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NAÍSA CRISTINE PASSOS

Padrões de contaminação por microplásticos em peixes recifais na RESEX-Mar de Arraial do Cabo

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Dissertação de mestrado apresentada ao Instituto de Estudos do Mar Almirante Paulo Moreira e à Universidade Federal Fluminense, como requisito parcial para a obtenção do grau de Mestre em Biotecnologia Marinha.

Orientador: Prof. Dr. Carlos Eduardo Leite Ferreira

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RESUMO

Apesar de fornecerem serviços ecossistêmicos críticos ao planeta e às populações costeiras, os ecossistemas marinhos têm sido continuamente impactados por diversas atividades antropogênicas. Neste cenário, a onipresença e o pequeno tamanho dos microplásticos (MP's) em diferentes escalas geográficas nos sistemas marinhos, tornam-os um dos poluentes mais críticos nas cadeias alimentares. As características dos detritos plásticos, associadas aos atributos ecológicos da espécie, são fatores importantes que influenciam as taxas de ingestão, ainda pouco estudadas para recifes rochosos. Este trabalho teve como objetivo estudar comparativamente a contaminação ambiental e a ingestão de MP's por diferentes espécies de peixes recifais, analisando diferentes atributos ecológicos das espécies de peixes em um costão rochoso em Arraial do Cabo, RJ. Da mesma forma, os MP's foram quantificados em matrizes ambientais (água, matriz de algas epilíticas - MAE e sedimentos) para compreender as principais fontes de contaminação na cadeia alimentar. Os peixes foram coletados com arbalete e imediatamente levados ao laboratório para retirada do trato gastrointestinal para quantificação da presença de microplásticos. Os microplásticos encontrados foram quantificados, medidos e classificados quanto à forma e cor. O tipo e a quantidade de polímeros foram comparados entre os compartimentos pelágico e bentônico, e entre espécies de peixes recifais de diferentes grupos tróficos. MP's foram encontrados em todas as matrizes ambientais e espécies analisadas. O maior número de detritos plásticos foi encontrado no MAE. *Stegastes fuscus* e *Abudefduf saxatilis* foram as espécies com maior número médio de MP's/ind. O tamanho do corpo, da boca e do intestino influenciam a ingestão de MP's. “Grubbers-excavating” e “nibblers” tendem a ingerir menos MP's. Não encontramos relação significativa entre o nível trófico e a ingestão de MP's. Essas descobertas podem ser aplicadas em estratégias de manejo que contribuam para a conservação dos ambientes recifais.

Palavras-chave: ingestão de detritos, peixes recifais, microplásticos, tamanho corporal, poluição marinha.

ABSTRACT

Despite providing critical ecosystem services to the planet and coastal populations, marine ecosystems have been continually impacted by diverse anthropogenic activities. In this scenario, the ubiquity and small size of microplastics at different geographic scales on marine systems make them one of the most critical pollutants in food webs. The characteristics of plastic debris, associated with the ecological attributes of the species, are important factors influencing ingestion rates, which are still understudied for rocky reefs. This work aimed to comparatively study the environmental contamination and the ingestion of microplastics by different species of reef fishes, analyzing different ecological attributes of fish species on a rocky shore in Arraial do Cabo, RJ. Likewise, microplastics were quantified in environmental matrices (water, epilithic algal matrix EAM- and sediment) to understand the main sources of contamination in the food web. The fish spearfished and immediately taken to the laboratory to remove the gastrointestinal tract to quantify the presence of microplastics. The microplastics found were quantified, measured and classified according to shape and color. The type and quantity of polymers were compared between the pelagic and benthic compartments, and among reef fish species from different trophic groups. MPs were found in all environmental matrices and analyzed species. The largest number of MPs was found in the EAM. *Stegastes fuscus* and *Abudefduf saxatilis* were the species with the highest average number of MPs/ind. Body, mouth and intestine size influence the intake of MPs. “Grubbers-excavating” and “nibblers” tend to ingest fewer MPs. We found no significant relationship between trophic level and MP intake. These findings can be applied in management strategies that contribute to the conservation of reef environments.

Keywords: debris ingestion, reef fish, microplastics, body size, marine pollution.

SUMÁRIO

1. INTRODUÇÃO GERAL

Todos os anos, aproximadamente 400,3 milhões de toneladas de plástico são produzidas no mundo (PlasticEurope, 2023; UNEP, 2023). Amplamente utilizado devido a combinação de características únicas como baixo custo, versatilidade e durabilidade, detritos plásticos são encontrados em todos os lugares do globo terrestre (Worm et al., 2017). Sua durabilidade é uma das razões pelas quais os mesmos são considerados importantes marcadores do Antropoceno (Brandon et al., 2019; Bourzac, 2023; Lewis e Maslin, 2015; Webb et al., 2012).

A maioria dos plásticos é composta por polímeros de hidrocarbonetos não renováveis, como petróleo e gás natural. O polipropileno (PP), polietileno (PE), cloreto de polivinila (PVC), poliuretano (PUR), polietileno tereftalato (PET) e poliestireno (PS), são os polímeros mais utilizados no mundo (Worm et al. 2017). Em menor escala, o plástico também é produzido a partir de outros polímeros como celulose, amido, óleo vegetal e açúcares (British Plastics Federação, 2019).

Desde a década de 50, aproximadamente 85% de todo plástico produzido no mundo teve como destino final aterros sanitários ou o ambiente natural e apenas 10% desse material tem sido reciclado (Geyer et al., 2020; UNEP, 2023). Dos plásticos que são descartados de forma incorreta, anualmente, um volume entre 4,8 a 12,7 milhões de toneladas chegam nos oceanos do mundo todo, contaminando ecossistemas marinhos costeiros e oceânicos, rasos e profundos, e, consequentemente, as cadeias tróficas marinhas (Geyer et al., 2017; Sarkar et al., 2022; Savoca et al., 2021; Wootton et al., 2021). A maior parte destes resíduos é de origem terrestre que, após serem descartados incorretamente, são transportados através de sistemas fluviais até o mar (Jambeck et al., 2015; Rech et al., 2014). Outra parcela menor é de origem marinha, como por exemplo da indústria da pesca, navegação e aquicultura (Andrady, 2011; GESAMP, 2015).

Dos polímeros descartados e encontrados no ambiente marinho, parte são de maior tamanho, sendo classificados como macroplásticos (Eriksen et al., 2014). Por exemplo, linhas e redes de pesca, quando descartadas incorretamente no ambiente, continuam ‘capturando’ peixes e invertebrados (Carvalho-Souza et al., 2018; Gregory, 2009), fenômeno conhecido como pesca fantasma (*ghostfishing*). Partículas de menor tamanho (< 5mm), classificadas como microplásticos (MP’s), passaram a ser o foco de diversas pesquisas devido o seu potencial impacto negativo na vida marinha e até mesmo na saúde humana (Cox et al., 2019; GESAMP, 2015; Rochman et al., 2015). Os microplásticos podem se originar de duas fontes distintas: primária ou secundária. Os de origem primária se referem ao material produzido de forma intencional para uso direto ou como matéria-prima para outros produtos, como abrasivos e esfoliantes; enquanto os de origem secundária são originados após a fragmentação e degradação de macroplásticos, por meio de processos naturais como o intemperismo e a radiação UV (Arthur et al., 2009; Napper e Thompson, 2016; Thompson et al. 2004). Esta fragmentação gera diferentes formas nas quais os microplásticos são encontrados, como fragmentos, pelotas, filmes e fibras, sendo esta última a mais comum (Markic et al., 2019; Remy et al., 2015).

Os impactos causados por plásticos nos ecossistemas marinhos e biodiversidade associada podem variar de acordo com a abundância, forma, tamanho, cor e composição destes polímeros (Li et al., 2016; Santos et al., 2016). Os microplásticos, devido ao seu pequeno tamanho, são facilmente ingeridos por diferentes animais marinhos. Sua ingestão é relatada globalmente em peixes habitando sistemas aquáticos diversos (Wootton et al., 2021), incluindo espécies com diferentes estratégias de alimentação (Cardozo-Ferreira et al., 2021; Macieira et al., 2021). A ingestão de MP’s é também reportada para diversos níveis tróficos ao longo da cadeia trófica marinha, desde zooplâncton (Cole et al., 2013; Setälä, Fleming-Lehtinen and Lehtiniemi, 2014) até invertebrados (Hall et al., 2015; Rotjan et al., 2015), peixes de interesse comercial (Savoca et al., 2021), até grandes mamíferos marinhos ameaçados de extinção (Lusher et al., 2015; Remy et al., 2015). Os MP’s possuem ainda potencial capacidade de transferência entre os diferentes níveis tróficos por meio da predação de espécies contaminadas. Por exemplo, espécies filtradoras ingerindo microplásticos dispersos na coluna d’água, espécies herbívoras ao ingerir algas com microplásticos associados e através da incorporação de sedimento por espécies bentônicas (Farrell e Nelson, 2013; Germanov et al., 2019; Gutow et al., 2016; Miranda et al., 2016; Nelms et al., 2018; Saikumar et al., 2023; Tahir et al., 2019; 2020).

A ingestão de MP’s pode resultar em alterações fisiológicas, como a diminuição da capacidade de alimentação, devido a obstrução do sistema digestivo, ou serem translocados para o sistema circulatório (Browne et al., 2008; Germanov et al., 2018; Murray et al. 2011). Além dos impactos físicos diretos, eles também são capazes de lixiviar substâncias químicas e servir como meio de transporte para microrganismos patogênicos (bactérias, fungos e parasitas), bem como serem vetores de introdução de espécies exóticas (Sérvulo et al., 2023; Zettler et al., 2013; Zhang et al., 2022). As substâncias carreadas pelos polímeros, são os aditivos, produtos químicos adicionados intencionalmente aos plásticos durante a sua fabricação ou processamento para conceder a eles diferentes características e atender a necessidades específicas, como a incorporação de antioxidantes para proteger os polímeros contra condições oxidativas e plastificantes para melhorar a flexibilidade (Hahladakis et al., 2018; Hansen et al., 2013).

Diversos compostos químicos já foram classificados como perigosos, afetando a saúde dos seres humanos e dos animais com impactos na reprodução, desenvolvimento e possíveis riscos de câncer e mutações (Campanale et al., 2020; Lithner et al., 2011; Meng, Sun and Su, 2023). Estes contaminantes podem entrar no ambiente marinho durante todas as fases do ciclo de vida do plástico, expondo os organismos através da ingestão direta ou indireta pelo contato com água, ar, sedimentos e presas contaminadas (Tekman et al., 2022). Além das substâncias adicionadas nos polímeros de maneira intencional, estes detritos também possuem a capacidade de agregar compostos tóxicos (e.g. ftalatos e POPs) já presentes no ambiente, se tornando uma fonte adicional de contaminação para os organismos (Teuten et al., 2009).

