Microplastic ingestion by planktivorous fish in the Canary Current

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Abstract

Marine plastic debris is present worldwide at all depths in the ocean water column and can affect all marine habitats and the organisms living in it. In the last decade, microplastics (MPs) have become a subject of intense investigation due to the increasing concerns about their negative impact on wildlife and possible toxicity to living organisms (including humans). In the ocean MPs can be easily ingested by numerous marine organisms because of their small size (<5 mm). The Northwest African upwelling system is an important fishery region. This study is the first one to reveal the presence of MP particles in the stomach contents of two zooplanktivorous fish species in the Canary Current region: bogue (*Boops boops*) and Atlantic chub mackerel (*Scomber colias*). From 64 fish samples examined, 21 fish stomachs (33%) contained microplastic fragments.

Key words

Microplastics, Canary Current, ingestion, stomach dissection, contamination, *Scomber colias, Boops boops*

Contents

1. Introduction

Plastics are synthetic organic polymers developed in the beginning of 20th century, produced by the polymerization of monomers extracted from oil or gas, accounting for approximately 8% of global oil production (Thompson et al., 2009). As being a practical material, mass production of plastics started in 1940s and 1950s and has increased since then with world production reaching 311 megatons (Mt) in year 2014 with around 19% coming from Europe (Plastics Europe, 2015).

Plastics form most of the marine litter worldwide (Derraik, 2002), accounting for 60-80% (up to 95% in some regions) of the total (Moore, 2008). Plastics are present from the ocean surface to the seabed (Ivar Do Sul and Costa, 2014) and can significantly affect all marine habitats and the organisms living in them (Cole et al., 2011). Jambeck et al. (2015) estimate that 4.8-12.7 million metric tons of plastic waste entered the ocean in 2010, however Thompson et al. (2006) suggested that up to 10% of the world's plastic production ends up in the marine environment where it can accumulate and be further modified. Around 80% of the marine plastic litter has a terrestrial source, while about 18% is attributed to the fishing industry (Andrady, 2011).

Marine plastic debris can be hazardous to the environment. It has been documented that plastic litter can harm marine organisms in various ways including entanglement, external wounds and ingestion resulting in gut blockage or chemical pollution (Collard et al., 2015; Cole et al., 2011; Moore, 2008; Teuten et al., 2009). Hydrophobic pollutants (which are often persistent, bioaccumulative and toxic) may get adsorbed onto plastic debris and thus endanger the environment (Cole et al., 2011; Thompson et al., 2009). Canary Islands and north-west Africa is an important fishery region. This study attempts to reveal microplastics ingestion by local zooplanktivorous fish species.

1.1 Microplastics

Microplastics (MPs) were described during the seventies (Carpenter & Smith 1972), but since the beginning of the new millennium they have become an object of intense study (Andrady, 2011; Cole et al., 2011; Ivar Do Sul and Costa, 2014) due to the increasing concerns about their negative impact on wildlife and their toxicity on living organism including humans (Wright et al., 2013). There is a range of definitions of MPs varying in different studies, with diameters <1 mm (Browne et al., 2007), <2 mm (Ryan et al. 2009), <5 mm (Arthur et al., 2008; Betts 2008), and <10 mm (Graham et al. 2009). Here, we consider microplastics any plastic particle which has at least two out of its three dimensions smaller than 5 mm (fragments or primary-sourced) which is the most recognized definition of the National Oceanic and Atmospheric Administration (NOAA) (Rocha-Santos and Duarte 2015; Wright et al., 2013). Most of the MPs are found in the surface layer of the ocean due to their positive buoyancy (Ivar Do Sul and Costa, 2014) and are believed to accumulate in the center of subtropical gyres. Nevertheless, their transport through the ocean, including deep waters, is largely unknown (Hidalgo-Ruz et al., 2012).

Microplastics are diveded into primary MPs and secondary MPs acording to their origin. Primary-sourced microplastics are small pellets already produced with a size smaller than 5 mm. They are present in various domestic and cosmetic products such as face cleaners (Fendall et al., 2009) where they have replaced traditionally used natural ingredients (almond shells, oatmeal or pumice) or in cleaning synthetic blasting technology (Andrady, 2011; Derraik, 2002; Gregory, 1996) involving blasting acrylic or polyester microplastic scrubbers to remove rust or paint from metallic surfaces (engines parts). These particles are usually re-used many times before being discarded. This practice can result in heavy-metal contamination (Cole et al., 2011; Derraik, 2002; Gregory, 1996). All these particles can be released through the waste water system and eventually reach the marine environment.

Physical, biological and chemical processes can over time reduce the structural integrity of plastic debris, resulting in fragmentation, and giving rise to secondary MPs (Cole et al., 2011; Browne et al., 2007). These processes include polymer photo degradation by solar UV radiation, thermooxidative degradation, ozone-induced

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degradation, mechanochemical degradation and biodegradation (Andrady, 2011; Singh and Sharma, 2008; Wang et al., 2016). Plastic degradation is an extremely slow process taking hundreds to thousands of years (Wang et al., 2016). Plastic litter occurs on beaches, surface water and deep water, nevertheless weathering rates in these sites are very different (Andrady, 2011). Due to the deficiency of solar UV and low temperatures in the marine environment the photo degradation is much slower than in terrestrial environments (Cole et al., 2011; Wang et al., 2016). Plastic debris at beaches, however, is directly exposed to sunlight and oxygen, leading to embrittlement, crack formation and eventual fragmentation. As a result, beaches are probably the most common source of secondary MPs in the ocean (Andrady, 2011; Wang et al., 2016). Coasts can receive plastics from both terrestrial and marine sources. It has been shown that presence of elevated number of microplastics on a shoreline can alter the chemical and physical properties of the beach sediments, resulting in different permeability of the sediment and heat absorbance. This could have an impact on marine biota in different ways, including effects on temperature based sex-determination in turtle eggs (Carson et al., 2011; Cole et al., 2011).

1.2 Sources and distributions of microplastics

Indiscriminate disposal of waste material leads to direct or indirect transfer of plastic litter to the marine environment. Most of the plastics encountered at the sea are coming from terrestrial sources via rivers, waste-water systems and being blown off-shore (Moore 2008). Although waste-water treatment plants are able to filter some of the microplastics and plastic debris, there is still a considerate amount of microplastics that is not being captured by these filtration systems (Browne et al., 2007; Fendall and Sewell, 2009; Gregory, 1996). Plastics that enter river systems – directly or indirectly - will eventually end up in the sea. Extreme weather conditions such are strong storms, hurricanes or flooding can temporarily increase this transfer of terrestrial debris from the land to the sea (Barnes et al., 2009).

Commercial fishing and marine-industries can be a source of direct plastic pollution as macroplastics or as secondary microplastics after long-term degradation in the marine

environment. Lost or discarded fishing gear is the source of most of the marine debris (Andrady, 2011). Discarded fishing lines and nylon nets, known as "ghost nets" are usually floating and drifting in the water column due to their neutral buoyancy. There they capture a large variability of marine species. In 1987, the <u>Protocol to the International Convention for the Prevention of Pollution from Ships</u> came out, taking effect in 1988. It banned disposing plastic litter in the sea (MARPOL Annex V; Ninaber, 1997). Nevertheless shipping remains a dominant marine source of microplastics due to lack of enforcement and education (Derraik, 2002).

Plastics consist of many polymers and depending on their density and shape can float at the surface, be neutrally buoyant in the water column or sink to the bottom. Those polymers that float at the surface have positive buoyancy (e.g. PE and PP). These plastic particles may be transported long distances at the sea water surface and be washed ashore eventually (Andrady, 2011; Thompson et al., 2009). Some of the polymers that are denser than seawater (e.g. PVC) tend to settle to the sediment and once there, they can be transported by ocean currents (Engler 2012). Additionally, there is evidence that plastic debris rapidly accumulate microbial films which permit other organisms (algae and invertebrates) to colonise the surface. This biofouling process can cause originally buoyant (micro)plastics to sink (Andrady, 2011; Barnes et al., 2009; Cole et al., 2011; Derraik, 2002). Sedimentation and shore deposition may therefore play an important role in temporal variability of microplastics in marine environment.

