Marine Environmental Research 169 (2021) 105327

Contents lists available at ScienceDirect

Marine Environmental Research

journal homepage: http://www.elsevier.com/locate/marenvrev

A fresh look at microplastics and other particles in the tropical coastal ecosystems of Tamandaré, Brazil

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ARTICLE INFO

Keywords:
Polypropylene
Polyethylene
Nylon
Relative microplastics concentration
ZooScan
FTIR

ABSTRACT

Plankton organisms, biogenic particles, inorganic mineral particles, and microplastics are the four main components of particulate organic matter in aquatic ecosystems. We propose a new index, the Relative Microplastics Concentration (RMC, in %), considering that microplastics are more deleterious when food is scarce. A total of 112 plankton net samples were collected in estuarine, coastal and shelf environments of Tamandaré, Brazil. Particles were identified by image analysis (ZooScan) and FTIR. Higher concentrations of total microplastics, PP (Polypropylene) and PE (Polyethylene) in the estuary indicate an oceanward decreasing gradient from terrestrial sources. Higher concentrations of nylon fibres were found offshore. Yet, RMC indicated that the Bay had the most severely impacted ecosystems (RMC: 2.4% in the estuary, 5.1% in the Bay, and 2.0% on the shelf), for total microplastics and PP & PE. Shelf ecosystems were most severely impacted with nylon fibres. RMC analysis provided a new perspective into the impact of microplastics on tropical coastal food webs.

1. Introduction

Plankton organisms, biogenic particles, inorganic mineral particles, and microplastics are the four main components of particulate organic matter in aquatic ecosystems (Fleming et al., 2019; Bowers and Binding, 2006; Nakajima et al., 2010; Vroom et al., 2017; Lins Silva et al., 2019). However, there are no published studies available yet, which consider these four components in a synoptic way and compare their distribution patterns.

Suspended particles are key elements in marine ecosystems, mainly because of their role in fueling food webs and biogeochemical cycles, such as the biological "carbon pump" (Schumann and Rentsch, 1998; Schwamborn et al., 2002, 2006; Checkley et al., 2008). Recent studies showed that non-organismic particles (particles that do not constitute a single living organism, e.g., detritus, aggregates, sand grains and microplastics) constitute a significant portion of common plankton samples, leading to an overestimation of plankton biomass in the oceans (Nakajima et al., 2010; Ohman et al., 2012; Lins Silva et al., 2019). In estuarine and coastal waters, ignoring the huge contribution of particles may lead to a severe underestimation of seston biomass by traditional wet weight-based methods (Lins Silva et al., 2019). Coastal tropical environments, such as mangroves and coral reefs, receive many terrigenous materials. Also, nearshore coastal ecosystems may show high concentrations of anthropogenic particles, such as microplastics (Chong et al., 2001; Barnes et al., 2009).

Zooplankton, biogenic particles and microplastics are traditionally studied by means of sampling with fine-meshed nets and manual counts under a microscope (Blanchot et al., 1989; Neumann-Leitão et al., 1998). Since manual sorting, identification, and quantification of particles and plankton are a time-consuming task, image analysis has become a popular tool in the last ~30 years. Benchtop imaging devices, such as the ZooScan (Grosjean et al., 2004), provide good quality images, with almost perfect focus. Fourier-Transformed Infrared Spectroscopy (FTIR) can be used to identify polymers and to investigate the chemical composition and weathering of microplastics (Dutra et al., 1995; Munajad et al., 2018). Extensive protocols for the preparation of microplastic samples have been developed, including slow and complex dehydration procedures, to allow a reliable interpretation of FTIR spectra (Dutra et al., 1995; Pinho and Macedo 2005). Different sample preparation techniques have been applied, since the methods to separate polymers from other components depend on the diversity of particle types (Dutra et al., 1995; Pinho and Macedo 2005; Munajad et al.,

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https://doi.org/10.1016/j.marenvres.2021.105327
Received 14 December 2020; Received in revised form 30 March 2021; Accepted 1 April 2021

2018). Few studies have used FTIR to analyze microplastics taken from plankton net samples (Di Mauro et al., 2017; Cincinelli et al., 2017), and none have yet combined ZooScan and FTIR to distinguish plankton organisms, biogenic particles and microplastics.

Although there are few studies that estimated the chemical composition and characterization of these particles (McCave et al., 2001; Cincinelli et al., 2017), there are no studies about the origin (anthropogenic or natural) and type (chemical markers) of suspended particles in tropical marine environments.

In spite of the vast recent literature on this subject, there is no practical approach available for the assessment of contamination with microplastics, that explicitly considers the relative contribution of these pollutants, with regard to the available food (suspended particles and plankton) in the water column. This is probably due to the fact that most studies on microplastics destroy and digest biogenic particles and plankton in the samples with acids or enzymes, previous to counting microplastics. The few studies that actually consider microplastics/zooplankton ratios (Cole et al., 2013; Botterell et al., 2019), but do not consider non-organismic biogenic particles, such as plant detritus (Schwamborn et al., 2006), carcases (Silva et al., 2020), and marine aggregates (Kvale et al., 2020).

In the present study, we suggest a new approach and a new index (RMC) to analyze contamination with microplastics. Furthermore, we used this new approach to test the hypothesis that particle type, concentration and volume (in absolute and relative units), differ between ecosystems (mangrove estuary, coral reef-lined bay and continental shelf), thus helping to reveal sources and sinks of biogenic and anthropogenic particles. Also, we aim at discerning the ecosystems that are most impacted by different types of microplastics.

2. Methods

2.1. Study area

The sampled areas range from highly turbid, “brown” estuarine waters, lined by mangroves, to clear “green” waters at nearby coral reefs, and oligotrophic “blue“ waters at mid-shelf. The Rio Formoso Estuary (8° 39’ - 8° 42′S and 35°10’ - 35° 05′W) extends over 12 km and along its route, it receives wastes from domestic sewage and sugar cane industry (Fidem, 1987; CPRH, 1999). The Rio Formoso Estuary is located ~4 Km North of the Tamandaré Bay. The estuarine channels are entirely bordered by mangroves with muddy sediments that are rich in organic matter, which appear to be the most important source of suspended matter in the estuarine area (Silva et al., 2003; Vasconcelos et al., 2004).

