Total weight.

Species		*Haemulon squamipinna*	*Chaetodon ocellatus*	*Syacium micrurum*	*Alphestes afer*
SL cm (min-max)		10.7 - 14.4	8.5 - 10	8.6 - 20.5	11.6 - 16.4
TW g (min-max)		30.3 - 75.8	33.3 - 56.4	10.1 - 149.3	32.5 - 118.2
Sample size		7	10	10	20
MPs FO%		71%	50%	70%	80%
MP size (mm)		0.07 ± 0.10	0.28 ± 0.72	0.24 ± 0.45	0.63 ± 0.92
MPs		1 ± 0.81	0.70 ± 0.82	0.90 ± 0.73	1.45 ± 1.09
Shape	Fibre	0.28 ± 0.48 (29%)	0.40 ± 0.69 (30%)	0.20 ± 0.42 (20%)	0.60 ± 0.68 (50%)
	Fragment	0%	0%	0.40 ± 0.69 (30%)	0.25 ± 0.44 (25%)
	Film	0.71 ± 0.75 (57%)	0.30 ± 0.48 (30%)	0.20 ± 0.42 (20%)	0.45 ± 0.82 (30%)
	Foam	0%	0%	0%	0%
	Pellet	0%	0%	0.10 ± 0.31 (10%)	0.15 ± 0.48 (10%)
Colour	Blue	0.71 ± 0.75 (57%)	0.50 ± 0.52 (50%)	0.30 ± 0.48 (30%)	0.45 ± 0.51 (45%)
	White	0.14 ± 0.37 (14%)	0%	0.10 ± 0.31 (10%)	0.35 ± 0.58 (30%)
	Black	0.14 ± 0.37 (14%)	0.20 ± 0.42 (20%)	0.50 ± 0.70 (40%)	0.65 ± 0.81 (45%)
	Green	0%	0%	0%	0.05 ± 0.22 (5%)
	Red	0%	0%	0%	0%

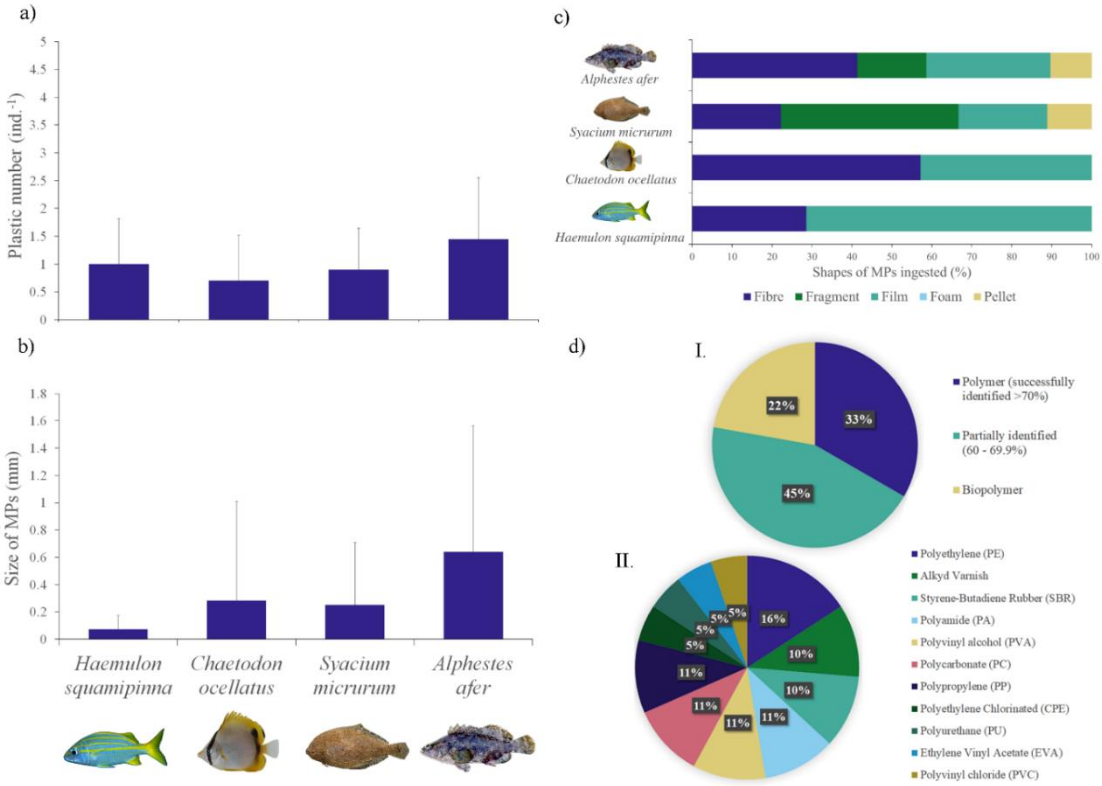


Figure 2. a) Mean number (± standard deviation), b) mean size (mm) of microplastics ingested per fish species, c) shapes of microplastics found in the coastal fishes are expressed as a percentage (%), and d) polymers identified: I. particle composition in the detected samples, and II. the abundance of the found polymers.

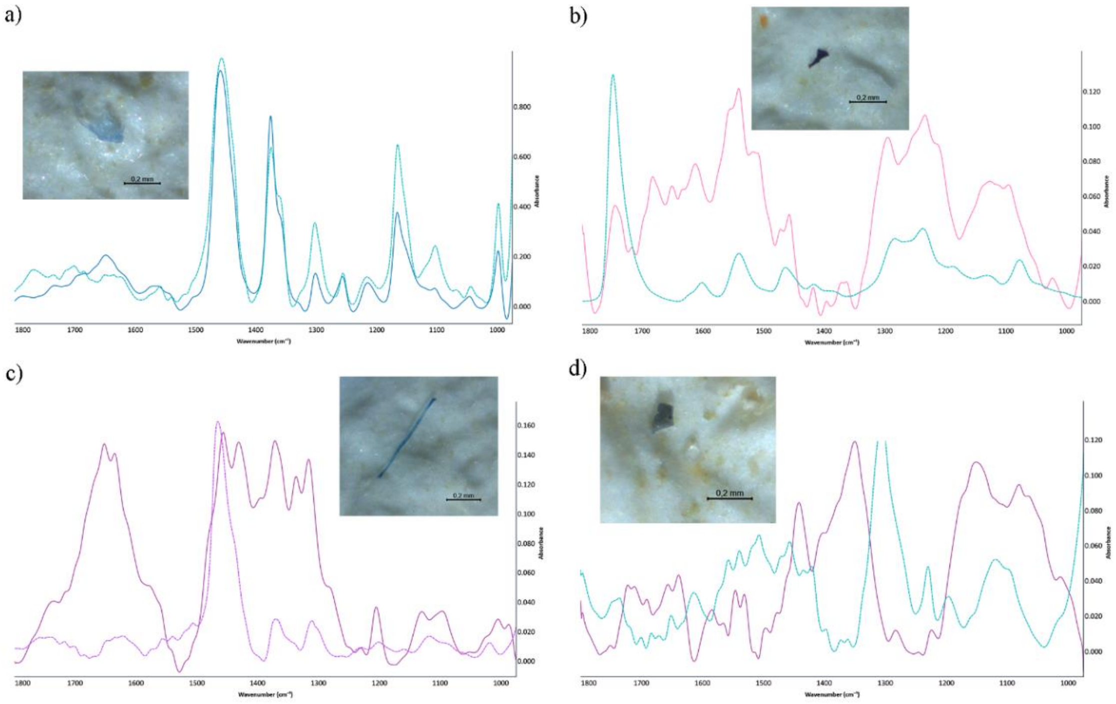


Figure 3. Polymers and shapes detected in the analysed sample, with the reference spectrum: a) blue fragment - polypropylene (PP), b) black film – Alkyd varnish, c) blue fibre - polyethylene (PE), and d) black fragment - styrene-butadiene rubber (SBR).

Discussion

This study provides new insights into the contamination of coastal fishes in Northeast Brazil, SWTA. Despite many studies evaluating microplastic contamination in fish species, there are limited reports on reef species (Baalkhuyur et al., 2018; Garnier et al., 2019; Macieira et al., 2021; Nie et al., 2019). Our results confirmed high contamination in reef species inhabiting the area, with a frequency of occurrence (FO%) of 70%.

Even though all the fish species were detected with MPs, no statistically significant differences were observed in the contamination rate and particle size among the species analysed, which varied between 0.70 ± 0.82 part. ind.$^{-1}$ (mean number) for *C. ocellatus* and 1.45 ±1.09 part. ind.$^{-1}$ for *A. afer*. The number of found MP is similar to that found in reef fish species at Guarapari Islands in Southeast Brazil (Macieira et al., 2021) and from Moorea Island in French Polynesia (Garnier et al., 2019).

The four species here analysed are demersal and share similarities, such as their feeding habits and habitat occupation. They are classified as mobile invertebrate feeders, which primarily feed on benthic invertebrates, catching prey on the bottom or in the water column (Pereira et al., 2015; Pinheiro et al., 2018). Despite having active swimming ability, they spend most of their time in association with the substrate. In fact, species which rely on substrate resources may be more susceptible to MPs available in the sediment (Justino et al., 2021) since they may accidentally ingest these particles by mistaking them for prey or foraging on the substrate. Overall, the MPs detected in the species in this study were, on average smaller than 1 mm. Besides the accidental ingestion of MPs mistaken for prey, these tiny particles can be passively captured while fish breathe (Li et al., 2021).

In general, fibres and films were the most frequent shapes of MPs detected in this study. Fibre is the MP shape most frequently associated with fish ingestion in different marine ecosystems (Ferreira et al., 2016; Foekema et al., 2013), as it was also observed in reef fish species (Baalkhuyur et al., 2018; Macieira et al., 2021). The possible sources of fibres in these regions can be either the fragmentation of fishing gear or the textile industry, which release fibres through the flow of domestic sewage from the continent and reach the continental shelf. However, not only fisheries but also touristic activities in reef areas may be impacting such habitats. The second most frequent MP shape was film, which can be originated from maintenance or fragmentation by abrasion between the boats sailing in these regions that release paint particles. Also, the most abundant colours were blue, black and white, as observed in other studies, and are similar to the typical colour used in fishing nets (Baalkhuyur et al., 2018; Ferreira et al., 2016).

The types of polymers identified in our study corroborate the primary source of MPs as fishing activities in the region. Among the various encountered polymers, the most abundant types were PE, PA and PP, which are used in fishing nets and fabrics and are frequently reported in fishes (Lima et al., 2021; Nie et al., 2019). In addition, we identified alkyd varnish, which is widely used in paints of boats, varnishes and synthetic enamels and as thermosetting plastics that can be moulded (Polymer DataBase, 2022). Finally, we identified other thermoplastics as PVA and PC; and the SBR, which is used in manufacturing car tires and as a substitute for natural rubber (Polymer DataBase, 2022). MP particles can adsorb pollutants available in the environment and release toxic additives to organisms (Fauvelle et al., 2021; Teuten et al., 2009). The impacts of MP ingestion may include digestive damage, decrease predation efficiency, or induce toxic effects (Barboza et al., 2018; de Sá et al., 2015; Moore, 2008). Furthermore, MPs can host a wide diversity of microorganisms such as viruses and bacteria (Pinheiro et al., 2021), which may increase disease proneness on coral reefs (Lamb et al., 2018).

Hence, the wide variety of MP polymers available in the reef can pressure this ecosystem, which is crucial to maintaining marine biodiversity. On the continental shelf, reef ecosystems are considered of great importance for the conservation of species (Eduardo et al., 2018). Moreover, in spite of the considerable ecological importance of the fish species analysed here, species of the genus *Haemulon* spp. are an important fishery resource and are exploited extensively by the traditional community in the northeast region of Brazil (Cardoso de Melo et al., 2020; Frédou et al., 2006). Similarly, to the contamination of *H. squamipinna* observed in this study, fish resources worldwide have already been reported contaminated with MPs, and the incidence of plastic ingestion by fish is increasing (Savoca et al., 2021). The contamination of reef species may lead to future problems for the fish stocks in the region and issues in the ecosystem functioning. Furthermore, the presence of MPs in fishery stocks, which are essential to traditional communities, can negatively impact human health in the future.

Acknowledgements

Our research was supported by the LMI TAPIOCA program CAPES/COFECUB (88881.142689/2017-01); FUNBIO and HUMANIZE under the grant “Programa Bolsas Funbio - Conservando o Futuro 2019 (02/2019)”; FACEPE (Fundação de Amparo à Ciência e Tecnologia do Estado de Pernambuco); and MicroplastiX Project/JPI-Oceans/CONFAP/FACEPE (n°APQ-0035-1.08/19). We thank the CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico), which provided research grants to Flávia Lucena Frédou (CNPq n° 308554/2019-1); FACEPE for granting a scholarship (BFP-0049-5.06/20) and CAPES for granting a scholarship at doctoral level for the first author.

REFERENCES

Andrady, A.L., 2011. Microplastics in the marine environment. Mar. Pollut. Bull. 62, 1596–1605. https://doi.org/10.1016/j.marpolbul.2011.05.030

Arthur, C., Baker, J., Bamford, H., 2009. Workshop on the Occurrence , Effects , and Fate of Microplastic Marine Debris. Group 530.

Baalkhuyur, F.M., Bin Dohaish, E.J.A., Elhalwagy, M.E.A., Alikunhi, N.M., AlSuwailem, A.M., Røstad, A., Coker, D.J., Berumen, M.L., Duarte, C.M., 2018. Microplastic in the gastrointestinal tract of fishes along the Saudi Arabian Red Sea coast. Mar. Pollut. Bull. 131, 407–415. https://doi.org/10.1016/j.marpolbul.2018.04.040

Barboza, L.G.A., Vieira, L.R., Branco, V., Figueiredo, N., Carvalho, F., Carvalho, C., Guilhermino, L., 2018. Microplastics cause neurotoxicity, oxidative damage and energy-related changes and interact with the bioaccumulation of mercury in the European seabass, Dicentrarchus labrax (Linnaeus, 1758). Aquat. Toxicol. 195, 49–57. https://doi.org/10.1016/j.aquatox.2017.12.008

Boerger, C.M., Lattin, G.L., Moore, S.L., Moore, C.J., 2010. Plastic ingestion by planktivorous fishes in the North Pacific Central Gyre. Mar. Pollut. Bull. 60, 2275–2278. https://doi.org/10.1016/j.marpolbul.2010.08.007

Bruzaca, D.N.A., Justino, A.K.S., Mota, G.C.P., Costa, G.A., Lucena-Frédou, F., Gálvez, A.O., 2022. Occurrence of microplastics in bivalve molluscs Anomalocardia flexuosa captured in Pernambuco, Northeast Brazil. Mar. Pollut. Bull. 179, 113659. https://doi.org/10.1016/j.marpolbul.2022.113659

Cardoso de Melo, C., Soares, A.P.C., Pelage, L., Eduardo, L.N., Frédou, T., Lira, A.S., Ferreira, B.P., Bertrand, A., Lucena-Frédou, F., 2020. Haemulidae distribution patterns along the Northeastern Brazilian continental shelf and size at first maturity of the most abundant species. Reg. Stud. Mar. Sci. 35, 101226. https://doi.org/10.1016/j.rsma.2020.101226

de Sá, L.C., Luís, L.G., Guilhermino, L., 2015. Effects of microplastics on juveniles of the common goby (Pomatoschistus microps): Confusion with prey, reduction of the predatory performance and efficiency, and possible influence of developmental conditions. Environ. Pollut. 196, 359–362. https://doi.org/10.1016/j.envpol.2014.10.026

Eduardo, L.N., Frédou, T., Lira, A.S., Ferreira, B.P., Bertrand, A., Ménard, F., Frédou, F.L., 2018. Identifying key habitat and spatial patterns of fish biodiversity in the tropical Brazilian continental shelf. Cont. Shelf Res. 166, 108–118. https://doi.org/10.1016/j.csr.2018.07.002

Fauvelle, V., Garel, M., Tamburini, C., Nerini, D., Castro-Jiménez, J., Schmidt, N., Paluselli, A., Fahs, A., Papillon, L., Booth, A.M., Sempéré, R., 2021. Organic additive release from plastic to seawater is lower under deep-sea conditions. Nat. Commun. 12. https://doi.org/10.1038/s41467-021-24738-w

Ferreira, G.V.B., Barletta, M., Lima, A.R.A., Dantas, D. V., Justino, A.K.S., Costa, M.F., 2016. Plastic debris contamination in the life cycle of Acoupa weakfish (Cynoscion acoupa) in a tropical estuary . ICES J. Mar. Sci. J. du Cons. 73, 2695–2707. https://doi.org/10.1093/icesjms/fsw108

Ferreira, G.V.B., Justino, A.K.S., Eduardo, L.N., Lenoble, V., Fauvelle, V., Schmidt, N., Junior, T.V., Frédou, T., Lucena-Frédou, F., 2022. Plastic in the inferno: Microplastic contamination in deep-sea cephalopods (Vampyroteuthis infernalis and Abralia veranyi) from the southwestern Atlantic. Mar. Pollut. Bull. 174, 113309. https://doi.org/10.1016/j.marpolbul.2021.113309

Foekema, E.M., De Gruijter, C., Mergia, M.T., Van Franeker, J.A., Murk, A.J., Koelmans, A.A., 2013. Plastic in north sea fish. Environ. Sci. Technol. 47, 8818–8824. https://doi.org/10.1021/es400931b

Frédou, T., Ferreira, B.P., Letourneur, Y., 2006. A univariate and multivariate study of reef fisheries off northeastern Brazil. ICES J. Mar. Sci. 63, 883–896. https://doi.org/10.1016/j.icesjms.2005.11.019

Garnier, Y., Jacob, H., Guerra, A.S., Bertucci, F., Lecchini, D., 2019. Evaluation of microplastic ingestion by tropical fish from Moorea Island, French Polynesia. Mar. Pollut. Bull. 140, 165–170. https://doi.org/10.1016/j.marpolbul.2019.01.038

IBGE, 2011. Atlas geográfico das zonas costeiras e oceânicas do Brasil. IBGE.

Jambeck, J.R., Geyer, R., Wilcox, C., Siegler, T.R., Perryman, M., Andrady, A., Narayan, R., Law, K.L., 2015. Plastic waste inputs from land into the ocean. Science (80-.). 347, 768–771. https://doi.org/10.1126/science.1260352

Justino, A.K.S., Ferreira, G.V.B., Schmidt, N., Eduardo, L.N., Fauvelle, V., Lenoble, V., Sempéré, R., Panagiotopoulos, C., Mincarone, M.M., Frédou, T., Lucena-Frédou, F., 2022. The role of mesopelagic fishes as microplastics vectors across the deep-sea layers from the Southwestern Tropical Atlantic. Environ. Pollut. 300, 118988. https://doi.org/10.1016/j.envpol.2022.118988

Justino, A.K.S., Lenoble, V., Pelage, L., Ferreira, G.V.B., Passarone, R., Frédou, T., Lucena Frédou, F., 2021. Microplastic contamination in tropical fishes: An assessment of different feeding habits. Reg. Stud. Mar. Sci. 45, 101857. https://doi.org/10.1016/j.rsma.2021.101857

Lamb, J.B., Willis, B.L., Fiorenza, E.A., Couch, C.S., Howard, R., Rader, D.N., True, J.D., Kelly, L.A., Ahmad, A., Jompa, J., Harvell, C.D., 2018. Plastic waste associated with disease on coral reefs. Science (80-.). 359, 460–462. https://doi.org/10.1126/science.aar3320

Li, B., Liang, W., Liu, Q.-X., Fu, S., Ma, C., Chen, Q., Su, L., Craig, N.J., Shi, H., 2021. Fish Ingest Microplastics Unintentionally. Environ. Sci. Technol. 55, 10471–10479. https://doi.org/10.1021/acs.est.1c01753

Lima, A.R.A., Barletta, M., Costa, M.F., Ramos, J.A.A., Dantas, D. V., Melo, P.A.M.C., Justino, A.K.S., Ferreira, G.V.B., 2016. Changes in the composition of ichthyoplankton assemblage and plastic debris in mangrove creeks relative to moon phases. J. Fish Biol. 89, 619–640. https://doi.org/10.1111/jfb.12838

Lima, A.R.A., Ferreira, G.V.B., Barrows, A.P.W., Christiansen, K.S., Treinish, G., Toshack, M.C., 2021. Global patterns for the spatial distribution of floating microfibers: Arctic Ocean as a potential accumulation zone. J. Hazard. Mater. 403. https://doi.org/10.1016/j.jhazmat.2020.123796

Lins-Silva, N., Marcolin, C.R., Kessler, F., Schwamborn, R., 2021. A fresh look at microplastics and other particles in the tropical coastal ecosystems of Tamandaré, Brazil. Mar. Environ. Res. 169, 105327. https://doi.org/10.1016/j.marenvres.2021.105327

Macêdo, S.J., Muniz, K., Montes, M.F., Eskinazi Leça, E., Neumann Leitão, S., & Costa, M.D., 2004. Hidrologia da região costeira e plataforma continental do estado de Pernambuco. Oceanografia: um cenário tropical. Bagaço, Recife.

Macieira, R.M., Aparecida Silva Oliveira, L., Cardozo-Ferreira, G.C., Ribeiro Pimentel, C., Andrades, R., Luiz Gasparini, J., Sarti, F., Chelazzi, D., Cincinelli, A., Carvalho Gomes, L., Giarrizzo, T., 2021. Microplastic and artificial cellulose microfibers ingestion by reef fishes in the Guarapari Islands, southwestern Atlantic. Mar. Pollut. Bull. 167. https://doi.org/10.1016/j.marpolbul.2021.112371

Meijer, L.J.J., van Emmerik, T., van der Ent, R., Schmidt, C., Lebreton, L., 2021. More than 1000 rivers account for 80% of global riverine plastic emissions into the ocean. Sci. Adv. 7, eaaz5803. https://doi.org/10.1126/sciadv.aaz5803

MMA, 2006. Programa REVIZEE: Avaliação do potencial sustentável de recursos vivos na zona econômica exclusiva: relatório executivo. Brasília, DF.

Monteiro, R.C.P., Do Sul, J.A.I., Costa, M.F., 2020. Small microplastics on beaches of fernando de noronha island, tropical atlantic ocean. Ocean Coast. Res. 68, 1–10. https://doi.org/10.1590/S2675-28242020068235

Moore, C.J., 2008. Synthetic polymers in the marine environment: A rapidly increasing, long-term threat. Environ. Res. 108, 131–139. https://doi.org/10.1016/j.envres.2008.07.025

Morais, L.M.S., Sarti, F., Chelazzi, D., Cincinelli, A., Giarrizzo, T., Martinelli Filho, J.E., 2020. The sea anemone Bunodosoma cangicum as a potential biomonitor for microplastics contamination on the Brazilian Amazon coast. Environ. Pollut. 265. https://doi.org/10.1016/j.envpol.2020.114817

Nie, H., Wang, J., Xu, K., Huang, Y., Yan, M., 2019. Microplastic pollution in water and fish samples around Nanxun Reef in Nansha Islands, South China Sea. Sci. Total Environ. 696, 134022. https://doi.org/10.1016/j.scitotenv.2019.134022

Nóbrega, M.D., Lessa, R., Santana, F.M., 2009. Peixes marinhos da região Nordeste do Brasil. Editora Martins & Cordeiro, Fortaleza.

Pereira, P.H.C., Barros, B., Zemoi, R., Ferreira, B.P., 2015. Ontogenetic diet changes and food partitioning of Haemulon spp. coral reef fishes, with a review of the genus diet. Rev. Fish Biol. Fish. 25, 245–260. https://doi.org/10.1007/s11160-014-9378-2

Pinheiro, H.T., Rocha, L.A., Macieira, R.M., Carvalho-Filho, A., Anderson, A.B., Bender, M.G., Di Dario, F., Ferreira, C.E.L., Figueiredo-Filho, J., Francini-Filho, R., Gasparini, J.L., Joyeux, J.C., Luiz, O.J., Mincarone, M.M., Moura, R.L., Nunes, J. de A.C.C., Quimbayo, J.P., Rosa, R.S., Sampaio, C.L.S., Sazima, I., Simon, T., Vila-Nova, D.A., Floeter, S.R., 2018. South-western Atlantic reef fishes: Zoogeographical patterns and ecological drivers reveal a secondary biodiversity centre in the Atlantic Ocean. Divers. Distrib. 24, 951–965. https://doi.org/10.1111/ddi.12729

Pinheiro, L.M., Agostini, V.O., Lima, A.R.A., Ward, R.D., Pinho, G.L.L., 2021. The fate of plastic litter within estuarine compartments: an overview of current knowledge for the transboundary issue to guide future assessments. Environ. Pollut. 116908. https://doi.org/10.1016/j.envpol.2021.116908

Polymer DataBase, 2022. Polymer DataBase [WWW Document]. URL https://polymerdatabase.com/ (accessed 4.29.22).

R Core Team, 2020. R: A language and environment for statistical computing. R Foundation for Statistical Computing.

Rochman, C.M., Hoh, E., Hentschel, B.T., Kaye, S., 2013. Long-term field measurement of sorption of organic contaminants to five types of plastic pellets: Implications for plastic marine debris. Environ. Sci. Technol. 47, 1646–1654. https://doi.org/10.1021/es303700s

Savoca, M.S., McInturf, A.G., Hazen, E.L., 2021. Plastic ingestion by marine fish is widespread and increasing. Glob. Chang. Biol. 27, 2188–2199. https://doi.org/10.1111/gcb.15533

Teuten, E.L., Rowland, S.J., Galloway, T.S., Thompson, R.C., 2007. Potential for plastics to transport hydrophobic contaminants. Environ. Sci. Technol. 41, 7759–7764. https://doi.org/10.1021/es071737s

Teuten, E.L., Saquing, J.M., Knappe, D.R.U., Barlaz, M.A., Jonsson, S., Björn, A., Rowland, S.J., Thompson, R.C., Galloway, T.S., Yamashita, R., Ochi, D., Watanuki, Y., Moore, C., Viet, P.H., Tana, T.S., Prudente, M., Boonyatumanond, R., Zakaria, M.P., Akkhavong, K., Ogata, Y., Hirai, H., Iwasa, S., Mizukawa, K., Hagino, Y., Imamura, A., Saha, M., Takada, H., 2009. Transport and release of chemicals from plastics to the environment and to wildlife. Philos. Trans. R. Soc. B Biol. Sci. 364, 2027–2045. https://doi.org/10.1098/rstb.2008.0284

Thompson, R.C., Olson, Y., Mitchell, R.P., Davis, A., Rowland, S.J., John, A.W.G., McGonigle, D., Russell, A.E., 2004. Lost at Sea: Where Is All the Plastic? Science (80-.). 304, 838. https://doi.org/10.1126/science.1094559

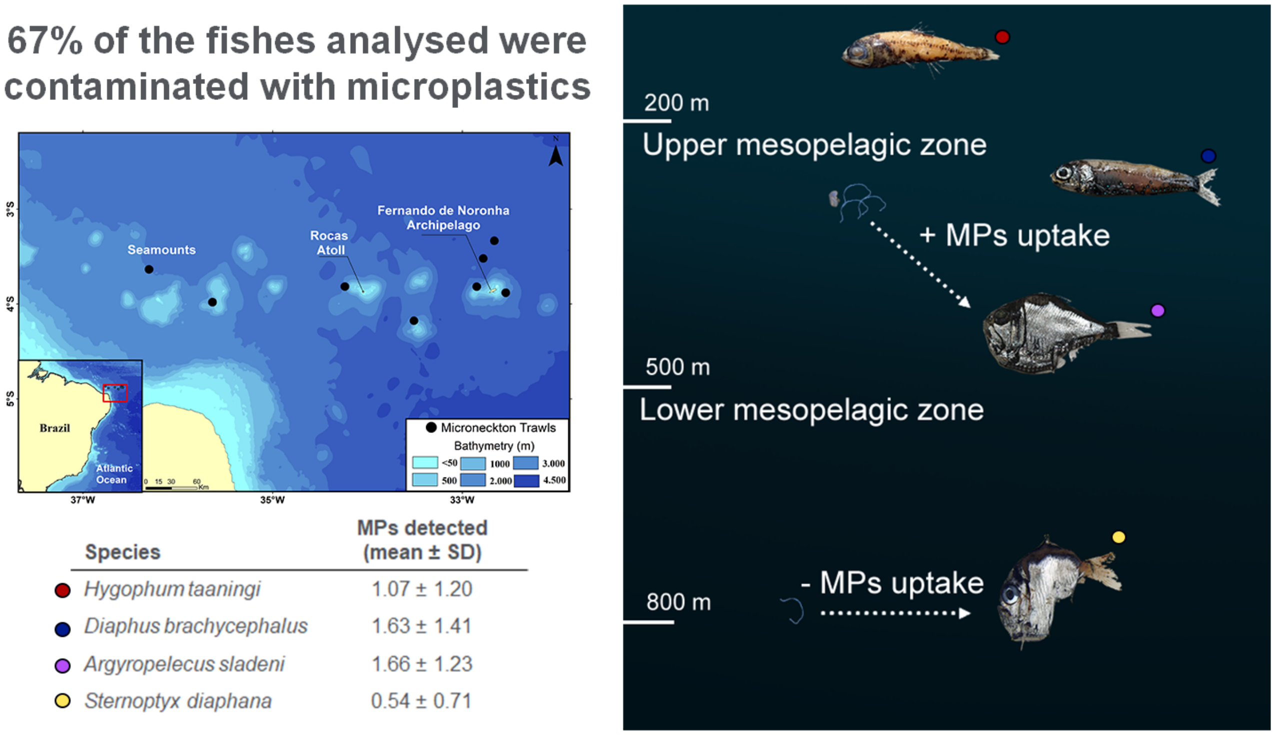
Vendel, A.L., Bessa, F., Alves, V.E.N., Amorim, A.L.A., Patrício, J., Palma, A.R.T., 2017. Widespread microplastic ingestion by fish assemblages in tropical estuaries subjected to anthropogenic pressures. https://doi.org/10.1016/j.marpolbul.2017.01.081

CHAPTER 3

Article "The role of mesopelagic fishes as microplastics vectors across the deep-sea layers from the Southwestern Tropical Atlantic"

Manuscript submitted on 1 November 2021 and published on 11 February 2022 in the Environmental Pollution.

https://doi.org/10.1016/j.envpol.2022.118988



67% of the fishes analysed were contaminated with microplastics

Species	MPs detected (mean ± SD)
🔴 *Hygophum taaningi*	1.07 ± 1.20
🔵 *Diaphus brachycephalus*	1.63 ± 1.41
🟣 *Argyropelecus sladeni*	1.66 ± 1.23
🟡 *Sternoptyx diaphana*	0.54 ± 0.71

The role of mesopelagic fishes as microplastics vectors across the deep-sea layers from the Southwestern Tropical Atlantic

Anne K. S. Justino[1,2*], Guilherme V. B. Ferreira[1], Natascha Schmidt[3], Leandro N. Eduardo[1,4], Vincent Fauvelle[3], Véronique Lenoble[2], Richard Sempéré[3], Christos Panagiotopoulos[3], Michael M. Mincarone[5], Thierry Frédou[1], Flávia Lucena-Frédou[1]

[1] Universidade Federal Rural de Pernambuco (UFRPE), Departamento de Pesca e Aquicultura (DEPAQ), Rua Dom Manuel de Medeiros, s/n, 52171-900, Recife, Brazil.
[2] Université de Toulon, Aix Marseille Univ., CNRS, IRD, MIO, Toulon, France.
[3] Aix Marseille Univ., Université de Toulon, CNRS, IRD, MIO, Marseille, France.
[4] Institut de Recherche pour le Développement (IRD), MARBEC, Univ. Montpellier, CNRS, Ifremer, IRD, Sète, France.
[5] Universidade Federal do Rio de Janeiro (UFRJ), Instituto de Biodiversidade e Sustentabilidade (NUPEM), Macaé, RJ, Brazil.

