

Michael Manthopoulos

**Presence of microplastic in commercial
bivalves along the Portuguese coast,
comparing different aquaculture systems.**



UNIVERSIDADE DO ALGARVE

Faculdade de Ciências e Tecnologia

2022

a64241 - Michael Manthopoulos

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along the Portuguese coast, comparing
different aquaculture systems.**

Mestrado em Biologia Marinha

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2022

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Acknowledgements:

First and foremost, I would like to thank Professor Dr. Maria João da Anunciação Franco Bebianno for accepting to supervise my thesis project and giving me the opportunity to participate in the collection of data in the scope of an ongoing project “PlasticSea”. I would also like to recognise Justine Nathan for her directions and assistance as the co-supervisor of this project.

I am grateful to CIMA (Centro de Investigação Marinha e Ambiental) for allowing me to conduct the analysis of data within their laboratory facilities, at the University of the Algarve, in the campus of Gambelas.

ABSTRACT

Plastic pollution is a hazard that has entered many aspects of life and is becoming increasingly distressing on human health. The consumption of marine animals, unfortunately, is now associated with the consumption of plastic, including in aquaculture species. Bivalves are traditionally grown in aquaculture facilities in Portugal for national and international consumption. During the cultivation of these animals, plastic is used in nets and sacks for the growth process and in collection and processing. As more evidence appears for negative impacts of plastic consumption on human health, it is becoming more important to expand the global knowledge base of how much plastic is present in our food. The aim of this thesis as part of the PlasticSea project was to investigate the presence of plastic in three species of bivalves in Portuguese aquaculture: Mediterranean mussels (*Mytilus gollaprovincialis*), clams (*Ruditapes decussatus*) and Pacific Oysters (*Crassostrea gigas*). Five different locations were selected from the Portuguese coast in the regions of Aveiro, Sado and the Algarve. 15 organisms from each location were analysed to identify plastic, remarking their size, colour, and type. 54 particles of plastic were successfully identified from 90 organisms (0.6 particles/ individual), of which the most common colour was black and most common type was fragments. Results were compared to coastal organisms from different natural environments which showed that there was no significant difference between the amount of plastic ingested by each category (natural or aquaculture), but aquaculture specimens are more likely to ingest black plastic, feasibly from aquaculture equipment. Further studies such as spectroscopy analysis would be needed to determine if this is true. This data will help to identify and eliminate the reasons of occurrence of microplastics in marine organisms such as bivalves.

Key-Words: Micro particles; Polymer; oyster; mussel; clam; aquaculture; Portugal

RESUMO

A produção de plástico para utilização em todos os sectores da sociedade está a aumentar, e como resultado da má gestão desse produto, a poluição pelo plástico está a aumentar. Este é um perigo que tem afetado muitos aspetos da vida humana e está a tornar-se cada vez mais preocupante para o ambiente e para a saúde humana. Um dos ambientes que tem gerado muita preocupação devido ao problema deste tipo de poluição é o oceano. A entrada do plástico no oceano é proveniente de muitas fontes, incluindo o lixo da pesca e o lixiviado de fontes terrestres. No oceano, grandes fragmentos de plástico, tais como redes de pesca, podem causar danos aos animais através do emaranhamento, o que também é chamado de pesca fantasma. Estes grandes fragmentos degradam-se e decompõem-se mecanicamente pela ação das ondas ou também quimicamente pela degradação UV, e resultando em microplásticos (MPs). Outra fonte de MPs é originada diretamente a partir de partículas plásticas em cosméticos ou pellets, e estas podem ter tamanho menor que 5 mm. Devido ao pequeno tamanho, os microplásticos são facilmente confundidos como alimento para muitas espécies no oceano ou ingeridos inconscientemente. Quando ingerido por organismos marinhos o plástico pode ter consequências destrutivas, o que pode resultar, por exemplo, na redução da capacidade de alimentação e transferência de substâncias nocivas, tais como químicos e poluentes orgânicos persistentes (POPs), para os seus tecidos. Mundialmente, muitos estudos têm sido conduzidos sobre a presença de plástico nos organismos marinhos e seus impactos. A presença de plástico em organismos marinhos está a surgir com mais frequência provando a capacidade dos organismos de acumula-lo. O consumo de frutos do mar e espécies aquícolas, tanto provenientes da pesca quanto da aquicultura, infelizmente, estão agora associados ao consumo de plástico. Os bivalves, em Portugal, são tradicionalmente cultivados em instalações de aquicultura para o consumo nacional e internacional, durante o cultivo destes organismos, o plástico é utilizado em redes e sacos para o processo de crescimento, na colheita e processamento, contudo, ainda não se sabe, se e, até que ponto o plástico tem impacto nos bivalves. À medida que aparecem mais provas dos impactos negativos do consumo de plástico na saúde humana, torna-se ainda mais importante expandir o conhecimento global sobre a quantidade e efeitos do plástico nos nossos alimentos e assim elaborar estratégias para mitiga-los.

Em vista disto, esta tese integrada no projecto PlasticSea teve por objetivo investigar a presença de plástico em três espécies de bivalves na aquicultura portuguesa, mexilhões (*Mytilus gollaprovincialis*), amêijoas (*Ruditapes decussatus*) e ostras do Pacífico (*Crassostrea gigas*). Recolheram-se amostras destes bivalves em cinco locais da costa portuguesa, um em Aveiro (aquicultura entremarres), um no Sado (aquicultura entremarres), e três na região do Algarve (EPPA (aquicultura costeira), Ria Formosa (aquicultura entremarres), e Sagres (aquicultura offshore). Os organismos foram recolhidos em cada área de amostragem (15 indivíduos em cada área) utilizando técnicas para evitar qualquer contaminação plástica e posteriormente analisados em laboratório. Todos os organismos foram medidos, pesados e dissecados. O tecido molde de cada organismo foi digerido com KOH e incubados durante pelo menos 48 horas em estufa à temperatura de 50 °C. Uma vez digeridas, as amostras foram colocadas numa solução hipersalina (NaCl) com densidade de 1,2 para recolher microplásticos flutuantes. Esta solução contendo as partículas flutuantes foi então filtrada com uma bomba de vácuo utilizando filtros de acetato de celulose de 5 µm. Os filtros foram analisados com auxílio de um microscópio para identificar as partículas de plástico presentes em cada amostra. Foram identificadas como plástico as partículas que não reagiram com HCl e que reagiram com uma agulha quente. As partículas identificadas como plástico foram registradas, observando-se também o seu tamanho, cor, e tipo.

No presente estudo foram identificadas 54 partículas de plástico em 90 organismos (0,6 partículas/ indivíduo), das quais a cor mais comum foi o preto e o tipo mais comum fragmentos. Foram efetuadas análises estatísticas uni e multivariadas com o foco em responder a pergunta da investigação sobre a presença de microplástico na aquicultura. A presença de plástico foi identificada em todas as áreas estudadas em pelo menos um organismo. Em comparação com as amostras recolhidas o local com a maior presença de plástico foi no estuário do Sado, o que muito provavelmente é resultado de estar localizado perto de uma área povoada e poluída do país. No presente estudo 35,6% de todos os organismos apresentaram partículas de plástico nos seus tecidos, o que corrobora com outros estudos sobre a mesma espécie alvo. Os resultados foram comparados com organismos costeiros de onze locais no ambiente natural do Algarve, o que mostrou que não havia diferença significativa entre a quantidade de plástico ingerida por cada categoria (natural ou aquicultura). Contudo, a análise estatística mostrou uma diferença entre locais como Portimão, que era o sítio costeiro com a maior

quantidade de plástico nos organismos amostrados. Outro resultado encontrado foi que os espécimes de aquicultura são mais propensos a ingerir plástico preto, possivelmente de poluição externa ou de equipamento de aquicultura como estruturas de plástico preto usados para cultivar ostras em sistemas entremarres no Sado e em Aveiro. Outros estudos como a análise espectroscópica seriam necessários para determinar se isto é verdade, comparando os resultados dos espectros do plástico do equipamento no campo com o que se encontra nos tecidos dos organismos. Os resultados fornecem possíveis provas de que o equipamento plástico utilizado em aquicultura contribui para a ingestão de resíduos plásticos por organismos que são vendidos para o consumo humano. Estes dados ajudarão os decisores a identificar e eliminar as razões da ocorrência de microplásticos em organismos marinhos, tais como os bivalves. Uma sugestão feita é que o método com menor exposição ao plástico está a utilizar a aquicultura offshore onde o plástico não se pode acumular. A quantidade de plástico nos nossos alimentos pode estar a afeitar a saúde humana, pelo que é urgente melhorar a qualidade dos alimentos. Globalmente, este estudo demonstra a quantidade desnecessária de plástico no oceano e os efeitos adversos que os seres humanos estão a contribuir no ambiente marinho, mostrando que é necessário fazer mais, desde governos a indivíduos, para ajudar a enfrentar o problema da poluição pelo plástico.

Palavras-chave: Micro partículas; Polímero; ostras; mexilhão; amêijoas; aquicultura; Portugal

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LIST OF ABBREVIATIONS

CIMA -Centro de Investigação Marinha e Ambiente

CP -Cellophane

EPPO -Estação Piloto de Piscicultura em Olhão

FTIR -Fourier- transform infrared spectroscopy

GHG -Greenhouse gas

IPMA -Instituto Portugues do Mar e Ambiente

MDS -Multi-Dimensional Scale

MP -Micro-plastic

Mt -Million tones

NP -Nano-plastic

PA -Polyamide

PC -Polycarbonate

PE -Polyethylene

PET -Polyethylene terephthalate

POP -Persistent organic pollutant

PP -Polypropylene

PS -Polystyrene

PVC -Polyvinyl chloride

SGM -Sagremarisco

VRSA -Vila Real de Santo António

1 INTRODUCTION

1.1 Plastic in the environment

Plastic compounds are polymers which have now been synthesised by mankind for more than 100 years. This time frame is significantly short when looking at the impact that this material has had on the environment and how it has changed biomes. Overall, polymeric micro-particles are created to be very persistent thus resilient to biodegradation, and allows them to accumulate in the natural environment which eventually disturbs eco-physiological processes (ECHA, 2019).

Globally detected in soils, air, oceans and the integrated biota, plastic composites are considered to be contaminants in the natural environment by the scientific community (Rochman & Hoellein, 2020). A large proportion of items made of plastic are discarded, sometimes intentionally and often washed until the oceans, up to the point where they are now mentioned omnipresent, polluting at all biological levels of the marine world (Sharma & Chatterjee, 2017). Although the presence of plastic is globally detected in all kinds of water bodies, the concentration of plastic items varies greatly between locations. About 80% of plastic pollution in the oceans is land-sourced due to poor lifecycle management and inefficient disposal of plastic items (Martins & Sobral, 2011). Plastic Europe declares that from the nearly 370 million tonnes (Mt) of plastic produced worldwide in 2020 less than 10% of it was collected in their end-of-life. Of which, 24% were disposed in landfills while only one third was sent to recycling facilities (Lusher et al., 2017; Plastics Europe, 2021). The already very high level of plastic production and consumption is growing each year by nearly 100Mt more than ten years ago. There was a slight decrease in production during the first half of 2020 due to the COVID-19 pandemic, but also a fast “recovery” afterwards as the demand for plastic, especially single-use items increased greatly (Plastics Europe, 2021).

