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Microplastics FTIR characterisation and distribution in the water column and digestive tracts of small pelagic fish in the Gulf of Lions.

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Authors' contributions
D.B and C.S designed the study. C.S participated in the sampling survey. C.L, D.B and A.N performed the laboratory analyses. C.L, O.H, C.S and D.B analysed the results. C.L, D.B and C.S wrote the manuscript. All authors approved of the final version of the present manuscript.

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Abstract

This study aims at quantifying and characterising microplastics (MP) distribution in the water column of the NW Mediterranean Sea as well as MP ingestion by the 2 main planktivorous fish of the area, sardine and anchovy.

Debris of similar sizes were found in all water column samples and in all but 2 fish guts (out of 169). MP were found in 93% of water column samples with an average concentration of 0.23 ± 0.20 MP.m^{-3}, but in only 12% of sardines (0.20 ± 0.69 MP.ind^{-1}) and 11% of anchovies (0.11 ± 0.31 MP.ind^{-1}). Fibres were the only shape of MP encountered and polyethylene terephthalate was the main polymer identified in water columns (61%), sardines (71%) and anchovies (89%).

This study confirms the ubiquity of MP in the Mediterranean Sea and imparts low occurrence in fish digestive tracts.

Key words: microplastics, Mediterranean, *Sardina pilchardus, Engraulis encrasicolus*, FTIR

1. Introduction

The first modern plastic, called Bakelite, was made in 1907 (Crespy et al., 2008). Nowadays, all market sectors such as packaging, building, automobile, textile, or cosmetics make use of it. Since its conception, the worldwide global demand in plastic has increased every year, reaching 335 million tons in 2016 (PlasticsEurope, 2018); a number that could be much higher as this estimation does not consider some widely-used plastic fibre types such as polyethylene terephthalate, polyamide, polypropylene and polyacryls. Such a massive use of plastics along with mismanagement raises important environmental issues (Barnes et al., 2009). In particular, plastic is the main litter type in marine environments (Andrady, 2011; Bergmann et al., 2015) and no single place can escape plastic anymore (Barnes, 2005): from coasts to open oceans (Cózar et al., 2014; Desforges et al., 2014), or even more remote places such as sea ice from Northern latitudes (Obbard et al., 2014) and the deep ocean (Chiba et al., 2018). As such, at least 268 940 tons of plastics currently float at the surface of the ocean (Eriksen et al., 2014) and 206 kg of plastic are still discharged in it every second (Jambeck et al., 2015). The simplest definition of marine plastic debris is based on three size categories. Macroplastics include plastic pieces measuring more than 2.5 cm, mesoplastics cover the size range from 0.5 cm to 2.5 cm and microplastics (MP) refer to pieces measuring less than 0.5 cm (Galgani et al., 2013). They could display different shapes such as fibre, fragment, film or microbead (Hidalgo-Ruz et al., 2012). MP are scattered in all marine areas and are more abundant than macroplastics as they account for about 92% of total plastic (Eriksen et al., 2014). Because of their small size, MP can be ingested by a wide range of organisms and trophic levels. *In situ* ingestion data were thus reported for various organisms such as copepods (Desforges et al., 2015), bivalves (Van Cauwenberghe and Janssen, 2014), shrimps (Devriese et al., 2015), cetaceans (Lusher et al., 2015), seabirds (Codina-García et al., 2013; Amélineau et al., 2016), and fish (Nadal et al., 2016; Pazos et al., 2017). Further, MP can be mixed up with bioresources or even mistaken for prey especially in species, such as planktivorous ones, for which prey and MP exhibit similar sizes (Amélineau et al., 2016; Ory et al., 2017). In the North Pacific gyre, 34% of planktivorous fish had ingested MP (Boerger et al., 2010), while in Tokyo Bay, MP were ingested by 77% of Japanese anchovies sampled (*Engraulis japonicus*; Tanaka and Takada, 2016).

While some accumulation zones around the oceanic gyres such as the 7th continent in the North Pacific Ocean are now regrettably infamous (Eriksen et al., 2014), our knowledge on other areas is still very scarce. Yet, according to models (Lebreton et al., 2012), the Mediterranean Sea might be an accumulation zone of plastic debris, due to the long residence time of its waters (Lacombe et al., 1981) and few exports to the Atlantic Ocean (Cózar et al., 2015). Cózar et al.

(2015) found that in the Mediterranean Sea, 83% of the total abundance of plastic were MP. Consistently, MP were identified in 90% of the sea surface water samples collected in the North Western Mediterranean Sea (Collignon et al., 2012). This pollution could be an important threat for biodiversity considering that the Mediterranean Sea holds 4% to 18% of all known marine species (approximately 17 000) despite a very small spatial cover (<1% of the global marine waters in terms of area and volume) and has a high level of endemic wildlife (i.e. 10% of the 635 fish species recorded – Coll et al., 2010).

Although the origins of marine plastic litter are not yet well understood due to a lack of studies focusing on freshwater and terrestrial environments, it was assessed that 80% of inputs are land-based (Andrady, 2011). In particular, rivers play an important role in discharging plastic in the ocean; an estimation based on floating plastic in river indicated that between 1.15 and 2.41 million tons of plastic are entering the ocean each year by this pathway (Lebreton et al., 2017; Schmidt et al., 2017). As such, areas with important river discharge, such as the Gulf of Lions in which the Rhone river flows, are of particular interest to study. Indeed, the Rhône river is the main freshwater input in the Mediterranean Sea (Struglia et al., 2004) with a mean annual discharge of 1700 $m^3.s^{-1}$ (http://hydro.eaufrance.fr; last access : 30/11/2018). The Gulf of Lions is a 10 000 km² area with a large continental shelf, in the Northwestern part of the Mediterranean Sea and it is acknowledged as the most productive area of this sea (Bethoux, 1981) as well as a substantial fishing area. The two main small pelagic species occurring in the Gulf are *Sardina pilchardus* and *Engraulis encrasicolus* (Palomera et al., 2007). Along the Mediterranean coasts, sardines and anchovies are widely consumed iconic species. Moreover, in 2015, 15.7% of European sardines and 79.9% of European anchovies of the worldwide captures were caught in the Mediterranean and Black sea (©FAO, 2018, Capture: quantity (t), www.fao.org/figis, last access: 23/01/18). These two Clupeiforme species have trophic similarities in terms of prey types (mostly consisting of copepods – Le Bourg et al., 2015). Although they can both use filtration and particulate feeding (Costalago and Palomera, 2014; Plounevez and Champalbert, 2000), sardines are thought to favour filtration whereas anchovies select particles (Van der Lingen et al., 2006). Therefore, sardines might be more prone to MP ingestion, as their main feeding strategy is non-selective (Collard et al., 2017). On the contrary, particulate feeders could be either able to distinguish prey from plastic particles or will mistake plastics for prey, as their feeding strategy is active. Hence, ingestion of MP may not be the same between these two species owing to their different feeding strategy.

