"SPlasH! - Stop to plastic in H_2O!" an EU Project to investigate the state of the port environment

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Abstract

Plastic debris are one of the main contaminants in marine environment, coming from several anthropic sources that are mainly located along the coast. This is the reason why a lot of plastics can be found in coastal environments and especially in those areas that are strongly affected by human activities, such as ports. In this frame, the project "SPlasH! – Stop to plastic in H_20!", part of the European Program Interreg Italy-France 2014-2020 Maritime, aims to focus on port areas and their roles as plastic sources. Part of this project consists in monitoring the marine environment of three different ports of Genoa, Olbia and Toulon, collecting samples from sediment, water column and biota, and investigating their content in terms of microplastics. The study of water currents and physical parameters is used to understand the dynamics of microplastics within the ports. In this article, the results on the first sampling of sediment and water column in Genoa are presented. In addition, the stomatal content of Mugilidae fish, from industrial fisheries in Mediterranean Sea, was investigated to have preliminary information for further studies on fish from port areas. SPlasH! project will produce information and techniques that can be useful for different stakeholders to understand how, where and when it is necessary to reduce the impact of the growing issue of plastic pollution.

Introduction

The presence of plastic represents a threat to the whole environment and its global production has increased significantly over the past decades, reaching more than 348 million tonnes in 2018, of which Asia accounts for 50.1% of the total amount, followed by Europe (18.5%) and NAFTA-North American Free Trade Agreement (17.7%) [1]. In the last years, the volume of plastic materials collected for recycling and energy recovery rapidly increased, overcoming landfill for the first time in Europe (EU28+NO/CH) in 2016 [1]. However, an unknown amount of plastic is not collected and it remains into the environment, being transported by winds and rivers and finally reaching the sea [2]. The issue of plastic marine littering was firstly highlighted as an aesthetic problem, but it soon became clear that it could also endangered living organisms, as causing choking [3]. This aspect is largely known due to a great media interest, because the consequences can be easily seen on organisms that are more common for the large audience, as fishes, birds, turtles and marine mammals [4]. Unfortunately, one of the most worrying aspects cannot be seen with the naked eye, which is the occurrence of microplastics.

Microplastics (MPs) are defined as plastic particles that are smaller than 5 mm in size [5] and they represent a large part of plastics that can be found in coastal zones, accounting for over 80% of the ones collected on beaches [6]. MPs can be classified as primary or secondary MPs, whereas the first ones are purposely manufactured as tiny plastic particles which are used for different applications, such as toothpastes, resin pellets, exfoliating scrubs, antifouling, abrasives, air-blasting media, drugs, etc. [5, 7-9]. Instead, secondary MPs arise from photolytic, mechanical and biological degradation of bigger plastic items, respectively due to sunlight, waves action and fungi/bacteria [5-6]. About big plastic items that are likely to be affected by fragmentation, the focus falls on packaging, which is material designed for immediate disposal [10], followed by carrier bags, footwear, cigarettes lighters and other domestic items [3].

MPs can be found in all marine environmental compartments, such as sediment, water column and biota [11]. In fact, MP vertical transport depends on particle density, in turn dependent on the polymer composition: for example, polyvinyl chloride (PVC, 1.40 g cm^{-3}), nylons (1.01-1.12 g cm^{-3}) and polyethylene terephthalate (PET, 1.385 g cm^{-3}) are characterized by a higher density compared to other materials like polyethylene (PE, 0.92-1.28 g cm^{-3}), polypropylene (PP, 0.90 g cm^{-3}) and polystyrene (PS, 1.05 g cm^{-3}) [5]. In addition, MPs can be trapped in marine snow, which is composed by faecal pellets, larvacean houses, phytoplankton, microbes, particulate organic matter (POM) and inorganics, thus increasing their settling rates; furthermore, this incorporation increases the size of MPs, enhancing their bioavailability for living organisms [12]. The same process happens when MPs increase in density due to biofouling, making sinking to the bottom and accumulation in sediment more likely to occur [5]. It is well known that MPs can be ingested by different organisms which live in the marine environment, as seabirds, mammals, fish and invertebrates, and this can be proved by their occurrence in gastrointestinal tract [13-16]. Furthermore, MPs can be transferred from gut to other tissue, entering the lymphoid system and consequently the circulatory system [6]. The ingestion of MPs by living organisms is proved to affect their health status, resulting in reduced feeding, increased mortality, decreased growth rates, decreased hatching success and reduced fecundity [9, 17]. The main problem related to MP presence into marine habitats is the fact that they contain or efficiently absorb organic chemicals, such as polychlorinated biphenyls, polycyclic aromatic hydrocarbons (PAHs), petroleum hydrocarbons, organochlorine pesticides, polybrominated diphenyl ethers, alkylphenols and bisphenol A, that can be released in the external environment or in organism tissues after degradation processes, potentially affecting organism health status [18-19]. MPs can be also contaminated by heavy metals, for example in the case of microbeads that are used for stripping paints from metallic surfaces and cleaning engine parts by blasting; furthermore, these microbeads can be recycled up to ten times, decreasing their size during the process [20]. Besides pollutants, the transport of MPs can be related to the introduction of non-indigenous species to new locations and distribute algae associated with red tides [3], as well as oviposition by insects, such as *Halobates micans* and *H. sericeus*, which can affect their abundance and dispersion [8].

