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**Microplastics in gastrointestinal tracts  
of *Trachurus trachurus* and *Scomber  
colias* from the Portuguese Coastal  
waters**

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**Quantification of microplastics in gastrointestinal tracts of commercial fish from the Portuguese coastal waters**

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## Abstract

Plastic and microplastics are ubiquitous in the marine environment, their presence and possible ingestion can lead to harmful consequences in the marine environment and biota. To provide a quantification of microplastics ingested by commercial fish from the Portuguese Coastal waters and public awareness of the potential harm to human health, a total of 164 samples of *Trachurus trachurus* and *Scomber colias* were collected from Figueira da Foz and Sesimbra fishing ports. After dissection and evaluation, 67% of the individuals were found to have ingested microplastics.

A total of 399 microplastics were registered, with an average (mean  $\pm$  SD) of  $3.63 \pm 3.16$  microplastics per contaminated fish. Fibres and fragments were the type of MP found in fish, fibres were recurrently found among individuals, 79% comparing to 21% of fragments. Fish collected from Figueira da Foz fishing port, comprise 62% of the total MPs detected, and, *S. colias*, the Atlantic mackerel ingested 54% of the total microplastics.

Kruskal Wallis H test were performed to identify significant statistical differences between sampled groups and Spearman correlation to link any possible relationship between biometric parameters and number of microplastics ingested per fish. Intra-species comparisons identified significant differences in the number of fibres ingested and in the gastrointestinal tract weights, while inter-species comparisons revealed differences in the amounts of fibres and fragments ingested among individuals. Slight Spearman correlations were registered between the biometric parameters and the number of fibres and fragments ingested.

Keywords: Microplastics; Ingestion; Fish; Fibres; Fragments



## Resumo

Para estimar a quantidade de microplásticos ingeridos por peixes da costa Portuguesa e estabelecer alguma informação acerca do potencial de perigo à saúde humana, 82 amostras de *Trachurus trachurus* e 82 amostras de *Scomber colias* (n=164) foram recolhidas das lotas de Figueira da Foz e Sesimbra. Após dissecação e digestão dos tractos gastrointestinais, foi determinado que 67% das amostras evidenciaram ingestão de microplásticos, e cerca de 54 indivíduos estavam livres de contaminação.

O plástico e os microplásticos são persistentes no ecossistema marinho, a sua presença e possível ingestão pode levar a consequências prejudiciais no ecossistema marinho e biota, poluentes orgânicos persistentes, com capacidade de adsorção à superfície dos microplásticos, capazes de bioacumular ao longo da cadeia alimentar marinha, representam a maior ameaça.

Foi registada uma distribuição média de  $(3.63 \pm 3.16)$  microplásticos por peixe contaminado, um total de 399 microplásticos foram identificados. Os tipos de microplástico encontrados nos peixes variaram entre fibras e fragmentos, sendo que as fibras compuseram o contaminante mais recorrente, 79% enquanto os fragmentos 21%. Os peixes recolhidos na lota da Figueira da Foz, compreendem 62 % do total de microplásticos identificados, enquanto que, em relação à espécie, *S. colias*, ingeriu 54% do total de microplásticos identificados.

O teste de hipóteses de Kruskal-Wallis foi testado para identificar diferenças estatísticas significativas entre as populações amostradas e a correlação de Spearman permite avaliar a significância das correlações encontradas entre os parâmetros biométricos e os hábitos de ingestão de microplásticos. Comparações intra-espécies identificaram diferenças estatísticas significativas para o número de fibras ingeridas e o peso do trato gastro-intestinal, enquanto que as comparações inter-espécie identificaram diferenças nas quantidades de fibras e fragmentos ingeridos entre os indivíduos amostrados. Correlações de Spearman, embora fracas foram também registadas entre os parâmetros biométricos e a quantidade de fibras e fragmentos ingeridos.

Palavras – chave: Microplásticos; Ingestão; Peixe; Fibras; Fragmentos



# Table of contents

<b>1. Introduction</b>	<b>- 1 -</b>
1.1. Problem Definition	- 1 -
1.2. Objectives	- 1 -
<b>2. State of the Art</b>	<b>- 3 -</b>
2.1. Marine litter	- 3 -
2.2. Plastic in marine litter	- 3 -
2.3. Microplastics	- 5 -
2.4. Impacts in marine environment and biota	- 6 -
2.5. Persistent Organic Pollutants	- 7 -
2.6. Regulations and Legal Instruments	- 8 -
<b>3. Materials and Methods</b>	<b>- 11 -</b>
3.1. Study area	- 11 -
3.2. Samples collection	- 11 -
3.3. Species characterization	- 12 -
3.4. Laboratory procedures	- 12 -
3.4.1. Biometric parameters	- 12 -
3.4.2. Dissection	- 13 -
3.4.3. Digestion	- 13 -
3.4.4. Filtration	- 14 -
3.4.5. Stereoscopic observation and microplastics identification	- 15 -
3.5. Statistical Analysis	- 15 -
<b>4. Results</b>	<b>- 17 -</b>
4.1. Biometric parameters	- 17 -
4.2. Microplastics	- 17 -
4.3. Statistical Analysis	- 21 -
<b>5. Discussion</b>	<b>- 23 -</b>
<b>6. Conclusions</b>	<b>- 27 -</b>
<b>7. References</b>	<b>- 29 -</b>



## Figure Index

Figure 1 – Plastic production trends, A) Plastic production evolution since 1950 (1.5MTons) to 2015 (322MTons), B) Plastic production patterns in the World and in Europe from 2005 to 2015, adapted from PlasticsEurope 2015 and 2016.....	- 4 -
Figure 2 – Plastic demand by polymer type (%) in the year of 2015.....	- 5 -
Figure 3 - Location of the fishing ports where samples were landed; A) Figueira da Foz port; B) Sesimbra port.....	- 11 -
Figure 4 – Biometric parameters obtained from fish, A) Procedure for standard and total length measurement, B) Determination of the sample wet weight. ....	- 13 -
Figure 5 – Dissection process demonstration, A) dissected sample with the organs inside, B) dissected sample after extraction of the organs. ....	- 13 -
Figure 6 – Digestion procedure, A) GI tract content, B) Samples covered with aluminium foil in digestion process.....	- 14 -
Figure 7 - Filtration phase, A) Vacuum filtration apparatus system, B) Individual storage of filters .....	-14 -
Figure 8 – Stereoscopic microscope Leica system used for filters observation .....	- 15 -
Figure 9 - A - Length (cm) and B - wet weight (g), of <i>Trachurus trachurus</i> (Tt) and <i>Scomber colias</i> (Sc). Error bars indicate SD (standard deviation), collected in Figueira da Foz(FF) and Sesimbra(S), n = 82 for both species.....	- 17 -
Figure 10 - Selected fibre and fragment. A) example of material considered as fibre, B) example of material considered as fragment.....	- 17 -
Figure 11 - Proportion (%) of total MPs (fibres and fragments) identified in the fish sampled. ....	- 18 -
Figure 12 - Different fibres identified among the fish sampled; A) Blue fibre identified in Atlantic chub mackerel from Figueira da Foz, 3.09mm in size, B) Red fibre identified in Atlantic chub mackerel from Figueira da Foz, 2.49mm in size, C) Blue and transparent fibre identified in Atlantic chub mackerel from Sesimbra, 1.56mm in size, D) Blue fibre identified in Horse mackerel from Figueira da Foz, 2.12mm in size.....	- 19 -
Figure 13 – Fragments identified among the fish sampled. (A) Green Fragment, 870µm in size, (B) light blue fragment, 436 µm in size, (C) grey fragment, 634 µm in size, and (D) small blue fragment, 96 µm in size, (A, B and C) fragments from Atlantic chub mackerel collected in Figueira da Foz and (D) fragment from Horse mackerel collected in Figueira da Foz .....	- 20 -
Figure 14– Diagram displaying MPs distribution among contaminated fish, A) 63 fish samples ingested fibres, B) 18 fish samples ingested fragments, C) 29 fish samples ingested fibres and fragments, 54 fish samples were free of contamination. ....	- 21 -
Figure 15 – Number of fibres and fragments of both species from both locations and GI tract weight, the first two were observed on Leica stereoscope and the last one, measured during laboratory procedure. Error bars indicate SEM (standard error of the mean), A) average number of fibres per fish, B) average number of fragments per fish, C) Average weight of GI tract per fish; (FF) – Figueira da Foz, (S) – Sesimbra. (Tt) – <i>T. trachurus</i> , (Sc) – <i>S. colias</i> , (a, b) – intra-species significant statistical differences, (α, β) – significant statistical differences between species.....	- 22 -



## Table Index

Table 1 - Information about the fish collected (Docapesca, product sheet 2017).....	12 -
Table 2 - Fibres and fragments identified in both species and locations.....	18 -
Table 3 – Fibres length variation per color, n = 136 .....	18 -
Table 4 - Quantification of MPs photographed based on color and size (n = 183)...	20 -
Table 5 – Number of MPs ingested versus number of fish that effectively ingested those quantities. ....	21 -
Table 6 – Information about MPs distribution among species and ports, A) comparisons between locations, B) comparisons between species. ....	21 -



# 1. Introduction

## 1.1. Problem Definition

Global plastic production and consumption have been increasing since 1950, and plastic accumulation in the terrestrial and marine environment continues to increase. It is estimated that 80 – 85% of the marine litter is composed by plastic materials (Auta et al., 2017), which are very persistent in the marine environment, and has harmful effects in both ecosystems and biota. This is caused by their properties and constitution, which provide them resistance to persist in the marine environment.

Microplastics can induce physical or chemical harm in fish due to their ingestion. Microplastics are capable to adsorb persistent organic pollutants, and their bioaccumulation through the marine food web is starting to be addressed by the scientific community. The potential risk for human health by consuming contaminated fish flesh is still a speculative hypothesis.

It is necessary to enhance the scientific knowledge and people awareness regarding the quantifications of microplastics in different commercial fish and shellfish. This way, this thesis aims to contribute to the scientific knowledge of microplastics quantification in two commercial fish, *Scomber colias* and *Trachurus trachurus*, from the Portuguese Coastal waters.

## 1.2. Objectives

To enhance the scientific knowledge about microplastics in the Portuguese Coastal waters and consequent ingestion by two different species the following objectives were established.

- Quantification of microplastics in the contents of the fish gastro intestinal tract;
- Differentiate the typologies of microplastics identified;
- Intra and inter-species comparisons of ingested microplastics;
- Comparisons between the two locations where species were sampled;



## **2. State of the Art**

### **2.1. Marine litter**

Marine litter is classified as a complex issue with relevant implications for the marine and coastal environment, as well as for the human activities all over the globe. A few measures at regional, national and international levels are being implemented to reverse this tendency but until now the problem continues to grow (Portman et al. 2017; Ryan, 2015; Jambeck et al., 2015; UNEP, 2009). A wide range of evaluations in different geographic locations were made and all identified marine litter in beaches, at the water surface, water column and at the seafloor (Galgani et al., 2015).