The time for eventual biodegradation of plastics is estimated in order of centuries (Moore, 2008). Law et al. (2010) didn't find any significant changes in the amount of microplastics in the Northwest Altantic in the past twenty years, despite the increase of marine plastic debris, while Cózar et al., (2014) revealed that the global load of plastics on the open ocean surface is much lower than the abundance expected, compared to the amount of plastic litter that has been released to the ocean. These observations support the hypothesis of substantial losses from the ocean surface (Wang et al., 2016) suggesting that the deep ocean could serve as a sink for a large amount of plastic trash.

1.3 Impacts of microplastics on marine organisms

Due to their small size and abundance, microplastics are potentially ingested by a wide range of organisms and MPs ingestion has been observed in various invertebrate and vertebrate species, including fishes (reviewed in Ivar Do Sul et al., 2014). However, most of the research on invertebrates is restricted to controlled laboratory experiments. Microplastics can be ingested accidently by confusing them for the prey or also as a result from eating lower trophic organisms that have consumed microplastics themselves (Browne et al., 2008; Cole et al., 2011).

Once ingested, microplastics may be egested, retained or may block the digestive tract, cause pseudo-satiation leading to decreased food consumption (Derraik, 2002; Thompson, 2006), get absorbed to the gut or be translocated into other tissues (Wang et al., 2016). Browne et al. (2008) observed that microplastics ingested by *Mytilus edulis* were translocated from the gut to the circulatory system and persisted there for several weeks. Microplastic ingestion in *Mytilus edulis* is commonly studied and transference of microplastics from *M. edulis* to higher trophic levels has been observed. The implication for the rest of the food web, including humans is concerning (Farrell and Nelson, 2013; Wegner et al., 2012). There are several studies that reveal microplastics ingestion in various fish species in different parts of the world (Carson, 2013) including planktivorous fish in the North Pacific Central Gyre (Boerger et al., 2010); planktivorous fish of North Atlantic (Collard et al., 2014); various small pelagic fish in North Pacific (Davison and Asch, 2011); pelagic and demersal species from the English Channel (Lusher et al., 2013), marine catfish on Brazilian coast (Possatto et al., 2011); fish from markets in Indonesia and California (Rochman et al., 2015), and fish species of the Mediterranean Sea (Nadal et al., 2016; Romeo et al., 2015) etc. Davison and Asch (2011) estimate the ingestion rate of plastic debris by mesopelagic fish in the North Pacific to be from 12,000 to 24,000 tons per year. Apart from the potential harm caused by ingesting the microplastics themselves they can be toxic due to the inherent contaminants leaching from them or due to pollutants adhered to them in the marine environment.

Persistent organic pollutants (POPs) such as polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs) and dichlorodiphenyltrichloroethane (DDTs)

can be adsorbed onto microplastics, mainly) due to a greater affinity of these pollutants for the hydrophobic surface of plastics compared to seawater (Wang et al. 2016). Plastic materials contaminated by persistent organic pollutants (POPs) are found worldwide including coastal areas and remote ocean habitats (Hirai et al., 2011), accumulating the concentration of these pollutants by orders of magnitude (Wang et al., 2016). Many POPs are considered toxic, leading to endocrine disruption, mutagenesis or carcinogenesis and can bioaccumulate and biomagnify through the food chain (Cole et al., 2011; Wang et al., 2016). Rochman et al. (2013) found greater concentrations of PCBs and polybrominated diphenyl ethers (PBDEs) in fish fed with marine plastic than those fed with virgin plastic particles, which indicates that plastic litter serve as an accumulation point and a pathway for the adsorbed POPs into the food web. PCBs can lead to reproductive disorders and alteration of hormone levels and have a harmful effect on marine organism even at low levels (Derraik, 2002; Lee et al., 2001).

Uptake of some metals (e.g. Ag, Cd, Pb, Al, Fe, Mn, Hg) has been reported, although the mechanism of metal uptake is largely unknown (Ashton, 2010; Wang et al., 2016). Beached plastic pellets showed greater metal adsorption capacities than virgin pellets (Holmes et al., 2012; Turner and Holmes, 2015).

The process of polymerization during the plastics production is never entirely complete and there are some monomers left (such as styrene and bisphenol-A, BPA) which can migrate from the polymer matrix into surrounding compounds (Moore, 2008). Polymers are often mixed with chemical additives such are phthalates, polybrominated diphenyl ether (PBDE), tetrabromobisphenol A (TTBPA) and others, typically to enhance their performance and extend their life by providing resistance to heat, oxidative damage and microbial degradation (Browne et al., 2007; Cole et al., 2011). Some of many of these additives can have potentially adverse effects on animals and humans (Andrady and Neal, 2009) resulting in endocrine disruption, reproduction and development alteration and carcinogenesis (Barnes et al., 2009; Cole et al., 2011). Oehlmann et al. (2009) showed that phthalates and bisphenol A (PBA) have an effect on reproduction in studied animal groups, especially molluscs, crustaceans and amphibians. There is a growing evidence that BPA may have an adverse effect on human population (Rochester, 2013). PBDE and TTBPA have been shown to disrupt thyroid hormone homeostasis (Thompson et al., 2009).

Furthermore plastic debris could serve as a possible pathway for the invasion of various non-native marine species (bacteria, diatoms, barnacles etc.) as these could travel long distances rafting on floating plastic debris to environments where they were previously absent (reviewed in Derraik, 2002).

2. Materials and Methods

2.1   Fish samples

For this study we chose two zooplanktivorous fish species of the Canary Current which usually feed near the surface; demersal to semipelagic bogue *Boops Boops* (Linnaeus, 1758) and pelagic Atlantic chub mackerel *Scomber colias* (Gmelin, 1789). *Scober colias* is a coastal pelagic species feeding on small pelagic fish (sardines, anchovy, sprat etc.) and invertebrates. *Boops boops* is a gregarious demersal, semi-pelagic species living in various bottom types including sand, mud, rock and seaweed. It usually appears in depths above 150 m but can be found as deep as 350 m (Pollard et al., 2014). In total, 64 fish were sampled, 58 *Scomber colias* and 6 *Boops boops*.

Fish were caught in two different fishing regions in Gran Canaria and Lanzarote. Fish were bought in April – June 2016 from local fishermen in 1) San Cristóbal, Gran Canaria (43 samples), 2) Puerto del Carmen – La Tiñosa, Lanzarote (21 samples) (fig. 1). The fishing areas are located in the south-east part of both of the islands near the coast, approximately above the 100 m isobath. The fish samples were between 21.5 cm – 44 cm, in size and 114.5 g – 830 g, in weight.
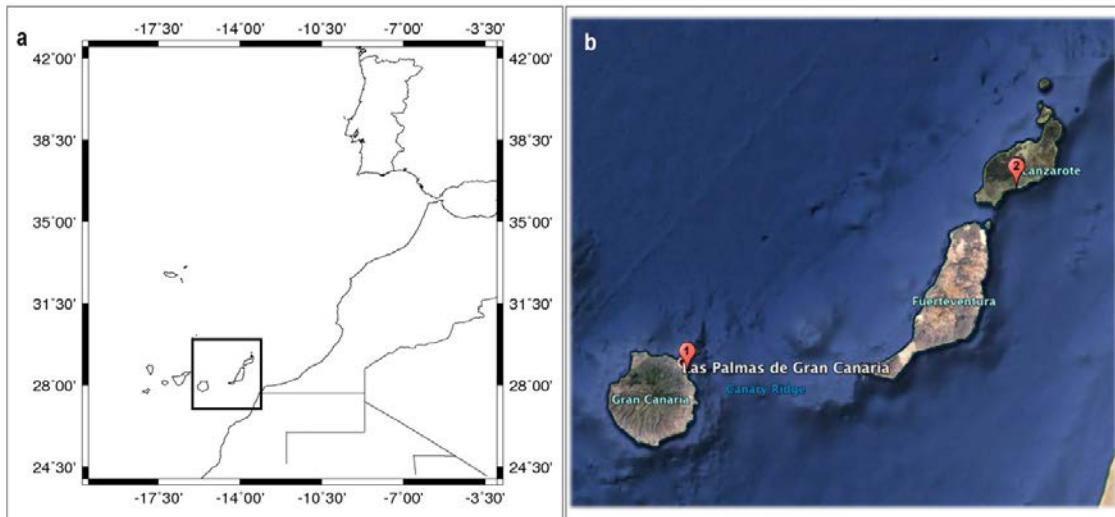
Fig. 1. a) Location of Canary Islands; b) Location of fish markets: 1- San Cristóbal, Gran Canaria; 2- Puerto del Carmen-La Tiñosa, Lanzarote.

