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Assessing plastic pollution in Brazilian aquatic ecosystems: an integrated study of pollution in organisms and environments

Maringá 2024

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Tese apresentada ao Programa de Pós-Graduação em Ecologia de Ambientes Aquáticos Continentais do Departamento de Biologia, Centro de Ciências Biológicas da Universidade Estadual de Maringá, como requisito parcial para obtenção do título de Doutora em Ecologia e Limnologia.

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Avaliando a poluição plástica em ecossistemas brasileiros: um estudo integrado da poluição em organismos e ambientes

RESUMO

Plásticos são contaminantes onipresentes no planeta, acumulando-se dos polos ao equador, desde ambientes pristinos em grandes altitudes até bacias oceânicas profundas. Investigou-se como a ingestão de plásticos por peixes de água doce é influenciada pelas variações sazonais de uma planície de inundação, bem como as tendências e lacunas da pesquisa brasileira em relação a poluição plástica. Investigou-se também a possível relação entre atividades antropogênicas e as quantidades de plástico encontradas nos ambientes aquáticos brasileiros. Das 23 espécies de peixes analisadas na planície de inundação do alto rio Paraná, nove ingeriram plásticos, e as partículas encontradas são associadas com a atividade pesqueira e o lixo doméstico. A sazonalidade promovida pelo ciclo hidrológico desempenhou um papel importante nas quantidades de plásticos ingeridas por essas espécies, onde o maior número de partículas foi registrado durante o período de cheias. Em relação as tendências e lacunas da pesquisa brasileira sobre a poluição plástica, encontrou-se um grande número de publicações para ambientes marinhos, microplásticos e peixes. Ambientes de água doce e invertebrados permanecem pouco estudados no país. Considerando a distribuição dos estudos dentro dos biomas brasileiros, Pantanal e Cerrado foram os biomas com o menor número de publicações, um fato preocupante visto os importantes rios e bacias que estes abrigam. Por fim, os modelos não encontraram correlações entre a quantidade de plásticos e as atividades antropogênicas dos municípios brasileiros. A ausência dessas relações pode estar relacionada às limitações do conjunto de dados, especificamente devido ao baixo número de estudos com dados disponíveis. Entretanto, outros fatores influenciaram as quantidades de plásticos encontradas. Em amostras bióticas, o número de plásticos ingeridos esteve associado ao grupo animal, sendo os répteis, aves e peixes os mais afetados. Para amostras abióticas, o tipo de ambiente foi um fator influente. Em amostras de sedimento o número de plásticos foi maior para ambientes estuarinos e de água doce. Para as amostras de água, o número de plásticos foi maior apenas para ambientes de água doce. Devido aos efeitos negativos da poluição plástica e à importância econômica e ecológica das espécies afetadas, os resultados desse estudo representam um passo importante na avaliação dos impactos gerados nas populações de peixes de água doce pela ingestão de plásticos. Espera-se que estes resultados contribuam para o direcionamento de novas pesquisas em relação a poluição plástica nos ambientes aquáticos brasileiros, e que estes estudos expandam nosso conhecimento sobre a dinâmica dos plásticos em ambientes de água doce, assim como sua interação com diferentes organismos.

Palavras-chave: poluição aquática; ambientes de água doce; ingestão de plástico; revisão sistemática; impactos antropogênicos.

Assessing plastic pollution in Brazilian aquatic ecosystems: an integrated study of pollution in organisms and environments

ABSTRACT

Plastics are pervasive contaminants worldwide, accumulating from the poles to the equator, spanning pristine environments to deep ocean basins. This work explores how seasonal variations in a floodplain influence the ingestion of plastics by freshwater fish and systematically examines trends and gaps in Brazilian research related to plastic pollution. Additionally, the potential relationship between anthropogenic activities and the amounts of plastic found in Brazilian aquatic environments was investigated. Among the 23 fish species analyzed in the Upper Paraná River floodplain, nine were ingested plastics, and the particles were associated with fishing activity and domestic waste. Seasonality played a crucial role in the amounts of plastic ingested by these species, with the highest number of particles recorded during the wet season. As for trends and gaps in Brazilian research on plastic pollution, a substantial number of publications related to marine environments, microplastics, and fish was identified. Conversely, freshwater environments and invertebrates are underexplored. Considering the distribution of studies within Brazilian biomes, the Pantanal and Cerrado had the lowest number of publications — an alarming trend considering the significant rivers and basins they house. Finally, the models were unable to find strong and significant correlations between the number of plastics and anthropogenic activities in Brazilian municipalities. The lack of significant relationships may be attributed to the limitations of our dataset, specifically due to the low number of studies. Nevertheless, other factors influenced the quantities of plastic detected. In biotic samples, the number of ingested plastics was influenced by the animal group, with reptiles, birds, and fish being the most affected groups. For abiotic samples, the type of environment emerged as a significant factor. In sediment samples, the quantity of plastics was higher in estuarine and freshwater environments. In water samples, the quantity of plastics was higher exclusively in freshwater environments. The finds of this work contribute to new research on plastic pollution in Brazilian aquatic environments, expanding the understanding of the dynamics of plastics in freshwater environments and their interaction with various organisms.

Keywords: aquatic pollution; freshwater environments; ingestion of plastics; systematic review; anthropogenic impacts.

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1 GENERAL INTRODUCTION

Plastic is a pervasive contaminant that has become ubiquitous in our world, accumulating from the poles to the equator, from pristine freshwaters in high altitudes to deep ocean basins (Barnes et al., 2009; González-Pleiter et al., 2020; Borrelle et al., 2020). Now, we are living in the "Plastic Age", where plastic infrastructure and products are widely used and have a significant impact on all facets of our lives (Osborn & Stojkovic, 2014). Nowadays, plastics are manufactured for several applications in many industries, such as cosmetics, fisheries, and automobilists (Barnes et al., 2009; Lebreton et al., 2017). The wide applications of this material are due to several characteristics they may present, like resistance, durability, and flexibility (Thompson et al., 2009; Geyer et al., 2017). However, the same characteristics that make plastics materials useful are responsible for the accumulation and prevalence of this pollutant in many ecosystems, especially aquatic ones.

Generally plastic pollutants often accumulate in aquatic environments, which result in several effects on ecological, social, and economic aspects (Lima et al., 2020; Thushari & Senevirathna, 2020). These effects can include the quality deterioration of drinking water and food safety, declining in touristic activities, and threats to biodiversity (Carvalho et al., 2015; Ferraz et al., 2020; Thushari & Senevirathna, 2020; Kasavan et al., 2021). Plastic pollution in marine environments have been well documented in the scientific literature. However, freshwater and estuarine environments are polluted in a similar proportion, and still lack substantial information (Blettler et al., 2018; Garcia et al., 2020). In fact, rivers and estuaries transport plastics from land based sources to the open ocean but also act as depositories of this pollutant for certain periods of time (Lima et al., 2020; van Emmerik et al., 2022).

Animal species inhabiting these environments are also affected by plastic pollution, mainly by entanglement or ingestion of particles (Sigler et al., 2014, Blettler & Mitchell, 2021). The interactions between aquatic biota and plastics have motivated the assessment of different taxa, and many species had been used as bioindicators of plastic pollution, depending on its biological and ecological aspects (Reboa et al., 2022). Sessile species, such as mollusks, and threatened species, such as sea turtles, are considered excellent bioindicators (Fossi et al., 2018). Among other animal groups affected by plastic pollution, fish has raised as good bioindicator. They are of the most studied taxa worldwide, due their commercial interest, ecological role, wide spatial distribution, and

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diverse feeding habits (Azevedo-Santos et al. 2019; Reboa et al., 2022). Globally, the ingestion of plastics was reported for 427 fish species, in which only 17% are from freshwater environments (Azevedo-Santos et al., 2019). These data highlights that both freshwater environments and species are somewhat neglected regarding plastic pollution.

Like in many other regions of the world, in some Brazilian locations garbage and solid waste are disposed in improper areas, such as dump sites and river margins, due to the lack of adequate supervision (Azevedo-Santos et al., 2021; Sodré et al., 2023). In 2010, Brazil established the National Solid Waste Policy (Brazil, 2010), a law that aimed to meet several goals of increasing recycling and reducing solid waste, including plastics. However, despite this political approach, the nation does not adequately monitor the management of over 60% of its plastic waste output and only 1% of the plastics produced is properly recycled (Pincelli et al., 2021; Trindade et al., 2023). In addition to the absence of monitoring, in the last years Brazilian government (from 2018 to 2022) did not adopt the international recommendations proposed to reduce and manage plastic waste (Lima et al., 2020).

Due to its hazardous and pervasive effects, plastic pollution was included in two initiatives of United Nations: The Sustainable Development Goals (SDG, goal 14 – Life bellow water) and in the Ocean Decade (2021 - 2030) (da Costa et al., 2022), which motivate scientists around the world to improve and expand our knowledge about this pollutant. Even with the global increase in research about plastic pollution in the last decade (Kasavan et al., 2021), we still face many challenges in understand the dynamics and interactions of this pollutant in aquatic environments, especially in Brazil. Large-scale and national datasets cover wide spatial and temporal scales and may be powerful tools to address the problem of plastic pollution in a holistic approach (van Emmerik et al., 2023). This kind of approach may provide a better understanding of the knowledge gaps and research bias, helping to direct the focus of future research and the actions of stakeholders.

This thesis consists of three papers assessing the dynamics and interactions between plastic pollution and organisms and environments. The first paper investigates the ingestion of plastics by carnivore fish from the Upper Paraná River floodplain, assessing the correlation between species and different particle types and the possible relationships between the abundance and occurrence of plastics in diet and seasonality. In the second paper, the current state of research about plastic pollution in Brazilian aquatic environments was assessed through a systematic literature review, evaluating the number of publications on several aspects related to this topic. Finally, in the third paper, we investigated the influence of local anthropogenic activities, animal groups and type of environment on the abundance of plastics reported for Brazilian aquatic environments.

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2 PLASTIC INGESTION BY CARNIVORE FISH IN A NEOTROPICAL FLOODPLAIN: SEASONAL AND INTERSPECIFIC VARIATIONS

ABSTRACT

Some studies have shown that freshwater ecosystems are polluted in a similar proportion to marine ecosystems, however, there are many gaps to be filled in this topic. Here we investigated whether plastics were consumed by carnivore fishes in a Neotropical floodplain and whether it was connected to seasonality (dry and wet seasons). We also evaluated the association between each type of plastic and the fish species. We analyzed the gastrointestinal contents of 23 species and assessed the occurrence and number of plastic particles. Plastics were obtained through chemical digestion and the spectrum of each sample, using a FT-IR imaging microscope. We performed a correspondence analysis (CA) with plastic data to assess the relationship between each type of plastic and the fish species. We also performed linear regression models to assess the relationships of occurrence and number of plastics ingested with seasonality. Nine species had plastics in their gastrointestinal contents, and they were identified as Polyvinyl Alcohol (PVA), Polyamide (PA), Polyethylene (PE), Polystyrene (PS) and Polypropylene (PP). The number of plastics had a positive relationship with the wet season, while the occurrence did not show a significant relationship with any season. These results are particularly important when considering the socioeconomic relevance and the ecological importance of this trophic guild.

Keywords: Freshwater Environment; Aquatic Pollution; Anthropic Impacts; Upper Paraná River; Feeding Ecology; Top Predators.

2.1 Introduction

As cities grow, activities near or within aquatic environments tend to intensify and become point sources of solid waste, mainly plastic, for these environments (Collicutt et al. 2019). Assessing the potential risks of pollutants released into the environment is an important aspect for management and conservation, especially of aquatic ecosystems (Tlili et al. 2016), since many pollutants are often discharged close to aquatic environments. Plastic materials have become very popular due to their low production and marketing cost, high durability and strength (Thompson et al. 2009). Once they enter aquatic environments, plastics can persist for decades due to their physical characteristics (Barnes et al. 2009). These characteristics, allied to the technological advance, promote an increase in its production. In 2021, 390 million tonnes were produced worldwide and this amount is expected to double over the next two decades (Geyer et al. 2017; PlasticsEurope 2022).

Some studies have shown that freshwater ecosystems are polluted in a very similar proportion to marine ecosystems (Biginagwa et al. 2016; Slootmaekers et al. 2019). Despite this, there are still few studies and many gaps to be filled in this topic, especially in floodplain systems (Blettler et al. 2017; Scheurer and Bigalke 2018), which are characterized by fluvial variation. Large fluctuations in water level are usually monomodal and predictable in large floodplains because they are part of the wet and dry seasons cycle (Junk et al. 2014). Because plastics are carried to water bodies through river drainage and atmospheric deposition, as is the case of microplastics, particles with size ranging between 1µm and 5 mm (Barnes et al. 2009; O'Connor et al. 2022), the presence of this material can show a seasonal pattern, such as an increase in its concentration in rainy seasons (Pazos et al. 2017; Pelamatti et al. 2019). Stormwater runoff can be a significant source of macroplastics and microplastics during rainfall events, and the increase in flow during floods can relocate sediments, and consequently particles that were already sedimented (Hurley et al. 2018; Treilles et al. 2022), making them available to the fishes and other organisms.

Plastic ingestion has already been reported for approximately 427 fish species worldwide (Azevedo-Santos et al. 2019), and has been much discussed in the last decade for species in marine environments (Dantas et al. 2012; Ory et al., 2017, Cardozo et al. 2018; Ferreira et al. 2018). Recent studies also report plastic ingestion by freshwater species (Silva-Cavalcanti et al. 2017; Andrade et al. 2019; Garcia et al. 2020; Lima et al.

2021). It is also known that different dietary habits can directly impact the intake of plastics by fish (McGoran et al. 2018; Andrade et al. 2019), since differences in prey utilization can increase the probability of accidental ingestion of these particles. Ingestion of plastics can lead to significant reductions in fish populations (Boerger et al. 2010), given that the effects of the interaction of plastic and wildlife are many. In general, larger particles, such as macroplastics (particles bigger than 20 mm) and mesoplastics (particles with size ranging between 5 and 20 mm) (Barnes et al. 2009), can cause lacerations and also cause animals to starve, as they are not digested and accumulate in the gastrointestinal tract, causing a constant feeling of satiety (Rummel et al. 2016; Cardozo et al. 2018). Microplastics, of primary or secondary origin (due to fragmentation of larger particles, Lehtiniemi et al. 2018), can have toxic effects, since these residues adsorb pollutants from the environment, including persistent bioaccumulative and toxic substances (PBTs), persistent organic pollutants (POPs), drugs and heavy metals (Barnes et al. 2009; Rochman et al. 2013; Prata 2018). With plastics as vectors, these substances can accumulate in organs and tissues, leading to lethality, or passing to the next trophic levels, impacting the entire trophic chain (Rochman et al. 2013; Silva-Cavalcanti et al. 2017).

Carnivore fish species represent one of the main consumer groups in freshwater ecosystems and occupy the highest trophic position (Barbosa et al. 2017), in addition to playing a fundamental ecological role in the communities in which they are inserted. In Neotropical aquatic environments, carnivores fish represent a high percentage of the total biomass (Pereira et al. 2017). For many carnivore fish species, piscivory is an obligatory habit, while for others it can be an opportunistic habit, depending on the availability of resources (Pereira et al. 2017). As top predators in the chain, carnivore fish species regulate and stabilize fish community, modifying the composition and balance in the food chain, promoting implications like the cascade effect (Barbosa et al. 2017; Pereira et al. 2017; Soe et al. 2021). In addition to their ecological role, many carnivore fish species are also important resources for fisheries, whether economic or recreational (Rojo et al. 2019). For these reasons, many studies have shown that carnivore fish species are the most threatened by industrial-scale fishing (Myers and Worm 2003), mainly in marine environments, and more recently by the ingestion of plastics debris (Dantas et al. 2012; Ferreira and Barletta, 2016). The ingestion of plastics by these species can take different routes, depending on the feeding behavior of each species.

Some studies address that carnivore fish species only ingest plastics passively or indirectly, through the consumption of prey from other trophic levels (Ribeiro et al. 2019; Parker et al. 2020), with contamination being transferred from prey to the predator (Erikson and Burton 2003). However, these particles can also be ingested during predation, when predators feed on small prey aggregated in places with greater "availability" of plastics, consuming accidentally, since the predator's focus will be on multiple targets (Romeo et al. 2015). Considering the important role of carnivore fish for the ecosystem and for economic activities, this study aims to evaluate the ingestion of plastics in carnivore fish species from a floodplain ecosystem, answering the following questions: i) Is there ingestion of plastic particles by the carnivore fish species of the upper Paraná River floodplain? ii) Is there a correlation between the species and the different types of plastics ingested? iii) Is there a relationship between plastic ingestion (occurrence and number of particles) and the seasonality (dry and wet seasons) promoted by the hydrological regime?