Tamandaré Bay (8° 44’ - 8° 47′ S and 30° 0.5’ - 35.07′ W) is a coastal embayment lined by several parallel sandstone reefs with high sedimentation rates and low hydrodynamics, which promote water imprisonment, functioning as an open coastal lagoon (Rebouças, 1966; Camargo et al., 2007). The coastal dynamic is influenced by three large rivers: Rio Formoso, which drains from the north, and Mamucaba and Una rivers, draining into the Bay from the south. Intertidal reef tops are predominantly covered by zoanthids, calcareous algae, and hydrocorals, with several endemic coral species (Amaral and Ramos, 2007; Santos et al., 2015). The main economic activities in this region are agriculture (mostly sugar cane plantations), tourism and fisheries (Moura and Passavante, 1993; Araújo and Costa, 2004). Marine erosive processes observed in this area are related to anthropogenic interventions, such as urban expansion (CPRH, 1999).

The continental shelf off Pernambuco is relatively narrow (~35 km wide), with shallow depths. The shelf break is at ~50 m depth, with warm waters, high salinity and a sedimentary base composed of biogenic, terrestrial and carbonate sediments (Manso et al., 2003). The adjacent inner shelf off Tamandaré has a sandstone linear beach rock parallel to the coast, which is a substrate for the development of algae and corals, and also an effective protection against wave energy (Manso et al., 2003; Camargo et al., 2007).

The rationale for the choice of the study areas was to investigate three tropical coastal ecosystems (a highly turbid mangrove estuary, a coral reef-lined bay, and a nearshore shelf area) that may receive considerable amounts of anthropogenic particles and other pollutants, but are not located within any atypically polluted geographical setting. These three areas were chosen since they are relatively pristine (i.e., compared to other coastal areas, such as coastal megacities), without any large cities or large industrial complexes nearby. They are included within two large Marine Protected Areas (State Decree, nº 19.635, March 13, 1997 and Federal Decree, s/n, October 23, 1997).

The climate is hot and humid, with distinct rainfall seasons and wind energy patterns. Wind speed at sea level exhibits a characteristic seasonality (Silva et al., 2011), with a distinct windy season from July to October (strong SE to E winds) and a calm season from January to May (weak north-easterlies). Rainfall is more intense between March and August, with peaks in July, i.e., the rainy season, while the dry season is generally from September to February (Ferreira et al., 2003; Grego et al., 2009; Venekey et al., 2011).

2.2. Plankton sampling and hydrological parameters

A total of 112 plankton samples were analyzed. Sampling occurred in regular bimonthly intervals, during two years, between April 2013 and May 2015 during ESPLAN and INCT AmbTropic campaigns. During each field campaign, twelve fixed stations were sampled, three in the Rio Formoso Estuary, three in Tamandaré Bay, and six on the continental shelf, distributed along a straight transect (three stations at the nearshore shelf and three at mid-shelf), from the reef line to the mid-shelf (Fig. 1). Tows were performed using a conical-cylindrical plankton net with a 300-μm mesh (diameter: 60 cm), by means of subsurface horizontal tows during 5 min at a speed of 2–3 knots, during ebb (Rio Formoso Estuary) and flood tides (Tamandaré Bay and Shelf). The upper rim of the net opening was maintained at the surface with a float (sampling depth: 0–0.6 m). Sampling was conducted during daytime, at the same tidal phase, during new moon spring tides. A calibrated flow meter (Hydro-Bios, Kiel) was coupled to the mouth of the net to estimate the filtered volume. Samples were immediately preserved in formaldehyde (4% final concentration), buffered with sodium tetraborate (5 g L^{-1}), as described by Omori and Ikeda (1984). A CTD probe (CastAway, YSI) was used to obtain vertical profiles of salinity and temperature. Water transparency was estimated from the Secchi-disk depth (Preisendorfer, 1986).

2.3. Image acquisition and analysis

A ZooScan device (Hydroptic model ZSCAN03) was used to digitise the plankton samples with 2400 dpi resolution, following the protocol established by Grosjean et al. (2004; http://www.zooscan.obs-vlfr.fr/). Each sample was separated into two size fractions (>1000 μm and <1000 μm) to avoid underestimating large organisms and particles, considering that large objects are less abundant (Gorsky et al., 2010). Samples obtained in the Rio Formoso Estuary and in Tamandaré Bay were diluted in filtered water in a beaker, according to the total number of particles in the sample, and then carefully mixed before the extraction of a 10 ml fraction, to obtain a sub-sample of approximately 2,000 particles for subsequent scanning (Grosjean et al., 2004). For samples obtained on the continental shelf, a Motoda box splitter was used (Motoda, 1959). The number of objects contained in each scanned fraction ranged from 40, for the size fraction >1000 μm, up to 8,000 objects, for the size fraction <1000 μm.

Images were processed using the ZooProcess software (Version 7.25), written in the ImageJ macro language (https://imagej.nih.gov/ij/), developed for plankton image analysis. The Plankton Identifier (PkID) software (version 1.3.4), was used for semi-automatic classification. First, we built a training set, wich was then used for Random Forest, an