Apart from the aesthetic degradation of the environmental perception created by the presence of plastic debris in nature that disturbs sight-seeing for instance, large plastic debris are also hazardous for the wild marine organisms that can get wounded through entanglement or suffer from asphyxia due to ingestion (Cole et al., 2011; Ivar Do Sul & Costa, 2014). Furthermore an additional concern is the effects of weathering and

degradation causing the plastic debris to breakdown into small particles down to the micro and nano-metre size range which makes them biologically available to interact with the natural world, and believed to trigger serious issues within organism functions (Avio et al., 2017). In terms of plastic weight found in the oceans the majority of it is still in the form of large items, referred to as macro-plastic, and defined by measuring more than 5 mm from the fragments widest angles. The smaller, so called microplastics (MPs) (<5 mm) were first detected in the environment in the 1970s (Carpenter & Smith, 1972) and now represent an emerging hazard for marine organisms as the breakage into MP particles exponentially increase with the quantity of plastic debris discarded (Sharma & Chatterjee, 2017). Examples of studies about interactions between MPs and the environment are listed in Table 1.1 .

Table 1.1 : Evidence of impacts of microplastics

Subject	Location	Types of plastic	Procedure	Results	Reference
Human safety	London	Micro- and macroplastics debris	Survey & questionnaires 42 participants	81% witness Pollution daily Good understanding of risk but little concern (n=27 feel importance of pp) policy maker and industrials responsibility, not consumers.	(Henderson & Green, 2020)
Biodiversity	Global	MPs pollution	Litt.review	Source control (less production, consumption) solutions to limit MP pollution the 5Rs!	(Ivar Do Sul & Costa, 2014)
Shoreline sediment Pollution	18 costal worldwide with diverse population density	MP pollution	Abundance of particles in 250L sediments samples 18 sites, 5 samples per site	Relation of abundance of MP particles and populations density. Sewage-disposal key source of MP to marine habitat. polyester fibre was dominant, suggesting source from washing clothes and cleaning products. Ultrafiltration of sewage wastewaters is efficient to reduce MP contamination. Sediment is a sink for MP	(Browne et al., 2011)

<p>Bivalves (species of oysters clams and mussels)</p>	<p>China research</p>	<p>MPs in literature</p>	<p>Bibliometric & meta-analysis related to</p>	<p>Distribution, molecular mechanisms, and toxicity. The presence and level of oxidative damage induce by MP particles in bivalves is time dependent. Catalase activity reveals as a reliable biomarker to study sublethal effects of MP in bivalves.</p>	<p>(Li et al., 2022)</p>
<p>Bivalves</p>	<p>Vigo, Spain Cadiz, Spain</p>	<p>MPs and NPs</p>	<p>Review of laboratory experiments, mesocosm bioassays and field studies to assess MPs and NPs effects on bivalve species</p>	<p>Main uptake of MPs in bivalves is through the gills. MPs can be transported to the digestive gland and haemolymph. Particle size influences the percentage of gut retention. Bivalves expel higher percentages of large and spherical particles. No mortality of bivalves induced by MP. Changes in biomarkers related to oxidative stress when bivalves are exposed to MPs recorded, cause DNA damage. The high level of plastic ingestion in bivalve directly and indirectly influenced humans health.</p>	<p>(Sendra et al., 2021)</p>

MPs can enter the ocean environment via a variety of pathways. Primary MPs refers to industrial manufacturing of particulate polymers for direct usage such as pre-production pellets or nurdles, or microbeads used in assemblage of consumer goods, cosmetics or even to supplement chemical solutions used in industrial agriculture (De Witte et al., 2014). Secondary MPs are the result of the degradation of primary, larger plastic items from natural processes. When these plastic debris are wrongly discarded, either by consumers, producers or fisheries (fishing gear, consumer goods & packaging, toys, cigarette butts), they eventually reach the ocean and break down until they reach micro sized particles of 5 mm or smaller, which are molecularly persistent thus hardly biodegradable (De Witte et al., 2014; Henderson & Green, 2020). Until lately there was no certainty that plastic decomposition and degradation would cause wider impacts as it remains in a form that could be inert. However, a recent study showed that UV rays from the sun initiate photochemical mechanisms that cause the disintegration and release of other tenacious toxic chemicals present in plastic mixtures (Walsh et al., 2021).

A large proportion (about 65%, the rest is recycled) of the end-of-life plastic that was collected still ends up in incinerators or in landfills where it can still disperse and contaminate the environment through emanations or leakage as micro plastic particles (Millican & Agarwal, 2021). Many being invisible to the human eye, it is a difficult challenge to establish realistic measurements of their presence and their concentration in the various marine ecosystems, which is expected to increase exponentially due to the disintegrating processes previously mentioned coupled with the fact that we keep producing hundreds of million tonnes of it every year whilst still mismanaging and dumping plastic waste (Koelmans et al., 2017).

Once in the ocean, these MP particles, depending on their specific gravity of polymers, either float or sink in seawater. Polypropylene (PP), polyethylene (PE) and polystyrene (PS) all float in seawater, allowing MP to spread globally through marine currents, whereas more dense materials such as nylon and polyester sink (Andrady, 2011). Pelagic organisms such as fish that feed at the surface can confuse these particles for food and directly ingest them (Clere et al., 2022). Benthic feeders are exposed to plastics that drop to the bottom and permeate in the sediment (Avio et al., 2017). Accumulation of plastic in the guts of organisms can cause blockage and lead to malnutrition, reducing the overall physical fitness, making them more vulnerable to predation (Ivar Do Sul & Costa, 2014). Sometimes the gastric blockage can even cause starvation. Bivalves can be found in the

sediment or attached to substrates including rocks and manmade structures, these organisms are thus potentially exposed to MP due to their feeding strategies that is unselective (Trestrail et al., 2021). Oysters and mussels for example, are filter-feeders filtering seawater, whereas clams are suspension-feeders, nourishing straight from the sediment. Here, in the shallow coastal waters or in harbours, there can be an accumulation of microplastic particles which move through the water column and settle in the sediment. Bivalves feed by filtering the water or sediment, which reduces in general their selectivity of nourishment. Therefore, they can inadvertently ingest MPs present in their environments and accumulate them in their tissues (Cozzolino et al., 2021). Examples of MP presence in bivalves species are listed in Table 1.2. Once present in their bodies, it is expected to cause negative effects on the organisms themselves but maybe also affect future generations well-being (Clere et al., 2022; Sussarellu et al., 2016)

Table 1.2 : Evidence of microplastics in bivalve species.

Species	Location	Types of Plastic part.	Presence of plastics in the organisms	results	Reference
Oyster (<i>Crassostrea gigas</i> , <i>Crassostrea angulate</i> , <i>Crassostrea hongkongensis</i> and <i>Crassostrea sikamea</i>)	China	Ploymeric types CP-PE-PET-PP- PS-PA-PC-PVC	84% of organisms n=1218 items 902.82 ± 782.99 µm,	Cultured oysters Average abundance was 0.62 items/g, 2.93 items/individuals.	Teng et al., (2019)
Oyster and mussel + sediments, coastal waters, and wastewaters	North Sea Coast of Nederland	MP fibres	Concentrations between 11 and 105 particles/g d.w	Higher concentration in filter feeding organisms	(Leslie et al., 2013)
<i>Mytilus edulis</i> <i>Mytilus galloprovincialis</i>	Belgium	Black, red, blue, purple, translucent, transparent, orange, green fibres, with the most common size class being 1000–1500 lm	2.6 to 5.1 fibres/10 g of mussel.	The uptake of plastic films and spherical or granular microplastics from their natural environment was not observed for the assessed mussels.	(De Witte et al., 2014)

<i>Mytilus edulis</i> <i>Crassostrea gigas</i>	Germany Brittany	MP	microparticles were present in both species tested. environmental collection: 0.36 ± 0.07 particles g^{-1} (wet weight), 0.47 ± 0.16 particles g^{-1} ww. After depuration period: 0.24 ± 0.07 particles g^{-1} ww. 0.35 ± 0.05 particles per gram (ww)	After the depuration period only the largest MP particles were expelled by the organism	(Van Cauwenberghe & Janssen, 2014)
<i>Mytilus edulis</i>	French-Belgian-Dutch coastline	MP of low and high density PE, PS	MP present in all organisms collected in the field 0.2 ± 0.3 microplastics g^{-1}	Calculation on microplastic retention efficiency demonstrated that a mussel will be exposed, and potentially take up, approximately 10 particles per day.	(Van Cauwenberghe et al., 2015)
Bivalves	Worldwide	MP	100% of the farmed mussels Contained MP	Farmed blue mussels contain more microplastics than wild mussels from adjacent area. Concentrations are still relatively low	(Bråte et al., 2014)
<i>Ruditapes decussatus</i> <i>Cerastoderma</i> spp. <i>Polititapes</i> spp.	Ria Formosa Lagoon, southern Portugal	88% of the MPs found were synthetic fibers (blue). Dimension: >0.1-1mm and 1-5mm. Polymer: PE, PS	<i>Ruditapes decussatus</i> presented the highest abundance (18.4 ± 21.9 MP items g^{-1} WW) <i>Cerastoderma</i> spp. (11.9 ± 5.5 MP items g^{-1} WW) <i>Polititapes</i> spp. (10.4 ± 10.4 MP items g^{-1} wet weight)	The abundance of MPs (items per soft tissue weight) did not significantly differ among species	(Cozzolino et al., 2021)
<i>Mytilus</i> spp.	Portuguese coast	Particles size: 36-4439 μm Fibers (50%) Films (20%) Spherules (18%)	0.54 to 3.0 items g^{-1}	No significant difference among sites	(Marques et al., 2021)

Organisms that are intentionally exposed or accidentally ingest MPs have shown some level of damage to their tissue (see examples listed in Table 1.3) affecting cells enzymatic activities and molecular structures (Paul-Pont et al., 2016). In fish, there is evidence of MPs damaging growth development and the regeneration process (Tarasco et al., 2022). More precisely, effects on bivalves have been observed during experimental exposure damaging the DNA, neurologic systems, promoting oxidative stress and affecting reproductive activity (Li et al., 2022; Ribeiro et al., 2017; Sussarellu et al., 2016). A laboratory exposure assessment of the clam species *Scobricularia plana* used biomarkers to evaluate the physical damage to the tissues of the organisms and alterations to their genetics, showing DNA and oxidative damage among other impacts (Ribeiro et al., 2017). Pollution of the aquatic ecosystems from MPs where they are globally detected is additionally acting as a sink gathering at the surface, displaced through the water column or trapped in benthic sediment (Browne et al., 2011), as such particles are persistent in the environment accumulating in various organisms along the food chain (Cole et al., 2011), eventually causing a potential hazard to human health when consuming seafoods such as fishes, crustaceans or bivalves in which such pollutants accumulate (Ribeiro et al., 2017; Teng et al., 2019). In addition, plastic compounds are usually associated with persistent organic pollutants (POPs), which are known to have effects on higher trophic levels in the food chain we belong to. POPs, along with other harmful chemicals and compounds, also bind to the surface of plastic as it is hydrophobic increasing the concentration of contaminants. As plastic particles degrade into smaller pieces the surface area to volume ratio increases, therefore increasing the number of pollutant compounds that can be adsorbed and when ingested by organisms, becomes bioavailable for accumulation throughout the trophic levels (Courtene-Jones et al., 2017).