2.2 Material and Methods

2.2.1 Study area and sampling

The studied area was the Upper Paraná River floodplain, located between the confluence of the Paranapanema and Ivinhema rivers (Brazil) (Agostinho et al. 2008), which constitutes the last undammed stretch of the Paraná River in Brazilian territory, with 230 km of extension. It presents a great diversity of habitat, which includes the alluvial plain with numerous secondary channels, connected and isolated lagoons and the main channels of the Paraná, Baía and Ivinhema rivers, in addition to a high diversity of terrestrial and aquatic organisms (Agostinho et al. 2004, 2007). In this region fishing, tourism, and agriculture activities are developed throughout the year (Tomanik et al. 2009).

The fish used in this study were collected by the Long Term Ecological Research program (Brazil LTER –PIAP) from the Upper Paraná River floodplain. Fish were sampled quarterly from March 2019 to March 2020, in nine locations (rivers and lagoons), located in the Paraná, Baía and Ivinhema rivers (Figure 1). The wet period usually occurs from November/December to April/May, with maximum hydrometric levels prevailing between January and March, and the dry period occurs between June and October with minimum values between July and September (Agostinho et al. 2004). Fish were

collected with gillnets with different mesh sizes, which were exposed for 24h, and checked every 8 hours. Then, they were anesthetized with benzocaine and euthanized according to ethical practices (CEUA nº 1420221018 (ID 001974)). Measurements of total and standard length, and weight were taken. After that, the stomachs and intestines were removed and stored in 4% formalin for further analysis.

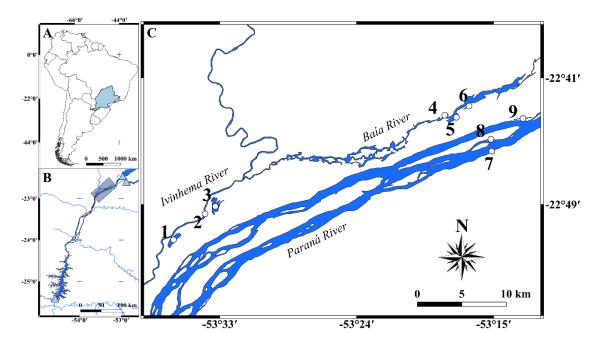


Fig 1. (a) The blue area represents the Paraná River basin. (b) Upper Paraná River. The triangles represent the reservoirs upstream and downstream of the study area. (c) Upper Paraná River floodplain. White dots represent sampling sites. 1- Ventura Lake, 2 - Ivinhema River, 3- Patos Lake, 4 – Guaraná Lake, 5- Baía River, 6: Fechada Lake, 7: Paraná River, 8: Pau Véio Backwater, 9: Garças Lake. EPSG: 4326.

2.2.2 Gut content and plastics analysis

In order to evaluate the presence of plastics, individuals with different degrees of stomach repletion (RD) were analyzed, considering individuals with RD 0 (empty stomach), RD 1 (with up to 25% of the stomach full), RD 2 (from 25 - 75% full stomach) and RD 3 (75 - 100% full stomach) (Pelicice and Agostinho 2006; Kovalenko et al. 2009). To determine the composition of the diet and to verify if the carnivore ingested plastic particles, the contents of the gastrointestinal tracts were analyzed under stereoscopic and optical microscope. For the quantification of the diet, the occurrence and the volumetric

methods were used (Hyslop 1980). The plastic particles found were also counted. The plastic fragments were stored in 70% alcohol for further analysis.

To isolate the plastic particles from residual organic matter and natural fibers, the samples of gastrointestinal content analyzed were digested with 10% potassium hydroxide solution (m/v) at 60°C for 24h (Rochman et al. 2015). The samples were filtered with a vacuum pump on a fiberglass filter (porosity of 1.2 μ m), and dried for 24h at 60°C. For the quantification and characterization of plastic particles, the filters were inspected under a stereoscopic and optical microscope. To avoid airborne contamination in gut analysis and in the digestion process based in Lusher et al (2017), we used 100% cotton lab coats and disposable latex gloves, the laboratory instruments were sanitized in distilled and filtered water. Finally, Petri dishes with fiberglass filters were placed at the beginning of each work session, in order to assess possible air contamination, so that the fibers and other particles with the same color and size contained in the filter and the found in the analyzed material were disregarded.

Each plastic item was classified according to shape (fragment, fiber or film) and size (microplastics 1 μ m -5 mm, mesoplastic 5-20 mm and macroplastics \geq 20 mm) (Barnes et al. 2009; O'Connor et al. 2022). To identify the polymer, we used the spectrum of each sample. Measurements for sample characterization were obtained using a Fourier transform infrared imaging microscope (LUMOS II, Bruker Optik GmbH). The spectra were acquired in Attenuated Total Reflectance (ATR) mode, in the spectral range of 4000-680 cm⁻¹, 8x objective, 4 cm⁻¹ resolution and 100 scans. The data were baseline corrected and the plastics were identified with the help of the OPUS 8.5 software polymer library (Bruker Optik GmbH).

2.2.3 Data analysis

Data analysis was performed only with species that presented plastics in their gastrointestinal contents and we considered all the particles found for the analysis, without separating for types or colors. To assess the relationships between the species and the ingested plastics, we applied a Chi-square test and a correspondence analysis (CA) on the plastic count matrix (number of ingested particles). The Chi-square test was applied to verify if there is a significant correlation between the different plastics identified and

the analyzed species. Following this, the correspondence analysis was performed to identify which species are correlated with each identified polymer.

To evaluate the relationship between the number and occurrence of ingested particles between different hydrological seasons, linear models were used. Before modeling, all assumptions were verified and met (linearity, normality of residuals, homogeneity of variances and overdispersion). A significance level of p < 0.05 was used for all analyses. To assess the relationship between the number of ingested plastics (number of plastic particles) and seasonality, we applied a zero-inflated regression with negative binomial errors (ZINB) on the number of ingested plastics in each species for each period. This technique performs optimally for dealing with overdispersion problems and zeros in the response variable, modeling "zeros" and "counts" differently (Martin et al. 2005; Zeileis et al. 2008). To evaluate the relationship between the occurrence of ingested plastics and seasonality we applied a generalized linear model with binomial distribution to the data on the presence and absence of plastics in each species for each period. All statistical analyzes were performed in the software R 4.0 (R Core Team 2020) using the packages "pscl", "factoextra", "FactoMineR" and the graphics were made using the package "ggplot2".

2.3 Results

In total, 394 individuals were analyzed, distributed in 23 species (Table 1). The diet of the analyzed species consisted predominantly of fish, as expected, however, some species also consumed shrimp and bivalves (Table S1).

Species	SL	Se	eason	Total	
	(cm)	Dry	Wet		
Astronotus crassipinnis	17.0 - 19.8	2	4	6	
Ageneiosus inermis	31.0 - 48.0	1	2	3	
Acestrorhynchus lacustris	13.0 - 24.5	12	34	46	
Ageneiosus ucayalensis	22.5		1	1	
Crenicichla jaguarensis	18.1		1	1	

Table 1. List of species and number of individuals analyzed in the Upper Paraná River floodplain, by season. SL = standard length.

Cichla kelberi	15.8 - 42.8	9	18	27
Cichlasoma paranaense	9.6 – 12.6	3	5	8
Galeocharax gulo	8.3 - 21.8	3	1	4
Hoplias spp.	16.5 – 36.3	44	46	90
Hemisorubim platyrhynchos	22.2 - 43.7		3	3
Hoplerythrinus unitaeniatus	16.3 – 21.7	5	1	6
Pseudoplatystoma corruscans	54.5 - 74.0	4	5	9
Potamotrygon falkneri	48.6		1	1
Pinirampus pirinampu	41.0 - 56.5		6	6
Plagioscion squamosissimus	9.5 - 46.0	5	26	31
Rhamdia quelen	16.8		1	1
Rhaphiodon vulpinus	29.0 - 56.5	5	13	18
Salminus brasiliensis	62.0 - 63.1		3	3
Salminus hilarii	17.5 - 25.2	1	2	3
Sorubim lima	35.0 - 46.0	1		1
Serrasalmus maculatus	9.0 - 26.5	1		1
Serrasalmus marginatus	7.8 - 24.9	25	77	102
Zungaro jahu	55.0	1		1

Of the 23 species analyzed, nine had plastics in their gastrointestinal contents (Table 2), and the highest number of ingested particles was found in *Serrasalmus marginatus* and *Hoplias* spp. (Table 2). Of the five samplings, only in June 2019 sampling plastics were not detected (Table S1). The volumetric percentage of plastics ingested was low for most species (Table S1), except for those that presented an empty gastrointestinal tract, with plastics detected in the digestion step as the only items consumed. This is the case of *Acestrorhyncus lacustris* and *Cichlasoma paranaense* (Table S1). The occurrence (%) of plastics ranged from 2.65% to 100% for the different species sampled (Table S1). The plastics found in the gastrointestinal contents were classified in mesoplastics and microplastics. Mesoplastics found were white films identified as polyvinyl alcohol (Table 3, Fig 2 and S1, PVA) and transparent polyamide fragments (Table 3, Fig 2 and S1, PA). Microplastics found were transparent films of polystyrene (Fig 2 and S1, PS), white films

of polypropylene (Fig 2 and S1, PP) and blue and black fibers identified as polyethylene (Table 3, Fig 2 and S1, PE). PE fibers and PA fragments were the most abundant types of plastic found (26 particles both) (Table 2).

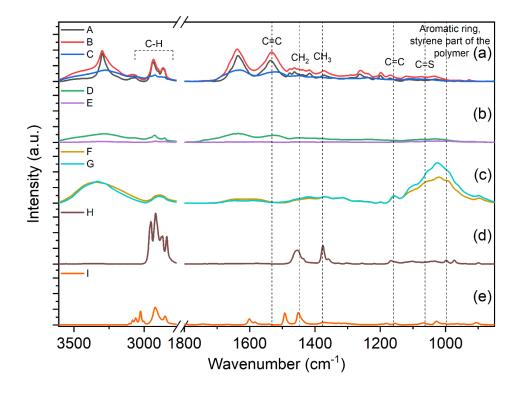


Fig 2. Spectra of plastics identified using the polymer library in the OPUS 8.5 software (Bruker Optik GmbH). Samples A, B and C were identified as polyamide, samples D and E were identified as polyethylene, samples F and G were identified as polyvinyl alcohol, sample H was identified as polypropylene and sample I as polystyrene.

Species	Sampling	PA	PVA	PE	PS	PP	Total
	mar/19	0	0	0	0	0	0
	jun/19	0	0	0	0	0	0
Acestrorhyncus lacustris	sep/19	2	0	0	0	0	2
	dez/19	0	0	1	0	0	1
	mar/20	0	0	0	0	0	0
Cichlasoma paranaense	sep/19	0	0	0	0	0	0
	mar/20	0	0	1	0	0	1
	jun/19	0	0	0	0	0	0
Galeocharax gulo	dez/19	0	0	1	0	0	1
	mar/20	0	0	2	0	0	2

Table 2. Number of plastics found in different species of carnivores fish species in the Upper Paraná River floodplain. PA – Polyamide, PVA – Polyvinyl Alcohol, PP – Polypropylene, PS – Polystyrene, PE – Polyethylene.

	mar/19	0	0	0	0	0	0
TT 1'	jun/19	0	0	0	0	0	0
Hoplias spp.	sep/19	0	1	3	0	0	4
	dez/19	0	0	3	0	0	3
	mar/20	0	0	4	0	0	4
	mar/19	0	0	0	0	0	0
Plagioscion squamosissimus	sep/19	0	0	0	0	0	0
	dez/19	0	0	0	0	0	0
	mar/20	0	0	1	0	0	1
Rhamdia quelen	nov/19	0	0	1	0	0	1
	mar/19	0	0	0	0	0	0
D 1 1 1 1 1	jun/19	0	0	0	0	0	0
Raphiodon vulpinus	sep/19	0	0	2	1	0	3
	dez/19	0	0	1	0	0	1
	mar/20	0	0	0	0	0	0
	mar/19	0	0	0	0	0	0
Serrasalmus maculatus	jun/19	0	0	0	0	0	0
	dez/19	0	0	1	0	0	1
	mar/20	1	0	0	0	1	2
	mar/19	12	0	0	0	0	12
Serrasalmus marginatus	jun/19	0	0	0	0	0	0
	sep/19	1	0	3	1	0	5
	dez/19	4	1	0	0	0	5
	mar/20	6	0	2	0	0	8
Total		26	2	26	2	1	57

Table 3. Number of plastics by shape and color in the diet of carnivore fish species in the Upper Paraná River floodplain.

Туре	Dry	Wet
Fragments		
Transparent	3	23
Fibers		
Black	5	2
Blue	3	16
Film		
Transparent	2	
White	2	1

The chi-square test, performed prior to the correspondence analysis, identified a high and significant correlation between the different polymers identified and the analyzed species (X-squared = 57.23; p = 0.004). Correspondence analysis (CA), performed in order to investigate associations between species and polymers, explained approximately 86.9% of the variation in the data (Fig 3). It is possible to observe a strong relationship between *S. marginatus* and *A. lacustris* with the PA fragments and between *C. paranaense*, *G. gulo*, *Hoplias* spp., *R. quelen* and *R. vulpinus* with the PE fibers (Fig 3). Finally, *S. maculatus* showed a moderate association with polypropylene fragments, as it was the only species in which this polymer occurred and with low number (Fig 3). PS and PVA were also the least abundant polymers (Table 2) and, for this reason, they did not show a strong association with the analyzed species (Fig 3).

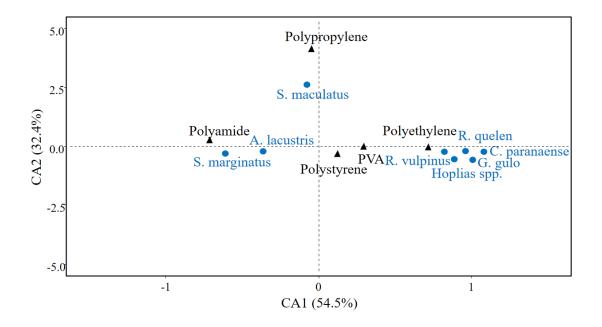


Fig 3. Correspondence Analysis (CA) for the interactions between the polymers found in the gastrointestinal contents and the carnivore species analyzed in the Upper Paraná River floodplain. *A. lacustris = Acestrorhynchus lacustris; C. paranaense = Cichlasoma paranaense; G. gulo = Galeocharax gulo; Hoplias spp.; R. quelen = Rhamdia quelen; R. vulpinus = Rhaphiodon vulpinus; S. maculatus = Serrasalmus maculatus; S. marginatus = Serrasalmus maculatus.*

The average number of plastic particles consumed per species was higher in the wet season (1.95 ± 0.64) when compared to the dry season (1.16 ± 1.85) (Fig 4a). The

relationship between the number of plastics and seasonality, evaluated through a zeroinflated model (Count-model), was much greater and significant for the wet season (intercept, p = 0.0198) than for the dry season (dry, p = 0.82). (Table 4). The absence of plastics (Zero-model) was not significant for any of the two periods (Table 4). As for the number, the average occurrence of plastics consumed by species was higher in the wet season (13.42% ± 5.12) when compared to the dry season (6.06% ± 3.11) (Fig 4b). The relationship between the presence of plastics and seasonality was greater for the wet season than for the dry season (Table 5). However, none of the predictors was significant (intercept, p = 0.827; dry, p = 0.295). Thus, despite a higher average of plastic particles found in the wet season, there is no seasonal statistical difference in the occurrence of plastics in the gastrointestinal content of the evaluated species.

