microplastics

Article

Accumulation Evaluation of Potential Microplastic Particles in *Mytilus galloprovincialis* from the Goro Sacca (Adriatic Sea, Italy)

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Abstract: Microplastics (MPs; <5 mm) are present throughout the marine environment and are recognized as an emerging threat to aquatic ecosystems. Filter feeding organisms, such as mussels, are considered as bioindicators of MP pollution and are useful to evaluate the potential risks of MPs to human health. The work presented shows data on potential MPs found in *Mytilus galloprovincialis* samples collected from the Adriatic Sea during two sampling sections (1st sampling: December 2019 and 2nd sampling: May 2020). The mussels were subjected to digestion with H_2O_2 individually and filtered and the MP elements found were observed using a stereomicroscope and ultimately categorized by shape, size class and color, with the aid of a digital camera and data acquisition software. The highest MP concentrations were observed in the mussels collected in December 2019 (1.11 microplastic items per gram wet weight of mussels' tissue), highlighting the possible influence of the following two main factors: greater river discharges following adverse weather events and higher river water pollution due to industrial activities. Indeed, the second sampling was performed after the Italian lockdown, due to the COVID-19 emergency. MP fibers (50–80%) were the most abundant type of MPs identified, followed by fragments (10–40%), granules (1.5–2.5%), non-categorized shape (1–2%) and foam (<1%). The color black (50–70%) and sizes smaller than 500 μm were the most dominant characteristics recorded both in the 1st sampling (50–70%) and the 2nd survey (30–50%). These data could be overestimated, due to the lack of polymer identification. The results of this study provide further data on the importance of bivalves as environmental bioindicators with regard to the pollution of MPs in the Adriatic Sea, supporting their instrumental role as environmental bioindicators for MP pollution.

Keywords: bio-monitoring; mussels; microplastics; Adriatic Sea

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1. Introduction

Fragments of plastics smaller than 5 mm, commonly called microplastics (MPs), are located both in terrestrial and aquatic environments worldwide [1–3] and constitute a major modern-day global environmental problem [4]. A remarkable amount of this plastic debris comes from continental sources, which generally enters the marine environment through rivers [5,6], and secondarily from offshore activities, in particular the loss of fishing nets and litter released during sea activities, including tourism [5,7].

In the midst of plastic litter, MPs, due to their small size, have become of particular scientific interest with regard to the environment, and additionally the health of animals and humans alike. MPs can be up taken by a wide range of marine organisms by different processes, even though the main exposure route is considered ingestion [8–10]. MP particles are ingested intentionally by fish that mistake them for food, due to their size and similar appearance to plankton [11,12]. Conversely, filter feeders unintentionally feed upon the MPs as they strain water containing suspended matter, as well as food particles [10,13].

To date, several studies demonstrated that many aquatic organisms ingest MPs and taking into consideration that the global food supply of seafood, both from capture and aquaculture production, was over 125 × 10^6 tonnes in 2009, consequences for human food safety need to be considered [14–28]. Ingested MPs can result in numerous adverse health impacts on aquatic organisms, including increased immune response, decreased food consumption, weight loss, alteration of endocrine system functions, intestinal damage and decreased growth rate [25,29–32].

Additionally, MPs may contain organic pollutants and heavy metals, which were added during production, or through absorption when present in seawater, which can become bioavailable to organisms after the ingestion of these plastic particles [14,33,34], thus, increasing the risks to human health [35,36].

Mytilus spp., which are common in temperate coastal seas throughout the globe, are optimal sentinels with biological features that make them ideal subjects to perform the bio-monitoring of marine coastal waters [1,3,37]. As a medium-size organism, they provide a suitable amount of tissue for analysis, and because they are hardy, they are easily collected and cultured [1,3,38]. Mussels are benthic extensive filter feeding organisms with the selective mechanism of suspension feeding, which leads to an accumulation of MPs, chemical pollutants and microorganisms in mussels [27,39,40]. Mussels have been widely used for biomonitoring studies in marine environments due to several advantages, such as broad geographical distribution, easy accessibility and high tolerance for a considerable range of salinity. Mussels have also been used in MP research, including fielding investigations, as well as laboratory exposure experiments [14].

Since *Mytilus* spp. feed predominately on phytoplankton by filtering large volumes of water through their ciliated gills, the probability of them ingesting MPs becomes very high. Furthermore, because they are a seafood that often enters the human food chain [41], their MP ingestion and resultant effects have become of even more interest and concern. Numerous worldwide studies on MPs in *Mytilus* spp. have taken place [3,21,36,39,40,42–44]. These studies are encouraging for both their relevance of Mytilus spp. as environmental sentinels for MPs, and for their important role in improving the inter-study comparability through the standardization of methods for MP identification and quantification [1]

Despite the recently increasing number of worldwide studies that analyze marine organisms [1,3,18,19,22,28,45–47], to date, there is insufficient evidence to determine the importance of mussels in the transfer of MPs into the food chain to gauge the human health risks associated with their consumption [41,48].

Although legislative bodies globally are profiling solutions by discouraging the use of plastic materials [49], both further studies to contribute to the definition of baseline levels of MPs in marine biota, with the prospect of improving environmental bio-monitoring programs for the assessment of MP pollution [50], and harmonized plastic observation systems for regular reporting, monitoring, and evaluation of sources, pathways, fluxes, and mass balances of MP pollution in all types of ecosystems to support plastic waste management policies and regulations [49,51]) are necessary. Moreover, policy- and governance-based countermeasures must focus on food quality assurance to minimize human exposure due to the ingestion of MPs [52,53].

