PANAMJAS
Pan-American Journal of Aquatic Sciences

Status of Brazilian research on microplastics present in aquatic ecosystems: freshwater

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Abstract. Microplastics are small fragments of polymers. Due to their size (0.1 µm to 5 mm), they may pollute various natural resources, such as air, soil, and water bodies. There are few studies focusing on the relation between microplastics and freshwater ecosystems in the world. In Brazil, the situation is quite similar to the rest of world: data are scarce and reveal little about the real dimension of this anthropogenic phenomenon. In view of the relevance of this topic for the scientific community, the present review aims to trace the state of the Brazilian research so far, and point out the research gaps in the area, such as the lack of research with sediment samples, and results regarding the toxicity of secondary microplastics, which are more common in the environment than the primary ones. A bibliographic survey was conducted in online databases to identify all scientific articles published in Brazil until December 2020. Eighteen articles were found and analyzed. We concluded that the number of articles has shown a significant increase, mainly in 2020, with scientific productions very relevant for the academic community. However, greater investments and incentives are still needed to consolidate the development of this research area in Brazil.

Key words: Plastics, Pollution, Water, Brazil.

Resumo: **Status da pesquisa brasileira em microplásticos presentes em ecossistemas aquáticos: água doce.** Microplásticos são pequenos fragmentos de polímeros. Devido ao seu tamanho (0.1 µm to 5 mm), podem poluir diversos recursos naturais, como ar, solo e corpos d'água. Existem poucos estudos focando na relação entre microplásticos e ecossistemas de água doce no mundo. No Brasil, a situação é bastante similar ao restante do mundo: os dados são escassos e pouco revelam sobre a real dimensão desse fenômeno antrópico. Tendo em vista a relevância do tema para a comunidade científica, a presente revisão visa traçar o estado da pesquisa brasileira até o momento e apontar lacunas de pesquisas na área, como a escassez de pesquisas com amostras de sedimentos e de resultados quanto à toxicidade de microplásticos secundários, os quais são mais comuns no meio ambiente que os primários. O levantamento bibliográfico foi realizado em bases de dados online para identificar todos os artigos científicos publicados no Brasil até dezembro de 2020. Dezoito artigos foram encontrados e analisados. Concluímos que o número de artigos tem apresentado aumento significativo, principalmente em 2020, com produções científicas de repercussão positiva para comunidade acadêmica. No entanto, ainda são necessários maiores investimentos e incentivos para consolidar o desenvolvimento dessa área de pesquisa no Brasil.

Palavras-chave: Plástico, Poluição, Água, Brasil

Introduction

The excesses of the consumerism era and free trade policies by which quality of life is often confused with unrestricted consumption have produced alarming quantities of solid waste largely composed of plastic materials (Hoornweg & Bhada-Tata 2012). Plastics are chemical structures capable of polluting a wide range of natural resources and cause devastating consequences for the environment and public health (Thompson *et al*. 2009). Reducing the excessive consumption of plastic, which is often associated with deficient solid waste management systems, is a global and urgent task (Backhaus & Wagner 2018), especially at the present moment when it is known that the presence of plastics in the environment is much larger than that visible to the naked eye.

Currently, more people are concerned about the huge amounts of plastic artifacts disposed of improperly in the environment (Henderson & Green 2020). Such artifacts undergo a constant process of fragmentation and slowly transform into countless microplastic particles (Backhaus & Wagner 2018), which are polymer fragments with sizes ranging between 0.1 μm and 5 mm (Carr *et al*. 2016, Jahnke *et al*. 2017). Due to their small size, they are present in the air, soil, sewers, and water bodies such as lakes, rivers, and oceans (Rillig 2012, Andrady 2017, Koelmans *et al*. 2019, Li *et al*. 2020).

Microplastic debris is classified as primary or secondary. Primary microplastics are the direct release of small particles by the industry through microspheres present mainly in cleaning, hygiene, and cosmetics products (Carr *et al*. 2016). Secondary microplastics originate from the fragmentation of larger polymers, known as macroplastics, during and after the degradation process (Jahnke *et al*. 2017, Schür *et al*. 2020).

