ORIGINAL RESEARCH
published: 13 April 2022
doi: 10.3389/fmars.2022.786026

Evolution of the Distribution and Dynamic of Microplastic in Water and Biota: A Study Case From the Gulf of Gabes (Southern Mediterranean Sea)

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OPEN ACCESS

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Specialty section:
This article was submitted to Marine Pollution, a section of the journal Frontiers in Marine Science

Received: 29 September 2021
Accepted: 28 February 2022
Published: 13 April 2022

Citation:
Ben Ismail S, Costa E, Jaziri H, Morgana S, Boukthir M, Ben Ismail MA, Minetti R, Montarsolo A, Narizzano R, Sammari C, Faimali M and Garaventa F (2022) Evolution of the Distribution and Dynamic of Microplastic in Water and Biota: A Study Case From the Gulf of Gabes (Southern Mediterranean Sea). Front. Mar. Sci. 9:786026. doi: 10.3389/fmars.2022.786026

Marine plastic pollution represents a major problem owing to its increasing presence in the environment, persistence and ability to spread in every compartment in the form of small plastic particles, namely microplastics (MPs). Studies concerning MPs abundance in the Mediterranean Sea are growing, but their occurrence in the Southern regions remains largely unexplored. In this study, distribution, abundance, size, and polymer type of microplastics were investigated in surface water samples collected with a Manta net (200 μm mesh size) and in 118 marine specimens of commercial interests, including fishes, crustaceans, and mollusks, during Spring and Autumn 2019 EU H2020 Claim Project sampling Campaigns in the Gulf of Gabes (Southern Mediterranean Sea). Laboratory characterization showed significant plastic pollution concentrations, with an average abundance of 312,887 and 77,110 items/km^2 in surface water samples collected in Spring and Autumn, respectively. A 3D hydrodynamic and Tracking Model was used to identify dispersal and transport pathways of the floating plastics, reporting a seasonal variability observed in MPs distribution between I (Spring) and II Campaign (Autumn). Despite the high values of MPs abundance found in surface water samples, an overall low frequency of ingestion among studied species was observed, with a maximum value of 20% of individuals (in *Scomber scombrus*) found with ingested MPs. The present study contributes to expand our state of knowledge regarding MPs pollution level in water and biota samples collected in the Gulf of Gabes, an area of particular interest for its biological resources, but still little investigated.

Keywords: microplastic (MP), Mediterranean Sea, surface waters, plastic pollution, biota, hydrodynamic dispersion

INTRODUCTION

After World War II, with the skyrocketing rise in the production and consumption of plastic products, the plastic flow into the environment appears to have been unstoppable and accelerating. Plastic products have become ubiquitous in everyday life. With an estimated global production of 368 million tons (PlasticEurope, 2020), 4.8–12.7 million tons of plastic are estimated to be released into the marine environment every year (Lebreton et al., 2019). The Mediterranean Sea is a semi-enclosed basin with limited exchanges with the Atlantic Ocean (Tanhua et al., 2013). With an high density coastal population, influx of freshwater from densely populated river catchments and a contribution of 15–30% to the global shipping activity (Compa et al., 2020), the Mediterranean Sea has been recognized as one of the most impacted regions in the world by plastic pollution (Cózar et al., 2015; Suaria et al., 2016; Zayen et al., 2020).

Once in the aquatic environment, due to a combination of chemical, mechanical, and biological processes, plastic debris tends to break down into smaller micrometric debris, namely microplastics (MPs). Most commonly, MPs have been defined as synthetic organic polymer particles, less than 5 mm in size that may differ in shape, color and chemical composition (Duis and Coors, 2016). Microplastic pollution has been reported worldwide, in different environmental compartments, including water, soil, air, and biota (Cincinelli et al., 2019; Zhou et al., 2020). Owing to their small size, MPs are potentially bioavailable to a wide range of organisms, having the potential to interact across trophic levels. There is evidence of MPs ingestion, accumulation and transfer by organisms along the food chain (Jiang et al., 2020). The ingestion of small plastics is known to cause direct adverse effects (e.g., entanglement, suffocation) and to expose organisms to plastic-associated chemical (e.g., POPs, PAHs) or microbial agents sorbed to surface (Mammo et al., 2020). MPs ingestion has been reported in different aquatic organisms, from small zooplanktonic invertebrates up to large marine mammals (Fossi et al., 2012). MPs has been identified also in species of commercial interests, including fishes, bivalve mollusks and crustaceans (Mercogliano et al., 2020), thus raising concern for potential risks to food safety and human health (Bakir et al., 2020).

Although during the last years there has been a virtual explosion of research on MPs pollution, especially in the Mediterranean Sea, there is a significant data gap for the Southern part of the basin (Anastasopoulou and Fortibuoni, 2019; Missawi et al., 2020; Wakkaf et al., 2020). According to its physical, biogeochemical, and biological characteristics, the Gulf of Gabes in Southeast Tunisia has been identified as one of the Mediterranean Sea 11 consensus eco-regions (Ayata et al., 2017). Strongly impacted by hydrodynamics, with tides and anticyclonic winds playing a major role (Béjaoui et al., 2019; Zayen et al., 2020), the Gulf of Gabes is highly productive (D'Ortenzio and Ribera d'Alcala, 2009; Ben Brahim et al., 2010), being an important nursery for several fish species (Hattour et al., 2010; Derbel et al., 2012; Enajjar et al., 2015). Overall, the area contributes approximately 40% of the national fish production in Tunisia (DGPA, 2015), thus being an anomaly in an area, the SE Mediterranean Sea, that is known to be oligotrophic (Béjaoui et al., 2019).

Despite being an important resource for biological marine resources, the growing urbanization and industrialization of the shoreline, notably in the northern (Sfax city) and central (Gabes city) regions of the Gulf of Gabes, is compromising the marine environment quality (Darmoul et al., 1980; Ayadi et al., 2014; Rabaoui et al., 2015; El Zrelli et al., 2017). Many untreated industrial and domestic wastewaters originating from the Ghannouch-Gabes industrial complex and the local municipal wastewater treatment plant, respectively, are discharged into the open sea on a regular basis, worsening coastal pollution (El Zrelli et al., 2018; Zayen et al., 2020), including plastic contamination.

Within this context, the aim of this study was to generate a baseline characterization regarding MPs pollution level in the Gulf of Gabes, an area of particular interest for its high biological and economical value but still poorly considered. More specifically, this study intended to (i) assess the evolution of MPs occurrence in coastal water samples collected during two different sampling periods (Spring/Autumn), including dynamic of MPs dispersion and accumulation in the studied area, and the correlation with water masses circulation; (ii) evaluate MPs ingestion by different marine species of commercial interests, including fishes (El Zrelli et al., 2017), crustaceans and mollusks, collected from the studied area.

MATERIALS AND METHODS

Study Area and Samples Collection

Water sampling activities were performed onboard the vessel of the fishing school of Gabes during Spring and Autumn 2019 EU H2020 Claim Project sampling Campaigns, namely I and II Campaign, respectively. Six coastal stations in the central part of the Gulf of Gabes (**Figure 1** and **Table 1**) were selected. The stations are located near ports (stations 4 and 5), Ghannouch Gabes industrial complex (station 7 and 8), and rivers (station 16 and 17).