2.2   Laboratory analysis

Since studying MPs in fish stomach contents is a relatively new scientific research area there is no standardized method that would be generally recognized, therefore we followed various suggestions mentioned in several studies focusing on this topic. Each fish was measured and weighted prior to stomach dissection. The whole stomachs were kept in ethanol (70%) or formol (4%) until the following procedure. The stomach content was extracted using classical dissection tools and put into KOH (10%) solution for 24 h in 60°C as described in (Dehaut et al. 2016) and filtered through a 50 µm sieve. The content was visually examined using OPTIKA stereomicroscope. The process is illustrated in fig. 2.
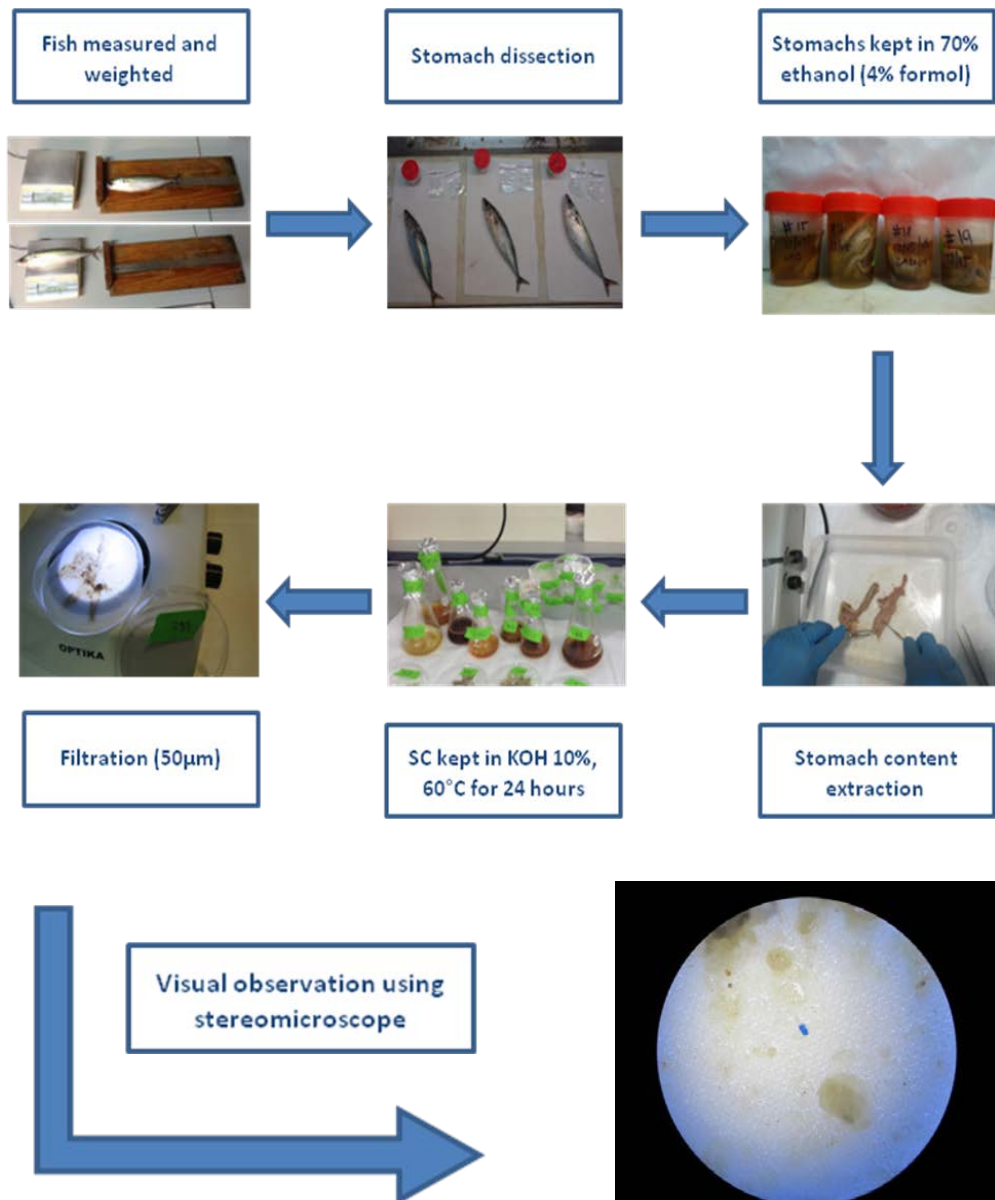
Fig. 2. The process of stomach extraction and stomach content dissolution in order to reveal any microplastic particle.

All samples were visually examined using OPTIKA stereomicroscope and all potential MP particles were photographed and divided into fibres or fragments. The stomach content filtration and final sample observation was performed under a laboratory hood in order to reduce any air-born fibre contamination.

2.3   Statistical analysis

Data were analyzed using R Studio program version 0.99.902.

To confirm normality, the data were analyzed with the Shapiro-Wilk test. The distribution of data (fish weigh, MP, fibres and fragments abundance) was not normal and statistical differences between the fishing areas of Gran Canaria and Lanzarote were assessed using the non-parametric test of Wilcoxon-Mann Whitney.

To study the correlation between fish weight and microplastic abundance we obtained the regression equation using a confidence limit of 95% and the Pearson correlation coefficient.

3. Results

From 64 fish samples examined, 21 fish stomachs (33%) contained fragments, ranging from 0.05 mm to 4 mm in size, with one fragment reaching 8 mm. We provide the results also for fibres, which were found very commonly in the samples, although there is a possibility of air contamination which couldn't be eliminated. Fifty fish samples (78%) contained at least one piece of fibre, ranging from 0.1 mm to 14 mm in length. The highest number of fibres was 7 in one stomach. If we take into account both fibres and fragments, 55 fish samples (86%) contained a potential MP of some type. Only 9 samples (14%) were free of fibres and fragments (fig. 3).
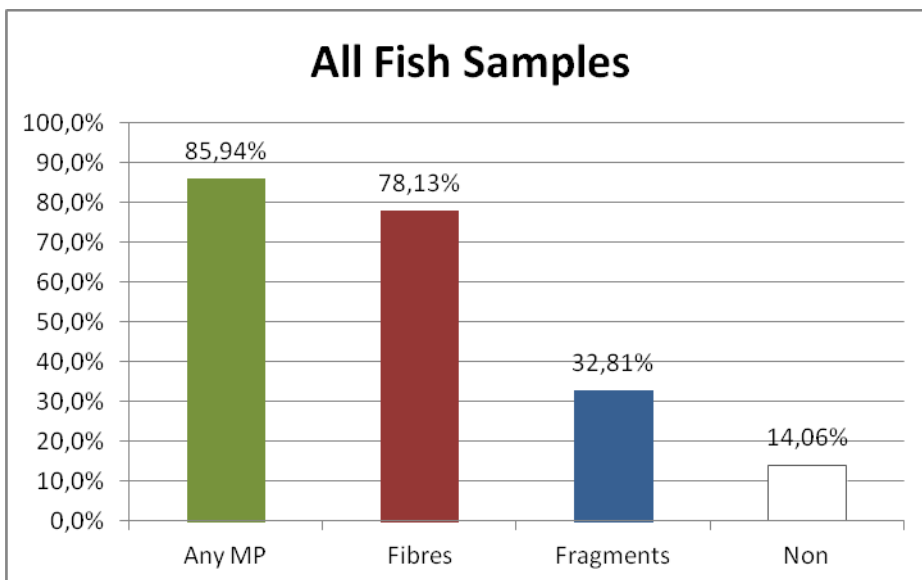

Fig. 3. Abundance of any kind of MP, fibres and fragments in the studied fish samples.