The principal aim of the research reported herein was to investigate the possibility of pollutants from the Po River flowing into the northern Adriatic Sea (the Goro Sacca), as evidenced by the presence of MPs in native mussels (*Mytilus galloprovincialis*).

In order to assess the Po river's pollutant contribution with regard to the transport of the plastic litter directly into the sea, this study categorized and quantified the extent of microplastic contamination in mussels before and after the Italian lockdown period, due to the COVID-19 emergency that led to the interruption of various human activities.

These activities were part of the Interreg Italy-Croatia NET4mPLASTIC project (New Technologies for macro and Microplastic Detection and Analysis in the Adriatic Basin).

The outcome of this study will provide a further contribution to the knowledge of the presence, distribution and typology of MPs in native mussels in the Adriatic Sea, in order to support the assessment of the ecological and health risks associated with MPs.

2. Materials and Methods

2.1. Study Area and Sampling

The northern Adriatic Sea receives significant freshwater input from several rivers along the north-eastern coast of Italy. The most important is the Po, which is the country's largest river with a length of 673 km, averaging daily 1500 m^3/s, with streamflow ranging between 100 m^3/s and 11.550 m^3/s [54].

As the longest river in Italy, the Po river drainage area (74,000 km^2) encompasses much of the northern region of the country, with >20 million inhabitants, and includes many large cities, as well as areas of intensive industrial and agricultural activities [55]. The Po river collects wastewater and rainwater from one of the most heavily industrialized areas of Europe, thus contributing to the anthropogenic pressure through large loadings of organic and inorganic chemicals, nutrients, and garbage, including those of a plastic nature [56,57]. The river splits into many sub-rivers before flowing into the Adriatic Sea, the main recognized arms of which are the Po di Maistra, dellaPila, delle Tolle, di Gnocca (or dellaDonzella) and di Goro [55].

Moreover, the northern Adriatic Sea is also subject to heavy marine traffic from merchant ships, supplier vessels for offshore activities (e.g., gas extraction), ferries, fishing vessels, and recreational craft [58].

The mussels were sampled, in the framework of the NET4mPLASTIC project, from a long-line type mussel culture farm in the Goro Sacca, specifically from two stations located at 2.5 and 3.5 nautical miles (nmi) off the Po delta (Figure 1). The study area was chosen because it is affected by different anthropogenic inputs. Nets used for mussel's socks in this farm were made of polypropylene white fibers. The samplings were carried out in early December 2019 (before the Italian lockdown) and in late May 2020 (after the Italian lockdown). The first sampling period was decided based on Po's meteorological and discharge conditions just before sampling. Indeed, from 22 November to 3 December 2019, a significant flood event occurred for the Po river, characterized by a flow with a peak equal to about 8000 m^3/s at the station of Pontelagoscuro. This flood is comparable to that of November 2014, but lower than those of 1994 and 2000.

Po river discharge's data of both sampling periods were obtained from ARPA-ER [59] (https://www.arpae.it (accessed on 27 August 2020)) and are given in Table 1.

Table 1. Data of Po river's average flow rate (m^3/s) in the periods relating to the two samplings.

Period	December 2019	May 2020
Week before sampling	6643.68	1527.07
Week of sampling	4504.70	1037.93

The second sampling period instead coincides with the reopening of the various intensive industrial and agricultural activities located in the Po basin following the lockdown carried out in Italy, due to the world emergency caused by COVID-19.

For each sampling, the collected organisms were measured to determine biometrics (maximum length, cm; weight, g) and later were analyzed individually rather than in pools to improve data representativeness and statistical significance of the collected data.

Fifty native individual bivalves were collected from each sampling station and period, with a total of two-hundred mussels collected. Mussel samples were collected from each sampling station. In both sites a global sample was created gathering mussels collected from three different levels of water column (deepest part, middle and from 50 cm from the surface).

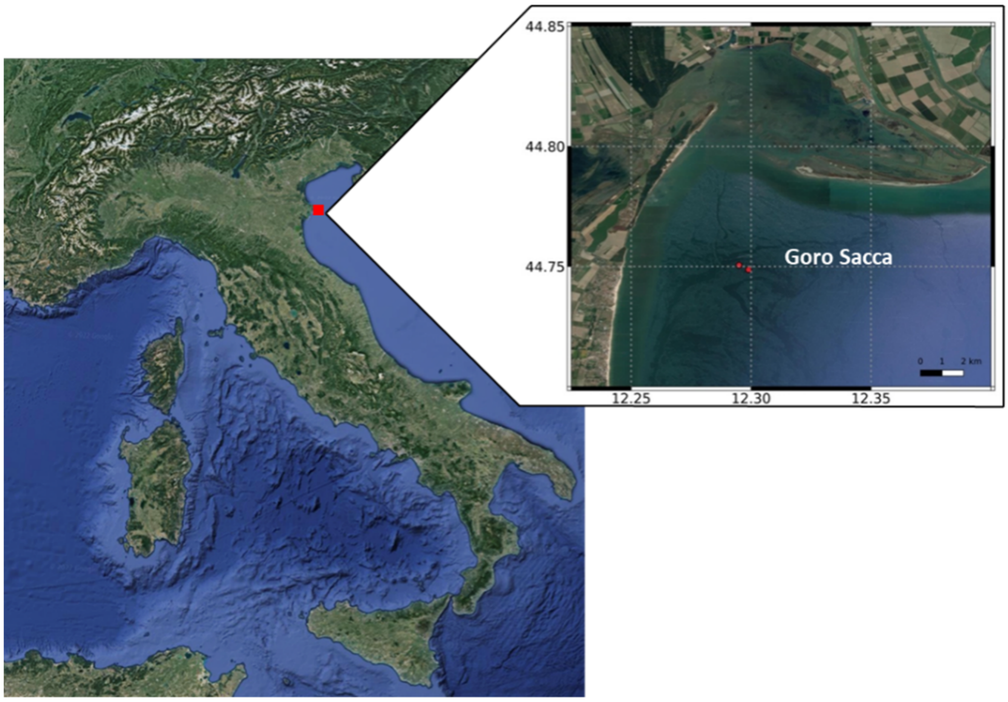


Figure 1. Map of Italy with indication of the sampling site (Goro Sacca-northern Adriatic Sea) with details of the two sampling sites.

