CIESM Workshop Monographs

Marine litter in the Mediterranean and Black Seas

Tirana, Albania, 18 - 21 June 2014

CIESM Workshop Monographs ◊ 46.

To be cited as:
CIESM 2014. Marine litter in the Mediterranean and Black Seas. CIESM Workshop Monograph n° 46 [F. Briand, ed.], 180 p., CIESM Publisher, Monaco.

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A collection founded and edited by Frédéric Briand.

Plastic fragments on the surface of Mediterranean waters

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Abstract

The Mediterranean Sea is one of the most affected by floating plastic debris however few studies were performed on the impact of microplastics fragments. Data obtained up now suggests that neustonic microplastics are widespread in the North and Central Western Mediterranean Sea. The highest density (300,000 particles/ km^2) detected during a survey in Ligurian Sea is of the same order of magnitude as that found in the North Pacific Gyre. Microplastics debris composed mainly of polyamides (53%), polystyrenes, polyolefins and polyesters, were present in all Manta tows. The size distribution frequency showed that fragments are of small size (1-2.5mm). The average ratio between microplastics and mesozooplankton surface area was 0.2 for the whole survey. Copepods were the most abundant organisms in the surface layer but neustonic mollusks and cladocerans were also abundant. Due to of the fragmentation, small microplastics may be ingested by organisms commonly unaffected by the larger marine debris. The magnitude, distribution and especially the potential impact of microplastics on the environment and their interactions with the biota need to be better assessed.

Keywords: microplastic particles, zooplankton, Ligurian Sea, Mediterranean Sea

Introduction

Microplastics are now ubiquitous in the marine environment, found in seawater at the surface and at depth, in high seas and coastal waters from the equator to the poles. Several studies have reported high concentrations of surface microplastics in many of the world's oceans (see review of Barnes *et al.*, 2009) with a large impact on Gyres as North Pacific Ocean (Doyle *et al.*, 2011, Goldstein *et al.*, 2013), the central Northern Pacific Ocean (Moore *et al.*, 2001), the Sargasso Sea (Law *et al.*, 2010) and along coastlines and estuaries (Lima *et al.*, 2014). In the marine environment a wide range of organisms, from plankton to larger vertebrates such as fish, turtles or whales are "confronted to this abiotic plankton" and may ingest them (Wright *et al.*, 2013). The potential confusion with plankton by filter feeders in the neuston, their association with chemical contaminants and their possible role of vector of microorganisms make these particles potentially "harmful" for the ecosystem (MSFD, 2013). In addition, disintegrated plastic can be absorbed by marine life and therefore likely incorporated into the pelagic marine food webs with multiple unknown consequences (Teuten *et al.*, 2009; Fotopoulou *et al.*, this volume). Thus understanding the mechanisms by which microplastics are distributed, transported and enter our food chain is essential to assess its effects on habitat degradation and to develop policies for their management.

Scientific investigation on the impact of neustonic microplastics (0.3–5 mm) in Mediterranean Sea is recent, existing data originate mainly from summer cruises performed from 2010 to 2013 in the Ligurian and Sardinian Seas (Collignon *et al.*, 2012; Fossi *et al.*, 2012; de Lucia *et al.*, 2013). There is only one annual survey describing the variations in microplastics and the neuston zooplankton from August 2011-2012 in the bay of Calvi (Collignon *et al.*, 2013). Additionally Fossi *et al.* (2012, 2014a and this volume) reported on the impacts of microplastics on large filter feeding marine organisms such as Mediterranean fin whale and basking shark. They showed that the presence of harmful chemicals in Mediterranean fin whales was linked with intake of plastic derivatives by water filtering and plankton ingestion. Here we report on the distribution and concentration of floating microplastics and zooplankton using a Manta collector in the Ligurian Sea in the summer of 2013. Surface floating microplastics abundance and area per square kilometer were calculated and compared to the abundance of the neustonic zooplankton.