There were found more fragments in fish brought from Lanzarote in comparison to Gran Canaria. Only 11% of the fish samples from Gran Canaria contained microplastic fragments. On the other hand 76% of fish samples from Lanzarote contained microplastic fragments as can been seen in fig. 4 and fig. 5.
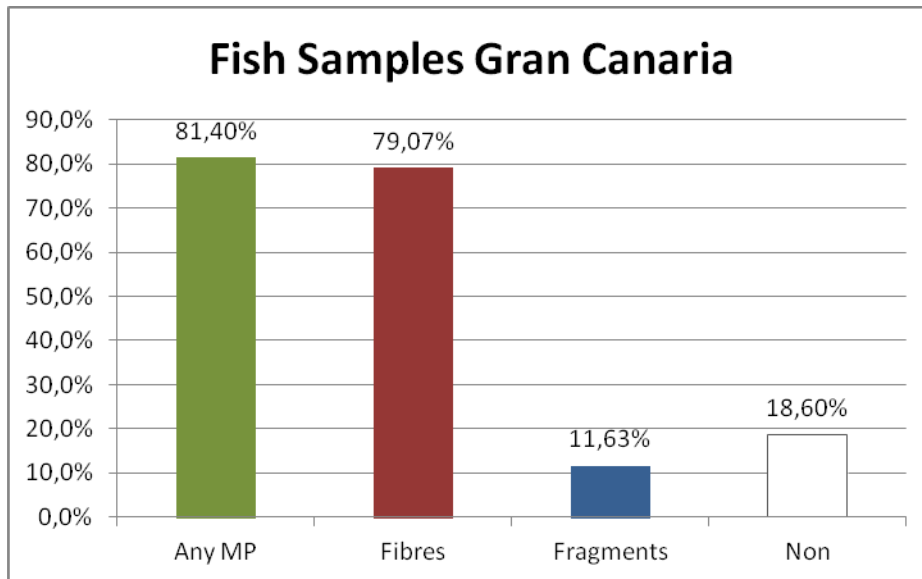
Fig. 4. Abundance of all kinds of MP, fibres and fragments in fish samples from Gran Canaria.
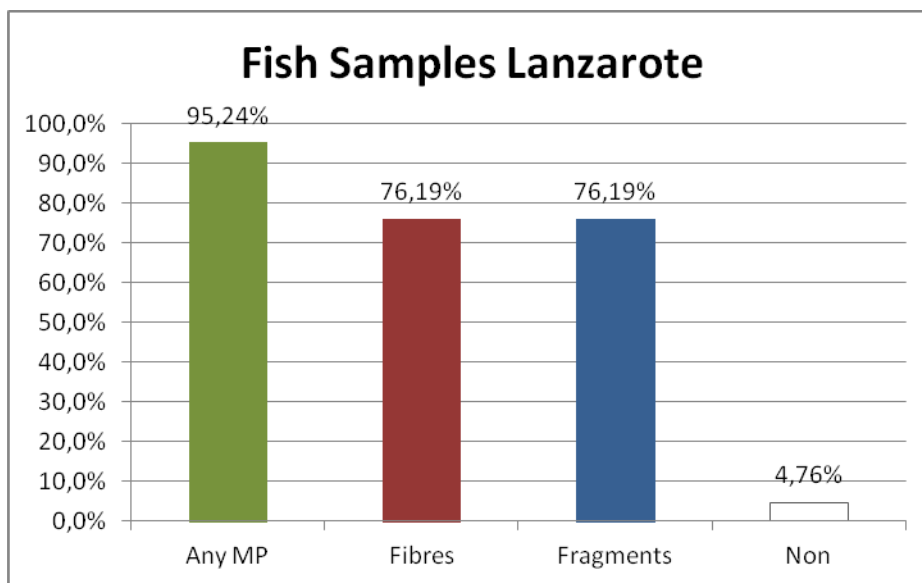

Fig. 5. Abundance of all kind of MP, fibres and fragments in fish samples from Lanzarote.

In total, 132 fibres were observed in 50 fish samples. The fibres were of the following colours: dark blue, light blue, black/dark, red, green, grey, yellow/brown, purple and orange. The most common colour was blue (43% of all fibres) as seen in fig. 6.
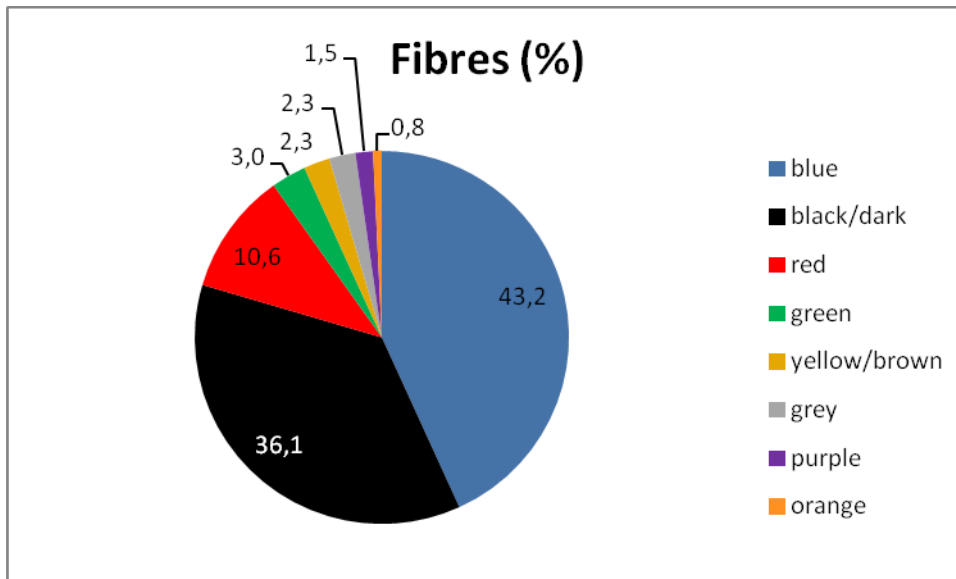
Fig. 6. Colour composition of recovered fibres.

In total, 29 fragments were found within 21 fish samples and of the following colours: blue, white, green and transparent. The most abundant fragment colour was blue (79%) of all fragments; fig. 7).
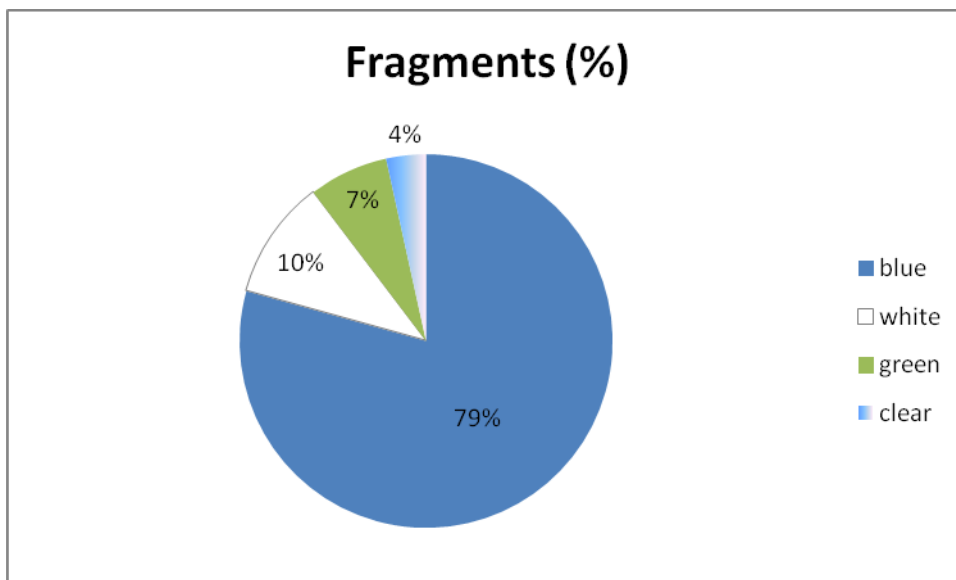

Fig. 7. Colour composition of found MP fragments.

Nine fish samples (14%) contained blue paint chips that were soft, unstable, and formed a mash after mechanical pressure was applied. The paint chips in several of

these samples were spread and mixed with the rest of the organic matter. These samples are included in the group of stomach samples containing fragments. A complete list with fish samples, their size, weight and number of fibres/fragments found within each sample can be found in Annex 1.

