UNIVERSIDADE FEDERAL DO RIO GRANDE- FURG

PROGRAMA DE PÓS-GRADUAÇÃO EM OCEANOGRAFIA BIOLÓGICA

PLÁSTICOS NA SUPERFÍCIE OCEÂNICA DO SUL DO BRASIL E ANTÁRTICA: CONCENTRAÇÕES, CARACTERÍSTICAS E COMUNIDADES EPIPLÁSTICAS

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Tese apresentada ao Programa de Pós-Graduação em Oceanografia Biológica da Universidade Federal do Rio Grande-FURG, como requisito parcial à obtenção do título de Doutor em Oceanografia Biológica.

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"O essencial na educação não é a doutrina ensinada, é o despertar".
Ernest Renan

Dedico esta tese a todos os cientistas do Brasil, resistentes em desenvolver suas pesquisas no atual cenário político do país.

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ESTRUTURA DA TESE

A presente tese está estruturada em duas partes: 1) parte introdutória escrita em língua portuguesa composta por resumo, palavras-chave, introdução, hipóteses, objetivos, material e métodos, síntese dos resultados e conclusões; 2) três anexos escritos em língua inglesa que correspondem aos artigos oriundos da tese, intitulados:

1. “Floating plastics at the sea sruface in Southern Brazil: concentration, carachteritcs and inhabitants” (artigo a ser submetido ao periódico *Environmental Pollution*)

2. “Diverse groups of Fungi associated with plastics in surface waters of the Western South Atlantic and the Antarctic Peninsula” (artigo submetido ao periódico *Molecular Ecology*)

3. “Plastics in sea suface waters around the Antarctic Peninsula” (artigo publicado no periódico *Scientific Reports*).

RESUMO

Visto o rápido e crescente acúmulo de plásticos nos oceanos e seus impactos negativos aos ecossistemas marinhos, torna-se fundamental ampliar o entendimento sobre este tema para propor soluções. Este estudo visou quantificar, caracterizar e avaliar as fontes de plásticos em águas marinhas superficiais da costa Sul do Brasil e da Antártica, bem como descrever as comunidades que esses materiais abrigam (comunidades epiplásticas, também conhecidas como plastisfera), através de microscopia eletrônica de varredura e um perfil *multibarcoding* (genes 16S, regiões V4 e V9 do 18S e ITS2). Plásticos foram amostrados no ambiente oceânico na interface oceano-ar, utilizando rede manta (malha de 330 µm), em dez pontos na costa Sul do Brasil e em doze pontos no entorno da Península Antártica. Foram encontrados plásticos de diferentes tamanhos (micro e mesoplásticos), formatos (fragmento, linha, esfera, filme e espuma), cores, maleabilidade (rígido e flexível) e composição polimérica. Para ambas as localidades, houve dominância de microplásticos (< 5mm), com o formato "fragmento" sendo a categoria mais abundante, seguido de "linha". Itens flexíveis foram mais abundantes e os plásticos amostrados neste estudo apresentaram nove cores (branco, azul, amarelo, verde, cinza, laranja, vermelho, preto, marrom), com maior ocorrência de plásticos de cor branca. Análises de espectroscopia revelaram que polietileno, poliamida, poliuretano e polipropileno foram os polímeros mais comuns, com alguns apresentando degradação e outros não. No Brasil, as maiores concentrações de plástico foram em estações próximas à costa, na desembocadura de estuários (Rio Itajaí-Açu e Lagoa dos Patos), bem como em uma estação oceânica localizada próxima a uma área de intensa atividade pesqueira, indicando que rios/lagunas e a pesca são importantes fontes de plástico para a região. Na Antártica, o modelo de dispersão de partículas mostrou que, por pelo menos sete anos (tempo considerado no modelo), os plásticos foram oriundos de latitudes acima de 58ºS. Isto pode indicar que atividades locais (como atividades de turismo, pesquisa e pesca) são as principais fontes de plásticos na região. A diversidade de organismos presentes no biofilme dos plásticos mostrou diferentes grupos de procariotos e eucariotos, incluindo microorganismos (como bactérias e fungos) previamente descritos como patógenos e biodegradadores de plásticos. Esta foi a primeira descrição de fungos de plastisfera no Hemisfério Sul e torna-se necessário melhor investigar os impactos desse grupo, com foco em suas funções ecológicas.

Palavras-chave: plásticos, poluição marinha, *metabarcoding*, plastisfera

ABSTRACT

Given the rapid and increasing accumulation of plastics in the oceans and their negative impacts on marine ecosystems, it is essential to increase understanding on this topic in order to propose solutions. This study aimed to quantify, characterize and evaluate potential sources of plastics in surface marine waters of Brazil and Antarctica, as well as describe the communities that these materials host (plastisphere) by scanning electron microscopy (SEM) and a multibarcoding profile (genes 16S, 18S and ITS2 V4 and V9 regions). Plastics were sampled at the ocean-air interface using a manta net (330 µm mesh) at ten points along the southern coast of Brazil and twelve points around the Antarctic Peninsula. Our study revealed plastics of different sizes (micro, mesoplastic), shapes (fragment, line, sphere, film, foam), colors, malleability (rigid or flexible) and polymer composition. For both localities, there was dominance of microplastics (<5mm), with the "fragment" format being the most abundant category, followed by "line". Flexible items were more abundant and the plastics sampled in this study showed nine colors (white, blue, yellow, green, gray, orange, red, black, brown), with a greater abundance of white plastics. Spectroscopy analysis revealed that polyethylene, polyamide, polyurethane and polypropylene were the most abundant polymers, and presented different degrees of degradation. In Brazil, the highest concentrations of plastic were in stations near the coast, at the mouth of estuaries (Itajaí-Açu River and Lagoa dos Patos), as well as at an oceanic station located near an area of intense fisheries, indicating rivers/lagoons and fishing as important sources of plastic for the region. In Antarctica, the particle dispersion model showed that for at least seven years (time considered in the model) plastics did not originate from latitudes lower than 58º S. This may indicate that local activities (attributed to tourism, research and fishing activities) are the main sources of plastics. The diversity of organisms present in plastic biofilms showed different groups of prokaryotes and eukaryotes, including microorganisms (such as bacteria and fungi) previously described as pathogens and plastic biodegraders. This is the first description of plastisphere fungi in the Southern Hemisphere, and we highlight the need to further investigate the impacts of this group, focusing on their ecological functions.

Key-words: plastics, marine pollution, metabarcoding, plastisphere.

INTRODUÇÃO

Nas últimas décadas vem ocorrendo um aumento significativo da poluição marinha por plásticos, estimando-se que existam entre 15-51 milhões de itens plásticos flutuando na superfície de todos os oceanos (van Sebille et al., 2015). Plásticos são definidos como polímeros sintéticos preparados por polimerização de monômeros derivados de petróleo ou gás, com adição de diversos aditivos químicos, sendo versáteis, leves, duráveis, de baixo custo, resistentes à corrosão e com propriedades de isolamento térmico e elétrico (Richard C. Thompson, Swan, Moore, & Vom Saal, 2009). Devido às suas características, os plásticos tem sido amplamente utilizados em diversas aplicações, tendo substituído outros materias como vidro, papel e madeira na confecção de produtos (Andrady & Neal, 2009).

Os primeiros relatos de plásticos no ambiente marinho ocorreram no início dos anos 70 (Carpenter & Smith, 1972) e desde então suas potenciais consequências ecológicas tem sido crescentemente reportadas (SAPEA, 2019; Galloway, Cole, & Lewis, 2017; Gall & Thompson, 2015; Vegter et al., 2014; Ryan, Moore, van Franeker, & Moloney, 2009; Thompson, Moore, vom Saal, & Swan, 2009). Os plásticos nos oceanos tem recebido crescente atenção da comunidade científica e, uma vez que a produção anual deste material continua aumentando, com 348 milhões de toneladas em 2017 (Plastic Europe, 2018), agências governamentais em todo o mundo estão dando maior atenção para o adequado manejo e redução de plásticos nos ecossistemas marinhos (GESAMP, 2019; Hopewell, Oosterhuis, Papyrakis, & Boteler, 2014; Dvorak, & Kosior, 2009). Apesar disso, milhões de toneladas desses polímeros sintéticos entram nos oceanos anualmente e ainda existem muitas lacunas sobre as abundâncias, características e consequências da poluição por plásticos neste ambiente (Beaumont et al., 2019; Khatmullina & Chubarenko, 2019; Haward, 2018).

As fontes de plástico para os oceanos descritas até o momento são predominantemente de origem continental, com menor contribuição de plásticos de origem marítima (Krantzberg 2019; Li et al. 2016; GESAMP 2015). Recentemente, também foi reportado o transporte aéreo de partículas plásticas para o oceano Ártico (Bergmann et al., 2019). Dentre as fontes continentais, foi estimado que entre 1,15 – 2,41 milhões de toneladas de plásticos entram anualmente nos oceanos a partir de rios, de acordo com um modelo global baseado no gerenciamento de resíduos, densidade populacional e informações hidrológicas (Lebreton et al., 2017). Além do transporte pela

drenagem urbana, há também o descarte direto de plásticos na zona costeira por usuários de praia; atividades náuticas como pesca, transporte de carga e de pessoal (turismo marítimo) são as principais fontes de plásticos de origem matítima para os oceanos (GESAMP 2015).

Diversos estudos sobre plásticos nos oceanos estão focados nos efeitos físicos diretos que eles causam na biota, incluindo mortes por ingestão, emaranhamento e asfixia, problemas já reportados para mais de 800 espécies marinhas de diferentes grupos, incluindo aves, tartarugas, peixes, mamíferos e invertebrados (Wilcox, Mallos, Leonard, Rodriguez, & Denise, 2016; Gall & Thompson, 2015; Cole et al., 2011; Ivar do Sul et al., 2011). Outro potencial impacto dos plásticos nos oceanos é sua atuação como um substrato artificial para a fixação e desenvolvimento de diferentes grupos de organismos aderidos à sua superfície, sendo chamados de "epiplásticos" (Reisser et al., 2014). A natureza hidrofóbica dos plásticos estimula a formação de biofilme e possibilita o estabelecimento de complexas comunidades, também conhecidas como "Plastisfera" (Zettler et al 2013). A plastisfera pode ser constituída por diversas espécies de vírus, bactérias, algas e invertebrados (Kirstein, Wichels, Krohne, & Gerdts, 2018; De Tender et al., 2017; Oberbeckmann, Lo, & Labrenz, 2015; Reisser et al., 2014; Zettler et al., 2013), incluindo organismos patogênicos e potencialmente degradadores de hidrocarbonetos (Oberbeckmann & Labrenz, 2020; Paço et al., 2017; Kirstein et al., 2016). Essas comunidades epiplásticas podem estimular a ingestão intencional de plásticos por organismos marinhos, já que atua como um atrativo devido ao seu cheiro e características semelhantes ao alimento (Amaral-Zettler et al., 2015).

A colonização de plásticos nos oceanos também pode resultar na dispersão de espécies entre ambientes. Plásticos menos densos que a água do mar (e.g. polietileno e polipropileno) flutuam e são transportados por ventos e correntes, com altas taxas de dispersão nos oceanos (Fazey & Ryan, 2016). Esses materias podem carrear organismos de um local para outro, incluindo espécies invasoras que naturalmente não conseguiriam se dispersar (Carlton et al., 2017; Mccormick, Hoellein, Mason, Schluep, & Kelly, 2014; Barnes, 2002). Rech et al. (2016) ressaltam a ameaça dos plásticos nos oceanos como vetor de transporte de espécies não-nativas e sugerem a necessidade urgente de ampliação do conhecimento neste tema. Estes autores consideram que pouco é conhecido sobre as comunidades epiplásticas, porém seus efeitos ecológicos podem ser deletérios, com risco de invasões biológicas. Este risco já foi demonstrado por Carlton et al. (2017), que registraram centenas de espécies de invertebrados e peixes sendo transportadas do oeste

ao leste do oceano Pacífico em materiais sintéticos (incluindo plásticos) após o tsunami de 2011, ocorrido no Japão.

Uma vez nos oceanos, os plásticos vão se fragmentando em partículas cada vez menores sob condições de intemperismo físico-químico-biológico como, por exemplo, radiação ultravioleta, ação de ondas e ingestão pela biota (Andrady, 2011). Alguns microplásticos (considerados neste estudo como plásticos com tamanho entre 1mm e 5mm) são originalmente produzidos nesta categoria de tamanho, a exemplo das microesferas de cosméticos, sendo chamados de microplásticos primários; os microplásticos denominados de secundários constituem-se de itens maiores que são fragmentados devido às intempéries mencionadas anteriormente (GESAMP 2015; Cole et al. 2011; Andrady 2011). Além de se fragmentarem em tamanhos menores, no ambiente marinho muitos tipos de plásticos perdem suas características primárias de cor ao longo do tempo, tornando-se mais “esbranquiçados” ou “amarelados” (Endo et al., 2005). Em adição às alterações dessas características externas, as alterações químicas da cadeia poliméria dos plásticos pode ser verificada baseada no sinal emitido em análises de espectroscopia (ex: estiramento C=O em alguns comprimentos de onda do espectro), podendo ser relacionadas com o seu tempo de exposição no mar (Jin, Christensen, Egerton, Lawson, & White, 2006).

Tanto os plásticos quanto as comunidades epiplásticas ainda não estão bem caracterizados em águas costeiras e oceânicas do Sul do Brasil e da Antártica. Essas duas regiões estão ligadas por correntes oceânicas, a exemplo da Corrente Circumpolar Antártica (ACC) que se ramifica em direção ao equador através da Corrente das Malvinas (*Falkland current*) e entra no Oceano Atlântico Sul, chegando à costa brasileira (Gille & Gordon, 2019). Devido ao aporte de águas ricas em nutrientes, a região Sul do Brasil apresenta uma alta produção de pescado oriundo da pesca marinha extrativista do país (336.451,5 toneladas em 2011) (Boletim Estatístico da Pesca e Aquicultura, 2011). A Antártica tem sua importância principalmente relacionada com a regulação do clima global (Monaghan, 2009) e, apesar de ser uma região remota, não está isenta da poluição marinha por plásticos (Waller et al., 2017). Considerando que os plásticos podem ser transportados por longas distâncias via correntes oceânicas, os organismos epiplásticos aderidos a eles também podem ser dispersos entre regiões; os impactos ecológicos e econômicos destes processos precisam ser avaliados em detalhes, subsidiando informações para estratégias de redução dos efeitos negativos em ambas as regiões.

Um estudo recente identificou uma variedade de plásticos na superfície do mar no entorno da Península Antártica, com predominância de diatomáceas e bactérias em suas comunidades epiplásticas (Lacerda et al., 2019). Alguns grupos de organismos aderidos aos plásticos, contudo, ainda não foram bem caracterizados em ambientes marinhos globais, como é o exemplo dos fungos (Kettner, Rojas-Jimenez, Oberbeckmann, Labrenz, & Grossart, 2017). Este grupo apresenta espécies previamente descritas como patogênicas e biodegradadoras de plásticos (Russell et al., 2011; Shah et al., 2008). Além disso, muitas espécies de fungos atuam na ciclagem de nutrientes (Crowther, Boddy, & Jones, 2012) e apresentam a capacidade de sobreviver a condições extremas de temperatura, salinidade e radiação UV (Richards, Jones, Leonard, & Bass, 2012), podendo ter vantagens de adaptação e sobrevivência em plásticos flutuantes na superfície dos oceanos por longos períodos.

O Grupo de Especialistas nos Aspectos Científicos de Proteção do Ambiente Marinho (GESAMP) - corpo das Nações Unidas que atua na prevenção, redução e controle de degradação do ambiente marinho - recomenda a "avaliação da importância de plásticos como um vetor de transferência de organismos" (GESAMP, 2015). Isto reforça que a caracterização da diversidade dessas comunidades é essencial para a criação de estratégias eficientes de prevenção e manejo (Oberbeckmann & Labrenz, 2020; Rech et al., 2016). Pesquisas na área de microplásticos-microbiologia estão em estágio de crescente desenvolvimento (Ivar do Sul, Tagg & Labrenz, 2018), onde a diversidade da plastisfera tem sido comumente caracterizada através de microscopia eletrônica de varredura (De Tender et al., 2017; Reisser et al., 2014; Carson, Nerheim, Carroll, & Eriksen, 2013) e, mais recentemente, através de técnicas de genômica ambiental, principalmente por sequenciamento simultâneo de vários genes de assinatura (*multibarcoding*) que permitem gerar perfis minuciosos dessas comunidades (Oberbeckmann & Labrenz, 2020; Kettner, Oberbeckmann, Labrenz, & Grossart, 2019; Kirstein et al., 2018; Xu et al., 2019, De Tender et al., 2017; Dinsdale et al., 2008).

Estudos mostram que os plásticos estão cada vez mais presentes nas linhas de costa em todo o planeta (Iñiguez, Conesa, & Fullana, 2016; Li et al., 2016; Barnes et al., 2009), porém pouco ainda se conhece sobre a abundância e distribuição de plásticos tanto em águas superficiais marinha no Brasil (Castro, Silva, & Araújo, 2018; Oliveira & Turra, 2015; Ivar do Sul & Costa, 2007) quanto na Antártica (Lacerda et al. 2019; Waller et al. 2017; Ivar do Sul et al. 2011). A Antártica, embora seja uma região remota e existam medidas que regulem o descarte e manejo do lixo através do protocolo ambiental do

Tratado Antártico (1993), não está isenta da entrada de plásticos em seus ecossistemas marinhos, devido à crescente presença de atividades antropogênicas na região (Lacerda et al. 2019; Waller et al. 2017; Ivar do Sul et al. 2011). Em ambos os locais, a poluição por macroplásticos pode impactar os organismos marinhos devido ao emaranhamento, provocando lesões cutâneas e dificuldade de locomoção e respiração, bem como ingestão de plásticos (de diferentes categorias de tamanho) por diversas espécies marinhas, que pode causar impactos como lesões internas, sensação de saciedade, desnutrição, alterações fisiológicas e comportamentais, assim como a potencial contaminação química pelo contato com compostos tóxicos usados na fabricação dos plásticos ou adsorvidos do ambiente (Wilcox et al., 2016; Gall & Thompson, 2015; Teuten et al., 2009). Esta contaminação por recursos pesqueiros pode afetar a saúde humana: os compostos tóxicos dos plásticos ingeridos podem bioacumular nos tecidos dos organismos e serem transferidos para níveis tróficos superiores por biomagnificação, incluindo os seres humanos como consumidores de topo (Setälä, Fleming-Lehtinen, & Lehtiniemi, 2014; Teuten et al., 2009). Este tema, contudo, ainda necessita de mais investigações.

Um dos temas de estudo prioritário e de efeito preocupante da poluição marinha por plásticos em ambas as regiões é a introdução de espécies não-nativas presentes nas comunidades epiplásticas (Castro et al., 2018; 2015 Barnes, 2002; Convey, Barnes, & Morton, 2002). Estes efeitos na região Antártica são especialmente preocupantes, considerando as características ecológicas únicas desse ambiente, com teias tróficas curtas e poucos elos, sendo fortemente afetado e com baixa capacidade de recuperação em casos de desequilíbrios ecológicos provocados pela ocorrência de plásticos no ambiente marinho local (Lacerda et al., 2019). Por fim, alguns grupos bactérias que vivem aderidas aos plásticos, juntamente com os fungos, já foram descritas com capacidade de biodegradação destes polímeros (Oberbeckmann & Labrenz, 2020; Urbanek, Rymowicz, & Mirończuk, 2018; Paço et al., 2017; Russell et al., 2011), reforçando a necessidade de ampliar o conhecimento sobre a diversidade de organismos que esses materiais abrigam e que podem ter aplicações na remediação da problemática da poluição por plásticos nos oceanos (Oberbeckmann & Labrenz, 2020).

A presente tese está alinhada aos Objetivos de Desenvolvimento Sustentável (ODS) 14 -Vida Debaixo D'água (http://www.estrategiaods.org.br/os-ods/ods14/) da Organização das Nações Unidas. Este estudo se alinha também com a Estratégia Nacional de Ciência, Tecnologia e Inovação 2016-2022 do Ministério de Ciência e Tecnologica do Governo Brasileiro, no âmbito dos seus Planos de Ação para os Oceanos, para a Antártica, e para a

Popularização e Divulgação da Ciência e Tecnologia (https://www.mctic.gov.br/mctic/opencms/ciencia/SEPED/Publicacoes/ENCTI/PlanosDeAcao.html). Em adição a isso, os objetivos desta pesquisa atendem às demandas prioritárias estabelecidas pelo Comitê Nacional de Pesquisas Antárticas, no documento "Ciência Antártica para o Brasil: um plano de ação para o período 2013 – 2022" (https://www.mctic.gov.br/mctic/opencms/ciencia/SEPED/Antartica/2022/Ciencia_Antartica_para_o_Brasil__Plano_de_Acao_2013__2022.html) e pelo Plano Nacional de Combate ao Lixo no Mar do Ministério do Meio Ambiente - Governo Federal do Brasil (PNCLM-MMA 2019).

HIPÓTESES

O presente estudo tem as seguintes hipóteses:

(1) A costa sul do Brasil apresenta maior concentrações de plásticos em águas marinhas superficiais próximo à costa do que em oceano aberto, devido ao aporte continental desses materiais;

(2) A Antártica recebe aporte de plásticos em sua superfície oceânica oriundos de fontes locais;

(3) As características dos plásticos (tamanho, formato, maleabilidade, cor e composição polimérica) indicam fontes diversas nas regiões de estudo;

(4) As categorias dos plásticos, bem como a localidade, são fatores determinantes na composição das comunidades epiplásticas;

(5) Existem diversos táxons de fungos associados aos plásticos na costa Sul do Brasil e na Antártica.

OBJETIVOS

Este estudo visou quantificar, caracterizar e estimar as fontes de plásticos na superfície oceânica do Brasil e da Antártica, bem como caracterizar a diversidade dos organismos epiplásticos nestas regiões. Os objetivos específicos foram:

i) Avaliar a abundância, tipos e prováveis fontes de plásticos encontrados em águas da superfície oceânica do Brasil e da Antártica;

ii) Avaliar a diversidade de procariotos e e ucariotos da plastisfera nas duas regiões de estudo, através de Microscopia Eletrônica de Varredura (MEV) e *Multibarcoding* de genes do rRNA;

iii) Verificar se existe variação latitudinal na composição das comunidades da plastisfera em águas marinhas do Brasil;

iv) Explorar a diversidade e funções ecológicos de fungos encontrados na plastisfera do Brasil e da Antártica.

Os objetivos foram explorados ao longo dos três artigos científicos anexados à esta tese.

MATERIAL E MÉTODOS

Amostragem

As amostras foram coletadas em dez pontos entre 26° S e 34° S de latitude na costa do Brasil, em outubro de 2016, e em doze pontos no entorno da Península Antártica, entre 61° S e 64° S, em fevereiro de 2017 (Figura 1), durante expedições científicas dos Projetos TALUDE e INTERBIOTA, respectivamente. As amostragens foram realizadas com arrastos na superfície do mar (interface oceano-ar), utilizando rede Manta (101 cm x 21 cm de abertura e malha de 330 µm), para coleta dos plásticos.