Material and methods

Sampling

Surface floating microplastics were collected in the Ligurian Sea (NW Mediterranean Sea) in the framework of the participative science activities of ExpéditionMED association (www.expeditionmed.eu) from July 6th to August 6th 2013. Sampling was performed at different locations across the Northern (Liguro-Provençal) current and front, near urban centers, harbors, and in the open sea. Eco-volunteers were involved in assisting scientists in sampling. Throughout the cruise, weather conditions were calm (Beaufort Sea State from 0 to 2) and nets were towed in calm sea conditions. The samples were collected with a 330 µm Manta trawl with the size of the rectangular net opening of 60 x 20 cm (Fig. 1A). The net was towed at an average speed of 2.5 knots during 30 or 60 min at the top 10cm of the sea surface. The particles abundance was calculated per square kilometer. The content of the collector was sieved through a 150 µm mesh and fixed in 2 % buffered formalin.

Sample processing

In the lab, samples were gently mixed and transferred into a 2 L glass jar in order to separate by gravity the floating particles from the sedimented material containing zooplankton and organic tissues. This process was repeated from 3 to 6 times until no microplastic was observed in the supernatant. Two fractions were then obtained: the sediment and the supernatant with plastic particles. Both fractions were rinsed with filtered seawater before manual sorting of the plastic particles. Using a dissecting microscope, plastic particles were removed from preserved organic material in both fractions obtained by density separation. Microplastics and zooplankton were enumerated, sorted and measured by imaging analysis using the Zooscan system (Gorsky *et al.*, 2010; Goldstein *et al.*, 2013, Fig. 1B).

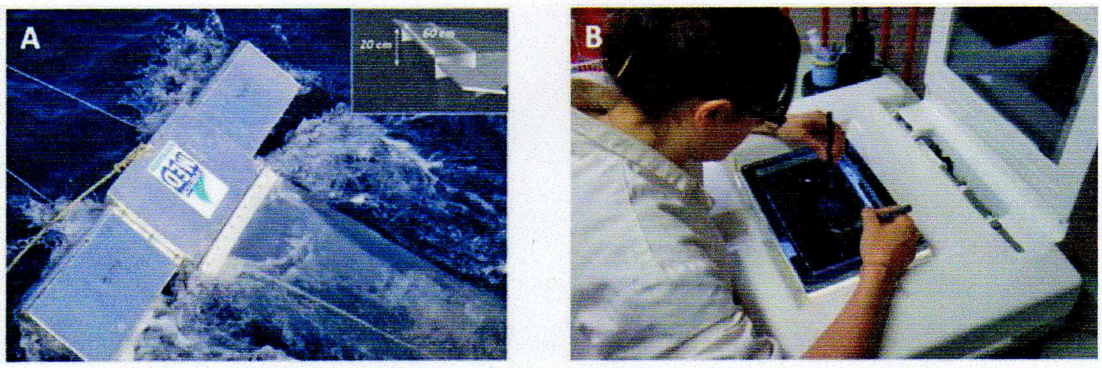

Figure 1. Methods for collection and analysis of microplastics. Samples were collected using (A) a Manta trawl net with 333µm mesh size and 60 x 20 cm opening. Trawl shots were performed at 2.5 knots from 30 to 60 min covering a known area at the top 10cm of the sea surface. (B).Back to the lab, plastic particles and zooplankton were enumerated, sorted and measured by digital imaging analysis using a data acquisition system and the Zooscan.

Zooscan analysis

Zooplankton and plastic particles were rinsed with 0.2 µm filtered seawater and then digitally imaged with a Zooscan digital scanner with a resolution of 2400 dpi; each pixel is about 10 microns wide. Image post-processing was performed with the Zooprocess & Plankton Identifier software that enumerates and gives a large set of morphological parameters for each object such as the ferret length (approximately equivalent to particle length), circularity and two- dimensional surface area (mm), etc. For zooplankton the biovolumes, concentration and surface areas were calculated and taxonomic identification was also determined (Fig. 2). After analysis, zooplankton samples were reconditioned in 4 % formalin solution whereas microplastic particles were split and conserved for further chemical analysis.