During both Campaigns, a 200 μm Manta net (0.6 m width 0.2 m height) was towed at an average speed of 3 knots for 15 min for each sampling transect, yielding a total of 12 surface water samples. To gather all particles into the cod-end, the net was rinsed completely with seawater from the outside after each sampling operation. The samples were then transferred from the cod-end to 500 mL glass containers using a 200 μm mesh stainless steel sieve, fixed with 70% ethanol, and transported to the CNR laboratory for analysis.

Regarding biota samples, a total of nine species of commercial interests were purchased from local fishermen in different periods (Spring/Autumn). Organisms were selected since they were representative of different species (including fishes, crustaceans and mollusks), habitats (pelagic, benthic, demersal, and benthopelagic) and trophic levels (TL). The list of collected species are given in **Table 2**. All the samples were kept at −20°C and stored for subsequent MPs analyses.

Surface Water Samples Analysis

A total of 12 surface water samples were analyzed at the CNR IAS Laboratory in Genoa (Italy) using a stereomicroscope (Olympus SZX7, 8x-56x) coupled with a digital camera (Nikon, DSL3). All potential particle items were identified and manually sorted out from the sample and categorized by color, shape (fragment, fiber, pellet, film, and foam) and size (macroplastics: > 5 mm; microplastics, MPs: 5–3 mm; 3–1 mm and < 1 mm) following the criteria reported by Imhof et al. (2012) and Morgana et al. (2018). Then, each particle was manually transferred onto a microscope

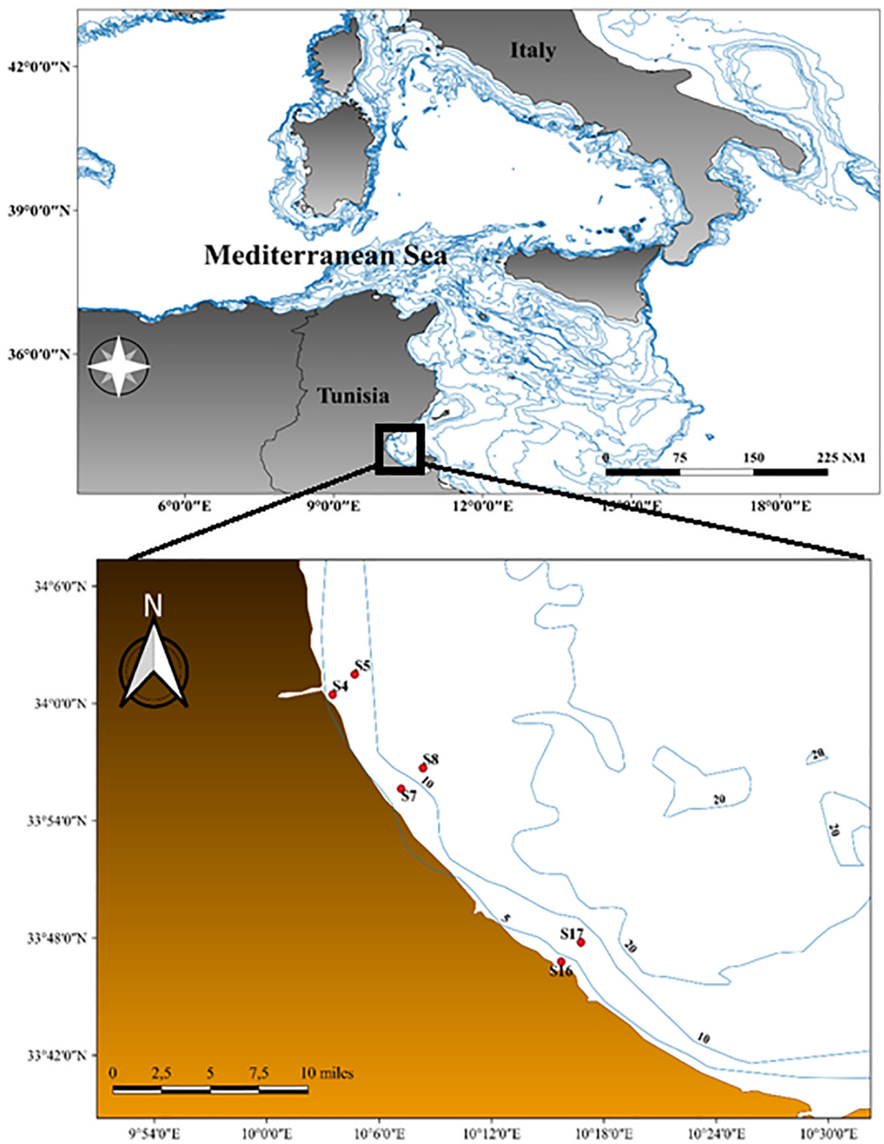

FIGURE 1 | Map showing the locations of the sampling stations in the Gulf of Gabes.

slide for the subsequent chemical analysis. A PerkinElmer *Spectrum Two* Fourier Transform Infrared (FT-IR) spectrometer, coupled with Universal ATR (UATR) was used to define the chemical nature of the isolated items. After analysis, the spectrum created by FT-IR from each particle analyzed was compared to the reference spectra library supplied by Perkin Elmer, with a > 70% similarity threshold. The MPs abundance in surface water samples was expressed as items/m^3 and then converted to items/km^2

TABLE 1 | Gulf of Gabes sampling stations.

Sampling stations	I Campaign	II Campaign	Latitude	Longitude
4	23/04/2019	07/11/2019	34°00′386″N	010°03′449″E
5	23/04/2019	07/11/2019	34°00′494″N	010°05′959″E
7	23/04/2019	07/11/2019	33°55′507″N	010°07′044″E
8	23/04/2019	07/11/2019	33°56′691″N	010°08′333″E
16	25/04/2019	20/11/2019	33°46′789″N	010°15′701″E
17	25/04/2019	20/11/2019	33°47′769″N	010°16′824″E

Biota Samples Analysis

Out of 9 species, a total of 118 specimens were analyzed for their MPs content at CNR laboratory in Genoa (Italy). For each species, the feeding habitat and Trophic Level were established by Fishbase or FAO database[1] as reported in **Table 2**. For each individual, morphometric characteristics were measured,

[1] www.fishbase.org

TABLE 2 | Species (common name), Feeding Habitat (FH), Trophic Level (TL), Sampling area and season, number of analyzed organisms, frequency (%) of ingestion, MPs ingested per individual obtained in the present study (in bold) and from literature.

