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Floating plastics and their associated biota in the Western South Atlantic

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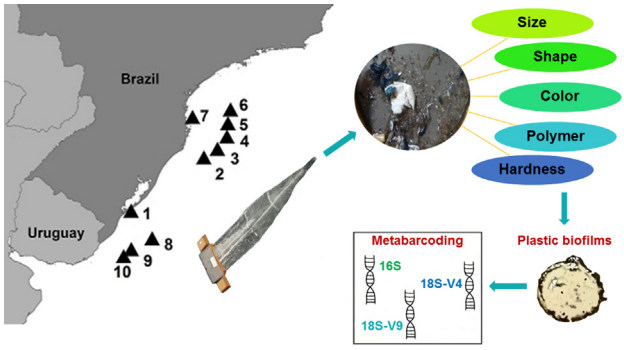
HIGHLIGHTS

- An average of 4461 plastic items.km^{-2} was found in the Western South Atlantic.
- Microplastic fragments and lines, of nine colours and seven polymers, were dominant.
- Rivers and fisheries are likely major sources of plastics to the region.
- Plastics had a high diversity of associated prokaryotes and eukaryotes.
- Plastic-associated organisms included potential biodegraders and pathogens.

GRAPHICAL ABSTRACT



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ABSTRACT

The lack of information about plastic pollution in many marine regions hinders firm actions to manage human activities and mitigate their impacts. This study conducted for the first time a quali-quantitative evaluation of floating plastics and their associated biota from coastal and oceanic waters in South Brazil. Plastics were collected using a manta net, and were categorized according to their shape, size, malleability and polymer composition. Multi-marker DNA metabarcoding (16S, and 18S V4 and V9 rRNA regions) was performed to identify prokaryotes and eukaryotes associated to plastics. We found 371 likely plastic particles of several sizes, shapes and polymers, and the average concentration of plastics at the region was 4461 items.km^{-2} (SD ± 3914). Microplastics (0.5 - 5 mm) were dominant in most sampling stations, with fragments and lines representing the most common shapes. Diverse groups of prokaryotes (20 bacteria phyla) and eukaryotes (41 groups) were associated with plastics. Both the community composition and richness of epiplastic organisms were highly variable between individual plastics but, in general, were not influenced by plastic categories. Organisms with potential pathogenicity (e.g. *Vibrio* species. and *Alexandrium tamarense*), as well as potential plastic degraders (e.g. *Ralstonia*, *Pseudomonas*, and *Alcanivorax* species), were found. The information generated here is pivotal to support strategies to prevent the input and mitigate the impacts of plastics and their associated organisms on marine environments.

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1. Introduction

Although plastic pollution is receiving increasing scientific attention, there are still many gaps in our understanding of factors that are crucial to solve this issue, such as the abundance, characteristics and consequences of plastics in the ocean (Wilcox et al., 2016; Beaumont et al., 2019; Rochman and Hoellein, 2020). Most plastics found at sea are from continental sources (land-based), with a lower contribution from maritime (Li et al., 2016; Krantzberg, 2019) and atmospheric (Sridharan et al., 2021a) sources; however, this can vary according to location (Jang et al., 2014). Riverine systems are one of the main continental sources of plastics to the ocean, and it has been estimated that between 1.15 and 2.41 million tonnes of plastics enter the ocean annually from rivers (Lebreton et al., 2017). Among maritime sources, nautical activities such as fishing, shipping, offshore mining and illegal dumping at sea are important contributors of plastics (GESAMP, 2019), with an estimated 0.6 million tonnes entering the ocean annually (Boucher and Billard, 2019). Most plastics at sea are small, being classified as microplastics (0.001 - 5 mm); those that reach the ocean in this size are classified as primary microplastics, whereas those that originate from the breakdown of larger items are called secondary microplastics (Cole et al., 2011).

Once in the ocean, plastics can cause habitat degradation and physically impact marine biota by entanglement, asphyxiation and ingestion (Gall and Thompson, 2015; Kühn and van Franeker, 2020), which has been reported for over 1200 species (Santos et al., 2021) – including several that are commercially exploited (Neto et al., 2020). When ingested, plastics can cause lesions or physical obstruction of the digestive tract, fecalomas, and false sense of satiety (Kühn et al., 2015; Kumar et al., 2021); they can also transfer their associated toxic compounds to animals (Diepens and Koelmans, 2018), with potential biomagnification of such compounds to higher trophic levels (Teuten et al., 2009; Meyer-Rochowa et al., 2015). Entanglement in plastics can result in drowning, lesions and infection, as well as restrict movements and foraging ability of marine biota (Kühn et al., 2015; Kumar et al., 2021).

Plastics in the ocean also provide durable surfaces for the attachment of many species, and can therefore support complex ecological communities (Reisser et al., 2014; Kirstein et al., 2018; Amaral-Zettler et al., 2020). Mature communities may have a wide range of associated bacteria (Pinto et al., 2019), fungi (Lacerda et al., 2020), microalgae (Nava and Leoni, 2021), and several metazoan groups (Reisser et al., 2014; Kirstein et al., 2018; Amaral-Zettler et al., 2020). Floating plastics can transport organisms over large distances, with unknown consequences that could include species introductions and bioinvasions (Barnes, 2002; Barnes and Milner, 2005; Fazey and Ryan, 2016; Rech et al., 2016; Carlton et al., 2017). Biofilm-covered plastics can also appear and smell like food items, stimulating their ingestion by other organisms (Amaral-Zettler et al., 2015). Although the ecological impacts of plastic biofilms in the ocean are still unclear, it is known that plastic-associated communities can include primary producers, predators, symbionts and saprotrophic groups (Amaral-Zettler et al., 2020; Lacerda et al., 2020). The prokaryotic and eukaryotic groups that live in plastic biofilms can also include potential pathogenic (Zettler et al., 2013; Kirstein et al., 2016; Amaral-Zettler et al., 2020) or hydrocarbon-degrading organisms (Muthukumar et al., 2011; Paço et al., 2017; Delacuvellerie et al., 2019; Oberbeckmann and Labrenz, 2020; Zhang et al., 2021). Some pathogenic species are generally sparse in the open ocean, and their association with plastics raises the concern as to whether the increasing amount of plastics in this environment provides greater opportunities for pathogens to be transported and transmitted to hosts, leading to increased outbreaks of disease (Bowley et al., 2020). It has been suggested that the characteristics of plastics (e.g. polymer composition) influence the diversity of colonizing groups. However, studies have shown contradictory evidences: while some show significant difference in the species composition of distinct plastic polymers (Debroas et al., 2017; Bhagwat et al., 2021), others conclude that plastic composition does not determine its associated communities (Bryant et al., 2016; Dussud et al., 2018; Oberbeckmann and Labrenz, 2020; Oberbeckmann et al., 2021). Indeed, biogeography seems to be the most important factor driving the community composition on plastics in aquatic systems (Oberbeckmann and Labrenz, 2020).

In the Western South Atlantic, studies on floating plastics are scarce; this includes the Brazilian coastline, whose extensive length (~ 8000 km) hinders thorough monitoring of plastic pollution. In fact, studies on this subject in Brazil were for many years focused on beaches (Wetzel et al., 2004; Ivar do Sul and Costa, 2007; Portz et al., 2011; Carvalho and Baptista Neto, 2016; Ramos et al., 2021) and estuaries (Possatto et al., 2015; Krelling and Turra, 2019), and there is little information on plastics at the sea surface (Black et al., 2020; Videla and Araujo, 2021; Lins-Silva et al., 2021). Additionally, few of these studies have characterized the species inhabiting plastics at the region. To our knowledge, only two studies in the open ocean of the Brazilian coast has assessed the plastisphere through DNA analysis, one identifying the microbial communities of plastics experimentally deployed in the deep sea (Agostini et al., 2021), and the other focused on fungi from floating plastics (Lacerda et al., 2020); however, no studies have used a DNA multibarcoding approach to identify both prokaryotes and eukaryotes associated to plastics in surface waters of the Western South Atlantic so far.

Only recently the Brazilian Ministry of the Environment created the "National Plan to Combat Litter at Sea" (Ministério do Meio Ambiente, Brasil, 2019), which recommends the conduction of research on plastics at the Brazilian coast. In addition, this action is also suggested in one of the Sustainable Development Goals (SDG) established by the United Nations (SDG 14 - Life Below Water). Most plastisphere studies worldwide have targeted prokaryotes (Oberbeckmann and Labrenz, 2020; Wright et al., 2020a), with a very small number focusing on eukaryotes, especially those on plastics from the open ocean (Kirstein et al., 2018; Amaral-Zettler et al., 2020). Moreover, studies that have evaluated the eukaryotic communities that live on plastic debris sampled in the ocean clearly identify many taxa (Amaral-Zettler et al., 2020), which reinforces the need to understand the holobiome of plastics, and their ecological interactions in marine environments (Oberbeckmann et al., 2021). Therefore, the aim of our study was to evaluate the abundance and characteristics of plastics, including the diversity of their associated organisms, sampled at surface oceanic waters in the Brazilian coastline. We hypothesized that 1) the sea surface in South Brazil is highly polluted by plastics due to the input of both continental (e.g. mismanaged waste, direct disposal by tourism) and maritime sources (e.g. ship traffic, intense fishing activities); 2) there are numerous prokaryotic and eukaryotic groups living associated with plastics from coastal and oceanic regions along South Brazil, forming complex communities; and 3) the characteristics of plastics influence the community composition of their associated organisms at the region. Such information is pivotal for establishing strategies to prevent inputs and mitigate the impacts of plastics on marine ecosystems at local and global scales.