Water temperature and salinity were measured at the beginning of each sampling using a multi-parametric probe (IDROMAR mod. IM5235, Idromarambiente, Genova, Italy).

Directly after sampling, the bivalves were kept in an icebox and transported to the laboratory, where they were rinsed with deionized filtered water (0.22 µm) for the removal of most of the sediment grains, biofilm and debris. The samples were then placed in aluminum foil to best limit any possible plastic contamination and stored at −20 °C for future analysis, that is, MP extraction, characterization, and quantification analyses.

2.2. Sample Preparation and Extraction

The shell length and wet weight (ww) of each organism were recorded (Table 2).

Table 2. Characteristics of the sampling sites and mussels collected from the Goro Sacca. Biometric values expressed as mean ± standard error.

Site	Geographic Position	Sampling Period	Collected Mussels	Shell Length (cm)	Soft Tissue Weight (g/Individual)
Goro Sacca **Station 1** (2.5 nmi)	44°44.920′ N 12°17.936′ E	December 2019	50	5.32 ± 0.36	2.84 ± 0.74
	44°44.920′ N 12°17.936′ E	May 2020	50	6.97 ± 4.3	7.46 ± 1.87
Goro Sacca **Station 2** (3.5 nmi)	44°45.031′ N 12°17.699′ E	December 2019	50	4.81 ± 0.36	2.64 ± 0.59
	44°45.031′ N 12°17.699′ E	May 2020	50	6.24 ± 0.48	7.90 ± 1.61

Mussels were thawed at room temperature. In order to degrade organic matter and enable detection of the microplastic particles, the soft tissues of each individual mussel were subjected to hydrogen peroxide digestion according to the Mathalon and Hill procedure [39] and Bessa et al. [60] with minor modifications, which included increased digestion time until 7 days and elimination of density separation by NaCl′s phase, since only a small amount of organic matter would remain.

Briefly, each mussel was dissected, and the inner contents were emptied into a conical flask. Next, 20 mL of 15% H_2O_2 per gram of mussel soft tissues was added to each conical flask. The bottles were covered (with aluminum foil) and placed in an incubator at 65 °C for 5–7 days, depending on the digestion status of the soft tissue.

If the organic matter was not fully removed, approximately 1–2 mL of 15% H_2O_2 was added until almost all of the organic matter was digested.

The digestates were diluted with 100 mL of deionized filtered water (0.22 μm), stirred and subjected to filtration with a 2.7 μm pore size, 47 mm diameter glass microfiber filter (Whatman GF/D, GE Healthcare, Maidstone, UK) using a vacuum system. Prior to analysis, the filters were stored for 24 to 48 h at room temperature in clean, covered glass petri dishes and left to dry.

To preclude any possible contamination with airborne microfibers, a blank samples extraction without tissue was performed simultaneously, as described by Baldwin et al. [61]. Procedural blank samples are used at all stages to exclude the MP elements that are similar to those found in blank samples from the count, as they are considered as airborne contamination.

2.3. MP Observation and Quantification

Optical analysis of microplastics on the filters was performed using a stereomicroscope (Leika MZ6, Leica Microsystem Ltd., Heerbrugg, Switzerland), with a maximum resolution of approximately 25 μm.

Using a digital camera (JVC-C1381, JVC, Yokohama, Japan) and the data acquisition software (Leica IM500 version 1.5, Leica Microsystem Ltd., Heerbrugg, Switzerland), the observed microplastics items were photographed, counted, measured (based on the longest diagonal) and categorized according to size class (<15 μm, 15–50 μm, 50–100 μm, 100–500 μm and >500 μm), shape (fragment, pellet, fiber, film, foam, granule and not categorized shape) and color (white, black, red, blue, clear, green, and other colors).

Potential plastic particles were verified with a hot needle test, as described by Devriese et al. [17].

In order to facilitate comparisons to the current scientific literature, microplastic abundance was expressed for each sampling station both as (a) an average number of microplastic items per individual and (b) average number of microplastic items per gram wet weight of mussel soft tissue.

2.4. Statistical Analysis

All results are presented as mean ± standard error of the mean. The statistical analyses of data were performed using R sofware version 3.6.3 (R Core Team, 2020, R Foundation for Statistical Computing, Vienna, Austria).and Excel (2016, Microsoft, Silicon Valley, CA, USA). Normality of the data set was tested with the Shapiro–Wilk test. Then, non-parametric tests were used if the data were not normally distributed.

The Mann–Whitney U test was applied to determine differences in microplastic numbers among the mussel sampling sites and sampling periods.

The Spearman rank correlation test was performed to test any correlation between the size of the mussels and the number of microplastics.

The beta distribution with 95% confidence intervals was used to compare types of ingested microplastics (shapes and colors) among the mussel sampling sites and sampling periods.