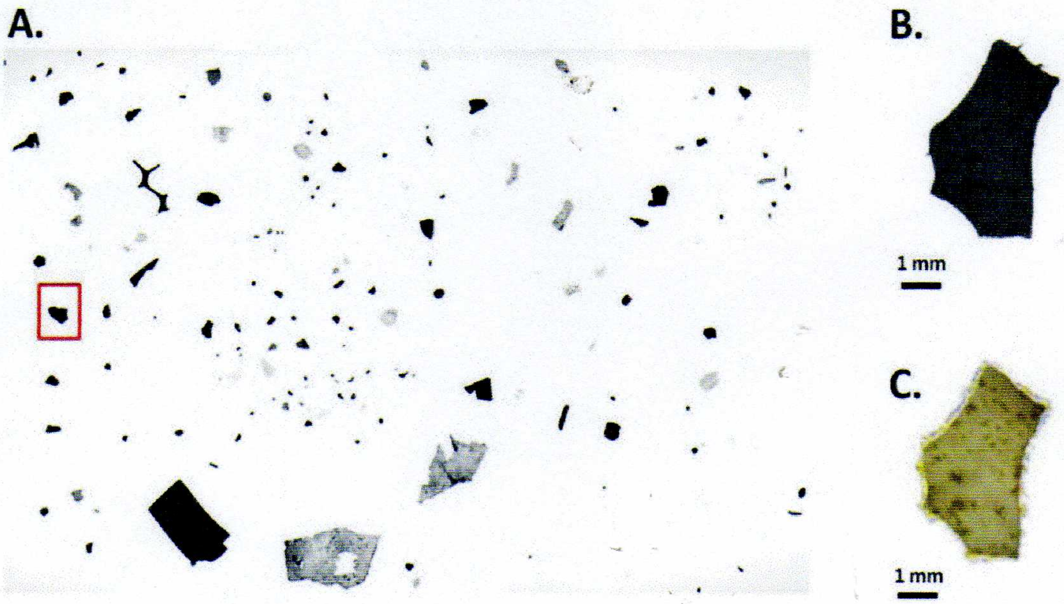

Figure 2. Plastic fragments imaged by the Zooscan (A & B) and by stereo microscope (C).

Results and discussion

We were able to enumerate plastic fragments and determine their size spectra. In our survey, microplastic were present in all Manta tows varying from 1.3 x 10^4 to more than 3.6 x 10^5 plastic debris per km^2 observed in a coastal station. This result suggests that plastic fragments are widespread in the Ligurian Sea as all studied 35 stations between Toulon and Genoa had detectable plastic micro-debris.

The average concentration was 1.03 x 10^5 plastic micro-debris per km^2 (Table 1A, Fig.3 A). The area covered by plastic represented in average 4.2 x 10^5 mm^2 / km^2 with a maximum of 1.72 x 10^6 mm^2 / km^2 and a minimum of 8.58 x10^4 mm^2 / km^2 (Fig.3 B). The average abundance value in our study is comparable to averages obtained in the same areas by Collignon *et al.* (2012) although maximal concentration of this study is lower (Table 1). Concerning the other studies, they are made with different sampling devices or mesh aperture, and results are expressed in cubic meters (Table1B). Overall, the average abundance of microplastics found in Ligurian Sea is higher than in Sardinian Sea with levels approximately seven times higher in the samples from the same survey in both sites (Fossi *et al.,* 2014a).

The spatial distribution of microplastics varies greatly across the Ligurian Sea spanning over one order of magnitude among the studied stations; high concentration and surface area of debris were detected in the inshore coastal stations, and were lower in the bays with open sea connections (Figs. 3, 4). Our results corroborate those of Collignon *et al.* (2012), which showed higher concentration of microplastics along shorelines adjacent to densely populated areas. In fact, higher densities of debris in coastal waters were correlated with proximity of human population centers (Browne *et al.*, 2011). This could explain the differences in the seasonal distribution observed by Collignon *et al.* (2013) in the Bay of Calvi, Corsica. In this touristic location, Collignon *et al.* (2013) found the highest abundance of microplastic during the summer with a decreasing concentration in autumn and levels close to zero in winter and spring. In the bay of Oristano (Sadinian Coast) while de Lucia *et al.* (2013) showed that spatial location of the different sampling sites can influence the abundance of microparticles, no significant difference in particle concentration was found between the coastal and offshore stations.