* Corresponding author: anne.justino@ufrpe.br

Abstract

Microplastics (MPs; <5 mm) are a macro issue recognised worldwide as a threat to biodiversity and ecosystems. Widely distributed in marine ecosystems, MPs have already been found in the deep-sea environment. However, there is little information on ecological mechanisms driving MP uptake by deep-sea species. For the first time, this study generates data on MP contamination in mesopelagic fishes from the Southwestern Tropical Atlantic (SWTA) to help understand the deep-sea contamination patterns. An alkaline digestion protocol was applied to extract MPs from the digestive tract of four mesopelagic fish species: *Argyropelecus sladeni*, *Sternoptyx diaphana* (Sternoptychidae), *Diaphus brachycephalus*, and *Hygophum taaningi* (Myctophidae). A total of 213 particles were recovered from 170 specimens, and MPs were found in 67% of the specimens. Fibres were the most common shape found in all species, whereas polyamide, polyethylene, and polyethylene terephthalate were the most frequent polymers. The most contaminated species was *A. sladeni* (93%), and the least contaminated was *S. diaphana* (45%). Interestingly, individuals caught in the lower mesopelagic zone (500–1000 m depth) were less contaminated with MPs than those captured in the upper mesopelagic layer (200–500 m). Our results highlight significant contamination levels and reveal the influence of mesopelagic fishes on MPs transport in the deep waters of the SWTA.

Keywords: Marine Pollution, Plastic ingestion, Myctophidae, Sternoptychidae, Oceanic islands.

Introduction

Since its invention, plastic production has risen considerably, reaching up to 348 million tons (Mt) in 2017 (PlasticsEurope, 2018), with a prognosis to hit 1100 Mt by 2050 (Geyer, 2020). Vast quantities of plastic materials are mismanaged or illegally discarded in marine ecosystems (Koelmans et al., 2017; Ostle et al., 2019). Land-based sources contribute to about 80% of plastics entering the oceans (Andrady, 2011) via riverine discharges (Meijer et al., 2021). In marine ecosystems, plastic debris is weathered by natural processes (*e.g.*, hydrodynamics, solar radiation and interaction with biota (Jambeck et al., 2015; Thompson et al., 2004) and eventually fragmented into microplastics (MPs, < 5 mm; Arthur et al., 2009).

MPs are widely distributed all over the marine environment, from urban coastal areas (Lins-Silva et al., 2021) to remote regions such as the Arctic and Antarctic polar seas (Lusher et al., 2015; Waller et al., 2017). MPs accumulate in the ocean gyres (Jiang et al., 2020) due to the interaction of winds and rotatory ocean currents. In the Atlantic Ocean, remote islands are known to be contaminated with MPs, as is the case of Falklands and Ascension Islands (Green et al., 2018); the Canary Islands (Álvarez-Hernández et al., 2019); Abrolhos Archipelago, Fernando de Noronha Archipelago, and Trindade Island (Ivar do Sul et al., 2013, 2014). In the short term, these islands might retain MPs in the nearshore due to the actions of winds, waves, vortices, and eddies surrounding the islands (Lima et al., 2016). Nevertheless, not only the sea surface is impacted by MPs, but also the deep sea, which has been pointed out as a major MPs reservoir (Woodall et al., 2014). Indeed, MPs have already been observed in the subsurface waters, sediments, and fauna of the deep sea (Lusher et al., 2016; Courtene-Jones et al., 2017; Choy et al., 2019; Jamieson et al., 2019; Kane et al., 2020). However, processes involved in the dispersion and fate of MPs into deeper ocean layers are still poorly understood.

MPs can be transported from the surface to deep waters through interaction with marine communities. For example, giant larvaceans can pack MPs filtered in the surface into faecal pellets that quickly sink to the seafloor (Katija et al., 2017; Choy et al., 2019). MPs incorporation into marine snow is hypothesised to be the main sinking mechanism for buoyant polymers (Kvale et al., 2020). Additionally, many deep-sea species undertake epipelagic vertical migrations to feed (Eduardo et al., 2020a) and may act as biological plastic transporter whenever contaminated with MPs (Ferreira et al., 2022). Although the role of mesopelagic fishes in the vertical movement of MPs in the water column has been proposed, it is still not well understood (Lusher et al., 2016; Savoca et al., 2021). Thus, widespread MPs pose several threats to marine biota (Galloway et al., 2017), as they can easily be mistaken with prey and ingested by marine species (Boerger et al., 2010). Furthermore, they might be transferred from prey to predator through trophic interactions (Ferreira et al., 2016, 2019; Nelms et al., 2018). Once ingested, MPs can cause digestive damage, decrease predatory efficiency, and induce toxic effects (Teuten et al., 2007; Moore, 2008; de Sá et al., 2015; Barboza et al., 2018). Moreover, MPs can adsorb and concentrate pollutants available in the ocean (*e.g.*, persistent organic pollutants and heavy metals; Oehlmann et al., 2009; Ashton et al., 2010; Rochman et al., 2013c; Jamieson et al., 2017) or release their additive burden (Paluselli et al., 2019; Fauvelle et al., 2021), and may be bioaccumulated and biomagnified in the food web (Teuten et al., 2009; Batel et al., 2016).

The mesopelagic layer (200–1000 m) hosts remarkable marine biodiversity that plays a pivotal role in sequestering carbon, recycling nutrients, and acting as a key trophic link between primary consumers and higher trophic levels (*e.g.*, larger fishes, mammals, and seabirds; Drazen and Sutton, 2017; Eduardo et al., 2020a). Additionally, many mesopelagic species migrate vertically to the upper ocean layers to feed at night and return to deep waters during daylight, contributing to the connection between shallow and deep-sea ecosystems (Davison et al., 2013; St. John et al., 2016; Eduardo et al., 2020b).

MP ingestion by mesopelagic fishes has been already reported all over the world, as observed in the North Pacific Central Gyre (Boerger et al., 2010), North Pacific Subtropical Gyre (Davison and Asch, 2011), North Atlantic (Lusher et al., 2016; Wieczorek et al., 2018), Mediterranean Sea (Romeo et al., 2016), South China Sea (Zhu et al., 2019), and in the South Atlantic, around the Tristan da Cunha and St. Helena islands (McGoran et al., 2021). However, this group is still poorly investigated in deep waters due to sampling difficulties (*e.g.*, high sampling cost and operational complexity), especially in the least developed countries (Howell et al., 2020). To date, no study has investigated MP contamination in fishes inhabiting the mesopelagic zone of the Southwestern Tropical Atlantic (SWTA). Located in the SWTA, the Fernando de Noronha Archipelago (FNA) is essential for the conservation of the marine biodiversity in the tropical oceanic region, as it serves as a shelter, reproduction and nursery area for several species, including the mesopelagic fishes (Lima et al., 2016; Eduardo et al., 2020a; Martins et al., 2021).

Hatchetfishes (Sternoptychidae) and lanternfishes (Myctophidae) are among the most abundant and widespread mesopelagic fish groups in the world (Gjøsaeter and Kawaguchi, 1980; Eduardo et al., 2020a, 2021). These groups present an essential linkage between the epipelagic producers and deep-sea predators since they represent a key energy source in the mesopelagic zone (Eduardo et al., 2020b, 2020a, 2021).

Within the SWTA, four species in the mesopelagic compartment are outstanding in terms of abundance and/or vertical migration: the sternoptychids *Argyropelecus sladeni* Regan, 1908 and *Sternoptyx diaphana* Hermann, 1781; and the myctophids *Diaphus brachycephalus* (Tåning, 1928) and *Hygophum taaningi* Becker, 1965. These species are zooplanktivorous, feeding primarily on fish larvae, amphipods, gelatinous, and euphausiids (Drazen and Sutton, 2017; Eduardo et al., 2020a; Eduardo et al., 2021). Furthermore, they all perform diel vertical migration, ascending to the epipelagic zone at night mainly to forage and avoid predators (Eduardo et al., 2020a; Eduardo et al., 2021). However, these species present strong niche segregation, belonging to functional groups with different diet preferences, isotopic composition, and vertical distribution (Eduardo et al., 2020a; Eduardo et al., 2021). These ecological differences, therefore, might also influence MP uptake.

In this study, we identify the patterns of MP contamination in mesopelagic fishes from the SWTA and their relationship with different ecological habits. Specifically, this study aims (*i*) to describe the occurrence of MP contamination in four mesopelagic species from the SWTA, (*ii*) to identify the main shapes and polymer nature of the ingested particles, and (*iii*) to investigate whether there are differences in MP ingestion rates according to depth and period (day or night).

Materials and Methods

Study area

The study area is located along the Fernando de Noronha Ridge, SWTA, with oligotrophic and warm waters influenced by the South Equatorial Current (SEC) and South Equatorial Undercurrent (SEUC) (Assunção et al., 2020), specifically the Fernando de Noronha Archipelago (FNA), Rocas Atoll (RA), and adjacent seamounts (**Figure**). These areas are important for marine biodiversity and are recognised as an EBSA

"Ecologically and Biologically Significant Marine Area" (CBD, 2014). Furthermore, FNA is inserted in a Marine Protected Area (MPA), with a National Marine Park (PARNAMAR) and an Environmental Protection Area (EPA), which is classified as a UNESCO natural heritage. The RA is also inserted in an MPA, and it is situated at the top of a submarine mountain chain, with its base at 4000 m depth, located 148 km west of the Fernando de Noronha Archipelago (Soares et al., 2010).

Sample collection and laboratory procedures

Mesopelagic fishes were collected using a micronekton trawl (body mesh: 40 mm, cod-end mesh: 10 mm) during the day and at night, from 90 to 800 m depth for 30 min at 2–3 kt (Eduardo et al., 2020b). Samples were collected along the Fernando de Noronha Ridge during the scientific survey ABRACOS 2 (Acoustics along the BRAzilian COaSt), carried out from 9th April to 6th May 2017, onboard the French RV *Antea* (Bertrand, 2017). After each sampling, the specimens were labelled, frozen, and subsequently identified.

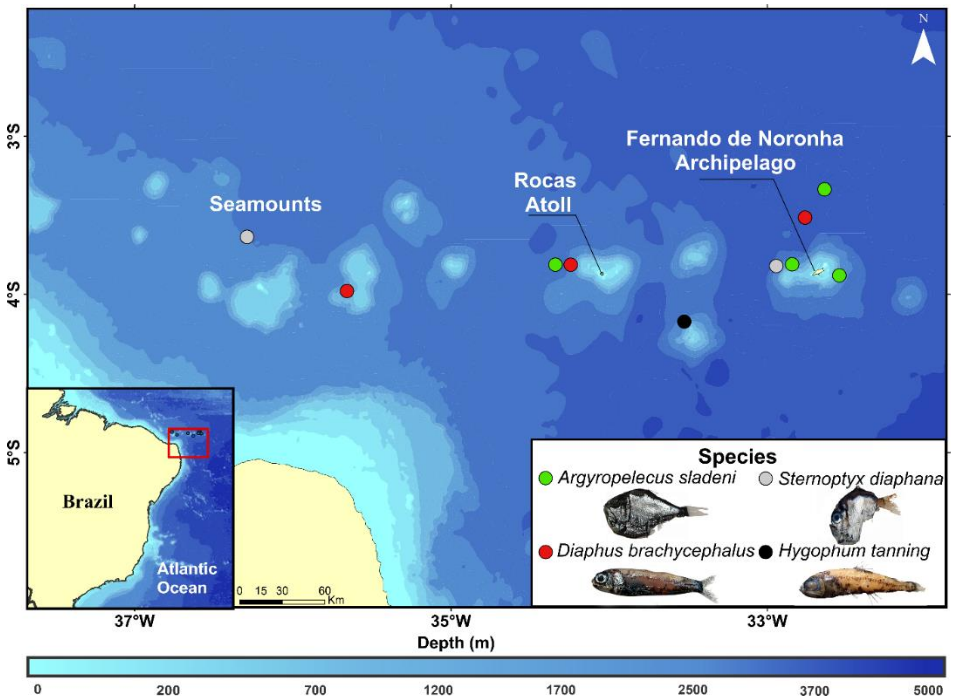


Figure 1. Fernando de Noronha Ridge, off northeastern Brazil (STWA). Sampling stations for each species are indicated by coloured circles.

Four mesopelagic species were selected for this study: *Argyropelecus sladeni* (*n* = 15); *Sternoptyx diaphana* (*n* = 33); *Diaphus brachycephalus* (*n* = 69); and *Hygophum taaningi* (*n* = 53). Specimens were measured (nearest 0.1 cm of total length and standard length), weighed (nearest 0.01 g of total weight), and dissected (Table I). The digestive tracts (stomach and intestine) were carefully removed, weighed, and frozen again for the digestion analysis.

Contamination control

Before the extraction procedures, several steps were carefully carried out to ensure quality assurance/quality control (QA/QC) and avoid possible airborne and cross-contamination, following the protocol described by Justino et al. (2021). This QA/QC includes using 100% cotton lab coats, face masks, and disposable gloves in a cleaned and reserved room, with a limited flow of people during the whole process. Additionally, all solutions were filtered using a vacuum pump system (equipped with laboratory glassware) through a 47 mm GF/F 0.7 µm pore size glass fibre filter (Whatman). Extraction tools were cleaned with ethanol 70%, rinsed with filtered distilled water and checked for contamination.

Before starting the chemical digestion, blank procedures were done for each set of 10 samples. For the blanks, a beaker was filled with 50 mL of NaOH (1 mol L^{-1}) solution, covered with a glass lid, and then treated with the same protocol applied to the samples (see next section). A total of 4 particles were observed in the blank procedures, of which two were filaments (one red and one white), and two resembled paint chips (blue). The red filament was further identified as polylactic acid (PLA), and the blue particle resembled a paint chip as styrene-butadiene rubber (SBR). Particles identified in the samples with any similarity to those observed in the blanks were excluded from further analysis.

Microplastic extraction protocol

An alkaline digestion protocol using sodium hydroxide (NaOH) was used for extracting MPs from the digestive tract of fish (Justino et al., 2021). Digestive tract samples were rinsed with filtered distilled water to remove any particles adhering to the external tissue before being placed in a beaker and submerged in NaOH (1 mol L^{-1}; PA 97%) solution (the proportion used was 1:100 (w/v), i.e. 1 g of digestive tract weight for 100 mL NaOH solution), covered by a glass lid and oven-dried at 60 °C for 24 h. After that, samples were filtered using a vacuum pump system through a 47 mm GF/F. After filtration, samples were carefully set in a Petri dish and covered. These filters were oven-dried again at 60 °C for 24 h. Then, filters were visually examined for MPs identification using a stereomicroscope (Zeiss Stemi 508, with 40–50 times magnification with a size detection limit of 0.07–5 mm). The particles suspected to be MPs were photographed

(Axiocam 105 Color), counted, and measured in length (mm) (Zeiss Zen 3.2). MPs were categorised according to their shape (Figure ; Justino et al., 2021) as fibres (filamentous shape), fragments (irregular shape), films (flat shape), foams (soft with an irregular shape), or pellets (spherical shape).

Laser Direct Infrared (LDIR) Analysis of MPs polymers

A subset (10% of the total particles extracted) of samples was selected to identify the main types of MPs polymers using the LDIR analyser Agilent 8700 Chemical Imaging System using the Microplastic Starter 1.0 library. The LDIR analyser scans the particles (size range 20–5000 µm) in an automatic mode and obtains a spectral curve using a wavelength range of 1800–975 cm^{-1}. The information is collected with the Clarity image software (© Agilent version 1.3.9) and compared with the polymer spectrum library (~400 references spectra). A particle was considered as identified if the accordance of its spectrum with the reference spectrum was ≥ 70% (Ourgaud et al., In prep).

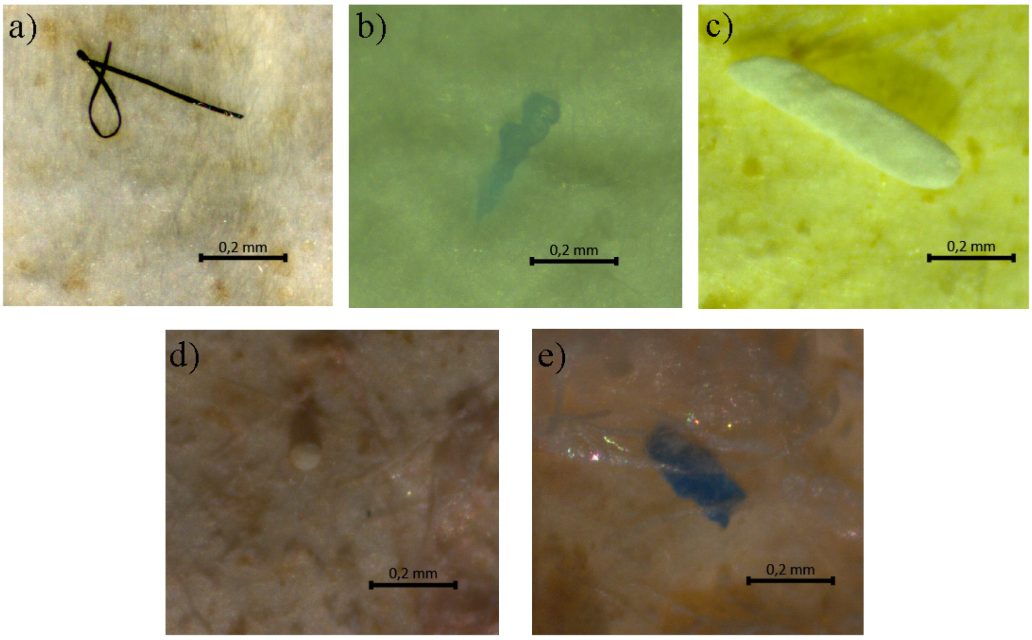


Figure 2. Shapes of microplastics identified in the mesopelagic fishes: a) fibre; b) fragment; c) foam; d) pellet; e) film.

Data analysis

Kruskal-Wallis test was used to verify whether ingested MPs presented significant differences among species (*A. sladeni*, *S. diaphana*, *D. brachycephalus*, and *H. taaningi*) considering the number and size of MPs. We also used Kruskal-Wallis to test whether the total number of MPs ingested varied according to depth. When the Kruskal-Wallis test presented significant differences, *post hoc* pairwise comparisons, Dunn's test was used to investigate the sources of variance (Dunn, 1964). Mann-Whitney tests were applied to determine differences in the MPs ingested according to the period (day or night). A Spearman's correlation test was used to evaluate the relationship between MPs ingestion and biological parameters of fishes (standard length and total weight). All statistical analyses were performed with the software R version 3.6.3 (R Core Team, 2020) and were conducted considering a level of significance of 5%.

Results

A total of 213 microplastic (MPs) particles were recovered from the 170 analysed specimens (frequency of occurrence 67%). MPs were presented in 93% of *Argyropelecus sladeni*, 75% of *Diaphus brachycephalus*, 62% of *Hygophum taaningi*, and 45% of *Sternoptyx diaphana* specimens (Table I). According to the number of MPs, ingestion significantly differed between species (chi-squared = 20.437, df = 3, $p < 0.05$), with *A. sladeni* being the most contaminated (1.66 ± 1.23 MPs ind.$^{-1}$), followed by *D. brachycephalus* (1.63 ± 1.41 MPs ind.$^{-1}$), *H. taaningi* (1.07 ± 1.20 MPs ind.$^{-1}$), and *S. diaphana* (0.54 ± 0.71 MPs ind.$^{-1}$) (Table I). Dunn's *post hoc* test showed that *S. diaphana* differed from *A. sladeni* and *D. brachycephalus*. Additionally, there was no relationship between the MPs ingested by fish species and the biological parameters (standard length and the total weight) (Spearman's rank correlation, $p > 0.05$).

In general, the mean size of ingested MPs also varied according to the species (chi-squared = 12.247, df = 3, $p < 0.05$). *Argyropelecus sladeni* (0.74 ± 0.53 mm ind.$^{-1}$) showed the longest size of MPs ingested, followed by *H. taaningi* (0.49 ± 0.80 mm ind.$^{-1}$), *D. brachycephalus* (0.44 ± 0.53 mm ind.$^{-1}$), and *S. diaphana* (0.36 ± 0.82 mm ind.$^{-1}$), with significant differences observed between *A. sladeni* and *S. diaphana* (Table I). Overall, fish MP contamination levels were not significantly different between day or night sampling, regardless of species (chi-squared = 1.4024, df = 1, $p > 0.05$), and by species individually ($p > 0.05$). However, ingestion differed among the sampling depths (chi-squared = 18.80, df = 6, $p < 0.05$). Fishes were generally most contaminated at 230 m (1.73 ± 1.25 MPs ind.$^{-1}$), followed by 430 m (1.66 ± 0.57 MPs ind.$^{-1}$), and 610 m (1.62 ± 1.44 MPs ind.$^{-1}$), and less contaminated at 800 m (0.57 ± 0.75 MPs ind.$^{-1}$) (**Figure**). Statistically significant differences were observed between depths of 800 and 230 m and between depths of 800 and 610 m ($p < 0.05$). Regarding the shape of MPs ingested by fishes, most were fibres (64%), followed by fragments (19%), pellets (6%), films and foams (4%). However, the shape of ingested MPs did not vary between the species (chi-squared = 3.1683, df = 4, $p > 0.05$). Fibres were mainly observed in *S. diaphana* (83%), *A. sladeni* (76%), *H. taaningi* (63%), and *D. brachycephalus* (58%), followed by fragments in *D. brachycephalus* (23%), *H. taaningi* (21%), *A. sladeni* (12%) and *S. diaphana* (11%). Pellets were found in *H. taaningi* (12%), *S. diaphana* and *D. brachycephalus* (5%), and films were found in *A. sladeni* (12%), *D. brachycephalus* (5%), *H. taaningi* (1%). Foams were only found in *D. brachycephalus* (7%) and *H. taaningi* (1%) (**FigureErro! Fonte de referência não encontrada.Erro! Fonte de referência não encontrada.**).

Overall, plastic polymers were identified in 80% of particles from the subset of samples. Natural particles identified as cellulose were observed in 15% of all particles, and 5% were unidentified. The most common polymers found were polyamide (PA) at 25% abundance, followed by polyethylene (PE) and polyethylene terephthalate (PET), with a similar abundance at 19%. The other polymers contributed to a similar percentage of 6-7% and included the ethylene-vinyl acetate (EVA), polyvinylchloride (PVC), styrene-butadiene rubber (SBR), polylactic acid (PLA), alkyd varnish and chlorinated polyisoprene (**Figure**).

Table I. Biological aspects and sampling data of the species analysed. Abbreviations: SL, standard length; TW, total weight; FO%, frequency of occurrence; SD, standard deviation.

Family/Species	Sampling		Biometry		Microplastics occurrence		
	n	Depth (m)	SL (cm) range	TW (g) range	FO%	MPs mean ± SD	Length (mm) mean ± SD
Sternoptychidae							
Argyropelecus sladeni	15	430; 610; 615; 800	3.00–5.85	0.70–3.18	93	1.66 ± 1.23	0.74 ± 0.53
Sternoptyx diaphana	33	615; 800	1.92–3.06	0.18–0.97	45	0.54 ± 0.71	0.36 ± 0.82
Myctophidae							
Diaphus brachycephalus	69	230; 610; 700	2.51–4.98	0.34–2.15	75	1.63 ± 1.41	0.40 ± 0.55
Hygophum taaningi	53	90	4.13–5.99	1.14–2.68	62	1.07 ± 1.20	0.49 ± 0.80

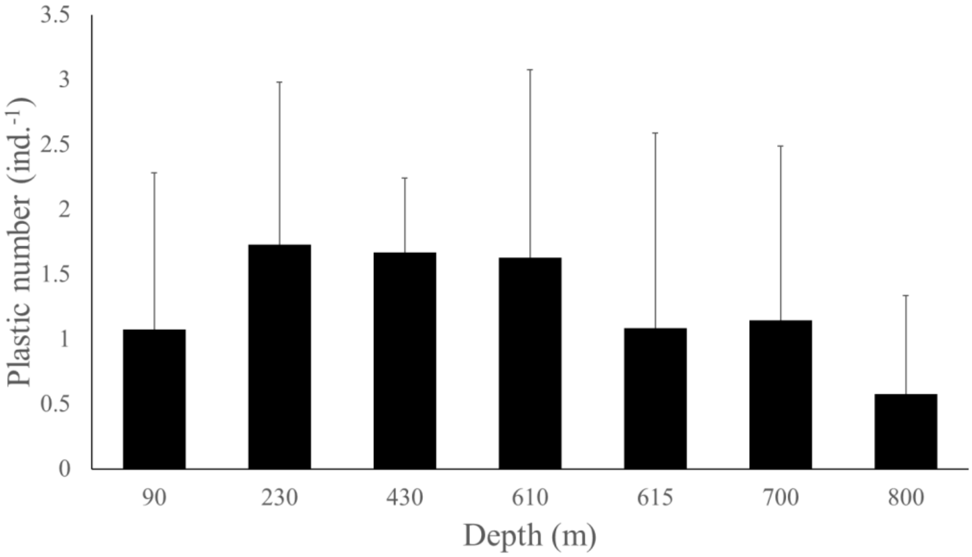


Figure 3. Mean number (± standard deviation) of MPs ingested per depth strata.

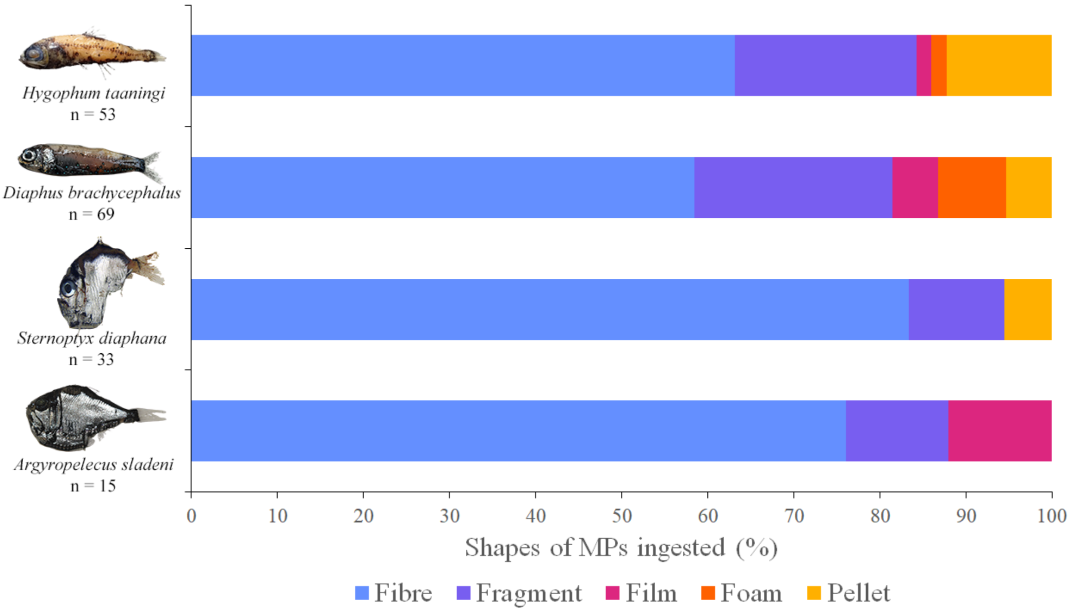


Figure 4. Relative abundances (%) of MP shapes ingested per fish species.

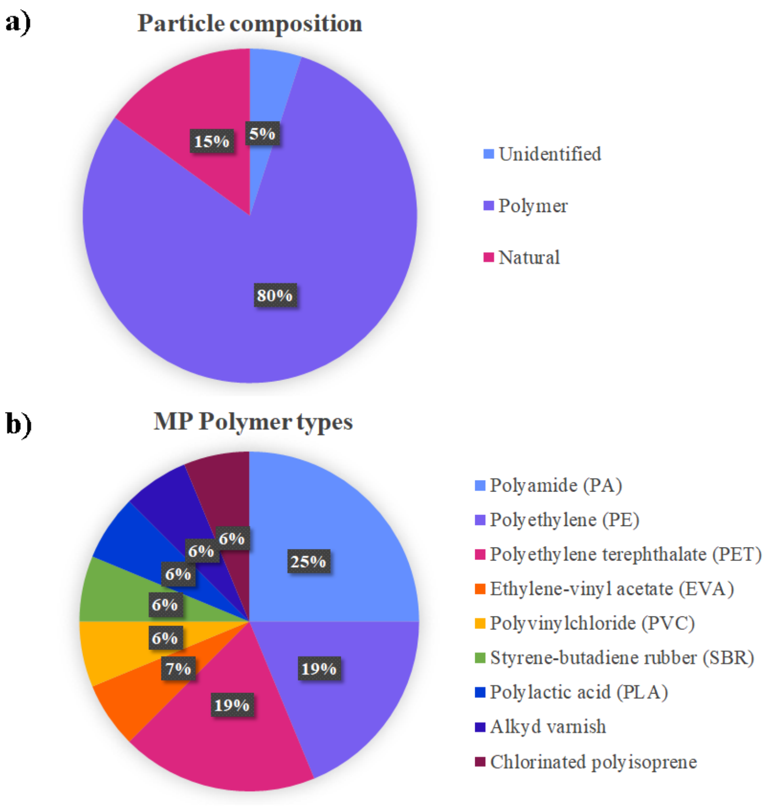


Figure 5. Polymers identified using the LDIR analyser. a) Particle composition in the samples analysed, and b) Percentage of microplastic polymers found in the samples.