Costões rochosos abrigam uma enorme diversidade de peixes, cada espécie desempenhando papéis importantes no funcionamento do ecossistema (Ferreira, Gonçalves e Coutinho, 2001; Halpern e Floeter, 2008). O tamanho corporal, hábito alimentar, habitat e o nível trófico das espécies marinhas, podem influenciar na susceptibilidade à ingestão de microplásticos e no tempo de retenção destas partículas no trato gastrointestinal (Costa et al., 2023; Covernton et al., 2021; Cardozo-Ferreira et al., 2021; Germanov et al., 2018; Salerno et al., 2021). Porém, alguns estudos relacionando as diferentes estratégias alimentares e o nível trófico das espécies com o número de microplásticos ingeridos não são conclusivos (Güven et al., 2017; Markic et al., 2019). Em contrapartida, predadores ativos com hábitos alimentares bentônicos ingerem mais microplásticos quando comparado com animais herbívoros e filtradores (Savoca et al., 2021), indicando que o nível trófico/modo de forrageamento da espécie pode influenciar na ingestão de partículas plásticas. O uso das características morfológicas e ecológicas de cada espécie pode auxiliar na compreensão de como a comunidade se estrutura e em como as espécies respondem aos impactos antrópicos (Beauchard et al., 2017).

A contaminação do habitat também pode ter um impacto significativo na quantidade de microplásticos ingerida por peixes, com peixes pelágicos sendo mais susceptíveis a ingerir microplásticos que espécies bentônicas (Güven et al., 2017; Jovanović, 2017). As taxas de contaminação parecem diminuir com a profundidade, com espécies demersais consumindo mais partículas plásticas quando são encontradas em profundidades mais rasas, porém o contrário foi reportado para espécies pelágicas (Savoca et al., 2021).

No Brasil, o lixo encontrado nas praias ao longo do litoral é composto na sua grande maioria por plástico (Andrades et al., 2020). Devido ao número alto de espécies de peixes contaminadas por MP's no Brasil, o país pode ser considerado um "hotspot" de poluição plástica no mundo, ficando atrás apenas da China (Kibria, 2023). A Reserva Extrativista Marinha (RESEX-Mar) do Arraial do Cabo é uma das unidades de conservação federais mais visitadas do Brasil (Brasil, 2022). Dentre os principais resíduos sólidos encontrados nas praias da região, também predominam os plásticos (Motta e Terra, 2011; Silva et al., 2018). A região de Arraial do Cabo é marcada por eventos de ressurgência que favorecem uma maior produtividade e biodiversidade associada (Cordeiro et al., 2016; Giglio et. al., 2017; Valentin, 2001). Entretanto, a ressurgência também pode induzir a ressuspensão de microplásticos depositados no fundo recifal, levando estes microplásticos para a coluna d'água ou áreas mais rasas (Gao et al., 2024). Estudos prévios já confirmaram a presença de detritos plásticos em peixes, pinguins e tartarugas marinhas, indicando a contaminação da cadeia trófica em Arraial do Cabo (Awabdi et al., 2013; Cardozo-Ferreira et al., 2021; Lima et al., 2018; Pinto et al., 2007).

Identificar atributos ecológicos associados à ingestão de partículas plásticas é de suma importância para compreender o impacto desse agente estressor nos ecossistemas como um todo. Esta abordagem permite reduzir e mitigar as consequências causadas por esses detritos no ecossistema, nos animais e na saúde humana (Cardozo-Ferreira et al., 2021; Macieira et al., 2021; McNeish et al., 2018; Pantos, 2022; Santos et al., 2016; Savoca et al., 2021). Este estudo teve como objetivo identificar as espécies de peixes recifais mais suscetíveis à contaminação por microplástico através de atributos ecológicos das espécies que possam estar associados à ingestão de partículas plásticas, além de verificar as possíveis fontes dos microplásticos ingeridos, visando compreender as vias de contaminação em diferentes níveis da cadeia trófica recifal e auxiliar na elaboração de medidas de manejo que contribuam para a conservação dos ambientes recifais da RESEX-Mar do Arraial do Cabo.

2. OBJETIVOS

2.1 OBJETIVO GERAL

Investigar as fontes, composição e abundância de microplásticos ingeridos por peixes recifais com diferentes modos de forrageamento da região de Arraial do Cabo, de modo a gerar subsídios para fomentar o manejo, conservação e conscientização para os impactos dos microplásticos no ecossistema.

2.2 OBJETIVOS ESPECÍFICOS

- Investigar a abundância, tamanho, forma e cor de microplásticos em 5 espécies de peixes recifais;

- Quantificar a disponibilidade dos microplásticos em três principais compartimentos (sedimento, matriz de algas epilíticas - MAE, água) de forrageamento por peixes nos costões rochosos;

- Analisar as características ecológicas e morfológicas das espécies que podem influenciar na ingestão de microplásticos;

3. HIPÓTESES

- Espécies que se alimentam na MAE apresentarão maior ingestão de microplásticos do que aquelas que se alimentam na coluna d’água ou no sedimento;
- A MAE é a matriz ambiental com maior concentração de microplásticos, devido à sua maior complexidade e capacidade de reter partículas;

- Espécies com corpo, área bucal e trato gastrointestinal (TGI) maiores são propensas a ingerir mais microplásticos.

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Capítulo Único

Patterns of microplastic contamination on reef fishes at the RESEX-Mar in Arraial do Cabo

Formatado para a revista “Marine Pollution Bulletin”

1. INTRODUCTION

Anthropogenic impacts frequently threaten marine ecosystems (Andrello et al., 2022; Bellwood et al., 2004). Among the many threats, plastic debris poses a global issue, being spread all over the Earth's surface, having becoming a major threat to marine biodiversity (Andrady et al., 2011; Derraik, 2002; GESAMP, 2015; Loganathan and Kizhakedathil, 2023; Worm et al., 2017). Every year, between 4.8 and 12.7 million tons of plastic enter the oceans, breaking into smaller particles after degradation (Sarkar et al., 2022). Microplastics (plastic particles <5mm) are ubiquitous in the marine realm. The small size increases their bioavailability, facilitating the ingestion and transfer throughout marine food webs (Cardozo-Ferreira et al., 2021; Wootton et al., 2021).

The impacts of plastic debris on marine life vary according to its abundance, shape, size, color, and composition (Li et al., 2016; Santos et al., 2016). The different forms, physicochemical properties, and possible associations, such as the aggregation of toxic compounds (e.g., phthalates and POPs), should all be considered while assessing the environmental impacts of microplastic particles (Teuten et al., 2009). Recently, the term "microplastome" was defined to represent the collection of different particles and their particularities found in a sample (Li et al., 2024). The microplastome can influence the behavior and fate of microplastics (MPs) in the ocean and consequently their bioavailability to marine organisms (Botterell et al., 2019; Li et al., 2024; Wright et al., 2013).

Microplastics contamination affects a wide variety of habitats within the marine environment (Andrady 2011). In addition to their availability in different habitats, morphological and behavioral characteristics of species can influence species’ susceptibility to ingest these particles (Cardozo-Ferreira et al., 2021; Costa et al., 2023; Ockenden et al., 2021). Despite the growing number of studies on microplastic ingestion by marine species (Cardozo-Ferreira et al., 2021; Costa et al., 2023; Covernton et al., 2021; Germanov et al., 2018; Salerno et al., 2021), research linking it to biotic (i.e., species traits) and abiotic (i.e., environmental matrices) characteristics yield controversial results. For example, Savoca et al. (2021) indicated that fish classified as active predators are more likely to ingest plastic when compared to grazing herbivores and filter-feeding species, suggesting a link between the trophic level and the ingestion of this debris. On the other hand, other works found no relationship between microplastic contamination and the trophic guilds (Dantas et al., 2020) or the trophic level (Güven et al. 2017; but see Siddique et al., 2024). Yet, morphological characteristics such as body size and food acquisition mode may be related to the ingestion of microplastics (Cardozo-Ferreira et al., 2021; Parolini and Romano, 2024), even within the same trophic guild (Cardozo-Ferreira et al, 2021). In contrast, Güven et al. (2017) found no relationship between body size and the presence of ingested plastic debris, but highlight the influence of the type of habitats explored by fishes, a feature typically linked to feeding behavior. Another trait recently associated with the ingestion of plastic particles is the mouth-body ratio, or oral gape, with larger mouths being related to higher ingestion of these particles (Siddique et al., 2024). Many of the controversial results in this topic may lie on the geographical variation, including the contribution of environmental variables and / or local pollution to the contamination of the marine biota (Parolini and Romano, 2024).

The lack of studies that relate the intake of MPs with the ecological and morphological characteristics of the species limits the understanding of their effect on the trophic chain and their true ecological risk (Costa et al., 2023; Horton, 2022). This knowledge is even more limited for reef fish species (Cardozo-Ferreira et al., 2021; Garnier et al., 2019; Macieira et al., 2021). Aiming to understand the influence of biotic and abiotic factors in the ingestion of plastic debris, we (i) quantified different microplastic types present in three main substrates as sources of microplastics in subtropical reefs (water, epilithic algal matrix EAM-, and sediment); (ii) compared ingestion of MPs by five reef fish (*Abudefduf saxatilis*, *Halichoeres poeyi*, *Pseudupeneus maculatus*, *Sphoeroides greeleyi* and *Stegastes fuscus*); and (iii) analyzed the association between morphological/behavioral attributes and the ingestion rates comparatively among fish species. We hypothesize that (1) the EAM is the substrate type with the highest concentration of microplastics, due to its higher complexity and capability to retain particles; (2) species feeding on the EAM will display higher ingestion of microplastics than those feeding on the water column or sand bottom; and (3) species with larger body, mouth area and gastrointestinal tract are prone to ingest more microplastics.

2. MATERIALS AND METHODS

2.1 Study Area

The studied site is located within the Arraial do Cabo Marine Extractive Reserve (RESEX-Mar), a protected area of multiple use with delimited areas for most activities (mostly fishing and tourism). The RESEX-Mar guarantees the self-sustainable exploration and conservation of renewable natural resources, traditionally used for artisanal fishing by the population of Arraial do Cabo (Brazil, 1997).

Praia dos Anjos (22°58'20.3"S, 42°01'14.6"W) (Figure 1) is located in the bay area and is the closest beach to the urban center and also where the city’s harbor is located. The harbor presents a large flow of tourists, especially in the austral summer, from where the typical nautical tours depart. It is possible to observe numerous vessels on the shore of Praia dos Anjos (Figure 2). This beach also has a rainwater drainage channel, which for many years domestic sewage was dumped on this beach without adequate treatment, leading to sporadic reports of improper bathing capacity at the beach (INEA, 2024) (Figure 2).