Species (Common name)	FH	TL	Area	Season	n. organisms	%frequency of ingestion	MPs/individual	References
Scomber scombrus (Atlantic mackerel)	PN	3.6	**Gulf of Gabes**	**Spring**	**10**	**20**	**2.5**	**This study**
			Northern Adriatic Sea	Summer	10	30	1.3 ± 0.58	Neves et al., 2015
			Central Adriatic Sea	Summer	10	70	1.3 ± 0.5	Avio et al., 2020
Pagellus erythrinus (Common pandora)	BP	3.5	**Gulf of Gabes**	**Spring** **Autumn**	**10** **10**	**–**	**–**	**This study**
			Croatian Sea	Spring/Autumn/Summer	30	50	1 (± 1.6)	Anastasopoulou et al., 2018
			NE Ionian Sea		19	42	0.8 (± 1)	
			Ionian Sea	Summer	80	42.1	1.66	Digka et al., 2018
			Tyrrhenian Sea	–	–	6.7	1	Savoca et al., 2019
			Cost of Turkey	Summer	54	22	1.08	Güven et al., 2017
			Southern Adriatic Sea	Summer	6	66.6	1.5 ± 0.6	Avio et al., 2020
Lithognatus mormyrus (Sand steenbras)	D	3.4	**Gulf of Gabes**	**Spring** **Autumn**	**8** **10**	**–**	**–**	**This study**
			East Mediterranean	Summer	46	35	1.89	Güven et al., 2017
			Southern Adriatic Sea	Summer	7	14	1	Avio et al., 2020
			Turkish coast		55	34.3	1.7	Gündoğdu et al., 2020
Sardinella aurita (Round sardinella)	P	3.2	**Gulf of Gabes**	**Autumn**	**17**	**6**	**1**	**This study**
			Coast of Egypt	–	33	100	–	Shabaka et al., 2019
			Southern Adriatic Sea	Summer	9	33	1	Avio et al., 2020
Pomatomus saltatrix (Bluefish)	P	4.5	Gulf of Gabes	Spring	10	–	–	This study
Mullus surmuletus (Surmullet)	D	3.4	**Gulf of Gabes**	**Autumn**	**10**	**10**	**1**	**This study**
			Croatian Sea	Spring/Autumn/Summer	30	70	1.8 (± 1.9)	Anastasopoulou et al., 2018
			Ionian Sea	Summer	80	46.25	1.83	Digka et al., 2018
			Mediterranean Coast	–	19	60		Bellas et al., 2016
			Turkish coast	Summer	38	32.8	1.3	Gündoğdu et al., 2020
			NW Iberian Shelf	Spring Autumn	15	60%	1.56 ± 0.53	Filgueiras et al., 2020
Metapenaeus monoceros (Speckled shrimp)	D	n.a	**Gulf of Gabes**	**Spring**	**18**	**–**	**–**	**This study**
Penaeus kerathurus (Caramote prawn)	D	n.a	**Gulf of Gabes**	**Autumn**	**20**	**–**	**–**	**This study**
Sepia officinalis (Common cuttlefish)	DN	4.27	**Gulf of Gabes**	**Spring**	**3**	**–**	**–**	**This study**
			Portugal	–	39 (mean)	100%	1	Oliveira et al., 2020

PN, pelagic-neritic; P, pelagic; BP, bentho-pelagic; D, demersal; n.a., not available.

including: Total Length (TL), Standard Length (SL), Weight (W), Gutted Weight (GW) for fish; Cephalothorax Length (CL), Weight (W), Gutted Weight (GW) for crustacean; Dorsal mantle length (DML), Weight (W), Gutted Weight (GW) for cuttlefish. In addition, visible deformations and external conditions were observed and for the individuals without tail, the TL was not measured. For each organism, the digestive tract was removed with surgical forceps and a scalpel (previously cleaned with deionized water), placed into a glass Petri dish, and put in an oven dried at 50°C for 24 h. The sample was carefully covered with aluminum foil to prevent airborne contamination. According to protocol recommended by literature (Avio et al., 2015; Bour et al., 2018; Bessa et al., 2019; Cau et al., 2019), a density separation with a NaCl hypersaline solution (density > 1.2 g/cm^3) added to the dried sample was used to extract the MPs. After that, the supernatant was filtered using 0.45 μm nitrate cellulose filters (SARTORIUS) using a filtration system coupled with a vacuum pump. The sample was then placed in a Petri dish with a 15% H_2O_2 solution for partial digestion of remaining organic materials before being dried in the oven (at 50°C overnight). The filter membranes were observed at the stereomicroscope (Olympus SZX7, 8x-56x) coupled with a digital camera (Nikon, DSL3). All putative MP particles were manually sorted out from the sample and categorized by the same criteria (colors, shape, and size) applied during water sample analysis. In order to confirm the chemical nature of the isolated items, a μ-Raman analysis was carried out in collaboration with the Regional Agency for Environmental Protection- Liguria (ARPAL). The number of MPs found in biota samples was expressed as the percentage of organisms for each species found with ingested MPs. This value represents the frequency of occurrence of plastic ingestion.

Contamination Control

In order to avoid potential contamination during sample analysis, several precautions need to be taken into account as recommended by scientific literature and guidelines (Gago et al., 2018; Bessa et al., 2019; GESAMP, 2019; Morgana et al., 2021). All consumables were taken directly out from their packaging and all equipment was carefully rinsed with Milli-Q before and after use. Samples and equipment were covered with aluminum foil where possible. In addition, filter blanks were run in parallel to verify airborne contamination occurring during both water and fish sample processing. Particles or fibers detected on filter blanks were analyzed for color and size and then compared to those found in the analyzed samples in order to avoid false results.

Modeling Influence of Hydrodynamics on Microplastic Distribution

To understand the role of the hydrodynamics on MPs distribution, we adopted a particle tracking approach based on a two-step procedure. First a three-dimensional hydrodynamic model (ROMS, Shchepetkin and McWilliams, 2005)[2] was forced by all the forcing likely to contribute to the hydrodynamics of the Gulf of Gabes. Secondly, a 3-h 3D velocity fields extracted from ROMS are then offline coupled to the Lagrangian particle tracking model Ichthyop (Lett et al., 2008),[3] which is used to track the floating virtual microplastic particles. In the Ichthyop model, virtual microplastic particles behave as a Lagrangian drifter under the effect of horizontal and vertical advection, horizontal and vertical dispersion, and also a buoyancy force due to the difference between the particle and surrounding water density. Ichthyop was used by many authors as a Lagrangian particle tracking model to study the coastal accumulation of microplastic particles (Atwood et al., 2019; Miladinova et al., 2020; Soto-Navarro et al., 2020; Bouzaiene et al., 2021). The modeled area including the Gulf of Gabes extends from 32.5 to 35.5°N and from 10 to 12.5°E. For a more accurate depiction of small-scale processes, a high spatial resolution of nearly 1 km (1/96°) in both longitudinal and latitudinal directions was adopted, which is largely below the first internal Rossby radius of deformation (10 km, Send et al., 1999). Such configuration allowed not only a better resolution of the main small-scale patterns of the circulation but also a good representation of the bathymetry. The spatial discretization on the vertical uses the generalized sigma coordinates which follow the bathymetry variations of the seabed and allow to have the same number of vertical levels. In this study, we used 25 vertical levels, a sufficient resolution since the bathymetry in the Gulf of Gabes does not exceed 40 m. The model bathymetry is deduced from Smith and Sandwell (1997) topography database by a bilinear interpolation onto the model grid. The model was initialized using the MEDATLAS (MEDAR/MEDATLAS Group, 2002) monthly climatology of observed temperature and salinity fields. The open boundaries are prescribed from the daily output (elevation, velocity, temperature, and salinity) of MED12 simulations with a spatial resolution of 1/12° (Lebeaupin Brossier et al., 2013) through a one-way off-line nesting. The model simulations were forced by wind, atmospheric pressure, heat fluxes, and also tides since their effects could not be neglected.