Discussion

This study confirmed that the mesopelagic fishes from the SWTA are contaminated with MPs. The four species analysed here exhibited a high MP detection frequency in their digestive tract (67%). These findings bring new information into the contamination of the deep sea and shed light on the potential role of marine organisms in MPs sinking.

Worldwide, few studies have documented plastic ingestion by mesopelagic fishes. For example, in the North Pacific Gyre, Davison and Asch (2011) reported an MP detection frequency of 9.2% of the fishes sampled, whereas Boerger et al. (2010) found 35% in the same area. In the Mediterranean Sea, Romeo et al. (2016) found MPs in 2.7% of sampled lanternfishes, whereas Zhu et al. (2019) reported the presence of MPs in more than 90% of the deep-sea fishes sampled in the South China Sea. In the Islands of Tristan da Cunha and St. Helena, McGoran et al. (2021) found 73.3% of species contaminated with MPs; and in the North Atlantic, Lusher et al. (2016) found 11% of individuals contaminated, in contrast with Wieczorek et al. (2018) which detected MPs in 73% of the mesopelagic fish specimens from the same area. The substantial divergence in the frequency of occurrence of MPs recovered in mesopelagic fishes may be due to several factors such as ecological behaviour, site-specific oceanographic differences, laboratory procedures, and sampling methods. However, differences in the extraction methods, an issue previously addressed by Wieczorek et al. (2018), might also influence the contamination rate. A lack of standardisation of the protocols for MPs extraction in organisms is the main issue for comparing studies on plastic contamination. The scientific community emphasises the importance of employing reliable and replicable research methods (Hermsen et al., 2018; Markic et al., 2020; Müller, 2021), not only concerning the choice of a suitable extraction method for MPs (*e.g.*, digestion and QA/QC protocols), but also an adequate sample size (> 10; Justino et al., 2021) and size detection threshold of the particles, which is determinant in the number of plastics recovered (Savoca et al., 2021). Such decisions are important to avoid the bias of over/underestimation due to cross-contamination and loss of samples and were carefully considered in the present study.

The wide availability of MPs is expected to threaten biodiversity throughout the marine environment. Plastic debris is found all along the coastal zone, continental slope, around oceanic islands, seamounts, and even in the deepest parts of the ocean (Cai et al., 2018; Monteiro et al., 2018; Lins-Silva et al., 2021; Pinheiro et al., 2021). Differences in the ecological habits, such as feeding strategy and migration, might influence the MP uptake by marine species. A clear distinction was observed in our study between the number of MPs ingested by species. For example, *A. sladeni* exhibited the highest number of particles (mean of 1.66 ± 1.23 MPs ind.$^{-1}$; FO=93%), while *S. diaphana* exhibited the lowest number (0.54 ± 0.71 MPs ind.$^{-1}$; FO=45%). A distinct pattern from that recorded in previous studies on mesopelagic fishes, where two of the most up-to-date references did not observe any differences between species and depths (Lusher et al., 2016; Wieczorek et al., 2018).

The difference observed in MPs ingestion might be explained by the species vertical migration behaviour. For example, in our study area, *A. sladeni* is mostly distributed at 400–500 m during the daytime, mainly feeding on fish larvae and ostracods (Eduardo et al., 2020a). On the other hand, *S. diaphana* is found chiefly in deeper waters (700–900 m), primarily feeding on amphipods (Eduardo et al., 2020a). Likewise, in the daytime, *D. brachycephalus* is mainly distributed in the upper mesopelagic layer at 200–500 m, while *H. taaningi* was predominantly found in deeper waters (700–1000 m) (Eduardo et al., 2020a, 2021). However, the *H. taaningi* analysed in this study were only caught in the epipelagic zone, probably captured during migration towards superficial areas. Even though all species analysed in this study performed diel vertical migration (DVM), we did not observe any significant differences in the MP concentration in specimens sampled day or night. However, differences in MP number were observed depending on the depth strata.

Indeed, the most contaminated species (*A. sladeni* and *D. brachycephalus*) were mainly caught in the upper mesopelagic layer (230–430 m), and *S. diaphana*, which ingested a lower number of MPs particles, was captured in the lower mesopelagic layer (800 m). Therefore, we suggest that when migrating to the upper layers, these species interact with MPs and, when returning, they probably act as vectors of MPs to the deeper ocean layers (**Figure**). For instance, in the study area, myctophids constitute 85% of the viperfish diet, the most abundant mesopelagic micronektivore fish species (Eduardo et al., 2020b). To our best knowledge, there is no information on MP in sediment and bottom organisms for the SWTA region, making the real impact of MP and their transportation into the deep sea speculative. However, coupling the data gathered in the present study with the widely acknowledged fact that mesopelagic species transport carbon to deep waters (Davison et al., 2013; Drazen and Sutton, 2017; Eduardo et al., 2020a), it seems that these species may also be transporting MPs to the deep sea.

Furthermore, our data support previous hypotheses that the deeper layers are less contaminated (Kvale et al., 2020; Zobkov et al., 2019). In Monterey Bay, California, Choy et al. (2019) also observed a similar pattern: a peak concentration of MPs in the mesopelagic zone at a range of 200–600 m depth. Additionally, the size of MPs ingested was also influenced by the depth in which species were caught (Ferreira et al., 2022). *Argyropelecus sladeni* ingested the longest MPs, whereas *S. diaphana* ingested significantly smaller MPs, coinciding with surveys investigating MP size in the water column (Dai et al., 2018; Zobkov et al., 2019). The ingestion of smaller size plastics was also observed in deep-water species in the North-East Atlantic (Pereira et al., 2020). The sinking of MPs is associated with biological activities such as biofouling, marine snow, faecal pellets, and plastic pump, contributing to the dispersion of smaller particles in the deeper layers (Van Sebille et al., 2020). We corroborate previous findings by linking MP size to depth since we found the smallest particles in species inhabiting the deepest layers.

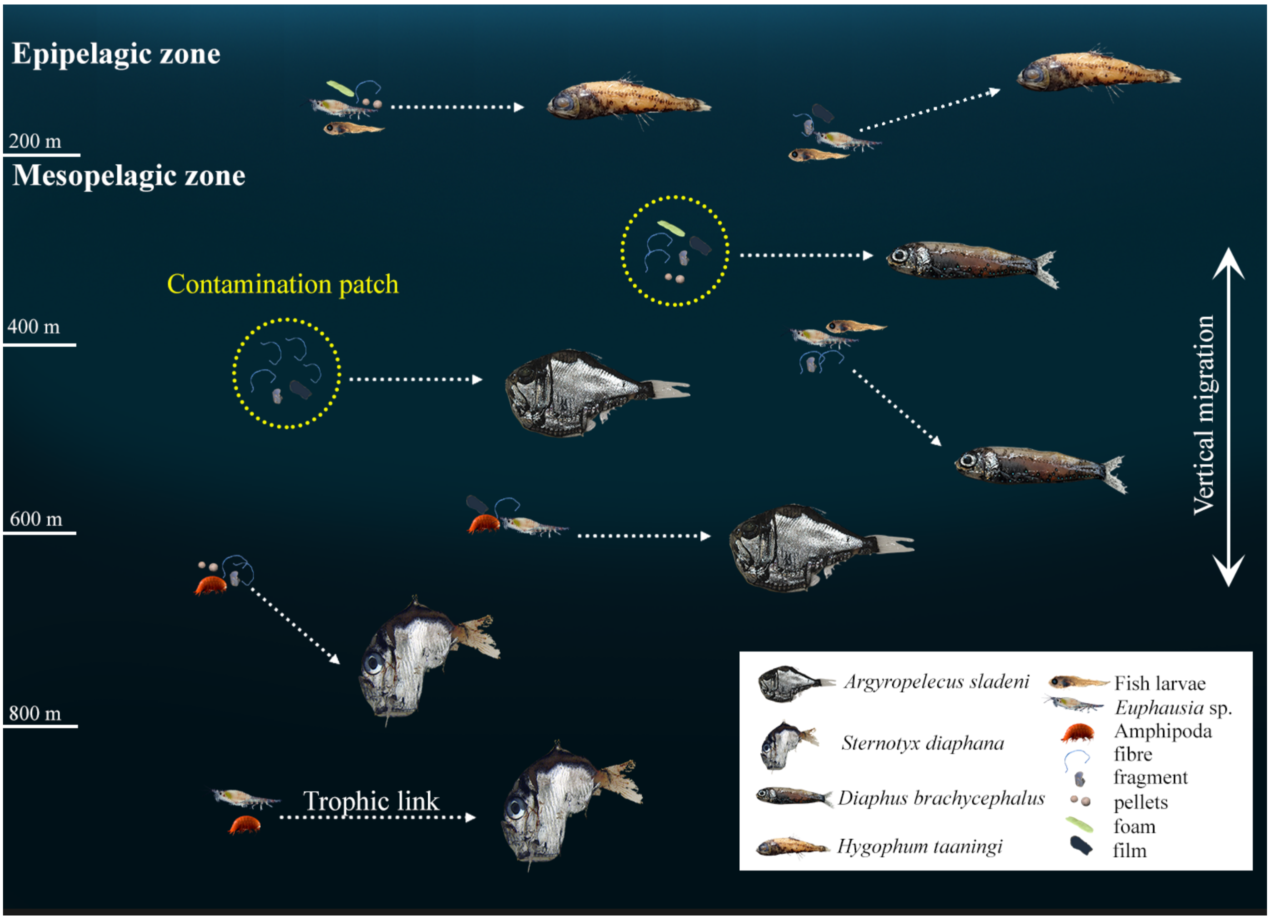


Figure 6. Schematic representation of the microplastic ingestion by mesopelagic fishes in the Southwestern Tropical Atlantic. White dotted arrows indicate the ingestion by trophic link, and yellow dotted circles the probable microplastic accumulation zone.

In our study, fibres were the common MP shape for all species (64%), and polyamide (PA), polyethylene (PE), and polyethylene terephthalate (PET) were the most common polymers identified, which are mainly used in the fishery and the textile industry (Lima et al., 2021). Previous research has already found lower density polymers as polyethylene in mesopelagic fishes (Wieczorek et al., 2018); these buoyant microplastics can be ingested by fish when they migrate towards epipelagic areas, thereby transporting these particles to deeper areas. Sources of fibres are related to the release of untreated water from the washing machine into aquatic environments (De Falco et al., 2019) and extensive fishery activities (Chen et al., 2018; Xue et al., 2020). Despite FNA including MPAs, this archipelago has a high influx of tourists and extensive subsistence and recreational fishing activities (Lopes et al., 2017). Nets and fishing lines are known to degrade and fragment in the environment by physical factors, such as solar radiation (Andrady, 2011). Indeed, microfibres are the most common type observed in marine ecosystems (Kanhai et al., 2018; Lima et al., 2021) and recorded in the FNA and nearby islands (Ivar do Sul et al., 2014; Lima et al., 2016). Additionally, the Equatorial Atlantic is not perceived as an accumulation zone of fibres in surface water masses, decreasing the sinking of this type of MPs to deeper layers where fishes were captured (Lima et al., 2021). However, in the short-term, these islands might retain MPs in the nearshore due to the actions of winds, waves, vortices, and eddies surrounding the islands (Lima et al., 2016; Gove et al., 2019). The most contaminated species were captured around the FNA, suggesting that proximity to the MPs sources also influences ingestion rates.

Fibres are reported as the most ingested shape by mesopelagic fishes (Wieczorek et al., 2018; McGoran et al., 2021) and were also found in deep-sea amphipods in the Mariana trench (Jamieson et al., 2019); these tiny zooplankton act as energy sources in the oceanic trophic web. All fish species analysed here are zooplanktivorous, and amphipods are one of their main prey (Eduardo et al., 2020a, 2021). In the Mediterranean Sea, Romeo et al. (2016) observed similarities in the size of MPs and the size of the copepods, prey of lanternfishes, suggesting active and selective ingestion of MPs. We observed a similar pattern, as the dimensions of the MPs found in the SWTA were similar to those of common prey of the species (< 2 mm), *e.g.*, amphipods and fish larvae in this region (Figueiredo et al., 2020). Through experiments, Li et al. (2021) demonstrated that fish could capture MPs passively by breathing but that some of them are also ingested inadvertently due to the similarity between their prey or the tiny sizes, which are hard to distinguish. Thus, MPs in mesopelagic fishes analysed here might be accidentally consumed when confused as prey or by trophic transfer through ingestion of contaminated prey. However, due to methodological limitations in our study, we cannot state that these species interacted with MP by ingestion through food or swallowed by accident.

Regardless of the uptake routes (ingestion or breathing) of MPs in the mesopelagic fishes, the contamination rates (MP extracted from the digestive tract) observed in this study can be used as an indicator for the levels of MP available in the environment. The less contaminated species, *S. diaphana* captured in the deepest region, is evidence of the lower availability of MP particles in these areas. Additionally, this fact is corroborated by the smaller dimensions of MP extracted from *S. diaphana*, as expected for greater depths.

MPs' wide availability in the deep ocean layers may be harmful to the marine community, which is poorly investigated, but already interacts with these anthropogenic particles. In addition to organic additives (phthalates, OPEs, bisphenols) contained in plastics (Paluselli et al., 2019; Fauvelle et al., 2021), the surface of MPs can adsorb organic pollutants such as polychlorinated biphenyls (PCBs) and polycyclic aromatic hydrocarbons (PAHs; Rochman et al., 2013a), the latter process being enhanced by a longer transit time of MPs in meso- and bathypelagic waters (Rochman et al., 2013b; Jamieson et al., 2017). All of these compounds may very likely migrate into their surrounding environment, such as the digestive tract of biological species. Besides, MPs ingestion can cause adverse effects in fishes, such as physical injuries and blockage of the digestive tract, or even developmental, reproductive and locomotor toxicity (Teuten et al., 2009; Bhagat et al., 2020). Additionally, smaller MPs can bioaccumulate in tissues (Lee et al., 2019; Sökmen et al., 2020).

Conclusions

This study was the first to assess microplastic (MP) contamination in mesopelagic fishes in the Southwestern Tropical Atlantic (SWTA). The four species analysed here were contaminated with MPs in their digestive tract. The primary polymer types identified were polyamide (PA), polyethylene (PE), and polyethylene terephthalate (PET). Ingestion rates of MPs varied between species and depth. However, no difference between day or night sampling was observed. Thus, even though all species interact at some level with MPs, individuals caught at the lower mesopelagic zone seem to be less exposed to MPs than those captured in the upper mesopelagic layer.

Mesopelagic fishes may act as a vector of MP to the deep sea as they perform vertical migrations, presenting an important link between epipelagic and lower mesopelagic layers (Lusher et al., 2016; Savoca et al., 2021). They also play an essential role in the energy transfer in the ecosystem, transferring the energy of primary and secondary consumers to the top oceanic predators, which are valuable for the fishery stocks. So, the presence of MPs in the SWTA mesopelagic ecosystem will likely pose several risks to marine ecosystems if high contamination is confirmed in the near future.

Further research on MP contamination is needed, especially concerning the deep-sea community, whose crucial role in the marine ecosystem functioning has been proven. Additionally, including the effects of oceanographic parameters (*e.g.*, oceanic currents, microturbulence, salinity) and ecological interactions (*e.g.*, prey-predator interaction) into the evaluation of MPs uptake is also needed since there are many factors involved in the transport, sinking, and uptake of MPs in the deep ocean. Finally, the pressure of anthropogenic impacts is rapidly increasing in the SWTA, so there is an urgent need to comprehend how contaminations occur and affect the ecosystem to establish mitigation measures.

Acknowledgements

We thank the crew of the RV *Antea* and the French oceanographic fleet for funding the at-sea survey ABRACOS2 (http://dx.doi.org/10.17600/17004100). All authors thank the members of the BIOIMPACT (UFRPE) and LIZ (UFRJ) labs, for their support in the sampling procedures. This study was supported by the LMI TAPIOCA, program CAPES/COFECUB (88881.142689/2017-01); FUNBIO and HUMANIZE under the grant “Programa Bolsas Funbio - Conservando o Futuro 2019 (02/2019)”; FACEPE (Fundação de Amparo à Ciência e Tecnologia do Estado de Pernambuco); and MicroplastiX Project/JPI-Oceans/CONFAP/FACEPE (APQ-0035-1.08/19). We also thank CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior) for granting a doctoral scholarship to Anne Justino; FACEPE for granting a scholarship to Guilherme Ferreira (BFP-0049-5.06/20); and CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico), for providing research grants to Michael Mincarone (314644/2020-2), Thierry Frédou (307422/2020-8), and Flávia Lucena-Frédou (308554/2019-1). Christos Panagiotopoulos and Richard Sempéré received financial support for LD-IR acquisition via the Region-SUD (SUD-PLASTIC project, Grant No 2019_02985 DEB 19-573) the CNRS and IFREMER.

REFERENCES

Álvarez-Hernández, C., Cairós, C., López-Darias, J., Mazzetti, E., Hernández-Sánchez, C., González-Sálamo, J., Hernández-Borges, J., 2019. Microplastic debris in beaches of Tenerife (Canary Islands, Spain). Mar. Pollut. Bull. 146, 26–32. https://doi.org/10.1016/j.marpolbul.2019.05.064

Andrady, A.L., 2011. Microplastics in the marine environment. Mar. Pollut. Bull. 62, 1596–1605. https://doi.org/10.1016/j.marpolbul.2011.05.030

Arthur, C., Baker, J., Bamford, H. (eds.), 2009. Proceedings of the International Research Workshop on the Occurrence, Effects, and Fate of Microplastic Marine Debris. NOAA Tech. Memo. NOS-OR&R-30. https://marinedebris.noaa.gov/proceedings-international-research-workshop-microplastic-marine-debris

Ashton, K., Holmes, L., Turner, A., 2010. Association of metals with plastic production pellets in the marine environment. Mar. Pollut. Bull. 60, 2050–2055. https://doi.org/10.1016/j.marpolbul.2010.07.014

Assunção, R.V., Silva, A.C., Roy, A., Bourlès, B., Silva, C.H.S., Ternon, J.F., Araujo, M., Bertrand, A., 2020. 3D characterisation of the thermohaline structure in the southwestern tropical Atlantic derived from functional data analysis of in situ profiles. Prog. Oceanogr. 187, 102399. https://doi.org/10.1016/j.pocean.2020.102399

Barboza, L.G.A., Vieira, L.R., Guilhermino, L., 2018. Single and combined effects of microplastics and mercury on juveniles of the European seabass (*Dicentrarchus labrax*): Changes in behavioural responses and reduction of swimming velocity and resistance time. Environ. Pollut. 236, 1014–1019. https://doi.org/10.1016/j.envpol.2017.12.082

Batel, A., Linti, F., Scherer, M., Erdinger, L., Braunbeck, T., 2016. Transfer of benzo[*a*]pyrene from microplastics to *Artemia nauplii* and further to zebrafish via a trophic food web experiment: CYP1A induction and visual tracking of persistent organic pollutants. Environ. Toxicol. Chem. 35, 1656–1666. https://doi.org/10.1002/etc.3361

Bertrand, A., 2017. ABRACOS 2 cruise, RV *Antea*. https://doi.org/10.17600/17004100

Bhagat, J., Zang, L., Nishimura, N., Shimada, Y., 2020. Zebrafish: An emerging model to study microplastic and nanoplastic toxicity. Sci. Total Environ. 728, 138707. https://doi.org/10.1016/j.scitotenv.2020.138707

Boerger, C.M., Lattin, G.L., Moore, S.L., Moore, C.J., 2010. Plastic ingestion by planktivorous fishes in the North Pacific Central Gyre. Mar. Pollut. Bull. 60, 2275–2278. https://doi.org/10.1016/j.marpolbul.2010.08.007

Cai, M., He, H., Liu, M., Li, S., Tang, G., Wang, W., Huang, P., Wei, G., Lin, Y., Chen, B., Hu, J., Cen, Z., 2018. Lost but can’t be neglected: Huge quantities of small microplastics hide in the South China Sea. Sci. Total Environ. 633, 1206–1216. https://doi.org/10.1016/j.scitotenv.2018.03.197

CBD, 2014. Ecologically or Biologically Significant Marine Areas (EBSAs). Special places in the world’s oceans. Volume 2: Wider Caribbean and Western Mid-Atlantic Region. Secretariat of the Convention on Biological Diversity, Montreal, 86 pp.

Chen, M., Jin, M., Tao, P., Wang, Z., Xie, W., Yu, X., Wang, K., 2018. Assessment of microplastics derived from mariculture in Xiangshan Bay, China. Environ. Pollut. 242(B), 1146–1156. https://doi.org/10.1016/j.envpol.2018.07.133

Choy, C.A., Robison, B.H., Gagne, T.O., Erwin, B., Firl, E., Halden, R.U., Hamilton, J.A., Katija, K., Lisin, S.E., Rolsky, C., Van Houtan, K.S., 2019. The vertical distribution and biological transport of marine microplastics across the epipelagic and mesopelagic water column. Sci. Rep. 9, 7843. https://doi.org/10.1038/s41598-019-44117-2

Courtene-Jones, W., Quinn, B., Gary, S.F., Mogg, A.O.M., Narayanaswamy, B.E., 2017. Microplastic pollution identified in deep-sea water and ingested by benthic invertebrates in the Rockall Trough, North Atlantic Ocean. Environ. Pollut. 231, 271–280. https://doi.org/10.1016/j.envpol.2017.08.026

Dai, Z., Zhang, H., Zhou, Q., Tian, Y., Chen, T., Tu, C., Fu, C., Luo, Y., 2018. Occurrence of microplastics in the water column and sediment in an inland sea affected by intensive anthropogenic activities. Environ. Pollut. 242, 1557–1565. https://doi.org/10.1016/j.envpol.2018.07.131

Davison, P., Asch, R.R.G., 2011. Plastic ingestion by mesopelagic fishes in the North Pacific Subtropical Gyre. Mar. Ecol. Prog. Ser. 432, 173–180. https://doi.org/10.3354/meps09142

Davison, P.C., Checkley, D.M., Koslow, J.A., Barlow, J., 2013. Carbon export mediated by mesopelagic fishes in the northeast Pacific Ocean. Prog. Oceanogr. 116, 14–30. https://doi.org/10.1016/j.pocean.2013.05.013

De Falco, F., Di Pace, E., Cocca, M., Avella, M., Di, E., 2019. The contribution of washing processes of synthetic clothes to microplastic pollution. Sci. Rep. 9, 6633. https://doi.org/10.1038/s41598-019-43023-x

de Sá, L.C., Luís, L.G., Guilhermino, L., 2015. Effects of microplastics on juveniles of the common goby (*Pomatoschistus microps*): Confusion with prey, reduction of the predatory performance and efficiency, and possible influence of developmental conditions. Environ. Pollut. 196, 359–362. https://doi.org/10.1016/j.envpol.2014.10.026

Drazen, J.C., Sutton, T.T., 2017. Dining in the deep: The feeding ecology of deep-sea fishes. Ann. Rev. Mar. Sci. 9, 337–366. https://doi.org/10.1146/annurev-marine-010816-060543

Dunn, O.J., 1964. A note on multiple comparisons using rank sums. Technometrics 6, 241–252. https://doi.org/10.1080/00401706.1965.10490253

Eduardo, L.N., Bertrand, A., Mincarone, M.M., Santos, L. V., Frédou, T., Assunção, R. V., Silva, A., Ménard, F., Schwamborn, R., Le Loc'h, F., Lucena-Frédou, F., 2020a. Hatchetfishes (Stomiiformes: Sternoptychidae) biodiversity, trophic ecology, vertical niche partitioning and functional roles in the western Tropical Atlantic. Prog. Oceanogr. 187, 102389. https://doi.org/10.1016/j.pocean.2020.102389

Eduardo, L.N., Lucena-Frédou, F., Mincarone, M.M., Soares, A., Le Loc'h, F., Frédou, T., Ménard, F., Bertrand, A., 2020b. Trophic ecology, habitat, and migratory behaviour of the viperfish *Chauliodus sloani* reveal a key mesopelagic player. Sci. Rep. 10, 20996. https://doi.org/10.1038/s41598-020-77222-8

Eduardo, L.N., Bertrand, A., Mincarone, M.M., Martins, J.R., Frédou, T., Assunção, R.V., Lima, R.S., Ménard, F., Le Loc'h, F., Lucena-Frédou, F., 2021. Distribution, vertical migration, and trophic ecology of lanternfishes (Myctophidae) in the Southwestern Tropical Atlantic. Prog. Oceanogr. https://doi.org/10.1016/j.pocean.2021.102695

Fauvelle, V., Garel, M., Tamburini, C., Nerini, D., Castro-Jiménez, J., Schmidt, N., Paluselli, A., Fahs, A., Papillon, L., Booth, A.M., Sempéré, R., 2021. Organic additive release from plastic to seawater is lower under deep-sea conditions. Nat. Commun. 12. https://doi.org/10.1038/s41467-021-24738-w

Ferreira, G.V.B., Justino, A.K.S., Eduardo, L.N., Lenoble, V., Fauvelle, V., Schmidt, N., Junior, T.V., Frédou, T., Lucena-Frédou, F., 2022. Plastic in the inferno: Microplastic contamination in deep-sea cephalopods (*Vampyroteuthis infernalis* and *Abralia veranyi*) from the southwestern Atlantic. Mar. Pollut. Bull. 174, 113309. https://doi.org/10.1016/j.marpolbul.2021.113309

Ferreira, G.V.B., Barletta, M., Lima, A.R.A., Dantas, D. V., Justino, A.K.S., Costa, M.F., 2016. Plastic debris contamination in the life cycle of Acoupa weakfish (*Cynoscion acoupa*) in a tropical estuary. ICES J. Mar. Sci. 73, 2695–2707. https://doi.org/10.1093/icesjms/fsw108

Ferreira, G.V.B., Barletta, M., Lima, A.R.A., Morley, S.A., Costa, M.F., 2019. Dynamics of marine debris ingestion by profitable fishes along the estuarine ecocline. Sci. Rep. 9, 13514. https://doi.org/10.1038/s41598-019-49992-3

Figueiredo, G.G.A.A., Schwamborn, R., Bertrand, A., Munaron, J.M., Le Loc'h, F., 2020. Body size and stable isotope composition of zooplankton in the western tropical Atlantic. J. Mar. Syst. 212, 103449. https://doi.org/10.1016/j.jmarsys.2020.103449

Galloway, T.S., Cole, M., Lewis, C., 2017. Interactions of microplastic debris throughout the marine ecosystem. Nat. Ecol. Evol. 1, 0116. https://doi.org/10.1038/s41559-017-0116

Geyer, R., 2020. Production, use, and fate of synthetic polymers. In: Letcher, T.M. (ed.). Plastic waste and recycling: Environmental impact, societal issues, prevention, and solutions. Academic Press, https://doi.org/10.1016/b978-0-12-817880-5.00002-5

Gjøsaeter, J., Kawaguchi, K., 1980. A review of the world resources of mesopelagic fish. FAO

Fish. Tech. Pap. 193, 123–134.