To our knowledge, only two publications broach description of MP at sea surface in the Gulf of Lions (Collignon et al., 2012; Schmidt et al., 2018) but none of them has described MP abundance in the water column or even the polymer types encountered. Here, our goal was (i) to estimate the amount and chemical nature of MP in the integrated water column of the Gulf of Lions, from bottom to surface, using zooplankton net tows, (ii) to assess MP, in terms of quantity and polymer types, in digestive tracts of two main commercial fish species, (iii) to compare MP in the water column with MP from fish digestive tracts and to investigate whether MP ingestion differed between sardines and anchovies thus translating different feeding behaviours.

2. Methods

2.1 Field Sampling

Water column and fish samples used in MP quantification were collected during the PELMED survey (PELagiques en MEDiterranée) in July 2015. This annual survey takes place in the Gulf of Lions in summer in order to assess small pelagic fish populations. Sampling locations and catches of small pelagic species are shown in Figure 1.

For this study, 169 small pelagic fishes (85 European sardines -*Sardina pilchardus*- and 84 European anchovies -*Engraulis encrasicolus)* were sampled using a pelagic trawl net (vertical opening between 15 and 20 m). Individuals were collected at 17 stations in which the two species co-occurred. At each station, five individuals per species (except for station 15, where only 4 anchovies were available) were entirely and directly frozen on board to avoid plastic contamination. Fish trawls lasted for an average of 44 minutes (31- 67 min) at 4 knots and at an average depth of 77 m (from 36 m to 112 m). Zooplankton and debris in the water column were collected after each fish trawl, in the middle of the surface trawled, towing vertically a WP2 plankton net (200 µm mesh), from bottom (or 100 m when bathymetry was higher) to surface. The average maximum depth of water column station was 67 m. A flowmeter was used to calculate the volume of filtered water. Among the 17 stations mentioned above, water column sampling did not occur in 3 of them due to unfavourable weather conditions. Water column samples were stored in 125 ml vials and fixed with formaldehyde 4% allowing identification of the zooplanktonic species and estimation of MP abundance.

2.2 Digestive tract dissection

Fish were thawed back at the laboratory, weighed and measured (total length from the tip of the snout to the tip of the tail). Body condition (K) was calculated according to Le Cren (1951) and Van Beveren et al. (2014), given that this index is the most adapted to fit these species. However, Fulton’s index (F) described by Froese (2006) was also calculated in order to compare with Compa et al.’s study (2018).

$$\text{For sardines: } K_1 = \frac{\text{Weight}}{5.9 \times 10^{-3} \times \text{Length}^{3.1}}$$

$$\text{For anchovies: } K_2 = \frac{\text{Weight}}{3.86 \times 10^{-3} \times \text{Length}^{3.2}}$$

$$\text{Fulton's index } = 100 \times \frac{\text{Weight}}{\text{Length}^3}$$

During dissection, sex of individuals was determined by macroscopic observations (male, female, immature). Sex-ratio (SR) was calculated according to the following equation:

$$SR=\frac{\text{Number of males}}{\text{Number of males + Number of females}}$$

Then, the entire digestive tract – from oesophagus until the end of intestine – was isolated and put in a glass petri dish with filtered sea water (0.2 µm). Oesophagus, stomach, pyloric caeca and intestine were cut off and completely emptied of their content with tweezers.

2.3 Debris visual sorting

Microplastics were extracted using a common methodology of visual sorting under a stereomicroscope for both water and fish samples (Nikon SMZ25 and Zeiss stemi 2000-c for water and fish samples respectively, ranges of magnification from x6.5 to x50 in both cases) to avoid methodological biases. Visual characteristics described by Zhao et al. (2016) were used to recognise debris that could be MP. Briefly, identification of non-biological particles was based on surface characteristics, morphology and physical response. No cellular or organic structure, such as segmentation or ornamentation, must be seen. Fibre diameter should be equal along all ends and not tapered.

Collected debris were then placed in Eppendorf tubes with filtered sea water and classified into two categories, fibres and fragments, based on their shape (Zhao et al., 2016). Colours were also reported and then gathered in four categories: light, dark, blue (frequent colours) and other (rare colours such as red, pink, yellow or orange).

2.4 Size of debris

After homogenisation, each Eppendorf tube was emptied and debris were observed under a Leica M80 stereomicroscope connected to a camera (x7.5 – x60). The Leica Application Suite (V4.5.0) was used to take photos of the debris collected both in the water column and in the fish digestive tracts. Photos were then analysed using Image J (V1.50d) to measure the size of each debris in pixel. Finally, distances in pixels were converted in millimetres through a conversion table established using a photography of a micrometric slide for each magnification. Quantification and comparison of the smallest size class (0 – 250µm) could be affected by the 200 µm mesh size of the plankton net, although this size class was found in very small quantity in fish guts as well (<1%).

2.5 Fourier Transform InfraRed (FTIR) spectroscopy

Polymer types of debris were then characterised by using Fourier Transform InfraRed spectroscopy (PerkinsElmer) in ATR (Attenuated Total Reflection) mode. Infrared light was configured at wavelengths ranging from 4000 to 600 cm^{-1}. Debris were put individually on the diamond of the spectroscope and then pressed. Given this handling, only a subsample of 1085 debris of the 2165 visually sorted debris was investigated. To define the subsample, debris of each sample were first examined under a stereomicroscope and pooled if they were strongly similar in shape, colours, curves and borders.

Spectra were recorded by the software Perkin Elmer version Spectrum (10.4.3; 2014). The quality of each spectrum was assessed by checking peaks, transmittance range, libraries and recurrence of results to be considered as exploitable. Spectra were then compared to spectral libraries (Table S1, supplementary material) to establish a list of potential polymer correspondences. Spectrum identifications were directly validated if they showed a percentage of correspondence superior to 70%. Spectra with lower matches were visually examined for polymer identification and compared with those obtained within the same sample.