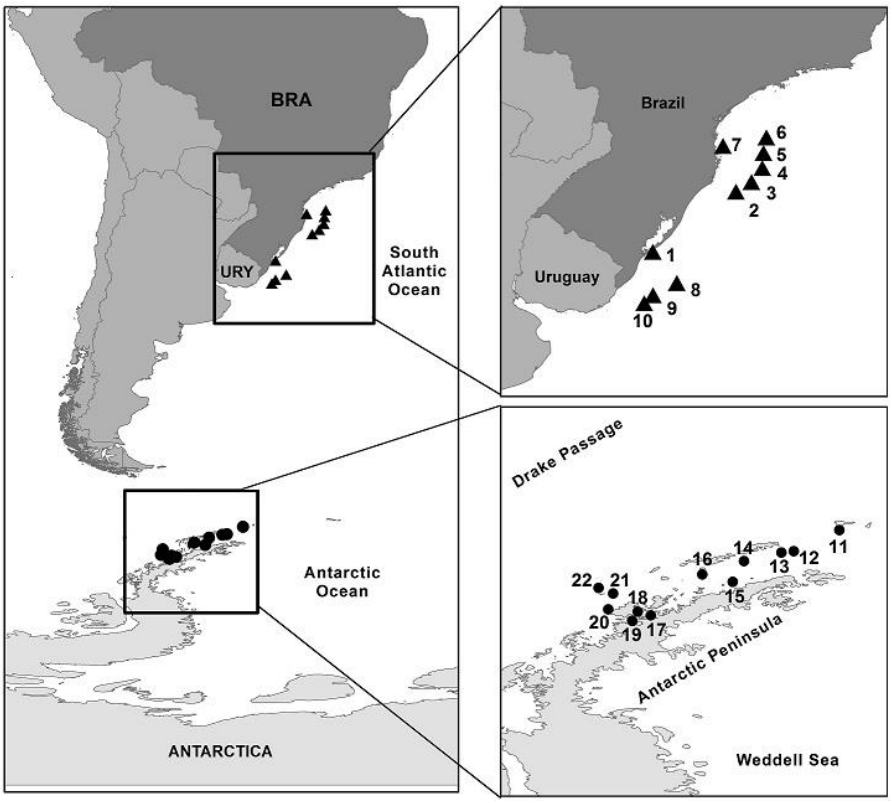

Figura 1. Estações de coleta de plásticos na superfície oceânica do Brasil (dez estações entre 26º S e 34º S) e da Antártica (doze estações entre 61ºS e 64ºS).

Os arrastos tiveram duração entre 15-55 minutos, com velocidade da embarcação de 2-3 nós. A duração dos arrastos, bem como informações sobre o estado do mar (escala Beaufort), profundidade local, direção e velocidade do vento (em nós) foram obtidas em cada ponto de amostragem. Após os arrastos, as amostras foram imediatamente acondicionadas em embalagens de alumínio e congeladas a -20°C para preservação do DNA do biofilme dos plásticos.

Quantificação e caracterização dos plásticos

Triagem e quantificação

Em laboratório, cada amostra foi descongelada separadamente e colocada em um recipiente estéril preenchido com água salgada estéril artificial (salinidade 35, temperatura ~ 4° C) para separação manual dos plásticos flutuantes e biomassa (ex: zooplâncton e outros organismos presentes na superfície do mar no momento da coleta e que foram capturados junto aos plásticos durante os arrastos) (Reisser et al. 2013). Cada amostra foi examinada visualmente e, quando encontrados, os plásticos foram recolhidos usando uma pinça estéril para posterior caracterização. Considerando que a técnica de inspeção visual possui limitações na identificação dos microplásticos (Hanvey et al., 2017), neste estudo foram considerados como microplásticos os plásticos com tamanho entre 1-5mm.

A área arrastada em cada ponto foi calculada com base na velocidade do arrasto (*trawl vel*, considerando 1 nó= 0.514 $m.s^{-1}$), tempo (*t*, em segundos) e a largura da abertura da rede manta (*1* metro), expressa através da seguinte equação:

$$Área = trawl\ vel * t * 1$$

O volume amostrado foi calculado considerando a área de arrasto e a submersão total da extremidade posterior da rede de manta (21 cm de profundidade). Para estimar a concentração de plásticos na superfície do mar, o número de plásticos por área arrastada foi extrapolado para itens.km^{-2} em cada ponto de amostragem. A concentração média dos plásticos foi calculada para cada região (Brasil e Antártica). O peso dos plásticos foi obtido e extrapolado para g.km^{-2} apenas para a Antártica, pois não foi possível pesar os itens plásticos amostrados no Brasil.

Identificação dos tipos e estimativa de fontes

Cada plástico encontrado foi contabilizado e medido em sua maior seção transversal (comprimento total) usando um paquímetro digital. Em termos de tamanho, os plásticos foram classificados em microplástico (< 5 mm) e mesoplástico (5 - 200 mm) (adaptado de Eriksen et al. 2014). Os itens plásticos também foram categorizados de acordo com formato (fragmento, linha, pellet, espuma, esfera e filme) (adaptado de GESAMP 2019), maleabilidade (rígido ou flexível) e cor (de acordo com os 12 termos básicos de cores do departamento Nacional de Normas do Conselho de Cores da Inter-Society/ ISCC-NBS). Para as amostras da Antártica, cada item foi também pesado em balança digital (precisão de 0,00001 g). A composição polimérica dos plásticos foi determinada para itens selecionados aleatoriamente (45 itens do Brasil e 28 itens da Antártica) através de espectroscopia no infravermelho por transformada de Fourier (FTIR), com equipamento SHIMADZU, modelo Prestige 21, utilizando módulo de refletância difusa, 24 varreduras e resolução de 4 cm^{-1}. As amostras foram submetidas a um longo período (até seis meses) de secagem em sílica ou em dissecador. Os espectros gerados foram comparados com padrões já estabelecidos dos principais componentes desse material (ex: polietileno, polipropileno, poliamida, poliestireno), utilizando o programa Agiltron RSQI (http://www.agiltron.com). Através dos espectros também foi analisado se os polímeros estavam degradados (Jin et al., 2006). Fragmentos de tinta foram caracterizados apenas pelas classes de formato, cor, tamanho e composição polimérica, conforme proposto por Song et al. (2014). Tintas foram identificadas de acordo com: 1) características visuais da partícula, consistindo de lascas finas, planas e flexíveis; e 2) espectros FTIR de 21 amostras de fragmentos de tintas.

Para o Brasil, as fontes foram inferidas a partir dos tipos de plásticos (dentre as diferentes categorias descritas acima), sendo relacionadas com potencias fontes de aporte de plásticos previamente descritas na literatura, tais como proximidade da costa e desembocaduras de rios/lagoas (origem continental) ou áreas de pesca (origem marítima) (Andrady, 2011). Para a Antártica, além de relacionar os tipos de itens com as potenciais fontes descritas anteriormente, foi utilizado um modelo de dispersão de partículas na superífice do mar, onde partículas virtuais foram rastreadas usando a estrutura OceanParcels (Lange & Sebille, 2017) em campos de velocidade de superfície do HYCOM + NCODA Global Analysis 1/12° (Bleck, 2002), na qual a deriva de Stokes foi adicionada a partir do WaveWatchIII (Rascle & Ardhuin, 2013). Em cada um dos 12

locais, 100 partículas virtuais foram liberadas no dia da amostragem (durante fevereiro de 2017) e rastreadas no tempo por sete anos previamente à coleta (até 2010), com rastreamentos armazenados diariamente. Para simular o movimento da escala de subgrade, uma difusão browniana de 10 m^2/s foi adicionada. O código para essas simulações está disponível em https://github.com/OceanParcels/AntarcticPeninsulaPlastic.

Caracterização da plastisfera

Extração de DNA do biofilme dos plásticos

O DNA genômico total foi extraído individualmente de cada item plástico utilizando kit de extração de DNA PowerSoil (www.qiagen.com), de acordo com as instruções do fabricante. A eficiência desse kit para extração de DNA do biofilme de plásticos já foi previamente comprovada (Debeljak et al., 2017). Os fragmentos plásticos foram transferidos para tubos *eppendorf* e as extrações foram realizadas de acordo com as instruções do fabricante, com exceção da primeira etapa (digestão), em que foi adicionado 10 µl (1000 U/µl) de lisozima para melhorar a eficiência da extração (Debeljak et al., 2017), e última etapa em que o DNA foi eluído em 30 µl de tampão de eluição. A qualidade e a concentração do DNA extraído foram verificadas por espectrofotometria no Biodrop DUO (Harvard Bioscience ™).

Amplificação dos genes 16S, 18S (regiões V4 e V9) e ITS2 e sequenciamento de alto rendimento

Para amplificar o gene 16S foram utilizados os iniciadores (*primers*) 515f (GTGCCAGCMGCCGCGGTAA) e 808r (GGACTACHVHHHTWTCTAAT) (modificado) com as seguintes condições de PCR: desnaturação em 94° C por 5 min, seguida de 32 ciclos de 95° C por 30 s, 53° C por 45 s e 72° C por 90 s, com extensão final a 72° C por 7 min. Para a região V9 do gene 18S do rRNA (Amaral-Zettler, McCliment, Ducklow, & Huse, 2009) foram utilizados os *primers* 1391F (5´-GTACACACCGCCCGTC-3´) e Euk B (5´-TGATCCTTCTGCAGGTTCACCTAC-3´) e a PCR foi realizada nas seguintes condições: 95º C para 5 min, seguidos de 35 ciclos de 95º C por 30 s, 55º C por 45 s e 72º C por 90 s, com extensão final de 5 min a 72º C; para amplificar a região V4 do 18S rRNA (Stoeck et al., 2010), os *primers* utilizados foram TAReuk454FWD1 (5´-CAGCASCYGCGGTAATTCC-3´) e TAReukREV3 (5´-

ACTTTCGTTCTTGATYRA-3´) e as condições de PCR foram: 95ºC por 7 min, seguidas de 40 ciclos de 95º C por 30 s, 48º C por 45 s e 72º C por 90 s, com uma extensão final de 7min a 72ºC. Finalmente, para amplificar a região ITS2 (Ihrmark et al., 2012), foi utilizada uma abordagem de PCR *semi-nested*. A primeira PCR foi realizada utilizando o *primer* específico para fungos ITS1f (5'-CTTGGTCATTTAGAGGAAGTAA-3') e o *primer* geral ITS4 (5'-TCCTCCGCTTATTGATATGC-3'). Os produtos da primeira PCR foram diluídos 1:50 e 1 µl usado na segunda PCR com os *primers* gITS7 (5´-GTGARTCATCGARTCTTTG-3´) e ITS4; a primeira e a PCR *semi-nested* foram realizadas com as mesmas condições de termociclagem: 94º C durante 5 min, seguidas de 30 ciclos de 94º C por 30 s, 53º C por 45 s e 72º C por 90 s, com extensão final de 7 min a 72º C.

Todas as PCRs foram realizadas em reações de 25 µL usando a polimerase GoTaq Flexi G2 contendo 5 µL de Gotaq FlexiBuffer, 2 µL de MgCl2 (2mM), 0,5 µL de DNTPs (10 mM, conc. final) (Thermo-scientific, UK), 0,5 µL de primers (*forward* e *reverse*, 0,2 mM cada), 0,125 da enzima Taq DNA polimerase, 1 µL de molde de DNA e 15 µL de água ultrapura. O sucesso das amplificações foi confirmado com eletroforese em gel de agarose 2%. Controles negativos contendo 1 µL de água ultrapura ao invés do molde de DNA foram executados em todas as etapas de PCR e foram também sequenciados. Todos os *primers* continham adaptadores para o fluxo de trabalho do Illumina Nextera XT, além de 12 bases no *primer forward* para aumentar a diversidade das sequências na plataforma do Illumina. As amplificações foram purificadas usando microesferas Ampure XP de 0,8x, de acordo com as instruções do fabricante (Beckman Coulter). Os produtos foram então quantificados usando um kit Quanti-Fluor de alta sensibilidade Promega (Promega, Reino Unido) e diluídos para 10 ng/µL. Os amplicons 16S, 18S V9 e V4, e ITS2 foram então agrupados em concentrações equimolares e a indexação foi realizada utilizando o kit de código de barras Illumina Nextera, de acordo com as instruções do fabricante. As bibliotecas indexadas foram agrupadas, quantificadas e diluídas para 4 pM e sequenciadas no Illumina Miseq usando o kit de sequenciamento V3 2x 300bp Illumina (Illumina®).

Microscopia Eletrônica de Varredura (MEV)

Imagens de microscopia eletrônica de varredura (MEV) foram obtidas do biofilme de plásticos e tintas da Antártica para identificação morfológica dos organismos da plastisfera. Anteriormente à obtenção das imagens, as amostras foram desidratadas em

etanol, sendo em seguida fixadas em uma placa de alumínio com fita de carbono e revestidas com uma camada de 20-30 nm de ouro. Os organismos epiplásticos foram observados utilizando um microscópio JEOL (JSM 6610LV, JEOL, Tokyo), operado em 10-20 kV, a uma distância de trabalho de 10-26 mm. Os itens foram avaliados com magnificações entre 20x e 40000x para identificação dos diferentes organismos. As imagens obtidas foram avaliadas e os organismos epiplásticos identificados em grupos (por exemplo: bactérias, diatomáceas, etc.) no menor nível taxonômico possível.

Análises de dados

A concentração de plásticos foi relacionada com os seguintes fatores: ponto de amostragem, tamanho, formato, cor, flexibilidade, composição polimérica, estado do mar (escala Beuford), direção e velocidade do vento (em nós). As análises estatísticas foram feitas no R studio 1.1.456 (*R core team*, 2015), utilizando o pacote *vegan* (Oksanen et al., 2019). As sequências dos genes amplificados foram agrupadas utilizando o pacote de comparação e agrupamento do programa QIIME ver. 1.3.0 (Caporaso et al., 2010). As sequências de 16S, 18S V4 e V9 foram classificadas de acordo com a taxonomia atribuída às unidades taxonômicas operacionais (OTUs) com base no pacote mais recente do banco de dados SILVA (Quast et al., 2013). Para o ITS2, a taxonomia das OTUs foi obtida a partir do banco de dados de sequências em cluster UNITE ITS 99% (Kõljalg et al., 2005). Os gráficos foram gerados no *R Studio* v 1.1.456 utilizando o pacote *ggplot2* (Wickham, 2009) para verificar a variabilidade dos grupos de organismos de diferentes amostras. As OTUs que apareceram em apenas uma amostra e com menos de duas sequências foram excluídas das análises. Para avaliar a composição das comunidades da plastisfera e riqueza de OTUs associadas a itens individuais de plástico de cada categoria e em cada local, as tabelas de OTUs foram rarefeitas para 5000 leituras para o 16S, 300 leituras para 18S rRNA V4, 500 leituras para 18S rRNA V9 e 1000 leituras para ITS2.

Para avaliar a variação latitudinal da composição das comunidades epiplásticas no Brasil, os pontos de amostragem foram agrupados em duas localidades, denominadas: Santa Catarina (SC, pontos 2-7) e Rio Grande do Sul (RS, pontos 1 e 8-10). Um teste de Kruskal-Wallis foi realizado para verificar diferenças da riqueza de OTUs por amostra entre as variáveis (localidade e categorias de plásticos) para cada marcador. Matrizes de dissimilaridade entre as amostras foram construídas usando um índice binário de Jaccard e as diferenças estatísticas entre a composição das comunidades epiplásticas em relação

à localidade e categorias de plásticos foram testadas com PERMANOVA com 999 permutações.

Diagramas de Venn foram criados para verificar as ordens de fungos exclusivas/compartilhadas entre o Brasil e a Antártica, através da ferramenta Venny (http://bioinfogp.cnb.csic.es/tools/venny/). Potenciais guildas tróficas foram atribuídas às OTUs de fungos usando o FUNGuild, considerando apenas as correspondências prováveis e altamente prováveis para cada taxa (Nguyen et al., 2016). Os dados das sequências de fungos estão disponíveis no Arquivo Europeu de Nucleotídeos, sob o código de acesso ao projeto PRJEB35146. As sequências dos demais grupos taxonômicos serão disponibilizadas a partir da publicação dos futuros artigos oriundos desta tese.

SÍNTESE DOS RESULTADOS

Plásticos na superfície oceânica do Brasil

Foram observados 371 itens plásticos de diferentes tamanhos, formatos, maleabilidade, cores e composição polimérica, indicando serem itens de fontes potencialmente distintas. A concentração média de plásticos na região foi estimada em 8922 itens.km^{-2} (± 3466) e as estações mais próximas à costa apresentaram as mais altas concentrações, conforme hipotetisado neste estudo. Isto mostra que o aporte de plástico de origem continental, devido à drenagem urbana por corpos de água, bem como pelo descarte nas praias, é um meio de introdução de plásticos na costa do Brasil. Adicionalmente, a segunda mais alta concentração de plásticos (comparada aos outros pontos de amostragem) foi uma estação oceânica localizada em uma zona de intensa atividade pesqueira no Rio Grande do Sul, mostrou que a pesca é um fator importante no aporte de plásticos, em especial de linhas (predominantemente constituídas de poliamida, também conhecido como *nylon*), para águas oceânicas da região.

Microplásticos foram dominantes na maioria das estações de coleta, com menor contribuição de mesoplásticos, o que era esperado devido à fragmentação desses materiais sob condições de intemperismo nos oceanos. Plásticos nos formatos fragmento e linha foram os mais abundantes (98%). Em relação à maleabilidade, houve dominância de itens flexíveis em relação aos rígidos e, exceto para a categoria *pellet*, todos os itens rígidos foram fragmentos. Foram encontrados plásticos em nove cores (branco, azul, amarelo, verde, vermelho, cinza, laranja, marrom e preto), com dominância de itens brancos. Plásticos de diversas composições poliméricas foram identificados, tais como polietileno,

poliamida, polipropileno, poliuretano, poliestireno, polietileno tereftalato e acetato de celulose, estando alguns muito degradados e outros não. Os fragmentos de tintas presentes nas amostras foram contabilizados (total de 613 itens), porém não foram incluídos nas análises estatísticas dos plásticos, uma vez que atualmente não existe um consenso na comunidade científica em termos de incluir essas partículas em estimativas de plásticos flutuantes na supeíficie do mar.

A análise *multibarcoding* da plastisfera revelou diversos grupos de procariotos e eucariotos vivendo aderidos aos plásticos. Dentre os procariotos, foram identificados archae e bactéria, com este último apresentando uma variedade maior de filos, com altas abundância e frequência de ocorrência de *Proteobacteria*, *Cyanobacteria*, *Bacteriodetes* e *Firmicutes*. Os dois marcadores usados para caracterização de eucariotos mostraram diferenças na identificação dos grupos, com alguns táxons exclusivos para cada, reforçando a necessidade de abordagens *multibarcoding* para determinar a diversidade de espécies da plastisfera. O conjunto de dados do 18S V9 mostrou maior abundância e diversidade de táxons, comparado à resolução do 18S V4. Os grupos mais abundantes e frequentes observados através do 18S V4 foram alveolata e cnidaria; outros grupos como rodófita, fungo, rhizaria, stramenopiles, crustáceos, tunicados e clorófitas apresentaram abundância e/ou frequência de ocorrência alta, e nenhum grupo eucariótico foi frequente em todas as amostras. O conjunto de dados do 18S V9, entretanto, revelou os grupos rizaria, rodófita, chetognata e cnidaria como os mais abundantes, com menor contribuição de stramenopiles, alveolata, chelicerata, fungos, moluscos e peixes (possivelmente ovos). Rizaria apresentou-se como o grupo mais frequente para o 18S V9 (presente em todas as amostras); este também foi o grupo mais frequente no 18S V4.

A composição de espécies da plastisfera em relação às categorias de plásticos e localidade (SC x RS) foi variável, de acordo com cada marcador molecular. Para o 16S, não foram encontradas diferenças estatísticas entre nenhuma categoria de plástico e localidade. Contudo, para o 18S V4 e V9, existiu diferença estatística significativa entre a composição das comunidades epiplásticas em termos de localidade para ambos os marcadores. Com relação às categorias de plástico, as únicas que mostraram significância estatística em termos de diversidade de OTUs foram "formato" para o 18S V4 e "tamanho" para o 18S V9. Este estudo revelou que as águas marinhas superficias do Brasil apresentam plásticos de diferentes categorias e, além de origens diversas, a dominância de microplásticos secundários, bem como o alto grau de degradação em algumas amostras e a presença de um biofilme bem estabelecido (comunidade diversa

composta desde microoganismos até invertebrados) indica que alguns desses materias estavam no ambiente oceânico por tempo suficiente para sofrerem fragmentação e alteração de suas características primárias.

Multibarcoding de fungos do Brasil e da Antártica

Através de *multibarcoding* (análise do ITS2 e regiões V4 e V9 do 18S), foram detectados diversos táxons de oito filos de fungos (Ascomycota, Basidiomycota, Mucoromycota, Aphelidiomycota, Chytridiomycota, Cryptomycota, Zoopagomycota e Blastocladiomycota) na plastisfera das duas regiões estudadas, totalizando 64 ordens taxonômicas. Alguns grupos de fungos identificados neste estudo ainda não haviam sido descritos associados a plásticos, tais como táxons pertencentes aos filos Aphelidiomycota, Mucoromycota, Zoopagomycota e Blastocladiomycota. Este estudo mostrou, pioneiramente, as funções tróficas dos fungos da plastisfera, onde foi observada uma variada assembléia de táxons predominantemente saprotróficos, porém com outros táxons caracterizados como simbiotróficos ou patógenos de plantas/animais. Dentre os fungos do Brasil e da Antártica, encontramos diversos gêneros marinhos cosmopolitas presentes em plásticos das duas regiões, como *Aspergillus*, *Cladosporium* e *Wallemia*, porém também foram observados táxons exclusivos em cada região, além de uma variação de Chytridiomycota e Aphelidiomycota entre as duas localidades. Fungos previamente reportados como biodegradadores de plásticos (via experimentos em laboratório) foram observados em plásticos coletados no ambiente oceânico das duas regiões avaliadas neste estudo.

Plásticos na superfície do mar no entorno da Península Antártica

A concentração média de plásticos no entorno da Península Antártica foi estimada em 1.794 itens.km², com um peso médio de 27,8 g.km². A proporção de micro (1-5mm) e mesoplásticos (5-200 mm) foi similar, representando 54% e 46% dos plásticos, respectivamente. Na Antártica houve ocorrência de plásticos nos formatos fragmento, esfera e linha, com partículas rígidas e flexíveis. Foram encontrados plásticos em sete cores (branco, amarelo, azul, verde, vermelho, preto e marrom), com predominância de plásticos de cor branca; plásticos dessa cor também foram os mais frequentes, presentes em dez dos doze pontos de amostragem. A composição polimérica desses materiais foi

majoritariamente poliuretano, seguida de poliamida, polietileno, poliestireno e polipropileno.

