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Lara Mesquita Pinheiro

Microplásticos, suas interações com organismos bentônicos e distribuição nas praias da
Ilha da Trindade (Brasil)

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2017

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Dissertação apresentada ao Programa de Pós-Graduação em Oceanografia do Departamento de Oceanografia da Universidade Federal de Pernambuco, como requisito para obtenção do grau de Mestre em Oceanografia.

Orientadora: Profa. Dra. Monica Ferreira da Costa

Co-orientadora: Profa. Dra. Juliana A. Ivar do Sul

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BANCA EXAMINADORA

Profa. Dra. Monica Ferreira da Costa
(Orientadora/Presidente/Titular interna PPGO)

Prof. Dr. Pedro de Souza Pereira
(UFPE/Titular interno-PPGO)

Profa. Dra. Monica Lucia Botter Carvalho
(UFRPE/Titular externo)

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“Voltei
Mais uma vez voltei pra teus braços
Tenho o corpo fechado
Minha vida é o mar”

Dorival - Academia da Berlinda

Abstract

The intense pollution on marine and coastal environments have important aspects such as the production and inappropriate disposal of plastic items. These widely used polymers usually accumulate and degrade on those environments forming particles smaller than 5mm called microplastics. These particles present many risks to both coastal environment and biota such as ingestion, blockage of digestive and/or respiratory pathways and toxicological effects caused either by the polymer or by associated pollutants. This work had two objectives corresponding to two chapters of this document: (1) to perform a literature review about microplastic interaction with the coastal environment, focusing on the benthic compartment; (2) to characterize microplastic pollution on sandy beaches of Trindade island, on Espírito Santo state. In the first chapter, 52 articles were analysed, addressing seven animal phyla. This number of works on this issue is relatively small, and mainly laboratorial. It was found that the effects of microplastic ingestion are being reported since the beginning of this century. In general, it was shown that factors such as microplastic characteristics, laboratory methodologies, microplastic concentration and distribution on the sediment are determinant on this type of work. Therefore, there is lack of methodology standardization for microplastic analysis in sediment, as well as a more relevant ecological approach that involves both field and laboratory experiments. In the second chapter, microplastics were isolated from sediment samples from Trindade island using a density separation method. It was found that this island, despite its remote location, is widely contaminated with microplastics smaller than 1mm. Microplastics were found in the shape of fragments and fibres, with densities of up to 311 fragments or 333 fibres per m². Microplastic deposition dynamics in sediment is strongly related to current, wind and tidal systems. However, factors affecting this dynamic for microplastics smaller than 1mm remains unclear. Considering that Trindade island has high ecological importance, these results show that future studies are extremely necessary to determine the risks to which the island's coastal ecosystem is submitted to.

Keywords: Benthic fauna. Oceanic islands. Plastic pollution. Saline flotation. Sandy beaches. Small microplastics.

Resumo

A intensa poluição dos ambientes costeiros e marinhos têm como importante aspecto a produção e descarte inadequado de itens plásticos. Esses polímeros amplamente utilizados pela sociedade comumente acumulam e se degradam nestes ambientes, formando partículas menores do que 5 milímetros chamadas de microplásticos. Tais partículas apresentam diversos riscos ao ambiente costeiro e à biota, como ingestão, bloqueio de vias digestivas e/ou respiratórias e efeitos toxicológicos causados pelos polímeros em si ou por poluentes associados. Este trabalho teve dois objetivos que correspondem aos dois capítulos desse documento: (1) realizar revisão bibliográfica sobre a interação dos microplásticos com o ambiente costeiro, focando no compartimento bentônico; (2) caracterizar a poluição por microplásticos nas praias arenosas da Ilha de Trindade, no estado do Espírito Santo. No primeiro capítulo, 52 artigos foram analisados, abordando sete filões de animais. Esse número de trabalhos tratando dessa problemática é relativamente pequeno, e na sua maioria de laboratório. Viu-se que os efeitos da ingestão de microplásticos por organismos bentônicos vem sendo reportados desde o começo do século. No geral, viu-se que fatores como as características dos microplásticos, metodologias de laboratório, concentração e sua distribuição dos microplásticos no sedimento são determinantes nesse tipo de trabalho. Portanto, falta uma padronização de metodologias para análise dos microplásticos em sedimento, assim como uma análise ecológica mais relevante que envolva experimentos de campo e laboratório. No segundo capítulo, microplásticos foram isolados de amostras de sedimento da ilha de Trindade. Viu-se que a ilha, apesar da sua remota localização, está amplamente contaminada com microplásticos menores que 1mm. Microplásticos foram encontrados tanto no formato de fragmentos quanto de fibras, com densidades de até 311 fragmentos e 333 fibras por m² de sedimento. A dinâmica da deposição de microplásticos em sedimento é fortemente ligada aos sistemas de corrente, ventos e maré. Entretanto, fatores que afetam essa dinâmica para microplásticos na faixa de tamanho menor que 1mm permanece incerto. Considerando que a ilha de Trindade é um ambiente de grande importância ecológica, esses resultados mostram que estudos futuros são necessários para determinar os riscos aos quais o ecossistema costeiro da ilha está submetido.

Palavras-chaves: Fauna bentônica. Flutuação salina. Ilhas oceânicas. Pequenos microplásticos. Poluição por plásticos. Praias arenosas.

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1 INTRODUÇÃO GERAL

A poluição dos ambientes marinhos e costeiros por lixo antropogênico é crescente no mundo inteiro, representando um problema de grande importância (GALGANI; HANKE; MAES, 2015). Estima-se que mais de 40% da população mundial habita em regiões costeiras, i.e. a 100km da costa (BOLLMAN et al., 2010). Como consequência, enormes quantidades de lixo acabam sendo jogadas nos oceanos todo ano (JAMBECK et al., 2015).

A maior parte desse lixo é composta de plástico (BARNES et al., 2009). Esses polímeros sintéticos, indispensáveis para o atual modelo de sociedade, são derivados da polimerização de monômeros extraídos do petróleo ou gás natural (VIKAS; DWARAKISH, 2015). Isso garante que esse material apresente leveza, durabilidade, flexibilidade e baixo custo (RYAN, 2015). Consequentemente, itens plásticos são extremamente difíceis de serem degradados e por esse motivo têm causado inúmeros problemas no ambiente marinho (BARNES et al., 2009).

Jambeck e colaboradores (2015) estimaram que em 2010, 1,5 a 4,5% do plástico produzido no mundo teve como destino final os oceanos. Isso representa cerca de 4 a 12 milhões de toneladas de plástico por ano se tornando disponíveis no mar para interação com a biota e com o meio abiótico. Tais evidências levaram as autoridades mundiais e a comunidade científica a reconhecer a seriedade do problema do plástico no mundo (NATIONAL RESEARCH COUNCIL, 2009).

Os tipos mais comuns de plásticos encontrados no ambiente são polietileno (PE), polipropileno (PP), poliestireno (PS), poliéster, poliamida, policloreto de vinila (PVC), politereftalato de etileno (PET) e poliuretano (HIDALGO-RUZ et al., 2012). A diferença de densidades específicas de cada tipo de polímero em relação a da água do mar faz com que diferentes itens se encontrem em diferentes posições no compartimento ambiental costeiro e marinho (Tabela 1). Uma vez no mar, plásticos flutuando na água são transportados pela ação de ventos e correntes superficiais, podendo ser carregados por grandes distâncias e se acumular em todos os ambientes marinhos do mundo, incluindo-se costas e o fundo oceânico (ZALASIEWICZ et al., 2016).

Tabela 1: Principais tipos de plástico encontrados no ambiente marinho, suas aplicações e densidades. Adaptado de Hidalgo-Ruz et al. (2012).

	TIPO DE PLÁSTICO	APLICAÇÕES COMUNS	DENSIDADE (g cm ⁻³)
Densidade menor que a água do mar (1,03 g cm ⁻³)	Poliétileno (PE)	Sacolas plásticas, embalagens de latinhas	0,917-0,965
	Polipropileno (PP)	Cordas, tampas de garrafa, cintas	0,90-0,91
	Poliestireno (PS)	Caixas de isca, flutuadores, copos descartáveis, utensílios	1,04-1,1
	Poliamida ou nylon	Cordas, redes	1,02-1,05
Densidade maior que a água do mar (1,03 g cm ⁻³)	Resina de poliéster + fibras de vidro em tecidos	Tecidos	1,24-2,3
	Acrílico	Substituição ao vidro, luminárias, material de desenho	1,09-1,2
	Policloreto de vinila (PVC)	Filmes, tubos, recipientes	1,16-1,58
	Politereftalato de etileno (PET)	Garrafas, cintas, engrenagem	1,37-1,45
	Poliuretano	Pneus, mobílias, colchões, assentos	1,2

Fonte: A autora

O acúmulo de plásticos nos oceanos traz sérias consequências aos organismos marinhos. Efeitos como emaranhamento e ingestão de itens plásticos já foram amplamente reportados em diversos grupos animais (WANG et al., 2016). Plásticos podem servir também como carreadores de substâncias hidrofóbicas que aderem à sua superfície como poluentes orgânicos persistentes (POPs) que podem trazer efeitos tóxicos aos organismos e ao ambiente (BAZTAN et al., 2014; ROCHMAN, 2013). Além disso, uma vasta microbiota também pode se associar à superfície dos plásticos, podendo representar riscos de invasão de espécies exóticas (KIESSLING; GUTOW; THIEL, 2015) e de patogenicidade (KIRSTEIN et al., 2016).

Outro problema associado a presença de itens plásticos nos ambientes costeiros e marinhos é que eles podem sofrer processos de degradação, dando origem a partículas menores de plástico chamadas de microplásticos (BROWNE; GALLOWAY; THOMPSON, 2007). Esses fragmentos menores que 5mm podem ser classificados de acordo com sua origem em primários ou secundários. Microplásticos secundários são originados da fragmentação de itens maiores, enquanto que microplásticos originados de tecidos sintéticos usados na fabricação de roupas, microesferas utilizadas em cosméticos e indústrias petroquímicas na forma de pellets (BOUCHER; FRIOT, 2017) são chamados de microplásticos primários.

Outra classificação para microplásticos foi recentemente proposta por Hanvey et al. (2017) baseado em outras classes de tamanho. Microplásticos na faixa de 1 a 5

milímetros são classificados como microplásticos grandes, enquanto que microplásticos menores do que 1 milímetro podem ser chamados de microplásticos pequenos (HANVEY et al., 2017).

A presença de microplásticos no ambiente marinho foi detectada pela primeira vez nos anos 1970 (CARPENTER; SMITH, 1972). Entretanto, apenas recentemente estudos vêm retratando a ampla distribuição desse poluente nos ambientes marinhos e costeiros e seus efeitos negativos no ambiente e nos organismos (IVAR DO SUL; COSTA, 2014). Esses efeitos são agravados pela alta relação superfície/volume que essas partículas apresentam, podendo então carregar quantidades significativamente maiores de poluentes associados (TEUTEN et al., 2009).

Devido ao seu pequeno tamanho, microplásticos podem ser ingeridos por uma grande variedade de organismos marinhos. Os efeitos dessa ingestão já foram demonstrados tanto em vertebrados, como aves marinhas, tartarugas e mamíferos (LUSHER et al., 2015; PROVENCHER et al., 2016); peixes pelágicos e demersais (DAVISON; ASCH, 2011; LUSHER; MCHUGH; THOMPSON, 2013) tanto quanto em vários invertebrados (IVAR DO SUL; COSTA, 2014). A toxicidade dos microplásticos pode ser causada tanto pela ingestão das partículas em si – danos físicos - quanto por contaminantes associados a eles - toxicidade (IVAR DO SUL; COSTA, 2014; ROCHMAN et al., 2015).