The mean number of all types of MP was 2.5 ± 0.25 (mean $\pm$ SE) per fish. The number of fibres per fish was 2.06 ± 0.24 (mean $\pm$ SE) and number of fragments per fish was 0.45 ± 0.10 (mean $\pm$ SE) (Fig. 8).
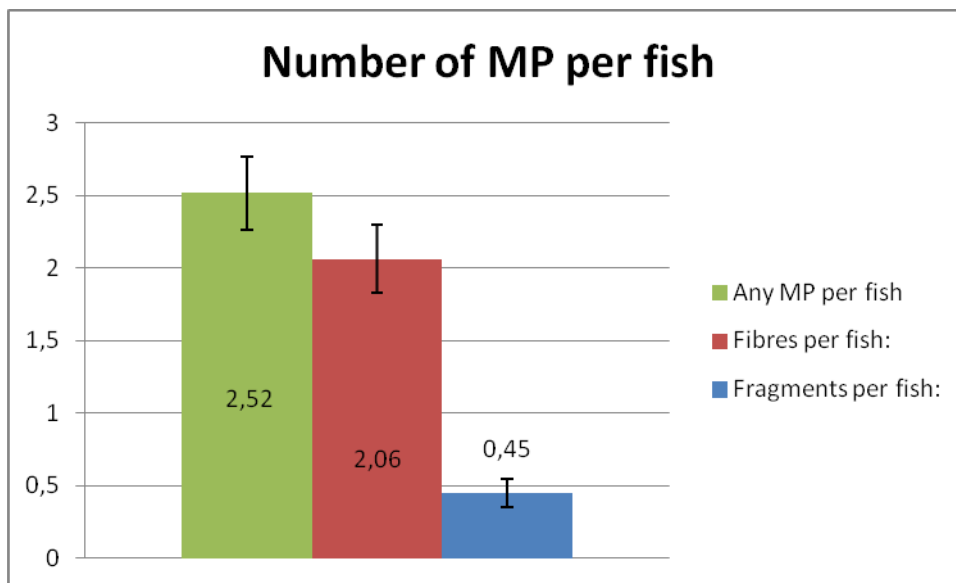

Fig. 8. Mean number of all types of MP, fibres and fragments per fish.

In order to compare the fish samples from Gran Canaria to those from Lanzarote, the non-parametric Wilcoxon-Mann Whitney test was applied. There were no significant differences observed in the total number of MPs and in the number of fibres between the Gran Canaria and Lanzarote fish samples (fig. 9 and 10, p-value = 0.12 and p-value = 0.6565 respectively). However, there was a significant difference in number of fragments per fish between these areas (p-value = 1.455e-07), where samples from Gran Canaria contained 0.12 ± 0.05 (mean $\pm$ SE) pieces of fragments per fish while samples from Lanzarote contained 1.14 ± 0.21 (mean $\pm$ SE) pieces of fragments per fish (fig. 11).
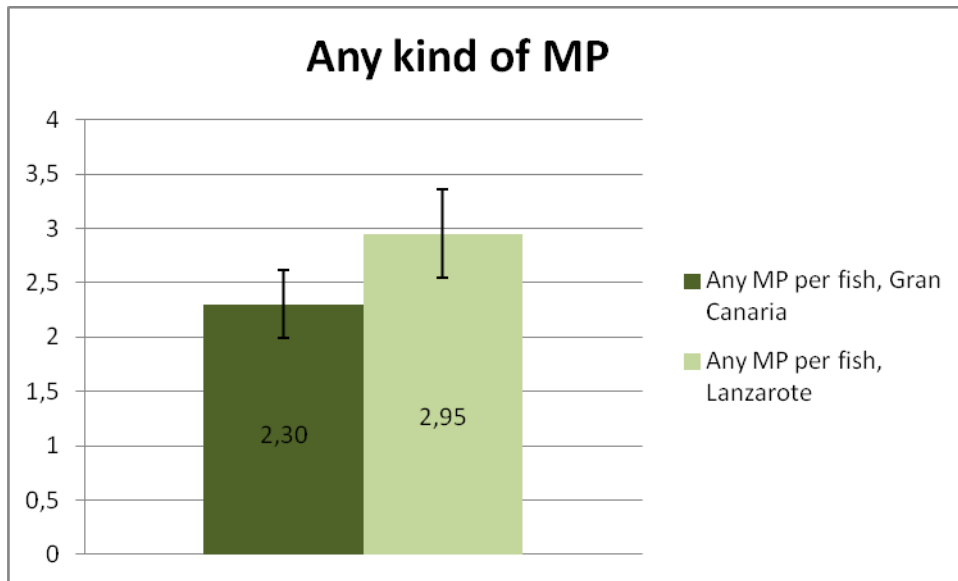
Fig. 9. Comparison of the mean number of all types of MP per fish between two of the Canary Islands (Gran Canaria and Lanzarote). There was no significant difference found (p-value = 0.12).
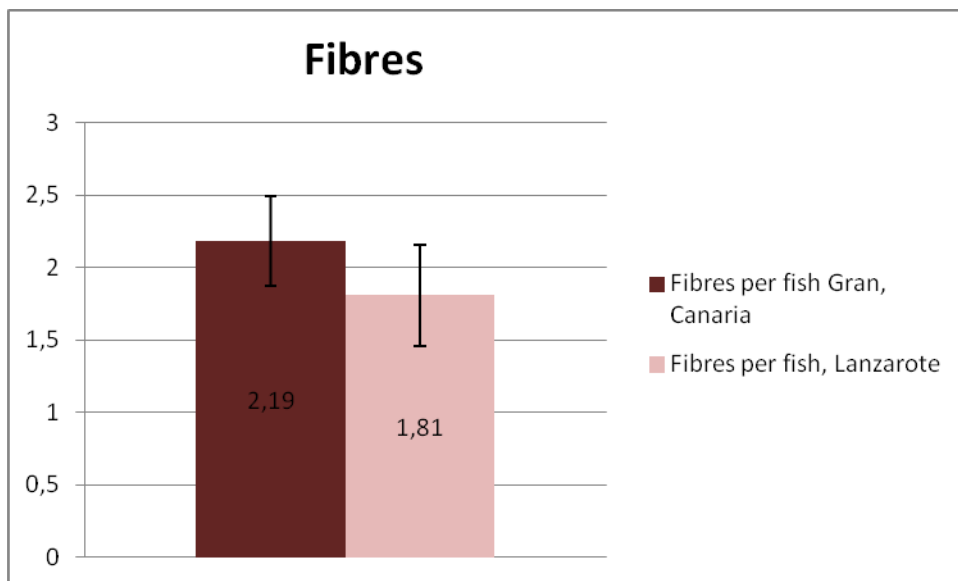

Fig. 10. Comparison of the mean number of fibres per fish between the islands of Gran Canaria and Lanzarote. There was no significant difference found (p-value = 0.6565).
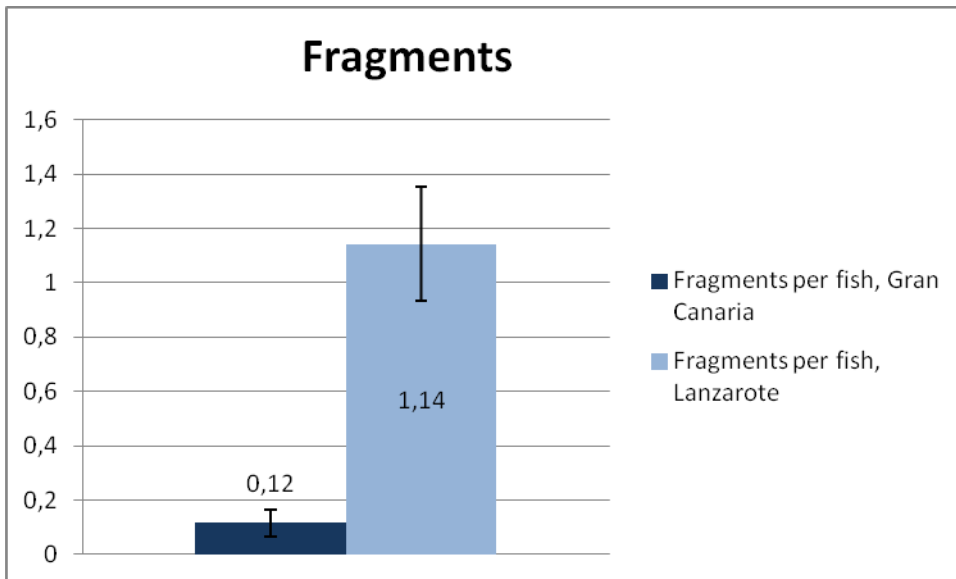
Fig. 11. Comparison of the mean number of fragments per fish between the islands of Gran Canaria and Lanzarote. Fish from Lanzarote were significantly more contaminated by MP fragments (p-value = 1.455e-07).