When some materials are discarded, abandoned or even lost in public spaces and natural environments, they can become litter and may be produced by different sources, which are frequently associated to human activities (Veiga et al., 2016). Due to transportation mechanisms like winds, oceanic tides, river flows among others, land based litter can reach the Ocean surfaces, suffer breakdown and further distribution, while sea based litter may experience deterioration and dissipate to deep waters. It is estimated that 80% of marine litter originates from land-based sources. (Veiga et al., 2016; Jambeck et al., 2015)

Marine litter is not strictly correlated with the most densely populated regions, since it is a global issue and there are a wide range of transport mechanisms which may influence their distribution. Although some authors (Barnes et al., 2009 and Browne et al., 2011) consider that most polluted areas by marine litter occur near the coastline, because of the high populational density and human activities, marine litter is also observed in remote places far from obvious pollution sources (Obbard et al., 2014, Pichel et al., 2012 and Waller et al., 2017) and human activities. According to Pasternak et al (2016) marine litter could result from poor or inappropriate solid waste management strategies, product design and consumer choices.

Anthropogenic litter in the sea surface, seafloor and beaches has been increasing notably. The scientific community first started to become aware about the issue between the 1960s and 1970s, and recognized marine litter as a global problem later in the 1990s. Nowadays marine litter is easily detected in all the oceans (Ryan, 2015). The breakdown of larger debris, result in small particles, further contributing to the numerous floating litter items that can be transported over long distances due to oceanic flows and winds (Barnes et al., 2009).

Several researches were made until the present days proving marine debris distribution across worldwide waters and implications with marine organisms are also reported extensively, however proper quantifications about marine debris annual input are not know. Scientific knowledge still displays some limitations for this type of evaluation once all the variables can be highly influenced from innumerable external factors.

### **2.2. Plastic in marine litter**

Modern plastics expansion increased in the last 50 years. The success of plastic as a material has been massive. Plastic has unique properties, it is cheap, strong and durable, can be used in a broad range of temperatures, can be chemical and light resistant and still very strong and tough, however, easily manipulated by man (Andrady & Neal, 2009). According to a couple of authors (Therzi et al., 2013, Topçu et al., 2013) which surveyed the compositions of marine litter collected from different geographic locations indicate plastic materials has the main category identified.

Human needs and habits enhanced the need of certain type of materials. Plastics emerge with unique characteristics hence their mass production starts. Since 1950 the global trend of plastic production and consumption increases. In this very premature stage their production was about 1.5M tons, about 50 years later in 2004 the production rapidly increased to 225M tons. Nowadays, worldwide plastic production is above 320M tons. Concerning to the global and European tendency, for the period of 2004 – 2014, global production was increasing while for Europe the production patterns were constant with slight variations per year (Figure 1). China occupies the first place in the ranking with (27.8%), followed by Europe and NAFTA with (18.5%) each, regarding to thermoplastics and polyurethanes production (PlasticsEurope, 2015 and 2016).

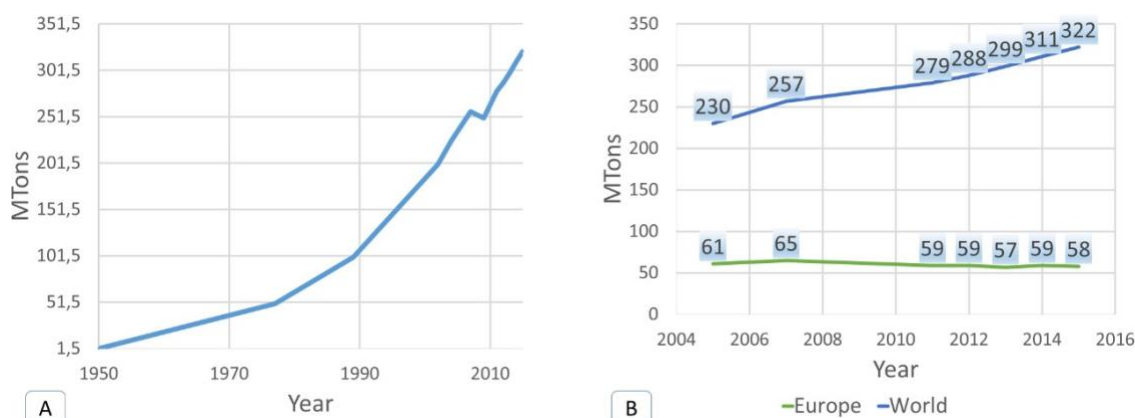


Figure 1 - Plastic production trends, A) Plastic production evolution since 1950 (1.5Mtons) to 2015 (322Mtons), B) Plastic production patterns in the World and in Europe from 2005 to 2015, adapted from PlasticsEurope 2015 and 2016

This rapid growth and mass production of plastic material lead to the development of different polymers and other constituents used to enhance plastic versatility, durability among other properties. There are plenty of different plastic polymers and combinations that could be made, resulting in plastic materials, however there is the big six category, which comprehends the group of polymers more produced: Polypropylene (PP), Low Density Polyethylene and High Density Polyethylene (LDPE and HDPE), Polyvinyl chloride (PVC), Polyethylene terephthalate (PET) and Polystyrene (PS) (Figure 2). While the market sectors where these material is applied the most, corresponds to Packaging (39.9%), Others (22.4%) and Building and Construction (19.7%), the category others includes: consumer and household goods, furniture, sports, health and safety departments among others (PlasticsEurope, 2015 and 2016).

Plastic debris in the sea follow an irregular distribution, for many reasons, including local winds and current conditions but patterns reveal the ubiquity of plastics, greater loads are detected close to urban areas and touristic regions due to human activities (Barnes et al., 2009). Developed regions of the globe display steady consumption patterns of the most used plastic types in their different applications areas (Andrady and Neal, 2009). Plastics have become more powerful in the consumer marketplace since their commercial development in the 1930s and 1940s (Jambeck et al., 2015) By themselves, their use have not shown potential, moreover when mixed with other materials called 'additives' their performance enhance significantly (Andrady & Neal, 2009).

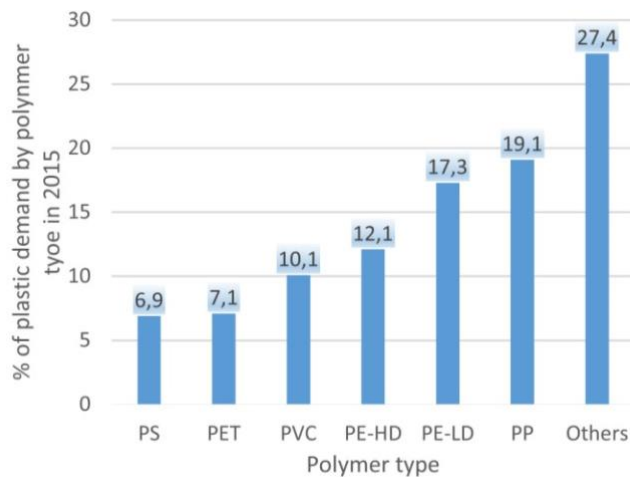


Figure 2 - Plastic demand by polymer type (%) in Europe for the year of 2015, Source: PlasticsEurope 2015 and 2016

Plastic constitute about 80 – 85% of the marine litter (Auta et al, 2017). Furthermore, while plastics resins production had increased by 25 times in the past 40 years, the proportion of material recovered was less than 5%, leading to an accumulation in the coastal and marine environment (Sutherland et al., 2010). Regarding to the exclusive characteristics provided by this material, degradation rates differ consonant to the environment they are exposed to. At land environment, including beaches photo - oxidative degradation occurs at faster rates than in marine environments, because of the water surfaces which are a good heat sink. Weathering is another process that turns plastics in smaller dimensions, however the rates of efficiency are dependent on the sunlight, temperature, oxygen levels and fouling. The rates of degradation of plastics decrease in depth once the environment conditions are different and could not perform the same efficiency has in land (Andrady, 2015).

### 2.3. Microplastics

In the present study microplastics (MPs) were described as plastic particles with less than 5 mm in size (Arthur et al., 2009). This material is widespread in all environments, mostly due to their lightweight and natural factors as winds, currents, tides and other extreme natural events. According to the International Marine Organization, (2015) microplastics were defined as plastic particles in a range of 1nm - 5mm, although, these range definitions for the lower boundary are adjustable according to the purposes of the research and technical limitations. For this study the lower size particle observed measured 20 µm.

Microplastics can be classified as primary or secondary regarding to their pathway of formation: they occur in the marine environment by direct release of plastic particles 'pellets and microbeads' and from the fragmentation of larger debris, in marine environment their presence is felt from the poles to the equator and various shapes of microplastics were identified among studies from spherical particles, long textile fibres to different shape of fragments easily differentiated from the natural environment particles (Thompson et al., 2015). Primary microplastics are regularly found in different commercial products like exfoliating facial scrubs, toothpastes and other cosmetics and can enter the marine ecosystem through industrial or domestic drainage systems (Auta et al., 2017). According to Browne et al., (2011) washing clothes machines represent an important source of contamination of the wastewater system due to leakages of fibres.

MPs display worldwide distribution based in the diverse researches proceeded. Fish guts assessed by (Foekema et al., 2013; Lusher et al., 2013; Rochman et al., 2015; Romeo et al., 2015; Bellas et al., 2016; Rummel et al., 2016) and by other investigators provided

a list of vulnerable species to MPs ingestion: Indian mackerel, Shortfin scad, Herring, Rabbitfish, Whiting, Horse mackerel, Haddock, Cod, Atlantic Horse mackerel, Poor cod, John Dory, Red gurnard, Dragonet, Redband fish, Solenette, Thickback sole, Swordfish, Bluefin, Albacore, Dab, Flounder, Dogfish, European hake, red Mullet and few others.

This way, information about MP ingestion levels by fish are being reported in different water bodies, North and Baltic Sea, Mediterranean Sea and coasts, Portuguese coastal waters, the English Channel, Indonesia and California coastal waters. Coastal environments as beaches are also highly impacted (Martins and Sobral, 2011; Antunes et al., 2013, Auta et al., 2017), by MPs due to their characteristics and trend to accumulate in these environments.

These particles due to their chemical characteristics promptly adsorb contaminants from the water. Persistent Organic Pollutants (POPs) are one of the principle pollutants which stick to the MPs surface. Additives, used in the plastic production stage, to improve their characteristics and enhance their performance, may leach out of the degrading particles and can be chemically hazardous for organisms which may ingest them. Leachable compounds, as residual monomers and oligomers, were also found in the environment, by disassociation from the plastic items which deteriorate with time (Cole et al., 2011).

#### **2.4. Impacts in marine environment and biota**

Plastic litter occurrence is evident in different locations, and Jambeck et al., (2015) estimated that 99.5 million tons of plastic waste produced in the coastal areas of the world, of which, about 32 million tons were classified as mismanaged and an estimated load of 4.8 to 12.7 million tons entered the oceans in 2010. Plastic materials are known to accumulate in ocean gyres (Law et al., 2010) which may increase fragmentation and mixture of different plastic materials. Furthermore, microplastics ingestion in marine environment depends on characteristics, such as: size, density, abundance and color (Wright et al., 2013), although they are very persistent and degradation is very slow. Concerning the distribution of microplastics in the water column, they are spread among the different layers: surface waters and pelagic and benthic domains, as reported in several surveys examining different organisms including fish species (Foekema et al., 2013; Lusher et al., 2013 and Rochman et al., 2015).

