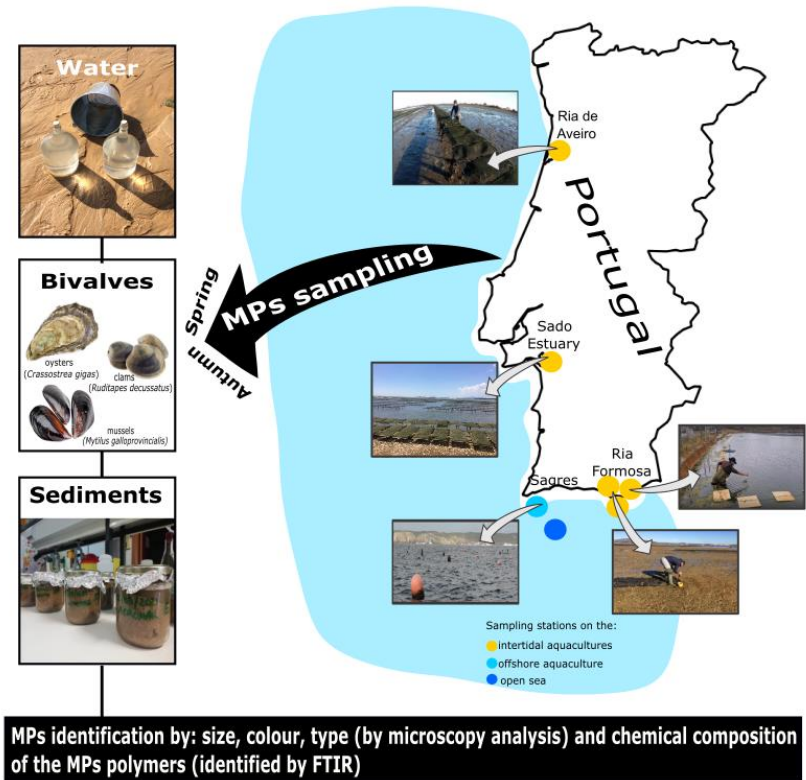


Aquaculture in the crossroad of microplastic contamination



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ABSTRACT

Plastic pollution threatens life and human health, with microplastics (MP) linked to seafood consumption. MPs enter aquaculture through the environment and from aquaculture gear. During aquaculture production, plastic is used in nets and sacks for the growth process and in collecting and processing so it becomes important to expand the knowledge about how much MPs are present in seafood. The aim was to investigate the presence of MPs in three bivalve's species; oysters (*Crassostrea gigas*), clams (*Ruditapes decussatus*), and mussels (*Mytilus galloprovincialis*) produced in offshore and intertidal aquaculture in two different climate conditions. Water, bivalves and sediments were collected from each site and abundance size, colour, type and composition of the MPs polymers analysed. The most common colour in offshore aquaculture was blue while in intertidal was black, and the type was fragments. Sixty per cent of bivalves did not have MPs in their tissues Bivalves from offshore aquaculture was less impacted by MPs probably due to the hydrographic conditions and distance from the coast. Most of MPs ingested by bivalves were related to the plastic type used in aquaculture materials. Transformative solutions and/or procedures to eliminate plastic from aquaculture equipment are needed, and depuration might be a practical solution.

Keywords: microplastics, aquaculture, bivalves, oysters, mussels, clams, *Crassostrea gigas*, *Mytilus galloprovincialis*, *Ruditapes decussatus*

Introduction

Aquaculture produces high-quality protein for human consumption and because the demand of seafood continues to rise, it is growing faster than any other food production sector. This industry achieved 35.5 million tons in 2022 that corresponds to 296 million US dollars, employ 22.1 million people worldwide and exceeded fisheries capture for the first time (FAO, 2024). The global marine molluscs aquaculture production reached 18.9 million of tons in 2022 and oyster, clams, cockles, ark shells and mussels represent 71% of the production (7.0, 4.5 and 1.9 million tons respectively) while in the European Union, mussels represent more than one third of the aquaculture production (FAO, 2024). Bivalve aquaculture supports direct and indirect jobs, provides nutritious food and

contributes to international trade. This increase in aquaculture production needs transformative solutions to achieve better economic value, social benefits and environmental sustainability.

The aquaculture industry, which operates inland, coastal, and marine environments, employs various practices for shellfish farming. Mussels can be cultivated using long-line systems or rafts. Research suggests that long-line bivalve cultivation has a relatively low environmental impact compared to other aquaculture methods (Garrido Gamarro, & Costanzo, 2022). However, plastic materials such as fishing nets, ropes, and floating buoys are used in offshore cages and raft culture (Dao et al., 2023). In mussel farms utilizing long-line systems, mussels grow on ultraviolet-resistant polypropylene ropes suspended parallel to each other. These setups rely on plastic floating buoys and ropes to facilitate natural growth conditions (Mascorda-Cabre et al., 2021; Imasha & Babel, 2023). Oysters, on the other hand, can be cultivated in bottom trays (Song et al., 2024) or in mesh plastic bags placed on trestles, or with rubber tires or fibrocement materials serving as supports for oyster beds (Dao et al., 2023).

Although, the development of technologies and equipment that use plastic, made bivalve aquaculture more efficient in recent years, bivalves may be exposed to plastic particles, particularly micro and nanoplastics (particles less than 100 nm), in the water column or in the sediments and ingest these plastics particles that may have negative impact on biosecurity and food health. Although the risk assessment of MPs is a complicated task due to the number of chemical components of the plastic composition, there is a need for a rational framework to assess the impact of plastic debris in aquaculture production (Koelmans et al., 2017).

Microplastics (MPs) (<5 mm) enter the ocean via a variety of pathways. Once in the ocean, MP particles either float in seawater or sink, depending on the specific density of type MP polymers, and benthic feeders are exposed to plastics that drop to the bottom and permeate in the sediments (Avio et al., 2017a). MPs can be a vector of other contaminants thereby resulting in cumulative effects to these species (c et al., 2020; Islam et al., 2021). Found in different sizes and at different levels of biological organization, MPs and/or associated contaminants accumulate in tissue of marine organisms, where they ultimately be ingested by humans and pose a threat to the marine environment and human health (Barbosa et al., 2024). Therefore, MP pollution results in significant economic costs as well as ethical, aesthetic, and ecological impacts (Shumway et al., 2023).

Bivalves are commercially valuable species that can be found in the sediments or attached to substrates, including rocks and man-made structures or can be produced in aquaculture. These organisms are thus potentially exposed to MPs due to their feeding strategies that is unselective (Trestrail et al., 2021). Bivalves feed by filtering the water and/or sediments, which reduces in general their selectivity of nourishment. Therefore, they can inadvertently ingest MPs present in the marine environment assuming MPs as food or prey and accumulate them in their tissues (Cozzolino et al., 2021) where MPs are expected to cause negative physiological effects such as inflammation, effects on feeding behaviour, energy metabolism, growth, development, reproduction, immune toxicity and genetic damage that also can compromise future generations well-being (Li et al., 2015; Akhbarizadeh et al., 2018; Sendra et al., 2021; Clere et al., 2022).

Microplastics in global aquaculture environments primarily originate from land-based sources (which represents more than 80% of plastic reaching the ocean) (UN, 2021), tourism, shipping, fishing, aquaculture, and atmospheric deposition (Dong et al., 2020). Over time, plastic debris accumulates in the water due to weathering, erosion, abrasion, and collisions involving aquaculture equipment (Dao et al., 2023). In some cases, plastic gear used in aquaculture serves as a significant source of MPs (Chen, Li and Wang, 2021). Studies have confirmed MP contamination across all aquaculture systems (Chen, Li and Wang, 2021), primarily due to the degradation of plastic fishing gear, as well as from food and medicine. A global review on MP incidence in oysters (*Crassostrea gigas*) found that 94.4% of sampled oysters across the world contained an average of 1.41 ± 0.33 MPs g⁻¹ wet weight (w.w.). This highlights the importance of assessing the impact of MPs on various marine aquaculture systems (Song et al., 2023).

Microplastics were detected in multiple mollusk species from shellfish farms (Mathalon & Hill, 2014; Chen, Li and Wang, 2021; Dao et al., 2023; Song et al., 2023; Wootton et al., 2023; Fraissinet et al., 2024), often at higher levels than in the surrounding environment (Chen, Li and Wang, 2021). However, comparisons between farmed and wild bivalves show inconsistent results. Song et al. (2023) reported that bivalves from various aquaculture systems, including inland facilities, had consistently higher MP concentrations than their wild counterparts. In China, bivalves such as *Ruditapes philippinarum*, *Mercenaria mercenaria*, *Meretrix meretrix*, and *Scapharca broughtonii* from inland aquaculture facilities exhibited higher MP levels than those from non-aquaculture areas (Song et al., 2023). Similarly, *Mytilus edulis* cultivated in Halifax

Harbor, Nova Scotia, showed higher MP contamination compared to wild mussels (Mathalon & Hill, 2014). The same trend was observed in blue mussels and oysters farmed in Vancouver (Murphy, 2018). However, this pattern did not hold for *Venerupis philippinarum* from Baynes Sound, where no significant differences in MP levels were detected between farmed and wild clams (Davidson & Dudas, 2016). On the other hand, Wootton et al. (2023) analyzed MPs in the Pacific oyster (*C. gigas*) across eight aquaculture farms in southern Australia, finding that 92.3% of farmed oysters contained MPs. Interestingly, this percentage was slightly lower than that observed in wild-caught oysters (95.7%). Although wild oysters had higher MP concentrations (2.18 ± 0.77 MPs g^{-1} w.w.) compared to farmed oysters (1.03 ± 0.33 MPs g^{-1} w.w.), the difference was not statistically significant. Furthermore, MP levels in farmed bivalves were directly related to MP concentrations in surrounding water. Qu et al. (2019) found a significant relationship between MPs in surface seawater and farmed mussels along the Chinese coast. These findings contrast with Scott et al. (2019), who observed a correlation between MPs in mussels and sediment surface concentrations along the southwest coast of the United Kingdom. These discrepancies may be attributed to differences in bivalve feeding strategies.