De acordo com o modelo oceanográfico de dispersão de partículas na superfície oceânica, foi observado que, por pelo menos sete anos (período da modelagem), os plásticos amostrados foram oriundos de latitudes superiores a 58° S, indicando fontes locais (ex. turismo, pesquisa e atividades pesqueiras) como principais responsáveis pela introdução de plásticos em águas marinhas no entorno da Península Antártica. Contudo, vale ressaltar que os plásticos também podem ter sido perdidos/descartados em latitudes mais baixas, sendo dispersados e acumulados na região anteriormente aos sete anos modelados, devido, por exemplo, ao aprisionamento no gelo e praias, com posterior reintrodução no oceano local. Os sistemas de vótices e correntes oceânicas na região poderiam reter partículas plásticas em seu fluxo, criando uma zona de acumulação de plásticos ao redor da Península e do continente Antártico como um todo. Fragmentos de tinta foram 30 vezes mais abundantes (n = 2805) do que os plásticos e foram frequentes em todas as amostras. Embora as partículas de tinta não tenham sido incluídas nas estimativas de concentração de plásticos na superfície oceânica no entorno da Península Antártica, elas podem ter impactos semelhantes aos dos plásticos nos ecossistemas marinhos e por isso foram analisadas neste estudo.

CONCLUSÕES

Visto a rápida e crescente expansão na utilização de plásticos ao longo das últimas décadas, assim como seus graves impactos ambientais, é evidente a necessidade de se conhecer as concentrações e características dos resíduos plásticos no ambiente marinho, incluindo as comunidades que esses materiais abrigam. No Brasil foram observadas concentrações de plástico mais altas do que na Antártica. Para a costa brasileira, a maior concentração de plásticos nos pontos mais próximos à costa, bem como em um ponto localizado em uma área de intensa atividade pesqueira, permitindo inferir que a Lagoa dos Patos e o rio Itajaí-Açu (fontes continentais), assim como a frota pesqueira (fonte marítma) são fontes dos diversos tipos de plásticos encontrados em águas marinhas do Brasil. Na Antártica, tanto o modelo de dispersão de partículas por correntes superficiais quanto os tipos de plásticos permitiram inferir que a poluição na região tem fontes predominantemente locais, que podem ser resultado direto de atividades antrópicas (pesquisa, turismo e pesca) no oceano Austral. A abundância de plásticos encontrados em

uma região remota como a Península Antártica mostra a extensão da influência humana e a potencial irreparabilidade de seus impactos nos oceanos, reafirmando uma necessidade de diminuir a produção e o consumo de plásticos e aumentar práticas adequadas de descarte e gerenciamento desses materiais no mundo.

A diversidade de organismos que habitam este novo substrato marinho, incluindo organismos que podem ser dispersados entre os oceanos, bem como microorgaismos potencialmente patogênicos e/ou com a capacidade de degradar hidrocarbonetos (a exemplo dos fungos e bactérias), pode ter grandes impactos ecológicos e econômicos. Grupos diversos de organismos epiplásticos foram encontrados em ambas as regiõe, com dominância de bactérias, stramenopiles, fungos e alveolatas na plastisfera da costa brasileira; na plastisfera Antártica, bactérias fungos e diatomáceas foram os grupos mais frequentes. Dentre os organismos epiplásticos descritos, alguns já foram previamente reportados com potencias patógenos, a exemplo de bactérias do gênero *Vibrio* e fungos do gênero *Acremonium*. Também foram identificados organismos previamente descritos como biodegradadores de plásticos (em condições de laboratório). Contudo, ainda é preciso avaliar "se" e "como" os microorganismos da plastisfera atuam na degradação desses polímeros no ambiente marinho. Esta foi a primeira descrição de fungos da plastisfera no Hemisfério Sul, onde espécies de nove filos foram identificadas, destacando a necessidade de investigar os impactos desse grupo em outros organismos e nos ecossistemas marinhos, com foco em suas funções ecológicas.

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ANEXO 1

Floating plastics at the sea surface in Southern Brazil: abundance, types and inhabitants

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Floating plastics at the sea surface in Southern Brazil: abundance, types and inhabitants

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Abstract

The lack of information about plastic pollution in many regions around the world hinders concrete actions to manage and mitigate its impacts. In Southern Brazil, plastic amounts, types and potential sources, as well as their inhabitants, are poorly studied. In this work, we conducted a quali-quantitative evaluation of marine plastic pollution at the region. We found 371 plastic items of different sizes, shapes, malleability, colors and polymer compositions, indicating they have different uses and possibly different sources. The average concentration of plastics in the region was estimated at 8922 items.km^{-2} and the stations closest to the coast, as well as one oceanic station located at an intense fishery area, presented the highest plastic concentrations. This possibly indicates that land-based waste and fisheries are the main sources of plastics, mainly secondary microplastic fragments and nylon lines, to Brazilian marine waters. Microplastics were dominant in most sampling stations, with fragments and lines being the most common shapes (representing 98% of the dataset). Regarding malleability, there was dominance of flexible items. Plastics were found in nine colors (white, blue, yellow, green, red, gray, orange, brown and black), with predominance of white/white-ish items. Polymeric compositions of plastics were polyethylene, polyamide, polypropylene, polyurethane, polystyrene, polyethylene terephthalate and cellulose acetate, with some plastics being degraded. Multibarcoding analysis of the plastisphere revealed several groups of prokaryotes and eukaryotes living on the surface of plastics. This study generated important information to support strategies to prevent the input and mitigate the impacts of plastics on marine environments in Brasil.

Introduction

The marine pollution by plastic is increasing exponentially, and represents a serious problem to the oceans. As the production of plastics continues to increase, with an estimated 348 million tonnes being produced in 2017 (Plastic Europe 2018), government agencies around the world are attempting to reach a proper sources-to-sink management of plastics (GESAMP, 2019; Oosterhuis et al., 2014). However, despite local initiatives, plastics continue to enter the oceans in large amounts. It is estimated that in 2010 alone at least 4.8-12.7 million tons of plastics reached coastal and marine environments (Jambeck et al., 2015); as production rises, likely so does this estimate. Although the issue is receiving increasing scientific attention, there are still many gaps on our understanding of the abundances, characteristics and consequences of plastics in the oceans (Beaumont et al., 2019; Maximenko et al., 2019; Wilcox et al., 2016).

The direct physical effects of plastics on marine biota are commonly registered, and entanglement, asphyxiation and ingestion by over 800 species has been reported (Gall & Thompson, 2015; Gregory, 2009; Wright, Thompson, & Galloway, 2013). Plastics in the marine environment (mainly in small sizes) can also possibly act in the transfer of toxic compounds to the animals that ingest them (Diepens & Koelmans, 2018; Provencher, Ammendolia, Rochman, & Mallory, 2018), with potential bioaccumulation of such compounds to higher trophic levels (Benno Meyer-Rochowa, Valérie Gross, Steffany, Zeuss, & Erren, 2015; Setälä et al., 2014; Teuten et al., 2009). Plastics in the oceans also provide durable surfaces for the attachment of many species (Reisser et al., 2014), coined "epiplastic" organisms, and plastics that float can potentially transport these organisms over large distances with unknown consequences (Barnes, 2002; Carlton et al., 2017; Fazey & Ryan, 2016; Masó, Garcés, Pagès, & Camp, 2007; Milner, 2005; Rech et al., 2016). The hydrophobic nature of plastics stimulates biofilm formation and allows the establishment of complex communities, currently known as the "plastisphere" (Quero & Luna, 2017; Zettler et al., 2013).

The plastisphere can host a wide range of prokaryotic and eukaryotic groups (Carpenter & Smith, 1972; Kirstein et al., 2018; Muthukumar, Aravinthan, Lakshmi, & Venkatesan, 2011; Oberbeckmann et al., 2015; Reisser et al., 2014), including pathogenic and potential hydrocarbon degrading organisms (Delacuvellerie, Cyriaque, Gobert, Benali, & Wattiez, 2019; Kirstein et al., 2016; Oberbeckmann & Labrenz, 2020; Oberbeckmann et al., 2015; Paço et al., 2017; Zettler et al., 2013). The diversity of these

epiplastic organisms has been characterized through morphological (by microscopy) and genetic (using specific and general molecular markers) analyses (Amaral-Zettler et al. 2015; Antonio et al. 2019; Lacerda et al. 2019; Oberbeckmann and Labrenz 2020; Reisser et al. 2014; Zettler, Mincer, and Amaral-Zettler 2013), with molecular tools showing a more detailed resolution of taxonomic groups. The role of biofilm on microplastics, however, is still unclear, especially considering if the plastisfere is different than natual-particle associated assemblages (e.g wood and silicate)(Oberbeckmann & Labrenz, 2020).

While estimates show that more than 5 trillion plastics are floating in the oceans around the planet (Eriksen et al., 2014), little is known about the abundance and also the characteristics of plastics floating at the ocean surface of the Brazilian coast, mainly due to the intrinsic difficulty in monitoring its extensive area (approximately 7,500 km). According to a review (Ivar do Sul & Costa, 2007), until 2007, studies involving plastic debris and its interaction with biota were conducted mainly at beaches and estuaries in the states of Pernambuco (Costa, Ivar, Christina, Ângela, & Paula, 2009; Ivar & Costa, 2013; Ramos, Barletta, & Costa, 2012; Silva-Cavalcanti, Barbosa de Araújo, & Ferreira da Costa, 2009), Rio Grande do Sul (Bugoni, Krause, & Petry, 2001; Fillmann, Moller Jr, Ciotti, & Odebrecht, 1995; Portz, 2011; Rizzi et al., 2019; Tourinho, Ivar do Sul, & Fillmann, 2010) and São Paulo (Fisner, Majer, Balthazar-Silva, Gorman, & Turra, 2017; Moreira, Balthazar-Silva, Barbosa, & Turra, 2016; Santana, Ascer, Custódio, Moreira, & Turra, 2016). Since then, other investigations on the subject have been conducted at all regions of the country (Cardozo et al., 2018; Castro et al., 2018; de Carvalho & Baptista Neto, 2016; Dolislager, 2018; Krelling & Turra, 2019; Santana et al., 2016), but none have used molecular metabarcoding to characterize the species that inhabit the surface of plastics in Brazilian marine waters.

We are currently in the "Decade of Ocean Science for Sustainable Development" (2021–2030) as proclaimed by the United Nations, in which the Sustainable Development Goal (SDG) Target 14.14 aims to prevent and reduce marine pollution of all kinds by 2025, including plastics (GESAMP, 2019). In addition to insufficient knowledge on the subject, concrete actions to improve plastic waste management in coastal/marine areas in Brazil are hindered by a lack of adequate sanitation planning and infrastructure (Oliveira & Turra 2015). Only recently (2019) the Brazilian Environmental Ministry created the "National Plan to Combat Litter in the Sea" (PNCLM 2019). In addition, regarding the "gaps in knowledge and themes for future research" on microplastic pollution in aquatic

systems in Brazil, Castro et al. (2018) suggest, among eight topics, to: i) evaluate possible sources, determining trends and abundances; and ii) investigate the potential role of plastics in the dispersal of organisms. In order to contribute towards this understanding, this study aimed to quantify and characterize plastics at the sea surface along the southern coast of Brazil, and analysed the diversity of epiplastic organisms, evaluating possible transport of species, as well as the presence of potential pathogens and plastic biodegraders. This information is fundamental for the creation of strategies to prevent the input and mitigate the impacts of plastics on marine ecosystems.

Methodology

Sampling

Plastics were collected at the ocean-air interface in ten stations along the Southern Brazilian coastline, between latitudes 26º S and 34º S (Figure 1), as part of the TALUDE project (see acknowledgment section for details on this project). At each station, trawls using a Manta net (100 cm × 21 cm mouth, 330µm mesh) were performed in triplicate for 11-17 min, at a speed of 2.5-3 knots. After sampling, volume-reduced samples were collected in an aluminium tray (Figure 1) and samples were frozen in -20º C in aluminium bags.

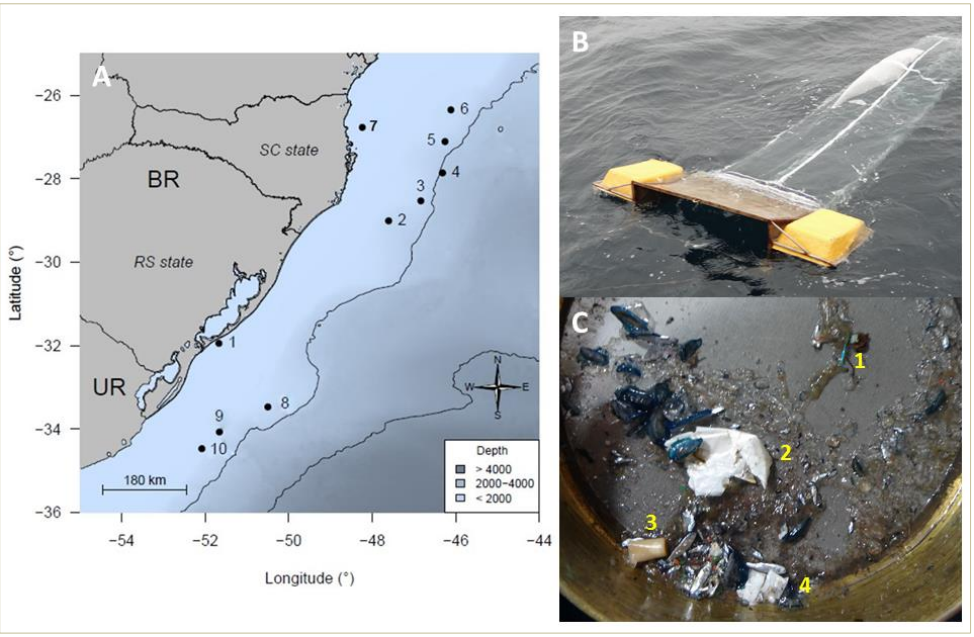

Figure 1. A: Sampling areas (10 stations) of floating marine plastics along the Southern Brazilian coast; B: Manta net trawling at the ocean-air interface to sample plastics; C: Different types of plastics (1 - fishing line, 2 - chocolate pack, 3 - cigarette butt and 4 - fragment) present in one sample from station 8.

At the start and end of each trawl we noted the geographical coordinates, time, depth and wind speed. 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 meter), and was expressed by the equation:

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

Quanti-qualitative characterization of plastics

In the laboratory, samples were thawed separately and placed in a sterile container filled with artificial an sterile salt water (salinity 35, temperature ~ 4°C) for manual separation of floating plastic pieces and biomass (Reisser et al., 2013). A trained observer visually examined each sample for at least 2 hours, and the plastics were picked up using sterile forceps, measured over their total length and classified according to their size (microplastic: 1-5 mm and mesoplastic: 5-200 mm; adapted from Eriksen et al. 2014), shape (fragment, foam, line, pellet and film) (GESAMP, 2019), malleability (hard/flexible), and colour (according to the 12 basic colour terms of the Inter-Society Colour Council National Bureau of Standards/ISCC-NBS), that are called "plastic categories" in this manuscript. Each plastic piece was placed individually in a micro-centrifuge tube with absolute ethanol (reagent grade, MERK) to preserve the genetic material until biofilm DNA extraction, and thirty-two were randomly chosen for genetics analysis. Polymer composition 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.

Biofilm genetic analysis

Plastic pieces were rinsed in sterile artificial seawater to remove loosely associated organisms (organisms that could have co-occurred with plastics during sampling) before DNA extraction. The total DNA of plastic biofilms was extracted using a PowerSoil DNA extraction kit (Qiagen) (Debeljak et al., 2017), with some modifications from the manufacturers' instructions: in the first step we added 10 µl (1000 U/µl) of lysozyme to improve the extraction (Debeljak et al., 2017) and in the last step DNA was eluted in a lower volume (20-30 µl). The quality and concentration of extracted DNA were checked by spectrophotometry using Biodrop DUO (Harvard Bioscience™). We used primers 515f and 808r to amplify the 16S V4 region, TAReuk454 and TAReukRev3 to amplify the 18S V4 region (Stoeck et al., 2010), and finally 1391f and EukB to amplify the 18S V9 region (Amaral-Zettler et al., 2009).

PCR conditions for 16S were: initial denaturation at 94º C for 5 min, followed by 32 cycles of 95º C for 30 s, 53º C for 45 s and 72º C for 90 s, with a final extension at 72º C for 7 min. For 18S V4, the PCR conditions were: 95º C for 7 min, followed by 40 cycles of 95º C for 30 s, 48º C for 45 s and 72º C for 90 s, with a final extension at 72º C for 7 min. Finally, PCRs for 18S V9 were carried out under the following conditions: 95º C for 5min, followed by 35 cycles of 95º C for 30 s, 55º C for 45 s and 72º C for 90 s, with a final extension of 72º C for 5 min. All PCRs were carried out in 25 µL reactions using GoTaq Flexi G2 epolymerase containing 5 µL of Gotaq FlexiBuffer, 2 µL of $MgCl_2$ (2 mM), 0.5 µL of DNTPs (10 mM, final conc.) (Thermo-scientific, UK), 0.5 µL forward and reverse primers (0.2 mM each one), 0.125 U Taq DNA polymerase, 1 µL of DNA template and 15 µL of ultrapure water. Successful amplification was confirmed with electrophoresis in agarose gel. Negative controls containing 1 µL of ultrapure water instead of template were run in PCR steps and sequenced, and all confirmed lack of contamination. The PCR primers contained adapters for the Illumina Nextera XT workflow as well as 12 random bases on the forward primer to increase sequence cluster diversity on the Illumina slide. Amplifications were purified using 0.8x Ampure XP beads (Beckman Coulter) as per the manufacturer's instructions. Products were quantified using a Promega high sensitivity Quanti-Fluor kit (Promega, UK), and diluted to 10 ng/µL. All amplicons were then pooled in equimolar concentrations and indexed using an Illumina Nextera barcoding kit according to the manufacturer's instructions. Indexed libraries were pooled, quantified and diluted to 4 pM and run on Illumina Miseq using the V3 2x 300 bp Illumina sequencing kit.

Data analysis

Statistical analyses were conducted in R studio 1.1.456 using the *vegan* package (Oksanen et al., 2019) for multivariate analysis of data. DNA sequences were analysed as described by Taylor & Cunliffe (Taylor & Cunliffe, 2015) 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. The resulting fastq files had their 12 random base spacers removed using USEARCH –fastq_strip_left command. Cutadapt was used to separate each individual primer set from the data set. For each primer set fastq files were then quality filtered (low quality, expected error > 0.5 and short sequences < 200 bp), with a truncated length of 370 bp for 16S, 370 bp for 18S V4 and 150 bp for 18S V9, 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, Haas, Clemente, Quince, & Knight, 2011), and OTUs were then mapped back to the original reads and an OTU table was produced. Both 18S V4 and V9 sequences were classified against the 97% clustered SILVA database (Quast et al., 2013) using UCLUST (Edgar, 2010). 16S sequences were classified with 98% similarity also against the SILVA database. OTUs appearing in only one sample and with less than two reads were excluded from analyses.

Bar charts based on the frequency of occurrence of prokaryotes (phylum level) and eukaryotes (separated by "groups") for each molecular marker were produced in R studio 1.1.456 (R Development Core Team) with the *ggplot2* package (Wickham, 2009). Differences in community composition of epiplastic communities were analyzed using the binary Jaccard index. These differences were visualized by constructing non-Metric Multidimensional Scaling (nMDS) plots, using the Jaccard distance matrix as input. PERMANOVA was used to test for significant differences between plastic categories and sampled region, divided into two geographical "provinces" corresponding to the states of Santa Catarina (SC) and Rio Grande do Sul (RS). Significances ($p < 0.05$) of these variables were calculated with 9999 permutations. Only the variables with significant difference were fitted on the nMDS plots.

Results

Plastic concentration and types

We found a total of 371 plastic particles of different sizes, shapes, colours and polymer composition. The mean concentration of plastics at the sea surface was 8922 items.km², varying from 2989.6 items.km² in station 5 to 19267.3 items.km² in station 1. As expected, microplastics (1-5 mm) were dominant in most sampling stations except station 9 (Figure 2), followed by mesoplastics (5-200 mm).

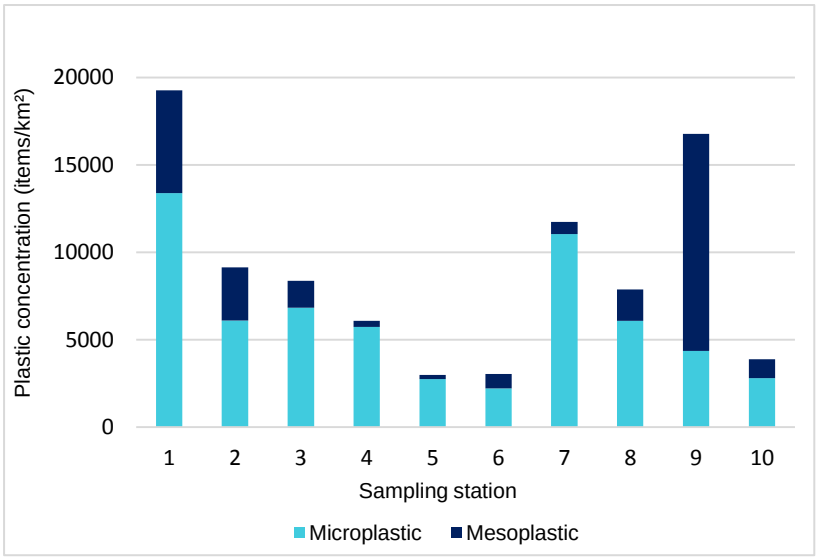

Figure 2. Plastic concentration at the sea surface of ten sampling stations along the Southern Brazilian coast, categorized according to their size class as microplastic (< 5mm, light blue) and mesoplastic (5 - 200mm, dark blue).

The most common plastic shape was fragment (65%), followed by line (33%); other shapes (pellet, foam and film) were less than 1% each. While fragments were predominat among microplastics (>80%) lines, the second most abundant plastic shape, were predominalty mesoplastics (70%). Pellets, foam and film were categorized as microplastics according to their size. Sampling staions showed relatively different plastic shapes and sizes (Figures 1 and 3) but with the predominace of microplastic fragments (more than 50%). None of the stations displayed all categories of plastic shapes, but station 8 had the largest variety of shapes (fragment, line, foam and film).

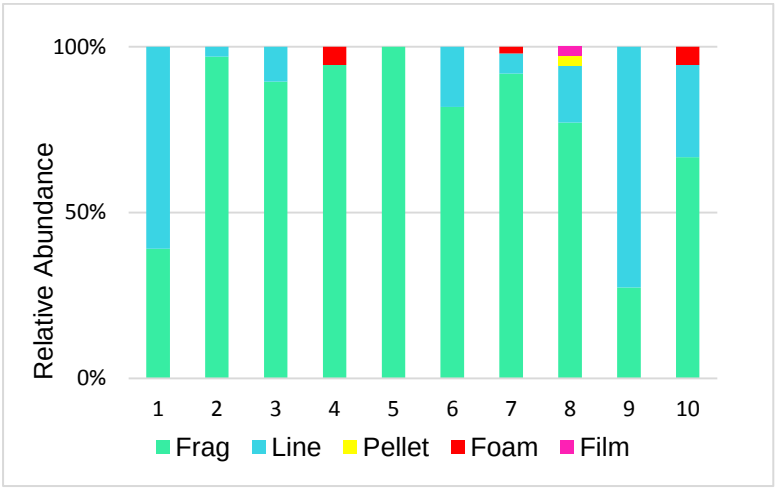

Figure 3. Shapes of plastics sampled at surface waters of ten stations along the South Brazilian coast, separated by sampling stations (Frag= fragment).