Due to plastic commercial purposes, MPs are very durable and therefore persistent [5]; in addition, they are often characterized by a density lower than seawater that allows them to easily float on the surface, or, if they are denser than seawater, they may still be transported by underlying currents [19]. Thus, MPs remain into the marine environment for a long period of time and they can be transported over long distances by ocean currents, winds, river outflow and drift [7]. This is the reason why MPs can be found all over the world, from Arctic [21] to Antarctic [22]. Despite MPs can be directly released in the marine environment due to maritime activities, such as shipping and aquaculture, the main inputs are land based, including not only the direct discharge through beach littering, sewage treatment plants and urban or agricultural runoff, but also the transport by rivers and atmospheric dust [23]. Part of the plastic that enters the marine environment from land reaches the open ocean thanks to offshore currents, but most of it remains near the coast line for a long time, due to processes of beaching, drifting and settling [19, 23]. For this reason, it is particularly interesting to investigate MP issue in an environment affected by the presence of coastal populations (<50 km from coastline) [2], which are widely present in the top waste-producing countries [10].

Within the European Program Interreg Italy-France 2014-2020 Maritime, the project "SPlasH! – Stop to plastic in H_2O!" aims to investigate several aspects related to MP occurrence in marine environments, focusing on port areas. Three partners collaborate for the realization of the project: University of Genoa, University of Toulon and European Research Institute (ERI). Three different ports are selected in both Italy and France, in the cities of Genoa (IT), Olbia (IT) and Toulon (FR). The whole project will last two years (2018-2020) and the monitoring program started in Genoa, during December 2018. Further studies will be conducted in the ports of Olbia and Toulon. In this project, ports have been selected because of their role and importance in human activities, both commercial and industrial; consequently, port areas are widely affected by contaminants coming from sewages, shipping yards and traffic and accidental losses [20, 24-26]. Cross-border cooperation is a very important aspect of "SPlasH!" project, aiming to standardize protocols of MP sampling and analysis, and to better understand the dispersion pathways of these contaminants. The developing of an innovative prototype for MP sampling and a dispersion model will help to partially reach these goals. The other fundamental part of the project is related to the characterization of ports as both receptors and sources of MPs, applying a monitoring program that investigates the occurrence of MPs in different environmental compartments, such as sediment, water column and biota.

In the present study, we present the results on the first winter sampling of sediment and water in the selected port area in Genoa. In addition, a preliminary investigation has been performed on stomatal content of Mugilidae fish from industrial fisheries in Mediterranean Sea; these data will help further studies on Mugilidae fish from port areas both in Genova and Olbia.

Study area

In this work, the study area is the Port of Genoa (**Fig. 1**), which is situated at the apex of the Ligurian Sea in the north-western Mediterranean Sea and has a total area of about $7 \cdot 10^6$ m^2 and 47 km of shipping lanes, landing $2.2 \cdot 10^6$ TEU (Twenty-foot Equivalent Unit) of containers, $51.3 \cdot 10^6$ tons of goods, and 6,000 vessel moorings in 2015 [27]. Several anthropic activities are likely to affect the marine environment within the study area (Fig. 1): commercial and industrial activities are mainly located in the western zone (Sampierdarena channel), while the part dedicated to shipyards occur in the eastern area; finally, ferry, tug and cruise docks, with the solid garbage collection site from vessels, are located in the inner part of the port (Old Port Basin) [24]. Furthermore, the Port of Genoa is closely connected to the urban area of the city, thus suffering of urban runoff and sewage discharges [28]. In this context, MPs are proved to come from sewages inputs, for example due to the washing process of synthetic clothes which release polyester and acrylic fibres that cannot be blocked by wastewater treatment plants [29]. Finally, the port is characterised by the presence of urban streams and rivers that contribute to the supply of MPs to the marine environment, collecting several discharges from their catchment areas [30-31]. In particular, the mouth of Polcevera Torrent is located within the Port of Genoa and supplies a large quantity of detrital minerals in the port, along with the contribute of Bisagno Torrent, whose mouth is located just outside the eastern part of the study area [27]. Furthermore, Polcevera Torrent collects contaminants from the "Valpolcevera" sewage treatment plant and 27 industrial discharges, due to several activities, such as farming, mining, oil companies, paint and mechanical equipment production, gas stations and car washes [32].

The marine environment of the Port of Genoa has been the subject of several studies in recent years from ecological, chemical-physical, hydrodynamic and mineralogical [27, 33-34] point of view. Moreover, the port has been subjected to a capital dredging from 2009 and 2014 to guarantee access to the port even for larger and deeper vessels [35-36].

The main environmental conditions characterising the Port of Genoa are summarised here: winds come mainly from the NE (the most frequent wind) and the SE, with a mean velocity of 3.1 $m \cdot s^{-1}$ from both directions [27]; water masses generally tend to concentrate in the inner part of the port when wind blows from the S, and flow out of the port when wind blows from the N [35]; sea temperature varies from 12 to 14 °C in winter and from 14 to 26 °C in summer, in according with seasonal variations, such as salinity, which is at its maximum in summer and winter (37–38) and minimum in early spring and autumn (36–37) depending of fresh water supplies [33-34].