Table 1.3: Evidence of MP impacts on bivalves health

Species	Location	Types of Plastic part.	Mean Particle size / n samples	Results	Reference
Clams <i>Scobricularia plana</i>	South Portugal Laboratory	PS microparticles	20 µm n=60	Effects on antioxidant capacity, DNA damage, neurotoxicity and oxidative damage	(Ribeiro et al., 2017)

Mussel <i>Mytilus spp.</i>	Laboratory exposure (Nantes, Fr.)	Polystyrene microbeads in combination with Fluoranthene	6 µm with concentration 32µg/L 12 treatments with 24 mussel/treat	micro-PS led toxic effects at tissue, cellular and molecular levels, and controlled fluoranthene kinetics and toxicity in mussels	(Paul-Pont et al., 2016)
Oyster (<i>Crassostrea gigas</i>)	Laboratory exposure (France)	Micro polystyrene PSspheres (2 and 6µm at 0.023mg/L)	PSspheres (2-6 µm at 0.023mg/L)	38% oocytes with -5% diameter, and reduce sperm velocity. Less offspring -41% Minor larval development - 18% (vs control) Feeding & absorption disruptions when exposed to micro-PS	(Sussarellu et al., 2016)

1.2 Human Health

Bivalves are a commercially valuable species. Development of technologies and equipment that use plastic have made bivalve aquaculture more efficient in recent years but there may also be potentially negative impacts of using plastic in the production of seafood, as bivalves may be more exposed to plastic particles in the water column or sediment and therefore ingest such contaminants more frequently. If the plastic or associated contaminants accumulate in the tissue of organisms, they would ultimately be ingested by humans once consumed. Found in different sizes and at different levels of biological organisation plastic compounds present a threat to the environment and thus to human health. Moreover, plastic pollution presents high level of costs, economic, but also ethical, aesthetic and ecological. Although the risk assessment is a complicated task that needs a rational framework to assess the impacts of plastic debris as proposed by Koelmans et al., (2017). Authors explain how complicated it is to assess the effects of plastic pollution itself due to other chemical components that are linked to debris of plastic.

1.3 Study area

Portugal is in southwestern Europe and most of the population live near the coastal region, where they rely on the ocean for a lot of their food. Aquaculture is a big industry in Portugal contributing greatly to the economy. Bivalve aquaculture supports many jobs in the country, provides food and contributes to international trade therefore is an important area of study. The Portuguese coast is vulnerable to plastic debris accumulation, high fishing activity, increasing recreational activities, one of the major route for commercial and touristic cruises pass by the Portuguese waters and many currents accumulating plastic debris and thus MPs along the coastline (Martins & Sobral, 2011). The increasing amount of plastic in the ocean may cause aquaculture organisms to become contaminated so it is important to monitor this level in Portugal for both ocean and human health. Commercial aquaculture stations are used in this study from three regions in Portugal: The Algarve, Aveiro and Sado Estuary.

2 OBJECTIVES

This study aims to assess the level of plastic in the tissues of three commercial bivalve species from multiple locations across the Portuguese coast from different types of aquacultures to establish conclusions about the general amount of plastic pollution in our food and if there are any accumulation zones or better practices for bivalve farming. Then also to compare between commercial bivalve species that have different feeding strategies. The following questions were created to reach conclusions on this topic.

How much plastic is present in bivalves of aquaculture in Portugal?

Is there a difference in the amount of plastic between organisms from natural and aquaculture sites?

Is there a difference in the amount of plastic between different aquaculture types?

Does the feeding strategies between different bivalve species affects the amount of plastic ingested?

Does equipment used in aquaculture contribute to the ingestion by organisms?

3 MATERIAL AND METHODS

3.1 Sampling

As a part of the PlasticSEA project, sampling campaigns were conducted by different teams according to the different bivalve environments, either natural coastal area or in aquaculture facilities. CIMA sampled bivalves from the different natural sites, Sagremarisco (SGM) provided samples from aquacultures and IPMA (Instituto Português do Mar e da Atmosfera) with inshore aquacultures specimens. Sampling campaigns were held during the year 2021 in spring (March). From each site, 25 individuals were randomly collected from a defined area. Mussels (*Mytilus gollaprovincialis*), clams (*Ruditapes decussatus*) and Pacific Oysters (*Crassostrea gigas*) were the bivalves species selected for this project. 16 sites along the coast of Portugal were sampled, 11 coastal sites and five aquaculture sites (Figure 3.1). This project will focus on the aquaculture sites to determine variances between location, species and type of aquaculture but compare overall results to those of the coastal regions to identify differences caused by aquaculture practices.

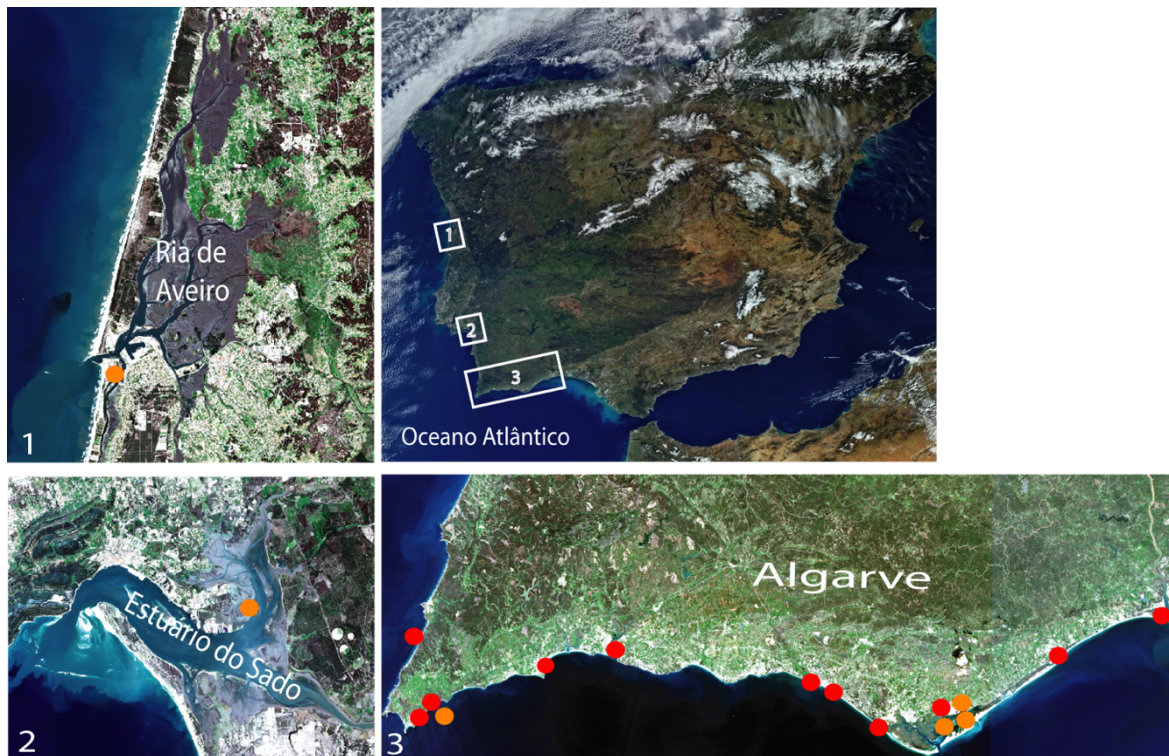


Figure 3.1: Map of sampling locations along the coast of Portugal. Orange points are Aquaculture stations and red points are coastal sampling sites.

Mussels were collected from natural coastal sampling sites, which are all located in the Algarve ranging from the west coast to the border with Spain. These sites are Praia da Barriga, Sagres harbour, Praia da Barranco, Lagos, Portimão, Vilamoura, Forte Novo (Quarteira), Faro, Olhão, Tavira and Vila Real de Santo António (VRSA) as shown in Figure 3.1. Oysters were collected from three aquaculture facilities which use plastic growing bags placed inside the water, attached to structures, two being intertidal aquaculture farms in the Sado estuary near Setúbal and the Ria do Aveiro and one site at an inshore aquaculture farm in Estação Piloto de Piscicultura em Olhão (EPPO), Ria Formosa. Cultured mussels were collected from two aquaculture locations, one in an offshore aquaculture facility in Sagres, Finisterra, which uses longlines on which the mussels are grown in depths between 5 to 12 meters. The second, from an intertidal aquaculture site, located in the Ria Formosa near to and hereafter referred to as Fortaleza, where mussels grow on a manmade substrate. Clam samples, which are seeded in the sediment within a maintained area were also (Figure 3.2).



Figure 3.2: Photos of collection of samples from each aquaculture location. a) Oysters from an intertidal farm in Ria do Aveiro. b) Oysters from an intertidal farm in Sado Estuary near Setúbal. c) Mussels from offshore aquaculture longlines in Sagres collected by divers at a depth of 5 m. d) Oysters from an inshore aquaculture farm in EPPO. e) Clams and mussels from an intertidal aquaculture farm in Fortaleza, Ria Formosa.

Sampling strategies such as materials, storage of samples and methodologies to assess MP pollution in bivalves followed a precise protocol determined from the project team according to the “Manual for good methodological practices to assess MP contamination in water, salt, sediment and bivalves” issued by CIMA (JPI oceans standardize protocol). Thus, sampling was conducted using no plastic equipment to avoid contamination. From each of the aquaculture facilities 20-25 farmed bivalves of commercial size were provided by the producers, Oysters (*Crassostrea gigas*) from Aveiro, Setúbal and EPPO-IPMA, Clams (*Ruditapes decussatus*) from Fortaleza and mussels (*Mytilus galloprovincialis*) from Finistera and Fortaleza. The organisms were wrapped in aluminium foil, transported in a thermal box to the laboratory and frozen until further analysis.