Portugal located in Southwestern Europe, where most of the population live near the coast rely on the ocean for their seafood. Aquaculture is a growing industry in Portugal and can be an important contribution to the blue economy. The Portuguese coast is vulnerable to plastic debris accumulation due to terrestrial activities, maritime transport, fishing industry, tourism and recreational activities and oceanographic processes (Martins & Sobral, 2011; Vital et al., 2021). Although most of the plastic detected in the ocean are from land-based sources, another MPs source may related to the major routes of commercial and touristic cruises that pass through the Portuguese waters and are transported by ocean currents to the coastline (Martins & Sobral, 2011). The levels of plastic in the ocean and particularly in coastal areas have an impact in offshore and intertidal aquaculture facilities (Chen, Li and Wang, 2021). Moreover, plastic equipment and materials used in aquaculture represent an additional source of MPs along with that of land-based sources. Both these sources can contaminate the organisms produced by aquaculture. So, it is important to identify MPs levels in offshore and intertidal aquaculture facilities to access the impact of MPs in the ocean, in marine food and in human health. For that purpose, the levels of MPs in mussels (*M. galloprovincialis*) from an offshore facility in the South coast of Portugal and in oysters (*C. gigas*), clams (*Ruditapes decussatus*) and mussels from intertidal aquaculture

facilities from three Portuguese regions: Aveiro lagoon, Sado Estuary and Ria Formosa lagoon was accessed in two different climatic conditions (Spring and Autumn) and compared between sites, number, colour, and types of MPs polymers found in bivalves and in water and sediments of the aquaculture facilities. Furthermore, an evaluation was made to assess the main contribution of MPs and thus propose recommendations for best transformative aquaculture practices to reduce MPs contamination.

Material and methods

Sampling site location

Two sampling campaigns were held in Spring and Autumn in offshore and intertidal aquaculture facilities along the Portuguese coast, in the Aveiro lagoon and Sado estuary both in the West coast and off Sagres and the Ria Formosa lagoon in the South Coast of Portugal. Sampling sites are indicated in Figure 1. Water, bivalves, and sediments were collected from each aquaculture facility. The bivalve's species selected were the most representative of the Portuguese aquaculture production (INE, 2024) namely the pacific oyster (*C. gigas*), the Mediterranean mussel (*M. galloprovincialis*), and the clam (*R. decussatus*) and from each site these commercial bivalves were randomly collected.

Offshore aquaculture

The offshore mussel farm in the South Coast of Portugal is installed in the coastal area (Figure 1). The structure is deployed between the depth of 18 m in the North to 32 m in the South. The longlines are made of polyethylene terephthalate (PET) ropes of 55 mm \varnothing which are anchored to the seabed and kept afloat by flotation poly propylene (PP) plastic buoys. The longlines are placed parallel to the coast and follow along the direction of the currents, offering the least resistance to the flow. From the longline, continuous ropes or “droppers” made of nylon (polyamide (PA)) are used to grow mussels, at depths of 5-12 meters. The mussels' seed can be bought or captured from the wild using special rope collectors and are then socked around the culture rope with cotton mesh and protected with plastic (PET) anti-grazing nets. Because these coastal waters are of Class A, there is no need for depuration. The mussels are harvested and then processed into three

pasteurized products (mussel meat, half-shell and whole mussel). These products are sold mainly to the international market but can also be found at the national market.

Water samples (10 L) were collected in Spring and Autumn from the offshore aquaculture longline mussel's farm at the surface, at 10 and at 20 m depth, and compared to water samples collected at the same depth in the open sea. At the surface water was collected with a metal bucket and at the other depths with a Niskin bottle and then transferred to two 5L glass bottles. Prior to sampling, the material was rinsed three times with water from each sampling site. Water temperature and salinity were measured with a multiparameter probe (WTW ProfiLine Conductivity Meter LF 197-S) and current direction, visually estimated, and the wind speed and direction were determined with a compass and anemometer (see supplementary Table S1).

Thirty farmed mussels *M. galloprovincialis* of commercial size (n=30, 6.9±2.9 cm; 10.3±2.8 g) were randomly collected by divers directly from the longlines at a depth of 5 m below surface and wrapped up in aluminium foil, transported in a thermal box to the laboratory and frozen at -20°C, until further analysis. In addition, eight replicates of five centimetres of surface of sediments were collected at the bottom of the offshore mussel farm by divers with a stainless-steel square section collector (47 mm x 47 mm) performing 111 cm³ each. The sediment samples were transferred to glass flasks with metal lid and transported to the laboratory. The procedure for the analysis of colour, size, and type of MPs in water, mussel tissues and sediments are described below.

Intertidal Aquaculture

The sites selected for sampling water, sediments and bivalve species from intertidal aquaculture are represented in Figure 1. Ria de Aveiro is a coastal lagoon in the North of Portugal and is an important aquaculture area with the influence of tides from the ocean but there is also a freshwater influence. In this specific case, oysters (*C. gigas*) are farmed above ground in trestles inside mesh black plastic bags that need to be turned daily by hand.

The Sado Estuary located South of Lisbon is regulated by tidal influence and the upstream freshwater flow from the Sado River, which has been in high hydric stress in recent years (Biguino et al., 2021). The Sado estuary had a long history on the oyster's production with natural oyster banks, which have nearly disappeared some years ago due to tributyltin contamination (Phelps &

Page, 2010). The aquaculture site is in a sand bank island in the middle of the estuary where the oysters (*C. gigas*) are grown above ground in trestles inside mesh black plastic bags, which need to be turned manually. Additionally, oysters also grown in Flip/Flop systems that use the water movement to shake the oysters.

The Ria Formosa is a coastal lagoon of tidal influence without significant freshwater inputs. This area is the most important for Portuguese bivalve aquaculture. The clam (*R. decussatus*) is the most important species and represent 27% of the total aquaculture production in Portugal in 2019 (INE, 2021). Production areas are leased in the intertidal, and clams are produced directly in the sediments which needs constant human intervention, through the addition of sand and gravel to avoid the development of anoxic conditions in the sediments. After depuration the clams are sold as fresh product at local and national markets.

The Aquaculture Research Station (EPPO-IPMA) of the Portuguese Institute of the Sea and the Atmosphere located in the Ria Formosa Lagoon, is a marine core facility devoted to marine fish aquaculture and marine biology. The oysters (*C. gigas*) were reared in integrated multitrophic aquaculture system with fish species (meagre - *Argyrosomus regius*, white seabream *Diplodus sargus* and two-banded seabream - *Diplodus vulgaris*) in earthen pond, under semi-intensive regime. Oysters are reared in bags maintained at water surface with floaters (pond depth around 1.5 m).

From each of the aquaculture facilities, 10 L of water were collected in Spring and Autumn with a metal bucket and then transferred to two 5L glass bottles using a metal funnel. Moreover, 5 cm surface of sediment's samples were collected with a stainless-steel square section collector (47 mm x 47 mm). The sediments were transferred to glass flasks with a metal lid and capacity of 350 cm³. The water and sediment samples were stored until further development.

Bivalves of commercial size were provided by the aquaculture producers in the two contrasting climate conditions (Spring and Autumn). Clams (n=30, 3.6 ±0.01 cm, 3.5±0.1 g) seeded in the sediments within a maintained area from the Ria Formosa Lagoon were collected. Cultured mussels (n=25, 5.3 ± 0.4 cm, 4.4 ±1.0 g) that grow on a manmade substrate, were also collected from an intertidal aquaculture site from the same lagoon. Oysters (n= 105, 10.3 ± 2.5 cm, 21.2±2.9 g) were collected from three aquaculture facilities which use plastic growing bags placed inside

the water, attached to structures, two being aquaculture farms (in the Ria of Aveiro ($n=35$, 9.3 ± 0.5 cm, 15.5 ± 0.5 g) and the Sado estuary ($n=35$, 9.3 ± 1.2 cm, 18.5 ± 0.1 g)) and the other from IPMA Aquaculture Research Station ($n=35$, 12.4 ± 0.7 cm, 29.5 ± 2.6 g) in the Ria Formosa lagoon. The organisms were wrapped up in aluminium foil, transported in a thermal box at 4°C to the laboratory and frozen until further analysis.

A multiparameter probe (WTW ProfiLine Conductivity Meter LF 197-S) was used to measure water temperature, and salinity at all sites. The current direction visually estimated and the wind speed and direction determined with a compass and anemometer (see supplementary Table S1).

MPs identification

In the laboratory, the water was filtered with cellulose acetate filters with a mesh size of 5 μm using a pump filtration system consisting of a Vacuum pump (Pall®) attached to a three-way filtering ramp system, to collect the MPs particles present in the water samples. Filters were stored in glass petri dishes until further microscopic analysis. MPs levels present in water are expressed as number of MPs per litter

Bivalves were measured using a calliper and weighed. The soft tissues were separated from the shell, placed in 250 ml Erlenmeyer flasks, weighed and digested for MPs analysis according to the method described by Avio et al. (2015; 2017b). For the digestion, tissues were covered with a 10% solution of potassium hydroxide (KOH) at a ratio 5:1 ml per gram (soft tissue wet weight). The flasks were placed in an oven at 50°C for two days or until the digestion of the tissues was completed. Blanks holding KOH solution were also placed in the oven for further analysis to check the level of external contamination in the laboratory during the analysis. Once the digestion was completed, the solution was transferred to 100 ml glass cylinders and a 100 ml of a pre-filtered sodium chloride (NaCl) solution (1.2 g cm^{-3}) was added to each sample to collect the plastic particles present through density separation. 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 were expected to float. The solution collected in the glass beakers was filtered as described above for the water samples. The levels of MPs in bivalves are expressed per individual and per gram of bivalve's tissue weight.

The analysis of the sediments for MPs assessment includes drying, organic matter digestion, separation of size fractions (granulometry) and isolation of MPs from sediments through density separation. After collection, sediments were placed in an oven, at 40 °C until fully dried. Sediments with high organic matter content were treated with 100 ml of H₂O₂ (30 %) to oxidise organic material. Increments of 50 ml of H₂O₂ were added, until the organic matter was fully oxidised and remained in the fume cupboard until completely dried. After the oxidation of the organic content, all samples were separated in a Retsch AS 200 digit shaker using a series of sieves with mesh sizes 500, 355, 250, 180, 125, 90 and 63 µm. Each fraction was weighed and placed in a paper envelope until further analysis. For the density separation, a hypersaline sodium chloride (NaCl) solution, with the density of 1.2 g cm⁻³ was used. In this procedure, 80 ml of the NaCl was added to the sediments and placed inside 1 L glass beaker. The addition of the hypersaline sodium chloride solution to the sediments promotes the MP content to float and rise to the top. The solution was then stirred, decanted for 60 min and the supernatant collected by using a glass test tube to cause the mixture to overflow. The tube was then rinsed with distilled water to ensure collection of MPs. These steps were repeated for maximizing extraction performance (Avio et al., 2017b). The supernatant was collected and filtered with the same apparatus and filter features as described for the seawater and mussel samples. MPs levels in sediments are expressed in MPs per cm⁻².

