Quantification of microplastic ingestion in *Scomber colias* (Gmelin, 1789) along the Mediterranean coast.

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Contents

Abstract

Pollution by microplastics in the ocean is a topic that nowadays is receiving more attention from the scientific community. The effects and the implications that microplastic pollution has on marine organisms have already been reported throughout the world.

In the present study was quantified the amount of microplastic litter found in the gastrointestinal contents of a commercial species (*Scomber colias*) from the Mediterranean Sea. From the total number of sampled fish (63) microplastic ingestion was identified in 14 individuals (22.22%). A total of 20 microplastics items were identified in the *Scomber colias* using stereomicroscope techniques.

Samples were obtained from five different locations along the Mediterranean coast of Spain; Gerona, Barcelona, Denia, Almería and Granada (Motril). The station that presented species with higher values of microplastic intake was Barcelona with a percentage of occurrence of 42.9% and a mean of 0.71(±0.42) microplastic per fish, followed by Gerona with 28.6% of occurrence and a mean of 0.5(±0.25) microplastic per fish. Denia and Almeria had 21.4% of occurrence of intake and a mean value of 0.21(±0.11) and 0.29(±0.16) microplastic per fish respectively. The lowest value was determined in Granada with a 7.1% of occurrence and a mean of 0.07(±0.07) microplastic per fish. The highest intake values corresponded to the localities that had larger individuals. The 64.3% of the microplastics identified were of fragment type, and 21.4% were filaments. Finally, in less proportion some granules and films were identified and almost all the microplastics found were blue or black.

This study provides new data according to ingestion values on a commercial species in the Mediterranean region, remarking the existing problem and associated impacts which microplastic litter have on marine environment and organisms.

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1. Introduction

1.1. Plastic pollution ('the plastic age')

During the past century there has been an exponential increase in the demand and production of plastic (Lusher, 2016), and the plastic waste is increasing worldwide. In the early 70s, the first appearance of plastic debris in the oceans was recorded without much attention from the scientific community. But now more recent studies have provided new data, stressing evidence of the ecological consequences of plastic debris and the problem of this type of anthropogenic pollution in the oceans (Andrady, 2011).

The properties of the plastics such as versatility, strength and lightweight, make them perfect material for multiple applications in the global industry. Also, their low cost production, excellent oxygen and moisture barrier properties make them ideal form for packaging, especially for food products replacing conventional material such as glass, metal or paper (Andrady and Nepal, 2009). There are several types of plastic materials which are used in this packaging: Polyethylene (PE), Polypropylene (PP), Polystyrene (PS), Poly (ethylene terephthalate) (PET), Poly (vinyl chloride) (PVC), Polyurethane (PU) and Styrene-Butadiene Rubber (SBR) (GESAMP, 2016). These plastics may contain chemicals added during manufacturing processes or sorbed to their surface once in the marine environment which may be harmful for marine organisms and the environment.

In recent years, an increasing trend towards investigating and monitoring the contamination of the environment by microplastics has been observed worldwide; the presence of smaller particles of plastic in oceans worldwide, which are not visible to the naked eye and undetectable without the need of optical magnifying instruments. Microplastics (MPs) are defined as plastic fragments in ranging size for a few µm to 500 µm (5 mm) (Arthur et al., 2009). The concern for the amount of plastic and microplastic waste in the ocean has grown rapidly in recent years, it is important to know that most of the plastic litter produced by anthropogenic activities enter into the marine environment causing large problems in marine organisms and have been overlooked for a long time (Nadal et al., 2016).

Several studies have revealed that the load and presence of plastics is increasing in marine ecosystems around the world, possibly causing alterations at the species, community or even ecosystem level (Alomar and Deudero, 2015). Nevertheless, due to their persistent nature, microplastic abundance in the marine environment will only increase (GESAMP REPORTS AND STUDIES, 2O16).

Microplastics can be classified as primary and secondary. Primary microplastics are produced intentionally at a microscopic scale, either as precursors to other products (for example, plastic pellets, (Costa et al., 2010)) or for direct use such as abrasives in cleaning or cosmetic products (Browne, 2015). On the other hand, secondary microplastics are the result of the degradation of larger plastics items to smaller items due to their degradation and fragmentation in the marine environment (Mathalon and Hill, 2014). Consequently these can enter the marine environment through river systems, coasts, directly from ships or be transport induced by the wind in the atmosphere (GESAMP REP. AND STUDIES, 2O16), and therefore be potentially accessible to marine organisms.

1.2. Plastic pollution in the Mediterranean Sea.

The Mediterranean Sea, a semi-enclosed sea, is one of the most affected seas worldwide in terms of plastic litter, and high concentrations of plastics are probably linked to its nature and oceanographic regimes (Deudero and Alomar, 2014). The Balearic Islands, located in the western Mediterranean, due to their large influx of tourism are highly exposed to anthropogenic actions that impact coastal habitats (Alomar and Deudero, 2015). The presence of microplastics in shallow coastal waters (Alomar et al., 2016), in deep waters (Woodall et al., 2014) and in surface waters has already been demonstrated (Faure et al., 2015). Microplastics had already been detected in coastal sediments in the island of Mallorca and in the Marine Protected Area (MPA) of Cabrera. (Alomar, Estarellas, Deudero. 2016).

The United Nations Environment Program (UNEP) has estimated that 6.4 million tons of litter enter the oceans every year, with 62 million plastic litter items currently floating on the surface of the entire Mediterranean basin (Suaria and Aliani, 2014). In the Mediterranean Sea, Pham et al. (2014) have already reported that plastics are the most prevalent litter items found on deep sea floors, while Suaria and Aliani (2014) have stated that plastic objects account for 82% of all man-made floating items. A review of the marine litter in the Mediterranean stated that the most abundant marine litter founded are polymers derived from plastic (Alomar and Deudero, 2015).

For this reason studies related to the interactions of plastics and organisms are a clear priority nowadays and even more in the Mediterranean. A recent study shows high concentrations of plastic in the Mediterranean and Black Sea, as well as their interactions with marine species (Figure 1).