3.2 Laboratory analysis

3.2.1 Identify microplastic

Samples were defrosted in the laboratory ready for analysis. Organisms were measured for length using callipers and weighed in a balance to obtain overall, shell and tissue weight in grams. Organisms were dissected using a scalpel. Once physical measurements were taken from each specimen, the soft tissue was entirely separated from the shell, placed and weighed in separate and labelled (250 ml) Erlenmeyer flasks for digestion. The tissue was covered with Potassium Hydroxide (KOH) at 10% solution using a ratio with at least 5:1 ml per gram (soft tissue wet weight). Flasks were placed in the oven at 50°C for two days or until digestion of tissue was complete. Blanks containing KOH solution were also placed in the oven for further analysis to check the level of external contamination in the laboratory during the experiment. Once completely digested the samples were transferred to 100 ml glass cylinder, which stand inside 1 L glass beakers to collect the plastic particles through density separation method (Munno et al., 2018; Teng et al., 2019). Pre-filtered sodium chloride (NaCl) solution at a density of 1.2 was added to the digested solution. Samples were covered with aluminium foil and left to settle for around 30 minutes. Due to the higher density of the saline water in relation to the plastic density polymer particles are expected to float. Accordingly, a glass test tube was inserted into the top of the cylinders to cause the superior part of the solution, which should contain most of the MPs present in the original sample, to overflow into the glass beaker below. Samples were agitated using a glass rod, more NaCl solution added and left for another 30 minutes to settle. The same process is repeated to overflow the floating particles as shown in picture Figure 3.4 C. What is collected in the glass beakers is then transferred to a pump filtration system consisting of a Vacuum pump (Pall®) attached to a three-way filtering ramp, 3 Glass filter holders, 3 Glass filter flasks (250 ml), attached to the filter holders with metal clamps. Cellulose acetate filters with mesh size 5 µm or smaller were used to collect the plastic particles present in the samples. Filters were stored in aluminium envelopes until further microscopic analysis.

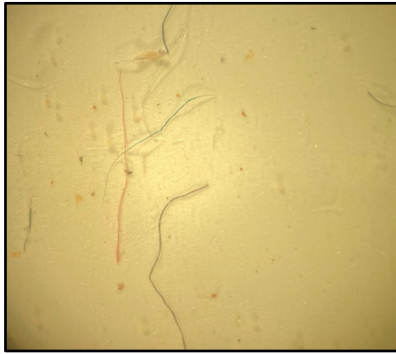


Figure 3.3: Blank #1 of filtered KOH solution during the assessment of Fortaleza clams.

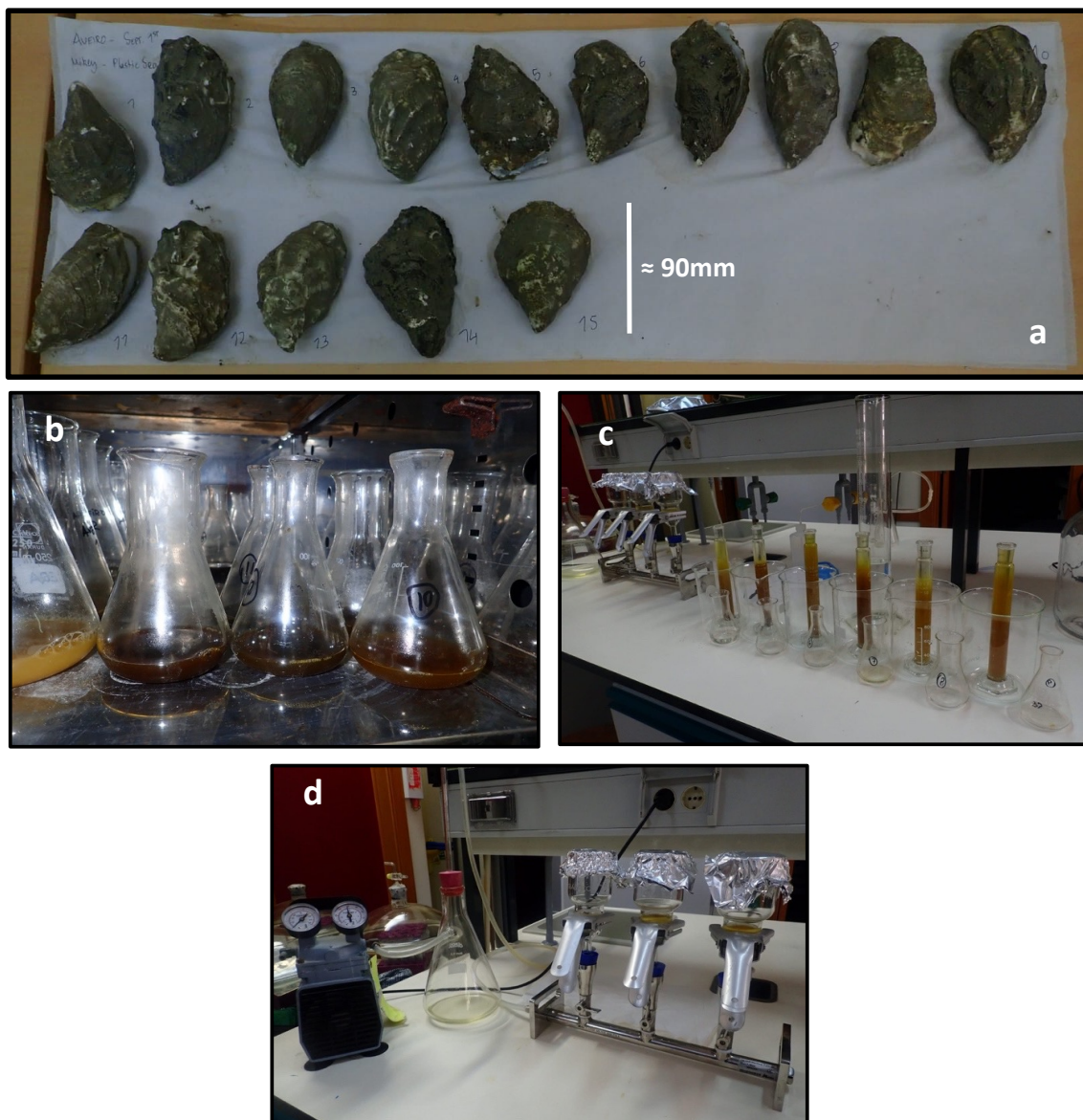


Figure 3.4: Laboratory methods a) Shells of dissected oysters from Aveiro. b) Dissected soft tissue with KOH solution digesting in the oven. C) Floatation process of digested samples in glass cylinders with NaCl solution. d) Filtration system of pump and three filters with samples in.

Using the Leitz Laborlux 11 at a magnification of x40 or x100, filters then underwent microscope analysis to search for MPs. Once a particle was identified as potential plastic, it was exposed to hydrochloric acid (HCl) which dissolves organic carbonate compounds. If the particle did not dissolve, it was exposed to a heated needle as plastic compounds melt or bend in the presence of heat. If the particle reacted to the heat or melted, then it is assumed that it is plastic. These particles are then photographed, measured for size using a ruler on the ocular lens of the microscope and categorised by type (fragment or fibre) and colour.

3.2.2 Contamination prevention

To prevent contamination from airborne plastic present in the laboratory entering the samples the following measures were followed. Samples were always stored in non-plastic containers such as glass or aluminium foil. They were covered whilst defrosting, during movement around the lab and in the floatation process. Natural fibres fabrics were worn in the lab to prevent direct contamination from clothing. All solutions (NaCl, KOH) were filtered before use through a 0.45µm cellulose acetate filter. Blank samples were used and analysed. Only natural fibres were found as contamination on these samples but in relatively large amounts (see blank filter in figure 3.3).

3.3 Statistical Analysis

To support and validate the differences between the analysed samples some statistical analyses were performed in the present study, such as the Kruskal Wallis non-parametric variance analyses and, Multi-Dimensional Scaling (MDS). Subsequently, a Dunn's post-hoc pair-wise analysis was applied to highlight which are the significant differences between samples. These statistical analyses were performed using the software Primer6 and Past3. The outliers were previously removed and, to respect the prerequisites of MDS analysis, data were normalized (Anderson, 2001, 2005; Clarke & Warwick, 2001; Elliott & Hynan, 2011; Ostertagová et al., 2014). The MDS analyses are grouping analyses based on the formation of groups according to the similarity between the samples, in this case using an Euclidian Distance similarity matrix. In other words, this analysis links the samples into hierarchical groups, based on the chosen variables (in the present, colours, MP size and MP concentration).

4 RESULTS

In this present study we aim to identify the MP particles in organisms from aquaculture sites to compare with the organisms previously analysed from natural coastal sites for the PlasticSea project. In total 256 organisms were considered but we mainly focus on the aquaculture samples. A total of 90 individuals from aquaculture sites were analysed, consisting of 15 clams, 30 mussels and 45 oysters from the farming sites chosen for sampling from which a total of 54 particles were identified as MPs. Examples of particles are represented in Figure 4.1.

4.1 Microplastic contents

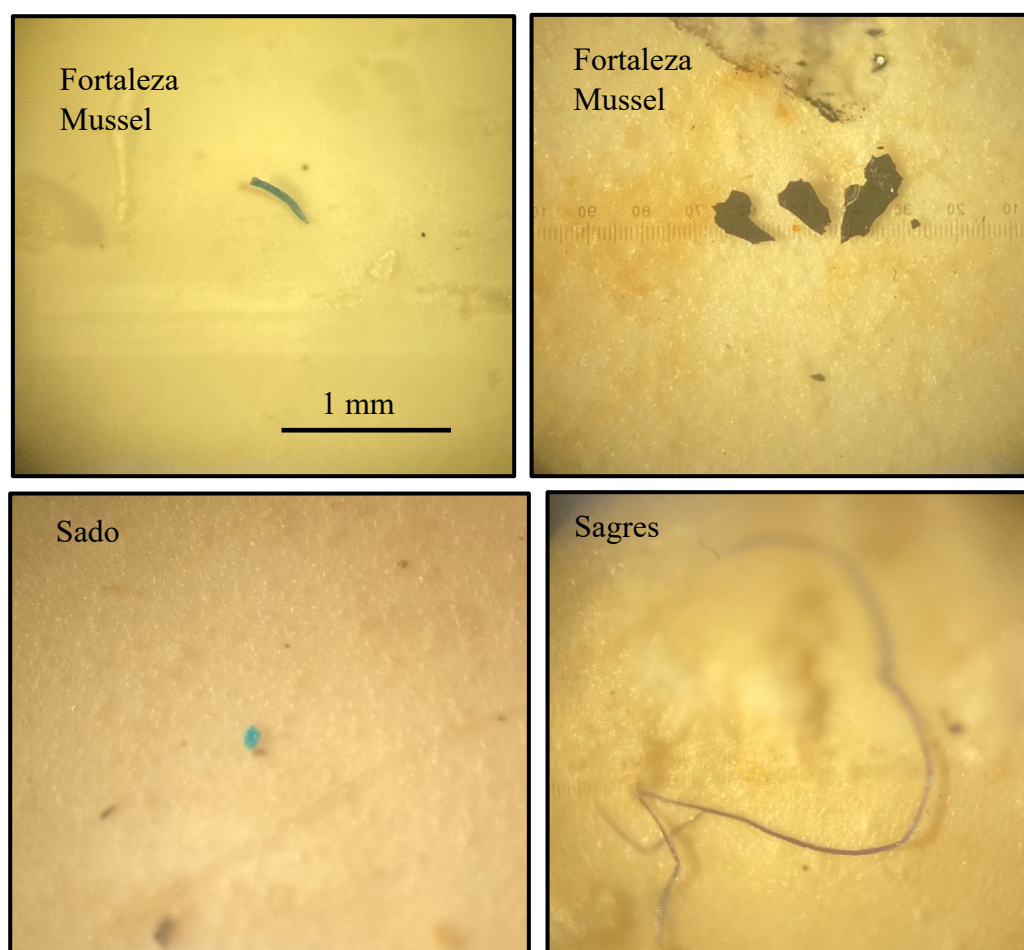


Figure 4.1: Examples of plastic particles found in organisms. Blue fibre and black fragment from Fortaleza mussels, blue fragment from Sado oysters and black fibre from Sagres mussels.