Since the first signs of microplastics in the environment, oceans have been the primary choice in terms of concern. There are several academic studies on the interaction of this pollutant with marine organisms (Su *et al*. 2018). In aquatic freshwater ecosystems, the presence and impacts of microplastics are still unclear because studies are still at an early stage considering that microplastics in freshwater and sediments began to be studied only in 2010 (Wagner & Lambert 2017). As Brazil is considered an important holder of global biodiversity hotspots, it is necessary to obtain evidence and understand how local biodiversity is threatened, and one of the ways to do this is analyzing it from the perspective of microplastic pollution.

This review aims to investigate the status of Brazilian research on the presence and impacts of microplastics in freshwater ecosystems and point out the advances and research gaps in this area, subsidizing scientific divulgation.

Materials and methods

We performed a comprehensive literature search in scientific databases, including Science Direct (Elsevier), Web of Science, and Google Scholar. The inclusion criteria were (i) research conducted in the Brazilian territory and (ii) research on freshwater environments, excluding studies on estuarine regions.

We opted to perform keyword searches, which would allow ranking as much relevant data records as possible, and avoid the exclusion of pertinent and compatible articles. Therefore, we used keywords with a wide reach, such as “microplastics Brazil” and “microplásticos Brasil”.

Technical reports, monographs, dissertations, theses, conference proceedings, and review articles were excluded. We selected all articles according to the inclusion criteria published or available for consultation until December 2020.

Results

Overview of the studies selected: Based on the bibliographic survey, we identified 69 articles written in Brazil in different aquatic environmental matrices (Fig. 1).

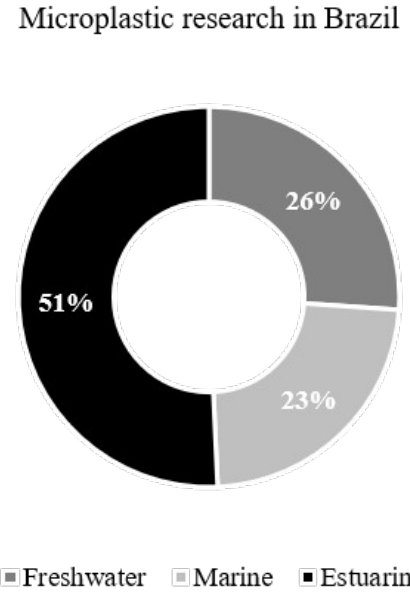

Figure 1. Percentage of microplastics scientific articles according to environmental matrices in Brazil (n = 69).

Eighteen scientific articles were develop in freshwater and were included in this review, which fifteen articles are on ecotoxicology of organisms (Daphnia, fish, bivalves, and amphibians) and three on the presence of microplastics in a water column.

Research groups and home institutions: The research groups identified in this review are in research centers, mainly at universities in nine Brazilian states (Fig. 2). The authors and their home research institutions are in Table I.

Scientific journals: The articles about microplastics in freshwater environment were published in eight scientific journals. All are international journals (Table II) and within the same field of knowledge: Environmental Sciences. The impact factor is usually considered an indicator of the quality of journals and articles (McKiernan *et al*. 2019), although there is no consensus as to these values being considered good or bad (Chorus & Waltman 2016, Wouters *et al*. 2019).

Thematic axes: Toxicological tests help to provide data and insights that guide and contribute to the understanding of the mechanisms involved in the toxicity of microplastics to biota. In this research,the articles assessed the effects of exposure to microplastics on sensitive organisms at different endpoints. The articles evaluated the planktonic crustaceans, fish, bivalves, and amphibians and investigated the presence of microplastics in the water column (Table III).

The main highlights of all studies analyzed according to the organisms or the sample analyzed are shown below and in Table IV.

Daphnia magna: D. magna is a model organism well-established in the literature to study many biological processes due to several factors, including its importance in the freshwater food chain and easy handling and maintenance in laboratory condition, besides its simple and short life cycle. Regarding microplastics, it serves as a link between primary producers and final consumers. Thus, its response in tests have a great relevance as a warning indicator for this pollutant (Eerkes-Medrano *et al.* 2015, Imhof *et al.* 2017).