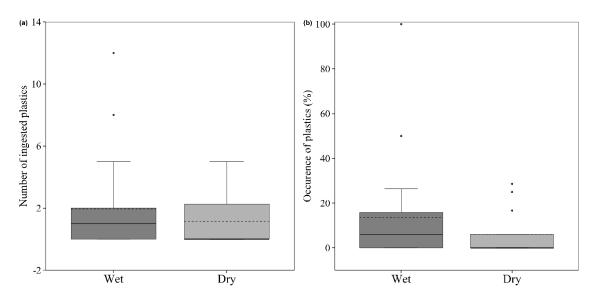


Fig 4. a) Number and b) Percentage of occurrence of plastics consumed by species in the wet and dry periods in the Upper Paraná River floodplain. The lower and upper ends of the boxplot represent the 25th and 75th quartiles, respectively. The horizontal and dotted lines within each box represent the median and mean, respectively, excluding outliers, which are represented by black dots.

	Parameter Estimation	Std. Error	Z-value	<i>p</i> -value
Count-Model				
Intercept	0.6703	0.2877	2.33	<i>0.019</i> 8*
Dry	0.1565	0.7232	0.216	0.8287
Log (theta)	-0.2667	0.4764	-0.56	0.5756
Zero-Model				
Intercept	-8.921	92.038	-0.097	0.923
Dry	8.879	92.025	0.096	0.923

Table 4. Results of the zero-inflated model with negative binomial distribution for errors (ZINB)
 for the differences between the wet and dry periods in the number of ingested plastics.

Table 5. Results of the generalized linear model with binomial distribution for the differences between the wet and dry periods in the presence and absence of ingested plastics.

	Parameter Estimation	Std. Error	Z-value	<i>p</i> -value
Intercept	0.09531	0.43693	0.218	0.827
Dry	-0.78846	0.75227	-1.048	0.295

2.4 Discussion

In this study, of the 23 species of carnivores analyzed, nine species had plastics in their gastrointestinal contents. The identified polymers were Polyvinyl Alcohol (PVA), Polyamide (PA), Polyethylene (PE), Polystyrene (PS) and Polypropylene (PP) and there was a high and significant correlation between the identified polymers and the analyzed species. The quantity of these polymers had a positive and significant relationship with the flood seasons, while the occurrence did not show a significant relationship with the flood seasons.

Studies related to marine environments often consider rivers as major sources of plastic for the oceans (Meijer et al. 2021). Rivers are described as efficient routes for transporting waste to marine environments, taking not only materials generated in coastal

areas to the open sea, but also transporting waste generated in continental areas over large distances (Castro-Jimenez et al. 2019; Meijer et al. 2021). Several factors contribute to the direct or indirect entry of waste into rivers, and population density and urban development close to these environments are the most expressive (Schirinzi et al. 2020). Another important point is that the largest rivers in the world are located in developing countries, where large discharges, the size of the basins and poor sanitary conditions are factors that increase the amount of plastic waste present in rivers (Blettler et al. 2019). This work brings one of the first reports on the occurrence and ingestion of plastics in the Upper Paraná River floodplain, a stretch that, despite being located within environmental protection areas, is still an integral part of the Upper Paraná River basin, located in one of the most populous and urbanized regions of Brazil (Rudke et al. 2019).

The five different types of plastics found in the gastrointestinal contents of the species evaluated may reflect the reality of the activities carried out around the environments in which they were sampled (Dantas et al. 2012; Ramos et al. 2012; Cardozo et al. 2018; Monteiro et al. 2022). PE, PS and PP are non-biodegradable polymers commonly used in packaging, toys and other household items (Farias et al. 2018; Freire 2019; Turner 2020; Monteiro et al. 2022). Their presence in the samples may reflect an increase in local urbanization and domestic activities and highlights the lack of management and inadequate disposal of plastic waste in this region. PE fibers were found only in blue and black colors, and their ingestion could be related with a visual attraction, since they are less contrasted and bright than other colors (Ory et al. 2018; Ríos et al. 2022). Garcia et al (2020) found a greater amount of fibers, mainly due the presence of a textile factory near the sampling sites, however, this is not the case for our study area. The presence of these fibers could be due to effluents of households (Hernandez et al. 2017) and the wear of fishing gears over time (Monteiro et al. 2022). PS and PP were the less abundant polymers and were found in film form. They are commonly used in packaging (Monteiro et al. 2022) and probably are a secondary microplastic product of an advanced breakdown process (Blettler et al. 2019). These polymers were found in transparent and white colors, and their ingestion could be accidental, once fishes avoid these colors (Ory et al. 2018; Ríos et al. 2022).

PA fragments, often referred to in the literature as "nylon", were also abundant in the samples. The incidence of these fragments is a clear example of the relationship between the proximity of the source of pollution and the environmental consequences (Possato et al. 2011). PA is commonly used in the manufacture of fishing gear, which indicates an important contribution of fishing activity to plastic pollution (Dantas et al. 2012, 2019). PA fragments have a strong impact on fish due to their large size and their adherence to the gastrointestinal tract (Cardozo et al. 2018). Among the various negative effects caused by this polymer in fish, the sensation of constant satiety and subsequent starvation stand out, making them easy prey and with worse nutritional and body condition (Possato et al. 2011; Rummel et al. 2016; Cardozo et al. 2018; Dantas et al. 2019).

The presence of polyvinyl alcohol (PVA) in the gastrointestinal contents is a curious fact. PVA is a water-soluble polymeric resin, mainly used in the treatment of textile materials and papers (Al-Mamun and Chen 2022). Furthermore, it is commonly used in fishing activities, where the bait is placed in PVA bags, which dissolve in water while the baits remain concentrated on the surface (Al-Mamun and Chen 2022). The occurrence of this polymer may be associated with sport/recreational fishing activities that occur in the region, and may also indicate neglected pollution. Despite being a biodegradable polymer, PVA can be considered an emerging form of plastic pollution, integrating the so-called "liquid plastics" or Water-Soluble Polymers (WSPs) (Nigro et al. 2022). These polymers, which are soluble under certain temperature or pH conditions, may become insoluble if these conditions change (Nigro et al. 2022), and remain in the environment. Although no ecotoxicological effect of PVA has been found on aquatic organisms (Nigro et al. 2022), the fact that particles of this polymer are available and ingested by the biota, possibly due to its insolubility under certain conditions, draws attention.

Meso and microplastics were found in similar amounts in the gastrointestinal contents of the analyzed species, indicating that polymers of different sizes are available to the biota. The mesoplastics found (PA and PVA) are directly related to fishing activities and used in specific fabrications (Cardozo et al. 2018; Dantas et al. 2019; Al-Mamun and Chen 2022), can be considered primary microplastics since they are not the result of the fragmentation of macroplastics due to natural causes (Garello et al. 2021), unlike the microplastics found (PE, PS and PP), which are certainly of secondary origin (Weinstein et al. 2016; Blettler et al. 2019). Plastic items such as bags, bottles, lids and packaging constitute a large part of the macrowaste floating in rivers and their polymeric composition and environmental conditions are determining factors in the

depolymerization process (Castro-Jiménez et al. 2019). Through the physical actions of floods and winds (Garello et al. 2021), microbial activity and ultraviolet rays (Wagner and Lambert 2018), these polymers can degrade and fragment into small pieces that are transported to different areas due to their low density and polymeric composition (Monteiro et al. 2022).

The gastrointestinal contents of fish can be an important indicator of the extent of pollution in individuals foraging areas (Dantas et al. 2019), and the different forms of foraging can represent important routes of ingestion of plastics. The polyamide fragments were highly correlated with the piranhas (S. marginatus and S. maculatus) and the dogfish (Acestrorhynchus lacustris), and despite being classified as carnivore species, they have very different foraging strategies. The predation behavior of piranhas consists mostly of biting other fish, leading to the acquisition of small fragments of muscles and fins, rather than killing their prey and ensuring rapid renewal and stability of food resources (Ferreira et al. 2014; Alves et al. 2017). However, this opportunistic form of feeding and the voracity of piranha species (Deprá et al. 2021) can enhance the consumption of PA fragments because they can prey on small prey caught in gillnets, which become easy targets, leading to PA intake during the feeding process. Besides the strong artisanal fisheries in this region, it is important to point out that the nets used in our samplings are made of PA and can attract these species since the captured fish are vulnerable to piranha attacks. In this case, our sampling methods could be a source of PA in these species as well.

A. lacustris, in turn, pursues its prey, feeding on small fish that inhabit and forage in macrophyte stands and the water column (Hahn et al. 1999; Silva and Goiten 2009). Among the species preyed upon by *A. lacustris* are some Characiformes such as species of the genera Hyphessobrycon, Serrapinus and Astyanax (Silva and Goiten 2009), species commonly found in macrophyte stands (Prado et al. 2016; Quirino et al. 2021). In addition to fish, the stomachs analyzed in this study also contained shrimp and plant remains, indicating the foraging activity of *A. lacustris* within or close to macrophyte stands. Although fishing activities in the region are not common near these stands, the PA fragments accidentally ingested by *A. lacustris* may come from "ghost nets", resulting from incorrect disposal or loss of fishing nets. A recent study by Azevedo-Santos et al. (2022) reports a large number of occurrences of these nets in the Upper Paraná River and also highlights that as the nets degrade, fish can ingest these plastic fragments. Due to the transport dynamics of rivers, these nets can end up trapped in macrophyte stands and the riparian vegetation (Newbould et al. 2022), where they undergo a process of fragmentation and become available to several organisms.

The other evaluated species showed high consumption of microplastics, mainly PE. This is an interesting result, since even less abundant species, such as C. paranaense and Galeocarax gulo, with few individuals sampled, presented plastics in their gastrointestinal contents (Table S1). These plastics were probably ingested during predation, as fish can consume up to three times as much plastic when they are near food resources (Rios et al. 2022). The carnivores species analyzed generally consume small fish, many of them inhabit macrophyte stands, where they are usually aggregated in search of shelter and food (Warfe and Barmuta 2006; Pereira et al. 2017). In addition small fish are usually aggregated (Romeo et al. 2015), making it difficult to distinguish among plastic particles. The increase in voracity during the predation action can result in an active consumption of plastic particles, without their discrimination of real food (Rios et al. 2022). However, the ingestion of these microplastics can also result from a secondary ingestion, *i.e.*, the ingestion of plastics that have already been ingested by prey. The transfer of microplastics between trophic levels has already been demonstrated in some studies (Kim et al. 2018; Nelms et al. 2018). Considering the importance of piscivores, not only ecological, but also socioeconomic, the study of plastic ingestion by this group is quite important. As mentioned earlier, many of these species are targeted by fisheries, whether recreational or professional (Rojo et al. 2019), and the largest fisheries in continental waters are located in the most polluted rivers (Lebreton et al. 2017; Blettler et al. 2017, 2019).

Plastic pollution found on the margins and floodplains of rivers can have different origins, such as the dumping of garbage in inappropriate areas, leakage of industrial waste, in addition to natural conditions such as wind, surface runoff and natural disasters that carries waste (Schirinzi et al. 2020; van Emmerik et al. 2022). However, regardless of their origin, the combination of the dynamics and characteristics of the rivers are fundamental factors for the deposition of plastics on the margins and floodplains, since these can be trapped in the riparian vegetation, sediment or can still be transported to areas downstream (van Emmerik et al. 2022). Plastics can accumulate on river margins for long periods, and despite large emissions to the oceans, most of this waste remains retained, progressively polluting aquatic ecosystems (Tramoy et al. 2020; Meijer et al.

2021). Here we found a positive and significant relationship between the number of ingested particles and the wet season. Although there is no quantitative assessment of the impact of floods (that usually occurs in wet season) on plastics, some studies have found great amounts of plastics in periods with high discharges into rivers (Castro-Jimenez et al. 2019; Tramoy et al. 2021). Temporal variations in river discharge, especially floods, have a major impact on the transport and mobilization of both macro and microplastics (Meijer et al. 2021).

Garbage that remains trapped on riverbanks or entangled in vegetation can be remobilized by wind or water runoff (Schirinzi et al. 2020). In wet seasons, when river margins, floodplains and tidal areas are flooded, the plastics that accumulate in these "reservoirs" under normal conditions could be also remobilized (Garello et al. 2021; van Emmerik et al. 2022), in addition to easy entry of new plastics into the watercourse through surface runoff (Castro-Jiménez et al. 2019). Floods can produce the so-called "washing effect" on plastics, where they leave the margins and beaches of rivers, leaving a clean sediment and entering water bodies (Garello et al. 2021). Factors such as flow regulation by dams, overflow and runoff during rainfall events upstream of the basin are quite important, as they release the accumulated garbage in the basin through pulses in relatively short periods (Castro-Jiménez et al. 2019). The Upper Paraná River floodplain is in the last stretch free of dams after a long cascade of reservoirs in Brazilian territory, however the water flow in this stretch is still regulated by the Porto Primavera dam, affecting the duration and intensity of the flood events (Moi et al. 2020), factors that may be crucial for the number of plastics reported here.

In summary, we found nine different species of piscivores ingesting plastics and the amount of ingested plastics has a positive correlation with the wet period. This is particularly important when considering their socioeconomic relevance, since many of these species are key fisheries resources, as well as their ecological importance, given the function predators that this trophic guild plays as top predators. Given the terrible impacts generated on fish populations by the ingestion of plastics, more studies are needed to elucidate the interactions of biota and these polymers. In addition, this is one of the few studies conducted on a floodplain and the first for the Upper Paraná River plain on this topic, which highlights the need for future studies in these environments, due to their unique hydrological dynamics and their importance for biodiversity.

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3 EXPLORING PLASTIC CONTAMINATION IN BRAZILIAN AQUATIC ENVIRONMENTS: A SYSTEMATIC REVIEW OF RESEARCH TRENDS AND GAPS

ABSTRACT

Plastic pollution in aquatic environments is a global concern that has drawn the attention of numerous scientists in the last two decades. Studies on this subject are scarce in developing countries, where socio-economic aspects related to waste discard are often not regulated or monitored. In this paper, a systematic review of the current state of scientific research regarding the presence of plastics in Brazilian aquatic environments was conducted, to identify the trends and gaps in this topic. A total of 207 articles were found and the number of publications about plastic pollution has increased over the years. Studies on marine areas had the highest number of published studies (57.7%), and they are concentrated in the Atlantic Forest biome. However, freshwater environments exhibited a huge publication scarcity, being the least studied environment (12%). Biomes with high portions of inland waters, such as Pantanal and Cerrado, exhibited few or no publications. Studies with biotic samples were more abundant (48.6%), compared to abiotic ones, and fish were the most studied group, with 40.6% of the studies. On the other hand, invertebrates together represented less than 25% of biotic publications. The evidence found in this systematic review highlights how freshwater environments are deeply neglected in Brazilian research, likewise invertebrates. Due to the huge territorial extension of the Brazilian biomes and its unique biodiversity, there is no reason for perpetuating this negligence, especially considering the severe threat of the ongoing lack of waste management in many regions of the country.

Keywords: Plastic pollution; Brazil; Aquatic organisms; Particle size; Aquatic ecosystems; Biome.

3.1 Introduction

Plastic pollution in aquatic environments is a global concern that has drawn the attention of numerous scientists in the last two decades (Kasavan et al., 2021). Nowadays, plastic pollution affects a variety of ecosystem types, including freshwater, estuarine, and marine ecosystems, from the poles to deep ocean basins (Borrelle et al., 2020). Recently, the United Nations addressed the problem of plastic pollution in its global agenda for the Sustainable Development Goals (SDG, goal 14 – Life below water) and in the Ocean Decade (2021 – 2030) (da Costa et al., 2022), which has motivated environmental awareness and raised several recycling campaigns worldwide (Borrelle et al., 2020). Moreover, due to the relevance of this topic, plastic pollution has gained considerable attention, especially in marine environments, although freshwater ecosystems still a huge lack of scientific knowledge (Blettler et al., 2018; Blettler & Mitchell, 2021).

Plastic in aquatic environments has several ecological, economic, and social implications for society. It depreciates the quality of drinking water (Ferraz et al., 2020) and food safety from fisheries, which may lead to several social and economic problems, especially in developing countries (Thushari & Senevirathna, 2020). Plastic litter on coastlines diminishes the landscape quality and reduces the attraction for recreational activities, leading to decrease in tourism revenue (Kasavan et al., 2021). Plastics can also be trapped in fishing gears, negatively impacting fisheries productivity and profitability by increasing the expenditure on repairs and reducing fishing efficiency (Reinert et al., 2017; Kasavan et al., 2021; Pinheiro et al., 2021). Wildlife is often injured due to entanglement or ingestion of plastics (Sigler, 2014), which may lead to significant reductions in the populations of several animal groups (Boerger et al., 2010; Carvalho et al., 2015; Kasavan et al., 2021). Several species, such as sea turtles, marine mammals, and all types of sea birds, are negatively affected by the interaction with plastic litter, which may result in severe injuries or physical restrictions, ultimately leading to death (Carvalho et al., 2015; Thushari & Senevirathna, 2020).