Plastic was found in all three species assessed and all locations. The percentage of organisms that contain plastic micro-particles among each species, oysters, mussels and clams is 44%, 30% and 20% respectively. The location with the most plastic was oysters from Sado with nineteen particles. Clams from Fortaleza and mussels from Sagres offshore aquaculture had three particles each, which represents the lowest number of particles found in all aquaculture stations (Figure 4.2). From all the organisms studied, 35.6% contained plastic in their tissues with an average of 0.6 particles per individual. Oysters and mussels from the intertidal aquacultures had the largest shares of contaminated organisms compared to the clams. This backs up the fact that mussels from the offshore aquaculture in Sagres and clams from the intertidal environment had the lesser amount of particles and thus smaller shares of organisms with plastic (Figure 4.3).

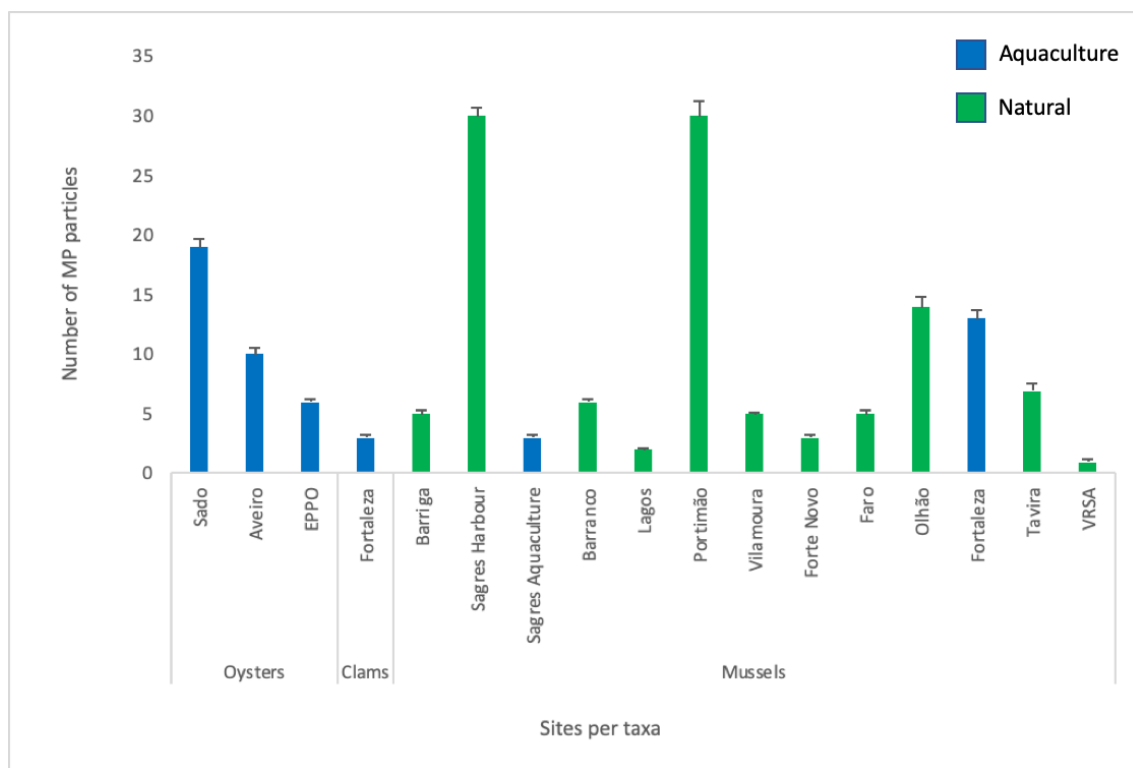


Figure 4.2: Number of MP particles for each site, grouped in taxa, with standard deviations error bars.

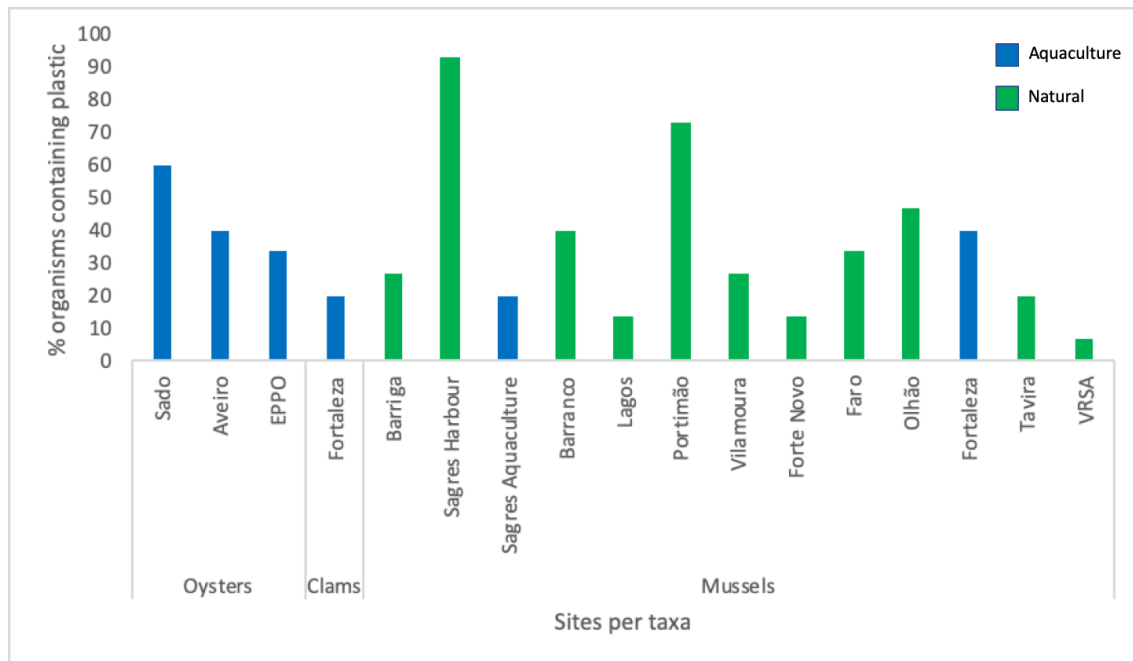


Figure 4.3: Percentage of the amount of organisms with detected MP particles. Blue bars represent aquaculture sites.

As the size of organisms varied greatly between locations and species, especially between oysters, which had an average tissue weight of 16.89 g and clams which had an average weight of 3.98 g, the number of particles per gram of weight was calculated and shown in Figure 4.4. Standard deviation error bars indicate the variability in number of pieces per gram between organisms of a same site. This graph indicates a lower concentration of particles in aquaculture, although the culture of mussels in Fortaleza appears to have more particles per gram due to the low weight of these samples.

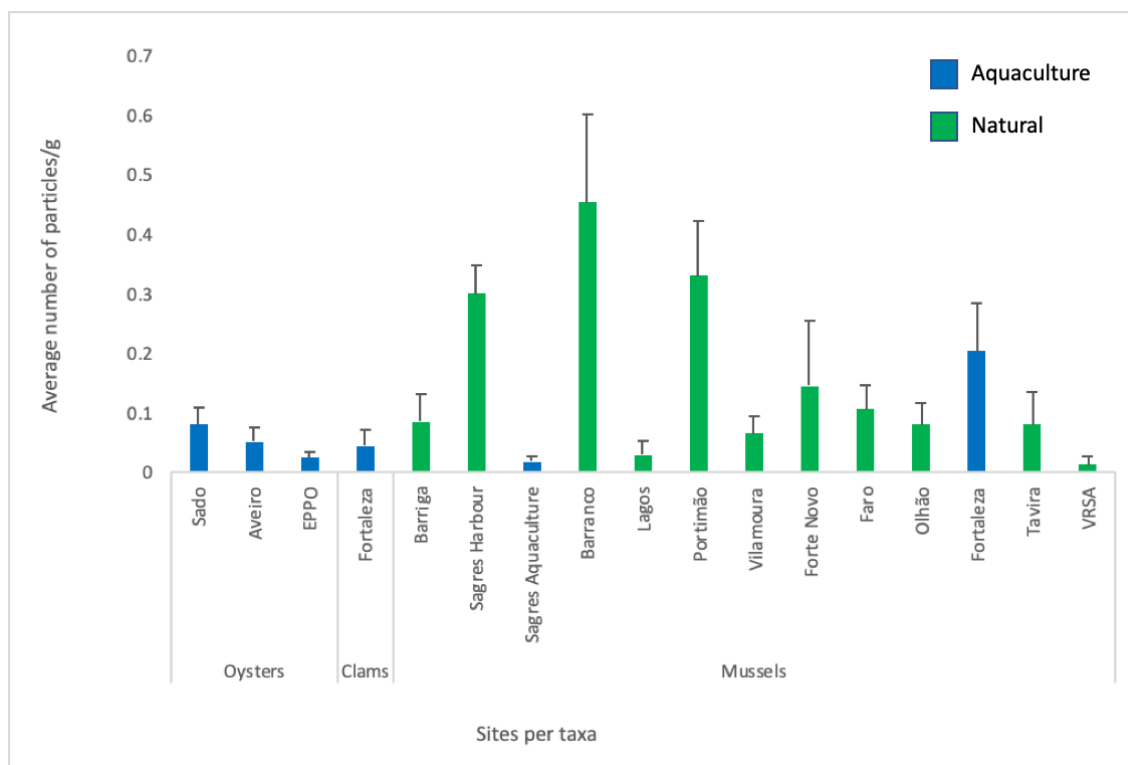


Figure 4.4: Average number of particles per gram per sites. Standard deviation errors (horizontal bars). Blue is aquaculture sites and green is natural coastal sites

4.2 Colours of MP particles

The most abundant colour found in aquaculture and across species was black (54%). Different types of aquacultures had different numbers of particles but a similar distribution of colours. Except for the offshore aquaculture in Sagres which had a majority of blue particles as shown in Figure 4.6. Accordingly, looking at Figure 4.5 the majority of black particles were identified in Oysters and mussels which corresponds to the intertidal aquacultures, except for mussels from the offshore farm in Sagres, which had a majority of blue particles.