Microplásticos são tratados como poluentes ubíquos dos ambientes aquáticos e marinho no mundo inteiro (WRIGHT; THOMPSON; GALLOWAY, 2013). Há trabalhos com microplásticos em água doce (WAGNER et al., 2014), sedimentos de praia (LOZOYA et al., 2016) até o fundo oceânico (WOODALL et al., 2014); em águas costeiras (LI et al., 2016) e de mar aberto (GOLDSTEIN; TITMUS; FORD, 2013) e até em ambientes isolados como ilhas oceânicas (IVAR DO SUL; COSTA; FILLMANN, 2014; YOUNG; ELLIOTT, 2016) e regiões polares (WALLER et al., 2017).

2 OBJETIVO GERAL

O objetivo geral desse trabalho de dissertação foi caracterizar a poluição por microplásticos em praias da Ilha de Trindade, Oceano Atlântico (20° 31' 29" S, 29° 19' 29" W).

Os objetivos específicos foram então:

1. realizar revisão bibliográfica sobre a interação entre microplásticos e a fauna bentônica, especialmente de sedimentos inconsolidados;
2. analisar amostras de sedimentos de praias da Ilha da Trindade para diferentes frações de tamanho dos microplásticos primários e secundários.

3 MICROPLASTICS AND BENTHIC FAUNA: HOW DO THEY INTERACT?

3.1 Introduction

Plastics are an essential part of societal life from past decades to the present. They are durable, flexible and resistant to heat, and so indispensable everywhere in the world. However, its indiscriminate disposal has been causing consequences to both terrestrial and marine environments (BROWNE; GALLOWAY; THOMPSON, 2007; HUERTA LWANGA et al., 2016). Then, the interest of the scientific community increased substantially in the last years mainly regarding microplastic pollution (COLE et al., 2011; IVAR DO SUL; COSTA, 2014).

Microplastics are plastics particles smaller than 5 millimetres that originate from the degradation and fragmentation of larger items (secondary microplastics) and from cosmetics such as facial scrubs and toothpastes for example (primary microplastics) (COLE et al., 2011; THOMPSON et al., 2004). They are now treated as a new category of pollutant, and so different monitoring strategies and ecological effects approaches are being reported in the literature (AVIO; GORBI; REGOLI, 2016). Environmental and food safety authorities in different countries are also gathering efforts to assess microplastics pollution in water, biota and sediments (e.g. NOAA Marine Debris Program; UK/EU Marine Strategy Framework Directive).

Microplastics have been ingested by organisms from different marine trophic levels, from top predators such as birds, turtles and mammals (LUSHER et al., 2015; PROVENCHER et al., 2016), to pelagic (CHOY; DRAZEN, 2013; DAVISON; ASCH, 2011) to demersal fishes (LUSHER; MCHUGH; THOMPSON, 2013) and invertebrates (IVAR DO SUL; COSTA, 2014).

The small size of microplastics indicates that they can be ingested by small organisms, from benthos and plankton and being potentially transferred to other trophic levels, where they can cause substantial damage to entire ecosystems and reaching seafood products. Benthic environments, especially loose unconsolidated sediments that allow movement between grains, are both a sink and source of microplastics to organisms in marine food webs (BROWNE et al., 2011). Benthic fauna living in or on the sediment, from shores to the deep sea, are then in potential risk of interaction with microplastics,

mainly near developed coasts (BOLLMAN et al., 2010; VIKAS; DWARAKISH, 2015). It is also relevant to know if and how these plastics are transferred to successive trophic levels characterizing its biotransference (SANTANA; MOREIRA; TURRA, 2016).

It is therefore crucial to understand how organisms inhabiting and feeding in benthic habitats interact and are affected by microplastic pollution (ANDRADY, 2011; WRIGHT et al., 2013). The available literature is a valuable source to identify potential gaps in ecological studies related to the interactions between benthic fauna and microplastics. Therefore, the aim of this literature review was to assess factors that interfere on microplastic interaction with benthic fauna on the sediment. This work expects to list and analyse the main research gaps to delineate future studies in the topic.

3.2 Background Literature

Articles were searched in Scopus (<https://www.scopus.com>) and Web of Science (<https://www.webofknowledge.com/>). Keywords (*microplastic* and *ingestion*; *microplastic* and *benthic*) were used in two independent searches for articles published until May 2017. For this work, all plastic particles <5mm were considered “microplastics”, although some authors consider other categories that include smaller size limits (HANVEY et al., 2017).

The hundreds of articles recovered were then sorted for redundancies and filtered to select only the most relevant literature (53 documents). Articles attending one of the following criteria was analysed: (i) if ingested microplastics are observed and/or quantified in gut contents and/or gills of marine benthic animals; (ii) if microplastic are related to biological effects; (iii) if tools/techniques were used during research or laboratory work are reported and; (iv) quality of documents (preferred peer-reviewed papers). Selected papers were then analysed according to: 1) year of publication; 2) experimental approach (field or laboratory work); 3) animal group assessed; 4) microplastic sizes and concentrations; 5) exposure time, when laboratory experiment; and 6) effects of microplastic ingestion to organism development and survival. Each one of these approaches are discussed here in terms of achievements and suggestions for future works.

3.3 Publication Timeline

Eighty percent of the analysed papers were published in the last 5 years, showing a recent and rapid increase of interest on aspects related to microplastic ingestion by benthic biota (Table 2), as also observed for other topics on microplastic studies (e.g. IVAR DO SUL & COSTA 2014). Hart et al. (1991) were the first to describe plastic ingestion by echinoderm (planktonic stage larvae) during laboratory experiments with concentration of 2.4 microspheres μl^{-1} in seawater. This was followed by others (BOLTON; HAVENHAND, 1998; BRILLANT; MACDONALD, 2000, 2002; LEI; PAYNE; WANG, 1996) which used microplastics as a tool to describe and analyse physiological aspects of molluscs and annelids. Although synthetic microparticles were not the focus of experiments at that time, potential impacts to organism have been reported and consequently bring new insights to subsequent studies on microplastic ingestion and accumulation in the digestive tract of benthic species.

Table 2: Selected articles on microplastic ingestion by benthic fauna. C: carnivore; FF: filter feeder; D: detritivore; O: omnivore; P: predator; S: scavenger; DF: deposit feeder; SF: suspension feeder; Can: cannibal; G: grazer; H: herbivore; L: laboratory; F: field; A: acrylic; PE: polyethylene; HDPE: high-density polyethylene; LDPE: low-density polyethylene; PS: polystyrene; PP: polypropylene; PVC: polyvinyl chloride; PA: polyamide; PES: polyester; PET: polyethylene terephthalate; CF: cellophane; PLA: polylactic acid; DB: divinylbenzene; PMA: polymethylacrylate ; PVA: polyvinyl-alcohol; DW: dry weight; SW: seawater; WW: wet weight. NA: -. Bold in “feeding type” indicate information from the article; other feeding types were consulted at WoRMS (2017) and FishBase (2017) websites.

	FEEDING TYPE	TAXA	SETTINGS	POLYMER	SHAPE	SIZE	EXPOSURE	CONCENTRATION	REF. *
EPIFAUNA	C FF	Crustacea Mollusca	L	PS	microspheres	0.5 µm	up to 21 days	50 µl (411 million particles)	1
	C, P	Chordata	F	A, PA, PES, LDPE, PS, Rayon	fragment, fibre, bead, film	0.13 – 14.3 mm	-	1 – 15 pieces per individual; average 1.90 ± 0.10 pieces per individual;	2
	C, P	Chordata	F	PA, PET, PES, Nylon, A, PE	fibres	not informed	-	not informed	3
	O, P, S C, P	Crustacea Chordata	L, F	PE, PP	balls, strands	5 mm	24 hours	not informed	4
	O, P	Crustacea	L	PP	fibres	500 µm	4 weeks	0% (0 mg), 0.3% (0.6 mg), 0.6% (1.2 mg), 1% (2.0 mg) to 2g food	5
	O, P	Crustacea	L	carboxilated or aminated PS	microspheres	8 µm	1, 16, 24 hours	10 ⁻⁶ or 10 ⁻⁷ microspheres l ⁻¹	6
	FF	Mollusca	L	PE, PS	microspheres	<100 µm	7 days	1.5g l ⁻¹ SW	7
	FF	Annelida	L	not informed	microspheres	3 or 10µm	20 minutes	5 particles µl ⁻¹	8
	C, P	Mollusca	F	not informed	pellets, fishing line	not informed	-	not informed	9
	FF	Mollusca	L	PS	beads	5, 10, 20 µm	1 hour	10000 particles ml ⁻¹	10
	FF	Mollusca	L	DB	beads	16 – 18 µm	1 hour	5 x 10 ³ particles ml ⁻¹ or 15000 particles	11
	FF	Mollusca	L	PS	microspheres	2 - 16 µm	3 hours, 12 hours	0. 51 g l ⁻¹	12
	DF	Mollusca	L	amino-PS	microspheres	50 nm	30 minutes – 4 hours	1, 5, 50 µg ml ⁻¹	13

FF	Crustacea	L	PE	microspheres	unknown	up to 72 hours	0.1 g	14
FF	Mollusca	F	not informed	fragments, fibres, film	not informed	-	0.07 – 5.47 particles g ⁻¹	15
FF	Mollusca	F	not informed	fibres	200 – 1500 µm	-	2.6 to 5.1 fibres per 10 g of mussel	16
FF	Mollusca	L	PP	pellets	not informed	48 hours	0.5, 1 and 2 ml of pellets	17
FF, DF	Echinodermata	L	PVC, nylon	fragments, resin pellets	0.25–15 mm; 0.25–1.5 mm; 4 mm	20 -25 hours	10g PVC fragments, 65g PVC resin pellets, 2g nylon line fragments per 600 ml silica	18
FF	Cnidaria	L	PP	fragments	10 µm–2 mm	48, 12, 3 hours	0.395 g l ⁻¹ , 0.197 g L ⁻¹ , 0.24 g L ⁻¹ ,	19
SF	Echinodermata	L	PE	microspheres	10–45 µm	up to 5 days	1, 10, 100, and 300 spheres ml ⁻¹ freshwater	20
G, SF, FF H, C, O FF, DF FF C	Mollusca, Crustacea, Echinodermata, Porifera, Cnidaria	F	not informed	fibres, pieces, pelets	average 231 µm	-	5.82 x 10 ³ – 73.6 x 10 ³ particles g ⁻¹ DW	21
FF	Mollusca	L	not informed	microspheres	0.5, 1.0, 1.5, 2.0, 3.1, 4.0, and 5.1 µm	up to 2 hours	25 – 33 mg l ⁻¹ ; 5, 13, 27, 43, and 64 mg·L ⁻¹ ; 7.4, 12.2, 27.4, 37.2, 49.7, and 83.5 mg·l ⁻¹	22
FF	Mollusca	F	PE, PET, PA	fibres, fragments, pellets	5 µm to 5 mm	-	2.1 – 10.5 items g ⁻¹ ; 4.3 – 57.2 items per individual	23
FF	Mollusca	F	CP, PET, PES, PE, PA, others	fragments, spheres, flakes, fibres	< 250 µm to > 1 mm	-	0.9 - 7.6 items per individual	24
FF	Mollusca	F	not informed	fibres	> 8 µm	-	20-80 particles per 10 g sediment	25
FF	Mollusca	L	PVC	microspheres	1–50 µm	up to 91 days	0, 0.0216, 0.216 and 2.160 mg ml ⁻¹	26
FF	Mollusca	F	not informed	fragments, fibres	not informed	-	not informed	27
FF	Mollusca	F	not informed	microparticles	5 – > 25 µm	-	0.36 ± 0.07 particles g ⁻¹ WW; 0.47 ± 0.16 particles g ⁻¹ WW	28
FF	Mollusca	L	HDPE	powder	0 - 80 µm	up to 96 hours	2.5 g l ⁻¹	29

