



## Large-scale monitoring and risk assessment of microplastics in the Amazon River

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### ABSTRACT

Microplastics (MPs) are one of the most widespread contaminants worldwide, yet their risks for freshwater ecosystems have seldom been investigated. In this study, we performed a large monitoring campaign to assess the presence and risks of MPs in Amazonian freshwater ecosystems. We investigated MP pollution in 40 samples collected along 1500 km in the Brazilian Amazon, including the Amazon River, three major tributaries, and several streams next to the most important urban areas. MPs in the 55–5000  $\mu\text{m}$  size range were characterized (size, shape, color) by microscopy and identified (polymer composition) by infrared spectroscopy. Ecotoxicological risks were assessed using chronic Species Sensitivity Distributions for effects triggered by food dilution and tissue translocation using data alignment methods that correct for polydispersity of environmental MPs and bioaccessibility. This study shows that MPs are ubiquitous contaminants in Amazonian freshwater ecosystems, with measured concentrations (55–5000  $\mu\text{m}$ ) ranging between 5 and 152  $\text{MPs}/\text{m}^3$  in the Amazon River and its main tributaries, and between 23 and 74,550  $\text{MPs}/\text{m}^3$  in urban streams. The calculated Hazardous Concentration for the 5% of species ( $\text{HC}_5$ ) derived from the SSDs for the entire MP range (1–5000  $\mu\text{m}$ ) were  $1.6 \times 10^7$   $\text{MPs}/\text{m}^3$  (95% CI:  $1.2 \times 10^6 - 4.0 \times 10^8$ ) for food dilution, and  $1.8 \times 10^7$   $\text{MPs}/\text{m}^3$  (95% CI:  $1.5 \times 10^6 - 4.3 \times 10^8$ ) for translocation. Rescaled exposure concentrations (1–5000  $\mu\text{m}$ ) in the Amazon River and tributaries ranged between  $6.0 \times 10^3$  and  $1.8 \times 10^5$   $\text{MPs}/\text{m}^3$ , and were significantly lower than the calculated  $\text{HC}_5$  values. Rescaled concentrations in urban streams ranged between  $1.7 \times 10^5$  and  $5.7 \times 10^8$   $\text{MPs}/\text{m}^3$ , and exceeded both calculated  $\text{HC}_5$  values in 20% of the locations. This study shows that ecological impacts by MP contamination are not likely to happen in the Amazon River and its major tributaries. However, risks for freshwater organisms may be expected in near densely populated areas, such as the cities of Manaus or Belem, which have limited wastewater treatment facilities.

### 1. Introduction

Environmental pollution from plastic has been considered a global threat due to its persistence and potential impacts on a wide range of living organisms, including humans (Rochman et al., 2013; Yang et al., 2022). Plastic materials break down into smaller particles, microplastics (MPs, 1–5000  $\mu\text{m}$  in length; GESAMP, 2019), which can travel long

distances and have been detected in a wide range of environmental compartments, including air, water and soil (Li et al., 2018; González-Pleiter et al., 2021; Jacques and Prosser 2021). Urban wastewater is considered one of the major pathways for MPs into aquatic ecosystems, as it has been found to contain up to 10,000  $\text{MPs}/\text{L}$ , most of them being fibres and fragments (Schell et al., 2020; Ngo et al., 2019). In general, over 90% of MPs found in wastewater are retained by

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wastewater treatment plants (Schell et al., 2020; Iyare et al., 2020). However, only 52% of the global sewage is treated: 74% in high-income countries and 4.2% in developing countries (Jones et al., 2021). This suggests that untreated wastewaters can be a major source of MPs in areas with limited or unexisting access to sewage treatment facilities.

The Amazon River is the largest river in the world. It discharges more than  $5 \times 10^{12} \text{ m}^3$ /year into the Atlantic coast, representing about 17% of the total volume of freshwater entering the global ocean (Callède et al., 2010). Currently, about 30 million people are living in the Amazon basin (WWF, 2020), most of whom live in large cities that are undergoing continuous expansion (Córtes et al., 2020). It is estimated that only 8–12% of the population that live in the urban areas of the Brazilian Amazon have access to basic sanitation systems (ANA, 2020). Therefore, the majority of urban wastewaters as well as urban runoff waters are discharged untreated into the river, constituting a major pathway for MPs. Moreover, large amounts of plastic are dumped on the banks of the Amazon River (Lucas-Solis et al., 2021), which can break down due to erosion and microbial processes, contributing to an increase in MP exposure levels.

Recent studies have reported the occurrence of MPs in environmental samples collected in the Amazon basin. For example, Santos Queiroz et al. (2022) reported high MP exposure levels in water samples collected along the Amazon Continental Shelf (320–13,000 MPs/m<sup>3</sup>), while Gerolin et al. (2020) denoted an increase in MP concentrations in sediment samples collected downstream of the urban area of Manaus (Brazil). Moreover, some studies have reported the occurrence of MPs in the digestive tract of fish (Andrade et al., 2019; Pegado et al., 2018; 2021) as well as in anemones (Morais et al., 2020) collected from the Amazon River estuary, suggesting that the Amazon River is an important entry route for MPs into the Atlantic Ocean. In fact, the study by Lebreton et al. (2017) suggested that the Amazon River is one of the world's top-ten polluting rivers in terms of plastic emissions into the ocean. This study by Lebreton et al. (2017) was based on indirect extrapolations from a limited number of monitoring studies (mostly conducted in Europe) and data on mismanaged plastic waste generation, and overlooked regional social and environmental dynamics (Meijer et al., 2021), therefore further investigations of MP pollution in the Amazon are needed.

MPs can affect a wide range of aquatic organisms through different effect mechanisms, including blockage of the digestive tract, food dilution, sorption to the skin, entanglement, shading or even by translocation to the inner parts of the body and organs (de Sá et al. 2018; Schell et al., 2022). Few studies have attempted to assess the risks posed by MP pollution to aquatic organisms by comparing measured exposure concentrations with Species Sensitivity Distributions (SSDs) built with laboratory toxicity data (Burns and Boxall, 2018; Besseling et al., 2019; Everaert et al., 2020). However, many of these comparisons are substantially flawed since they compare exposure and effect data from studies that target different MP size ranges. Furthermore, they compare the results of toxicity studies that are usually performed with monodisperse particle distributions (i.e., one single polymer type, with a given shape/size) with the polydisperse particle distributions obtained from field exposure assessments. Recently, Koelmans et al. (2020) and Kooi et al. (2021) have proposed a methodology to re-scale exposure and effect data towards the full MP size range (1–5000  $\mu\text{m}$ ) so that effect threshold concentrations and risk calculations can be aligned for the polydisperse distribution of environmental MPs. Despite the fact that the methods have become accessible to a wide audience through the ToMEX web application (Thornton Hampton et al. 2022), only a limited number of studies have implemented this methodology (Koelmans et al., 2020; Coffin et al., 2022; Redondo-Hasselerharm et al., 2023). Koelmans et al. (2020) compared a global distribution of MP samples taken in the water column of freshwater ecosystems with the HC<sub>5</sub> (hazardous concentration for 5% of species), which is usually considered as threshold concentration for unacceptable ecological effects, and concluded that risks may be expected at a reduced number of locations (1.5%), which were

close to anthropogenic pollution sources. In a similar study, Redondo-Hasselerharm et al. (2023) concluded that MP risks to freshwater benthic organisms are likely to occur in some hotspot areas and provided threshold concentrations for the risk assessment of MPs in the sediment compartment.

To date, the risks of MP contamination for Amazonian freshwater ecosystems have not been evaluated. The Amazon constitutes one of the most important biodiversity biomes, containing about 3500 species of fish (Junk et al., 2007). Former investigations on the impacts of urban wastewater into Amazonian freshwater ecosystems have revealed unacceptable risks posed by pharmaceuticals and pesticides in rivers and streams close to the major urban areas (Rico et al., 2021; 2022), and have indicated the presence of large amounts of plastic debris accumulated in the shoreline of these urban areas. Therefore, it is expected that MPs constitute yet another threat for Amazonian freshwater biodiversity, for which specific risk assessment and management are needed.

Therefore, the main objectives of this study were to characterize MP exposure in several areas of the Amazon River with different levels of anthropogenic impact, and to assess MP risks for Amazonian freshwater ecosystems. For this, we characterized MP exposure in water samples collected along a longitudinal gradient of over 1500 km, including the Amazon River and its main tributaries, as well as small rivers and streams receiving wastewater from four important cities of the Brazilian Amazon. MP risks for freshwater biodiversity at the catchment scale were calculated on the basis of SSDs for effects triggered by food dilution and tissue translocation using data-alignment methods to correct for the polydispersity and bioaccessibility of MPs in environmental samples.