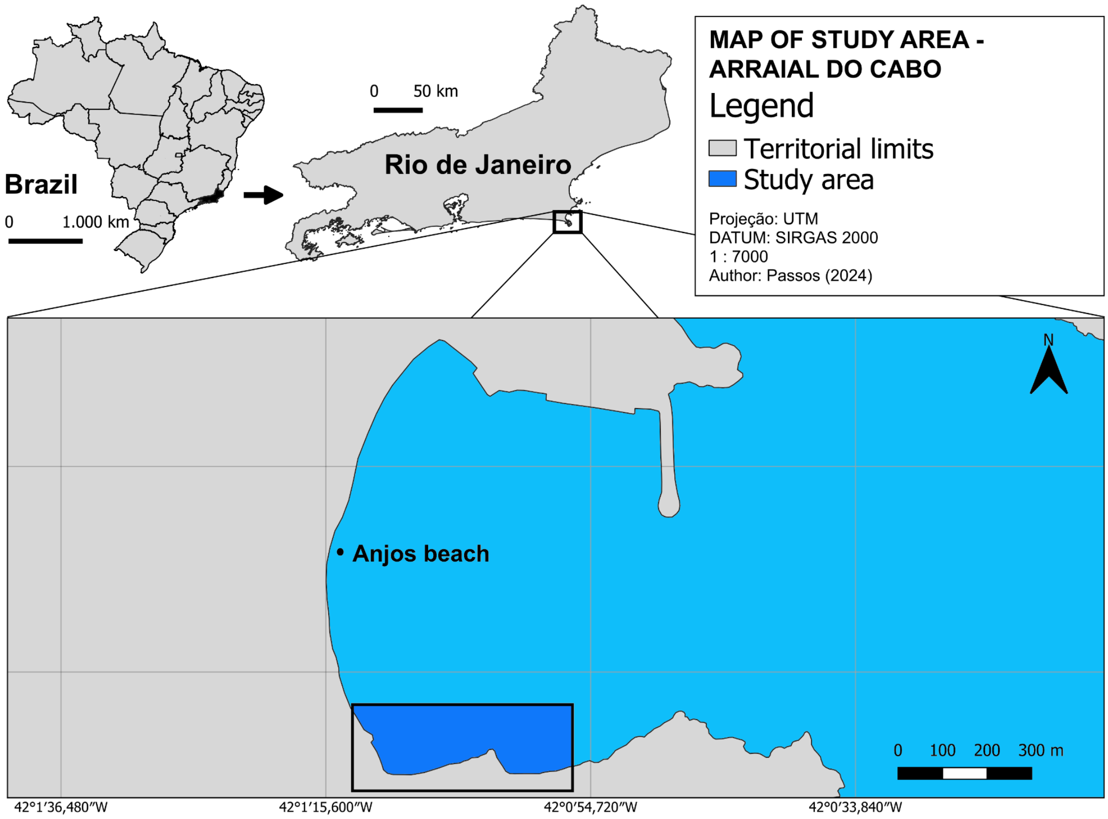

Figure 1: Map of the study area. Bottom polygon demarks the sampling area.

Figure 2: (A) e (B) Numerous vessels on the shore of Praia dos Anjos Cove and (C) Rainwater drainage channel. Photos: (A) and (B) Projeto Costão Rochoso Collection - Alta Productions, (C) Naísa C. Passos.

The rocky shore of Praia dos Anjos were selected for sampling due to the intense movement of tourists, high sewage dumping and oceanographic factors such as the prevailing NE winds which contributes to accumulated dumping on the beach, being considered the most impacted site in Arraial do Cabo. Litter is commonly found along the beach, on the water surface, and even associated with benthic invertebrates, predominantly plastic waste (Figure 3).

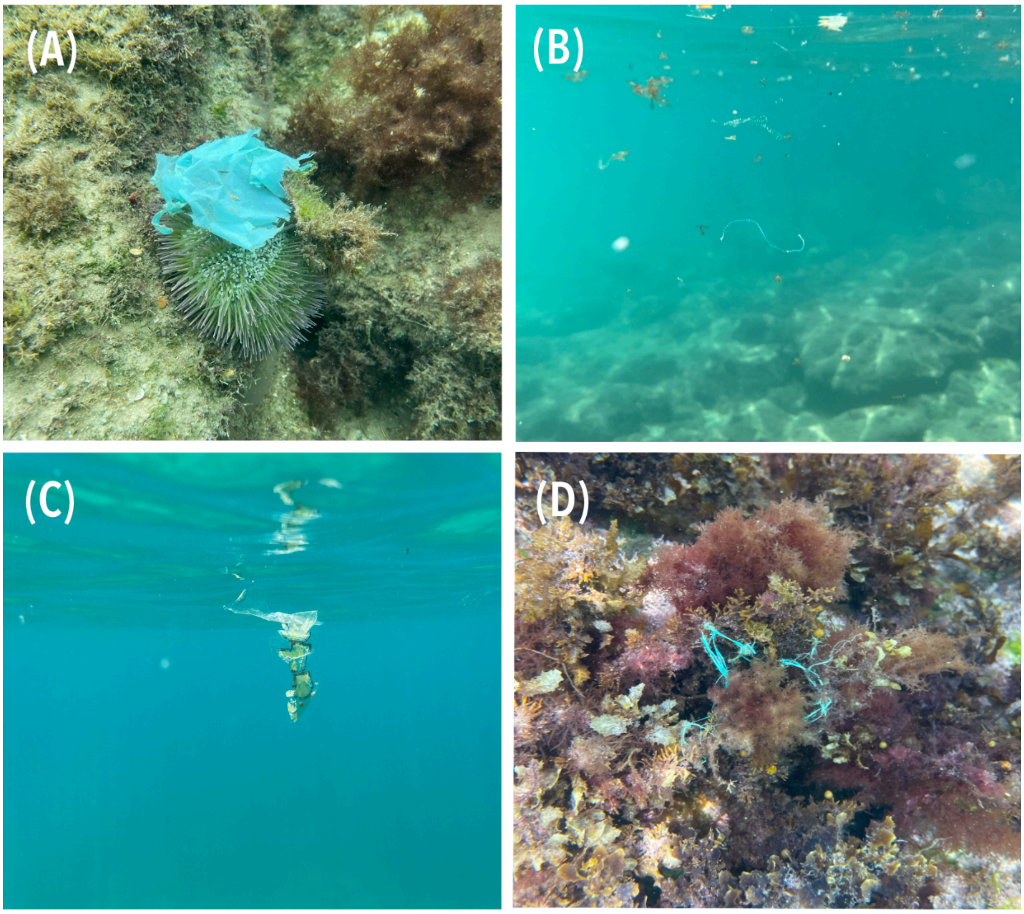

Figure 3: (A) Plastics on the surface of the purple hedgehog (*Lytechinus variegatus*); (B) and (C) Plastics (Fiber and Film) in the water column; (D) Fibers tangled in algae. Photos: Naísa C. Passos.

2.2 Fish traits

Five reef fish species (*Abudefduf saxatilis*, *Halichoeres poeyi*, *Pseudupeneus maculatus*, *Sphoeroides greeleyi*, and *Stegastes fuscus*) were selected for this study, representing species with high abundance and foraging pressure over benthic community, but also encompassing different trophic groups (herbivores, omnivores and invertivores), food acquisition modes, and morphologies that influence on ingestion rates (Figure 4).

Fish species were collected from the rocky shore of Praia dos Anjos, located on the opposite side of the harbor (Figure 1). Specimens were spearfished between November and December 2023 and January 2024, under environmental licenses (SISBIO No. 87825-1), and immediately transported to the laboratory.

Species were classified based on diet (omnivores, herbivores and mobile invertivores - Ferreira et al. (2004) and food acquisition mode (surface pickers, grazers, nibblers, and grubbers excavating - Sazima et al., 1986). To test for the relationship between ingestion of plastic debris and fish characteristics, besides the food acquisition mode, we chose morphological and ecological traits (total length, oral gape, Zihler’s index, and trophic level). Total length and oral gape were measured at the laboratory from each individual, Zihler’s index was calculated based on the following formula: $ZI = Intestinal\ length\ (mm)/(10\ .\ \sqrt[3]{Weight\ (g)}\ )$. Gastrointestinal tract also measured in the laboratory and the trophic level was obtained from FishBase (Froese and Pauly, 2024).

Herbivores typically present longer gastrointestinal tracts than carnivore fishes (Kapoor et al., 1976), which could increase exposure to microplastics and their pollutants (Lei et al., 2018; Roch et al., 2021). We used Zihler's index to assess the relationship between intestinal length and the ingestion of microplastics as it accounts for body mass, a better alternative to represent body shape (Duque-Correa et al., 2024; Zihler, 1981) instead of the relative size of intestine with body length, which ignores the different body shapes of fish that can influence on ingestion rates.

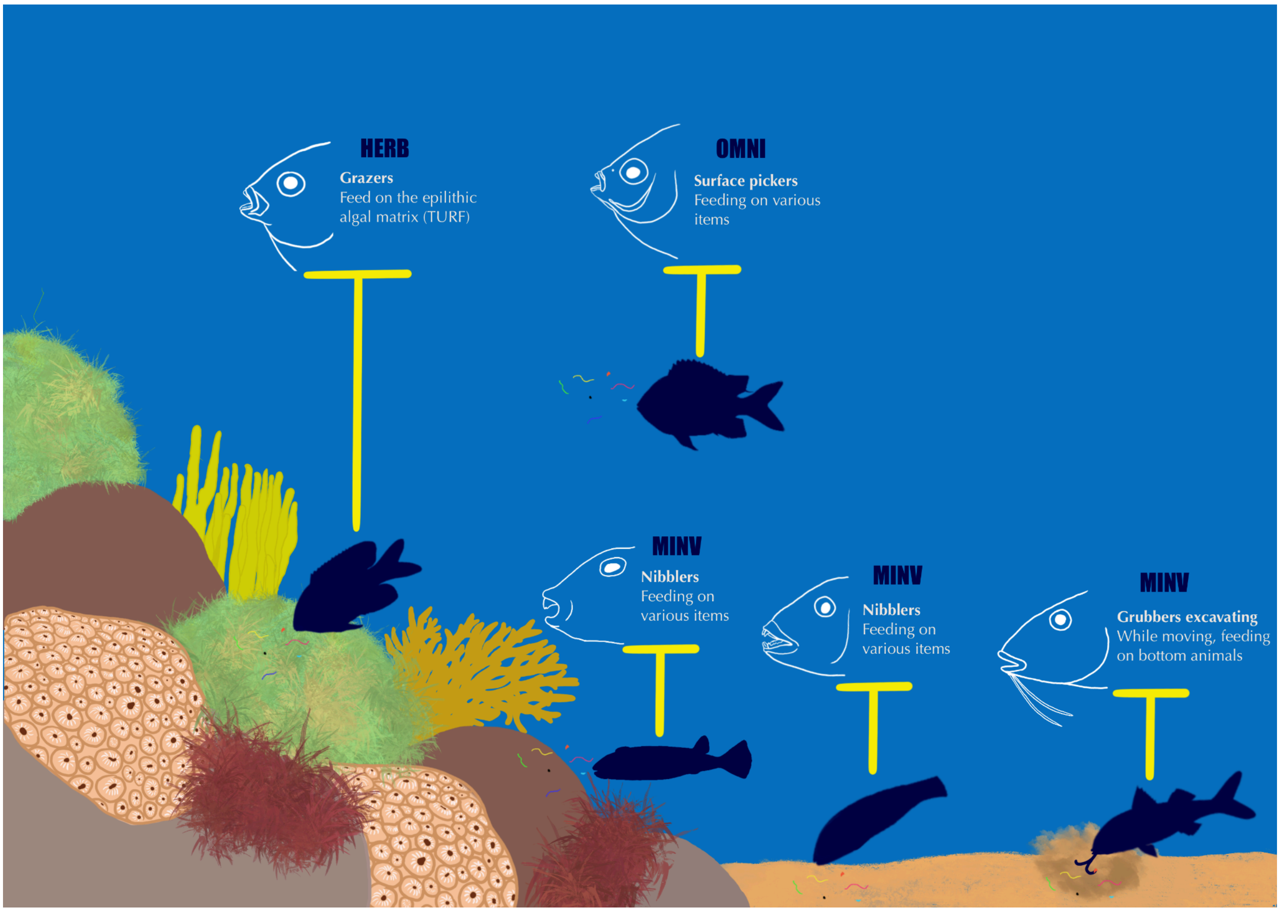

Figure 4: Dietary group, food acquisition mode and mouth shape of selected fishes collected from RESEX-Mar Arraial do Cabo.