Plastic degradation in marine environment can occur due to several mechanisms: (1) biodegradation, (2) Photodegradation, (3) Thermo-oxidative degradation and (4) hydrolysis, all these processes result in a considerable reduction of the average molecular weight of the polymer (Andrady, 2011).

(1) Biodegradation could represent a solution for low plastic quantities and low molecular weight situations, once they do not display capacity to degrade high molecular weight polymers;

(2) degradation started by the UV solar radiation is very efficient for particles exposed in land, including at beaches on the surface sediment;

(3) After initiated the degradation can occur without direct exposure to UV radiation for some time, an autocatalytic degradation reaction sequence can hold the process if there is oxygen in the system;

(4) It is not usually an efficient mechanism in seawater;

MPs in the ocean are distributed in different layers, surface waters, water column, seafloor, shoreline and in biota, interactions between the different layers and fluxes of MPs are not fully understood yet, further sampling for larger period of times and layers modelling might be necessary (GESAMP, 2016).

Coastal and marine birds, Sea turtles, marine mammals, fish, crabs and squids compose the 267 different species identified by Laist in 1997. Persistency of plastic materials has proved to be a threat to the biota, since the last review in 1997 an increase of 40% was

reported, all combined a total of 663 different species were, by entanglement or/and ingestion, somehow, affected by plastic or other synthetic debris (GEF, 2012). An estimative was made and from all this encounters between different species and marine debris, 80% of the reported situations were associated to plastic materials while only 11% related to MPs (GEF, 2012).

Organisms can be impacted by plastic debris consist in entanglement and ingestion. This first route of physical harm can be fatal, leading to organisms death, entanglement is widely reported in different parts of the globe and for a great variety of species and is mostly reported in marine mammals which may be more susceptible (Laist, 1997; Galgani et al., 2010; GEF, 2012). Otherwise, plastic or/and MP ingestion has started to be reported more recently, more knowledge in this area is necessary for further comprehension about routes of harm, quantifications for a better understanding of the problem dimension and classification for easy interpretations between studies from other researches.

Ingestion of microplastics has been recorded from the baseline of the marine food web through plants such as microalgae and zooplankton, hence its transfer to higher trophic levels animals in the food web is a strong hypothesis (Cole et al., 2016; Auta et al., 2017; Vroom et al., 2017).

Research conducted by Foekema et al., 2013; Lusher et al., 2013; Rochman et al., 2015, Neves et al., 2015; Romeo et al., 2016; Bellas et al., 2016 and Rummel et al., 2017, confirmed ingestion of microplastic particles by commercial, pelagic and demersal fish from different geographic locations, these authors focused, as this study, in the quantification and typology of the MP present, and physical harm was not reported. Possible effects report to blockage of gastric enzyme production, low feeding stimulus, reduced growth rates, low hormone levels, delayed ovulation and reproduction failure (Galgani et al., 2010), blockage of the digestive tract or injuries caused by microplastic shape can lead to death (Wright et al., 2013).

Simultaneously with physical harm MPs may display chemical harm to the organisms which feed on them. Andrady (2011, 2017) described some of the possible repercussions as leaching of residual monomers from manufacture as well as additives used to enhance plastics performance, the persistent organic pollutants present in the water represent a serious issue, once they absorb and accumulate in microplastics, hence their transfer and bioaccumulation through the marine food web may pose a threat to human health.

## **2.5. Persistent Organic Pollutants**

After the second world war, followed a period of industrial revolution which conceded space for the development of synthetic toxic chemicals, many of them were beneficial in crops production, pest and disease control, and industry. Otherwise their side effects were not a concern in the early stages. However, there are persistent organic pollutants (POPs) intentionally made for a variety of applications and those which are by-products of reactions from some industrial processes. From all the kinds and varieties of POP, Polycyclic aromatic hydrocarbons (PAHs), Polychlorinated biphenyls (PCBs) and Dichloro – diphenyl – trichloroethanes (DDTs) are the most characterized groups in plastic debris.

PAHs comprise two or more benzene rings, made of carbon and hydrogen atoms and can occur in a wide variety of different combinations. They are mostly colorless or yellowish with solid aspect and can be found naturally in the environment from natural disasters such as volcanic activities, forest fires, among others (EPA, 2008) or be man-made, in situations like automobile emissions and cigarette smoke (Abdel-Shafy and Mansour, 2016). There are more than 100 possible combinations of PAHs and 7 of them reported individually in the EPA Priority Chemical List. They are used to produce coloring agents, plastics and pesticides and their presence is felt in soils, waters and air and they are susceptible to suffer long range transportation due to winds and currents (EPA,

2008). PAHs are commonly used in manufacture of pigments, pesticides, agrochemicals, pharmaceuticals, resins, plastics, among others (Abdel-Shafy and Mansour, 2016).

PCBs are man-made from organic chemicals, consisting of carbon, hydrogen and chlorine atoms, physical and chemical properties are determined by the position occupied by the chlorine atoms in the PCB molecule, in consistency these compounds vary from oil to waxy solid form. Commercial uses were related to oil use in motors and hydraulic systems, transformers, cable insulation and several old electrical devices and appliances containing PCBs, although production was banned in 1979 (EPA, 2017). PCBs are considered a worldwide concern for humans and the environment due to their persistence, bioaccumulation and toxicity (Ivanescu, 2015). Seawater close to industrial facilities tend to display higher levels of PCBs, hence contamination of resin pellets and toxic effects towards marine organisms could be superior in industrialized areas (Mato et al., 2001 and Endo et al., 2005)

DDTs are organochlorines, composed of carbon, hydrogen, chlorine and sometimes other elements, they were principally used in crops as pesticide and for insect control, EPA ban their usage by 1972, in developed countries because of their harm to humans and the environment, it persists, accumulates and can cause adverse health effects in wildlife and humans, possible effects in animals are related to tremors, incoordination, toxicity, convulsions, among others (NPIC, 1999).

Most of these chemical pollutants display hydrophobic behaviors, hence they look after plastics, microplastics, sediment and other materials to sink on. Particularly microplastics due to their properties, constitute an important pathway for persistent organic pollutants (POPs) adsorption and further contamination. MPs and larger plastic debris analysis, collected from multiple beaches, corroborate the presence of POP in their constitution (Ogata et al., 2009; Antunes et al., 2013; Frias et al., 2010; Herrera et al., n.d.). Organic pollutants adsorbed to microplastics is widely documented and the ingestion of these contaminated particles by marine organisms could lead to significant levels of exposure (Hirai et al., 2011).

A comparison between the results found by Frias et al. (2010) and the values registered by the International Pellet Watch were analyzed to identify possible conformities. For DDTs, the values registered by IPW are in the interval considered in the study of Portuguese Coast, PAHs are found in higher concentrations than those considered by IPW, otherwise PCBs represent lower concentrations comparing with IPW registrations.

Rochman et al, 2013 evidence the possibility of transfer of POPs through contaminated MPs ingested by fish and their transfer and bioaccumulation through the marine food web is confirmed by Murray and Cowie, (2011) and Trouwborst, (2011). However, further knowledge is necessary to estimate the rate of contaminant that effectively passes from the plastic particle to the organism who ingested it and further transfer through the food chain. At the moment, the potential risk for human health by ingestion of plastic contaminated fish, is unknown.

## **2.6. Regulations and Legal Instruments**

A group of international, European and national conventions, policies and laws were gradually implemented due to necessity for the conservation and protection of marine environments against marine litter and microplastics.

The United Nations Environment Program, Regional Seas Program (UNEP), started at 1974, comprises a collaborative international program, now with 140 countries, to protect marine environment and their resources. UNEP supports 14 conventions, one of them named OSPAR, which comprises Marine Litter Regional Action Plan and seeks to prevent and reduce marine litter pollution in the North-East Atlantic and its impact in marine organisms, habitats and all the related aspects and activities.

The London Convention 1972 (IMO), active since 1975, is considered the first convention which globally considers controlling and protect the marine environment from the human

activities. This convention aims to promote an effective control of possible pollution sources and prevent pollution through regulation and policies.

The International Convention for the Prevention of Pollution from Ships (MARPOL 73/78) is the leading international convention in this matter, the convention was adopted by 1973 but the protocol activated in 1978 due to 76-77 tankers accidents. This convention comprises 6 technical annexes concerning certain areas with strict control on discharges. The annex IV, in place since 2003, is the last one approved related to marine environment pollution and it pretends to control the pollution of the sea by ships sewage discharges, with the same or more importance, the annex V, in place from 1988/89, which bans the disposal of all forms of plastics in the sea.

In Portugal the directive nº 478/71 defined the Portuguese public water domain, which were responsible for the coastal environment management. In 1992, due to the decree law no. 201/92, the management of the coastal environment were transferred to the Ministry of Environment. With these developments, in 1993 appeared the (POOC) Plan for sustainable management of coastal environments. A minister's council 129-R/2002, resulted in the project 'Finisterra' which comprises an integrated planning for all the Portuguese coastal environment, interventions such as environmental values protection, ecosystem services, mitigate possible threats and requalification of deteriorated ecosystems.

For the POPs considered in the present study, a group of directives are in force to prevent their deliberation and further contamination, once they are considered very persistent. The directive no. 277/99, which states the precedents to follow, for used PCBs elimination, visioning their destruction. PAHs directives in force in national territory are transposed from European directives. The European parliament emitted 2005/69/CE directive, which pretends to aware companies to be perceptive about several dangerous substances production and their commercialization.

Several other directives are established in the following years with European and national commitment, searching for a positive development in the marine environment protection related to control and recycle several types of waste, define re-usable and non re-usable materials, and improve adequate platforms for waste generated in ships disposal, avoiding direct releases in the marine environment.

MSFD (Directive 2008/56/EC) transposed for the Portuguese legislation by the Decree Law no. 108/2010 – Marine Strategy Framework Directive (2010), which introduced 11 qualitative descriptors to achieve good environmental status in all water bodies by 2020, the descriptor no. 10, particularly concerning the marine litter (Galgani et al., 2010).

The Agenda 21, adopted by more than 178 nations, is the product of Earth Summit in Rio 1992, pretends to promote the sustainable development and comprises the environmental protection and equilibrium. This Agenda implementation is responsibility of the national governments, through national strategies, plans, policies and procedures.

The Ocean Clean-up Program, founded in 2013, it is developing feasible methods for the ocean clean up, initially designing mechanisms for larger plastic debris.

The international Coastal Clean-up Program, supported by the Ocean Conservancy, which represents the largest campaign of coastal clean-up. <https://oceanconservancy.org/trash-free-seas/international-coastal-cleanup/>, and Beat the MicroBead application, designed to offer people information about personal care and cosmetics products, if contains synthetic microbeads <https://www.beatthemicrobead.org>.

In Portugal, non-governmental entities such as associação portuguesa do lixo marinho (APLM) and few others are responsible for the people awareness and coastal clean-up campaigns. These entities are versatile and contribute with different type of awareness according to the target community, from children, students, to fishermen and the community in general.