## 2. Material and methods

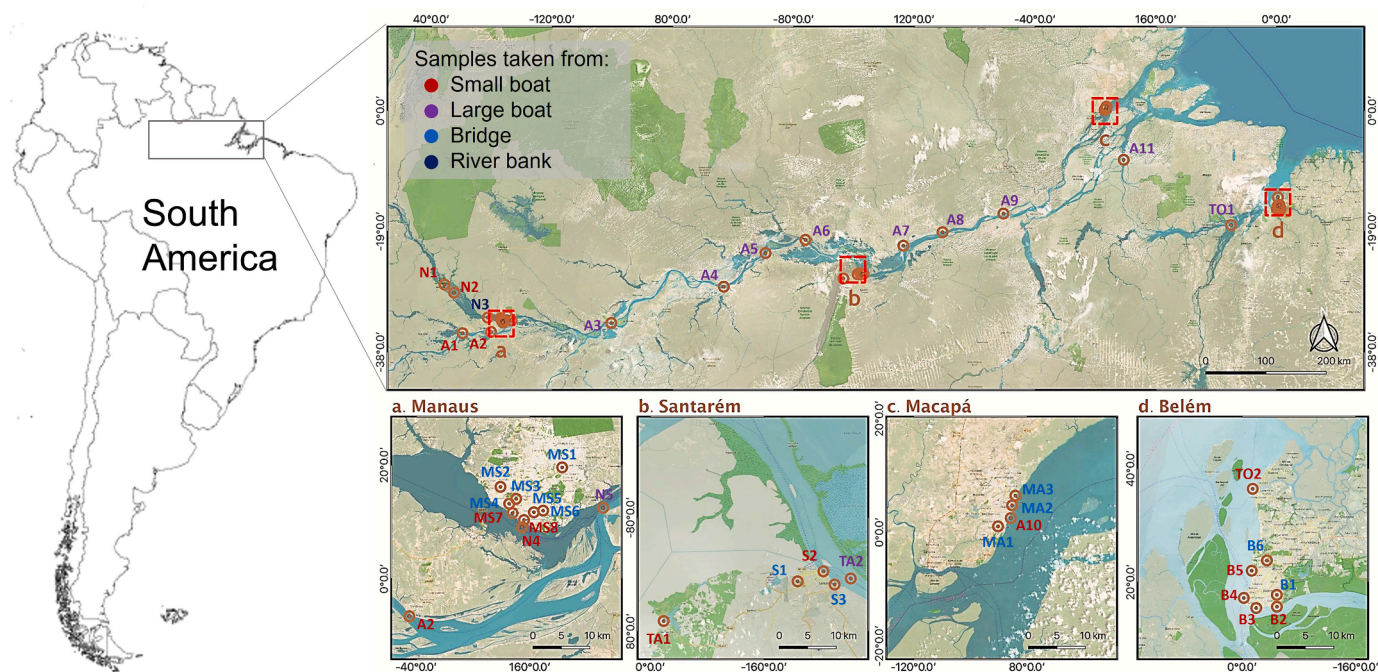
### 2.1. Microplastic sampling

Our study included 40 water samples taken during November–December of 2019 (end of the dry season) in the Amazon River ( $n = 11$ ), in three major tributaries: Negro, Tapajós, Tocantins ( $n = 9$ ), and in the urban streams of Manaus, Macapá, Santarém and Belém ( $n = 20$ ) (Fig. 1). Two of the samples in the Negro River were taken in the Anavilhanas National Park (N1 and N2), and two samples in the dilution area of Manaus (N4 and N5). The sampling locations in Macapá, as well as in the Tocantins River and Belém, were subject to some tidal effects. In these locations, the sampling was preferably done during the low tide moments; however, in one sample taken in Belém (B2), we observed clear mixing of stream waters with up-stream tidal currents.

Sampling in the Amazon River and in the tributaries was performed from small boats that remained in the same location as long as possible or from large passenger boats that were in continuous movement (Fig. 1). Sampling in urban areas was predominantly performed from bridges or small boats. A given amount of water was pumped from the middle of the river section (approximate water depth: 0.5–1 m) and filtered over a plankton net (55  $\mu\text{m}$ ). After filtration, the samples were stored in glass containers (0.5 L) and filtered over a filter paper (GF/A,  $\emptyset$  47 mm, pore size 0.7  $\mu\text{m}$ ). Then, the filter was folded and introduced into aluminum envelopes until further analysis. The amount of sampled water was decided based on the expected level of anthropogenic impact in the sampling location and ranged between 0.3–4.6 m<sup>3</sup> (see Table S1).

### 2.2. Microplastic extraction

Sample processing depended on the material observed on the filters. If the filter content was mainly composed of organic matter, the material was rinsed into Erlenmeyer flasks with filtered reverse osmosis (RO) water. The content of the Erlenmeyer flasks was then left to settle overnight and the overlying water was filtered onto filter paper (Whatman, GF/A,  $\emptyset$  47 mm, pore size 0.7  $\mu\text{m}$ ). The sedimented material was passed through a 53  $\mu\text{m}$  stainless steel sieve to eliminate fine



**Fig. 1.** Map of study area and sampling locations. Sampling from small boats was performed, as far as possible, from the same location; while sampling from large boats was performed while the boat was moving downstream. For these, the dots represent the location in which the sampling started, while the exact GPS coordinates of the start and end of the sampling are provided in Table S1. Figure adapted from Rico et al. (2021).

material, mainly clay, below the study's assessed MP sizes. The material retained in the sieve was transferred to Erlenmeyer flasks and treated to remove organic matter with  $\text{H}_2\text{O}_2$  30% (v/v) at 40 °C on a shaker (100 rpm) until the reaction stopped. This was followed by two density separations using a sodium iodide solution (density: > 1.8 g/ml). After each density separation, the overlaying material was filtered onto separate filter papers and retained for analysis.

Samples containing a large amount of sandy sediment were first dried at 45 °C. Each sample was then transferred into clean pre-rinsed polyethylene tubes and subjected to two density separations using a NaI solution (density: > 1.8 g/ml). The first density separation extract of each sample contained a large amount of organic material. For this reason, the extract was filtered through a 53  $\mu\text{m}$  stainless steel sieve and followed by organic matter removal as described above. The overlaying material was filtered onto separate filter papers. The content remaining after organic matter removal and the extract from the second density separation were each filtered onto separate filters (GF/A,  $\varnothing$  47 mm, pore size 0.7  $\mu\text{m}$ ) and retained for analysis.

### 2.3. Microplastic analysis

All filters were first analysed visually for MPs using a Nikon SMZ 745T stereomicroscope at 20–50x magnification. When the filters were too loaded with MPs after a first visual check, only a small fraction of the filter (1/8 or 1/4) was analysed and extrapolated to the rest of the filter. This subsampling was performed by taking several segments from the filter paper, to ensure that subsamples were as representative of the distribution of MPs on the original filter as possible. Suspected MP particles were photographed using an Infinity 1 camera. The long and the short axis of each MP were measured using the Infinity Analyze (v.6.5.4) software package, following calibration using a measurement standard. For a proportion of the particles (c.a. 40%), the particle depth was estimated with the microscope. For the remaining particles, the depth was calculated based on the ratio between the short axis and the estimated depth of the particles of the same shape for which the depth was estimated in the microscope. Each particle was classified according to shape into beads, fibres, fragments, films, or glitter particles. Some

fibres formed clumps that could not be separated and were therefore reported as such.

As the dataset contained a huge number of MPs (>12,000 particles were sized and classified according to their shape), only a proportion (12%) of these suspected MP particles could be analysed for their polymer composition using Fourier Transform Infrared Spectroscopy (FTIR). Particles were characterized using a Perkin Elmer Spotlight 400  $\mu\text{FTIR}$  in transmission mode. The particles were first compressed using a diamond compression cell (DC-3, Perkin Elmer) before being loaded onto the machine to improve spectral quality. Four co-scans were taken at a spectral resolution of 4  $\text{cm}^{-1}$  for each particle measurement. A new background scan was made each time the diamond compression cell was loaded onto the machine (approximately every 1–10 particles). Each spectrum was compared to a series of commercial (PerkinElmer Polymer library, Agilent Polymer library), open source (Pimpke et al., 2018), and in-house libraries. A minimum hit quality index (HQI) of 70% was used as a threshold to accept a match for polymer identification. In addition, the reliability of each match was assessed by manually checking the characteristic peaks of each spectrum. After evaluation, the particles that did not comply with the above criteria (18%) were excluded from the analysis.