Filters containing the MPs present in seawater, bivalves or sediments samples underwent microscope analysis using the Leitz Laborlux 11 at a magnification of x40 or x100 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 was plastic. These particles were then photographed, measured, and categorised by type (fragment, fibre or film), size and colour. The type of plastic polymer of the selected particles was then identified by Fourier-transform infrared spectroscopy (FTIR) as described in below.

Identification of MPs with FTIR

FTIR technology was used after visual identification of microplastic samples with microscopy (Edo et al., 2020). After all the MPs samples being classified according to size, colour and shape,

a subsample of 60 MP particles found in seawater, bivalve tissues and sediments was selected for FTIR analysis, so that each polymeric compound could be adequately identified. Each selected particle was manually isolated from the filter and placed on a KBr matrix for FTIR analysis. FTIR spectra were recorded with a micro-FTIR Thermo Scientific IN10 ®, using an MCTA detector. Reflection and Attenuated Total Reflection techniques were the main acquisition modes used. The measured parameter for the micro-transmission mode were the following: resolution of 4 cm⁻¹, 16 scans and 4000 – 650 cm⁻¹ in the middle infrared spectral range. The database used for the interpretation of the spectral bands, was assessed through using OMNIC™ software. Only results above 70% were considered for positive identification.

Quality Control

Sampling strategies such as materials, storage of samples and methodologies to assess MPs pollution in water, bivalves and sediments followed a precise protocol conducted with no plastic equipment to avoid contamination. To prevent contamination from airborne plastics, 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 laboratory and in the flotation process. Natural fibers fabrics were worn in the laboratory to prevent direct contamination from clothing. All solutions (NaCl, KOH) were filtered before use through a 0.45 µm cellulose acetate filter. Blank samples were prepared and analysed to detect the presence of MPs, but only natural fibres were found in the blank samples.

Statistical Analysis

To support and validate the differences between the analysed samples, the non-parametric Kruskal Wallis variance test was applied. Subsequently, a Dunn´s post-hoc pair-wise analysis was run to highlight which are the significant differences between seasons. One way ANOVA was also applied to detect differences between seasons, sites and species. These statistical analyses were performed using the software Primer6. A significant level was set up at a 95 % confidence level ($p < 0.05$)

Results

MPs in offshore aquaculture

The number of MPs detected in water at the offshore aquaculture and at open sea, collected at three different depths is presented in Figure 2. In spring, the number of MPs decreased with depth. At the surface (0.6 MPs L^{-1}), MPs concentrations were higher at the farm compared to the open ocean (0.1 MPs L^{-1}). However, at 20 meters depth, no MPs were detected (Figure 2). In open ocean, no MPs were detected at 10 m depth in Spring (Figure 2). On the contrary, in Autumn the number of MPs in the offshore aquaculture increase with depth, and in the open ocean MPs were only detected at 10 m depth although 2-fold higher than in the offshore aquaculture at similar depth (Figure 2). Moreover, MPs levels in offshore aquaculture in Spring were higher than in Autumn from the surface until 10 m depth. Regarding the type of MPs present in the water column in Spring, they were all fragments while in Autumn 75% of MPs were fragments and 25% fibres at 10 m depth. In what concerns colour, blue was the dominant colour in Spring being 67% and 50% at surface and at 10 m depth while the other colours of MPs were black (32%) at the surface and white (50%) at 10 m depth. In the open sea the dominant colour at the surface was also blue but at the bottom the colours were completely different (red, pink, and black) with 33% each. Interestingly, in the Autumn, blue was dominant at the surface and at 20 m depth but black was the only colour detected at 10 m depth at the offshore aquaculture or the open sea. Moreover, the size of the MPs in water (Figure 3A) was also seasonal dependent and the higher percentage of MPs was in the range $100 - 499 \mu\text{m}$ in both seasons although higher in Autumn. In addition, the MPs polymers in water of the offshore aquaculture were PE, PP and acrylic (equally distributed in Spring) and in Autumn PE, PP present (although with a lower percentage) while the higher percentage was PET. In the open ocean MPs polymers detected were PP and alkyd resin (Figure 4).

The numbers of MPs detected in sediments were 0.4 MPs cm^{-2} . All MPs in the sediments were fibres and the higher percentage was in the size range 1-5 mm (56%), although no MPs smaller than $500 \mu\text{m}$ were present (Figure 3C). The colours were transparent (89%) and pink (11%). Moreover, PET was the plastics polymer type detected in the sediments.

In mussels, no MPs were detected in 87% and 73% of mussels collected in Spring and Autumn respectively while MPs abundance increase from 13% to 27%. In mussels with MPs ingested, most

were detected in Autumn where 13% of mussels had one MP, 50% had two MPs, and 38% had three MPs ingested. Whereas in Spring, mussels had only one MP ingested. The size range of MPs in mussels was similar to that in water (Figure 3B). In Spring, the size of MPs ranged between <100 μm and 100-499 μm while in Autumn was between <100 μm and <5 mm. The size range of 100-499 μm was also higher in Autumn compared with that in Spring. The type of MPs ingested, in both seasons were all fragments and the percentage of colour was similar to that of water in Spring (blue and black). However, in Autumn, transparent was the dominant colour (50%) followed by white (25%), blue, and green (12.5%). In mussels, the predominant polymer type was PP, and rayon was also detected.

MPs in intertidal aquaculture

MPs present in water of the aquaculture facilities were low, ranging in Spring from 0 to 0.20 MPs L^{-1} in the Sado estuary and the Aveiro lagoon in Autumn while in the Ria Formosa lagoon MPs 0.10 MPs L^{-1} were detected in Spring but absent in Autumn. A similar pattern was observed in water from EPPO, where only 0.1 MP L^{-1} were detected in Spring. The type of MPs in water of the Sado Estuary and in the Aveiro lagoon were fragments while in the Ria Formosa lagoon in Spring were fibres. The size range of MPs were 15 –675 μm in the Aveiro lagoon, 300 - 1100 μm in the Sado estuary and n.d.- 1000 μm in the Ria Formosa lagoon. The colour of MPs was blue and white in the Aveiro lagoon, transparent and grey in the Sado estuary, blue in EPPO and red in the Ria Formosa lagoon. Regarding the type of plastic polymer, all particles were PE.

The higher number of MPs in the sediments was in the Aveiro aquaculture (0.95 MPs cm^{-2}), followed by 0.72 MPs cm^{-2} in the Ria Formosa lagoon in the area where *R. decussatus* were reared and lower in the Sado aquaculture (0.05 MPs cm^{-2}) while in the Ria Formosa lagoon, where the mussels were cultivated, and at EPPO MPs levels were similar (0.36 MPs cm^{-2}). Most of the MPs were fragments that range from 36% in Aveiro to 96% in the Ria Formosa, mainly from where the clams were collected (Figure 5). Films were only detected in Aveiro and EPPO aquaculture facilities (Figure 5). Unfortunately, no data is available from the Sado estuary due to the sample lost. The colour of the MPs detected in sediments were different among sites (Figure 5). Transparent MPs was the colour with the highest amount in the Aveiro facility (41%), where a wide range of colours of MP was observed compared to the other aquaculture sites (Figure 5). In EPPO however, most of the particles were blue (50%), followed by transparent (38%) and yellow.

Regarding the area of the Ria Formosa where the clams and the mussels were collected there were some colour similarities (blue, green and red) although with different percentages between the areas where clams and mussels were collected. Blue was the most representative colour ranging from 63% in the area where the clams were produced compared to 69% in the area where the mussels were reared (Figure 5). Regarding MPs size in the sediments of the different aquaculture sites (Table 1), the highest percentage was in the range of 1-5 mm in Aveiro followed by EPPO but in the Sado estuary all the MPs were in the size range of 100 – 499 μm , while in the Ria Formosa lagoon where clams and mussels were produced the size range of MPs was smaller (< 100 μm and 500 – 999 μm) and highest percentage was in the range < 100 μm . In what concerns the type of polymer of MPs in the sediments at Aveiro and EPPO aquaculture facilities, PE, PP and PET were common although at different percentages while PE polypropylene was also detected in Aveiro and PVC also present in EPPO (Figure 6). Selected FTIR spectra are in Figure S1 on Supplementary material.

From all the organisms collected during the two seasons, 60 to 73% of oysters from Aveiro, 40 to 73% of oysters from the Sado estuary and 67 to 73% of the oysters from EPPO did not contain any MPs in their tissues. Even so, MPs were found in all species (a total of 69 MPs particles ingested by these species) and at all locations and seasons and the percentage of MPs ingested by each species and season is in Figure 7A. Spring was the season where the highest percentage of MPs was detected in oysters from all sites (range from 33% (Aveiro) to 60% (Sado)) as well as in mussels (40%) from the Ria Formosa lagoon while for clams the highest percentage of MPs ingested was in Autumn (27%). The percentage of MPs ingested in Autumn was similar among species (Figure 7A).

The species and location with most MPs ingested were oysters from Sado Estuary with 19 MPs detected in Spring, followed by oysters from Aveiro (9 MPs) and from EPPO (7 MPs). In Spring, mussels from the Ria Formosa lagoon had 13 MPs ingested and clams from the same area had only two MPs ingested, which represents the lowest number of MPs found from inshore aquaculture sites. However, in Autumn all the oysters had lowest levels of MPs (4-5 MPs) while those of clams were higher (6 MPs). The average number of MPs ingested per species is in Figure 7B. MPs ingested by mussels in Spring (2.4 ± 1.4 MPs/individual) were higher compared to the other species although not significantly different ($p > 0.05$) and a maximum of four MPs were ingested by two mussels. The highest average of MPs per oyster was in the Sado estuary (1.8 ± 0.2

MPs/oyster) in both seasons compared to the other sites although not significant ($p > 0.05$) (Figure 7B). In Spring oysters have ingested higher levels of MPs compared to Autumn although levels were not significantly different among seasons ($p > 0.05$), while in clams' differences among seasons were significantly different ($p > 0.05$) (Figure 7B). In Spring one oyster from Sado estuary has five MPs ingested while an oyster from Aveiro had three MPs. Oysters from EPPO and clams from the Ria Formosa had only two MPs ingested per organism. Statistical analysis revealed that there were no significant differences in MPs detected in oysters between sites or seasons nor between oysters and clams or mussels in Spring ($p > 0.05$). However, there were significant differences between seasons in MPs ingested by clams ($p < 0.05$).