2.3 Environmental matrices

Three reef realms, here indicated as environmental matrices, were sampled for sources of microplastic contamination: water, sediment and the epilithic algal matrix (EAM). For water collection, a plankton net (70 µm) was towed along the coastline for a distance of 170 meters for 10 minutes, positioned at a depth of 30 cm from the surface. For EAM and sediment samplings, three points were randomly selected along the rocky shore, distancing 30 meters between them. EAM and sediment samplings were performed using a 30x30 cm quadrant with the aid of a stainless steel spatula. Sediment collection involved only the removal of the superficial layer (1 cm depth). All the material for the three matrices was stored in glass jars, transferred to the laboratory and frozen.

2.4 Sample processing

In the laboratory, fish individuals were weighted (g), measured for standard length, total length, mouth width, and mouth depth (cm), and dissected. The gastrointestinal tract (GIT) was carefully removed from each individual, stored in aluminum containers, and frozen for further analysis. Water samples were filtered through nitrocellulose filters (0.45µm) using a vacuum pump, and the filters were stored in Petri dishes. EAM and sediment samples were dried in an oven (50°C) until reaching constant weight. Subsamples of 5 g of EAM were separated into Petri dishes for subsequent analysis.

An adaptation of Zhang et al.'s methodology (2019) was used to extract MPs from the sediment. A subsample (100g) of each sediment sample was weighed on a precision balance and transferred to properly identified beakers which were filled with 700 ml of a concentrated saline solution (NaCl: 140g.L). Homogenization of the solution was achieved by agitating the contents for 2 minutes with an aluminum spatula. The beakers were covered with aluminum foil, and after 24 hours at room temperature, the supernatant was filtered using a vacuum pump and nitrocellulose filter (0.45µm). The filters were stored in Petri dishes and dried for 24 hours in an oven at 50°C.

The stomach contents, EAM samples, and filters from water and sediment samples were evaluated for the presence of microplastics through visual examination on a Petri dish using a BEL Photonics stereomicroscope with a zoom range of 0.7-4.5X. All suspicious particles were measured (maximum length), photographed, classified according to color and shape following Markley et al. (2024): fiber, pellet, fragment, film, rubber, foam, and fiber bundle. Particles were stored on glass slides for later polymer identification.

2.5 Contamination control

To mitigate the risks of cross-contamination, several steps were undertaken before and during the processing and analysis of samples to ensure quality control (QA/QC). The workspace was vacuumed and cleaned with 70% alcohol. All materials used were predominantly stainless steel and glass. Prior to each analysis, all materials used for visual examination were checked under a magnifying glass for the presence of MPs. Milli-Q water used for washing materials or when necessary was filtered with a nitrocellulose membrane (0.45μm). All utensils were washed with neutral detergent and rinsed three times with filtered ultrapure water. A 100% cotton lab coat dyed violet and nitrile gloves were worn during all procedures. For every dissection batch (10 individuals), a blank control was simultaneously conducted (a nitrocellulose membrane was exposed inside an open glass Petri dish during the fish dissection). During the processing of environmental matrix samples, a blank was also filtered with between 50-500ml of ultrapure water for every sample filtration. Only two fibers of the same coloration as the lab coat were found in the blanks.

2.6 Statistical analysis

The count and length of microplastics found in the species' gastrointestinal tract did not show normal distribution, therefore we used non-parametric Kruskal-Wallis and Dunn's multiple comparisons tests (with Benjamin-Hochberg *p*-adjustment to avoid Type I errors), using base R 'stats' (R Core Team 2024) and 'FSA' (Ogle et al., 2019) packages for among species comparisons. On the other hand, the count and length data for microplastics obtained from environmental matrices (water, EAC, sediment) have shown normal distribution. Therefore, we used analyses of variance (ANOVA), followed by Tukey multiple comparisons test from the 'stats' package.

To evaluate the relationship between our response variable (microplastic abundance) and the functional traits, we conducted Poisson generalized linear model (GLM) due to the commonly observed Poisson distribution in count data (Zuur et al., 2009). However, prior to GLM, we used the function 'check_zeroinflation()' from 'pscl' package (Jackman, 2020) which revealed a probable zero-inflation, i.e. a high proportion of zero counts in our data (49.3%). ZIP models permit a mixture of causal factors to be evaluated and help better predict outcomes when data has a large number of zeros due to both the rarity of an event and false negatives. The ZIP models were fitted as a two-part modeling approach: a Bernoulli distribution with a logit-link function and estimates the probability of observing a zero, and a Poisson distribution through a log-link function (Zuur et al., 2009). As most species have unique ecologies, the traits *food acquisition mode*, *species ID*, *trophic level*, and *Zihler's index* are collinear and preclude model convergence. Collinearity was checked through the function 'check_collinearity()' from the 'pscl' package. When collinearity was considered high (Variance Inflation Factor – VIF > 10), variables were not put in the same model. Therefore, we ran three different ZIP GLM models, all having the concentration of microplastics into species' gut as the response variable. The zero-inflated Poisson (ZIP) GLM were conducted to estimate how the following traits influence on the number of microplastics ingested: *Individual TL*, *oral shape* and *food acquisition mode* (with four levels: Grazers, Grubbers excavating, Nibblers and Surface pickers) (M1); *individual TL*, *oral shape* and *species ID* (five levels: *Abudefduf saxatilis*, *Halichoeres poeyi*, *Pseudupeneus maculatus*, *Sphoeroides greeleyi* and *Stegastes fuscus*) (M2), and the *individual TL*, *oral shape*, *Zihler's index* and *trophic level* (M3). The Bernoulli component was needed in the three models to account for the probability of false zeros. We included the variable 'food acquisition mode' as the Bernoulli component in M1, and the variable 'species' in the models M2 and M3.

The model selection was based on multimodel inference approach (Anderson 2008) using the function 'dredge' from the 'MuMIn package' (Barton, 2020). In this approach, models are ranked based on Akaike information criteria (AIC), delta AIC (Δi) and Akaike weights (wi) (Anderson, 2008). Models were selected based on Δi, which precludes that those models with Δi < 3 have similar strength. Akaike weights (wi) is the probability of a certain model being the best among the set of models. As among the best models (Δi < 3) all the variables were important to reach the best fit, all variables were used in the different models. We used Vuong tests (Vuong, 1989) to compare the zero-inflated models with the standard Poisson and negative-binomial regression models.

All analyses were conducted in the R environment (R Core Team, 2024). Plots were produced using base R, 'ggplot2' (Wickham, 2016), 'colorspace' (Zeileis et al., 2020) and 'cowplot' (Wilke, 2020).

3. RESULTS

3.1 Abundance and characteristics of microplastics in environmental matrices

Microplastics were found in all samples of the environmental matrices. In the sediment, we found an average of 0.18 ± 0.03 items per gram (items/g), in the EAM 22.13 ± 5.41 items/g, and in water 9.64 ± 3.46 items/m³. The 55 plastic particles found in the sediment were classified according to shape as fibers (78.2%), film (12.7%), fragments (5.5%), and fiber bundle (3.6%) (Figure 5). The colors found were transparent (38.2%), black (21.8%), blue (21.8%), gray (7.3%), pink (5.5%), white (3.6%) and red (1.8%), with transparent being the most common color (Figure 5). In water samples, 261 particles were found, being classified according to shape as fibers (75.1%), film (14.6%), fragment (8.4%), rubber (1.5%) and foam (0.38%). The microplastics found in water samples were of the colors black (25.7%), blue (24.9%), transparent (19.2%), gray (7.7%), white (6.5%), pink (6.1%), green (5.0%), yellow (2.3%), red (2.3%), and orange (0.4%) (Figure 5). The predominant color was black. EAM was the environmental matrix with the highest quantity of plastic debris found (n = 332). Fibers were predominant (88.9%), followed by fragments (4.5%), film (3.6%), fiber bundle (1.8%), and rubber (1.2%). The colors found were transparent (35.2%), black (27.1%), blue (13.9%), red (6.9%), pink (5.4%), gray (3.3%), yellow (3.0%), green (2.4%), orange (2.1%) and white (0.6%) (Figure 5). The most common color was transparent as in the water samples.

The total number of MPs in the sediment was significantly lower than in the EAM (ANOVA; *p* = 0.008) and in the water (ANOVA; *p* = 0.029) samples. However, no difference was found between EAM and water. The average size of MPs found in water samples was 2.40 ± 4.25 mm, while in the EAM they measured 2.08 ± 2.31 mm, and in sediment 1.48 ± 0.94 mm, but such differences were not significant.

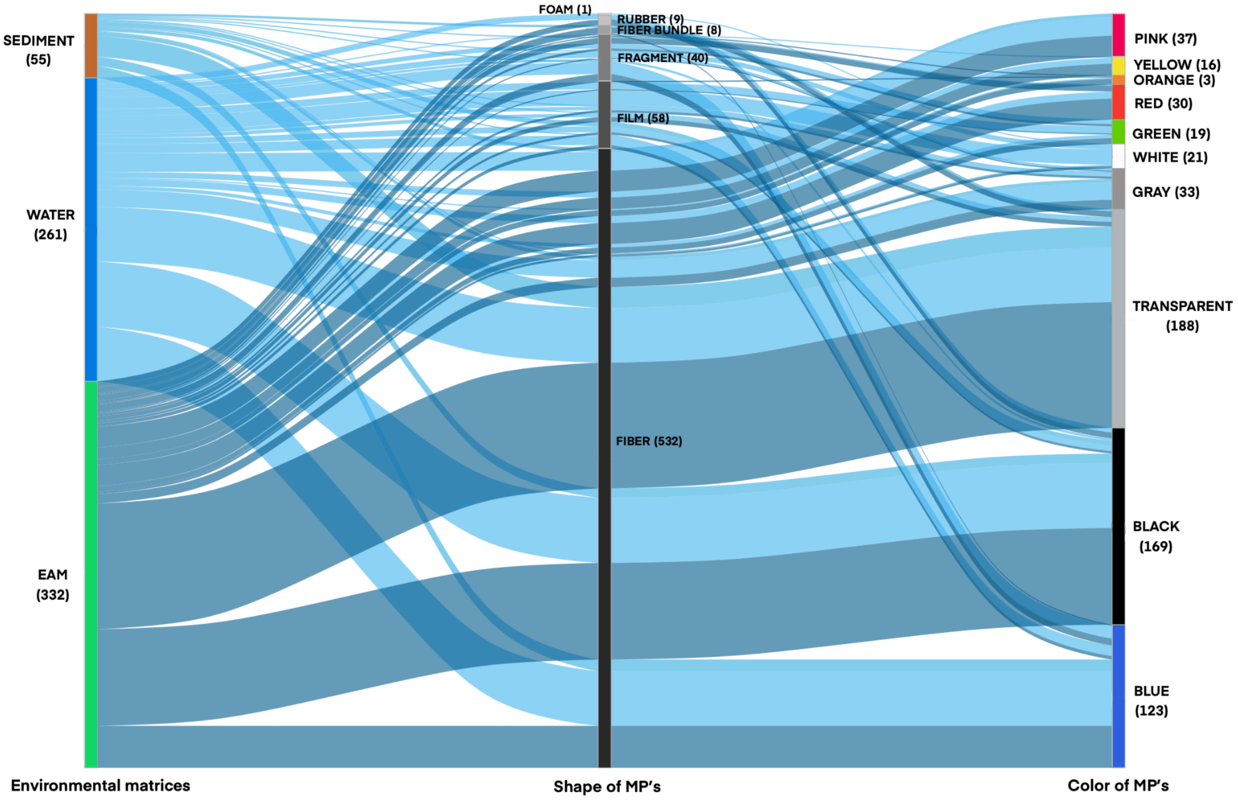

Figure 5: Comparative type and color of microplastics (Alluvial Diagram) among environmental matrices (Sediment, Water, and EAM).