### 2.4. Quality assurance/quality control

Except for the pumping device, all materials used in the field were non-plastic and had been prewashed with distilled water. Furthermore, organic cotton clothing was used during sampling. All collected samples were transported from Brazil to Norway in aluminum foil envelopes. Then, they were processed and analysed at the Microplastic Laboratory of the Norwegian Institute for Water Research (NIVA) in a positive pressure room with HEPA-filtered (class H13) air input. The contamination reduction measures implemented in this laboratory included the use of natural fiber clothing and lab coats, removal of loose fibres using a lint roller upon entry to the laboratory, and regular removal of dust from all laboratory areas. Furthermore, all processing steps in which samples were exposed to the laboratory environment (e.g., during rinsing of filters) were done in a laminar flow cabinet. All laboratory water or



solutions used in the sample processing were pre-filtered (0.22  $\mu\text{m}$  for reverse osmosis water, 1.2  $\mu\text{m}$  for NaI and  $\text{H}_2\text{O}_2$ ) immediately before use. Glass containers and polyethylene tubes used during sample processing were rinsed three times with filtered reverse osmosis water before use.

Three blanks were included with each set of samples that was processed simultaneously. These blanks represent combined procedural, container and solution blanks and were treated identically to the field samples. All suspected MPs observed in these blanks were visually and chemically characterized in the same way as the field samples. Most of the particles identified in the blanks were non-plastic (Table S2), so no further corrections to the field samples were applied. The method used here has been previously validated using spiked samples for a range of MP particle types. The extraction efficiencies and recovery rates are provided in Hurley et al. (2018) and Crossman et al. (2020).

## 2.5. Microplastic mass estimation and exposure concentrations

The mass of the MPs was determined based on the volume of the measured particles and the density of the identified polymers. Particle volume was calculated based on the assigned morphology and the size of the analysed axes, using the following approach: beads were treated as spheres; fibres as cylinders; and films, fragments, and glitter as ellipsoids. Information on polymer densities is shown in Table S3. The mass of the MPs for which the polymer composition was not determined with FTIR was assigned to 1  $\text{g cm}^{-3}$  for beads, 1.3  $\text{g cm}^{-3}$  for fibres, 1.1  $\text{g cm}^{-3}$  for films and fragments, and 1.4  $\text{g cm}^{-3}$  for glitter, based on the mean density of the MPs with identified polymer type in the current dataset. MPs were grouped into four size classes according to their longest measured axis (55–150  $\mu\text{m}$ ; 150–300  $\mu\text{m}$ ; 300–1000  $\mu\text{m}$  and 1000–5000  $\mu\text{m}$ ). The particles with the longest axis beyond the 55–5000  $\mu\text{m}$  range were excluded from the analysis. Microplastic exposure concentrations in the different samples were estimated based on the number of particles (MPs/L) and the mass of the MP particles (mg/L) by dividing the MP particles or the total mass by the water volume passed over the sampling nets.

## 2.6. Ecological risk assessment

To assess MP risks for aquatic organisms we first rescaled the measured exposure concentration data (55–5000  $\mu\text{m}$ ) to the standard MP size range (1–5000  $\mu\text{m}$ ) following the approach described by Koelmans et al. (2020). The approach is based on the application of a Correction Factor (CF) to the measured particle concentration:

$$CF = \frac{5000^{1-\alpha} - 1^{1-\alpha}}{x_2^{1-\alpha} - x_1^{1-\alpha}}$$

where,  $x_1$  and  $x_2$  are the minimum (55  $\mu\text{m}$ ) and maximum (5000  $\mu\text{m}$ ) values of the targeted size range in the current study. The  $\alpha$  is the exponent of the power law distribution fitted to the measured MP concentrations based on particle size (i.e., length of the longest axis). The fitting of the power law distribution was done separately for the samples taken in the Amazon River and tributaries and those taken in urban areas, as it was expected they would show potential differences related to major MP sources, environmental fate, or fragmentation processes. In both cases the fitting of the power law distribution was successfully done. The fitting was done according to the Maximum Likelihood Estimation (MLE) method described in Kooi et al. (2021) based on 100 bootstraps and using the powerlaw package (Gillespie, 2014). The  $\alpha$  value for the Amazon River and tributaries was 2.77, while the  $\alpha$  value for the samples taken in urban streams was 3.23. Based on these  $\alpha$  values, the calculated CFs for the Amazon River and tributaries and for the urban areas were 1202 and 7604, respectively.

For the characterization of the ecotoxicological effects of MPs in the aquatic environment, SSDs were constructed, and the  $\text{HC}_5$  and their 95%

Confidence Intervals (CIs) were calculated. For this purpose, a literature search was conducted (on publications until September 2022) using the Web of Science (WOS) and ProQuest databases to obtain toxicity data for aquatic organisms exposed to MPs via water. The following strings were used: (effect OR impact OR toxicity) AND (microplastic(s) OR plastic particle OR fiber OR fragment OR film) AND (freshwater OR marine OR aquatic) AND (species OR organism OR invertebrate OR crustacean OR fish). From the studies collected, we selected chronic toxicity data ( $\geq 3$  days for phytoplankton,  $\geq 7$  days for zooplankton,  $\geq 21$  days for macrophytes and fish,  $\geq 28$  days for benthic invertebrates) for endpoints linked to effects at the individual level (e.g., survival, growth, reproduction, etc.) following Rico et al. (2019). The No Observed Effect Concentration (NOEC) was used as the species effect threshold for the construction of the SSDs, and only those studies in which a NOEC and a Lowest Observed Effect Concentration (LOEC) could be obtained were included in the analysis, excluding studies where no statistically significant effects were found and studies reporting non-dose dependent effects. When the NOEC was given in mg/L only, and the information provided in the study was insufficient to calculate the equivalent NOEC in  $\text{MPs/m}^3$ , it was converted to  $\text{MPs/m}^3$  using the method described by Leusch and Ziajahromi (2021). Then, the NOECs for animal species were aligned to the environmentally relevant and bioaccessible effective concentration following the calculations described in Redondo-Hasselerharm et al. (2023). These included data transformations for  $\text{MPs/m}^3$  for ingested particle volume and surface area as Ecologically Relevant Metrics (ERMs), which correspond to the effect mechanisms of food dilution and tissue translocation, respectively (Redondo-Hasselerharm et al., 2023). For food dilution, when the length of the largest MP axis was larger than the mouth opening of the test organism, it was considered biologically unavailable. For tissue translocation, the particles with a length above the cut off value of 83  $\mu\text{m}$ , proposed by Mehinto et al. (2022), were considered biologically unavailable, except for the species with a mouth opening size smaller than 83  $\mu\text{m}$ , for which the mouth opening size determined bioaccessibility. Following Koelmans et al. (2020) and Redondo-Hasselerharm et al. (2023), no alignments were applied to the microalgae data included in the SSDs. This is because effect mechanisms for algae and macrophytes are still unclear. A summary of the characteristics of the toxicity data used to build the SSDs, including the original and rescaled NOECs can be found in Table S4; while the maximum ingestible sizes of MP used for each species to perform the data alignment can be found in Table S5.

Two separate SSDs were constructed with the rescaled NOECs (in  $\text{MPs/m}^3$ ) that correspond to the effect mechanisms of food dilution (using ingested particle volume as ERM) and tissue translocation (using particle surface area as ERM). For the construction of the SSDs, the geometric mean was calculated for those species with more than one toxicity value, corresponding to different studies, test materials or endpoints assessed. The SSDs were constructed based on a log-normal distribution using the ssdtools package in Rstudio (version 4.1.3) (Thorley and Schwarz, 2018). The  $\text{HC}_5$  values and their 95% CI were calculated using parametric bootstrapping (based on 1000 bootstrap iterations). Finally, to characterize the ecological risks of MPs, the rescaled (1–5000  $\mu\text{m}$ ) MP exposure concentrations in the Amazon River and its tributaries, and in the urban areas, were plotted as two separate cumulative frequency distributions, together with the calculated  $\text{HC}_5$  values and their 95% CIs. Samples with concentrations within the 95% CI were expected to have a high probability to result in ecotoxicological effects. Additionally, when the  $\text{HC}_5$  was exceeded, the Potentially Affected Fraction (PAF) of species was calculated with the SSD parameters. The SSD and cumulative frequency distribution graphs were made with ggplot2 in Rstudio (version 4.1.3) (Wickham, 2016).