Gove, J.M., Whitney, J.L., McManus, M.A., Lecky, J., Carvalho, F.C., Lynch, J.M., Li, J., Neubauer, P., Smith, K.A., Phipps, J.E., Kobayashi, D.R., Balagso, K.B., Contreras, E.A., Manuel, M.E., Merrifield, M.A., Polovina, J.J., Asner, G.P., Maynard, J.A., Williams, G.J., 2019. Prey-size plastics are invading larval fish nurseries. Proc. Natl. Acad. Sci. 116, 24143–24149. https://doi.org/10.1073/pnas.1907496116

Green, D.S., Kregting, L., Boots, B., Blockley, D.J., Brickle, P., da Costa, M., Crowley, Q., 2018. A comparison of sampling methods for seawater microplastics and a first report of the microplastic litter in coastal waters of Ascension and Falkland Islands. Mar. Pollut. Bull. 137, 695–701. https://doi.org/10.1016/j.marpolbul.2018.11.004

Hermsen, E., Mintenig, S.M., Besseling, E., Koelmans, A.A., 2018. Quality criteria for the analysis of microplastic in biota samples: A critical review. Environ. Sci. Technol. 52, 10230–10240. https://doi.org/10.1021/acs.est.8b01611

Howell, K.L., Hilário, A., Allcock, A.L., Bailey, D.M., Baker, M., Clark, M.R., Colaço, A., Copley, J., Cordes, E.E., Danovaro, R., Dissanayake, A., Escobar, E., Esquete, P., Gallagher, A.J., Gates, A.R., Gaudron, S.M., German, C.R., Gjerde, K.M., Higgs, N.D., Le Bris, N., Levin, L.A., Manea, E., McClain, C., Menot, L., Mestre, N.C., Metaxas, A., Milligan, R.J., Muthumbi, A.W.N., Narayanaswamy, B.E., Ramalho, S.P., Ramirez-Llodra, E., Robson, L.M., Rogers, A.D., Sellanes, J., Sigwart, J.D., Sink, K., Snelgrove, P.V.R., Stefanoudis, P. V, Sumida, P.Y., Taylor, M.L., Thurber, A.R., Vieira, R.P., Watanabe, H.K., Woodall, L.C., Xavier, J.R., 2020. A blueprint for an inclusive, global deep-sea ocean decade field program. Front. Mar. Sci. 7, 584861. https://doi.org/10.3389/fmars.2020.584861

Ivar do Sul, J.A., Costa, M.F., Barletta, M., Cysneiros, F.J.A., 2013. Pelagic microplastics around an archipelago of the Equatorial Atlantic. Mar. Pollut. Bull. 75, 305–309. https://doi.org/10.1016/j.marpolbul.2013.07.040

Ivar do Sul, J.A., Costa, M.F., Fillmann, G., 2014. Microplastics in the pelagic environment around oceanic islands of the western Tropical Atlantic Ocean. Water. Air. Soil Pollut. 225, 2004. https://doi.org/10.1007/s11270-014-2004-z

Jambeck, J.R., Geyer, R., Wilcox, C., Siegler, T.R., Perryman, M., Andrady, A., Narayan, R., Law, K.L., 2015. Plastic waste inputs from land into the ocean. Science 347(6223), 768–771. https://doi.org/10.1126/science.1260352

Jamieson, A.J., Brooks, L.S.R., Reid, W.D.K., Piertney, S.B., Narayanaswamy, B.E., Linley, T.D., 2019. Microplastics and synthetic particles ingested by deep-sea amphipods in six of the deepest marine ecosystems on Earth. R. Soc. Open Sci. 6, 180667. https://doi.org/10.1098/rsos.180667

Jamieson, A.J., Malkocs, T., Piertney, S.B., Fujii, T., Zhang, Z., 2017. Bioaccumulation of persistent organic pollutants in the deepest ocean fauna. Nat. Ecol. Evol. 1, 24–27. https://doi.org/10.1038/s41559-016-0051

Jiang, Y., Yang, F., Zhao, Y., Wang, J., 2020. Greenland Sea Gyre increases microplastic pollution in the surface waters of the Nordic Seas. Sci. Total Environ. 712, 136484. https://doi.org/10.1016/j.scitotenv.2019.136484

Justino, A.K.S., Lenoble, V., Pelage, L., Ferreira, G.V.B., Passarone, R., Frédou, T., Lucena-Frédou, F., 2021. Microplastic contamination in tropical fishes: An assessment of different feeding habits. Reg. Stud. Mar. Sci. 45, 101857. https://doi.org/10.1016/j.rsma.2021.101857

Kane, I.A., Clare, M.A., Miramontes, E., Wogelius, R., Rothwell, J.J., Garreau, P., Pohl, F., 2020. Seafloor microplastic hotspots controlle by deep-sea circulation. Science 368(6495), 1140–1145. https://doi.org/10.1126/science.aba5899

Kanhai, L.D.K., Gårdfeldt, K., Lyashevska, O., Hassellöv, M., Thompson, R.C., O'Connor, I., 2018. Microplastics in sub-surface waters of the Arctic Central Basin. Mar. Pollut. Bull. 130, 8–18. https://doi.org/10.1016/j.marpolbul.2018.03.011

Katija, K., Choy, C.A., Sherlock, R.E., Sherman, A.D., Robison, B.H., 2017. From the surface to the seafloor: How giant larvaceans transport microplastics into the deep sea. Sci. Adv. 3, e1700715. https://doi.org/10.1126/sciadv.1700715

Koelmans, A.A., Kooi, M., Law, K.L., Van Sebille, E., 2017. All is not lost: Deriving a top-down mass budget of plastic at sea. Environ. Res. Lett. 12, 114028. https://doi.org/10.1088/1748-9326/aa9500

Kvale, K., Prowe, A.E.F., Chien, C.T., Landolfi, A., Oschlies, A., 2020. The global biological microplastic particle sink. Sci. Rep. 10, 16670. https://doi.org/10.1038/s41598-020-72898-4

Lee, W.S., Cho, H.J., Kim, E., Huh, Y.H., Kim, H.J., Kim, B., Kang, T., Lee, J.S., Jeong, J., 2019. Bioaccumulation of polystyrene nanoplastics and their effect on the toxicity of Au ions in zebrafish embryos. Nanoscale 7. https://doi.org/10.1039/c8nr90280a

Li, B., Liang, W., Liu, Q.-X., Fu, S., Ma, C., Chen, Q., Su, L., Craig, N.J., Shi, H., 2021. Fish Ingest Microplastics Unintentionally. Environ. Sci. Technol. 55, 10471–10479. https://doi.org/10.1021/acs.est.1c01753

Lima, A.R.A., Barletta, M., Costa, M.F., 2016. Seasonal-dial shifts of ichthyoplankton assemblages and plastic debris around an Equatorial Atlantic archipelago. Front. Environ. Sci. 4, 56. https://doi.org/10.3389/fenvs.2016.00056

Lima, A.R.A., Ferreira, G.V.B., Barrows, A.P.W., Christiansen, K.S., Treinish, G., Toshack, M.C., 2021. Global patterns for the spatial distribution of floating microfibers: Arctic Ocean as a potential accumulation zone. J. Hazard. Mater. 403, 123796. https://doi.org/10.1016/j.jhazmat.2020.123796

Lins-Silva, N., Marcolin, C.R., Kessler, F., Schwamborn, R., 2021. A fresh look at microplastics and other particles in the tropical coastal ecosystems of Tamandaré, Brazil. Mar. Environ. Res. 169, 105327. https://doi.org/10.1016/j.marenvres.2021.105327

Lopes, P.F.M., Mendes, L., Fonseca, V., Villasante, S., 2017. Tourism as a driver of conflicts and changes in fisheries value chains in Marine Protected Areas. J. Environ. Manage. 200, 123–134. https://doi.org/10.1016/j.jenvman.2017.05.080

Lusher, A.L., O'Donnell, C., Officer, R., O'Connor, I., 2016. Microplastic interactions with North Atlantic mesopelagic fish. ICES J. Mar. Sci. 73, 1214–1225. https://doi.org/10.1093/icesjms/fsv241

Lusher, A.L., Tirelli, V., O'Connor, I., Officer, R., 2015. Microplastics in Arctic polar waters: The first reported values of particles in surface and sub-surface samples. Sci. Rep. 5, 14947. https://doi.org/10.1038/srep14947

Markic, A., Gaertner, J.-C., Gaertner-Mazouni, N., Koelmans, A.A., 2020. Plastic ingestion by marine fish in the wild. Crit. Rev. Environ. Sci. Technol. 50, 657–697. https://doi.org/10.1080/10643389.2019.1631990

Martins, K., Pelage, L., Justino, A.K.S., Frédou, F.L., Júnior, T.V., Le Loc'h, F., Travassos, P., 2021. Assessing trophic interactions between pelagic predatory fish by gut content and stable isotopes analysis around Fernando de Noronha Archipelago (Brazil), Equatorial West Atlantic. J. Fish Biol. 99, 1576-1590. https://doi.org/10.1111/jfb.14863

McGoran, A.R., Maclaine, J.S., Clark, P.F., Morritt, D., 2021. Synthetic and semi-synthetic microplastic ingestion by mesopelagic fishes from Tristan da Cunha and St Helena, South Atlantic. Front. Mar. Sci. 8, 633478. https://doi.org/10.3389/fmars.2021.633478

Meijer, L.J.J., van Emmerik, T., van der Ent, R., Schmidt, C., Lebreton, L., 2021. More than 1000 rivers account for 80% of global riverine plastic emissions into the ocean. Sci. Adv. 7, eaaz5803. https://doi.org/10.1126/sciadv.aaz5803

Monteiro, R.C.P., Ivar do Sul, J.A., Costa, M.F., 2018. Plastic pollution in islands of the Atlantic Ocean. Environ. Pollut. 238, 103–110. https://doi.org/10.1016/j.envpol.2018.01.096

Moore, C.J., 2008. Synthetic polymers in the marine environment: A rapidly increasing, long-term threat. Environ. Res. 108, 131–139. https://doi.org/10.1016/j.envres.2008.07.025

Müller, C., 2021. Not as bad as it seems? A literature review on the case of microplastic uptake in fish. Front. Mar. Sci. 8, 672768. https://doi.org/10.3389/fmars.2021.672768

Nelms, S.E., Galloway, T.S., Godley, B.J., Jarvis, D.S., Lindeque, P.K., 2018. Investigating microplastic trophic transfer in marine top predators. Environ. Pollut. 238, 999–1007. https://doi.org/10.1016/j.envpol.2018.02.016

Oehlmann, J., Schulte-Oehlmann, U., Kloas, W., Jagnytsch, O., Lutz, I., Kusk, K.O., Wollenberger, L., Santos, E.M., Paull, G.C., VanLook, K.J.W., Tyler, C.R., 2009. A critical analysis of the biological impacts of plasticizers on wildlife. Philos. Trans. R. Soc. B Biol.

Sci. 364, 2047–2062. https://doi.org/10.1098/rstb.2008.0242

Ourgaud M., Phuong N. N., Papillon L., Brach-Papa C., Galgani, F., Panagiotopoulos C., Sempéré R. Identification and quantification of microplastics in the marine environment using Laser Direct Infra-Red (LDIR). In prep.

Ostle, C., Thompson, R.C., Broughton, D., Gregory, L., Wootton, M., Johns, D.G., 2019. The rise in ocean plastics evidenced from a 60-year time series. Nat. Commun. 10, 1622. https://doi.org/10.1038/s41467-019-09506-1

Paluselli, A., Fauvelle, V., Galgani, F., Sempéré, R., 2019. Phthalate Release from Plastic Fragments and Degradation in Seawater. Environ. Sci. Technol. 53, 166–175. https://doi.org/10.1021/acs.est.8b05083

Pereira, J.M., Rodríguez, Y., Blasco-Monleon, S., Porter, A., Lewis, C., Pham, C.K., 2020. Microplastic in the stomachs of open-ocean and deep-sea fishes of the North-East Atlantic. Environ. Pollut. 265, 115060. https://doi.org/10.1016/j.envpol.2020.115060

Pinheiro, L.M., Agostini, V.O., Lima, A.R.A., Ward, R.D., Pinho, G.L.L., 2021. The fate of plastic litter within estuarine compartments: An overview of current knowledge for the transboundary issue to guide future assessments. Environ. Pollut. 279, 116908. https://doi.org/10.1016/j.envpol.2021.116908

PlasticsEurope, 2018. Plastics – the facts 2017: An analysis of European plastics production, demand and waste data. Association of Plastics Manufacturers, Brussels, 41 pp.

R Core Team, 2020. R: A language and environment for statistical computing. R Foundation for Statistical Computing.

Rochman, C.M., Browne, M.A., Halpern, B.S., Hentschel, B.T., Hoh, E., Karapanagioti, H.K., Rios-Mendoza, L.M., Takada, H., Teh, S., Thompson, R.C., 2013a. Classify plastic waste as hazardous. Nature 494, 169–171. https://doi.org/10.1038/494169a

Rochman, C.M., Hoh, E., Hentschel, B.T., Kaye, S., 2013b. Long-term field measurement of sorption of organic contaminants to five types of plastic pellets: Implications for plastic marine debris. Environ. Sci. Technol. 47, 1646–1654. https://doi.org/10.1021/es303700s

Rochman, C.M., Hoh, E., Kurobe, T., Teh, S.J., 2013c. Ingested plastic transfers hazardous chemicals to fish and induces hepatic stress. Sci. Rep. 3, 3263. https://doi.org/10.1038/srep03263

Romeo, T., Pedà, C., Fossi, M.C., Andaloro, F., Battaglia, P., 2016. First record of plastic debris in the stomach of Mediterranean lanternfishes. Acta Adriat. 57, 115–124. https://doi.org/10.3389/fmars.2021.672768

Savoca, M.S., McInturf, A.G., Hazen, E.L., 2021. Plastic ingestion by marine fish is widespread and increasing. Glob. Chang. Biol. 27, 2188–2199. https://doi.org/10.1111/GCB.15533

Soares, M.O., Paiva, C.C., Godoy, T., Silva, M.B., Castro, C.S.S., 2010. Gestão ambiental de ecossistemas insulares: O caso da Reserva Biológica do Atol das Rocas, Atlântico Sul Equatorial. Rev. Gestão Costeira Integr. 10, 347–360. https://doi.org/10.5894/rgci214

Sökmen, T.Ö., Sulukan, E., Türkoğlu, M., Baran, A., Özkaraca, M., Ceyhun, S.B., 2020. Polystyrene nanoplastics (20 nm) are able to bioaccumulate and cause oxidative DNA damages in the brain tissue of zebrafish embryo (*Danio rerio*). Neurotoxicology 77, 51–59. https://doi.org/10.1016/j.neuro.2019.12.010

St. John, M.A., Borja, A., Chust, G., Heath, M., Grigorov, I., Mariani, P., Martin, A.P., Santos, R.S., 2016. A dark hole in our understanding of marine ecosystems and their services: perspectives from the mesopelagic community. Front. Mar. Sci. 3, 31. https://doi.org/10.3389/fmars.2016.00031

Teuten, E.L., Rowland, S.J., Galloway, T.S., Thompson, R.C., 2007. Potential for plastics to transport hydrophobic contaminants. Environ. Sci. Technol. 41, 7759–7764. https://doi.org/10.1021/es071737s

Teuten, E.L., Saquing, J.M., Knappe, D.R.U., Barlaz, M.A., Jonsson, S., Björn, A., Rowland, S.J., Thompson, R.C., Galloway, T.S., Yamashita, R., Ochi, D., Watanuki, Y., Moore, C., Viet, P.H., Tana, T.S., Prudente, M., Boonyatumanond, R., Zakaria, M.P., Akkhavong, K., Ogata, Y., Hirai, H., Iwasa, S., Mizukawa, K., Hagino, Y., Imamura, A., Saha, M., Takada, H., 2009. Transport and release of chemicals from plastics to the environment and to wildlife. Philos. Trans. R. Soc. B Biol. Sci. 364, 2027–2045. https://doi.org/10.1098/rstb.2008.0284

Thompson, R.C., Olson, Y., Mitchell, R.P., Davis, A., Rowland, S.J., John, A.W.G., McGonigle, D., Russell, A.E., 2004. Lost at sea: Where is all the plastic? Science 304, 838. https://doi.org/10.1126/science.1094559

van Sebille, E., Aliani, S., Law, K.L., Maximenko, N., Alsina, J.M., Bagaev, A., Bergmann, M., Chapron, B., Chubarenko, I., Cózar, A., Delandmeter, P., Egger, M., Fox-Kemper, B., Garaba, S.P., Goddijn-Murphy, L., Hardesty, B.D., Hoffman, M.J., Isobe, A., Jongedijk, C.E., Kaandorp, M.L.A., Khatmullina, L., Koelmans, A.A., Kukulka, T., Laufkötter, C., Lebreton, L., Lobelle, D., Maes, C., Martinez-Vicente, V., Morales Maqueda, M.A., Poulain-Zarcos, M., Rodríguez, E., Ryan, P.G., Shanks, A.L., Shim, W.J., Suaria, G., Thiel, M., van den Bremer, T.S., Wichmann, D., 2020. The physical oceanography of the transport of floating marine debris. Environ. Res. Lett. 15, 023003. https://doi.org/10.1088/1748-9326/ab6d7d

Waller, C.L., Griffiths, H.J., Waluda, C.M., Thorpe, S.E., Loaiza, I., Moreno, B., Pacherres, C.O., Hughes, K.A., 2017. Microplastics in the Antarctic marine system: An emerging area of research. Sci. Total Environ. 598, 220–227. https://doi.org/10.1016/j.scitotenv.2017.03.283

Wieczorek, A.M., Morrison, L., Croot, P.L., Allcock, A.L., MacLoughlin, E., Savard, O., Brownlow, H., Doyle, T.K., 2018. Frequency of microplastics in mesopelagic fishes from the Northwest Atlantic. Front. Mar. Sci. 5, 39. https://doi.org/10.3389/fmars.2018.00039

Woodall, L.C., Sanchez-Vidal, A., Canals, M., Paterson, G.L.J., Coppock, R., Sleight, V., Calafat, A., Rogers, A.D., Narayanaswamy, B.E., Thompson, R.C., 2014. The deep sea is a major sink for microplastic debris. R. Soc. Open Sci. 1, 140317. https://doi.org/10.1098/rsos.140317

Xue, B., Zhang, L., Li, R., Wang, Y., Guo, J., Yu, K., Wang, S., 2020. Underestimated microplastic pollution derived from fishery activities and “hidden” in deep sediment. Environ. Sci. Technol. 54, 2210–2217. https://doi.org/10.1021/acs.est.9b04850

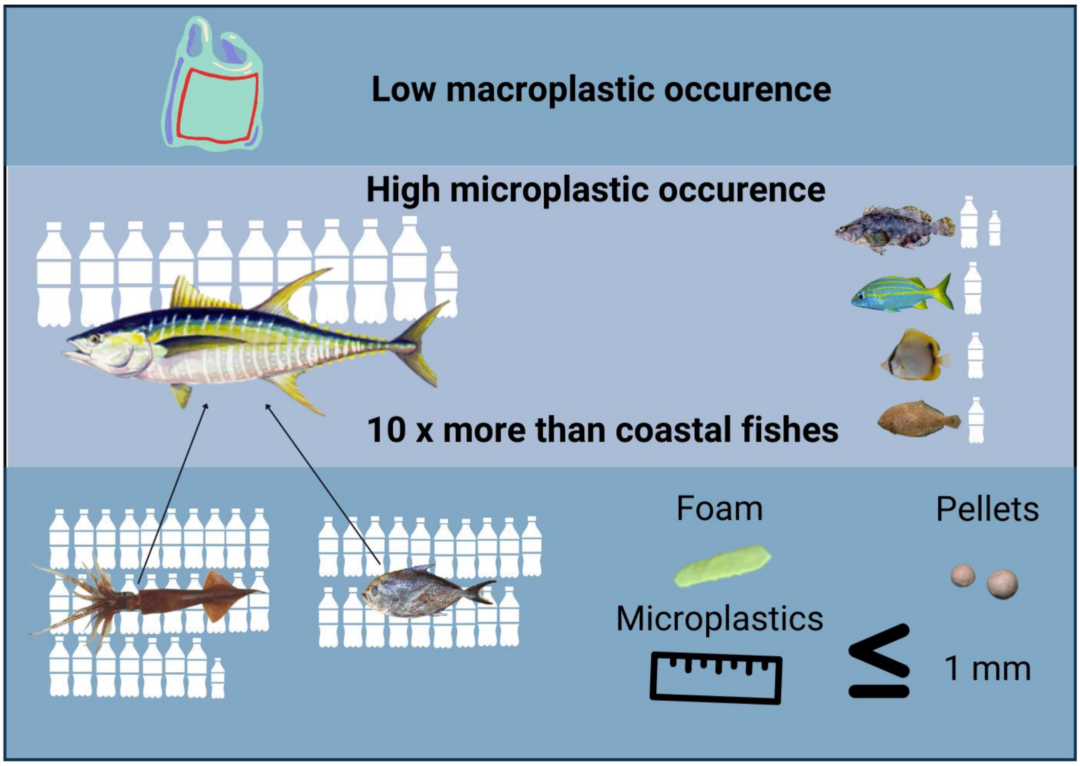
Zhu, L., Wang, H., Chen, B., Sun, X., Qu, K., Xia, B., 2019. Microplastic ingestion in deep-sea fish from the South China Sea. Sci. Total Environ. 677, 493–501. https://doi.org/10.1016/j.scitotenv.2019.04.380

Zobkov, M.B., Esiukova, E.E., Zyubin, A.Y., Samusev, I.G., 2019. Microplastic content variation in water column: The observations employing a novel sampling tool in stratified Baltic Sea. Mar. Pollut. Bull. 138, 193–205. https://doi.org/10.1016/j.marpolbul.2018.11.047

CHAPTER 4

Article "From prey to predators: evidence of microplastic trophic transfer in tuna and tuna-like species in the Southwestern Tropical Atlantic"

Manuscript to be submitted after thesis defence in the journal Environmental Pollution.



From prey to predators: evidence of microplastic trophic transfer in tuna and tuna-like species in the Southwestern Tropical Atlantic

Anne K. S. Justino[1,2*], Guilherme V. B. Ferreira[1], Vincent Fauvelle[3], Natascha Schmidt[3], Véronique Lenoble[2], Latifa Pelage[1], Karla Martins[1], Paulo Travassos[1], Flávia Lucena-Frédou[1]

[1] Universidade Federal Rural de Pernambuco (UFRPE), Departamento de Pesca e Aquicultura (DEPAQ), Rua Dom Manuel de Medeiros, s/n, 52171-900, Recife, Brazil.
[2] Université de Toulon, Aix Marseille Univ., CNRS, IRD, MIO, Toulon, France.
[3] Aix Marseille Univ., Université de Toulon, CNRS, IRD, MIO, Marseille, France.

* Corresponding author: anne.justino@ufrpe.br

Abstract

Plastic pollution is present in most marine environments; however, contamination in pelagic predators, including species of economic interest, is still poorly understood. This study aims to access the macro- and microplastic contamination in tuna and tuna-like species and verify whether a trophic transfer occurs from prey to tunas captured by two fleets in the Southwestern Tropical Atlantic (SWTA). We combined different methodological approaches to analyse the intake of macro- and microplastics. In addition to examining the plastics in the fish' stomachs, we investigated the contamination in the prey retrieved from the guts of predators. A low frequency of occurrence (3%) of macroplastic was detected in the tuna and tuna-like species; conversely, we observed a high frequency of microplastic in the tuna's stomachs (100%) and prey analysed (70%). We evinced the trophic transfer of microplastics by analysing the ingestion rate of particles in prey retrieved from the tuna stomachs. In the 34 analysed prey, we detected 355 microplastic particles. The most contaminated prey were cephalopods and fishes of the Bramidae family. The most frequent microplastic shapes in both prey and tuna stomachs were foams, pellets and fibres (< 1 mm). A variety of polymers were identified; the most frequent were styrene-butadiene rubber (SBR), polyamide (PA), polyethylene terephthalate (PET) and polyethylene (PE). Our findings enhance scientific knowledge of how the ecological behaviour of marine species can affect microplastics intake.

Keywords: Tropical fishery, Plastic Pollution, Predators, Microplastic, South Atlantic.

Introduction

Plastic pollution is one of the most preoccupying environmental issues of the 21st century, with a production that has drastically increased and is expected to hit 1100 million tons (Mt) by 2050 (Geyer, 2020). To tackle the issue of plastic pollution on an international level, representatives at the fifth session of the United Nations Environment Assembly (UNEA-5.2) recently endorsed a landmark resolution to forge an international legally binding agreement by 2024 (UNEP, 2022). However, even if all available solutions to minimise the impact of plastic on the environment were to be applied, annual emissions of plastic into the environment could only be reduced by 79% by 2040 (Lau et al., 2020). Considerable amounts of continental plastic materials are improperly managed and transported by riverine discharges into marine ecosystems (Koelmans et al., 2017; Meijer et al., 2021). Moreover, once present in the environment, plastic waste is weathered by natural processes (*e.g.*, solar radiation, hydrodynamics and interaction with biota) (Jambeck et al., 2015; Thompson et al., 2004). The particles derived from the breakdown of larger plastics are referred to as microplastics (< 5 mm) (Arthur et al., 2009).

Microplastics are widespread in all marine ecosystems, from coastal to polar regions, and are vertically distributed in the water column (Choy et al., 2019; Lins-Silva et al., 2021; Waller et al., 2017). Due to their tiny size and wide availability, microplastics can easily be mistaken as prey (Boerger et al., 2010) and further pose several threats to marine biota when ingested (Galloway et al., 2017), such as damage to the digestive system, decrease of the predation efficiency, and induction of toxic effects (Barboza et al., 2018; de Sá et al., 2015; Moore, 2008; Teuten et al., 2007).

Microplastics are likely to be transferred from prey to predators through trophic transfer (Eriksson and Burton, 2003). Laboratory studies have confirmed the trophic transfer of plastics from mussels to crabs (Farrell and Nelson, 2013) and among planktonic organisms with different trophic levels (mesozooplankton to macrozooplankton) (Setälä et al., 2014). In a study carried out in the South Pacific, it was observed that the prey ingested by tuna was contaminated with microplastic, probably associated with the trophic transfer between predator-prey (flying fish) in the natural environment (Chagnon et al., 2018). Indeed, predators are hypothesised to ingest more plastics than other species due to the large prey intake and a momentary build-up of particles in the stomachs (Ferreira et al., 2019).

Currently, although a large amount of information on microplastic contamination in marine fishes is available (Savoca et al., 2021), knowledge on tuna and tuna-like species, which are among the leading fisheries stocks (FAO, 2020), is still scarce. Microplastic contamination has been reported in the common dolphinfish (*Coryphaena hippurus*) from the Mediterranean Sea (Schirinzi et al., 2020), skipjack tuna (*Euthynnus affinis* and *Katsuwonus pelamis*) in Indonesia (Andreas et al., 2021; Lessy and Sabar, 2021), and dolphinfish in the eastern Pacific Ocean (Li et al., 2022).

In the Southwestern Tropical Atlantic (SWTA), tropical tuna and tuna-like fisheries contribute significantly to the regional economy and provide an essential source of income and livelihood for fishers (FAO, 2020; Silva et al., 2018). The industrial fleets, which represent most of the catches, target mainly the yellowfin tuna (*Thunnus albacares* Bonnaterre, 1788) and the bigeye tuna (*Thunnus obesus* Lowe, 1839) and occur in offshore areas (Silva et al., 2016). Meanwhile, artisanal and recreational fisheries occur close to ocean islands, such as in the Fernando de Noronha Archipelago (FNA), mainly targeting the barracuda (*Sphyraena barracuda* Walbaum, 1792), the wahoo (*Acanthocybium solandri* Cuvier, 1832) and the yellowfin tuna (Martins et al., 2021).

In the South Atlantic, some data regarding the ingestion of plastic debris by pelagic predators are available: in the southeast-south of Brazil (Neto et al., 2020), on the coast of Salvador, in northeast Brazil (Miranda and de Carvalho-Souza, 2016), and reports for the Western Equatorial Atlantic (de Mesquita et al., 2021; Menezes et al., 2019; Vaske-Júnior and Lessa, 2004). However, despite their vast importance as a source of wealth and food security worldwide, to our best knowledge, there is no information regarding the microplastic contamination in tuna and tuna-like species in the SWTA.

Analysing microplastics in larger predatory fishes is a great challenge, mainly due to laboratory procedures. The use of standard techniques for the digestion of stomachs to separate the organic matter and the plastic items (*e.g.*, alkaline and acid digestion) is very time-consuming and must be done with caution to avoid cross-contamination and over/underestimation (Justino et al., 2021). Our study used combined methodologies to analyse the contamination of macro- and microplastics in tuna and tuna-like species targeted by industrial, recreational and artisanal fisheries operating in the SWTA. In addition to examining the microplastics in the stomachs, we investigated the contamination of the prey found inside the guts (Chagnon et al., 2018; Ferreira et al.,

2019) to verify if there might be a trophic transfer of microplastics in the pelagic predators, also considering potential differences concerning contamination rates (number of microplastic extracted in the digestive tract) among the target species, fleets and their analysed prey (prey groups and species).

Materials and Methods

Study area and sampling

The study area is located along the Southwestern Tropical Atlantic (SWTA) (Fig. 1). The climate there is tropical, with well-defined rainy (March to July) and dry (August to February) seasons, and the warm and oligotrophic waters are influenced by the South Equatorial Current (SEC) and South Equatorial Undercurrent (SEUC) (Almeida, 2006; Assunção et al., 2020). The Fernando de Noronha Archipelago (FNA), registered on the UNESCO world heritage, is located in this area at ~360 km from the Northeastern Brazilian coast and inserted in a Marine Protected Area (MPA) with a National Marine Park (PARNAMAR) and an Environmental Protection Area (EPA).

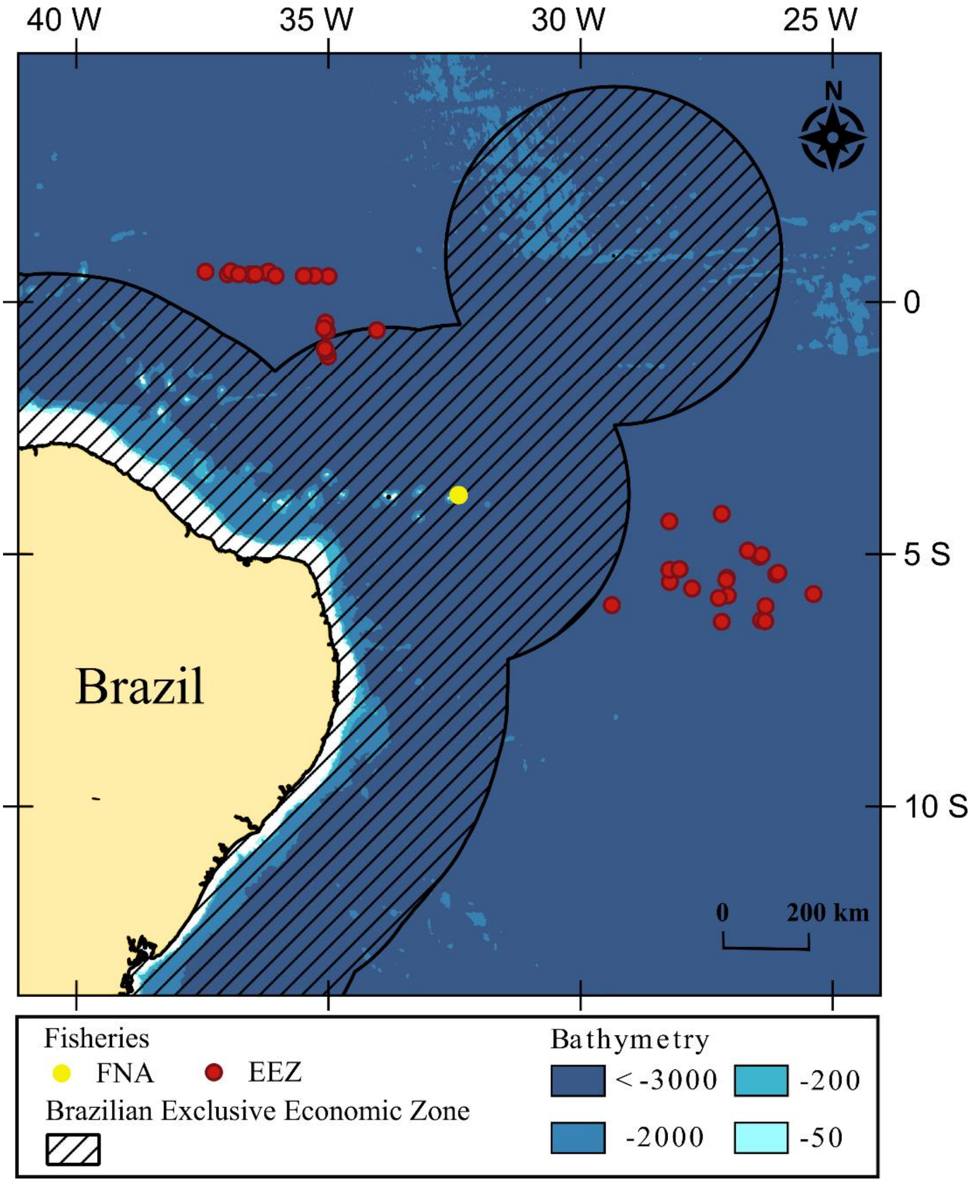


Figure 1. Map with the fishing fleets' collection points along the Southwestern Tropical Atlantic (SWTA). Red dots show that the fishery operates outside the Brazilian Exclusive Economic Zone (EEZ), and the yellow dot indicates the captures in the Fernando de Noronha Archipelago (FNA).