3.2 Abundance and characteristics of microplastics ingested by fish

A total of 69 individuals from five species were sampled (Table 1). All analyzed species exhibited microplastics in their gastrointestinal tracts. Out of the total collected from the 5 species, 35 (50.7%) specimens ingested plastic debris. The abundance of microplastics varied among species. *Stegastes fuscus* was the species that ingested the most MPs (n=50 debris), followed by *Abudefduf saxatilis* (n=37), *Sphoeroides greeleyi* (n=16), *Pseudupeneus maculatus* (n=13), and *Halichoeres poeyi* (n=10). The amount of ingested particles was higher in *A. saxatilis* than in *H. poeyi* (Kruskal-Wallis: Z-statistic = 3.011, p = 0.026) and *S. greeleyi* (Kruskal-Wallis: Z-statistic = 2.800, p = 0.017). The same occurred when comparing *S. fuscus* (Kruskal-Wallis: Z-statistic = -2.922, p = 0.017) and *H. poeyi* and *S. greeleyi* (Kruskal-Wallis: Z-statistic = -2.661, p = 0.019). The mean ingestion rate (number of microplastics (MP's) per individual) ranged from 0.48 to 3.36 MP's/individual. To facilitate the comparison of our results with other studies, we also expressed microplastic ingestion in the frequency of occurrence ($\%FO = (Ni/n) \times 100$), where “Ni” is the number of individuals that presented MPs and “n” is the total number of individuals analyzed (Miranda-Peña et al., 2023) (Table 2).

TABLE 1 | Total number (n) of specimens, mean (range) total length (mm) and total weight (g), Zihler’s index, trophic level, oral gape, and ecological characteristics of each species.

	Species				
	Abudefduf saxatilis n=11	***Stegastes fuscus n=16***	***Sphoeroides greeleyi n=10***	***Halichoeres poeyi n=21***	***Pseudupeneus maculatus n=11***
Fish traits					
Total length (mm)	172.53 (155-187)	124.69 (97-147)	142.68 (107-255)	148.50 (92-173)	136.54 (66-196)
Total weight (g)	94.09 (41-142)	53.39 (31-78)	79.42 (32-328)	36.93 (10-73)	40.01 (3-101)
Zihler’s index	156.1 (106.8-206.6)	92.8 (48.7-155.3)	77.8 (38.6-254.1)	34.0 (12.0-56.3)	41.0 (6.5-76.4)
Trophic level	3.8 ± 0.2	3.3 ± 0.23	3.5 ± 0.4	3.7 ± 0.2	3.7 ± 0.2
Oral gape	0.02 (0.02-0.02)	0.02 (0.02-0.02)	0.05 (0.04-0.05)	0.04 (0.04-0.06)	0.06 (0.04-0.07)
Dietary group	OMNI	HERB	MINV	MINV	MINV
Food acquisition mode	Surface pickers	Grazers	Nibblers	Nibblers	Grubbbers excavating

Individual TL, mode of food acquisition and oral gape were used in a model to relate anthropogenic debris ingestion to species characteristics.

TABLE 2 | Frequency of occurrence (%FO), mean ingestion rate (MP/ind.), shape and most common colors of microplastics found in species’ gut contents.

	$\%FO$	**Mean ingestion rate (MP/ind.)**	**Shape of MPs**	**Most common color**
Species (n)				
Stegastes fuscus (16)	75%	3.13	Fiber, film	Black
Abudefduf saxatilis (11)	72.7%	3.36	Fiber, film, rubber, fragment	Black
Pseudupeneus maculatus (11)	45.5%	1.18	Fiber	Gray
Halichoeres poeyi (21)	38.1%	0.48	Fiber	Blue and transparent
Sphoeroides greeleyi (10)	20%	1.60	Fiber	Transparent

A total of 126 plastic particles were found in the analyzed gut contents, among which 95.2% were fiber, 3.17% film, 0.79% fragment, and 0.79% rubber (Figure 6). The size of the particles ranged from 0.18 to 25 mm (mean 3.57%); the majority of MPs (78.6%) were smaller than 5 mm, while (21.4%) were larger than 5 mm. No significant difference was found in the length of MPs among species

(Kruskal-Wallis test: $p > 0.05$). Particles of blue (30.2%), black (27%), transparent (25.4%), red (4.8%), green (4.8%), gray (4.0%), pink (3.2%), and orange (0.8%) colors were found (Figure 6).

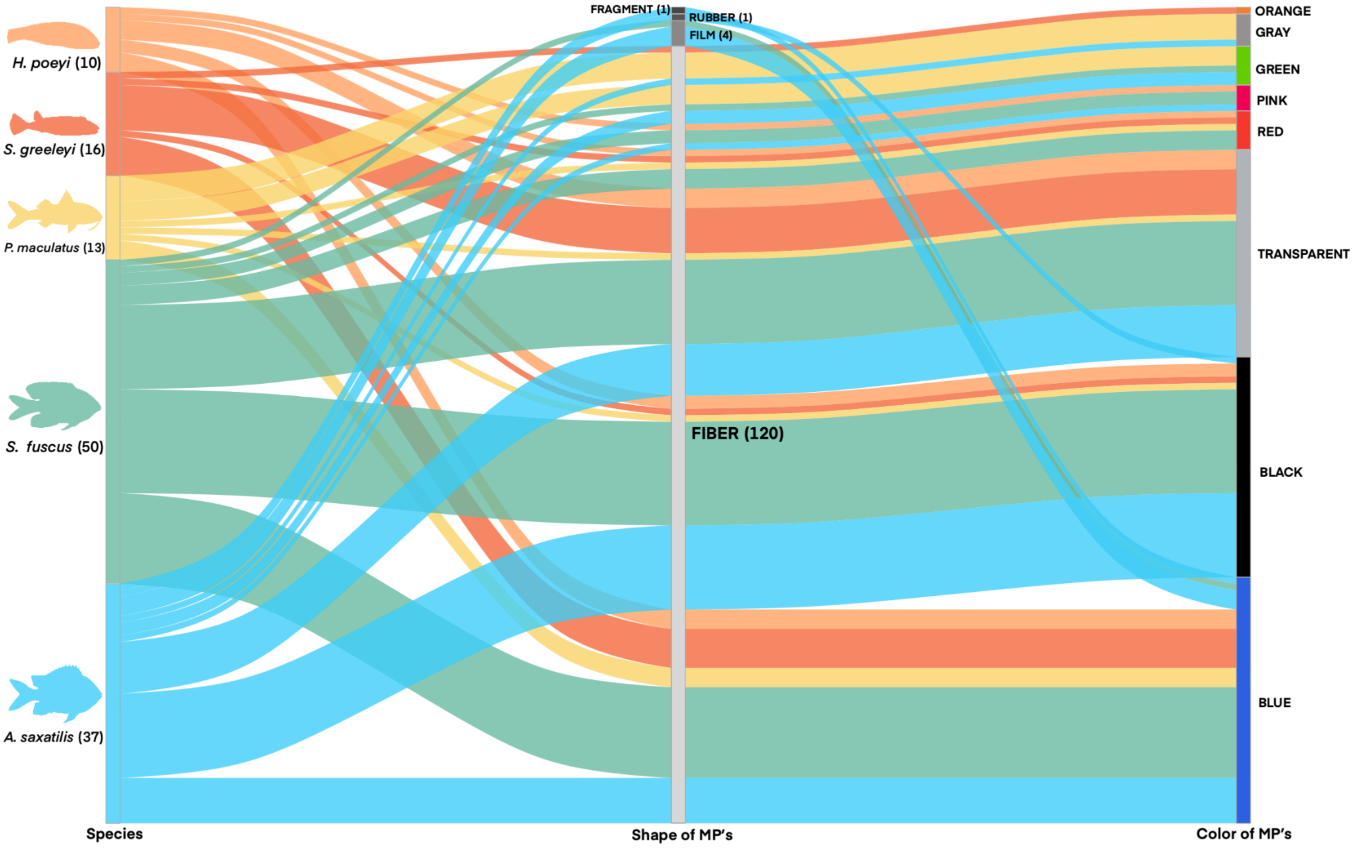

Figure 6: Comparative type and color of microplastics (Alluvial Diagram) among reef fish species

3.3 Relationship between fish characteristics and microplastic ingestion

In M1 and M2, ZIP models did not show significant improvement over the standard Poisson models (M1: Vuong z-statistic = 1.150, $p = 0.125$; M2: Vuong z-statistic = 1.483, $p = 0.069$), besides data have shown highly over-dispersed due to a large proportion of zero-counts within the data (49.3%). Therefore, for these two models, we show the results of the less complex models, the GLM Poisson regressions (Tables 3 and 4; Figures 7 and 8). ZIP models for M1 and M2 can be seen in the supplementary material. On the other hand, the ZIP model M3 was significantly improved over the standard Poisson model (M3: Vuong z-statistic = 2.181, $p = 0.015$).

The standard Poisson model M1 returned positively significant for body size, indicating that the larger the individual, the higher the number of plastic debris found in the gut content (Table 3; Figure 7). Moreover, in the Poisson GLM model the food acquisition modes grubbers-excavating and nibblers were negatively associated to ingestion of MPs, indicating that species employing these feeding modes tend to ingest fewer plastic debris (Table 3; Figure 7).

TABLE 3 I Parameters estimates of the Model 1 (M1) for the standard Poisson generalized linear model for the association between traits of the reef fishes and number of debris ingested as the response variable.

Variable	**Estimate**	**SE**	***z*-Value**	***p***
Poisson GLM				
Intercept	-7.448	3.125	-2.383	**0.017**
log Body size	1.288	8.567	2.271	**0.023**
log Oral shape	3.409	2.245	1.519	0.129
Food acquisition mode (grubbers excavating)	-1.310	0.346	-3.785	**<0.001**
Food acquisition mode (nibblers)	-1.477	0.249	-5.927	**<0.001**
Food acquisition mode (surface pickers)	-0.232	0.289	-0.801	0.423

Showing coefficient estimates of explanatory variables, standard error (SE), test statistic (z-value), and the *p*-value (*p*). Coefficient in bold indicates that p-value is significant at $p < 0.05$ level. Reference level for this regression was set as “Grazers” for food acquisition mode.

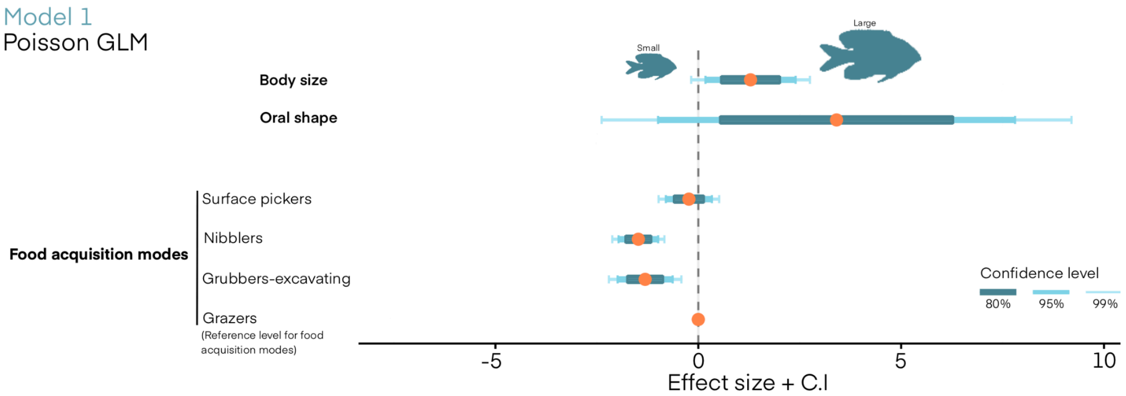

Figure 7: Effect sizes and confidence intervals at three levels (80, 95, and 99%) of significance resulted from the Poisson generalized linear model testing the effect of body size, oral shape, and food acquisition mode on the number of debris ingested.