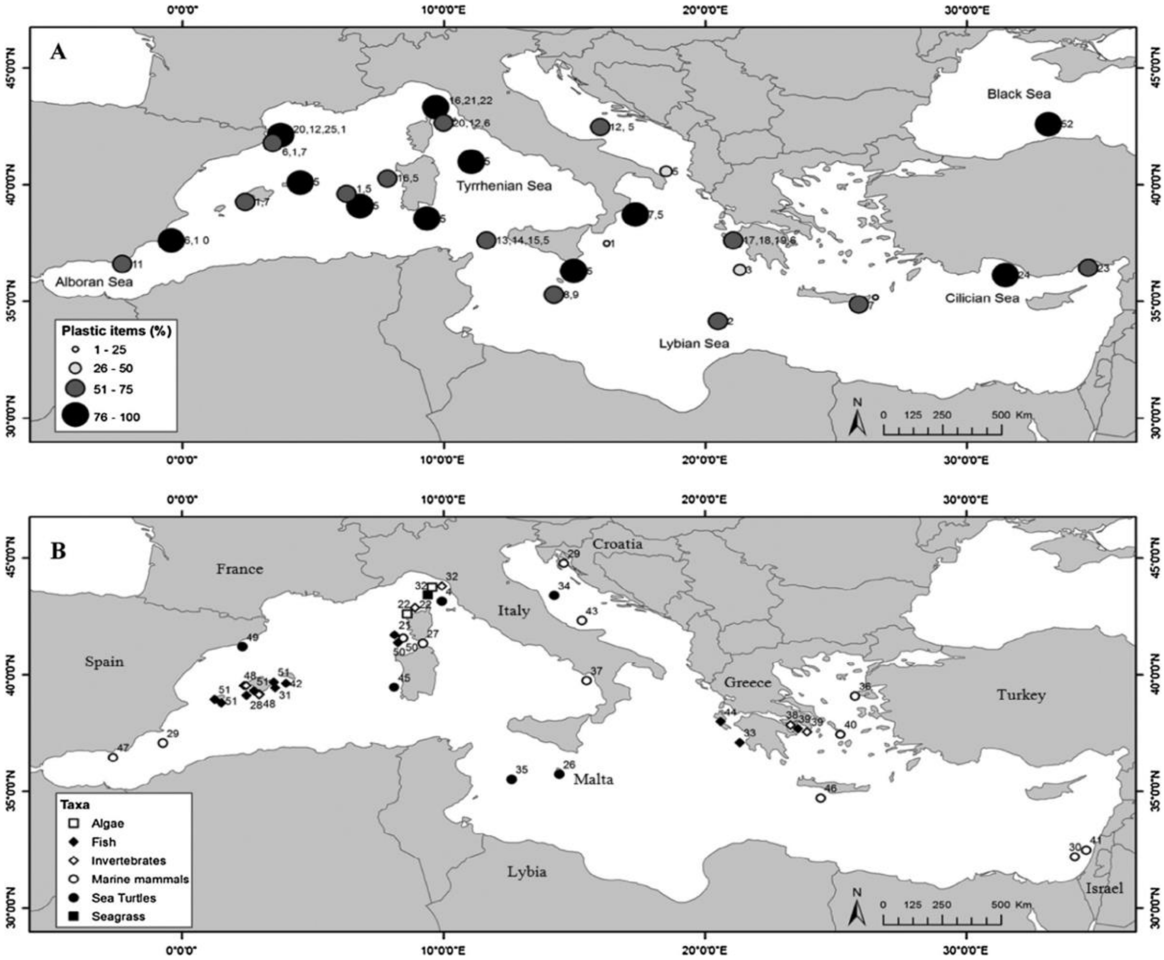


Figure 1. Location of geographic regions in the Mediterranean and Black Sea where research has been conducted on (a) plastic (%) and (b) marine litter and biota. Extracted from Deudero and Alomar, (2015).

1.3. Ingestion of microplastics

Due to their small dimensions (>5 mm), microplastics become available for ingestion to a wide range of marine organisms. Ingestion has already been demonstrated for organisms at the base of the food chain; a large variety of planktonic organisms, such as copepods, euphasiacea (krill) and larval stages of molluscs, decapods and echinoderms (Cole et al., 2013; Hart, 1991; Lee et al., 2013) will take up microplastics while feeding.

Microplastics can either be ingested directly or indirectly through the consumption of lower trophic level prey (Farrell and Nelson, 2013). Moreover, the ingestion of microplastics does not only cause physical harm but can also act as vectors of additives incorporated during manufacture (e.g. polybrominated diphenyl ethers (PBDE)) and organic pollutants sorbed from the surrounding seawater (e.g. Polychlorinated biphenyls (PCBs)) (Teuten et al., 2009).

Even though the existing data is too limited to determine a realistic natural concentration of microplastics in seawater, the potential for ingestion by commercially important species, however, remains a cause for concern.
Microplastics could cause in marine organisms; mortality, reduction of the alimentary activity, inhibition of the growth and correct development, endocrine alterations, alteration of the energy, oxidative stress, immunity, dysfunction of the neurotransmission and even genotoxicity (Avio et al., 2015; Rochman et al., 2014; Wright et al., 2013).

In addition, there are recent studies that have shown that plastic is being ingested by both pelagic and benthic fish species. (Neves et al., 2015; Romeo et al., 2015; Lusher et al., 2013). More specifically in the Balearic Islands a study has been carried out with a species similar to our target species, *Boops boops*, in this study they report a high presence of microplastics in the gastrointestinal tract of the bogue, a species with a semipelagic habitat very frequent in the Balearic Islands and the Mediterranean Sea. The study shows that almost 70% of bogues have plastic polymer fibers smaller than 5 mm in their stomachs (Nadal et al., 2016).

Recently another study of the Mediterranean Sea, evidenced the ingestion of MP in the sampled striped red mullet (*Mullus surmuletus*) (Alomar et al., 2017). They stated that 27.3% of sampled fish had microplastic in their gastrointestinal tract although they did not found evidence of oxidative stress associated to the ingestion.

1.4. Target species

For the purpose of the study *Scomber colias* has been selected as the study species. *Scomber colias* belongs to the family Scombridae, and is abundant in warm or hot waters, inhabitating the continental slope from the surface to 300 m deep. The species performs seasonal migrations mainly for spawning, wintering and feeding (Usami, 1973, Collette and Nauen, 1983).

In general, this species feeds essentially on decapod euphausia and crustaceans. In addition, it feeds on small pelagic fishes such as anchovy, pilchard, sardines, sprat and silversides. This species may live up to 13 years, and has a length at 50% maturity of approximately 18 cm corresponding to an approximate age of two years (Hattour, 2000). Spawning occurs in water temperatures between 15ºC and 20ºC (Collete and Nauen, 1983). The spawning was carried out in coastal waters after a migration from the feeding area (Baird, 1977; Angelescu, 1979, 1980).

The interest of studying this pelagic-coastal species lies mainly in the fact that it is an important link in trophic webs related to large marine predators, such as tuna fish (Ramos et al., 1990). Frequent in the Mediterranean, Black Sea and Red Sea (Radokov, 1972; Rodríguez-Roda, 1982; Ben-Tuvia, 1983), it is also the only species of the genus Scomber found in the Atlantic and Pacific oceans.

Scomber colias is a pelagic species of great importance in the world of fisheries, occupying the third place of the most captured species in the year 1978 (Castro, 1991). According to the FAO Global Capture Production data base, in the 2015 were captured 1.492.495 tonnes of *S.colias* (Atlantic chub mackerel). With a variation of 6.8% more than in the 2014.

Figure 2. Target species *Scomber colias*. (Gmelin, 1789).