Tuna and tuna-like species were collected by the industrial, recreational, and artisanal fleets that operated along the SWTA in 2018 and 2019. The industrial fleet operates with longlines mainly outside the Brazilian Exclusive Economic Zone (EEZ). The main target species are *T. albacares* and *T. obesus*, usually destined for exportation and the important centres of fishery trades. On the other hand, artisanal and recreational fleets based in the FNA operate using rods and reels, and the main species caught are *S. barracuda*, *A. solandri*, and *T. albacares.* In FNA, the catches usually supply the island solely. We relied on onboard observers who recorded information about the fisheries. The specimens were labelled, measured (nearest 0.1 cm of fork length), weighed (kg of total weight), and dissected onboard. The stomachs were carefully removed and frozen at -18°C and kept in freezers until laboratory analysis.

In both fisheries (EEZ and FNA), a total of 350 samples of tuna and tuna-like species were collected: *T. albacares* (yellowfin tuna, YFT; n = 102); *T. obesus* (bigeye tuna, BET; n = 63); *S. barracuda* (barracuda, BAR; n = 136); and *A. solandri* (wahoo, WAH; n = 49).

Contamination control

Several steps were carried out before the extractions (macro- & microplastics) to ensure quality assurance and quality control and to avoid cross-contamination, following the protocol proposed by Justino et al. (2021). The protocol includes using 100% cotton lab coats and disposable latex gloves in a dedicated workspace with a limited flow of people. Moreover, all the utilised solutions were filtered through a glass fibre filter (47 mm GF/F 0.7 µm pore size, © Whatman) using a vacuum pump system equipped with laboratory glassware. We implemented procedural blanks for each set of 10 samples for the microplastics analysis. The blanks received the same treatment as samples. We excluded the particles found in the samples with similarity (colour and shape) to those observed in the blanks from the study. A total of three particles were detected in all observed blanks, two blue fibres and one black fibre. All particles were identified as cellulosic and excluded from further analysis.

Laboratory procedures and plastic extraction

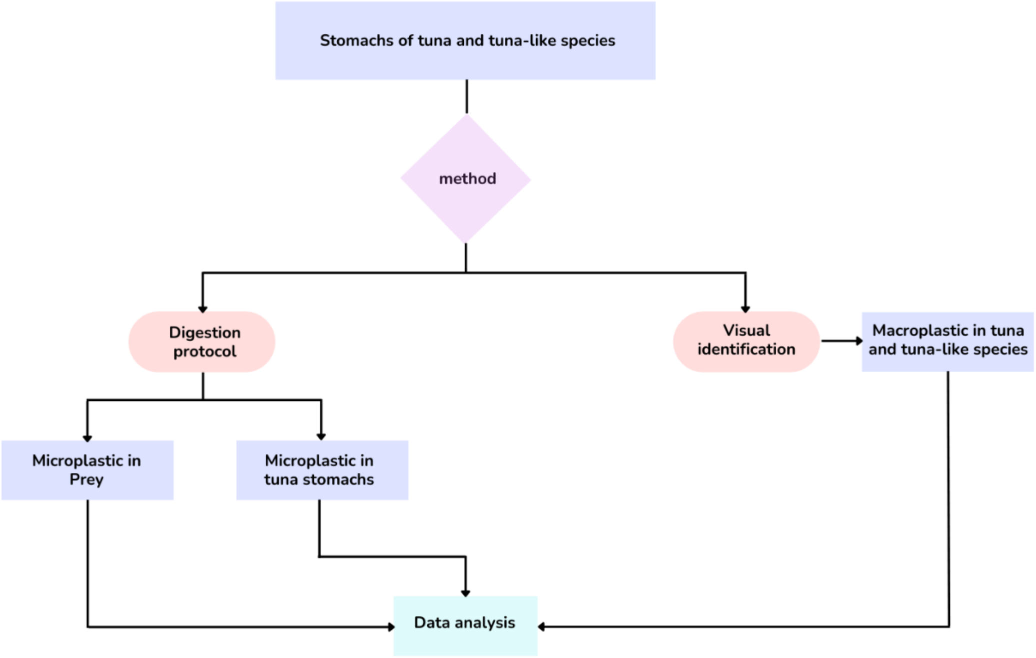


Figure 2. Flowchart illustrating the experimental strategy applied to detect macro- and microplastics in tuna and tuna-like species from the SWTA. Macroplastics from 341 stomachs were detected by visual identification. Microplastics from 9 *Thunnus albacares* stomachs and 34 prey were identified by Laser Directed InfraRed after an alkaline digestion protocol.

Macroplastic and prey identification in tuna and tuna-like species

For the macroplastic (> 5 mm) analysis, a total of 341 stomachs were analysed, and all anthropogenic materials identified were photographed and counted. At the same time, prey in good condition found in the stomachs of tuna and tuna-like species, which had the digestive tract intact, were separated for subsequent analysis.

Tuna prey was measured, weighed, and stored for microplastic (< 5 mm) analysis (see next section). The prey items were identified at the lowest taxonomic level possible and classified into large groups (mainly fishes and cephalopods) (Humann and DeLoach, 2002; Vaske-Júnior, 2006). In the case of prey identified as fish, only the digestive tract (stomach and intestine) was used for digestion, whereas for cephalopods, beaks and pen were removed, and then the whole animal was digested (Ferreira et al., 2022).

Microplastics detection in tuna stomachs and prey

Nine *T. albacares* stomachs from EEZ were separated for microplastic analysis regarding the predator. The stomachs were carefully eviscerated, and only the gut content was placed in a beaker for the microplastic extraction. The material found inside the stomachs analysed was in an advanced degree of digestion and could not be taxonomically identified.

Microplastic extraction from prey and *T. albacares* stomachs was performed with the help of an alkaline digestion protocol using sodium hydroxide (NaOH, 1 mol L^{-1}; PA 97%) (Justino et al., 2021). The prey samples (digestive tract of fish and the whole cephalopod) and the gut content of *T. albacares* stomachs were carefully washed before analysis with filtered distilled water to remove any particles attached to the external tissue. Then, samples were placed in a beaker and submerged in the NaOH solution (the proportion used was 1:100 w/v), covered by a glass lid and oven-dried at 60 °C for 24 h. After that, samples were filtered through a 47 mm GF/F 0.7 µm pore size glass fibre filter (© Whatman) using a vacuum pump system. After filtration, samples were carefully set in a Petri dish and covered; these filters were oven-dried again at 60 °C for 24 h. Finally, the filters were visually inspected for microplastics using a stereomicroscope (© Zeiss Stemi 508, with a size detection limit of 0.04 mm). All the particles suspected to be microplastics were counted, photographed (© Zeiss Zen 3.2; Axiocam 105 Color), and measured in length (mm). Microplastics were first categorised according to their shape as fibres (filamentous shape), fragments (irregular shape), films (flat shape), foams (soft with an irregular shape), or pellets (spherical shape) (Justino et al., 2021).

Polymer analysis

A random subset (15% of total microplastic extracted) of samples was selected to identify the main polymers using the Laser Direct Infrared (LDIR) analyser Agilent 8700 Chemical Imaging System with the Microplastic Starter 1.0 library. The LDIR analyser works by scanning the particles (size range 20–5000 µm) within a wavelength range of 1800–975 cm^{-1} (Ourgaud et al., *In prep*.). The information is collected with the Clarity image software (© Agilent version 1.3.9) and compared with the polymer spectrum library (~400 references spectra). We confirmed the polymer type of a particle when the identification match was >70% (Ferreira et al., 2022; Justino et al., 2022).

Data analysis

As the data on microplastic particles did not meet parametric assumptions, a Kruskal-Wallis test was used to verify whether detected particles in tuna prey presented significant differences among the fleets, predator species, prey groups, and prey species according to microplastic mean number and size. When significant differences were detected, a *post hoc* pairwise comparison, Dunn's test, was used to investigate the sources of variance (Dunn, 1964). A Spearman's correlation test was used to verify the relationship between microplastics and the predator's biometry. All statistical analyses were performed with the software R version 3.6.3 (R Core Team, 2020) and were conducted considering a significance level of 5%.

Results

Macroplastics in tuna and tuna-like species

Macroplastics were found in 10 of the 341 examined stomachs, presenting, regardless of species, a low frequency of occurrence (FO= 3%) in both FNA (FO= 4) and EEZ (FO= 1%). Among the species, *A. solandri* (FO= 8%, FNA) individuals were the most contaminated, followed by *S. barracuda* (FO= 3%, FNA) and *T. albacares* (FO= 2%, EEZ; FO= 3%, FNA). No plastics were found in *T. obesus* samples. A single particle resembling a plastic bag was observed in a *T. albacares* caught in the EEZ, whereas for the FNA catches, we observed mostly fibres, plastic tape, fibre fishhook, and artificial bait (Fig. 2).

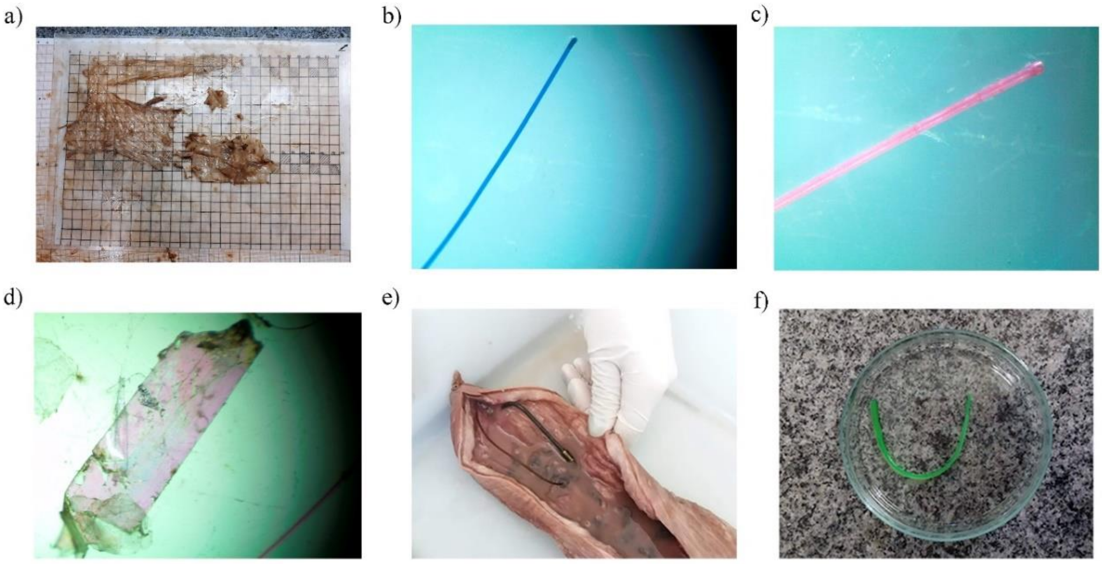


Figure 3. Macroplastics detected in the tuna and tuna-like species from the Southwestern Tropical Atlantic: a) plastic bag, b) blue filament, c) pink filament, d) plastic tape, e) nylon fishhook, and f) fragment of artificial bait.

Microplastics in the stomachs of T. albacares

A total of 93 microplastic particles were recovered from the nine stomachs of *T. albacares* (FO= 100%) captured outside the EEZ, with an average of 10.33 ± standard deviation 14.06 particles per individual^{-1} and mean size of 0.77 ± 0.92 mm ind.$^{-1}$. The analysed *T. albacares* ranged from 40 to 145 cm in fork length and weighed from 1 to 47.8 kg. However, there was no relationship between the number and size of detected particles and the size of tuna (Spearman's rank correlation, $p > 0.05$). Overall, regarding the shapes of the microplastics, the most abundant were foams (61%), followed by fibres (22%), films (10%), pellets (6%) and fragments (1%). The colours white and blue were the most predominant (Table 1).

Table 1. Summary of results regarding the mean (±standard deviation) number (particles individuals^{-1}), size (mm), and FO% (frequency of occurrence) of microplastics extracted from *Thunnus albacares* stomachs, according to shape and colours.

		Number	Size	FO%
	MPs	10.33 (± 14.06)	0.77 (± 0.92)	100
Shape	Fibre	2.22 (± 3.11)	0.89 (± 0.98)	66
	Fragment	0.11 (± 0.33)	0.06 (± 0.19)	11
	Film	1 (± 0.86)	0.10 (± 0.19)	66
	Foam	6.33 (± 10.92)	0.09 (± 0.13)	44
	Pellet	0.66 (± 1.32)	0.04 (± 0.07)	33
Color	White	7.44 (± 10.52)	0.94 (± 1.15)	77
	Black	0.22 (± 0.44)	0.45 (± 0.44)	22
	Blue	1.66 (± 2.39)	0.20 (± 0.21)	55
	Yellow	0.11 (± 0.33)	0.61 (± 0)	11
	Red	0.66 (± 1.65)	0.96 (± 0.71)	22

Microplastics in tuna prey

Among all the analysed stomachs, we recovered 34 tuna prey items with their organs intact. The main prey items found in tuna caught outside the EEZ were identified as Cephalopoda, Bramidae, Exocoetidae, Gempylidae, and Teleostei (unidentified fish). The prey found in the FNA catches were identified as Cephalopoda and the fish families Exocoetidae, Gempylidae, Acanthuridae, Dactylopteridae, Diretmidae, and Hemiramphidae (Table 2). In the recovered samples, 355 microplastics were detected in tuna prey. According to the mean number of microplastics, ingestion significantly differed between tuna prey species (chi-squared = 20.636, df = 11, $p < 0.05$), the Cephalopoda from the EEZ was the most contaminated prey with an average of 27.33 ± 30.98 part. ind.$^{-1}$, followed by Bramidae from the EEZ (19.45 ± 31.15 part. ind.$^{-1}$). Overall, the prey of tuna and tuna-like species caught in the FNA were less contaminated than EEZ prey (Fig. 4). However, the number and size of microplastic found in the prey did not vary statistically significantly between the areas (chi-squared = 3.2216, df = 1; chi-squared = 0.11138, df = 1, $p > 0.05$).

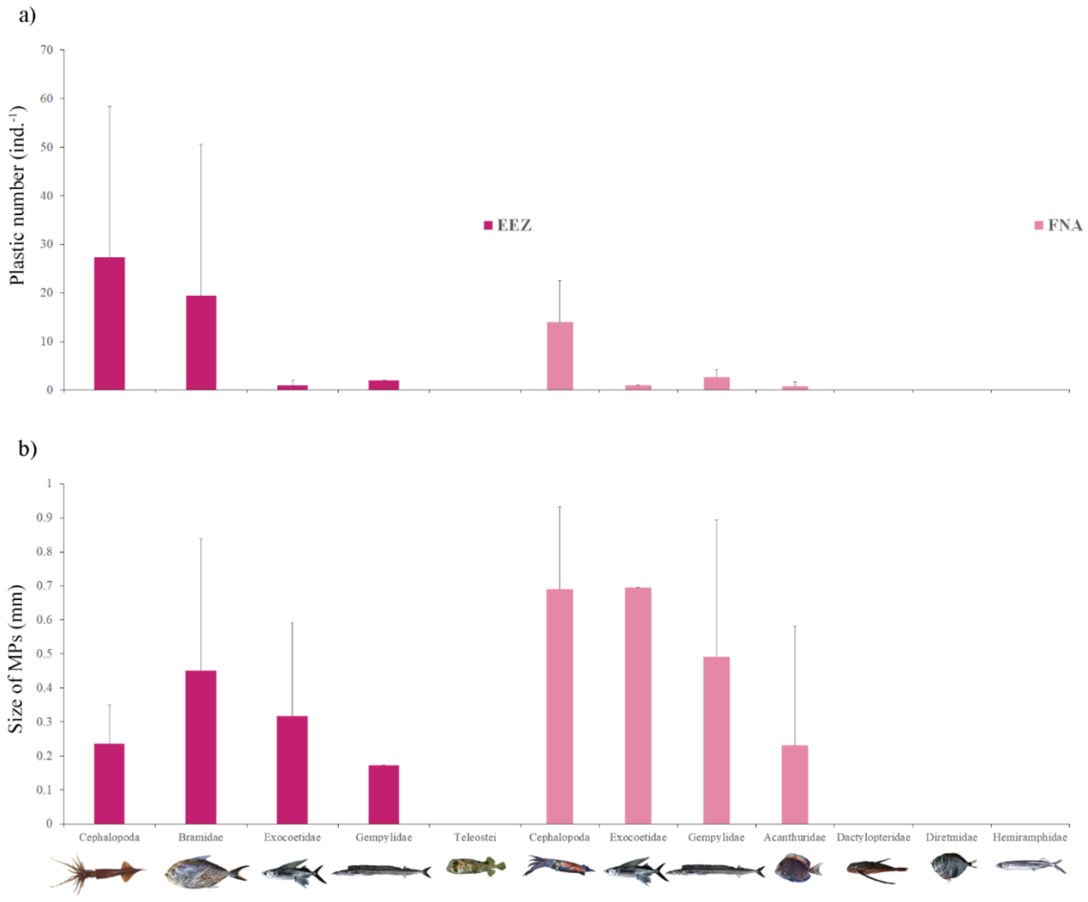


Figure 4. a) mean number (±standard deviation) and b) mean size (length mm) of microplastics found in the prey species.

Nevertheless, when we grouped prey into larger groups, fish and cephalopods, we also observed significant differences in contamination rates (chi-squared = 13.226, df = 3, $p < 0.05$). Fish predated in FNA were less contaminated (1 ± 1.34 part. ind.$^{-1}$) than cephalopods predated outside of the EEZ (27.33 ± 30.99 part. ind.$^{-1}$) and the cephalopods from the FNA (14 ± 8.54 part. ind.$^{-1}$), and also fish from the outside of the EEZ (13.75 ± 26.96 part. ind.$^{-1}$) (Fig. 5). Dunn's *post hoc* test showed that the number of microplastics detected in fish predated on FNA differed from that found in the cephalopods of EEZ and FNA.

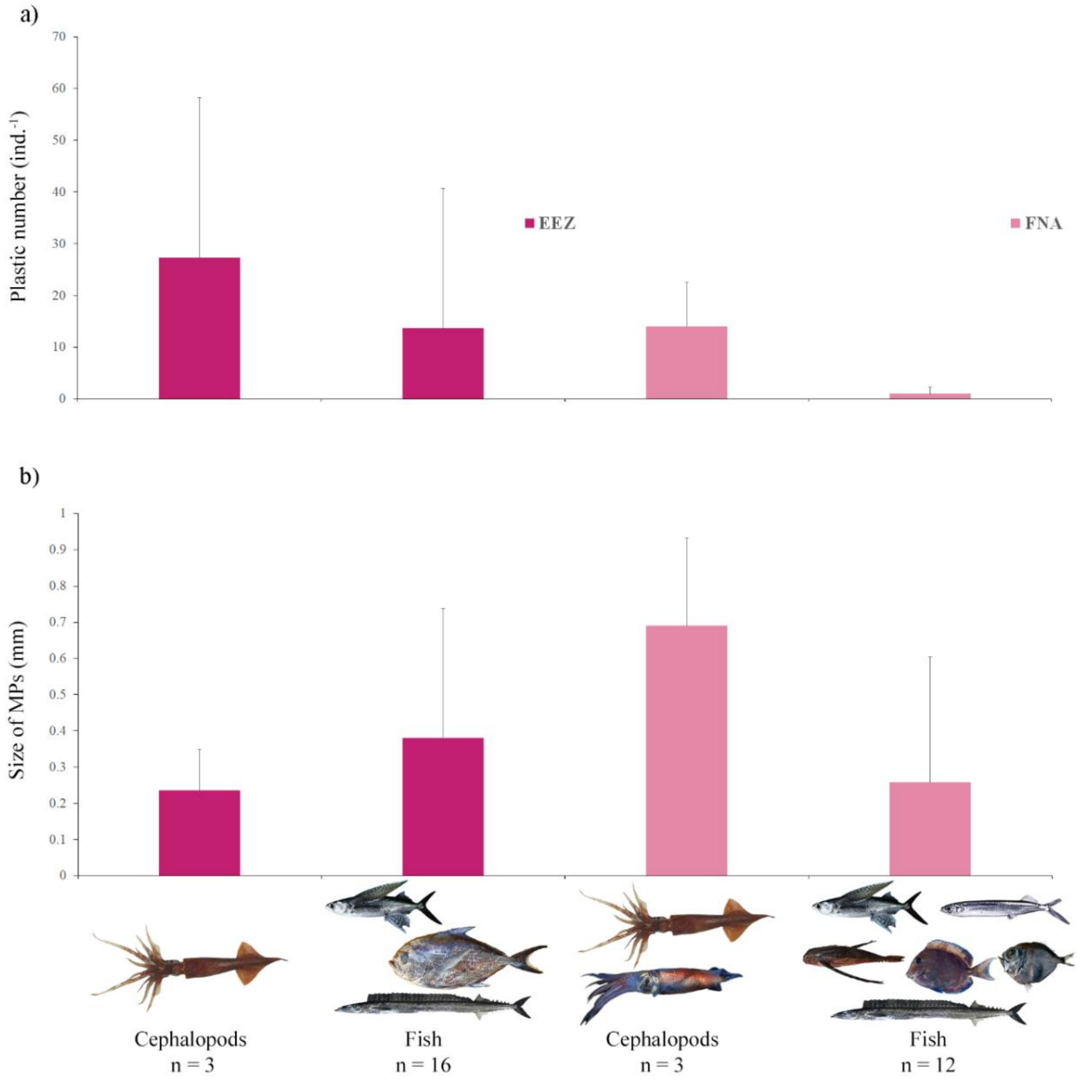


Figure 5. a) mean number (±standard deviation) and b) mean size (length mm) of microplastics detected in grouped prey.

Prey as a data proxy for tuna contamination

We used the prey contamination data as a proxy to access the contamination rate (microplastics extracted in the prey) of tuna and tuna-like species. The data on microplastic number detected in prey significantly differed between the tuna species (chi-squared = 9.3041, df = 3, $p < 0.05$). The *T. albacares* captured outside the EEZ were the most contaminated (22.08 ± 32.85 part. ind.$^{-1}$) (75%), followed by the specimens of this species captured in the FNA (18.5 ± 4.94 part. ind.$^{-1}$) (100%) and *T. obesus* captured outside of the EEZ (5.14 ± 4.70 part. ind.$^{-1}$) (85%). The least contaminated species was *S. barracuda* caught in the FNA (1.30 ± 1.70 part. ind.$^{-1}$) (53%). However, the mean size of microplastics did not vary significantly between species (chi-squared = 3.3636, df = 3, $p > 0.05$), and in general, particles were small (< 1 mm) (Fig. 6, Table 2).

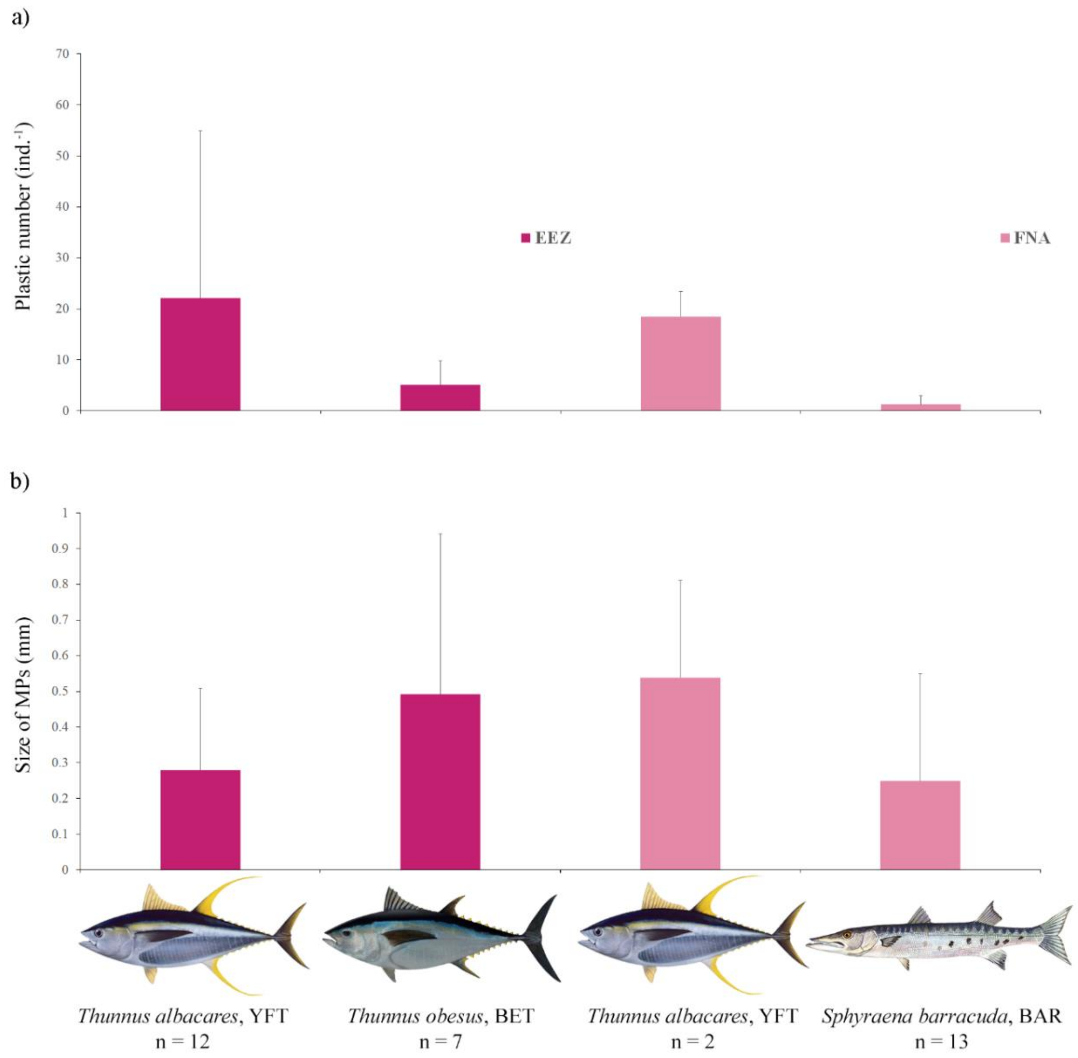


Figure 6. a) mean number (±standard deviation) and b) mean size (length mm) of microplastics detected per tuna and tuna-like species captured in the SWTA.

Table 2. Summary of results regarding the mean (±standard deviation) number (particles individuals^{-1}), size (mm), and FO% (frequency of occurrence) of microplastics (MPs) extracted from tuna prey. ∑ = sum of MPs found in prey. The ecological importance of prey was obtained in the literature for the area and is expressed as frequencies in number (%N), weight (%W) and FO%. Reference set as: α = Silva et al., 2019; β = Martins et al., 2021. n/d = unidentified.

Predator	Prey (group/taxa)	Sampling		Microplastics (MPs) occurrence in prey				Ecological importance of prey			
		Number of prey	Fishery	∑	FO%	MPs mean ±SD	Length (mm) mean ±SD	%N	%W	%FO	Reference
Thunnus obesus Bigeye tuna (BET)	Fish; Bramidae	4	EEZ	22	100	5.50 (± 4.04)	0.78 (± 0.34)	6.17	14.24	20	α
	Fish; Gempylidae	1	EEZ	2	100	2	0.17 (± 0.04)	0.11	0.08	0.95	α
	Fish; Teleostei n/d	1	EEZ	-	-	-	-	9.31	9.69	23.81	α
	Cephalopod; Cephalopoda	1	EEZ	12	100	12	0.11 (± 0.05)	8.58	14.92	26.6	α
Thunnus albacares Yellowfin tuna (YFT)	Fish; Bramidae	7	EEZ	192	71	27.4 (± 37.4)	0.25 (± 0.26)	6.31	6.41	11.36	α
	Fish; Exocoetidae	3	EEZ	3	67	1 (± 1)	0.31 (± 0.27)	33.18	82.97	47.16	α
	Cephalopod; Cephalopoda n/d	2	EEZ	70	100	35 (± 39.5)	0.29 (± 0.04)	10.05	2.61	23.29	α
	Cephalopod; *Abralia veranyi*	1	FNA	22	100	22	0.41 (± 0.25)	0.26	0.02	2.94	β
	Cephalopod; *Ornitotheuthis antillarum*	1	FNA	15	100	15	0.87 (± 0.85)	8.85	3.19	26.47	β
Sphyraena barracuda Barracuda (BAR)	Fish; Exocoetidae *Exocoetus volitans*	1	FNA	1	100	1	0.69	18.18	46.27	11.67	β
	Fish; Gempylidae *Gempylus serpens*	3	FNA	8	100	2.66 (± 1.52)	0.49 (± 0.40)	2.39	1.11	0.83	β
	Fish; Achanturidae *Acanthurus* sp.	4	FNA	3	50	0.75 (± 0.95)	0.23 (± 0.35)	4.78	12.49	2.5	β
	Fish; Dactylopteridae *Dactylopterus volitans*	2	FNA	-	-	-	-	3.83	1.48	2.5	β
	Fish; Diretmidae *Diretmus argenteus*	1	FNA	-	-	-	-	0.48	0.49	0.83	β
		1	FNA	-	-	-	-	0.96	5.27	0.83	β

	Fish; Hemiramphidae *Oxyporhamphus micropterus* Cephalopod; *Ornitotheuthis antillarum*	1	FNA	5	100	5	0.77 (± 0.73)	1.91	0.25	0.83	β

Regarding the shapes of microplastics identified in the prey and used as a proxy for tuna contamination, we observed that *T. albacares* captured outside the EEZ ingested mainly pellets (61%), fragments (24%), foams (12%), fibres (2%) and films (1%) (Fig. 7). The *T. obesus* from the EEZ ingested mostly foams (75%), followed by fibres (14%), pellets and fragments (6%) (Fig. 7). Meanwhile, in *T. albacares* caught in the FNA, ingested mostly pellets (51%) and fibres (43%), whereas films represented only 5% of the total (Fig. 7). For the *S. barracuda*, the main shapes observed were fibres (76%), followed by fragments (12%), pellets and foams (6%) (Fig. 7).