In the standard Poisson model M2, body size and oral shape were significantly and positively related to the number of debris found in the guts, while *H. poeyi* and *Pseudupeneus maculatus* were negatively associated with it (Table 4; Figure 8), being less prone to the ingestion of plastic debris.

TABLE 4 I Parameters estimates of the Model 2 (M2) for the standard Poisson generalized linear model for the association between traits of the reef fishes, species ID, and number of debris ingested as the response variable.

Variable	**Estimate**	**SE**	***z*-Value**	***p***
Poisson GLM				
Intercept	-9.121	3.212	-2.840	**0.005**
log Body size	1.062	0.533	1.992	**0.046**
log Oral shape	7.346	2.613	2.811	**0.005**
Species (*Halichoeres poeyi*)	-1.171	0.419	-5.180	**<0.001**
Species (*Pseudupeneus maculatus*)	-1.493	0.424	-3.522	**<0.001**
Species (*Sphoeroides greeleyi*)	-0.492	0.315	-1.565	0.117
Species (*Stegastes fuscus*)	0.015	0.290	0.050	0.960

Showing coefficient estimates of explanatory variables, standard error (SE), test statistic (z-value), and the *p*-value (*p*). Coefficient in bold indicates that p-value is significant at $p < 0.05$ level. Reference level for this regression was set as "*Abudefduf saxatilis*" for species.

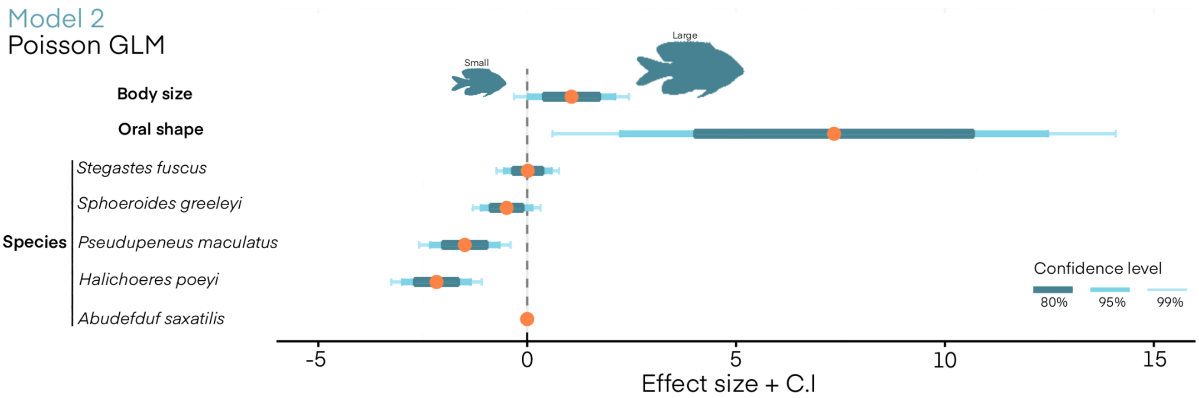

Figure 8: Effect sizes and confidence interval at three levels (80, 95, and 99%) of significance resulted from the zero-inflated Poisson generalized linear model testing the effect of body size, oral shape, and species ID on the number of debris ingested.

The ZIP GLM M3 returned positively significant between the Zihlers' index and the number of plastic debris found in species gut content (Table 5; Figure 9). The longer the gut, higher the ingestion of plastic debris. The other traits (body size, oral shape and trophic level) had not significant relationship with plastic debris ingestion. The Bernoulli component in M3 revealed a positive and significant relationship between the ingestion of plastic debris and the species *S. greeleyi.*

TABLE 5 I Parameters estimates of the Model 3 (M3) for the final zero-inflated generalized linear model (ZIP GLM) for the association between traits of the reef fishes and number of debris ingested as the response variable.

Variable	**Estimate**	**SE**	***z*-Value**	***p***
Count process (Poisson distribution)				
Intercept	-0.224	2.848	-0.079	0.937
log Body size	0.377	0.978	0.386	0.700
log Oral shape	-0.926	1.946	-0.476	0.634
log Zihlers` index	0.664	0.256	2.595	**0.001**
Trophic level	-0.768	0.678	-1.133	0.257
Zero-inflation model coefficients (binomial logit link)				
Intercept	-1.009	0.692	-1.459	0.145
Species (*Halichoeres poeyi*)	1.111	0.883	1.257	0.209
Species (*Pseudupeneus maculatus*)	0.444	1.152	0.386	0.700
Species (*Sphoeroides greeleyi*)	2.307	1.062	2.172	**0.030**
Species (*Stegastes fuscus*)	-0.278	0.966	-0.288	0.774

Showing coefficient estimates of explanatory variables, standard error (SE), test statistic (z-value), and the *p*-value (*p*). Coefficient in bold indicates that p-value is significant at $p < 0.05$ level. Reference level for this regression was set as "*Abudefduf saxatilis*" for species.

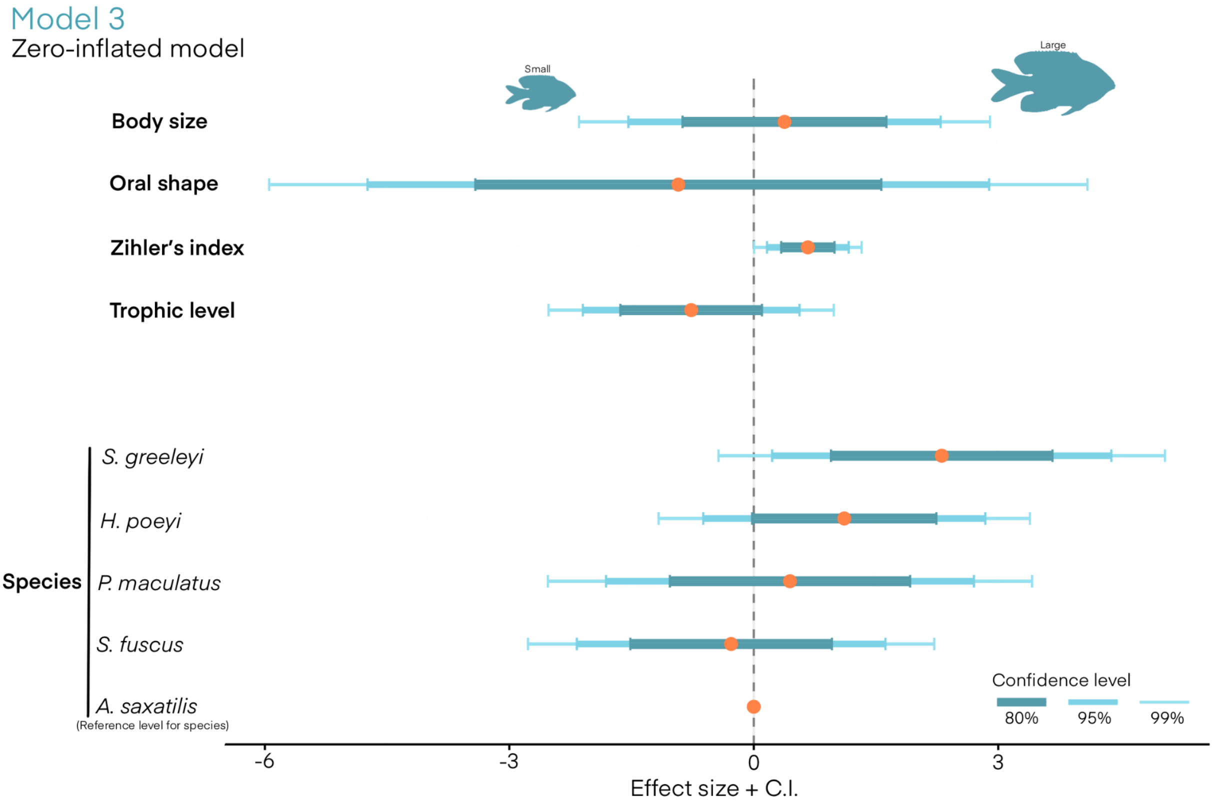

Figure 9: Effect sizes and confidence interval at three levels (80, 95, and 99%) of significance resulted from the zero-inflated Poisson model testing the effect of body size, oral shape, Zihler's index, and trophic level on the number of debris ingested.

4. DISCUSSION

Contamination by microplastics is a growing concern in marine ecosystems due to their widespread distribution and persistence in the environment (Worm et al., 2017). Microplastics are now considered a global threat, with virtually no region of the planet escaping this contamination (GESAMP, 2015; Jambeck et al., 2015; Loganathan and Kizhakedathil, 2023). In this study, we found the ubiquity of these contaminants in subtropical rocky reefs of the Southwestern Atlantic. We found MPs contamination in all three environmental matrices (water, sediment and epilithic algal matrix - EAM) and the five reef fish species analysed.

The concentration of MPs found in the GIT of fish varied among species, which was expected given fish contrasting foraging modes. We found ingestion rates between 0.48 to 3.36 MPs/individual, values close to those found in recent reviews for marine and freshwater fish (Markic et al., 2019 - 2.6 MPs/ind.; Parolini and Romano, 2024 - 3.79 MPs/ind.; Wootton et al., 2021 - 2.8 MPs/ind.). Besides interspecific variation, contamination in fishes may also vary among different locations and methodologies used at the time of collection (Cardozo-Ferreira., 2021; Martí et al., 2020; Parolini and Romano, 2024). This variability can be explained by abiotic and biotic factors, such as the physical and chemical characteristics of MPs and their different availability in the environment (Gray et al., 2017; Lehtiniemi et al., 2018; Li et al., 2016; Santos et al., 2016). Notwithstanding, Cardozo-Ferreira et al. (2021) found similar values for herbivorous reef fishes in the same area of our study, ranging from 1.84 to 10.65 MPs/ind.

Fibers were the most common type of microplastic found in the environmental matrices and reef fishes analyzed. This result is corroborated by several authors, who found the predominance of fibers in different environments (Markic et a., 2019), from deep (Woodall et al., 2014) to shallow marine habitats (Remy et al., 2015), but also in estuaries (Abbasi et al., 2018) and reefs (Macieira et al., 2021), including a wide range of marine fish species (Azevedo-Santos et al., 2019). The elongated shape of fibers facilitates their entanglement in the gastrointestinal tract of species (Kolandhasamy et al., 2018).

We found that in both the biota and environmental matrices the most common colors of MPs were blue, black and transparent. Similar results were found in reviews on plastic ingestion worldwide (Martí et al., 2020; Parolini and Romano, 2024; Ugwu et al., 2021). The predominance of these colors in fish can be explained by their greater availability in the environment, as observed in our environmental matrices, as well as the colors that are most easily confused with food, the color blue for example is easily mistaken by planktonic organisms (Ory el at., 2017). Yet, the perception of plastic colors should vary among species with different feeding habits, where species approaching preys from below (or floating plastic debris in the water column) should ingest darker debris, while those approaching the food resources from above (e.g. investing in the substrate) should ingest paler plastic fragments (Santos et al., 2016). Blue fibers can originate from fishing/boating gears, such as synthetic boat ropes (Cardozo et al., 2018; Cardozo-Ferreira et al., 2021), which are very common in the touristic region of Arraial do Cabo.