### 3. Materials and Methods

#### 3.1. Study area

At Figueira da Foz, fish samples were collected by commercial fishing vessels from areas near Praia da Leirosa and São Pedro de Moel, which are affected by the mouth of Mondego river and the respective estuarine region. The Mondego estuarine area is characterized by the North arm which receives most of the marine tidal water and presents the main navigation channel and the South arm, which is highly affected by the nutrient inputs upstream coupled with high residence time of water (Dolbeth, 2010). The high nutrient loads are related to agriculture fields upstream the estuary and aquacultures downstream, other activities as paper, cellulose and extractive industries also occur (Pinto et al., 2009). However, the primary production has been decreasing while recreational activities and tourism related have been increasing significantly, hence several anthropic pressures are identified (Pinto et al., 2010).

Sesimbra fish samples were collected by commercial fishing vessels in offshore waters, where microplastics may occur in lower concentrations, away from coastal and river influence. The lower estuarine region of the major river (Sado) is a Natural Reserve, however several industries are there implemented, agriculture and salt works were also observed. The city of Setubal and the copper mines present in the Sado watershed use the estuary for effluents discharge (Caeiro et al., 2009).

#### 3.2. Samples collection

*Scomber colias* and *Trachurus trachurus* species were made available by Docapesca, the public national entity responsible for the first sell of fish and shellfish at those fishing ports. Samples were collected in 24<sup>th</sup> April in Sesimbra (B) and 2<sup>nd</sup> June 2017 in Figueira da Foz (A), two of the four ports, with major relevance regarding fish capture and commercialization (Docapesca, 2015). Figure 3 illustrates the location of the fishing ports where the samples were landed.

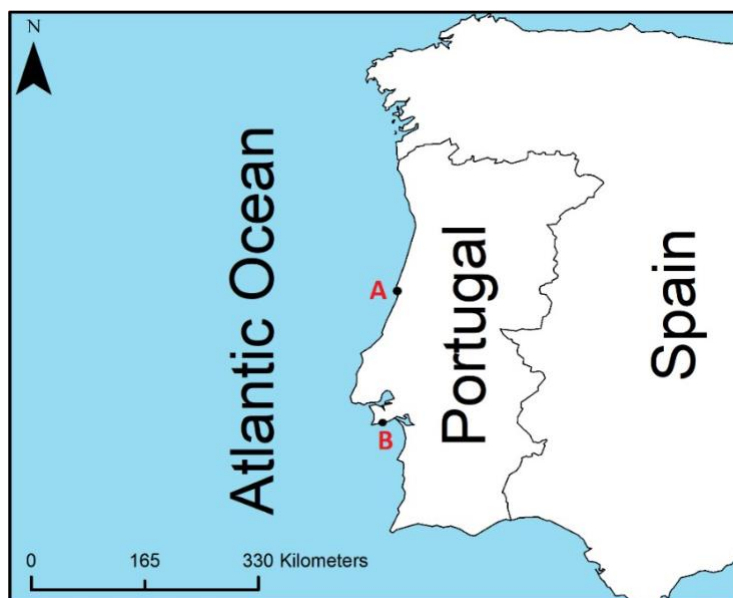


Figure 3 – Location of the fishing ports where samples landed; A) Figueira da Foz port; B) Sesimbra port.

Two species of fish were analyzed for ingested MPs: Horse mackerel (*Trachurus trachurus*) n=41 and Atlantic chub mackerel (*Scomber colias*) n=41 from each location (A and B), making a total of 164 fish. General information about the species collected is presented in Table 1, regarding to fish origin, Food and Agriculture Organisation (FAO) provides a worldwide map identifying the FAO fishing areas. Fish were collected frozen,

and immediately transported to the laboratory, in appropriate ice coolers and frozen at (-18 °C) in laboratory until dissection for analyses of the gut content.

Table 1 - Information about the fish collected (Docapesca, product factsheet 2017).

Scientific name	Commercial name	Family	Origin	Minimum capture size (cm)	Fishing gear	Observations
<i>Trachurus trachurus</i>	Horse mackerel	Carangidae	FAO zone 27; FAO zone 37	15	Purse seines; Trawl nets; Gill nets	Classified as vulnerable (VU) by IUCN Red List
<i>Scomber colias</i>	Atlantic chub mackerel	Scombridae	FAO zone 27; FAO zone 37	20	Purse seines; Bottom trawling	Classified as least concern (LC) by IUCN Red List

### 3.3. Species characterization

Both species collected are characterized for being pelagic oceanodromous, however Horse mackerel display benthopelagic behavior (FAO, 2005). The geographic distribution and depth ranges are similar. The Atlantic chub mackerel is distributed in the North Atlantic Ocean and Mediterranean Sea, ranging from 0 – 200 m in depth usually, in winter moves to deeper waters and in spring to neritic zones, while the Horse mackerel is distributed from Norway to South Africa, including the Mediterranean Sea and Eastern Atlantic, more common in depths from 100 – 200m respectively. The feeding behaviors of Atlantic chub mackerel are based on zooplankton (fish larvae, small crustaceans and pteropods) and Horse mackerel feeds on crustaceans (copepods), shrimps, small fish and squids. Horse mackerel individuals trend to be in demersal waters during the day and at night they rise to the surface for feeding, Atlantic chub mackerel is in the pelagic zone and occurs in schools close to surface waters feeding on the living organisms and other organic particles present in these areas (FAO, 2005).

### 3.4. Laboratory procedures

To prepare samples for posterior microplastics identification and extraction several main steps were processed before: Registration of the biometric parameters followed by (1) dissection to excise the gastro-intestinal (GI) tract, (2) digestion to eliminate organic matter, (3) filtration of the digested content and (4) observation of the filters to detect non-natural microparticles.

#### 3.4.1. Biometric parameters

Samples were measured (standard length, total length) and weighed (total wet weight) to obtain the biometric parameters (Figure 4). The total length was obtained measuring the fish from the mouth to the tail, however in some cases occurred that the fish tails were damaged. For all cases standard length was also measured, which comprised the

distance between the fish mouth and tail muscles and was considered the main length in this study.

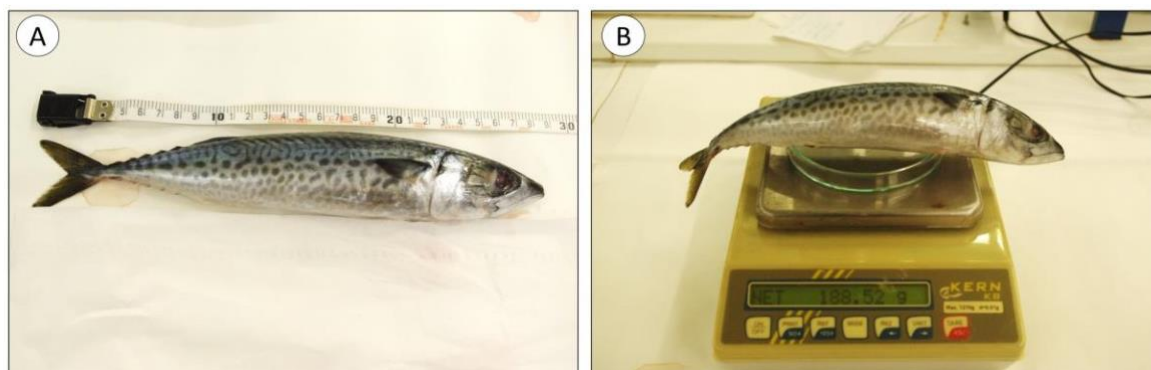


Figure 4 - Biometric parameters obtained from fish, A) Procedure for standard and total length measurement, B) Determination of the sample wet weight.

### 3.4.2. Dissection

With a scalpel, an incision was done from the anus to the upper part near the gills (Figure 5). The gastro-intestinal (GI) tract were extracted with tweezers and organs were weighted in a weighing scale (KERN KB), on Petri dishes, and kept in covered small glass jars. Dissections were carefully made to avoid any perforation of the internal organs. All the equipment used for the dissection was carefully cleaned with distilled water and MilliQ every time a fish was dissected to avoid possible contaminations.

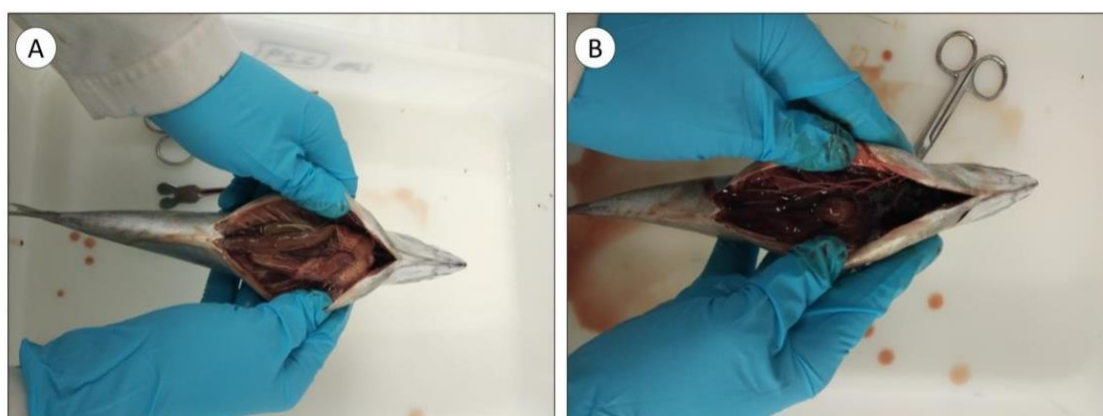


Figure 5 - Dissection process demonstration, A) dissected sample with the organs inside, B) dissected sample after extraction of the organs.

### 3.4.3. Digestion

The extraction technique involved chemical digestion of the soft tissue, using a base, potassium hydroxide, followed by filtration and stereoscopic analysis. GI tract digestion was adapted from Foekema *et al.* 2013. using a potassium hydroxide solution (KOH 10%). The solution was prepared under clean laboratory conditions. 100 g of potassium capsules were dissolved in 1000 mL of MilliQ water (Type II). After preparation, the solution was kept in glass opaque flask to avoid possible degradation.

Volumes of 15 to 25 mL of KOH 10%, depending on the amount of biological material, were added to each jar containing the GI tract (Figure 6). The jars were covered with

aluminum foil and stored at room temperature. The content was shaken twice a day to help the dissolution of the organic matter. This step was repeated until complete digestion could be observed, or most of the GI tract was dissolved, a process which took three days (72h) on average. Blank samples were also considered during the digestion procedure, one for each group of 6 samples.

This digestion method has been tested in a recent benchmark of protocols for extraction and characterization of microplastics in seafood, conducted by Dehaut et al., 2016. Six approaches were tested across 15 different plastic polymer types from mussels, crabs and fish tissues. The result showed, only one of the methods was fit for the procedure, KOH 10% method.