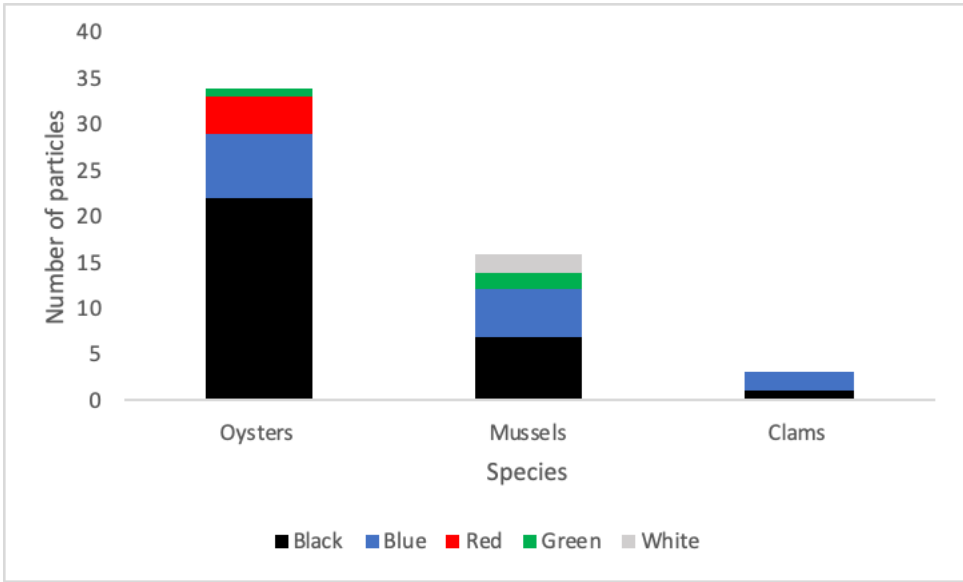


Figure 4.5: Number of particles by colours for each species

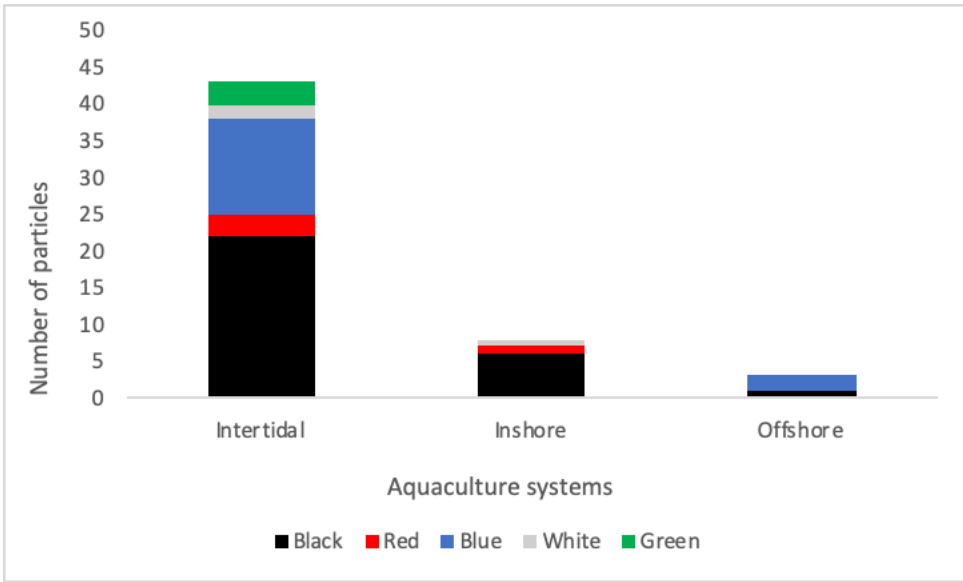


Figure 4.6: Number of particles by colour for each aquaculture systems.

Figure 4.7 shows a comparison between the colours found in aquaculture to those in coastal natural sites. We observe in this figure that there are more black particles in aquaculture than from the natural sites which will be further explored in section 5 of this study.

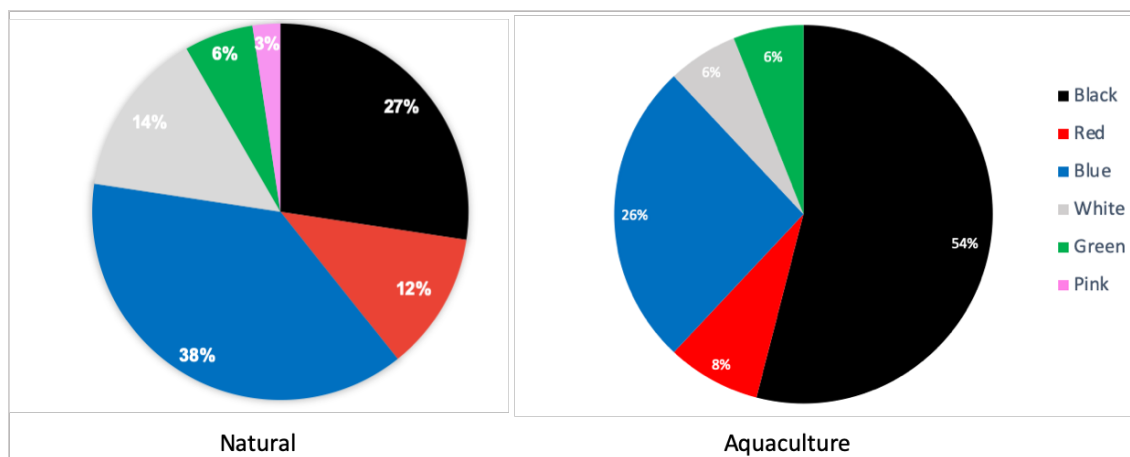


Figure 4.7: Pie chart comparing shares of colours between natural costal environment and Aquacultures environments.

4.3 Types of MP particles

Only two types of plastic were identified: fragments and fibres. Most of the plastic found (65%) was in the form of fragments. The only location that had more fibres than fragments was Sado as shown in Figure 4.8.

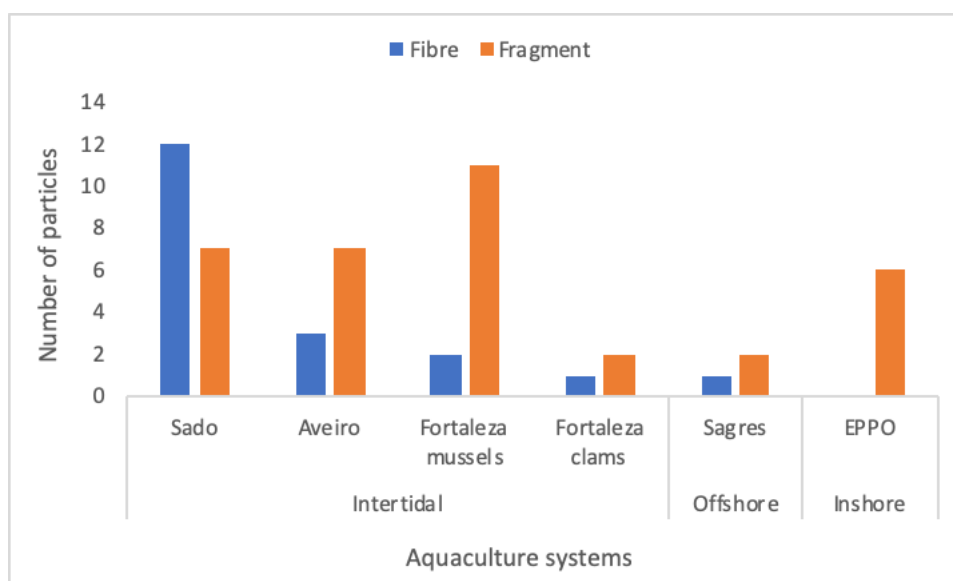


Figure 4.8: Types of particles in the different aquaculture systems.

4.4 Size of MP particles

The size of particles identified ranged between 0.025 mm and 3.75 mm. The average size of particles was 0.36 mm. Most particles were in the size range of 100-499 μm .

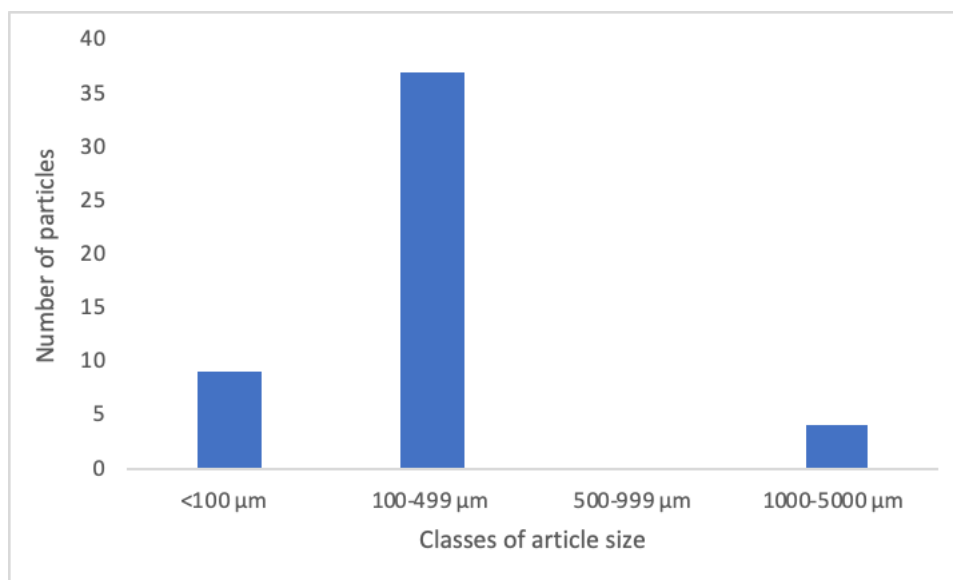


Figure 4.9: Number of particles per size class in the aquaculture facilities.

4.5 Statistical analysis

As the data is not normally distributed, according to the software used for statistical analysis, a non-parametric Kruskal Wallis variance analysis was applied. This analysis was applied to check if there were significant differences in MP concentration between the sites, aquaculture and coastal environments, and sampled organisms. When finding a significant difference, a Dunn's post hoc test was performed, these results are presented in Tables 4.2, 4.4, and 4.7.

Table 4.1 shows a non-significant difference in the micro-plastic content between the aquaculture sites. Even though there was no significant difference overall, in this case post hoc was also applied. The post hoc (Table 4.2) shows a slightly significant difference between Sagres and Aveiro and Sagres and Fortaleza, a difference which is possible also to observe in the previous graphs (See section 4.1).

Table 4.1: Kruskal Wallis applied to MP concentration results. Significant differences using a 95% significance level.

Kruskal-Wallis test for Aquaculture Sites	
H (chi2)	5.467
Hc (tie corrected)	7.289
p value	0.1214

Table 4.2: Dunn's post-hoc pairwise applied to the Kruskal Wallis results regarding aquaculture sites. Significant differences using a 95% significance level are shown in red.