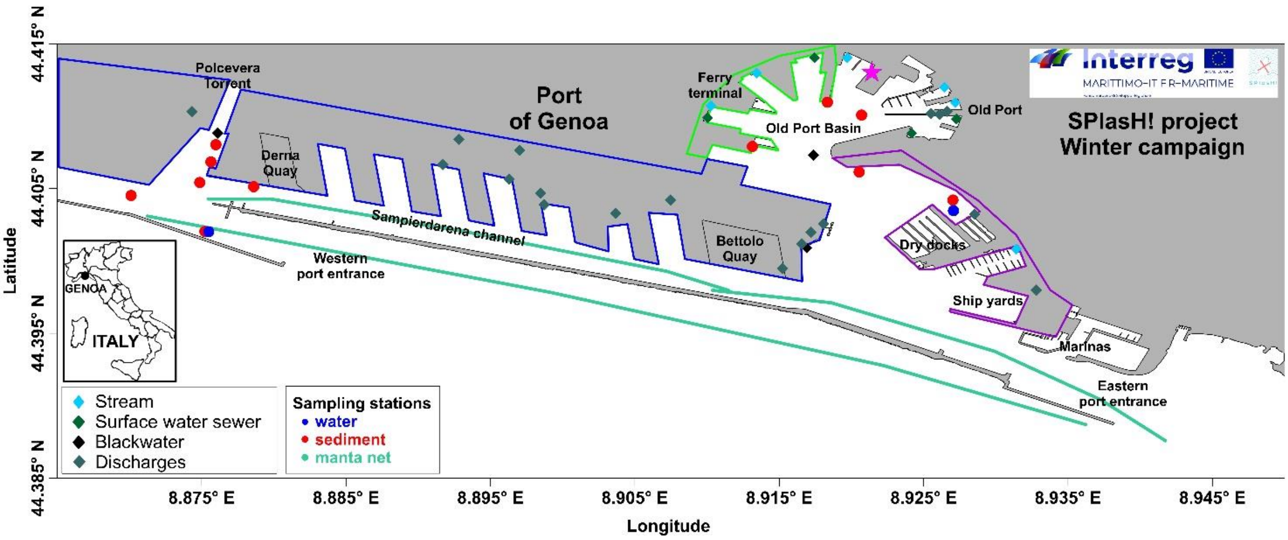

Fig. 1 Map of the Port of Genoa (north-western Italy), showing water (blue points) and sediment (red points) sampling stations and Manta net transects (light blue lines); blue line: commercial and industrial area; purple line: part dedicated to shipyards; green line: ferry, tug and cruise Terminal; pink star: site of the solid garbage collection deriving from vessels [24].

Materials and methods

The winter sampling campaign of the SPlasH! project started in the Port of Genoa (north-western Italy) during the period 13th-14th December 2018, with strong and persistent N wind, calm sea and clean sky. The sampling was performed in the area between the mouth of Polcevera Torrent and the Eastern Port Entrance (Fig. 1).

Superficial bottom sediments were sampled using a 5 L Van Veen grab at 11 different stations (Fig. 1): 5 stations were selected in the eastern area, between dry docks and the ferry terminal, and 6 stations were chosen in the western area, between the mouth of Polcevera Torrent and the Western Port Entrance. About 500 mL of sediment for each station were transferred in a wide-necked glass jar using a metal spoon, and then stored at 4 °C.

Samples from water column were collected using a 5 L Niskin bottle at 1 m of depth (sub-surface sampling); 2 sampling stations were selected, respectively in the eastern and the western area (Fig. 1), and 10 L of sea water were collected for each station and stored in glass bottles.

In laboratory, for each sediment sample, 200 mL of a pre-filtered and concentrated hypersaline solution (1.2 g cm^{-3}) was added to 50 mL of sediment and stirred for 2 min; mixture was then allowed to settle for 12 h, and this extraction was performed 3 times for each sample. Subsequently, the supernatant was collected on GF/C Glass Microfiber filters with 1.2 μm porosity. Water samples were filtered, using a vacuum pump, directly on the same type of filters used for sediment samples; one filter was employed each 2 L of water sample to subdivide the sample and allow an easier MP visual sorting under the microscope. Filters, from both sediment and water samples, were stored in glass Petri dishes; the removal of the organic matter was performed using hydrogen peroxide and letting dry for at least 24 h. Particles collected were examined using a binocular microscope (Leica Z16 apochromatic microscope of 0.57x–9.2x zoom range equipped with digital camera). Items on each filter were counted, dimensionally measured and classified into different categories according to the type (fragments, pellet, filaments, foamed plastics, granules, styrofoam, and others), shape (cylindrical, disks, flat, ovoid, spheroid, rounded, sub-rounded, sub-angular, angular, and others), colour (white, creamy-white, red, orange, blue, black, grey, brown, green, pink, red-brown, yellow and others) and appearance (lucid, opaque, crystalline, and transparent) [37]. About the dimensional aspect, items were divided according to the sediment grain-size classes: fine sediments (Ø<63 µm), very fine and fine sand (63<Ø<250 µm), medium sand (250<Ø<500 µm), coarse and very coarse sand (500<Ø<2000 µm), and gravel (Ø>2000 µm).