In the Mediterranean Sea, this is a common and locally abundant species that has fairly high, fluctuating catches. There has been a steady decline in landings of this species since the 1980s which is confirmed by anecdotal evidence from fishery experts. However, within the last 10 years (generation length of three years) the fluctuations have been inconclusive in terms of any trend. Current exploitation levels are intense with technological advances and because of the steady decline over the past 20 years this species is regionally considered Near Threatened based on population declines suspected to be approaching 30% based on A2d list of threatened species. Recent decreases in population trends may be parallel with recent increases in population of *Scomber scombrus* (L., 1758). (FAO, The IUCN Red list of threatened species 2011.)

There is a minimum size limit of 18 cm for all *Scomber* species in the European Union and Turkey. In the Mediterranean, a targeted management plan for this species is needed to reverse long term declining trends.

Given the evidence that fish species are susceptible of ingesting microplastics and the demonstrated concentrations of plastic pollution in the Mediterranean Basin, the aim of this study is to:

1) Quantify microplastic ingestion in a commercially valued fish species, *Scomber colias*, along the Spanish Mediterranean coast.

2) Analyse spatial differences according to microplastic ingestion in a same species along the Spanish Mediterranean coast.

2. Materials and Methods

2.1. Sample Collection and study area.

To study the intake of microplastics by the species *Scomber colias* (Gmelin, 1789) in the Mediterranean Sea, 63 samples were collected in the MEDIAS (Mediterranean Acoustic Surveys) oceanographic survey that focuses on the estimation and abundance of pelagic species in the Mediterranean Sea. Species were extracted with an experimental semi-pelagic trawling gear by scientific personnel during 2015. The gastrointestinal tract of each individual was extracted and stored in individual bags at a temperature of -18º C until posterior work at the laboratory of the Spanish Institute of Oceanography (IEO), Balearic Centre of Oceanography (COB).

Five stations were sampled (4, 13, 29, 43, and 48) covering the area of the Spanish Mediterranean coast. (Fig.3).

Station number 4 refers to the zone of Gerona, number 13 to Barcelona, station number 29 lies between Gandía and Denia, 43 near Carboneras (Almería) and finally the station 48 is located in the Alboran Sea (between Málaga and Granada). From now onwards sampling stations will be assigned as 4 (Gerona), 13 (Barcelona), 29 (Denia), 43 (Almería) and 48 (Granada (Motril)).

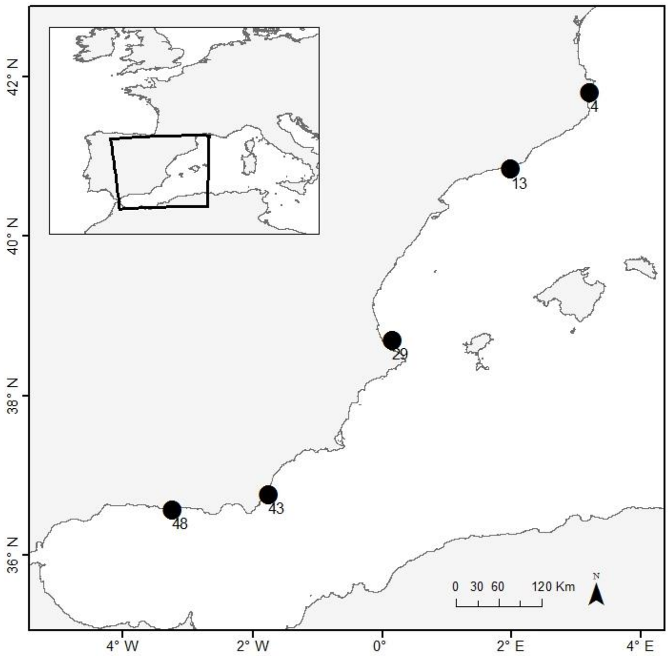


Figure 3. Distribution of sampling areas along the Mediterranean coast. The black dots indicate sites in which *Scomber colias* were captured: ((4 (Girona), 13 (Barcelona), 29 (Denia), 43 (Almería) and 48 (Granada (Motril))).

2.2. Laboratory work

All samples were evaluated prior to dissection, to obtain the basic biological parameters: Total length (TL), fresh weight (W), eviscerated weight (We), and weight of the gastrointestinal tract (Wg). In addition data on latitude, length and depth where samples were captured was provided from the MEDIAS survey. Also, Vacuity index (%VI)* was calculated to get more information about the state of the fish when eating microplastics and to compare results.
*Vacuity Index (%VI) = number of empty gastrointestinal tracts ×100/ total number of gastrointestinal tracts.

A total of 63 gastrointestinal tracts of *S. colias* were analyzed from 63 individuals with a size range between 130 cm and 360 cm of TL. The visual identification of the content in the entire gastrointestinal tract was made with a stereomicroscope, model Nexius Zoom with an increase from 6.7 x to 45 x. Several parameters are evaluated for each sample; the degree of repletion of the gastrointestinal tracts (1 being less repleted and 4 the most repleted), main preys, number of MPs found in the gastrointestinal tracts, type of MPs (filament, granular, fragment, and films) and colour of MPs were calculated. A camera (CMEX 3.0 MP) coupled to the stereomicroscope with calibration software, Image Focus 4.0. (Euromex Software) allowed to take images of the identified MPs. In their study Alomar et al., (2016) defined this protocol of identification.

During the processing of all samples, measures were adopted to avoid contamination (by air) (Woodall et al., 2015). During the analysis process, two petri dishes were placed at each side of the stereomicroscope and it was observed if they have any microplastic before and after each sample. In the case of finding any atmospheric contamination, these were stored in glass vials to account for the possible error. Throughout the analysis process, laboratory instruments were washed with alcohol to prevent contamination. In addition, hands and forearms were washed thoroughly and a 100% cotton lab coat was used throughout the entire analysis process.

2.3. Data analysis

To test normality of data, the Kolmogorov-Smirnov test and a p-value of 0.05 was applied since data sample was bigger than 50 (n = 63). All p-values were significant, that is, less than 0.05, therefore none of the quantitative variables had a normal distribution.

As our quantitative variables did not follow a normal distribution, the parametric tests as the *ANOVA* variance analysis could not be applied. In the case of nonparametric data, when the categorical variable has 2 categories was used the *Mann-Whitney U* test and in the case of 3 or more groups the *Kruskal Wallis* test was applied.
To study differences of microplastic ingestion between individuals of the five different sampling sites a non-parametric *Kruskall Wallis* test was carried out, since the data used had more than 2 categories.