2.6 Avoiding and quantifying contamination

Throughout the different manipulations, 100% cotton lab coats and nitrile gloves were worn. Working places and all labwares (dissection tools, glass petri dishes, stereomicroscopes, watch glasses, etc.) were cleaned with 75% ethanol. This procedure helps in removing debris from tools and reducing the risks of contamination. Furthermore, air circulation and access to the laboratory were limited.

In order to quantify airborne contamination, glass petri dish filled up with filtered sea water were set up close to the analysed sample and were used as contamination controls (17 controls were realised). Then, they were analysed with the same processes of detection, storage and characterization as other samples.

2.7 Statistical analysis

Results such as fish sizes, debris sizes or MP concentrations in water and digestive tracts are presented as mean values ± standard deviation and ranges in square brackets.

Size debris similarity between samples (water, sardines and anchovies) was estimated by calculating the overlap percentage of size class debris between two samples according to the equation: $\text{Overlap} = \frac{2\times(\text{sizes found in both sample only})}{\text{all sizes found in samples}}$

This index varies between 0 (when no common size is shared between the two samples) and 100% (when all sizes are common between the two samples).

All statistical analyses were run using R software (V. 1.0.143). When using individual data, mixed models were used with the sampling site as a random intercept to consider the non-independence of data of fish caught in the same trawl. Model selection was performed based on Akaike's Information Criterium (AIC). The model with the lowest AIC was selected, except when the difference between the two AIC was smaller than 2, in which case the most parsimonious model was selected (Burnham and Anderson, 2002). Debris sizes were compared using a linear mixed model (LMM) in which species was tested as an explanatory variable. The effect of variables such as species, length, body condition index and sex on the number of debris ingested was tested by using generalized linear mixed models (GLMM) with Poisson distribution.

Additionally, non-parametric statistical tests were run when parametric assumptions were not valid. Correlations between variables or samples were tested with Spearman's rank correlation, and a chi-squared test was used to compare polymer types and colour proportions between different types of samples. For MP ingestion data, a Wilcoxon–Mann–Whitney test was made to compare the number of MP ingested between species. Finally, to determine the potentially non-linear influence of some variables on the number of MP, a regression tree was built. Significance level was fixed at 0.05 for each statistical hypothesis testing.

3. Results

3.1 Pelagic trawls

Anchovy and sardine accounted for 78% of the total biomass of trawled fish in the 17 stations under study (Figure 1). Sardines were more present in coastal stations, while anchovies clearly dominated offshore. Sardines showed a mean length of 11.72 ± 1.00 cm and an average weight of 14.14 ± 4.02 g while anchovies were 11.25 ± 0.90 cm long and weighed 9.53 ± 2.78 g on average (Table S2, supplementary material). Mean body condition (K) was 1.15 ± 0.09 and 1.03 ± 0.11 respectively for sardines and anchovies. Sex ratio was balanced for anchovies (SR = 0.5) whereas there were slightly more males in sardine samples (SR = 0.6).

3.2 Amount, composition and size of debris

After visual sorting, debris were discovered in all water column samples and in 98.8% digestive tracts from sardines and anchovies. In the water column, the average concentration was 3.08 ± 3.04 debris.m^{-3}. Sardines ingested more debris than anchovies (8.56 ± 6.67 debris vs. 7.12 ± 4.81 debris per sardine and anchovy respectively; GLMM, Z = 2.037, p-value = 0.04, N = 169). On average, 0.88 ± 1.36 debris were found in the contamination controls. Debris sizes were very similar whether in the water column or in sardine and anchovy stomach (1.81 ± 1.42 mm [0.24 - 4.93 mm], 1.77 ± 1.67 mm [0.10 - 4.95 mm] and 1.81 ± 1.52 mm [0.21 - 4.99 mm] respectively in the water column, sardines' and anchovies' digestive tracts; LMM: null model being the best model, ΔAIC = 9.332, N = 1618; Figure 2). This was further confirmed by very high overlaps between all three size distributions (≥ 96%; Figure 2). The most represented size classes comprised debris between 0.5 and 1.5 mm (representing 47.4%, 46.8% and 48.3% of the debris ingested by anchovy and sardine or found in the water column respectively). A large majority of fibre-shaped debris was encountered (99.1%) and only a very small portion of debris was fragment-shaped (0.9%). Light colour debris prevailed in all three sample types (58 %, 69 % and 64% respectively for water column samples, sardines' and anchovies' digestives tracts). Dark debris were recovered in 20% of water column samples, 14% and 12% of sardines and anchovies' digestive tracts respectively, while blue debris accounted respectively for 15%, 10% and 15%. Other colours were rarely observed (water column: 7%, sardine: 7%, anchovy: 9%).

3.3 Microplastics characterisation

After FTIR analyses, 16% of sample showing an exploitable spectra were microplastics. Once extrapolated to all debris sorted, MP contribution to total debris amounted to 7.7% for the water column, 2.3% for sardines and 1.5% for anchovies (Table 1). None of the 17 controls showed airborne contamination by MP.

Exclusively fibre-shaped MP were reported in digestive tracts and water column samples. In total, 61 MP were quantified in water column samples, 17 in sardines' and 9 in anchovies' digestive tracts (Table 1). In the water column, MP were found in 93 % of samples at an average concentration of 0.23 ± 0.20 $MP.m^{-3}$, while they were ingested respectively by 12% and 11% of all sardines and anchovies studied. As such, we first studied data in the form of presence/absence to compare length and body condition index of fish with and without MP in their gut. There were no total length or body condition differences between sardines or anchovies that had ingested MP and those without MP in their digestive tract (Figure 3). Furthermore, the number of MP ingested was not correlated to body condition index (K) of sardines (S = 103320, p-value = 0.68, rho = -0.05, N = 85) nor to anchovies (S = 91009, p-value = 0.48, rho = 0.08, N = 84). Sardines ingested 0.20 ± 0.69 $MP.ind^{-1}$ on average, while anchovies ingested 0.11 ± 0.31 $MP.ind^{-1}$ (Table 1). Although sardines ingested twice as much MP per individual, this difference was not significant due to a very high inter-individual variability (Wilcoxon-Mann-Whitney, W = 3552, p-value = 0.73, N = 169). Furthermore, the abundance of ingested MP per station was neither correlated between species (S = 1165, p-value = 0.09, Rho = -0.43, N = 17) nor between species and water column (sardine: S = 484.18, p-value = 0.83, Rho = -0.06; anchovy: S = 550.17, p-value = 0.47, Rho = -0.21; N = 14).