	FF	Mollusca	L	PS	nanobeads	10 µm, 100 nm	45 minutes	1000 beads ml ⁻¹	30
	FF	Mollusca	L	PS	nanospheres	30 nm	8h	0, 0.1, 0.2, and 0.3 g l ⁻¹	31
	D, O, P	Crustacea	F	cellulose	fibres	0 – 6 mm	-	~1 fibre per organism	32
	FF C, O, H, Can	Mollusca Chordata Chordata	F	not informed	Fragments, fibres, films, foam, monofilaments	not informed	-	0 - 2.5 ± 6.3, 0 - 21 items per individual	33
	P	Crustacea	F	not informed	fragments, fibres	200-1000 µm	-	0.68 ± 0.55 particles g ⁻¹ WW (1.23 ± 0.99 particles per shrimp)	34
	H	Echinodermata	L	PE	pellets	not informed	24 hours	2 ml; 200 ml	35
	O, P	Crustacea	F	not informed	balls and strands	0.5 - 5 mm	-	not informed	36
	O, P, S	Crustacea	L	PS	microbeads, fragments, fibres	1-2,500 µm	3 days; 6 weeks	~120 microbeads mg of food ⁻¹ ; ~350 fragments mg of food ⁻¹ ; 0.3 mg g food ⁻¹	37
	SF, P	Crustacea	F	PE, PP, PS	fragments and monofilaments	< 0.5 mm	-	1 to 30 particles per individual	38
	SF	Echinodermata	L	PS - DB	microspheres	10, 20 µm	-	2400 per ml	39
	SF	Mollusca	L	PS	not informed	not informed	up to 65 days	not informed	40
	C O, P FF	Chordata Crustacea Mollusca	L	PVC	not informed	not informed	3 hours - 10 days	4.4×10 ¹⁰ particles, 0.5 g·L ⁻¹	41
INFAUNA	DF	Annelida	L	PS	microspheres	400-1300 µm	28 days	0-7.4% sediment DW	42
	DF	Crustacea	L	PS	microspheres	700-900 µm	2 months	108 and 1000 mg particles kg ⁻¹ dry sediment	43
	DF	Annelida	L	PVC	microspheres	230 µm	11 days	5%	44
	DF	Annelida	L	PLA, HDPE, PVC	fragments	1.4-378 µm	31 days	0.02, 0.2 and 2% of sediment WW	45
	O, DF	Crustacea	L	PE	microspheres	38-45 µm	24, 72, 120 hours	3.8% DW	46
	DF	Crustacea	L	PE	microspheres	10-45 µm	3, 6, 24, 48 and 168 hours	10% of the weight of the food	47

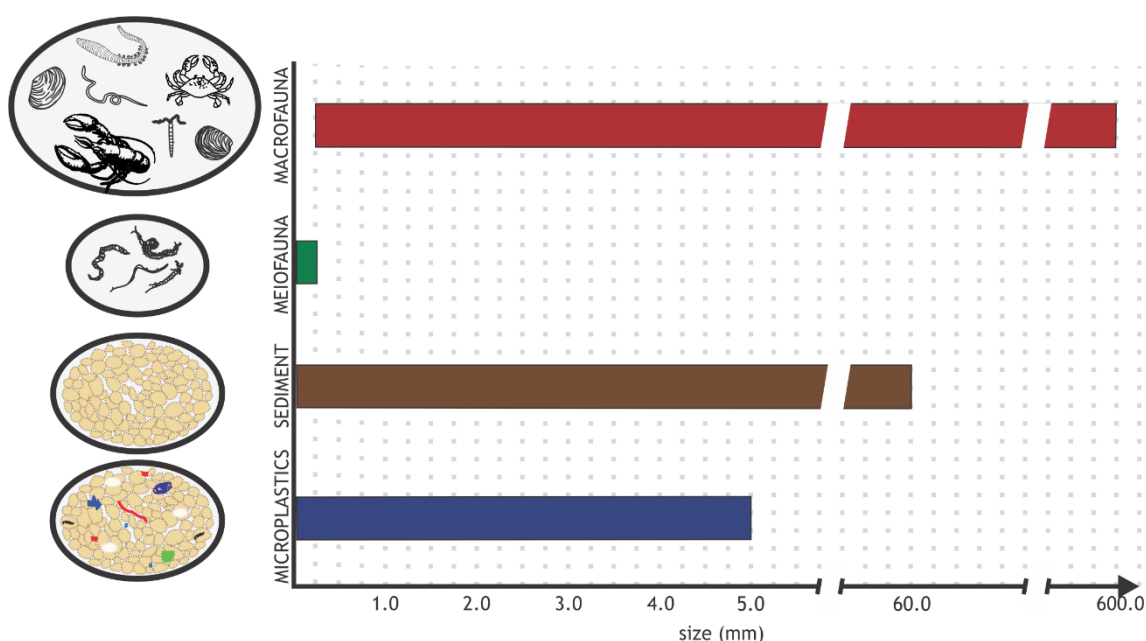
	DF	Annelida	L	PVC	microspheres	125-149 µm	48h; 4 weeks	0–5 % w/w	48
BOTH	DF FF	Annelida, Mollusca	L, F	PS	microspheres	10, 30, 90 µm	14 days	0.2 ± 0.3 particles g ⁻¹ 1.2 ± 2.8 particles g ⁻¹ / 110 particles g ⁻¹ sediment or water;	49
	G FF DF	Crustacea, Mollusca, Echinodermata	L	PLA, HDPE	microspheres	0.48-363 µm	60 days	0.8 or 80 µg l ⁻¹	50
	DF, FF DF, SF H, O, DF, P	Mollusca, Annelida Crustacea	L	PS	microspheres	10 µm	24 hours	5, 50, 250 beads ml ⁻¹	51
	FF, D DF	Crustacea, Annelida	L	A, PE, PP, PMA, PVA, PA, Nylon	fragments, fibres	20 – 2000 µm	not informed	1.5 g l ⁻¹ ; 1g per individual; 1g l ⁻¹	52

Fonte: A autora

* **References:** **1** FARRELL; NELSON, 2013; **2** LUSHER; MCHUGH; THOMPSON, 2013; **3** MCGORAN; CLARK; MORRITT, 2017; **4** MURRAY; COWIE, 2011; **5** WATTS et al., 2015; **6** WATTS et al., 2016; **7** AVIO et al., 2015; **8** BOLTON; HAVENHAND, 1998; **9** BRAID et al., 2012; **10** BRILLANT; MACDONALD, 2000; **11** BRILLANT; MACDONALD, 2002; **12** BROWNE et al., 2008; **13** CANESI et al., 2015; **14** CHUA et al., 2014; **15** DAVIDSON; DUDAS, 2016; **16** DE WITTE et al., 2014; **17** GANDARA E SILVA et al., 2016; **18** GRAHAM; THOMPSON, 2009; **19** HALL et al., 2015; **20** KAPOSÍ et al., 2014; **21** KARLSSON, 2014; **22** LEI; PAYNE; WANG, 1996; **23** LI et al., 2015; **24** LI et al., 2016; **25** MATHALON; HILL, 2014; **26** RIST et al., 2016; **27** SANTANA et al., 2016; **28** VAN CAUWENBERGHE; JANSSEN, 2014; **29** VON MOOS; BURKHARDT-HOLM; KÖHLER, 2012; **30** WARD; KACH, 2009; **31** WEGNER et al., 2012; **32** REMY et al., 2015; **33** ROCHMAN et al., 2015; **34** DEVRIESE et al., 2015; **35** NOBRE et al., 2015; **36** WÓJCIK-FUDALEWSKA; NORMANT-SAREMBA; ANASTÁCIO, 2016; **37** HÄMER et al., 2014; **38** GOLDSTEIN; GOODWIN, 2013; **39** HART, 1991; **40** HAU KWAN; KIT YU, 2017; **41** SANTANA; MOREIRA; TURRA, 2016 **42** BESSELINE et al., 2013; **43** BRENNECKE et al., 2015; **44** BROWNE et al., 2013; **45** GREEN et al., 2016; **46** TOSETTO; BROWN; WILLIAMSON, 2016 **47** UGOLINI et al., 2013; **48** WRIGHT et al., 2013; **49** VAN CAUWENBERGHE et al., 2015b; **50** GREEN, 2016; **51** SETÄLÄ; NORKKO; LEHTINIEMI, 2016; **52** THOMPSON et al., 2004.

In 2004, the first work specifically regarding the potential harmful effects of microplastic ingestion was published (THOMPSON et al., 2004). Organisms (amphipods, lugworms and barnacles) with different feeding strategies (detritivores, deposit feeders or filter feeders) were shown to be able to uptake microplastics from the sediments through laboratory experiments. This study opened discussions on the potential transference of microplastics between organisms from different levels within marine food webs.

Figure 1: Scale comparing microplastic sizes reported in articles used here to assess interactions between microplastics and marine benthic fauna.



Fonte: A autora

3.4 Laboratory and field studies: Conflicts and agreements

Laboratory experiments are an important tool to understand microplastics potential risks since they can mimic *in situ* conditions of benthic environments. The majority (66%) of the published papers reviewed here were experiments developed under controlled laboratory conditions, with the advantage to plan and control environmental variables, and therefore obtain reliable results adequate for statistical analysis.