We compared the mean weights of the fish samples from these two areas and found a significant difference (Wilcoxon-Mann Whitney test, p-value = 0.02286). It turned out that the fish samples we obtained from Lanzarote were, on average, 208g heavier than the fish samples from Gran Canaria (fig. 12). In order to reveal any possible influence of fish size on fragments abundance in the stomach contents, we analysed the fish weigh-fragments abundance correlation. No significant correlation was found (R^2=0.035; p-value=0.07; Pearson correlation coefficent =0.22).
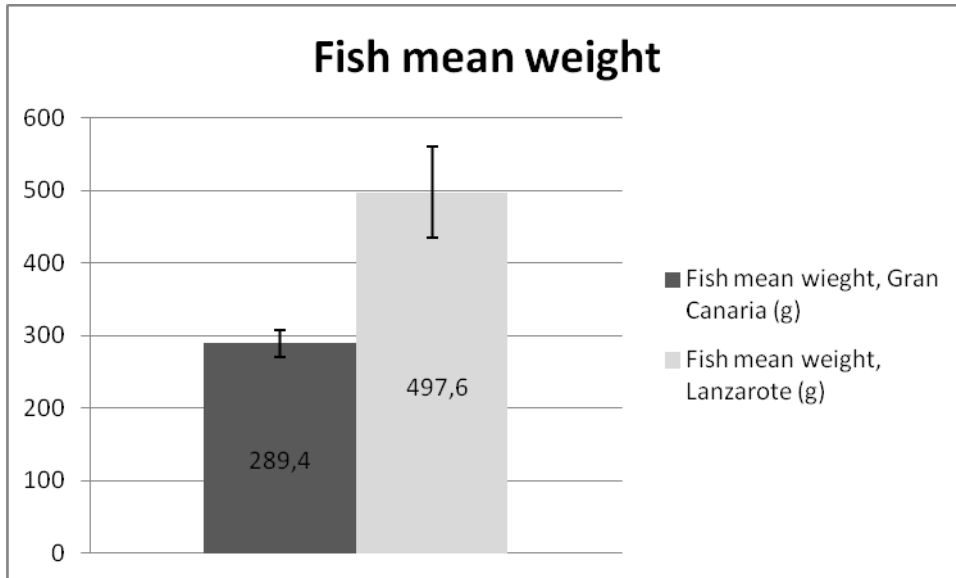
Fig. 12. Comparison of mean weights of the fish samples from Canaria and Lanzarote.

4.   Discussion

This study is the first to investigate MPs ingestion by fish species in the Canary Current. The methodological difficulties in the MPs isolation procedure can be one of the reasons why, up to date, there exist only a few studies addressing the occurrence of MPs in wild fish species populations. We chose the 10% KOH-digestion method for the samples because it was recommended as the best option out of five methods tested by Dehaut et al. (2016). Enzymatic digestion with proteinase-K results would perhaps result in better dissolution of the organic matter, nevertheless its use on larger organisms is not cost-effective (Avio et al., 2015).

Despite the measures taken to avoid the air-born fibre contamination we could not completely eliminate it. Thus, contamination could be the reason the abundance of fibres is relatively high in the fish samples. These results are, therefore, approximate and serve mostly as a trial for further studies in similar conditions. After excluding the fibres from the results, this study indicates that 33% of the fish stomach contents were contaminated by microplastics.

A few studies have been published on MPs ingestion by wild fish populations with quite variable results. Boerger et al. (2010) found microplastics in the guts of 35% of planktivorous mesopelagic fish caught in North Pacific central gyre, which has an MP abundance similar to the one found in our samples. Other studies reveal the same or lower abundance of MPs in fish stomach contents from other regions (Romeo et al., 2015; Possatto et al., 2011). Rochman et al. (2015) revealed anthropogenic debris in 28% of the fish in Indonesia and 25% of investigated fish samples in the US. Plastic fibres, fragments and films were also revealed in 13 stomachs (9%) of 141 mesopelagic fish from the North Pacific gyre (Davison and Asch, 2011). Moreover, the study of Nadal et al. (2016) reveal MP contamination in stomachs of more than 50% of the sampled fish (*Boops boops*) from the Mediterranean Sea.

A study from Baztan et al. (2014) about microplastic pollution in sediments of three of the Canary Islands (Lanzarote, La Graciosa and Fuerteventura) revealed that all studied beaches are exceedingly vulnerable to micro-plastic pollution despite being located in highly-protected natural areas. The majority of the plastics found within the study area was, according to the authors, generated far away and transported to the islands by ocean currents. During transit they were available for ingestion by marine organisms in the water column. (No similar study has been published so far in the island of Gran Canaria).

In the study of Boerger et al. (2010), the most commonly ingested plastics were of white (58.2%), clear (16.7%) or blue (11.9%) colour. On the contrary in our study, white and clear fragments formed only a small part of the fragments (10% and 4% respectively). With our methodology, not all of the organic material from the stomach contents was dissolved, which makes it more difficult to reveal plastic fragments of white, yellow/brown or transparent colour because these particles are similar, in colour, to the organic remains in the fish stomachs. Therefore, we believe that we have underestimated the abundance of light coloured MPs.

Due to diverse approaches in the methodology it is complicated to compare correctly the results from different studies. Visual inspection of the samples can be subjective and can vary depending upon the procedure used to separate MPs from the organic matter. In order to define the origin of the particles that differed in appearance from natural particulate material and to compare the results with similar studies,

techniques other than visual determination of the samples, would be needed. Examples are Raman spectroscopy or Fourier-Transform Infrared Spectroscopy (FT-IR). Unfortunately these instruments are expensive and not easily accessible.

In several samples, small round beads of yellow or light brown colour were present. They were suspected of being primary microplastic beads from face cleaners or similar products, but they turned out to be of organic origin, probably smaller fish eye lenses coloured by the digestion process (photo in Annex 2). In similar cases where visual observation wasn't sufficient to reveal whether we were observing a plastic particle or not, we used the hot needle test of Vandermeersch et al. (2015) because the hot needle would make the plastic sticky and leave a characteristic mark on the particle (photo in Annex 2).

5. Conclusions

This study of microplastics in the stomach contents of *Scomber colias* and *Boops boops* is the first one to determine the existence of plastic particles in fish from the Canary Current. 64 fish samples of wild populations of two pelagic species were studied and the results show that stomachs of 33% of the fish were contaminated by microplastics. There was a significant difference found in the number of ingested microplastic fragments between the different studied areas, suggesting that the level of MP fragment contamination is higher in fish from Lanzarote than in fish from Gran Canaria. However, to have data, statistically strong enough to support this hypothesis, more fish samples have to be investigated. Fibres are not included in these results due to possible air-born contamination that, despite precautions, could not be completely eliminated.

Upgrades in methodology are needed in order to determine the MPs with better accuracy and, more importantly, there is a need for standardized methodology, which could be used in all studies of MPs in fish stomachs contents, in order to be able to compare the results. This study is the initial part of a new investigation focusing on microplastic pollution in marine organisms from the Canary Current. Its aim is to know

the extent and possible consequences in this area and be able to develop a strategy to mitigate this problem.

Acknowledgement

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TFT REPORT

1.- Detailed description of the activities performed for TFT

Searching for literature related to the topic of microplastics, gathering and organizing information about plastic pollution, microplastic origin and its effects on marine environment, microplastic ingestion by marine species, especially fish and choosing an adequate methodology for dissolving the fish stomach content. My duties were to buy fish, do stomach dissections and also take out and freeze livers and muscle tissues of the fish for any future studies. I was responsible for the dissection process and following the methodology procedure, filtering the samples, looking for plastic contamination in the stomachs using the stereomicroscope and taking photos of the discovered microplastics. At FIMAR 2016 me and Paloma (master student) gave a short public presentation about the problematic of microplastics in the marine environment. I also prepared a poster with pre-liminary results of the microplastic ingestion study and presented it at the international microplastic congress which took place in Lanzarote in May 2016.