The main polymer types encountered in water column were polyethylene terephthalate (PET; 61%; Table 2) followed by polyamide (PA; 31%), polyvinyl chloride (PVC; 5%), polypropylene (PP; 2%) and polyacrylonitrile (PAN; 2%). In sardines' digestive tracts, 4 types of polymers were detected which first consisted in PET (71%), then polyethylene (PE; 18%), PA (6%) and PP (6%) while in anchovies only 2 kinds of polymers were uncovered and chemically identified: 89% of them being PET and 11% PE (Table 2). Polymer proportions were similar between water and sardine samples (χ^2 = 20.417, p-value = 0.67, N = 14), water samples and anchovy (χ^2= 15.225, p-value = 0.51, N = 14) and between species (χ^2= 5.1, p-value = 0.75, N = 17). MP from water column were mostly light coloured (51%) as in sardines' (44%) and anchovies' (59%) digestive tracts (Table 1). Proportions of recorded colours between water column and fish samples and between species were similar (sardine-water: χ^2= 29.75, p-value = 0.58; anchovy-water: χ^2= 19.542, p-value = 0.24, N = 14; sardine-anchovy: χ^2= 4.4968, p-value = 0.81, N = 17).

3.4 MP spatial distribution

Sardines from the North-eastern part of the Gulf of Lions seemed to have ingested more MP than those caught on the South-western part of the Gulf (Figure 4). The greatest mean ingestion of MP by sardines was at North-East station 26 (1 ± 2.24 $MP.ind^{-1}$ with one individual presenting up to 5 MP) while fish from several Western stations did not ingest any MP. The number of MP in sardines' digestive tracts was correlated with longitude (S = 76501, p-value = 0.02, rho = 0.25, N = 85). According to regression tree method, combined effects of longitude and distance to the shoreline described three groups. The first one gathered two stations in the Eastern part of the Gulf, located at less than 13.74 Km from the coastline, where ingestion was maximal (mean = 0.8 ± 1.6 $MP.ind^{-1}$). The second group was made up by two Eastern stations located at longitudes superior to 4.389 and at more than 13.74 Km from the coastline, where ingestion was twice higher than the average (mean = 0.4 ± 0.70 $MP.ind^{-1}$). The last group was formed by stations at longitude lower than 4.389, where ingestion was minimal (mean = 0.08 ± 0.32 $MP.ind^{-1}$). Still, there was no direct linear correlation between the number of MP ingested and latitude (S = 85278, p-value = 0.12, rho = 0.17, N = 85), distance from the shoreline (S = 111090, p-value = 0.44, rho = -0.08) or depth (S = 107300, p-value = 0.66; rho = -0.04).

On the contrary, ingestion of MP by anchovies seemed to be lower in North-eastern stations (Figure 4). Maximal ingestion was reported at stations 10 and 28 (0.4 ± 0.52 $MP.ind^{-1}$). Abundance of ingested MP was not correlated to longitude (S = 120710, p-value = 0.10, rho = -0.18, N = 84), latitude (S = 104740, p-value = 0.79, rho = -0.03), distance to the coast (S = 106330, p-value = 0.72, rho = -0.04) or depth (S = 104740, p-value = 0.83, rho = -0.01). No spatial pattern was clearly determined with the regression tree and mean ingestion was 0.11 ± 0.31 $MP.ind^{-1}$.

Spatial distribution of MP from water column samples was also heterogeneous (Figure 4). Minimal concentration occurred at coastal station 10 (0 $MP.m^{-3}$), located at the North Western part of the Gulf of Lions, while maximal concentration was observed at station 49 (0.7 $MP.m^{-3}$), which is more offshore and situated at the Eastern part of the studied area. Relying on the explanatory variables available, no spatial distribution pattern was indicated by the regression tree method. In addition, no correlation was shown between the concentration of MP and longitude (S = 416, p-value = 0.77, rho = 0.08, N = 14), latitude (S = 508, p-value = 0.69, rho = -0.11), distance to the coast (S = 327.86, p-value = 0.33, rho = 0.28) or depth (S = 248.96, p-value = 0.10, rho = 0.45).

4. Discussion

The Mediterranean Sea is prone to several anthropic pressures that could generate marine plastic pollution such as mass tourism, important density of coastal populations, fisheries activities or sea transports. Debris smaller than 5 mm were indeed found in all water column samples and in almost every fish digestive tract in relatively high abundance. Anthropogenic particles may thus be a considerable contaminant in the studied environment and could be frequently encountered by the local fauna. Debris' size and colour found in water column samples and digestive tracts of both species were almost identical (Fig. 2), suggesting that small pelagic fish could be good indicators of environmental conditions.

However, the presence of debris is not synonymous with microplastics occurrence. Indeed, characterisation of these particles by FTIR indicated that most identifiable debris were not MP (Remy et al., 2015). In this study, the contribution of MP to the sorted debris (i.e. percentage of MP among all debris) represents even smaller percentages than what has been observed before in fish digestive tracts (1.5 and 2.3% in this study *vs*. 7.7%; Zhao et al., 2016 and 29.2%; Obbard et al., 2014) or in the water (7.7% *vs*. 16.7% in the Arctic Ocean; Amélineau et al., 2016). These low contributions underline the importance of polymer determination to avoid MP overestimation after visual sorting despite strict guidelines. Nevertheless, spectral analysis is not yet systematically performed due to time issues and budgetary constraints, impairing the possibility to compare studies and to run meta-analyses.

Most of the debris and absolutely all MP isolated in this study were observed under the shape of fibres, confirming that fibre shape is highly present (Barrow et al., 2018) and likely ubiquitous in marine systems (Claessens et al., 2011; Kanhai et al., 2017). Across the world, fibres were already found at very high rates in seawaters (Lusher et al., 2014; Barrows et al., 2018), and in fish digestive tracts (Lusher et al., 2013; Nadal et al., 2016; Murphy et al., 2017; Pazos et al., 2017; Peters et al., 2017; Vendel et al., 2017). Nonetheless, fibres are not systematically considered as MP and are sometimes excluded from datasets as they may come from air contamination (Dris et al., 2016). Our results highlight the need for a particular attention to fibre shaped MP due to their ubiquity in aquatic environments and recurrent ingestion.