Studies about plastic pollution are scarce in developing countries, where socioeconomic aspects related to waste management are often not regulated or monitored (Sodré et al., 2023). Some regions of the world have long been disposing waste and garbage directly into river and, only recently, through the creation of modern legislation and policies, these practices are being avoided (Azevedo-Santo et al., 2021). Brazil is the fifth largest country in extension and the sixth most populous in the world. In terms of the production of plastic, Brazil figures in the global scenario as the fourth largest producer, with around 11.3 million tons of plastics produced per year, of which only 1% is appropriate recycled (Trindade et al., 2023). Besides the creation of the National Solid Waste Policy (Brasil, 2010), few advances were accomplished, and nearly 60% of plastic waste is discarded without monitoring (Sodré et al., 2023). All this plastic waste produced in Brazil is likely to be carried by natural water drainage, ending up in important water bodies, threatening the biodiversity and health security of these environments. Brazil is known for having a huge biodiversity distributed across six biomes: the Amazon Forest, Cerrado, Atlantic Forest, Caatinga, Pampa, and Pantanal. These biomes are recognized by their environmental relevance, especially the Amazon Forest and the two global biodiversity hotspots: Cerrado and Atlantic Forest (Mittermaier et al., 2004). Moreover, the country presents an extensive coastal area, harboring several important watersheds and rivers, which are pivotal for global freshwater security (da Silva et al., 2016).

In recent years, many studies have been developed about plastic pollution in Brazilian aquatic ecosystems, encompassing different types of environments (marine, estuarine, or freshwater) and matrices (animals, sediment or water surface). However, there is no overview of the results found by these studies (e.g., number of studies regarding different environments, animal group, plastic size, etc.), nor any indication of research gaps. Thereby, using a dataset compiled from scientific studies conducted within the country, we aimed to systematically review the current state of scientific research regarding the presence of plastics in Brazilian aquatic environments.

3.2 Methods

3.2.1 Search protocol

In accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) criteria, we conducted a systematic review to collect data from scientific research regarding the presence of plastic in Brazilian aquatic environments. The main procedures to identify, select, and analyze pertinent studies are described in this section. The search was carried out systematically in three online databases, namely "Web of Science", "Scielo", and "Scopus", in September 21st,2023. We used Boolean search operators in English: (microplastic* OR mesoplastic* OR macroplastic* OR plastic debris OR plastic fragments OR plastic ingestion) AND NOT (experiment OR exposure OR trial) AND (fish* OR invertebrate* OR reptile* OR bird* OR mammal* OR sediment OR water OR benthic OR fisheries OR aquatic organism). We entered these keywords and operators in "topic" for Web of Science and "all fields" for Scopus and Scielo. Additionally, we applied a restriction to type of publication ("article") to exclude synthesis, metanalysis and reviews papers. Also, to focus on Brazilian research, we applied a restriction to region ("Brazil"). In Scopus, we also applied a restriction to the "Environmental Sciences" area, to exclude studies related to material development and agriculture.

3.2.2 Data eligibility

We found a total of 874 articles in our search in Web of Science, Scopus, and Scielo databases. From these records, 145 were removed because they were duplicated. For the remaining 729 records, a manual checking of the results (paper by paper) was performed at the discretion of the authors of this study. This step is crucial to avoid study duplicates, papers outside the topic of this study, unclear or incomplete reports, etc. (Blettler et al., 2018). In this assessment, from the remaining 729 records, 522 were excluded, resulting in 207 records (Table S1).

From each of the reviewed papers we extracted: i) publication year; ii) the sampling location (municipalities and biomes); iii) type of environment (estuarine, freshwater, or marine); iv) plastic particle size (macroplastics (> 20 mm); mesoplastics (5-20 mm) or microplastics (< 5 mm); v) study object (biota, sediment, or water); and vi) animal group (poriferous, cnidarian, mollusk, annelid, crustacean, insect, fish, reptile, bird, or mammal).

3.3 Results and Discussion

A total of 207 publications about plastic pollution in Brazil aquatic environments were eligible in the search. The number of publications about this topic has increased along the years (Fig 1). A global bibliometric assessment conducted by Kasavan et al. (2021) showed that Brazil is one of the ten countries that most published articles about plastic pollution in aquatic ecosystems over the last 21 years. The country also follows the global publication trends on plastic pollution, in which few publications occurred in the 2000 to 2010 decade, followed by a rapid increase from 2011 to 2020 (Fig 1; Kasavan et al., 2021). The most prominent years were 2021 and 2022, exhibiting a total of 34 and 24 articles published, respectively (Fig 1). As for 2023, we found a total of 23 articles

published until the date of the search, although this number will probably increase until the end of the year. Considering the advances in research on plastic pollution in the last decade and the international agendas proposed to reduce the threat, it is worth highlighting that Brazilian previous government (2018-2022) did not adopt the international legislation about waste management, as well as stood against signing an international agreement aiming to limit the global volume of plastic waste (Lima et al., 2020). With this scenario, Brazil is still far from comprehensively assessing plastic pollution in its aquatic environments. Difficulties in achieving this goal could be related to the country's huge territorial extension, its large population, restrictions in funding, and the negligence of the country on this subject, to what can be associated with the small number of publications so far. Although plastic pollution in Brazil is still an understudied topic, the country hosts a variety of aquatic ecosystems that experience problems with this kind of pollution, indicating that efforts to understand the patterns of this contamination must be prioritized (Lima et al., 2020).

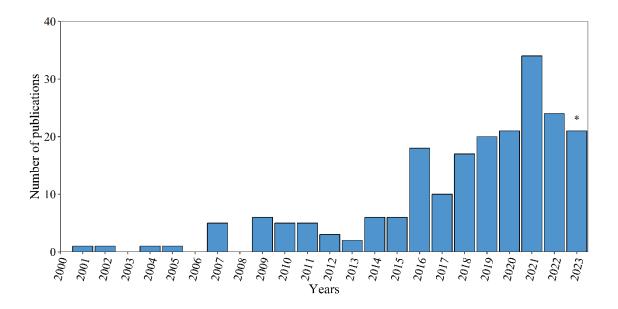


Fig 1. Number of publications per year about plastic pollution in Brazilian aquatic environments between 2000 and 2023. The asterisk represents the number of publications up to September 2023.

From all six biomes, only the Atlantic Forest may be considered a well-studied biome regarding plastic pollution. This is mainly because this biome covers a considerable portion of the Brazilian marine and coastal areas, where publications are concentrated (Fig. 2). These studies were concentrated in remarkable locations, such as the Goiana Estuary (14 studies), Todos os Santos Bay (11 studies), Guanabara Bay (11 studies), Santos Bay (11 studies), and Paranaguá Bay (nine studies).

Amazon Forest is the second most studied biome, with publications concentrated in the Pará River Estuary (five studies), São Marcos Estuary (three studies), and Xingu River (two studies) (Fig 2). Considering its huge territorial extension and ecological relevance, the number of publications about plastic pollution is minimal when compared to the Atlantic Forest. The Amazon Forest is internationally known for its high biodiversity and for providing essential ecosystem services (Levis et al., 2020). This biome harbors the Amazon hydrographic basin, which is the largest hydrographic system in the world, covering 4.7% of the world's land area and draining nearly 6,869,000 km² (Goulding et al., 2003; Giarrizzo et al., 2019). The Brazilian Amazon, receive nearly 182,000 tons of synthetic polymers per year, and the Amazon hydrographic basin is responsible for 10% of the total amount of plastic waste found in the ocean worldwide (Giarrizzo et al., 2019). The low portion of this basin harbor several tide-dominated rivers, in which studies on the influence of hydrodynamics over plastic import and export processes are scarce (Rosa et al., 2023). Some studies regarding plastic pollution have been conducted in the Amazon biome in the 2011 to 2020 decade, namely the publications of Andrade et al. (2019), Gerolin et al. (2020), and Morais et al. (2020). From 2021 onwards more studies were published, and several others are being developed (Oliveira et al., 2023; de Souza et al., 2023; Rosa et al., 2023; da Costa et al., 2023; Guimarães et al., 2023).

The remaining biomes, especially the ones who cover large portions of freshwater courses, are poorly studied regarding plastic pollution. Pampa biome exhibited a considerable number of publications in coastal areas, mainly in the Patos Lagoon (eight studies) (Fig 2). Pantanal is the least studied biome, with only three published studies on the entire dataset (de Faria et al., 2021; 2022; Camargo et al., 2022; Fig 2). Like Atlantic Forest and Pampa, most of the publications in Caatinga and Cerrado are from coastal environments. From all the biomes, Cerrado has the most concerning situation, once it encompasses the headwaters and the largest portions of Paraná-Paraguay, Tocantins-Araguaia, and São Francisco River basins (Latrubesse et al., 2019; Salmona et al., 2023). Specifically, the Tocantins-Araguaia hydrographic basin covers nearly 11% of Brazil's total area and most of this basin is located in the Cerrado, being one of the most significant

freshwater environments in the country (Latrubesse et al., 2019). Moreover, most of the rivers that compose the Tocantins-Araguaia basin are navigable (Ribeiro et al., 1995; Coelho et al., 2012; Latrubesse et al., 2019), a factor already known to contribute to plastic pollution in aquatic environments (van Emmerik et al., 2019; Kaptan et al., 2020; Thushari & Senevirathna, 2020). Despite that, not a single publication was found in the inland portion of this biome, only in its coastal areas.

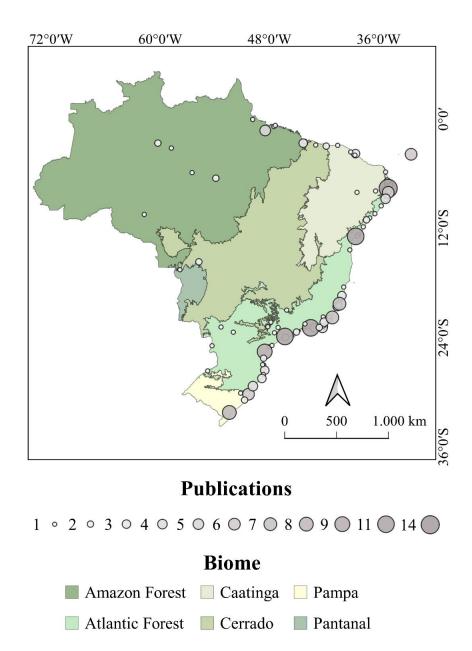


Fig 2. Number of publications about plastics per site in each Brazilian biome: Amazon Forest, Atlantic Forest, Caatinga, Cerrado, Pampa and Pantanal. Bigger circles indicate more publications. Map EPSG: 4326.

Among the 207 articles, marine environments were the most studied, with 120 published articles (57.7%), followed by estuarine environments, with 63 published articles (30.3%) (Fig 3). Freshwater environments revealed a huge publication scarcity, being the least studied environment, with only 25 published articles (12%) up to the final search date (Fig 3). Besides of the few studies found, it is globally estimated that freshwater environments are polluted in a similar proportion compared to marine environments, which raises concern about the lack of studies in the former environment (Blettler et al., 2018; Garcia et al., 2020). This unbalanced number of publications between marine/coastal and freshwater environments in Brazil highlights the global tendency of lack and bias in scientific research regarding plastic pollution in freshwater environments (Blettler et al., 2018). Rivers are known to conduit plastic to estuarine and marine ecosystems. In this process, the input rates of plastics from rivers to oceans range from around 1.15 to 2.41 million tons per year (Häder et al., 2020; van Emmerik et al 2022; 2023). Nevertheless, the dynamics involved in this process still needs to be evaluated. Until 2018, South America had contributed with 11.8% of the global publications about plastic pollution in freshwater environments, and Brazil was the country with higher number of studies in the continent (Blettler et al., 2018). However, compared to the rest of the world, Brazil still needs to improve the number of publications, especially in biomes with large and important river basins, such as the Amazon Forest and Cerrado.

Plastic pollution in estuarine environments has a considerable number of publications. Notably, we found that one sampling location, the Goiana Estuary in the Northeast region, have more publications than other estuarine areas in our assessment. Publications in this location covered several topics, from planktonic samples (Lima et al., 2014; 2015;2016a) to top predators (Dantas et al., 2012; Ferreira et al., 2019), which contributes to the knowledge about plastic dynamics through an ecosystemic approach. Estuaries are the main pathway exporting plastics from land to sea, connecting rivers to oceans, and having been systematically contaminated by plastics in both biotic and abiotic compartments (Lima et al., 2020; Pinheiro et al., 2021). Rivers and estuaries have a great retention capacity due to their rainfall and salinity dynamics, and, thus, often exhibit higher plastic densities compared to marine environments (Lima et al., 2020; van Emmerik et al., 2022). Nevertheless, such as freshwater environments, a remarkable

knowledge gap was evidenced and still needs to be further investigated for this type of environment, both in Brazil and worldwide.

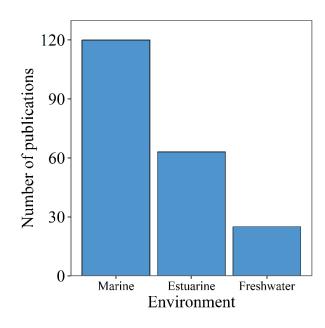


Fig 3. Number of publications about plastic pollution in Brazilian aquatic environments per environment type (n = 207).

Concerning the size of the assessed plastic particles, microplastics are much more studied than other sizes, with 113 publications (56.2%) (Fig. 4). Macro and mesoplastics presented less than half the number of publications of microplastics, with 49 (24.4%) and 39 (19.4%) publications, respectively (Fig 4). Besides the visual pollution caused by larger particles, microplastics has drawn the attention of the scientific community due to its high hazardous effects. Microplastics may be of primary origin, for example as those manufactured for the cosmetics and personal hygiene industry, or of secondary origin, when they originate from the fragmentation of large-sized particles (Barnes et al., 2009). This fragmentation process due to natural factors makes microplastics much more abundant and ubiquitous, especially in marine environments (Lima et al., 2016b). Blettler et al. (2018) found that for freshwater environments, microplastics are more studied than any other particle size. Independent of their origin, microplastics are one of the most hazardous materials to biota (Lima et al., 2016b). This threat relates to several direct and indirect effects, namely the potential physical harm, blockage, starvation, and chemical contamination due to organic and inorganic pollutants (Rochman et al. 2013; Lima et al., 2016a; Prata 2018), which can be transferred through food webs (Santana et al., 2017; Wendel et al., 2018) and has effects on humans that are still unclear (Li et al., 2021).

Although microplastics are often presented as the main threat, meso and macroplastics are also hazardous to biota since they may cause the blockage of the gastrointestinal tract, leading to starvation and ultimately to death by inanition (Rummel et al., 2016; Cardozo et al., 2018). Additionally, macroplastics can cause suffocation and entanglement (Andrades et al., 2021; Blettler & Mitchell, 2021), especially in larger animals, such as sharks (Sazima et al., 2002) and turtles (Mascarenhas et al., 2004). In summary, different particle sizes may result in different impacts on the aquatic environment, and this information highlights the importance of studies regarding the distribution and dynamics of different particle sizes. The disproportional results regarding particle sizes in Brazilian aquatic environments should be interpreted with concern, especially because larger plastic particles are a direct source of small sized ones.

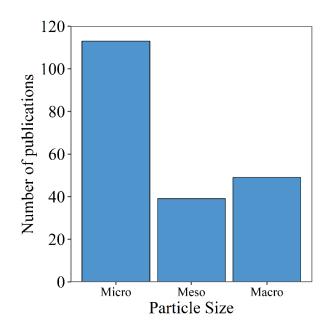


Fig 4. Number of publications per plastic size in Brazilian aquatic environments (n = 207).