In terms of malleability, most of plastic particles were flexible (75%). Mos all hard plastics were fragments, apart from one pellet. A range of colors was identified (white/transparent, yellow, blue, grey, orange, brown, black, green and red), with dominance of white/transparent and blue (44% and 32%, respectively). Plastics were composed of polyethylene (PE), polyamide (PA), polypropylene (PP), polyurethane (PU), polystyrene (PS) polyethylene terephthalate (PET) and cellulose acetate (CA) (Figure 4). The FTIR spectra showed that some polymers/particles were degraded, with PE, PET and PU presenting modifications in their spectra (Figure 4-A, C and D, respectively) when compared with the standard. However, some polymers did not show degradation, such as the PA particle shown in Figure 4-B, which was similar to the standard spectrum.

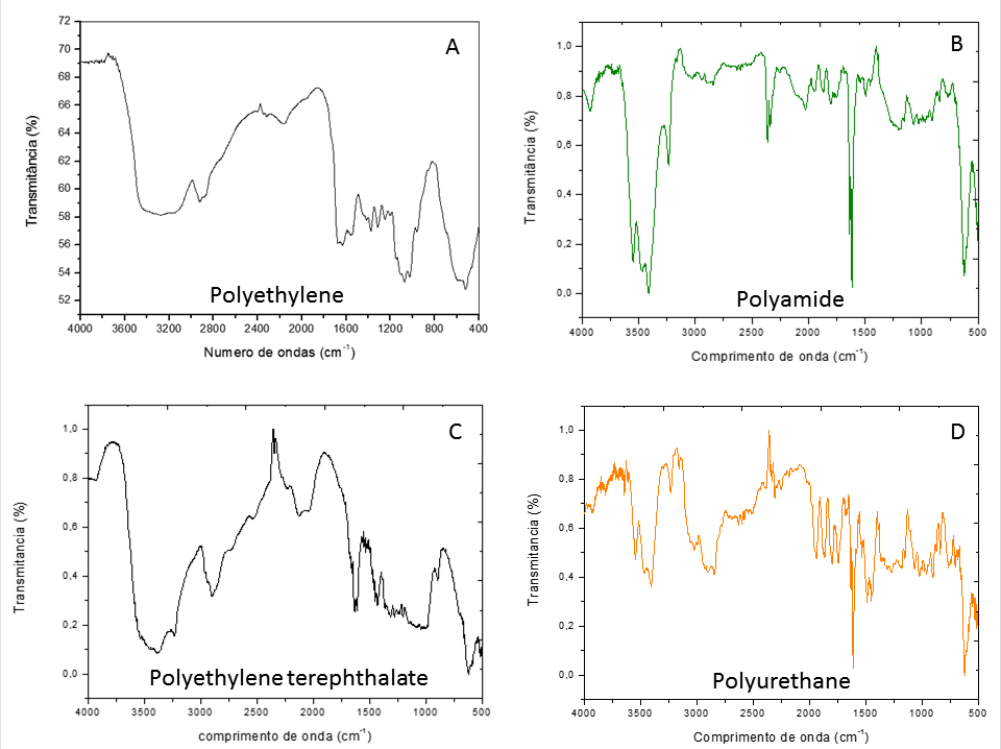

Figure 4. Polymer composition spectra of plastics sampled at surface waters off Southern Brazil, as detected by Fourier Transform Infrared Spectroscopy (FTIR).

Prokaryotic and eukaryotic diversity in the plastisphere

The 16S dataset showed 578,556 reads of 1507 prokaryotic OTUs (Operational Taxonomic Units), with 1492 OTUs classified as Bacteria and 15 as Archaea. All 32 analysed samples had Bacteria associated with plastic biofilms and 17 samples had Archaea OTUs. The number of OTUs per sample varied from 7-602 OTUs, with an average of 136 OTUs/sample. Bacteria presented a wide range of phyla (31 phyla) (Figure

5), with dominance of *Proteobacteria* (abundance of 52%), followed by *Cyanobacteria* (16%), *Bacteriodetes* (13%) and *Firmicutes* (4%). Six bacterial phyla presented abundances between 1-3% (*Verrucomicrobia*, *Planctomycetes*, *Tenericutes*, *Epsilonbacteraeota*, *Deinococcus-Thermus* and *Actinobacteria*) and the remaining phyla together represented less than 2% of the total 16S dataset. The four most abundant groups of Bacteria also showed a high frequency of occurrence (Proteobacteria FO 100%; Cyanobacteria FO 93%; Bacteriodetes FO 97% and Firmicutes FO 90%). Apart from *Proteobacteria*, *Deinococcus-Thermus* was the only group frequent in all the analysed plastics (FO 100%), albeit with low abundance (1.6%). The Archaea group was composed by phyla *Euryarchaeota* and *Thaumarchaeota*.

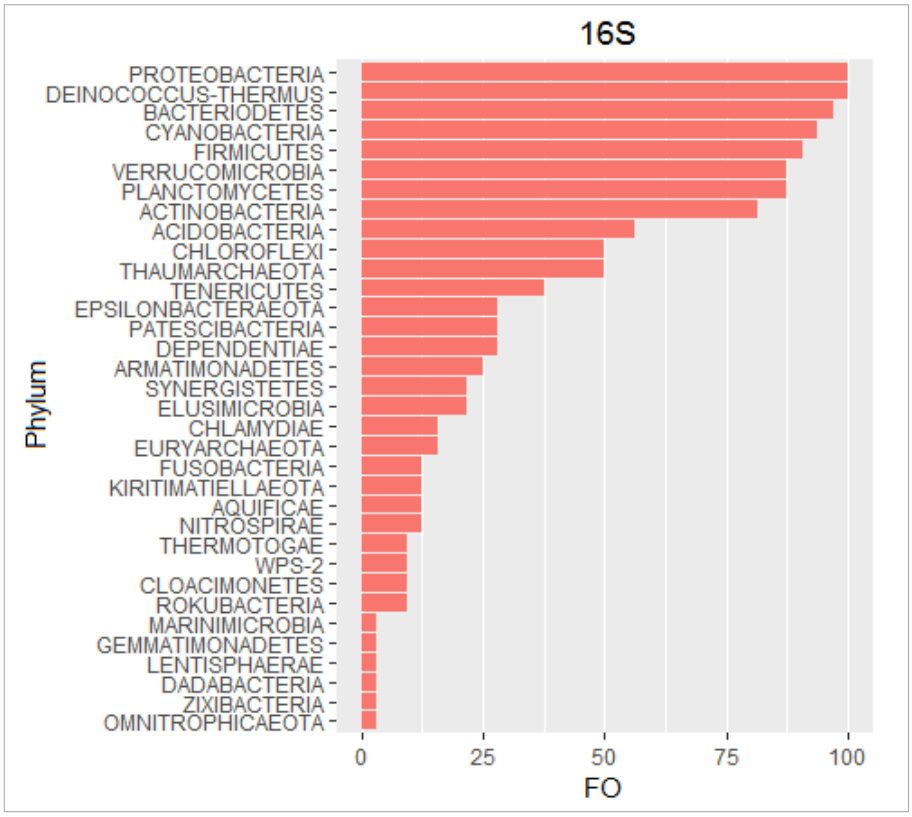

Figure 5. Frequency of occurrence (FO) of prokaryotes associated to floating marine plastics from Sothern Brazil, identified through analyses of the V4 region of the 16S rRNA.

For eukaryotes, the 18S V4 and V9 markers showed different resolutions, with few exclusive groups in each dataset (Figure 6). The 18S V4 dataset had 368,563 reads and the 18S V9 showed 969,673 reads, clustered into 388 and 775 OTUs, respectively. The number of OTUs per sample within the 18S V4 dataset ranged between 1-85 OTUs and within the 18S V9 between 6-331 OTUs. The most abundant groups in the 18S V4

dataset were Alveolata (38%), Cnidaria (19%) and Rodophyta (11%); Fungi, Rhizaria, Stramenopiles, Crustacea, Tunicata and Chlorophtya had abundances between 3-7%. All other groups represented, together, 3% of the 18S V4 reads. Alveolata was also the most frequent group (FO 89%), followed by Cnidaria, which also had a high frequency (FO 78%). Rhodophyta, however, was present in only 40% of samples despite being the third most abundant group. In addition, the groups Tunicata, Fungi, Chlorophyta, Stramenopiles, and Crustacea, which had low abundances, displayed FOs of 70% or more each. Rhizaria also had high FO (59%) but abundance of only 3%. No group was present in 100% of samples within the 18S V4 dataset.

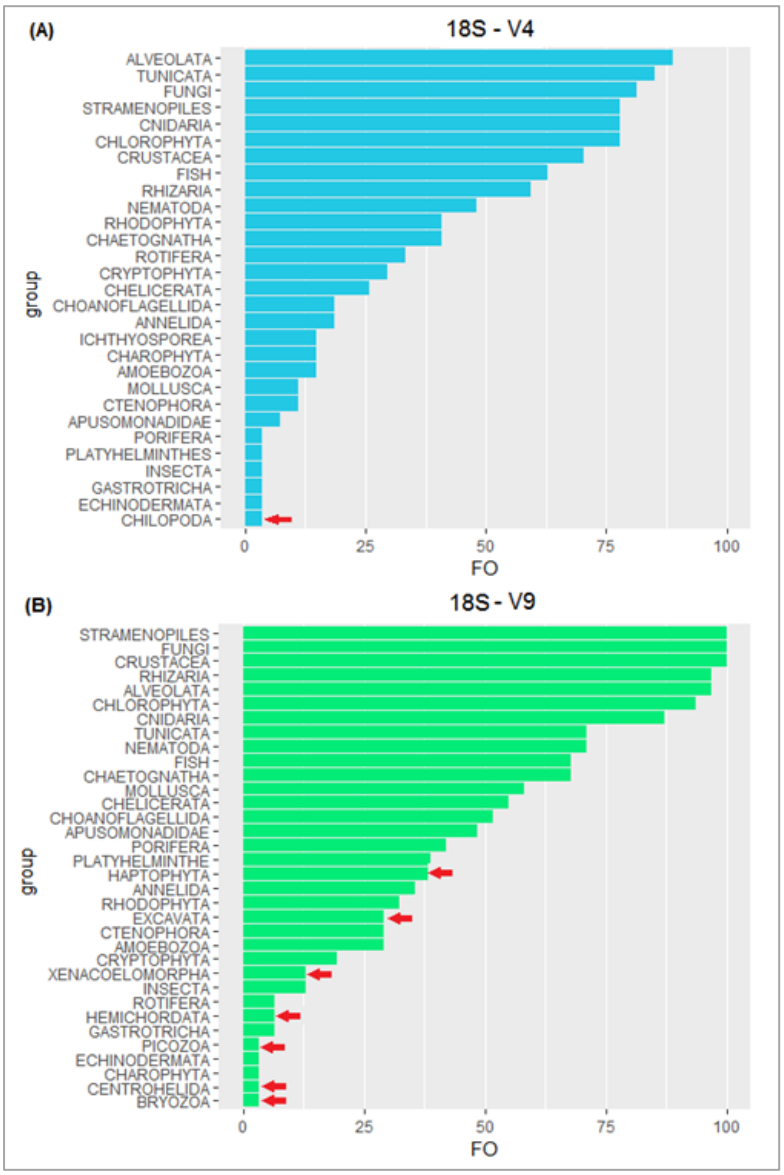

Figure 6. Frequency of occurrence (FO) of eukaryotes associated to floating marine plastics from Southern Brazil, identified through analyses of the rRNA 18S V4 (A) and V9 (B) regions. Red arrows indicate the exclusive groups within each dataset.

The 18S V9 marker showed a different resolution in the abundance and diversity of taxons, with the most abundant group being Rhizaria (37%), followed by Rodophyta and Chaetognatha (10% each) and Cnidaria (9%). Stramenopila, Alveolata, Chelicerata, Fungi and Mollusca had abundances between 3-6% and the other groups together represented 5% of the 18S V9 dataset (Figure 6). Rhizaria was the most abundant and one of the most frequent groups (FO 97%), corroborating results obtained with the 18S V4 marker. However, some groups presented low abundances but high frequencies: Fungi, Stramenopiles and Crustacea had FO of 100%, and Alveolata and Chlorophya FO of 90%. Invertebrates such as Cnidaria, Tunicata, Nemotoda, Chaetognatha, Mollusca and Chelicerate, as well as Choanoflagellida, were frequent in more than 50% of samples.

The diversity of the plastisphere in relation to plastic categories and geographic province was variable within each dataset. For 16S, we found no statistical significance between any category or geographical province. However, for 18S V4 and V9 datasets, there was significant difference in the community composition of the plastisphere in terms of province (PERMANOVA; p = 0.0025 for 18S V4 and p = 0.0263 for 18S V9). In relation to plastic categories, we found statistical significance in terms of shape for 18S V4 (PERMANOVA, F = 1.344; p = 0.0372) and size for 18S V9 (PERMANOVA: F = 1.249, p = 0.0495) (Figure 7).

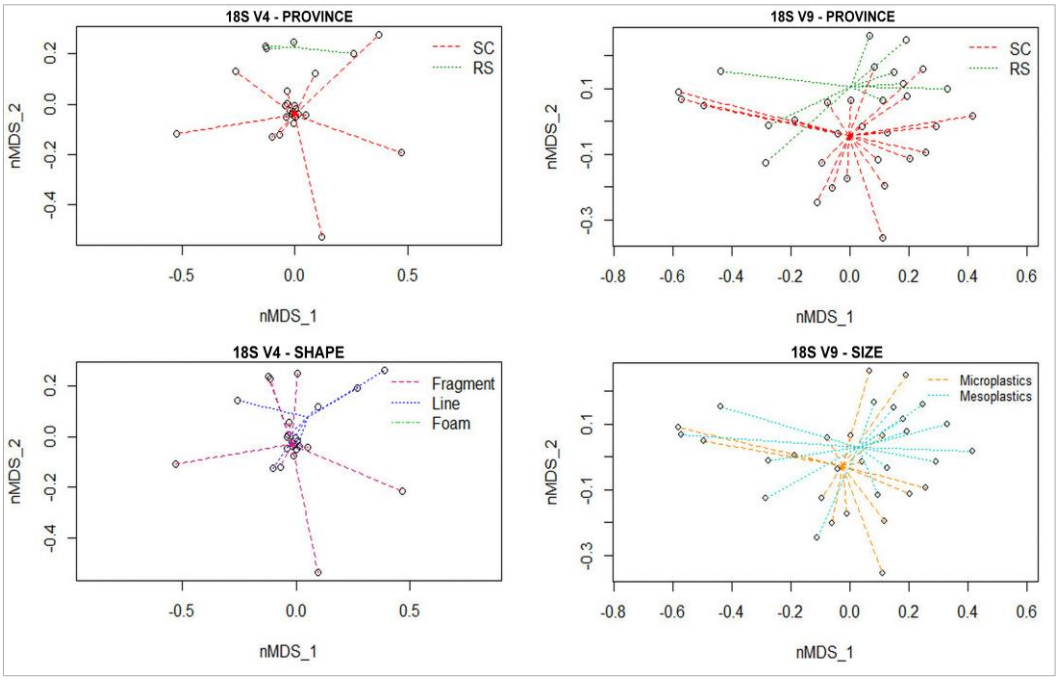

Figure 7. Non-metric multidimensional scaling (nMDS) ordination based on Jaccard matrix distance of eukaryotes living attached to floating marine plastics in Southern Brazil, detected through analysis of the rRNA18S V4 and V9 regions. Significant difference ($p < 0.05$) was found between

"provinces" (RS x SC states) for both markers (top plots). Regarding plastic categories, significant difference was found between shapes for 18S V4 (bottom left, p = 0.0372) and sizes for 18S V9 (bottom right, p = 0.0495).

Discussion

Concentrations and characteristics of marine plastics

Our results show that the sea surface off Southern Brazil is contaminated mainly by microplastics fragments and meso-sized lines. The broad range of size, shape, colors, malleability and polymer composition suggests that plastics are from different sources, as has been suggested by Pan et al. (2019). The dominance of microplastics, which is in accordance with what has been reported in studies of floating plastics worldwide at all ocean basins (Cozar et al., 2014; Eriksen et al., 2014), could be explained by the breakdown of larger plastics into small pieces, due to physical, chemical and biological mechanisms in the marine environment (Andrady, 2011; Mark A. Browne, Tamara Galloway, 2007). This is also corroborated by the dominancy of secondary over primary microplastics in our samples (Andrady, 2011; Cole et al., 2011; Rowland et al., 2004).

High amounts of plastics were found at stations close to the mouths of drainage basins such as to the Patos Lagoon (station 1 - highest concentration) and Itajaí-Açu (station 7 – third highest concentration), possibly due to large contributions of land-based plastic waste. It has been previously suggested that most plastic litter entering the oceans is the result of inadequate waste management on land (UNEP, 2016). In fact, rivers were estimated to carry between 1.15 and 2.41 million tonnes of plastic to the oceans every year (Lebreton et al., 2017). The Patos Lagoon and Itajaí-Açu River are large hydrographic basins (200,000 km² and 35,298 km², respectively) located near several urban and industrial development areas, and both have ports in their estuarine portions. Considering the inefficient waste management in Brazil (PNCLM 2019; Oliveira and Turra 2015), we highlight that these basins could be potential carriers of plastics (and waste in general) to the adjacent marine area.

The morphology of plastics can indicate, at some level, if they are from land-based sources and reach the ocean via urban runoff (mostly packaging fragments), or sea-based from fishing activities (net, line and rope fragments) (GESAMP 2015). However, microplastic properties (including density) can vary significantly according to the time and conditions (e.g. wind stress, waves, U.V. radiation) experienced in the marine environment (Galloway et al., 2017; Gewert, Plassmann, & Macleod, 2015; Khatmullina

& Chubarenko, 2019); it is therefore important to identify the degradation level of these materials to evaluate their behavior.

In our samples, there was a dominance of monofilament fishing lines at station 9, which presented the second highest concentration of plastics with more than 70% consisting of meso-sized PA lines, and some particles presented little or no signal of polymer degradation. This station is located within an of intense operation of the Southern Brazil fishing fleet, and the dominance of meso over micro-sized particles, as well as the presence of undegraded plastics could be an indicative of local origins from fisheries.

Characteristics such as size, color, degradation level and biofilm formation may also indicate if plastics have recently reached aquatic/marine environments, or have been there for a long time (GESAMP, 2019). In our samples, we were able to identify more white/light-colored plastics and FTIR spectra indicated that some plastics presented alterations in their primary characteristics, suggesting that these particles had likely been subject to weathering. The presence of biolfilms containing a diversity of groups (from microorganisms to invertebrates) on plastics may also indicate that these materials were in the ocean for some time, allowing the establishment of a developed community in the plastisphere. Although still poorly known, marine pollution by plastics in Southern Brazil has been shown to affect marine wildlife in different ways. For instance, plastic ingestion has been reported for sea turtles, birds (Bugoni et al., 2001; Rizzi et al., 2019; Tourinho et al., 2010), and several commercial species of fish (Dantas, Ribeiro, Frischknecht, Machado, & Farias, 2019; Lessa, Bismarck, & Gadig, 2009; Neto, 2019), leading to ecological and economic impacts.

The marine plastisphere in Southern Brazil: diversity and ecological impacts

Multi-marker metabarcoding of plastic biofilms found in marine waters along the Southern Brazilian coast revealed diverse communities dominated by Bacteria, Fungi, Rhodophyta, SAR goups (Stramenopiles, Alveolata and Rhizaria), and invertebrates such as Crustaceans, Cnidarians, Chaetognathes, and Tunicatas. Most taxa within the same groups found in our study have been previously described in the marine plastisphere of other regions around the world (Kirstein et al., 2018; Oberbeckmann & Labrenz, 2020; Oberbeckmann et al., 2015; Oberbeckmann, Osborn, & Duhaime, 2016; Reisser et al., 2014). However, groups such as Chelicerata, Xenacoelomorpha and Chilopoda, to the best of our knowledge, have never been reported associated with plastics. High throughput sequencing has been increasingly used to evaluate the diversity of the

plastisphere, and allows a more detailed identification of plastic-associated organisms when compared with methods based only on morphology (De Tender et al. 2017). We demonstrate that a multibarcode approach is more efficient than single marker analyses in identifying the community diversity of plastic biofilms, highlighted by the difference in eukaryote taxonomic detection between the 18S V4 and 18S V9 markers.

The prokaryotic community of sampled plastics was highly variable, and no statistical significance was found in terms of OTU richness over the latitudinal gradient. This is different than has been previously reported for plastic biofilms from the western North Atlantic and eastern North Pacific (Amaral-Zettler et al., 2015), where the authors found bacterial communities to significantly vary with latitude, but over larger range (12.0º N – 45.1º N). In the open North Atlantic and North Pacific Oceans, *Proteobacteria*, *Cyanobacteria* and *Bacteriodetes* were dominant (Bryant et al., 2016; Zettler et al., 2013) and in China's coastal waters *Proteobacteria* and *Bacteriodetes* were the most abundant groups (Xu et al., 2019), which is consistent with our findings for Southern Brazil.

The similarity of bacteria and archaea communities living on plastics sampled over 1000 km apart could be explained by the transport of plastics and their associated prokaryotes via ocean currents. The two major oceanic currents at our study region (Brazil and Malvinas/Falkland Currents) (Piola & Matano, 2019) have been reported to transport larval/planktonic organisms southwards and/or northwards, leading to a lack of population structure in organisms such as fish and crustaceans (Lacerda et al. 2016; da Silva Cortinhas et al. 2016). However, the diversity of plastic-associated eukaryotes between provinces in Southern Brazil was significantly different, which could indicate an actual difference or be a result of the distinct resolutions of the used molecular markers. Additionally, we found significant difference in community composition of eukaryotes in terms of plastic shape and size (depending on the molecular marker).

The role of plastics as a substrate for the establishment of species was first reported in 1972, when Carpenter & Smith (Carpenter & Smith, 1972) found diatoms and hydroids living in the biofilm of plastics in the Sargasso Sea. Since then, several studies had identified diverse epiplastic organisms (Kirstein et al. 2018; Lacerda et al. 2019; Oberbeckmann and Labrenz 2020; Oberbeckmann, Lo, and Labrenz 2015; Reisser et al. 2014; De Tender et al. 2017). Carlton et al (2017) reported that at least 289 taxa were transported attached to marine debris (mostly plastics) across the Pacific after the 2011 tsunami in Japan, dispersing from one continental margin to the other. Plastics can act as a verctor for both native and non native species (Barnes 2002, GESAMP 2015) and since

this material lasts for very long periods in the oceans, is pervasive and can travel slowly, the survival of colonists could be high and lead to biological invasions.