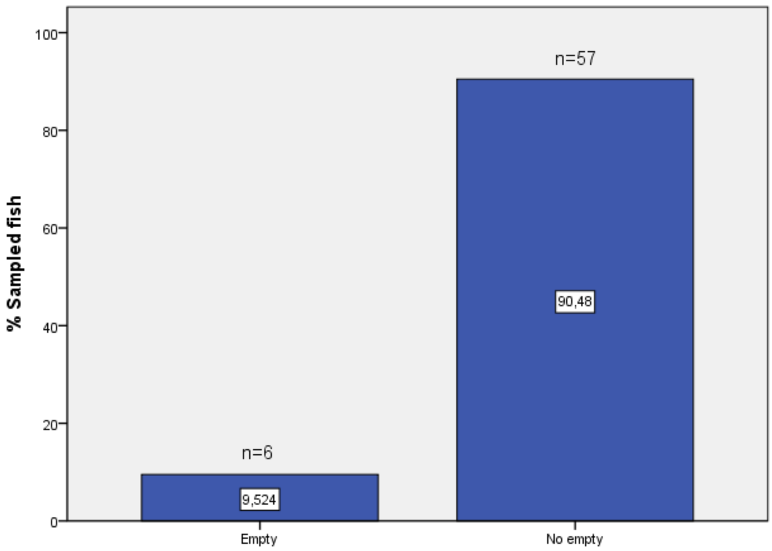
The *Kruskal Wallis* test was used as the method for exploring affinities between the variables and the five stations. For the statistical analysis of data we used the IBM SPSS Statistics 23 program.

3. Results

A total of 63 individuals of *Scomber colias* collected along the Mediterranean coast of Spain were studied. Individuals had a total length ranging from 130 to 360 cm and a fresh weight ranging from 14 to 448.2 g.

From the total of individuals sampled only 9.5% (n=6) had empty gastrointestinal tract, whereas 90.5 % (n=57) had non empty gastrointestinal tracts (Figure 4.A). In terms of containing microplastic in the gastrointestinal tract or not, from the total of individuals sampled, microplastics were not found in 77.8 % (n=49) of the gastrointestinal tracts, while microplastics were present in 22.2 % (n=14) of the total number of gastrointestinal tracts sampled (Figure 4.B).

A)



B)

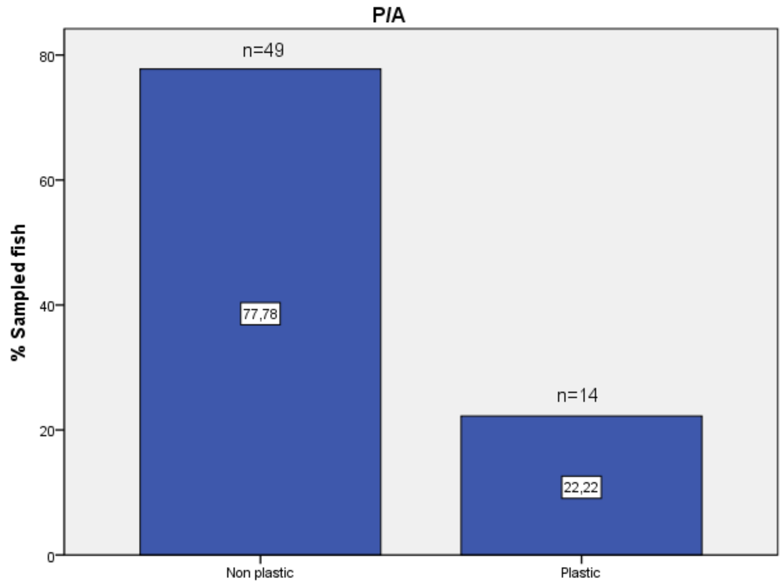


Figure 4. A) Total number of individuals with non-empty and empty gastrointestinal tracts and the percentage of sampled fish. B) Total number of individuals observed with and without (Presence/Absence) plastics in their gastrointestinal tracts.

The observation with stereomicroscope allowed determining the number of MPs items ingested among the different stations (Fishing stations). This allowed to calculate the percentage of occurrence, the mean of MPs for each individual and their standard error, and Vacuity Index (%VI). The highest value of % of occurrence was registered in Barcelona (42.9%) with mean values of 0.71(±0.42) MP ingested per fish, which matches with the highest value of Vacuity Index (28.6%), followed by Gerona with the second highest value of occurrence percentage (28.6%) and a mean value of 0.5(±0.25) MP ingested per fish and by Denia and Almeria with an occurrence percentage of 21.43% and with mean values of 0.21(±0.11) and 0.29(±0.16) MP ingested per fish respectively . The lowest value of % of occurrence was recorded in the Granada (Motril) station (7.1%) with a mean value of 0.07(±0.07) MP ingested per fish and where the Vacuity Index registered was 0 (Table 1).

Station	*Nº individuals sampled*	*Nº of MPs ítems*	*Ind. with MPs*	*% MP occurrence*	*Mean value of MP ingested (MPs/ind ± SE)*	*Vacuity Index (%)*
Gerona	**14**	**7**	**4**	**28.57**	**0,5 ± 0,251**	**7.14**
Barcelona	**7**	**5**	**3**	**42.86**	**0,714 ± 0,421**	**28.57**
Denia	**14**	**3**	**3**	**21.43**	**0,214 ± 0,114**	**14.28**
Almería	**14**	**4**	**3**	**21.43**	**0,286 ± 0,163**	**7.14**
Granada(Motril)	**14**	**1**	**1**	**7.14**	**0,071 ± 0,071**	**0**
Total	**63**	**20**	**14**	**22.22**	**0,317 ± 0,087**	**9.52**

Table 1. Total number of fish per station, total number of MPs items ingested, individuals with presence of MPs, % of microplastic occurrence, mean value of ingested MP (MPs/Ind ± SE) and Vacuity Index (%VI).

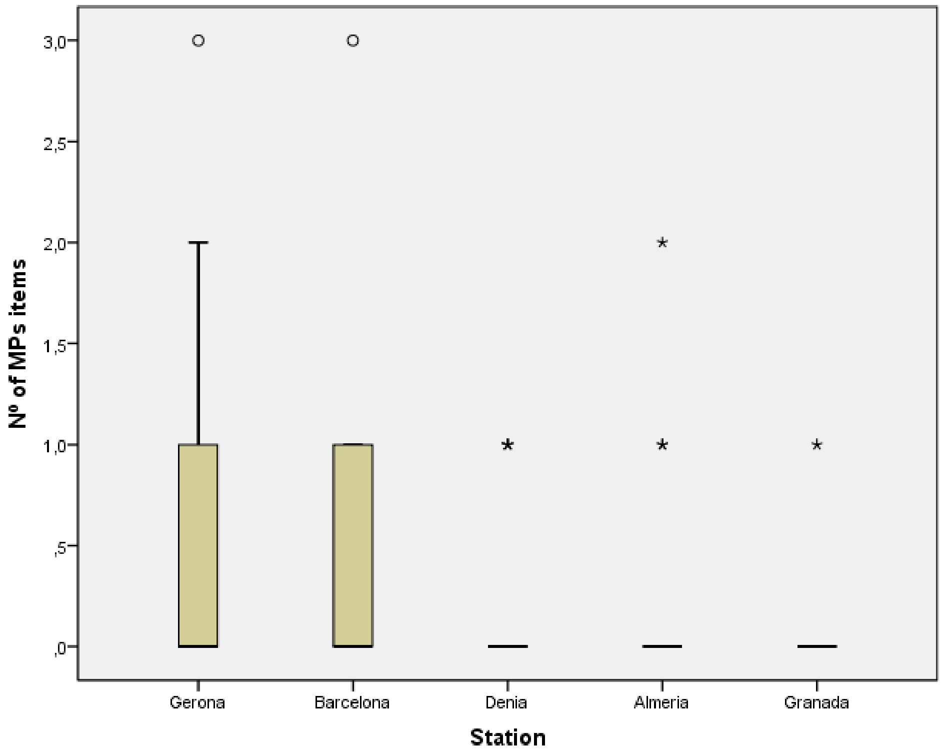


Figure 5. Boxplot of number of MPs items among the different sample stations. Bars represent standard deviation.