Table 1. (A) Microplastics and zooplankton abundance (items / km^2) and (B) Average microplastic concentration (items /m^3) in the Mediterranean Sea. Survey were performed between 2010 and 2013.

A	Microplastics (items / km²)			Zooplankton (items / km²)			Ref
	Mean	Max	Min	Mean	Max	Min	
	1.03E+05	3.66E+05	1.35E+04	2.96E+08	2.33E+09	7.75E+05	Our study
	1.16E+05	8.92E+05	0.00E+00	NA	NA	NA	Collignon, 2012
	6.20E+04	6.88E+05	0.00E+00	1.12E+08	9.86E+08	3.42E+06	Collignon, 2013

B	Microplastics (items /m3)		
	Location	Mean	Ref
	Ligurian Sea	0.373	Our study
	Ligurian Sea	0.116	Collignon, 2012
	Ligurian Sea	0.940	Fossi et al, 2012
	Sardinia Sea	0.130	Fossi et al, 2012
	Sardinia Sea	0.150	de Lucia et al 2014

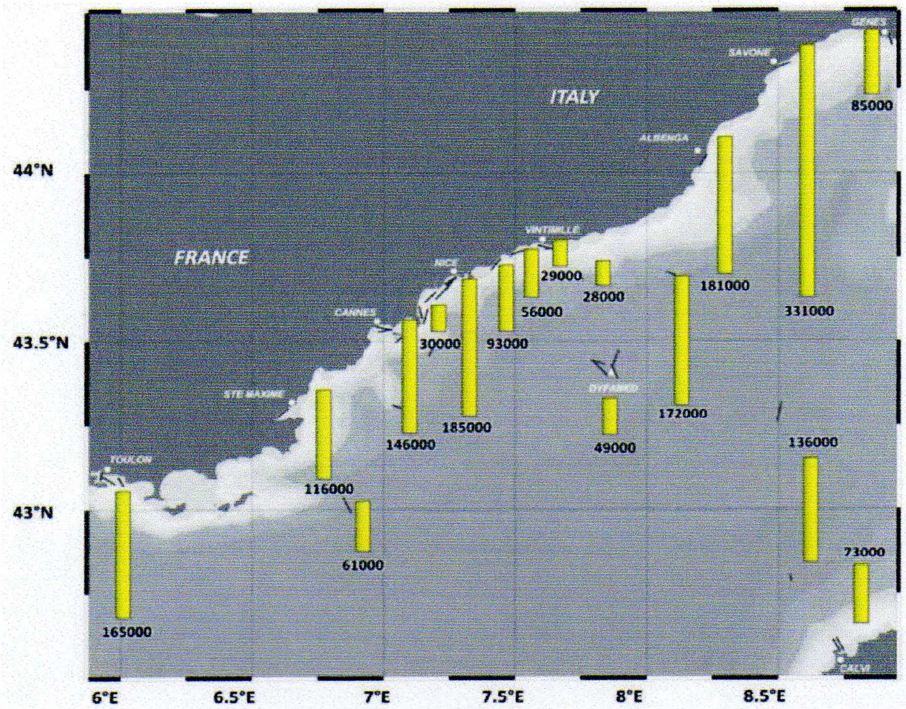

Figure 3. Distribution of the microplastic particles (size <0.5 cm) present in the top 10 cm of sea surface water collected in the Ligurian sea in July and August 2013 by ExpeditionMED.

