



First occurrence and composition assessment of microplastics in native mussels collected from coastal and offshore areas of the northern and central Adriatic Sea

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Abstract

In recent years, the occurrence of microplastics in the aquatic environment has gathered increasing scientific interest. Several studies have shown that the ingestion of microplastics may negatively influence the physiology of marine organisms having different feeding strategies, particularly in those species which cannot discriminate between food sources. Recent studies highlighted the potential for such particles to accumulate in the food web, posing risks to human health via the consumption of seafood. Furthermore, early findings also indicated the role of microplastics as vectors of chemical pollutants either used as additives during synthesis of the plastics or adsorbed directly from seawater, i.e., PAHs, PCB, and surfactants. Despite the importance of microplastics in adsorption and transport of hydrophobic pollutants, little is known about their distribution and accumulation in marine food webs, or their direct and indirect harmful effects. The Adriatic Sea represents a semi-enclosed basin with a low water recirculation rate and high anthropogenic pressures associated with unsustainable fishing and inputs of contaminants. The body burden, accumulation rates, polymer composition, and recurring morphotypes of microplastics in native blue mussels (*M. galloprovincialis*) were examined. Organisms collected offshore were compared to those collected in coastal areas. Microplastics were recovered from the soft tissues of all analyzed mussels. Coastal organisms showed a load of 1.06–1.33 fragments g⁻¹ (wet weight) and 0.62–0.63 fibers g⁻¹ (wet weight) while offshore organisms showed an accumulation of 0.65–0.66 fragments g⁻¹ (wet weight) and 0.24–0.35 fibers g⁻¹ (wet weight). The size class distribution revealed a marked prevalence of smaller particles (20 µm to 40 µm range) and the most recurring polymer type in analyzed organisms was PE followed by PP, PET, and equal amounts of PS, PLY, and PVC. A significant site-, time-, and oceanographic-related distribution trend was observed. Based on the findings presented here, there is a clear need to implement a seafood safety monitoring program to better understand actual human health-related risks.

Keywords Microplastics · Fragments · Fibers · Native mussels · Adriatic sea

Introduction

In the last decades, limited and/or inappropriate waste management practices and irresponsible human behavior in

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disposing of rubbish has resulted in large masses of single-use plastics released into the natural environment (Eriksen et al. 2018). Inevitably, the majority of these non-degradable and persistent solid plastics have entered the world's oceans (Alimi et al. 2018). The increasing use of polymers in everyday products has resulted in the widespread presence of plastic waste in the natural environment (Horton et al. 2017). Currently, the most widely used synthetic plastics are low- and high-density polyethylene (PE), polypropylene (PP), polyvinyl chloride (PVC), polystyrene (PS), polyamide (PLY), and polyethylene terephthalate (PET; Li et al. 2016). Once released into the environment, large plastic items can be degraded into microplastics (MPs) by UV degradation and physical fragmentation by means of wind and waves (Wright et al. 2013). The impact of plastic debris on marine

organisms depends on a combination of parameters that determine their partition and availability in the water column or in sediments. Typically, plastics' chemical-physical property such as the polymer's density together with the biofouling formation, occurrence of waves, and seawater currents, contributing with the vertical mixing, may influence their occurrence in the benthic or pelagic environments (Horton and Dixon 2018). Furthermore, due to their chemical-physical properties, co-occurring hydrophobic pollutants may adsorb onto plastic particles thus increasing their distribution (Gomiero et al. 2018). As the majority of these pollutants are persistent, bioaccumulative, and toxic, their interaction with marine debris has become of particular concern for both environmental and human health (Revel et al. 2018).

To date, reports of seawater concentrations of MPs (microplastics) are rather limited in the scientific literature, especially when compared to sediment concentrations. Indeed, due to the small size of many plastic fragments, sampling and extraction from seawater is challenging. Reported seawater concentrations exhibit large spatial variability of MP per cubic meter (Cózar et al. 2015; Enders et al. 2015; Ng and Obbard 2006; Van Cauwenberghe and Janssen 2014), being influenced by various factors, such as surface circulation and river discharges, which determine specific accumulation patterns as shown in Vianello et al. (2018) for the northern Adriatic Sea. Few studies have addressed the occurrence of floating plastic debris in the surface water of the Adriatic Sea (Gomiero et al. 2018b; Strafella et al. 2019). Suaria et al. (2016) pointed out values ranging from 0.4 ± 0.7 to 1.0 ± 1.8 items/m³ with polyethylene being reported as the predominant polymer with an overall relative abundance of 52% of the total amount of detected polymers, followed by PP (16%) and synthetic paints (7%). Even though the existing data are too limited to determine a realistic natural concentration of MP in seawater, the potential for ingestion by commercially important species collected in the Adriatic Sea has been already documented (Avio et al. 2015; Pellini et al. 2018; Renzi et al. 2018). Early studies have shown that MP can be ingested by marine organisms exhibiting a range of different feeding strategies, and particularly in those species which cannot discriminate the food source (Messinetti et al. 2017). Bivalves are of particular interest since their extensive filter-feeding activity exposes them directly to MP present in the water column. Models simulating physiological conditions in the gut suggest that both adsorbed pollutants and chemical additives of plastics might be released from ingested particles to organisms during digestion processes (Besseling et al. 2017). The uptake may both negatively influence the physiology of marine organisms and facilitate the transfer of pollutants from particle to organisms and from organisms to other organisms, eventually

affecting the entire marine food web (Gomiero et al. 2018; Pittura et al. 2018). Recent studies have focused their attention on the accumulation of these particles in the food web and on the direct consequences for human health as increasing amounts of seafood are being consumed (Van Cauwenberghe and Janssen 2014). To date, knowledge about microplastic characterization and distribution in marine environments is still fragmented.