2. Material and methods

2.1. Sampling of plastics at the sea surface

Plastics were collected at the ocean-air interface in October 2016 and 2017 at ten stations along the continental shelf off the coast of South Brazil, with two inshore sampling locations (Supplementary material, Table 1), between latitudes 26° S and 34° S (Fig. 1A), as part of the TALUDE project. We highlight that the two inshore locations were near the Patos Lagoon Estuary, with a drainage basin of about 200,000 km^2 (Seeliger and Odebrecht, 2010), and the Itajaí-Açu river, with an estuarine area of 15,000 km^2 (http://www.jornalmetas.com.br/valedasaguas/orio/a-maior-bacia-hidrografica-de-sc). Both estuaries have several urban and industrial centres along their margins, with ports and intense fishing activities. At each station, trawls using a

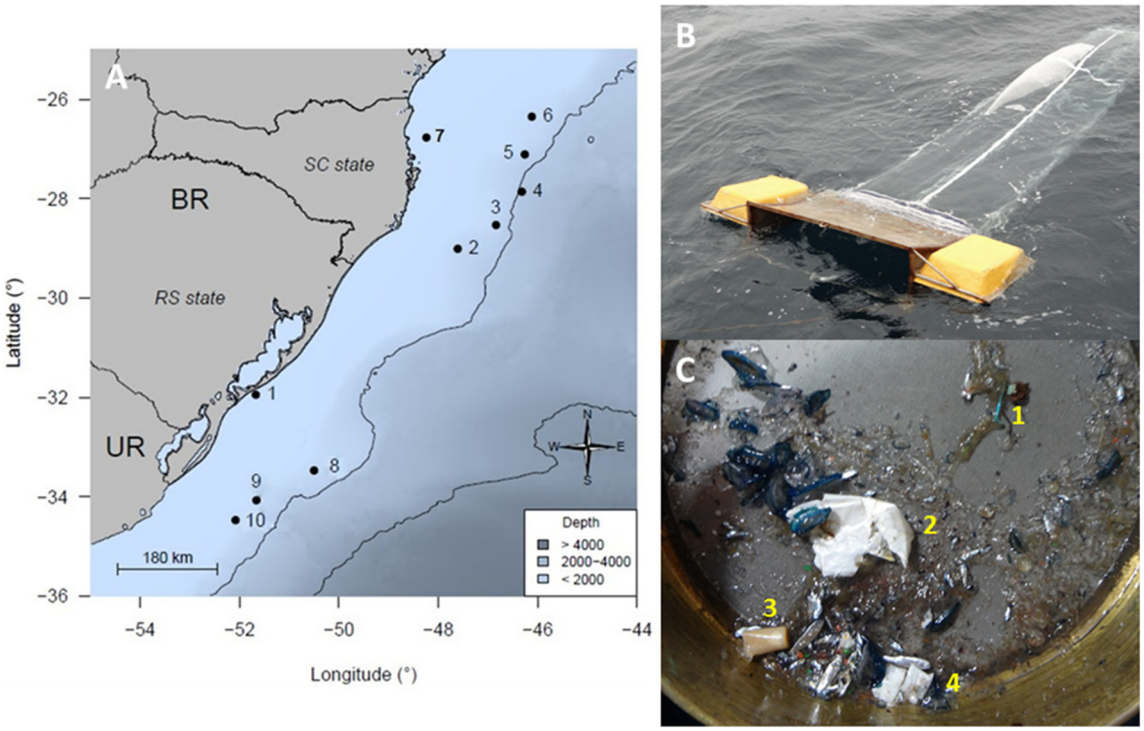

Fig. 1. A) Sampling stations of floating plastics along the South Brazilian coast; B) Manta net trawling at the ocean-air interface; C) Different types of plastics (1 - fishing line, 2 - chocolate wrapper, 3 - cigarette butt, and 4 - fragment) present in one sample.

Manta net (100 cm × 21 cm mouth, 330 μm mesh, Fig. 1B) were performed in triplicate for 11-17 min each, at a speed of 2.5–3.0 knots. After sampling, volume-reduced samples were collected in a sterile sieve with the same mesh size (Fig. 1C), and frozen in aluminium bags at -20 °C to preserve the DNA of plastic-associated biota.

At the start and end of each trawl, we noted the geographical coordinates and time. The trawled area was calculated based on trawl velocity (*trawl vel*, considering 1 knot = 0.514 m.s^{-1}), time (*t*, in seconds) and the Manta net width (1 m), using the equation:

$$Area = trawl\ vel * t * 1$$

To estimate the concentration of plastics at the sea surface, for each sampling point the number of items found in the trawled areas was extrapolated to items.km^{-2}, and the total average concentration was calculated. We used an Analysis of Variance (ANOVA) to check for differences in plastic concentrations in terms of categories and sampling regions (divided by states: sampling points 1, 8, 9, 10 in Rio Grande do Sul - RS; sampling points 2, 3, 4, 5, 6, 7 in Santa Catarina - SC).

2.2. Quanti-qualitative characterization of plastics

In the laboratory, samples were thawed separately and placed in a sterile container filled with artificial sterile salt water (salinity 35) for manual separation of floating plastic pieces and biomass (Reisser et al., 2013). A trained observer visually examined each sample with the naked eye for at least 2 h. Synthetic polymers were classified into paint particles and non-paint plastics (Song et al., 2014) (called only paints and plastics, respectively, from here on). All items were picked up using sterile forceps, and measured over their largest cross-section (total length) using a digital caliper (0.01-150 mm). According to their size, visible plastics ranging from ~0.5 mm up to 5.0 mm were classified as microplastics, and those with 5 mm to 200 mm as mesoplastics, adapted from Eriksen et al., 2014. We highlight that smaller particles could have been present, but cannot be detected by this evaluation method. Plastics were also classified according to their shape (fragment, foam, line, pellet and film) (GESAMP, 2019), polymer composition and malleability (hard or flexible), where items were considered flexible if they could be manually folded. The colours of paints and plastics were estimated following the 12 basic colour terms of the Inter-Society Colour Council, National Bureau of Standards/ISCC-NBS.

All plastic pieces were placed individually in a microcentrifuge tube with absolute ethanol (reagent grade, MERK) to preserve the genetic material until DNA extraction of plastic-associated organisms, and 32 were randomly chosen for genetic analyses. The polymer composition of 117 samples (31.53% of all particles) was determined through Fourier Transform Infrared Spectroscopy (FTIR) with a SHIMADZU spectrometer, model Prestige 21, using a diffuse reflectance module, 24 scans and 4 cm^{-1} resolution. Of the 32 samples destined for metabarcoding analysis, 21 were recovered from the PowerBead extraction tube after DNA extraction for polymer analysis. FTIR procedures and data analysis followed the standard practice ASTM E1252 (2013) (ASTM international). We did not perform any FTIR or metabarcoding analysis for paint particles.

2.3. Profiling of plastic-associated organisms

Plastic pieces were rinsed in sterile artificial seawater to remove loosely associated organisms (organisms that co-occurred with plastics during sampling) before DNA extraction. The total DNA of plastic biofilms was extracted using a PowerSoil DNA extraction kit (Qiagen) (shown to be efficient for this type of sample by Debeljak et al. (2017)), with some modifications from the manufacturers' instructions: in the first step we added 10 μl (1000 U/μl) of lysozyme (Debeljak et al., 2017), and in the last step the DNA was eluted in a lower buffer volume (20-30 μl) to increase DNA yield and concentration.

The quality and concentration of extracted DNA were checked by spectrophotometry using a Biodrop DUO (Harvard Bioscience™). We used primers 515f (5´-GTGYCAGCMGCCGCGGTAA-3´) and 806r (GGACTACNVGGGTWTCTAAT) to amplify the 16S V4 region (Walters et al., 2016); TAReuk454 (5´-CAGCASCYGCGGTAATTCC-3´) and TAReukRev3 (5´-ACTTTCGTTCTTGATYRA-3´) to amplify the 18S V4 region (Stoeck

et al., 2010); and 1391f (5´-GTACACACCGCCCGTC-3´) and EukB (5´-TGATCCTTCTGCAGGTTCACCTAC-3´) to amplify the 18S V9 region (Amaral-Zettler et al., 2009). PCR reactions and conditions for all molecular markers are detailed in Supplementary material (Table 2). The library preparation and sequencing on the Illumina Mi-seq platform followed the methods described in Lacerda et al. (2020).