However, these laboratory works normally use high concentrations of virgin (non-weathered) microplastics with specific size and polymer composition (Table 1), so they

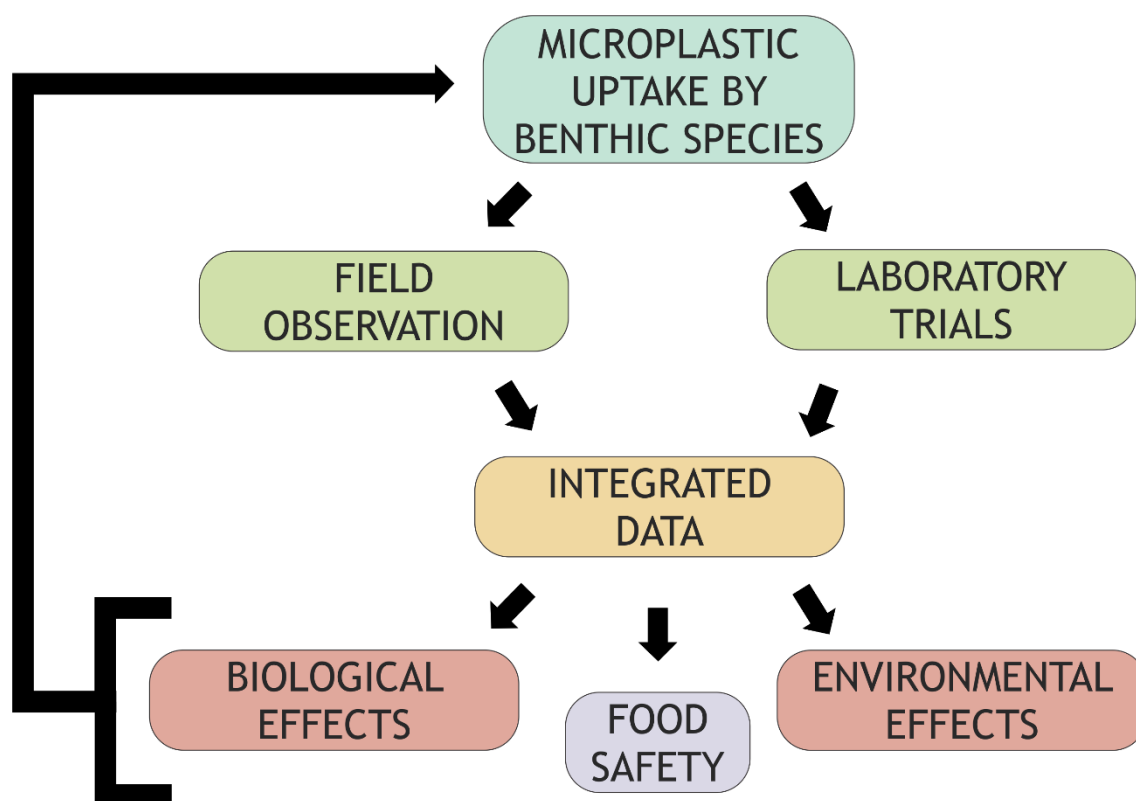
frequently do not represent environmentally relevant quantities of microplastic (PHUONG et al., 2016). Then, microplastics are frequently overestimated in terms of quantity and underestimated in terms of polymer diversity. The problem is that these high concentrations used in laboratory studies do not represent the real chances of contact and interactions between microplastics and benthic species in marine environment (LENZ; ENDERS; GISSEL, 2016). However, from a toxicological perspective, they are easier to be detect/manipulated during experiments and to potentially determine the lethal concentration (LC₅₀) for organisms.

On the other hand, field measurements are rare. When available, they normally report the number of items found in each organism or their concentration in tissues (dry or wet weight) (Table 1). However, physiological effects to organism were not reported. Although these observations focus on biological processes, they nicely portray microplastic uptake and can be used as basis for further characterization of these effects. Field works mainly analyse digestive tract contents of animals collected from the benthic zone and do not report any effect related to the ingestion event (DAVIDSON; DUDAS, 2016; GOLDSTEIN; GOODWIN, 2013; VAN CAUWENBERGHE et al., 2015b; WÓJCIK-FUDALEWSKA; NORMANT-SAREMBA; ANASTÁCIO, 2016).

Two articles merged field analysis of gut contents and laboratory experiments. Murray and Cowie (2011) found microplastics fragmented from fishing nets in the stomach of 83% of lobsters (*Nephrops norvegicus*) collected in the northern Clyde Sea. Then, they performed a laboratory experiment exposing lobsters to contaminated fishes (*Merlangius merlangus* and *Micromesistius poutassou*) that were fed with the same fibres when lobsters were observed to accumulate fibres from ingested fishes. This is until today one of the few studies to show microplastics transference between organisms. Van Cauwenberghe et al. (2015a) analysed microplastics in mussels (*Mytilus edulis*) and lugworms (*Arenicola marina*), finding 0.2 ± 0.4 particles g⁻¹ tissue and 1.2 ± 2.8 particles g⁻¹ tissue, respectively. Then, in the laboratory, they exposed these two species to 110 spheres ml⁻¹ of seawater (*M. edulis*) or sediment (*A. marina*). Both species were shown to ingest microplastics, although no clear effects on energy budget was observed.

Works integrating both field measurements and laboratory experiments must be encouraged as important tools to obtain relevant and updated data on this subject.

Figura 2: Suggested model for integrating approaches in order to study microplastic pollution effects on benthic communities.



Fonte: A autora

3.5 Microplastic types, shapes and sizes

Polymers used in laboratory feeding trials are similar polymers sampled in organisms and sediments (GALLOWAY, 2015). In laboratory studies, polystyrene is most commonly used, followed by polyethylene and polypropylene (Table 1). They have lower densities when compared with seawater (ANDRADY, 2011), but can reach sediments and become available to benthic species (e.g. CHUBARENKO et al., 2016).

Regarding shape, microplastics on experiments are commonly used as spheres, and rarely as fragments or fibres (e.g. HALL et al., 2015; WATTS et al., 2015). This is because it is easier to obtain spheres from chemical companies, while fibres and fragments have to be artificially produced/prepared in laboratory before experiments (WATTS et al., 2015). Also, it is harder to avoid chemical contamination from other pollutants when using microplastics harvested in nature in controlled experiments.

The most common size range of microplastics is from 5 to 45 micrometres (KAPOSI et al., 2014; TOSETTO; BROWN; WILLIAMSON, 2016; UGOLINI et al., 2013) but other sizes are also used (BESSELING et al., 2013; GRAHAM; THOMPSON,

2009; WATTS et al., 2015). Figure 1 shows a scale comparing animals and sediment sizes with the microplastic range. It is clear that microplastic size range is wider than the meiofauna, so this needs to be considered on experimental planning in order to fit animal size.

Size is also related to the animals' feeding selectivity and retention capacity can determine the particle size used in laboratory experiments. For example, the mussel *Mytilus edulis* seems to retain particles ranging from 10 to 30µm, while lugworms (*Arenicola marina*) retain relatively larger particles from 30 to 90µm (VAN CAUWENBERGHE et al., 2015b). Other works found influence of ingested microplastic size between species on particle ingestion, indicating a possible biological role for particle size in feeding selection (e.g. GRAHAM; THOMPSON, 2009). Further works are required to investigate potential correlation and to assess reasons for particle size selection.

Some manufactured microspheres fit in the nanometre scale (10-100nm) (WARD; KACH, 2009; WEGNER et al., 2012). This category is relatively new in the literature when compared to microplastics, as nanomaterials have been only recently in use and the concern about intrinsic biological effects of these particles' ingestion is raising (MATTSSON; HANSSON; CEDERVALL, 2015). Another aspect that delayed the appearance of nanoplastics in the specialized literature is related to analytical procedures and contamination issues (KOELMANS et al., 2015). This literature review found and reported some articles using plastic particles in this size class, and this is predicted as the next challenge regarding marine biota and plastics interactions.

3.6 Microplastic concentration units in laboratory studies

Environmental concentration of microplastic in sediment can vary widely among habitats (PHUONG et al., 2016). Therefore, it is hard to define how much plastic will actually be available and potentially ingested by an animal. In laboratory experiments, microplastics contamination is studied in water, in the case of filter feeding species, or sediments, in the case of deposit feeders (e.g. BRENNECKE et al., 2016; GANDARA E SILVA et al., 2016b). Some works also define microplastic quantities according to food weight (UGOLINI et al., 2013; WATTS et al., 2015) or number of particles per

experimental unit (tank or beaker) (CHUA et al., 2014; FARRELL; NELSON, 2013; NOBRE et al., 2015).

With the information given on the materials and methods section of articles, it is frequently not possible to compare units used, for example number of particles per area or volume of sediment with percentage of microplastics in sediment mass (CARSON et al., 2011; IVAR DO SUL; SPENGLER; COSTA, 2009; WRIGHT et al., 2013). Underwood et al. (2017) have criticized experimental designs and microplastic sampling in published works, as, in their point of view, many analytical aspects need to be considered. A standardized analysis (consensual protocol) could be an appropriate start but would require information gathering and effort from researchers on the subject.

3.7 Exposure time in laboratory experiments

Microplastics uptake can cause short- and/or long-term effects on animals. The time of exposure used in laboratory trials is expected to determine the type of effects observed. This literature review revealed that the time of exposure largely varied among the analysed works but the majority focused on acute, short-term effects for the organisms (20 minutes - 60 days) (table 1). Two articles have exposed benthic species to longer periods (> 2 months) (BRENNECKE et al., 2015; RIST et al., 2016). This is a paradox since long-term exposures are more realistic in natural environments. However, short-term experiments are important to understand potential harms that benthic fauna may suffer due to non-heterogenous distribution of microplastic over time and/or on and sediment column.

3.8 Model animal groups

Microplastic uptake have been reported for several animal groups, almost half with commercial importance and used for human consumption. Molluscs are the most studied group specially bivalves. Individually, the most studied species is *Mytilus edulis*, with 12 articles. *Arenicola marina* is in second place with 6 articles, followed by *Mytilus galloprovincialis* with 4 articles, *Carcinus maenas* and *Perna perna* with 3 articles each, *Crassostrea gigas*, *Merlangius merlangus*, *Micromesistius poutassou* and *Ostrea edulis* with 2 articles each and other 98 species with one article each (Table 3).

Table 3: Benthic species studied by the analysed papers, with number of works where each one appears.

SPECIES	STUDIES WHERE APPEAR
<i>Mytilus edulis</i>	12
<i>Arenicola marina</i>	6
<i>Mytilus galloprovincialis</i>	4
<i>Carcinus maenas</i>	3
<i>Perna perna</i>	3
<i>Crassostrea gigas</i>	2
<i>Merlangius merlangus</i>	2
<i>Micromesistius poutassou</i>	2
<i>Ostrea edulis</i>	2
<i>Placopecten magellanicus</i>	2
Other species (98)	1

Fonte: A autora

M. edulis is abundant in coasts and easy to obtain and to manipulate. Also, it is already used as an important bioindicator of chemical/biological pollution in aquatic habitats, as they are passive filter feeders and therefore most likely to portray marine pollution realistically. These animal models are able to indicate microplastic pollution in both spatial and temporal scales, as environmental quantification depends on many abiotic factors such as wind, currents, etc. (FOSSI et al., 2017). Benthic species, specially filter feeders, have been described to be at high risk of microplastic pollution (SETÄLÄ; NORKKO; LEHTINIEMI, 2016), and therefore should be prioritized as key models in both field and laboratory studies on microplastic pollution.

Crustaceans and annelids are also commonly studied. Animals within these groups present different feeding mechanisms (i.e. filter feeders, detritivores and deposit feeders) but can uptake and retain microplastics in their digestive and/or respiratory system. Only two articles analysed ingested microplastics on benthic vertebrate organisms (i.e. demersal fishes) (LUSHER; MCHUGH; THOMPSON, 2013; MCGORAN; CLARK; MORRITT, 2017).

Molluscs, crustaceans and annelids are at lower levels on the marine trophic chain and potentially represent entry points of microplastic particles into food webs, when they can bioaccumulate on higher trophic levels predators (IVAR DO SUL; COSTA, 2014).

There are two compartments from where benthic species can uptake microplastics depending on the animal's feeding behaviour: the sediment and the water column. Filter feeders from the epifauna, for example, will ingest microplastics suspended in the water

right above the sediment, while deposit feeders from the infauna will ingest microplastics in the sediment. Also, microplastics on the sediment can be resuspended by mechanical forces and become available on the water column again (BALLENT et al., 2016). Therefore, different feeding behaviour (e.g. filter feeder, deposit feeder) simply in different feeding matrices (e.g. water, sediment) to be considered in both laboratory and field experiments.