In the Mediterranean Sea, 90% of the stations sampled at the surface layer of the North Western part presented MP (Collignon et al., 2012); 81% in the Central Western part of the sea, (Panti et al., 2015) and 100% in the Eastern Mediterranean waters (van der Hal et al., 2017). This is in accordance with our study that also displays a high occurrence of MP in water column samples suggesting that MP are spread all around the Gulf of Lions.

Despite such a broad occurrence, MP concentration seems to vary very importantly across regions, ranging from 0.021 ± 0.015 MP.m^{-3} along the Portuguese coasts (Frias et al., 2014) to 7.68 ± 2.38 MP.m^{-3} in the Israeli Mediterranean surface water (van der Hal et al., 2017), although polymer identification was not performed in the last study and concentrations may thus be overestimated to a certain extent. Such high differences across the world could be explained by (i) differences in methodology to identify MP (e.g. polymer analysis or not), (ii) surface waters sampling versus integration of the entire water column, (iii) oceanographic processes such as local currents or winds, and (iv) socio-geographical factors such as coastal geography, coastal population, and distance from plastic source input (Barnes et al., 2009). Focusing on studies which performed FTIR identification, MP concentrations varied between 0.021 ± 0.015 MP.m^{-3} in Portugal (sea-surface and 25 m depth samples combined; Frias et al., 2014) and 3.74 ± 10.4 MP.m^{-3} in sea surface waters that surround Japan (Isobe et al., 2015). In comparison, MP concentration observed in our study was low to intermediate (0.23 ± 0.20 MP.m^{-3}), a result similar to the only study conducted in the Mediterranean Sea which integrated the water column and used FTIR analysis (0.22 ± 0.57 MP.m^{-3} in the North Tyrrhenian sea in spring; Baini et al., 2018) as well as other studies which have sampled only the surface waters in Sardinia (0.17 ± 0.3 MP.m^{-3} using WP2 plankton net; Panti et al., 2015 and 0.15 MP.m^{-3} using Manta trawl; de Lucia et al., 2014).

Our results thus indicate that the concentration of MP could be as important in the water column as in the surface layer (de Lucia et al., 2014; Baini et al., 2018). This reinforces previous work, which showed that MP could be present in the entire water column and that maximal concentration could be found below the surface (between 30 and 60 m during summer in the Baltic Sea, Gorokhova, 2015). Knowledge about MP in the water column is still scarce and more data are needed to have a better understanding on the vertical distribution of MP, the factors influencing it and on describing interactions with pelagic organisms. In particular, physical and hydrodynamic features could affect vertical distribution such as thermo/halocline, with the combined effect of biofouling (Lobelle and Cunliffe, 2011) or ingestion (Cole et al., 2013). Further, polymer types have different density (Hidalgo-Ruz et al., 2012) and thus should be distributed at different depths in the water column. At sea surface, polymers with low density such as PE and PP are commonly characterised (Frias et al., 2014; Enders et al., 2015; Suaria et al., 2016; Ory et al., 2017). Here, PP and PE (use in packaging) contributions were low probably due to the small water volume collected at sea surface by the vertical plankton tow. On the contrary, polymers like PET and PVC have higher densities (Morét-Ferguson et al., 2010) and are generally less recovered at the surface. In our study, PET, a polymer commonly used for soft drink bottles, was largely dominant in the water column samples, which is consistent with the sampling method (vertical tows). Similarly, PA was the second most abundant polymer found in our study, which may result from the important fisheries activities in this area and its high density (Bjordal et al., 2002).

Finally, in our study, the highest MP concentrations were found in the eastern part of the Gulf, in accordance with the high concentration of plastic predicted by a recent model in this area (Liubartseva et al., 2018). Nonetheless, no general spatial pattern was discerned in our results and none of the studied parameters (longitude, latitude, depth, distance to the coast) seemed to drive the spatial distribution of MP in the water column of the Gulf of Lions. In the literature, different and even opposite relationships have been found between MP concentration in the sea and geographical or physical parameters. For instance, MP concentration could either decrease with increasing distance to the coast (Desforges et al., 2014; Panti et al., 2015), or remain the same (de Lucia et al., 2014; Baini et al., 2018; Gorokhova, 2015). In the Gulf of Lions, the absence of clearly defined spatial pattern could be due to several parameters such as the complex water circulation forming small eddies inside the Gulf (http://marc.ifremer.fr/resultats/courants/modele_mars3d_mediterranee, last access : 10/05/2018), other currents such as the Ligurian Current (Ourmieres et al., 2018), recurrent strong winds arising in summer (Millot, 1999) or storm events (Collignon et al., 2012).

Despite being present in all but one water sample, microplastics were recovered in only one out of eight or nine fish on average, resulting in a relatively low average concentration of MP in digestive tracts for both species. Such an intermediate level of ingestion is in agreement with a recent similar study (same methods, season and year) in a neighbouring area (Compa et al., 2018). In the North Sea, even lower occurrence of MP were described in fish digestive tracts (0.25% and 2.60%; Foekema et al., 2013; Hermsen et al., 2017), while higher occurrences have been found in the Portuguese coast (19.80%; Neves et al., 2015), the Balearic Islands (27.30%; Alomar et al., 2017) and in Tokyo bay (77%; Tanaka and Takada, 2016). This variation in ingestion might of course appear between fish species due to differences in their ecology, spatial distribution and feeding behaviour. Here, both species ingested comparable abundance of MP from similar polymer type and colours, suggesting that their feeding behaviour might be similar and that they are representative of MP occurring in their environment.

Further, previous studies suggested that a single species could also display different levels of MP ingestion (from 0.20 to 2.4 $MP.ind^{-1}$ for sardines and 0.11 to 0.85 $MP.ind^{-1}$ for anchovies; see Table 3). Methodological differences could explain part of this variation. Indeed, sample sizes varied between 7 and 105 organisms in these studies and one of these studies considered MP until

10 mm rather than 5 mm. Nevertheless, the heterogeneity of MP concentrations ingested by fish, shown in Table 3, could be due to the to the magnitude of pollution of surrounding waters that probably plays an important part in ingestion. For instance, Japanese anchovies from the heavy polluted area of Tokyo bay displayed the highest ingestion observed (2.3 ± 2.5 MP.ind^{-1}; Tanaka and Takada, 2016). Looking at a more local scale, no correlation was highlighted between the concentration of MP in fish digestive tracts and in the water column or any of the spatial parameters investigated in our study or in Compa et al. (2018). Small pelagic fish are constantly moving and can be distributed all around the Gulf of Lions (Saraux et al., 2014), so they potentially did not ingest MP at the site they were collected, making correlations harder to underline. Nonetheless, according to the regression tree, longitude and distance to the coast might have an effect on MP ingestion in sardines. Indeed, the two groups presenting the highest MP ingestion were the closest to the Rhone river mouth and therefore to a plastic input source, although the small number of stations in this area prevents conclusive results.