Castro *et al*. (2020) conducted a study on *D. magna* with neonates. The results showed that the concentrations of microplastics assessed did not cause immobility or affect the reproductive cycle and body length. This result is consistent with other studies (Rehse *et al.* 2016, Eltemsah & Bøhn, 2019, Jaikumar *et al.* 2019) and also indicates that smaller fragments represent a greater harm to the digestive system, which may in turn compromise the entire metabolism.

Fish: Fish are often used in research on environmental contaminants. This practice is already well established in laboratory experiments mainly due to the position these organisms occupy in the trophic level. Therefore, the response of these animals is an indicator of environmental quality and eventual risk of human harm due to the consumption of contaminated fish (Thiele *et al*. 2019). Because of economic and environmental interests, the impacts possibly caused by the exposure of fish to plastic fragments have been increasingly studied (Prokić *et al*. 2019).

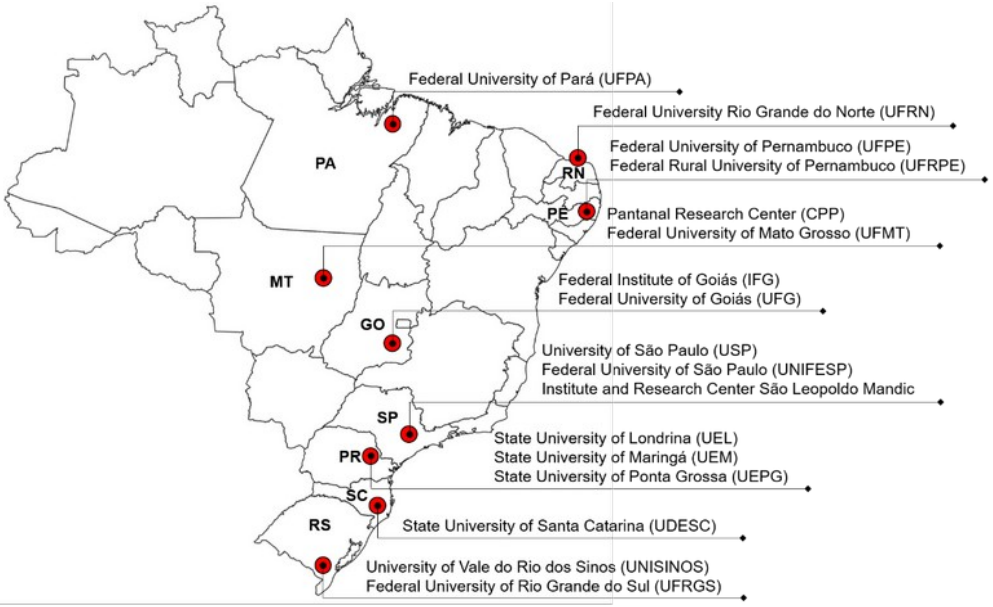

Figure 2. Map of home institutions of researchers which publish scientific articles about microplastics in freshwater environments in Brazil, between 2018 and 2020.