Sampling of water surface was performed using a suitcase Manta net (designed and produced by Markus Eriksen, 5 Gyres Institute) with a 60 cm x 16 cm rectangular mouth, and a mesh size of 300 µm. The net was trawled at the distance of 20 m from the boat, in order to avoid turbulence, along multiple transects for at least 30 min each, at the speed of 2.5 knots (Fig. 1). The volume of filtered water was calculated using a flowmeter. Then, Manta net was gently rinsed with sea water, flushing all the samples inside a collector. In a 300 µm sieve, items larger than 5 mm (both plastic and non-plastic items) were removed. Samples were finally stored in a glass jar at -20 °C to prevent biological development. Samples were processed in the Mediterranean Institute of Oceanography (University of Toulon). A binocular loupe (Leika) was used for all observations and identifications of items based on shape and appearance. Collected items were analysed using a Zooscan V4 (version 2.4.0, Hydroptic, France). This technology was initially designed for plankton identification [38], but it has been adapted to MP research in the last years [39]. Dried items were digitally analysed by Zooscan with a resolution of 2400 dpi. After this step, number of items, maximum length and surface were determined by EcoTaxa, a web application (https://ecotaxa.obs-vlfr.fr/). Classification of the analysed items was performed with the same classification method described for sediment and water samples.

The stomach content of fish was investigated through a baseline evaluation conducted on 9 samples from the family *Mugilidae* (*Liza* spp), coming from industrial fisheries in Mediterranean Sea. Fishes were photographed and length and weight taken. Stomachs were excised, collected in glass jars covered with 70% ethanol and stored at 4 °C. In laboratory, stomachs were washed with pre-filtered water to eliminate any items present on the outside of the stomach, dried at room temperature, and then opened after weighing; their content was transferred in a Petri dish with 70% ethanol. In order to remove the organic component, samples were treated with 30% hydrogen peroxide for a time period between 24 and 72 h at 62 °C; after that, they were filtered, adding 1 L of pre-filtered water each, on a GF/C Glass Microfiber filter with 1.2 µm porosity. Finally, filters were analysed under microscope with the same method described for sediment and water samples.

The hydrological data were collected using a conductivity–temperature–depth (CTD) multiparametric probe (MAR-365 IdromarAmbiente) equipped with a turbidimeter (Turner Designs), in 27 sampling stations distributed throughout the port basin (**Fig. 2**). A Teledyne RD Instruments 300-kHz Workhorse® over-the-side-mounted current meter (Vertical Acoustic Doppler Current Profiler, V-ADCP) with bottom-track function, using a 316L stainless-steel bracket, was used to collect the current velocity and direction data (Fig. 2). Teledyne RD Instruments software "WinRiver® II" running on a notebook was used to collect and display the data in real time. Navigational data received from an external global positioning system (GPS) were collected to geo-localise measurements. CTD and ADCP data were processed using Surfer® 13 software (Goldens Software LLC).

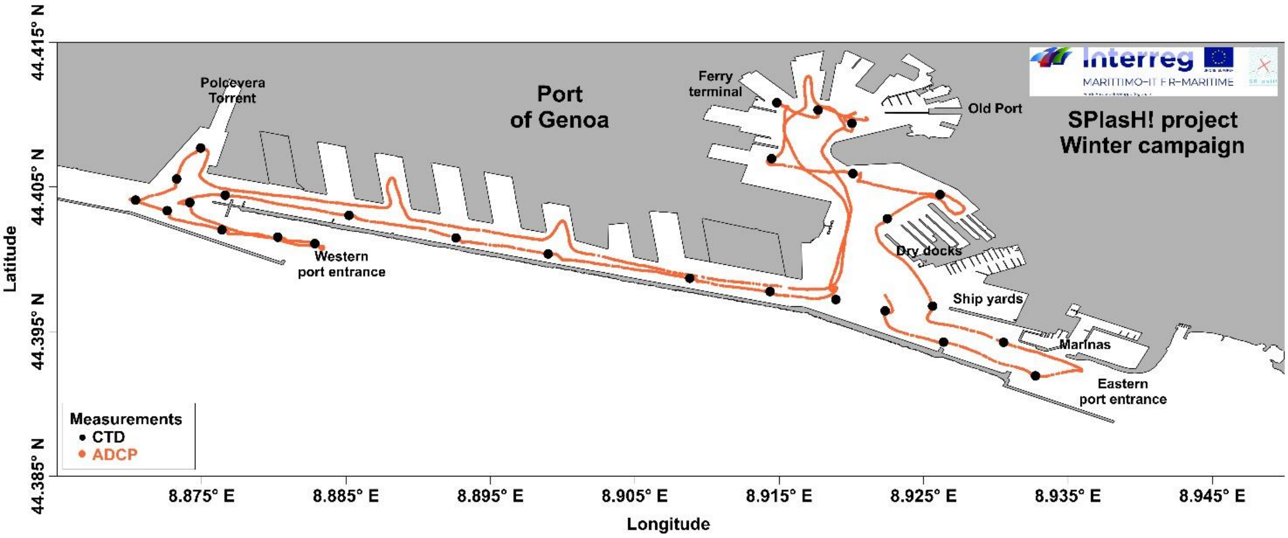

Fig. 2 CTD sampling stations (black points) and ADCP transect (orange line).