The Kruskal–Wallis test was used to determine the differences between the microplastic size classes found and organism lengths.

The analyses with $p < 0.05$ were considered statistically different.

3. Results

3.1. Abundance of Microplastics

A total of 200 *M. galloprovincialis*′ specimens, of which 100 samples were collected in December 2019 (before the COVID-19 emergency and after a flood) and the remaining 100 samples were collected in May 2020, were analyzed.

In the first sampling, a total of 220 microplastic items were observed in 79 mussels, and in the second sampling, 101 microplastic items were present in the 54 mussels collected (Table 3). The detection frequency of ingested microplastics in the mussels was 72% and 86% in the first survey, in contrast to 54% in the second survey (Table 3).

The average number of microplastics observed in the mussels collected on December 2019 from the station at 3.5 nmi from the coast was 0.55 ± 0.56 microplastic items per gram wet weight of mussel tissue, whereas the highest concentration was observed at the station nearer to the coast (2.5 nmi), amounting to 1.11 ± 0.92 microplastic items per gram wet weight of mussel tissue (Table 3). The photographs of microplastics found in bivalves are shown in Figure 2.

In May 2020, the total amount of microplastics observed in the mussels collected along the two stations was generally lower than what was observed during the first survey, with concentrations of 0.17 ± 0.21 and 0.12 ± 0.13 microplastic items per gram wet weight of mussels' tissue at 3.5 and 2.5 nmi, respectively, from the coast (Figure 3).

The mean values of the microplastic particles per gram wet weight of mussels' tissue of each sampling showed significant differences between the two time samplings (Mann–Whitney test, p value > 0.05) (Table 3; Figure 4).

Table 3. Frequency of abundance of ingested microplastics (% of individuals containing microplastics) and abundance of microplastics in mussels sampled in the Goro Sacca (northern Adriatic Sea). Microplastic abundance (mean ± SE) is expressed as (a) an average number of microplastic items per individual (b) average number of microplastic items per gram wet weight of mussel tissue.

Sampling	Station 1 at 2.5 nmi (December 2019)	Station 2 at 3.5 nmi (December 2019)	Station 1 at 2.5 nmi (May 2020)	Station 2 at 3.5 nmi (May 2020)
Number of mussels examined	50	50	50	50
Number of mussels containing microplastics	43	36	27	27
Microplastics frequency of occurence	86%	72%	54%	54%
Microplastics number	151	69	55	46
Microplastics mean size	1892.7 µm	1343.9 µm	1162.02 µm	496.15 µm
Microplastics size class range more present	>500 µm	>500 µm	100–500 µm	100–500 µm
Minimum particle size	21.6 µm	38 µm	22 µm	25 µm
Microplastic Abundance				
(a) Average number of microplastic items per individual	3.02 ± 2.28	1.38 ± 1.24	1.1 ± 1.46	0.92 ± 1.04
(b) Average number of microplastic items per gram wet weight of mussel tissue	1.11 ± 0.92	0.55 ± 0.56	0.17 ± 0.21	0.12 ± 0.13

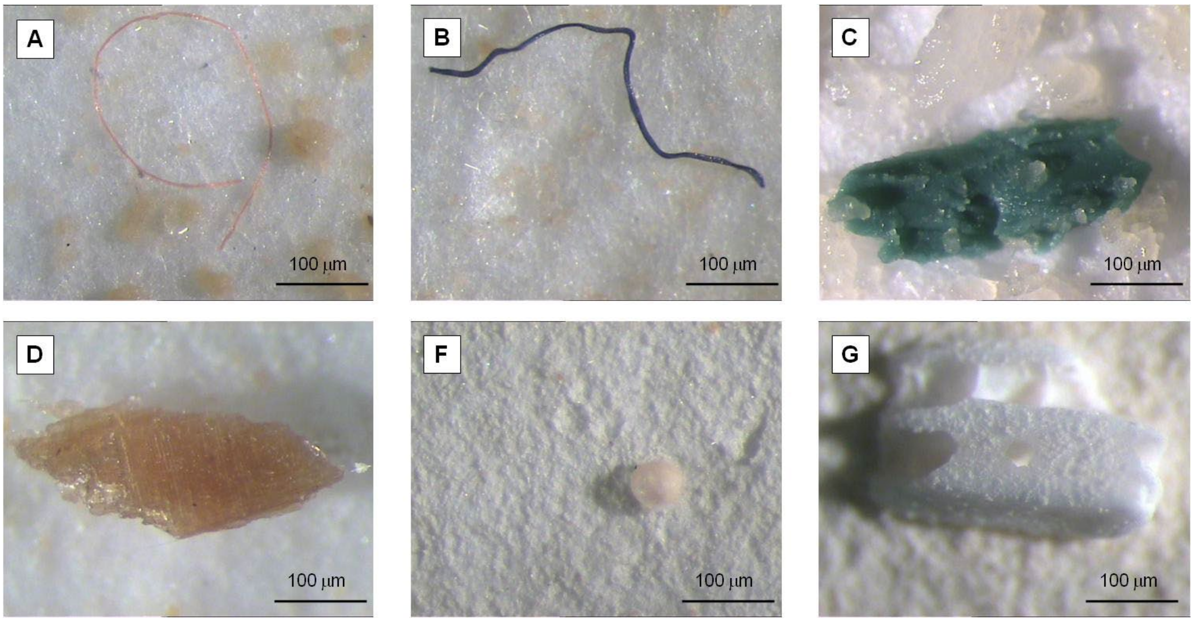
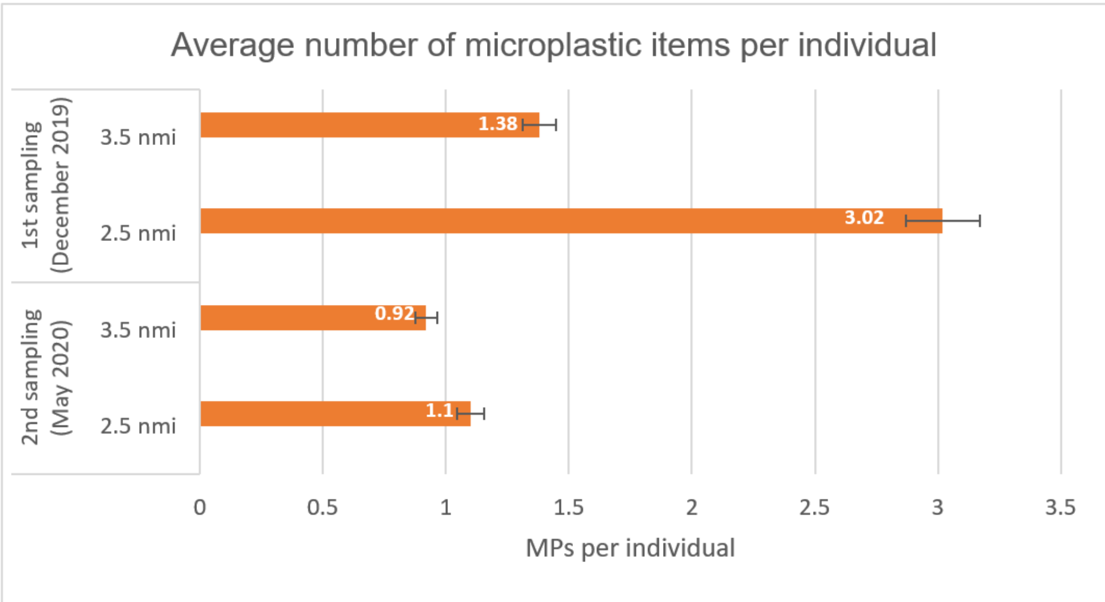