Besides spatial distribution, MP ingestion should also be affected by fish vertical distribution. While small pelagic fish can use the entire water column, they usually stay relatively low in the water column in summer (all trawls used in this study were close to the bottom). As such, pelagic fish may come in contact more frequently with denser polymers than floating MP. This might explain why PET was the most ingested polymer type in this study and others (Alomar et al., 2017; Compa et al., 2018). Here, as in Compa's et al. (2018) and Rummel's et al. (2016), PE was the second MP type occurring in fish gut despite its low density, suggesting that other phenomena might modify their vertical distribution or attractiveness. Actually, polymers could be colonised by microorganisms and biofouling may increase MP density and mass, thus enhancing their bioavailability for pelagic fish (Morét-Ferguson et al., 2010). Moreover, this biological activity could also lead to a dimethylsufide signature (DMS) acquirement (e.g. for PE and PP; Savoca et al., 2016). The smell emitted by DMS might play a role in trophic interactions by signalling prey availability as shown for procellariform seabirds (Savoca et al., 2016) and suggested for fish (Savoca et al., 2017). Once ingested, harmful effects of MP on fish are unclear. Laboratory experiments indicate that predatory performance could be affected (de Sá et al., 2015), detoxification system induced (Alomar et al., 2017) and even that neurotoxic effects could appear (Oliveira et al., 2017). Some studies used body condition to estimate the state of health of wild caught fish. For instance, omnivore fish can show lower body conditions when displaying high abundance of MP ingested (Mizraji et al., 2017). Compa et al. (2018) also revealed that sardines with lowest body condition (F) ingested more anthropogenic particles whereas no relationship was described for anchovies. Here, regardless of the body condition index used (K and F), ingestion of MP was not related to the body condition of any of the two studied species as described in North Sea fish (Foekema et al., 2013). In the Gulf of Lions, sardine and anchovy have been smaller and thinner for a decade (Van Beveren et al., 2014). Here, our results point out that MP ingestion is not responsible for this issue and does not even appear to work in synergy. The main hypothesis for these changes thus remains a shift in plankton community affecting these species' diet (Brosset, 2016; Saraux et al., 2018).

Overall, the average MP concentration recorded in the water column was lower than in accumulation zones but it was comparable with concentrations assessed in the Mediterranean Sea (sea surface and water column) and small pelagic populations are not ingesting high concentration of MP. Further, we showed that in order to monitor MP in seawater and in organisms as advised by the MSFD framework, standardised methods for sampling, extracting and identifying MP need to be developed.

With several questions still being unresolved, it is clear that not only the scientific community is concerned. All stakeholders, such as legislators, manufacturers and citizens must think about using plastics in a more environmentally responsible way. An easy concept, named the "5R", can summarise actions that are possible to take: Reduce, Reuse, Recycle, Redesign, Recover (Thompson et al., 2009).