Regarding the research object, biota was the most studied subject, with 101 published articles (48.6%) (Fig 5). The first studies on plastic ingestion by biota in Brazil date to the early 2000, specifically with marine turtles (Bugoni et al., 2001; Mascarenhas et al., 2004). Organisms can be used as bioindicators for many pollutants. This assumption is based on the hypothesis that cumulative effects of environmental changes are both integrated or reflected in the taxonomic response of the species and/or in the diversity of

pollutants present in the organisms (Holt & Miller, 2011; Bonanno & Vymazal, 2017; Bonanno & Orlando-Bonaca, 2018). Different organisms are cited in the literature as good bioindicators of plastic pollution, and its efficiency is evaluated through biological and ecological criteria (Reboa et al., 2022).

Regarding environmental matrices, sediment samples were more studied than water samples, with 75 (36%) and 32 (15.4%) articles published respectively (Fig 5). The first publication with sediment samples also dating to the early 2000 decade (Santos et al., 2005) and most of the studies on these samples were performed on sandy beaches (e.g., Costa et al., 2009; Araújo et al., 2018; Ribeiro et al., 2021). Water samples, otherwise, followed the increase in publications in the decade from 2010 to 2020. This disparity in the number of publications regarding the different study objects may also be a result of the growing interest of scientists in the plastic pollution topic, which resulted in the elaboration of novel methods and standardizations to evaluate this pollutant.

In this regard, the first reports of plastics in aquatic environments resulted from the accidental encounter of this pollutant in the analyzed samples (Wehle & Coleman, 1983). However, the popularity and the global concern of the topic "plastic pollution in aquatic environments" has increased in the last decade (Kasavan et al., 2021), which has probably motivated scientists to design studies directly focused on evaluating plastic pollution. Moreover, the elaboration and popularization of methods and standardizations to evaluate plastic pollution, such as the ones proposed by Lusher et al. (2013) and Rochman et al. (2015), probably allowed scientists to expand their investigation possibilities and further compare their results.

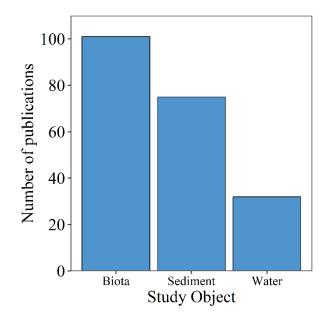


Fig 5. Number of publications about plastic pollution in Brazilian aquatic environments per study object (n = 207).

Among the articles published with biota, fish were the most studied group, with 43 published articles (40.6%) (Fig 6). Studies analyzing plastic ingestion by fish included a wide variety of species, encompassing different sizes and trophic positions, including small omnivores such as the species from Gerreidae and Characidae families and top predators represented by some shark species. Fish studies also assessed commercially important species, such as the skipjack tuna Katsuwonus pelamis and the whitemouth croaker Micropogonias furnieri in the marine environment and the spotted pim Pimelodus maculatus and the drum Plasgiocion squamosissimus in the freshwater. Fishes have emerged as an advantageous taxon to assess plastic pollution mainly due to their wide spatial distribution, diverse feeding and habitat guilds, and commercial importance (Reboa et al., 2022. Fossi et al., 2018). Until 2019, 427 fish species had been assessed for plastic ingestion globally, encompassing marine (54.6%), estuarine (22.2%) and freshwater (17.1%) environments (Azevedo-Santos et al., 2019). The data from Brazil follows the same tendency, where most studies assess the ingestion of plastic particles in marine species (44.4%), with estuarine and freshwater environments also having considerable percentages (31.1% and 24.4%, respectively), which highlights that the differences between environments in Brazil, for fish studies at least, are not so distinct.

Large sized animals belonging to groups such as marine turtles, birds, and mammals are also considered good bioindicators of plastic pollution (Bonanno & Orlando-Bonaca, 2018; Fossi et al., 2018; 2020), mainly due to its high trophic position and its ecological importance as umbrella and sentinel species (Hazen et al., 2019). The second most studied group were birds, with 19 published articles (17.9%) mostly depicting marine species (Fig 6). The Procellariformes order was the most studied in Brazil, with most publications being from Petry et al. (2007, 2010, 2017). Only two publications were found on freshwater birds, one related to the ingestion of particles (Sazima & D'Angelo, 2016) and the other associated with the use of plastics in nesting activities (Azevedo-Santos et al., 2022), which evidence the lack of information about freshwater bird species. Reptiles came in third, with 15 published articles (14.1%), represented exclusively by marine turtles (Fig 6). Also, regarding this animal group, we found the oldest publication in the studies analyzed in this review (2001). The studies encompassed five of the seven species of sea turtles found along the Brazilian coast (Carvalho et al., 2015). However, most studies reported the ingestion of plastic debris only for Chelonia mydas. The publications of Bugoni et al. (2001), Reis et al. (2010), and Poli et al. (2014) were the only ones that addressed the topic of other turtle species. Given the importance of this group and its conservation urgency, Brazilian scientists should expand their research to address other marine turtle species as well. Only six papers were found about plastic ingestion by mammals in Brazil (Fig 6), representing 5.7% of the animal publications, and this group was mainly represented by dolphins and whales. Contrary to fish and invertebrates, studies are more complex to design for the aforementioned groups, especially for mammals, once their sampling is often conditioned to the encounter of dead stranded animals or their carcasses, as is the case of the studies conducted in Brazil (Tourinho et al., 2010; Bortolotto et al., 2016; Padula et al., 2023). This is probably why studies with aquatic mammals are not very abundant in Brazil.

Invertebrates were also reported ingesting plastics and the most studied group was mollusks with 11 published articles (10.4%), followed by crustaceans with six published articles (5.7%). Mollusks are known for being good bioindicators of many pollutants, mainly due to its feeding habits and general characteristics (Cunha et al., 2017; Dirrigl et al., 2018; Bonanno & Orlando-Bonaca, 2018). In our assessment, they were often reported ingesting microplastics in marine and estuarine environments (Santana et al., 2016; Bruzaca et al., 2022; Costa et al., 2023). Most of the publications with crustaceans

were in marine environments, except for the study of Guimarães et al. (2023) that addressed the freshwater shrimp *Macrobrachium amazonicum*. Annelids, represented by marine polychaetas, and insects each had two published articles (3.8%) (Fig 6). Finally, cnidarian and poriferous had only one published article each (1.9%). The fewer publications for these groups highlight the huge gap in understanding how plastic pollution affects aquatic invertebrates in Brazil. Even the most studied invertebrate groups, mollusks and crustaceans, are poorly documented for plastic interaction when compared to other animal groups, which is particularly concerning given its important social and economic role, as they serve as fishing resources directly destinated to human consumption (Rochman et al., 2015). Furthermore, preserved specimens of sessile organism, such as sponges, can be used as bioindicators of plastic pollution on a large temporal scale (Soares et al., 2022), allowing comparations with old samples and the elaboration of historical datasets about plastic pollution.

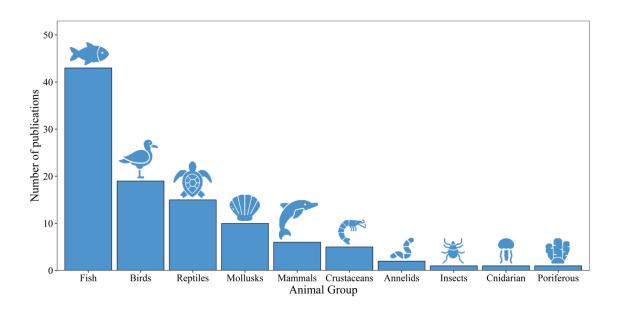


Fig 6. Number of publications about plastic ingestion by different animal groups of aquatic organisms in Brazilian aquatic environments (n = 101).

3.4 Conclusion

In summary, Brazil still needs several improvements in assessing plastic pollution in aquatic environments. The evidence found in this systematic review highlights how freshwater environments are deeply neglected in Brazilian research, likewise larger plastic particles and invertebrates. In fact, the research trends found here are similar to the global research tendencies of plastic pollution in aquatic environments. However, due to the huge territorial extension of the Brazilian biomes and its unique biodiversity, there is no reason for perpetuating this negligence, especially considering the severe threat represented by the ongoing lack of waste management in many regions of the country. As stated here, Cerrado, Caatinga and Pantanal biomes are poorly studied regarding plastic pollution, which makes them priority. Future research should address the aforementioned questions, and especially freshwater scientists should design studies to appropriately and proportionately assess plastic pollution in important biomes and watercourses of Brazil.

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4 THE INFLUENCE OF LOCAL ANTHROPOGENIC ACTIVITIES, ANIMAL GROUP AND ENVIRONMENT TYPE IN PLASTIC POLLUTION IN BRAZILIAN AQUATIC ENVIRONMENTS

ABSTRACT

Anthropogenic activities drive massive amounts of plastic entering aquatic environments. However, its management is poor, and its impacts are not properly estimated, as they are often assessed as categorical variables. The aim of this paper was to assess the relationship between anthropogenic activities and the amount of plastics reported in Brazilian aquatic environments and the difference in the amount of plastics reported according to the animal groups and environment types. A total of 874 articles were found in our search, of which 105 articles presented enough information for our quantitative synthesis. Demographic density, urban land cover, and farming land cover were selected as anthropogenic variables. Linear mixed effects models were used to assess the relationships between the number of plastics reported and the anthropogenic variables, animal group and environment type. Based on the models, anthropogenic variables did not influence the quantity of plastic found in Brazilian aquatic environments. However, the animal group was an important factor that influenced the quantities of ingested plastic by aquatic organisms, while the type of environment (estuarine, freshwater or marine) influenced the number of plastics in the abiotic samples. The absence of relationships with anthropogenic variables does not invalidate the importance of these predictors since it could be related to the limitations of our dataset. However, these results should be used as an incentive for developing new studies and increasing the investment in science and research about plastic pollution in Brazilian aquatic environments.

Keywords: Plastic debris; Anthropic impact; Brazil; Aquatic habitats; Environmental Pollution.

4.1 Introduction

Plastics have become very popular in the last decades, especially due to their low production costs and commercialization facilities allied with their high durability and resistance (Thompson et al., 2009). In 2022, about 400 million tons of plastics were produced worldwide, and this amount is supposed to increase in the next decades (Geyer et al., 2017; Plastics Europe, 2023). The presence and accumulation of plastics in aquatic environments are attributed to several factors, related to increased production and improper disposal of this waste (Barboza & Gimenez, 2015). Improper waste management is one of the most important causes of plastic pollution in aquatic ecosystems, and, specifically in the southern hemisphere, it is still represented by improper dumping and lower rates of wastewater treatment (Guerrero et al., 2013; Blettler et al., 2019; Sodré et al., 2023). Thereby, without efficient waste management systems, the disposal near water courses often leads to waste accumulation in aquatic ecosystems (Pazos et al., 2017).

Anthropogenic activities mainly drive huge amounts of mismanaged land-based plastic entering aquatic environments (Kasavan et al., 2021). Domestic and industrial effluent discharges, tourism, fisheries, aquaculture, construction of artificial structures, and harbor operations are some of the many anthropogenic activities that contribute to the presence and abundance of plastics in aquatic environments (Cardozo et al., 2018; Thushari & Senevirathna, 2020; Monteiro et al., 2022). The increase in the intensity of these activities usually leads to the increase of plastic pollution (Wagner et al. 2014; Dantas et al., 2019; Luo et al., 2019; Garcia et al., 2020; Schirinzi et al. 2020; van Emmerik et al., 2023). However, the magnitude of the impact of these factors is poorly quantified in the literature.

Many studies suggest anthropogenic activities as precursors of plastic pollution, but few quantify their impacts, as they are often assessed as categorical variables (Garcia et al., 2020; Nobre et al., 2021; Augusto et al., 2023). High population densities are often associated with higher amounts of plastic pollution (Lestari & Trihadiningrum, 2019), especially in developing countries with poor waste management rates (Blettler et al., 2019). Urbanization is also related to plastic pollution. Garcia et al. (2020) assessed the ingestion of microplastics by fishes from urbanized and rural streams in Southern Brazil and, while their results evidenced plastic pollution in both types of streams, fish from urbanized ones ingested more plastics. Additionally, a study by Luo et al. (2019) suggests that microplastic abundance tends to increase in urban areas. Farming activities, such as agriculture and livestock, also contribute to plastic pollution in aquatic environments. Croplands, for example, can be considered reservoirs of plastic debris because of the accumulation of agricultural films, single-use irrigation pipes, and plastic packaging of pesticides and fertilizers, which can enter the aquatic ecosystems via surface runoff and irrigation (Lechthaler et al., 2020; Zhang et al., 2020; Çevik et al., 2022).

Plastics are ubiquitous in aquatic environments, polluting from pristine freshwater environments to deep ocean basins (González-Pleiter et al., 2020; Borrelle et al., 2020). Despite the anthropogenic factors that directly cause this type of pollution, the quantity of plastics found in different environments is conditioned to its intrinsic characteristics. Nowadays, many studies highlight that plastics are concentrated in oceans and marine areas, and most of them come from land-based sources or even launched directly into it (Lebreton et al., 2017; Thushari & Senevirathna, 2020). The distribution and accumulation of plastic waste in these environments is also related to climatic conditions such as storms, hurricanes, and flooding (Thushari & Senevirathna, 2020). Rivers and estuaries are part of the transport routes of this pollutant to marine areas, being also affected by this pollution while retaining these particles for long periods, mainly due to its unique hydrodynamics characteristics (Lima et al., 2020; van Emmerik et al., 2022). Furthermore, these dynamics promote changes in the availability of plastics, which can be ingested and interact with living organisms (Lima et al., 2015; Vecchi et al., 2021).

Plastics may interact with wildlife in many ways, depending on the plastics size and the characteristics of the organisms. In the first case, larger particles, such as macro and meso debris, may be used as nest materials, be ingested, or cause entanglement and suffocation (Blettler & Mitchell, 2021). When ingested, these particles may cause internal injuries, blocking the gastrointestinal tract and leading to starvation due to a constant sensation of saciety (Rummel et al., 2016; Cardozo et al., 2018; Blettler & Mitchell, 2021). In addition to starvation, micro debris may also have toxic effects, since they adsorb pollutants from the environment, including heavy metals and pharmaceuticals (Barnes et al., 2009; Rochman et al., 2013; Prata, 2018). Some characteristics can influence the amount of plastics ingested by these organisms, such as trophic and habitat guilds (Yofukuji et al., 2024). Species that present opportunistic feeding habits, for example, are more likely to ingest plastics when compared to more selective species (Mizraji et al., 2017; Cardozo et al., 2018). Similarly, omnivorous species may have their rate of ingestion increased due to their foraging behavior (Mizraji et al., 2017; Garcia et al., 2020).

Globally, Brazil is the fifth largest country in extension, the sixth most populous, and the fourth largest producer of plastics (Trindade et al., 2023). Additionally, urbanization has exponentially grown in Brazilian coastal areas in the past decades, and a considerable portion of the inland areas are destinated to agriculture and livestock (Barbosa et al., 2017; Silva et al., 2017). Thereby, considering the current knowledge gap on plastic pollution research in Brazil and especially the effects of anthropogenic activities on this matter, we aimed to assess: i) the relationship between anthropogenic activities of the municipalities and the amount of plastics reported in Brazilian aquatic environments and ii) the difference among animal groups (for biotic samples) and environment types (for abiotic samples) on the amount of plastics reported in Brazilian aquatic environments. To achieve this goal, we systematically analyzed a comprehensive dataset compiled from scientific studies conducted within the country, covering various biotic and abiotic subjects.

4.2 Methods

4.2.1 Search protocol and synthesis design

In accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) criteria, we carried out a systematic review to collect data from scientific research on the presence and abundance of plastic in Brazilian aquatic environments. The search was carried out systematically in three online databases, namely "Web of Science", "Scielo", and "Scopus", from January 12th to September 21st,2023. We used Boolean search operators in English: (microplastic* OR mesoplastic* OR macroplastic* OR plastic debris OR plastic fragments OR plastic ingestion) AND NOT (experiment OR exposure OR trial) AND (fish* OR invertebrate* OR reptile* OR bird* OR mammal* OR sediment OR water OR benthic OR fisheries OR aquatic organism). We entered these keywords and operators in "topic" for Web of Science and "all fields" for Scopus and Scielo. We applied a restriction to the type of publication ("article") to exclude synthesis, metanalysis and reviews papers. Also, to focus on Brazilian research, we applied a restriction to region ("Brazil"). In Scopus, a restriction to the "Environmental Sciences" area was applied, to exclude studies related to material development and agriculture.