Results

Fig. 3-5 shows the results about the items collected and analysed in water surface, water column, sediment samples and fishes, and dynamics. 78 items were found in water surface samples collected with Manta net: 60 within the breakwater and 18 outside the breakwater. Most of them were bigger than 2000 µm, both within (63%) and outside (61%) the breakwater; fragments (**Fig. 6**) prevailed regarding the shape, with a percentage of 78% and 61%, respectively within and outside the breakwater. Spheres were always absent in water surface samples. About the samples collected in the water column, 754 items were analysed, mostly in the size range 125-250 µm (33%) with a prevalence of filaments (31%) (Fig. 6). Sediment samples presented 1,692 items; most of them fell in the size range 63-125 µm (36%) and were predominantly granules (33%) (Fig. 6); in detail, granules prevailed off the Polcevera mouth, fragments prevailed in the Old Port Basin, while sphere and filaments were equally distributed all over the port.

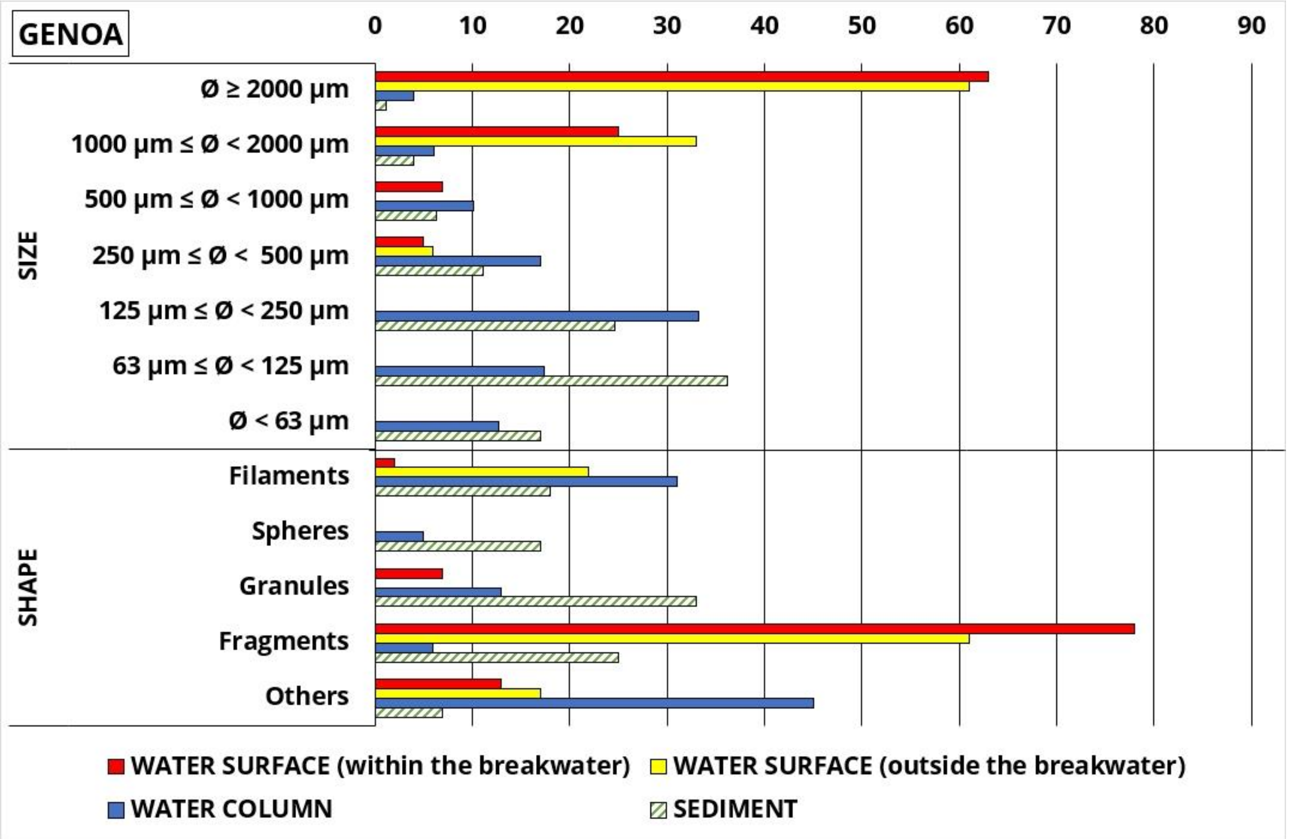

Fig. 3 Graphic showing percentages of size ranges (above) and shapes (below) of the analysed items, collected from water surface within the breakwater (red columns), water surface outside the breakwater (yellow columns), water column (blue columns) and sediment (white columns with green stripes) samples; items from the water surface samples are absent below 300 µm of size, due to the mesh size of the Manta net used for sampling.

Results from the analysis performed of fish from industrial fisheries in Mediterranean Sea are showed in Fig. 4. 165 items were collected from stomatal content of Mugilidae fish: most of them fell in the size range of 63-125 µm (27%), and a prevalence of filaments (39%) and fragments (37%) can be highlighted about the shape (Fig. 6).