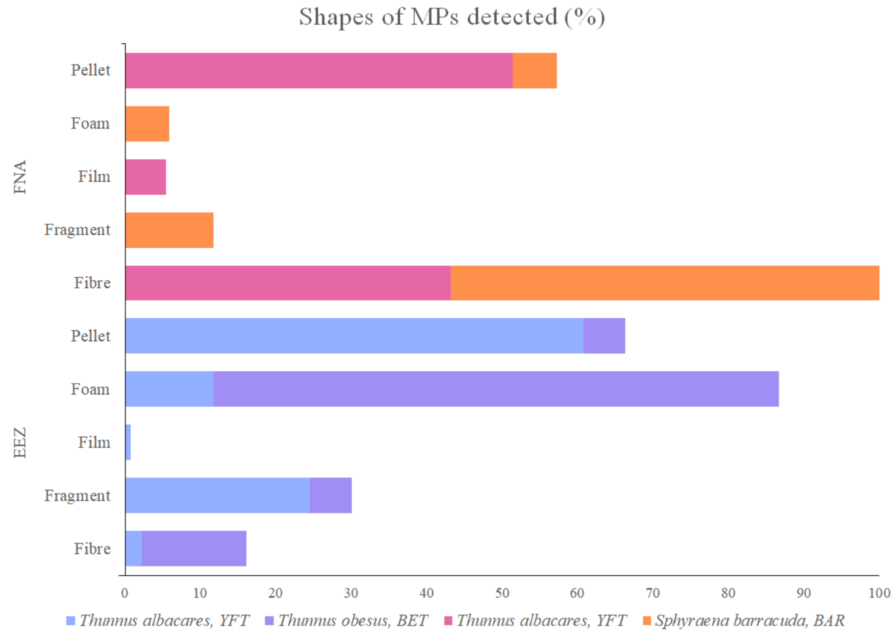


Figure 7. Shapes of microplastics found in tuna's prey are expressed as a percentage (%).

Identified polymers

Overall, plastic polymers were successfully identified in 35% of particles from the subset of samples analysed by LDIR. Particles that were identified between 60-69.9% similarity to the reference spectrum were considered partially identified and comprise 34% of the samples (Fig. 8). The lower similarity between partially identified particle spectra to reference spectra might be explained by the advanced weathering of the particles. LDIR is still a novel technique for identifying plastic polymers in environmental samples; the number of reference spectra of weathered polymers will increase in the future, thereby diminishing the percentage of partially identified particles. Biopolymers identified as cellulose and natural polyamide were observed in 19% of all particles, and 12% were unidentified. A wide range of polymers was identified, but the most commons were Styrene-Butadiene Rubber (SBR) with 17% abundance, followed by Polyamide (PA) with 15%, Polyethylene Terephthalate (PET) and Polyethylene (PE) with a similar abundance of 12%, and Polyurethane (PU) with 10%. The other polymers, such as Low-density polyethylene (LDPE), Polyvinyl chloride (PVC), Acrylonitrile Butadiene Styrene (ABS), Alkyd Varnish, Polypropylene (PP), Polystyrene (PS), Polymethylmethacrylate (PMMA), Polytetrafluoroethylene (PTFE), and Chlorinated Polyisoprene contributed with a similar abundance of 2-5% (Fig. 8).

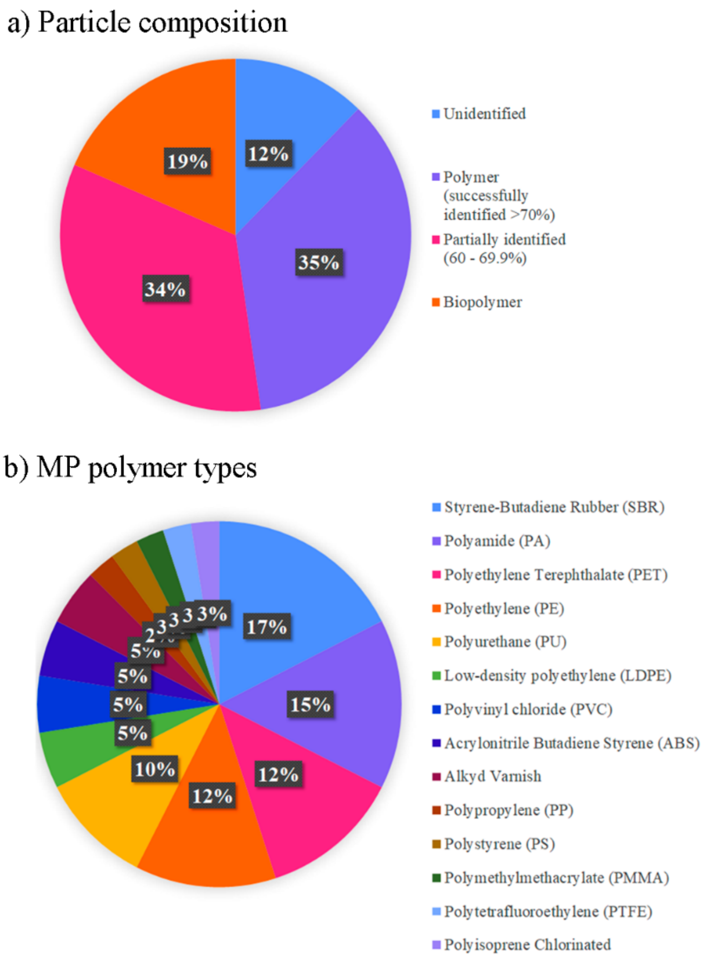


Figure 8. a) Particle composition in the analysed samples, and b) Percentage of identified polymers.

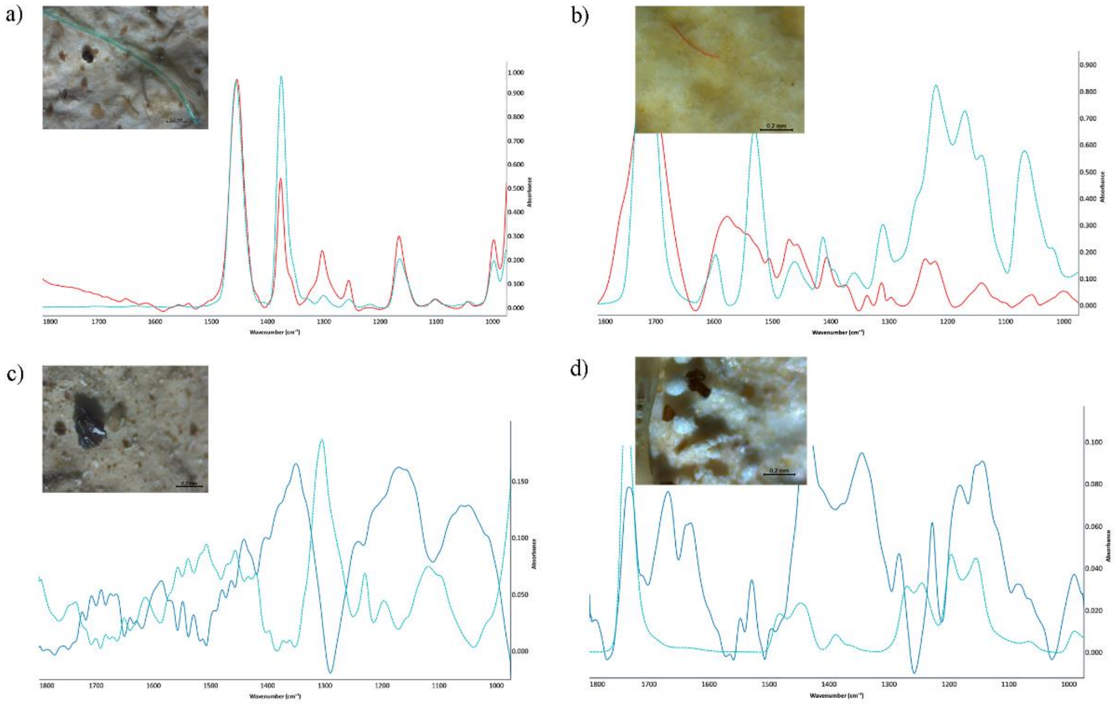


Figure 9. Particles and the polymers identify by the LDIR analysis (the particle signature is displayed as a continuous line and the reference spectrum as a dotted line). a) green fibre - polypropylene (PP), b) red fibre - polyurethane (PU), c) foam - styrene-butadiene rubber (SBR), and d) pellets - polymethylmethacrylate (PMMA).

Discussion

Macroplastic in tuna

In general, we found a low frequency of macroplastic contamination (FO= 3%) in tuna and tuna-like species caught in the Southwestern Tropical Atlantic (SWTA). This low frequency of occurrence has also been observed in other studies analysing the ingestion of marine debris in *T. albacares*, *T. obesus* and *Katsuwonus pelamis* (FO= 0, 0 and 0.75%, respectively) from the Western Atlantic (de Mesquita et al., 2021), in *T. albacares* from the South Pacific subtropical gyre (2%) (Chagnon et al., 2018), and in *K. pelamis*, *T. albacares*, *C. hippurus*, and *T. obesus* (FO= 0, 0, 2 and 9%, respectively) from the North Pacific subtropical gyre (Choy and Drazen, 2013). Researchers often suggest that the lower intake of large plastics by pelagic fishes can be explained due to geographical location (de Mesquita et al., 2021) since plastics tend to accumulate in oceanic gyres (Cózar et al., 2014; Jiang et al., 2020) and areas that do not have a convergence zone would accumulate fewer plastics. Conversely, even in the most polluted regions of the ocean (*e.g.*, North Pacific subtropical gyre), some species may exhibit a low frequency of ingested macroplastics (Choy and Drazen, 2013). Therefore, the contamination of marine species does not seem to be solely linked to the availability of plastic debris since plastic debris is ubiquitous, and the interaction of marine organisms with plastic waste might be associated with the ecological behaviour of species (Justino et al., 2022).

In the present study, among tuna caught by industrial fleets outside the EEZ, only *T. albacares* presented a single plastic bag. However, concerning the tuna and tuna-like species caught in the FNA, *A. solandri* was the most contaminated species (FO= 8%), followed by *S. barracuda* (FO= 3%) and *T. albacares* (FO= 3%) captured in the same area. The items extracted from the fishes were filaments, nylon fishhooks, artificial bait, and plastic tape. The availability of macroplastics in the vicinity of the Archipelago is mainly due to the intense tourist activity and fishery in the region and the island's topography. Islands can accumulate plastic material on the water surface due to the island effect (Lima et al., 2016). Besides that, these predator species are generalists-opportunists, feeding mainly on fishes, cephalopods and crustaceans (Martins et al., 2021). Thus, their foraging habits in the islands are closely associated with coastal regions, which may be subjected to higher amounts of plastic waste due to their proximity to urban centres. In addition, some of these plastic items may serve as a habitat for microorganisms due to biofouling (Pinheiro et al., 2021) and could be attractive to fishes, which may accidentally ingest them by confusion with prey items. In the tropical and oligotrophic waters away from the islands, tuna species need to forage over large areas in search of feeding resources; as a result, they might be even more exposed to plastic pollution (Roch et al., 2020). However, the low presence of macroplastics in individuals caught by the industrial fisheries outside the EEZ may be related to the fast evacuation rate (~10-12h) of these tuna (Magnuson, 1969; Olson and Boggs, 1986) and strategies of regurgitation (Li et al., 2021). Ingestion of plastics can lead to several sub-lethal effects for the individual, such as digestive damage, gut blockage, decreased predatory efficiency and starvation (de Sá et al., 2015; Menezes et al., 2019; Moore, 2008).

Microplastics in tuna stomachs

Microplastics were detected in the stomachs of *T. albacares* captured outside the EEZ in a high number (mean of 10.33 ± 14.06 particles per ind.$^{-1}$; FO= 100%). However, we did not find any correlation between the number and size of particles detected and tuna size. This high number may be related to the fact that, as opportunistic predators, due to the intake of large amounts of contaminated prey, the momentary build-up of microplastics increases before egestion (Ferreira et al., 2019; Justino et al., 2021). In addition, for tropical tuna, it is vital to have the ability to process large quantities of food in a brief period when food is available (Olson and Boggs, 1986). Hence, it may lead to numerous microplastics accumulating through trophic transfer, as was observed in our study (see next section). The tiny sizes and shapes of microplastics found in the stomachs of yellowfin tuna observed in our study strongly suggest that this accumulation is probably due to particles ingested and transferred by the prey. The length of microplastics found in the stomachs was generally smaller (mean size of 0.77 ± 0.92 mm ind.$^{-1}$) than previously observed for other oceanic predator species, such as the *C. hippurus* captured in the Eastern Pacific Ocean (Li et al., 2022). Besides accidental ingestion, these particles of such small size can be unintentionally swallowed when fish breathe (Li et al., 2021). However, to analyse the relevance of microplastic uptake through breathing, particles need to be detected in both gills and stomachs.

Trophic transfer of microplastics

The presence of microplastic in ingested prey of tuna and tuna-like species suggests that trophic transfer of microplastics might occur in fishes from the SWTA. From the analysed prey items found in fishes, we detected 355 microplastic particles. Differences in the contamination rate were observed between the ingested prey. Cephalopoda outside the EEZ was the most contaminated prey (27.33 ± 30.98 part. ind.$^{-1}$), followed by Bramidae fishes (19.45 ± 31.15 part. ind.$^{-1}$), also predated in the EEZ. Compared with prey that was preyed upon outside the EEZ, in FNA, they were less contaminated (microplastic number). For example, when grouping the data, fishes predated in the FNA had an average of 1 ± 1.34 MPs (part. ind.$^{-1}$). In the FNA, previous studies have reported the ubiquitous presence of microplastics in the water column, mainly fibres (Ivar Do Sul et al., 2014), and also extracted in the digestive tract of deep-sea fish (Justino et al., 2022). In fact, in our study, fish that were predated in the FNA had more fibres in their stomachs when compared with fishes predated outside the EEZ. Islands can retain these particles near shore due to the action of waves and winds, which might explain these findings (Gove et al., 2019). However, when we compare the shapes of microplastics in the prey, we observe a clear difference between the areas. Prey predated outside the EEZ had most foams and pellets in their digestive tract.

Tuna that prey in areas far from islands may be feeding in deeper layers. Observations of the behaviour of *T. obesus* in the Pacific Ocean have recorded that this species performs daily vertical migration to forage, diving at night at 100 m depth and between 400-500 m during daytime (Dagorn et al., 2000). On the other hand, *T. albacares* spends most of its time in shallow waters (75 m); however, they can dive deeper than 500 m in some cases (Dagorn et al., 2006). We have observed some preys in our samples that are commonly found in deeper areas like Cephalopods (*e.g*., *A. veranyi*), Bramidae and Gempylidae (Ferreira et al., 2022; Klautau et al., 2020; Roper et al., 1984). These prey items had already been reported for the tuna species analysed in the study area, emphasising their importance as a resource for the tuna (Silva et al., 2019). It is the first time that microplastic contamination has been registered for the preys Bramidae and Gempylidae, highlighting the lack of information for some important groups that serve as a source of energy for fish stocks. The high contamination rate in individuals caught outside the EEZ and the different shapes of particles found may be due to differences in the feeding habits of the prey. Indeed, the fish prey groups outside the EEZ (Gempylidae and Bramidae) are also opportunistic predators, like tunas, and feed mainly on cephalopods and other fish species (Froese and Pauly, 2022).

In our study, using prey as bioindicators to verify the presence of microplastics, we observed that the planktivorous fishes (*e.g*., Exocoetidae), which feed mainly in the epipelagic zone, were less exposed to microplastics than deep-sea fishes and organisms that feed on marine aggregates (*e.g*., Cephalopods; Hoving and Robison, 2012). Fishes of the family Exocoetidae are among the essential energy sources for tunas (Martins et al., 2021; Silva et al., 2019) and were already reported to be contaminated with plastics (Chagnon et al., 2018; Gove et al., 2019). Here, we observed a contamination rate of one particle per individual, similar to the one observed in the Pacific Ocean for a species of the family Exocoetidae (1.5 particles per fish; Chagnon et al., 2018). Cephalopods were the prey that registered the highest contamination rate in our study. In recent research conducted in the SWTA, Ferreira et al. (2022) reported a high contamination rate in deep-sea cephalopods, which was attributed to the feeding strategy of the species, which usually feed on fish, zooplankton and marine snow. Marine snow is an organic matter aggregate that can be originated from the release of substances by decomposed organisms or other organic matter in marine environments (Tansel, 2018). Additionally, marine aggregates serve as an important energy source for various organisms, including midwater zooplankton (Steinberg et al., 1994). Incorporating microplastics into marine snow is hypothesised to be an important microplastic sinking mechanism (Kvale et al., 2020). Furthermore, most marine organisms can egest microplastics, a possible route for their incorporation into marine aggregates (Wright et al., 2013).

For the Cephalopods, Bramidae and tuna, we observed plastics of sizes < 1 mm (suggesting that these particles had already been quite degraded through weathering) and were mostly foams and pellets (shapes mainly associated with aggregates). Therefore, we assume that these microplastics may have been accidentally ingested by the prey when foraging in deeper waters and transferred along the trophic chain to predators. However, we emphasise the importance of studying marine aggregates and verifying the association of microplastics and the main types of polymers present.

Characterisation of microplastic polymers

Among the polymers identified in our study, we found various polymer types in tuna stomachs and prey items. Nevertheless, the main polymers were identified as SBR, PA, PET and PE. The SBR polymer is often used in manufacturing car tires and as a substitute for natural rubber due to its resistance to abrasion (Polymer DataBase, 2022). Tiny particles generated from the abrasion of car tires against the road surface are widely available in the environment but still rarely reported as microplastic contaminants in environmental studies (Arias et al., 2022; Knight et al., 2020; Kreider et al., 2010). Tire wear particles (TWP) could be one of the likely sources of SBR particles in the marine environment. While it is still unclear how these particles reach the oceans, possible pathways could be atmospheric fallout, wastewater effluent, rivers, and oceanic currents (Knight et al., 2020; Luo et al., 2021). SBR was also found in the gastrointestinal tract of tuna-like species (*C. hippurus*) in the Mediterranean Sea (Schirinzi et al., 2020), in mesopelagic fishes from the SWTA (Justino et al., 2022), and mussels *Mytilus* spp. from the Norwegian sea (Bråte et al., 2018). Moreover, the other polymers (PA, PET and PE) are primarily used in the textile industry and fishing activities (Lima et al., 2021) and are frequently reported in marine species (Justino et al., 2022; Li et al., 2022; Schirinzi et al., 2020).

In addition to the polymers themselves, there is a significant concern about the additives released from these particles, and the associated hazards are still poorly understood. For example, researchers found a compound (6PPD) derived from tire wear particles, which induced acute mortality in coho salmon in the Pacific Northwest (Tian et al., 2021). Moreover, the leachate of TWP can be toxic to organisms (Yang et al., 2022). In addition to additives, microplastics can adsorb and concentrate other pollutants (*e.g.*, heavy metals and persistent organic pollutants), which are widely available in the ocean (Ashton et al., 2010; Rochman et al., 2013) and can be bioaccumulated and biomagnified in the food web (Batel et al., 2016; Teuten et al., 2009).

Moreover, the exposure of marine organisms to microplastic contaminants is of major concern for human health, which depends on fishery resources. For example, microplastics have already been detected in canned tuna (Akhbarizadeh et al., 2020; Diaz-Basantes et al., 2022). The fact that the tuna is ingesting such small particles serves as an additional warning to society, which is already exposed to these particles through various pathways, such as the atmosphere, water, salt and seafood (Bruzaca et al., 2022; Karami et al., 2017; Pratesi et al., 2021; Wang et al., 2020). The degradation of microplastics into progressively smaller particles, such as nano plastics, can increase health risks due to their ability to accumulate in tissues such as the brain and cause oxidative DNA damage in the regions where they bioaccumulate (Sökmen et al., 2020). Furthermore, plastic particles have recently been detected in human blood (Leslie et al., 2022). In our study, it was impossible to quantify nanoparticles due to the methodology used, so we merely report microplastics here (0.04-5 mm). However, due to the potential risks of bioaccumulation of nanoparticles, and their associated risks with other pollutants available in the environment, we emphasise the importance of further investigations of the degradation of polymers and their impact on marine organisms, and we reaffirm the urge for a debate on measures to establish appropriate limit values for safe consumption.

Conclusions

This is the first study to assess microplastics contaminating tuna and tuna-like species and to observe strong evidence of microplastic trophic transfer in tunas from the South Atlantic Ocean. We found a low frequency of occurrence of macroplastic in the four species analysed. Conversely, we observed a high abundance of microplastics in the stomachs of *T. albacares,* including ingested prey.

The low occurrence of macroplastics is probably due to the species' rapid egestion and the regurgitation of items. On the other hand, the high contamination rate by microplastic may be due to the opportunistic behaviour of predatory species and its potential to accumulate these particles through trophic transfer. This study verified the possibility of trophic transfer of microplastics by analysing the ingestion rate of particles in prey found in tuna. Ingestion rates differed significantly between the prey species, and the most contaminated prey were the Cephalopods and fishes of the Bramidae family caught outside the EEZ. The ecological habits of organisms can explain the high number of microplastics found in the prey. Cephalopods from deeper waters usually feed on marine snow that may contain aggregated microplastics. On the other hand, the Bramidae are opportunistic predators such as the tunas. Additionally, predators generally feed on a large amount of available prey, accumulating the particles prior to egestion and thus transferring them into the trophic chain. The most frequent shapes of microplastics found in both prey and tuna stomachs were foams and pellets with sizes < 1 mm. A variety of polymers were identified; the most frequent were SBR, PA, PET and PE.

Our findings enhance scientific knowledge of how the ecological behaviour of marine species can affect the intake of microplastics. Moreover, it alerts the current contamination level of apex predators, such as tunas, which can pose severe risks to human health, given their worldwide high socio-economic value stocks. The information provided here may be used to monitor microplastic contamination in fish stocks and help decision-makers establish future mitigation strategies.

Acknowledged

Our research was supported by the LMI TAPIOCA program CAPES/COFECUB (88881.142689/2017-01); FUNBIO and HUMANIZE under the grant "Programa Bolsas Funbio - Conservando o Futuro 2019 (02/2019)"; FACEPE (Fundação de Amparo à Ciência e Tecnologia do Estado de Pernambuco); and MicroplastiX Project/JPI-Oceans/CONFAP/FACEPE (n°APQ-0035-1.08/19). We thank the CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico), which provided research grants to Flávia Lucena-Frédou (CNPq n° 308554/2019-1); FACEPE for granting a scholarship (BFP-0049-5.06/20) and CAPES for granting a scholarship at doctoral level for the first author. We also thank to the Secretariat of Aquaculture and Fisheries (SAP) funded the Project of Scientific and Technical Support for the Development of Tuna Fishing in Brazil

(PROTUNA; Process Nb. 445810/2015-7) under which this study was carried out. We wish to thank Christos Panagiotopoulos and Richard Sempéré for access to the LDIR Chemical Imaging System. CP and RS received financial support for the LDIR acquisition via the Region-SUD (SUD-PLASTIC project, Grant No 2019_02985 DEB 19-573) and the CNRS.

REFERENCES

Akhbarizadeh, R., Dobaradaran, S., Nabipour, I., Tajbakhsh, S., Darabi, A.H., Spitz, J., 2020. Abundance, composition, and potential intake of microplastics in canned fish. Mar. Pollut. Bull. 160, 111633. https://doi.org/10.1016/j.marpolbul.2020.111633

Andreas, Hadibarata, T., Sathishkumar, P., Prasetia, H., Hikmat, Pusfitasari, E.D., Tasfiyati, A.N., Muzdalifah, D., Waluyo, J., Randy, A., Ramadhaningtyas, D.P., Zuas, O., Sari, A.A., 2021. Microplastic contamination in the Skipjack Tuna (*Euthynnus affinis*) collected from Southern Coast of Java, Indonesia. Chemosphere 276. https://doi.org/10.1016/j.chemosphere.2021.130185

Arias, A.H., Alfonso, M.B., Girones, L., Piccolo, M.C., Marcovecchio, J.E., 2022. Synthetic microfibers and tyre wear particles pollution in aquatic systems: Relevance and mitigation strategies. Environ. Pollut. https://doi.org/10.1016/j.envpol.2021.118607

Arthur, C., Baker, J., Bamford, H., 2009. Workshop on the Occurrence , Effects , and Fate of Microplastic Marine Debris. Group 530.

Ashton, K., Holmes, L., Turner, A., 2010. Association of metals with plastic production pellets in the marine environment. Mar. Pollut. Bull. 60, 2050–2055. https://doi.org/10.1016/j.marpolbul.2010.07.014

Almeida, F. F. M. (2006). Ilhas oceânicas brasileiras e suas relações com a tectônica atlântica. Terra Didática, 2,3–18.

Assunção, R. V., Silva, A.C., Roy, A., Bourlès, B., Silva, C.H.S., Ternon, J.F., Araujo, M., Bertrand, A., 2020. 3D characterisation of the thermohaline structure in the southwestern tropical Atlantic derived from functional data analysis of in situ profiles. Prog. Oceanogr. 187, 102399. https://doi.org/10.1016/j.pocean.2020.102399

Barboza, L.G.A., Vieira, L.R., Branco, V., Figueiredo, N., Carvalho, F., Carvalho, C., Guilhermino, L., 2018. Microplastics cause neurotoxicity, oxidative damage and energy-related changes and interact with the bioaccumulation of mercury in the European seabass, Dicentrarchus labrax (Linnaeus, 1758). Aquat. Toxicol. 195, 49–57. https://doi.org/10.1016/j.aquatox.2017.12.008

Batel, A., Linti, F., Scherer, M., Erdinger, L., Braunbeck, T., 2016. Transfer of benzo[*a*]pyrene from microplastics to *Artemia* nauplii and further to zebrafish via a trophic food web experiment: CYP1A induction and visual tracking of persistent organic pollutants. Environ. Toxicol. Chem. 35, 1656–1666. https://doi.org/10.1002/etc.3361

Boerger, C.M., Lattin, G.L., Moore, S.L., Moore, C.J., 2010. Plastic ingestion by planktivorous fishes in the North Pacific Central Gyre. Mar. Pollut. Bull. 60, 2275–2278. https://doi.org/10.1016/j.marpolbul.2010.08.007

Bråte, I.L.N., Hurley, R., Iversen, K., Beyer, J., Thomas, K. V., Hovingdal, C.C., Green, N.W., Olsen, M., Lusher, A., 2018. Mytilus spp. as sentinels for monitoring microplastic pollution in Norwegian coastal waters: A qualitative and quantitative study. Environ. Pollut. 243, 383–393. https://doi.org/10.1016/j.envpol.2018.08.077

Bruzaca, D.N.A., Justino, A.K.S., Mota, G.C.P., Costa, G.A., Lucena-Frédou, F., Gálvez, A.O., 2022. Occurrence of microplastics in bivalve molluscs Anomalocardia flexuosa captured in Pernambuco, Northeast Brazil. Mar. Pollut. Bull. 179, 113659.

https://doi.org/10.1016/j.marpolbul.2022.113659

Chagnon, C., Thiel, M., Antunes, J., Ferreira, J.L., Sobral, P., Ory, N.C., 2018. Plastic ingestion and trophic transfer between Easter Island flying fish (*Cheilopogon rapanouiensis*) and yellowfin tuna (*Thunnus albacares*) from Rapa Nui (Easter Island) *. Environ. Pollut. 243, 127–133. https://doi.org/10.1016/j.envpol.2018.08.042

Choy, C.A., Drazen, J.C., 2013. Plastic for dinner? Observations of frequent debris ingestion by pelagic predatory fishes from the central North Pacific. Mar. Ecol. Prog. Ser. 485, 155–163. https://doi.org/10.3354/meps10342

Choy, C.A., Robison, B.H., Gagne, T.O., Erwin, B., Firl, E., Halden, R.U., Hamilton, J.A., Katija, K., Lisin, S.E., Rolsky, C., S. Van Houtan, K., 2019. The vertical distribution and biological transport of marine microplastics across the epipelagic and mesopelagic water column. Sci. Rep. 9. https://doi.org/10.1038/s41598-019-44117-2

Cózar, A., Echevarría, F., González-Gordillo, J.I., Irigoien, X., Úbeda, B., Hernández-León, S., Palma, Á.T., Navarro, S., García-de-Lomas, J., Ruiz, A., Fernández-de-Puelles, M.L., Duarte, C.M., 2014. Plastic debris in the open ocean. Proc. Natl. Acad. Sci. 111, 10239–10244. https://doi.org/10.1073/pnas.1314705111

Dagorn, L., Bach, P., Josse, E., 2000. Movement patterns of large bigeye tuna (*Thunnus obesus*) in the open ocean, determined using ultrasonic telemetry. Mar. Biol. 136, 361–371. https://doi.org/10.1007/s002270050694

Dagorn, L., Holland, K.N., Hallier, J.P., Taquet, M., Moreno, G., Sancho, G., Itano, D.G., Aumeeruddy, R., Girard, C., Million, J., Fonteneau, A., 2006. Deep diving behavior observed in yellowfin tuna (*Thunnus albacares*). Aquat. Living Resour. 19, 85–88. https://doi.org/10.1051/alr:2006008

de Mesquita, G.C., Menezes, R., da Cunha-Neto, M.A., Dantas-Neto, A.B., da Silva, G.B., 2021. Feeding strategy of pelagic fishes caught in aggregated schools and vulnerability to ingesting anthropogenic items in the western equatorial Atlantic Ocean. Environ. Pollut. 282, 117021. https://doi.org/10.1016/j.envpol.2021.117021

de Sá, L.C., Luís, L.G., Guilhermino, L., 2015. Effects of microplastics on juveniles of the common goby (*Pomatoschistus microps*): Confusion with prey, reduction of the predatory performance and efficiency, and possible influence of developmental conditions. Environ. Pollut. 196, 359–362. https://doi.org/10.1016/j.envpol.2014.10.026

Diaz-Basantes, M.F., Nacimba-Aguirre, D., Conesa, J.A., Fullana, A., 2022. Presence of microplastics in commercial canned tuna. Food Chem. 385, 132721. https://doi.org/10.1016/j.foodchem.2022.132721

Dunn, O.J., 1964. A Note on Multiple Comparisons Using Rank Sums. Technometrics 6, 241–252. https://doi.org/10.1080/00401706.1965.10490253

Eriksson, C., Burton, H., 2003. Origins and Biological Accumulation of Small Plastic Particles in Fur Seals from Macquarie Island. AMBIO A J. Hum. Environ. 32, 380–384. https://doi.org/10.1579/0044-7447-32.6.380

FAO, 2020. The State of World Fisheries and Aquaculture 2020, INFORM. FAO, Rome. https://doi.org/10.4060/ca9229en

Farrell, P., Nelson, K., 2013. Trophic level transfer of microplastic: *Mytilus edulis* (L.) to *Carcinus maenas* (L.). Environ. Pollut. 177, 1–3. https://doi.org/10.1016/j.envpol.2013.01.046