The number of particles expressed per gram of whole tissue weight of species is shown in Figure 7C. This figure indicates a lower average of MPs per gram of oysters (range from 0.13 ± 0.11 MPs g^{-1} w.w. in Sado estuary in Spring and 0.04 ± 0.01 MPs g^{-1} w.w. in EPPO on Autumn) compared to clams (0.64 ± 0.29 MPs g^{-1} w.w. in Autumn) and mussels (0.50 ± 0.26 MPs g^{-1} w.w.) (Figure 7C). These changes are a result of the fact that oysters are larger and heavier (22.2 ± 2.9 g) than clams (3.5 ± 0.1 g) and mussels (4.4 ± 1.0 g). One way ANOVA revealed that there were no significant differences between the number of MPs per gram of tissues among oysters or among oysters and clams in Spring ($p > 0.05$) but there were significant differences between oysters from all sites and clams collected in Autumn and with mussels collected in Spring ($p < 0.05$). Similarly for clams there were significant differences among seasons ($p < 0.05$).

Different species had different colours of MPs ingested. (Figure 8). The dominant colour in oysters in both seasons was black, with different percentages among sites and seasons (Figure 8). The other colours of MPs ingested by oysters were blue (in Aveiro and Sado estuary) and red (in Aveiro and EPPO). Orange was only detected in oysters from EPPO (14%) and from *R. decussatus* in Spring, while green was only present in oysters from the Sado estuary and from mussels. Regarding the type of MPs, only fragments and fibres were ingested by bivalves. In oysters, most of the MPs found in Spring were fragments ranging from 78% in the Aveiro lagoon to 100% in EPPO while in the Sado estuary the majority were fibres (63%). In clams and mussels, the MPs fragments represent 100% and 85% respectively. In Autumn, fibres were dominant in oysters from EPPO (100%) while in those from Aveiro and Sado estuary most of the MPs were fragments (67% and 63% respectively) but in clams, fragments and fibres were equally distributed. The size of particles found in the distinct species ranged between 75 μ m and 5 mm. Most particles detected in

bivalves from both seasons were in the size range of 100 - 499 μm with the highest percentage in oysters from Aveiro in Autumn and from EPPO and mussels in Spring (Table 2). In clams, however, the size was equally distributed between 100 - 499 μm and 1-5 mm in Spring and between <100 μm and 500 – 999 μm in Autumn.

DISCUSSION

The impact of MPs in aquaculture becomes a significant concern as aquaculture is a key source of high-quality food for human consumption. Additionally, bivalves play a crucial role in providing ecosystem services across many regions of the world. Aquaculture contributes to food quality and safety on a global scale by supplying premium products. Plastics are commonly used in aquaculture gear in various farming operations, due to its inherent properties (resistance, durability, cost, etc.). Farmed bivalves are then exposed to plastics present in the water and during farming (Fraissinet et al, 2024). Therefore, it is essential to know if aquaculture activities represent an additional source of MPs. The knowledge about the presence, distribution and ingestion of MPs in bivalve aquaculture production in Portugal is still very limited (Botelho et al., 2023). Because of their small size, MPs can be ingested by a wide variety of marine organisms. Ingestion may be direct or indirect via trophic transfer (Schmidt et al, 2018). Bivalves cannot distinguish between food and MPs particles therefore being a MPs pathway for human uptake (Barbosa et al. 2024). In this work, MPs were identified and quantified in six aquaculture facilities of economically important seafood products distributed across three regions in Portugal: two of mussels (*M. galloprovincialis*) (offshore and inshore), four of oysters (*C. gigas*) and one of clams (*R. decussatus*) production over two different seasons. The results indicate that bivalves from aquaculture facilities in Portugal do ingest MPs with various types, shapes, colour and polymer composition. However, the level of incidence was lower than 66% at most sampling sites. Still, the species that had the highest presence of MP particles ingested were oysters (44%), followed by mussels (40%) and clams (27%) (Figure 7A). When these levels are expressed per individual there were no significant differences between species, sites or seasons except for clams (Figure 7B). This suggests that plastic ingestion may vary between species, regardless of their location. This variation can be linked to their feeding strategies, as both oysters and mussels feed by filtering the water, making them more prone to accumulate the MPs in their tissues. In contrast, clams are

suspension feeders that obtain food from water and particles within the sediments (Ribeiro et al., 2017). This could explain the lower level of MPs detected in *R. decussatus*.

MPs in offshore Aquaculture

Abundance, type, colour and polymer composition of MPs in water, sediments and mussels were detected in an aquaculture facility offshore on the Southwest coast of Portugal. MPs in the water column were seasonal dependent, with higher numbers at the surface, and similar levels at 10 m depth. These levels were higher than in the open sea (Figure 2). This might be explained by the reduced water velocity that occurs inside the farm (Newell and Richardson, 2014; Mascorda-Cabre *et al.*, 2024). Mascorda-Cabre *et al.* (2024) identified that the current speed can increase near the bottom, that could explain lower concentrations of MPs detected in Spring in the bottom. The coastal area where the offshore aquaculture is established had slightly higher levels of MPs in the water column (Figure 2) compared to inshore aquaculture (Figure 7A) and to other areas of the South coast of Portugal (PlasticSea project, data not shown). This indicates that the levels of MPs in water were similarly impacted offshore and inshore. The number of MPs detected in mussels *M. galloprovincialis* were also seasonal dependent (Figure 2B) and lower than in mussels from inshore aquaculture (Figure 7B), and in wild mussels from the South and North coast of Portugal (Vital et al., 2021, Barbosa et al., 2024). Mussels at this aquaculture site are cultivated between 5 and 12 m depth, and MPs were also detected in the water column at these depths. The number of MPs ingested per mussel in Autumn were higher (two to three MPs ingested per mussel) than in Spring (only one MP ingested per mussel). These levels are lower than those found in mussels from inshore aquaculture in Spring (50% had one MP ingested, 33% had four MPs and 17% two) (Figure 7B). A comparison with the levels of MPs found in mussels and in other bivalve species from aquaculture facilities in different areas of the world are shown in Table 3. The lower number of MPs in mussels from offshore aquaculture can be related to the number of MPs in the water column at the depth where mussels are reared. On the contrary, Chen, Li and Wang (2012) found that there were more MPs in aquaculture facilities than in the surrounded environment. The type of MPs ingested by offshore mussels were fragments, while in mussels from inshore facilities, fragments represented only 85%.

The particle size range of MPs ingested by mussels was similar to those found in water, but quite different from those in the sediments (Figure 3) showing that MPs are directly ingested by mussels from the water column. In fact, according to Dambrosio *et al.* (2023) MPs in mussels reflect, for number, shape and size, the MPs in the surrounding water of the growth site. No mussels were collected from inshore aquaculture in Autumn, so the size of MPs ingested in mussels from the offshore facility could only be compared with mussels the inshore site in Spring where 86% were from the size range between <100 and 100-499 μm and no particles of higher size were detected (Table 2).

The colour differences between aquaculture types shows that a high proportion of MPs found in the offshore site was similar to the water column's MPs proportion. So, colour need to be included beside number, shape and size in the list proposed by Dambrosio *et al.* (2023) to assess MPs impact. Blue MPs were also found in mussels from inshore aquaculture, but black was the dominant colour followed by blue, green and transparent at similar amounts. MPs in the offshore aquaculture site probably have their origin in the open ocean where the oceanographic conditions and wind influence the transport of MPs in the water column (Schmidt *et al.*, 2018; Rosas *et al.*, 2021). The blue gear used in the longlines from which the mussels are grown could also be a potential source of MPs to the mussels. Moreover, ocean modelling in the South coast of Portugal indicates that MPs do not stay close to their source and are quickly transported to adjacent coastal areas and the North Atlantic Gyre is the main pathway hydrodynamic of MPs in the water column in the region (Rosas *et al.*, 2022).

The type of MPs ingested by mussels was predominantly PP, but rayon was also detected while those present in water were PE, PP, PET and acrylic. PE is a polymer with lower density than seawater and is generally present in seawater surface. PET was the only polymer type detected in the sediments. PET is a thermoplastic polymer of the polyester family (Käppler *et al.*, 2015) that in the sediments might result from the sinking of MPs incorporated in mussels biodeposits (Piarulli and Airoldi, 2020) or result from the reduction of water velocity in the aquaculture facility that might enhance heavier particles to settle (Mascorda-Cabre *et al.*, 2024) or even result from the degradation of the longlines anchored to the seabed. However, the other type of polymers used in the offshore facility were PE and PP but also nylon. PE and PP have a high production and applications (Plastics Europe, 2023) and have been detected in several bivalve species worldwide. The nylon used is green so the green MPs detected in the mussels might have a source in the

aquaculture equipment. On the other hand, the buoys used are made of PP and this might support previous findings that aquaculture gear (Hossain et al., 2024) might be the source of this type of polymer detected in water and ingested by the mussels.

Offshore mussels are processed into three commercial food products (mussel meat, half-shell and whole mussel) for human consumption. Therefore, it is desirable, in the future, to identify the levels of MPs in the mussel farming final products, ready for human consumption, to ensure good food quality and food safety to avoid potential risks for the health of consumers.

MPs in intertidal aquaculture

From the species collected from the five inshore aquaculture facilities, the species that have ingested the highest percentage of MPs in Spring were oysters *C. gigas* from the Sado estuary (60%) and the lowest were clams from the Ria Formosa lagoon. In Autumn, however, the percentage was similar among species (27%) (Figure 7A). This could be linked to the feeding strategy of these bivalve species (Ribeiro et al., 2017). The Sado River represents a vast discharge of water to the estuary that passes through a highly populated area where a lot of waste can reach the water sources and potentially contribute to the number of MPs present. The number of MPs per species from inshore aquaculture facilities of the three Portuguese regions suggests that MPs ingestion is independent of location and season because no significant differences were observed between the number of MPs ingested by each bivalve species per site and season except for *R. decussatus* (Figure 7B; Table 3). Botelho et al. (2023) found out that in the Aveiro lagoon, *M. galloprovincialis* and *Cerastoderma edule* showed seasonal differences in MPs ingestion with lower levels in winter which was not the case of oysters collected from an aquaculture facility in the Aveiro lagoon. Similarly, Mladinich et al. (2024) detected 0-2 MPs/individual in the gut of *C. virginica* from an aquaculture in Niantic Bay, USA (Table 3).