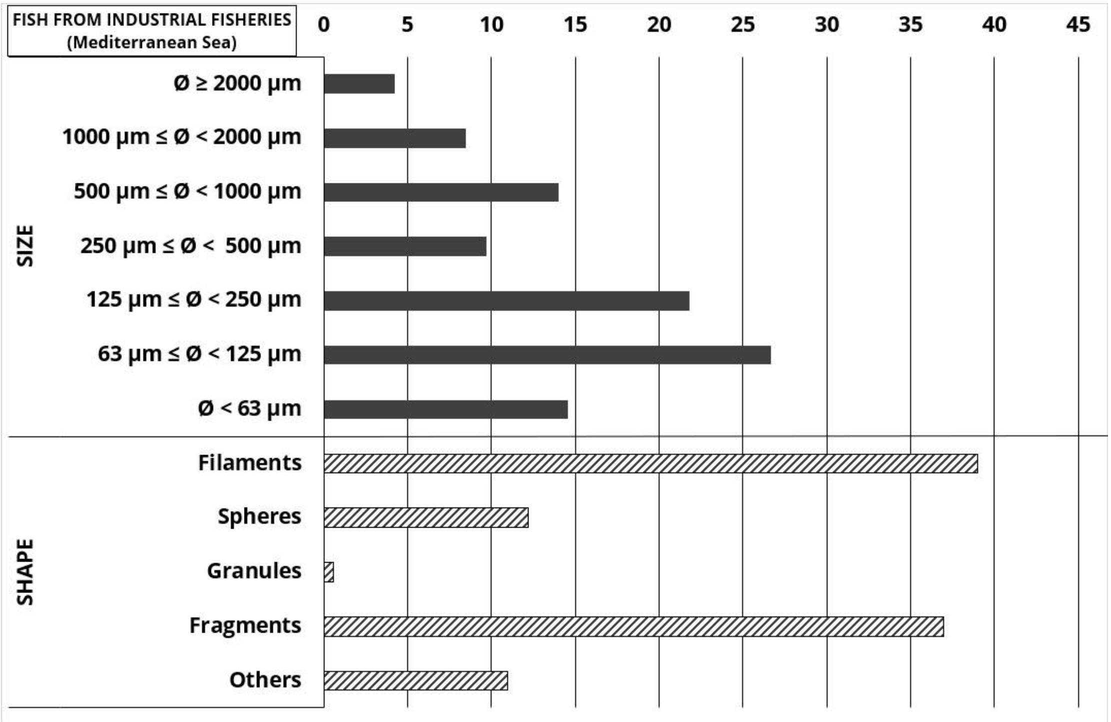

Fig. 4 Graphic showing percentages of size ranges (above) and shapes (below) of the analysed items, collected from fish samples from industrial fisheries in Mediterranean Sea.

Results from the CTD probe during the winter campaign in the Port of Genoa showed temperature values in a relatively wide range in the water column, between 11.5 and 14.5 °C accordingly with the season, showing the presence of relatively cooler water in the inner port area. Salinity highlighted the presence of a relatively thin surface water lens at the stations off the Polcevera mouth indicating the presence of a reduced fresh water input from the torrent and had values between 32.5 and 39.3. Turbidity showed values <8 FTU at all stations with uniform and low surface values as confirmation of a general calm situation and an absence of turbid torrent inputs. In the presence of strong and persistent N-wind, both surface and bottom currents (Fig. 5; mean speed of 8.4 and 7.4 cm s^{-1}, respectively) tended to move eastwards in the Sampierdarena channel and at the western entrance tending to exit the basin towards the open sea, and in the eastern area, from the Old Port Basin to the Eastern Port entrance, tending to exit towards the open sea.