Figure 2. Photographs of different types of microplastics found in collected mussels: fibers (**A**,**B**), fragments (**C**,**D**), granule (**F**) and foam (**G**).

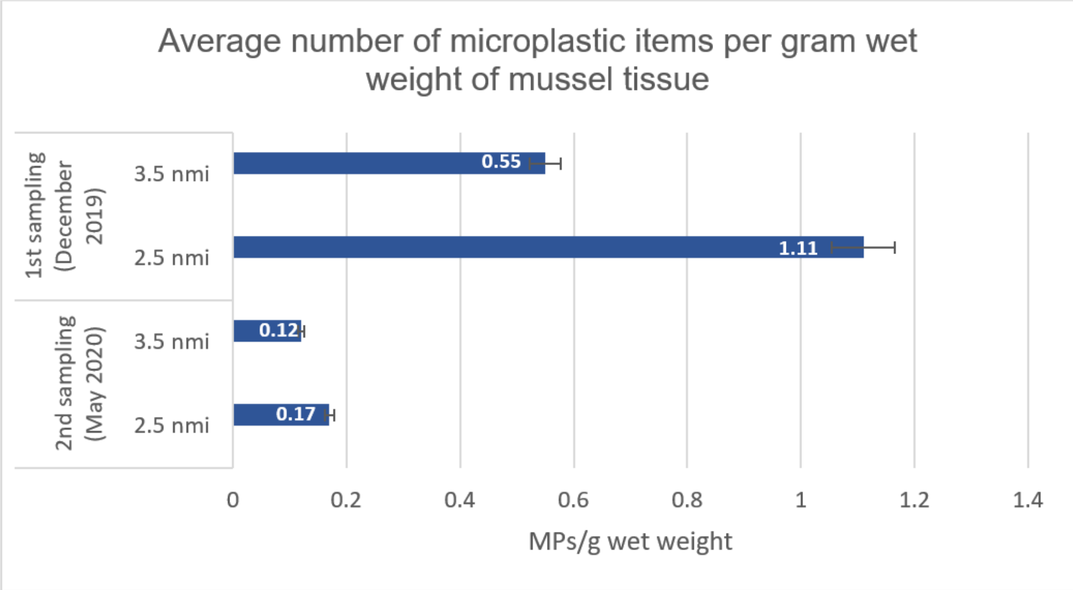
Instead, there were no statistically significant differences in the average number of microplastics between the sampled mussels at 2.5 and 3.5 nmi from the coast. Therefore, the average number of microplastics per individual was similar between each mussel sampling station (Mann–Whitney test, p value > 0.05).

The procedural blank samples were completely free of any microplastic contamination.



(**a**)

Figure 3. *Cont.*



(**b**)

Figure 3. Microplastic items (mean ± standard error) found in collected mussels from the Goro Sacca (northern Adriatic Sea). Results expressed (**a**) by individual (items/individual) and (**b**) by soft tissue wet weight (items/g wet weight).

3.2. Shape, Color, and Size Class of Microplastics

The characteristics of the microplastics were presented by averaging their composition of each sampling station.

Microplastics of different shapes, such as fiber, fragment and granules, were observed in the soft tissues of the analyzed bivalve mollusks.

Fiber particles were the most abundant shape (50–80%), followed by fragments (10–40%). In contrast, granules (1.5–2.5%), non-categorized shape (1–2%) and foam (<1%) accounted for less than 2.5% or were not detected (Figure 5).