The marine plastisphere can also host pathogenic organisms such as the bacteria *Vibrio spp.* (Kirstein et al., 2016; Zettler et al., 2013) and the protozoan *Halofolliculina spp* (Goldstein, Carson, & Eriksen, 2014). Some OTUs in our samples closely matched previously reported pathogens of humans or marine organisms: seven *Vibrio* OTUs, and fungi *Acremonium sp*. Another possible impact of organisms associated with plastics is the increase in attractiveness for ingestion due their smell and characteristics similar to food (Amaral-Zettler et al., 2015; Procter, Hopkins, Fileman, & Lindeque, 2019). Once ingested, the presence of pathogens in plastic biofilms could lead to the transmission of harmful organisms to animals from low to high trophic levels, since these materials (mainly microplastics) are easily ingested over all levels of marine food webs (Setälä et al., 2014; Wilcox et al., 2016).

Marine plastics can also harbor potential hydrocarbon degraders such as some groups of Bacteria and Fungi (Oberbeckmann & Labrenz, 2020; Paço et al., 2017; Sangeetha Devi et al., 2015; Shah et al., 2008b; Urbanek, Rymowicz, & Miro, 2018). It was recently suggested that the family *Sphingomonadaceae* (*Proteobacteria*) has became one of the - "if not the most" - important microplastic-associated group, due to two main characteristics: their ability to degrade hydrocarbons and the formation of carotenoids, which protect bacterial cells from oxidative stress caused by UV light at the ocean surface (Oberbeckmann & Labrenz, 2020). In the marine plastisphere from Southern Brazil we found 33 OTUs within the *Sphingomonadaceae* family, and these groups should be further investigated to evaluate their ability to degrade plastics in the open ocean. Fungi are also receiving increasing attention in terms of their role in plastic biofilms and ecosystems (Kirstein et al. 2018; Paço et al. 2017; De Tender et al. 2017), and the use of fungi-specific primers in future studies has been suggested to increase our insights into their composition in the plastisphere (Kirstein et al., 2018). We highlight that although we detected a large variety of organisms living in the plastisphere, it is possible that not all groups were interacting with the plastic itself, and only occurr in association (e.g. symbiosis, infections and degradation of organic matter) with other organisms present in our samples (e.g. algae, coral, sponges and other eukaryotic organisms) (Kettner et al., 2019). This issue is still little understood and requires further investigation.

We found the highest concentration of plastics at stations close to the coast and at a high-use fishery area, suggesting that marine plastics at the region have land-based and

sea-based sources. To reduce gaps in our understanding of the transport of plastics between land and sea, we suggest that plastic pollution in the Patos Lagoon and Itajaí-Açu River should be monitored and quantified. This will aid in the search for effective solutions to this problem, since macroplastic removal from drainage basins using artisanal boom barriers or drainage nets can be a more efficient and cost-effective measure when compared to removal from the marine environment (Blettler & Wantzen, 2019). Finally, diverse communities of prokaryotes and eukaryotes were found living in the marine plastisphere of Southern Brazil, which could potentially lead to environmental impacts considering the transport of species between regions and the pathogenic microorganisms found in our samples. The diversity of the plastisphere, as well as its impacts, should be further characterized via *in situ* studies conducted at different regions and ocean compartments. Finally, our findings could also be relevant to other fields such as public health, when considering the effects of plastic pollution and their associated organisms on marine wildlife and humans.

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Competing Interests

The authors declare no financial and non-financial competing interests

Author Contributions

ALdFL MCP conceived the research on plastics. ERS obtained funding for surveys and conceived the oceanographic survey design. ALdFL conducted sampling. ALdFL JDT FK LSR performed lab work and analyzed the data. ALdFL wrote the first draft of the paper, and all authors contributed to discussing and editing the manuscript.

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ANEXO 2

Diverse groups of Fungi associated with plastics in surface waters of the Western South Atlantic and the Antarctic Peninsula.

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Versão de artigo submetida ao periódico *Molecular Ecology*

Diverse groups of Fungi associated with plastics in surface waters of the Western South Atlantic and the Antarctic Peninsula.

Running title: Plastisphere Fungi of the Southern Hemisphere

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Abstract

Marine plastic pollution has a range of negative impacts for biota and the colonization of plastics in the marine environment by microorganisms may have significant ecological impacts. However, data on epiplastic organisms, particularly fungi, is still lacking for many ocean regions. To evaluate plastic associated fungi and their geographic distribution, we characterised the diversity of this group on plastics sampled from surface waters of the western South Atlantic (WSA) and Antarctic Peninsula (AP), using DNA metabarcoding of three molecular markers (ITS2, 18S rRNA V4 and V9 regions). Numerous taxa from eight fungal phyla and a total of 64 orders were detected, including groups that had not yet been described associated with plastics. There was a varied phylogenetic assemblage of predominantly saprotrophic taxa within Ascomycota and Basidiomycota. We found a range of marine cosmopolitan genera present on plastics in both locations, i.e *Aspergillus, Cladosporium, Wallemia*, with a number of taxa being unique to each region. We also found a high variation of Chytridiomycota and Aphelidiomycota between locations. This is the first description of fungi from the plastisphere within the Southern Hemisphere, and highlights the diversity of this group on marine plastics as well as the need to further investigate their potential impacts plastic on other organisms and marine ecosystems.

Introduction

The oceans contain an estimated 50 trillion pieces of floating plastics (van Sebille et al., 2015). Plastics in the oceans have a significant impact on marine wildlife through ingestion, entanglement and asphyxiation (Gall & Thompson, 2015; Gregory, 2009; Li et al., 2016). One important consequence of marine plastic pollution is the creation of floating artificial ecosystems, referred to as the "Plastisphere" (Zettler et al., 2013). The hydrophobic nature of plastics stimulates biofilm formation and allows the establishment of a succession of both micro and macro organisms (Carpenter & Smith, 1972; Oberbeckmann, Loeder, Gerdts, & Osborn, 2014; Reisser et al., 2014). As a result, a variety of prokaryotic and eukaryotic groups (epiplastic organisms) have been found living within biofilms in the Plastisphere (Barnes, 2002; Oberbeckmann & Labrenz, 2020; Oberbeckmann et al., 2014), but only recently have these organisms received attention (De Tender et al., 2017; Kirstein et al., 2018). The impacts of these biofilms may include the attachment and spread of pathogenic organisms or invasive species (Barnes, 2002; Reisser et al., 2014; Zettler et al., 2013) or the increase in palatability of plastics (Coppock et al., 2019; Hodgson, Bréchon, & Thompson, 2018; Procter et al., 2019), possibly leading to higher ingestion by organisms.

One group of epiplastic organisms that have been given little focus are fungi. Molecular surveys have revolutionised our understanding of fungi in the marine environment, with a high diversity of both filamentous and yeast-like Ascomycota and Basidiomycota being identified (Richards et al., 2015, 2012), as well as saprotrophic and parasitic Chytridiomycota and basal fungi in both planktonic and benthic systems (Amend et al., 2019; Gladfelter, James, & Amend, 2019; Grossart et al., 2019; Orsi, Biddle, & Edgcomb, 2013; Ortega-Arbulú, Pichler, Vuillemin, & Orsi, 2019; Richards et al., 2015; Taylor & Cunliffe, 2014, 2016). In the ocean, fungi occupy a complex range of niches and many may have species-specific associations (Zuccaro et al., 2014), living with or parasitizing diatoms, seagrasses, seaweeds (Taylor & Cunliffe, 2016), coral, fish and crustaceans (Loque et al., 2010). In plastic biofilms, little is known about the functional role of fungi, but several fungal taxa from marine and terrestrial environments are known to be able to degrade plastic polymers (Paço et al., 2017; Russell et al., 2011). Fungi are early colonisers of plastics, and may be important in biofilm formation (Tender et al., 2017). Recent studies have directly linked fungi to the degradation of organic matter (Cunliffe, Hollingsworth, Bain, Sharma, & Taylor, 2017; Ortega-Arbulú et al., 2019), and in the open ocean fungi may attach to marine snow particles (Bochdansky, Clouse, &

Herndl, 2017; Duret, Lampitt, & Lam, 2019). As marine plastics are often covered in polysaccharide-rich diatom biofilms (Lacerda et al., 2019), this could explain the attachment and associationof fungi on plastics (Kettner et al., 2017; Oberbeckmann et al., 2016). Other marine debris such as wood also harbour fungi, suggesting that this group is well adapted to live on different types of floating marine substrates (Kettner et al., 2019, 2017).

Bacterial and algal communities on plastics show structuring by location, linked to variation in environmental and physical variables of coastal waters (Amaral-Zettler et al., 2015; Oberbeckmann et al., 2014). A study looking at fungal colonization of plastics showed highly variable community compositions between coastal and offshore waters of the North Sea (De Tender et al., 2017). However, the abundance and diversity of fungi in coastal waters varies seasonally in response to environmental variables such as particulate organic carbon, which could also affect how these organisms colonize plastics (Duan et al., 2018; Taylor & Cunliffe, 2016). Therefore, further study is needed to show whether plastic associated fungal communities display such structuring by location(Kettner et al., 2019).

Assessing the biodiversity of the plastisphere is fundamental to increase our knowledge on plastic-colonising organisms and their biogeography (Amaral-Zettler et al., 2015; Oberbeckmann et al., 2015; Zettler et al., 2013). Plastic pollution, as well as their fungal communities, is not well characterized in oceanic waters of the South Atlantic and Southern Oceans. These two regions are linked by the Antarctica Circumpolar Current (ACC), which branches towards the equator (Falkland/Malvinas Current) and enters the South Atlantic Ocean reaching the Brazilian coast. Subsurface ocean currents have also been show to link these regions, with waters from the South Atlantic reaching the coast of Antarctica (Wichmann, Delandmeter, & Sebille, 2019). Considering that plastics could be transported by oceanic currents (Eriksen et al., 2014), the attached epiplastic organisms may also be dispersed between areas. Antarctica is often viewed as a pristine environment, yet our recent study identified a range of plastic types, with a dominance of diatom and bacteria biofilms, in seawater around the Antarctic Peninsula (AP) (Lacerda et al., 2019).

Within Antarctic coastal waters a diversity of fungi species have been recorded (Bridge & Spooner, 2012; Rosa et al., 2019), with several endemic taxa being found associated with macroalgae (Loque et al., 2010). In the western South Atlantic (WSA), only a few studies have described the fungal diversity in marine ecosystems, with more

focus on using fungi as indicators of pollution in the marine environment (Azedo Loureiro, De Queiroz Cavalcanti, Neves, & De Oliveira Passavante, 2005; Hagler & Mendonça-Hagler, 1981). Molecular techniques can reveal a much higher diversity of fungi, particularly parasitic and pathogenic taxa which are difficult to isolate without host taxa (Richards et al., 2012). Several studies looking at eukaryotic diversity on plastics have used a general 18S ribosomal RNA (rRNA) marker to profile communities with high-throughput sequencing, identifying a variety of fungal taxa within the datasets (Kettner et al., 2019; Kirstein et al., 2018; Oberbeckmann et al., 2016). However, the 18S rRNA gene has limited taxonomic resolution for some fungal groups (Richards et al., 2012; Stoeck et al., 2010; Taylor & Cunliffe, 2014). Using fungi-specific primers, such as those targeting the Internal Transcribed Spacer regions (ITS) of the rRNA operon, ensures a high proportion of sequence reads from fungi and provide good resolution for higher fungi within Ascomycota and Basidiomycota (Andreakis et al., 2015; Rämä et al., 2016).

This study is the first description of the taxonomic composition of fungi from the Plastisphere of oceanic surface waters within the Southern Hemisphere. We aimed to evaluate the diversity of fungi attached to plastics and their geographical distribution between two Southern Hemisphere locations, the WSA and the AP. To profile the total fungal community, a multi-barcode approach was used on marine micro and meso plastics from subtropical waters of WSA and polar waters around the AP. We amplified the 18S V4 and V9 hypervariable regions using general eukaryotic PCR primers, which are commonly used markers for profiling eukaryotic communities but can result in different community profiles and taxonomic resolutions (Nguyen et al., 2016). We also used fungal specific primers to amplify the ITS2 region. We hypothesised that 1) multiple taxa of fungi are associated with plastics in the WSA and AP, as has been suggested in other areas such as the North Sea and 2) there is a marked geographical difference in fungal community composition associated with plastics between WSA and the AP region, as noted for algal and bacterial communities in the Plastisphere of the Northern Hemisphere.

Materials and methods

Plastic sampling and characterization

Plastic samples were collected from surface waters of two oceanic regions, the subtropical western South Atlantic (WSA) and around the Antarctic Peninsula (AP), as

part of the TALUDE and INTERBIOTA Projects. At each sampling point, manta trawls (mesh with 330µm) were performed for 15-55 min at the air-ocean interface, at a speed of 3 knots. Samples were collected at ten stations at WSA and twelve stations around the AP (Figure 1). Within the WSA we sampled from stations close to the Brazilian coast and stations near the shelf break in a latitudinal gradient from 26°S to 34°S. In Antarctica, sampling stations were situated to the west of the AP from 61°S to 64°S. After sampling, the collected material was placed in aluminium bags and immediately frozen at -20° C.

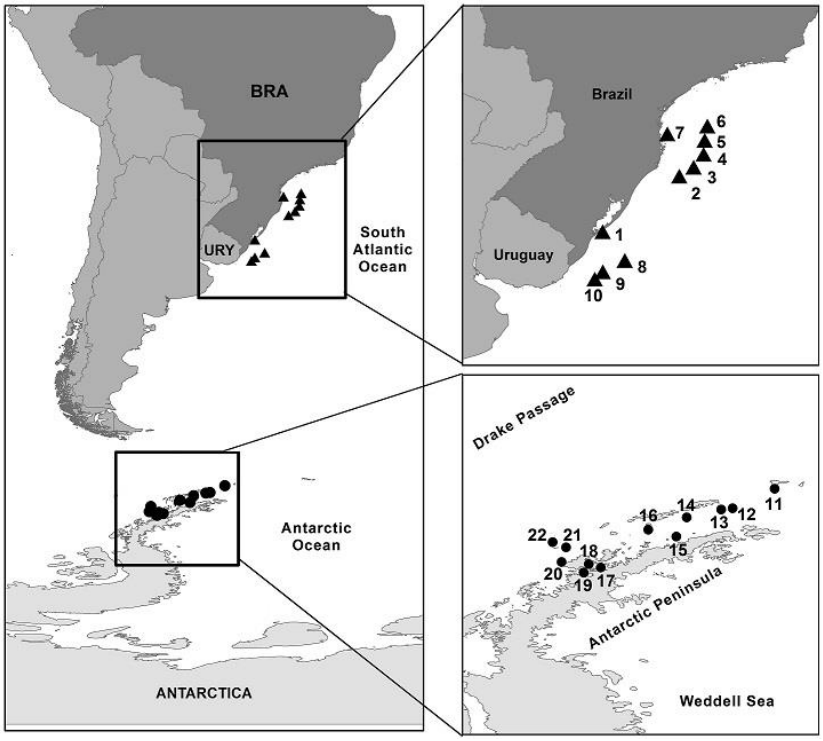

Figure 1. Sampling stations of marine plastics from surface waters in the western South Atlantic (WSA) and Antarctic Peninsula (AP).

In the laboratory, each sample was separately thawed and placed it in a sterile container filled with artificial sterile salt water (salinity 35, temperature ~ 4° C) for manual separation of floating plastic pieces and biomass (Reisser et al., 2013). Each sample was visually examined and when found, plastics were picked up using sterile forceps and measured over their largest cross-section (total length). We found a total of 449 pieces of plastic (371 from WSA and 78 from the AP) (Lacerda et al., 2019) and 58 plastics were randomly chosen for metabarcoding analysis, of which 32 were from WSA and 26 from AP. Plastics with size between 1 - 5 mm were classified as microplastic (17 plastics in WSA and eight plastics from AP) and plastics between 5 - 200mm were

classified as mesoplastic (15 plastics in WSA and 18 plastics from AP) (GESAMP, 2015). Each item was classified according to format as fragment (19 in WSA and 13 in AP), line (11 in WSA and nine in AP), and sphere (two in WSA and four in AP). Each piece of plastic was placed individually in a micro-centrifuge tube with ethanol P.A. (MERK) and frozen at -20°C to preserve biofilm DNA.

DNA extraction

Plastics were rinsed in sterile artificial seawater to remove loosely associated organisms before DNA extraction. The total DNA of the biofilm formed on the marine plastics was extracted using PowerSoil DNA extraction Kits (Qiagen). This kit has previously been shown to be suitable in recovering microbial DNA from the surface of plastics (Debeljak et al., 2017). Plastic fragments were transferred to extraction tubes and the extractions were carried out according to the manufacturers' instructions, with the exception of the first step (digestion), in which we added 10 µl (1000 U/µl) of lysozyme to improve extraction efficiency (Debeljak et al., 2017), and the last step where DNA was eluted in a lower buffer volume (30 µl). The quality and concentration of the extracted DNA were checked by spectrophotometry using a Biodrop DUO (Harvard Bioscience™).

Polymerase chain-reaction (PCR) and high-throughput sequencing

To amplify the V9 region of the 18S rRNA gene (Amaral-Zettler et al., 2009) the primers 1391F (5´-GTACACACCGCCCGTC-3´) and Euk B (5´-TGATCCTTCTGCAGGTTCACCTAC-3´) were used, and PCR was carried out under the following conditions: 95º C for 5min, followed by 35 cycles of 95º C for 30 s, 55º C 45 s and 72º C 90 s, with a final 5 min extension at 72º C; to amplify the V4 region of the 18S rRNA gene (Stoeck et al., 2010) we used the primers TAReuk454FWD1 (5´-CAGCASCYGCGGTAATTCC-3´) and TAReukREV3 (5´-ACTTTCGTTCTTGATYRA-3´), and PCR conditions were: 95º C for 7 min, followed by 40 cycles of 95º C for 30 s, 48º C 45 s and 72º C 90 s, with a final 7 min extension at 72º C.

Finally, to amplify the ITS2 region (Ihrmark et al., 2012), a semi-nested PCR approach was used. The first PCR was carried out using the fungal specific forward primer ITS1f (5´-CTTGGTCATTTAGAGGAAGTAA-3´) and general primer ITS4 (5´-TCCTCCGCTTATTGATATGC-3´). PCR products from the first PCR were diluted 1:50 and 1 µl used in the second PCR with primers gITS7 (5´-GTGARTCATCGARTCTTTG-3´) and ITS4. Both PCRs were run with the same conditions of 94º C during 5 min,

followed by 30 cycles of 94º C for 30 s, 53º C 45 s and 72º C 90 s, with a final 7 min extension at 72º C.

All PCRs were carried out in 25 µL reactions using GoTaq Flexi G2 polymerase containing 5 µL of Gotaq FlexiBuffer, 2 µL of $MgCl_2$ (2 mM), 0.5 µL of DNTPs (10 mM, final conc.) (Thermo-scientific, UK), 0.5 µL of primers (forward and reverse, 0.2 mM each), 0.125 U of Taq DNA polymerase enzyme, 1 µL of DNA template and 15 µL of ultrapure water. Successful amplification was confirmed with gel electrophoresis. Negative controls containing 1 µL ultrapure water instead of template were run with all PCR steps and were taken through to sequencing. All PCR primers contained adapters for the Illumina Nextera XT workflow as well as 12 random bases on the forward primer to increase sequence cluster diversity on the Illumina slide (see Supplementary Table 1 for full primer constructs).

Amplifications were purified using 0.8x Ampure XP beads as per the manufacturer's instructions (Beckman Coulter). Products were then quantified using a Promega high sensitivity Quanti-Fluor kit (Promega, UK), and diluted to 10ng/µL. 18S V9, V4 and ITS2 amplicons were pooled in equimolar concentrations and indexing was performed using an Illumina Nextera barcoding kit as per the manufacturer's instructions. Indexed libraries were then pooled, quantified and diluted to 4 pM and run on Illumina Miseq using the V3 2x 300bp Illumina sequencing kit (Illumina®).

Data analysis

Sequences were analysed using a combination of USEARCH v8 (32Bit) (Edgar, 2010) and QIIME v 1.8.0 (Caporaso et al., 2010). Forward and reverse reads were merged using USEARCH. Cutadapt was used to separate each individual primer set from the dataset, removing primers and adapters. For each primer set fastq files were quality filtered (low quality, expected error > 0.5 and short sequences < 200bp), with a 200 bp minimum length for ITS2 and length truncated for 18S (370 bp for V4 and 150 bp for V9) and then converted to FASTA files. The FASTA files were dereplicated, abundance sorted and had their singleton sequences removed. Operational Taxonomic Units (OTUs) were clustered using the UPARSE clustering algorithm at 97% (Edgar, 2013). Chimeras were filtered using UCHIME (Edgar et al., 2011), and OTUs were then mapped back to the original reads and an OTU table produced. For ITS2 sequences, taxonomy was assigned to OTUS using BLAST against the UNITE ITS 99% clustered sequence database (7.1 2016-11-20 release) (Kõljalg et al., 2005) and a custom ITS database

containing ITS sequences across all taxonomic groups downloaded from Genbank which was used to identify non-fungal OTUs. The custom ITS database was prepared from ITS sequences downloaded from the National Center for Biotechnology Information database (NCBI – accessed 17/05/2018). Sequences were filtered to contain full length ITS and partial ITS2 fragments > 100 bp and no more than two consecutive N bases. The database was then dereplicated of identical sequences and clustered at 99% before both FASTA files and taxonomy were manually formatted for input into QIIME. The 18S V4 and V9 sequences were classified against the 99% clustered SILVA 132 database (Quast et al., 2013) using UCLUST (Edgar, 2010).

Fungal OTUs were separated from all datasets and detailed taxonomy checked and confirmed by BLAST against the full NCBI database. OTUs appearing in only one sample and with less than two reads were excluded from downstream analyses. Potential trophic guilds were assigned to fungal OTUs using FUNGuild considering only probable and highly probable matches for each taxa (Nguyen et al., 2016). Frequency graphs were constructed based on the frequency of occurrence of fungi (number of plastic pieces on which the fungal order was present) at each location, separated by marker. To visualise shared and unique orders of fungi between locations, Venn diagrams were constructed using Venny (http://bioinfogp.cnb.csic.es/tools/venny/).

To assess OTU richness of fungi associated with individual fragments of plastic at each location, OTU tables were rarefied to 1000 reads for ITS2, 300 reads for 18S rRNA V4 and 500 reads for 18S rRNA V9. Statistical analysis was performed in R studio 1.1.456 (R Development Core Team) using the *vegan* (Oksanen et al., 2019) package. A Kruskal-Wallis test was performed to check for differences between OTU richness per sample between variables (location, size class and shape) for each marker gene. Dissimilarity matrices between the samples were constructed using a Binary Jaccard index, statistical differences between locations and metadata groups were tested with PERMANOVA with 999 permutations. Sequence data is available in the European Nucleotide Archive under the project accession code PRJEB35146.

Results

High-throughput sequencing of plastic biofilms

PCR amplification and sequence library generation success was variable between markers. The ITS2 sequence dataset was comprised of 32 samples from the western South

Atlantic (WSA) and 26 from the Antarctic Peninsula (AP), with fungi representing 60% and 80% of all reads from the total dataset from WSA and AP, respectively. The ITS2 dataset had a total of 135 fungal OTUs, with 122 detected in WSA and 97 in AP. The 18S V4 dataset had 45 samples, 28 from WSA and 17 from AP, with fungi representing 2.2% of the eukaryote dataset of the WSA and 4.3% of the AP 18S V4 detected a total of 59 fungi OTUs, with 55 in WSA and 24 in AP (Table 1). The 18S V9 marker generated data for all 58 plastic samples, and fungi represented respectively 2.9% and 5.6% of all reads. The 18S V9 dataset had a total of 110 fungi OTUs, with 76 detected in WSA and 78 detected in AP (Table 1).