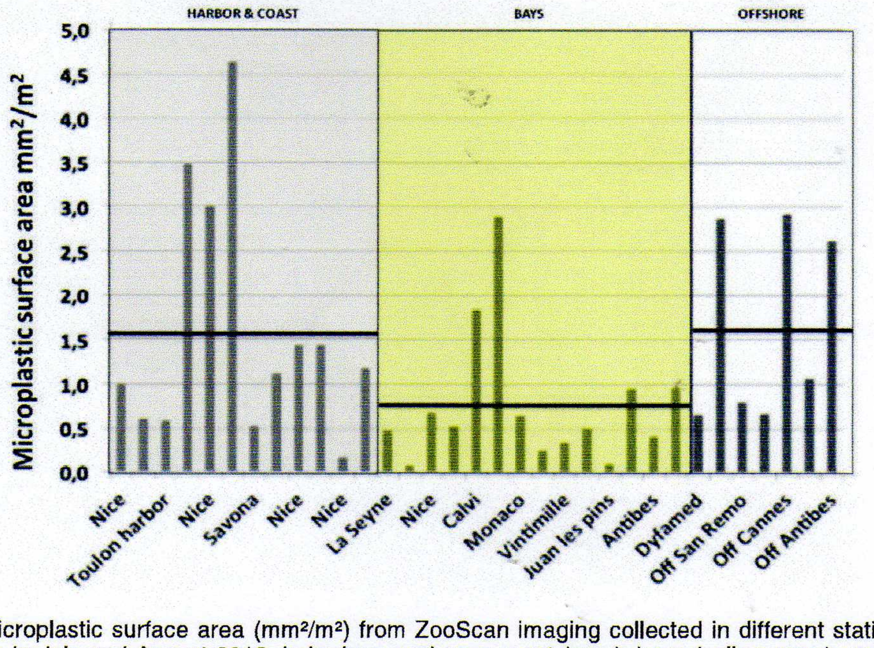

Figure 4. Microplastic surface area (mm^2/m^2) from ZooScan imaging collected in different station of the Ligurian sea in July and August 2013, in harbors and near coast (grey), bays (yellow zone) and offshore (white). Horizontal lines = mean values.

Data collected during our survey completed the results obtained from the other sampling surveys in the north and central Western Mediterranean (Collignon *et al.*, 2012; 13; Fossi *et al.*, 2012; de Lucia *et al.*, 2014b) and corroborate the initial insight of a significant distribution of fragments floating in a basin wide surface layer in the Western part of Mediterranean Sea, even if we cannot extrapolate to the other areas, as it is known from several studies that microplastic patterns in the ocean are patchy (Gordstein *et al.*, 2013). Maximum concentrations detected in several studies in this area are of the same order of magnitude as those found in the Pacific and Atlantic North subtropical gyre by Law *et al.* (2010; 2014). These authors analyzed data from more than 20 years in the North Atlantic gyre (6135 surface plankton tows) and 11 years in the eastern Pacific Ocean (2,529 surface plankton tows) and estimated a maximum concentration of 100,000 and 500,000 pieces of plastic km^2 respectively in the two gyres that correspond to centers of accumulation resulting from the convergence of ocean surface currents predicted by several oceanographic numerical models. Compared to others areas, the concentration detected in the Med surveys are lower than in the South California Current and Bering Sea (Gilfiland *et al.*, 2009; Doyle *et al.*, 2011) and in the California Coast (Moore *et al.*, 2002); but higher than in the Caribbean Sea, the Gulf of Maine and in the North Atlantic Gyre (Law *et al.*, 2010). The spatial heterogeneity in microplastic distribution is show to be linked to mesoscale and regional oceanographic conditions, such as gyres, eddy formation, upwelling and convergences areas (Law *et al.*, 2010; Collignon *et al.*, 2012; Ribic *et al.*, 2012; Goldstein *et al.*, 2013). On a smaller spatial scale, wind patterns affect the distribution of debris by differentially moving or mixing particles of different densities (Browne *et al.*, 2011; Kukulka *et al.*, 2012). Collignon *et al.* (2012) observed that concentrations of neustonic plastic particles are five times higher before than after a strong wind event. In areas as the North Pacific Subtropical Gyre (NPSG) Goldstein *et al.*, 2013 found an inverse relationship between wind and plastic concentration suggesting that microplastic is mixed down in high wind conditions, and is thus undersampled in the neuston tow.