A particle-tracking 3D model (Ichthyop; Lett et al., 2008) was used to investigate the influence of hydrodynamics in the spatial distribution of MPs through an offline coupling to the high resolution (1/96°) hydrodynamic model (ROMS). The Ichthyop model allows researchers to investigate how physical and biological factors influence ichthyoplankton dynamics. In this study, we are interested in identifying the role of 3D marine currents in the MPs dispersion and accumulation. The surface currents play a potential role in the distribution of floating marine particles (Kubota, 1994; Martinez et al., 2009; Miladinova et al., 2020) and, according to Kubota (1994), Stokes drift has a negligible impact on debris transport. For these reasons, Stockes drift was not considered, and a backward simulation was carried out to determine MPs potential sources that have accumulated at the sampling stations and how these have reached the stations. In each station, 1,000 particles considered as passive tracers, were advected backward in time for 2 months and the positions of each particle was recorded every 3 h.

[2]http://myroms.org

[3]https://doi.org/10.5281/zenodo.4243813

RESULTS

Quality Control

In the control membranes, only textile microfibers, mainly in blue and black colors, were found, with an average value of 3 fibers/h. Consequently, the final MPs abundances in water and biota samples reported in this study were given as subtracted of blank values (MPs in control membranes), in order to avoid overestimation (Avio et al., 2020).

Surface Water Samples

Throughout the I Campaign and II Campaign, microplastic particles were found in all sampled stations (**Table 3**). During the

TABLE 3 | Abundance of microplastics (items/km^2) found in the samples collected during the I Campaign (Spring) and II Campaign (Autumn) in the Gulf of Gabes.

	Abundance (items/Km^2)	
Sampling stations	**I Campaign**	**II Campaign**
4	2.1×10^5	2.2×10^5
5	4.8×10^5	2.3×10^4
7	6.8×10^5	1.4×10^5
8	6.2×10^4	2.2×10^4
16	2.4×10^5	1.8×10^4
17	1.9×10^5	3.6×10^4

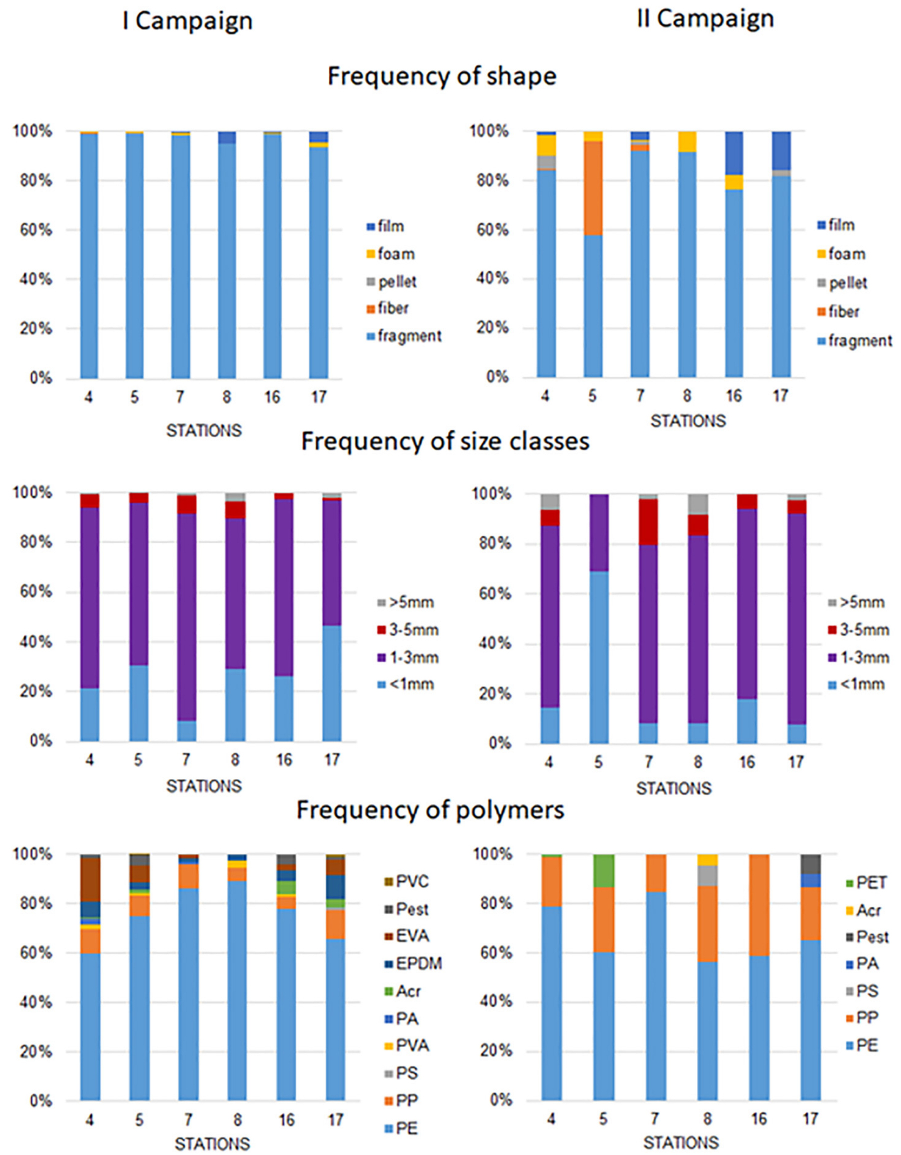

FIGURE 2 | Frequency (%) of particle shape, size classes and polymers extracted from water samples collected in Gulf of Gabes during I and II Campaign. PE, Polyethylene; PP, Polypropylene; PS, Polystyrene; PVA, Polyvinyl.

I Campaign, the abundance of MPs ranged between 6.2×10^4 items/km^2 (station 8) and 6.8×10^5 items/km^2 (station 7), with an average of $3.1 \pm 2 \times 10^5$ items/km^2. Within the II Campaign, a mean value of $7.8 \pm 8 \times 10^4$ items/km^2 was found, with a minimum of 1.8×10^4 items/km^2 (station 16) and a maximum of 2.2×10^5 items/km^2 (station 4). **Figure 2** reported results on MPs characterization. Bar graphs showed the relative contribution (%) of each shape, size class and polymer considering the overall MPs isolated from water samples collected during the I and II Campaign. Different MPs shapes were detected, with fragments being the most abundant plastic-shape from both the I and II Campaign (97 and 80%, respectively), while fibers, pellets, films, and foams constituted only a small fraction of the total. Identified particle size varied between 0.2 and 9.4 mm, with an average size of 1.56 mm ($\pm$ 0.22) and 1.83 ($\pm$ 0.59) mm for the I and II Campaign, respectively. Most of the sorted items belonged to 1–3 mm class size, specifically 67% (I Campaign) and 69% (II Campaign). The chemical composition of sorted items (>700 μm) was confirmed by FT-IR analysis. Throughout both Campaigns, 11 different polymer typologies were identified. Polyethylene (PE) made up the majority of MPs, with 75% (I Campaign) and 67% (II Campaign), respectively, followed by polypropylene (PP) (8 and 26% from samples of the I and II Campaign, respectively). Less frequent polymers included (<6%): polystyrene (PS), polyvinyl alcohol (PVA), polyamides (PA), acrylic (Acr), ethylene-vinyl acetate (EVA), polyvinyl chloride (PVC), ethylene propylene diene monomer (EPDM), polyesters (mainly PET).