3.9 How ingestion affects benthic fauna

Toxic effects of microplastic ingestion in benthic fauna have been listed in many articles (e.g. IVAR DO SUL; COSTA, 2014). Laboratory experiments are usually performed to obtain information about potential physiological effect on benthic organisms. Reported harmful effects include changes in metabolic rate (GREEN et al., 2016); reduction of feeding activity and loss of energy budget and/or weight (BESSELING et al., 2013; KAPOSI et al., 2014; WATTS et al., 2015); lower filtration and respiratory rates (RIST et al., 2016; WATTS et al., 2016; WEGNER et al., 2012) ; oxidative stress (AVIO et al., 2015; BROWNE et al., 2013; CANESI et al., 2015); inflammatory responses (AVIO et al., 2015; VON MOOS; BURKHARDT-HOLM; KÖHLER, 2012; WRIGHT et al., 2013); and changes in survival rates and behaviour (TOSETTO; BROWN; WILLIAMSON, 2016).

Microplastics can also enter through the animals' gills causing physical effects such as blockage or injury as reported by only a few studies. Watts et al. (2016) showed no significant effect on gill function of the shore crab *Carcinus maenas* in the presence of microplastics, as well as Wegner et al. (2012) to the mussel *Mytilus edulis*. Further work on mechanical effects of microplastic on ventilatory structures are needed.

Overall, it seems that consequences to the energy budget are well established in some species, but other mechanisms involved in inflammatory responses and oxidative stress caused by microplastic ingestion are still unclear (VAN CAUWENBERGHE et al., 2015b). Also, physical effects of microplastics on gills and other ventilation structures such as blockage are under studied so far. Furthermore, the analysed articles have approached environmental effects suffered by the organisms but not in all its extent. A holistic approach is extremely necessary to understand the real danger that this type of

pollution represents for entire ecosystems, which involves both field observations and laboratory trials to assess its effects (Figure 2).

3.10 Effects at community level

One work deserved special attention due to its remarkable approach. In 2016, Green (2016) designed an outdoor mesocosm system that used intact sediment cores to evaluate the effects of microplastic ingestion on the European flat oyster *Ostrea edulis* and on the benthic community. The results showed that oysters fed with biodegradable microplastics had their respiration rate increased after 60 days of exposure, but the main effects were on the benthic assemblage. Twenty-six species of macrofauna were identified and the analysis showed that there taxa diversity in control environment was higher than those with low (0.8 mg l^{-1}) concentration of microplastics, and also higher on low (0.8 mg l^{-1}) than high (80 mg l^{-1}) concentration of microplastics. Also, there was a decrease in the number of individuals and biomasses of some species on the mesocosm with microplastics, which decreased even more on the high microplastic concentration environment.

Other factor that is related to animals' exposure to microplastics is bioturbation, which includes animals' movements in the sediment. These movements cause particle transport of particles including microplastics in the sediment, which has been recently reported as a research priority (GESAMP, 2016). Näkki et al. (2017) found a correlation between microplastic vertical distribution in the sediment caused by bioturbation actions such as ingestion and movement by the Baltic clam *Macoma balthica*. In general, this type of work represents an approximation of how laboratory works can be used to determine the effects of microplastic pollution in a given ecological compartment such as the benthos. Strategies such as simulating natural environments by collecting sediment cores and adapting it to controlled laboratory conditions must be reproduced in order to obtain meaningful results on this matter.

3.11 Conclusions

Studies regarding microplastics ingestion by benthic organisms are a relatively new field to be explored by microplastic researches. Standardized protocols, for instance,

is a mandatory issue, as it can be useful to compare study results and then contribute more significantly to marine pollution and toxicological research. Goals might be regulations on the use/discard of microplastics to the environment.

After reviewing the literature presented here, it is clear that there is a lack on studies using ecologically relevant approaches such as experiments integrating environmental factors and variables controlling microplastics availability, microplastics interactions with the biota and effects. Laboratory experiments are efficient tools to elucidate effects on population and community level. Also, studies involving biological effects for different ontogenetic phases are important to study since some edible species need a more complete assessment to be part of food safety policies.

As a final suggestion, studies focusing on the resulting microplastics distribution and preservation in sediments after interaction with the biota will be important since this pollutant is a strong candidate for serving as an indicator of anthropogenic interference in benthic habitats.

4 CHARACTERIZATION OF SMALL MICROPLASTIC POLLUTION ON TRINDADE ISLAND (TROPICAL ATLANTIC)

4.4 INTRODUCTION

The marine environment is susceptible to changes since anthropogenic effluents have the ocean as their final destination (FENDALL; SEWELL, 2009). Consequently, tons of pollutants, including litter, continue to be found on the sea each year (JAMBECK et al., 2015). Among litter categories, all plastic types are the most expressive in quantity (ZALASIEWICZ et al., 2016), commonly representing more than half of total litter amounts (BARNES et al., 2009). Recent estimations shows that 1.5-4.5% of all the plastic produced globally ended up in the ocean only in 2010 (JAMBECK et al., 2015).

Plastics are derived from the polymerization of monomers extracted from oil or natural gas, and present interesting characteristics such as durability and flexibility (COLE et al., 2011). Therefore, plastics are not easily biodegraded and rapidly accumulated in the marine environment (BARNES et al., 2009). Entanglement of biota and ingestion by animals are some of the well-known effects of macroplastics pollution (AVIO; GORBI; REGOLI, 2016), but more attention is now given to smaller size categories of plastics called microplastics.

Microplastics derive from primary or secondary sources (COLE et al., 2011). Primary-sourced microplastics are released in the environment as particles smaller than 5mm. Usually they come from cosmetics such as microbeads in exfoliants, from petrochemical industries such as pellets, and from washing machines in the form of synthetic fibres (BOUCHER; FRIOT, 2017). On the other hand, secondary-sourced microplastics are originated from the breakdown of larger plastic items in coastal and marine environments (COOPER; CORCORAN, 2010), and include hard and soft fragments, paint chips and fibres (COSTA; BARLETTA, 2015).

A recent way to classify microplastics based on their size has been proposed by Hanvey (2017) (Table 4). Particles with size between 1 and 5 millimetres are called large macroplastics, while particles smaller than 1mm can be called small microplastics. In turn, nanoplastics are particles smaller than 1000nm.

Table 4: Plastic litter terminology proposed by Hanvey et al. (2017).

Size range	Proposed terminology
>20 cm	Macroplastic
5-20 cm	Mesoplastic
1-5 mm	Large microplastic
1-1000 μm	Small microplastic
<1000nm	Nanoplastic

Fonte: A autora

Many published works demonstrated physical effects related to microplastics ingestion in both vertebrates and invertebrates (reviewed in IVAR DO SUL; COSTA, 2014). Chemical and toxicological effects can also occur because they can carry significant amounts of persistent organic pollutants (POPs) such as polychlorinated biphenyls (PCBs), organochlorine pesticides (OCPs) and polybrominated diphenyl ethers (PBDEs) (GESAMP, 2016; KARAPANAGIOTI et al., 2011; VAN CAUWENBERGHE et al., 2015a) that will be released to the organism after ingestion and transit along the digestive tract. Finally, microbiological effects can also be listed as a significant risk related to microplastics ingestion (KIRSTEIN et al., 2016).

Most microplastics research reporting processes involving it as pollutants dates from 1990s onwards. While a reasonable number of papers have assessed plastic pollution on oceanic islands of the Atlantic (reviewed by MONTEIRO; IVAR DO SUL; COSTA, in press), only a few are available on microplastic pollution on their coastal sediments (e.g. DEKIFF et al., 2014; LIEBEZEIT; DUBAISH, 2012; YOUNG; ELLIOTT, 2016).

Trindade island is an important insular environment on the tropical Atlantic Ocean. Previously, large microplastics (1-5mm), mostly fragments, were reported both floating around the island (IVAR DO SUL; COSTA; FILLMANN, 2014) and deposited on sandy beaches (IVAR DO SUL; COSTA; FILLMANN, 2017). Now, this work analyses beach sediment samples from Trindade island in order to identify, characterize and classify the fraction corresponding to the small microplastics size.

4.5 METHODS

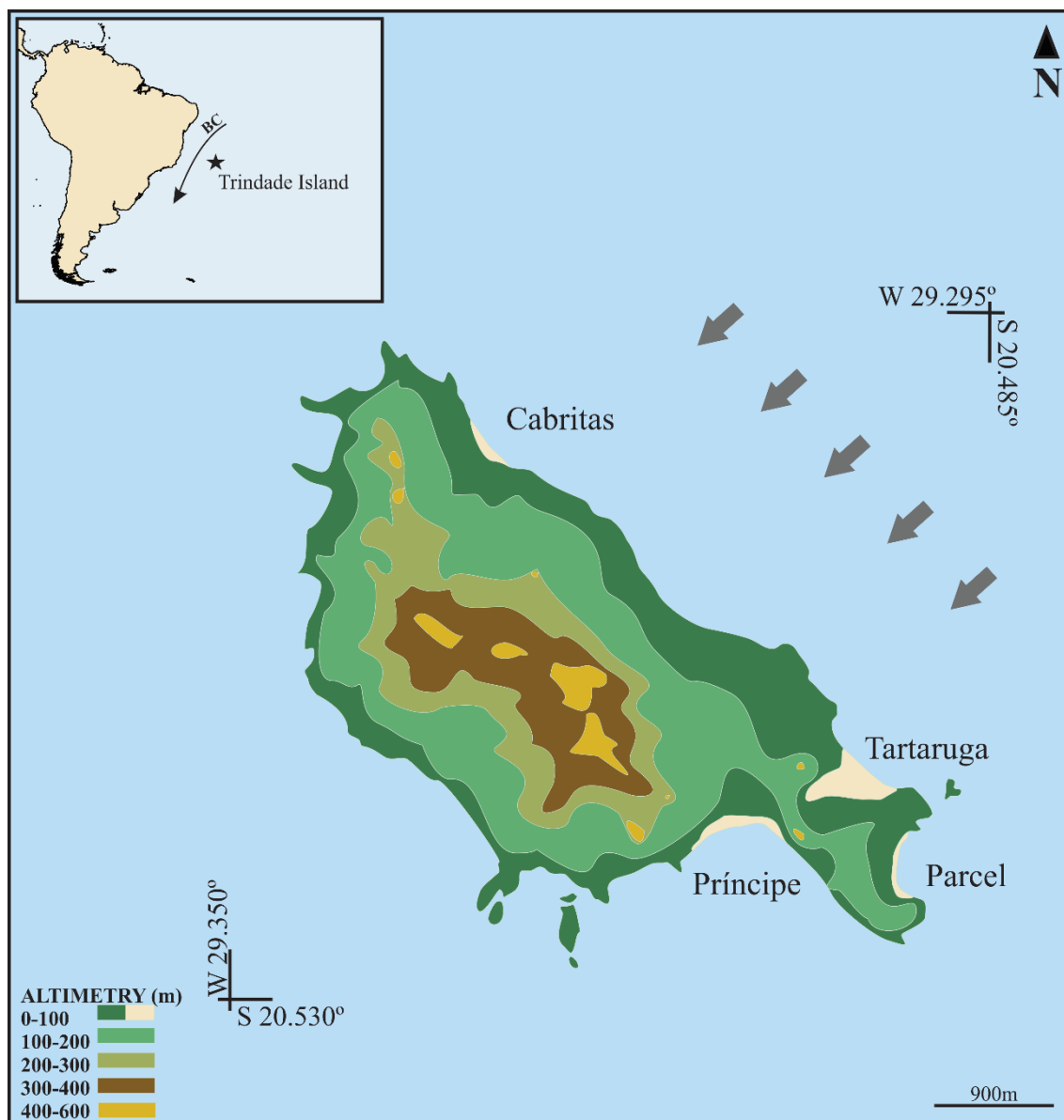
4.5.1 Study site

Trindade island (20° 31' 29" S, 29° 19' 29" W) (Figure 3) is located 1,160km east from the Brazilian coast, and it is inhabited only by militaries and scientists (<100 people). The Brazilian government develops a research programme in Trindade (<https://www.mar.mil.br/secirm/portugues/trindade.html>) in order allow scientific studies that assess local biodiversity and oceanographic features.