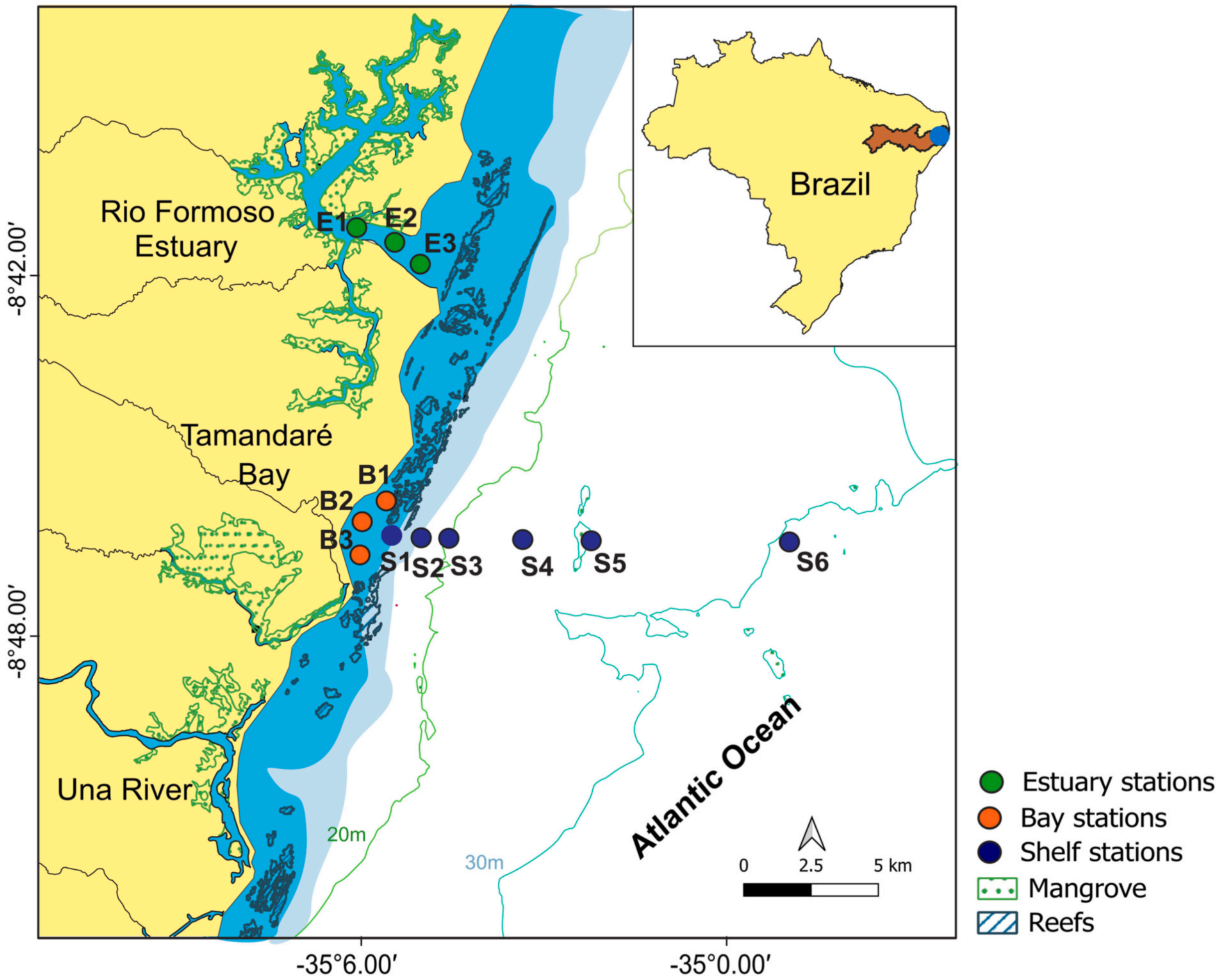

Fig. 1. Map showing the study area and stations where sampling was conducted in three environments (Rio Formoso Estuary, Tamandaré Bay, and on the adjacent continental shelf), from April 2013 to May 2015. Map inlet (above): map of Brazil showing the State of Pernambuco (dark brown) and the location of the Tamandaré coastal region (blue circle). Plankton samples and abiotic parameters were obtained bimonthly between April 2013 and May 2015. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

algorithm for the automatic classification of vignettes into predefined categories. Finally, all vignettes were manually validated to correct for misclassification. Size parameters were converted from pixels to micrometers, according to the scanner resolution (size of 1 pixel at 2400 dpi: 10.58 μm). To minimize possible contamination of the samples on board and in the laboratory, we applied a series of protocols and blanks, e.g., we used filtered water (20 μm mesh filters), and all sample flasks and materials were thoroughly washed before placing the sample. All particles were classified according to their shape and gray levels, based on the ZooScan images (vignettes). For the classification according to gray level, three different categories were created: dark, opaque and transparent particles, whose shape could be globulose or flat.

Particle volume (mm^3) was usually estimated as the ellipsoid volume, based on the lengths of major and minor axes of the equivalent ellipse (i.e., an ellipse with the same area and similar height/width ratio as the original vignette, Vandromme et al., 2012 and Stemmann and Boss, 2012), except for flat particles. Flat particles were considered as having a flat shape and their volume (mm^3) was calculated based on the surface area (the “area_exc” parameter, in mm^2) multiplied by the thickness (mm) of each particle type (Grosjean et al., 2004). The thickness was measured under a stereomicroscope (Zeiss, Stemi SV6 model) in 30 randomly chosen plankton samples. For each sample, 50 particles were taken from three different categories (opaque, dark, and transparent flat particles), and classified according to their gray level. For opaque and dark flat particles, mean thickness was 781 μm, whereas for transparent flat particles, mean thickness was 319 μm (Lins Silva et al., 2019).

2.4. Fourier-Transformed Infrared Spectroscopy analysis (FTIR)

The specific density of plankton and biogenic particles has a broad range, but usually lies between 1.03 and 1.10 $g.L^{-1}$ (Goldemberg and Saldana-Corrêa, 2010). On the other hand, man-made polymer particles have a much narrower range of specific density values (Mark, 1999). The density of these polymers varies only little over time, e.g., their buoyancy may decrease due to rapid colonization with microorganisms (Cole et al., 2011). The most common polymers, which are polyethylene (PE), polypropylene (PP), polystyrene (PS), polyvinyl chloride (PVC), polyamide (PA), polyethylene terephthalate (PET), polyvinyl alcohol (PVA). They present specific ranges of density values: PE: 0.93–0.98 $g.L^{-1}$, PP: 0.89–0.91, PS: 1.04–1.11, PVC: 1.2–1.45, PA: 1.13 to 1.5, PET: 1.38–1.39, PVA: 1.19–1.35. (Avio et al., 2016). Thus, their density can be useful to separate biogenic particles and microplastics.

The particles preparation had two objectives: to separate biogenic particles from microplastics according to their density, and to remove water from microplastics. Vacuum filtration (Whatman cellulose acetate filters 1.2 μm pore size) was used to remove the particles from the original sample. Samples were put in a separatory funnel (100 ml of solution + particles), in contact with controlled density liquids, mixed and let to rest for 30 min. The liquids were: ethanol (C_2H_5OH) +

methanol (CH_3OH) + sodium bromide solution (NaBr) solution (0.88 g/cm^3), ethanol + sodium chloride (NaCl) solution (P.A – A.C.S; 0.81 g/cm^3), and absolute ethyl alcohol (P.A – A.C.S; CH_3CH_2OH.; 0.79 g/cm^3). The decanted fraction passed to the next step. The supernatant fraction was dried in a dry oven at 50 °C for 24 h to six months, depending on the water contents in the sample. After that, additional two steps were realized intending to dissolve any polymer particle which was not removed from biological particles in the previous three steps. Particles went into two solvents: acetone (P.A – A.C.S; $CH_3(CO)CH_3$ 0.78 g/cm^3), and chloroform (P.A – A.C.S; $CHCl_3$,1.48 g/cm^3). Again the decanted fraction and supernatant fraction were dried in a dry oven at 50 °C for periods that varied from 24 h to six months, depending on the water content of the sample.