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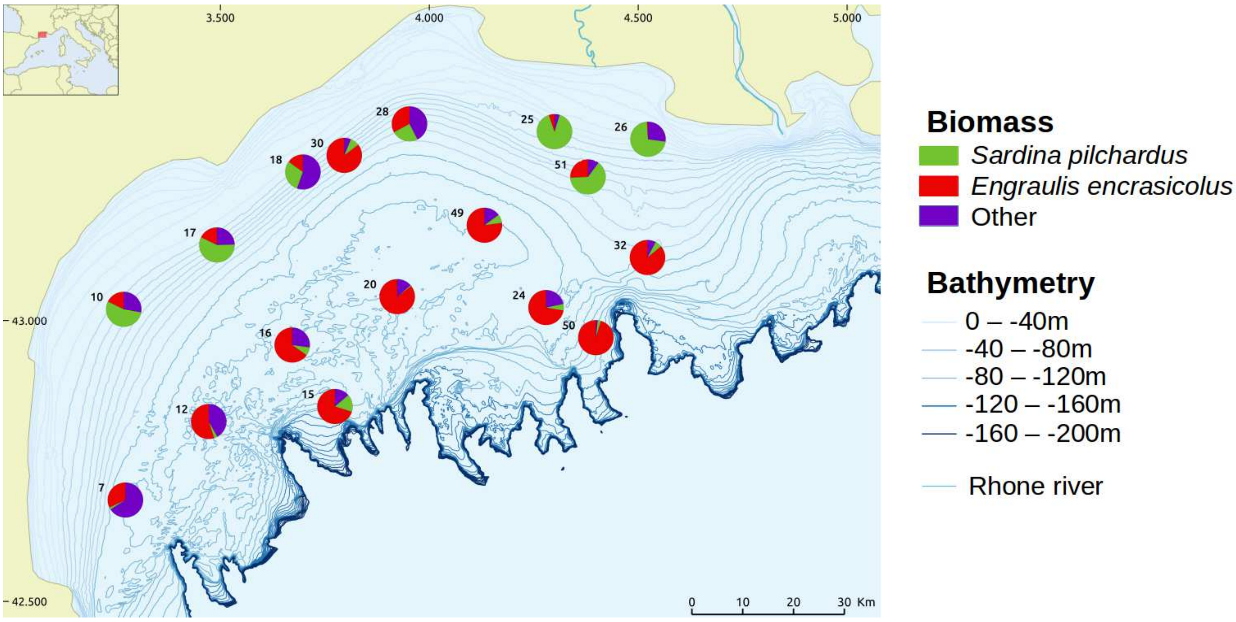
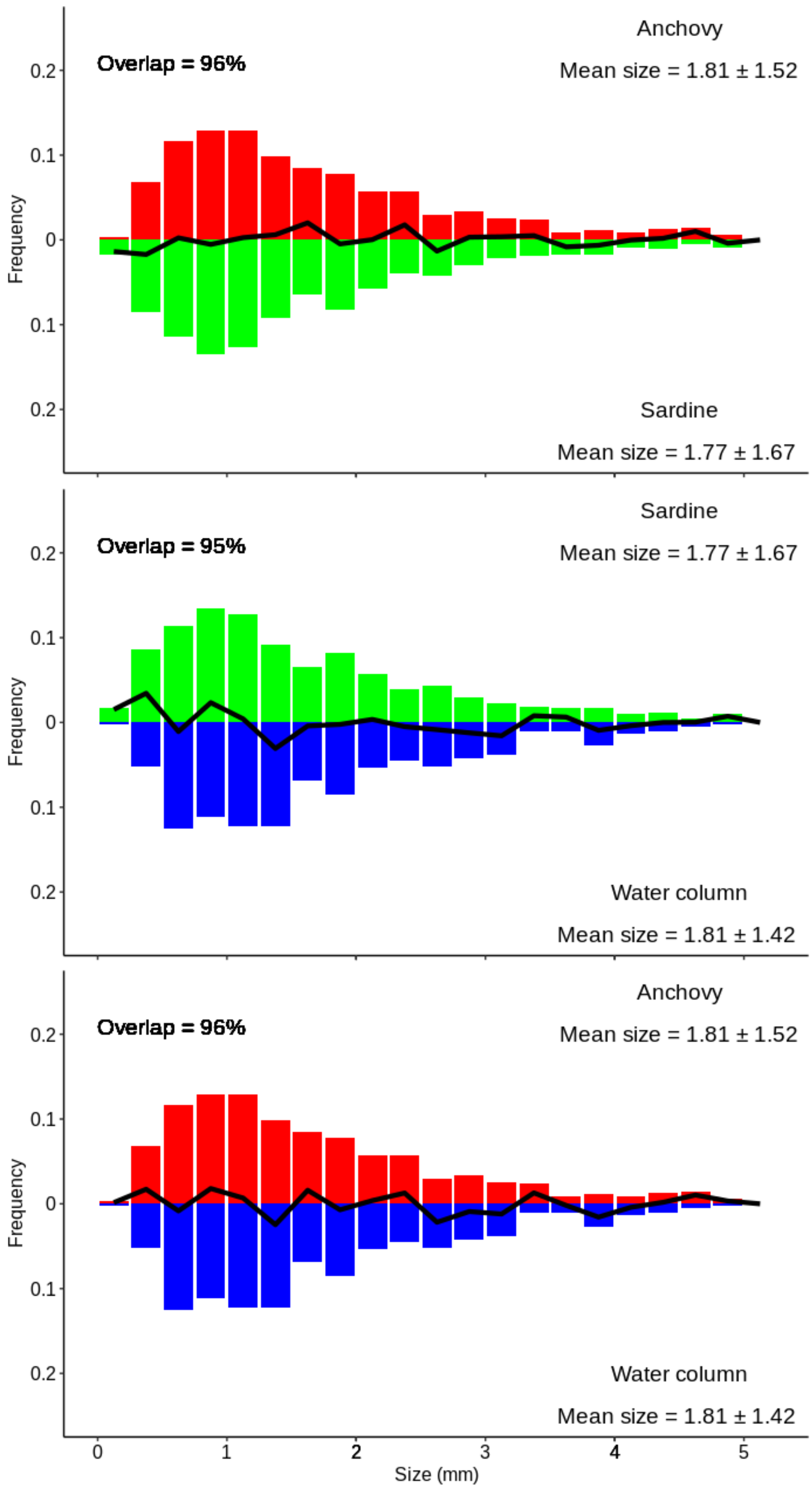


3.500
4.000
4.500
5.000
43.000
42.500
28
30
18
25
26
51
17
49
32
10
20
24
50
16
15
12
7
0 10 20 30 Km
Biomass
Sardina pilchardus
Engraulis encrasicolus
Other
Bathymetry
0 – -40m
-40 – -80m
-80 – -120m
-120 – -160m
-160 – -200m
Rhone river

Figure 1



Anchovy
Overlap = 96%
Mean size = 1.81 ± 1.52
Frequency
Sardine
Mean size = 1.77 ± 1.67
Sardine
Overlap = 95%
Mean size = 1.77 ± 1.67
Frequency
Water column
Mean size = 1.81 ± 1.42
Anchovy
Overlap = 96%
Mean size = 1.81 ± 1.52
Frequency
Water column
Mean size = 1.81 ± 1.42
Size (mm)