We found a total of 874 articles in our search in Web of Science, Scopus, and Scielo databases (Fig 1). From these records, 145 were removed because they were duplicated. For the remaining 729 records, articles should mandatorily present the average number of plastics found (detailed in Data extraction section), the sampling stations' geographical information, and the year when samplings were carried out. Based on these criteria, 510 records were excluded after the abstract screening because they were out of scope or out of Brazil (Fig 1). After evaluating the full text of the remaining articles, we removed 114 articles due to lack of data or because they were out of scope, resulting in 105 articles with available data for inclusion in our quantitative synthesis (Fig 1, Table S1). For the model construction, this final number of articles was posteriorly separated between biotic (animal groups) and abiotic (sediment and water) samples.

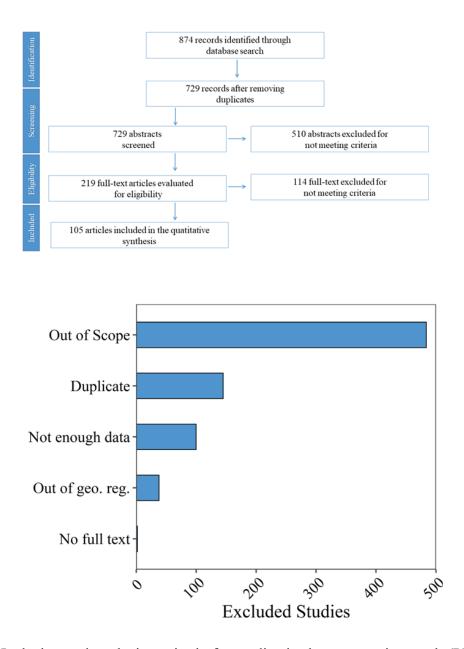


Fig 1. Inclusion and exclusion criteria for studies in the systematic search (PRISMA; Moher et al. 2009). Out of scope = Articles not related to plastics in aquatic environments. Also include reviews and other meta-analysis; Not enough data = Articles without complete information. It includes articles in which were not possible to acquire data from tables, graphs or supplementary material, and others that presented few or no information about the sampling locations or sampling period; Duplicate = Same record from different databases; Out of geo. reg. = About plastics, but not in Brazilian territory; No full text = Not able to find the full text.

4.2.2 Data extraction

For the remaining 105 studies, we extracted the following information: i) the mean number of reported plastics (for animal taxa: plastics per individual and for sediment and water samples: plastics per sample unit); ii) the method used to measure the abundance of plastics; iii) the municipalities; iv) the sampling year; v) the type of environment (estuarine, freshwater, and marine); and vi) the study object (biota, sediment, or water). When the target of the study was the biota, we also extracted the vii) specific animal group analyzed (poriferous, cnidarian, mollusk, crustacean, fish, reptile, or bird).

4.2.3 Anthropogenic variables

The demographic density, urban land cover, and farming land cover were selected as anthropogenic variables. The demographic density of Brazilian municipalities was obtained from population estimates provided by the Brazilian Geography and Statistics Institute (Instituto Brasileiro de Geografia e Estatística – IBGE). The estimates are available for 2000, 2005, 2009, 2015, and 2020. For the remaining sampling years, whenever the study sampling year was in the interval of available years, a mean was calculated. Finally, demographic density was converted into inhabitants/km². The yearly land use data for each municipality, considering all sampling years, was obtained through the Mapbiomas project website, collection 8 (Souza et al., 2020; Mapbiomas, 2023). We selected the farming and urban types of land use, and data was converted into percentage to standardize the metrics and avoid extreme values. For both metrics, we calculated a mean value when study area comprised more than one municipality.

4.2.4 Data analysis

To reduce data variation and improve the quality of model residuals, the mean number of plastics reported was transformed in Log + 1, and extreme values (outliers) were removed. We used linear mixed effects models to assess the relationships between the anthropogenic variables and the mean abundance of plastics reported in the studies. For the biota, 34 articles were used and the model was constructed with demographic density, urban land cover, farming land cover and the specific animal group as predictor variables and the mean abundance of plastics as the response variable. As counting was the most frequent method to assess plastic ingestion by biota, and to make comparisons possible, articles that reported plastic ingestion by other metrics, such as volume, density, or occurrence, were removed from the model. Considering the high variation in plastic quantities among studies and the unbalanced number of publications for each type of environment (freshwater, estuarine, and marine), we included these variables as random effects in our model with studies identity nested within the type of environment.

For sediment (18 articles) and water samples (11 articles), the model was constructed with demographic density, urban land cover, farming land cover and type of environment as predictor variables and the mean abundance of plastics as the response variable. Density was the most frequent method used to report the abundance of plastics in these studies, thereby, articles that used other methods, such as occurrence and count, were removed from the model. Considering that the plastic quantities also presented high variation among studies, we included the study ID as a random effect in our model.

The three models were constructed using the Gaussian distribution and the model assumptions were graphically checked for the normality and homoscedasticity of the residuals. Model quality was assessed with marginal r^2 (the proportion of variation explained by fixed effects) and conditional r^2 (the proportion of variation explained by fixed and random effects) (Nakagawa and Schielzeth, 2013). For each model, a significance threshold of p < 0.05 was adopted. All regression models and graphs were created in R 4.2.1 (R Core Team, 2022) with the "lme" function for the linear mixed model from "lme4" (Bates et al. 2015) and "ggplot2," respectively.

4.3 Results

4.3.1 Biota

The anthropogenic variables selected for this model showed a very small and nonsignificant association with the mean abundance of plastic ingested by biota (Table 1, Fig 2). Regarding the animal group, birds (represented by intercept) and reptiles showed higher and positive estimates and were significant predictors in our model (Table 1, Fig 2). Conversely, fish showed a negative and significant estimate. The model explanation by fixed effects was higher than by random effects ($R^2c = 0.56$; $R^2m = 0.34$), which means that animal group was an important predictor in our model. **Table 1.** Results of the linear mixed models of the relationships between anthropogenic variables (demographic density, urban land cover, and farming land cover) and animal group (poriferous, cnidarian, mollusk, crustacean, fish, reptile, or bird) and the mean of plastics ingested by the biota. Bold values indicate significant predictors. DF= degree of freedom.

	Value	Std.Error	DF	t-value	p-value
Intercept (Bird)	0.7122362	0.197161	42	3.612455	<0.01
Demographic Density	0.000003	0.000237	42	0.012796	0.9899
Land Cover Urban (%)	0.0044659	0.018424	42	0.242389	0.8097
Land Cover Farming (%)	-0.0002687	0.002032	42	-0.1322	0.8955
Cnidarian	-0.2579351	0.317932	21	-0.81129	0.4263
Crustacean	-0.2093219	0.279159	21	-0.74983	0.4617
Fish	-0.3368378	0.131374	21	-2.56396	0.0181
Mollusks	0.1925086	0.221927	21	0.867441	0.3955
Poriferous	-0.4130791	0.654493	21	-0.63114	0.5348
Reptile	0.2789495	0.125855	21	2.216439	0.0378

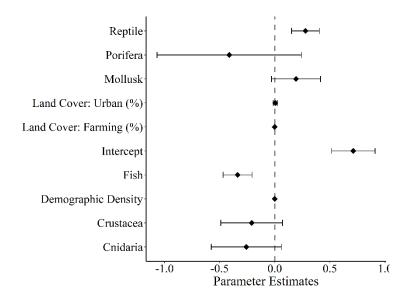


Fig 2. Estimates of the parameters for the means of the number of plastics ingested by animals in relation to the model predictors. The black line represents the confidence interval. The variables were significant if they did not present confidence intervals crossing the dashed line.

4.3.2 Sediment

The anthropogenic variables selected for this model also showed small and nonsignificant associations with the mean abundance of plastic present in the sediment samples (Table 2, Fig 3). Regarding the type of environment, estuarine (represented by the intercept) and freshwater environments had the highest estimate and were significant predictors (Table 2, Fig 3). These results suggest that estuarine and freshwater environments present higher amounts of plastics in the sediment when compared with marine ones. For this model, random effects (study identity) explained more of the data variation than the fixed effects ($R^2c = 0.96$; $R^2m = 0.07$), which suggests a great variation among the studies.

Table 2. Results of the linear mixed models of the relationships between anthropogenic variables (demographic density, urban land cover, and farming land cover) and the type of environment (marine, freshwater, and estuarine) and the mean of plastic reported for sediment samples. Bold values indicate significant predictors. DF= degree of freedom.

	Value	Std.Error	DF	t-value	p-value
Intercept (Estuarine)	1.578572	0.312449	65	5.052253	<0.01
Demographic Density	0.0000291	0.000121	65	0.239555	0.8114
Land Cover: Urban (%)	-0.00301	0.018464	65	-0.16284	0.8712
Land Cover: Farming (%)	0.000047	0.003582	65	0.013113	0.9896
Freshwater	1.400346	0.687623	41	2.036504	0.0482
Marine	-0.22926	0.347252	41	-0.66022	0.5128

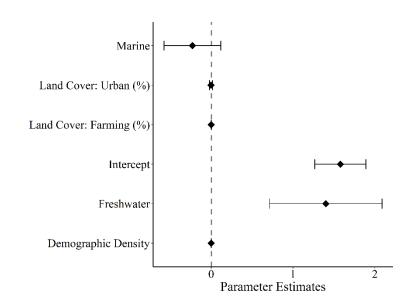


Fig 3. Estimates of the parameters for the means of plastics reported for sediment samples in relation to the model predictors. The black line represents the confidence interval. The variables were significant if they did not present confidence intervals crossing the dashed line.

4.3.3 Water

Like the other models, the anthropogenic variables selected for this model showed small and non-significant associations with the mean abundance of plastic present in water samples (Table 3, Fig 4). Only the freshwater environment presented a positive and significant estimate. The model explanation by fixed effects was higher than by random effects ($R^2c = 0.80$; $R^2m = 0.72$), which means that the type of environment was an important predictor in our model.

Table 3. Results of the linear mixed models of the relationship between anthropogenic variables (demographic density, urban land cover, and farming land cover) and the type of environment (marine, freshwater, and estuarine) and the mean of plastic reported for water samples. Bold values indicate significant predictors. DF= degree of freedom.

	Value	Std.Error	DF	t-value	p-value
Intercept (Estuarine)	0.14694	0.26876	10	0.546733	0.5965
Demographic Density	0.000598	0.000713	4	0.838896	0.4487
Land Cover: Urban (%)	-0.03676	0.06588	4	-0.55802	0.6066
Land Cover: Farming (%)	0.004592	0.0036	4	1.275366	0.2712
Freshwater	1.375205	0.340016	4	4.044529	0.0155
Marine	-0.368	0.270619	4	-1.35985	0.2455

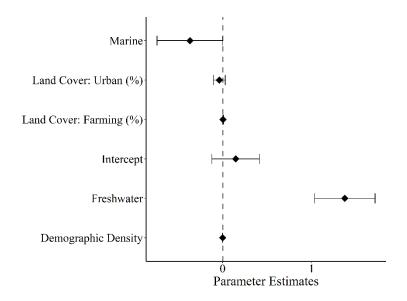


Fig 4. Estimates of the parameters for the means of plastics reported for water samples in relation to the model predictors. The black line represents the confidence interval. The variables were significant if they did not present confidence intervals crossing the dashed line.

4.4 Discussion

Contrary to our expectations, we did not observe the influence of anthropogenic variables of the municipalities on plastic quantity found in Brazilian aquatic environments. However, the animal group was an important factor that influenced the quantities of ingested plastic by aquatic organisms. For abiotic samples, the type of environment was an important predictor and the number of plastics in the sediment samples was higher in freshwater and estuarine environments and for water samples only in the freshwater environments.

The anthropogenic variables included in our synthesis (demographic density, urban land cover, and farming land cover) were not significant, and neither exhibited a strong correlation with the mean number of reported plastics. Demographic density and land use are often considered essential predictors of litter and plastic levels in other regions of the world (Schuyler et al., 2022). For example, at local scales, population density is strongly and positively correlated with litter abundance (van Emmerik et al., 2023). Likewise, urbanization close to water bodies is often described as a potential source of plastics to the environment (Lima et al., 2020), and higher litter levels can be found in economically and socially disadvantaged neighborhoods (van Emmerik et al.,

2023). When urbanized areas are compared with more conservated ones, there is usually more plastic in the anthropized environment (Luo et al., 2019; Garcia et al., 2020). Regarding the farming land cover indicator, it is estimated that 6.5 million tons of plastics are used annually in the farming sector (Lechthaler et al., 2020). Rural areas subjected to intensive agriculture activities usually present incorrect disposal of contaminated plastics, such as pesticide packing (Azevedo-Santos et al., 2021). Moreover, plastic films used in greenhouses and mulch packing can be introduced into the soil, subsequently ending in water bodies due to surface runoff (Pelamatti et al., 2019; Lechthaler et al., 2020; Çevik et al., 2022).

With 211 million inhabitants, Brazil is a very populous country where most of the population lives in or near aquatic environments. Additionally, agriculture and livestock are some of the most important economic activities in Brazil (Silva et al., 2017; Withers et al., 2018). However, even with these characteristics, the models could not find strong and significant correlations between these anthropogenic activities and the mean number of plastics in aquatic environments. The absence of these relationships does not invalidate the importance of these predictors, as it could be related to the limitations of the dataset. The dataset used was limited due to the lack of information in the published articles, since many of them do not report the mean of plastics found neither provide information for estimate it. Furthermore, many Brazilian aquatic environments and important water bodies did not present studies about plastic pollution, especially considering freshwater environments. Regarding these facts, more studies about plastic pollution should be developed in the country to fill the lacunes and research should encompass as many aquatic environments as possible, both in number and type of environments. Finally, we only considered our predictors on a local scale, *i.e.*, we took data from the municipalities surrounding sampling areas, and did not consider larger scale levels, such as the draining areas of rivers or watersheds of the analyzed locations.

On the scale selected, anthropogenic variables did not present a significant relationship with the quantities of plastics in biotic or abiotic samples. In fact, depending on the scale of the evaluation, these variables may not represent the impact of other point sources of plastics on aquatic environments (Klein et al., 2015). Also, it is possible that other anthropogenic variables may better address our questions, such as the Human Footprint or the Human Development Index, which may provide better explanations regarding the quantities of plastics reported for Brazilian aquatic environments. Future

studies should explore the influence of these anthropogenic variables in different spatial scales, for example, using watersheds.

Although no relationship was found concerning the anthropogenic variables, other predictors were significant in our models. For plastics ingested by biota, the animal group was an important predictor for the quantities of reported plastics. Specifically, fishes, reptiles, and birds were the taxa in which significant relationships occurred. These three taxa are the most studied in the country (unpublished data), and this probably influenced the results. Based on the model, the amount of plastics ingested by reptiles and birds is higher than that of fish. Reptiles and birds included in our analysis are mainly represented by marine species, and the ingestion of plastics by these taxa is often associated with the fact that they ingest particles that are similar in shape, color, or odor to the individuals' natural food items, a process named confounding factor (Schuyler et al., 2014; Savoca et al., 2017; Rossi et al., 2019). The diet of marine turtles, such as the green turtle Chelonia mydas, which was the most evaluated species in our data set, frequently consumes jellyfish (González-Carman et al., 2014; Andrades et al., 2019; Nunes et al., 2021). In aquatic environments, plastic bags may look like their natural prey, which makes them more likely to be ingested by turtles (Schuyler et al., 2014; Nunes et al., 2021). Marine birds are susceptible to ingesting plastics due to feeding on small prey that concentrate on the surface of ocean waters, where plastics float and accumulate (Vanstreels et al., 2021). Plastics in marine environments may serve as substrate for the growth of biofilm, which emits a similar olfactory signature to that of some natural food items (Savoca et al., 2017). Another explanation for the higher estimate of plastics ingested by birds is the fact that some of the reported species, especially seabirds, may be considered top predators (Hazen et al., 2019) and, therefore, may accumulate plastic particles from feeding on other animals from lower strata of the food chain (Di Beneditto & Oliveira, 2019). Some studies have already addressed the possibility of trophic transference of plastic particles, mainly microplastics, between different trophic levels, and these studies suggest that the higher trophic levels are likely to accumulate these particles through the trophic pyramid (Santana et al., 2017; Justino et al., 2023).