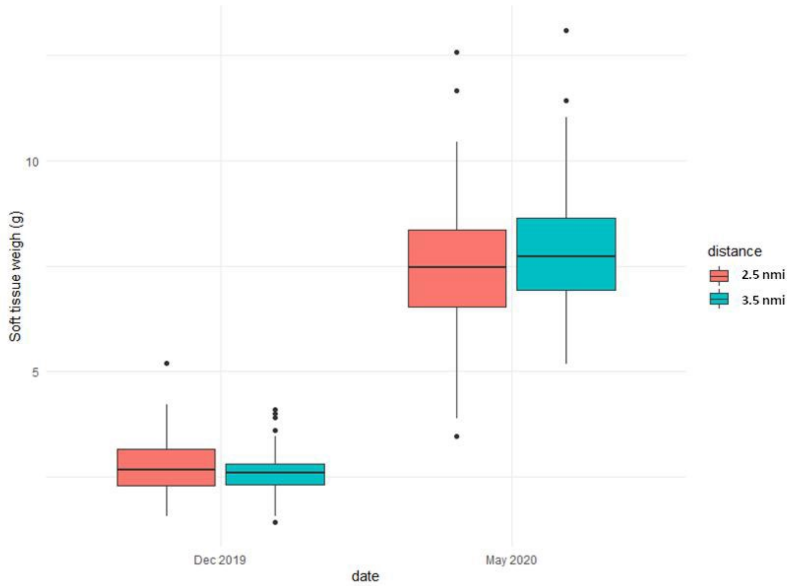


Figure 4. The box plot describes the mean values of microplastic particles per gram wet weight of mussels' tissue as a function of distances from the coast (at 2.5 and 3.5 nmi) and sampling period (December 2019 and May 2020), underlining the significant differences between the two time samplings.

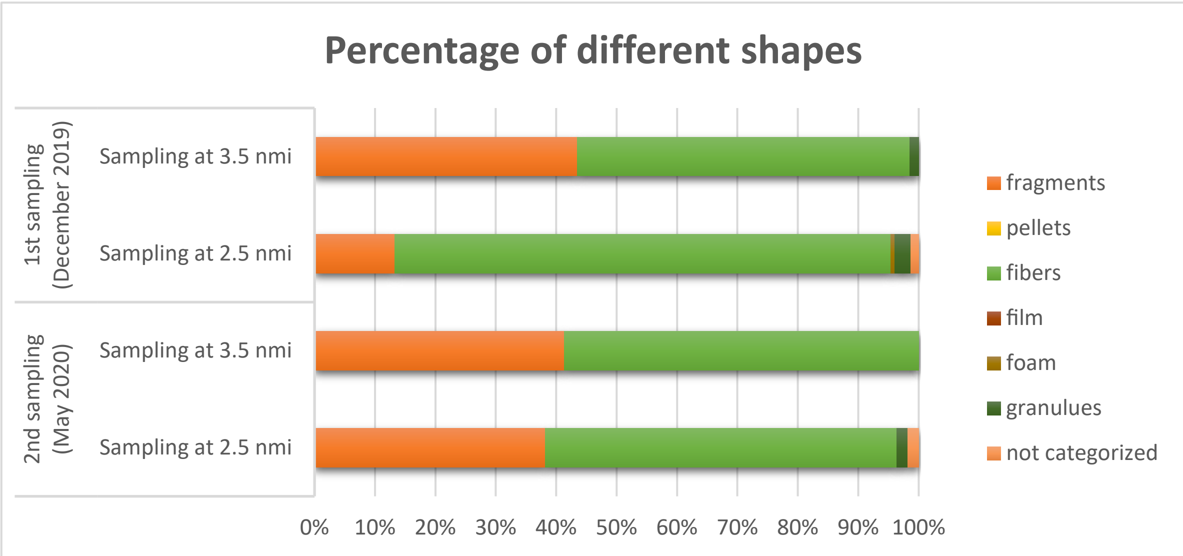


Figure 5. Percentage of different shapes of microplastics found in the bivalves of 1st and 2nd sampling.

Although the most common color among all the encountered MPs was black (50–70%), microplastics particles were also found to be transparent (5–15%), red (2–15%), green (5–13%), white (2–13%) and blue (2–10%) (Figure 6).