2.4. Analysis of metabarcoding data

DNA sequences were analysed using a combination of USEARCH v7.0.1090 (32Bit) (Edgar, 2010) and QIIME v 1.8.0 (Caporaso et al., 2010). Forward and reverse reads were merged using USEARCH. Each primer set was separated using Cutadapt (Martin, 2011), removing primers and adapters. For each primer set, fastq files were quality filtered, removing reads with expected error > 0.5 and short sequences <200 bp. Reads were then truncated to 250 bp, 370 bp and 150 bp for 16S, 18S V4 and 18S V9, respectively, and converted to FASTA files. The FASTA files were dereplicated, abundance sorted and had their singleton sequences removed. OTUs (Operational Taxonomic Units) were clustered using the UPARSE clustering algorithm at 97% (Edgar, 2013). Chimeras were filtered using UCHIME (Edgar et al., 2011), OTUs were mapped back to the original reads, and an OTU table was produced. Both 16S and 18S sequences were classified against the SILVA 132 database (Quast et al., 2013) using UCLUST (Edgar, 2010). OTUs with less than four sequence reads per cluster were excluded from the downstream analyses. In addition, prokaryotic OTUs present in less than three samples were also excluded from the 16S dataset. Within the 16S dataset, contaminants such as Eukarya, mitochondria, and unknown domain were removed; within both 18S datasets some Eukarya sequences (Chloroplastida, some fungi species and large metazoans such as Salpida, Chelicerata, Chilopoda, and Eutelostomi) were also manually removed during analysis, since they likely represented contamination. Abundant and frequent OTUs, as well as those identified as being potentially pathogenic, hydrocarbon or plastic degraders were further classified against the full NCBI database.

2.5. Analyses of epiplastic communities

The prokaryotic and eukaryotic communities obtained with each molecular marker were analysed separately. To assess both alpha and beta diversity, OTU tables were rarefied to 1000 reads for 16S, 500 reads for 18S V4 and 700 reads for 18S V9. Differences in alpha and beta diversity (OTUs richness and community composition) among plastic categories (size, shape and polymer composition) and locations (SC and RS) were evaluated. Categories were considered as the following levels: size (fixed factor- microplastic/mesoplastic), shape (fixed factor- fragment/line) and polymer composition (fixed factor- polyamide/polyethylene/polypropylene/polystyrene/polyurethane). A Kruskal-Wallis test was performed to check for differences between OTU richness per sample between plastic categories and locations for each marker. The beta diversity was measured as the average distance from the individual plastic to the category's median, using the binary Jaccard index (Anderson et al., 2006).

The Jaccard dissimilarity matrix was used to produce nonmetric multidimensional scaling (NMDS) plots. Tests of multivariate homogeneity of group dispersions (PERMDISP) were implemented to ensure that differences in communities were not a within-group variation (Anderson, 2001). A Permutational Multivariate Analysis of Variance (PERMANOVA), with fixed factors and 9999 permutations was used to check if the community beta diversity differs among plastic categories. A significance level of $p < 0.05$ was considered, and all statistical analyses were done with the *vegan* package (Oksanen et al., 2019) in R studio 1.1.456 (R Development Core Team). The *ggplot2* package (Wickham, 2009) was used in R studio to build dot plots with number of OTUs (richness), NMDS plots, as well as balloon plots showing the frequency of occurrence of prokaryotes (phylum level) and eukaryotes (separated by groups across the tree of life) for each molecular marker.

3. Results

3.1. Plastic and paint concentration and types

We found a total of 371 likely plastic particles of different sizes, shapes, colours, malleability, and polymer compositions. The mean concentration of plastics at the sea surface along South Brazil was 4461 items.km^{-2}, varying from 2989 items.km^{-2} at the oceanic station 5 to 19,267 items.km^{-2} at the coastal station 1. The two highest plastic concentrations were at stations located in Rio Grande do Sul waters (stations 1 and 9, Fig. 2 - I). Microplastics (< 5 mm) represented 68% of the plastic items and were dominant in all the sampling stations, except at station 9; mesoplastics (5 - 200 mm) represented 32% of the dataset. There was significant difference (ANOVA; $F = 11.924$, $p = 0.014$) between the concentration of micro and mesoplastics at the Santa Catarina region (Fig. 2 - I).

The most common plastic shape was fragment (65%), followed by line (33%); other shapes (pellet, foam and film) represented less than 1% each of the total. While fragments were dominant among microplastics (80%), lines – the second most abundant plastic shape – dominated in the mesoplastic category (70%). Pellet, foam and film were all microplastics. Sampling stations had plastics of different shapes and sizes (Fig. 2 - I and III), but microplastic fragments prevailed in over half of the stations. There was significant difference in the concentration of lines between the RS and SC regions ($p = 0.036$). None of the stations had all plastic shapes, but station 8 had the highest variety (fragment, line, foam and film) (Fig. 2 - III).

In terms of malleability, most plastics were flexible (75%). The majority of hard plastics were fragments, apart from one pellet. White/transparent, yellow, blue, grey, orange, brown, black, green and red plastics were observed, with a dominance of white/transparent and blue items (44% and 32%, respectively). FTIR spectra of the 117 particles revealed that all were plastics, composed of polyamide (PA, 48%), polyurethane (PU, 21%) polyethylene (PE, 9%), polystyrene (PS, 9%), polypropylene (PP, 7%), polyethylene terephthalate (PET, 7%), cellulose acetate (CA, 3%), and ethylene-vinyl acetate (EVA, 1%) (Fig. 2 - IV).

We found 613 paint chips in our samples, which corresponded to almost twice the abundance of common plastics. The mean concentration of paint fragments along South Brazil was 16,442 items.km^{-2}, varying from 3243 items.km^{-2} at station 10 to 31,843 items.km^{-2} at station 2 (Fig. 2 - II). Paint chips displayed sizes ranging from 0.5 to 8 mm – with most particles falling in the micro size class – and were green, orange, yellow, white, red and blue.

3.2. Prokaryotic and eukaryotic diversity on plastics

After sequence processing and quality filtering, the 16S dataset contained 21 samples with 408,805 reads comprised into 444 prokaryotic OTUs. The 21 plastic samples had Bacteria associated with biofilms and 17 had Archaea OTUs. The Archaea group was composed by phyla Euryarchaeota and Thaumarchaeota, but none of the Archaea OTUs had four or more reads per sample, and thus were not included in further analyses.

The number of prokaryotic OTUs per sample varied from 30 to 288 OTUs (mean 109 ± 12.8 SD). Representatives from 20 phyla of Bacteria were found associated with plastics (Fig. 3), with dominance of Proteobacteria over the entire dataset (abundance of 64%, composed by Alpha, Delta and Gammaproteobacteria), followed by Bacteroidetes (14%), Cyanobacteria and Firmicutes (6% each). Six bacterial phyla presented abundances of between 1 and 3% (Verrucomicrobia, Planctomycetes, Epsilonbacteraeota, Actinobacteria, Thermotogae and Chloroflexi), and the remaining phyla together represented less than 2% of the total 16S dataset (Fig. 3 - I). The prokaryotic community of sampled plastics was highly similar in terms of OTU richness over the latitudinal gradient (26° S – 34° S).