The island has 9.28 km² and elevates up to 5.500 m from the seafloor (CALLIARI et al., 2016) in the Vitória-Trindade chain. It has quite irregular topography, with elevations of up to 600 m (ALMEIDA, 1961). It is mainly under the influence of the Brazil Current, with high water salinity (37) and temperatures (27°C) (GASPARINI; FLOETER, 2001). The climate in the region is classified as tropical oceanic, with mean annual temperature of 24°C. The prevailing winds in the equatorial south Atlantic are from southeast trade (average 6.6 m s⁻¹), but the strongest winds in Trindade come from extra-tropical cyclones originated from south and southeast winds (CALLIARI et al., 2016). Waves predominantly come from the south (33.7%), southwest (23.4 %), east (18.1 %), north (10.3 %) and southeast (10.1 %) (CALLIARI et al., 2016).

Beaches in Trindade are basically composed of sand with calcareous algae fragments. It also reflects the mineralogy of adjacent rocks formation, which includes volcanic originated material such as tephras of phonolite with high percentages of heavy minerals (CALLIARI et al., 2016).

Figura 3: Map of Trindade island with main beaches locations, on the tropical Atlantic Ocean ($20^{\circ} 31' 29''$ S, $29^{\circ} 19' 29''$ W). Grey arrows indicate prevailing wind and wave direction (IVAR DO SUL; COSTA; FILLMANN, 2014). BC: Brazilian Current.



Fonte: A autora

4.5.2 Fauna of Trindade island

Along the centuries, Trindade has suffered an important and difficult to estimate loss in its biodiversity due to introduction of exotic species to the island. Goats brought in for food supply have eradicated plant species, and initially it brought more attention to the vegetation rather than fauna. However, the island has recovered many species since the goats have been removed (ALVES; MARTINS, 2004). This eradication directly affects associated fauna (SOTO, 2009), but still there is a rich fauna mainly composed of crabs, seabirds, marine turtles, fishes and many known invertebrates (ALVES, 1998).

Trindade also serves as a nesting site for the green turtle (*Chelonia mydas*), an endangered species according to the IUCN Red List. It is the biggest reproductive site for green turtles in Brazil and the seventh in the Atlantic, with 3600 annual nests (ALMEIDA et al., 2011).

The ichthyofauna in Trindade has six endemic species and at least 1 endemic subspecies (GASPARINI; FLOETER, 2001). This unique fish biodiversity is explained by the island's location and the Vitória-Trindade chain structure (PINHEIRO et al., 2017). There are also four endemic species of marine sponges around the island (MORAES et al., 2006). Eight species of seabirds are residents on the island, but there are also species that are visitants, migrants and occasional visitants. Two subspecies of frigates (*Fregata minor nicolli* and *Fregata ariel trinitatis*) are endemic to Trindade island (LUIGI et al., 2009).

4.5.3 Sampling procedure

A total of 26 samples from four beaches (Cabritas, Parcel, Príncipe and Tartaruga) (Figure 3), collected during the austral summers of 2011/2012 and 2012/2013, were analysed for the presence of microplastics. Samples were collected from the most recent strandline, recognized as an area of significant short-term deposition (DAVIES; GILLHAM, 2004; WILLIAMS; MICALLEF, 2009).

In order to assess the entire extent of the beach, samples were collected from the middle of the bay (M) and on the edges (namely northern (N) and southern sides (S)) according to their position on the beach (Table 3).

Table 5: Details of samples collected on each beach. Sampling occurred in the middle of the bay (M) and on the northern (N) and southern (S) sides of each beach.

Beach	Collection date	Location
Cabritas	January 2012	M
	January 2012	S
	January 2012	N
	February 2011	M
	February 2011	N
	January 2012	M
	January 2012	N
Parcel	January 2011	S
	December 2011	N
	December 2011	S
	January 2011	N
	December 2011	N
	December 2011	M
	January 2011	M
Príncipe	February 2011	N
	February 2011	M
	February 2011	S
	December 2011	S
	December 2011	N
	December 2011	M
	February 2011	S
	February 2011	N
Tartaruga	February 2011	M
	December 2011	N
	February 2011	S
	December 2011	S

Fonte: A autora

Samples corresponded to the first two centimetres of 900cm² quadrats and were collected with a small shovel. In the laboratory, they were oven-dried at 100°C and sieved through a 1mm mesh. This work analysed the fraction <1mm, which from now on will be called small microplastics according to the terminology proposed by Hanvey et al. (2017) (Table 4).

Table 6: Beaches length and sediment characteristics of Trindade island. Adapted from Ivar do Sul et al. (2017).

Beach	Beach length (m)	Sediment Grain size	Classification of sorting
Cabritas	350	Medium sand	Moderate
Parcel	200	Coarse sand	Moderate
Tartaruga	200	Medium sand	Moderate
Príncipe	200	Coarse sand	Well-sorted

Fonte: A autora

4.5.4 Sample treatment and analysis by saline flotation

Microplastics were isolated from sediments using a previously established protocol (Pinheiro et al., unpublished data) based on a literature compilation (HIDALGO-RUZ et al., 2012; MARTINS; SOBRAL, 2011). A NaCl solution (1.2 g L^{-1}) was used in which polymers with lower densities such as polystyrene, polyethylene and polypropylene will float and could be collected by filtration of the supernatant. To eliminate salt contamination bias, the saline solution was filtered and analysed every new solution (blanks). During extraction, precautions such as minimal air exposure and appropriate laboratory clothing were used to avoid external contamination.

Briefly, in a 2L beaker, 1L of saline solution was added to each sample and put under agitation for 30 minutes. The mixture was then let to rest for 30 minutes to allow sediment settling. The supernatant was carefully filtered (mesh size $2 \mu\text{m}$) by vacuum filtration. Each sample was washed with the saline solution three times to guarantee plastics extraction. Filters were stored in Petri dishes and oven-dried at 40°C to be analysed under a stereomicroscope (Carl Zeiss Stemi 2000-C, objective 1.0x) equipped with an AxioCam ERc 5s associated with the ZEN lite 2.3 (blue edition) software from Carl Zeiss Vision. Microplastics were reported in total quantities (number of fragments or number of fibres per sample), density (fragments m^{-2} or fibres m^{-2}), type (fragments, fibres), total area (mm^2) and colour.

4.5.5 Statistical analysis

Data were analysed using ActionStat 3.2.60.1118 software as part of the R 3.3.2 program. Normal distribution of the data was tested using Kolmogorov-Smirnov test. As the data did not fit as normal requisites, Kruskal-Wallis tests were performed to test

significant differences among microplastic quantities, densities and areas and beaches ($\alpha=0.05$).

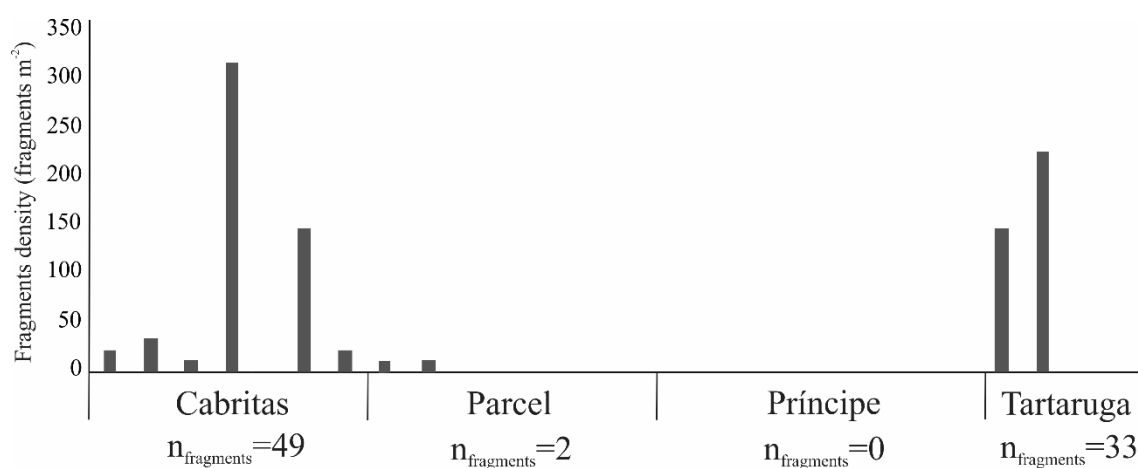
4.6 RESULTS

Small microplastics were successfully isolated from sediment samples from sandy beaches of Trindade island using a previously established protocol based on density separation (Pinheiro et al., unpublished data). No contamination from the table salt was identified, and the possibility of airborne contamination was kept to minimal levels. Nearly 630 microplastics were extracted, measured and analysed.

4.6.1 Small microplastic fragments

Eighty-four small microplastic fragments were found distributed in 10 of the 26 samples (Figures 4 and 5). Cabritas, Parcel and Tartaruga beaches were contaminated with small microplastic fragments but no fragment was found on Príncipe beach. Cabritas beach had the highest quantity and density, followed by Tartaruga and Parcel beaches, respectively. No significant difference was found among beaches considering microplastic densities ($p=0.079$) (Figure 4) or areas ($p=0.080$).