Table I. Authors and their home institutions

Authors	Home institutions
Andrade *et al*. 2019 Ribeiro-Brasil *et al*. 2020	UFPA: Federal University of Pará
Araújo *et al*. 2020a, c Araújo & Malafaia 2020 Araújo & Malafaia 2021	IFG: Federal Institute of Goiás
Araújo *et al*. 2020b	IFG: Federal Institute of Goiás UFG: Federal University of Goiás Institute and Research Center São Leopoldo Mandic UEL: State University of Londrina
Bertoldi *et al*. (accepted)	UFRGS: Federal University of Rio Grande do Sul
Castro *et al*. 2020	USP: University of São Paulo
Faria *et al*. 2019	UFMT: Federal University of Mato Grosso CPP: Pantanal Research Center UEPG: State University of Ponta Grossa UFPE: Federal University of Pernambuco
Ferraz *et al*. 2020	UNISINOS: University of Vale do Rio dos Sinos
Garcia *et al*. 2020	UEL: State University of Londrina UEM: State University of Maringá UDESC: State University of Santa Catarina
Guimarães *et al*. 2020	IFG: Federal Institute of Goiás UFG: Federal University of Goiás USP: University of São Paulo
Malafaia *et al*. 2020 Oliveira *et al*. 2021	IFG: Federal Institute of Goiás UFG: Federal University of Goiás
Moreschi *et al*. 2020	UFMT: Federal University of Mato Grosso UEPG: State University of Ponta Grossa CPP: Pantanal Research Center
Roda *et al*. 2020	UEL: State University of Londrina
Silva-Cavalcanti *et al*. 2017	UFRPE: Federal Rural University of Pernambuco UFRN: Federal University of Rio Grande do Norte UNIFESP: Federal University of São Paulo

In all studies, the presence of microplastics in the stomach contents of fish was observed, which was attributed to the levels of pollution in each study area, thus, the more degraded the environment, the higher the concentrations found. In addition, the authors point out that the microplastics are originated from degradation of bags, plastic bottles, fishing gear, improperly discarded in the watershed and drained by rivers and streams.

Studies have also been carried out to evaluated neurotoxicity, cardiotoxicity, teratogenic effects, and external morphotoxicity of zebrafish (*Danio rerio*) embryos (Malafaia *et al*. 2020). Another aspects analyzed, referred to the possibility of association of microplastics with other pollutants, such as copper (Cu) (Roda *et al*. 2020) and the potential transfer of microplastics between two species of fish (Araújo *et al*. 2020c).

Ribeiro-Brasil *et al*. (2020) and Silva-Cavalcanti *et al*. (2018) classified microplastics into types and noticed that fibers were significantly more common than fragments. In two different approaches from the other studies, Guimarães *et al*. (2020) and Oliveira *et al*. (2021) analyzed the response of fish to interaction with naturally aged microplastics and microplastics of natural origin (bioplastics), respectively. These methodological variations allowed the authors to register that both of these

Table II. List of articles about microplastics in freshwater environment in Brazil, scientific journals and their respective impact factors.

Authors	Journal	Impact factor
Moreschi *et al*. 2020	Case Studies in Chemical and Environmental Engineering	-
Castro *et al*. 2020	Journal of Environmental Science and Health, Part A	1.53
Garcia *et al*. 2020	Water Air and Soil Pollution	1.90
Roda *et al*. 2020	Comparative Biochemistry and Physiology, Part A	2.14
Faria *et al*. 2019	PeerJ Preprints	2.35
Ferraz *et al*. 2020	Water	2.54
Araújo *et al*. 2020c Bertoldi *et al*. (accepted) Malafaia *et al*. 2020	Science of the Total Environment	5.58
Andrade *et al*. 2019 Ribeiro-Brasil *et al*. 2020 Silva-Cavalcanti *et al*. 2017	Environmental Pollution	5.71
Araújo *et al*. 2020a, b Araújo & Malafaia 2020 Araújo & Malafaia 2021 Guimarães *et al*. 2020 Oliveira *et al*. 2021	Journal of Hazardous Materials	7.65

materials may induce physiological changes in the tested organisms. Therefore, these lines of research need to be continued so that the results can be better understood and explained.

In general, data on plastics in fish stomachs are still scarce and need further analysis, since smaller microplastic particles can be transferred from the stomach to other body parts, which may affect the functioning of muscles and other organs (Karami *et al.* 2017).

Bivalves: Freshwater bivalves have great ecological importance. As filter feeders, this class of animals is responsible for transferring nutrients from the water column to the sediment (Smaal *et al*. 2019). Which leads us to question the role of these animals in the distribution of microplastics in the environments in which they are located.

Only one study was conducted with bivalves (Moreschi *et al*. 2020), but as a great candidate to be used as a biological indicator, complementary studies should be done in order to contribute to a better understanding of the impacts caused by this pollutant on these animals.