Size and area distribution of microplastic particles

The ongoing analysis of individual particles (n=2,578), showed that the median size values of microplastics during the whole survey was 2.5 mm, however, we found a broad range of sizes (0.10 to 200 mm) with an asymmetrical frequency distribution skewed toward smaller diameters (size class 1-2.5 mm), (Fig.5 A). The median microplastic surface area was 2.3 mm^2, with microplastic areas ranging from 0.1 to 200 mm^2 (Fig. 5B). In our survey microplastic particles detected in the coastal station, in the bays, and offshore regions showed the same size pattern, 52 % of the total particles analyzed are microplastics smaller than 2.5mm in diameter (Fig. 6A). The majority of the microplastics (53 %) are smaller particles with a surface smaller than 2.5 mm^2 (Fig. 6B). There was also an increase in circularity (roundness) of microplastic with smaller particle size. The dominance of smaller particles in the neustonic samples suggests that the dominant pathway of microplastic formation is fragmentation from large plastics.

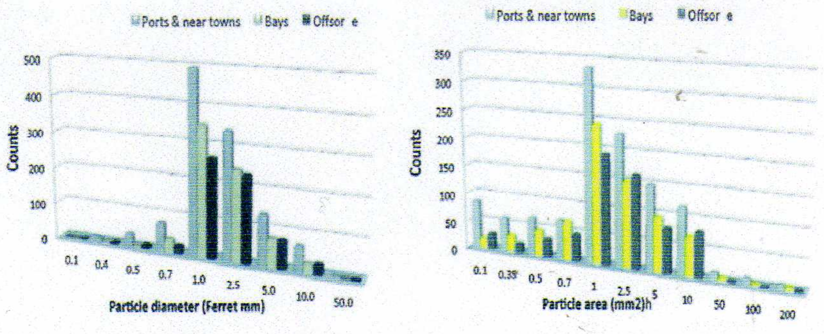

Figure 5. Microplastic size spectra. A-Histogram of frequency distribution of particle diameter (ferret mm). B-Histogram of particle surface area (mm^2).

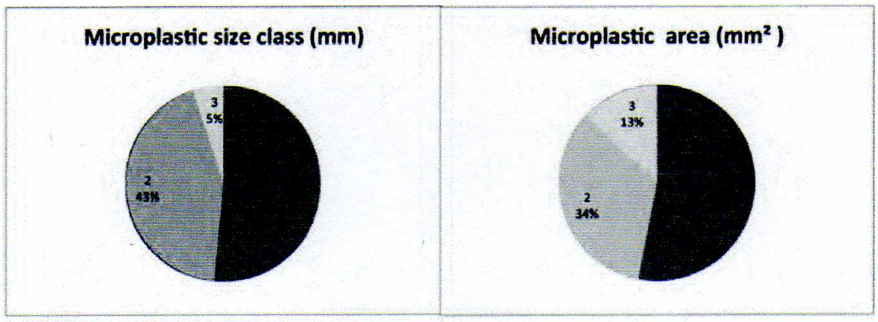

Figure 6. Proportion of the total abundance of microplastic for each size class and area during the whole study: A- Microplastic size class (1) <2.5mm; (2)2.5-5,0mm; (3)>5.0mm. B- Microplastic area (1)<2.5 (2) 2.5-5.0mm; (3) >5.0mm.