2.- Received training

I received general instructions on the procedures in the laboratory, learning how to extract the gastro-intestinal tract of fish and learn about organic material dissolving techniques. From Alicia I received an introduction to statistics using the R studio. I also started to use Mendeley program for organizing the bibliography.

3.- Integration and involvement within de department and relationship with the staff

I felt integrated within the investigation group. Alicia always helped me at the beginning of every step of the investigation and Ico the technician was always keen to help me find all the tools I needed in the laboratory. Overall there was a good atmosphere and a pleasant working environment. As a member of EOMAR I participated in FIMAR 2016 in Las Palmas and also took part in the first international congress about microplastics in Lanzarote.

4.-Most significant negative and positive aspects of the TFT development

Most positive aspects of my TFT development was gaining new knowledge about the topic of microplastics, being involved in activities of the investigation group (congress, FIMAR) and gaining contacts with people interested in marine conservation. Most negative aspect was the lack of time. I feel the outcome of this study could have been much better if I had time to do it properly and investigate higher number and variety of fish.

5.- Personal assessment of the learning achievement throughout the TFT fulfillment

I have submerged myself in the problematic of microplastic and plastic pollution in general and I have discovered further impacts of this issue than I expected. I especially appreciated getting in touch with people having the same interests and the opportunity to be part of the first international congress on microplastics in Lanzarote.

Annex I. Table 1. Complete overview of the fish samples. (LZ = Lanzarote)

Sample N°	Date	Species	Origin	Weight (g)	LENGHT (cm)	N° Fibres	N° Fragments	N° MPs Total
1	29/04/2016	*Scomber colias*	San Cristobal, Gran Canaria	265,4	28,5	0	1	1
2	29/04/2016	*Scomber colias*	San Cristobal, Gran Canaria	209,5	26,0	3	0	3
3	29/04/2016	*Scomber colias*	San Cristobal, Gran Canaria	193,2	25,5	7	0	7
4	29/04/2016	*Scomber colias*	San Cristobal, Gran Canaria	192,0	25,5	3	1	4
5	29/04/2016	*Scomber colias*	San Cristobal, Gran Canaria	284,2	29,0	7	0	7
6	29/04/2016	*Scomber colias*	San Cristobal, Gran Canaria	229,6	26,5	2	0	2
7	29/04/2016	*Scomber colias*	San Cristobal, Gran Canaria	200,2	25,5	1	0	1
8	12/05/2016	*Scomber colias*	San Cristobal, Gran Canaria	274,9	29,0	6	1	7
9	12/05/2016	*Scomber colias*	San Cristobal, Gran Canaria	295,6	29,5	1	0	1
10	12/05/2016	*Scomber colias*	San Cristobal, Gran Canaria	293,5	28,5	4	0	4
11	12/05/2016	*Scomber colias*	San Cristobal, Gran Canaria	175,7	25,0	4	0	4
12	12/05/2016	*Scomber colias*	San Cristobal, Gran Canaria	419,3	32,0	5	0	5
13	12/05/2016	*Scomber colias*	San Cristobal, Gran Canaria	502,6	34,0	3	0	3
14	12/05/2016	*Scomber colias*	San Cristobal, Gran Canaria	276,1	27,5	1	1	2
15	12/05/2016	*Scomber colias*	San Cristobal, Gran Canaria	481,9	33,0	1	0	1
16	12/05/2016	*Scomber colias*	San Cristobal, Gran Canaria	285,3	29,0	0	0	0
17	12/05/2016	*Scomber colias*	San Cristobal, Gran Canaria	409,2	31,5	0	0	0
18	12/05/2016	*Scomber colias*	San Cristobal, Gran Canaria	393,5	31,0	2	0	2
19	12/05/2016	*Scomber colias*	San Cristobal, Gran Canaria	534,8	34,5	1	0	1
20	12/05/2016	*Scomber colias*	San Cristobal, Gran Canaria	543,9	34,0	5	0	5
21	12/05/2016	*Scomber colias*	San Cristobal, Gran Canaria	449,9	31,5	0	0	0
22	12/05/2016	*Scomber colias*	San Cristobal, Gran Canaria	533,1	33,5	2	0	2
23	12/05/2016	*Scomber colias*	San Cristobal, Gran Canaria	445,3	32,0	1	0	1
24	01/06/2016	*Scomber colias*	San Cristobal, Gran Canaria	284,3	27	1	0	1
25	01/06/2016	*Scomber colias*	San Cristobal, Gran Canaria	196,0	25,5	2	0	2
26	01/06/2016	*Scomber colias*	San Cristobal, Gran Canaria	252,3	25,5	5	0	5
27	01/06/2016	*Scomber colias*	San Cristobal, Gran Canaria	191,7	25	0	0	0
28	01/06/2016	*Scomber colias*	San Cristobal, Gran Canaria	166,8	22,5	1	1	2
29	01/06/2016	*Scomber colias*	San Cristobal, Gran Canaria	120,6	22,5	1	0	1
30	01/06/2016	*Scomber colias*	San Cristobal, Gran Canaria	181	24,5	0	0	0
31	01/06/2016	*Scomber colias*	San Cristobal, Gran Canaria	120,5	23	2	0	2
32	01/06/2016	*Scomber colias*	San Cristobal, Gran Canaria	140,2	23	0	0	0
33	01/06/2016	*Scomber colias*	San Cristobal, Gran Canaria	119,8	22	1	0	1
34	01/06/2016	*Scomber colias*	San Cristobal, Gran Canaria	114,5	21,5	0	0	0
35	01/06/2016	*Scomber colias*	San Cristobal, Gran Canaria	128	23	2	0	2
36	01/06/2016	*Scomber colias*	San Cristobal, Gran Canaria	300,8	28	2	0	2
37	01/06/2016	*Scomber colias*	San Cristobal, Gran Canaria	311,8	27	0	0	0
38	01/06/2016	*Scomber colias*	San Cristobal, Gran Canaria	316,8	27,5	4	0	4
39	01/06/2016	*Scomber colias*	San Cristobal, Gran Canaria	387,7	28,5	6	0	6
40	01/06/2016	*Scomber colias*	San Cristobal, Gran Canaria	375,3	29	1	0	1
41	01/06/2016	*Scomber colias*	San Cristobal, Gran Canaria	328,9	27,5	3	0	3
42	01/06/2016	*Scomber colias*	San Cristobal, Gran Canaria	253,7	27	3	0	3
43	01/06/2016	*Scomber colias*	San Cristobal, Gran Canaria	264	26	1	0	1
44	15/04/2016	*Scomber colias*	Puerto Carmen - La Tiñosa, LZ	230	28	1	3	4
45	19/04/2016	*Scomber colias*	Puerto Carmen - La Tiñosa, LZ	440	33	6	1	7
46	20/04/2016	*Boops boops*	Puerto Carmen - La Tiñosa, LZ	100	21	4	2	6
47	20/04/2016	*Boops boops*	Puerto Carmen - La Tiñosa, LZ	100	19	1	1	2
48	20/04/2016	*Boops boops*	Puerto Carmen - La Tiñosa, LZ	100	19	0	1	1
49	20/04/2016	*Boops boops*	Puerto Carmen - La Tiñosa, LZ	80	18,5	0	1	1
50	25/05/2016	*Boops boops*	Puerto Carmen - La Tiñosa, LZ	110	20	2	1	3
51	25/05/2016	*Boops boops*	Puerto Carmen - La Tiñosa, LZ	90	18,5	2	0	2
52	25/05/2016	*Scomber colias*	Puerto Carmen - La Tiñosa, LZ	~700	35-40	3	1	4
53	25/05/2016	*Scomber colias*	Puerto Carmen - La Tiñosa, LZ	~700	35-40	2	1	3
54	25/05/2016	*Scomber colias*	Puerto Carmen - La Tiñosa, LZ	~700	35-40	1	3	4
55	25/05/2016	*Scomber colias*	Puerto Carmen - La Tiñosa, LZ	~700	35-40	3	3	6
56	25/05/2016	*Scomber colias*	Puerto Carmen - La Tiñosa, LZ	~700	35-40	1	1	2
57	25/05/2016	*Scomber colias*	Puerto Carmen - La Tiñosa, LZ	~700	35-40	0	2	2
58	25/05/2016	*Scomber colias*	Puerto Carmen - La Tiñosa, LZ	~700	35-40	1	1	2
59	25/05/2016	*Scomber colias*	Puerto Carmen - La Tiñosa, LZ	~700	35-40	0	1	1
60	03/06/2016	*Scomber colias*	Puerto Carmen - La Tiñosa, LZ	720	41	0	0	0
61	03/06/2016	*Scomber colias*	Puerto Carmen - La Tiñosa, LZ	730	41	4	1	5
62	03/06/2016	*Scomber colias*	Puerto Carmen - La Tiñosa, LZ	720	41	2	0	2
63	03/06/2016	*Scomber colias*	Puerto Carmen - La Tiñosa, LZ	830	44	2	0	2
64	03/06/2016	*Scomber colias*	Puerto Carmen - La Tiñosa, LZ	600	40	3	0	3
					In Total:	**132**	**29**	**161**