Table 1. Number of sequence reads, total number of OTUs and unique and shared OTUs in amplicon sequence libraries for ITS2, 18SV4 and 18SV9 markers amplified from DNA extracted from plastics sampled at the western South Atlantic (WSA) and Antarctic Peninsula (AP).

Marker	Number of fungi reads		Total number of fungi OTUs		Shared OTUs	Unique OTUs	
	WSA	AP	WSA	AP		WSA	AP
ITS	1,062,118	729,369	122	97	84	38	13
V4	9,586	9,683	55	24	20	35	4
V9	43,183	20,836	76	78	44	32	34

Taxonomic composition (Order level) of fungi in the Plastisphere

Across all the marker genes a total of 64 different fungal orders were associated with plastics, with 32 being shared between the two main sampling locations, 21 exclusive to WSA, and 11 unique to AP (Figure 2).

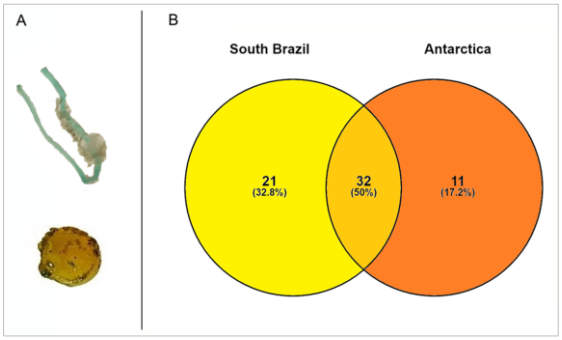

Figure 2. (A) Biofilm on the surface of floating marine plastics: nylon line (top) and pellet (bottom); (B) shared and exclusive taxonomic orders of fungi in the Plastisphere of the western South Atlantic (WSA) and Antarctic Peninsula (AP), identified through multi-profile analyses of combined rRNA ITS2 and 18S V4 and V9 regions.

The ITS2 dataset detected a total of 34 orders, with differences in occurrence depending on the location. For instance, orders Spizellomycetales (Chytridiomycota), Tubeufiales (Ascomycota), Gloeophyllales, Corticiales, Trechisporales and Russulales (Basidiomycota), and Diversisporales (Glomeromycota) were detected only in plastic samples from WSA, while Botryosphaeriales, Ophiostomatales and Myrmecridiales (Ascomycota), and Ustilaginales (Basidiomycota) Blastocladiales (Blastocladiomycota) were present only in samples from AP. The 18S V4 detected 25 fungi orders, with Lobulomycetales and Chytridiales (Chytridiomycota), Glomerales (Glomeromycota), Rozellida and LKM11 (Cryptomycota) detected only in WSA and only by the 18S rRNA V4 marker.

In WSA this order was detected in 18 samples. Eurotiales and Helotiales were the most frequent orders detected with the ITS2 marker in AP, each present in 23 samples. In WSA, Pleosporales was the most frequent and abundant order, present in 31 plastic pieces, followed by Eurotiales (29 plastics), and Wallemiales (23 plastics) (Figure 3; Supplementary information).

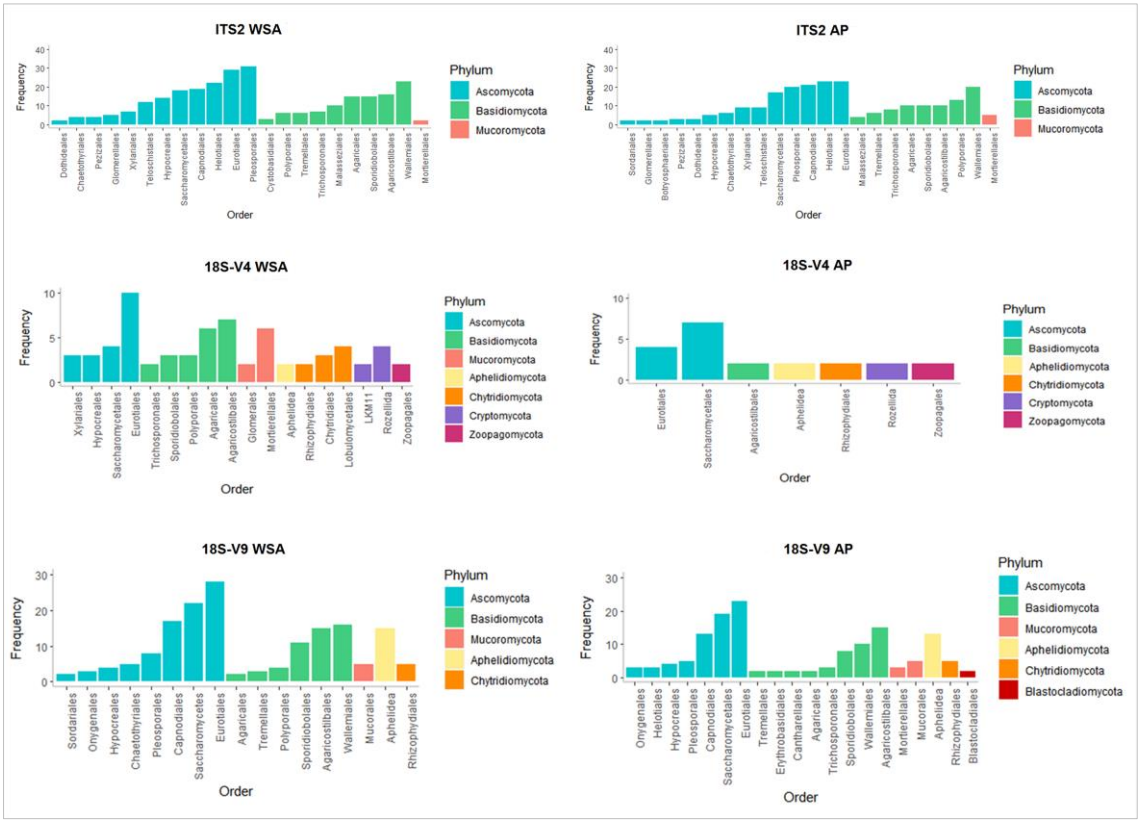

Figure 3. Frequency of occurrence of fungal taxonomic orders identified on floating marine plastics from the western South Atlantic and (WSA) and Antarctic Peninsula (AP) using metabarcoding with ITS2 region and 18S V4 and V9 rRNA markers.

Saccharomycetales was the most frequently found order (7 plastics) within the 18S V4 dataset from AP samples. In waters around AP, the 18S V4 found only six orders with a frequency of occurrence in two or more samples (Eurotiales, Saccharomycetales, Agaricostilbales, Aphelidea, Rozellida, Rhizophydiales and Zoopagales), but all were present in less than ten samples. In WSA the 18S V4 dataset identified more orders than in AP, with a higher frequency of Eurotiales, (10 plastics) and Agaricostilbales (7 plastics), followed by Agaricales and Mortierellales (6 plastics) (Figure 3).

The 18S V9 dataset contained sequences from 43 orders of fungi. The most frequent fungi orders associated with plastics in 18S V9 from waters of the WSA and AP were Eurotiales (23 plastics WSA, and 28 plastics AP), Saccharomycetales, Agaricostilbales, Capnodiales, Aphelidea, and Wallemiales (Figure 3). Both the 18S V4 and V9 were able to detect a higher number of Chytridiomycota taxa, as well as other groups that were not observed not all with ITS2 marker, such as Cryptomycota, Zoopagiomycota, Aphelidiomycota, and Blastocladiomycota (Figure 3).

Comparison of fungal OTU richness and community composition per plastic fragment between regions

The number of fungal taxa associated with each plastic fragment was highly variable in both study regions. In WSA, ranges were 7-23 OTUS for ITS2 (average 13.8 +/- 4.1 SE), 1-12 OTUs for 18S V4 (average 4.5 +/- 2.9 SE) and 2-25 OTUs for 18S V9 (average 12 +/- 5.4 SE). In AP, the range was 7-20 fungal OTUs for ITS2 (average 13.6 +/- 2.7 SE, 1-12 OTUs for 18S V4 (average 2.4 +/- 3.0 SE), and 3-28 OTUs for 18S V9 (average 9.5 +/- 7.0 SE) (Figure 4). Overall, the fungal specific ITS2 detected a higher number of OTUs per plastic fragment than the general 18S V4 and V9 regions. For the 18S V4 marker there was higher number of OTUs per plastic fragment in the WSA than in the AP (Kruskal-wallis, $p = 0.01$). We saw no significant correlation between fungal OTU richness and size of plastic fragments. Fungal community composition was highly variable between individual plastic fragments and many samples had low richness, therefore we did not find significant difference in the community composition of fungi between plastics of distinct size classes (micro and mesoplastic), shapes (line, sphere and fragment), and sampling station (PERMANOVA, $p > 0.05$).

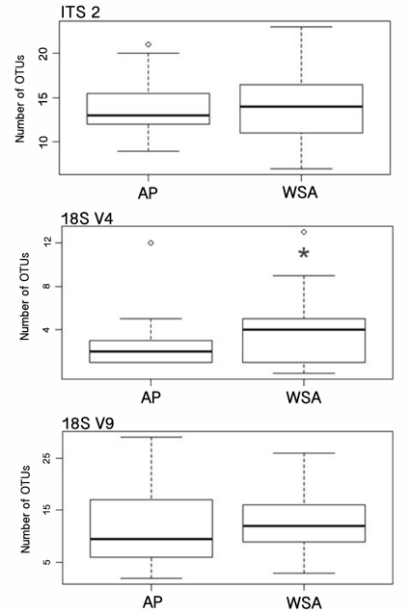

Figure 4. Mean number of observed OTUs per plastic sample from the western South Atlantic (WSA) and Antarctic Peninsula (AP), obtained from rarefied ITS2 (1000 sequence/sample), 18S V4 (300 sequences/sample) and 18S V9 (500 sequences/sample) amplicon sequence libraries. *denotes statistical significant difference (Kruskal-Wallis, $p<0.01$).

Phylogeny and potential function of the most frequent fungal OTUs within the plastisphere of the WSA and AP

Aspergillus was the most frequent or second most frequent OTU associated with plastics from WSA and AP in all marker gene datasets (Table 2). Phylogenetic assignment of OTUs matching *Aspergillus* to species level was variable between marker genes: for example, for the WSA ITS2 the most frequent OTU match with 100% similarity was *A. vitricola*, isolated from masonry or artworks. However, for 18S V4, the OTU with 100% match was *A. restricus* from the deep sea, and for the 18S V9 the OTU with 100% match was *A. wentii*, isolated from dried marine fish.

Table 2. Top five most frequent fungi OTUs from ITS2 and 18S V4 and V9 amplicon libraries obtained from marine plastic biofilms from the western South Atlantic (WSA) and Antarctic Peninsula (AP), showing: Order/OTU Number; Guild – the potential trophic guilds of each OTU (Saprotroph (S), Pathotroph (P) Symbiotroph (SY), Pathotroph-Saprotroph-Symbiotroph (PSSY), Pathotroph-Saprotroph (PS), Pathotroph-Symbiotroph (PSY), Not assigned (NA)); comparisons with sequenced environmental and cultured isolates (accession number, similarity (ID %), and source/highlights); Fre. – frequency of occurrence of the OTU on plastics; and. Abun. – relative abundance of the OTU in the total dataset (%).

ITS		Environmental sample			Cultured isolate					
Order and OTU #	*Guild*	*Source*	*ID %*	*Accession*	*Species*	*ID %*	*Accession*	*Source*	*Fre.*	*Abun.*
WSA										
Eurotiales_OTU6	S	Seagrass rhizosphere	100	MH364700.1	*Aspergillus vitricola*	100	MK367420.1	Cathedral Fresco	25	4%
Wallemiales_OTU1	S	Marine sediment	100	GU370753.1	*Wallemia mellicola*	98	FJ770080.1	Sponge parasite	23	14%
Agaricostilbales _OTU2	S	Seawater	100	KU163776.1	*Sterigmatomyces halophilus*	100	KY105557.1	Soil	13	12%
Capnodiales _OTU10	PSSY	Marine sediment	100	MH368301.1	*Cladosporium cladosporioides*	100	MH244422.1	Coral associate	13	6%
Sporidiobolales_OTU13	PS	Seagrass	100	MH364421.1	*Rhodotorula mucilaginosa*	100	MH231247.1	Sea water	13	<1%
AP										
Wallemiales_OTU1	S	Marine sediment	100	GU370753.1	*Wallemia mellicola*	98	FJ770080.1	Sponge parasite	19	9%
Eurotiales_OTU6	S	Seagrass rhizosphere	100	MH364700.1	*Aspergillus vitricola*	100	MK367420.1	Cathedral Fresco	17	9%
Capnodiales_OTU10	PSSY	Marine sediment	100	MH368301.1	*Cladosporium cladosporioides*	100	MH244422.1	Coral associate	15	3%
Agaricostilbales _OTU2	S	Seawater	100	KU163776.1	*Sterigmatomyces halophilus*	100	KY105557.1	Soil	10	10%
Saccharomycetales_OTU5	S	Hydrothermal vent	100	KX430947.1	*Meyerozyma guilliermondi*	100	KY401420.1	Brackish water	10	14%
18S rRNA V4										
WSA										
Eurotiales_OTU181	S	Seafloor	100	KR072832.1	*Aspergillus restrictus*	100	EU723495.1	Deep sea	10	1%
Agaricostilbales_OTU62	S	Acid hot spring	98	EF682445.1	*Sterigmatomyces halophilus*	100	NG062686.1	Anoxic seawater	6	9%
Mortierellales_OTU123	NA	Marine sediment	98	JX110992.1	*Mortierella hyalina*	99	JQ040259.1	Plant root	6	4%
Agaricales_OTU67	PSY	Seawater	98	GU824934.1	*Pachylepyrium sp*	98	HQ832429.1	Coastal grassland	6	5%
Hypocreales_OTU95	S	Soil	99	AY969172	*Trichoderma amazonicum*	99	NG_062836.1	Plant Endophyte	3	3%
AP										
Saccharomycetales_OTU46	S	Deep-sea	100	JF308274.1	*Meyerozyma guilliermondii*	100	MK355207.1	Compost	6	10%
Eurotiales_OTU181	S	Seafloor	100	KR072832.1	*Aspergillus restrictus*	100	EU723495.1	Deep sea	4	<1%
Sporidiobolales_OTU130	S	Antarctic ocean	100	MK003687.1	*Rhodotorula evergladensis*	100	NG_063017	Seawater	2	1%
Zoopagales_OTU79	NA	Sulfidic spring	87	KT072153.1	*Zoopage sp.*	90	MG920182.1	Rotifer parasite	2	2%
Agaricostilbales_OTU62	S	Acid hot spring	98	EF682445.1	*Sterigmatomyces halophilus*	100	NG062686.1	Air	2	<1%
18S rRNA V9										
WSA										
Eurotiales_OTU19	S	Marine sediment	100	GU474197.1	*Aspergillus wentii*	100	AB002063	Dried fish	27	44%
Capnodiales_OTU97	S	Soil	99	EU490070.1	*Cladosporium sp.*	100	MH102092.1	Seaweed	17	2%
Wallemiales_OTU44	S	Seawater	100	JF826393.1	*Wallemia mellicola*	100	AY741380.1	Hypersaline water	16	13%
Eurotiales_OTU489	S	Antarctic snow	99	KR131435.1	*Aspergillus penicillioides*	99	AF548066	Air	16	3%
Agaricostilbales_OTU42	S	Seawater	93	FJ153656.1	*Sterigmatomyces halophilus*	100	NG062686.1	Anoxic seawater	15	12%
AP										
Eurotiales_OTU19	S	Marine sediment	100	GU474197.1	*Aspergillus wentii*	100	AB002063	Dried fish	21	38%
Agaricostilbales_OTU42	S	Sea water	93	FJ153656.1	*Sterigmatomyces halophilus*	100	NG062686.1	Anoxic seawater	15	6%
Capnodiales_OTU97	S	Savanna soil	99	EU490070.1	*Cladosporium sp.*	100	MH102092.1	Seaweed	13	2%
Saccharomycetales_OTU213	S	Activated sludge	100	AB901846.1	*Geotrichum sp.*	100	MF381141.1	Freshwater	10	4%
Wallemiales_OTU44	S	Sea water	100	JF826393.1	*Wallemia mellicola*	100	AY741380.1	Hypersaline water	10	5%

Several of the most frequent OTUs were closely matched to isolates from saline or low water activity environments. For instance, in the 18S V9 dataset we observed the OTU Wallemiales_OTU44, with 100% match to *Wallemia mellicola* from hypersaline waters, and the OTU Agaricostilbales_OTU62 that was 100% matched to *Sterigmatomyces halophilus,* which has been described from multiple marine environments (Table 2). Frequently detected OTUs were also found to have high similarity to some isolates associated with marine flora and fauna, such as Capnodiales_OTU10 in the ITS2 dataset, which highly matched a *Cladosporium sp.* isolate from a soft coral, and Capnodiales_OTU97 that was closely matched to a *Cladosporium sp.* isolate from the seaweed *Fucus sp.* (Table 2). Several of the most frequents OTUs appeared in both the WSA and AP, such as those with high similarity to *Aspergillus spp., Cladosporium spp*. and *Wallemia spp*. Many of the OTUs had high similarity to sequences reported in environmental molecular surveys from marine and freshwater, marine sediments, deep sea, sewage and wastewater (Table 2).

The majority of functional assignments using FUNGuild for the top five most frequent OTUs from each location and each marker were Probable/Highly Probable Saprotrophs, with a few OTUs being assigned uncertain function or being absent in the current taxa database (Table 2). Looking across the whole dataset, the majority of OTUs were likely Saprotrophs, with some being assigned as Symbionts and Possible/Probable Animal and Plant Pathogens (Supplementary Material 2).

Discussion

This study is the first to focus on fungi associated with plastics in the Southern Hemisphere, as well as the first to evaluate such fungi using molecular techniques within coastal and oceanic regions of WSA and AP. It also takes a different approach evaluating eukaryotic communities associated with plastics, by sampling micro and mesoplastics from the environment instead of conducting colonisation experiments (Kettner et al., 2019, 2017; Oberbeckmann et al., 2016); our approach has previously been used for bacteria (Amaral-Zettler et al., 2015), but never for fungi. The analysis of particles sampled directly from the environment could lead to different results when compared with sessile experiments, since plastics could have travelled over long distances and suffered weathering during their dispersal, leading to changes in epiplastic communities.

We detected fungal taxa in our dataset that have never been reported within the marine plastisphere, such as Apheliomycota, Zoopagomycota, Mucoromycota and

Blastocladiomycota. Additionally, this is the first report of Cryptomycota on open ocean plastics; this group had previously been described only on plastic samples from coastal waters (Kettner et al., 2017) but not in the open ocean. In agreement with studies in the North Sea (Oberbeckmann et al., 2016, De Tender et al., 2017) Ascomycota and Basidiomycota were shown by all markers to be highly frequent plastic colonisers in both sampling sites. However, other studies in coastal waters of the North (Kirstein et al., 2018) and Baltic (Kettner et al., 2017). Seas showed that Chytridiomycota were the most abundant fungal group within plastic biofilms. In our dataset, Chytrids were detected, particularly from samples from AP, but presented low frequency despite often having a high relative abundance in samples where they were present. In Arctic systems Chytrids have been reported to the most abundant fungi group in ice cores (Hassett, Ducluzeau, Collins, 2017; Hassett & Gradinger, 2016) and seawater (Comeau, Vincent, Bernier, & Lovejoy, 2016), yet no data on this group using molecular markers exists for Antarctic systems.

Our study demonstrated the benefit of using a combination of marker genes for epiplastic fungi. Not only did this approach provide a greater overall detection of fungal groups, but for some taxa it highlighted differences in the taxonomic resolution between markers: for example, the most frequently encountered *Aspergillus* OTU in each dataset, which showed similarity to terrestrial isolates using ITS2 but similarity to marine isolates using 18S. Our 18S primers were general and while this did limit numbers of fungal reads they still detect many groups and provided better resolution than ITS2. Fungal specific 18S primers could be used to representation of fungi but these may have difficulties detecting all fungal groups (Banos et al., 2018). Our study further highlights that there are critical limitations in the detection and accurate classification of marine fungi (Banos et al., 2018; Godinho et al., 2013; Richards et al., 2012).

We did not observe geographical differences within ITS2 and 18S V9 at the community level for epiplastic fungi between the WSA and AP. This is contrary to patterns previously observed for bacterial communities (Amaral-Zettler et al., 2015) in the Atlantic and Pacific Oceans, and eukaryotic communities in the North and Baltic Seas (Kettner et al., 2019; De Tender et al., 2017). However, a colonisation experiment in the North Sea also showed highly variable patterns in fungal communities between a harbour and a coastal site (De Tender et al., 2017). Microplastic-associated bacterial communities have been hypothesized to rapidly adapt their composition to changing environments as they move over long distances (Oberbeckmann & Labrenz, 2020).

Many of the fungal taxa found on the plastics were present at both regions, e.g., the genera *Aspergillus* and *Cladosporium* and the order *Pleosporales*, and indeed these genera are cosmopolitan within the marine environment and found in many locations (Hassett, Vonnahme, Peng, Jones, & Heuzé, 2019; Richards et al., 2012; Rosa et al., 2019). Some of the fungi OTUs identified in the specific and general datasets were common to both WSA and AP, e.g. *Sterigmatomyces halophilus, Aspergillus versicolor* and *Cladosporium sp*. All of these OTUs have been previously reported in other marine environments (Bovio et al., 2017, eds.), with *S. halophilus* being one of the most abundant and frequent OTUs in both regions. The waters of the AP are significantly colder than those of WSA, yet it seems that there is no temperature barrier to many of the plastic associated fungal taxa. Many of the fungi we found have been detected in Arctic systems using molecular methods (Hassett et al., 2019; Hassett & Gradinger, 2016) and some groups have been detected previously in Antarctic waters using isolation and culturing (Gonçalves et al., 2017). We also found a range of taxa reported from the marine environment but not previously found on plastics. For example, an OTU matching *Meyerozyma guilliermondii* was found in high abundance and frequency in plastics from the AP; this fungal taxa has been previously reported associated with invertebrate species (Duarte et al., 2013; Godinho et al., 2019) and macroalgae (Godinho et al., 2013).

The WSA is influenced by the Malvinas (Falkland) current, which originates in the Southern Ocean (Antarctica) and flows superficially northwards, reaching the studied region mainly during early autumn (when samples were collected) and winter (Fillmann et al., 1995; Matano et al., 2010; Zavialov et al., 1998). The transport of microplastics from South Atlantic to the Southern Ocean can also occurs due the transport by near surface ocean currents (Wichmann, Delandmeter, & Van Sebille, 2019). Therefore, plastics and their associated organisms could be transported between areas. Many fungal taxa, particularly benthic fungi, may have their dispersal capacity amplified by attachment to floating plastics and the transport of spores between locations mediated by plastics could result in bioinvasions (Amaral-Zettler et al., 2015; Barnes, 2002; Carlton et al., 2017).