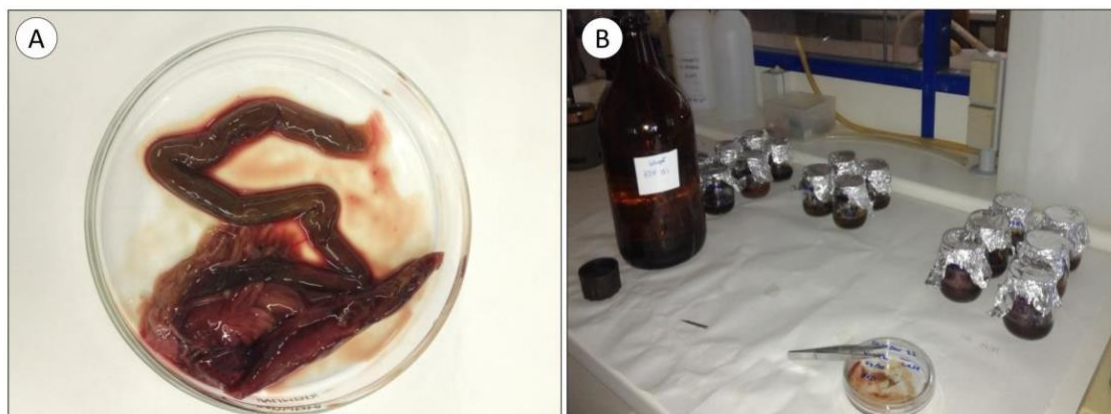


Figure 6 - Digestion procedure, A) GI tract content, B) Samples covered with aluminum foil in digestion process.

Another approach by (Kuhn et al., 2017) approves the use of KOH 10% 1M for the quantification of microplastics from small fish, 74 different particles were evaluated, 57 conventional plastics, 6 biodegradable plastics and 11 other debris. As reported by Dehaut et al., (2016) this assessment confirms the degradation of acetate cellulose, besides that, only 2 biodegradable plastics (polylactic acid (PLA) and cradonyl) suffered degradation as well, PLA was completely dissolved by the solution.

#### 3.4.4. Filtration

Filtration of the digested solution was performed with a vacuum filtration apparatus system (Figure 7) onto Fioroni & Whatman glass fiber filters (~1 $\mu$ m pore size). Distilled water was added to enhance the filtration performance. Three to five filters on average were used for each sample, depending of the amount of digested content. Filters were stored in Petri dishes individually and dried in an oven at 40°C for 24h.

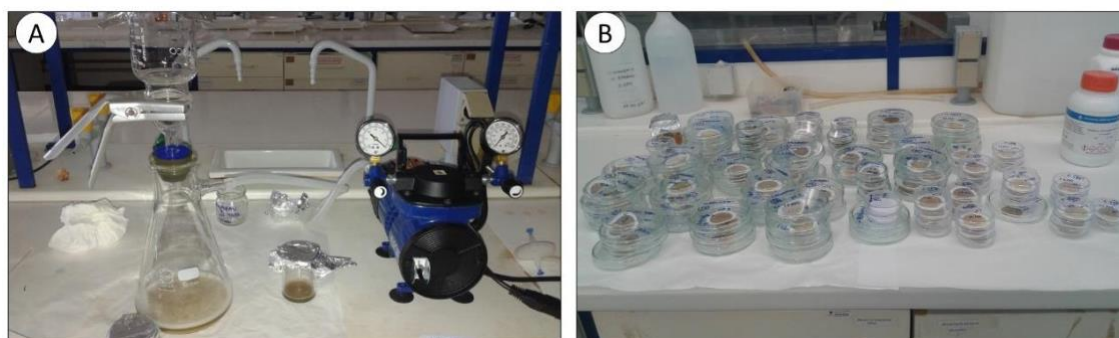


Figure 7 - Filtration phase, A) Vacuum filtration apparatus system, B) Individual storage of filters

### 3.4.5. Stereoscopic observation and microplastics identification

Filters were observed under a stereoscopic microscope Leica® equipped with a DFC480 digital camera (Leica Microsystems), Figure 8. MPs were analyzed and divided by type (fibres and fragments), measured and counted. MPs were photographed, and measurements were made using the Image J® software.

Blank samples were also considered during the filtration procedures and filters observation steps to detect any contaminations by airborne fibres, two blanks were considered for each group of 12 samples. Special care was taken with all the clothing, equipment and material to avoid possible contamination of the samples.



Figure 8 – Stereoscopic microscope Leica system used for filters observation

### 3.5. Statistical Analysis

After invalidation of at least one of the assumptions for the parametric tests, namely homogeneity of variances, as confirmed by (Levene's and Kolmogoroff-Smirnoff's tests), non-parametric tests were performed. Kruskal-Wallis H test was used for comparisons between multiple independent variables of fish sampled: Standard length, wet weight, GI tract weight, no. fibres and no. fragments ingested. Spearman correlation test were performed to identify if correlations between biometric parameters and microplastic ingestion were significant. A significance level  $\alpha = 0.05$  was established for all the analysis. Spearman correlations were considered significant if  $(R > |0.26|$  for  $p < 0.05$ ). This R coefficient were taken from critical values of the Spearman's ranked correlation table, for a  $n = 41$ . All the statistical analysis was performed using Statistica® software.



## 4. Results

### 4.1. Biometric parameters

Biometric data registered for each species were similar in both locations (Figure 9). The weight and standard length of the Horse mackerel (*T. trachurus*) collected ranged from 82.21 g – 175.17 g with a mean of 124.74g and 22.8 – 25.1 cm in length with an average 21.01 cm, respectively. The Atlantic chub mackerel (*S. colias*), weight registered ranged from 99.21 – 258.68 g, with an average of 174.22g and length from 22.2 – 28.1 cm, average 24.68 cm. These measurements refer to 82 individuals of each species (total n = 164).

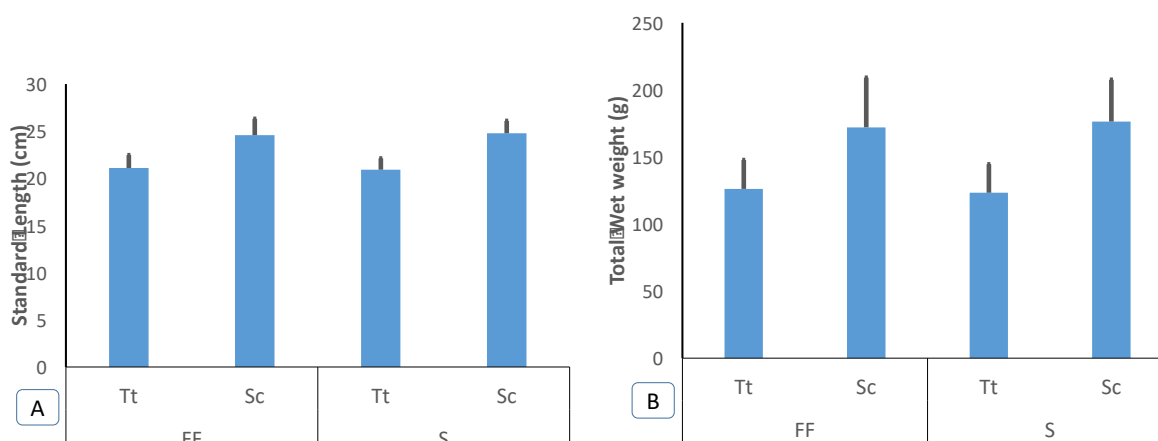


Figure 9 - Biometric parameters, A - Length (cm) and B - wet weight (g), of *Trachurus trachurus* (Tt) and *Scomber colias* (Sc). Error bars indicate SD (standard deviation), collected in Figueira da Foz(FF) and Sesimbra(S), n = 82 for both species.

### 4.2. Microplastics

Observation and identification of different types of microplastics allowed to establish two categories: fibres and fragments (associated to breakage of larger plastic debris). Figure 10 presents selected fibres and fragment analyzed in this study. Fibres and fragments have shown variations in color, length and shape.

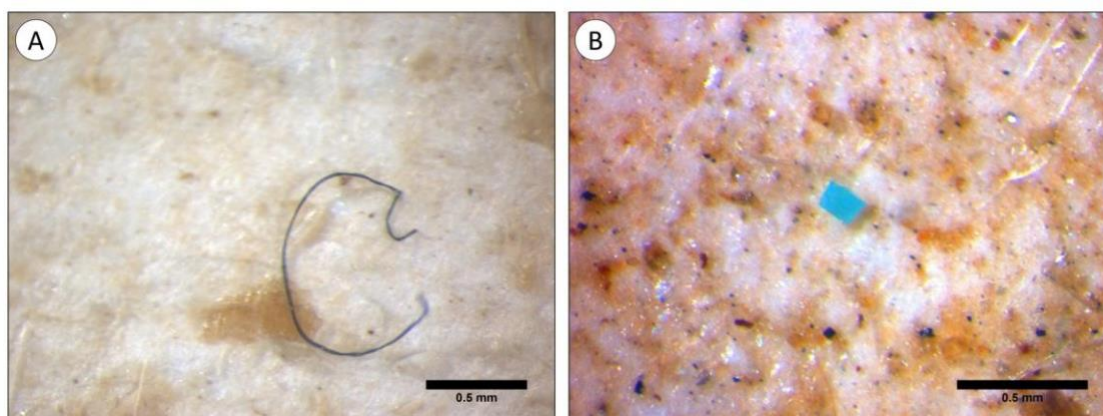


Figure 10 - Selected fibre and fragment. A) example of material considered as fibre, B) example of material considered as fragment

Microplastics distribution is presented in Table 2 according to typology (fibre, fragment) and color variation. In a total of 399 MPs, 79%, were fibres, the predominant type, while fragments corresponded to 21% of the total MPs analyzed (Figure 11).

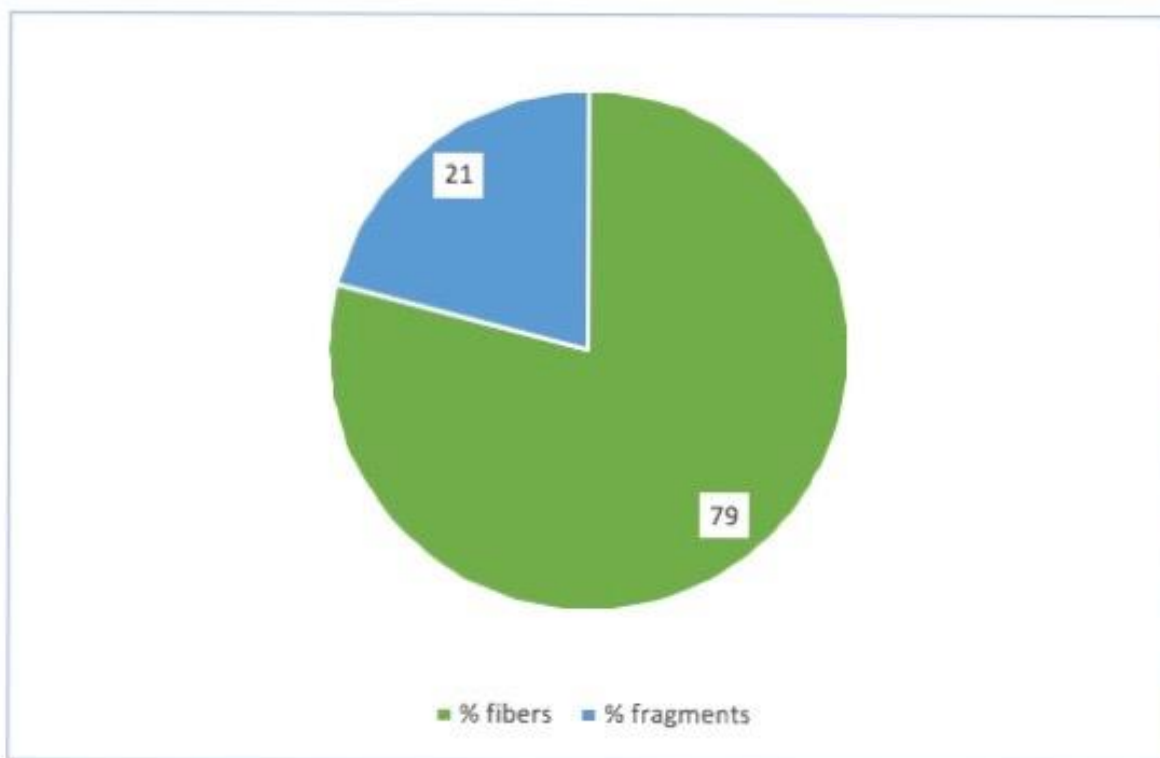


Figure 11 - Proportion (%) of total MPs (fibres and fragments) identified in the fish sampled.