Annex II

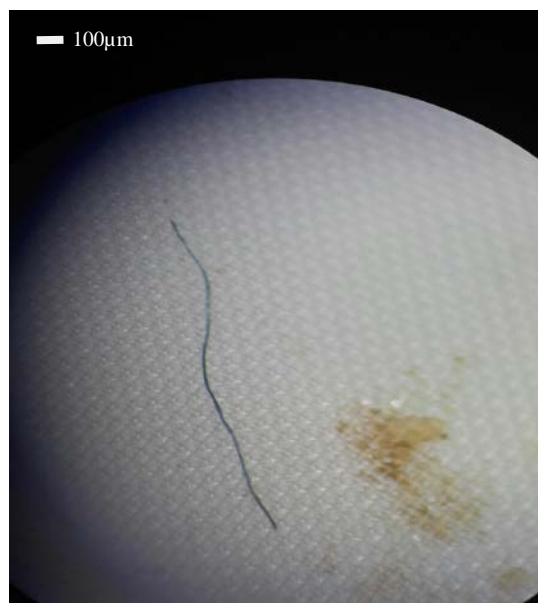Examples of MPs found in fish stomachs. Filtering sieve 50 µm.

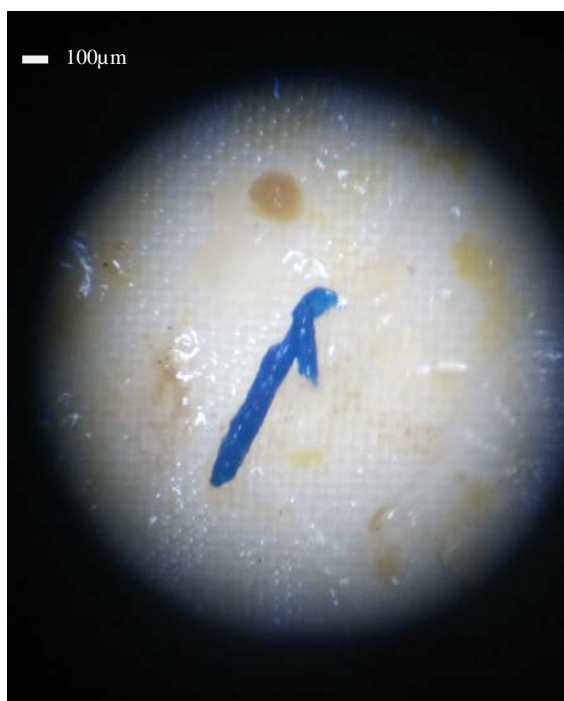Fig. 13. MP fragment.

Fig. 14. Fibre

Fig. 15. MP fragment
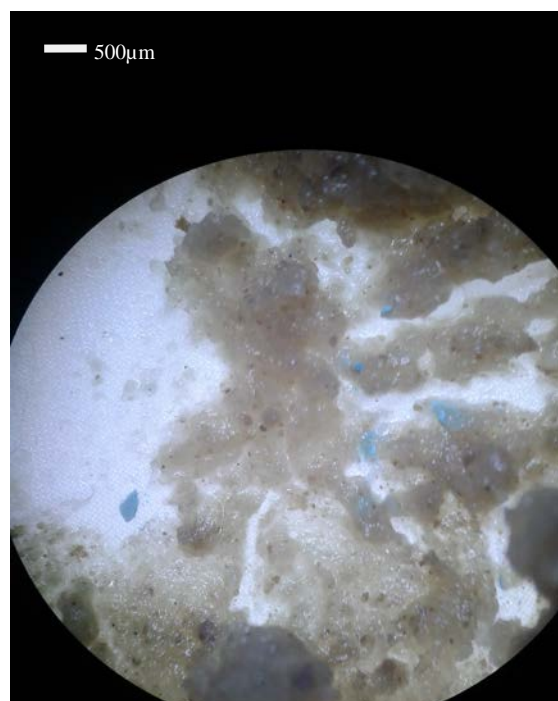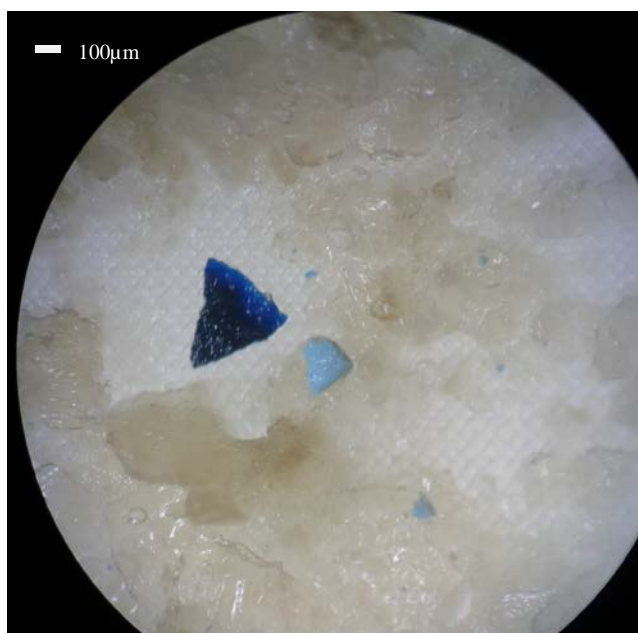

Fig. 16. Blue paint residues

Annex II

Fig. 17. MP fragment and paint residues

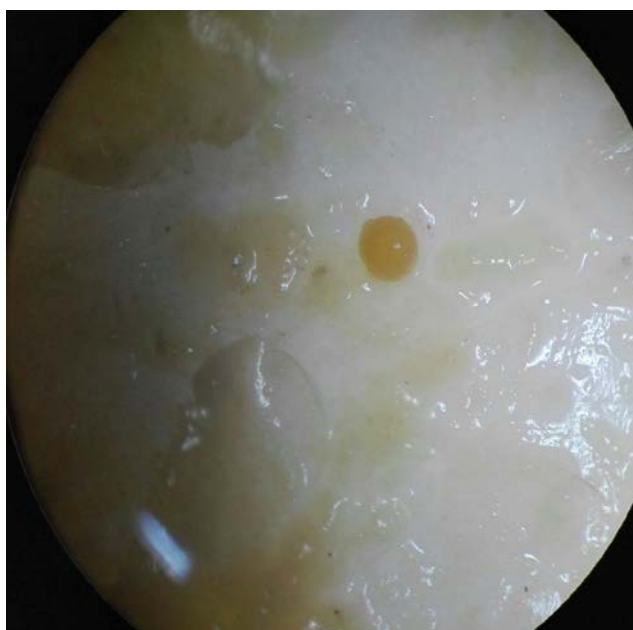
Fig. 18. Hot needle test on a white MP fragment

Fig. 19. A microsphere suspected to be a plastic micro bead. Hot needle test and following inspection of the stomach contents of various fish samples revealed we were dealing with small fish eyes' vitreous bodies.