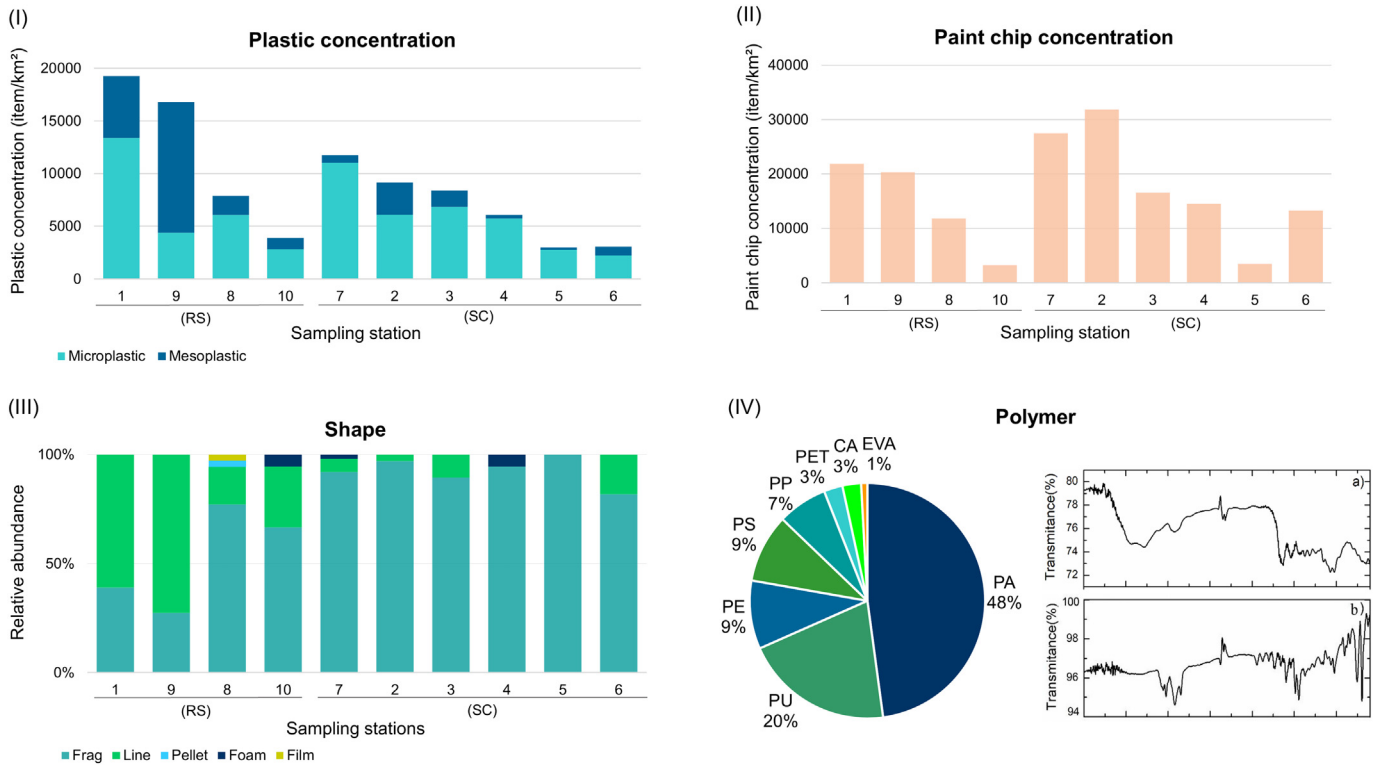

Fig. 2. (I) Plastic concentration (items/km^2) at the sea surface of ten sampling stations along the Southern Brazilian coast (Rio Grande do Sul – RS and Santa Catarina – SC states), categorized according to their size as microplastic (< 5 mm, light blue) and mesoplastic (5 - 200 mm, dark blue); (II) Paint chip concentration (items/km^2) at the sea surface of ten sampling stations along the Southern Brazilian coast (RS and SC states); (III) Relative abundance of plastic shapes (Frag = fragment, Line, Pellet, Foam and Film); (IV) Polymer composition of 117 sampled plastics: PA = Polyamide, PU = Polyurethane, PE = Polyethylene, PS = Polystyrene, PP = Polypropylene, PET = Polyethylene terephthalate, CA = Cellulose acetate, EVA = Ethylene-vinyl acetate (left panel), and examples of polymer FTIR spectra: a) degraded PA; b) degraded PS (right panel). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

The four most abundant bacterial groups also showed a high frequency of occurrence (Proteobacteria, Bacteroidetes, and Firmicutes FO 100%; Cyanobacteria FO 95%) (Fig. 3 - II). Within the Proteobacteria phylum, Gammaproteobacteria was the most abundant group (47% abundance, FO 100%), followed by Alphaproteobacteria (16% abundance, FO 100%) and Deltaproteobacteria (less than 1% abundance, FO 71%). Within Gammaproteobacteria, we found six *Oceanospirillales* OTUs, which had an abundance of less than 1% in the 16S dataset. The most abundant prokaryotic OTU, representing 23% of the dataset (OTU_1), was classified as uncultured bacteria (uncultured *Ralstonia*) by SILVA, and it also matched many uncultured bacteria from environmental samples (mostly sediments) on NCBI (Genbank MN723154.1) (Table 1).

Some bacteria OTUs in our samples closely matched potential pathogens such as *Vibrio parahaemolyticus* from shrimp (100%, Genbank

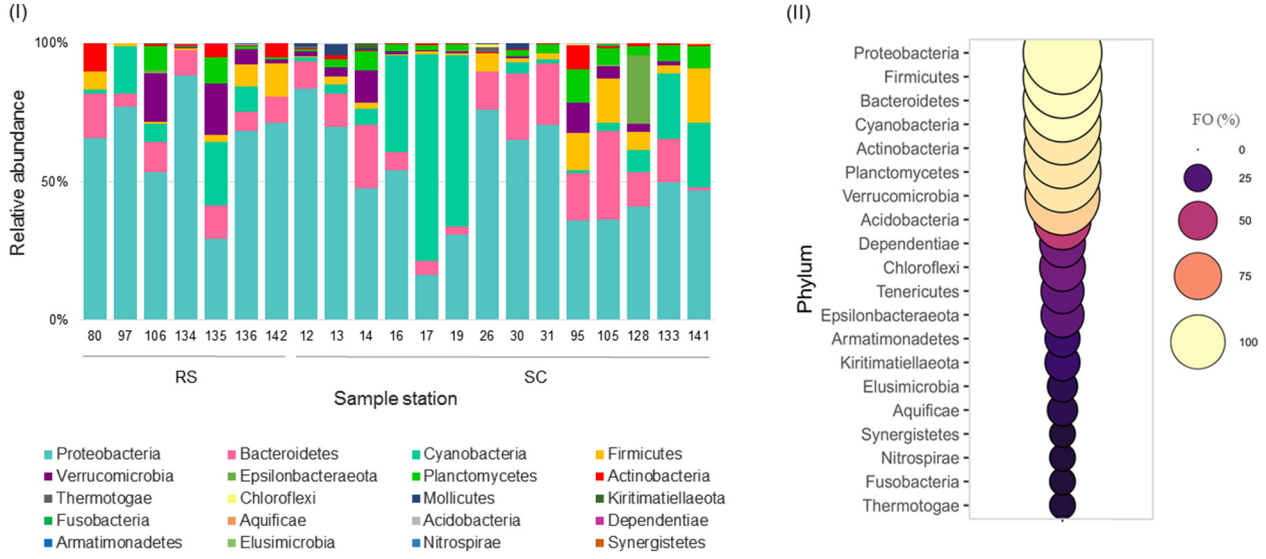

Fig. 3. I) Relative abundance and II) Frequency of occurrence (FO %) of prokaryotes associated with floating marine plastics from Southern Brazil (Rio Grande do Sul – RS and Santa Catarina – SC), identified through high-throughput amplicon sequencing of a partial fragment of the 16S rRNA gene.

reference MT549171.1), and *Escherichia coli* from wastewater (100%, Genbank reference CP055438.1), as well as many *Pseudomonas* species. We also found bacterial groups previously described as plastics and/or hydrocarbon degraders, such as polycyclic aromatic hydrocarbons (PAH), a common plastic-associated contaminant (Adams et al., 2007; Diepens and Koelmans, 2018): *Ralstonia* sp. (Biki et al., 2021; Ryan et al., 2007), *Erythrobacter* species (Gao et al., 2015), *Bacillus* species (Wright et al., 2020b), *Alcanivorax* and *Arenibacter* species (Delacuvellerie et al., 2019; Sekiguchi et al., 2011), *Pseudomonas* species (Balasubramanian et al., 2010), and *Colwellia* sp. (Urbanek et al., 2018).

The most abundant OTUs had closest matches to sequences from environmental sequence surveys in the full NCBI database, and some showed low matches to any cultured representative (e.g. OTU_12, Table 1). Most OTUs had matches to taxa observed in untreated water, as well as marine sediments and coastal waters, including species associated with marine organisms such as coral, fish, sponge, ascidian and shrimp (Table 1).

Two 18S markers were used to analyse the diversity of eukaryotic life on the sampled plastics. After sequence processing and quality filtering, the 18S V4 dataset contained 22 samples with 189,466 reads, whereas the 18S V9 dataset contained 28 samples with 876,534 reads, clustered into 337 and 655 OTUs, respectively. The number of OTUs per sample within the 18S V4 dataset ranged from 5 to 65 (average 31 $\pm$ 3.2 SD), and within the 18S V9 from 18 to 231 (average 68 $\pm$ 8.1 SD). The 18S V4 and V9 markers detected different taxonomic groups, with some exclusive groups in each dataset: Telonema and Ichthyosporea were shown only with the 18S V4 marker, whereas Bryozoa, Haptophyta, Excavata, Centrohelida, Picozoa, and Hemichordata (among others) were present only within the 18S V9 dataset (Fig. 4).

No eukaryotic group had FO 100% within the 18S V4 dataset: Chlorophyta, Charophyta and Cnidaria had FO 95% each, followed by Fungi with FO 91%, and Diatom, Dinoflagellata, and 'Other Stramenopiles' with FO 77% each. In addition, the groups Chrysophyta, Cercozoa, Ciliophora, Nematoda, Chaetognatha, Syndiniales, and 'Other Alveolata' displayed FO > 50% each. The remaining groups identified by this marker had FOs lower than 50% each (Fig. 4). The 18S V9 marker showed a different community composition in terms of taxa diversity. Some groups presented higher frequencies of occurrence: Fungi had FO 100%, followed by Chlorophyta with FO 96%, and Diatom, 'Other Stramenopiles' and Cnidaria with FO 93% each, while Cercozoa, Crustacea and Ciliophora had FO 86% each. Invertebrates such as Tunicata, Nemotoda, Chaetognatha, and Mollusca occurred in over 50% of samples within the 18S V9 dataset (Fig. 4).