When MPs levels are expressed per gram of tissue to avoid the effect of size, age and physiological status of the bivalve's species, there was a decreasing trend in the number of MPs in *C. gigas* from the Sado estuary (0.06-0.13 MP g⁻¹ w.w.) to Aveiro lagoon (0.09-0.12 MP g⁻¹ w.w.) and EPPO (0.03-0.06 MP g⁻¹ w.w.) (Figure 7C, Table 3). MPs levels in *C. gigas* were not significantly different between sites and seasons but were lower than those of clams in Autumn and mussels in Spring (Figure 7C). Wootton et al. (2022) also did not find significant differences in *C. gigas*

cultivated across different regions in Australia. Phuong et al. (2019) detected levels slightly higher in *C. gigas* from Pen-Be and Aiguillon Bay in France (0.18 ± 0.16 MP g⁻¹ w.w.) (Table 3) while levels of MPs in oyster's aquaculture facilities in Australia (0.09 ± 0.01 MPs g⁻¹ w.w.) (Wootton et al., 2022, Table 3) were of the same order of magnitude those detected in oysters from the Aveiro lagoon were lower than in wild oysters. As also shown in Table 3, MPs were detected in other oyster species and in different regions across the world and Mladinich et al. (2024) also detected similar levels of MPs in the gut of *C. virginica* from an aquaculture in Niantic Bay, USA ($0-1.3$ MP g⁻¹ w.w.).

Oysters (*C. gigas*) are a commercially important species farmed above the ground in trestles inside mesh black plastic bags that need to be turned daily by hand or grown in Flip/Flop systems that use the water movement to shake the oysters. Black was the major colour of the MPs ingested by oysters from the different aquaculture facilities in both seasons although blue was also present (Figure 8). Black MPs were absent from the water samples and were not detected in the sediments of EPPO (Figure 5). While no sediments samples were available for Sado Estuary this needs to be confirmed in this aquaculture facility. These results suggests that the black MPs ingested by oysters in different aquacultures may originate from aquaculture equipment itself, rather than from the water and sediments of the surrounding environment. Oyster aquacultures from most of the sites use black plastic mesh bags or baskets which might be an additional source of MPs. It is possible that the effect of weathering or sunlight, wave abrasion and handling, MPs will be generated, contributing to the number of MPs consumed by oysters (Walsh et al., 2021). Wootton et al. (2022) also detected that MPs present in oysters produced in Southern Australia, identified by FTIR, indicate that aquaculture gear (materials linked to the bags or structures where oysters grow) was a source of MPs. Moreover, most of the type of MPs detected were fragments, except in oysters from Sado Estuary aquaculture in Spring where size range was also smaller. Bendell et al. (2020) showed that there is a relationship between types of polymers detected in shellfish culture, and the anthropogenic polymeric materials being used. However, this needs to be confirmed in the case of oysters, because the identification of MPs polymers was not carried out, but PE was detected in water from those sites and PE, PP, PET and PVC were detected in the sediments (Figure 6). Moreover, in *M. galloprovincialis* collected in the Aveiro lagoon over a year, mussels have ingested PE, PU, PAN and PES (Botelho et al., 2023). On the other hand, polyester, PET and PP

were the most abundant type of polymers detected in an oyster aquaculture from USA where oysters were cultivated in bottom trays (Mladinich et al., 2024).

Oyster aquaculture offers a low greenhouse gas (GHG) emission compared to traditional animal protein production (terrestrial livestock) (Ray et al., 2019). It is a good alternative against land-based husbandry with lower ecological foot print (Ray et al., 2019). However, aquatic farming seems to be more vulnerable to contamination especially nearby coastal areas such as the present sites. Inshore aquaculture offers the advantage of controlled conditions for growth, but it also implies higher risks of environmental pollution.

Regarding MPs ingestion by *R. decussatus* there were seasonal differences in the number of MPs per clam, but they were not significantly different when expressed per gram of tissue. Cozzolino et al. (2021) reported for *R. decussatus* from the Ria Formosa lagoon up to 18.4 particles g⁻¹ that was significantly higher than those reported in the present study (0.19 ± 0.01 MPs g⁻¹). These discrepancies could be due to the inclusion of all fibres found by Cozzolino et al. (2021) which was not the case in the present study which only included data from fibres that reacted to a hot needle to prove plasticity. In the present case most of the MP's colours ingested by clams and mussels were like those found in water and in sediments indicating that aquaculture gears are not an additional source of MPs. Similarly, Qu et al. (2019) also found a significant relationship between MPs in water and mussels. Fragments were also higher in water, sediments and ingested in the mussel *Perna viridis* in an aquaculture in Thailand (Imasha and Babel, 2023). Marques *et al.* (2021), who studied mussels (*Mytilus* spp.) on the Portuguese coast also found no significant differences between sites and detected a similar contribution of fragments and fibres. However, they also found spherical plastic structures that were not found in the present case.

Oysters filter substantial amounts of water (3-7 L h⁻¹ g⁻¹ (Bayne, 2017)) and can egest MPs between 2-48 h after ingestion (Ward and Kach, 2009). Ward et al. (2019) reported that 90% of MPs ingested by oysters were egested within two days. On the other hand, mussels filtered about 8x10⁶ L h⁻¹ (Newell & Ricardson, 2014). Farmed *C. gigas* and *M. edulis* were able to eliminate 25 and 33% of ingested MPs after 72h of depuration (Van Cauwenberghe & Janssen, 2014). Moreover, *C. virginica* was able retain larger particles whose size was <500 µm and eject 30 to 40% of smaller spheres along with all the higher spheres previously ingested. These particles with size <100 µm can block the passage of food by blocking the intestinal passage in *C. gigas* (Corami et al., 2020).

Similarly, mussels can eject MPs as demonstrated by the farmed brown mussel *Perna perna* which was able to reduce the number of MPs ingested after 93 h depuration (Birnstiel et al., 2019). However, in the clam *Scrobicularia plana* previously exposed to polystyrene MPs (20 µm; 1 mg L⁻¹), MPs were not eliminated after 7 days of depuration indicating that this time was not long enough to eliminate the MPs injected (Ribeiro et al., 2017). Therefore, although there is a need to control MPs pollution within aquaculture systems, depuration of bivalve species produced in aquaculture can be a transformative solution to improve bivalve quality and minimise the transfer of MPs to humans.

Although the level of incidence of MP was around 30 to 40%, all types of aquaculture species production analysed contained MPs. The presence of MPs in seafood items is a cause of concern about seafood safety consumption and its impact on human health. Since there is a relationship between the type and number of MP in water and the incidence of MPs in shellfish, it will be important to monitor the presence of MP in water, and above a certain level suggest that shellfish must be submitted to depuration. Meanwhile, biomonitoring MP pollution in aquaculture facilities should continue to determine the evolution of MPs released to the marine environment (Bendell et al., 2020). As, MPs can release plastic additives to seawater and be another source of pollution to aquaculture species. (Vilke et al., 2024), it will be desirable to assess, besides the ingestion and polymer size, colour, type and chemical composition of MPs, if plasticizers and other chemical substances that leachate from plastic into seawater are accumulated in aquaculture species. In *M. galloprovincialis* when exposed to fishing nets MPs leachate the antioxidant and biotransformation systems was affected and became a threat to mussel's health (Vilke et al., 2024). So, there is a need to put in place more sustainable practices and include them in the best practices guidelines used by the aquaculture industry.

The present data indicate that offshore aquaculture is a good option when it comes to reduce the number of MPs in food from bivalve production. However, this might not be suitable for every bivalve species. It is recommended to promote the reduction of plastics in the aquaculture industry, particularly offshore, to develop environmentally friendly innovative solutions like replacement to plastic-free material as alternative to plastic products for aquaculture equipment. Although still in infancy (e.g. replacement of plastics by bio-based or biodegradable polymers (Millican & Agarwal, 2021; Arantzamendi et al., 2023; Hossain et al., 2024)) if proved environmentally friendly the use of these products needs to be implemented to prevent MPs release and reduce the

number of MPs available to be ingested by shellfish. Another alternative solution to mitigate MPs ingestion by humans is to depurate mussels before their transformation to commercial products for human consumption.

CONCLUSIONS

Bivalves from aquaculture in Portugal do ingest MPs and there is a direct relation between MPs in water and MPs ingested by bivalves. The number of plastic particles did not appear to be significantly different between the aquaculture species but differences in MP particles colours and types were observed. More than half of MPs particles ingested by oysters were black. These colour differences could be related to the type of plastic use in aquaculture equipment or present in the marine environment. Clams, which have a different feeding strategy than the two other bivalve species (oysters and mussels) ingest less MPs. Offshore aquaculture seems to be the best option when it comes to reduce the amount of plastic in the sea food. Therefore, for the future it would be important to reduce the use of plastic in aquaculture equipment and use good practices to avoid plastic sources to the marine environment and particularly to aquaculture facilities to prevent negative impacts. Meanwhile a possible transformative solution is to depurate bivalves to prevent long-term negative impacts to health.