As the p-value exceeds 0.05 (not significant), the hypothesis that there is no statistically significant difference between stations in the number of microplastics was accepted. Although in the graphs it can be observed that there are differences in MP intake between the stations of Gerona and Barcelona, no statistically significant difference was found due to not having enough data (Figure 5).

The distribution of the total length (T.L) (Figure 6) and the fresh weight (W) (Figure 7) of samples were analysed among stations to see if the distribution had significant differences.

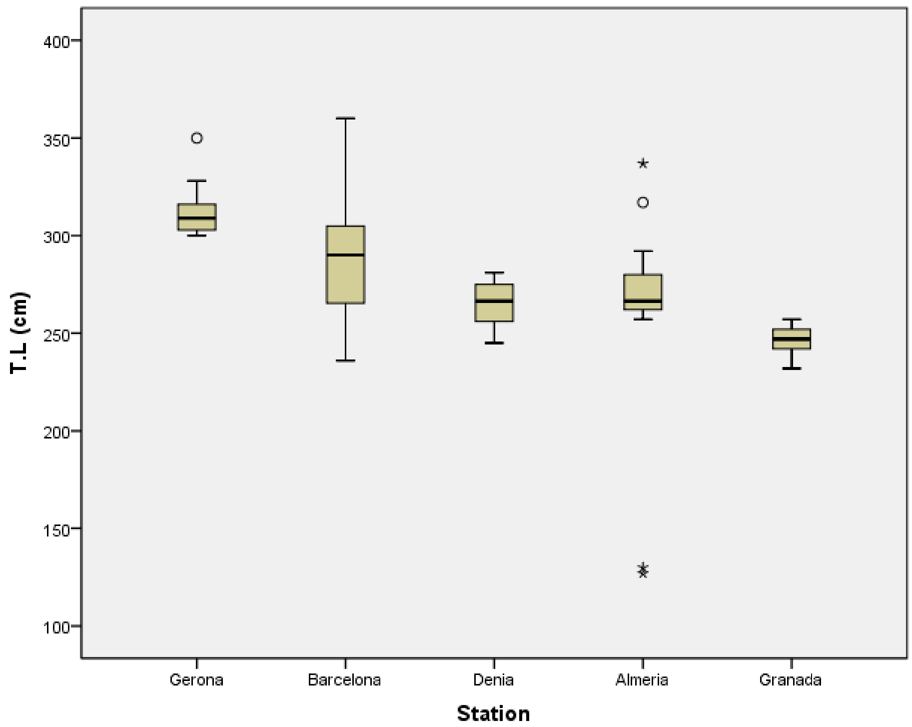


Figure 6. Boxplot showing the different sizes (Total length (cm)) of samples between stations. Bars represent standard deviation.

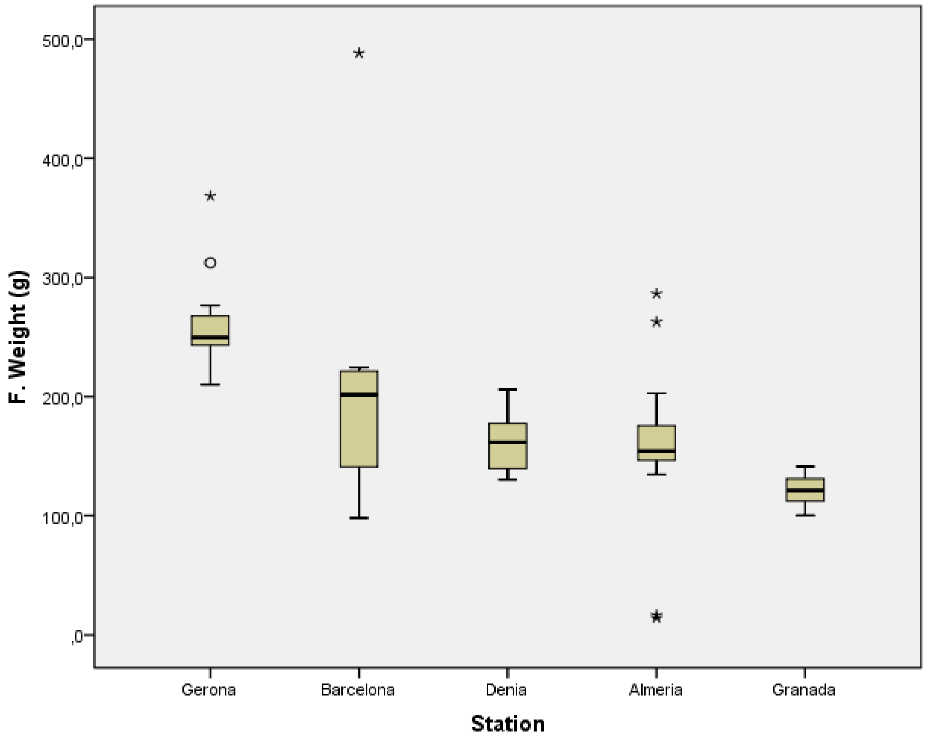


Figure 7. Boxplot showing the different weights (Fresh weight (g)) of samples between localities.

As you can observe in Figures 6 and 7, Gerona and Barcelona present the highest values of T.L and Weight. Denia and Almeria stations present more or less individuals of the same size and the lowest values was recorder to Granada (Motril) station.

As the p-value is less than 0.05 (significant), with the *Kruskal-Wallis* test, according to Total length (cm) and Fresh weight (g) among stations the hypothesis that there is a statistically significant difference between two categories was accepted. There would be significant differences between the stations of Gerona and Granada (Motril) or Barcelona and Granada (Motril).

From the total of samples, a total of 20 MPs items were found, of which: 28.6% were black, 21.4% were blue, 7.1% was green, 14.3% were orange, 7.1% was red, 7.1% was transparent and 14.3% were white. According to type of microplastics found we had four different types defined; filament, granular, film and fragments. Then it is shown: the frequency that colours of MPs found had, percentage of colors identified in samples and the different type of microplastics found in the samples also with their frequency and percentage of occurrence. In the summary table you could see the results obtained (Table 2, Table 3, Figure 8 and Figure 9).