In the Southern California Current, using the same Zooscan method, Gilfilland *et al.,* (2009) found the same median size (2.62 mm) and area (2.24 mm^2) values of particles. In the North Pacific Gyre plastic particles less than 3 mm accounted for 82 % by number of the plastic observed (Moore *et al.,* 2001) with an area approximately of 1 mm^2 (Goldstein *et al.,* 2012). However, the size of microplastics observed in our survey is smaller than size values obtained by Collignon *et al.* (2013), which found that 54 % of particles were large within a size class of 2-5 mm. Data from the Malaspina 2010 circumnavigation, showed that the size distribution of surface microplastics, when analyzed separately by ocean, is around 2 mm with a pronounced gap below 1 mm sizes observed (Cozar *et al.,* 2014). The progressive fragmentation of plastic objects from millimeter to micrometer scale should lead to a gradual increase of fragments toward small sizes, rendering the very small pieces undetectable using convectional sampling nets, and/or may be transferred to the ocean interior. These findings (concerning the size distribution of floating plastic) provide strong support to the hypothesis launched by Cozar *et al.* (2014) of substantial size-selective losses of plastic on a large scale of the surface ocean. The tendency observed in our study of increasing particle concentration with decreasing size suggests that a continual fragmentation of plastic items may occur in Mediterranean waters. This agrees with the general trend observed in global environments postulated by Barnes *et al.* (2009) that the average size of plastic particles seems to be decreasing, while abundance of such particles is increasing due to continuous breaking down. This could have important consequences regarding ingestion by small planktonic organisms serving as prey for larger animals.

Chemical characterization of microplastic

The chemical characterization of plastic items was performed simultaneously. Fourier Transform Infrared Spectroscopy (FT-IR) spectra of various samples were recorded with a FT-IR spectrometer (Shimadzu 8400 M), using 4 cm^{-1} resolution and 40 scans. Thermogravimetric analysis (TGA) was carried out on each microplastic sample. Finally, the thermal characteristics of microplastics, i.e. glass transition temperatures (Tg), cristallinity and melting temperatures (Tm), were collected using differential scanning calorimetry (DSC). Preliminary results from our ongoing analyses using the combination of the characterization techniques allow us to initiate and to develop an unambiguous methodology for classifying microplastic samples collected in the NW Mediterranean Sea. A data bank containing the spectra of main marine microplastics has been established (Fig. 7A). These spectroscopic results associated with the thermal data obtained using TGA and DSC revealed that the most frequent plastic types were polyamides (comprising 52 % of the plastic present), polystyrenics (different kinds of polystyrene and also copolymers of polystyrene), polyolefins (polyethylene and polypropylene) and polyesters (Fig. 7B). As shown by numerous recent studies, polyethylene, polypropylene and polystyrene were usually the most abundant types of debris (Frias *et al.*, 2014; Cozar *et al.*, 2014) but in that special case, the plastic predominantly recovered is polyamide. Note that polymers such as polyamides and polyesters are denser than seawater and their presence in our samples indicates that the transport of debris is influenced by factors other than density alone, as previously noted by Sadry *et al.* (2014). One likely explanation could be the introduction of some of these fibres via the sewage outlets onto shorelines and/or their re-suspension in water column as a result of turbulent mixing induced by wind and tidal currents (Browne *et al.*, 2011). Further work on this question is in progress.

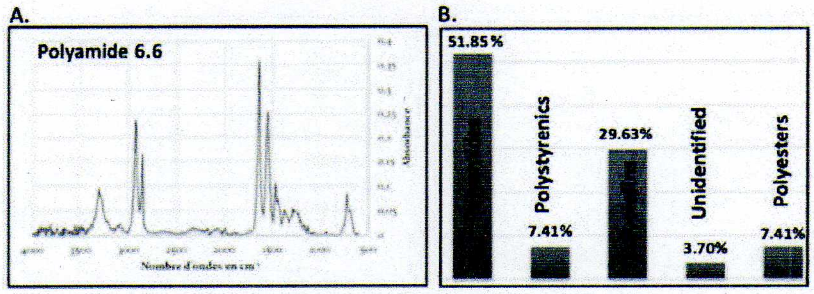

Figure 7. Chemical characterization of microplastic particles. As an example, IR spectrum of polyamide 6,6 (Nylon®) was shown (A). Distribution of plastic polymers in the microplastic samples is shown.