FTIR was used to analyze the typology of all biogenic and abiogenic (*i.e.,* microplastics) particles that were previously classified with the ZooScan. For this purpose, a subset of 30 plankton samples (10 samples randomly chosen from each study area), was selected to allow a detailed verification of the typology of all particles (biogenic particles and microplastics). A Shimadzu Fourier Transform Infrared spectrophotometer, model Prestige 21, with a diffuse reflectance module, was used to acquire the infrared spectra for all samples. We performed 24 scans per sample, with 4 cm^{-1} resolution, according to the ATSM SP E1252 – 98 normative. All spectra were treated using the OriginPro 8 software. The identification of the polymers was made by comparing the sample spectra with standard spectra contained in the Hummel Polymer and Additives library (Silverstein et al., 2007). FTIR was also used to evaluate the weathering status of particles (fresh *vs* weathered polymers).

2.5. Statistical analyses

ZooScan data and images were used for quantifying concentrations (counts per cubic meter) and volumes (mm^3 per cubic meter) of zooplankton, microplastics and other particles. Additionally to quantifying absolute concentrations, we propose a new index of microplastics contamination, the Relative Microplastics Concentration (RMC, in %). RMC is calculated as

$$\text{RMC (\%)} = 100 * (m / (z + p + m)),$$

where m = microplastics concentration, z = zooplankton, and p = "other particles" (e.g. biogenic particles detritus, sand grains, etc.). The rationale behind the proposed RMC is that the relative concentration is an index for the probability of encountering microplastics by any organism feeding in the water column at a given location. Also, it provides a straightforwardly intelligible percentage of microplastics among all food particles (living and nonliving) available for planktivores.

All data sets were tested for normality and homoscedasticity using Kolmogorov-Smirnov and Levene tests, respectively, prior to analysis. Since the data sets were not normally distributed, even after log10(x+1) transformation, non-parametric methods were applied to the 11 particle types tested and key parameter ratios, such as the ratio "total microplastics"/"total zooplankton" and RMC. A multivariate two-way PERMANOVA (Anderson, 2001) was used to test for significant effects of three factors: i) seasons (dry *vs* rainy), ii) areas (Estuary, Bay and Shelf) and iii) their interaction (seasons *vs* areas). For each particle type, a separate PERMANOVA was conducted (10,000 permutations), based on an euclidean distance matrix, (Anderson, 2017), applying the function "adonis" within the "vegan" R package (Oksanen et al., 2017). For variables that displayed significant PERMANOVA results for the factor "study areas" ($p < 0.05$), *post-hoc* tests for pairwise multiple comparisons of mean rank sums (i.e., non-parametric Kruskal-Nemenyi tests, Nemenyi, 1963, Hollander and Wolfe, 1999; Demsar, 2006), were conducted. These pairwise K–N tests (e.g., "Bay *vs* Estuary"), were conducted using the function "posthoc.kruskal.nemenyi.test" in the R package "PMCMR" (Pohlert, 2020). All analyses were conducted within the R environment for statistical computing and graphics, version 3.6.3 (R Core Team, 2020).

3. Results

3.1. Hydrographic features

The three study areas (Rio Formoso Estuary, Tamandaré Bay and Continental Shelf) showed characteristic hydrographic features (Fig. 2). Significantly ($p < 0.0001$) lower transparency was found in the Estuary (Secchi depth range: 1.2–3.5 m; mean: 1.8 m) than in the Bay (1.0–5.5 m; mean: 3.1 m) and on the Shelf (1.0–19.0 m; mean: 5.3 m). Euhaline conditions were found in the Bay (salinity range: 35.0–36.5; mean: 35.9) and on the Shelf (33.8–37.4; mean: 36.3). Conversely, mesohaline to euhaline conditions were observed in the Estuary (27.0–36.4; mean: 32.1). Temperature and salinity varied seasonally, with a gradual increase during the dry season, from September through February (Suppl. Table 1).

Estuarine waters were often brown and turbid, and transparency was significantly lower ($p = 0.0006$) in the rainy season than in the dry season, when particles, macroalgae and DOM (Dissolved Organic Matter) were more abundant. In the dry season, there was an intrusion of offshore transparent waters into the estuary. In the Estuary, temperature and salinity did not differ significantly between seasons (dry *vs* rainy).

In the Bay, water transparency was significantly higher in the dry season ($p = 0.02$), when low wind intensity and low rainfall led to calm,

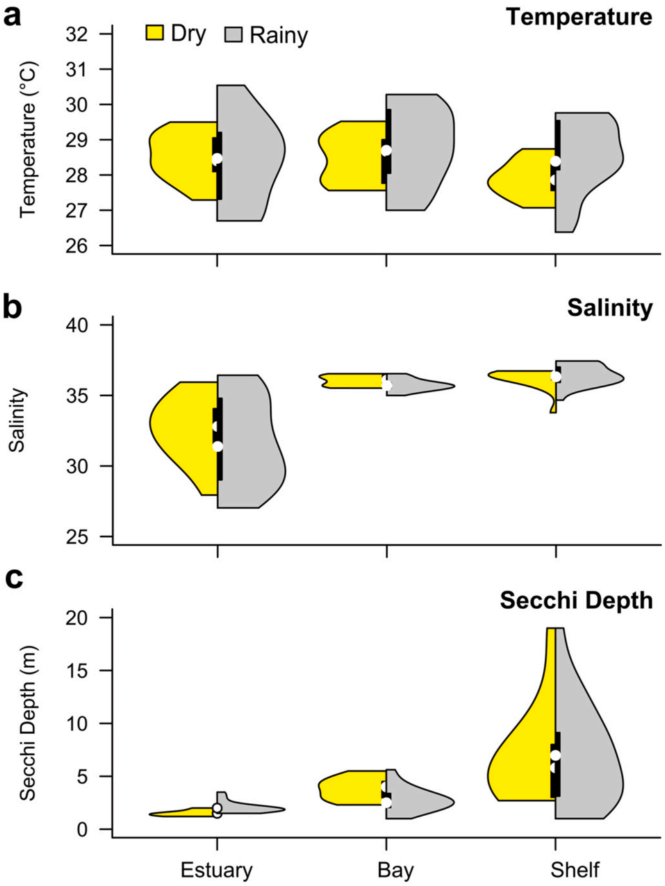

Fig. 2. Vioplots (Kernel density distributions with boxplots) of environmental variables (salinity, temperature, and Secchi depth) in estuarine, bay and shelf waters. Yellow color represents the dry season and gray color represents the rainy season. Bimonthly sampling was conducted from 2013 to 2015 in the Rio Formoso Estuary, Tamandaré Bay and on the adjacent Continental Shelf. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

transparent “blue” waters. Conversely, “green” waters (Secchi depth range: 1.5–4.5 m, mean: 3.0 m) were dominant during the rainy season, due to nutrient inputs from stronger wind turbulence and increased runoff from adjacent rivers. On the Shelf, water transparency was highly variable, but often showed transparent, “blue” waters, with >5 m Secchi depth.