The most frequent eukaryotic OTUs detected by both molecular markers also matched many uncultured eukaryotic OTUs from the marine environment (seawater and sediments), as well as with cultured species from coastal zones (e.g. coastal water, intertidal pools), and symbionts with marine sponge and radiolarian (Table 2). Some harmful eukaryotes were found in the plastisphere from the Western South Atlantic, such as dinoflagellates of genus *Alexandrium*, whose species are known for being toxic to marine life and humans (Donald et al., 2012). Fungi groups found in our samples were composed of some parasites/pathogens, as well as hydrocarbon degraders – these groups are detailed in Lacerda et al. (2020).

Richness of OTUs per plastic fragment was variable between individual plastic items, and some samples had low richness. There was no significant difference in the OTU richness of plastics of distinct categories (Fig. 5), as well as according to location (Kruskal-Wallis, $p > 0.05$), except within the 16S dataset where we found significant difference in bacterial OTUs richness (Kruskal-Wallis, $p = 0.006$) between RS and SC. Likewise, the community composition was highly variable between plastic items within each DNA marker dataset (Fig. 6). There was significant difference in community composition according to size class within the 18S V9 dataset (PERMANOVA: $F = 1.276$, $p = 0.04$), and according to location in both the 18S V4 (PERMANOVA: $F = 1.988$, $p < 0.001$) and V9 (PERMANOVA: $F = 1.495$, $p = 0.009$) datasets.

4. Discussion

4.1. Concentration, types and potential sources of plastics and paint particles

Studies conducted across the globe have revealed numerous types of plastics floating in the ocean (Cózar et al., 2014; Eriksen et al., 2014; Cózar et al., 2017; Avio et al., 2017), with a diversity of associated fauna (Reisser et al., 2014; Goldstein et al., 2014; Carlton et al., 2017). However, the lack of studies conducted in some regions hinders the mitigation of this problem, as it is hard to remediate what is not known. Here we present the first description of the concentration and characteristics – including associated prokaryotic and eukaryotic communities detected through DNA metabarcoding – of floating plastics in the open ocean of South Brazil.

We found plastics in a variety of sizes, shapes, colours, malleability and polymer compositions, suggesting that they were used in different applications (Rochman et al., 2019), and could be both continental and ocean-originated (Pan et al., 2019). The dominance of microplastics, which is in accordance with what has been reported in studies of floating plastics worldwide in all ocean basins (Cózar et al., 2014; Eriksen et al., 2014), could be explained by the higher numerical abundance of this size class due to the breakdown of larger plastics in the marine environment (Browne et al., 2007; Andrady, 2011). Due to the limitation of the method we used for plastic identification (naked eye inspection of samples), we did not classify each item specifically into primary/secondary microplastic categories. However, we can affirm that most items were secondary microplastics based on their physical features. This predominance of secondary over primary microplastics suggests that the breakdown of plastics is likely occurring due to physical, chemical and biological mechanisms such as solar U.V. radiation, abrasion through wave action, and biofouling (Andrady, 2011; Cole et al., 2011).

Table 1
Ten most abundant prokaryotic OTUs identified through 16S amplicon libraries from the marine plastisphere of the Western South Atlantic.

OTU number	R.A.	FO%	Environmental sample			Cultured			
			Source	ID%	Genbank accession	Species	ID%	Genbank accession	Source/highlights
OTU_1	23%	81	Freshwater	100	MN072796.1	*Ralstonia* sp.	100	MN723154.1	Soil
OTU_5	6%	67	*Soil*	100	MT318452.1	*Acinetobacter lwoffii*	100	MT323129.1	*Rainbow trout* (fish)
OTU_6	5%	90	Marine sponge	100	MT464708.1	*Synechococcus* sp.	100	KU867931.1	Coastal water
OTU_2	5%	76	Water (marsh)	100	AY652491.1	*Bacteroides* sp.	99	JQ317253.1	Cat fish
OTU_12	3%	24	*Porites compressa* (Coral)	90	FJ930300.1	*Alkalibacter saccharofermentans*	85	NR042834.1	Soda Lake
OTU_32	2%	81	Polluted seawater	100	MW559885.1	*Vibrio parahaemolyticus*	100	MW829316.1	Shrimp and marine fish
OTU_9	2%	43	Floodplain lake water	100	MF439455.1	*Alkalibacterium* sp.	100	MH044645.1	*B. schlosseri* (ascidian)
OTU_22	2%	62	Marine water	99	KX935277.1	*Salinimonas* sp.	100	CP064795.1	Marine sediment
OTU_34	2%	48	Surface sewater (China)	100	KC002482.1	*Afipia* sp.	100	EF371496.1	Yellow Sea
OTU_21	2%	52	Coastal sewater	100	LC496437.1	*Loktanella* sp.	100	LR722710.1	Coastal water

Notes: OTU number; Relative Abundance (R.A.); Frequency of occurrence (FO%); Environmental Samples (Source of uncultured bacterium) and Cultured samples (Species); Similarity (ID %); Genbank accession number; and Source/Highlights.

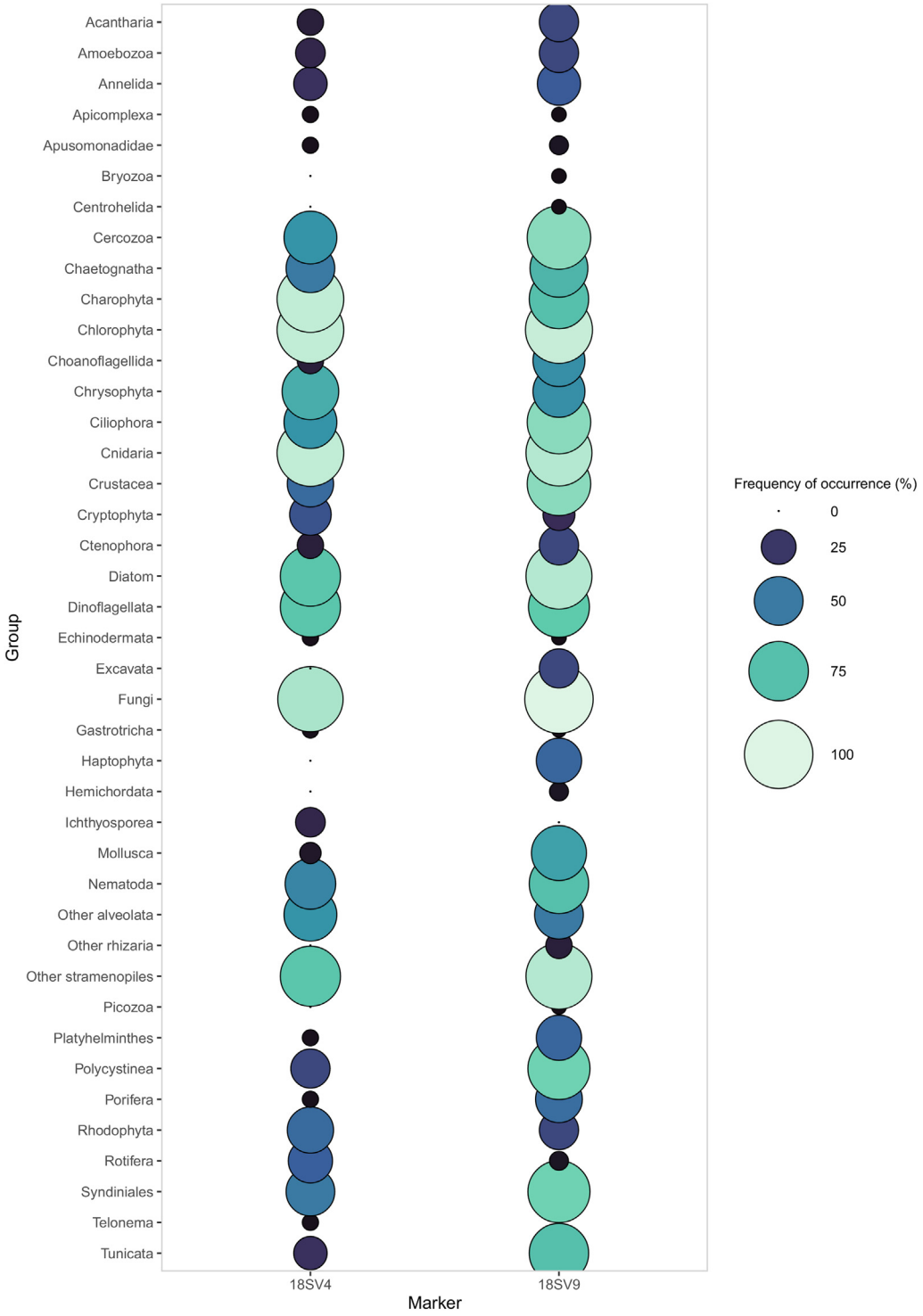

Fig. 4. Frequency of occurrence (%) of eukaryotic groups associated with floating plastics from Southern Brazil, identified through high-throughput amplicon sequencing of partial fragments of 18S rRNA gene regions V4 and V9.