Fishes are the most studied group in Brazil, and recently, they have been considered a good bioindicator group due to their ecological characteristics, such as the habitat and trophic guilds, which allow the sampling of different habitats and larger temporal scale assessments (Fossi et al., 2018; Reboa et al., 2022). The lower estimate of

plastics ingested by this group, when compared to reptiles and birds, may be attributed to the larger variation in the feeding habits of fishes. Species of different trophic guilds and feeding strategies could present different likelihoods of plastic ingestion. For example, opportunistic fish species, such as the Atlantic Big Eye *Priacanthus arenatus*, are more likely to ingest plastics than species with specialized diets (Cardozo et al., 2018). Omnivorous and detritivorous fish consume a wide variety of resources, which increases the chances of actively or accidentally capturing plastics (Mizraji et al., 2017; Lusher et al., 2017; Garcia et al., 2020). Similarly, carnivorous species, in addition to accidental ingestion, can consume plastics indirectly through the ingestion of prey from other trophic levels (Ribeiro et al., 2019; Parker et al., 2021; Cardozo et al., 2023). Therefore, our data was composed of several fish species, from marine to freshwater environments, which probably comprised different trophic guilds. In this case, even if omnivorous and opportunistic fish consumed more plastics, other trophic guilds may have diminished the estimates in the models.

Regarding sediment samples, the type of environment was an important predictor, in which estuarine and freshwater environments presented positive and significant estimates. Rivers and estuaries have a high capacity to retain plastic particles, mainly due to their rainfall and salinity dynamics, and, therefore, higher plastic densities are expected when comparing these systems with marine environments (Lima et al., 2020). The estimated values for estuarine environments were slightly higher than for freshwater, which suggests that the quantities of plastic in sediment samples are higher for estuarine environments. The abundance of plastics in river sediment depends on several factors, such as plastic characteristics, river channels, and flow dynamics (Xu et al., 2020), and is expected that more particles sink into sediment when subjected to a slower water flow (Kapp & Yeatman, 2018). Likewise, the plastics present in the sediments of estuarine environments are derived from river input; thereby, they can be entrapped and accumulate on low river flows before entering the oceans when runoff increases seaward (Pinheiro et al., 2021). In this case, rivers and estuaries may be considered plastics reservoirs, since some portions of sediments may never reach the oceanic areas, and plastics accumulate in the sediment, consequently increasing the abundance of this pollutant (Xu et al., 2020).

For the model of water samples, the type of environment was also an important predictor, specifically for freshwater environments, that presented a positive and significant estimative. Plastics can enter freshwater environments directly through improper disposal of solid waste, or from the surface runoff, that carries out plastics from land-based areas (van Emmerik et al., 2022). So, it is reasonable to expect a higher abundance of plastics in water samples of freshwater environments, since these environments are the first to receive land-based plastics on their way to marine areas (Meijer et al., 2021). Thereby, likewise for sediments, the abundance and distribution of plastics in surface waters may depend on plastic characteristics and river dynamics (Ryan, 2015; da Costa et al., 2022).

Besides the non-significance of the anthropogenic variables, other predictors influenced the quantities of reported plastics in the studies. For the biotic models, animal group was a significant predictor, where reptiles and birds ingested more plastics than fishes. We cannot extrapolate any conclusions for other groups, since they were not significant in our models, probably due to the low number of publications. For abiotic models, the type of environment was a significant predictor. Plastics were more abundant in sediments of estuarine and freshwater environments, suggesting the role of these environments as plastic reservoirs. For water samples, plastics were more abundant in freshwater environments, which may suggest that the abundance of plastics is higher in the surface of these environments. Nonetheless more studies are needed to solidify these conclusions.

Despite some limitations on the dataset, to the best of our knowledge, this is the first study to quantitatively address the impact of anthropogenic factors on plastic pollution of aquatic environments on a national scale, with a dataset composed by research developed in Brazil. The results obtained should be used as an incentive for developing new studies and increasing the investment in science and research about plastic pollution in aquatic environments, given that it is a current topic of international concern. These investments will surely allow scientists to expand their knowledge about plastic pollution in the critical and megadiverse Brazilian aquatic environments, providing subsidies for public policies and biodiversity conservation.

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5 CONCLUDING REMARKS

The aim of this thesis was assessing different facets of plastic pollution in aquatic environments, specifically how the ingestion of plastics by carnivore fish is influenced by seasonality, the current state of research about this topic and their gaps, and finally the relationship between plastics and anthropogenic activities.

Of the 23 fish species analyzed, nine species had plastics in their gastrointestinal contents, and the particles found are mainly associated with fisheries and domestic litter, reflecting the activities carried out around the Upper Paraná River floodplain. The results revealed that freshwater carnivore fish ingested different types of particles, which is probably related to behavioral and biological features of each species. The variations in hydrometric level, *i.e.* seasonality, influenced the number of plastics ingested by these species, resulting in higher uptake of plastics in the wet season. This probably reflects the increase in availability of plastics in wet season due to transport and mobilization of macro and microplastics from river margins. These results contribute to understand the mechanisms involved in the ingestion of particles by freshwater fish and the fate of plastics in dynamics systems as floodplains, that remains as a gap in the literature.

Based on a systematic approach, we found evidence of knowledge gaps in the research developed in Brazil. In our assessment, freshwater environments are the least studied environment, a fact evidenced not only by the low number of publications, but also in the unbalanced distribution of studies among the biomes. Biomes with significant portions of inland waters, such as Pantanal and Cerrado, exhibited few studies, while biomes that harbor great portions of coastal areas, such as Atlantic Forest, exhibited many studies. From these results, it is clear the need to focus research on biomes with large and important river basins. Additionally, invertebrates are also a neglected group in Brazilian research, representing less than 25% of the studies with animals. This find is particularly worrisome given the social and economic importance of many invertebrate species. On the other hand, marine environments likewise fishes are the most studied groups in Brazil, which follows the global trends in plastic research.

Regarding the relationship between plastics and anthropogenic activities, our models could not find strong and significant correlations between these predictors and the quantities of plastics. The absence of significant relationships could be related to the limitations of our dataset. Other predictors influenced the quantities of plastics in our studies. For the biotic models, animal group was a significant predictor, where reptiles and birds ingested more plastics than fishes. For abiotic models, the type of environment was a significant predictor. In sediment samples, plastics were more abundant in estuarine and freshwater environments, while for water samples they were more abundant in freshwater environments. Even with these finds, more studies are needed to improve the dataset and solidify these conclusions, since few articles were used for models due to the lack of data or the presence of extreme values.

In conclusion, our findings make a substantial contribution to the knowledge about plastic pollution in aquatic ecosystems, highlighting both the influence of hydrological dynamics and the huge research gaps of the studies developed in Brazil. The first study in the Upper Paraná River floodplain about plastic pollution was developed in this thesis. Due to their unique hydrological dynamics and their importance for biodiversity, more studies are needed for this region, and based on our results, should explore more the role of floods in plastic dynamics. The study also highlights the importance of the seasonality in the occurrence and abundance of plastics ingested by carnivore species. Due to the pervasive effects of plastic pollution and the economic and ecological importance of the analyzed species, the study represents an important step in the assessment of the impacts generated on freshwater fish populations by the ingestion of plastics. More studies are needed to elucidate the interactions of biota and these polymers and their possible impacts on human health. The inland portions of many biomes are being neglected regarding plastic pollution, and giving the international concern about this pollutant, this negligence must be addressed, especially regarding seasonality, since it plays an essential role in the dynamics of this pollutant. The results also indicate that other issues, like large size particles and invertebrates, should be better investigated, indicating new directions for future studies.

APPENDIX A - Plastics in the gastrointestinal content of carnivore species.

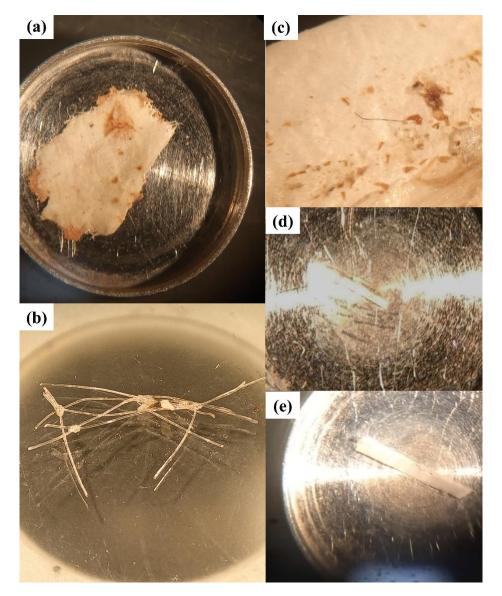


Fig S1. Plastics found in the gastrointestinal content of piscivorous species of the Upper Paraná River floodplain. (a) Polyvinyl alcohol (PVA), (b) Polyamide (PA), (c) Polyethylene (PE), (d) Polystyrene (PS) and (e) Polypropylene (PP).

Table S1. Volumetric percentage (V) and occurrence (O) of food items in the diet of piscivorous species analyzed in the Upper Paraná River floodplain. The plastic column is highlighted in bold. Fis = fish, Shri = shrimp, Biv = bivalve, Pla = plant, Det = detritus, Aq Ins = aquatic insects, Ter Ins = terrestrial insects, OIn = Other invertebrates, Plas = plastics.

Sampling	Species	%	Fis	Shri	Biv	Pla	Det	Aq Ins	Ter Ins	OIn	Plas
	A. lacustris	V	100.00								
	71. <i>tacustris</i>	0	100.00								
	C. kelberi	V	100.00								
	C. Keiberi	0	100.00								
	Hoplias spp	V	100.00								
	riopitas spp	0	100.00								
	P. pirinampu	V	98.90			1.09					
Mar/2019	1. рипатри	0	100.00			100.00					
	Danuamanianimum	V	75.00	25.00							
	P. squamosissimus	0	100.00	100.00							
		V	0.99	99.01							
	R. vulpinus	0	50.00	100.00							
	S. marginatus	V	91.94			6.70		0.35	0.49		0.0
	5. marginatus	0	94.74			34.21		2.63	2.63		15.7
	S.maculatus	V	69.07			23.62					
	5.maculalus	0	100.00			33.33					
	A. inermis	V	0.06								
	A. inermis	0	100.00								
	A la quatria	V	99.53	0.47							
	A. lacustris	0	100.00	16.67							
		V	91.43	8.57							
	C. kelberi	0	66.67	33.33							
	~ .	V	30.77	69.23							
	G. gulo	0	33.33	66.67							
		v	96.71	3.29							
	Hoplias spp	0	80.00	20.00							
Jun/2019		V	60.00	40.00							
• • • • • • • • •	H. unitaeniatus	0	100.00	100.00							
		v	100.00	100.00							
	P. corruscans	, O	100.00								
		v	100.00								
	R.vulpinus	o O	100.00								
		v	100.00	50.00		33.33					
	S.maculatus	~				33.33 100.00					
		0 V	04.40	100.00							
	S.marginatus	V	94.49 77.78			5.22					
		0 V	99.98			33.33					0.0
	A. lacustris	v O	33.33								0.0. 16.6
	C 1 11 ·	v	55.55	100.00							10.0
	C. kelberi	0		16.67							
Sep/2019	C. paranaense	V			99.01				0.98		
	e. pa. anaense	0		0175	50.00	12.14		0.04	50.00		0.0
	Hoplias spp	V O		84.75 7.69		13.14 5.13		0.06 2.56			0.00 2.50
	H. unitaeniatus	v	75.00	1.09		5.15		2.50		25.00	2.30

		0	25.00						25.00	
	P. corruscans	V	100.00							
		O V	33.33	81.65		15.50				
	P. squamosissimus	Ŏ		100.00		60.00				
		v	99.99							0.01
	R.vulpinus	0	100.00							25.00
	S. marginatus	V	87.86			10.53				0.36
	5. marginanas	0	85.71			28.57				28.57
	A. inermis	V								
		O V			71.25	21.57	1.25			
	A. crassipinus	Ŏ			100.00	75.00	25.00			
		v			100.00	10.00	25.00			100.0
	A. lacustris	0								7.69
	C. kelberi	V	70.83	20.83		7.69				
	C. Kelberi	0	23.08	15.38		7.69				
	G. gulo	V		99.83						0.17
	0	O V	15 56	100.00 54.43						100.0
	Hoplias spp	v O	45.56 25.00	34.43 16.67						0.01 4.17
		v	100.00	10.07						4.17
Dec/2019	H. unitaeniatus	0	100.00							
	H. platyrhynchos	V								
	11. piurymynenos	0								
	P. corruscans	V								
		O V	26.62	63.38						
	P.squamosissimus	v O	36.62 18.18	54.55						
		v	72.18	27.76						0.06
	R. vulpinus	Ó	40.00	20.00						20.00
	S. maculatus	V	78.92			4.10			14.38	0.01
	5. macutatus	0	50.00			40.00			10.00	10.00
	S. marginatus	V	79.41	2.02		12.79		1.74	1.98	0.10
	0	0 V	78.95 94.29	10.53		36.84 5.41		5.26	10.53	26.32
	A. lacustris	0 V	94.29 25.00			12.50				
		v	100.00			12.50				
	C. kelberi	0	50.00							
	C. paranaense	V								100.0
	C. purunuense	0		_						50.00
	G. gulo	V		86.88		12.50				0.63
	0	V V	100.00	50.00		25.00				25.00 0.01
	Hoplias spp	v O	100.00 17.65							5.88
		v	100.00							2.00
	H. platyrhynchos	0	100.00							
Mar/2020	P. pirinampu	V	100.00							
171 a 1/2020	1 . pri mumpu	0	20.00							-
	P. squamosissimus	V	84.79	15.21						0.01
		O V	35.71 86.72	35.71 13.28						7.14
	R. vulpinus	ŏ	57.14	28.57						
		v	66.67	20.07		25.00				
	S. brasiliensis	ò	33.33			33.33				
	S. hilarii	V	100.00							
	s. muaru	0	50.00							
	S. maculatus	V	80.66			15.63			0.77	0.04
		0	75.00			50.00			12.50	25.00
	S. marginatus	V	87.72			10.91			5.00	0.02
	5	0	80.00			50.00			5.00	10.0

APPENDIX C - List of the studies included in the systematic review.

Table S1. Key metadata for studies included in our systematic review about plastic pollution in Brazilian aquatic environments. We used the abbreviation *et al.* for studies with 3 authors or more.