Amphibians: Most studies on microplastics focus on invertebrates and insects and few of them have analyzed the response of vertebrate animals, except for fish (Eerkes-Medrano *et al.* 2015). Lately, interest has grown in conducting studies on amphibians, which are animals highly susceptible to changes in the environment. Amphibians have suffered a decrease in population due to high rates of pollution in aquatic ecosystems (Slaby *et al.* 2019). Some studies have proven the lethality of several pollutants in the dynamics of amphibians, but little is known about absorption, accumulation, and biological consequences of microplastics for these animals (Ripple *et al.* 2019, Slaby *et al.* 2019).

Four toxicity tests were performed with *Physalaemus cuvieri* tadpoles (Araújo & Malafaia 2020, Araújo *et al*. 2020a, Araújo *et al*. 2020b, Araújo & Malafaia 2021), and all occurred under similar conditions, such as plastic, size of microplastics, concentration tested and time of exposure (Table III). In general, the studies aimed to analyze and discuss the behavioral toxicity induced by exposure to the microplastics.

Overall, the results showed behavioral toxicity caused by microplastics in tadpoles. Even though further research is needed to evaluate the toxicity of microplastics to the biological functions of amphibians, these results highlight the problem of the presence of these pollutants of freshwater environments.

Table III. Parameters used in the ecotoxicological bioassays under laboratory conditions for exposure of microplastics on freshwater organisms and the findings of field experiments (specifying the organisms or sample on trial, location for field experiments, polymer type, fragments size, concentration, and duration). Ref: references; p: particle.

Organism / Sample	Organism group	Location	Polymer	Size (µm)	Concentration	Duration (hours)	Ref
Serrasalmid family	Fish	Xingu River	Polyethylene Polyvinyl chloride Polylactic acid Polypropylene Polyethylene terephthalate Polymethyl methacrylate Rayon	>1000	–	–	1
Physalaemus cuvieri	Amphibian	–	Polyethylene	35	60 mg/L	168	2
Physalaemus cuvieri	Amphibian	–	Polyethylene	35	60 mg/L	168	3
Poecilia reticulata *Danio rerio*	Fish	–	Polyethylene	35	60 mg/L	48	4
Physalaemus cuvieri	Amphibian	–	Polyethylene	35	60 mg/L	168	5
Physalaemus cuvieri *C. macropomum x P. Brachypomus**	Amphibian Fish	–	Polyethylene	35	60 mg/L	168	6
Freshwater	–	Lake Guaíba	–	5 – 5000	11.9 – 61.2 p/m^{-3}	–	7
Daphnia magna	Zooplankton	–	Polyethylene	40 – 48	20 mg/L 40 mg/L 80 mg/L 160 mg/L 320 mg/L	96	8
Freshwater	–	Cuiabá River	–	192 ± 142	9.6 $p/100L^{-1}$	–	9
Freshwater	–	Sinos River	–	>200	330.2 p/L^{-1}	–	10
Varied species (13 spp.)	Fish	Ivaí River	–	–	13 – 21 p/fish	–	11
Danio rerio	Fish	–	Polystyrene	150	4 x 10^4 p/m^3*** 4 x 10^6 p/m^3***	120	12
Danio rerio	Fish	–	Polyethylene	38	6.2 mg/L 12.5 mg/L 25 mg/L 50 mg/L 100 mg/L	24 48 72 96 120 144	13
Anodontites trapesialis	Bivalve	–	Polyethylene	55 – 110	75 mg/L	3 – 192	14
Danio rerio	Fish	–	Polylactic acid	1 – 8	3 mg/L 9 mg/L	120	15
Varied species (14 species)	Fish	Guamá and Acará-Capim Rivers	–	200 – 5000	5.6 p/fish	–	16
Prochilodus lineatus	Fish	–	Polyethylene	10 – 90	0.02 mg/L	24 96	17
Hoplosternum littorale	Fish	Pajeú River	–	<5000	3.6 p/fish	–	18