This study is focused on the Adriatic Sea, a semi-enclosed basin characterized by a low water recirculation rate and elevated anthropogenic pressures associated with unsustainable fishing and high inputs of environmental contaminants. The presence of microplastics in native blue mussels (*Mytilus galloprovincialis*) collected from offshore and coastal areas has been investigated. Being widely distributed, sessile and filtering large volumes of seawater to mussels represent an ideal indicator species for the occurrence of microplastics in aquatic environments. The results of this study provide a practical contribution toward increasing our knowledge on the presence, distribution, and typology of MP in natural populations of mussels, a representative species for the water column of the Adriatic sea as well as an important human food resource.

Materials and methods

Study area

The Adriatic Sea is a semi-enclosed basin dominated by a peculiar combination of oceanographic currents, with one flowing toward the SE along the western coast named the West Adriatic Current (WAC) and a second flowing NE along the eastern coast (East Adriatic Current, EAC). Strong periodic winds such as the Sirocco (SE), Tramuntana (N), Mistral (NW), and locally Bora (NNE) contribute to wave formation and surface water circulation. The Adriatic Sea has been divided into three distinctive sub-basins (El-Geziry and Bryden 2010; Zavatarelli and Pinardi 2003). The investigated area spreads from the northern section (average depth of about 45 m) to the central sector (average depth of about 140 m) within a depression reaching 260-m depth named Pomo pit.

In the area, several anthropogenic activities coexist such as heavy marine traffic from commercial and tourist vessels, offshore activities, ferry boats, and trawl-fishing, while in the coastal areas, a continuous intensification of mussel farms along the Italian coast and fish farms along the Croatian coast is underway. Furthermore, the Italian coast line is dominated by several rivers which collect wastewater and rainwater from one of most heavily industrialized area of Europe thus contributing toward the anthropogenic pressure through large loadings of organic and inorganic chemicals, nutrients, and garbage including plastic litter.

Sample collection

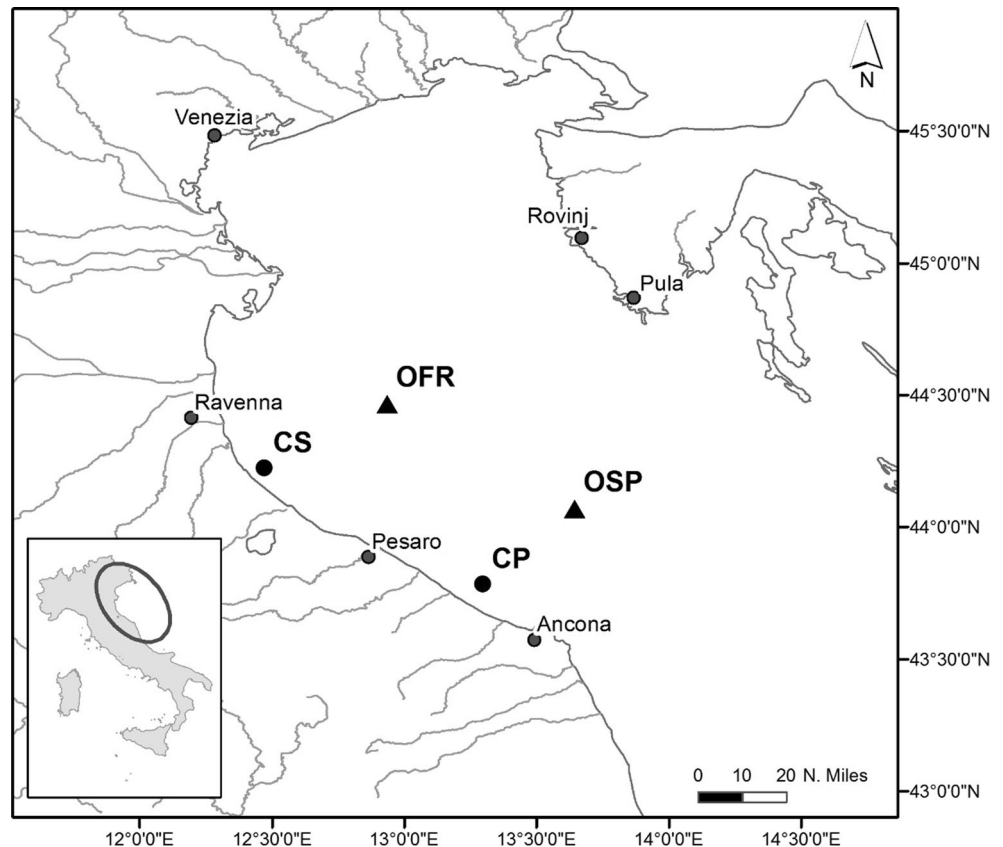
The survey was performed in 2013, collecting native mussels, *M. galloprovincialis*, from two artificial reefs located 20 nautical miles (NM) offshore Rimini (Italy, OFR; Fig. 1) and 30 nm offshore Pesaro (Italy, OFP) in the northern Adriatic Sea and from two coastal areas located 1 NM offshore Cesenatico (CS, Italy) and in the Conero natural park (CP, Ancona, Italy). Bivalves were individually processed for biometric analysis, recording length, width, thickness, and weight, before being transported to the laboratory where they were split into aliquots. Mussels were placed in large aluminum cans to limit at best any possible plastic contamination deriving by the use of plastic bags. Cans were sealed and placed in a cold room on board of the TecnoPesca research vessel. A sample of approximately 100 specimens from each site were stored at $-20\text{ }^{\circ}\text{C}$ for MP extraction, characterization, and quantification analyses.