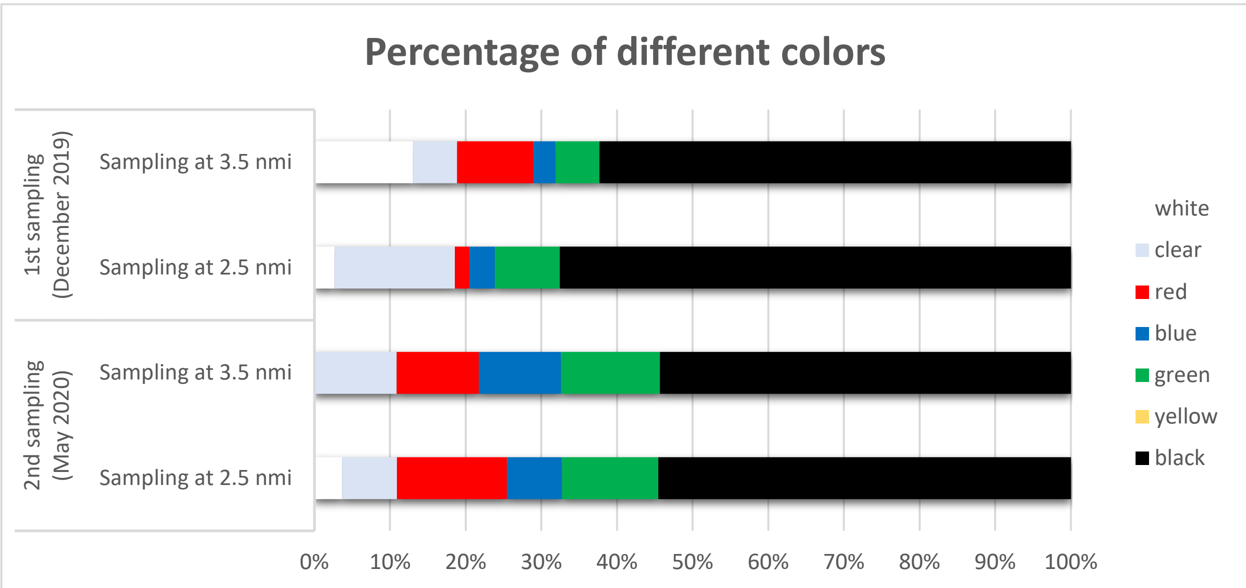


Figure 6. Percentage of different colors of microplastics found in the bivalves of 1st and 2nd sampling.

No significant differences were detected between the samplings in relation to colors (beta distribution 95% confidence intervals overlap).

Microplastics were classified into five size categories (<15 µm, 15–50 µm, 50–100 µm, 100–500 µm and >500 µm). In the samples collected in the 1st sampling (December 2019), microplastics of >500 µm were the most abundant size class, 73.51% and 46.38%, respectively, at 2.5 and 3.5 nmi from the coast. Instead, the intermediate size class (100–500 µm) made up 54–35% of the microplastic items found in the 2nd survey (May 2020). The size class frequency distribution of the isolated MPs is presented in Figure 7.

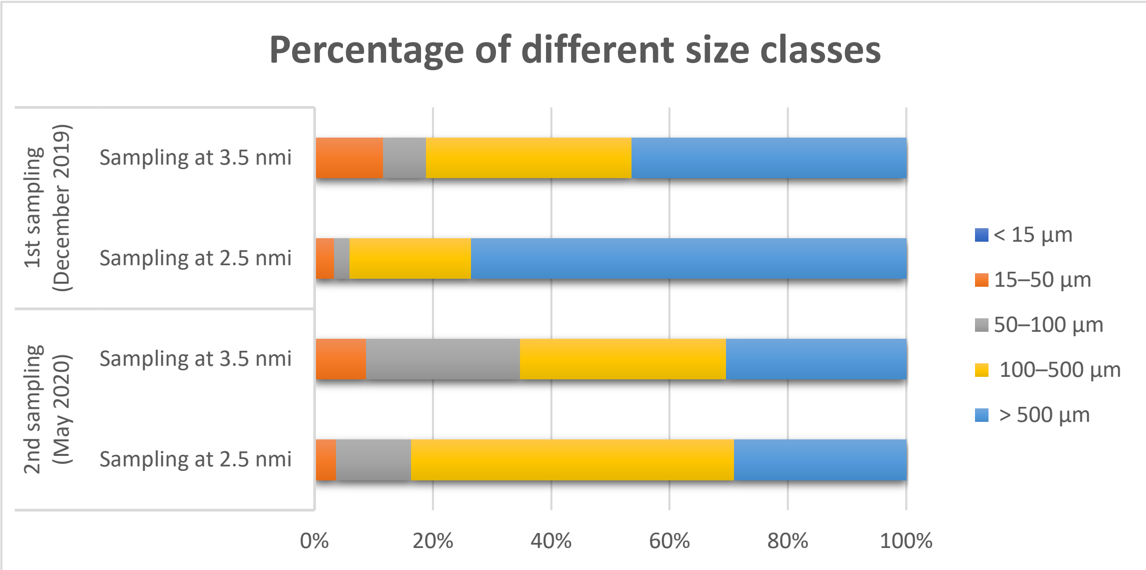


Figure 7. Percentage of different size classes (<15 µm, 15–50 µm, 50–100 µm, 100–500 µm and >500 µm) of microplastics found in the bivalves of 1st and 2nd sampling.

No correlation was found between microplastic size and organism length (Kruskal–Wallis test, p value > 0.05).

4. Discussion

The present investigation analyzed microplastic ingestion in native mussels (*M. galloprovincialis*) from a long-line farm located in the Goro Sacca in order to both gain knowledge on microplastic distribution and abundance in the northern Adriatic Sea and evaluate the two possible influencing factors, including weather events and industrial activities.

Microplastic extraction from mussels' soft tissue was performed using the procedure described by Mathalon and Hill [38] and Bessa et al. [60], which employs an oxidative agent (H_2O_2). There are some advantages to using this agent. Firstly, treatment with H_2O_2 digests organic matter with an efficacy of <95% [20,62], and a recovery rate of different plastic polymers of about 95%. Secondly, H_2O_2 has a minor effect on the chemical and physical state of plastic compared to other digesting solutions, such as HCl, HNO_3, NaClO, and NaOH [13,62].

The statistically significant difference (Mann–Whitney test, p value > 0.05) in the abundance of potential microplastics detected between the two samplings conducted could be attributed to the following two factors: (a) exceptional weather events, such as the one that occurred between the end of November and the beginning of December 2019, during which a significant flood of the Po river occurred, thus underlining that microplastics are transported to the sea via rivers and rainwater [63,64], resulting in an increase in their levels in coastal regions near populated and industrialized areas [65]; (b) reduction in the anthropogenic activities generally carried out in the Po basin area following the lockdown carried out in Italy, due to the global emergency caused by COVID-19, thus highlighting that there are a variety of anthropogenic activities that produce microplastics on land and this is reflected in the surrounding ecosystem [66].