Ferreira, G.V.B., Barletta, M., Lima, A.R.A., Morley, S.A., Costa, M.F., 2019. Dynamics of Marine Debris Ingestion by Profitable Fishes Along The Estuarine Ecocline. Sci. Rep. 9, 13514. https://doi.org/10.1038/s41598-019-49992-3

Ferreira, G.V.B., Justino, A.K.S., Eduardo, L.N., Lenoble, V., Fauvelle, V., Schmidt, N., Junior, T.V., Frédou, T., Lucena-Frédou, F., 2022. Plastic in the inferno: Microplastic contamination in deep-sea cephalopods (*Vampyroteuthis infernalis* and *Abralia veranyi*) from the southwestern Atlantic. Mar. Pollut. Bull. 174, 113309. https://doi.org/10.1016/j.marpolbul.2021.113309

Froese, R., Pauly., D., 2022. FishBase [WWW Document]. World Wide Web Electron. Publ. URL www.fishbase.org

Galloway, T.S., Cole, M., Lewis, C., 2017. Interactions of microplastic debris throughout the marine ecosystem. Nat. Ecol. Evol. 1. https://doi.org/10.1038/s41559-017-0116

Geyer, R., 2020. Production, use, and fate of synthetic polymers, Plastic Waste and Recycling. Elsevier Inc. https://doi.org/10.1016/b978-0-12-817880-5.00002-5

Gove, J.M., Whitney, J.L., McManus, M.A., Lecky, J., Carvalho, F.C., Lynch, J.M., Li, J., Neubauer, P., Smith, K.A., Phipps, J.E., Kobayashi, D.R., Balagso, K.B., Contreras, E.A., Manuel, M.E., Merrifield, M.A., Polovina, J.J., Asner, G.P., Maynard, J.A., Williams, G.J., 2019. Prey-size plastics are invading larval fish nurseries. Proc. Natl. Acad. Sci. U. S. A. 116, 24143–24149. https://doi.org/10.1073/pnas.1907496116

Human, P. & Deloach, N. (2002). Reef fish identification, Florida, Caribbean, Bahamas. 3 ed. Florida: New World Publication, 2002, 481p

Hoving, H.J.T., Robison, B.H., 2012. Vampire squid: Detritivores in the oxygen minimum zone. Proc. R. Soc. B Biol. Sci. 279, 4559–4567. https://doi.org/10.1098/rspb.2012.1357

Ivar Do Sul, J.A., Costa, M.F., Fillmann, G., 2014. Microplastics in the pelagic environment around oceanic islands of the western Tropical Atlantic Ocean. Water. Air. Soil Pollut. 225. https://doi.org/10.1007/s11270-014-2004-z

Jambeck, J.R., Geyer, R., Wilcox, C., Siegler, T.R., Perryman, M., Andrady, A., Narayan, R., Law, K.L., 2015. Plastic waste inputs from land into the ocean. Science (80-.). 347, 768–771. https://doi.org/10.1126/science.1260352

Jiang, Y., Yang, F., Zhao, Y., Wang, J., 2020. Greenland Sea Gyre increases microplastic pollution in the surface waters of the Nordic Seas. Sci. Total Environ. 712, 136484. https://doi.org/10.1016/j.scitotenv.2019.136484

Justino, A.K.S., Ferreira, G.V.B., Schmidt, N., Eduardo, L.N., Fauvelle, V., Lenoble, V., Sempéré, R., Panagiotopoulos, C., Mincarone, M.M., Frédou, T., Lucena-Frédou, F., 2022. The role of mesopelagic fishes as microplastics vectors across the deep-sea layers from the Southwestern Tropical Atlantic. Environ. Pollut. 300, 118988. https://doi.org/10.1016/j.envpol.2022.118988

Justino, A.K.S., Lenoble, V., Pelage, L., Ferreira, G.V.B., Passarone, R., Frédou, T., Lucena Frédou, F., 2021. Microplastic contamination in tropical fishes: An assessment of different feeding habits. Reg. Stud. Mar. Sci. 45, 101857. https://doi.org/10.1016/j.rsma.2021.101857

Karami, A., Golieskardi, A., Keong Choo, C., Larat, V., Galloway, T.S., Salamatinia, B., 2017. The presence of microplastics in commercial salts from different countries. Sci. Rep. 7, 46173. https://doi.org/10.1038/srep46173

Klautau, A.G.C. de M., Cintra, I.H.A., Rotundo, M.M., Carvalho-Filho, A., Caires, R.A., Marceniuk, A.P., 2020. The deep sea teleost fish fauna of the brazilian north coast. Neotrop. Ichthyol. 18, 1–19. https://doi.org/10.1590/1982-0224-2020-0030

Knight, L.J., Parker-Jurd, F.N.F., Al-Sid-Cheikh, M., Thompson, R.C., 2020. Tyre wear particles: an abundant yet widely unreported microplastic? Environ. Sci. Pollut. Res. 27, 18345–18354. https://doi.org/10.1007/s11356-020-08187-4

Koelmans, A.A., Kooi, M., Law, K.L., Van Sebille, E., 2017. All is not lost: Deriving a top-down mass budget of plastic at sea. Environ. Res. Lett. 12. https://doi.org/10.1088/1748-9326/aa9500

Kreider, M.L., Panko, J.M., McAtee, B.L., Sweet, L.I., Finley, B.L., 2010. Physical and chemical characterisation of tire-related particles: Comparison of particles generated using different methodologies. Sci. Total Environ. 408, 652–659. https://doi.org/10.1016/j.scitotenv.2009.10.016

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

Lau, W.W.Y., Shiran, Y., Bailey, R.M., Cook, E., Stuchtey, M.R., Koskella, J., Velis, C.A., Godfrey, L., Boucher, J., Murphy, M.B., Thompson, R.C., Jankowska, E., Castillo, A.C., Pilditch, T.D., Dixon, B., Koerselman, L., Kosior, E., Favoino, E., Gutberlet, J., Baulch, S., Atreya, M.E., Fischer, D., He, K.K., Petit, M.M., Sumaila, U.R., Neil, E., Bernhofen, M. V, Lawrence, K., Palardy, J.E., 2020. Evaluating scenarios toward zero plastic pollution. Science (80-.). 369, 1455–1461. https://doi.org/10.1126/SCIENCE.ABA9475

Leslie, H.A., van Velzen, M.J.M., Brandsma, S.H., Vethaak, A.D., Garcia-Vallejo, J.J., Lamoree, M.H., 2022. Discovery and quantification of plastic particle pollution in human blood. Environ. Int. 163, 107199. https://doi.org/10.1016/j.envint.2022.107199

Lessy, M.R., Sabar, M., 2021. Microplastics Ingestion by Skipjack tuna (Katsuwonus pelamis) in Ternate, North Maluku - Indonesia. IOP Conf. Ser. Mater. Sci. Eng. 1125, 012085. https://doi.org/10.1088/1757-899x/1125/1/012085

Li, B., Liang, W., Liu, Q.-X., Fu, S., Ma, C., Chen, Q., Su, L., Craig, N.J., Shi, H., 2021. Fish Ingest Microplastics Unintentionally. Environ. Sci. Technol. 55, 10471–10479. https://doi.org/10.1021/acs.est.1c01753

Li, W., Pan, Z., Xu, J., Liu, Q., Zou, Q., Lin, H., Wu, L., Huang, H., 2022. Microplastics in a pelagic dolphinfish (*Coryphaena hippurus*) from the Eastern Pacific Ocean and the implications for fish health. Sci. Total Environ. 809, 151126. https://doi.org/10.1016/j.scitotenv.2021.151126

Lima, A.R.A., Barletta, M., Costa, M.F., 2016. Seasonal-dial shifts of ichthyoplankton assemblages and plastic debris around an Equatorial Atlantic archipelago. Front. Environ. Sci. 4, 56. https://doi.org/10.3389/fenvs.2016.00056

Lima, A.R.A., Ferreira, G.V.B., Barrows, A.P.W., Christiansen, K.S., Treinish, G., Toshack, M.C., 2021. Global patterns for the spatial distribution of floating microfibers: Arctic Ocean as a potential accumulation zone. J. Hazard. Mater. 403. https://doi.org/10.1016/j.jhazmat.2020.123796

Lins-Silva, N., Marcolin, C.R., Kessler, F., Schwamborn, R., 2021. A fresh look at microplastics and other particles in the tropical coastal ecosystems of Tamandaré, Brazil. Mar. Environ. Res. 169, 105327. https://doi.org/10.1016/j.marenvres.2021.105327

Luo, Z., Zhou, X., Su, Y., Wang, H., Yu, R., Zhou, S., Xu, E.G., Xing, B., 2021. Environmental occurrence, fate, impact, and potential solution of tire microplastics: Similarities and differences with tire wear particles. Sci. Total Environ. https://doi.org/10.1016/j.scitotenv.2021.148902

Magnuson, J.J., 1969. Digestion and food consumption by skipjack tuna (Katsuwonus pelamis). Trans. Am. Fish. Soc. 98, 379–392.

Martins, K., Pelage, L., Justino, A.K.S., Frédou, F.L., Júnior, T.V., Le Loc'h, F., Travassos, P., 2021. Assessing trophic interactions between pelagic predatory fish by gut content and stable isotopes analysis around Fernando de Noronha Archipelago (Brazil), Equatorial West Atlantic. J. Fish Biol. 99, 1576–1590. https://doi.org/10.1111/jfb.14863

Meijer, L.J.J., van Emmerik, T., van der Ent, R., Schmidt, C., Lebreton, L., 2021. More than 1000 rivers account for 80% of global riverine plastic emissions into the ocean. Sci. Adv. 7, eaaz5803. https://doi.org/10.1126/sciadv.aaz5803

Menezes, R., da Cunha-Neto, M.A., de Mesquita, G.C., da Silva, G.B., 2019. Ingestion of macroplastic debris by the common dolphinfish (*Coryphaena hippurus*) in the Western Equatorial Atlantic. Mar. Pollut. Bull. 141, 161–163. https://doi.org/10.1016/j.marpolbul.2019.02.026

Miranda, D. de A., de Carvalho-Souza, G.F., 2016. Are we eating plastic-ingesting fish? Mar. Pollut. Bull. 103, 109–114. https://doi.org/10.1016/J.MARPOLBUL.2015.12.035

Moore, C.J., 2008. Synthetic polymers in the marine environment: A rapidly increasing, long-term threat. Environ. Res. 108, 131–139. https://doi.org/10.1016/j.envres.2008.07.025

Neto, J.G.B., Rodrigues, F.L., Ortega, I., Rodrigues, L. dos S., Lacerda, A.L. d. F., Coletto, J.L., Kessler, F., Cardoso, L.G., Madureira, L., Proietti, M.C., 2020. Ingestion of plastic debris by commercially important marine fish in southeast-south Brazil. Environ. Pollut. 267. https://doi.org/10.1016/J.ENVPOL.2020.115508

Olson, R.J., Boggs, C.H., 1986. Apex Predation by Yellowfin Tuna (*Thunnus albacares*): Independent Estimates from Gastric Evacuation and Stomach Contents, Bioenergetics, and Cesium Concentrations. Can. J. Fish. Aquat. Sci. 43, 1760–1775. https://doi.org/10.1139/f86-220

Ourgaud, M., N, N.P., Papillon, L., Brach-Papa, C., Galgani, F., Panagiotopoulos, C., Sempéré, R., n.d. Identification and Quantification of Microplastics in the Marine Environment Using Laser Direct Infra-red (LDIR).

Pinheiro, L.M., Agostini, V.O., Lima, A.R.A., Ward, R.D., Pinho, G.L.L., 2021. The fate of plastic litter within estuarine compartments: an overview of current knowledge for the transboundary issue to guide future assessments. Environ. Pollut. 116908. https://doi.org/10.1016/j.envpol.2021.116908

Polymer DataBase, 2022. Polymer DataBase. URL https://polymerdatabase.com/ (accessed 4.29.22).

Pratesi, C.B., Almeida, M.A.A.L.S., Paz, G.S.C., Teotonio, M.H.R., Gandolfi, L., Pratesi, R., Hecht, M., Zandonadi, R.P., 2021. Presence and quantification of microplastic in urban tap water: A pre-screening in brasilia, Brazil. Sustain. 13, 6404. https://doi.org/10.3390/su13116404

R Core Team, 2020. R: A language and environment for statistical computing. R Foundation for Statistical Computing.

Rochman, C.M., Hoh, E., Hentschel, B.T., Kaye, S., 2013. Long-term field measurement of sorption of organic contaminants to five types of plastic pellets: Implications for plastic marine debris. Environ. Sci. Technol. 47, 1646–1654. https://doi.org/10.1021/es303700s

Roper, C.F.E., Sweeney, M.J., Nauen, C.E., 1984. Cephalopods of the world-FAO species catalogue. FAO Fisheries Synopsis.

Roch, S., Friedrich, C., Brinker, A., 2020. Uptake routes of microplastics in fishes: practical and theoretical approaches to test existing theories. Sci. Rep. 10, 3896. https://doi.org/10.1038/s41598-020-60630-1

Savoca, M.S., McInturf, A.G., Hazen, E.L., 2021. Plastic ingestion by marine fish is widespread and increasing. Glob. Chang. Biol. 27, 2188–2199. https://doi.org/10.1111/gcb.15533

Schirinzi, G.F., Pedà, C., Battaglia, P., Laface, F., Galli, M., Baini, M., Consoli, P., Scotti, G., Esposito, V., Faggio, C., Farré, M., Barceló, D., Fossi, M.C., Andaloro, F., Romeo, T., 2020. A new digestion approach for the extraction of microplastics from gastrointestinal tracts (GITs) of the common dolphinfish (*Coryphaena hippurus*) from the western Mediterranean Sea. J. Hazard. Mater. 397, 122794. https://doi.org/10.1016/j.jhazmat.2020.122794

Setälä, O., Fleming-Lehtinen, V., Lehtiniemi, M., 2014. Ingestion and transfer of microplastics in the planktonic food web. Environ. Pollut. 185, 77–83. https://doi.org/10.1016/j.envpol.2013.10.013

Silva, G.B. da, Hazin, H.G., Araújo, P.V. do N., 2018. Fishing operations to catch tuna on aggregated schools at the vicinity of a data buoy in the Western Equatorial Atlantic. Brazilian J. Oceanogr. 66, 335–338. https://doi.org/10.1590/s1679-87592018018206604

Silva, G.B. da, Hazin, H.G., Mourato, B.L., Hazin, F.H.V., Fonteles-Filho, A.A., 2016. Composição das capturas na pesca de atuns e afins em cardumes associados no atlântico oeste equatorial. Bol. do Inst. Pesca 42, 866–877. https://doi.org/10.20950/1678-2305.2016v42n4p866

Silva, G.B., Hazin, H.G., Hazin, F.H.V., Vaske-Jr, T., 2019. Diet composition of bigeye tuna (*Thunnus obesus*) and yellowfin tuna (*Thunnus albacares*) caught on aggregated schools in the western equatorial Atlantic Ocean. J. Appl. Ichthyol. 35, 1111–1118. https://doi.org/10.1111/jai.13949

Steinberg, D.K., Silver, M.W., Pilskaln, C.H., Coale, S.L., Paduan, J.B., 1994. Midwater zooplankton communities on pelagic detritus (giant larvacean houses) in Monterey Bay, California. Limnol. Oceanogr. 39, 1606–1620. https://doi.org/10.4319/lo.1994.39.7.1606

Sökmen, T.Ö., Sulukan, E., Türkoğlu, M., Baran, A., Özkaraca, M., Ceyhun, S.B., 2020. Polystyrene nanoplastics (20 nm) are able to bioaccumulate and cause oxidative DNA damages in the brain tissue of zebrafish embryo (*Danio rerio*). Neurotoxicology 77, 51–59. https://doi.org/10.1016/j.neuro.2019.12.010

Tansel, B., 2018. Morphology, composition and aggregation mechanisms of soft bioflocs in marine snow and activated sludge: A comparative review. J. Environ. Manage. 205, 231–243. https://doi.org/10.1016/J.JENVMAN.2017.09.082

Teuten, E.L., Rowland, S.J., Galloway, T.S., Thompson, R.C., 2007. Potential for plastics to transport hydrophobic contaminants. Environ. Sci. Technol. 41, 7759–7764. https://doi.org/10.1021/es071737s

Teuten, E.L., Saquing, J.M., Knappe, D.R.U., Barlaz, M.A., Jonsson, S., Björn, A., Rowland, S.J., Thompson, R.C., Galloway, T.S., Yamashita, R., Ochi, D., Watanuki, Y., Moore, C., Viet, P.H., Tana, T.S., Prudente, M., Boonyatumanond, R., Zakaria, M.P., Akkhavong, K., Ogata, Y., Hirai, H., Iwasa, S., Mizukawa, K., Hagino, Y., Imamura, A., Saha, M., Takada, H., 2009. Transport and release of chemicals from plastics to the environment and to wildlife. Philos. Trans. R. Soc. B Biol. Sci. 364, 2027–2045. https://doi.org/10.1098/rstb.2008.0284

Thompson, R.C., Olsen, Y., Mitchell, R.P., Davis, A., Rowland, S.J., John, A.W.G., McGonigle, D., Russell, A.E., 2004. Supporting material: Lost at sea: where is all the plastic? Science 304, 838.

Tian, Z., Zhao, H., Peter, K.T., Gonzalez, M., Wetzel, J., Wu, C., Hu, X., Prat, J., Mudrock, E., Hettinger, R., Cortina, A.E., Biswas, R.G., Kock, F.V.C., Soong, R., Jenne, A., Du, B., Hou, F., He, H., Lundeen, R., Gilbreath, A., Sutton, R., Scholz, N.L., Davis, J.W., Dodd, M.C., Simpson, A., McIntyre, J.K., Kolodziej, E.P., 2021. A ubiquitous tire rubber–derived chemical induces acute mortality in coho salmon. Science (80-.). 371, 185–189. https://doi.org/10.1126/science.abd6951

UNEP, 2022. End plastic pollution: Towards an international legally binding instrument, United Nations Environment Assembly of the United Nations Environment Programme Fifth Session.

Vaske-Júnior, T., Lessa, R., 2004. Feeding habits of the common dolphinfish *Coryphaena hippurus*, in Northeastern Brazil's Exclusive Economic Zone. Arq. Ciências do Mar 131–138. https://doi.org/10.32360/acmar.v37i1-2

Vaske, T., Jr. (2006). Guia de identificação de cefalópodes costeiros e oceânicos do atlântico sudoeste equatorial através das mandíbulas (bicos) (p. 61). Olinda, PE: Editora Livro Rápido.

Waller, C.L., Griffiths, H.J., Waluda, C.M., Thorpe, S.E., Loaiza, I., Moreno, B., Pacherres, C.O., Hughes, K.A., 2017. Microplastics in the Antarctic marine system: An emerging area of research. Sci. Total Environ. https://doi.org/10.1016/j.scitotenv.2017.03.283

Wang, X., Li, C., Liu, K., Zhu, L., Song, Z., Li, D., 2020. Atmospheric microplastic over the South China Sea and East Indian Ocean: abundance, distribution and source. J. Hazard. Mater. 389, 121846. https://doi.org/10.1016/j.jhazmat.2019.121846

Wright, S.L., Thompson, R.C., Galloway, T.S., 2013. The physical impacts of microplastics on marine organisms: a review. Environ. Pollut. 178, 483–492. https://doi.org/10.1016/j.envpol.2013.02.031

Yang, K., Jing, S., Liu, Yang, Zhou, H., Liu, Yan, Yan, M., Yi, X., Liu, R., 2022. Acute toxicity of tire wear particles, leachates and toxicity identification evaluation of leachates to the marine copepod, *Tigriopus japonicus*. Chemosphere 297, 134099. https://doi.org/10.1016/j.chemosphere.2022.134099

GENERAL CONCLUSION

Plastic pollution has been steadily increasing, and, despite the growing number of studies on the subject around the globe, some questions remain open, especially related to the microplastic (MP) contamination in developing countries in the global south with limited research funding. For example, which regions are the most contaminated by MPs? How are these particles moved and distributed from their continental sources to distant ocean areas? What factors could be influencing the ingestion of MPs by marine organisms? Can species ecology explain this behaviour? This thesis, organised in four chapters, was proposed in order to answer some of these questions, providing a comprehensive overview of the ecological processes related to MPs contamination in fish species inhabiting the Southwestern Tropical Atlantic (SWTA) region, considering different marine ecosystems (from the estuary to the deep sea) and trophic levels and interactions.

First of all, to analyse MP contamination in marine organisms, we adapted and optimised an alkaline digestion protocol (using NaOH 1 mol) to extract MPs, which was a low-cost, efficient, reliable and replicable research method (Hermsen et al., 2018; Karami et al., 2017; Kühn et al., 2017; Markic et al., 2020). In addition to the digestion protocol, methodological measures were carefully considered, such as the choice of adequate sample size (> 10) whenever possible, particle size detection threshold, and a quality assurance/quality control (QA/QC) protocol to avoid over/underestimation bias due to cross-contamination and sample loss (Markic et al., 2020; Savoca et al., 2021). This protocol was applied in the whole research here conducted.

Borderless plastic contamination: fishes acting as transporters of microplastics across marine ecosystems

Approximately 80% of ocean plastic comes from continental sources (Andrady, 2011; Meijer et al., 2021). However, complex oceanographic and meteorological processes are involved in the distribution of plastics in the estuarine gradient, making plastic pollution a transboundary problem (Krelling and Turra, 2019).

In the first chapter, we observed a high contamination rate (number of MPs found in the digestive tract of fishes) in the estuarine ecosystem in fishes from different trophic levels. Both the predator and the two prey analysed were contaminated with MPs. The feeding strategy explained the high contamination, mainly for juveniles *Centropomus* *undecimalis* (mean 3.3 ±SD 2.9 part. ind. $^{-1}$), which is an opportunistic predator feeding on a large variety of prey with a piscivorous tendency. The high availability of plastic in estuaries is also observed by the high frequency of estuarine fish species reported as contaminated by MPs (Bessa et al., 2018; Ferreira et al., 2016; Possatto et al., 2011). We also reported that the main shape of MPs found in estuarine fishes was fibres. Fibres comprise the most common shape found in aquatic environments worldwide and could be derived from both effluents discharged and the fragmentation of fishery gears transported through the rivers to the estuaries (Lima et al., 2021, 2020). The lack of adequate sewage treatment facilitates the dispersion of these particles that can accumulate in the sediment and interact with the species. Hence, it was possible to suggest two main routes of exposure for estuarine predators, highly contaminated estuarine habitats and ingesting contaminated prey.

In the coastal region along the continental shelf, four demersal fish species were analysed to characterise the MP contamination (Chapter 2). All species were contaminated with MPs. However, contamination rates were lower (mean ~1 part. ind. $^{-1}$) than those observed for estuarine species (Chapter 1, predator), justified by the diet of the analysed coastal species, mobile invertebrate feeders, feeding mainly on benthic invertebrates. Fibres were the most frequent shape found in all the analysed fishes from the coastal region, similar to the findings for the estuarine ecosystem (Chapter 1); however, followed by films, which indicated another MP shape available in the coastal area. Through a laser direct infrared analyser, it was possible to identify a wide variety of polymers in the coastal zone, and the most abundant were polyethylene (PE), alkyd varnish and styrene-butadiene rubber (SBR). The polymeric nature of the MP corroborates the origin of MPs from the fragmentation of fishing gears, car tires, materials and paints used on boats, which might be input by the continental sources or by the maintenance of fishing gear at sea.

Towards the oceanic region, this thesis addressed organisms from the pelagic (Chapter 4) and mesopelagic (Chapter 3) regions. Physical oceanographical processes (*e.g.*, actions of winds, waves, and eddies) can accumulate MPs in oceanic gyres and trapped particles nearshore in island regions (Jiang et al., 2020; Lima et al., 2016). However, biological interactions such as biofouling, faecal pellets, marine aggregates, and plastic pumps can transport these anthropogenic particles from one pool to another (*e.g.*, through pelagic to deep-sea layers) (Van Sebille et al., 2020). The third chapter evaluated four species from the two most abundant families in the mesopelagic fish group.

Mesopelagic fishes may act as an MP transporter through the deep-sea layers as they perform vertical migrations, presenting an essential link between the epipelagic and lower mesopelagic layers (Lusher et al., 2016; Savoca et al., 2021). Here, we confirm for the first time the contamination of deep-sea fishes in the SWTA.

Although all species were contaminated, it was possible to observe that species captured in the lower mesopelagic layers (500–1000 m depth) had a lower contamination rate (0.54 ± 0.71 part. ind.$^{-1}$) than those that had been captured in the upper mesopelagic layer (200–500 m) (1.66 ± 1.23 part. ind.$^{-1}$). Fibres were the most frequent shape ingested by fishes, similar to the observations for estuarine and coastal species (Chapters 1 and 2). The most abundant identified polymers were polyamide (PA), PE and polyethylene terephthalate (PET). It was possible to observe lower density polymers at higher depths, which corroborate the vertical migration hypothesis, although these polymers eventually can sink into the water column. We also observed that the size of the ingested MPs decreased with depth. These particles available in the ocean undergo physical weathering and biological degradation through the biofouling and intestinal digestion processes, diminishing progressively. Thus, due to the small size of these particles (< 1 mm), mesopelagic fishes could ingest MP, accidentally confounding their prey (zooplankton) or when breathing.

The mesopelagic fishes play an essential role in the energy transfer through the oceanic ecosystem, transferring the energy of primary and secondary consumers to the top oceanic predators, which are valuable for the fishery stocks (*e.g.*, tuna). Moreover, using fishes as bioindicators of contamination for the SWTA area, we observed a peak concentration of MPs in the upper mesopelagic zone at 200–500 m. Thus, it seems that the biological pump can mediate MPs contamination in the deep sea.

In the SWTA, the tropical tuna and tuna-like fisheries, exploited worldwide, is very important as a source of income and subsistence in Brazil (FAO, 2020). In addition to their commercial importance, tuna are oceanic predators essential for the functioning of the ecosystem. These species are generalists-opportunists, feeding primary on fishes, cephalopods and crustaceans (Martins et al., 2021). In the fourth chapter, we combined macro- and microplastic extraction methodologies to identify contamination in pelagic oceanic predators from the SWTA and verify the evidence of trophic transfer of MPs from the ingested contaminated prey to the predator. Tuna and tuna-like species were collected by two fleets which operate along the SWTA. The industrial fleet operates outside the Brazilian Exclusive Economic Zone (EEZ) and the artisanal fleet in the

Fernando de Noronha Archipelago (FNA). Overall, a low frequency of occurrence of macroplastics (> 5 mm) was observed. The low presence of macroplastics in fish may be related to the faster evacuation rate (Olson and Boggs, 1986), avoidance of particles, and the strategy to regurgitate these items (Li et al., 2021). Conversely, a significant contamination rate of MPs was found in the stomachs of *Thunnus albacares* captured outside the EEZ (mean 10.33 ± 14.06 part. per ind.$^{-1}$). Such a high number can be explained by the tunas' opportunistic predatory tendency, which increases the MPs intake when feeding on larger quantities of contaminated prey before the egestion (Ferreira et al., 2019). We also corroborate this hypothesis by linking it with observations from the shape of found MPs, mainly foams, different from the previous observations from the estuarine, coastal and mesopelagic environments (Chapters 1, 2 and 3).

In the oceanic region (Chapter 4), we extracted MPs from the prey found inside the tunas' stomachs to observe evidence of trophic transfer. From the tuna caught outside the EEZ, the main prey found contaminated by MPs were Cephalopods and fishes from the Bramidae family. For both analysed preys, it was detected a high number of MPs (27.33 ± 30.98 part. ind.$^{-1}$ and 19.45 ± 31.15 part. ind.$^{-1}$, respectively). This fact, related to the high number of MPs retrieved inside the tuna's stomachs, suggests that trophic transfer of MPs might occur from the prey to predators (Chagnon et al., 2018; Ferreira et al., 2019). Indeed, active predators ingest plastic most frequently than any other fish guild (Savoca et al., 2021), in agreement with the findings in this thesis (Chapters 1, 2, 3 and 4).

Tunas' prey (cephalopods and Bramidae) feeding habitat is related to the deeper waters, where they can accidentally feed on marine snow that may contain aggregated MPs. Moreover, in the case of the Bramidae fishes, the opportunistic behaviour may also contribute to their high contamination. Furthermore, the main shapes found in the contaminated tunas' prey were foams and pellets, the main shapes associated with marine aggregates. Moreover, the most abundant polymers identified were SBR, PA, PET and PE. The SBR polymer is used in manufacturing car tires and as a substitute for natural rubber due to its resistance to abrasion (Knight et al., 2020) and was also identified in samples from the coastal and mesopelagic region (Chapters 2 and 3); however, at a lower frequency for mesopelagic fishes. It is not yet clear how these SBR particles reach the oceans, but some pathways could be wastewater effluent, rivers, and atmospheric precipitation (Luo et al., 2021).

The transboundary nature of MPs pollution was confirmed in this thesis, where it was possible to demonstrate the contamination of the marine ecosystems evaluated along the SWTA region (Fig. 1). In addition, we could show that, although all fish species and ecosystems analysed here were proven to be contaminated with MPs, ecological patterns of fish – being predators more vulnerable to MPs contamination – are directly associated with the shape, amount, and frequency of MPs intake.

Final remarks

From the 342 individuals analysed in this thesis, it was possible to recover about 889 MP particles (70% of the frequency of occurrence). This high contamination indicates the vulnerability of marine ecosystems in the SWTA region and emphasises the risks associated with marine organisms and the health of the people who consume these fishery resources. Ingestion of MP contaminants can lead to adverse effects on aquatic organisms, *e.g.*, blockage of the digestive tract, decreased predatory efficiency, starvation, physical injuries and developmental, locomotor and reproductive alterations (Bhagat et al., 2020; de Sá et al., 2015; Moore, 2008; Teuten et al., 2009). In addition, MPs can adsorb pollutants available in the environment, such as persistent organic pollutants (POPs) and heavy metals and can also release their additive burden (Ashton et al., 2010; Fauvelle et al., 2021; Oehlmann et al., 2009). Such impacts may be bioaccumulated and biomagnified in the food web (Batel et al., 2016; Teuten et al., 2009). Although humans do not consume fish guts, ingesting increasingly smaller MP particles by fish stocks can be hazardous to human health, as MPs can bioaccumulate.