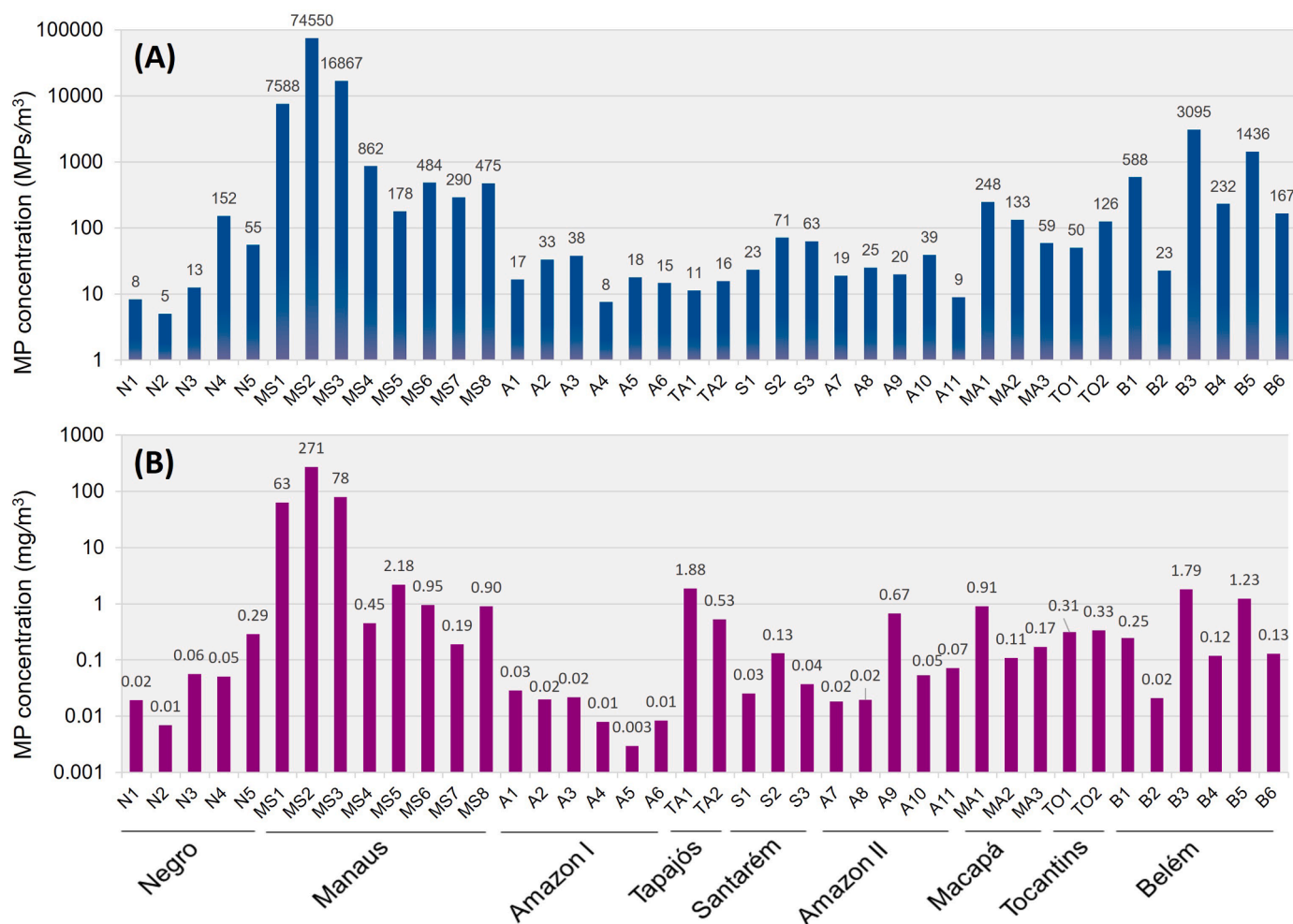


Fig. 2. Concentration of microplastics in water samples in terms of (A) number of particles and (B) mass. Please note that the y-axis is displayed on a logarithmic scale.

### 3. Results and discussion

#### 3.1. Microplastic exposure

MPs were found in all water samples analysed in this study. The concentrations in the Amazon River ranged from 8 to 39 MPs/m<sup>3</sup> (which corresponds to 0.003 to 0.67 mg/m<sup>3</sup>; Fig. 2). The concentrations in the Tapajós River, one of the tributaries, were similar to the Amazon River ones in terms of number of particles (11–16 MPs/m<sup>3</sup>) and slightly higher in terms of mass (0.53–1.9 mg/m<sup>3</sup>). The MP concentrations in the Negro River ranged from 5 to 152 MPs/m<sup>3</sup> (0.007–0.29 mg/m<sup>3</sup>), with the lowest values being found in the samples taken in the Anavilhanas National Park (N1 and N2) or a few km downstream (N3), and the highest values being found in the dilution area of Manaus (N4 and N5). The MP concentrations in the Tocantins River were 50–126 MPs/m<sup>3</sup> (0.31–0.33 mg/m<sup>3</sup>). Comparison of such results with other studies reporting MP concentrations in surface waters is hampered by the different sampling methods and size ranges evaluated in different studies. Dos Santos Queiroz et al. (2022) sampled MPs in the Amazon Continental Shelf by filtering water collected with aluminum buckets over a 64- $\mu$ m mesh during the rainy and dry periods. The concentrations reported by their study are notably higher (323–5733 MPs/m<sup>3</sup>) than the ones reported here for nearly the same period of the year. However, in their study, about 40% of the recorded particles were cellulose-based fibres, which were excluded from our study. Also, their sampling methods could be more suitable to collect free floating particles than ours. When focusing

on other studies that used water filtration methods and evaluated a similar size range as the one used here (i.e., 55–5000  $\mu$ m), it can be concluded that MP exposure levels in the Amazon River and its tributaries fall within those reported in the Seine River in France (3–108 MPs/m<sup>3</sup>; Dris et al., 2015) or the Tagus River basin in Spain (1–227 MPs/m<sup>3</sup>; Schell et al., 2021). Considering the characteristic of the Amazon catchment, the very large dilution factor and the relatively low population inhabiting the region, it is of particular concern that concentrations observed in the Amazon River already fall in the same range of those found in some of the most impacted rivers of Europe.

The MP concentrations in the streams crossing the main urban areas were, in general, between one to three orders of magnitude larger than those found in the Amazon River (Fig. 2). The largest concentrations were found in the streams of Manaus (178–74,550 MPs/m<sup>3</sup> or 0.45–271 mg/m<sup>3</sup>), followed by Belém (167–3095 MPs/m<sup>3</sup> or 0.12–1.79 mg/m<sup>3</sup>; excluding B2 which was highly influenced by the tides at sampling) and Macapá (59–248 MPs/m<sup>3</sup> or 0.11–0.91 mg/m<sup>3</sup>). However, the MP concentrations in the streams crossing the city of Santarém were found to be lower than in the other cities: 23–71 MPs/m<sup>3</sup> (0.03–0.13 mg/m<sup>3</sup>). Overall, the MP concentrations in the urban streams of Manaus, Belém and Macapá were similar to those reported in highly contaminated rivers, such as the Yangtze River in China (800–3089 MPs/m<sup>3</sup>; He et al., 2021); while some samples taken during heavy precipitation events in Manaus (MS2 and MS3) resemble contamination levels found in untreated urban wastewaters (Schell et al., 2020). The lower pollution status of the streams nearby Santarém, as compared to the other