Authors	Year	Environment	Study Object	Animal Group
Abude et al.	2021	Marine	Sediment	
Almeida et al.	2022	Marine	Sediment	
Alves et al.	2023	Estuarine	Sediment	
Alves & Figueiredo	2019	Estuarine	Sediment	
Amorim et al.	2020	Estuarine	Biota	Fish
Andrade et al.	2019	Freshwater	Biota	Fish
Andrades et al.	2019	Marine	Biota	Reptile
Andrades et al.	2020	Marine	Sediment	
Andrades et al.	2021	Estuarine	Biota	Fish
Araújo & Costa	2007	Estuarine	Sediment	
Araújo et al.	2018	Marine	Sediment	
Araújo & Costa	2021	Marine	Sediment	
Attademo et al.	2015	Marine	Biota	Mammal
Azevedo-Santos et al.	2022	Freshwater	Biota	Bird
Baptista-Neto et al.	2019	Estuarine	Sediment	
Barbieri	2009	Marine	Biota	Bird
Barbosa et al.	2019	Marine	Sediment	
Barrella et al.	2021	Estuarine	Sediment	
Bertoldi et al.	2021	Freshwater	Water	
Birnstiel et al.	2019	Estuarine	Biota	Mollusk
Bom et al.	2022	Marine	Sediment	
Bortolotto et al.	2016	Marine	Biota	Mammal
Brabo et al.	2022	Marine	Sediment	
Brandão et al.	2011	Marine	Biota	Bird
Brentano et al.	2020	Marine	Biota	Bird
Brentano & Petry	2020	Marine	Biota	Mammal

Bruzaca et al.	2022	Estuarine	Biota	Mollusk
Bugoni et al.	2001	Marine	Biota	Reptile
Camargo et al.	2022	Freshwater	Sediment	
Cardozo et al.	2018	Marine	Biota	Fish
Cardozo et al.	2023	Freshwater	Biota	Fish
Cardozo-Ferreira et al.	2021	Marine	Biota	Fish
Carvalho et al.	2021	Marine	Sediment	
Castro et al.	2020	Estuarine	Water	
Castro et al.	2016	Estuarine	Water	
Cavalcante et al.	2020	Marine	Sediment	
Clemente et al.	2018	Estuarine	Sediment	
Colabuono et al.	2009	Marine	Biota	Bird
Colabuono & Vooren	2007	Marine	Biota	Bird
Colferai et al.	2017	Marine	Biota	Reptile
Cordeiro & Costa	2010	Estuarine	Sediment	
Cordeiro et al.	2018	Estuarine	Sediment	
Corraini et al.	2018	Marine	Sediment	
Costa et al.	2018	Marine	Sediment	
Costa et al.	2010	Marine	Sediment	
Costa et al.	2021	Marine	Biota	Fish
Costa et al.	2019	Marine	Biota	Crustacea
Costa et al.	2023	Marine	Water	
Costa et al.	2023	Marine	Sediment Biota	Fish Mollusk Crustacea
Costa et al.	2011	Estuarine	Sediment	
da Costa et al.	2023	Freshwater	Water	
da Costa et al.	2022	Freshwater	Water	
da Costa et al.	2022	Marine	Biota	Bird
da Costa et al.	2023	Freshwater	Biota	Fish
da Costa et al.	2021	Marine	Biota	Annelid
da Costa et al.	2023	Marine	Biota	Mollusk
da Silva et al.	2022	Marine	Sediment	

	2012	Γ	F' 1	
Dantas et al.	2012	Estuarine	Fish	
Dantas et al.	2020	Marine	Biota	Fish
Dantas et al.	2019	Estuarine	Biota	Fish
de Araújo et al.	2007	Marine	Sediment	
de Carvalho et al.	2016	Marine	Sediment	
de Carvalho et al.	2015	Marine	Biota	Reptile
de Carvalho-Souza et al.	2016	Marine	Biota	Reptile
de Faria et al.	2021	Freshwater	Water	
de Faria et al.	2022	Freshwater	Water	
de Lemos Santana et al.	2022	Marine	Biota	Crustacea
de Melo Nobre et al.	2021	Marine	Sediment	
de Melo Nobre et al.	2021	Marine	Sediment	
de Oliveira et al.	2023	Marine	Sediment	
de Ramos et al.	2021	Marine	Sediment	
de Souza Petersen et al.	2016	Marine	Biota	Bird
de Souza et al.	2023	Freshwater	Water	
Denuncio et al.	2017	Marine	Biota	Mammal
Di Beneditto & Awabdi	2014	Marine	Biota	Fish
Di Beneditto & Oliveira	2019	Marine	Biota	Fish
Di Beneditto & Ramos	2014	Marine	Biota	Mammal
Di Beneditto & Siciliano	2017	Marine	Biota	Bird
do Sul & Costa	2013	Estuarine	Sediment	
do Sul et al.	2014	Marine	Water	
do Sul et al.	2013	Marine	Water	
do Sul et al.	2011	Marine	Sediment	
do Sul et al.	2009	Marine	Sediment	
dos Santos et al.	2020	Freshwater	Biota	Fish
Farias et al.	2018	Estuarine	Water	
Fernandes et al.	2021	Marine	Biota	Fish
Fernandino et al.	2016	Estuarine	Water	
Fernandino et al.	2015	Marine	Sediment	
Fernandino et al.	2016	Marine	Sediment	
Ferreira et al.	2019	Estuarine	Biota	Fish

Ferreira et al.	2016	Estuarine	Biota	Fish
Ferreira et al.	2019	Estuarine	Biota	Fish
Ferreira et al.	2018	Estuarine	Biota	Fish
Ferreira et al.	2022	Marine	Biota	Mollusk
Ferreira et al.	2023	Marine	Biota	Fish
Figueiredo & Vianna	2018	Estuarine	Water	
Fisner et al.	2017	Estuarine	Sediment	
Garcia et al.	2020	Freshwater	Biota	Fish
Garcia et al.	2020	Marine	Water	
Gerolin et al.	2020	Freshwater	Sediment	
Gonçalves et al.	2020	Marine	Biota	Crustacea
Gonçalves et al.	2020	Estuarine	Sediment	
Guebert-Bartholo et al.	2011	Estuarine	Biota	Reptile
Guimarães et al.	2023	Freshwater	Biota	Crustacea
Gusmão et al.	2016	Marine	Biota	Annelid
Justino et al.	2023	Marine	Biota	Fish
Justino et al.	2022	Marine	Biota	Fish
Justino et al.	2021	Marine	Biota	Fish
Krelling et al.	2017	Estuarine	Sediment	
Krelling et al.	2023	Estuarine	Sediment	
Krelling & Turra	2019	Estuarine	Water	
Leite et al.	2014	Marine	Sediment	
Lima et al.	2021	Marine	Sediment	
Lima et al.	2022	Marine	Sediment	
Lima et al.	2015	Estuarine	Water	
Lima et al.	2016	Marine	Water	
Lima et al.	2014	Estuarine	Water	
Lima et al.	2021	Freshwater	Biota	Fish
Lima et al.	2016	Estuarine	Water	
Lins-Silva et al.	2021	Estuarine	Water	
Lorenzi et al.	2020	Estuarine	Water	
Lorenzi et al.	2021	Estuarine	Water	
Machovsky-Capuska et al.	2020	Estuarine/Marine	Biota	Reptile

Macieira et al.	2021	Marine	Biota	Fish
Majer et al.	2012	Marine	Biota	Insect
Marques et al.	2018	Marine	Biota	Bird
Martinelli & Monteiro	2019	Marine	Sediment	
Mascarenhas et al.	2004	Marine	Biota	Reptile
Maynard et al.	2021	Marine	Sediment	1
Mengatto & Nagai	2022	Estuarine	Sediment	
Miranda & Carvalho-Souza	2016	Marine	Biota	Fish
Monteiro et al.	2022	Estuarine	Water	
Monteiro et al.	2020	Marine	Sediment	
Morais et al.	2020	Marine	Biota	Cnidarian
Moreira et al.	2016	Marine	Sediment	
Moreira et al.	2016	Estuarine	Sediment	
Neto et al.	2020	Marine	Biota	Fish
Neto et al.	2019	Marine	Sediment	
Nolasco et al.	2022	Marine	Water	
Novaes et al.	2020	Estuarine	Sediment	
Nunes et al.	2021	Marine	Biota	Reptile
Nunes et al.	2018	Marine	Biota	Fish
Oigman-Pszczol & Creed	2007	Marine	Sediment	
Olivatto et al.	2019	Estuarine	Water	
Oliveira et al.	2020	Freshwater	Biota	Fish
Oliveira et al.	2023	Freshwater	Water	
Padula et al.	2023	Marine	Biota	Mammal
Paes et al.	2022	Estuarine	Sediment	
Palombini et al.	2018	Marine	Sediment	
Pegado et al.	2021	Marine	Biota	Fish
Pegado et al.	2018	Estuarine	Biota	Fish
Perez et al.	2018	Marine	Sediment	
Petry et al.	2021	Marine	Biota	Reptile
Petry & Benemann	2017	Marine	Biota	Bird
Petry et al.	2007	Marine	Biota	Bird
Petry et al.	2009	Marine	Biota	Bird

Petry et al.	2010	Marine	Biota	Bird
Pinheiro et al.	2021	Estuarine	Sediment	
Pinheiro et al.	2021	Marine	Water	
Pinheiro et al.	2022	Estuarine	Sediment	
Pinheiro et al.	2019	Marine	Sediment	
Poli et al.	2014	Marine	Biota	Reptile
Poli et al.	2015	Marine	Biota	Reptile
Possatto et al.	2015	Estuarine	Sediment	
Possatto et al.	2011	Estuarine	Biota	Fish
Queiroz et al.	2022	Marine	Water	
Ramos et al.	2012	Estuarine	Biota	Fish
Ramos & Pessoa	2019	Estuarine	Sediment	
Ramos et al.	2022	Freshwater	Biota	Fish
Reis et al.	2010	Marine	Biota	Reptile
Ribeiro et al.	2022	Estuarine	Sediment	
Ribeiro et al.	2021	Marine	Sediment	
Ribeiro et al.	2021	Marine	Sediment	
Ribeiro et al.	2023	Estuarine	Biota	Mollusk
Rosa & Widmer	2021	Marine	Sediment	
Rosa et al.	2023	Estuarine	Water	
Rossi et al.	2019	Marine	Biota	Bird
Saldana-Serrano et al.	2022	Marine	Biota	Mollusk
Sampaio et al.	2018	Marine	Biota	Fish
Santana et al.	2016	Estuarine	Biota	Mollusk
Santos et al.	2017	Marine	Sediment	
Santos et al.	2009	Marine	Sediment	
Santos et al.	2005	Marine	Sediment	
Santos et al.	2016	Marine	Biota	Reptile
Santos et al.	2020	Estuarine	Biota	Fish
Sazima & D'Angelo	2016	Freshwater	Biota	Bird
Sazima et al.	2002	Marine	Biota	Fish
Schuab et al.	2023	Estuarine	Biota	Mollusk
Silva et al.	2018	Estuarine	Biota	Fish

Silva et al.	2018	Marine	Sediment	
Silva et al.	2019	Estuarine	Water	
Silva-Cavalcanti et al.	2009	Marine	Sediment	
Silva-Cavalcanti et al.	2023	Freshwater	Sediment	
Silva-Cavalcanti et al.	2017	Freshwater	Biota	Fish
Soares et al.	2022	Marine	Biota	Porifera
Tavares et al.	2016	Marine	Sediment	
Tavares et al.	2017	Marine	Biota	Bird
Tourinho et al.	2010	Marine	Biota	Reptile Bird
Toyama et al.	2021	Freshwater	Sediment	
Trindade et al.	2023	Estuarine	Water	
Trindade et al.	2023	Freshwater	Biota	Fish
Tsukada et al.	2021	Marine	Sediment	
Urbanski et al.	2019	Freshwater	Biota	Fish
Vanstreels et al.	2021	Marine	Biota	Bird
Vendel et al.	2017	Estuarine	Biota	Fish
Vieira et al.	2021	Estuarine	Biota	Mollusk
Zamprogno et al.	2021	Estuarine	Sediment	

APPENDIX D - List of the studies included in the synthesis.

Table S1. Key metadata for studies included in our synthesis of the influence of local anthropogenic activities, animal group and environment type in plastic pollution in Brazilian aquatic environments. We used the abbreviation *et al.* for studies with 3 authors or more.

Authors	Year	Environment	Study Object	Animal Group
Alves et al.	2023	Estuarine	Sediment	
Alves & Figueiredo	2019	Estuarine	Sediment	
Amorim et al.	2020	Estuarine	Biota	Fish
Andrade et al.	2019	Freshwater	Biota	Fish
Andrades et al.	2019	Marine	Biota	Reptile
Andrades et al.	2020	Marine	Sediment	
Araújo & Costa	2007	Estuarine	Sediment	
Araújo et al.	2018	Marine	Sediment	
Baptista-Neto et al.	2019	Estuarine	Sediment	
Barrella et al.	2021	Estuarine	Sediment	
Bertoldi et al.	2021	Freshwater	Water	
Birnstiel et al.	2019	Estuarine	Biota	Mollusk
Bom et al.	2022	Marine	Sediment	
Brabo et al.	2022	Marine	Sediment	
Bruzaca et al.	2022	Estuarine	Biota	Mollusk
Camargo et al.	2022	Freshwater	Sediment	
Cardozo et al.	2018	Marine	Biota	Fish
Cardozo et al.	2023	Freshwater	Biota	Fish
Carvalho et al.	2021	Marine	Sediment	
Castro et al.	2020	Estuarine	Water	
Castro et al.	2016	Estuarine	Water	
Cavalcante et al.	2020	Marine	Sediment	
Colferai et al.	2017	Marine	Biota	Reptile
Cordeiro et al.	2010	Estuarine	Sediment	
Cordeiro et al.	2018	Estuarine	Sediment	
Corraini et al.	2018	Marine	Sediment	
Costa et al.	2018	Marine	Sediment	
Costa et al.	2010	Marine	Sediment	
Costa et al.	2023	Marine	Water	
Costa et al.	2011	Estuarine	Sediment	
da Costa et al.	2023	Freshwater	Water	
da Costa et al.	2022	Freshwater	Water	
da Costa et al.	2022	Marine	Biota	Bird
da Costa et al.	2023	Freshwater	Biota	Fish
Dantas et al.	2019	Estuarine	Biota	Fish
de Faria et al.	2021	Freshwater	Water	

de Faria et al.	2022	Freshwater	Water	
de Lemos Santana et al.	2022	Marine	Biota	Crustacea
de Melo Nobre et al.	2022	Marine	Sediment	Clustacea
de Melo Nobre et al.	2021	Marine	Sediment	
de Ramos et al.	2021	Marine	Sediment	
Di Beneditto & Siciliano	2021	Marine	Biota	Bird
do Sul & Costa	2017	Estuarine	Sediment	DIIu
Farias et al.	2018	Estuarine	Water	
Fernandino et al.	2015	Marine	Sediment	F' 1
Ferreira et al.	2019	Estuarine	Biota	Fish
Ferreira et al.	2018	Estuarine	Biota	Fish
Ferreira et al.	2022	Marine	Biota	Mollusk
Figueiredo & Vianna	2018	Estuarine	Water	
Garcia et al.	2020	Freshwater	Biota	Fish
Garcia et al.	2020	Marine	Water	
Gerolin et al.	2020	Freshwater	Sediment	
Gonçalves et al.	2020	Estuarine	Sediment	
Guimarães et al.	2023	Freshwater	Biota	Crustacean
Justino et al.	2022	Marine	Biota	Fish
Krelling et al.	2017	Estuarine	Sediment	
Lima et al.	2022	Marine	Sediment	
Lima et al.	2015	Estuarine	Water	
Lima et al.	2016	Marine	Water	
Lima et al.	2014	Estuarine	Water	
Lorenzi et al.	2020	Estuarine	Water	
Lorenzi et al.	2021	Estuarine	Water	
Macieira et al.	2021	Marine	Biota	Fish
Martinelli Filho & Monteiro	2019	Marine	Sediment	
Mengatto & Nagai	2022	Estuarine	Sediment	
Monteiro et al.	2022	Estuarine	Water	
Monteiro et al.	2020	Marine	Sediment	
Morais et al.	2020	Marine	Biota	Cnidarian
Nunes et al.	2021	Marine	Biota	Reptile
Olivatto et al.	2019	Estuarine	Water	1
Paes et al.	2022	Estuarine	Sediment	
Pegado et al.	2021	Marine	Biota	Fish
Pegado et al.	2018	Estuarine	Biota	Fish
Petry et al.	2010	Marine	Biota	Reptile
Petry & Benemann	2021	Marine	Biota	Bird
Pinheiro et al.	2017	Estuarine	Sediment	Dird
Pinheiro et al.	2021	Marine	Water	
Pinheiro et al.	2021	Estuarine	Sediment	
Pinheiro et al.	2019	Marine	Sediment	Dant!1-
Poli et al.	2015	Marine	Biota	Reptile
Possatto et al.	2015	Estuarine	Sediment	F2: -1.
Possatto et al.	2011	Estuarine	Biota	Fish

Queiroz et al.	2022	Marine	Water	
Ramos et al.	2012	Estuarine	Biota	Fish
Ramos & Pessoa	2019	Estuarine	Sediment	
Ribeiro et al.	2021	Marine	Sediment	
Ribeiro et al.	2021	Marine	Sediment	
Ribeiro et al.	2023	Estuarine	Biota	Mollusk
Rosa & Widmer	2022	Marine	Sediment	
Rosa et al.	2023	Estuarine	Water	
Rossi et al.	2019	Marine	Biota	Bird
Saldana-Serrano et al.	2022	Marine	Biota	Mollusk
Santos et al.	2017	Marine	Sediment	
Santos et al.	2005	Marine	Sediment	
Silva et al.	2018	Marine	Sediment	
Silva-Cavalcanti et al.	2009	Marine	Sediment	
Silva-Cavalcanti et al.	2017	Freshwater	Biota	Fish
Soares et al.	2022	Marine	Biota	Porifera
Tavares et al.	2016	Marine	Sediment	
Toyama et al.	2021	Freshwater	Sediment	
Trindade et al.	2023	Estuarine	Water	
Trindade et al.	2023	Freshwater	Biota	Fish
Urbanski et al.	2019	Freshwater	Biota	Fish
Vendel et al.	2017	Estuarine	Biota	Fish
Zamprogno et al.	2021	Estuarine	Sediment	