Samples analysis

For MP quantification and characterization in wild mussels from the northern Adriatic Sea (Fig. 1), a total of 6 pools of 10 mussels each were selected from each of the sampled sites, excluding the specimens showing non-optimal condition (e.g., broken valves, complete open valves, and dead specimens). A

total of 60 individuals were therefore analyzed per site. All tools and glassware used for the digestion tests were pre-burned at $500\text{ }^{\circ}\text{C}$ for 5 h, carefully rinsed with bi-distilled water filtered through $1.2\text{-}\mu\text{m}$ GF/C grade glass fiber filters (Whatman, Milan, Italy). All reagents involved in every step of the sample preparation, extraction, and purification were pre-filtered through similar GF/C filters. After thawing in the laboratory, a pre-burned stainless steel scalpel was used to remove the shells from each of the sampled mussel. Each pool represented by 10 individuals was weighed, transferred in a 250-ml Pyrex bottle with 50 ml of a 5% w/v Tween 20 solution. The sample was gently stirred (20 rpm) by a stainless steel Jar test system (Velp Scientifica, Milan Italy) for 10 min and kept at $50\text{ }^{\circ}\text{C}$ for 2 h. Seventy milliliters of protease solution obtained by diluting protease concentrated enzyme (P3111 Sigma-Aldrich, Germany) 1:4 (v:v) in 0.1 M glycine buffer mixture at pH 9.0 was prepared. The obtained solution was added to the sample and incubated for 48 h at $50\text{ }^{\circ}\text{C}$. The solution was finally treated with 80 mL of 20% KOH solution to digest any remaining organic matter content. The sample was incubated at $50\text{ }^{\circ}\text{C}$ for 36 h before transfer to a separator funnel. MPs in the digested sample were separated by a density-based separation step using sodium iodide (NaI). The NaI solution was added to the sample to reach the final density of $d_{1/4} = 1.8\text{ g/cm}^3$. The mixture was then gently stirred for 30 min before being left to settle for 24 h. The supernatant

Fig. 1 Location of the sampling points in the central and northern Adriatic Sea. OSR, marine area offshore Rimini; OSP, marine area offshore Pesaro; CP, Conero natural park coastal area; and CS, Cesenatico coastal site



fraction containing the floating MPs was collected and filtered through an aluminum oxide filter (Anodisc) with 0.2- μm porosity. The filter was washed afterwards with 50 ml of MilliQ water to remove most of the saline fraction. The filter was dried at 40 °C for 24 h and analyzed for polymeric composition, size, and abundance of MP. Samples were both visually analyzed by a Leica M205C stereomicroscope coupled with a DFC495 digital camera and the LED5000 MCI illumination (Leica Microsystems, Milan) and characterized by a Bruker Hyperion 3000 μ -FTIR system. The entire surface of each filter was inspected. The spectrum was recorded and compared to the polymer database for each MP detected and all research scores in percentage were noted but only polymers matching reference spectra at greater than 85% were accepted. Each measurement was performed in reflection mode.

A visual assessment was applied to identify the morphotypes of microplastics according to the physical characteristics of the particles (Li et al. 2016). Microplastics were classified into two morphotypes including fibers and fragments. In brief, fiber was defined as microplastic with a slender and greatly elongated appearance while rounded particles as well as items showing a flake-like shape dominated by a thin layer or larger pieces of plastic debris were considered fragments.

Fragments were divided into four dimensional classes: $20\ \mu\text{m} < (D_1) < 40\ \mu\text{m}$; $40\ \mu\text{m} < (D_2) < 80\ \mu\text{m}$; $80\ \mu\text{m} < (D_3) < 100\ \mu\text{m}$; $< 100\ \mu\text{m} (D_4)$. Fibers were divided in two-dimensional classes according to the largest dimension: $10\ \mu\text{m} < (FD_1) < 100\ \mu\text{m}$ and $100\ \mu\text{m} < (FD_2) < 300\ \mu\text{m}$. Procedural blanks represented by the MilliQ water and all involved reagents were run to exclude plastic contamination in the analytical processes.