We identified a large amount of fibers in the EAM and water. Fibers tend to remain available in the water column for a longer time compared to other polymer forms, such as fragments. Although less dense than seawater (greater weight and volume), fragments sink more rapidly than fibers (Avio et al., 2017; Porter et al., 2018). However, when fibers reach the seafloor, they can either a) deposit directly in the EAM and become entangled, losing mobility, or b) deposit in the sediment and be subject to resuspension (either due to currents or animal movement), potentially becoming available again in the water column and/or entangled in the EAM. This effect can be enhanced by upwelling once MPs deposited on the ocean floor can resuspend into surface waters (Gao et al., 2024). In the specific case of Praia do Anjos, our sampling site, the predominant winds from the Northeast (which induce the occurrence of upwelling in Arraial do Cabo; Cordeiro et al. 2016; Paiva, 1993; Valentin et al., 2001) increase hydrodynamism and the occurrence of currents strong enough to resuspend and transport the suspended or deposited material from the substrate. This could explain the higher concentrations of microplastics in both the water and EAM than in the sediment.

Larger individuals tend to display higher ingestion of MPs (Alomar et al., 2017; de Vries et. al., 2020; Cardozo-Ferreira et al., 2021; Parolini and Romano, 2024). Body size was significantly associated with ingestion rate, as well as oral shape and relative gut length (Zihler’s index). Also, fishes with horizontally wider mouths tend to display a greater intake of MPs. Mouth size was also found to have positive relationship with debris ingestion in six tropical fish species (Siddique et al., 2024). Feeding is probably the main route of entry of MPs into fish (Markic et al., 2019; Savoca et al., 2021), therefore the body size and mouth size of fish directly influence the amount of food they need and can ingest to reach their energy requirements. It means that larger fish need to feed more often and in larger quantities than smaller individuals (Clarke e Johnston, 1999), just as fish with larger mouths are able to feed on larger portions of food at a time. These two factors increase the probability of ingesting MPs, whether this ingestion is intentional or not since the environment in which they are eating has a great availability of these particles.

The intestinal length of fish is one of the morphological characteristics associated with dietary specialization: herbivorous fish have longer intestines than invertivores or carnivores. Moreover, it is expected that fish that feed on a wider variety of foods (e.g. omnivores) have intermediate values of intestinal length (Duque-Correa et al., 2024; German et al., 2010; Zihler, 1981). Our results showed a positive relationship between the Zihler's index and the number of MP's found in the GIT, indicating that the larger the species' intestine, the greater the ingestion of plastic debris. *Stegastes fuscus* and *A. saxatilis* were the species with the highest concentration of MPs/ind., and also the ones with the greatest intestinal length. Herbivores as *S. fuscus* have longer GITs than carnivores, allowing a higher intake of low-quality foods and maintaining a minimum retention time in the gut to increase nutritional absorption (German et al., 2010; Lobato et al., 2014). The positive relationship between the size of the GIT and the ingestion of MP's can be explained by the high frequency of bites on the substrate while feeding, increasing the chances of occasionally ingesting plastic debris present in the environment. The daily feeding frequency of fish can also result in important changes in GIT transit and the time food remains in the species' intestine (Gilannejad et al., 2019). Yet, feeding rates and GIT transit time can vary greatly in fishes, mostly due to the high processing of inorganic material ingested by the different herbivorous fishes (Clements et al., 2009). Although most of the plastic debris that ingested are excreted (Grigorakis et al., 2017; Ohkubo et al., 2022), the longer these particles remain within individuals, the greater the chances of them causing health issues (e.g., gastrointestinal blockages and inflammatory responses) and the higher the probability of being transferred to predators (Pirsaheb, Hossin and Makhdoumi, 2020; Watts et al., 2015).

Regarding the influence of species' ecological characteristics on MP intake, many recent studies had taking into account different ecological attributes of different species (Costa et al., 2023; Cardozo-Ferreira et al., 2021; Ockenden et al., 2021; Zhang et al., 2022), but the results are still highly controversial. For example, Costa et al. (2023) working in sand beach subtropical habitats showed a positive relationship between pelagic planktivorous fish and the concentration of MPs in their digestive tract compared to omnivorous, carnivorous, demersal and benthic-pelagic species. In contrast, Zhang et al. (2022) working in subtropical estuaries found lower concentrations of MPs in pelagic fish compared to demersal fish, and a higher intake by omnivorous fish compared to carnivorous and planktivorous fish. A recent review showed that geographic factors, such as proximity to the coast and the tropics, which would influence the contamination rate of different areas, is more likely to explain the concentration of plastic debris in fish than the species' traits (Parolini and Romano, 2024).

The employed feeding methods are also decisive in identifying the most susceptible species ingesting plastic debris. The grazer herbivore *S. fuscus* and the omnivore surface-picker *A. saxatilis* showed higher ingestion rates and %FO than the nibblers and grubblers invertivores *H. poeyi* and *S. greeleyi*. The grazer *Stegastes fuscus* feeds directly on the EAM (Ferreira et al., 1998), while the surface-picker *A. saxatilis* feeds mainly in the water column closer to the surface. However, younger *A. saxatilis* have been observed feeding on the seabed, where the EAM is also part of their diet (Nunes et al., 2023). The higher contamination by species feeding on the EAM is possibly linked to the higher contamination of this environmental matrix. Due to its greater structural complexity, EAM has the capacity to retain plastic particles, which become entangled in the algal matrix, losing mobility, and becoming an important source of microplastics for the reef trophic chain. EAM is the most abundant substrate on all reefs in the world (Tebbett and Bellwood, 2021) and on reefs in Brazil (Aued et al., 2018) and serves as a food source for various fish and marine invertebrates (Ferreira, Gonçalves e Coutinho, 2001). This is the first time that MPs have been sampled in this environmental matrix and our results indicate that it is an important source of plastic debris in the reef environment.

The mobile invertebrate feeders *P. maculatus*, *H. poeyi* and *S. greeleyi*, differ in their food acquisition mode. While the former is a grubber-excavator that searches the substrate using barbels, the later two are nibblers that bite the targeted preys opportunistically, feeding on the invertebrates while foraging close to the bottom. Here we found a negative relationship between these two groups and the ingestion of MPs. These results go against a recent review that indicates that fish with benthic feeding habits and active predators ingested more MPs when compared to grazers and filter-feeding species (Savoca et al., 2021). This can be explained by the availability of MPs in the environment, once EAM and water had higher levels of microplastic contamination when compared with sediment.

The potential for bioaccumulation and/or biomagnification in marine fish species with different habits and attributes in the food chain is still understudied (Parolini and Romano, 2024). Some studies have demonstrated the potential for trophic transfer of MPs and harmful anthropogenic chemicals between organisms from different trophic levels (Rochman et al., 2013). For example, laboratory studies observed the transfer of plastic debris between planktonic organisms from one trophic level (mesozooplankton) to a higher level (macrozooplankton) (Setälä, Fleming-Lehtinen and Lehtiniemi, 2014) and between mussels and crabs (Farrell and Nelson, 2013). However, laboratory experiments may not represent the actual exposure conditions faced by species in the marine environment (Markic et al., 2020). Until recently, it was believed that marine species belonging to higher trophic levels could accumulate more MPs than those from lower trophic levels through bioaccumulation and/or bioamplification along the food chain (Carbery et al., 2018; Desforges et al., 2015; Miranda et al., 2016). However, recent works failed to support this hypothesis by demonstrating greater contamination in species at lower trophic levels (Capone et al., 2020; Covernton et al., 2021; Walkinshaw et al., 2020), or found no relationship between contamination and the trophic level (Güven et al. 2017; Parolini and Romano, 2024). Therefore, although bioaccumulation between different trophic levels was observed, bioamplification could not yet be confirmed (Miller et al., 2020). In our analyses, we also did not find a significant relationship between the trophic level of the species and contamination by MPs, although our range of trophic levels was small (3.3 to 3.8 - Froese and Pauly, 2024). Future works should focus on evaluating a wider array of species with more disparate trophic levels in order to better understand the trophic transfer of microplastic and its associated hazardous compounds on marine food webs.

5. CONCLUSIONS

Our study confirmed the ubiquity of MPs in the environment and in five species of reef fish from subtropical rocky shores inside a Marine Extractive Reserve in the South Atlantic. Water and EAM are the main sources of MP contamination, the species that feed in these matrices are also those that present higher concentrations of MPs/ind. Here we show for the first time microplastic contamination in the EAM, an important resource for reef species. The morphological characteristics of the species were decisive in the ingestion of plastic debris, the larger the body, mouth and intestine size of the fish, the greater the ingestion of MPs. Species with “grubbers-excavating” and “nibblers” feeding strategies tend to ingest fewer MPs. We found no significant relationship between trophic level and ingestion of plastic debris. Our results contribute to understanding the relationship between MP contamination in fish and major foraging substrates. We analysed data on the morphological and ecological characteristics of the species that influence the ingestion of these particles, providing a comprehensive analysis of MP contamination in the rocky reefs while indicating species more susceptible to contamination. These findings contribute to understanding MPs' contamination of coral reef food webs, highlighting that management strategies should address environmental microplastic contamination and its sources to mitigate and prevent further arrival and accumulation of plastic debris in coastal environments.

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7. SUPPLEMENTARY MATERIAL

Table S1: Parameters estimates of the Model 1 (M1) for the final zero-inflated and standard Poisson generalized linear model for the association between traits of the reef fishes and number of debris ingested as the response variable.

Variable	**Estimate**	**SE**	***z*-Value**	***p***
ZIP model				
Count process (Poisson distribution)				
Intercept	-4.300	3.963	-1.085	0.278
log Body size	1.304	0.800	1.630	0.103
log Oral shape	-0.929	2.132	-0.436	0.663
Food acquisition mode (grubbers excavating)	-0.874	0.442	-1.979	**0.048**
Food acquisition mode (nibblers)	-0.525	0.267	-1.969	**0.049**
Food acquisition mode (surface pickers)	-0.309	0.339	-0.910	0.363
Zero-inflation model coefficients (binomial logit link)				
Intercept	-1.236	0.648	-1.907	0.057
Food acquisition mode (grubbers excavating)	0.758	1.141	0.665	0.506
Food acquisition mode (nibblers)	1.848	0.765	2.417	**0.016**
Food acquisition mode (surface pickers)	0.205	0.955	0.214	0.830

Showing coefficient estimates of explanatory variables, standard error (SE), test statistic (z-value), and the *p*-value (*p*). Coefficient in bold indicates that *p*-value is significant at $p < 0.05$ level. Reference level for this regression was set as “Grazers” for food acquisition mode.