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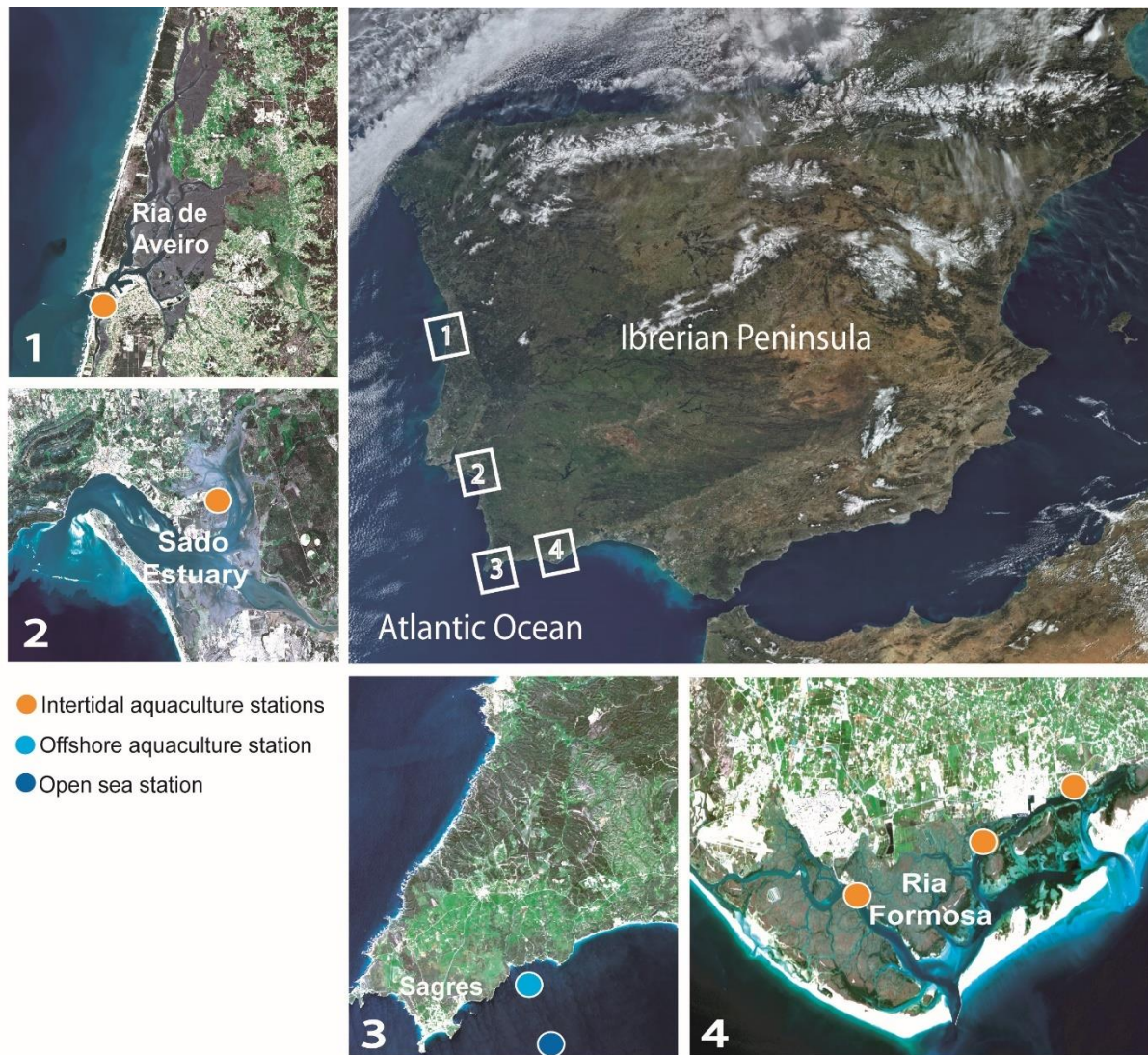


Figure 1. Geographic location of the aquaculture facilities along the Portuguese coast. Source of satellite images: modified image of the Iberian Peninsula [Cristina, 2022] from the Sentinel-3 OLCI mission [01-03-2016], and processed by the European Space Agency; the other satellite images related to Ria de Aveiro, Sado Estuary, offshore aquaculture off Sagres and Ria Formosa correspond to modified satellite images [Cristina, 2023] respectively from Sentinel-2A [24-02-2019], Sentinel-2B [21-03-2019], Sentinel-2B [24-02-2020] and Sentinel-2B [10-01-2019], and were processed by the European Space Agency.

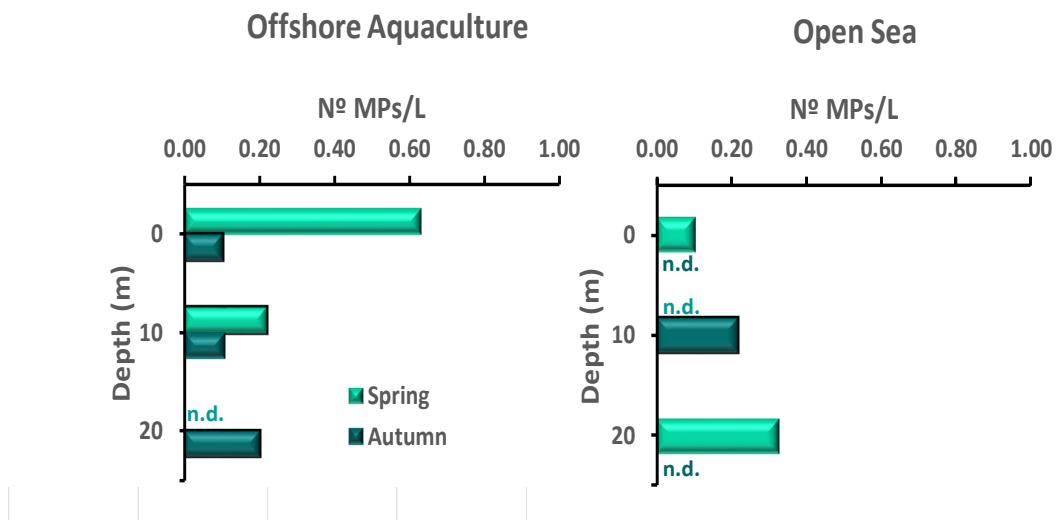


Figure 2 – Nº of MPs in water (MPs/L) from offshore aquaculture and in the open sea

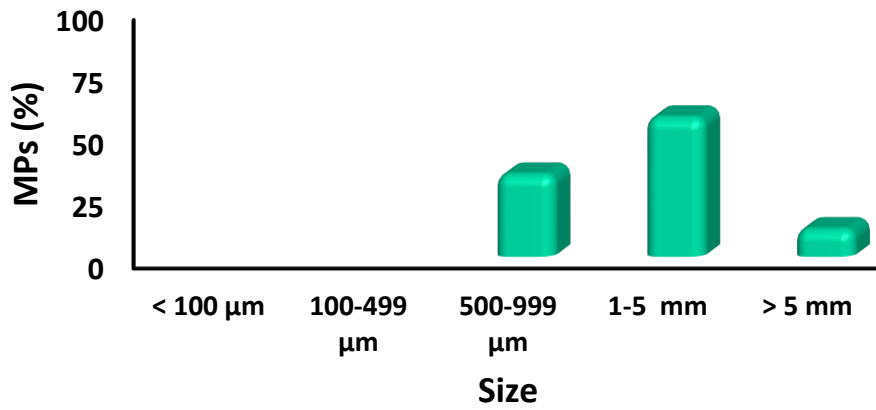
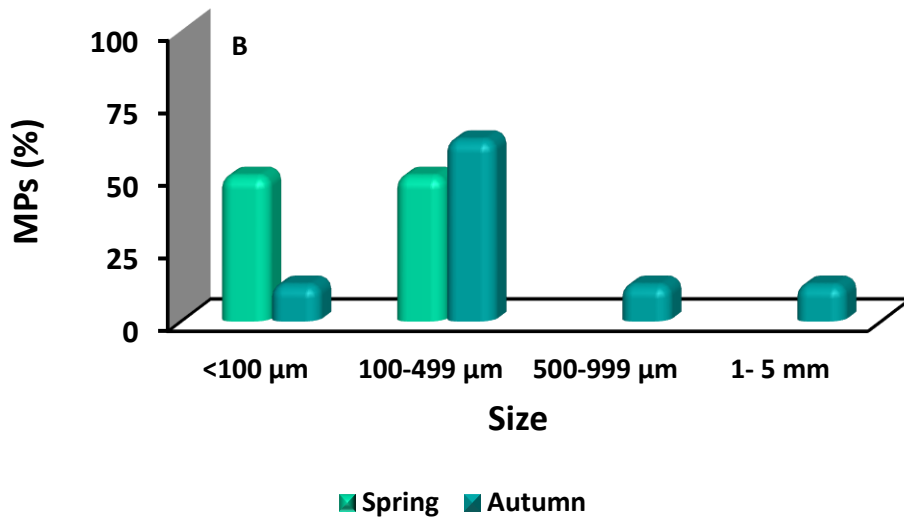
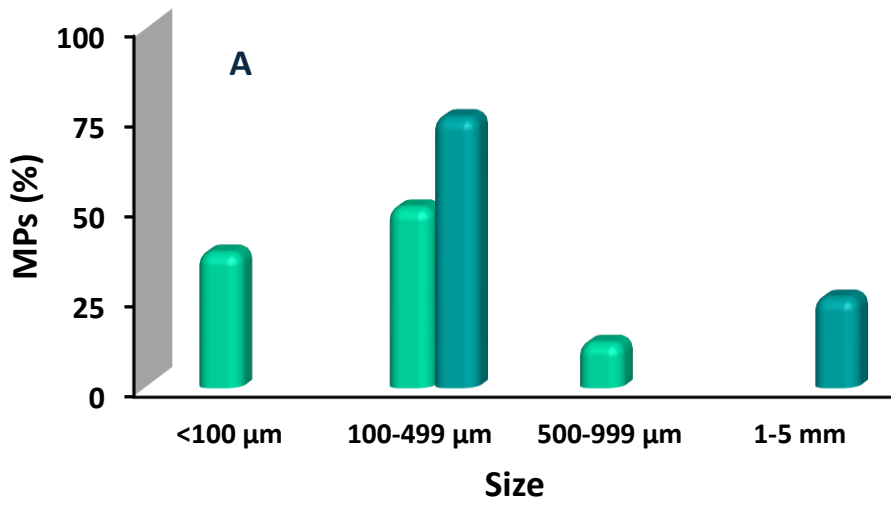


Figure 3 – Size range of MPs found in water (A), ingested by mussels (B) and in sediments (C) at the offshore aquaculture

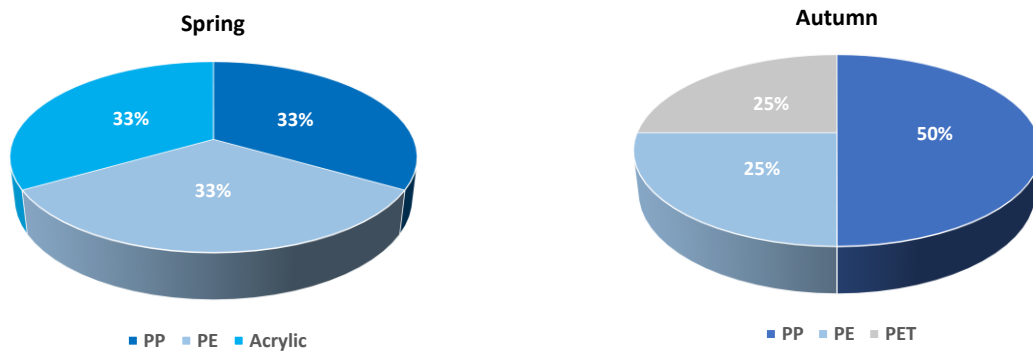
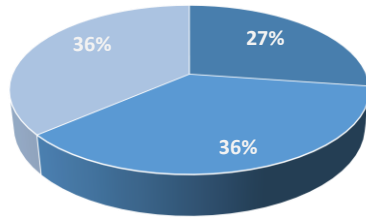


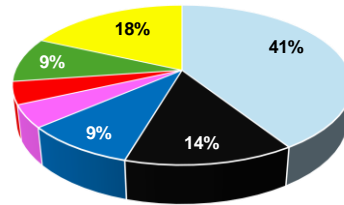
Figure 4 – Type of MPs polymers present in water of the offshore aquaculture.