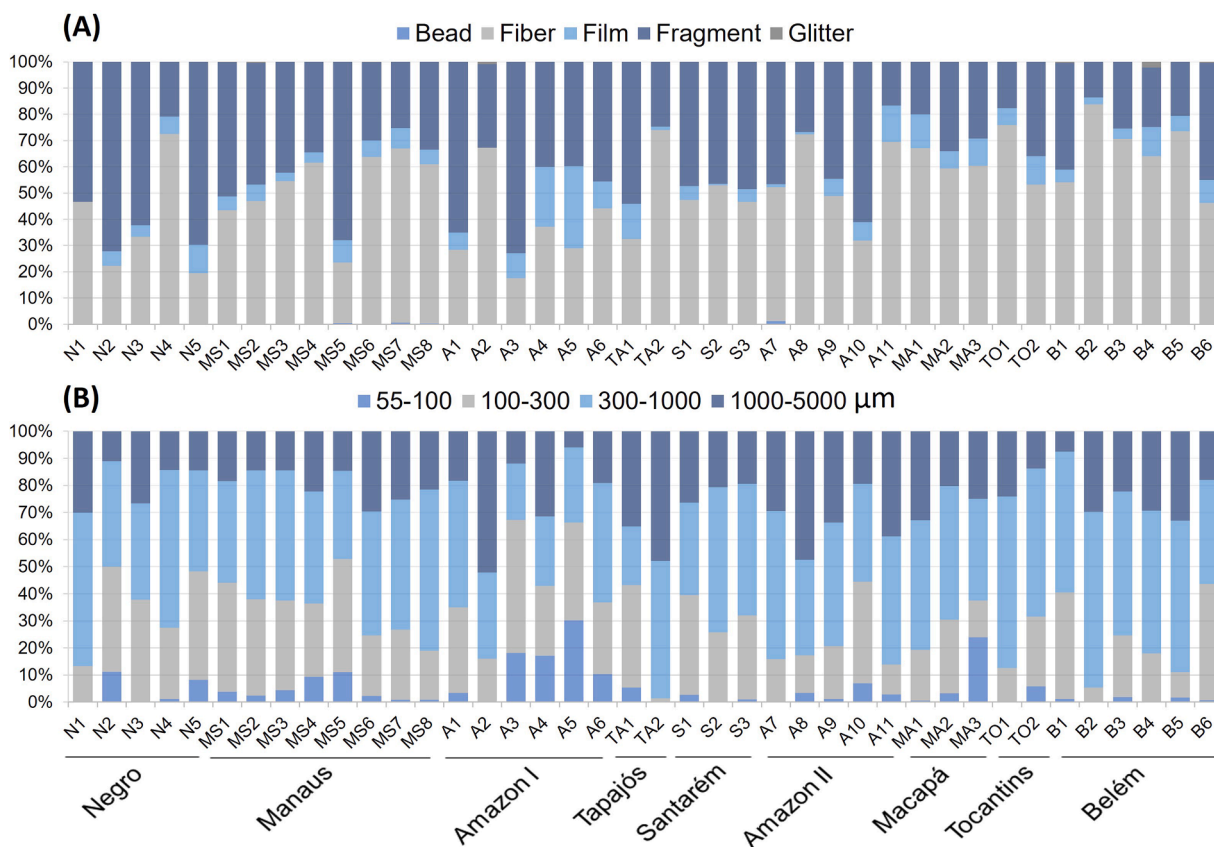


Fig. 3. Shape distribution (A) and size (longest axis) distribution (B) in the different samples.

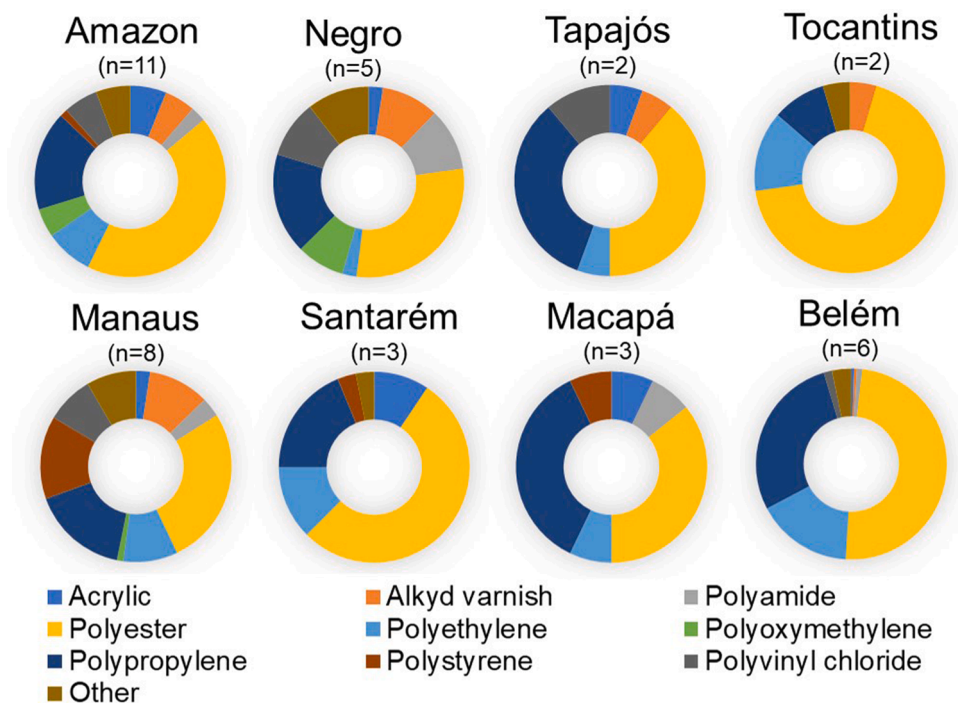
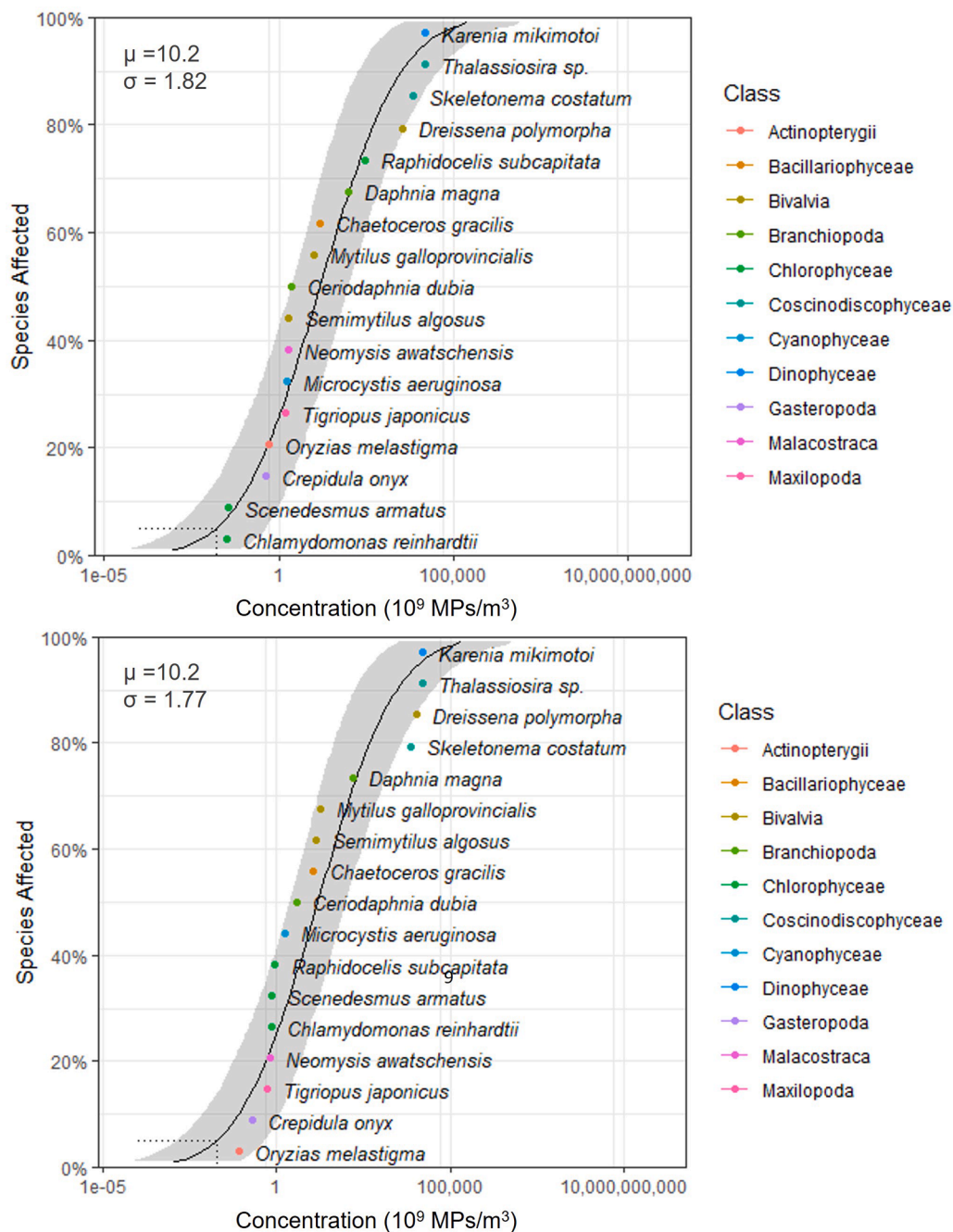


Fig. 4. Microplastic polymer composition in the different sample groups, which correspond to the Amazon River, the three major tributaries (Negro, Tapajós and Tocantins), and the small rivers and streams sampled in urban areas (Manaus, Santarém, Macapá and Belém).  $n$  = number of samples in each sample group.