Microplastic and zooplankton interactions

Macroscopic observation of different microplastic showed that marine organisms were associated with and transported by floating plastic fragments (Fig. 2C). In order to evaluate the potential interaction between microplastics on zooplankton we calculated a microplastic: zooplankton ratio. The average ratio between microplastics and mesozooplankton surface area was 0.2 for the whole survey. This ratio was based on the average surface in mm^2 of microplastic and the zooplankton surface area. The mean value indicates that the area occupied by plastic is neustonic biota is five times lower that zooplankton. Copepods were the most abundant organisms in the surface layer but neustonic mollusks and cladocerans were also abundant. To illustrate, the particles surface area of one sample collected in the Toulon harbor are represented by nearly 5 % of plastic particles, 22.74 % of copepods and 71.53 % of other organisms (Fig. 8).

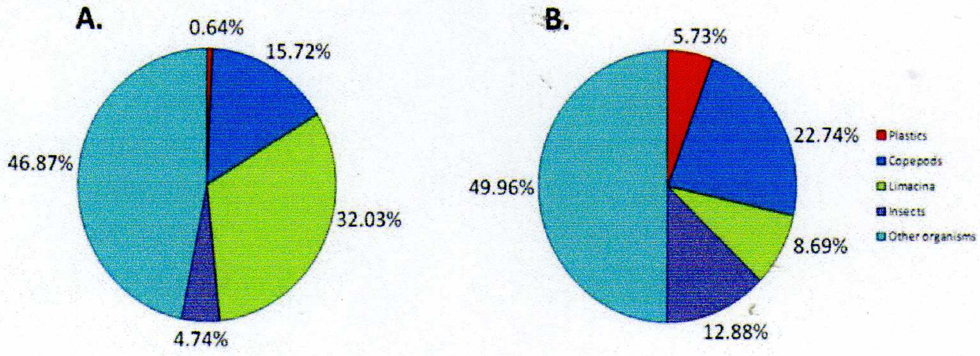

Figure 8. Proportion of the abundance (A) and the surface area (B) of plastic particles and of zooplankton for a sample collected in Toulon harbor the 7th July 2013.

This ratio is intermediary between the total dry weight ratio of 0.5 calculated by Collignon *et al.* (2012) and those calculated for three different size classes of zooplankton based on biovolumes (Collignon *et al.*, 2013). In the present case, the average number ratio between the abundance of small microplastics (0.2–2 mm) and zooplankton (e.g., copepods, cladocerans) are very low (0.002). This could imply that neustonic zooplankton rarely encounter or interact with small microplastic debris. On the other hand, the ratio calculated by Collignon *et al.* (2013) reached 2.63

for the large microplastics (2-5 mm) and zooplankton (decapod larvae, fish larvae). This would suggest a potential confusion for predators regarding planktonic prey of this size class.

The ratios published are often based on calculations between abundances or dry weight of both plankton and plastic particles (Moore *et al.*, 2001; 2002; Lattin *et al.*, 2004; Collignon *et al.*, 2012; Goldstein *et al.*, 2013). In the North Pacific Gyre, Golstein *et al.* (2013) obtained ratios varying from 0.01 to 10. Along the California Coast Moore *et al.* (2002) obtained a ratio of 0.6 and Lattin *et al.* (2004) in South California shore obtained a ratio of 0.3 for the size class of plastics smaller than 4.75 mm. These ratios are difficult to compare as different sizes of plastic were integrated in calculation and the areas studies have contrasted trophic state; in the California Current subjected to nutrient upwelling has a much higher biological productivity than the North Pacific central gyre. According to Doyle *et al.* (2011), this method is inappropriate due to high variance of both plastic and zooplankton in space and time, selective sampling by nets, and selective feeding by zooplankton.

Due to fragmentation, small microplastics may be ingested by organisms commonly unaffected by larger marine debris. The potential for ingestion of microplastic by the biota needs to be better assessed and efforts need to be made to establish an index for zooplankton encounter rates with various size ranges of microplastic in the marine pelagic environment. The first estimations obtained in our survey based on surface areas of both plastic and zooplankton give us an indication of the instantaneous encounter rate between plastic and zooplankton. This can be improved and proposed as an indicator to resolve at the small-scale microplastic and zooplankton interactions.