Aveiro



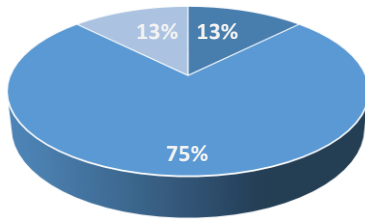
■ Fibre ■ Fragments ■ Films

Aveiro



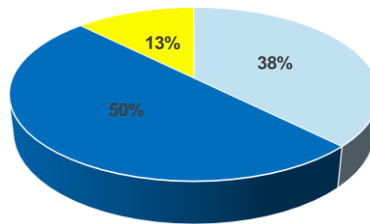
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EPPO



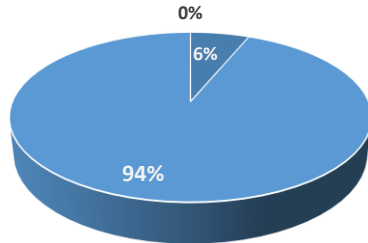
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EPPO



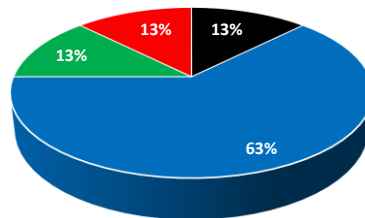
■ Transparent ■ Blue ■ Yellow

Ria Formosa (clams)



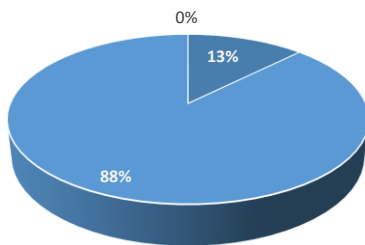
■ Fibre ■ Fragment ■ Film

Ria Formosa (clams)



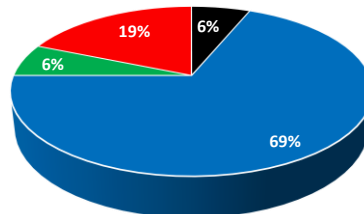
■ Black ■ Blue ■ Green ■ Red

Ria Formosa (mussels)



■ Fibre ■ Fragment ■ Film

Ria Formosa (mussels)



■ Black ■ Blue ■ Green ■ Red

Figure 5 – Percentage (%) of type and colour of MPs present in the sediments collected nearby the farmed bivalves (oysters, clams or mussels) of inshore aquaculture facilities.

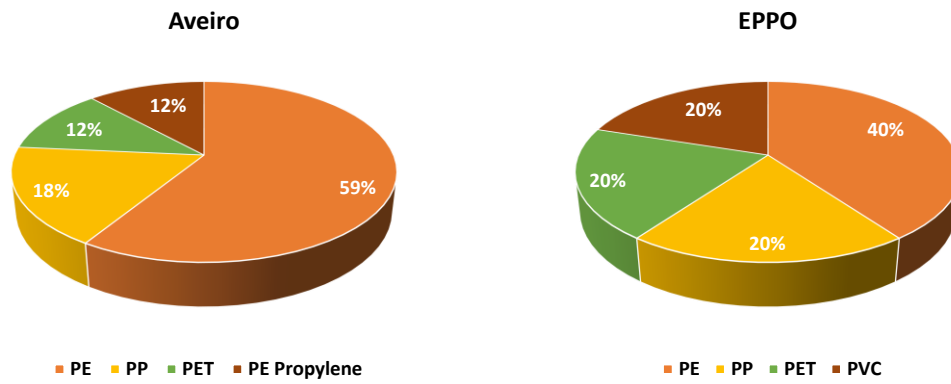


Figure 6 – Type of MPs polymers present in sediments from inshore aquaculture facilities

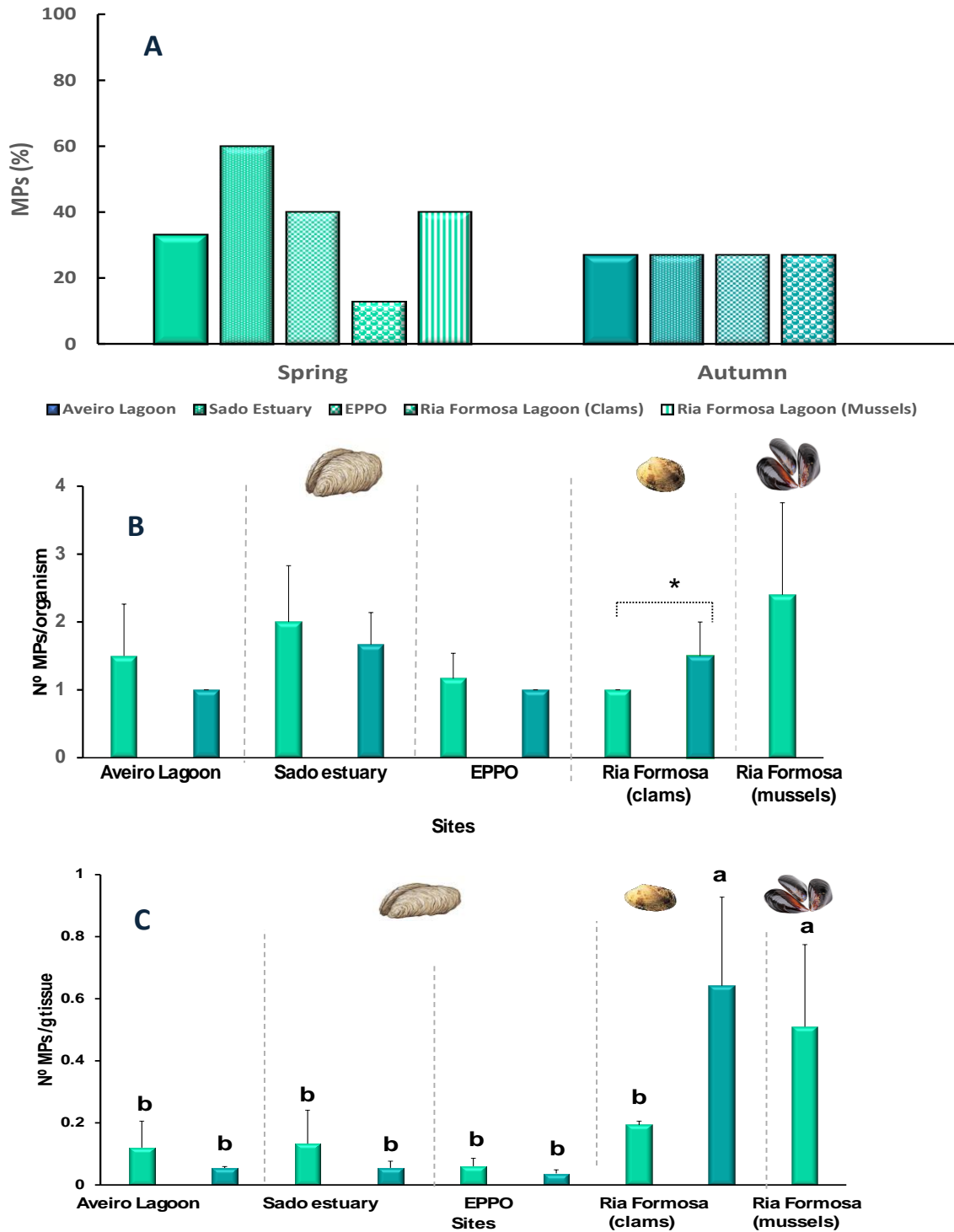


Figure 7 – (A) Percentage (%), (B) number of MPs per organism (mean \pm SD) and (C) number of MPs (mean \pm SD) per gram w.w. in oysters, clams and mussels tissues from different inshore aquaculture sites Spring () and Autumn(). ‘*’ indicates significant differences between seasons for clams ($p < 0.05$). Different

lower-case letters indicate significant differences between species and seasons, respectively ($p < 0.05$).

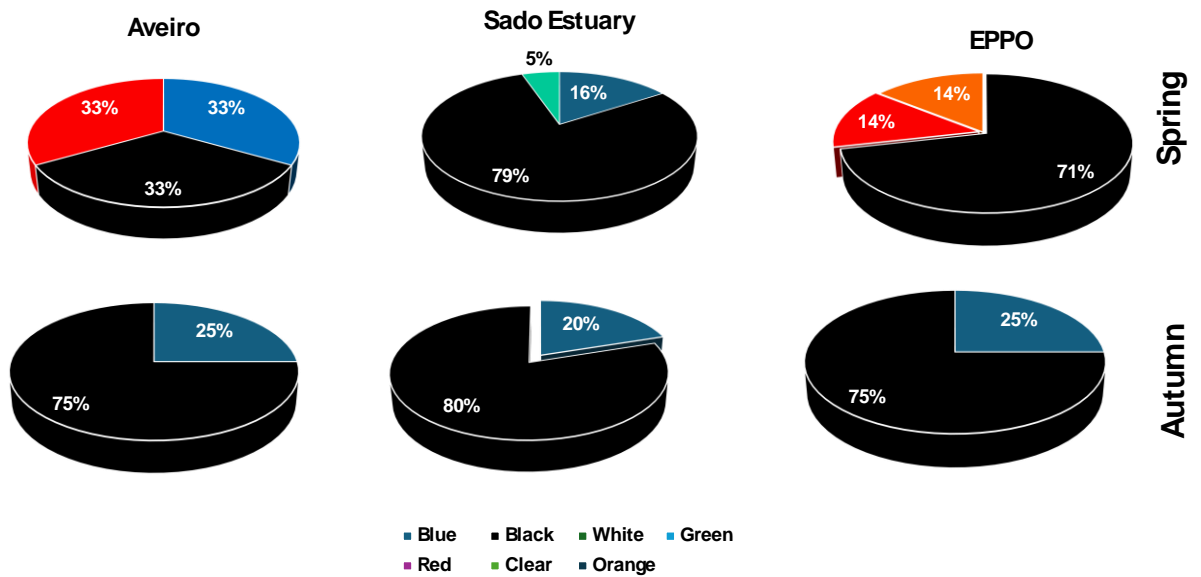


Figure 8 - Color of MPs (%) ingested by oysters collected in Spring and Autumn in inshore aquaculture facilities

Table 1 – Percentage of MPs size (%) in the sediments of inshore aquaculture facilities