Procedure validation

To evaluate the extraction efficiency of the adopted method, a set of spiked samples were prepared and analyzed. Polyethylene (LDPE, 100 μm), polypropylene (PP, 20 μm), and polyvinylchloride (PVC, 200 μm) were selected for the QA/QC test as they represent the most relevant fraction of polymers reported in the marine environment (Law and Thompson 2014). Plastic spheres of 99% purity were purchased from Goodfellow (Huntingdon, UK).

The recovery study was performed on five aliquots of pooled mussel samples (10 g; *w.w.*) individually spiked with ≈ 50 microspheres of the addressed polymers and processed according to the introduced extraction protocol (Tab. 1—Supplementary Material). Blank samples represented by only the reagents and MilliQ water used by the protocol were run to exclude any possible external source of MP contamination. Furthermore, wet sedimentation traps were placed in the lab during every phase of the sample's extraction, purification, and analysis phases to assess any possible micro-/nanoplastic

contamination from the atmospheric dust fallout. Wet traps were loaded with 1 L water pre-filtered through GF/C fiberglass filters MilliQ water which was collected, filtered through Anodisc filters, and analyzed for plastic content after the sample preparation phase.

Statistical analysis

Statistical data analysis was performed using Statistica 7.0 (StatSoft Inc., USA). All data were log-transformed. The homogeneity of variance and the distributions were analyzed using Levene and Kolmogorov-Smirnov tests, respectively. The analysis of variance (ANOVA) was used to check the abundance of the different size classes. When the ANOVA indicated significant differences, the groups were compared using Tukey's test to determine significant differences based on $p < 0.05$. Spatial distribution of the targeted polymer's classes was investigated by a principal component analysis. A non-parametric Mann-Whitney test was performed in order to identify differences in both plastics levels and in the occurrence of the main polymer classes between the different investigated geographical areas.

Results

In the PE, PP, and PVC spiked mussels, estimated recoveries ranged from 86 to 94% (tab. 1—Supplementary Material) which are in good agreement with the results from other recent studies (Li et al. 2016; Phuong et al. 2017; Dehaut et al. 2016) while the procedural blanks were completely free of any form of plastic contamination.

Biometric mussel analysis showed some significant differences with bivalves collected in the OFR site, these being bigger than those collected in the other areas ($p < 0.05$; tab.2—Supplementary Material). Higher growth rate in mussels in this area can be explained by the eutrophic conditions previously reported in the same area by other authors (Spillman et al. 2007). Most of the recovered MPs in processed filters were particle-shaped while fibers represented a minor fraction. Significantly higher levels of fragments were observed in the mussels collected in coastal areas (1.06–1.33 items/g WW) compared to organisms collected in offshore areas (0.65–0.66 items/g WW; $p < 0.05$; Fig. 2a). A similar trend was also observed for fibers with an average accumulation of 0.62–0.63 items/g in mussels collected in coastal areas and 0.24–0.35 items/g WW in organisms collected in offshore areas (Fig. 2b). Overall, 70 fragments were collected from mussels sampled in CS site followed by 56 fragments in CP, and 42 and 46 respectively in OFR and OSP sites. Among them, the size class distribution revealed a marked prevalence for smaller particles. The analysis pointed out D_1 (40–46%) as the dominant size class scored in the coastal areas, followed

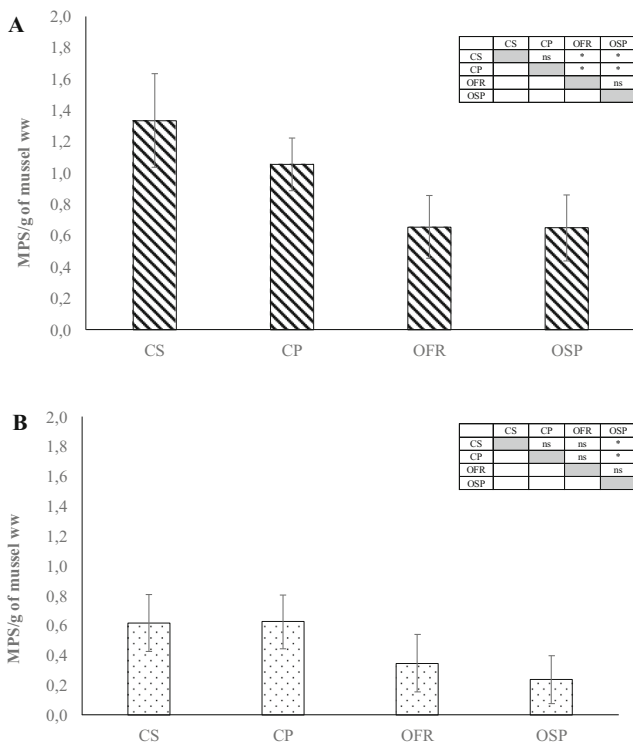
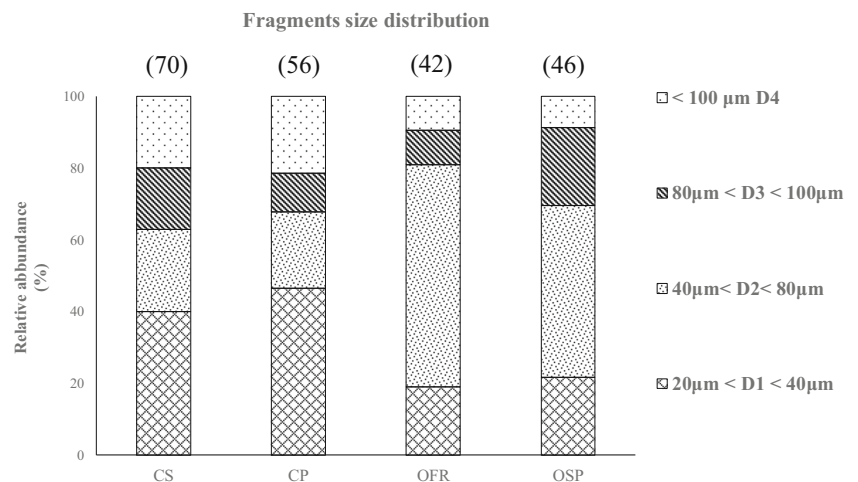