Nevertheless, we do not know yet what effects this exposure may have on human health. Moreover, some questions remain unclear, such as how long these particles stay in the digestive tract of fishes and their associated impacts. Further research is needed to identify plastic additives in the SWTA ichthyofauna, considering not only the trophic level but also the ontogenetic stages of fish and long-term monitoring. Moreover, studies that evaluate the effects of contamination on fish and its possible association with other contaminants (*e.g.*, POPs and metals) are still incipient. Our findings highlight the critical level of contamination of marine species, captured by both artisanal and industrial fleets in the SWTA region, from the estuary to the deep sea, essential sources of income and/or food.

Scientific information presented here is crucial as increasingly global initiatives are strengthened to end plastic pollution, such as the recent landmark resolution to forge an international legally binding agreement by 2024 at the fifth session of the United Nations Environment Assembly (UNEP, 2022). Additionally, the information provided here can be used to monitor microplastic contamination in marine ecosystems and fish stocks to help decision-makers establish future mitigation strategies. Moreover, it provides essential information for the UN Decade of Ocean Science for Sustainable Development (2021-2030) in the context of the UN 2030 Agenda on the Sustainable Development Goals (SDGs) addressed by Goal 14—life underwater. Finally, the information presented here can also be used to monitor Marine Protected Areas (MPAs) and indicate other priority areas for conservation and resource management, based on the data available on the MPs pollution.

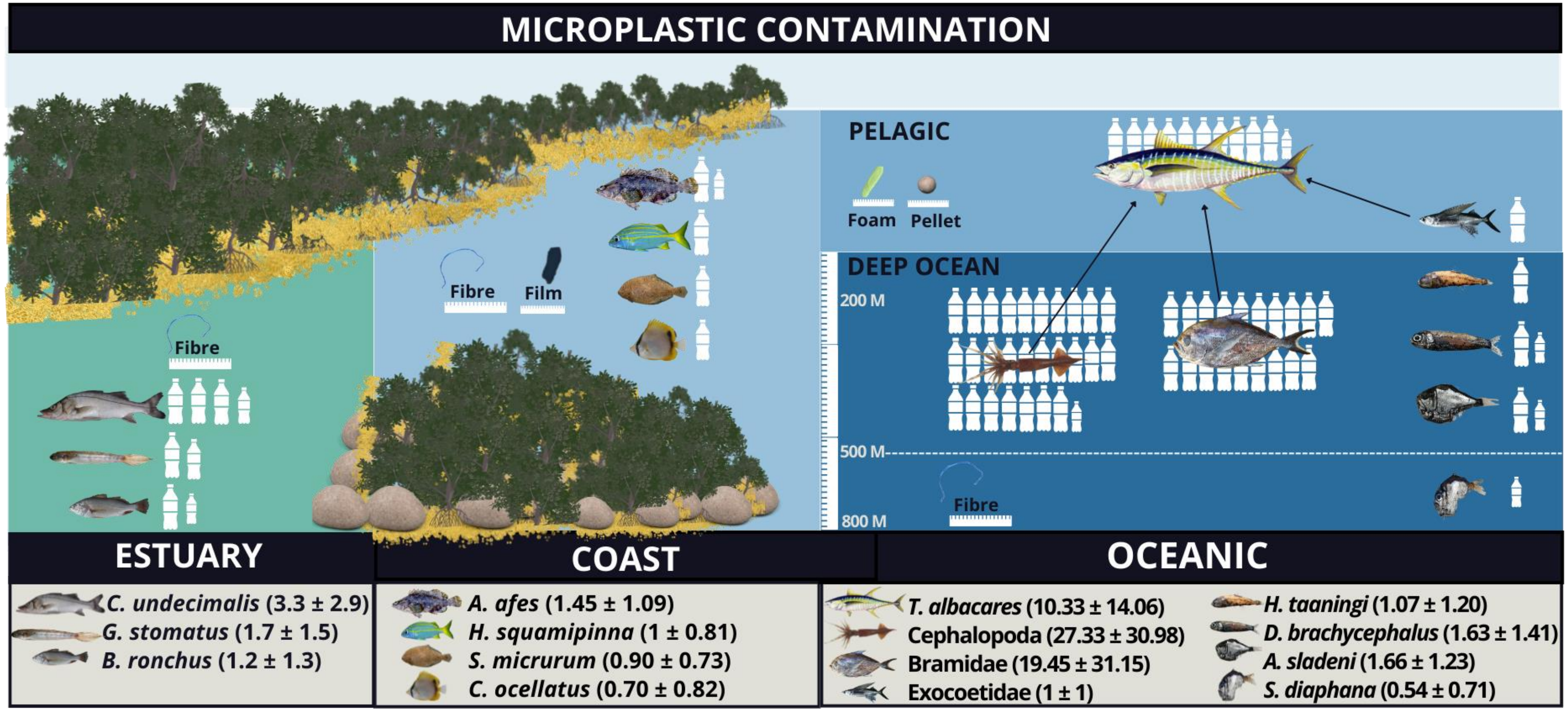


Figure 1. Schematic illustration with the synthesis of the thesis results on the microplastic contamination in fishes from different ecosystems, from the estuary to the deep sea along the Southwestern Tropical Atlantic.

REFERENCES

Andrady, A.L., 2011. Microplastics in the marine environment. Mar. Pollut. Bull. 62, 1596–1605. https://doi.org/10.1016/j.marpolbul.2011.05.030

Ashton, K., Holmes, L., Turner, A., 2010. Association of metals with plastic production pellets in the marine environment. Mar. Pollut. Bull. 60, 2050–2055. https://doi.org/10.1016/j.marpolbul.2010.07.014

Batel, A., Linti, F., Scherer, M., Erdinger, L., Braunbeck, T., 2016. Transfer of benzo[*a*]pyrene from microplastics to *Artemia* nauplii and further to zebrafish via a trophic food web experiment: CYP1A induction and visual tracking of persistent organic pollutants. Environ. Toxicol. Chem. 35, 1656–1666. https://doi.org/10.1002/etc.3361

Bessa, F., Barría, P., Neto, J.M., Frias, J.P.G.L., Otero, V., Sobral, P., Marques, J.C., 2018. Occurrence of microplastics in commercial fish from a natural estuarine environment. Mar. Pollut. Bull. 128, 575–584. https://doi.org/10.1016/j.marpolbul.2018.01.044

Bhagat, J., Zang, L., Nishimura, N., Shimada, Y., 2020. Zebrafish: An emerging model to study microplastic and nanoplastic toxicity. Sci. Total Environ. https://doi.org/10.1016/j.scitotenv.2020.138707

Chagnon, C., Thiel, M., Antunes, J., Ferreira, J.L., Sobral, P., Ory, N.C., 2018. Plastic ingestion and trophic transfer between Easter Island flying fish (Cheilopogon rapanouiensis) and yellowfin tuna (Thunnus albacares) from Rapa Nui (Easter Island) *. Environ. Pollut. 243, 127–133. https://doi.org/10.1016/j.envpol.2018.08.042

de Sá, L.C., Luís, L.G., Guilhermino, L., 2015. Effects of microplastics on juveniles of the common goby (Pomatoschistus microps): Confusion with prey, reduction of the predatory performance and efficiency, and possible influence of developmental conditions. Environ. Pollut. 196, 359–362. https://doi.org/10.1016/j.envpol.2014.10.026

FAO, 2020. The State of World Fisheries and Aquaculture 2020, INFORM. FAO, Rome. https://doi.org/10.4060/ca9229en

Fauvelle, V., Garel, M., Tamburini, C., Nerini, D., Castro-Jiménez, J., Schmidt, N., Paluselli, A., Fahs, A., Papillon, L., Booth, A.M., Sempéré, R., 2021. Organic additive release from plastic to seawater is lower under deep-sea conditions. Nat. Commun. 12. https://doi.org/10.1038/s41467-021-24738-w

Ferreira, G.V.B., Barletta, M., Lima, A.R.A., Dantas, D. V., Justino, A.K.S., Costa, M.F., 2016. Plastic debris contamination in the life cycle of Acoupa weakfish (Cynoscion acoupa) in a tropical estuary . ICES J. Mar. Sci. J. du Cons. 73, 2695–2707. https://doi.org/10.1093/icesjms/fsw108

Ferreira, G.V.B., Barletta, M., Lima, A.R.A., Morley, S.A., Costa, M.F., 2019. Dynamics of Marine Debris Ingestion by Profitable Fishes Along The Estuarine Ecocline. Sci. Rep. 9, 13514. https://doi.org/10.1038/s41598-019-49992-3

Hermsen, E., Mintenig, S.M., Besseling, E., Koelmans, A.A., 2018. Quality Criteria for the Analysis of Microplastic in Biota Samples: A Critical Review. Environ. Sci. Technol. 52, 10230–10240. https://doi.org/10.1021/acs.est.8b01611

Jiang, Y., Yang, F., Zhao, Y., Wang, J., 2020. Greenland Sea Gyre increases microplastic pollution in the surface waters of the Nordic Seas. Sci. Total Environ. 712, 136484. https://doi.org/10.1016/j.scitotenv.2019.136484

Karami, A., Golieskardi, A., Choo, C.K., Romano, N., Ho, Y. Bin, Salamatinia, B., 2017.

A high-performance protocol for extraction of microplastics in fish. Sci. Total Environ. 578, 485–494. https://doi.org/10.1016/j.scitotenv.2016.10.213

Knight, L.J., Parker-Jurd, F.N.F., Al-Sid-Cheikh, M., Thompson, R.C., 2020. Tyre wear particles: an abundant yet widely unreported microplastic? Environ. Sci. Pollut. Res. 27, 18345–18354. https://doi.org/10.1007/s11356-020-08187-4

Krelling, A.P., Turra, A., 2019. Influence of oceanographic and meteorological events on the quantity and quality of marine debris along an estuarine gradient. Mar. Pollut. Bull. 139, 282–298. https://doi.org/10.1016/j.marpolbul.2018.12.049

Kühn, S., van Werven, B., van Oyen, A., Meijboom, A., Bravo Rebolledo, E.L., van Franeker, J.A., 2017. The use of potassium hydroxide (KOH) solution as a suitable approach to isolate plastics ingested by marine organisms. Mar. Pollut. Bull. 115, 86–90. https://doi.org/10.1016/j.marpolbul.2016.11.034

Li, B., Liang, W., Liu, Q.-X., Fu, S., Ma, C., Chen, Q., Su, L., Craig, N.J., Shi, H., 2021. Fish Ingest Microplastics Unintentionally. Environ. Sci. Technol. 55, 10471–10479. https://doi.org/10.1021/acs.est.1c01753

Lima, A.R.A., Barletta, M., Costa, M.F., 2016. Seasonal-dial shifts of ichthyoplankton assemblages and plastic debris around an Equatorial Atlantic archipelago. Front. Environ. Sci. 4, 56. https://doi.org/10.3389/fenvs.2016.00056

Lima, A.R.A., Ferreira, G.V.B., Barrows, A.P.W., Christiansen, K.S., Treinish, G., Toshack, M.C., 2021. Global patterns for the spatial distribution of floating microfibers: Arctic Ocean as a potential accumulation zone. J. Hazard. Mater. 403. https://doi.org/10.1016/j.jhazmat.2020.123796

Lima, A.R.A., Silva, M.D., Possatto, F.E., Ferreira, G.V.B., Krelling, A.P., 2020. Plastic Contamination in Brazilian Freshwater and Coastal Environments: A Source-to-Sea Transboundary Approach, in: Handbook of Environmental Chemistry. pp. 427–460. https://doi.org/10.1007/698_2020_514

Luo, Z., Zhou, X., Su, Y., Wang, H., Yu, R., Zhou, S., Xu, E.G., Xing, B., 2021. Environmental occurrence, fate, impact, and potential solution of tire microplastics: Similarities and differences with tire wear particles. Sci. Total Environ. https://doi.org/10.1016/j.scitotenv.2021.148902

Lusher, A.L., O'Donnell, C., Officer, R., O'Connor, I., 2016. Microplastic interactions with North Atlantic mesopelagic fish. ICES J. Mar. Sci. 73, 1214–1225. https://doi.org/10.1093/icesjms/fsv241

Markic, A., Gaertner, J.-C., Gaertner-Mazouni, N., Koelmans, A.A., 2020. Plastic ingestion by marine fish in the wild. Crit. Rev. Environ. Sci. Technol. 50, 657–697. https://doi.org/10.1080/10643389.2019.1631990

Martins, K., Pelage, L., Justino, A.K.S., Frédou, F.L., Júnior, T.V., Le Loc'h, F., Travassos, P., 2021. Assessing trophic interactions between pelagic predatory fish by gut content and stable isotopes analysis around Fernando de Noronha Archipelago (Brazil), Equatorial West Atlantic. J. Fish Biol. 99, 1576–1590. https://doi.org/10.1111/jfb.14863

Meijer, L.J.J., van Emmerik, T., van der Ent, R., Schmidt, C., Lebreton, L., 2021. More than 1000 rivers account for 80% of global riverine plastic emissions into the ocean. Sci. Adv. 7, eaaz5803. https://doi.org/10.1126/sciadv.aaz5803

Moore, C.J., 2008. Synthetic polymers in the marine environment: A rapidly increasing, long-term threat. Environ. Res. 108, 131–139. https://doi.org/10.1016/j.envres.2008.07.025

Oehlmann, J., Schulte-Oehlmann, U., Kloas, W., Jagnytsch, O., Lutz, I., Kusk, K.O., Wollenberger, L., Santos, E.M., Paull, G.C., VanLook, K.J.W., Tyler, C.R., 2009. A critical analysis of the biological impacts of plasticisers on wildlife. Philos. Trans.

R. Soc. B Biol. Sci. 364, 2047–2062. https://doi.org/10.1098/rstb.2008.0242

Olson, R.J., Boggs, C.H., 1986. Apex Predation by Yellowfin Tuna (Thunnus albacares): Independent Estimates from Gastric Evacuation and Stomach Contents, Bioenergetics, and Cesium Concentrations. Can. J. Fish. Aquat. Sci. 43, 1760–1775. https://doi.org/10.1139/f86-220

Possatto, F.E., Barletta, M., Costa, M.F., Ivar do Sul, J.A., Dantas, D. V., 2011. Plastic debris ingestion by marine catfish: An unexpected fisheries impact. Mar. Pollut. Bull. 62, 1098–1102. https://doi.org/10.1016/j.marpolbul.2011.01.036

Savoca, M.S., McInturf, A.G., Hazen, E.L., 2021. Plastic ingestion by marine fish is widespread and increasing. Glob. Chang. Biol. 27, 2188–2199. https://doi.org/10.1111/gcb.15533

Teuten, E.L., Saquing, J.M., Knappe, D.R.U., Barlaz, M.A., Jonsson, S., Björn, A., Rowland, S.J., Thompson, R.C., Galloway, T.S., Yamashita, R., Ochi, D., Watanuki, Y., Moore, C., Viet, P.H., Tana, T.S., Prudente, M., Boonyatumanond, R., Zakaria, M.P., Akkhavong, K., Ogata, Y., Hirai, H., Iwasa, S., Mizukawa, K., Hagino, Y., Imamura, A., Saha, M., Takada, H., 2009. Transport and release of chemicals from plastics to the environment and to wildlife. Philos. Trans. R. Soc. B Biol. Sci. 364, 2027–2045. https://doi.org/10.1098/rstb.2008.0284

UNEP, 2022. End plastic pollution: Towards an international legally binding instrument, United Nations Environment Assembly of the United Nations Environment Programme Fifth Session.

Van Sebille, E., Aliani, S., Law, K.L., Maximenko, N., Alsina, J.M., Bagaev, A., Bergmann, M., Chapron, B., Chubarenko, I., Cózar, A., Delandmeter, P., Egger, M., Fox-Kemper, B., Garaba, S.P., Goddijn-Murphy, L., Hardesty, B.D., Hoffman, M.J., Isobe, A., Jongedijk, C.E., Kaandorp, M.L.A., Khatmullina, L., Koelmans, A.A., Kukulka, T., Laufkötter, C., Lebreton, L., Lobelle, D., Maes, C., Martinez-Vicente, V., Morales Maqueda, M.A., Poulain-Zarcos, M., Rodríguez, E., Ryan, P.G., Shanks, A.L., Shim, W.J., Suaria, G., Thiel, M., Van Den Bremer, T.S., Wichmann, D., 2020. The physical oceanography of the transport of floating marine debris. Environ. Res. Lett. https://doi.org/10.1088/1748-9326/ab6d7d

SCIENTIFIC PRODUCTION

Scientific Publications

Bruzaca, D.N.A., **Justino, A.K.S**., Mota, G.C.P., Costa, G.A., Lucena-Frédou, F., Gálvez, A.O., 2022. Occurrence of microplastics in bivalve molluscs *Anomalocardia flexuosa* captured in Pernambuco, Northeast Brazil. Mar. Pollut. Bull. 179, 113659. https://doi.org/10.1016/j.marpolbul.2022.113659

Ferreira, G.V.B., **Justino, A.K.S**., Eduardo, L.N., Lenoble, V., Fauvelle, V., Schmidt, N., Junior, T.V., Frédou, T., Lucena-Frédou, F., 2022. Plastic in the inferno: Microplastic contamination in deep-sea cephalopods (*Vampyroteuthis infernalis* and *Abralia veranyi*) from the southwestern Atlantic. Mar. Pollut. Bull. 174, 113309. https://doi.org/10.1016/j.marpolbul.2021.113309

Justino, A.K.S., Ferreira, G.V.B., Schmidt, N., Eduardo, L.N., Fauvelle, V., Lenoble, V., Sempéré, R., Panagiotopoulos, C., Mincarone, M.M., Frédou, T., Lucena-Frédou, F., 2022. The role of mesopelagic fishes as microplastics vectors across the deep-sea layers from the Southwestern Tropical Atlantic. Environ. Pollut. 300, 118988. https://doi.org/10.1016/j.envpol.2022.118988

Justino, A.K.S., Lenoble, V., Pelage, L., Ferreira, G.V.B., Passarone, R., Frédou, T., Lucena Frédou, F., 2021. Microplastic contamination in tropical fishes: An assessment of different feeding habits. Reg. Stud. Mar. Sci. 45, 101857. https://doi.org/10.1016/j.rsma.2021.101857

Martins, K., Pelage, L., **Justino, A.K.S**., Frédou, F.L., Júnior, T.V., Le Loc'h, F., Travassos, P., 2021. Assessing trophic interactions between pelagic predatory fish by gut content and stable isotopes analysis around Fernando de Noronha Archipelago (Brazil), Equatorial West Atlantic. J. Fish Biol. 99, 1576–1590. https://doi.org/10.1111/jfb.14863

Pelage, L., Bertrand, A., Ferreira, B.P., Lucena-Frédou, F., **Justino, A.K.S**., Frédou, T., 2021. Balanced harvest as a potential management strategy for tropical small-scale fisheries. ICES J. Mar. Sci. https://doi.org/10.1093/icesjms/fsab136

Passarone, R., Aparecido, K.C., Eduardo, L.N., Lira, A.S., Silva, L.V.S., **Justino, A.K.S**., Craveiro, C., Silva, E.F., Lucena-Frédou, F., 2019. Ecological and conservation aspects of bycatch fishes: An evaluation of shrimp fisheries impacts in northeastern brazil. Brazilian J. Oceanogr. 67, 1–10. https://doi.org/10.1590/s1679-87592019029106713

Manuscript Submitted for Publication

Ferreira, G.V.B., **Justino, A.K.S**., Eduardo, L.N., Martins, J. R., Ménard, F., Fauvelle, V., Schmidt, N., Lucena-Frédou, F. Influencing factors for microplastic intake in abundant deep-sea lanternfishes (Myctophidae). Journal of Hazardous Materials (July 2022).

Scientific outreach

Justino, A.K.S., 2022. Biodiversidade: Conservar para quê? Webinar FrancEcoLab, Ambassade de France au Brésil, Invited Talk to Webinar, Recife, BR (18 – 20 May 2022).

Justino, A.K.S., Frias, J., Müller, C., 2022. Webinar: Metodologias para análise de microplásticos. JPI Oceans, Invited Talk to Webinar, Brasil/Portugal (4 – 5 May 2022).

Justino, A.K.S., Ferreira, G.V.B., 2022. Matérias Plásticas: vidas selvagens. Institut de Recherche pour le Développement - IRD, Invited Talk, Recife, BR (18 – 19 April 2022).

Justino, A.K.S., Ferreira, G.V.B., 2022. In the Ocean, It’s Snowing Microplastics. The New York Times, Interview/Newspaper, New York, EUA (03 April 2022). https://www.nytimes.com/2022/04/03/science/ocean-plastic-animals.html

Justino, A.K.S., Ferreira, G.V.B., 2022. Como uma neve de microplásticos está cobrindo as profundezas do oceano. Um só Planeta, Época Negócios, Interview/Newspaper, São Paulo, BR (04 April 2022). https://epocanegocios.globo.com/Um-So-Planeta/noticia/2022/04/como-uma-neve-de-microplasticos-esta-cobrindo-profundezas-do-oceano.html

Justino, A.K.S., Ferreira, G.V.B., 2022. Oceanos estão ameaçados por ‘neve’ de microplásticos; entenda. Folha de São Paulo, Interview/Newspaper, São Paulo, BR (06 April 2022). https://www1.folha.uol.com.br/ambiente/2022/04/oceanos-estao-ameacados-por-neve-de-microplasticos-entenda.shtml

Justino, A.K.S., 2022. FrancEcoLab: um projeto de educação ambiental em língua francesa. Ambassade de France au Brésil, Radio or TV programme/Interview Brasilia, BR (22 March 2022). https://br.ambafrance.org/FrancEcolab-Brasil-um-projeto-de-educacao-ambiental-em-lingua-francesa

Justino, A.K.S., 2021. Pesquisadores encontram microplásticos em peixes no Litoral Norte de Pernambuco. Diário de Pernambuco, Interview/Newspaper, Recife, BR (14 July 2021). https://www.diariodepernambuco.com.br/noticia/cienciaesaude/2021/07/pesquisadores-encontram-microplasticos-em-peixes-no-litoral-norte-de-p.html

Justino, A.K.S., Ferreira, G.V.B., 2021. Poluição dos Oceanos: Microplásticos. Webinar FrancEcoLab, Ambassade de France au Brésil, Invited Talk to Webinar, Recife, BR (21 – 30 June 2021).

Justino, A.K.S., Lenoble, V., Pelage, L., Ferreira, G.V.B., Passarone, R., Frédou, T., Lucena-Frédou, F., 2020. Ingestion of microplastics by fishes of an estuarine trophic chain in the Western Atlantic. MICRO2020, Poster, Lanzarote, ES (23 – 27 November 2020). https://www.micro.infini.fr/publication.html?id=9YMPRVBJ

Justino, A.K.S., 2020. Microplástico, uma ameaça invisível. Mês da Educação & Meio Ambiente, UNIFESP, Invited Talk, São Paulo, BR (24 October 2020).

Justino, A.K.S., 2020. Microplásticos: qual o real tamanho desse problema? Pescando Ciência, Radio or TV programme/Interview Recife, BR (05 August 2020). https://www.youtube.com/watch?v=qkm_nun_eiI

Justino, A.K.S., 2019. Consumismo e sua relação com a poluição plástica. Dia Mundial da Limpeza e Campanha Mares Limpos, LMI-TAPIOCA, Invited Talk, Recife, BR (21 September 2019).

Justino, A.K.S., 2019. Microplásticos: até onde vão os impactos antrópicos? Ciclo de Palestras: Dia da Biodiversidade, PET-UFRPE, Invited Talk, Recife, BR (21 May 2019).

Anne JUSTINO

Laboratoire M.I.O, Université de Toulon - UTLN

Laboratório Bioimpact, Universidade Federal Rural de Pernambuco - UFRPE

De l'estuaire à la mer profonde : contamination microplastique des poissons de l'Atlantique tropical sud-ouest

Résumé en français

Les microplastiques (MPs ; 5 < mm) ont été identifiés dans la plupart des écosystèmes et organismes marins, ce qui suscite la préoccupation des scientifiques quant à leur impact sur la biodiversité et les écosystèmes. Cependant, certaines régions ne sont pas encore suffisamment documentées en ce qui concerne la caractérisation de la contamination par les MPs, comme la région de l'Atlantique tropical sud-ouest (ATSO). Afin de remplir ces lacunes, l'objectif général de cette thèse est de fournir une compréhension générale de la distribution et des modèles de contamination par les MP dans l'ichtyofaune de la région ATSO le long du gradient estuarien-océanique. Dans ce contexte, un protocole d'extraction rapide, efficace et d'un faible coût opérationnel, des MPs a été adapté aux organismes marins, tout en garantissant une probabilité minime de la contamination aéroportée dans les échantillons. La thèse est structurée en quatre chapitres sous forme d'articles, et une conclusion générale. Dans le premier chapitre, nous avons exploré une chaîne trophique estuarienne comprenant un poisson prédateur commercialement important et deux de ses principales proies. Ces espèces ont des stratégies alimentaires différentes, ce qui se retrouve dans la fréquence d'ingestion des MPs. Dans le deuxième chapitre, quatre espèces de poissons démersaux côtiers ont été étudiées pour caractériser l'ingestion de MPs chez les poissons de récif de la région ATSO. Aucune différence n'a été observée entre le taux d'ingestion de MPs par les espèces côtières, mais un large éventail de polymères a été identifié. L'écosystème océanique est abordé dans les deux derniers chapitres de la thèse, en se concentrant sur les espèces océaniques pélagiques et mésopélagiques. Le troisième chapitre caractérise la contamination de deux familles de poissons mésopélagiques abondantes dans l'ATSO, et aborde l'influence de ces espèces sur le transport des MPs dans l'océan profond. Ainsi, la contamination par les MPs des poissons d'eau profonde de la région a été identifiée pour la première fois et les zones d'accumulation probable de ces particules ont été discutées. Enfin, dans le quatrième chapitre, des prédateurs pélagiques importants pour la pêche industrielle et artisanale ont été étudiés. Outre la présence de MPs, le transfert trophique des MPs des proies aux prédateurs a été mis en exergue. Ce chapitre traite également de l'ingestion de macroplastiques (> 5 mm) provenant de l'intense activité de pêche et de tourisme dans l'Archipel de Fernando de Noronha. De façon générale, il a été possible, au travers de ce travail de thèse, de confirmer la contamination par les MPs des poissons de la région ATSO. Les comportements écologiques, tels que les stratégies d'alimentation et le niveau trophique, ont un impact sur la contamination. De plus, l'influence du comportement des espèces dans la colonne d'eau sur les zones d'accumulation des MPs a été mise en évidence. Il a également été possible d'observer qu'en général, la forme la plus fréquente de MP ingérés par les poissons sont les fibres. Cependant, les grands prédateurs pélagiques océaniques (thons) ont tendance à ingérer des pellets et des mousses, ce qui a également été observé chez leurs proies principales, indiquant un transfert trophique. En outre, les polymères les plus fréquemment retrouvés dépendent également des comportements écologiques des espèces ainsi que, dans les cas des thons, de la pression intense de la part des pêcheries (par exemple, filets de pêche et peinture des bateaux) et la forte activité touristique. Les informations disponibles ici peuvent servir de support pour discuter et développer des mesures visant à atténuer les impacts causés par la contamination par les MPs et la pollution plastique dans la région ATSO, contribuant ainsi à la conservation de la biodiversité et des écosystèmes marins.

Mot clés : Pollution plastique, Transfert trophique, Océan Atlantique, Ressources halieutiques, Écologie des poissons, Estuaires, Ecosystème mésopélagique, Thon.

From the estuary to the deep sea: microplastic contamination of fishes from Southwestern Tropical Atlantic

Résumé en anglais

Worldwide, microplastics (MPs; 5 < mm) have been identified in most marine ecosystems and organisms. The large availability of this contaminant has raised the concern of scientists about the impacts on biodiversity and ecosystems. However, despite being frequently reported, some regions still lack studies on the characterisation of MPs contamination. This is the case in the Southwestern Tropical Atlantic (SWTA) region. To fill this gap, this thesis aims to provide a general understanding of the distribution and patterns of MP contamination in the ichthyofauna inhabiting the SWTA region along the estuarine-oceanic gradient. Thus, a protocol for extraction of MPs for marine organisms was adapted to diminish the probability of airborne contamination in the samples, which would be practical and of low operational cost. The thesis is structured in four chapters presented as articles and a general conclusion. The first chapter explored an estuarine trophic chain, comprising a commercially important top predatory fish and two of its main prey. Both species have different feeding habits, which was also observed in the frequency of MP ingestion, which varied with feeding strategies. In the second chapter, four coastal demersal fish species were used to characterise the ingestion of MPs in reef fishes from the SWTA region. No differences were observed between the ingestion rate of MPs by coastal species; however, a wide range of polymers was identified. The ocean ecosystem is discussed in the last two chapters of the thesis, focusing on pelagic and mesopelagic oceanic species. The third chapter characterises the contamination of two abundant mesopelagic fish families in the SWTA and addresses the influence of these species on the transport of MPs in the deep ocean. In this chapter, MP contamination in deep-sea fishes in the region was identified for the first time and areas of possible accumulation of particles were discussed. Finally, in the fourth chapter, pelagic predators important for industrial and artisanal fisheries were evaluated for the presence of MPs. In addition to the characterisation of contamination, evidence of trophic transfer of MPs from prey to predators was observed. Furthermore, this chapter discusses the ingestion of larger particles (macroplastics > 5 mm) derived from the intense ocean fishing and tourism activity in the Fernando de Noronha Archipelago (FNA). Overall, it was possible through this thesis to confirm the contamination by MPs in fish from the SWTA region.

The ecological patterns, such as feeding strategies and trophic level, increased MP contamination probability. Furthermore, the influence of the species' behaviour in the water column in areas with an accumulation of MPs. In addition, it was also possible to observe that, in general, the most frequent shape of MPs ingested by fishes are fibres. However, large oceanic pelagic predators (tunas) tended to ingest pellets and foams, which was also observed in their primary prey, indicating trophic transfer. Moreover, a variety of polymers were identified, but the most frequent polymers in the coastal region were PE, Alkyd Varnish and SBR; for the mesopelagic fishes in the oceanic region were PA and PE. However, the most frequent polymers for tuna and prey were SBR, PA, and PET. The availability of these polymers in the SWTA region indicates intense pressure from fisheries (e.g., fishing nets and ship paint) and high tourist activity. The information available here can serve as a baseline to discuss and develop measures to mitigate the impacts caused by MPs contamination and plastic pollution in the SWTA region, thus contributing to the conservation of biodiversity and marine ecosystems.

Keywords: Plastic pollution, Trophic transfer, Atlantic Ocean, Fisheries resources, Fish ecology, Estuaries, Mesopelagic ecosystem, Tuna.