Table 2 - Fibres and fragments identified in both species and locations.

Fishing Port	Species	no. of samples	fibres	fragments	Total MPs
Figueira da Foz	<i>T. trachurus</i>	41	74	12	86
	<i>S. colias</i>	41	121	40	161
Sesimbra	<i>T. trachurus</i>	41	87	11	98
	<i>S. colias</i>	41	34	20	54

The number of fibres identified in the fish GI tract is similar for both species. Regarding fragments, more quantities were identified in the Atlantic chub mackerel GI tract comparing to Horse mackerel. Figueira da Foz reveal greater amounts of fibres and fragments comparing to Sesimbra. 136 fibres out of 316 were measured and separated in five groups, according to color variations: blue, black, transparent, green and red (Table 3).

Table 3 – Fibres length variation per color, n = 136

	Blue	Black	Transparent	Green	Red
Average size (mm) (mean ± SD)	1.07±0.98	0.81±0.93	1.49±0.85	1.16±0.27	1.32±1.33
Minimum size (mm)	0.17	0.10	0.44	0.09	0.15
Maximum size (mm)	5.51	4.37	3.64	3.82	4.91
n° of samples	53	36	17	18	12
Representativity (%)	39	26	13	13	12

Fibres identified among the individuals studied vary in shape, color, and size, Figure 12 displays the diversity observed. (A) represents hard rigid fibres, (B) and (C) flexible fibres from different colors and (D) thin fibres frequently identified. From the 136 fibres measured, an average of  $1.09 \pm 1.01$  mm (mean  $\pm$  SD) in length was registered, for  $n=136$ . The minimum observed was 0.09 mm and the maximum, 5.51 mm, only one fibre has been reported with more than 5 mm. For the total number of fibres identified ( $n = 316$ ), an average distribution of  $1.93 \pm 2.66$ ,  $n = 164$  per fish were observed, if considering only the fish contaminated a distribution of  $3.43 \pm 2.73$ ,  $n = 92$  fibres occur. This material was considered has the most prevalent, in the fish samples assessed.

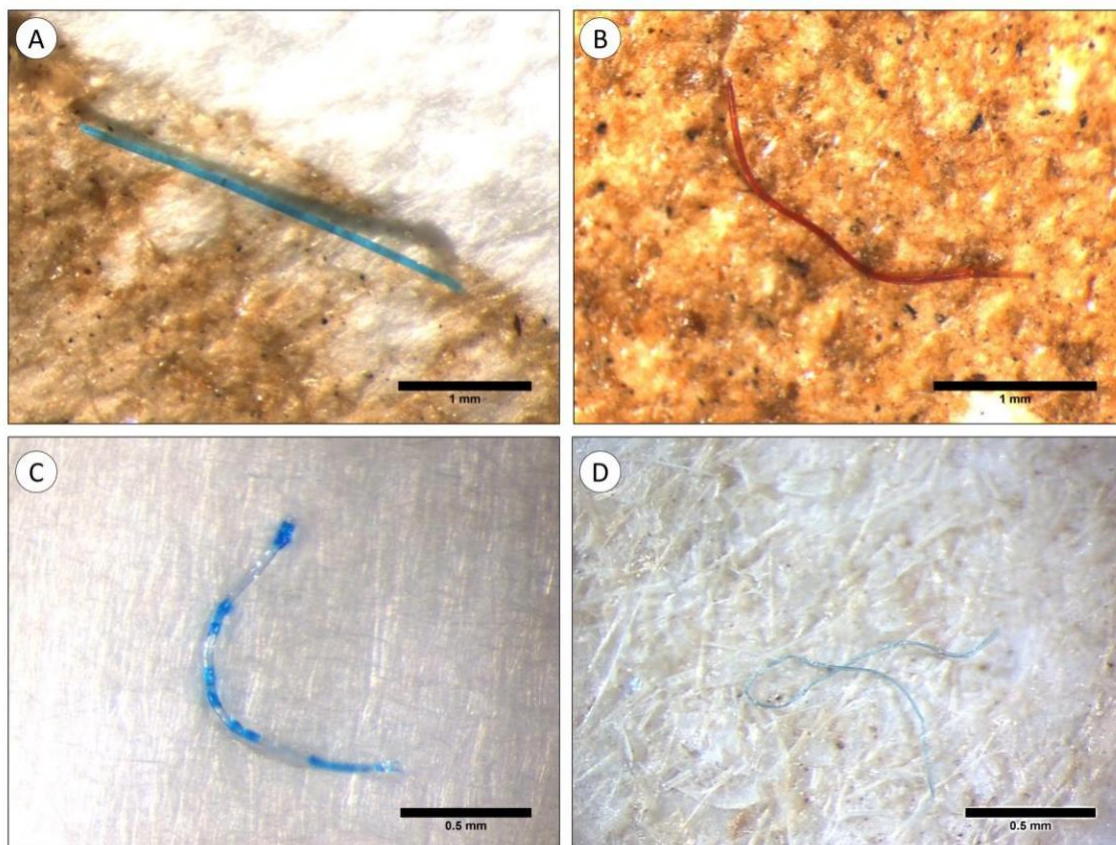


Figure 12 - Different fibres identified among the fish sampled; A) Blue fibre identified in Atlantic chub mackerel from Figueira da Foz, 3.09mm in size, B) Red fibre identified in Atlantic chub mackerel from Figueira da Foz, 2.49mm in size, C) Blue and transparent fibre identified in Atlantic chub mackerel from Sesimbra, 1.56mm in size, D) Blue fibre identified in Horse mackerel from Figueira da Foz, 2.12mm in size.

In a total of 47 fragments measured and photographed, 40 were blue with an average size of  $162 \pm 201$   $\mu\text{m}$  (mean  $\pm$  SD), the minimum size was 20  $\mu\text{m}$  and the maximum 891  $\mu\text{m}$ . Five grey fragments were also identified, ranging from 230 – 425  $\mu\text{m}$  with a mean size of  $499 \pm 226$   $\mu\text{m}$  and two black fragments. Different shapes of fragments were identified and are illustrated in Figure 13, (A) and (B) rigid fragments with different colors and shapes, (C) rigid sharp fragments and (D) flexible fragments. A total of 83 fragments were registered, and resulted on a mean distribution of  $0.51 \pm 0.99$  per fish, however only 47 fish were affected by fragments ingestion which correspond in average to a distribution of  $1.77 \pm 1.11$  fragments per contaminated fish.

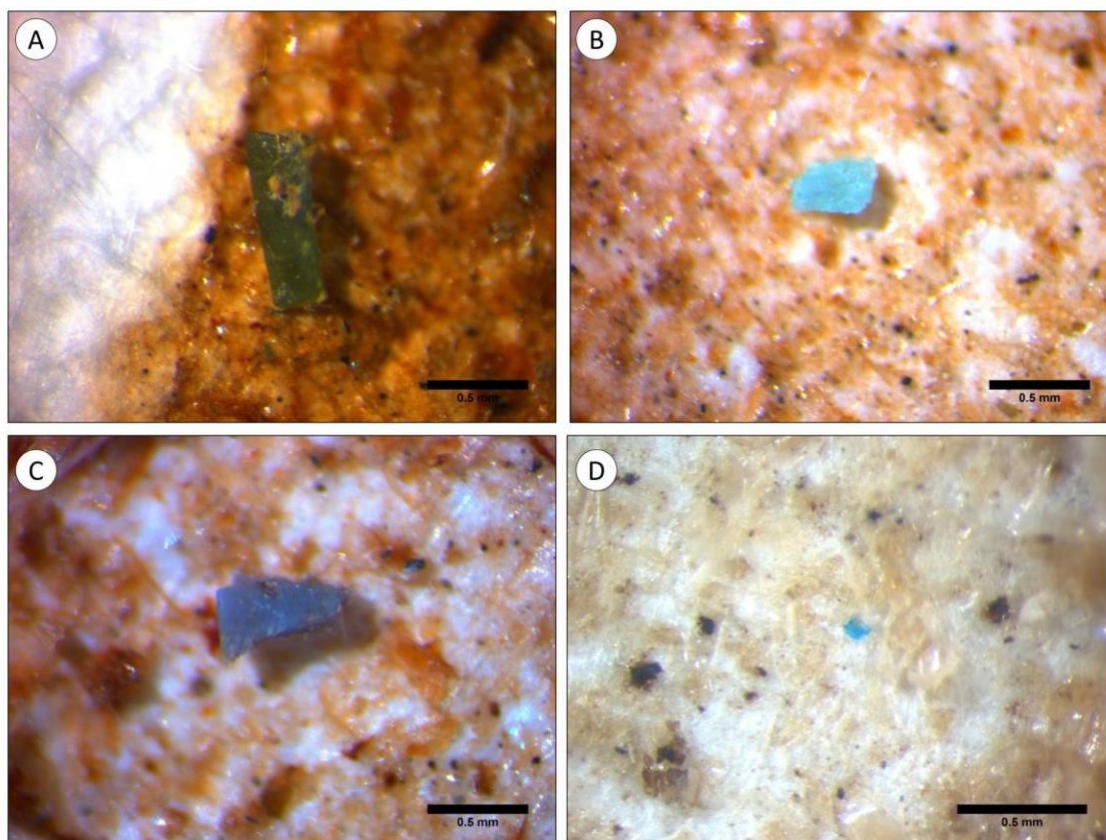


Figure 13 - Fragments identified among the fish sampled. (A) Green Fragment, 870  $\mu\text{m}$  in size, (B) light blue fragment, 436  $\mu\text{m}$  in size, (C) grey fragment, 634  $\mu\text{m}$  in size, and (D) small blue fragment, 96  $\mu\text{m}$  in size, (A, B and C) fragments from Atlantic chub mackerel collected in Figueira da Foz and (D) fragment from Horse mackerel collected in Figueira da Foz

A classification based in colour and size were considered in the 183 MPs photographed and measured to show the major trends in terms of disposition. MPs ranging from [0 – 1[ mm were the most common among the MPs photographed and it is seen the trend of MPs to decrease their presence while their sizes increase (Table 4).