Fig. 2 Characterization of microplastic particles extracted in the whole body of sampled mussels. Pooled samples of 10 individuals, 6 replicates per site. Reported results are no. of microplastic fragments (a) and fibers (b) observed in the investigated samples (no. of plastic fragments/g of tissue ww). **p* < 0.05; NS, not significant; OSR, marine area offshore Rimini; OSP, marine area offshore Pesaro; CP, Conero natural park coastal area; and CS, Cesenatico coastal site

by *D*₂ (21–22%) and *D*₄ (20–21%; Fig. 3). Different patterns of distribution were observed in the offshore marine areas with *D*₂ (48–62%) being the most dominant class followed by *D*₁ (19–21%) and *D*₄ (8–9%; Fig. 3). Six of the most recurring polymer types were detected in the sampled mussels (Fig. 4). PE was the most prevalent plastic type among all investigated sites. It was present in over 43% of the most south offshore

Fig. 3 Characterization of microplastic fragments extracted in the whole body of sampled mussels. Pooled samples of 10 individuals, 6 replicates per site. Reported results are size distribution of the extracted particles. OSR, marine area offshore Rimini; OSP, marine area offshore Pesaro; CP, Conero natural park coastal area; and CS, Cesenatico coastal site. On top of each histogram, the total number of scored fragments per site is reported in brackets



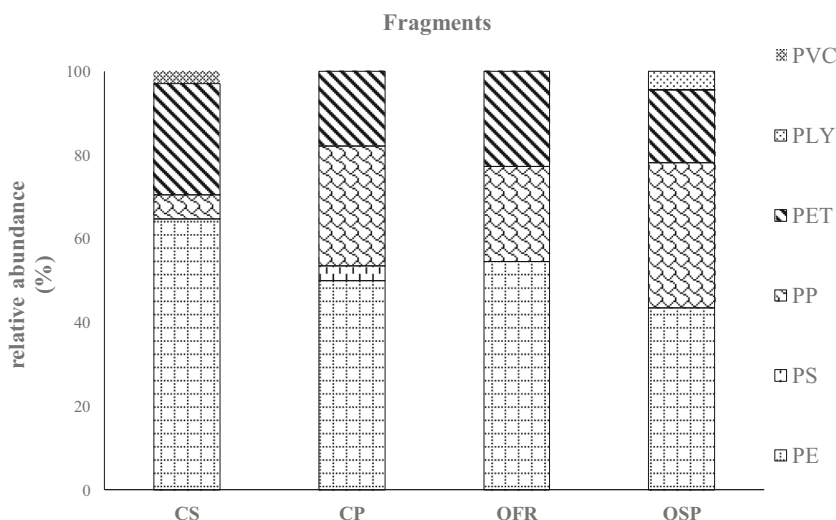
area (OSP) and up to 65% in the most northern coastal area (CS). The second most observed polymer was PP ranging from 9% (CS) to 35% (OSP) and PET which showed very little difference in level distribution among the investigated sites (17–26%). PVC, PS, and PLY were only reported in some of the investigated sampling sites with a low contribution (≈ 2%, ≈ 3%, ≈ 3% respectively). Regarding plastic fibers, a total amount of 32 items were scored in organisms collected in CS site followed by 33 in CP as well as 22 and 16 in OFR and OPS sites, respectively (Fig. 5).

On the other hand, the majority of fibers were associated with *FD*₁ class (10 μm ≤ *FD*₁ ≤ 100 μm) in CS and OFR (68–72%) sites, while an equal distribution of *FD*₁ and *FD*₂ (≈ 50%) was observed in CP and OSP sites (Fig. 6).

Similar to the particle-shaped fragments, six different polymer types were characterized from fibers extracted from samples of wild mussels. *PYL* was the most abundant plastic type among all investigated sites ranging from 60 to 65% (Fig. 6). The second most prevalent polymer was *PE* ranging from 26% (OSP) to 32% (CS) followed by *PP* which occurred only in CP (10%), OFR (14%), and OSP (16%). *PET* was present in minimal amounts (9%).