3.2. *Particle classification*

The 112 samples analyzed contained plankton and twelve types of non-organismic particles (biogenic particles and microplastics, see Suppl. Table 2). Particle size varied from 299 μm to 57 mm equivalent spherical diameter (ESD). FTIR spectroscopy allowed us to distinguish and typify four types of particles that were common in all sampling areas: i.) biogenic particles (vascular plant detritus, fragments of macroalgae, marine aggregates and exuviae), ii.) polypropylene (PP), iii.) polyethylene (PE), and iv.) polyamide (i.e., nylon fibres). Even though we detected and distinguished polypropylene and polyethylene particles with FTIR, we pooled these two categories (PP & PE) for our quantitative analyses, because they were not distinguishable in the ZooScan images. Also, when it was not possible to relate the ZooScan images with the infrared absorption spectrum with FTIR the classification of some types of particles was kept in the gray levels (see Suppl. Table 2).

Polymeric particles displayed well defined FTIR spectral peaks and bands, which allowed their identification, even when the particle showed extra peaks due the ageing process. Weathering bands were present to some extent in all polymer samples. There was no detectable contamination with fresh nylon (e.g. from the plankton nets). Conversely, the FTIR spectrum for biogenic particles showed persistent water bands and small peaks which can be considered as the resultant spectra from the sum of several compounds with complex chemical structure, consistent with biological samples (Suppl. Fig. 1).

Nine categories of non-zooplankton particles (Suppl. Table 2) could be classified and described according to shape and gray level descriptors, using digital images obtained with a ZooScan equipment: i.) flat dark particles, ii.) flat opaque particles, iii.) transparent particles, iv.) opaque particles, v.) marine aggregates, vi.) fragments of macroalgae, vii.) exuviae/carcasses and viii.) sand grains, and ix.) fibres. Multiples (touching objects) were not included in this list, since they are often composed of zooplankton organisms and aggregates. These categories of particles were based on gray level distributions and a matrix of shape descriptors. The combination of FTIR and Zooscan analyses allowed us to refine the identification process (PP & PE, nylon fibres, vascular plants), increasing the number of categories from nine to twelve (see Suppl. Table 2).

3.3. *Distribution of microplastics and other particles*

Three main groups of particles were found. The most abundant group was composed of biogenic particles (i.e., derived from vascular plant detritus, exuviae, carcasses, and marine aggregates), followed by opaque particles (origin unknown), and microplastics (e.g., nylon fibres, polyethylene and polypropylene - PP & PE) (Fig. 3). Non-zooplankton particles were abundant in all environments (Estuary, Bay and Shelf), usually comprising more than 50% of the plankton samples.

The concentration of particles generally decreased towards offshore, with high variability within areas. Total particle concentration ranged from 13.4 to 4903.7 particles m^{-3} in the Estuary, from 6.9 to 525.5 particles m^{-3} in the Bay and from 0.95 to 206.3 particles m^{-3} on the Shelf (Suppl. Table 3). While there was a striking and consistent effect of the spatial gradient, no consistent seasonal effects were detected for the total concentration of particles.

In the Estuary, the most abundant particles were vascular plant detritus (47% of all non-organismic particles, mean percentage, in units of concentration), most likely originating from the mangroves, followed by opaque (21%) and transparent particles (14%). In the Bay, the most abundant particles were opaque (25%), transparent (20%) and PP & PE (14%).

On the Shelf, marine aggregates (i.e., complex particles formed though coagulation of gelatinous detritus and plankton) were the dominant type of particles (35% of total particle concentration, on average), followed by vascular plant detritus (21%) and opaque particles (18%). Microplastics were also very abundant (14%) in Shelf waters (Fig. 3).

Significant effects of the factor “area” (Estuary vs Bay vs Shelf) were detected for the relative concentration of all particle types. Highest median concentration values were found in the Estuary, followed by Bay and Shelf waters ($p < 0.001$, PERMANOVA, Suppl. Table 4). The *post-hoc* pairwise tests revealed significant differences between areas in absolute concentration of most particle types (Suppl. Table 4).

3.4. *Contamination with microplastics*

Total concentrations of microplastics (PP & PE and Nylon, together) were highest in the Estuary, and decreased oceanward (Fig. 4a), similarly to other particles (Fig. 4b), such as biogenic particles (plant detritus, macroalgae, marine aggregates and exuviae), sand, opaque, opaque flat, transparent and transparent flat particles. However, PP & PE and nylon displayed marked differences in their spatial variability, reflecting their different origins and sinks. The concentration of PP & PE, which were the most abundant microplastics, had a maximum in the Estuary and decreased continuously towards the open ocean (Fig. 4c). Conversely, nylon fibres also showed maximum concentrations in the estuarine area, but higher concentrations at mid-shelf than in the Bay area (Fig. 4d). The concentration of zooplankton, similarly to the concentration of microplastics and the other particles, was highest in estuarine waters and decreased towards the Shelf (Fig. 4e). The Relative Microplastics Concentration (RMC, in %) had maximum values in the

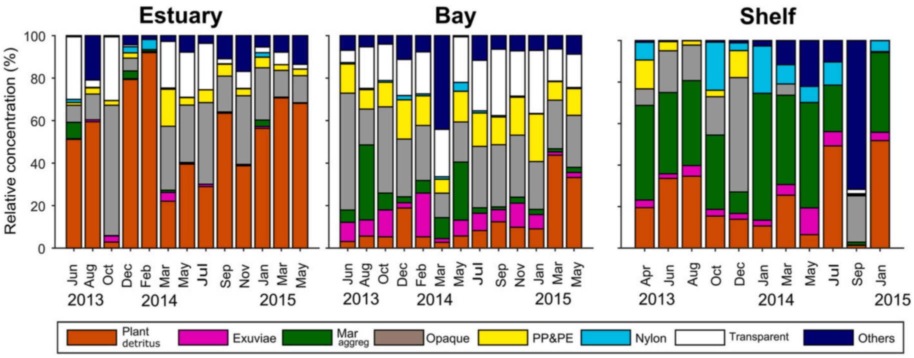

Fig. 3. Relative concentration of suspended particles in three areas (Rio Formoso Estuary, Tamandaré Bay and Continental Shelf) off Tamandaré, Brazil, n = 112. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