Biota Samples

Microplastic particle ingestion was characterized in 118 marine organisms (75 specimens of fish belonging to 6 species, and 43 specimens of invertebrates belonging to 3 species). For fish, SL (± SD) averaged 16.43 (± 2.48) cm, varying from 11.3 to 22.6 cm, and average BW was 94.78 g (± 43.85) ranging from 30.3 to 221.35 g. Out of 6 species collected in the I Campaign (Spring), only *Scomber scombrus* was found with ingested anthropogenic particles, specifically 20% of the analyzed organisms, containing between 1 and 4 particles (consisting of a glomerulus of fibers), with an average of 2.5 ± 2.1 items/individuals (**Table 4**). Within the II Campaign (Autumn), MPs were detected in *Sardinella aurita* and *Lithognatus mormyrus* with an ingestion frequency of 10%, with an average of 1 item/individual) (**Table 4**). Ingested MPs were mainly represented by fragments, with the exception of a knot of 4 fibers found in *S. scombrus*' gut. The size of ingested MPs ranged between 0.13 and 6.8 with an average size of 2.51 ± 3.71 mm (**Table 4**). The spectra obtained with DXR^{TM} 2 Raman Microscope allowed us to identify a mixture of dye additives (n = 2), Polyethylene (n = 1), and Ethylene-vinyl acetate (n = 1). A summary of the main plastic characterization output species is shown in **Table 4**.

The Impact of Hydrodynamics on Microplastic Dispersion

Because high kinetic energy can increase litter concentration or scattering, it's important to understand the distribution of kinetic energy and measure the contributions of mean kinetic energy (MKE) and eddy kinetic energy (EKE) to total kinetic energy. In order to do so, we calculated the logarithm of the EKE/MKE ratio. It is determined by the following parameter:

$\Phi = \log(EKE/MKE)$

According to the sign of Φ we can deduce if the EKE dominates MKE ($\Phi > 0$) or rather MKE dominates EKE ($\Phi < 0$).

The spatial distribution of the parameter Φ shows that the EKE can be, in logarithmic scale, around four times higher than MKE over a major part of the shelf and Boughrara lagoon (**Figure 3**). This means that the Gulf of Gabes is almost controlled

TABLE 4 | Seafood species with ingested MPs, morphometric measure (standard length SL and gutted weight GW) and plastic characteristics (shape, color, size, polymer). n.a, data not available.

Sampling campaign	Species	Morphometric measure		Plasticcharacteristic			
		SL (cm)	GW (g)	Shape	Color	Size	Polymer
I Campaign	*S. scombrus*	17.9	59.16	Fragment	Blue	0.13	Dye additive
		17.2	55.88	Fiber mix	Mix	n.a	Dye additive
II Campaign	*S. aurita*	16.9	58.9	Fragment	Transparent	0.68	PE
	M. surmuletus	12.8	37.35	Fragment	Transparent	6.8	EVA

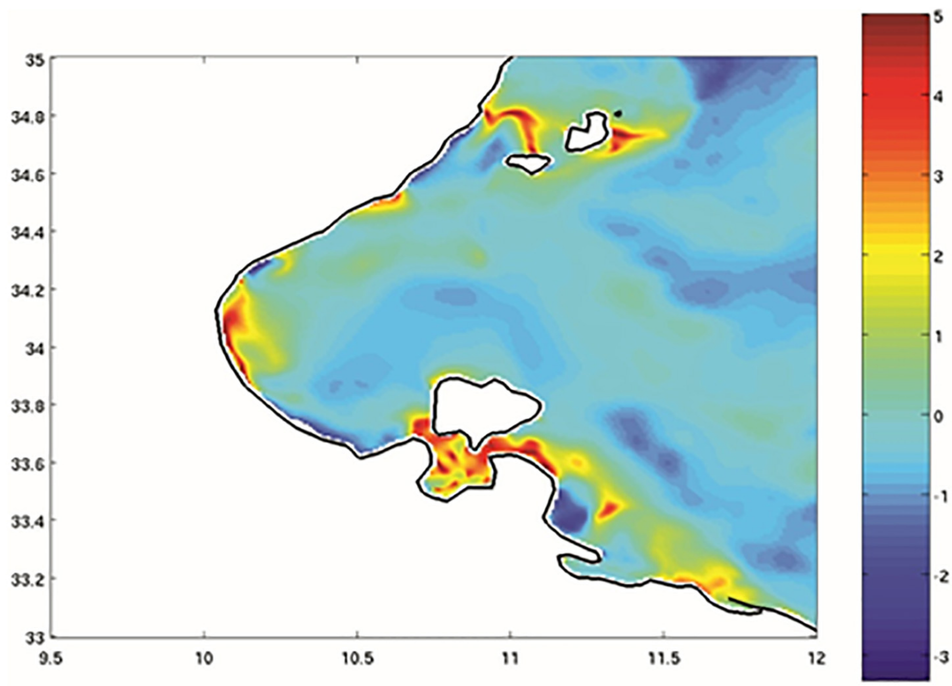

FIGURE 3 | The logarithmic of the ratio between EKE over MKE on the annual scale.

by fluctuating currents. The high values of EKE can be due to the effect of sudden reversals of the direction of currents in this region (**Figure 4**). The origin of water sampled in station 16 and 17 is different from the other stations and depends on seasons. During the autumn-winter period, it comes mainly from Tunisian coastal current, while during spring-summer period it could also come from the Libyan coast due to the current reversal between winter and summer (**Figure 4**). As a result, the hydrodynamic circulation in the Gulf of Gabes indicates that the MPs in the Gulf of Gabes could originate from many remote locations of the Mediterranean basin, as well as from coastal regions such as Sfax and Gabes, which are important industrial centers with ports.

The overall lower accumulation rates of MPs in November, compared to those in April (**Table 2**), may be explained by the fact that in winter the surface currents are more energetic (Boukthir et al., 2019), thus preventing the formation of offshore MPs accumulation patterns. Moreover, according to the surface currents distribution and the Lagrangian model used to track floating debris, the accumulation rates of MPs in April come from two potential zones (**Figure 4**), namely coastal industrial cities (Sfax and Gabes), a touristic city (Djerba Island) and even from remote regions such as Libyan coast. In contrast, during November it seems to originate mainly from Tunisian industrial cities due to the current reversal between autumn-winter and spring-summer periods (**Figure 4**) as mentioned by Boukthir et al. (2019). This behavior of the current reversal is coherent with the paths of two drifting buoys launched in November 2017 and July 2018. The sampling stations are in a convergence zone of current during April coming from the Tunisian coast and the Libyan coast after having bypassed the island of Djerba (**Figure 4**), which may advect MPs from surrounding Tunisian cities and from remote zones and consequently would favor the accumulation of plastic debris.