PCA ordination of the samples produced a two-dimensional pattern, with the first component explaining 64% of the total variance and the second component contributing with an additional 19% (Fig. 7). In detail, most of the separation between the different areas, i.e., OSR = marine area offshore Rimini, OSP = marine area offshore Pesaro, CP = Conero natural park coastal area, and CS = Cesenatico coastal site, occurred in the first axis. The PCA results show that a clear separation was observed among coastal and offshore areas with most of the discriminatory power held by component 1. The second component contributes to represent a limited geographical difference between the two sub-basins partially driven by the most recurrent size of the investigated particles.

Fig. 4 Characterization of microplastic fragments extracted in the whole body of sampled mussels. Pooled samples of 10 individuals, 6 replicates per site. Reported results are the size distribution of the detected fragments. OSR, marine area offshore Rimini; OSP, marine area offshore Pesaro; CP, Conero natural park coastal area; CS, Cesenatico coastal site; PE, polyethylene; PP, polypropylene; PVC, polyvinyl chloride; PS, polystyrene; PLY, polyamide; and PET, polyethylene terephthalate



Discussion

The present investigation is the first assessment of the levels and polymeric composition of microplastics in native populations of filter-feeding organisms (*M. galloprovincialis*) collected both in coastal and in offshore areas in the northern Adriatic Sea. The MP uptake in wild organisms serves as a starting point in assessing their potential exposure and potential effects of MP on organisms in the water column.

Despite significant progress in analyzing MP in field samples over recent years, the isolation and identification of MP from complex environmental matrices like biota remains challenging (Hidalgo-Ruz et al. 2012; Shim et al. 2017). Therefore, a goal of the present study was to validate a cost-effective and reliable method based on current best available knowledge and technology. Reported performances of ingestion efficiency and recovery rates are in good agreement with those recently reported by other authors (Phuong et al. 2017; Miller et al. 2017). The ingestion of microplastics of different

sizes and shapes by filter-feeding organisms was expected as this has been previously demonstrated for these organisms tested under laboratory (Cole et al. 2014; Von Moos et al. 2012) and natural conditions (Van Cauwenberghe and Janssen 2014; Phuong et al. 2017). In the present study, the most recurring polymer type in analyzed organisms was PE followed by PP, PET, and equal amounts of PS, PLY, and PVC roughly following the relative global stock production of polymers. Characterized by both an average density lower than seawater and extensive application in the disposable packaging industry, it is not surprising that PE and PP account for $\approx 75\%$ of the total amount of observed polymers. On the other hand, polyester, commonly referred to as PET in relation to packaging, makes up about 18% of world polymer production and is the fourth-most-produced polymer followed by PVC (Na 2013). The observed large contribution of floating polymers in the microplastic composition of organisms collected far from land supports their great mobility. Such a trend hampers any effort to classify and quantify input sources

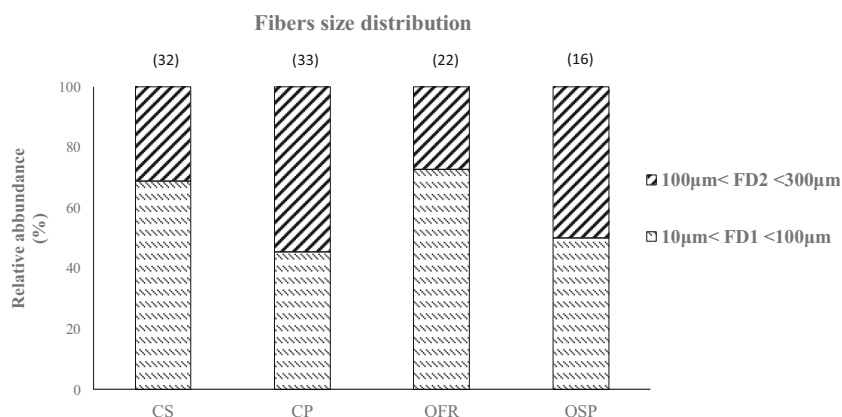


Fig. 5 Characterization of micro plastic fibers extracted in the whole body of sampled mussels. Pooled samples of 10 individuals, 6 replicates per site. Reported results are relative abundance of the investigated polymers in the extracted fibers form the analysed mussels.