Brazilian cities included here, has been documented in previous studies (Rico et al., 2021, 2022), and has been attributed to the lower population density and the higher dilution rate of its urban wastewaters.

MP contamination was dominated by fibres (51% of the total number of particles), followed by fragments (42%) and films (6%), while the presence of other particle types (i.e., glitter, beads) was negligible



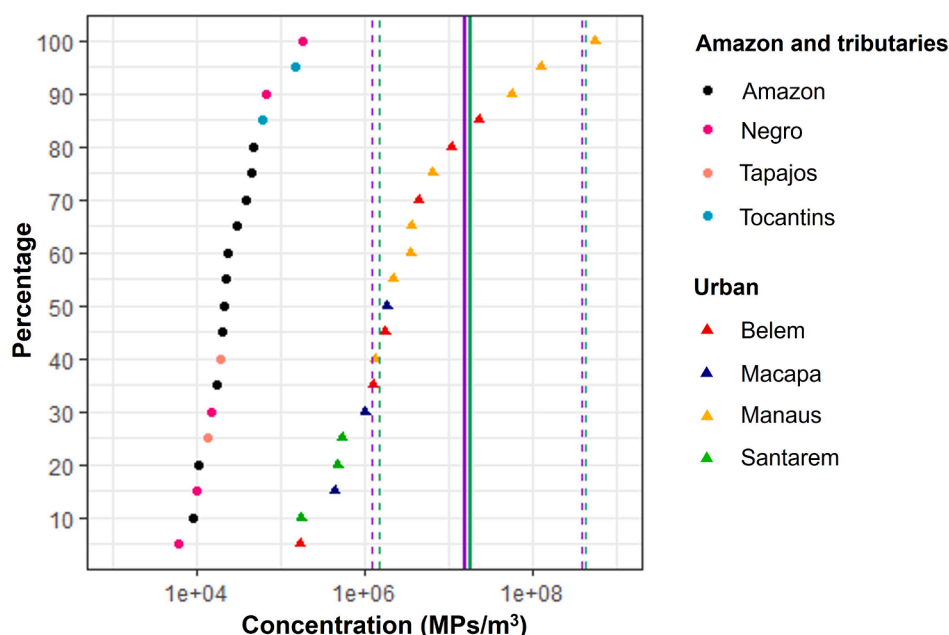
**Fig. 5.** Species Sensitivity Distributions (SSDs) for aquatic organisms exposed to microplastics under chronic exposure conditions corrected for bioaccessibility and polydispersity, and accounting for food dilution based on MP volume (above) and tissue translocation based on area (below) as ecologically relevant metrics.  $\mu$  and  $\sigma$  refer to the median and slope parameters of the SSDs, while the black line and gray area represent the Hazardous Concentration for the 5% of species (HC5) and 95% confidence interval, respectively. The markers show the rescaled no observed effect concentration (NOECs) of the species, and the color of the markers relates to the taxonomic class of each species.

(Fig. 3A). Based on the longest axis, the most abundant size category was 300–1000  $\mu\text{m}$  (47%), followed by 100–300  $\mu\text{m}$  (32%), 1000–5000  $\mu\text{m}$  (18%) and 55–100  $\mu\text{m}$  (3%) (Fig. 3B). The dominance of the 300–1000  $\mu\text{m}$  size category was largely influenced by the occurrence of fibres, which had a mean length of 923  $\mu\text{m}$  (SD 690  $\mu\text{m}$ ). Excluding fibres, the most abundant size range was 100–300  $\mu\text{m}$  (61%), followed by 300–1000  $\mu\text{m}$  (30%), 55–100  $\mu\text{m}$  (6%), and 1000–5000  $\mu\text{m}$  (3%). Overall, we could not identify a clear pattern regarding differences in MP shapes between sample groups (i.e., urban streams vs large rivers), which suggests that MP pollution in the Amazon and its main tributaries is most likely of urban origin. Similar size ranges and shapes have been reported in scientific reviews analysing MP monitoring results from global rivers (Koelmans et al., 2019; Li et al., 2020; Wang et al., 2021), although more accurate comparisons would require the derivation of continuous probability distributions for these parameters (see Kooi and Koelmans 2019). Also, similar MP types have been documented at the

Amazon Continental Shelf (dos Santos Queiroz 2022), except for the absence of foams, which may be related to the fact that these particles are typically floating and may have escaped from the water pumping systems used in our study (Zheng et al., 2021).

In total, we identified 32 different polymers through FTIR analysis (Table S3). Polyester was the most abundant polymer (32%), followed by polypropylene (18%), polystyrene (10%), polyethylene (9%) and alkyd varnish (9%; Fig. 4). Plastic particles were classified into 16 different colours. Most of the identified MPs were blue (30%), red (24%), green (13%) or black (12%; Figure S1). The polymer distribution found in this study slightly deviates from those reported in other studies (referring to rivers in other locations of the world), which report polyethylene, polypropylene, and polystyrene as the most abundant particles (Koelmans et al., 2019; Schell et al., 2020; Wang et al., 2021). Here polyester fibres were the most abundant ones in all sample groups. Polyester fibres are produced by the breakdown of synthetic textiles





**Fig. 6.** Cumulative frequency distributions of the rescaled microplastic exposure concentrations (1–5000  $\mu\text{m}$ ) in the Amazon River and its main tributaries (circles) and in the urban streams (triangles) plotted together with the calculated Hazardous Concentration for the 5% of species (HC5) (vertical solid line) and its 95% confidence interval (dashed lines) obtained from the Species Sensitivity Distributions (SSDs) for food dilution based on MP volume (purple) and tissue translocation based on area (green).

during laundry and are abundant in untreated wastewaters (Schell et al., 2021). These particles are usually well retained during wastewater treatment (Schell et al., 2021); however, the lack of wastewater treatment plant facilities in the Amazonian region is likely the reason for their high occurrence.

### 3.2. Ecological risk assessment

As the amount of studies assessing MPs on Amazonian species is very limited (Wen et al., 2018), risk calculations were performed using available chronic toxicity data for non-native species, assuming that the distribution of biological traits affecting MP uptake and sensitivity are comparable between Amazonian freshwater species and their marine and temperate counterparts. The SSDs used in our study contained chronic NOECs for 17 freshwater and marine species (belonging to 11 taxonomic groups), and were dominated by algae and invertebrates. The two calculated SSDs show differences in relative sensitivity among aquatic species depending on the ERM considered. For example, the two most sensitive taxa in the SSD using volume as ERM were two species of green algae (*Chlamydomonas reinhardtii*, *Scenedesmus amatus*) while the most sensitive taxa considering area were a fish (*Orizyias melastigma*) and a snail (*Crepidula onyx*; Fig. 5). However, the distribution parameters and the HC<sub>5</sub> value derived from these two chronic SSDs, which can be used as threshold concentrations for preventing effects on aquatic species assemblages, were relatively similar. The HC<sub>5</sub> for the chronic SSD considering volume as ERM was  $1.58 \times 10^7$  MPs/m<sup>3</sup> (95% CI:  $1.24 \times 10^6 - 3.96 \times 10^8$ ), while the same value for the chronic SSD considering area as ERM was  $1.84 \times 10^7$  MPs/m<sup>3</sup> (95% CI:  $1.53 \times 10^6 - 4.28 \times 10^8$ ).

The calculated threshold values are in line with the HC<sub>5</sub> provided by Burns and Boxall (2018;  $6.4 \times 10^7$  MPs/m<sup>3</sup>), but are at least one order of magnitude higher than the HC<sub>5</sub> values provided by Besseling et al. (2019;  $1.0 \times 10^6$  MPs/m<sup>3</sup>) and Everaert et al. (2020;  $6.1 \times 10^5$  MPs/m<sup>3</sup>). However, none of these threshold values were derived by making use of the re-scaling methods applied here. The HC<sub>5</sub> value provided by Koelmans et al. (2020), using the same re-scaling method for volume as ERM, resulted in  $7.5 \times 10^4$  MPs/m<sup>3</sup>, which is three orders of magnitude lower than the one calculated here. The difference between the HC<sub>5</sub> value provided by Koelmans et al. (2020) and ours is most likely related to the source data used. While Koelmans et al. (2020) used extrapolated NOECs estimated with the acute-to-chronic and the EC<sub>50</sub>-to-NOEC

assessment factors provided by ECHA (2008), which have not been validated for MPs, we only used experimental NOECs from studies showing statistically significant effects and derived under chronic exposure conditions.