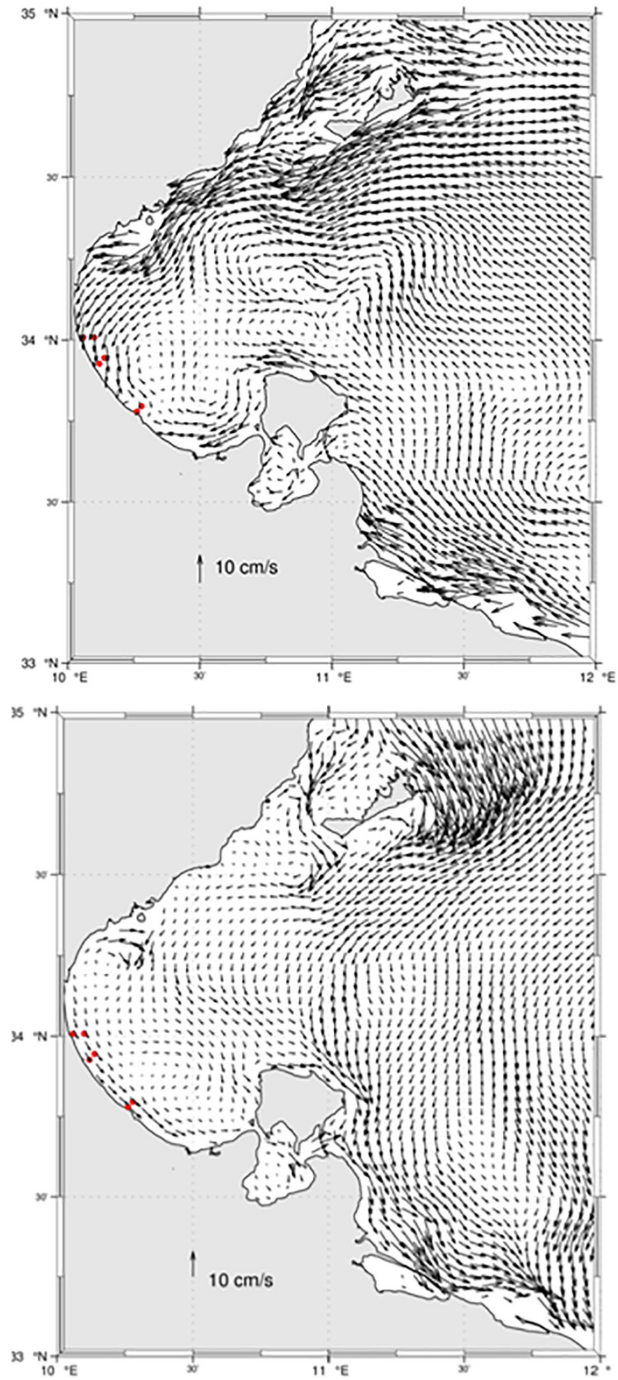

FIGURE 4 | The Gulf of Gabes surface circulation based on monthly mean velocity vectors averaged over upper 10 m in April (upper) and in November (lower). The sampling stations are indicated by red points.

DISCUSSION

The current study fills a data need on microplastic pollution in the Gulf of Gabes (Southern Mediterranean Sea) by characterizing the evolution of microplastic distribution and dynamics in surface water samples and analyzing MPs ingestion in commercially important species. The Gulf of Gabes is a rich coastal, marine, and freshwater ecosystem with major biological resources, but it is currently under severe anthropogenic strain, especially as a potential source of plastic pollution. Along Tunisian coasts, studies on MPs presence have been mainly carried out in sediment samples (Abidli et al., 2017, 2018; Chouchene et al., 2019; Missawi et al., 2020), whereas studies on MPs in waters are limited to few examples, including the waters from an urban lagoon (Bizerte lagoon, northern Tunisia, Wakkaf et al., 2020) and preliminary data from the Gulf of Gabes (Zayen et al., 2020).

In this work, we reported a remarkable MPs presence in all surface water samples, with an average abundance of $3.1 \pm 2 \times 10^5$ items/km^2 in Spring and $7.8 \pm 8 \times 10^4$ items/km^2 in Autumn. Our findings are in line with the recently published work of Zayen et al. (2020), showing comparable MPs abundance in surface water samples collected in Autumn 2017 from the Gulf of Gabes. Despite differences among studies conducted in the Mediterranean basin (e.g., net, sampling time, measure unit), overall our values are higher than MPs content found in mostly regions of the Mediterranean Sea (**Table 5**), thus confirming that the Gulf of Gabes is an area particularly sensitive to plastic pollution.

The sampled stations considered in this work were located close to coastline, in the proximity of important entry points for MPs (i.e., ports, rivers, industrial complex). The high level of anthropogenic pressure along the studied area contributes to explain the high concentration of MPs found. In this area, human activities, including industry, fishing, agriculture, and domestic waters, are likely to play a major role as MPs input (Fourati et al., 2018). In that regard, Liubasteva et al. (2018) have demonstrated by an exhaustive numerical modeling study that

TABLE 5 | MPs abundance values found in surface waters from Mediterranean Sea.

Area	Mean abundance ± SD (Max)	Shape and size	Polymer	References
Cretan Sea	119 ± 250 (1,160) g/km^2	Fishing line, cellophane, fragment	–	Kornilios et al., 1998
NW Mediterranean	0.116 (0.892) $items/m^2$	Filament, film 0.3–5 mm	Polystyrene	Collignon et al., 2012
Ligurian/Sardinian Sea	0.31 ± 1.0 (4.83) $items/m^2$	–	Phthalates	Fossi et al., 2012
Bay of Calvi	0.062 (0.688) $items/m^2$	Filament, film 2–5 mm	Polystyrene	Collignon et al., 2014
W Mediterranean	0.135 (0.42) $items/m^2$	Fragment, film < 5 mm	–	Faure et al., 2015
W Sardinia	0.15 (0.35) $items/m^2$	–	–	de Lucia et al., 2014
Ligurian Sea	0.103 (0.36) $items/m^2$	Fragment 1–2.5 mm	Polyamide Polystyrene Polyolefin Polyester	Pedrotti et al., 2014
Ligurian Sea	2.1×10^4–5.78×10^5 $items/m^2$	Fiber < 2 mm	Polyethylene Polypropylene Polyamide	Pedrotti et al., 2016
NW Sardinia	0.17 ± 0.32 (1.69) $items/m^2$	0.2–5 mm	–	Panti et al., 2015
Central W Mediterranean	$6.72 \pm 1.5 \times 10^4$ (1.04×10^4) g/km^2	Fragment 0.2–0.5 mm	Polyethylene Polypropylene	Suaria et al., 2016
Central and Western Mediterranean Sea	9×10^4–1.2×10^6 $items/m^2$	1–5 mm	–	Ruiz-Orejón et al., 2018
Coast of Turkey	1.63×10^4–5.2×10^5 $items/km^2$	Fiber 0.1–2.5 mm	Plastic copolymer (Polystyrene isoprene)	Güven et al., 2017
Northern Ionian Sea	0–1.6×10^6 $items/km^2$	Fragment 1–5 mm	Polyethylene	Digka et al., 2018
Gulf of Lion	6×10^3–1×10^6 $items/km^2$	1.48 ± 0.88 mm	–	Schmidt et al., 2018
Adriatic Sea	$3.15 \pm 5.68 \times 10^5$ $items/km^2$	Fragment 2.5–5 mm	Polyethylene Polypropylene	Zeri et al., 2018
Southern Mediterranean/Bizerte lagoon	453.0 ± 335.2 $items/m^3$	Fibers	Polyethylene polypropylene	Wakkaf et al., 2020
Southern Mediterranean/ Gulf of Gabes	2.5×10^4–1.1×10^5 $items/km^2$	Fragment, Film 0.2–1 mm	Polyethylene Polyethylene	Zayen et al., 2020
Eastern Mediterranean Sea	0.12–0.72 $items/m^3$	Fragment 1.71 ± 1.07 mm	Polyethylene	Adamopoulou et al., 2021
South-Western Mediterranean Sea	$1.01 \times 10^5 \pm 3.8 \times 10^4$ $items/km^2$	Fiber	Polyethylene	Setiti et al., 2021
Eastern Mediterranean/ Cyprus	Fragments: 4.19 ± 7.29 $items/m^3$ Fibers: 37.13 ± 21.33 $items/m^3$	Fragment 0.02–9.6 mm^2 Fiber < 2 mm	–	Vasilopoulou et al., 2021
W Mediterranean	3.52 ± 8.81 $items/m^3$	Fragment	Polyethylene	Fagiano et al., 2022
Southern Mediterranean/ Gulf of Gabes	$3.1 \pm 2 \times 10^5$ $items/km^2$ (I Campaign) $7.8 \pm 8 \times 10^4$ $items/km^2$ (II Campaign)	Fragments 1–5 mm	Polyethylene Polyethylene	This study