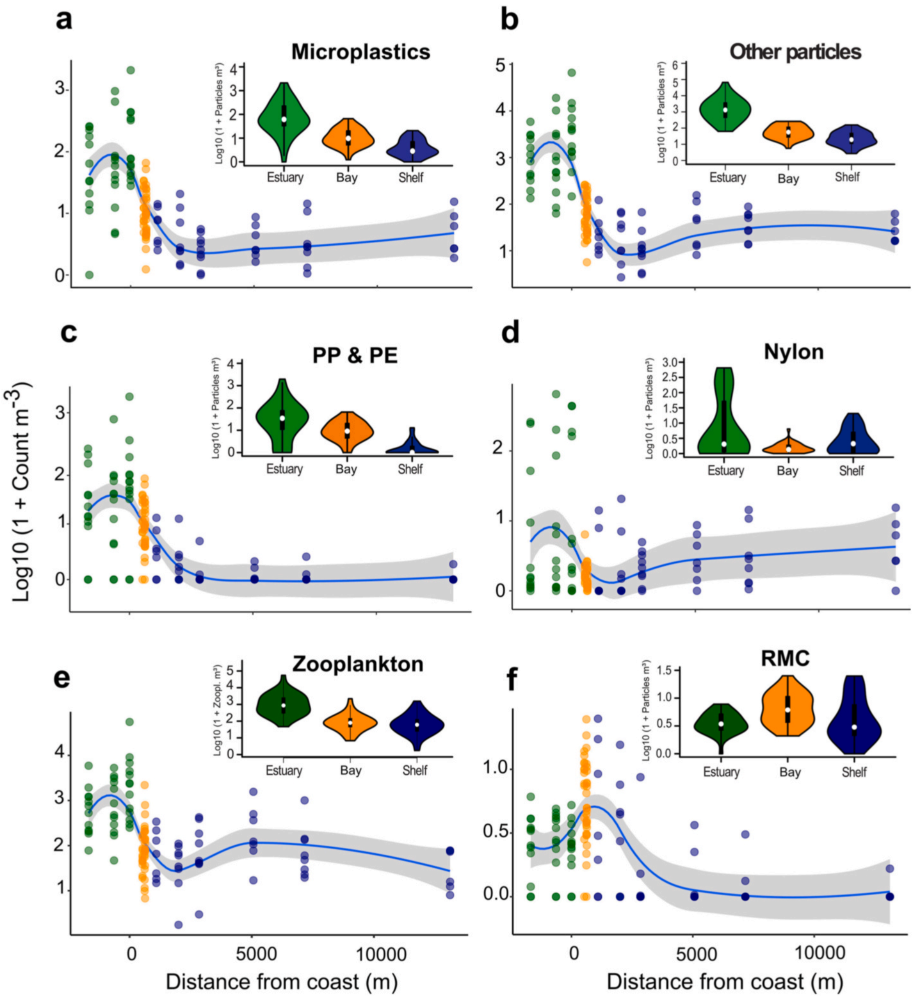

Fig. 4. Gradient of spatial distribution of the concentrations (counts m^{-3}) of total microplastics (a), other particles (plant derived + marine aggregates + macroalgae + exuviae + sand + opaque + opaque flat + dark flat + transparent + transparent flat) (b), PP & PE (polyethylene + polypropylene) (c), and Nylon (d), total zooplankton (e) and Relative Microplastics Concentration (RMC, %) = 100 * total microplastics/other particles + zooplankton + microplastics (f). All values have been log10(x+1) transformed prior to plotting.

Bay and nearshore Shelf areas, and lower RMCs in the Estuary. Minimum RMC values were found at the offshore shelf (Fig. 4f).

The study areas differed with regard to RMC (PERMANOVA, $p = 0.004$). Highest RMCs were recorded in the Bay (Fig. 5a), indicating a higher impact with microplastics in relation to the Estuary and Shelf. Significant differences were not observed for the relative volume of microplastics (RMV) between the areas (Fig. 5b), probably due to the additional variability introduced by volume calculations. For the microplastics ratio (PP & PE and Nylon)/other particles (in concentration units), there was a highly significant effect of the factor “area” (PERMANOVA, $p = 0.001$), with significantly lower proportions ($p < 0,001$) in the estuary and similar rates in the bay and shelf areas (Fig. 5c).

When testing the microplastics/zooplankton ratio (in concentration units), a significant effect of the factor “area” (PERMANOVA, $p = 0.013$) was observed, with highest values also being recorded in the Bay (Fig. 5d), similarly to RMC.

For the PP & PE/other particles + zooplankton + microplastics ratio (in concentration units), there was a highly significant effect of the factor “area” (PERMANOVA, $p = 0.001$) with highest values in the Bay, followed by Shelf and estuarine stations (Fig. 5e). Nylon fibres had a different spatial pattern. When testing the nylon/other particles + zooplankton + microplastics ratio (in concentration units), the highest relative concentration of nylon fibres was found in the Shelf area (PERMANOVA, $p = 0.002$; Fig. 5f).

4. Discussion

This study provides new insights into the distribution of microplastics and other suspended particles in tropical estuarine and marine environments, combining two different optical methods (ZooScan and FTIR). Through the use of a new approach and new indices, we quantified the contributions of different types of particles in the meso (>299 μm) size range, natural or not, as ecosystem components. This approach allows novel interpretations of the composition of robust, large-sized seston in the aquatic ecosystems. Also, the use of a new index (RMC) provided a new perspective into the impact of microplastics on the food particle spectrum available for planktivorous organisms, and consequently, on estuarine and marine ecosystems.