The alignment of the measured MP exposure concentrations to the 1–5000  $\mu\text{m}$  range based on Koelmans et al. (2020) and Kooi et al. (2021) suggests that the concentration of MPs in the Amazon River and its main tributaries will range between  $6.0 \times 10^3$  and  $1.8 \times 10^5$  MPs/m<sup>3</sup>, while in the monitored urban streams this concentration will range between  $1.7 \times 10^5$  and  $5.7 \times 10^8$  MPs/m<sup>3</sup>. The comparison of the data-aligned MP concentrations in the Amazon River and main tributaries with the calculated HC<sub>5</sub> values shows that MP exposure is far from posing an ecotoxicological risk for aquatic organisms, with a difference of 2 orders of magnitude between the highest measured concentration and the calculated HC<sub>5</sub> values (Fig. 6). However, MP concentrations in 20% of the samples (4 out of 20) taken near urban areas exceeded the calculated HC<sub>5</sub> values for volume and area. For volume, 65% of the samples (13 out of 20) fell within the HC<sub>5</sub> 95% CI, and one sample (5%) was above the upper CI. For area, 60% of the samples (12 out of 20) fell within the 95% CI of the calculated HC<sub>5</sub> (Fig. 6). The four samples that exceeded the HC<sub>5</sub> for volume and area, and therefore were considered to pose ecological risks, were obtained from the most populated areas: three in Manaus (MS2>MS3>MS1) and one in Belem (B3). The sample that exceeded the upper CI interval was collected in Manaus (MS2). The corresponding PAF of species for these samples ranged between 7% and 23% for food dilution as effect mechanism, and 7% and 22% for tissue translocation.

The results of this study show that small rivers and streams located near to the main urban areas of the Amazon have MP concentrations that are high enough to exert ecotoxicological effects on aquatic organisms due to food dilution and/or translocation across biological membranes. Although the fraction of species potentially affected was not very high (i. e., 7%–23%), the results of this study confirm the outcomes of previous studies that point at urban areas with limited sewage collection and treatment facilities as hotspots for MP contamination (Besseling et al., 2019; Koelmans et al., 2020; Schell et al., 2022). In the future, higher demographic pressure over these ecosystems is expected to increase (Cortes et al., 2020), potentially increasing MP emissions and risks. Therefore, the protection of aquatic biodiversity in the surroundings of urban areas of the Amazon would benefit from the construction of sewage treatment facilities that retain part of the MPs emitted. In

parallel, social awareness to reduce single use plastic consumption and disposal into Amazonian freshwater ecosystems should be increased.

### Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

### Data availability

Data will be made available on request.

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### Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:[10.1016/j.watres.2023.119707](https://doi.org/10.1016/j.watres.2023.119707).

### References

- ANA – Agência Nacional de Águas e Saneamento Básico, 2020. Atlas esgotos: atualização da base de dados de estações de tratamento de esgotos no Brasil / Agência Nacional de Águas. ANA, Bras. Accessed 12 September 2021 [www.saneamentobasico.com.br/](http://www.saneamentobasico.com.br/).
- Andrade, M.C., Winemiller, K.O., Barbosa, P.S., Fortunati, A., Chelazzi, D., Cincinelli, A., Giarrizzo, T., 2019. First account of plastic pollution impacting freshwater fishes in the Amazon: ingestion of plastic debris by piranhas and other serrasalmids with diverse feeding habits. *Environ. Pollut.* 244, 766–773.
- Besseling, E., Redondo-Hasselerharm, P., Foekema, E.M., Koelmans, A.A., 2019. Quantifying ecological risks of aquatic micro-and nanoplastic. *Crit. Rev. Environ. Sci. Technol.* 49 (1), 32–80.
- Burns, E.E., Boxall, A.B., 2018. Microplastics in the aquatic environment: evidence for or against adverse impacts and major knowledge gaps. *Environ. Toxicol. Chem.* 37 (11), 2776–2796.
- Callède, J., Cochonneau, G., Alves, F., Guyot, J.L., Guimarães, V., De Oliveira, E., 2010. Les apports en eau de l'Amazonie à l'Océan Atlantique. *Rev. Sci. l'eau/J. Water Sci.* 23 (3), 247–273.
- Coffin, S., Weisberg, S.B., Rochman, C., Kooi, M., Koelmans, A.A., 2022. Risk characterization of microplastics in San Francisco Bay, California. *Micro Nanoplast.* 2, 19.
- Côrtes, J.C., D'Antona, 'A.D.O., Ojima, R., 2020. Extended urbanization and rural reconfiguration in the Amazon: a theoretical-methodological proposal based on demographic and spatial indicators. *Rev. Bras. Estudos Urban. Reg.* 22, 22. V.
- Crossman, J., Hurley, R.R., Futter, M., Nizzetto, L., 2020. Transfer and transport of microplastics from biosolids to agricultural soils and the wider environment. *Sci. Total Environ.* 724, 138334.
- De Sá, L.C., Oliveira, M., Ribeiro, F., Rocha, T.L., Futter, M.N., 2018. Studies of the effects of microplastics on aquatic organisms: what do we know and where should we focus our efforts in the future? *Sci. Total Environ.* 645, 1029–1039.
- dos Santos Queiroz, A.F., da Conceição, A.S., Chelazzi, D., Rollnic, M., Cincinelli, A., Giarrizzo, T., Martinelli Filho, J.E., 2022. First assessment of microplastic and artificial microfiber contamination in surface waters of the Amazon Continental Shelf. *Sci. Total Environ.*, 156259.
- Dris, R., Gasperi, J., Rocher, V., Saad, M., Renault, N., Tassin, B., 2015. Microplastic contamination in an urban area: a case study in Greater Paris. *Environ. Chem.* 12 (5), 592–599.
- ECHA, 2008. Characterization of Dose [Concentration]-Response for Environment. Guidance on Information Requirements and Chemical Safety Assessment. European Chemicals Agency. Chapter R.8.
- Everaert, G., De Rijck, M., Lonnewille, B., Janssen, C.R., Backhaus, T., Mees, J., van Sebille, E., Koelmans, A.A., Catarino, A.I., Vandegehuchte, M.B., 2020. Risks of floating microplastic in the global ocean. *Environ. Pollut.* 267, 115499.
- Gerolin, C.R., Pupim, F.N., Sawakuchi, A.O., Grohmann, C.H., Labuto, G., Semensatto, D., 2020. Microplastics in sediments from Amazon rivers, Brazil. *Sci. Total Environ.* 749, 141604.
- GESAMP, 2019. In: Kershaw, P.J., Turra, A., Galgani, F. (Eds.), Guidelines or the Monitoring and Assessment of Plastic Litter and Microplastics in the Ocean. IMO/FAO/UNESCOIOC/UNIDO/WMO/IAEA/UN/UNEP/UNDP/ISA Joint Group of Experts on the Scientific Aspects of Marine Environmental Protection, p. 130. GESAMP Rep. Stud. No. 99. <http://www.gesamp.org/publications/guidelines-for-the-monitoring-and-assessment-of-plastic-litter-in-the-ocean>.
- Gillespie, C.S., 2014. Fitting heavy tailed distributions: the powerLaw package. *arXiv Prepr. arXiv1407.3492*.
- González-Pleiter, M., Edo, C., Aguilera, Á., Viúdez-Moreiras, D., Pulido-Reyes, G., González-Toril, E., Osuna, S., de Diego-Castilla, G., Leganés, F., Fernández-Piñas, F., Rosal, R., 2021. Occurrence and transport of microplastics sampled within and above the planetary boundary layer. *Sci. Total Environ.* 761, 143213.
- He, D., Chen, X., Zhao, W., Zhu, Z., Qi, X., Zhou, L., Chen, W., Wan, C., Li, D., Zou, X., Wu, N., 2021. Microplastics contamination in the surface water of the Yangtze River from upstream to estuary based on different sampling methods. *Environ. Res.* 196, 110908.
- Hurley, R.R., Lusher, A.L., Olsen, M., Nizzetto, L., 2018. Validation of a method for extracting microplastics from complex, organic-rich, environmental matrices. *Environ. Sci. Technol.* 52 (13), 7409–7417.
- Iyare, P.U., Ouki, S.K., Bond, T., 2020. Microplastics removal in wastewater treatment plants: a critical review. *Environ. Sci.: Water Res. Technol.* 6 (10), 2664–2675.
- Jacques, O., Prosser, R.S., 2021. A probabilistic risk assessment of microplastics in soil ecosystems. *Sci. Total Environ.* 757, 143987.
- Jones, E.R., Van Vliet, M.T., Qadir, M., Bierkens, M.F., 2021. Country-level and gridded estimates of wastewater production, collection, treatment and reuse. *Earth Syst. Sci. Data* 13 (2), 237–254.
- Junk, W.J., Soares, M.G.M., Bayley, P.B., 2007. Freshwater fishes of the Amazon River basin: their biodiversity, fisheries, and habitats. *Aquat. Ecosyst. Health Manag.* 10, 153–173.
- Koelmans, A.A., Nor, N.H.M., Hermsen, E., Kooi, M., Mintenig, S.M., De France, J., 2019. Microplastics in freshwaters and drinking water: critical review and assessment of data quality. *Water Res.* 155, 410–422.
- Koelmans, A.A., Redondo-Hasselerharm, P.E., Mohamed Nor, N.H., Kooi, M., 2020. Solving the nonalignment of methods and approaches used in microplastic research to consistently characterize risk. *Environ. Sci. Technol.* 54 (19), 12307–12315.
- Kooi, M., Koelmans, A.A., 2019. Simplifying microplastic via continuous probability distributions for size, shape, and density. *Environ. Sci. Technol. Lett.* 6 (9), 551–557.
- Kooi, M., Primpke, S., Mintenig, S.M., Lorenz, C., Gerdts, G., Koelmans, A.A., 2021. Characterizing the multidimensionality of microplastics across environmental compartments. *Water Res.* 202, 117429.
- Lebreton, L.C., Van Der Zwet, J., Damsteeg, J.W., Slat, B., Andrady, A., Reisser, J., 2017. River plastic emissions to the world's oceans. *Nat. Commun.* 8 (1), 15611.
- Leusch, F.D.L., Ziajahromi, S., 2021. Converting mg/L to particles/L: reconciling the occurrence and toxicity literature on microplastics. *Environ. Sci. Technol.* 55, 11470–11472.
- Li, C., Busquets, R., Campos, L.C., 2020. Assessment of microplastics in freshwater systems: a review. *Sci. Total Environ.* 707, 135578.
- Li, J., Liu, H., Chen, J.P., 2018. Microplastics in freshwater systems: a review on occurrence, environmental effects, and methods for microplastics detection. *Water Res.* 137, 362–374.
- Lucas-Solis, O., Moullet, G.M., Guamangallo, J., Yacelga, N., Villegas, L., Galarza, E., Rosero, B., Zurita, B., Sabando, L., Cabrera, M., Gimiliani, G.T., 2021. Preliminary assessment of plastic litter and microplastic contamination in freshwater depositional areas: the case study of Puerto Misahualli, Ecuadorian Amazonia. *Bull. Environ. Contam. Toxicol.* 107 (1), 45–51.
- Mehinto, A.C., Coffin, S., Koelmans, A.A., Brander, S.M., Wagner, M., Thornton Hampton, L.M., Burton, G.A., Miller, E., Gouin, T., Weisberg, S.B., Rochman, C.M., 2022. Risk-based management framework for microplastics in aquatic ecosystems. *Microplast. Nanoplast.* 2, 17.
- Meijer, L.J., Van Emmerik, T., Van Der Ent, R., Schmidt, C., Lebreton, L., 2021. More than 1000 rivers account for 80% of global riverine plastic emissions into the ocean. *Sci. Adv.* 7 (18), eaaz5803.
- Morais, L.M.S., Sarti, F., Chelazzi, D., Cincinelli, A., Giarrizzo, T., Martinelli Filho, J.E., 2020. The sea anemone *Bunodosoma cangicum* as a potential biomonitor for microplastics contamination on the Brazilian Amazon coast. *Environ. Pollut.* 265, 114817.
- Ngo, P.L., Pramanik, B.K., Shah, K., Roychand, R., 2019. Pathway, classification and removal efficiency of microplastics in wastewater treatment plants. *Environ. Pollut.* 255, 113326.
- Pegado, T., Brabo, L., Schmid, K., Sarti, F., Gava, T.T., Nunes, J., Chelazzi, D., Cincinelli, A., Giarrizzo, T., 2021. Ingestion of microplastics by *hypanus guttatus* stingrays in the Western Atlantic Ocean (Brazilian Amazon Coast). *Mar. Pollut. Bull.* 162, 111799.
- Pegado, T.S.S., Schmid, K., Winemiller, K.O., Chelazzi, D., Cincinelli, A., Dei, L., Giarrizzo, T., 2018. First evidence of microplastic ingestion by fishes from the Amazon River estuary. *Mar. Pollut. Bull.* 133, 814–821.
- Redondo-Hasselerharm, P.E., Rico, A., Koelmans, A.A., 2023. Risk assessment of microplastics in freshwater sediments guided by strict quality criteria and data alignment methods. *J. Hazard. Mater.* 441, 129814.
- Rico, A., Brock, T.C., Daam, M.A., 2019. Is the effect assessment approach for fungicides as laid down in the European Food Safety Authority Aquatic Guidance Document sufficiently protective for freshwater ecosystems? *Environ. Toxicol. Chem.* 38 (10), 2279–2293.