OSR, marine area offshore Rimini; OSP, marine area offshore Pesaro; CP, Conero natural park coastal area; and CS, Cesenatico coastal site. On top of each histogram, the total number of scored fibers per site is reported in brackets

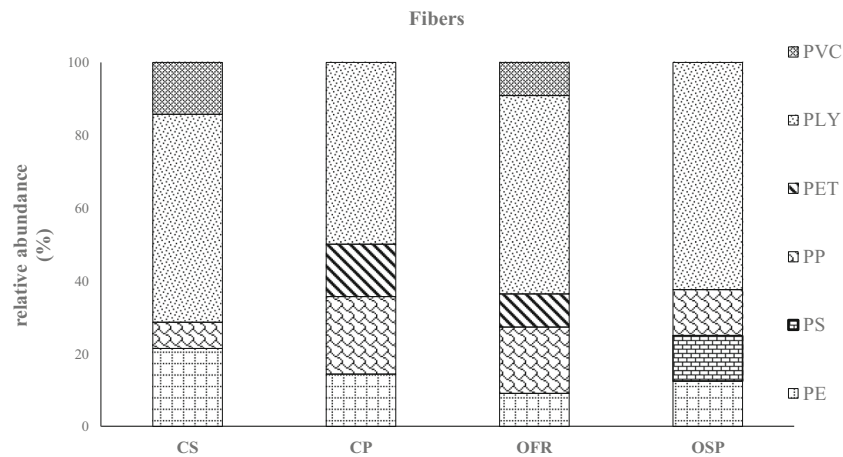


Fig. 6 Characterization of microplastic fibers extracted in the whole body of sampled mussels. Pooled samples of 10 individuals, 6 replicates per site. Reported results are the relative abundance of the investigated polymers in the extracted fibers of the analyzed mussels. OSR, marine

area offshore Rimini; OSP, marine area offshore Pesaro; CP, Conero natural park coastal area; CS, Cesenatico coastal site; PE, polyethylene; PP, polypropylene; PVC, polyvinyl chloride; PS, polystyrene; PLY, polyamide; and PET, polyethylene terephthalate

therefore limiting any realistic suggestion about an effective plastic emission reduction action (Suaria et al. 2016).

The observed polymer distribution in native mussels reflects the composition of floating and stranded macro-litter previously reported in the same investigated areas, pointing out a good correlation among the occurrence and composition of macro- and micro-litter (Munari et al. 2016; Suaria and Aliani 2014).

The highest microplastic particle accumulation was observed in organisms collected along coastal sites compared to the offshore areas. Such site-dependent distribution likely reflects the combination of the significant inputs from rivers and coastal-based anthropogenic activities (aquaculture farms, tourism, etc.) as well as hydrological and meteorological characteristics of the Adriatic Sea characterized by a superficial seawater circulation strongly dependent on the force and orientation of periodic winds (Carniel et al. 2009). In addition, generally higher MP levels were observed in mussels collected in the northern Adriatic sites, both close to the coast and in the offshore areas, compared to the central Adriatic sites. A distribution partially explained by the northern Adriatic gyre which might contribute to the accumulation and recirculation of floating plastic debris in a confined area located in the gulf of Venice (Kuzmić et al. 2006). The existence of a southern seawater stream, mainly parallel to the coast, could have contributed to the distribution of floating micro-litter over a larger area thus resulting in the lower accumulation of microplastics particles in the analyzed mussels (Liubartseva et al. 2016). Levels observed in bivalves collected from offshore areas in the present study were higher than those previously reported in mussels farmed in the southern sector of the North Sea (0.20–0.40 particles/g WW, Van Cauwenberghe and Janssen 2014) and in wild

organisms collected in the French Atlantic coast (0.23 particles/g, Phuong et al. 2017). On the other hand, MP levels observed in the coastal areas of the Adriatic Sea are comparable with those reported by De Witte et al. (2014) in wild mussels collected along the Belgian coastline, but generally lower than those reported by Li et al. (2016) following an extensive monitoring program along the East coast of China (1.5–5.0 particles/g). The size distribution of extracted microplastic particles in organisms collected in coastal areas showed a median class in a range of 20–40 μm. A comparable size distribution was observed by Van Cauwenberghe et al. (2015) in farmed mussels collected in the North Sea. In contrast, most of the bivalves collected in offshore marine areas within the present study showed a median size class within the range of 40–80 μm.

Overall, a significantly higher median size class has been estimated by the worldwide plastic distribution model developed by Cózar et al. (Cózar et al. 2014) for the surface of the open ocean. Nevertheless, the reported general shift is however in agreement with the current opinion that local oceanographic conditions may lead to speed up the progressive fragmentation of the plastic objects into smaller pieces thus leading to a gradual increase of fragments toward small sizes.

The accumulation of fibers in soft tissues was homogeneously distributed across the investigated areas due to large inter individual variability. The observed levels were one order of magnitude lower than those previously reported by Mathalon and Hill (2014) in the intertidal ecosystem surrounding Halifax Harbour (Atlantic coast of Canada). In all sites, PLY was the most dominant polymer of the fiber-shaped microplastics and it is the major component in ropes, lines, and fish nets (Fisher 1988; Wright et al. 2013).