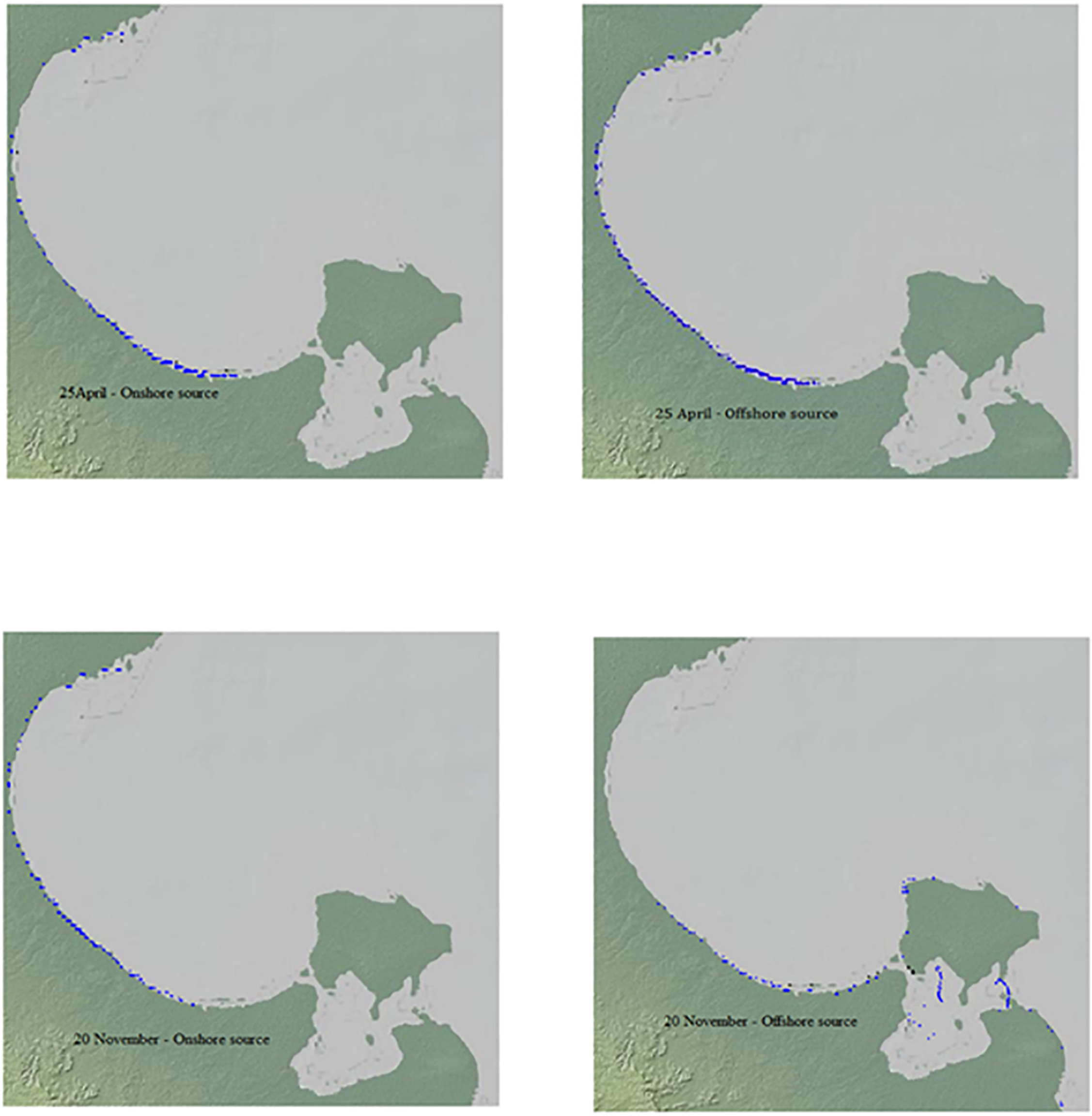

FIGURE 5 | Accumulation of virtual particles (blue points) coming from onshore and offshore virtual sources from numerical simulations after 2 months advection time in April and in November corresponding to the sampling period of the first and second campaign, respectively.

plastic emissions from Tunisia contribute more than 80% of their own coastline plastic pollution, defined as a "boomerang effect" (Liubasteva et al., 2018).

The approach used in this study to investigate the influence of hydrodynamics in the spatial distribution of MPs has been already applied in several studies and has given satisfactory results in terms of identifying potential areas of floating particles accumulation and their dispersion in the Mediterranean Sea (Macias et al., 2019) and in the Black Sea (Miladinova et al., 2020). The circulation in the Gulf of Gabes is influenced by the meteorological forcing and tides, being the most important tidal range in the Mediterranean Sea (2 m) as a result of a tidal resonance phenomenon (Abdennadher and Boukthir, 2006). Above all, the circulation is strongly forced by the mesoscale ATC with an Atlantic origin (Boukthir et al., 2019). The ATC is divided into two branches: one flows toward the gulf and the second one toward the south-east and forms a strong jet particularly in winter, namely the Atlantic Libyan Current (Sorgente et al., 2003; Ben Ismail et al., 2015). The main characteristics of the surface circulation scheme have been faithfully reproduced in our simulations.

The hydrodynamic models used in this study, on the other hand, suggest that the surface water samples taken at various locations came predominantly from outside the Gulf of Gabes and were advected by coastal current. As shown in **Figure 5**, the first source (the Atlantic Tunisian Current, ATC) entered the Gulf 2–3 months before the sampling date, and then carried to sampling stations by coastal currents (Tunisian Coastal

Current). As a result, while the majority of the water analyzed came from near the coasts, some of it is likely to have come from further away. Moreover, the models show that stations 16 and 17 are in a distinct convergence zone than the other stations and are substantially influenced by seasons. At most stations a difference of one order of magnitude was reported between I and II Campaign, that can be partly explained by the high rainfall recorded in November 2019 (during the II Campaign) in addition to the accumulation effects of currents features inversion highlighted by the hydrodynamic models. This evidence a seasonal effect on MPs distribution in the area, as indeed the Gulf of Gabes is characterized by important seasonal variation due to the surface current circulation. In that regard, the Lagrangian model used to track floating debris from two sources located offshore and onshore of the Gulf of Gabes shows that during April month (Campaign I) floating debris accumulates entirely along the Gulf of Gabes coastline and especially in the area where the sampling stations are located (**Figure 5**). On the other hand, during November (Campaign II), floating debris accumulated not only along the Gulf of Gabes coastline, but there are also transported toward the coast of the Djerba island and Boughrara lagoon (most in the south off the Gulf of Gabes) (**Figure 5**). This is explained, in part, why the accumulation rates of floating debris at all sampling stations are higher in April than in November.