Colonisation patterns of plastics can vary depending on which organisms are present at the moment in which plastics entered the marine ecosystem, particularly at coastal sites. Considering that many aquatic and terrestrial fungi are able to cope with increased salinity (Kettner et al., 2017), plastics from freshwater and terrestrial environments that reach the open ocean may be a also dispersal vector of fungal groups.

This may explain why there was a higher number of fungal orders detected on plastics from WSA than those from AP, and also why the mean number of OTUs per plastic piece was higher in WSA. The potential mix of terrestrial/freshwater and marine taxa could have increased the richness of plastic associated fungi in WSA. Indeed, several taxa from terrestrial and freshwater environments were found in WSA (i.e *Gibellula sp*., *Skeletocutis diluta*, *Glomus sp.*, *Cyberlindnera jadinii*, unclassified Russales), likely to the fact that the coastal stations of WSA were very close to the mouthes of large fluvial drainage basins (station 1, station 7).

Another novelty of this work is the assignment of potential trophic guilds using FUNGuild(Nguyen et al., 2016). The majority of OTUs classified were saprotrophs. Saprotrophic fungi are key regulators of nutrient cycles in terrestrial systems and it is believed that this is also true in marine ecosystems (Amend et al., 2019; Cunliffe et al., 2017; Grossart et al., 2019). It has been suggested that early biofilm colonizers on plastics, such as bacteria, can be attracted not to the plastic's surface, but to the biofilm that could increase their access to nutrients (Oberbeckmann & Labrenz, 2020). The same could apply for many saprotrophic fungi: once the early colonizers are established, secondary colonizers start to develop a diverse community living in association with other organisms and living off their exudates and organic matter.

Several OTUs in both WSA and AP that closely matched isolates from marine animals and plants, such as OTUs of genera *Cladosporium*, *Wallemia Aspergillus* and *Alternaria*, which have been found association with corals, sponges, macroalgae and marine plants (Ein-Gil et al., 2009; Godinho et al., 2013; Yarden, 2014). *Zoopage sp*, present in both WSA and AP samples, have been described as parasites of amoebas, nematodes and rotifers (Spatafora, 2017). Many other eukaryotic groups were detected in our data and this supports the suggestion that some fungi groups could be living associated with these organisms. OTUs within Chytridiomycota and Cryptomycota, which have been shown to be parasites of several algal groups (Gerphagnon et al., 2017; Kagami, et al., 2007) including diatoms, were also detected. A number of OTUs were found matching an undescribed Aphelidiomycota taxa, which are parasites of a range of organisms (Karpov et al., 2014; Letcher & Powell, 2019) and could be parasitizing the dense microalgal biofilms on the surface of plastics (Lacerda et al., 2019; Oberbeckmann et al., 2016).

The role of marine plastics as vectors for disease is still poorly understood (Dussud et al., 2018; Kettner et al., 2019; Masó et al., 2007; Oberbeckmann et al., 2018).

Considering the small size of most of our sampled plastics (1-5mm), they could be ingested by organisms from low trophic levels (Desforges et al., 2015; Setälä et al., 2014) to apex predators (Lusher et al., 2015; Wilcox et al., 2016). Several of the fungi taxa were identified as been possible or probable pathotrophs, such as *Acremonium sp.*, which have been described as parasites of the brown algae *Fucus serratus* (Zuccaro et al., 2014) as well as some animal groups.

Many of the taxa identified may have the ability to degrade plastics, such as members of the genus *Aspergillus*, which are able to degrade plastic polymers such as polyvinyl chloride, polyethylene (Shah et al., 2008a) and high and low density polyethylene (Gajendiran, Krishnamoorthy, & Abraham, 2016; Pramila & Ramesh, 2017; Sangeetha Devi et al., 2015). *Cladosporium*, found in our work and previously on plastic biofilms have also been shown to degrade plastics (Brunner et al., 2018; Shah et al., 2008a). We also found unclassified members of the *Pleosporales* in samples from WSA and AP, and some members of this order are able to degrade polyurethane (Russell et al., 2011). Most evidence of plastic biodegradation by fungi is obtained under laboratory conditions therefore further work should aim to isolate fungi from marine plastics to characterise their degradation potential.

In conclusion, this study reports a wide range of fungal taxa in coastal and oceanic waters of the Plastisphere of the western South Atlantic and Antarctic Peninsula, with some groups shared and some unique to each region. Our findings highlight the importance of combining molecular markers for a more robust profiling and characterization of fungal diversity. We report fungi groups that had not yet been described living on marine plastics and observed a highly variable phylogenetic assemblage of predominantly saprotrophic taxa, but also potential parasites, pathogens and hydrocarbon degraders. Considering the high diversity of fungi detected here and in previous studies of the plastisphere, there is a need to further investigate the functions and ecology of fungi living on marine plastics, as well as their potential interactions and impacts on other organisms and environments.

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Competing Interests

The authors declare no financial and non-financial competing interests.

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ANEXO 3

Plastics in sea surface waters around the Antarctic Peninsula

Ana L. d. F. Lacerda, Lucas dos S. Rodrigues, Erik van Sebille, Fábio L. Rodrigues, Lourenço Ribeiro, Eduardo R. Secchi, Felipe Kessler, Maíra C. Proietti

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Plastics in the seasurface waters around the Antarctic Peninsula

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Abstract

Although marine plastic pollution has been the focus of several studies, there are still many gaps in our understanding of the concentrations, characteristics and impacts of plastics in the oceans. This study aimed to quantify and characterize plastic debris in oceanic surface waters of the Antarctic Peninsula. Sampling was done through surface trawls, and mean debris concentration was estimated at 1,794 items.km^{-2} and an average weight of 27.8 g.km^{-2}. No statistical difference was found between the amount of mesoplastics (46%) and microplastics (54%). We found hard and flexible fragments, spheres and lines, in nine colors, composed mostly of polyurethane, polyamide, and polyethylene. An oceanographic dispersal model showed that, for at least seven years, sampled plastics likely did not originate from latitudes lower than 58ºS. Analysis of epiplastic community diversity revealed bacteria, microalgae, and invertebrate groups adhered to debris. Paint fragments were present at all sampling stations and approximately 30 times more abundant than plastics. Although paint particles were not included in plastic concentration estimates, we highlight that they could have similar impacts as marine plastics. We call for urgent action to avoid and mitigate plastic and paint fragment inputs to the Southern Ocean.

Introduction

Plastics make up about 90% of marine litter[1,2] and it is estimated that there are between 15 and 51 trillion plastic particles floating on the surface of the oceans[3]. Currently, plastics are widely distributed in the marine environment, in both hemispheres from the tropics to the poles, with accumulation zones along coastlines, the seafloor, and surface waters, especially in convergence zones such as vortexes and the center of subtropical gyres[2–6]. Marine plastics can have several impacts, such as degradation of habitats, impairment to navigation, contamination of environments, and direct effects on biota through ingestion, asphyxiation and entanglement; these direct effects have already been reported for at least 700 marine species[7–10]. Another worrying but still poorly understood effect of plastics in the marine environment is their role as an artificial substrate for the fixation of organisms[7]. The hydrophobic nature of plastics stimulates biofilm formation and allows the establishment of numerous organisms ("epiplastic" organisms)[13–15] that constitute a new marine ecosystem called the “Plastisphere”[12] that can harbor different groups including bacteria, viruses, fungi, micro and macroalgae, mollusks, cnidarians, crustaceans and fish[7,14,16,17].

The impacts of epiplastic organisms on the marine environment can be diverse. For instance, they may increase consumer attraction for plastics when they perceive this colonized material as a food item, since the biofilm on its surface may smell and look like food.[18]. Once ingested, plastics can obstruct or injure the gastrointestinal tract of animals, and possibly lead to death[10,19]. Exposure to plastics can also lead to a reduction (up to 45%) in the growth of microalgae[20]. In addition, the plastisphere has already been shown to contain pathogenic organisms, such as *Vibrio* bacteria, which can cause diseases to both marine animals and humans[12]. Recently, Arias-Andres et al.[21] analyzed aquatic microbial communities living adhered to plastics, and observed a greater transferal of plasmids carrying antibiotic resistance genes (via horizontal transfer) than in free-living communities. This may eventually transfer other genes that favor the establishment of new traits in bacterial communities by evolutionary changes.

The plastisphere can be especially impacting in terms of global biological invasions, through the dispersal of species between environments and regions via surface water transport[7,12,14]. This has already been shown by Carlton et al.[17], who report a transoceanic biological rafting event after the 2011 tsunami in Japan, with 289 species from 16 phyla crossing the Pacific Ocean from the Japanese coast to Hawaii and North America. Many plastics that enter the oceans are less dense than seawater, and float[22].

Additionally, factors such as ultraviolet (UV) radiation and interaction with atmospheric O_2 alter the physical and chemical properties of these materials, causing some plastics, originally denser than seawater, to decrease their density and float at the ocean surface[22,23]. By remaining at the surface, plastics can be transported via winds and currents over long distances and across ocean basins; the transoceanic dispersion of debris containing epiplastic organisms may cause changes in marine biogeographical patterns[17]. The dispersal of plastics and their inhabitants to remote areas of the planet, such as polar regions, has already been reported[24–26].

Antarctica, established as a World Heritage Site until 2041 and an international territory devoted to peaceful and scientific purposes[27], has suffered strong environmental impacts in recent years. Such impacts include the acidification of the surrounding ocean due to increase in atmospheric CO_2[28] and the growth of marine plastic pollution[25,29,30]. All studies on marine debris in surface waters, beaches and the seafloor in Antarctica highlight that this problem is still poorly understood and requires further evaluation in order to develop tangible and efficient strategies to prevent and mitigate marine plastic pollution in this remote and sensitive environment[24,25,29,30].

Sources of plastics in Antarctica can be diverse, including direct sources via disposal or inadequate management of waste produced by ships and research stations[30], and indirect sources such as transport by marine currents, which can carry plastics from distant areas located at lower latitudes[25,30,31]. Such varied sources can lead to a diversity in the types of plastics in Antarctica; for example, plastic fragments, fishing lines and different plastic packages have been reported in this region[25]. An example of the impact of plastic debris to marine animals in Antarctica is entanglement, reported mainly for mammals and birds and having affected over a thousand fur seals from 1989 to 2008[25]. Ingestion of plastics, as well as presence of this material in nesting areas, is also a common problem[25]. In this manner, our study aims to determine the concentrations, characteristics, and origins of plastic debris in oceanic surface waters around the Antarctic Peninsula.

Results

Abundance and characteristics of plastics

We found 78 plastic items with different abundances and characteristics at the sampling stations around the Antarctic Peninsula (Table 1). Plastic debris were found in all surface trawls. The total average concentration of plastics for the area was estimated

at 1,794 items.km^{-2}, with maximum densities of respectively 3,524 items.km^{-2} and 3,474 items.km^{-2} at stations 3 and 1, and a minimum of 755 items.km^{-2} at station 12. The total weight of the 78 plastic pieces was 1.21 g and the average weight was estimated at 27.8 g.km^{-2}, ranging from 0.21 g.km^{-2} at station 2 (weight of four plastic pieces) to 146 g.km^{-2} at station 8 (eight plastic pieces). Station 3 had the highest number of plastics (n = 13), which had an extrapolated weight of 50 g.km^{-2}. When expressed in terms of volume, plastic concentration was 0.000132 g.m^{-3} and 0.008 items.m^{-3}. We found no correlation between plastic abundance and environmental parameters (sea state, wind speed and local depth) at the sampling points ($p > 0.05$) (see Supplementary Fig. S1 online). The most common plastic format was 'fragment' (51.3%), with lower abundance of items in categories 'line' (42.3%), and 'sphere' (6,4%); in terms of flexibility, most items (83%) were categorized as flexible (Table 1). Fragments ranged from 1 mm to 67 mm; spheres ranged from 2 mm to 6 mm; and lines from 2 mm to 74 mm. There was an almost equal number of microplastics (54%) and mesoplastics (46%), and no macroplastics were found in this study. However, although there was no statistical difference between micro and mesoplastics ($p = 0.69$), we observed difference in the proportion of these two size categories between the sampling points, with microplastics predominating at sampling points 1 (more than 98% of plastics), 8, 9 and 12, and mesoplastics being more abundant at sampling points 3, 4, 5, 6 and 10. A more balanced proportion of the two size classes was found at stations 2, 7 and 11 (Figure 1).

Table 1. Number and weight of plastics found in twelve sampling points at surface waters around the Antarctic Peninsula, according to shape and flexibility.

Station	Number of plastics	Weight (g)	Shape (n)			Flexibility (n)	
			Fragment	Line	Sphere	Rigid	Flexible
# 1	6	0.02743	3	3	-	-	6
# 2	2	0.00052	-	2	-	-	2
# 3	13	0.18478	8	4	1	2	11
# 4	7	0.03719	4	1	2	3	4
# 5	7	0.06932	6	1	-	-	7
# 6	7	0.06039	4	2	1	3	4
# 7	4	0.00089	1	3	-	-	4
# 8	6	0.19845	3	3	-	-	6
# 9	8	0.57977	3	5	-	-	8
# 10	11	0.03610	5	5	1	3	8
# 11	4	0.00801	2	2	-	1	3
# 12	3	0.00779	1	2	-	1	2

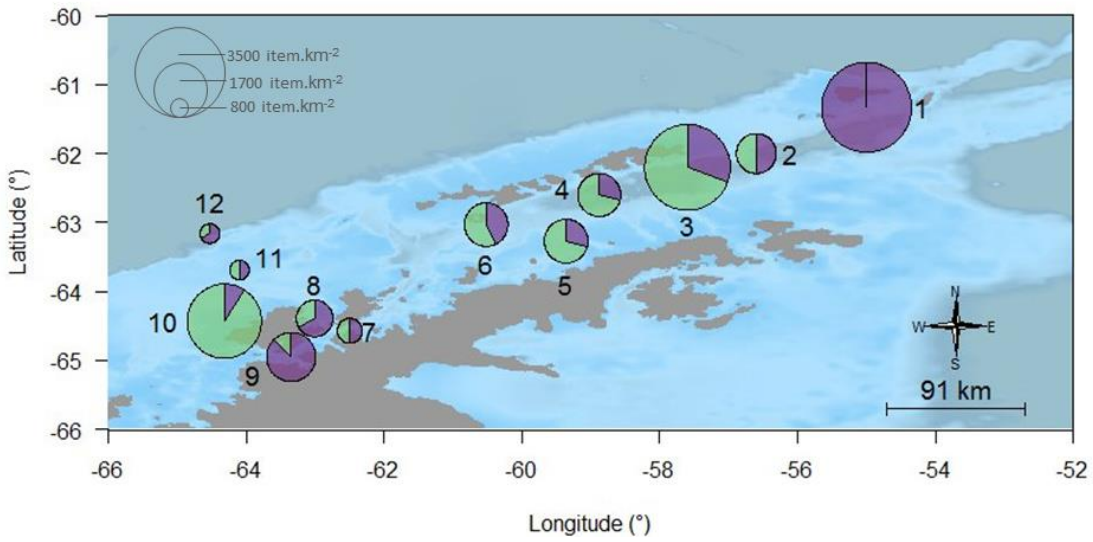

Figure 1. Abundance of micro (purple) and mesoplastics (green) per sampling point in Antarctic waters. The map was created using the *marmap* package and graphics were inserted using the *mapplots* package and *add.pie* function on *R* 3.5.0 (https://www.r-project.org).

In terms of color, the sampled plastics were white, yellow, blue, green, red, black and brown. The concentration of items, as well as the color of particles found at each sampling point, is shown in Figure 2. The color white was the most abundant (47%), and white plastics were present in all sampling points except point 2. The second most common color was black (23%), which was also present in almost all points with the exception of 4 and 12. White and black composed more fragments than the other plastic shapes. The other colors represented lower proportions, with blue, brown and green representing the same percentage (7%), followed by red (5%) and yellow (1%). Point 3 had the largest diversity of colors (Shannon's index = 1.304), while the lowest was observed at point 2 (Simpson's index = 0.000), where only black plastics were found.

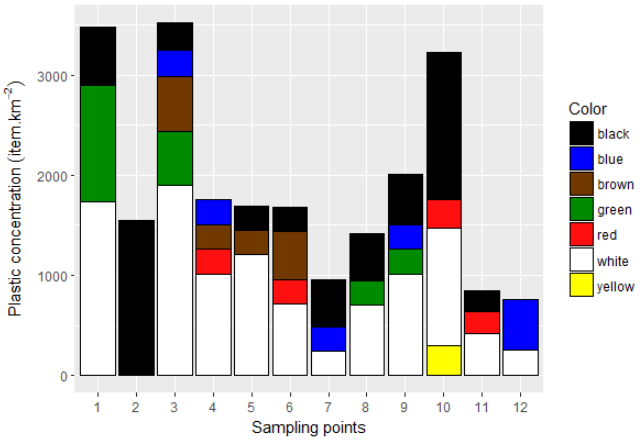

Figure 2. Concentrations and colors of plastics per sampling point off the Antarctic Peninsula.

Except for point 2, in general there was no dominance of a specific color in plastic items at sampling points, with point 6 presenting the highest equitability (that is, lower dominance) of different colors (Simpson's index = 0.6939), while points 5 (Simpson's index = 0.4489) and 12 (Simpson's index = 0.4444) presented lower equitability between the colors. Polymer analysis of 28 items showed that most plastics were composed of polyurethane (35%), followed by polyamide (25%), polyethylene (21%), polystyrene (11%) and polypropylene (8%). The majority of fragments were composed of polyurethane, and most lines were composed of polyamide.

Dispersal of plastics around the Antarctic Peninsula

Our backtracking dispersal model showed that the sampled plastics did not originate from latitudes lower than 58ºS for at least the past seven years, indicating that they have likely been around the Antarctic continent during this time or entered the Southern Ocean more recently through local sources (Figure 3, animation available in Figshare link: https://figshare.com/s/610834334d02b705abaf).

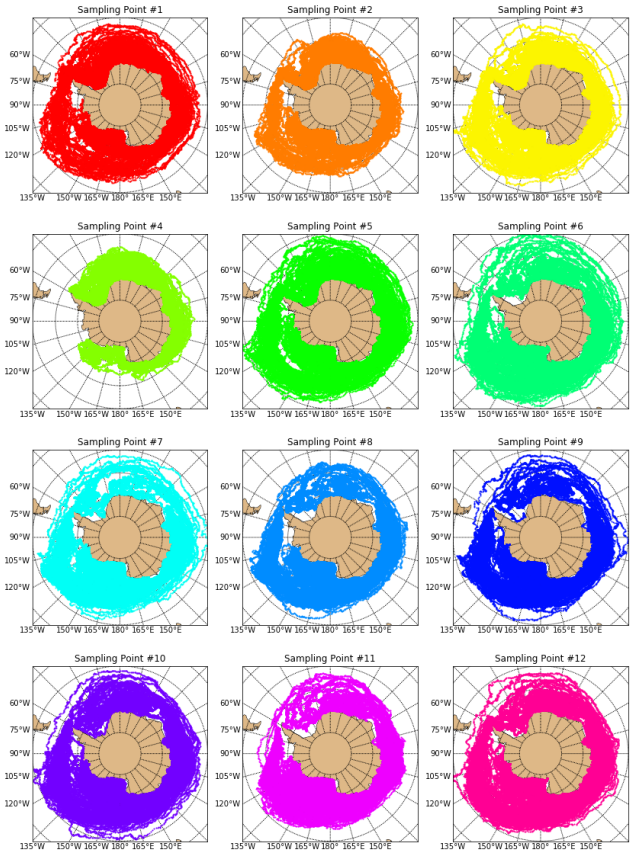

Figure 3. Dispersal model of plastic particles sampled with a manta net in surface waters of twelve points off the Antarctic Peninsula. The model backtracked seven years of dispersal, using ocean surface current data from HYCOM and Stokes drift data from WaveWatchIII.

During the modeling period (seven years before sampling in Feb 2017), it was observed that plastics sampled at most stations could have originated from anywhere poleward of 58ºS off Antarctica; meanwhile, plastics sampled at station 4 presented more restricted sources closer to the continent (Figure 3). The plastics sampled at the other localities presented more diffused origins throughout the Southern Ocean, and could have originated from any point around the Antarctic continent. We did not observe any major difference in terms of origins of plastics collected at the different points in the Southern Ocean.

Paint particles

We found a very high number of paint particles in the samples (n = 2805), ranging from 0.3 mm to 23 mm in seven colors: red, green, yellow, blue, orange, gray and white (Figure 4). Polymer composition analysis of 21 samples revealed that paint fragments were made of polyurethane, with varying degrees of degradation. To determine if part of the fragments were being released during sampling, we compared the spectra of red, green, and yellow paint from our samples with fragments taken from the ship (deck and hull, respectively green and red) and the net frame (yellow) (Figure 4). FTIR spectra showed that these paints were indeed derived from the hull, deck and net; red paint fragments from the hull were the most abundant, showed the largest size variation, and the highest amount of macro-sized fragments. Blue, orange, and gray paint fragments were not present on any external structure of our ship, and we therefore infer that the particles with these colors were already at the ocean surface, originating from other continental sources and/or nautical equipment.

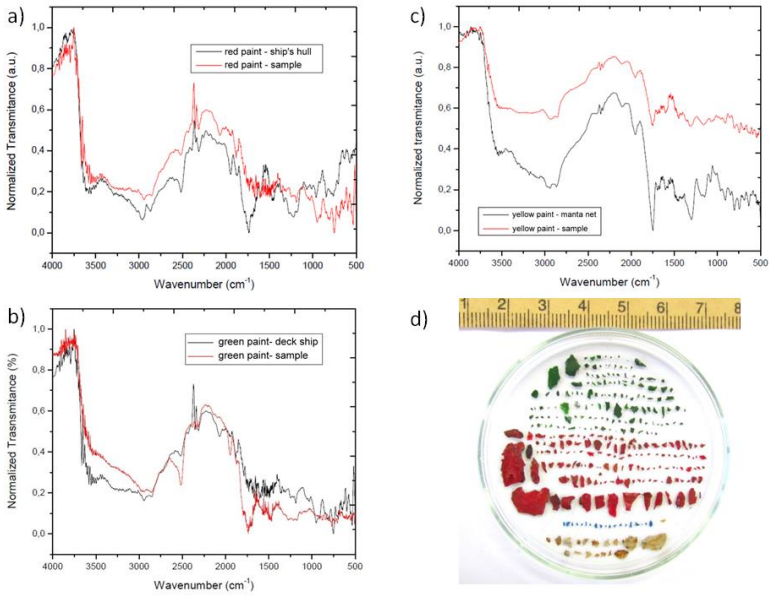

Figure 4. Fourier Transform Infrared (FTIR) spectra from (a) red, (b), green, and (c) yellow ocean paint fragments compared with those from the hull of the ship (red and green) and the manta net frame (yellow); (d) general appearance of paint pieces sampled around the Antarctic Peninsula.