- Rico, A., de Oliveira, R., de Souza Nunes, G.S., Rizzi, C., Villa, S., López-Heras, I., Vighi, M., Waichman, A.V., 2021. Pharmaceuticals and other urban contaminants threaten Amazonian freshwater ecosystems. *Environ. Int.* 155, 106702.
- Rico, A., de Oliveira, R., de Souza Nunes, G.S., Rizzi, C., Villa, S., Vizioli, B.D.C., Montagner, C.C., Waichman, A.V., 2022. Ecological risk assessment of pesticides in urban streams of the Brazilian Amazon. *Chemosphere* 291, 132821.
- Rochman, C.M., Browne, M.A., Halpern, B.S., Hentschel, B.T., Hoh, E., Karapanagioti, H. K., Rios-Mendoza, L.M., Takada, H., Teh, S., Thompson, R.C., 2013. Classify plastic waste as hazardous. *Nature* 494 (7436), 169–171.
- Schell, T., Hurley, R., Nizzetto, L., Rico, A., Vighi, M., 2021. Spatio-temporal distribution of microplastics in a Mediterranean river catchment: the importance of wastewater as an environmental pathway. *J. Hazard. Mater.* 420, 126481.
- Schell, T., Martinez-Perez, S., Dafouz, R., Hurley, R., Vighi, M., Rico, A., 2022. Effects of polyester fibers and car tire particles on freshwater invertebrates. *Environ. Toxicol. Chem.* 41 (6), 1555–1567.
- Schell, T., Rico, A., Vighi, M., 2020. Occurrence, fate and fluxes of plastics and microplastics in terrestrial and freshwater ecosystems. *Rev. Environ. Contam. Toxicol.* Volume 250, 1–43.
- Thorley, J., Schwarz, C., 2018. ssdtools An R package to fit species sensitivity distributions. *J. Open Source Softw.* 3, 1082.
- Thornton Hampton, L.M., Lowman, H., Coffin, S., Darin, E., De Frond, H., Hermabessiere, L., Miller, E., de Ruijter, V.N., Faltynkova, A., Kotar, S., Monclús, L., et al., 2022. A living tool for the continued exploration of microplastic toxicity. *Microplast. Nanoplast.* 2 (1), 1–11.
- Wang, Z., Zhang, Y., Kang, S., Yang, L., Shi, H., Tripathee, L., Gao, T., 2021. Research progresses of microplastic pollution in freshwater systems. *Sci. Total Environ.* 795, 148888.
- Wen, B., Zhang, N., Jin, S.R., Chen, Z.Z., Gao, J.Z., Liu, Y., Liu, H.P., Xu, Z., 2018. Microplastics have a more profound impact than elevated temperatures on the predatory performance, digestion and energy metabolism of an Amazonian cichlid. *Aquat. Toxicol.* 195, 67–76.
- Wickham, H., 2016. ggplot2: Elegant Graphics for Data Analysis. [WWW Document]. Springer-Verlag, New York.
- WWF, World Wide Fund for Nature, 2020. Inside the Amazon. Available at: [https://wwf.panda.org/knowledge\\_hub/where\\_we\\_work/amazon/about\\_the\\_amazon](https://wwf.panda.org/knowledge_hub/where_we_work/amazon/about_the_amazon).
- Yang, X., Man, Y.B., Wong, M.H., Owen, R.B., Chow, K.L., 2022. Environmental health impacts of microplastics exposure on structural organization levels in the human body. *Sci. Total Environ.*, 154025.
- Zheng, Y., Li, J., Sun, C., Cao, W., Wang, M., Jiang, F., Ju, P., 2021. Comparative study of three sampling methods for microplastics analysis in seawater. *Sci. Total Environ.* 765, 144495.