Microplastic items identified from water samples were mainly fragments, and this trend was confirmed during both the sampling Campaigns (**Figure 2**), which is in line with data from literature in other Mediterranean regions (**Table 5**). As suggested by Zayen et al. (2020), the highest presence of fragments could be due to the high rate of fragmentation of large plastic objects rather than MP primary input (i.e., direct release of particles in the form in which they are originally manufactured or from wastewater circuit discharge). In addition, the predominance of smaller MP (<1 or 1–3 mm; **Figure 2**) can be explained with the rapid breakdown of the particles occurring along the shoreline, considering that all stations sampled were located close to the coast. Considering MPs polymeric composition, a great variability of plastic types during both sampling campaigns were found, with a large predominance of PE, followed by PP, which is in line with other studies in the Mediterranean Sea (**Table 5**). PE and PP have the highest frequency due to their low density, which allows them to easily travel on surface water, as documented in literature studies (Pedrotti et al., 2016), as well as the fact that they are the most commonly used polymer types in various consumables such as packaging, domestic plastic waste, and various personal care and cosmetics products (Cole et al., 2011; PlasticEurope, 2020). To our knowledge only few studies focused on MPs occurrence in aquatic organisms sampled from the Gulf of Gabes area, specifically in the teleost *Serranus scriba* (Zitouni et al., 2020) and in the sea worm *Hediste diversicolor* (Missawi et al., 2020). Such lack of data limits comparisons with our results. To expand the current state of knowledge, this work assessed the presence of MPs in the digestive tracts of several species of commercial interests, including fishes, crustaceans and mollusks sampled in the Gulf of Gabes. Conversely to the high values reported in surface water samples, microplastics were found only in 3% of the total individuals analyzed, specifically in *S. scombrus* (20% of the analyzed specimen), *M. surmuletus* (10% of the analyzed specimen), and *S. aurita* (6% of the analyzed specimen). *S. scombrus* and *S. aurita* are pelagic, displaying planktivorous feeding habitat (Neves et al., 2015; Shabaka et al., 2019), while *M. surmuletus* is demersal, feeding on benthic organisms (Filgueiras et al., 2020; Gündoğdu and Çevik, 2020). The other species considered in this study included both pelagic and demersal organisms. From our results, it is difficult to relate organism feeding behavior to MPs ingestion. Previous studies have deeply investigated such correlation, but the results are often contrasting. For instance, some authors suggested that pelagic species ingest more MPs when compared to demersal species (Rummel et al., 2016; Güven et al., 2017), while on the contrary others reported that pelagic and demersal species did not differ in MP content (Neves et al., 2015; Lusher et al., 2017). Nevertheless, considering that sediments are considered as a major sink for MPs pollution (Woodall et al., 2014), many studies reported that demersal species have more probabilities of being in contact with microplastics than pelagic species (Filgueiras et al., 2020). For instance, Bellas et al. (2016) focused on MPs ingestion in three commercially, relevant demersal fish species (*Scyliorhinus canicula, Merluccius merluccius, Mullus barbatus*) from the Spanish Atlantic and Mediterranean coast, thus being selected as that are used as indicative species for marine pollution monitoring within the Spanish Marine Pollution Monitoring Programme (SMP). Besides, this study reported a frequency (16%) of MPs ingestion by *M. surmuletus,* that was comparable to our finding. To provide data on MPs ingestion in species of commercial interests is crucial considering their high economic importance worldwide, and representing the bulk of fish biomass, particularly in upwelling regions (FAO, 2016). Such species play a key role in pelagic food webs, by having an important effect on lower trophic levels (i.e., planktonic organisms) and, at the same time, controlling predatory fish (Cury et al., 2000; FAO, 2016). Among them, *S. scombrus* represent a widely studied species, and different frequencies of MPs occurrence (Nelms et al., 2018; Lopes et al., 2020) have been reported, both in Atlantic regions (Nelms et al., 2018; Lopes et al., 2020) as well as in Mediterranean Sea (Avio et al., 2020).

Overall, an average of 1 particle/individual was found in the digestive tracts, which is in line with data from literature (**Table 2**). All the identified MPs were fragments according to other works (Digka et al., 2018; Savoca et al., 2019; Avio et al., 2020), as well as being reflected in the high percentage of fragments found in seawater surface samples (Cole et al., 2011; Zitouni et al., 2020). In that regard, several investigations on MPs ingestion by aquatic vertebrates and invertebrates reported fibers as the most ingested particles (Avio et al., 2020). Also in our study, many fibers were identified during biota analysis, but these were not considered to avoid false results since we cannot exclude that contamination occurred during laboratorial analysis, although several precautional methodologies have been applied as recommended by literature (Rummel et al., 2016; Wesch et al., 2017; GESAMP, 2019). This conservative approach might in turn lead to underestimation of the plastic pollution level, thus contributing to explain, for example, differences found in this study with data from literature on MPs ingestion by aquatic organisms.

Ingested particles were constituted by PE and EVA, and two particles were identified as dye additives, thus confirming their anthropogenic origin. This data is in agreement with Zitouni et al. (2020) that have reported in *Serranus scriba* ingested particles made by a wide range of additives such as lubricants, stabilizers and plasticizers. These are known as "plasticizers," not properly plastic. The presence of these substances in the digestive tract of aquatic organisms is, however, an indication of plastic ingestion by organisms, probably attracted by their color Koelmans (2015), and for this reason these particles were included in the estimation of ingested items.

The low frequencies of MPs ingestion found in this study appear to be in contrast with the high level of plastic debris found in water samples. In that regard, the low number of analyzed individuals per species (10 on average) may explain the low frequencies found, as indeed previous works highlighted that a high number of individuals to be analyzed is essential to find a robust indicator regarding trends of ingested litter in a specific area (Neves et al., 2015; Avio et al., 2020). In addition, it should be also highlighted that these investigations should be considered a snapshot of MPs currently trapped as well as those yet to be egested or translocated (Parker et al., 2021), thus being representative of MPs contamination level at a given point in time and in space.

CONCLUSION

This research contributes to expanding our state of knowledge on microplastic pollution levels in a region, the Gulf of Gabes, that still poorly considered, but of great importance for its high ecological value. Our results confirmed the ubiquitous nature of MPs in the marine environment, by polluting the southern region of the Mediterranean basin.

This study reported a high level of MPs in surface water samples, with a seasonal variability in MPs distribution observed between the sampling campaigns. Despite high presence in waters, low frequencies of plastic ingestion by investigated species were reported, thus claiming for further and long-term investigations.

Considering the severe anthropogenic pressure insisting on the studied area, future work is recommended in order to define plastic pollution levels in the area and its reliable threat to marine ecosystems, that are essential to set effective management measures to face this emerging global threat.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

ETHICS STATEMENT

Ethical review and approval was not required for the animal study because the research was done on captured commercial fishes.

AUTHOR CONTRIBUTIONS

SB, EC, and SM wrote the manuscript initial draft. SB, HJ, MB, MAB, EC, SM, and RM collected and processed the samples. AM and RM processed the sample by chemical characterization. SB, EC, SM, MB, RM, FG, CS, and MF reviewed the manuscript. All authors contributed to the article and approved the submitted version.

FUNDING

This work was financially supported by the EC H2020 CLAIM project H2020-BG492 2016–2017 (grant agreement no. 774586).

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