All authors tested the concentration 0 mg/L, for the control sample during ecotoxicological tests. *Hybrid fish species resulted from the crossing between female *Colossoma macropomum* and male *Piaractus brachypomus* individuals. Ref: 1: Andrade *et al*. 2019; 2:

Araújo *et al*. 2020a; 3: Araújo *et al*. 2020b; 4: Araújo *et al*. 2020c; 5: Araújo & Malafaia 2020; 6: Araújo & Malafaia 2021; 7: Bertoldi *et al*. (accepted); 8: Castro *et al*. 2020; 9: Faria *et al*. 2019; 10: Ferraz *et al*. 2020; 11: Garcia *et al*. 2020; 12: Guimarães *et al*. 2020; 13: Malafaia *et al*. 2020; 14: Moreschi *et al*. 2020; 15: Oliveira *et al*. 2021; 16: Ribeiro-Brasil *et al*. 2020; 17: Roda *et al*. 2020; 18: Silva-Cavalcanti *et al*. 2017.

The study of microplastics accumulation is undoubtedly very relevant, but the observation of transference in the trophic chain (Araújo & Malafaia 2021) presents a great finding in terms of the ecological community since pollutants can reach the upper trophic level, and trigger behavioral changes.

Microplastics in water column: Studies on plastic fragments in water column samples play a very relevant role in research, since they are associated with degradation of environmental quality and reach public water systems, where they are proven to be present (Eerkes-Medrano *et al*. 2019, Koelmans *et al*. 2019).

The studies that focused on researching the presence of microplastics in freshwater analyzed samples from the following bodies of water: Cuiabá River (Faria *et al*. 2019), Lake Guaíba (Bertoldi *et al*.), and Sinos River (Ferraz *et al*. 2020). All three regions were polluted by the presence of microplastics, with samples from Sinos River having higher concentrations. The authors agreed that wastewater from industrial activities, agricultural inputs and outputs, and rainfalls can contribute to the process of contaminating the entire hydrographic basin with various pollutants, including microplastics. Besides that, bodies of water close to urbanized regions are more likely to have higher levels of microplastics.

Discussion

Advances and achievements in Brazilian research: The first original scientific article published in journals in the context of microplastics in freshwater in Brazil was published recently 2017 (Silva-Cavalcanti *et al*.) which only one study was reported, followed by two other publications in 2019 and twelve publications in 2020. Although there has been a significant increase in the number of publications, it is not yet possible to verify a solid evolution. Solid evolution will be achieve after a few more years of constant publications, bringing consistency and sufficient empirical evidence to reveal the levels of pollution by microplastics in the various Brazilian environmental matrices.

It should be noted, however, that these are not the first publications on microplastics in Brazil because studies on marine microplastics began in the 1970s (Gomes 1973). This first study was followed by several studies published since 2009 and, therefore, research already more advanced and even consolidated in Brazil. Nevertheless, marine and estuarine microplastics studies are more advanced and generally more robust than studies on other environmental matrices. Unlike the microplastics in freshwater environments, there are research groups focusing primarily on marine fragments. Seventy-four per cent of the research on microplastics in Brazil refers to studies in estuarine and marine environments (Figure 1).

Here, we analyzed 18 articles that addressed the topic of microplastics in several Brazilian locations. The studies come from nine Brazilian states and count on the participation and contribution of 17 universities and research centers and were published in nine scientific journals. As also observed by Castro *et al*. 2018, the contributions of the north and northeast regions are still less significant, and the choice of scientific journals continues to follow the same pattern of international journals with a high impact factor.

The studies have demonstrated, through different approaches, that biota is already in danger due to the presence of microplastics in freshwater environments. There is a directly proportional relationship between high levels of local pollution and the greatest negative effects on the many organisms on trial.