4.1. Hydrography

Estuarine areas are extremely productive habitats (French, 1997; Zarauz and Fernandes, 2008) and estuarine waters often have a turbid appearance and a brownish color as a result of large amounts of suspended particles, such as mangrove leaf detritus (Schwamborn et al., 2002, 2006; Lins Silva et al., 2019). The low transparencies observed in the Rio Formoso Estuary are similar to those observed in other turbid estuaries, such as the estuarine system of Santa Cruz Channel, Brazil (Flores-Montes et al., 1998; Schettini et al., 2016). The seasonally varying mixture of nutrient-rich river plumes and oligotrophic shelf waters explain the variability in water transparency regimes of Tamandaré Bay (Maida and Ferreira, 1995; Santos et al., 2010). On the adjacent continental shelf, water transparency was generally much

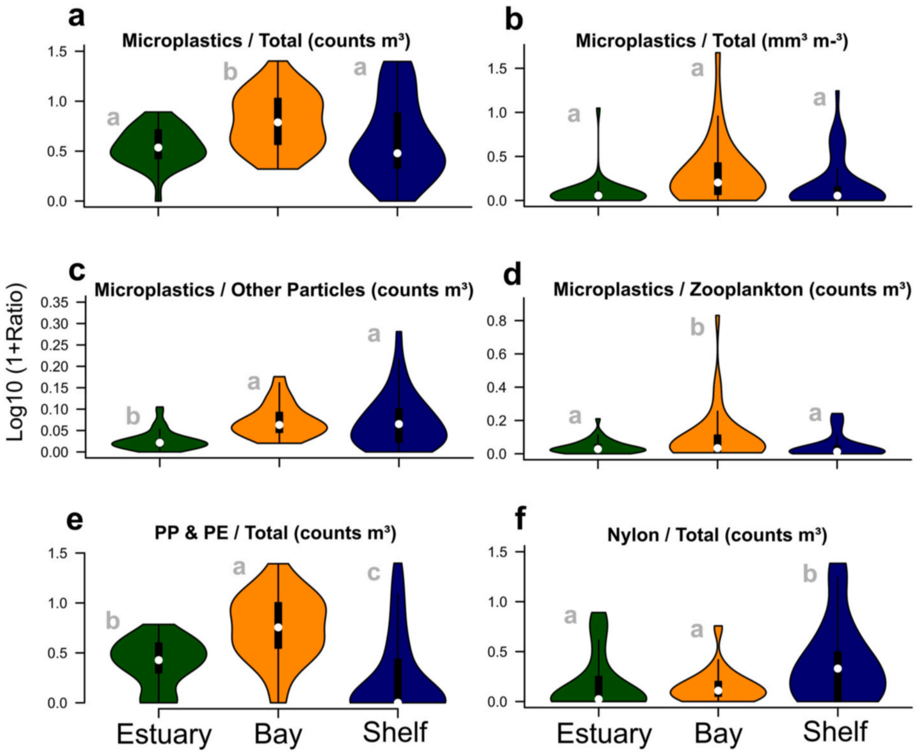

Fig. 5. Indices of microplastics contamination in units of concentration (counts m^{-3}) and volume (mm^{3} m^{-3}). Microplastics: Nylon + PP & PE; Total: other particles + zooplankton + microplastics; Other particles: non-plastic and non-zooplankton particles. Other particles are represented by plant derived detritus, marine aggregates, macroalgae, exuviae, sand, opaque, transparent, dark flat, opaque flat and transparent flat particles.

higher and extremely variable, with visibilities ranging from 1 to 19 m, depending on seasonal variations in rainfall and sediment resuspension driven by wind-induced turbulence (Rebouças, 1966; Moura and Passavante, 1993).

4.2. Spatial heterogeneity of microplastics and other particles

In this study, maximum values of total particle abundance and volume in the Estuary showed the importance of substantial inputs of detritus of riverine origin (Williams and Simmons 1997). Continental shelf regions generally display lower concentrations of microplastics than coastal areas, since most of these contaminants originate from the coast and river plumes and then are advected offshore by complex currents (Barnes et al., 2009). Similarly, our results revealed particular spatial patterns in particle distributions, with lower concentrations over the shelf, for different types of microplastics and biogenic particles (e.g., vascular plant detritus, macroalgae fragments, marine aggregates and exuviae).

The second most abundant group of particles were globose opaque particles. Still, their origin remains unclear. Unfortunately, the methodology used in this study did not permit the identification with certainty of the origin or typology of this group, which may also contain microplastics. Although opaque particles could be distinguished from PP&PE by the well defined contour of the latter and subtle differences in their gray levels, there is still some uncertainty regarding these particles. Thus, the real microplastics concentration could be even higher than reported in our data. Clearly, further studies are necessary to elucidate the origin of globose opaque particles in tropical coastal areas. This is the first attempt to use a laboratory-based semi-automatic optical method (i.e., the ZooScan approach) for this purpose.

4.3. Sources and sinks of microplastics

Microplastics may enter the seas from distinct land-based sources, from ships and other facilities at sea, from punctual and diffusive sources, and can travel long distances before they are stranded, degraded or buried in the sediment (Ryan et al., 2009).

Polyethylene and polypropylene are widely produced and used for many applications, and since approximately half the world's population resides within fifty miles of the coast, these materials have a high potential to reach the marine environment via rivers, wastewater systems, and by winds blowing offshore (Moore et al., 2001; Derraik, 2002; Thompson et al., 2005; Cole et al., 2011). In addition, polymers are highly persistent in the marine environment and their degradation is slow, even when exposed to strong UV radiation (Andrady 2003). The estimates on plastics longevity are highly variable but likely in the range of hundreds or even thousands of years, depending on the physical and chemical properties of the polymer (Teuten et al., 2007; Galgani et al., 2015). This feature is one of the main reasons for the high concentration and ubiquity of these pollutants worldwide, including tropical coastal seas.

Total microplastics, polyethylene and polypropylene displayed higher concentrations in the Estuary and in the Bay, when compared to the Shelf, indicating a dilution gradient, sedimentation and degradation oceanward. These polymers have lower densities than seawater, so they float until they are washed ashore or even sink because their density changes due to biofouling and leaching of additives (Galgani et al., 2015). This coastal-offshore gradient in the PP&PE concentration is indicative of coastal sources and continuous particles degradation and sedimentation.