Color of MPs					
		Frequency	%	Valid %	Accumulated %
Colors	black	4	28,6	28,6	28,6
	blue	3	21,4	21,4	50,0
	green	1	7,1	7,1	57,1
	orange	2	14,3	14,3	71,4
	red	1	7,1	7,1	78,6
	transparent	1	7,1	7,1	85,7
	white	2	14,3	14,3	100,0
	Total	14	100,0	100,0	

Table 2. Summary table for the different colors of MPs identified in fish samples.

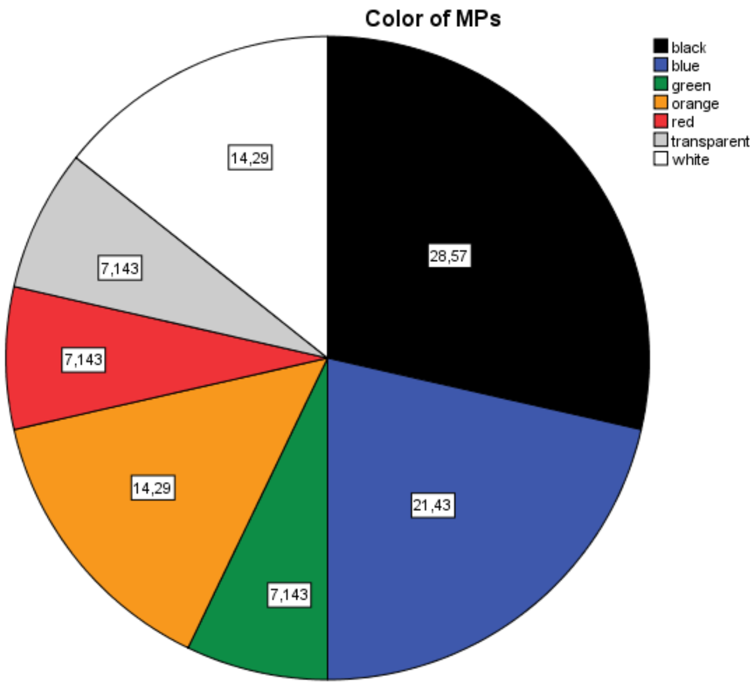


Figure 8. Pie chart showing the percentages of different colors of MPs identified in *Scomber colias*.

Type of MPs					
		Frequency	%	Valid %	Accumulated %
Types	filament	3	21,4	21,4	21,4
	film	1	7,1	7,1	28,6
	fragment	9	64,3	64,3	92,9
	granular	1	7,1	7,1	100,0
	Total	14	100,0	100,0	

Table 3. Summary table for the different types of MPs identified in fish samples.

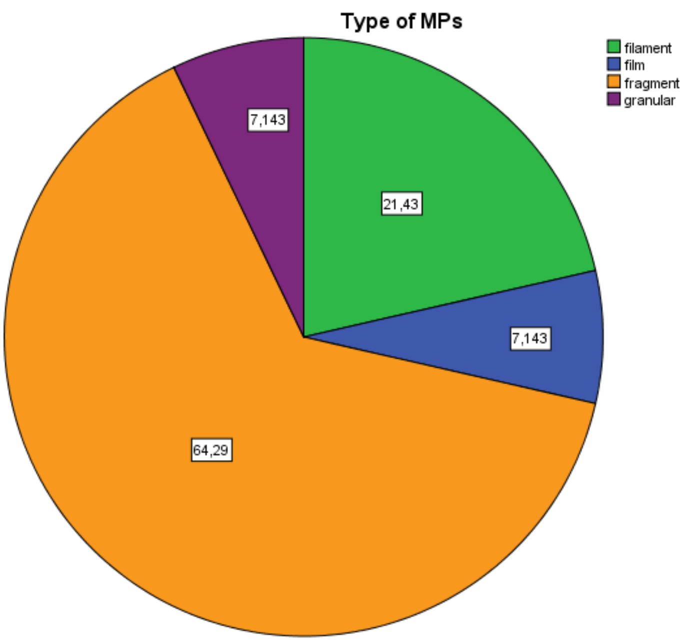


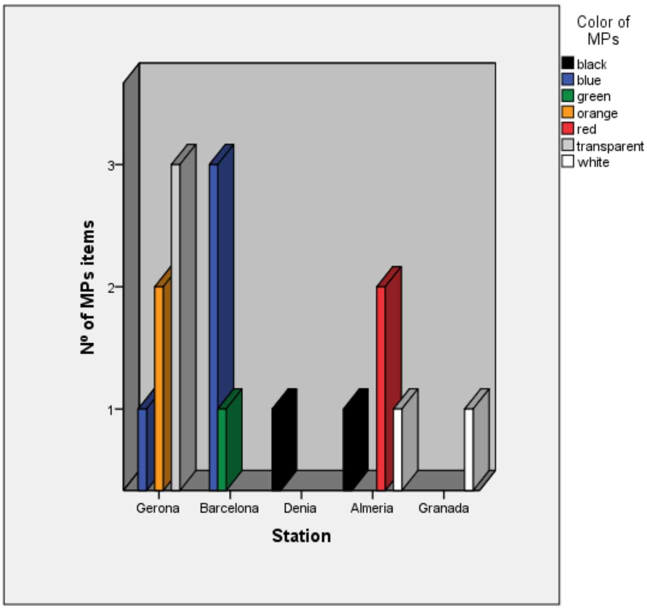
Figure 9. Pie chart showing the percentages for the different types of MPs identified in *Scomber colias*.

As it can be seen in the tables and charts the most abundant color found was black with a 28.6% of occurrence followed by blue with 21.4%, continued by orange and white with the same occurrence (14.3%). Finally other colours identified were: red, green and transparent with 7.1% of occurrence.

As for the type of microplastic most commonly observed (figure 9) these were the fragments with 64.3% of occurrence, followed by filaments with a 21.4% and then by film and granular type with 7.1%.

Color and type of MP identified was varied depending on the different fishing stations, the Gerona station had transparent as the most frequent color and filament was the most frequent type. In Barcelona station the results showed that the color blue and the film type were the most abundant. In Denia station only black color MPs were found as well as in the stations of Almería and Granada (Motril) only MPs of fragment type were found (Figure 10.A and 10.B).