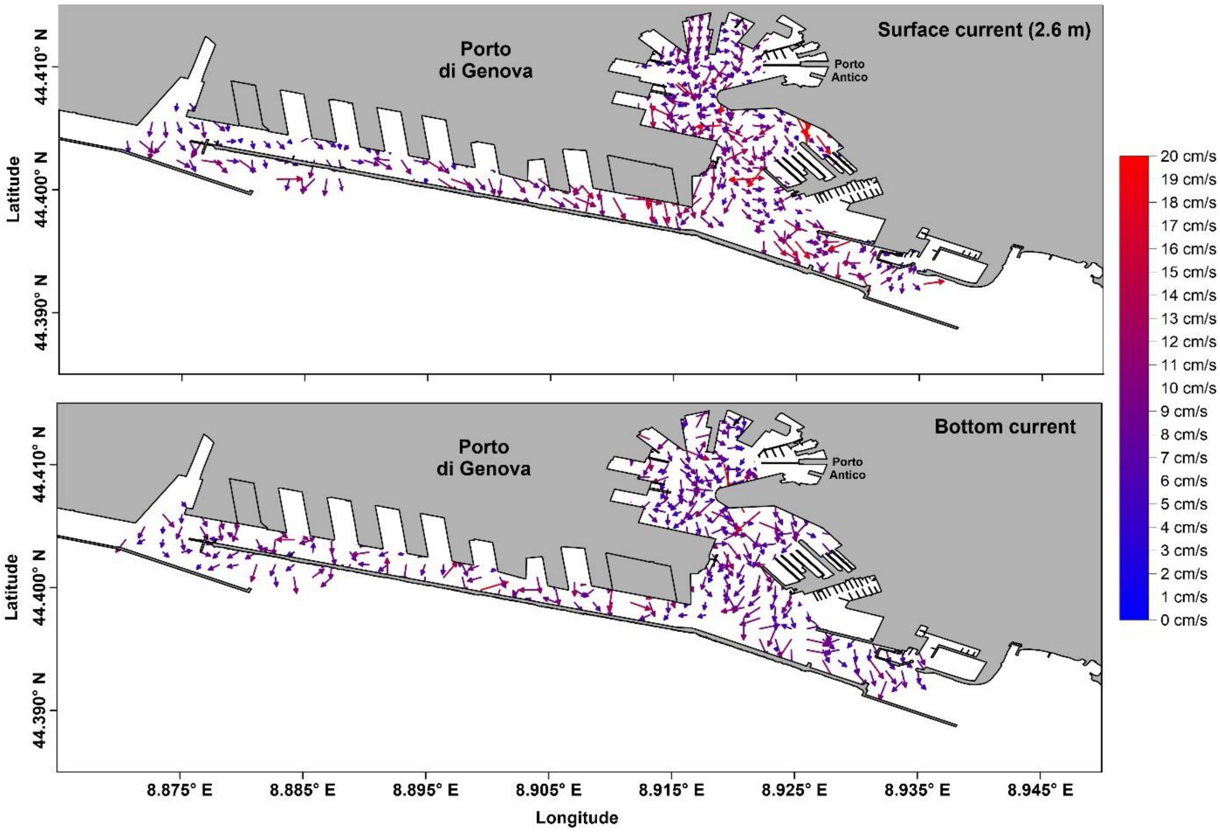

Fig. 5 Maps showing surface (above) and bottom (below) currents recorded during the winter campaign in the Port of Genoa.

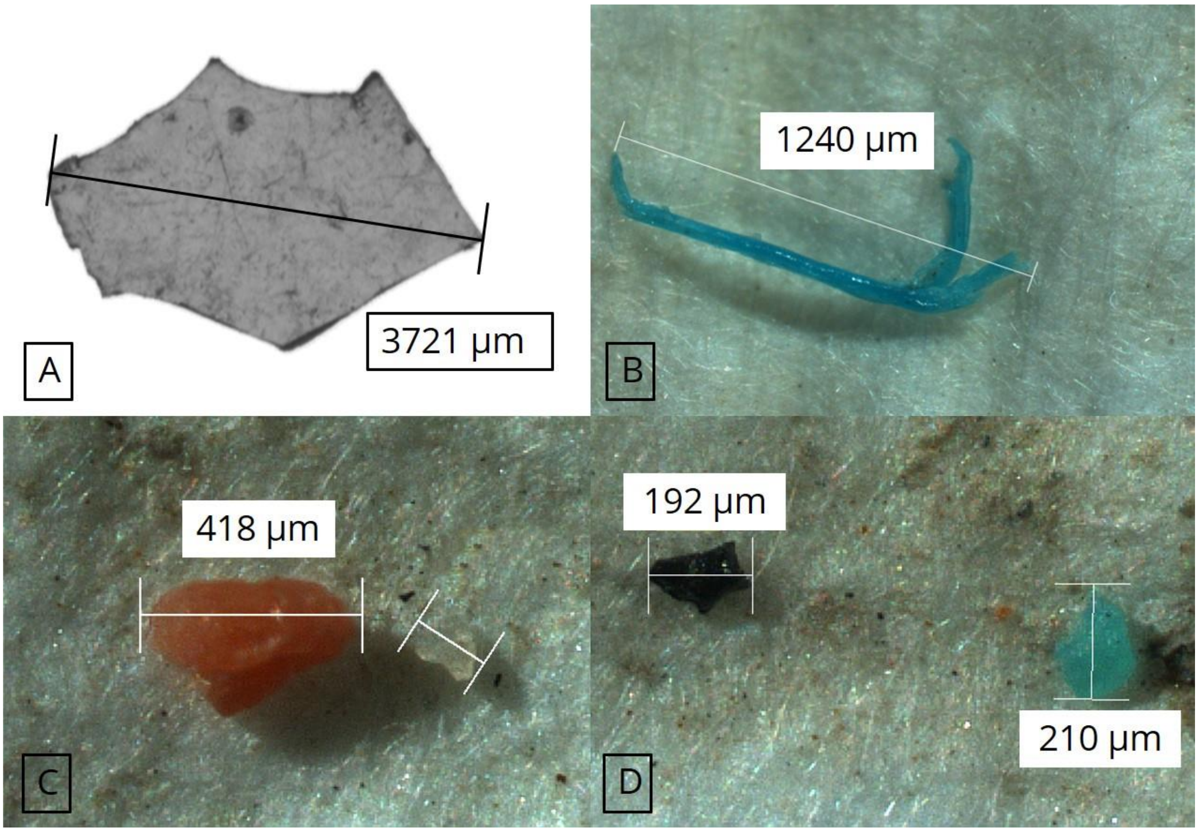

Fig. 6 Pictures of example of analysed items. A) Fragment from water surface sampling (Zooscan V4); B) filament from water column sampling (Leica Z16); C) granule from sediment sampling (Leica Z16); D) fragments from fish sampling (Leica Z16).