Dunn's post-hoc					
SITE	Aveiro	Sado	EPPO	Fortaleza	Sagres
Aveiro		0.4484	0.3653	0.8544	0.01366
Sado	0.4484		0.8592	0.5583	0.08056
EPPO	0.3653	0.8592		0.4585	0.1286
Fortaleza	0.8544	0.5583	0.4585		0.0202
Sagres	0.01366	0.08056	0.1286	0.0202	

Regarding all the sixteen sampled sites, the Kruskal Wallis analysis revealed a significant difference between them (Table 4.3). Following these results, a Dunn's post hoc was applied to see where these differences were found. Table 4.4 summarizes the results from the Dunn's test, and shows that Portimão and Sagres Harbour were the sites that most distinguish from the others regarding MP content.

Table 4.3: Kruskal Wallis applied to MP concentration results in all sample sites. Significant differences using a 95% significance level are shown in red.

Kruskal-Wallis test SITES	
H (chi2)	66,72
Hc (tie cor)	89
p value	1,52E-12

Table 4.4: Dunn's post-hoc pairwise applied to the Kruskal Wallis results regarding all sampled sites. Significant differences using a 95% significance level are shown in red.

Dunn's post-hoc					
SITES	Aveiro	Sado	EPPO	Fortaleza	Sagres_A
Aveiro		0,4466	0,3634	0,2073	0,01329
Sado	0,4466		0,8587	0,7035	0,07938
EPPO	0,3634	0,8587		0,8737	0,1271
Fortaleza	0,2073	0,7035	0,8737		0,1052
Sagres_A	0,01329	0,07938	0,1271	0,1052	
Sagres	0,01373	0,001124	0,001001	1,75E-05	1,09E-06
Barranco	0,1956	0,5587	0,6906	0,7564	0,2739
Forte Novo	0,00939	0,06275	0,1045	0,08156	0,9446
Lagos	0,004108	0,03202	0,05723	0,03968	0,7154
Portimão	0,0581	0,006727	0,005627	0,000233	1,10E-05
Tavira	0,1139	0,412	0,541	0,5746	0,3301
Vila Moura	0,06502	0,2642	0,3646	0,366	0,5318
VRSA	0,0011	0,01128	0,02276	0,01264	0,4658
Faro	0,09139	0,3297	0,439	0,4551	0,4658
Olhão	0,9097	0,378	0,3052	0,1588	0,009242
Barriga	0,06502	0,2642	0,3646	0,366	0,5318

However, when the same test was applied considering all the MP concentrations in all aquaculture and coastal sites, these do not present an overall significant difference. Table 4.5 shows this result. The non-significant difference in most of the sites, as shown in the previous table, could be explaining the value found here.

Table 4.5: Kruskal Wallis applied to MP concentration results regarding Yield (aquaculture and coastal). Significant differences using a 95% significance level.

Kruskal-Wallis test for Yield	
H (chi2)	0,01217
Hc (tie cor)	0,01624
p value	0,8986

Tables 4.6 shows the Kruskal Wallis results regarding the MP concentration in the different organisms (mussels, oysters, and clams) analysed. It's possible to say that no significant differences ($p=0,1256$) were found between them, however, the post hoc (Table 4.7) shows a slightly significant difference ($p= 0,0442$) between clams and oysters but not enough to make the MP concentration in the Taxa significantly different.

Table 4.6: Kruskal Wallis applied to MP concentration results regarding the different analysed organisms (mussels, oysters, and clams). Significant differences using a 95% significance level are shown in red.

Kruskal-Wallis test for Taxa	
H (chi2):	3,111
Hc (tie cor	4,15
p (same):	0,1256

Table 4.7: Dunn's post-hoc pairwise applied to the Kruskal Wallis results regarding the MP per Taxa (mussels, oysters, and clams). Significant differences using a 95% significance level are shown in red.

Dunn's post-hoc			
TAXA	Oysters	Mussels	Clams
Oysters		0,5791	0,0442
Mussels	0,5791		0,05872
Clams	0,0442	0,05872	

The MDS links on a two-dimensional map, in such a way that the distances between the samples on the map agree with the rank order of the matching similarities taken from the similarity matrix (Clarke & Warwick, 2001). Here variables are colour, type, and size and this MDS analysis for identified plastic particles was made to support the previous analysis and shows the same patterns observed in the Kruskal Wallis results. It's possible to observe in Figure 4.10 that Portimão samples is notably isolated from the other samples for example sample 12 (PO12) of Portimão was very small and pink particle which was rare. It is also notable that aquaculture samples make an aggregation with Sagres harbour as the substrate on which they grow is also manmade plastic structures. In most cases natural and aquaculture samples are grouped, corroborating the results presented previously in this section. Supporting the Kruskal Wallis analysis and showing that, the available data on this project, does not show a clear difference between MP concentration in aquaculture and natural environments.

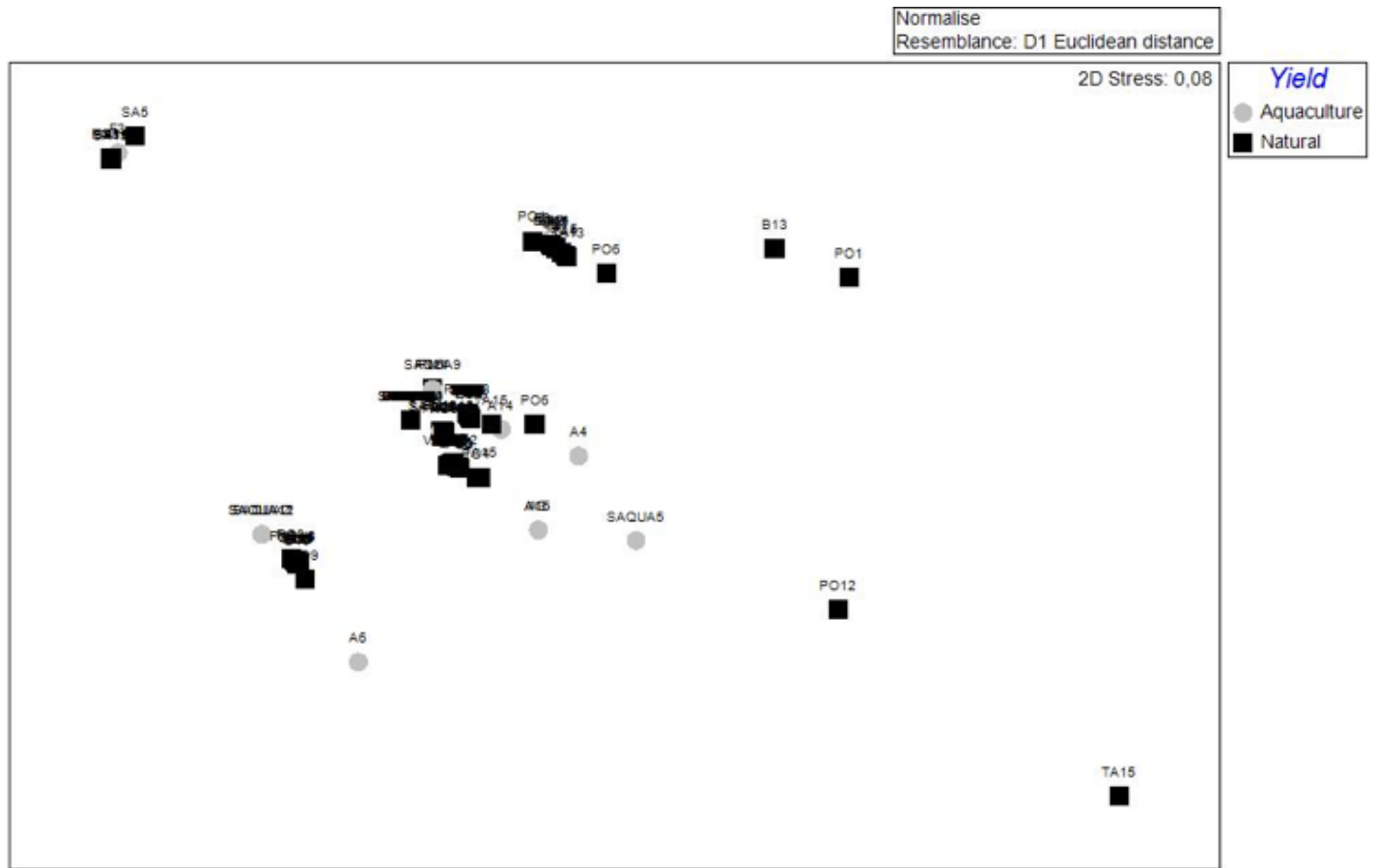


Figure 4.10: MDS ordering related to the samples present in the analysed sites. Black squares represent natural yield, and grey balls aquaculture yield.

5 DISCUSSION

This study successfully identified and quantified the amount of plastic in aquaculture farms for bivalves at five locations across Portugal. 35.6% of organisms contained plastic which is comparable to some studies, but a lot less than one study done by Cozzolino et al. (2021) on bivalves in the Ria Formosa, Algarve, which demonstrated up to 18.4 particles per gram of tissue compared to 0.13 particles/ g of tissue found in this study which could be due to the inclusion of all fibres found. While this study only included particles which reacted to a hot needle to prove plasticity. Marques *et al.* (2021), who studied mussels (*Mytilus* spp.) on the Portuguese coast also found no significant difference between sites and a similar contribution of fragments and fibres. However, they also found spherical plastic structures that were not identified in this study.

Although this study found that there was no significant different between abundance of MP in farmed organisms compared to wild organisms, some studies have found more plastics from aquaculture specimens, such as Bråte et al. (2014) found this to be the case in blue mussels worldwide. Plastic abundance in marine organisms is only likely to increase in the future, alongside increased plastic production. Though there is less production of plastic in Europe, it keeps increasing worldwide in order to satisfy European consumers demand, but delocalisation is not resolving the problem (Plastics Europe, 2021). Moreover when plastic production travels to regions of the world where they don't have the recycling power and perhaps poor legislation and management of industrial residues, MP is more likely to end up in the ocean and disperse globally. Aiming to reduce the problem, research in biodegradable polymers are ongoing to answer concerns about agricultural purposes of MPs directly released in the environment (Millican & Agarwal, 2021).

The species that had the highest presence of MP particles was oysters (44%), followed by mussels (30%) and clams (20%). This suggests that each species may be more or less likely to ingest plastic independent of their location. This could be linked to feeding strategy as both mussels and oysters feed by filtering the water and tend to accumulate more plastic in their tissues. Clams are suspension feeders nourishing from suspended particles within the sediment and may not be exposed to as many contaminants found in

the water column (Ribeiro et al., 2017). This could explain the lower level of MP identified in clams organisms. One could also consider the size of the animal itself since oysters are also relatively larger animals than clams they also probably absorb larger quantities of particles.

The site with the most particles in the tissues was Sado with nineteen particles. The Sado estuary river represents a vast discharge of water that passes through a highly populated area of the country where a lot of waste can reach the water sources and potentially contribute to the amount of plastic ingested by organisms. This could be the reason for the higher amount of plastic in the oysters from the Sado sample. The oysters here are grown in black plastic mesh sacks. 74% of the plastic from Sado was black, meaning that the ingested plastic may have come from the aquaculture equipment, but it cannot be proven as the origin of particles was not determined here.