Size	Aveiro	Sado	EPPO	Ria Formosa (clams)	Ria Formosa (mussels)
< 100 μm				56.3	50.0
100-499 μm	13.6	100.0	37.5	43.8	37.5
500-999 μm	22.7		12.5		12.5
1-5 mm	59.1		37.5		
> 5 mm	4.5		12.5		

Table 2 – Percentage of MPs size (%) found in oysters, clams and mussels from inshore aquaculture facilities

Size	Aveiro		Sado Estuary		EPP0		Ria Formosa (clams)		Ria Formosa (mussels)
	Spring	Autumn	Spring	Autumn	Spring	Autumn	Spring	Autumn	Spring
< 100 µm	11		21			25		50	13
100-499µm	67	100	79	80	100	50	50		73
500-999 µm				20				50	
1-5 mm	22					25	50		

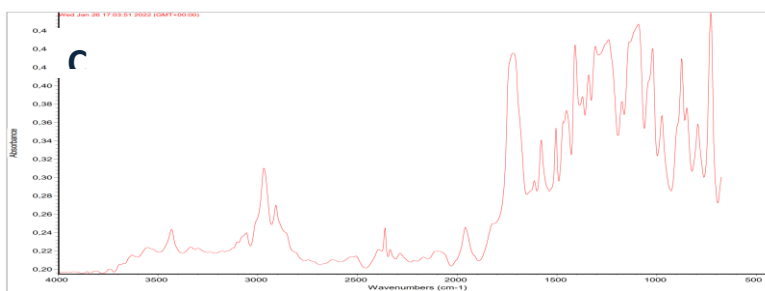
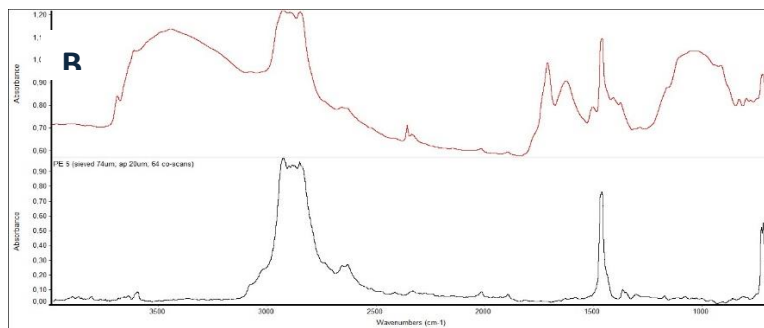
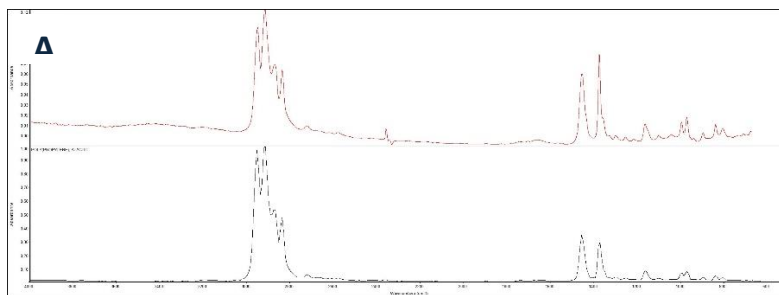
Table 3. Levels of MPs found in shellfish aquaculture sites around the world

Species	MPs/individual	MPs/g w.w.	Area	Country	Reference
<i>Crassostrea angulata</i>	0.67–8.33	0.02–0.33	Cha Va River	Vietnam	Dao et al., 2023
<i>Crassostrea gigas</i>		0.18 ± 0.16	Pen-Be and Aiguillon Bay	France	Phuonget al., 2018
<i>Crassostrea gigas</i>	1.0-1.5	0.09-0.12	Averio lagoon	Portugal	Present Study
<i>Crassostrea gigas</i>	1.7-2,0	0.06-0.13	Sado Estuary	Portugal	Present Study
<i>Crassostrea gigas</i>	1.0-1.2	0.03-0.06	EPPO	Portugal	Present Study
<i>Crassostrea gigas</i>		0.47 ± 0.16		France	Van Cauwenberghe and Janssen, 2014
<i>Crassostrea gigas</i>		0.47±0.16	Germany	Germany	Van Cauwenberghe and Janssen, 2014
<i>Crassostrea gigas</i>	0.49 ± 0.13	0.15 ± 0.04	Kangaroo Island	Australia	Wootton et al., 2023
<i>Crassostrea gigas</i>	1.25 ± 0.45	0.05 ± 0.02	Tansmania	Australia	Wootton et al., 2023
<i>Crassostrea gigas</i>	0,6		East coast of	USA	Rochman et al., 2015
<i>Crassostrea hongkongensis</i>		2.84 ± 0.44	Maowei Sea	China	Zhu et al., 2021
<i>Crassostrea virginica</i>	0–2	0–1.3	Niantic Bay, CT, USA	USA	Mladinich et al., 2024
<i>Mytilus galloprovincialis</i>			Offshore aquaculture, South Coast	Portugal	Present Study
<i>Mytilus galloprovincialis</i>	2.4 ± 1.4	0.50 ± 0.26	Ria Formosa Lagoon	Portugal	Present Study
<i>Mytilus edulis</i>		0.36±0.07		Germany	Van Cauwenberghe and Janssen, 2014
<i>Mytilus edulis</i>		0.2±0.3	Southern North Sea coastal waters	France, Belgium and The Netherlands	Van Cauwenberghe and Janssen, 2015
<i>Mytilus edulis</i>	34–178			Canada	Mathalon and Hill, 2014
<i>Mytilus edulis</i>		0.36 ± 0.07		Germany	
<i>Mytilus edulis</i>		1,4	Coast	UK	Li et al., 2018
<i>Mytilus edulis</i>		1.6		China	Li et al., 2016
<i>Mytilus galloprovincialis</i>	7.6±10.1			Spain	Rios-Fuster et al., 2022
<i>Mytilus galloprovincialis</i>		0.77–4.3	Aveiro lagoon	Portugal	Botelho et al., 2023
<i>Perna perna</i>	25,9	4,12	Rio de Janeiro	Brazil	Birmstiel et al., 2019
<i>Perna iridis</i>	3.2±1.6	2.4 ± 0.8	Sriracha farm	Thailand	Imasha and Babel, 2023
<i>Perna viridis</i>	7.32 ± 8.33	1.53 ± 2.04		Thailand	Imasha and Babel, 2021
<i>Perna viridis</i>	1.2 ± 0.2	0.3 ± 0.1	Phetchaburi farm	Thailand	Imasha and Babel, 2023
<i>Perna viridis</i>	2.2 ± 1.5			Thailand	Imasha and Babel, 2023
<i>Ruditapes decussatus</i>	1.0-1.5	0.19 ± 0.01	Ria Formosa Lagoon	Portugal	Present Study
<i>Ruditapes philippinarum</i>		5.93 ± 3.40	Lianyungang	China	Wang, Zang and Feng, 2023
<i>Venerupis philippinarum</i>	12	1,7	British Columbia	Canada	Davidson and Dudas, 2016
<i>Venerupis philippinarum</i>	9	0,9	British Columbia	Canada	Davidson and Dudas, 2016

Table S1. Sampling campaigns at the aquaculture facilities showing the geolocation, current and wind direction, sea surface temperature (SST - °C) and salinity.

	Site	Time	Latitude	Longitude	Current Direction	Wind Direction	SST	Salinity
Intertidal aquaculture	Ria Formosa (Sítio do Alcorão) – Clam aquaculture	-	37°00'18.22"N	7°51'01.63"W	-	-	-	-
	Ria Formosa (Fortaleza)– Clam aquaculture	09:33	37°01'23.99"N	7°48'41.4"W	-	South	-	-
	Ria de Aveiro – Oyster aquaculture	17:00	40°36'37.05"N	8°44'36.42"W	-	-	-	-
	Setúbal - Estuário do Sado – Oyster aquaculture	water - 13:15 Sediment - 14:30	38°29'28"N	8°44'53"W	Towards river mouth	330° Northwest (not strong)	17.9	34.1
	Ria de Aveiro – Oyster aquaculture	Sediment 8:20; Water 8:35	40°36'52"N (Water) / 40°36'36"N (Sediment)	8°44'26"W (water) / 8°44'58"W (sediment)	-	South	14.6	27.5
	Setúbal - Estuário do Sado – Oyster aquaculture	09:00	38°29'22"N	8°44'60"W	-	North	16.9	35.5
	Ria Formosa – Clam aquaculture	09:55	37°01'23.99"N	7°48'41.4"W	-	No wind	15.9	36.9
Inshore aquaculture	EPPO -IPMA- Olhão (Tank 3)	9:56	37°01'58.74"N	7°49'15.55"W	East to West	Southeast	17.9	-
	EPPO -IPMA- Olhão (Entrance)	10:34	37°01'47.63"N	7°49'12.22"W	-	Southeast	18	-
	EPPO -IPMA- Olhão (Tank 3)	10:00	37°01'58.74"N	7°49'15.55"W	-	Southeast	-	-
	EPPO -IPMA- Olhão (Tank 3)	10:30	37°01'47.63"N	7°49'12.22"W	-	Southeast	-	-

Offshore aquaculture	Sagres - Mussel aquaculture	9:50	37° 1' 59" N	8° 52' 19" W	North-south	NW	17.6	36.4
	Offshore - Sagres	-	37° 2' 1.97" N	8° 52' 37.0632" W	-	NW	17.9	36.5
	Sagres - Mussel aquaculture	9:20	37° 1' 59" N	8° 52' 19" W	Southeast	Southeast	16.3	36.6



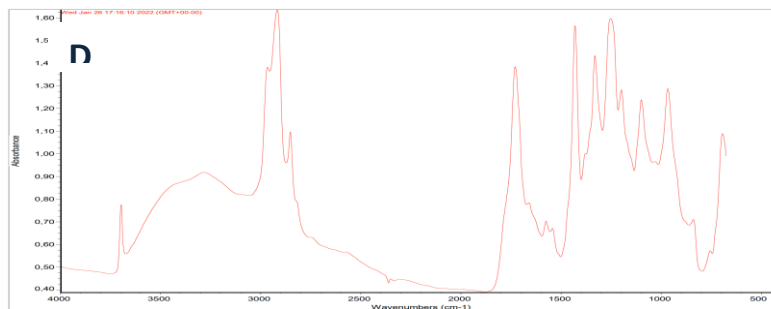


Figure S1- FTR spectra of (A) PP, (B) PE, (C) PET and (D) PVC.