504 Figure 2

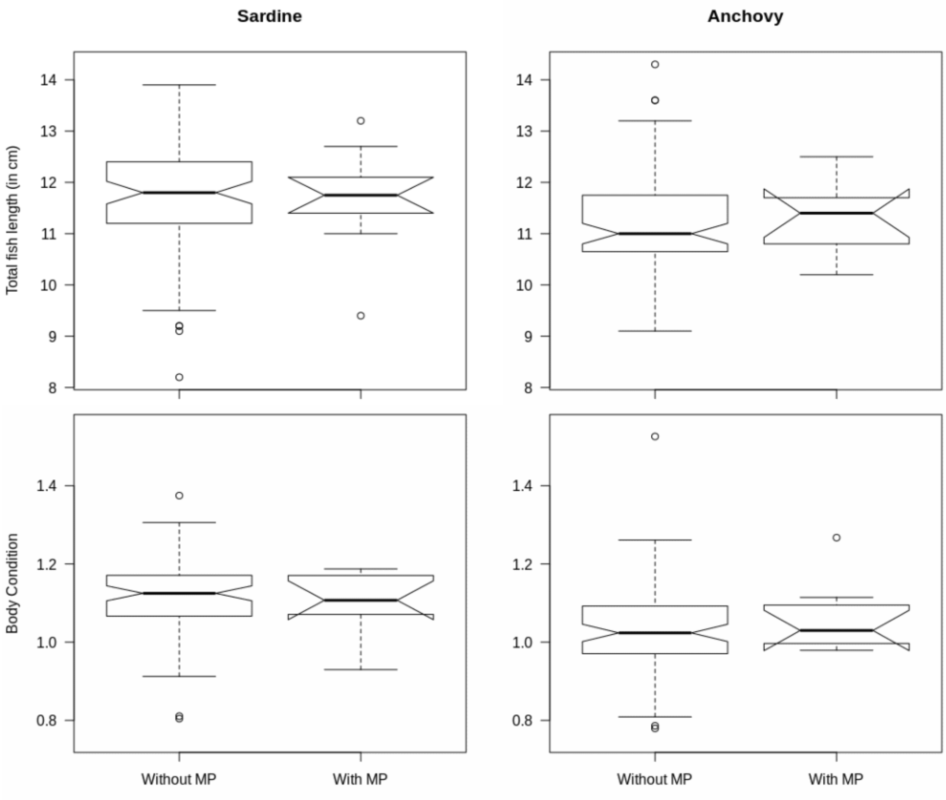
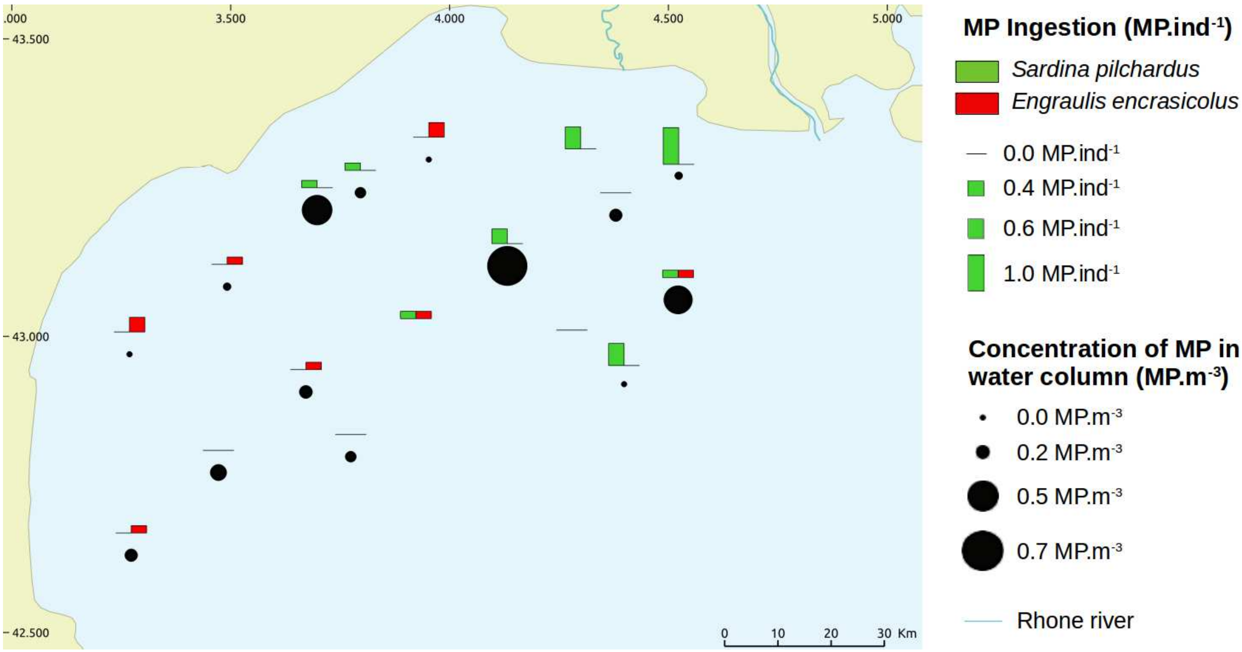

Sardine
Anchovy
Total fish length (in cm)
Body Condition
14
13
12
11
10
9
8
1.4
1.2
1.0
0.8
Without MP
With MP
Without MP
With MP

Figure 3



507
508 Figure 4

Table 1

	MP contribution (%)	Total number of MP determined	Occurrence of MP in total samples (%)	Concentration of MP (MP.m^{-3}) (MP.ind^{-1})	Colours (%)			
					Light	Blue	Dark	Other
Water column	7.7	61	93	0.23 ± 0.20	51	18	29	2
S. pilchardus	2.3	17	12	0.20 ± 0.69	59	12	12	17
E. encrasicolus	1.5	9	11	0.11 ± 0.31	45	22	22	11

Table 2

	PET (%)	PA (%)	PE (%)	PAN (%)	PVC (%)	PP (%)
Water column	61	31	0	2	5	2
S. pilchardus	71	6	18	0	0	6
E. encrasicolus	89	0	11	0	0	0

Table 3

Species	Sample size	Location	Year	Concentration (item.ind^{-1} ± SD)	Occurrence in fish	Authors
Sardina pilchardus	20	English Channel	2013	0.55	45 %	Collard et al. (2017)
	105	Spanish Western Mediterranean coasts	2015	0.21 ± 0.23*	15 %	Compa et al. (2018)
	7	Turkish Mediterranean coast	2015	2.14	57 %	Güven et al. (2017)
	12	Portuguese coasts	2013	0.00 ± 0.00	0 %	Neves et al. (2015)
	85	Gulf of Lions	2015	0.20 ± 0.69	12 %	This study
Engraulis encrasicolus	20	Gulf of Lions	2013	0.85	40 %	Collard et al. (2017)
	105	Spanish Western Mediterranean coasts	2015	0.18 ± 0.20*	14 %	Compa et al. (2018)
	84	Gulf of Lions	2015	0.11 ± 0.31	11 %	This study
Engraulis japonicus	64	Tokyo bay	2015	2.30 ± 2.50	77 %	Tanaka & Takada (2016)
Decapterus muroadsi	20	Easter Island	2015	2.50 ± 0.40	80 %	Ory et al. (2017)
Liza aurata	39	Turkish Mediterranean coast	2015	3.26	44 %	Güven et al. (2017)
Micromesistius poutassou	20	North Atlantic Ocean	2014	0.00 ± 0.00	0 %	Murphy et al.(2017)
Scomber japonicus	7	Turkish Mediterranean coast	2015	6.71	57 %	Güven et al.(2017)
	35	Portuguese coasts	2013	0.57 ± 1.04	31 %	Neves et al. (2015)
Scomber scombrus	13	Portuguese coasts	2013	0.46 ± 0.78	31 %	Neves et al. (2015)
Sprattus sprattus	141	North Sea	2013	0.01	0.7%	Hermsen et al. (2017)
Trachurus mediterraneus	98	Turkish Mediterranean coast	2015	1.77	68 %	Güven et al. (2017)
Trachurus trachurus	44	Portuguese coasts	2013	0.07 ± 0.25	7 %	Neves et al. (2015)

*Data corresponding to anthropogenic particles (microplastics and textile fibres not distinguished)