Table 4 - Quantification of MPs photographed based on color and size (n = 183)

class/color	Blue	Black	Green	Transparent	Red	Grey	Total
[0 – 1[mm	72	29	5	12	6	5	129
[1 – 2[mm	15	5	7	3	4	-	34
[2 – 3[mm	3	2	4	1	1	-	11
[3 – 4[mm	2	1	1	2	-	-	6
[4 – 5[mm	-	1	-	-	1	-	2
> 5mm	1	-	-	-	-	-	1

In overview, from the 164 fish sampled, 67% were affected by MP ingestion, distinct cases were observed, samples with fibres, fragments, both and without any. Regarding to the samples affected, 92 fish were reported to ingest fibres and 72 were clean, 47 fish ingested plastic fragments and the rest were clean (Figure 14). The number of MPs ingested by fish varied from 1 to 20, being 1 the most recurrent case (Table 5), species and locations assessed were also compared (Table 6).

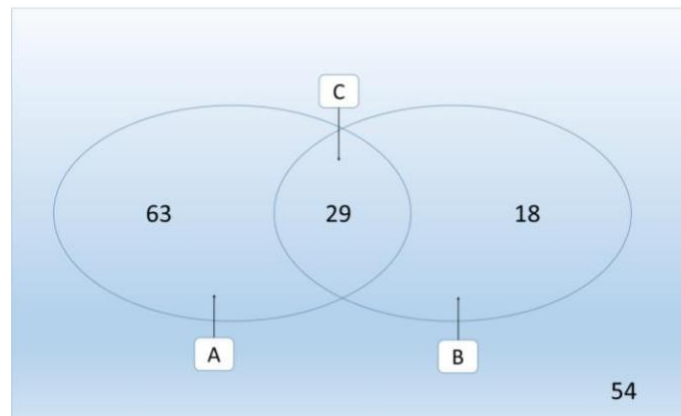


Figure 14 - Diagram displaying MPs distribution among contaminated fish, A) 63 fish samples ingested fibres, B) 18 fish samples ingested fragments, C) 29 fish samples ingested fibres and fragments, 54 fish samples were free of contamination.

Table 5 – Number of MPs ingested versus number of fish that effectively ingested those quantities.

no. MPs ingested	1	2	3	4	5	6	7	8	9	11	15	18	20
no. of fish samples	28	19	17	18	10	7	3	3	1	1	1	1	1
(%)	25	17	15	16	9	6	3	3	1	1	1	1	1

Table 6 – Information about MPs distribution among species and ports, A) comparisons between locations, B) comparisons between species.

A	Figueira da Foz	Sesimbra	B	<i>T. trachurus</i>	<i>S. colias</i>
no. of MPs	247	152	no. of MPs	184	215
mean ± sd n = 82	3.01±3.86	1.85±1.94	mean ± sd n = 82	2.24±2.05	2.62±3.88
(%)	62	38	(%)	46	54

### 4.3. Statistical Analysis

Comparisons between multiple independent variables were performed to identify any significant statistical differences. Figure 15 illustrates the mean distribution of fibres and fragments for both species and locations.

For the Horse mackerel samples, collected from both locations (see Figure 3), significant statistical differences were reported for the GI tract weight variable, between populations. On the other hand, between Atlantic chub mackerel populations, significant differences were found in the average amount of fibres ingested.

Comparisons between species were also made to identify possible unexpected or unknown behaviors. Comparing Horse and Atlantic chub mackerel from Figueira da Foz,

significant statistical differences were registered for the number of fragments ingested among populations. At Sesimbra, significant differences were reported between species regarding the number of ingested fibres per fish.

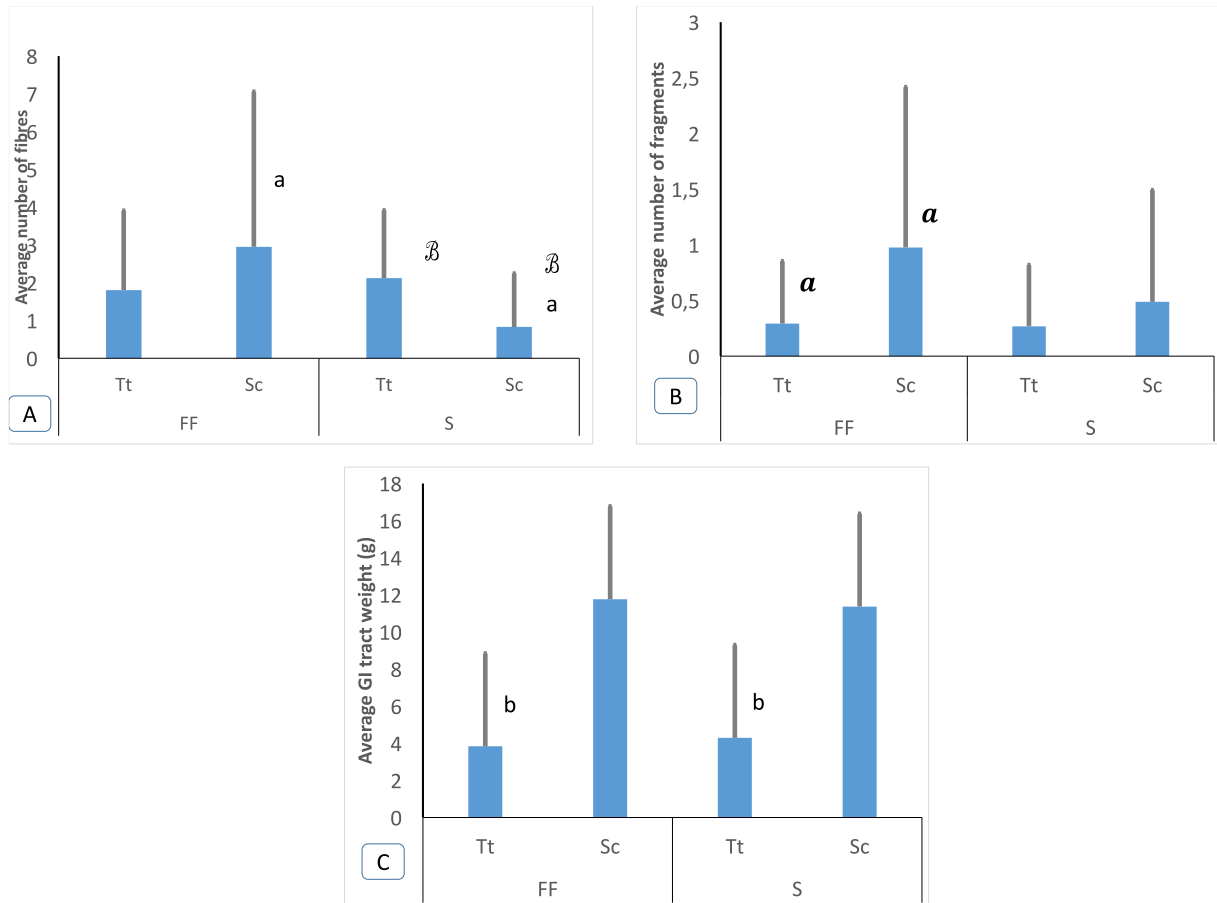


Figure 15 - Number of fibres and fragments of both species from both locations and GI tract weight, the first two were observed on Leica stereoscope and the last one, measured during laboratory procedure. Error bars indicate SD (standard deviation), A) average number of fibres per fish, B) average number of fragments per fish, C) Average weight of GI tract per fish; (FF) – Figueira da Foz, (S) – Sesimbra. (Tt) – *T. trachurus*, (Sc) – *S. colias*, (a, b) – intra-species significant statistical differences, (α, β) – significant statistical differences between species.

Reasonable correlations for the variables considered were accepted if verified the following condition ( $R > |0.26|$  and  $\alpha < 0.05$ ). Considering all the fish sampled, no significant correlations were identified between the biometric parameters and the respective total amount of MPs ingested. These correlation tests were also performed individually for the four populations in study, *T. trachurus*, from Figueira da Foz evidenced correlations between the wet weight and no. of fibres ingested ( $R = 0.32$ ;  $n=41$ ;  $\alpha < 0.05$ ) and between the standard length and no. of fibres ingested ( $R = 0.27$ ;  $n=41$ ;  $\alpha < 0.05$ ). In the other hand, *S. colias*, also from Figueira da Foz has not reported any correlations. In Sesimbra, no significant correlations were reported for the species, *T. trachurus*, while for *S. colias* several correlations were identified: correlation between the wet weight (g) and no. of fibres ingested ( $R = -0.36$ ;  $n=41$ ;  $\alpha < 0.05$ ) and also between the GI tract weight and no. of fragments ingested ( $R = -0.28$ ;  $n=41$ ;  $\alpha < 0.05$ ).

## 5. Discussion

The present investigation indicates that ingestion of microplastics, fibres and fragments, is common among the Horse and Atlantic mackerel collected from both locations which presented these small particles in their GI tract. Fibres were identified as the predominant category of MPs in both species. A total of 164 fish gut contents were examined, which resulted in the finding of 399 MPs, 316 fibres and 83 fragments. The results analysed describe weak correlations between the number of fibres or fragments ingested and the biometric parameters (weight and length) measured, thus it may suggest an aleatory distribution of MPs per fish.

The results observed in this study for fibres (79% of all MPs), are in accordance with previous studies, which pointed to fibres or fibrous material to be the prevalent type of microplastic found in fish (Lusher et al., 2013; Rochman et al., 2015 and Bellas et al., 2016). 140 fish were assessed by Rochman et al. (2015), from two distinct locations, Indonesia (n=76, 11 species) and USA (n=64, 12 species). The major microplastic materials found, were fragments (60% in Indonesia) and fibres (80% in USA). No fibrous material was recorded in the samples assessed from Indonesia. These differences were linked to different rates of plastic material consumption, which occurs, on a greater scale in the USA and because of the gap between the waste management systems of both countries. However, in the USA, the amount of fibres identified is around 80% (n=64) comparing to approximately 79% (n=164) in the present study. Regarding fragments, this type of material was predominant in Indonesia, 60% (n=76), while this study 21% was registered, (n=164). The present results are calculated for different total amounts of fish sampled. Despite that, it is important to enhance that more representative MPs found in fish, were classified as fibres and fragments in both studies.

Plastic ingestion by fish from the North Sea showed low values. From the 1203 fish assessed from 7 different species, only 5 presented contamination, 2.6% of the fish. No plastic materials were found in the Atlantic mackerel and grey Gurnard (Foekema et al., 2013). Concerning the total amount of fish sampled and the levels of contamination found, these results are categorically different from ours. A contamination of 67% of the fish samples appraised was registered in this study. Following Foekema et al. (2013), specie Atlantic mackerel, did not reveal any evidence of ingestion of microplastics, but this samples were only collected from the northern North Sea and it does not imply this species is free of microplastics in southern North Sea and in other locations, it will depend on the contamination level of each location and probability of consequent ingestion. The results found in this study confirms the ingestion of MPs by this species.