Research perspectives: The status of the Brazilian research on freshwater microplastics, represented by scientific articles published in indexed journals, reinforces the growth of this area and the potential contribution to the community that can be achieved, especially if there were more research groups focused exclusively on the topic discussed here. Castro *et al*. (2018) conducted a review analyzing Brazilian original studies on microplastics available up to October 2017 regardless of the environmental matrix. The authors pointed out that Brazilian publications represented 8.4% of the total of published studies in the world in the same period. Despite the increase in the number of publications in recent years, the expectations of the scientific community were higher than the reality until 2018, mainly caused by frequent budget cuts in Brazilian science (Castro *et al*. 2018). Notwithstanding, in 2020 the number of publications increased 600% compared to the previous year.

Table IV. Summary of the objectives and main results and conclusions of the eighteen articles on freshwater environments in Brazil. Ref: references.

Objectives	Main results and conclusion	Ref
Relationship between ingestion and trophic guild of microplastic	Ingestion rate was 29.2%; herbivorous specimens had less plastic in their stomachs. The trophic guilds did not differ in frequency/magnitude of plastic ingestion.	1
Hepatotoxicity of microplastics on *Physalaemus cuvieri*	There were some differences in nuclear erythrocyte morphometry and acute inflammatory response in liver tissue due to the accumulation of microplastics.	2
Changes on erythrocytes induced by microplastics on *P. cuvieri*	Microplastics induced mutagenic effects, damage to cells, affected survival and reproduction. The erythrocytes changes referred to cell size and nucleus.	3
Trophic transfer of microplastics by using two fish species	Accumulation was lower in animals exposed through feeding than by direct exposure. Trophic transfer, mutagenic and cytotoxic effects were observed.	4
Behavioral toxicity induced by exposure of *P. cuvieri* to microplastics	The accumulation of microplastics resulted on increase in anxiety and biomass, decrease in the locomotor activity, and a loss of antipredator behavior of tadpoles.	5
Bioaccumulation and trophic transfer of microplastics	Microplastics reached individuals of the last trophic level (third) and altered the animals' behavior and the anti-predatory response.	6
Microplastics in Lake Guaíba	Microplastics were found in all samples. The amount of microplastics is influenced by the geohydrological lake characteristics.	7
Analyze effects of exposure of *Daphnia magna* to microplastic in the short-term	Microplastic concentrations did not affect the reproductive cycle and body length, as well as the microplastic size assessed was not able to cause toxicological effects.	8
Presence of microplastics in Cuiabá river, Pantanal region	The Pantanal is contaminated with microplastic, with fibers being the type in greater quantity both in the urban tributaries and in the Pantanal.	9
Microplastic concentrations in raw and treated water from the Sinos River	The highest concentrations were measured at the sampling sites in the headwaters. - The mean microplastics concentration in drinking water was 105.8 p/L^{-1}.	10
Fish ingestion of microplastics in urbanized and non-urbanized streams	There is a greater microplastics intake in urbanized than in non-urbanized streams. Omnivorous fish ingested more microplastics than fish with other food diet.	11
Impacts of naturally-aged microplastics on zebrafish juveniles	The short-term exposure to naturally-aged microplastics induced its accumulation and predictive changes in REDOX imbalance, neurotoxicity and cytotoxicity.	12
Toxicity triggered by microplastic in zebrafish embryos	Acute exposure at low concentration impaired the development of organisms. The magnitude of toxicity depended on the exposure system: static or semi-static.	13
Filtration, assimilation, and elimination of microplastics by bivalves	Microplastics can be absorbed or assimilated in the branchia, in the gills and gut. Microplastics are possible inducers of problems in the reproductive system.	14
Neurotoxicity and behavioral changes caused by biomicroplastics in fish	Sublethal concentrations induced anxiety and decreased exploratory behavior. The biopolymer acted as anticholinesterase agent.	15
Ingestion of microplastics in the gastrointestinal tracts and gills of fish	Fragments were adsorbed significantly more in the gills than the gastrointestinal tract. Certain species are more susceptible to microplastics than others.	16
Effects of microplastics and the association with copper on fish	Fragments presented genotoxic, neurotoxic, and physiological effects, isolated and combined with copper; but there was no interactions between the two compounds.	17
Ingestion of microplastics by fish	83% of the analyzed organisms had plastic debris in the stomach. Types of plastic found: fiber (46.6%), soft plastic (36%), and hard plastic (17.4%).	18