Discussion and conclusions

In this study, results from the first sampling of "SPlasH!" project in the Port of Genoa were presented. Most of the items that were found in sediment came from the samples collected in the inner part of the port, compared to the ones sampled at the mouth of Polcevera Torrent. One of the possible reasons that explains this result is that the inner part of the port is strictly connected with the city and a lot of different anthropic activities occur in the area, in the first place the site of collection of solid waste deriving from vessels, while the western area only suffers from some commercial and industrial activities and the supply from the torrent. It is also possible that particles coming from a torrent are most likely to be affected by fragmentation processes, due to the hydrodynamic of the watercourse; thus, those items may become so small that is no longer possible to identify them, leading to an underestimation of the particles in the samples. The stronger hydrodynamic occurring along the torrent, compared to the one of sewage discharges, could also explain the abundance of granular shaped items found at the mouth of Polcevera Torrent, in contrast with the prevalence of fragments found in the samples collected in the inner part of the port (**Fig. 7**). From this set of evidences, it is possible to consider the port basin as a collector of MPs.

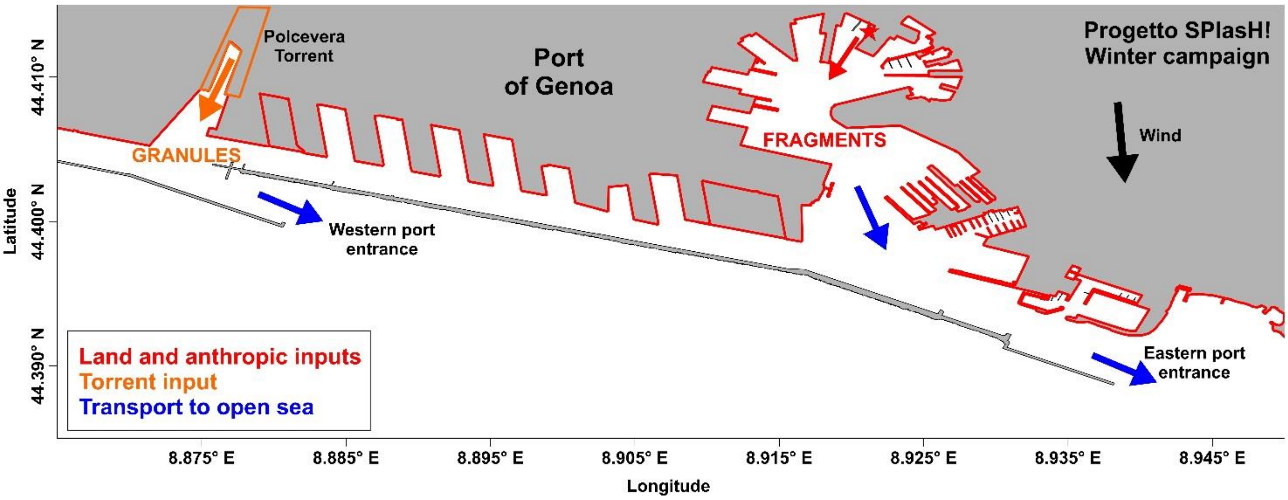

Fig.7 Map summarizing possible different inputs (in red for fragments and orange for granules) and spreading (in blue) direction of items, which highlights the prevalence of granules and fragments in the sediment samples in the western and in the eastern part of the Port of Genoa, respectively. Red star represents the site of the solid garbage collection deriving from vessels.

Water surface showed a prevalence of items in the samples collected within the breakwater, and so inside the port area, compared to the ones sampled outside the breakwater; this is what we expected, due to the presence of all the anthropic activities affecting the inner area of the Port of Genoa.

Data of current direction confirmed what already found in past measurements campaigns [35] and were very useful to characterize the Port of Genoa regarding the aspect of potential transport of MPs. In fact, in presence of strong and persistent North wind, suspended materials, including MPs, can be driven towards the open sea by marine currents (Fig. 7) in both the surface and bottom layer; this could be intensified after a long period of dead calm, causing the accumulation of particles within the port, which can then be spread outside the area all together when strong North wind occurs. The port, therefore, besides being considered as a collector of MPs, can at the same time be considered a source of MPs for the external marine environment, depending on weather conditions. Further investigations will apply numerical models to better characterize and predict MP transport within and outside port areas.

The investigation of polymeric composition on the analysed items is still in progress, thus it is not possible yet to certainly define them as MPs. It is necessary to be careful investigating MP content in environmental samples, because it is very easy to wrongly consider them as MPs when basing only on visual sorting, which can be affected by different confounding factors, such as the operator skills, the characteristics of the microscope used and the sample matrix; error rate of this type of analysis reported in literature ranges from 20% to 70%, increasing with decreasing particle size [40].

A baseline study on fish from Mugilidae family, coming from industrial fisheries of Mediterranean Sea, was performed. It is well known that Mugilidae can filter ingested material, thanks to their special gill rakers, and their stomach owns a grinding system, since they ingest relevant amounts of sediment in their diet [41]. Comparing data from literature, we did not expect to find items bigger than 250 µm, because this type of fish positively selects food particles of a lower size [42-43]. Actually, we found items bigger than 250 µm, but they were almost exclusively filaments, which can be very long in one dimension, but they also are very thin, thus being able to avoid the filtering system. These data will be useful to approach a further investigation on fish from project port areas, which will be collected during the next sampling campaigns.

In conclusion, data from the first sampling at the Port of Genoa gave us preliminary information that will be supported by further investigations in the same port area and in the two others port areas of the project, as Olbia and Toulon.

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