A survey in the English Channel, with 10 fish species, 5 pelagic and 5 demersal, in a total of 504 individuals, identified that 36.5% of them had ingested plastic particles. A total of 351 MPs were registered with a mean of  $(1.90 \pm 0.10)$  microplastic per contaminated fish, and which corresponded to 68.3% of fibres, 16.1% of fragments and 11.5% of beads Lusher et al., (2013). As in the results obtained for the English Channel, this study emphasizes the significant accumulation of fibres in the fish gut contents and indicates the presence of fragments among the individuals assessed, though, no beads were found. The average distribution (mean  $\pm$  SD) of MPs in the contaminated fish sampled (n=110) is  $3.63 \pm 3.16$  per fish and display higher mean distribution than the results presented by Lusher et al., (2013).

An investigation in the Mediterranean Sea, with three species of large pelagic fish for human consumption, swordfish, bluefin tuna and albacore (n = 123), identified 29 plastic fragments in 22 individuals and the colour of the fragments varied from transparent, white, grey, red and blue (Romeo et al., 2015). In our study 28.5% (n=47) of the fish sampled ingested plastic fragments while in the Mediterranean Sea 18% (n=22) was recorded, in terms of MPs distribution per individual, both studies showed similarities.

The colour of fragments detected in both study cases were similar, except for green colour particles, which were only found in our study.

As pelagic fish, demersal commercial fish, from Spanish & Mediterranean coasts were also target of analysis for the presence of MPs in their gut contents Bellas et al., (2016). A total of 212 fish, from three different species (Dogfish, European Hake and Red Mullet) were captured and examined. The results indicated that only 17.5% of the fish ingested MPs while in our study, 67%. In terms of the number of fish that effectively ingested MPs, a lower number of individuals affected (37) were reported compared to this study (110). In contrast, both studies are in accordance when considering the major microplastic contaminant present in the gut contents as fibres, 71% in the Spanish Atlantic and Mediterranean Coasts and 79% in our research. Fragments were also identified by Bellas et al., (2016) at a residual scale (1.6%) when compared to our study (21%). Six colours were identified: blue, black, transparent, green, red and grey from the samples dissected, while in Spanish waters were observed those, plus yellow, pink and brown colours.

A recent investigation in the North and Baltic Sea, (Rummel et al., 2016), examined circa of 290 GI's from 5 species, 3 demersal (dab, European flounder and Atlantic cod) and 2 pelagic (herring and Atlantic mackerel) regarding MPs consumption. They reported, that only 5.5% of 290 GI tracts had MPs (16 individuals) and a total of 23 plastic particles were found. These results are not in conformity with the ones found in our research, where 67% of the GI tracts were contaminated. Pelagic feeders displayed more quantities of particles ingested comparing to demersal ones, however this result might be influenced by the number of demersal and pelagic fish assessed, for a proper evaluation identical proportions should be considered.

Neves et al., (2015) reported the ingestion of microplastics in commercial, pelagic and demersal fish caught off the Portuguese Coast. 263 GI tracts were obtained from 26 species. A total of 73 MPs were recorded, which corresponded to 19.8% of the 263 individuals analysed. This study also represents lower levels of MPs detected among individuals surveyed, still, as demonstrated by other authors (Lusher et al., 2013; Rochman et al., 2015 and Bellas et al., 2016) it also reports fibres as the main microplastic material found in fish gut contents as found in the present study.

Microplastics were found among different species and locations, the respective abundance levels are very dependent of the concentration levels that fish are exposed to. Although similarities were found with different studies (Lusher et al., 2013, Bellas et al., 2016) regarding the most common MPs found in the fish gut contents, which are fibres, this was not always the case (Rochman et al., 2015, Romeo et al., 2015). These results are exposed to several natural and anthropogenic variables. In overview, industrial activities, agriculture, leisure activities and many other close to the areas where samples were collected might have influence in the water quality. In the other hand, natural variables such as winds, tides, migratory routes and seasonality are other factors that might affect MPs distribution in the waters.

For the groups of species studied, some differences were registered regarding to intra and inter-species comparisons, Horse mackerel collected from both locations revealed significant differences among their gut content weights, apparently the samples collected from Sesimbra presented higher values, this fact could be related to feeding behaviours, human pressures, such as Coastal pressure and fishing activities, or even marine environmental conditions. Between the populations of Atlantic chub mackerel, a difference was observed concerning to the number of fibres ingested which may suggest possible different levels of contamination in the locations, Figueira da Foz occur as the more contaminated, in this case. Fibres are identified as the principal contaminant in fish gut content, further research might be helpful to comprehend fish behaviour in their presence. Complementary to intra-species assessment, inter-species comparisons for

both locations were also performed to analyze possible distinct behaviours in microplastic feeding. In Figueira da Foz, Atlantic chub mackerel revealed almost 4 times more fragments ingested in comparison with Horse mackerel. As well as in Sesimbra, Atlantic chub mackerel presented, 4 times the amount of fibres detected in Horse mackerel guts. Analysing the information collected in this study, Atlantic chub mackerel appears as the most vulnerable to MPs contamination.

Spearman correlations were identified in the fish sampled, however *S. colias* from Figueira da Foz and *T. trachurus* from Sesimbra did not revealed any correlation. The correlations were found in *T. trachurus* from Figueira da Foz and *S. colias* from Sesimbra. These two populations registered correlation between the biometric parameters and the amounts of fibres or fragments ingested, which may suggest that mature fish, do display more MPs in their GI tract. This hypothesis is proved by slight correlations, although for a  $n = 41$  and with all the natural and anthropogenic variables that might influence the microplastic ingestion per fish, this hypothesis might lose strength.

Both species in this study are classified as pelagic, however their behaviours in the water column are slightly different, the Atlantic chub mackerel, is pelagic-neritic, feed in big schools near the surface and coastal waters, consuming food and non-natural particles which may occur, while the Horse mackerel, has a benthopelagic behaviour, during daytime they live in deep waters and rise to the surface at night for feeding. This difference in behaviour might influence the results observed. The different characteristics of the sampled locations could also influence the different amounts of MPs detected. In the case of Figueira da Foz, samples were collected from areas close to the coast, Praia da Leirosa (Atlantic chub mackerel) and São Pedro de Moel (Horse mackerel), which are influenced by the mouth of Mondego river and respective estuarine area, in Sesimbra samples were collected in off shore waters, more distant from the coastal area in comparison with Figueira da Foz.

Human activities such as agriculture (despite decreasing), aquaculture, paper and cellulose industries are established in the downstream region of the Mondego river which contribute with high loads of nutrients to the estuarine region, leading to eutrophication and consequent decrease of oxygen levels and biodiversity for the two last decades (Costa et al., 2013), but the key factor may be related with proximity to beaches and constant human pressures from leisure activities, among others, are more than reported has increasing significantly the quantities of plastic debris already in the environment (Thiel et al., 2013; Topçu et al., (2013); Pasternak et al., 2016 and Portman et al., 2017). A study carried by Antunes et al., (2013) quantified plastic pellets among beaches of the portuguese coast reporting greater accumulation in beaches near Figueira da Foz than near Sesimbra which may be relevant to the results found in the present study.

Samples from Sesimbra were collected in off-shore waters, further away from nearby estuarine and coastal activities area which may comprehend a threat. Though most of the estuary is in an area classified as Natural Reserve, harbour-associated activities, intensive agriculture, salt extraction and leisure activities are common, the city of Setubal also contributes with treated effluent to the Sado river and his effluents from copper mines exploration, which flows in direction of open sea (Caeiro et al., 2009). However, these activities are more harmful for the estuarine area and suffer dispersion when converging to the Atlantic Ocean and species collected could not display serious effects.

Ingestion of MPs by fish has been reported by several different authors (De Witte et al., 2014, Murray and Cowie, 2011) and may lead to physical consequences and/or toxic harm (Andrady, 2009; Wright et al., 2013; Kuhn et al., 2015), yet, an important concern about microplastics ingestion, is the potential transfer of the pollutants adsorbed in their surface through the food web and consequent biomagnification and bioaccumulation which could harm humans through their diet. MPs are optimum surfaces for

hydrophobic POPs to adsorb. In the other hand, is the possibility of residual monomers and additives to leach out from plastics ingested by marine organisms. All these compounds represent major threats to the biota of marine environments, however, bioaccumulation of these compounds and possible harm to human health are still speculative.

In the present study, ingestion of MPs has been confirmed for both species assessed. Considering the total amount of fish sampled ( $n=164$ ) a mean distribution (mean  $\pm$  sd) of  $2.43 \pm 3.10$  MPs per fish were registered, while if considering only the fish samples which ingested MPs, this distribution flows/trends to  $3.63 \pm 3.16$  items per fish. From the 399 MPs identified 79% belong to fibres and 21% to fragments, concerning to fish sampled 110 displayed MPs ingestion while 54 were clean of contaminants. It is estimated that 67% of our samples had ingested MPs, different quantities were observed in each GI tract digested, however in 25.5% of the contaminated samples, only 1 microplastic ingested were registered and it were the most representative class. The maximum number of MPs occurred in one *S. colias* sample from Figueira da Foz, with 20 (16fibres and 4fragments).

Microplastics due to their characteristics can be hazardous for biota and human health, their release occur through multiple pathways and natural factors may increase their further distribution. POPs present in the marine environment and consequent adsoption in MPs, additives and residual monomers which may leach out from MPs while ingested, comprise the primary concerns of MP ingestion by fish.

## 6. Conclusions

Relatively to the primary goal established in this thesis, it comprises to provide unknown information about microplastics quantification in the fish guts assessed from the Portuguese Coastal waters.

The fish GI tract digestion and examination proved as other studies did before, that ingestion of MPs by small fish is common among different pelagic species. *S. colias*, the Atlantic chub mackerel, displays greater amounts of fragments ingested, while both species ingested similar amounts of fibres. Regarding the locations, more microplastics were found in the fish guts from Figueira da Foz which may imply that these waters were possible more contaminated, although several natural and anthropogenic factors could lead to these results, due that this hypothesis is merely speculative. Presence of fibres was also more recurrent than fragments.

Ingestion of MPs is documented by several authors and concerning to multiple terrestrial and marine species. The microplastics adsorption property displays a dangerous pathway of POPs contamination, different environments were already assessed, and this contaminant were identified among different microplastic particles and its bioaccumulation through the marine food web is getting covered in the last years, existing already some scientific studies reporting this issue. Is also documented by laboratory procedures that several small marine organisms and fish have capability to egest these microparticles.

Fibres occur as the most common microplastic present in the fish gut contents analyzed, other researches support this trend, however in some studies, plastic particles were detected, and no signs of fibres were reported. This ingestion is compromised by several natural and anthropogenic variables which may conditionate the MP distribution in the marine environment and might affect the feeding behaviours of fish. Furthermore, these variables could lead to other restrictions in the marine environment and biota.

Several researches have been conducted to understand the microplastics ingestion by pelagic and demersal fish from different water bodies with different marine conditions. Regarding to the methods used in the fish organic material digestion, different methodologies were experimented across multiple studies, KOH 10% were the chosen for case. It is necessary to enhance the scientific knowledge about the concentrations of MPs in the water bodies, to establish a possible relationship between the environment conditions and the fish behaviours.

To evaluate the potential risk for human health by eating fish flesh which might be contaminated it is necessary to develop more and better methods for measurement of the concentrations of POPs in organisms from the basis of the food chain to the top. The lack of research concerning the concentrations that might bioaccumulate through the food web, limit significantly the evaluation of the potential risk to human health.



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