The mean concentration of floating plastics that we estimated for the Southern Brazil (4461 item.km^{-2}) is within the estimated density range of plastic pollution in the global ocean basins (from 1000 to 100,000 items.km^{-2}) (Eriksen et al., 2014). This concentration is much lower than the mean concentration of floating plastics in accumulation zones such as the North Pacific subtropical gyre (> 700,000 items.km^{-2}), as well as in the Mediterranean (> 800,000 items.km^{-2}) (Eriksen et al., 2014), and Portuguese coasts (40,822.58 item.km^{-2}) (Rodrigues et al., 2020), but it is around two times higher than what was reported for the Southern Ocean (Lacerda et al., 2019). However, we highlight that it is difficult to compare with other studies, since a variety of sampling and analysis methods are used when studying plastics in the ocean. In addition, the number of replicates from each site could also be a limiting factor in such comparisons.

A recent study evaluated microplastics in northeastern Brazil, and revealed polypropylene, polyethylene and nylon items contaminating estuarine, coastal and shelf waters (Lins-Silva et al., 2021). These authors report that the concentrations of microplastics were highest in

Table 2
Ten most frequent eukaryotic OTUs identified through 18S amplicon libraries (18S V4 and V9 regions) from the marine plastisphere of the Western South Atlantic.

OTU number	Group	FO%	Environmental sample			Cultured			
			Source	ID%	Genbank accession	Species	ID%	Genbank accession	Source/highlights
18S V4									
OTU_19	Cnidaria	64	Seawater	100	AY665134.1	*Muggieae atlantica*	100	AY937337.1	Seawater
OTU_41	Chlorophyta	55	Freshwater	100	HQ191320.1	*Ankyra judayi*	99	U73469.1	Culture (SAG 17.84)
OTU_5	Cnidaria	55	Seawater	99	KJ762819.1	*Aeginopsis laurentii*	100	KY007604.1	–
OTU_335	Chlorophyta	45	Intertidal sediment	99	EF100243.1	*Collinsiella tuberculata*	95	AY198125.1	Intertidal pools
OTU_181	Fungi	45	Seafloor	100	KR072832.1	*Aspergillus restrictus*	100	EU723495.1	Deep sea
OTU_25	Dinoflagellata	45	Radiolarian (symbiont)	100	U52353.1	*Brandtodinium nutricula*	100	MG905637.1	Radiolarian (symbiont)
OTU_40	Dinoflagellata	45	Tidal estuary	100	DQ386760.1	*Karlodinium veneficum*	94	KY979983.1	Coastal zone
OTU_77	Chrysophyta	45	Marine environment	98	EF527168.1	*Paraphysomonas* sp.	100	JQ967321.1	Drainage ditch
OTU_28	Chlorophyta	41	Seawater (Ross Sea)	100	KJ758236.1	*Thalassiosira* sp.	100	MW722949.1	–
OTU_2	Rhodophyta	41	Seawater	98	AJ626846.1	*Protomonostroma undulatum*	99	DQ821517.1	Shaw Island
18S V9									
OTU_19	Fungi	93	Marine sediment	100	GU474197.1	*Aspergillus wentii*	100	AB002063.1	Dried fish
OTU_3	Cercozoa	75	Seawater	100	KF130578.1	*Sphaeronectes haddocki*	100	KX421854.1	Monterey Bay
OTU_21	Cnidaria	71	Seawater	100	KF129695.1	*Nanomia bijuga*	100	AY937324.1	–
OTU_7	Ciliophora	64	Gulf Stream 15 m depth	86	KJ759360.1	*Tintinnopsis sp.*	82	JX178854.1	Coast (China)
OTU_24	Cnidaria	61	Seawater	100	HM799922.1	*Liriope tetraphylla*	100	KT722405.1	Coast (Brazil)
OTU_97	Fungi	61	Savanna soil	99	EU490070.1	*Cladosporium halotolerans*	100	MN859971.1	Marine sponge
OTU_18	Chaetognatha	57	–	–	–	*Sagitta enflata*	99	LC581989.1	Seawater (Japan)
OTU_489	Fungi	57	Antarctica snow	99	KR131435.1	*Aspergillus penicillioides*	99	AF548066	Air
OTU_44	Fungi	57	Seawater	100	JF826393.1	*Wallemia mellicola*	100	AY741380.1	Hypersaline water
OTU_33	Dinoflagellata	57	Radiolarian (symbiont)	100	U52353.1	*Brandtodinium nutricula*	99	U52357.1	Sargasso Sea

Notes: OTU number; Taxonomy identified by SILVA database (Group); Environmental Samples (Source) and Cultured samples (Species); Frequency of occurrence (FO%); Similarity (ID%), Genbank accession number; and Source/Highlights.

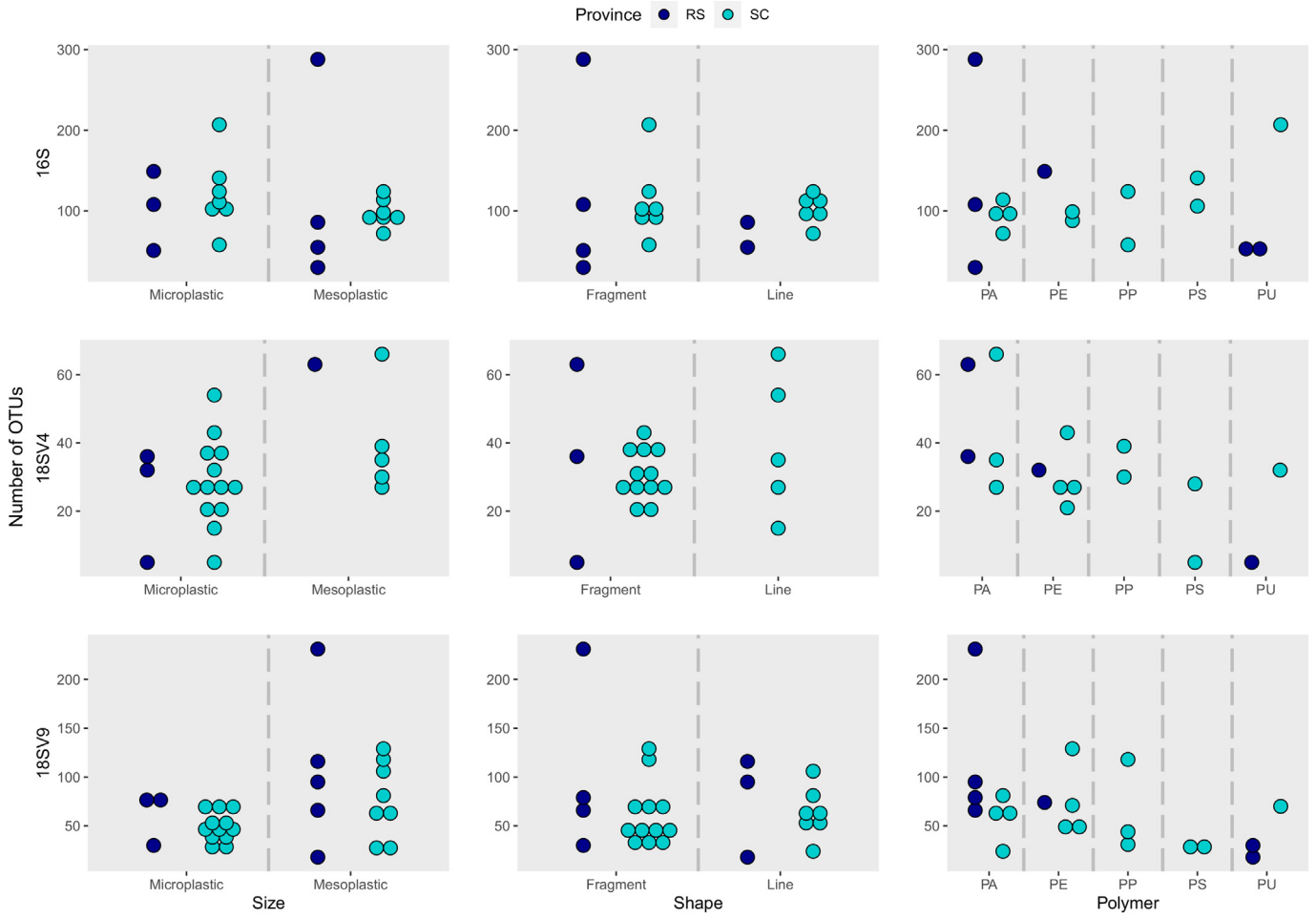

Fig. 5. Mean number of OTUs per plastic sampled in Southern Brazil, according to plastic categories (size, shape and polymer composition) by location (RS – Rio Grande do Sul, SC – Santa Catarina), obtained from rarefied 16S (1000 sequence/sample), 18S V4 (500 sequences/sample) and 18S V9 (700 sequences/sample) amplicon sequence libraries. Size: Microplastic and Mesoplastic; Shape: Fragment and Line; Polymer composition: PA (Polyamide), PE (Polyethylene), PP (Polypropylene), PS (Polystyrene), and PU (Polyurethane).