Ref: 1: Andrade *et al*. 2019; 2: Araújo *et al*. 2020a; 3: Araújo *et al*. 2020b; 4: Araújo *et al*. 2020c; 5: Araújo & Malafaia 2020; 6: Araújo & Malafaia 2021; 7: Bertoldi *et al*. (accepted); 8: Castro *et al*. 2020; 9: Faria *et al*. 2019; 10: Ferraz *et al*. 2020; 11: Garcia *et*

al. 2020; 12: Guimarães *et al*. 2020; 13: Malafaia *et al*. 2020; 14: Moreschi *et al*. 2020; 15: Oliveira *et al*. 2021; 16: Ribeiro-Brasil *et al*. 2020; 17: Roda *et al*. 2020; 18: Silva-Cavalcanti *et al*. 2017.

It is important to focus on the originality of studies on microplastics and amphibians, organisms that have been suffering a population decrease worldwide due to the increasing contamination of freshwater ecosystems. Besides that, studies on microplastics and *P. cuvieri* had never been recorded before. Such studies have shown relevant results regarding the toxicological effects microplastics can cause on vertebrate animals. Further research is needed to assess the biological effects of microplastics on these organisms in order to include new perspectives for future research on other animals as well. Thus, we suggest some issues that may be addressed in future works to fill gaps in knowledge on the topic discussed here.

Initially, the increase in the number of research groups focusing on limnological and microplastic studies is fundamental and decisive. Government grants for research and training programs to study microplastics in freshwater could strengthen the study area, contributing to consolidate study groups distributed throughout the Brazilian territory. Secondly, laboratory experiments should consider exploring more realistic scenarios regarding the concentration of microplastics. The use of microplastics, other than polyethylene such as polypropylene, polystyrene, polyethylene terephthalate and polyurethane, for example, which are present in large quantities in the environment need to be further investigated. The use of secondary microplastics is also a factor that should not be ignored since, unlike primary microplastics, secondary microplastics are not sterilized, have an irregular surface, and present a composition with high levels of chemical additives. Further chronic toxicity studies are also recommended considering that most studies are usually acute toxicity assays. Acute assays are useful, but they do not allow visualizing and evaluating long-term effects of contaminants or the consequences along generations of test organisms. The essential character of a greater volume of scientific publications is a way of filling the lack of local data and contributing to the global scenario. Finally, according to Nel *et al*. (2018), it is possible to identify microplastic pollution in freshwater sediments by conducting studies that determine in the local ecosystem the accumulation of microplastics after ingestion. However, given the importance of this environment for the maintenance of local biodiversity, we found no specific studies on this environment or on organisms that are found in it. Such studies are fundamental for collecting data and structuring new approaches.

Conclusion

The review conducted here shows that studies on microplastics in Brazilian freshwaters had a considerable development in the year 2020, but that more experimental and theoretical studies are needed to meet the needs of vulnerable environments. Globally, this field of research is new, however, based on those few but important studies, we conclude that the contamination of Brazilian freshwater bodies is already an ongoing reality. Even so, the real state of pollution will be known with further research.

The articles published in Brazilian freshwater environmental matrices mostly focused on microorganisms, vertebrates and water samples. All approaches proved to be efficient in achieving the proposed objectives, but many gaps are still open and need to be further investigated, including entire ecosystems. There are difficulties inherent to researching such a complex and recent issue, such as the lack of standardized and validated methodologies, which may delay the growth and development of the field. For this reason, it is essential to establish multidisciplinary collaborations to develop integrative projects seeking to obtain a greater understanding of the mechanisms of action and the impacts that microplastics may cause to the environment, biota, and humans.

Acknowledgments

The authors thank to Rufford Foundation for financial support through the Rufford Small Grant, the São Paulo State Research Support Foundation (FAPESP) (process 2019 / 10845-4), and the National Council for Scientific and Technological Development (CNPq) (process 303660/2016-3) for funding this research.

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Received: September 2020
Accepted: Jannuary 2021
Published: March 2021