Epiplastic communities

Diatoms (centric and pennate) and bacteria were the most abundant groups colonizing plastics and paint chips, but other microalgae and invertebrate groups were also found adhered to the surface of these marine debris. In terms of diatoms, we found species of the genus *Thalassiosira*, *Synedropsis*, *Chaetoceros*, *Navicula*, among others (Figure 5).

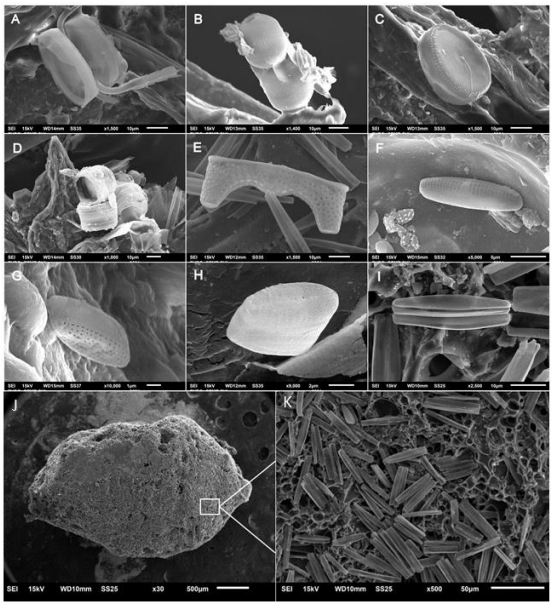

Figure 5. Diatoms found in the Antarctic Plastisphere. A: *Chaetoceros sp*.; B: *Melosira sp.;* C: *Thalassiosira* cf. *antarctica* Comber, D: *Thalassiosira sp*.; E: *Eucampia antarctica* (Castracane) Mangin

(resting spore valve); F: *Navicula sp.;* G: *Navicula* cf. *perminuta* Grunow; H: *Pseudogomphonema* cf. *kamtschaticum* (Grunow) Medlin; I*: Synedropsis sp.*; J: Extruded polystyrene foam piece covered with pennate diatoms of genus *Synedropsis* (overview with 30x magnification); and K: 500x magnification of the polystyrene foam for better visualization of microalgae.

We highlight the extruded polystyrene foam fragment completely covered with pennate diatoms of the genus *Synedropsis* (Figure 5J-K). Coccoid and filamentous bacteria were observed adhered to plastic fragments, lines and spheres (Figure 6A, B) and in the paint fragments we identified coccoid and elongated cells (Figure 6D, E), forming large colonies in some cases. We also identified other non-diatom microalgae species, as well as a chrysophyte and invertebrate organisms, in the Antarctic Plastisphere (Figure 6C,F).

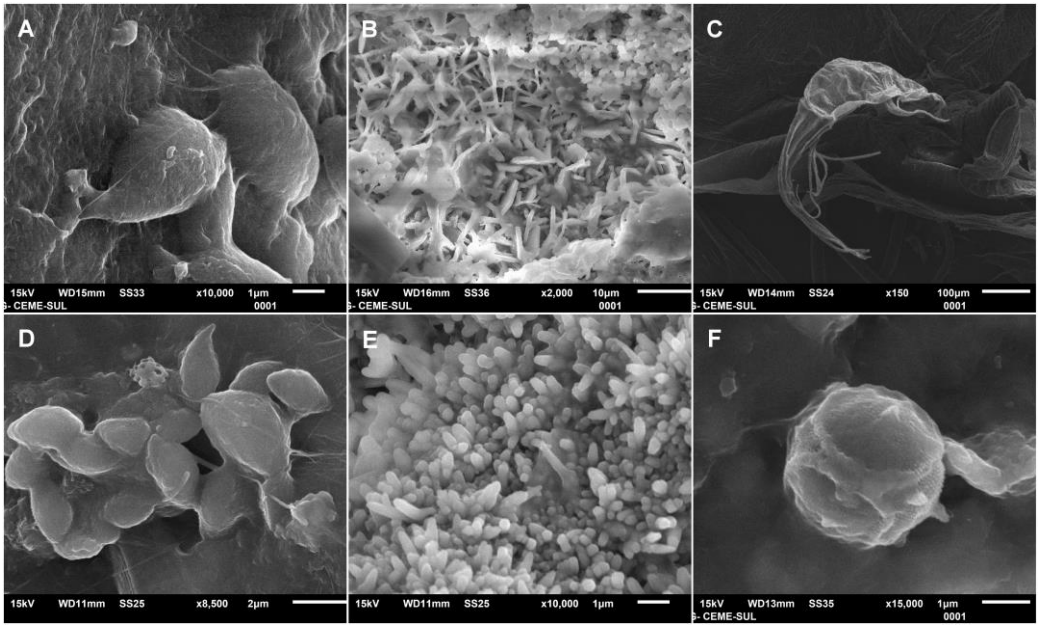

Figure 6. Groups of organisms attached to plastics and paint fragments sampled at surface waters in Antarctica. Coccoid (A) and elongated bacterial (B) colonies, and marine invertebrate (C) adhered to marine plastics; coccoid (D) and elongated cells of bacteria (E) and *Tetraparma*-like microalgae (Chrysophyceae - Parmales) (F) adhered to paint fragments.

Discussion

We estimated a mean concentration of 1,794 plastic items.km^{-2} around the Antarctic Peninsula. This concentration lies within the estimated density range of plastic pollution for more than 70% of the world's oceans: from 1000-100,000 pieces.km^{-2}, although closer to the lower end[5]. However, it is a low value when compared to accumulation zones such as the center of the subtropical gyres, e.g. the ‘Great Pacific

Garbage Patch' (with > 700,000 pieces.km^{-2})[2,5] or the Mediterranean (> 800,000 pieces.km^{-2})[5]. In any case, considering that Antarctica is an uninhabited area that has only the presence of vessels and research stations, and is an environment with unique biodiversity and ecological relations, this concentration of plastics is alarming and could cause serious environmental damage.

The concentration of plastics in surface waters of Antarctica is still poorly known. Barnes & Milner[32] reported from 0-1 items.km^{-2} of floating marine debris in the surroundings of the Antarctic Peninsula. Microplastics in waters of the Ross Sea have also been described, with similar concentrations (0.0032 to 1.18 particles.m^{-3} [33]) to what was found in the present study, likely due to the proximity between sampling areas. On the other hand, Isobe et al.[29] estimated higher average concentrations for two stations at the eastern portion south of the Polar Front close to the Antarctic continent, with 9.9 x 10^{-2} and 4.6 x 10^{-2} pieces.m^{-3}. Additionally, surface waters of the Southern Ocean have been estimated to have from 0.55 to 56.58 g.km^{-2} of plastic fragments[5]. The total average weight of plastics that we found (27.8 g.km^{-2}) lies within this estimated weight range of plastics at the surface of the Southern Ocean.

We report a predominance of fragments and lines smaller than 5 mm, showing that the majority of sampled particles are secondary microplastics originating from larger pieces that fragmented due to weathering in the marine environment[23]. In the "sphere" category we found two pellets that could likely be originated from lower latitudes[25] as there are no local sources of pellets, and according to our model they had probably been in Antarctica for at least seven years if we assume that they had been at the ocean surface for this time. These pellets may also have been retained in ice and/or beaches, being released into the ocean due to melting ice and meteorological events. The large number of nylon line fragments indicates that fishing activities, including IUU (illegal, unreported and unregulated) fishing, occurs around Antarctica and can be a source of plastics for the local environment[25].

Polyethylene and polypropylene are the two most common plastics found in other parts of the world's oceans[22,34], but not around the Antarctic Peninsula. At this region, we found more polyurethane and polyamide, supporting our hypothesis that the main sources of plastics to the Southern Ocean are local, as polyurethane is frequently used in insulation panels, high-resilience foam seating, electrical potting compounds, surface coatings and surface sealants[35], which are common at research and tourism vessels and research stations. Additionally, polyamide is a characteristic polymer of fishing nets and ropes[24].

The expanded polystyrene pieces found in our samples are also typically used in packaging and fishing gear[36]; fishing-related debris in Antarctic waters have been previously reported by Convey et al.[24] and Ivar do Sul et al.[25]. Single-use plastics made of polyethylene and polypropylene were also found in our samples, albeit in smaller numbers, and could represent a problem to the Southern Ocean.

Although the dispersal model showed that the plastics we collected had most likely been south of 58ºS for at least seven years or have been released through local sources more recently, it is worth mentioning that they could still have been lost/discarded at lower latitudes and dispersed to and accumulated in Antarctica. Isobe et al.[29] speculated that the absence of relatively "fresh" mesoplastics (from 5 to 20 mm) in their samples could be an indication that the sources of debris are far from the Southern Ocean. However, based on our dispersal model and what has been suggested in other studies[24,25,30,33], the main sources of plastic at the region, especially around the Antarctic Peninsula, most likely involve local research, tourism and fishing activities. Station 4, that presented sources closer to the continent, is located at a sheltered area close to the peninsula, and could be influenced by coastal currents that retain plastics.

Due to the isolation of the Antarctic Peninsula, and especially the islands around it, marine debris that reaches the coastline can accumulate for many years. These materials can then re-enter the oceans due to wind transport, ice melting, rising/falling sea levels and storms especially smaller particles that are easily carried[24]. Ocean current systems in the western portion of the Antarctic Peninsula, with several convergence zones, can explain the retention of plastics for years within this region, as suggested by Isobe et al.[29] and confirmed by our model. The Antarctic Circumpolar Current, which flows eastwards around the Antarctic continent[37], may also retain plastic particles within its flow, creating a plastic accumulation zone around the continent that can eventually be dislodged from the system due to events such as storms and vortex formation. At a smaller scale, the Bransfield Current system may form another accumulation zone at the western portion of the Antarctic Peninsula[38], keeping the plastic particles at the ocean surface of this area for years.

Paint fragments were present at all sampling stations and presented abundance (total n = 2805) of approximately 30 times that of plastics. A similar pattern was observed by Song et al.[39] in surface waters of South Korea's southern coast, where the authors found around 12 times more paint particles, in different sizes and colors, than plastics. We believe that the paint chips were already at the ocean surface at the time of sampling,

since the manta net is lowered using an A-Frame at approximately two meters from the ship and the net mouth does not touch the ship at any time, reducing the chance of contamination. Another indication that the paint fragments were in the ocean is the presence of paint colors that do not belong to our ship (e.g. blue, orange etc.). During sampling, we took care as to avoid the ship's wake, and considering that we found types of plastics and paint that were not present on our ship, and that biofilm was formed on most fragments, we can infer that most sampled items had already been in the environment for some time. However, it is possible that part of the sampled paint originated from our ship since most were of the colors of the hull (red) and deck (green) (confirmed by FTIR spectra). This could have occurred due to previous shedding, as the ship remains around the Antarctic Peninsula for five to six months every year, or from the net occasionally entering the wake due to wave influence. Although they are denser than seawater, paint particles can float due to water surface water tension[39]. A concerning characteristic of paints used on ships and nautical apparatus is the presence of metals such as Cu, Zn and Pb, and booster biocides used to prevent growth of marine organisms such as sessile invertebrates and algae[40,41]. The impacts of paint fragments in the marine environment may be similar to those of plastics in terms of ingestion and contaminant transfer/biomagnification[42], as well as attachment and transport of epiplastic organisms (Figure 6D-F).

Our results showed that plastic and paint fragments in Antarctic waters are a substrate for several species, with the identification of a variety of organisms living on the surface of marine plastics. We found more diatoms when compared to other taxonomic groups, which may be due to the sample preservation method, since freezing and rapid dehydration of the material may have ruptured cells or delicate structures of epiplastic organisms. Epibenthic diatoms (e.g. from genus *Synedropsis*; Figure 5) were common on floating plastics, which can increase the dispersal rates of these organisms and possibly alter the functioning of the system and compromise ecological relations. Arias-Andres et al.[21] showed that epiplastic organisms influence organic matter cycles in aquatic environments; this could also occur in Antarctica. In addition, although our model showed that it is unlikely that particles arrived from lower latitudes in the last seven years, we cannot discard the risk of bioinvasions resulting from the transport of epiplastic organisms from lower latitudes, or between different Antarctic biogeographic regions. Such risk is concerning for the biodiversity of the Southern Ocean, which is currently suffering from the invasion of species such as the crab *Hyas araneus*[43] and the mussel *Mytilus gallo-*

provincialis[44]. Environmental changes such as ocean acidification and sea surface temperature increase can lead to a greater chance of non-native species reaching and settling in Antarctica via plastics[45,46]. By growing on and interacting with marine plastic, the organisms of the plastisphere can become contaminated and transfer these contaminants to the organisms that ingest colonized plastics[47]. Epiplastic organisms could also impact the microflora of consumers, since infectious organisms may reach their hosts through plastic ingestion[12,14]. Studies on the microbial communities of marine plastics are still relatively recent, with central issues being focused on the colonization processes, diversity and stability of these communities[48]. Although SEM allows a detailed view of the surface of plastics, it is limited in terms of taxonomic resolution of organims[48]. This reinforces the need for studies using alternative identification tools, such as environmental DNA sequencing (i.e. metagenomic analyses), to characterize the Antarctic epiplastic communities, better revealing their components and ecological impacts.

As previously mentioned, marine plastic pollution can have several effects on the Antarctic ecosystem[24]. The interaction of marine organisms with plastics in Antarctica has already been described in some studies, which show that entanglement and ingestion affect different species of mammals and birds at the region[25]. The shallow waters of the Bransfield and Gerlache straits, where some sampling points were located, are nursery areas for organisms such as krill, a key component of the Antarctic food web[49]. Considering that most of the plastics sampled in this study fell into the 'microplastics' category, they could be ingested by and impact krill, as well as other primary consumers, affecting the marine trophic web[50].

Waller et al.[30] highlight that microfibers from synthetic clothes washed at research stations and vessels enter Antarctic waters, especially due to inadequate waste treatment systems and limited on-site inspection. We did not detect any microfibers due to our net's mesh size, but reaffirm from personal observation that wastewater may be a large source of microplastics at the area. Persistent organic pollutants used in the production of or adsorbed to marine plastics (and paint fragments), which are especially concentrated on microplastics, can have serious consequences such as alteration of growth and reproductive hormones, oxidative stress and reduction of fertility[47,51,52]. The ingestion of plastics by marine organisms has also been demonstrated to reduce energy reserves[9], potentially leading to their death. Another potential impact of marine plastics occurs during the physical and chemical breakdown of the polymer chain, when carbon

is released and transformed into CO_2[53], which can contribute to the creation of an anoxic environment and ocean acidification. Finally, plastic degradation can release other greenhouse gases such as methane and ethylene[54], possibly contributing to climate change.

The results obtained here show that the abundance of plastics in Antarctica is not comparable to high concentration areas such as the center of subtropical gyres or highly urbanized coastlines. However, due to the unique characteristics of this environment, it could be highly sensitive even to low levels of this type of pollution. Plastic pollution in the Southern Ocean has been described since the 1980s, with several studies raising concern regarding this issue[25], but in accordance with the global scenario, little has been done to effectively reduce the amount of plastics entering the Antarctic environment. If the prevention and mitigation of plastics continues to lag behind its production and inadequate management, we can expect increasing accumulation of plastic waste at the region, since plastic debris from local sources can be retained in Antarctica for long periods due to the closed system created by the Antarctic Circumpolar Current.

The abundance of plastic found at a remote and theoretically pristine region such as the Antarctic Peninsula shows the extent of human influence and the potential irreparability of its impacts on the oceans, reasserting an urgent need for decreasing the production and consumption of plastics, and increasing adequate discard and management practices. The concentration of plastics in Antarctic surface waters reinforces that, despite efforts to limit human use, this region is not exempt from marine plastic pollution. This is possibly due to the lack specific measures and enforcements for solid waste treatment at the area. Antarctica is a world heritage site and one of the most productive oceanic regions on planet, with unique biodiversity, which highlights the importance of elaborating and adopting strategies to conserve this environment. The abundance of paints from nautical vessels/apparatus shows that even the limited scientific and tourist activities are a potential source of pollution in Antarctica.

We call for urgent action to avoid plastic and paint fragment inputs to the Southern Ocean, with the implementation of adequate waste management and treatment. We suggest that environmental awareness initiatives with tourists, researchers, ship crews and fishers that use areas around Antarctica be expanded and obligatory. Additionally, further studies should be conducted at the region to increase our understanding of the impacts of plastics to the Antarctic ecosystem, e.g. in terms of entanglement, ingestion and contaminant uptake by marine animals. Finally, a more detailed description of epiplastic

communities in the Southern Ocean is fundamental to understand the impact of these organisms on the local and global marine environment.

Methods

Sampling

In February 2017, during the XXXVI Antarctic Operation and 7th expedition of project "Biological Interactions in Marine Ecosystems off the Antarctic Peninsula Under Different Impacts of Climate Change" (INTERBIOTA), samples were collected in surface waters, at the water-air interface, around the Antarctic Peninsula. These samples were taken at 12 points, between latitudes of 61º and 64ºS, using a manta net with a 100 cm x 21 cm mouth and a 330 μm mesh (Figure 7). At each point, the net was lowered carefully with a large A-frame at approximately 2 m from the windward side of the ship, and was trawled at a speed of 2.5-3.5 knots for between 15-55 minutes. After each trawl, the contents of the collection cup were placed in an aluminum bag and frozen at -40ºC for posterior sorting and analysis.

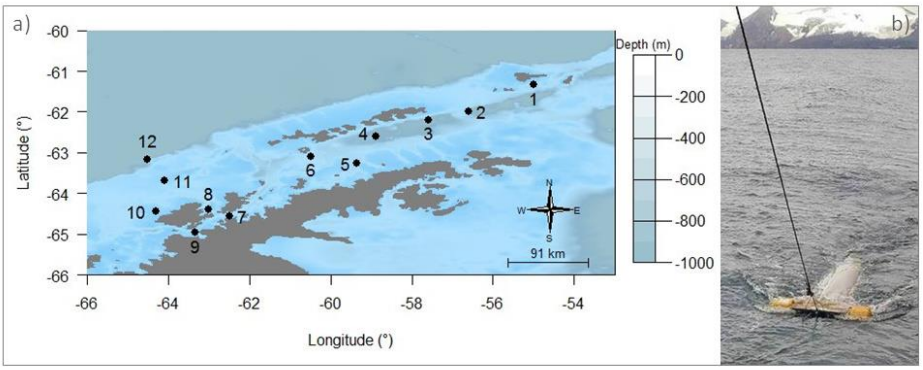

Figure 7. Sampling area of marine plastics in surface waters of twelve points off the Antarctic Peninsula (a), using a manta net (b). The map was created using the *marmap* package and *getNOAA.bathy* function on *R* 3.5.0 (https://www.r-project.org).

At the start and end of each trawl we noted the geographical coordinates, time, sea state (evaluated by the ship's officer in charge), local depth and wind speed. The trawled area of each point was calculated based on trawl velocity (considering 1 knots as 0.514 $m.s^{-1}$; *trawl vel*), time (seconds; *t*) and the manta net width (1 meter), and expressed by the equation:

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

The sampled volume was calculated considering the trawled area and full submersion (i.e. 21 cm depth) of the posterior end of the manta net.

Plastic count and characterization

In the laboratory, we thawed the sampled material of each point separately, and placed it in a sterile container filled with salt water (salinity 35, temperature ~4°C) for manual separation of floating plastic pieces and biomass[36]. Plastics were identified by naked eye (lower detection limit of approximately 500 microns) by a trained observer (ALdFL) picked up using forceps, and quantified and measured over their largest cross-section (total length) using a digital caliper. In terms of size, plastics were classified as microplastic (<5 mm), mesoplastic (5 - 200mm) or macroplastic (>200mm) (adapted from Eriksen et al, 2014)[5]. Each item was weighed with a digital scale (precision of 0.00001g) and also classified according to format (fragment, line, and sphere), flexibility (rigid, flexible) and color.

Polymer composition was determined for 28 plastic pieces selected randomly, through Fourier Transform Infrared Spectroscopy (FTIR) with an equipment SHIMADZU, model Prestige 21, using a diffuse reflectance module, 24 scans and 4 cm^{-} resolution. To estimate the concentration of plastics at the sea surface, the number and weight of plastics found in the trawled areas was extrapolated to items.km^{-2} and to g.km^{2} to each sampling point, respectively. The total average concentration and weight of plastics were also calculated. Paint fragments were not included in the concentration analyses and other statistics, being characterized only by color, weight and size classes, as proposed by Song et al.[39] (see section on paint particles in the results). Paint was identified by: 1) visual characteristics of the particle, which were thin, flat and flexible paint chips; and 2) FTIR spectra of 21 samples of paint chips, which indicated their primary polymer as polyurethane, commonly used in paint production.

Analysis of epiplastic communities

For evaluation of epiplastic communities through Scanning Electron Microscopy (SEM), items from different sampling points were selected from categories ‘fragment’ (n = 8), ‘sphere’ (n = 3) and ‘line’ (n = 3), in a total of 14 plastic pieces. Eight paint fragments were also selected for this analysis, totalizing 22 items. Before SEM, plastic and paint fragments were dehydrated in absolute ethanol (Reagent-grade, MERK). The items were fixed to an aluminum sheet with carbon tape and coated with a 20-30 nm gold layer. The epiplastic organisms were observed using a JEOL microscope (JSM 6610LV, JEOL,

Tokyo), operated at 10-20 kV at a working distance of 10-26 mm. For each fragment, the whole item was imaged at a magnification of 25x, followed by imaging at different magnifications (20x to 40,000x) to better record the diversity of organisms. A total of 100 images were evaluated, and the identified individuals were grouped into taxonomic groups (i.e. diatoms, bacteria, etc.) based on morphology, and we attempted to identify the organisms at the lowest possible taxonomic level with the aid of experts of each group.

Data analysis

To determine if environmental parameters influenced the abundance of plastics at each sampling point, linear regressions were performed between plastic concentration and the parameters local depth, sea state and wind speed, recorded during sampling. To evaluate diversity of plastic colors between the sampling points, we used Shannon's index, and to check if there was dominance of any colors between the points, we used Simpson's index. All statistical analyses were done in R (R Development Core Team, version 3.5.0), using the *car* and *vegan* packages.

Dispersal model

Virtual particles were tracked using the OceanParcels framework[55] in surface velocity fields from the HYCOM + NCODA Global 1/12° Analysis[56], on which Stokes drift was added from WaveWatchIII[57]. At each of the 12 locations, 100 virtual particles were released on the day of sampling, and then tracked back in time for seven years, with output stored daily. To simulate subgrid scale motion, a Brownian diffusion of 10 m^2/s was added. The code for these simulations is available at https://github.com/OceanParcels/AntarcticPeninsulaPlastic.

Data availability

The datasets generated during and/or analyzed during the current study are available in FIGSHARE (doi: 10.6084/m9.figshare.7491641) and from the corresponding author upon reasonable request.

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Author Contributions

ALdFL and MCP conceived the research. ERS and MCP contributed with sampling. EvS conducted the oceanographic modeling. ALdFL FK performed lab work. ALdFL LSR LR FK FLR ERS MCP analyzed the data. ALdFL wrote the first draft of the paper, and all authors contributed to editing the manuscript.

Competing Interests

The authors declare no financial and non-financial competing interests.