A)



B)

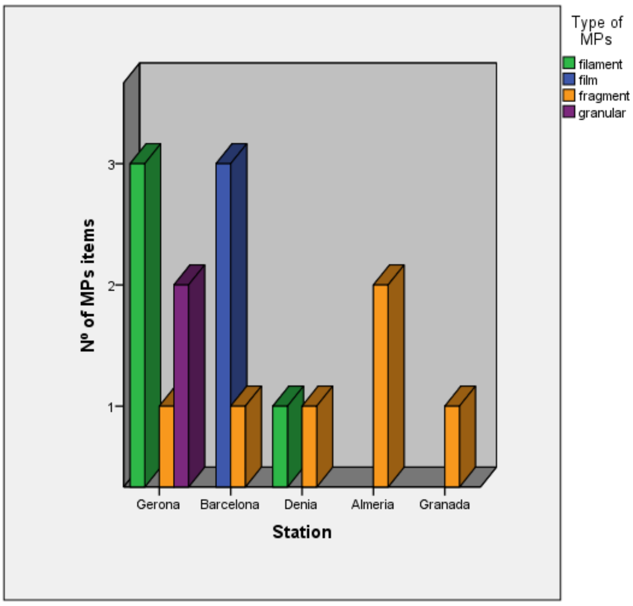


Figure 10. Chart bars of, A) Colors of MPs identified between the fishing stations. B) Types of MPs identified between the fishing stations.

4. Discussion

The ingestion of MP by fishes had already been documented, and as our target species is a commercial species, the data obtained will be of interest on the part of the entire community, not only the scientific.

In the Mediterranean Sea one of the studies that has contributed with data on a commercial species, was that of Nadal et al., (2016) which deals with a species with similar habits to our target species. In the study they found high concentrations of plastic fibers in the gastrointestinal tract of *Boops boops,* in 70% of bogues. Resulting in more than double that the value obtained in *Scomber colias,* and the other interesting fact is that in *Scomber colias* so many filaments or fibers were not found.

Microplastics have been identified in 14 of 63 individuals of the Atlantic chub mackerel *Scomber colias* in the continental shelf along the Mediterranean coast of Spain at about 60 m depth. This species is common in the continental slope distributed from the surface to 300 m deep. As we have already seen, in the Mediterranean Sea the presence of plastics and microplastics has been reported in coastal shallow waters (Alomar et al., 2016) in deep sea areas (Woodall et al., 2014) and in surface waters (Faure et al., 2015).

The study of the gastrointestinal contents of the whole population of mackerel without taking into account any type of differentiation between the size classes, allows to conclude that this type of fish predates all types of existing prey that may be accessible within its area of action (Castro, 1991). This way of feeding could explain why is predating on plastics.

Nevertheless, Lusher et al., (2013) showed in their study that this accidental ingestion of plastic marine litter mixed with food by fish is usually rare or infrequent, considered as sporadic events, which show no temporal or spatial trends and this ingestion probably happens accidentally during normal feeding activity. On the contrary, it is known that various species of fish feed on plastic litter, misleading plastic items with gelatinous preys, indicating that they ingest plastic selectively or in the case of planktivorous fishes it has been suggested that they ingest plastic fibres because they are the same colour as prey items (this is not empirically tested) (Boerger et al., 2010; Choy and Drazen, 2013).

In the present study, 22.22% of the studied fish had ingested microplastic litter with mean values of 0.32(±0.08) microplastics per fish. This ingestion is lower than previously observed by other authors, like Lusher et al., (2013), in the English Channel, where 36.5% of gastrointestinal tracts has ingested microplastic with an average number of 1.90(±0.10) pieces of plastic per fish. However, this values corresponds to both benthic and pelagic species and in our study we have investigated *Scomber colias* in the semipelagic area. Although, Nadal et al. (2016) found higher values for another semipelagic and commercial species (*Boops boops*) with an average value of 3.75(±0.25) plastic items per fish (731 filaments) in the coasts of Mallorca and Eivissa.

In the Canary Islands a study of two planktivorous fishes revealed the presence of microplastics in their gastrointestinal contents. This species are the Atlantic chub mackerel (*Scomber colias)* and the bogue (*Boops boops),* from 64 sampled fish they found evidenced of microplastics in 21 individuals (33%) from the Canary current (Stindlová et al., 2016). The values presented in the same species are higher than ours, this may be due to the fact that the Canary Islands area has larger accumulations of microplastics due to its position and collects all the litter carried by the Gulf Stream.

Those comparisons shows that although our values have not been as high, the concentrations of plastics in the Mediterranean are relevant and are being ingested by semipelagic fishes.

We had sampled individuals from 5 different stations along the Mediterranean coast of Spain, statistically significant differences were not found in the intake of microplastics between stations. Some interesting values were observed, for example from Barcelona (13) we only had 7 individuals and 3 of that had ingested microplastics, with an occurrence of 42.86%. Which could be indicating that being a highly developed area, influences the amount of waste that is concentrated on its coasts. The next area with highest values was Gerona (4) where we had 14 individuals of which 4 ingested microplastic, with an occurrence value of 28.57% (n=4). Then Denia (29) and Almeria (43) stations also with 14 individuals each, presented the same value of occurrence with a 21.43% (n=3). The lowest value was obtained for the Granada (48) station with only 1 individual of 14 with 1 microplastic item in his gastrointestinal tract. The percentage of occurrence was 7.14% this is probably related to the currents that move the waste towards other areas of concentration. Figure 1 shows that the Alboran Sea is one of the areas that had presented lower concentrations of plastic items (%). Another study of the Mediterranean but now of a demersal species of shark *Galeus melastomus* identified plastic in 21 of 125 individuals, with a mean value of 0.34(±0.07), that is pretty similar to our observed value. In addition, another study in Barcelona, western Mediterranean, about the red mullet (*Mullus surmuletus*) found higher values of intake with a 33 % of occurrence (Bellas et al., 2016) which indicates that probably there, species of demersal habitats are ingesting more plastics. In the Portuguese coast another study was carried out with commercial fish species and found evidenced of MP ingestion in 19.8% of the sampled fish, of which 63.5% was benthic and 34.5% pelagic species (Neves et al., 2015).

Recently another study of the Mediterranean Sea, evidenced the ingestion of MP in a 27.3% of the sampled striped red mullet (*Mullus surmuletus*) (Alomar et al., 2017). They stated that microplastic ingestion increases with total length of fish, which is consistent with our results.

Regarding the color and type of microplastics identified in the present study, colors black and blue were the most abundants as well as the fragments and filament types. More fragments than filaments were found, with values of occurrence of 64.3% and 21.4% respectively, which is interesting since in the majority of published studies it is the reverse, more filaments than fragments. For example Lusher et al., (2013) reported in their study that 68.3% of plastics were blue nylon fibres, also Possatto et al. (2011) reported 23% of blue nylon filaments in catfish. According to Lewis et al. (2004) the blue pigments were the most commonly used in the plastics industry, also the fishing nets are mostly of blue color, which explains why in most studies the most frequent plastics are blue.