The size distribution of extracted fibers in wild mussels collected in both coastal and offshore areas showed a size

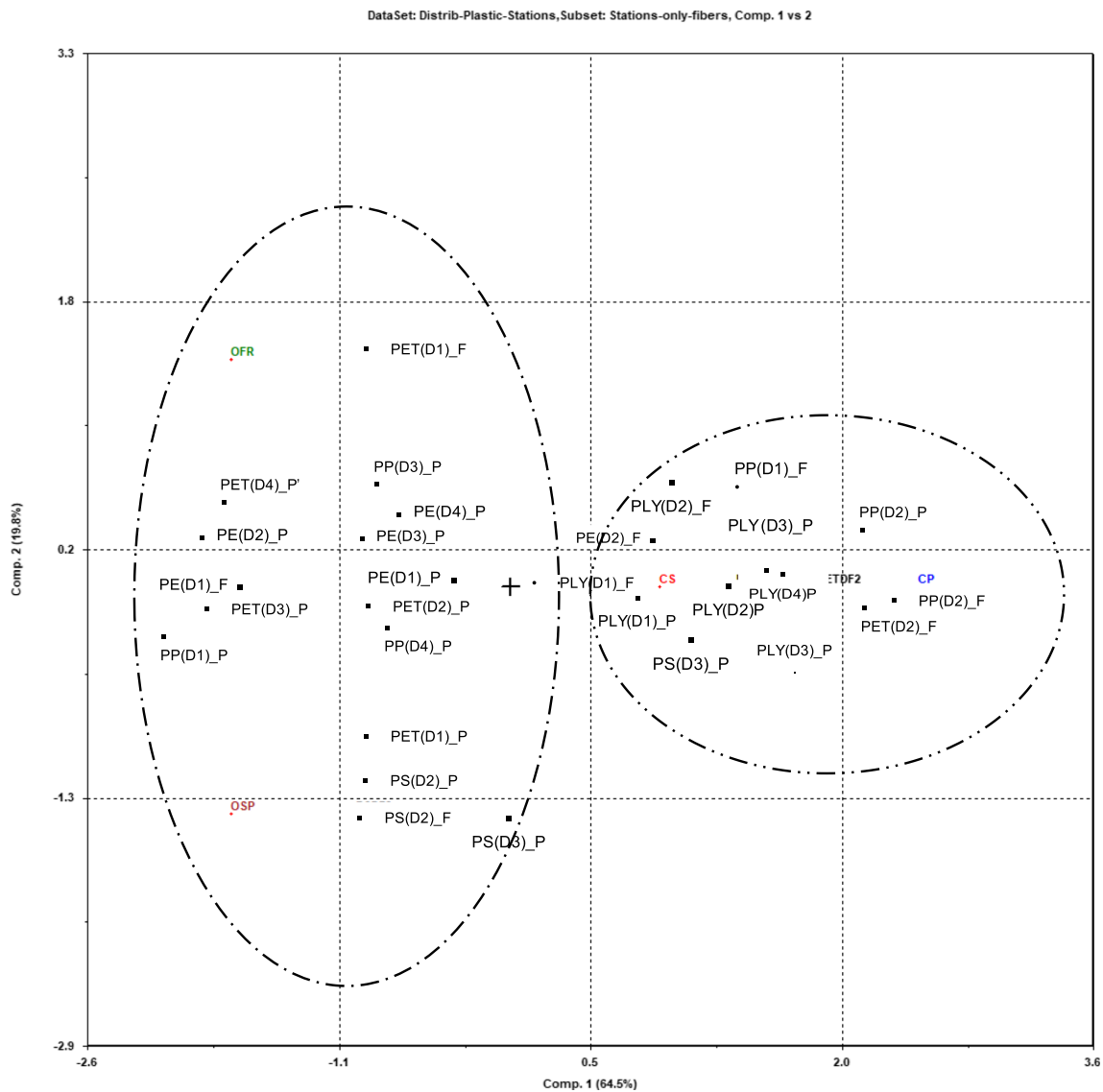


Fig. 7 Principal component analysis biplot. PCA ordination diagram microplastics densities grouped according to the dimensions. OSR, marine area offshore Rimini; OSP, marine area offshore Pesaro; CP, Conero natural park coastal area; CS, Cesenatico coastal site; PE, polyethylene; PP, polypropylene; PVC, polyvinyl chloride; PS, polystyrene; PLY, polyamide; and PET, polyethylene terephthalate.

Fragments (P) are divided into four dimensional classes: $20\ \mu\text{m} < (D1_P) < 40\ \mu\text{m}$; $40\ \mu\text{m} < (D2_P) < 80\ \mu\text{m}$; $80\ \mu\text{m} < (D3_P) < 100\ \mu\text{m}$; $< 100\ \mu\text{m} (D4_P)$. Fibers (F) were divided in two-dimensional classes according to the largest dimension: $10\ \mu\text{m} < (D1_F) < 100\ \mu\text{m}$ and $100\ \mu\text{m} < (D2_F) < 300\ \mu\text{m}$

range of 10–100 μm . Generally, larger fibers (200–1500 μm) were observed by de Witte et al. (2014) in *M. edulis*.

Conclusions

Our results demonstrated the ubiquity of plastic pollution in the northern Adriatic Sea. Reported levels and trends in the relative abundance of the investigated plastic polymers support increased research in this geographical area where hot spots of marine biodiversity and vital fish stocks coexist with different anthropogenic activities such as aquaculture, oil and

gas, and tourism. Moreover, in order to prevent any possible hazard for human health, it is relevant to figure out the MP uptake rates in mussels, as these represent an important food resource in several areas of the world. More effort needs to be focused on characterization of the sources for these plastics together with the fate and residence times of the various polymer now found within the marine environment.

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