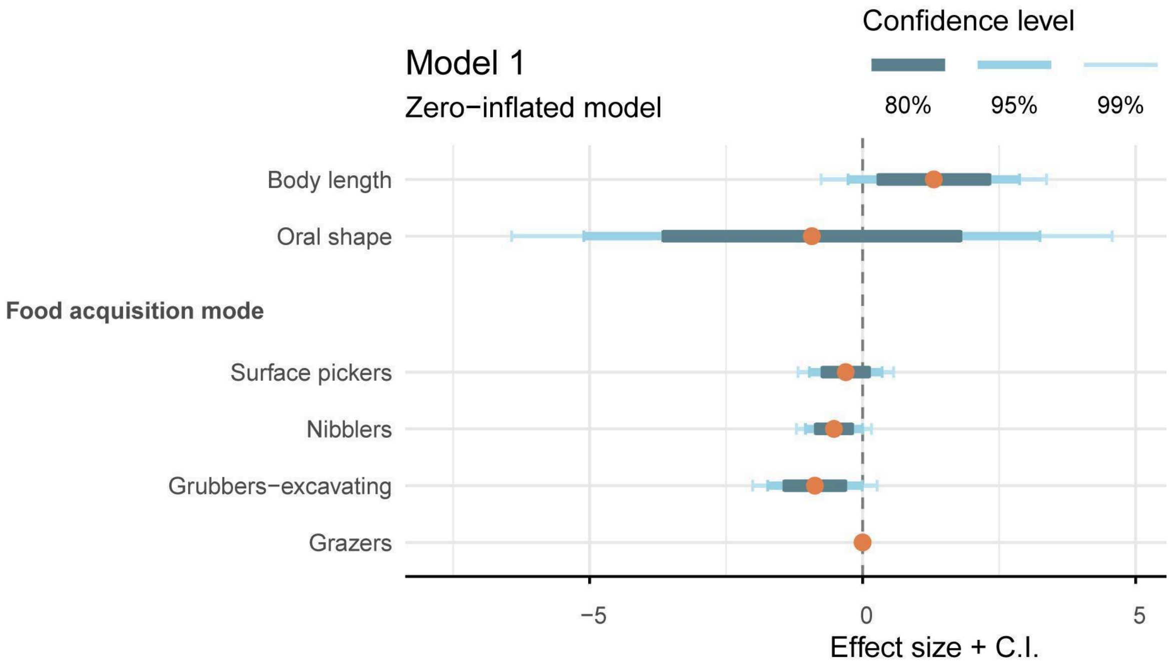

Figure S1: Effect sizes and confidence interval at three levels (80, 95, and 99%) of significance resulted from the zero-inflated Poisson generalized linear model testing the effect of body size, oral shape, and food acquisition mode on the number of debris ingested.

Table S2: Parameters estimates of the Model 2 (M2) for the final zero-inflated generalized linear model for the association between traits of the reef fishes, species ID, and number of debris ingested as the response variable.

Variable	**Estimate**	**SE**	***z*-Value**	***p***
ZIP model				
Count process (Poisson distribution)				
Intercept	-10.88	4.848	-2.245	**0.025**
log Body size	1.988	0.882	2.254	**0.024**
log Oral shape	3.157	2.748	1.149	0.251
Species (*Halichoeres poeyi*)	-1.765	0.757	-2.332	**0.020**
Species (*Pseudupeneus maculatus*)	-0.968	0.477	-2.028	**0.043**
Species (*Sphoeroides greeleyi*)	1.376	0.456	3.018	**0.003**
Species (*Stegastes fuscus*)	0.372	0.358	1.040	0.299
Zero-inflation model coefficients (binomial logit link)				
Intercept	-1.082	0.733	-1.475	0.140
Species (*Halichoeres poeyi*)	-0.653	3.723	-0.175	0.861
Species (*Pseudupeneus maculatus*)	0.079	1.503	0.053	0.958
Species (*Sphoeroides greeleyi*)	2.468	1.078	2.288	**0.022**
Species (*Stegastes fuscus*)	-0.344	1.056	-0.325	0.745

Showing coefficient estimates of explanatory variables, standard error (SE), test statistic (z-value), and the *p*-value (*p*). Coefficient in bold indicates that p-value is significant at $p < 0.05$ level. Reference level for this regression was set as “*Abudefduf saxatilis*” for species.

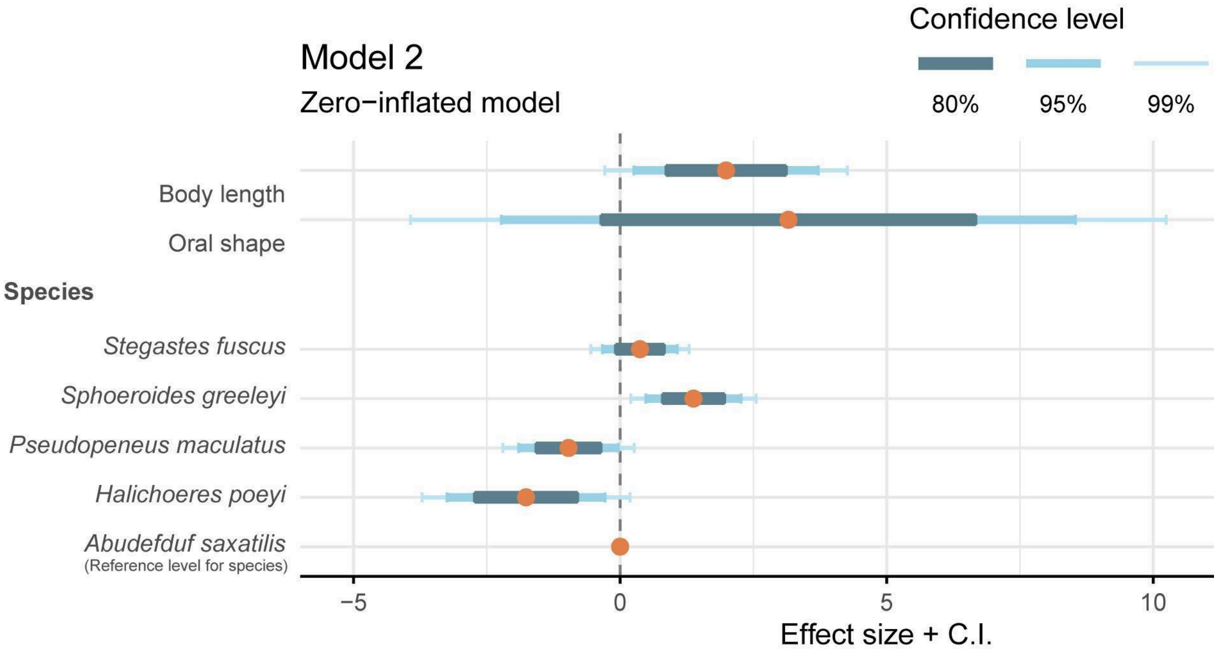

Figure S2: Effect sizes and confidence interval at three levels (80, 95, and 99%) of significance resulted from the zero-inflated Poisson generalized linear model testing the effect of body size, oral shape, and species ID on the number of debris ingested.

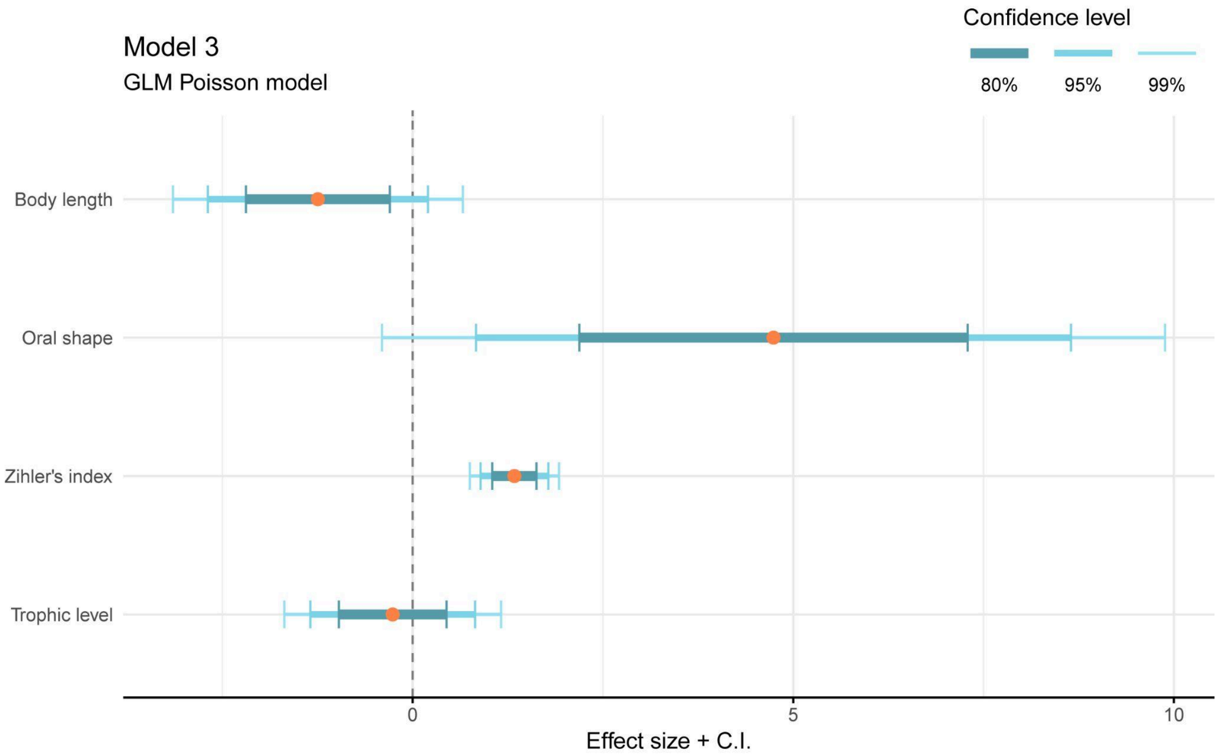

Figure S3: Effect sizes and confidence interval at three levels (80, 95, and 99%) of significance resulted from Poisson generalized linear model testing the effect of body size, oral shape, Zihler's index and trophic level on the number of debris ingested.

8. CONCLUSÕES GERAIS

No presente estudo nós confirmamos a contaminação por microplásticos em diversas camadas no ambiente marinho. Foram encontrados MP’s nas cinco espécies analisadas (*A. saxatilis, H. poeyi, P. maculatus, S. greeleyi* e *S. fuscus*) e nas três matrizes ambientais (sedimento, água e matriz de algas epilíticas - MAE). A fibra foi a forma mais abundante em todas as amostras e as cores azul, preto e transparente foram as mais comuns. A taxa média de ingestão variou entre 0,48 a 3,36 MP’s/ind., e as espécies *A. saxatilis* e *S. fuscus* tiveram as maiores taxas.

Aqui quantificamos pela primeira vez a contaminação por MP’s na MAE, importante substrato marinho. Dentre as matrizes ambientais, a água e a MAE apresentaram maior contaminação quando comparado ao sedimento, resultado que é condizente com o que foi encontrado nas espécies, indicando que as características dos peixes marinhos são também importantes para compreender a bioacumulação de MP’s em diferentes níveis da cadeia trófica.

O tamanho corporal dos indivíduos é uma característica importante na ingestão de MPs, espécies maiores tendem a ingerir mais detritos plásticos. Nossas análises também mostraram que as espécies com bocas e intestino maiores estão mais propensas a ingerir MP’s. Já as espécies com estratégias de alimentação “grubbers-excavating” e “nibblers” são menos propensas a ingerir estas partículas. Os resultados deste estudo podem ajudar a gerar medidas prioritárias de manejo, conservação e programas de interação social para a conscientização quanto aos efeitos dos microplásticos nos ecossistemas recifais e na RESEX-Mar de Arraial do Cabo.