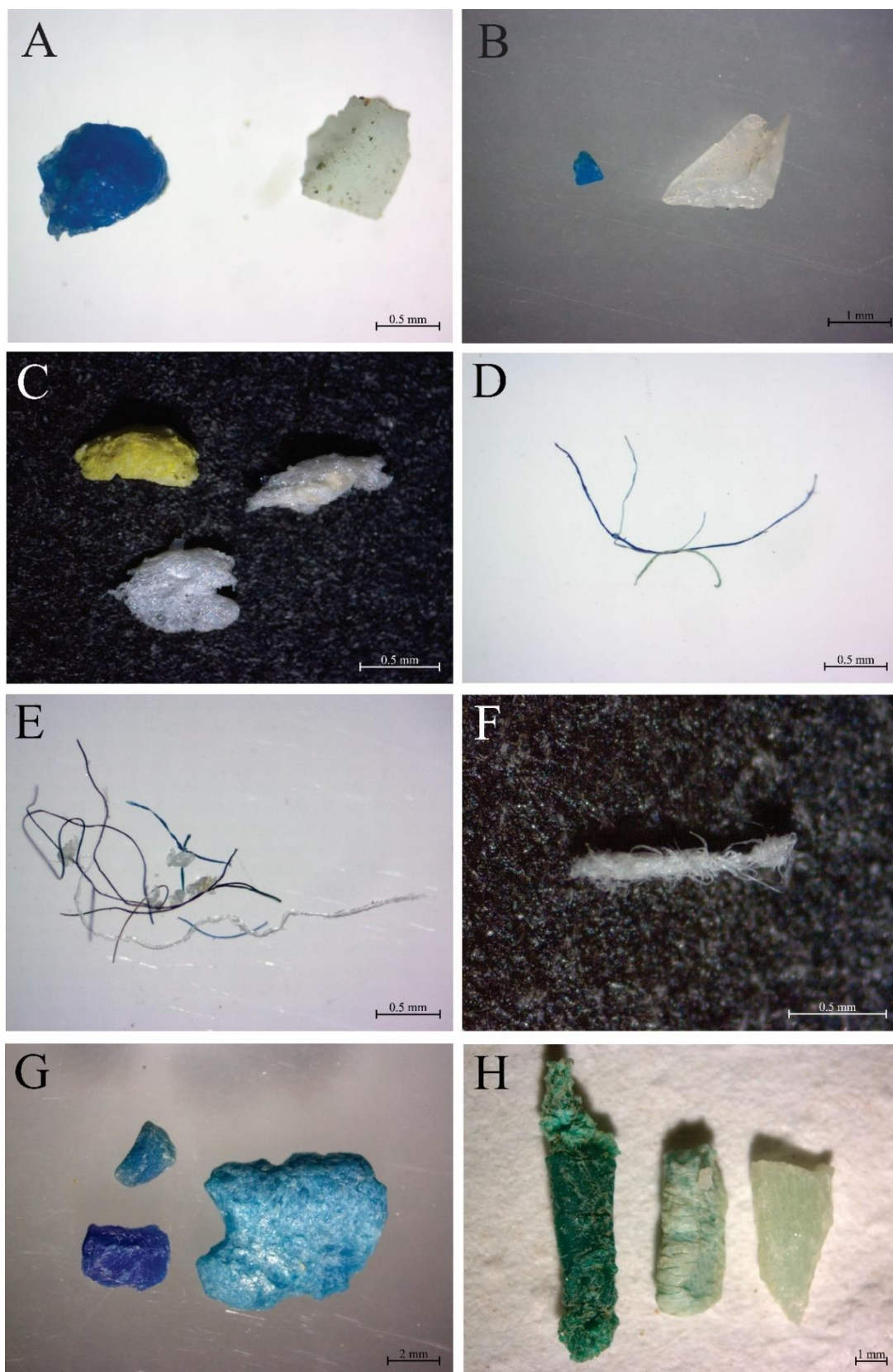
Figure 4: Total number and densities of small microplastic fragments on each sample (900 cm²) from four beaches (Cabritas, Parcel, Príncipe and Tartaruga) of Trindade island.



Fonte: A autora

Particles had a mean size of 0.45 ± 0.23 mm and were mainly smaller than 0.5mm (~70%). The total area of small microplastic fragments <1mm was of 10mm², representing approximately 0.01% of the total sampled area.

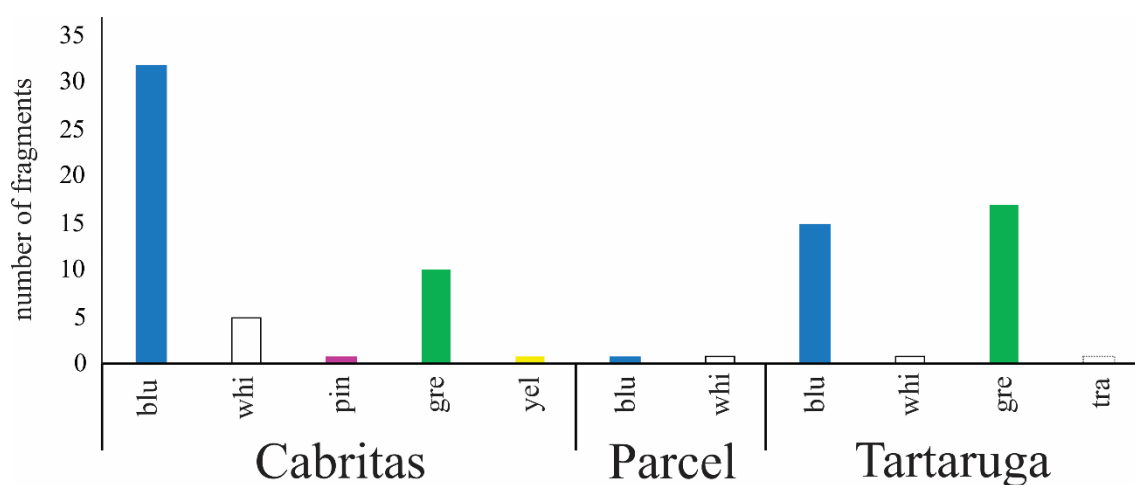
Figure 5: Plastic items found in samples from Trindade island. A-C: small microplastics; D-E: microplastic fibres; F: aggregated microplastic fibres; G, H: large microplastics.



Fonte: A autora

No clear pattern was found in relation to colours of small microplastics ($p=0.059$), although blue and green fragments were predominant on Cabritas and Tartaruga beaches, respectively. Other colours such as white, yellow and pink were also present on these beaches (Figure 6).

Figure 6: Colours of small microplastic fragments found on each sample from three beaches (Cabritas, Parcel and Tartaruga) of Trindade island. Codes for colours are: blu (blue), whi (white), pin (pink), gre (green), yel (yellow), tra (transparent).

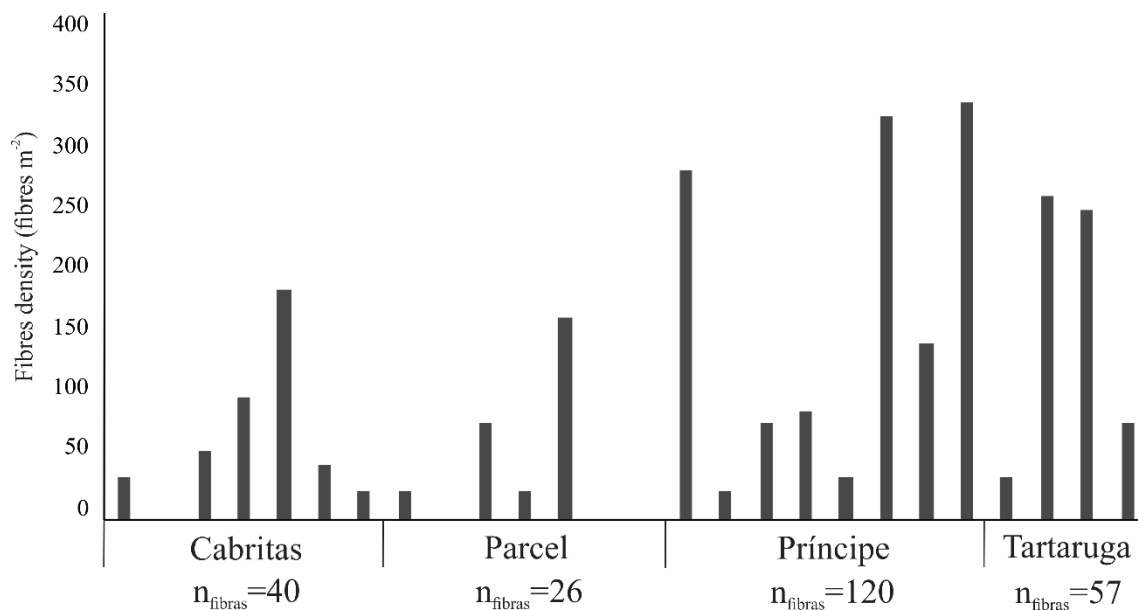


Fonte: A autora

4.6.2 Microplastic fibres

Microplastic fibres were identified in 22 of the 26 samples from Trindade island. All beaches were contaminated, and at least 243 fibres were found (i.e. some fibres, were tangled and could not be counted individually) (Figure 5F). Fibres were quantitatively the most common type of microplastic found on Parcel, Tartaruga and Príncipe beaches, but no significant difference was reported when compared to quantities of fragments and fibres ($p=0.4705$) (Figure 7). Microfibres were found in all samples, but no significant difference was found among beaches ($p=0.193$). Príncipe beach had the highest density for microplastic fibres, followed by Tartaruga, Parcel and Cabritas (Figure 7).

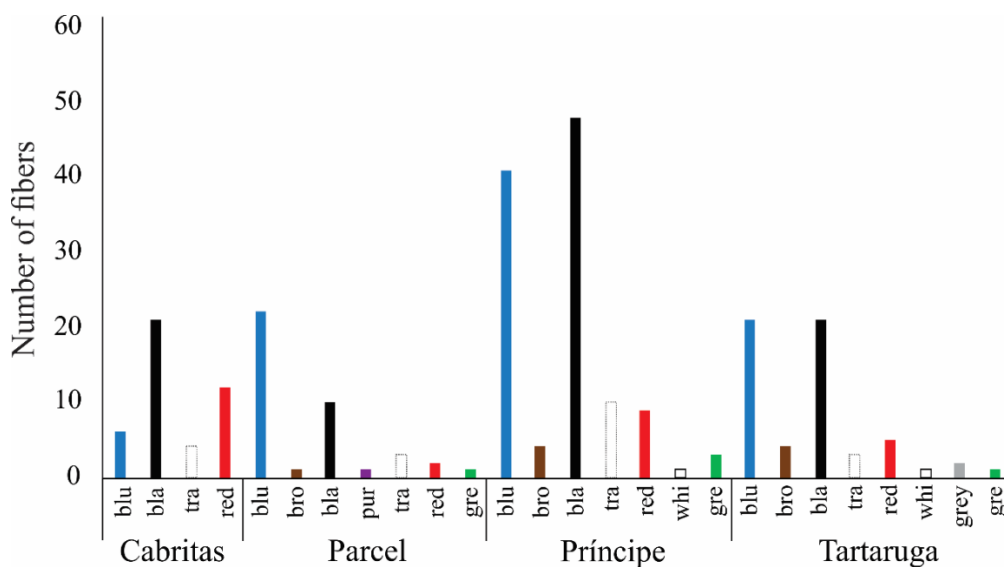
Figure 7: Total number and densities of microplastic fibres on each sample from four beaches (Cabritas, Parcel, Príncipe and Tartaruga) of Trindade island.



Fonte: A autora

Black was the most common colour for microplastic fibres, followed by red, transparent, brown, green, purple, white and grey, respectively (Figure 8). However, black was predominant in Cabritas and Príncipe. Tartaruga had the highest variety of colours (8), followed by Parcel (7), Príncipe (7) and Cabritas (4).

Figure 8: Colours of microplastic fibres found on each sample from four beaches of Trindade island (Cabritas, Parcel, Príncipe and Tartaruga). blu (blue), bla (black), tra (transparent), red (red), bro (brown), pur (purple), gre (green), whi (white), grey (grey).