Several studies around the world showed high concentrations of polyethylene and polypropylene in offshore waters. The high concentration of these types of microplastics observed in the Bay area were also observed in other marine systems. Pabortsava and Lampitt (2020), on the surface of the Atlantic Ocean, showed that both inputs and stocks of ocean plastics are much higher than those determined since the 1950s, and polypropylene and polyethylene were responsible for the highest mass concentrations among the investigated microplastics. Another study by Kedzierski et al. (2019) in the Mediterranean Sea proposed a protocol to determine the microplastics concentrations necessary to provide representative data, and also concluded that polyethylene and polypropylene were the main polymers on the samples. Same results were found by Cincinelli et al. (2017) in subsurface waters near-shore and off-shore the coastal area of the Ross Sea (Antarctica). These authors found predominantly polyethylene and polypropylene, among other types of microplastics, also using FTIR spectroscopy to determine particle typology.

On the other hand, the higher nylon concentrations over the mid-shelf indicate important nylon sources over the Shelf, e.g., derived from fisheries activities, and/or possibly a sink of nylon in the Bay area. Alternatively, the mid-shelf may receive inputs of large-scale estuarine plumes (e.g, from the nearby Una river), that may be rich in microplastics, PP & PE and nylon fibres.

4.4. Considering other particles and zooplankton during the evaluation of the impact of microplastics on marine food webs

Microplastics have been widely studied in recent years not only because of their increasing abundance in the water and in sediments, but also because they are being detected in numerous organisms throughout freshwater, estuarine and marine food webs (Browne et al., 2008; Collignon et al., 2012; Cole et al., 2013; Kaposi et al., 2014; Desforges et al., 2015). These microplastics can affect marine organisms whether chemically, due to the toxic effects of POPs (Persistent Organic Pollutants, Sobral et al., 2011) or physically, by damaging their digestive system (Cole et al., 2013). There are several laboratory studies that showed a variety of plankton organisms feeding on microplastics (Kremer and Madin, 1992; Cole et al., 2013; Botterell et al., 2019). These microplastics begin to accumulate in food webs (Setälä et al., 2014; Vandermeersch et al., 2015; Sun et al., 2017). There are few studies that assessed the effect of microplastic intake by zooplankton organisms on their feeding activity (Cole et al., 2013, 2015; Desforges et al., 2015; Sun et al., 2017). Cole et al., 2013 showed that the presence of microplastics drastically affects the ingestion of food items.

Yet, there are no published laboratory studies available that investigated the effect of the abundance of other food items (e.g., plankton and biogenic particles) on microplastic intake by zooplankton organisms. Numerous studies have shown that selective feeding by zooplankton on a given food item depends on the availability, abundance and exposure time of alternative food sources (Lenz, 1977; Paffenhöfer and Van Sant, 1985; Chong et al., 2001; Schwamborn et al., 2004, 2006; Feehan et al., 2017).

Clearly, the presence of other food items available for zooplankton will affect their intake of microplastics, and thus the impact of microplastics on marine food webs. Therefore, it is essential to consider the abundance and composition of plankton and biogenic particles when assessing the impact of microplastics on coastal environments.

4.5. Relative microplastics concentration (RMC) as a key index of contamination

The rationale behind the proposed RMC (Relative Microplastics Concentration) is that the relative concentration is an index for the probability of encounter and ingestion of microplastics in the water column at any given location.

The use of relative indices, such as RMC, assumes that a given consumer will most likely ingest a given particle (including microplastics), depending on its availability in relation to food particles (Schwamborn et al., 2006). For example, even a low absolute concentration of microplastics may be extremely deleterious for planktivores and suspension feeders, if all other available food particles are scarce, too, as observed in the Shelf area in the present study. Conversely, in the Rio Formoso Estuary, microplastics showed extremely high concentrations and volumes, but other particles and zooplankton were even more abundant. Thus, RMC was low in the Estuary and consequently, the probability of microplastics being ingested was relatively low in the mangrove-lined Estuary, for total microplastics, PP & PE and nylon fibres. Conversely, extremely low concentrations of zooplankton and other particles were found in the reef-lined Bay and at nearshore shelf stations, contributing to the maximum RMC values found in these two areas.

For nylon fibres, an unexpected pattern was observed, where maximum RMC (i.e., maximum impact) was observed in offshore shelf areas, indicating that these offshore areas were most severely impacted by nylon fibres.

5. Conclusions

This study provided new insights into the distribution of microplastics within the available food spectrum in tropical coastal areas. The composition of biogenic particles followed the expected pattern, with more plant matter (mangrove detritus) in the mangrove-lined estuary. Surprisingly, the impact of microplastics was more severe in offshore waters than at the river mouths (the sources of anthropogenic particles). In short, Bay and nearshore shelf waters were the most severely impacted areas by total microplastics and PP & PE, and offshore waters with nylon fibres, although absolute concentrations of all microplastics, total particles and zooplankton were much higher in the estuary. This indicates that the impact of these persistent pollutants may be more harmful in oligotrophic offshore waters, than in particle-rich estuarine waters, opening a new line of research.

CRediT authorship contribution statement

Nathália Lins-Silva: Conceptualization, Formal analysis, Funding acquisition. **Catarina R. Marcolin:** Conceptualization, Formal analysis, Funding acquisition. **Felipe Kessler:** Conceptualization, Formal analysis, Funding acquisition. **Ralf Schwamborn:** Conceptualization, Formal analysis, Funding acquisition.

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.

Acknowledgements

The authors thank the Brazilian National Research Council (CNPq) for the productivity fellowship granted to the last author. Fieldwork was funded by the ESPLAN project (CNPq grant no. 471038/2012-1) and by INCTAmbTropic (CNPq/CAPES/FAPESB). We also thank the Long Term Ecological Research Program PELD-TAMS (MCTI/CNPq), and to the Instituto Recifes Costeiros (IRCOS, supported by ICMBio, SOS Mata Atlântica and Toyota do Brasil Foundations), for logistical support with sampling. This paper was furthermore supported by the project TRIATLAS, which has received funding from the European Union's Horizon 2020 programme under grant agreement no. 817578 and by the MicroplastiX project funded by JPI Oceans. To the Tamandaré Municipality and CEPENE/ICMBio for logistical support. Many thanks to Marc Picheral (LOV, France) and Sylvain Fèvre (Hydroptic, France) for assistance with the ZooScan equipment and the ZooProcess software. Many thanks also to FURG's school of chemistry and food for logistical support. The authors thank the Brazilian ministry for the environment (MMA) for the SISBIO permits no. 38428–4 and 34067. We thank all people involved in the fieldwork and laboratory analyses, especially CEPENE's boat drivers, Sigrid Neumann Leitão, and all students and colleagues at the Zooplankton Laboratory at UFPE's Oceanography Department.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.marenvres.2021.105327.

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