The effects of microplastic ingestion by *Scomber colias* cannot be predicted in the present study. Nevertheless, it is known that living organisms, with plastic in their gastrointestinal tract, suffer several problems, such as internal injuries, ulcers and tumors or blockages of the digestive tract which produces a false sensation of satiation and reduces their feeding activity and this may lead to inanition (Gregory 2009; Possato et al., 2011). Rochmann et al. (2013) reported an experiment with fishes under different treatments, simulating the wildlife (individuals exposed to a mixture of polyethylene with chemical pollutants sorbed from the marine environment) to study the internal effects to fishes. Results suggested that plastic present in the marine environment serves as vector for the bioaccumulation of persistent bioaccumulative and toxic substances (PBTs), induces hepatic stress and produce cellular necrosis, lesions, fatty vacuolation, and severe glycogen depletion. A more recent study exposed individuals of Silver barb (*Barbodes gonionotus*) to polyvinyl chloride (PVC) fragments at increasing concentrations of 0.2, 0.5 and 1.0 mg/L for 96 h. They documented that were no evidence of histopathological damage to internal tissue after 96h but the intestinal mucosal epithelium thickened, and the activity of trypsin and chymotrypsin increased with increasing PVC exposure. This lack of damage may be attributed to the absence of contaminants associated with the PVC fragments and their relatively smooth surface. The increased whole body trypsin and chymotrypsin activities may indicate an attempt to enhance digestion to compensate for epithelial thickening of the intestine and/or to digest the plastics (Romano et al., 2017).

In their study Alomar et al. (2017) evaluated the potential of microplastic ingestion in causing oxidative stress in fish, but the results showed that MP ingestion causes no evidence of oxidative stress or cellular damage in fish’s liver.

Being a fairly recent field of research, a method for the identification, isolation and quantification of the intake of microplastics in fish has not yet been internationally standardized. If standardization of the methods is reached, the comparison between studies will improved (Lusher et al., 2015).

The results in the present study reveals microplastic ingestion by *Scomber colias* individuals and contributes with data to understand environmental status of the Mediterranean coast of Spain and the quality of fishes that are being consumed by humans in this area. The data presented here could represent a baseline for the implementation of the Marine Strategy Framework directive descriptor 10 in Spain (Bellas et al., 2016).

5. Conclusions

1. Of the total number of individuals analyzed, 22.22% (14 individuals) ingested microplastics in the Mediterranean coast, with mean values of 0.32(±0.08) microplastic per fish.

2. According to all the fishing areas in this study, the one that presented the highest percentage of occurrence was Barcelona, since of only 7 individuals sampled 3 had MPs in their gastrointestinal contents and 5 items of MPs were found in those 3 individuals. Being a large city, very populated with a large influx of tourism and an important industrial area, it is likely that the plastic waste in that area is quite high and thus affecting marine species. It is a possibility that the individuals of *Scomber colias* that feed in that area ingested more plastic particles than those feeding in other areas. In addition individuals from Barcelona were also the largest.

3. The stations that present higher values of Total length (T.L) and Fresh Weight (W) are Gerona and Barcelona, which coincides with the highest percentage of occurrence of intake. Granada is the station with lower values of T.L and W and again coincides with the lowest value of occurrence of intake, which indicates that larger individuals were ingesting more microplastic particles.

4. Other studies also showed the presence of MP particles in the gastrointestinal tract of the Atlantic chub mackerel (*Scomber colias*). This corroborate that microplastics (MPs) are being ingested by this species not only in the Mediterranean Sea.

5. The physiological effects of microplastic ingestion by *Scomber colias* cannot be predicted in the present study, although it is known that living organisms suffer several problems as a consequence of MP ingestion.

6. The most repeated microplastic colour found in our results was black and followed by blue.

7. The fragments, were the type of microplastic that were most commonly found in the total of our samples, unlike the majority of studies already published that always reported more filaments or fibres in the gastrointestinal tracts.

To conclude, it seems to me that studies like this will be important to open the eyes of those who still do not see the problem of plastic in our lives. We must make people aware from a young age, so that they avoid using the plastic unnecessarily and knowing about their chemical nature and persistence in the environment. Also to know the repercussions that this has on marine life and consequently on human health.

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Memoria Trabajo Fin de Titulo

Descripción detallada de las actividades desarrolladas durante la realización del TFT:

Durante mi estancia realizando el Trabajo de Fin de título en el Instituto Español de Oceanografía, Centro Oceanográfico de Baleares. Las tres primeras semanas estuve haciendo sobre todo revisión bibliográfica sobre la contaminación por micro plásticos, los plásticos en el Mediterráneo, las interacciones con la vida marina, sus implicaciones en el medio ambiente, etc. Y así fui poniéndome al día en la temática sobre la que iba a abordar mi trabajo.

Mi trabajo trataría sobre una especie comercial y de hábitat semipelágico la caballa o estornino (*Scomber colias*). Tras acabar con la revisión bibliográfica, comencé con la identificación visual bajo lupa de laboratorio, para ello cada día sacaba del congelador las muestras que iba a analizar y dejaba que se fueran descongelando. Pesaba la muestra en una balanza analítica y procedía a comenzar con el análisis visual, para ello colocaba el tracto gastrointestinal en una placa de cristal, y colocaba a cada lado de la lupa otras dos placas vacías que harían de blanco para contabilizar el posible error por contaminación aérea.

La formación recibida para la realización del TFT

Al tener las enseñanzas de la facultad muy recientes, creo que he puesto muchos de los conocimientos adquiridos durante la carrera en práctica. Sobre todo lo que tiene que ver con las asignaturas de Recursos vivos marinos y Pesquerías, donde aprendimos a realizar muestreos biológicos y análisis de contenidos estomacales. Por lo demás, el tema de identificación de MPs era algo nuevo para mí y he ido aprendiendo con la práctica y la ayuda de mis tutoras externas. Cada día descubría algo nuevo e iba mejorando mis habilidades con la lupa y demás instrumentos del laboratorio.

Aspectos positivos y negativos

Veo muchos aspectos positivos de haber podido venir a este centro a realizar las prácticas y el TFT, la experiencia que vas adquiriendo en lugares así no te la da nada, la oportunidad de compartir con otros profesionales y estudiantes de mi campo, me ha permitido aprender mucho sobre la contaminación marina por plásticos y sus interacciones con las especies marinas, los ecosistemas del mar Mediterráneo; que son algo desconocidos para mí ya que conozco más la región Macaronesica (Canarias). Y por supuesto como algo negativo tenía que tener, también me ha hecho darme cuenta de lo duro que es trabajar como investigador y el largo camino que conlleva. Sobre todo tratándose de una institución pública, donde no es fácil recibir subvenciones y no siempre son suficientes. En mi opinión es un trabajo muy poco valorado, teniendo en cuenta la implicación que requiere, y el trabajo incesante que realizan todos los investigadores de este centro.

Valoración personal

Para terminar, decir que en general valoro positivamente todo el aprendizaje conseguido a lo largo de la práctica, y realización del TFT, creo que me ha hecho aprender mucho sobre todo a comprender como se realiza un trabajo de investigación. Además creo que es en el TFT donde de verdad te das cuenta que has terminado los estudios y te toca poner los conocimientos en práctica, siempre se puede hacer mejor, pero el camino acaba de empezar.

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