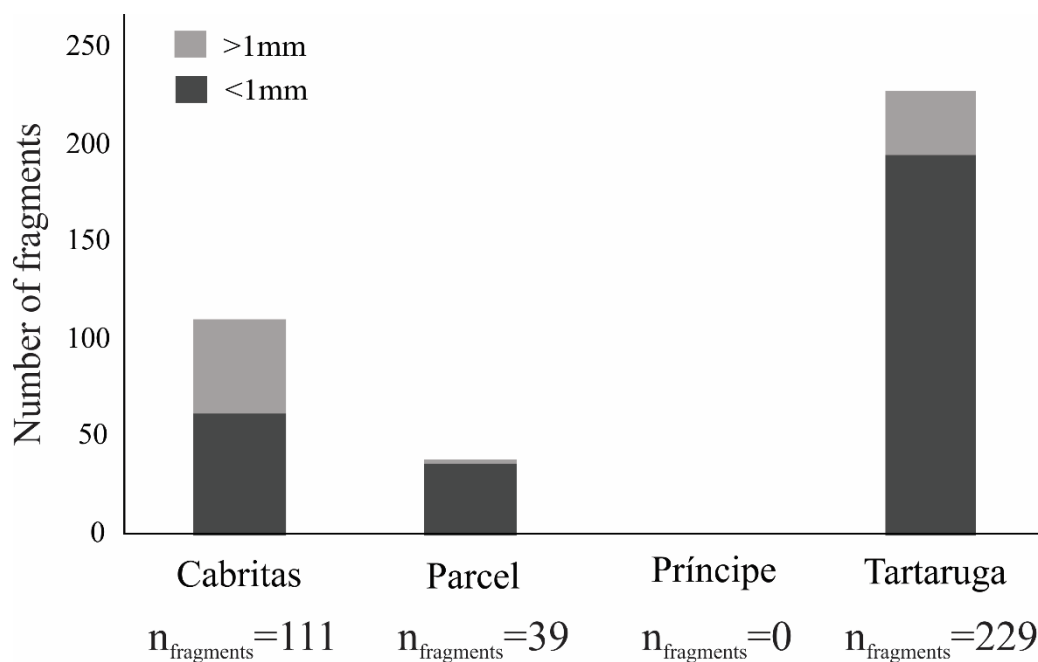
Fonte: A autora

4.6.3 Large microplastic and mesoplastic fragments

Twelve of the 26 samples analysed were not previously sieved through a 1mm mesh sieve and contained fragments bigger than 1mm, or large microplastics (Table 4). A total of 295 fragments from secondary origin were identified in these samples, with nearly half (~49%) considered mesoplastics (>5mm, mean size $5.93 \pm 4.29\text{mm}$).

Tartaruga was the most contaminated beach, with 196 fragments (Figure 9). However, most fragments (99.48%; $2166.6 \text{ particles m}^{-2}$) were in a single quadrat in the northern part of the beach, and could be considered an outlier. All pellets were found inside this quadrat. On Cabritas beach, 62 secondary particles were found, in a total area of 829.157 mm^{-2} .

Figure 9: Number of fragments (>1mm and <1mm) found in each of the four sampled beaches of Trindade island.



Fonte: A autora

A total of 37 fragments were found in Parcel, representing 1573.5 mm^{-2} of area. Again, no fragments were found on Príncipe beach. In general, there was no significant difference on plastic fragments between the analysed beaches of Trindade island, regarding density (particles m^{-2}) ($p=0.123$), quantity ($p=0.123$) and area (mm^2) ($p=0.124$).

Unlike sieved samples, white fragments were most present on non-sieved samples. Many of these appeared to be styrofoam fragments (28% of white fragments), distributed

in both Cabritas and Parcel beaches. Blue, transparent, black, yellow, red, green, pink, grey and beige fragments were also found on this survey.

4.7 DISCUSSION

Boucher and Friot (2017) state that 98% of microplastic fibres are originated on land by activities such as erosion of tyres and abrasion of synthetic fabrics and then released to the oceans. On the other hand, microplastic fibres can also originate from the abrasion of larger plastic items such as fishing nets (COLE, 2016). Nevertheless, fibres in Trindade island are more likely to be arriving onshore by wave and wind actions rather than being released from the island's human activities.

This specially applies for Príncipe beach. It is located on the leeward side of the island, where there are no human facilities, and still it had the highest number of microplastic fibres. In addition, fibres from seven different colours could be found on this beach indicating that they had various sources. This might be related to the fact that sampling occurred close to the period of sediment accretion, as described by Calliari et al. (2016). The beach profile on Príncipe suffer erosion between June and November, while there is sediment accretion and therefore higher sediment volume between March and April. Also, Príncipe is more exposed to storm waves when compared to the other beaches in Trindade (CALLIARI et al., 2016), which might be responsible for the transport of these fibres to this beach.

Beaches with higher contamination by secondary microplastics fragments were in the windward side of the island. Although they are not significantly more contaminated, this result indicate that wind and currents are important factors determining microplastic deposition on islands (COSTA; BARLETTA, 2015) and Trindade was no exception (IVAR DO SUL; COSTA; FILLMANN, 2017).

Presence of natural structures on the foreshore might influence microplastic deposition and removal on sandy beaches (VOUSDOUKAS et al., 2007; PINHEIRO et al., unpublished data). This applies for the beaches the windward side of Trindade island. Cabritas and Tartaruga beaches, for example, have continuous reef flats along the beach face, causing low sediment exchange between the beach face and the surf zone (CALLIARI et al., 2016). Hence, microplastics might easily accumulate on these areas. Variations in microplastic pollution between beaches of Trindade island are directly related to sediment dynamics, which can be explained by beach characteristics such as local hydrodynamics and beach profile. Príncipe beach has the most variable beach profile

among analysed beaches (CALLIARI et al., 2016), so sampling could have coincided with a sediment removal period which can explain the absence of microplastic fragments. This corroborate with Hinata and collaborators (2017), that stated that beach morphology is crucial to explain sediment flux and consequently microplastic residence time.

Shape, surface area and mean density of polymers can determine the dynamical properties of microplastics in the marine environment, influencing their movements and distribution within sediments and the seawater columns (CHUBARENKO et al., 2016). Vianello and collaborators (2013) also report sediment properties such as grain size and local hydrodynamics to affect plastic particle residence time and distribution. For small microplastics, however, factors affecting distribution within the marine environment are less known. Studies with larval dynamics and connectivity (e.g. D'AGOSTINI et al., 2015) might give some insights on how biophysical processes such as oceanic kinetic energy affect microparticles transport in the water and deposition on the strandline.

Trindade island is the biggest reproductive site for the green turtle *C. mydas*, with 4,808 nests during the 1999/2000 season alone (GROSSMAN et al., 2009). These nesting activities cause bioturbation of the sediment, which is another factor that can influence microplastic patterns on beaches (NÄKKI; SETÄLÄ; LEHTINIEMI, 2017) by changing microplastic distribution and accumulation in sediments. This might be significant because microplastics are suggested to alter sediment characteristics such as permeability and heat transfer, with effects to epi- and infaunal organisms and reptiles, the later having temperature-dependent sex determination (CARSON et al., 2011).

Comparisons with results from similar works can be hindered by some factors as reporting units (PINHEIRO et al, unpublished data). The units used to express microplastic concentration on beach sediments from oceanic islands vary a lot. Gregory (1983) have expressed microplastic concentration in particles per linear meter, finding up to 10000 microplastics per meter. In turn, McWilliams; Liboiron and Wiersma (2017) have reported microplastics in number or volume (m^3). All of these have only considered large microplastics ($>1mm$).

Small microplastics are not frequently reported and papers commonly do not analyse them as a separate category. Martins and Sobral (2011) and Mathalon and Hill (2014) have covered small microplastics, but it is not possible to calculate values of microplastic density for this category only. Van Cauwenberghe et al. (2013) and Vianello et al. (2013) analysed only small microplastics, but they expressed densities in items per mass of dry sediment, while Fischer et al (2015) and Imhof et al. (2013) expressed

densities in number of particles per area of sediment but did not separate large from small microplastics. Nevertheless, Costa et al. (2010) analysed small microplastics from an urban beach in Brazil, but the results found in Trindade are much lower.

Reporting microplastics as a bulk size class (everything <5mm) reduces the possibilities of ecological interpretations for the pollution phenomenon. Size classes have different effects, especially regarding the risk of ingestion by benthic fauna, and should be encouraged, at least using the division proposed (Table 4).

Although Cabritas, Parcel and Tartaruga beaches were contaminated with large microplastic fragments, the large majority of these fragments were found mainly in one single sample from Tartaruga beach. This result probably represents an outlier, as microplastic concentrations on this beach did not fit the values found by Ivar do Sul and collaborators (IVAR DO SUL; COSTA; FILLMANN, 2017). However, it is noticeable that important small-scale patchiness is possible and that it should be taken into consideration in planning future surveys.

The strandline acts as a pre-concentration microhabitat, facilitating the assessment of sources, sizes, colours and other characteristics of the stock available (DAVIES; GILLHAM, 2004). Therefore, this work reinforces the idea that sediment sampling on the strandline for microplastics assessment is an appropriate methodology to assess litter pollution on sandy beaches (SILVA-CAVALCANTI; DE ARAÚJO; DA COSTA, 2009), provided it is compared only to other similar works.

4.8 FINAL REMARKS

Microplastic pollution can virtually affect all coastal and marine environments, including isolated oceanic islands. Sandy beaches of Trindade island were contaminated with small microplastics, either fragments or synthetic fibres. Although these findings represent a snapshot of those beaches, it gives a baseline for future works to analyse temporal and spatial patterns of microplastic pollution on Trindade island.

Trindade island is an environment of high ecological importance. As biodiversity research in Trindade island is limited, it is not possible to accurately assess potential risks for local fauna with the data available. Nevertheless, microplastics represent a threat for local biota, especially small particles that can be ingested by virtually any species. It is then crucial to understand microplastic distribution and dynamics on coastal areas of

oceanic islands, and systematic surveys with different temporal scales are needed to determine microplastic transport dynamics. Also, future studies on local faunal biodiversity are also necessary to verify actual risks of microplastic pollution to the island's coastal ecosystem.

5 CONSIDERAÇÕES FINAIS

Este trabalho de dissertação apresentou alguns aspectos da interação dos microplásticos com o compartimento marinho bentônico e sua fauna, e descreveu a presença de microplásticos pequenos na ilha da Trindade, no Oceano Atlântico tropical.

A revisão bibliográfica mostrou ainda haver uma grande distância entre os resultados de experimentos de laboratório e os achados em campo sobre a ingestão de microplásticos pela fauna bentônica marinha. A atual forma como experimentos envolvendo microplásticos e o bentos são conduzidos precisa ser aperfeiçoada para refletir mais acuradamente as situações ambientais. Fatores cruciais como concentração, distribuição e bioturbação de microplásticos no sedimento precisam ser considerados e constantemente revistos em experimentos de laboratório e de campo para refletirem a evolução do problema no meio ambiente. Os compartimentos bentônico e planctônico possivelmente serão os alvos de monitoramentos regulares e exercícios de intercalibração no futuro, daí a importância de se achar um consenso sobre suas formas de avaliação o mais rapidamente possível.

O presente estudo também confirmou a poluição por pequenos microplásticos na ilha da Trindade, corroborando com a afirmação de que esses poluentes estão presentes até nos ambientes marinhos mais isolados. Entretanto, os fatores que influenciam a deposição e o transporte de partículas tão pequenas permanecem incertos. De qualquer forma, é de extrema importância que os diversos aspectos da poluição por microplásticos sejam caracterizados no ambiente bentônico para que futuras ações sejam propostas de forma a controlar a chegada descontrolada desses poluentes no ambiente marinho, assim como o tratamento de seus passivos.

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