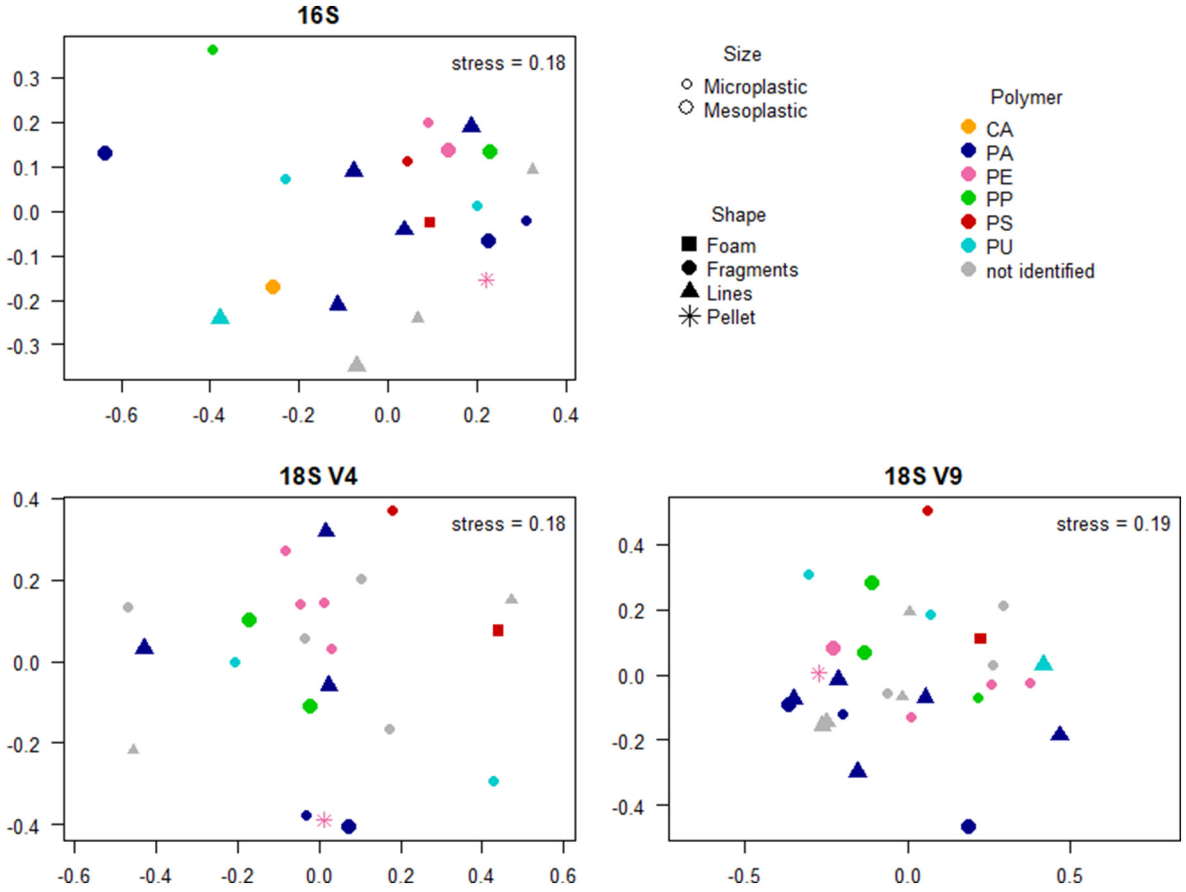

Fig. 6. Non-metric multidimensional scaling (NMDS) plots based on Jaccard distance matrix of prokaryotic and eukaryotic groups living associated with floating marine plastics in Southern Brazil, according to plastic size (micro and mesoplastic), shape (Foam, Fragments, Lines, Pellet), and polymer composition (CA: cellulose acetate, PA: polyamide, PE: polyethylene, PP: polypropylene, PS: polystyrene, PU: polyurethane).

the estuarine samples and decreased oceanward, likely due to a higher input of plastics from continental sources. We found high concentrations of plastics at stations close to the mouths of the Patos Lagoon (station 1 - highest concentration) and Itajaí-Açu (station 7 – third-highest concentration) estuaries, likely due to this large contribution of continental sources of plastic waste. In fact, it has been suggested that most plastic litter entering the oceans is the result of inadequate waste management on land (UNEP, 2016), and rivers alone have been estimated to carry millions of tonnes of plastic to the oceans every year (Lebreton et al., 2017). The Patos Lagoon and Itajaí-Açu estuaries are located near several urban and industrial development areas, with ports in their estuarine portions. Considering that waste management in Brazil is highly inefficient, with some regions lacking adequate waste collection, basic sanitation, landfill sites and recycling facilities, with recycling rates being generally low throughout the country (Ministério do Meio Ambiente, 2019; Oliveira and Turra, 2015), we suggest that these drainage basins are important carriers of plastics, and waste in general, to the adjacent marine area.

Monofilament fishing lines were common in our samples and were the dominant plastic type at sampling station 9, which had the second highest concentration of plastics. At this station, more than 70% of plastics consisted of *meso*-sized polyamide lines. As we mentioned previously, the manual sorting evaluation method could have limited the identification of smaller particles from all locations. Additionally, when smaller particles are biofouled or aggregate with larger and denser particles, they can sink to deeper waters (Peeken et al., 2018) that are not sampled with surface nets. However, considering that we used the same method to analyse all sampling locations, it is also possible that the dominance of meso over microplastics at this specific station – mostly made of polyamide, the main material used in fishing nets – is indeed due to the proximity of the source, since there are intense fishing activities close to station 9. This station is located at an area with an intense operation of gill, trawl and seine net fisheries by the Southern Brazil fishing fleet (FURG/MPA, 2018). In their evaluation of microplastics off Northeast Brazil, Lins-Silva et al. (2021) also found higher concentrations of nylon fibres in samples from the continental shelf than those from coastal/estuarine area. In this manner, it is important to act locally with fishermen to create effective mitigation actions.

Characteristics such as size, colour, and biofilm formation may also indicate the time that plastics have spent in aquatic/marine environments (GESAMP, 2019; Martí et al., 2020; Tu et al., 2020). As noted above, secondary microplastics predominated in our samples, indicating the breakdown of larger items; there was a dominance of white/light-coloured plastics, which could indicate discoloration over time due to weathering (Andrady, 2016). Additionally, FTIR spectra showed that some plastics presented alterations in their primary characteristics, also indicating that these particles had likely been subject to weathering: the presence or absence of peaks and bands typically assigned to oxygenated groups (-OH, -C=O, C-O-C) indicates different levels of degradation (Jin et al., 2006). Additionally, the presence of biofilms containing a diversity of groups (from microorganisms to invertebrates) corroborates that plastics were in the ocean for some time, allowing the establishment of a well-developed epiplastic community.

We found a high concentration of paint fragments in our samples, but since the focus of this paper was on floating plastics we did not include these paint chips into the estimates of floating plastics. There is still no consensus in the scientific community as to whether paint particles should be included in such estimates in the ocean, as they are denser than seawater and are expected to sink, but can be retained at the surface due to seawater surface tension (Song et al., 2014). In

addition, some of the paint fragments could have been a result of sampling. Green, orange and white paint chips were possibly originated from our research vessel (deck and hull), as the ship is painted in these colours; additionally, yellow paint particles could be from the yellow floaters of the manta net. However, red and blue paint fragments were not present on any external structure of our ship or net, and we therefore infer that these particles were already at the ocean surface, originating from other continental or maritime sources.

Although still poorly understood, marine pollution by plastics in Southern Brazil has been reported to impact marine mammals (Secchi and Zarzur, 1999) birds and sea turtles (Bugoni et al., 2001; Tourinho et al., 2010; Rizzi et al., 2019), as well as commercially exploited seafood such as fish, shrimp and crabs (Vaske et al., 2009; Dantas et al., 2019; Neto et al., 2020; authors' observation). The ingestion of plastics has been described as an evolutionary trap (Santos et al., 2021), and we highlight that such ingestion can lead to ecological as well as economic impacts at the region, especially if it affects fishery resources.