There are multiple techniques to identify types of plastic using spectroscopy including Fourier-transform infrared spectroscopy (FTIR) and Raman Spectroscopy, which provide a detailed spectrum of the chemical composition of a particle (Teng et al., 2019). Future studies could analyse particles in this way to provide a detailed description of the types of polymer found inside organisms and compare it to the equipment used in these aquacultures or to other potential source of pollution.

In the present study statistical tests found no overall difference between either type of aquaculture or between aquaculture and natural sites. However, considering the distinct sites there were some differences especially between Portimão and aquaculture sites (Table 4.4) as Portimão has a large amount of plastic and the aquaculture sites had significantly less. Sagres, Portimão and Olhão all had higher numbers of particles than aquaculture sites which can be associated to higher human activity of an important agglomeration. But as there were some coastal sites with very low levels including Lagos, Forte Novo and VRSA, the overall level of plastic in coastal organisms became lower.

Oyster aquaculture offers a low greenhouse gas (GHG) emission option in comparison to traditional animal protein production (terrestrial livestock). It is also a good alternative against land based husbandry with lower ecological foot print (Ray et al., 2019). However aquatic farming seems to be vulnerable to contamination especially nearby coastal

agglomerations such as the different site used in this study. Assessing systems of aquacultures, inshore aquaculture offers the advantage of controlled conditions for growth, but it also implies higher risks of environmental pollution concentrated in one area. And still, black particles of plastic were identified from the oysters sampled in EPPO where they used the same black plastic mesh sacks. Offshore aquaculture for mussels in Sagres had low levels of plastic, probably due to the quality of the water, in the water column where plastic cannot settle and aggregate to be ingested by the organisms. This type of aquaculture is probably the best option when it comes to reducing the amount of plastic in the food from bivalve aquaculture but cannot be implemented for every species such as clams, which require sediment and oysters, which would require a stronger substrate or attachment site.

There are more black particles in aquaculture sites which may be from the equipment used, therefore it is possible, due to weathering or sunlight (Walsh et al., 2021) that particles from the plastic used in aquaculture can contribute to the amount of plastic consumed by humans. In fact, Bendell et al. (2020) study shows that there is relation between types of polymer detected in shellfish culture, as well as for other fisheries, and the anthropogenic polymeric materials being used. Figure 4.6, which looks at the colour difference between types of aquacultures shows that a high proportion of plastic found in the offshore site, Sagres, was blue. This plastic could be from the open environment brought by currents, or from blue equipment used such as the longlines from which the mussels are grown. However, there were only three particles found for this site, the lowest of all locations, suggesting that plastic contamination in offshore aquaculture is lower to that in intertidal and inshore systems. Still, the presence of plastic in agricultural food items proves there is cause for concern to human health and food safety. Other studies that have also found plastic available for human consumption in aquaculture bivalves (Teng et al., 2019; Van Cauwenberghe & Janssen, 2014). While there is no evidence of mortality due to MP exposure in bivalves, nonetheless impacts on multiple biological levels were observed from the molecular (i.e. DNA damage, enzyme activity) up to the organismal functions such as digestion growth or reproduction (Sendra et al., 2021), eventually harming the existence of following generations. The likely impact of plastic on humans is still under investigation but importance lies with preventing serious incidents in the future.

6 CONCLUSIONS

The general observation from this study is that bivalves from aquaculture in Portugal do ingest microplastic. The first and obvious conclusion to make from this study is that about one third of cultured bivalves for commercial purposes contain microplastic particles and thus would eventually be ingested by humans upon consumption. Giving that more than half of the MP particles found in grown organisms were black, one could assume that these bits of plastic could come directly from the materials used in bivalve aquacultures inshore and in intertidal settings, it seems that offshore aquaculture diminishes the risk of microplastic accumulation and thus contamination of farmed bivalves, in contrary to intertidal aquacultures that is easily exposed to plastic pollution due to agglomeration and standing waters. Biomonitoring of levels of MP pollution using bivalves is effective to determine the evolution of MP released in environment (Bendell et al., 2020). All types of aquacultures contained plastic which brings concerns about seafood consumption in general, leading to the conclusion that more sustainable food sources should be selected over practical aquaculture.

The number of plastic particles did not appear to be significantly different between the natural sites sampled and the specimens issued from aquaculture. However, we can observe a difference in the MP particles colours and types. Colour differences could be related to the use of equipment in aquaculture. It would seem that clam species, which have a different feeding strategy than the two other bivalve species (oysters and mussels) ingest less MP. However, quite more oysters were analysed so in order to compare all species fairly, more clams would need to be analysed. Future studies should also include the use of spectroscopy analysis of plastic particles with the aim to identify the source of the pollution with the goal to eliminate it. More studies on more locations could help illuminate more details of the plastic problem such as other accumulation zones. However, the issue of plastic pollution has been effectively highlighted in this study as well as multiple others that look at presence and effects of plastic in the environment. Therefore, suggestions for the future would be to reduce plastic consumption in order to prevent more long-term negative impacts to the ocean and human health.

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8 APPENDIX

Table 8.1: Plastic particles in each organism analysed

Site	Organism #	Taxa	Length (mm)	Organism Weight (g)	Colour	Type	Size (mm)
Sado	1	Oysters	94	19.27	Green	Fragment	0.075
					Black	Fragment	0.2
Sado	2	Oysters	89	23.99			
Sado	3	Oysters	90	17.36	Black	Fibre	0.2
Sado	4	Oysters	81	18.50			
Sado	5	Oysters	94	18.56	Black	Fibre	0.325
Sado	6	Oysters	93	15.52			
Sado	7	Oysters	87	19.33	Blue	Fragment	0.05
					Black	Fibre	0.3
Sado	8	Oysters	84	15.16	Black	Fibre	0.175
					Black	Fibre	0.2
					Black	Fibre	0.175
Sado	9	Oysters	89	17.67	Blue	Fragment	0.025
Sado	10	Oysters	95	13.66			
Sado	11	Oysters	91	12.47			
Sado	12	Oysters	93	12.44	Black	Fibre	0.125
					Blue	Fragment	0.05
					Black	Fibre	0.225
					Black	Fragment	0.1
					Black	Fibre	0.15
Sado	13	Oysters	100	14.58			
Sado	14	Oysters	98	19.30	Black	Fibre	0.125
Sado	15	Oysters	95	17.42	Black	Fibre	0.225
					Black	Fibre	0.3
					Black	Fragment	0.1
Aveiro	1	Oysters	87	18.5868	Black	Fragment	0.3
Aveiro	2	Oysters	96	14.595			
Aveiro	3	Oysters	92	18.6804	Black	Fibre	2.5

Aveiro	4	Oysters	90	10.9215	Black	Fragment	0.275
Aveiro	5	Oysters	96	14.3766			
Aveiro	6	Oysters	93	9.9416	Red	Fragment	0.15
					Red	Fragment	0.175
					Red	Fibre	2.5
Aveiro	7	Oysters	91	11.4482			
Aveiro	8	Oysters	95	10.0037			
Aveiro	9	Oysters	83	15.1677			
Aveiro	10	Oysters	87	14.7604			
Aveiro	11	Oysters	84	13.1378			
Aveiro	12	Oysters	92	13.0499			
Aveiro	13	Oysters	88	11.183	Blue	Fragment	0.075
Aveiro	14	Oysters	98	15.2335	Blue	Fragment	0.125
					Blue	Fragment	0.175
					Blue	Fibre	1.625
Aveiro	15	Oysters	83	19.7601			
EPPO	1	Oysters	125	21.43			
EPPO	2	Oysters	108	14.97			
EPPO	3	Oysters	109	19.42			
EPPO	4	Oysters	102	13.19			
EPPO	5	Oysters	123	20.04			
EPPO	6	Oysters	108	26.85			
EPPO	7	Oysters	132	30.50	Clear	Fragment	0.2
EPPO	8	Oysters	126	26.59			
EPPO	9	Oysters	103	16.66	Black	Fragment	0.225
EPPO	10	Oysters	105	15.79			
EPPO	11	Oysters	110	8.74	Black	Fragment	0.175
EPPO	12	Oysters	127	19.90	Black	Fragment	0.15
EPPO	13	Oysters	133	20.13			
EPPO	14	Oysters	127	21.07			
EPPO	15	Oysters	112	18.57	Black	Fragment	0.375
					Red	Fragment	0.225
Sagres	1	Mussels	59	6.93			

Sagres	2	Mussels	59	7.48			
Sagres	3	Mussels	71	9.20			
Sagres	4	Mussels	83	14.14			
Sagres	5	Mussels	64	7.91	Blue	Fibre	3.75
Sagres	6	Mussels	71	7.72			
Sagres	7	Mussels	64	8.35			
Sagres	8	Mussels	75	14.03			
Sagres	9	Mussels	74	7.84			
Sagres	10	Mussels	70	10.56			
Sagres	11	Mussels	69	11.03			
Sagres	12	Mussels	62	7.36			
Sagres	13	Mussels	74	14.31	Black	Fragment	0.125
Sagres	14	Mussels	68	9.64			
Sagres	15	Mussels	73	11.81	Blue	Fragment	0.05
Fortaleza	1	Mussels	56	6.43			
Fortaleza	2	Mussels	51	3.52	Black	Fragment	0.15
					Black	Fragment	0.375
Fortaleza	3	Mussels	58	5.12	Green	Fragment	0.475
Fortaleza	4	Mussels	61	6.51			
Fortaleza	5	Mussels	58	4.63			
Fortaleza	6	Mussels	54	4.07			
Fortaleza	7	Mussels	56	4.36	Blue	Fragment	0.025
					Blue	Fragment	0.025
					Blue	Fibre	0.125
					Black	Fragment	0.125
Fortaleza	8	Mussels	57	5.20	Green	Fragment	0.1
					Black	Fibre	0.375
					White	Fragment	0.4
					White	Fragment	0.15
Fortaleza	9	Mussels	49	3.52			
Fortaleza	10	Mussels	55	4.78			
Fortaleza	11	Mussels	52	3.29	Black	Fragment	0.125
Fortaleza	12	Mussels	51	4.08			

Fortaleza	13	Mussels	48	3.71			
Fortaleza	14	Mussels	47	3.63			
Fortaleza	15	Mussels	49	3.23	Black	Fragment	0.1
Fortaleza	1	Clams	43	5.05			
Fortaleza	2	Clams	42	4.09			
Fortaleza	3	Clams	41	5.54	Orange	Fragment	0.25
Fortaleza	4	Clams	36	3.04			
Fortaleza	5	Clams	38	4.77			
Fortaleza	6	Clams	40	3.95			
Fortaleza	7	Clams	38	4.22			
Fortaleza	8	Clams	38	4.32			
Fortaleza	9	Clams	36	3.07			
Fortaleza	10	Clams	39	4.87	Blue	Fragment	0.075
Fortaleza	11	Clams	37	3.84			
Fortaleza	12	Clams	34	3.35	Red	Fibre	0.1
Fortaleza	13	Clams	34	2.23			
Fortaleza	14	Clams	33	3.48			
Fortaleza	15	Clams	37	3.89			