4.2. Epiplastic communities in the Western South Atlantic: diversity and ecological impacts

To date, relatively few studies have used molecular approaches to analyse both the diversity of plastic-associated prokaryotes or eukaryotes in environmental samples from the open ocean (Zettler et al., 2013; Bryant et al., 2016; Debroas et al., 2017; Dussud et al., 2018; Amaral-Zettler et al., 2020), and none have been performed in surface waters of the Western South Atlantic. Proteobacteria, Cyanobacteria and Bacteroidetes were among the most dominant bacterial taxa associated with plastics in this study. This was also observed for open waters of the North Atlantic (Zettler et al., 2013) and North Pacific oceans (Bryant et al., 2016), the Mediterranean coast (Davidov et al., 2020), and the Chinese coast (Jiang et al., 2018). These groups were also common in a global analysis of Bacteria in the plastisphere (Wright et al., 2020a). The dominant eukaryotic groups we found were Diatoms, Cilliates, Dinoflagellates, Radiolarian and Bryozoans, which are also among the most representative groups living associated with floating plastics in the North Atlantic (Zettler et al., 2013; Debroas et al., 2017) and North Pacific (Bryant et al., 2016) oceans. Our previous study on Fungi showed that this group is also highly frequent in plastics from the South Atlantic and Southern oceans (Lacerda et al., 2020).

There is still a lack of knowledge about "specific" organisms living on different plastics in aquatic environments, and although some studies – mostly those evaluating colonization – have reported that plastic types may drive the community composition of their associated biota (Zettler et al., 2013; Kirstein et al., 2019; Hansen et al., 2021), we did not observe this in samples from the natural environment. As previously described for epiplastic fungi in the Western South Atlantic (Lacerda et al., 2020), there was no difference between OTU richness or community composition among plastic polymers within prokaryotic and general eukaryotic datasets at the region. This finding supports the suggestions of Oberbeckmann and Labrenz (2020) and Oberbeckmann et al. (2021) that microorganisms opportunistically colonize different plastic substrates, and are not specific to plastic polymers. However, significant differences in community composition according to size class was observed within the 18S V9 dataset, which we believe could be due to the higher availability of physical space on mesoplastics for the settlement of larger-sized taxa.

The community composition found with the 18S markers was also significantly different according to location (RS versus SC). The two regions have similar environmental conditions, but some slight differences exist in temperature and rainfall (www.worlddata.info/america), as well as in productivity (Boletim Estatístico da Pesca e Aquicultura, 2011), which could be driving community differences as previously observed for microbial communities living on plastics (Amaral-Zettler et al., 2015, 2020; Basili et al., 2020). For instance, both prokaryotic and eukaryotic communities from the plastisphere in the North Sea varied among geographic regions (between 51°31.497 N and 53°31.918 N) (Oberbeckmann et al., 2016), and likewise the bacterial community composition significantly varied between the Pacific and Atlantic ocean basis (Amaral-Zettler et al., 2015). However, considering that RS and SC regions present quite similar environments, we believe the difference we found could be an artifact of uneven sample sizes between locations (4 for RS and 18 for SC for 18S V4, and 8 for RS and 20 for SC for 18S V9, respectively). We highlight that the differences in biofilm composition among geographic regions, as well as between experimental and environmental samples, should be considered limiting factors when comparing data.

It has been well documented that potentially pathogenic *Vibrio* species are associated with plastics (Zettler et al., 2013; Kirstein et al., 2016; Kesy et al., 2021), and we found a diversity and abundance of *Vibrio* OTUs. We also found OTUs in our samples that matched sequences of other potentially harmful taxa (e.g. *Alexandrium tamarense*), but it is important to highlight that the occurrence of *Vibrio* species and other harmful organisms on plastic biofilms does not confirm their pathogenicity (Amaral-Zettler et al., 2020; Oberbeckmann and Labrenz, 2020). The threat they pose to human and animal life is still not well known, but once ingested, plastic biofilms could potentially lead to the transmission of harmful organisms that can cause diseases to animals from low to high trophic levels, since these materials (mainly microplastics) are easily ingested over entire marine food webs (Setälä et al., 2014).

The ingestion of plastics by different marine species, including seafood, has been recorded worldwide. In fact, eight species of commercially exploited fish from South Brazil (Neto et al., 2020) have been shown to ingest plastics, and the authors suggest that biofilm may favour ingestion by increasing detectability/attractiveness of plastics. The interaction between plastic-associated pollutants and plastic biofilms has not been described, but it is known that pollutants could be biomagnified along marine trophic webs (Carbery et al., 2018). Since many groups of primary producers live on plastic surfaces, toxic compounds could bioaccumulate from the bottom of the food web, posing serious risk to marine biota of different trophic levels, including humans that consume seafood. It has been shown that plastics can host potentially pathogenic organisms (Zettler et al., 2013; Keswani et al., 2016; Kirstein et al., 2016; Bowley et al., 2020), but studies that evaluate the functional genes associated with pathogenicity have shown contrasting results as to if they pose (Bhagwat et al., 2021) or not (Oberbeckmann et al., 2021) a relevant risk to human health. Additional studies are needed to clarify possible risks of the plastisphere from different types of plastics, as well as from different geographic regions and the open ocean.

Marine plastics can also host some fungal and bacterial groups known to biodegrade these materials (Shah et al., 2008; Sangeetha et al., 2015; Paço et al., 2017; Urbanek et al., 2018; Lacerda et al., 2020; Oberbeckmann and Labrenz, 2020). It has been suggested that the Sphingomonadaceae (Proteobacteria) family is one of the most important microplastic-associated group, due to their ability to degrade hydrocarbons and form carotenoids, which protect bacterial cells from oxidative stress caused by U.V. light at the sea surface (Oberbeckmann and Labrenz, 2020). We found many OTUs of the Sphingomonadaceae family in the biofilm of plastics from the Western South Atlantic, including *Erythrobacter* sp. that is known for its ability to utilize polycyclic aromatic hydrocarbons (PAH) (Gao et al., 2015), and we therefore reinforce that these groups should be further investigated to evaluate their ability to degrade plastics and their pollutants in the open ocean (Oberbeckmann and Labrenz, 2020).

The transport of species that live attached on artificial substrates such as plastics is an important issue that should be deeply investigated, as it can lead to changes in the structure and functioning of natural communities (Sridharan et al., 2021b), once invasive species become well established (Póvoa et al., 2021). Mantelatto et al. (2020) indicated that transport by rafting over long distances on marine litter (including plastics) may be a mechanism of range expansion and secondary introduction

of two invasive species of corals, *Tubastraea coccinea* and *T. tagusensis*, in Southern Brazil. Based on the high diversity of groups within the plastisphere from the Western South Atlantic, which present different characteristics throughout their different life stages (e.g. free living adults, but with sessile spores, larvae or eggs), it is possible that at least some of these groups could be successful in colonizing new environments with favourable conditions.

Although we did not perform metabarcoding on paint chips, it was recently shown that their biofilm communities seem to be distinct from common microplastic biofilms (polypropylene, polyamide and polyvinyl chloride) in brackish systems, but it is not yet known what drives these differences (Tagg et al., 2019). Since ship coatings can be a prominent, even underestimated, source of microplastic pollution in some marine environments (European Commission DG, 2021), further work is needed to evaluate the ecology and composition of biofilms on paint chips in the ocean.

4.3. Final remarks

In our work, the highest concentrations of floating plastics were observed at stations close to the coast, near large drainage basins. This indicates that continental sources are important contributors of plastics, and to better understand the transport of plastics between land and sea, we suggest monitoring and quantification of plastic pollution in the Patos Lagoon and Itajaí-Açu estuaries should be conducted, aiding in the search for effective solutions for this problem. Additionally, high concentrations were found within a high-use fishery area, where polyamide (nylon) meso-sized lines were predominant, showing that fisheries are also an important source of plastics at the region. In this manner, we suggest that governments and NGOs encourage the implementation of sensibilization activities with fishers, tax incentives (e.g. tax discounts) and/or environmental certifications for returning gear, as well as incentives to properly dispose fishing gears on land.

The diversity of epiplastic communities, as well as their impacts, should be further characterized via in situ studies conducted at different regions and ocean compartments. Our study further highlights the utility of molecular techniques in revealing the biodiversity associated with plastics, and shows that a multi-marker approach is extremely important to detect different groups of eukaryotic organisms, and consequently better depict the plastisphere. Considering that the V9 region of the 18S marker allowed the identification of a higher diversity of eukaryotic taxa, and showed greater amplification success, we suggest that this molecular marker should be used in future studies on the diversity of plastic-associated eukaryotic organisms. However, the larger read length of the 18S V4 marker could provide a greater scope for more detailed phylogenetic analysis. Omics approaches to identify the function of genes present in the plastisphere should also be applied to investigate factors such as pathogenicity and biodegradation potential of plastic-associated organisms from environmental samples.

In summary, our study provides novel and fundamental regional information on plastic concentrations and their associated communities, which further integrates the current knowledge of global plastic pollution and the global plastisphere. This will aid our efforts to act towards prevention and mitigation strategies for plastic pollution, and to understand the broader ecology and impacts of the plastisphere.

CRediT authorship contribution statement

A.L.d.F.L. and M.C.P. conceived the research on plastics. A.L.d.F.L. conducted sampling. E.R.S. obtained funding for surveys and conceived the oceanographic survey design. A.L.d.F.L., J.D.T. and F.K. conceived labwork. A.L.d.F.L., J.D.T., and L.d.S.R. analysed the data. A.L.d.F.L. wrote the first draft of the paper, and all authors contributed to discussing and editing the manuscript.

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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2021.150186.

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