A negative significant correlation between mussel body weight and number of ingested particles was found (Spearman's r 0.293, p value < 0.0001), showing that larger mussels are prone to contain less microplastics than smaller sized mussels. This result possibly proves that they are able to filter more water in less time, and consequently purify themselves more easily from these pollutants. These data are in contrast to the work of Catarino et al. [67], who instead observed a positive significant correlation between mussel body weight and number of ingested particles, showing that larger mussels are prone to contain more microplastics than smaller sized mussels.

The high levels of fibers found in the examined mussels showed a concordance with most of the studies of mussels, which report a higher percentage of fibers than fragments [17,21,41].

Indeed, it is highlighted that fibers are the most common type of microplastics found in the marine environment and in shellfish [17,21,48]. These fibrous microplastics could originate from fisheries, recreational boating, laundry, domestic waste water and other anthropogenic activities [13,17].

Of course, having not carried out the identification of the type of polymer due to problems with the analyzed filters, could have broughtan incorrect interpretation of the data. It is very difficult to distinguish between natural and synthetic fibers by visual identification and several researchers [68,69] have already shown a high percentage of false positives. The limit could have overestimate the reported data, even though we tried to contain this by applying the hot needle test on all the particles detected with the stereomicroscope.

Although the color of the artificial lines used in the sampled mussel farm was white, black was the predominant color of the microplastic items found in the soft tissue of bivalves, which were likely derived from a variety of microplastics sources, including debris from industrial activity carried out on the mainland transported through rivers and offshore activities carried out in the marine environment [31,70]. In addition, the color of the microplastics found could be falsified (discolored or loss of their original colors) by environmental weathering processes [66,71].

We exclude any contamination with the cotton fibers of the garments used by the operators who carried out the analysis because the blank samples for the environmental control were always performed at the same time. In addition, the operators during the analysis wore white cotton clothing.

Our findings provide further evidence and support the observations of worldwide authors relating to mussels, that being filter feeder organisms show a remarkable capacity to ingest microplastics [13,19–22,36,38,40,42,56,66,72–74].

The results of this study were compared to other studies that used similar sample treatments and analytical methods and reported their findings in particles per g weight wet and particles per individual.

From these comparisons, it is evident that the microplastic levels found in this geographical region of study were lower than those recorded in *Mytilus* spp. specimens from the northern Ionian Sea coast (2.46–5.26 items/individual: Digka et al. [36]) and also to those observed in areas located in densely populated and industrialized regions, such as coastlines on the South China Sea (4.3–57.2 items/individual: Li et al. [20]; 1.5–7.6 items/individual: Li et al. [21]).

In addition, as indicated by Digka et al. [36], most studies involving mussels have not reported the frequency of microplastic ingestion, which is likely to be due to the use of pooled samples of animals rather than single individuals for microplastic extraction analysis.

In this study, the frequency of abundance of ingested microplastics was 72 and 86% in mussels found in the first survey, in contrast to 54% in the second survey. Frequencies of microplastic abundance in mussels reported in Digka et al. [36] (45.0–47.5%) and Avio et al. [72] (10–36%), are lower than those reported by this study (54 to 86%), and unlike the detection frequency reported by Cho et al. [66] in mussels from the Korean coasts, which was 96%.

The frequencies of microplastic abundance in mussels reported by this study (54 to 86%) is greater than those reported in Digka et al. [36] (45.0–47.5%) and Avio et al. [72] (10–36%) and similar to the detection frequency reported by Cho et al., [66] in mussels from the Korean coasts, which was 96%.

Unfortunately, these data are hardly comparable, as the identification of the polymers of the potential microplastic particles was not performed.

In addition, the observed differences, as already claimed by these authors, could be due to the use of different mussel sampling strategies, as well as the lack of standardized methods and protocols used by different research teams to allow comparability between studies.

In this study, mussels were sampled at three different depths (greater depth, intermediate and from 50 cm from the surface) in order to create a sampling that could be as representative as possible of the presence of microplastics in the water column. The mussels were subjected to digestion with H_2O_2 individually, filtered and the microplastic elements found were observed using a stereomicroscope (Leika MZ6, Leica Microsystem Ltd., Heerbrugg, Switzerland) and ultimately categorized by shape, size class and color, with the aid of a digital camera (JVC-C1381, JVC, Yokohama, Japan) and data acquisition software (Leica IM500 version 1.5, Leica Microsystem Ltd., Heerbrugg, Switzerland).

Particular attention should be paid to the comparison with the study of Gomiero et al. [56] regarding mussels taken from the Adriatic Sea. Gomiero et al. [56] observed in native mussels of the northern and central Adriatic Sea the highest microplastic particle accumulation in organisms collected along the coastal sites (1.06–1.33 fragment/g weight wet and 0.62–0.63 fibers/g weight wet), compared to the offshore areas (0.65–0.66 fragments/g weight wet and 0.24–0.35 fibers/g weight wet). This trend could be comparable to that observed by us, relating to the potential microplastics found in the mussels collected from stations at 2.5 and 3.5 nmi from the shoreline of the Goro Sacca in the December sampling. Indeed, it is easily deduced from this sampling that the quantity of microplastics found in the mussels collected nearer to coastal areas (1.11 items/g weight wet) were about twice as many as found in the mussels collected from the station at 3.5 nmi (0.55 items/g weight wet). According to Gomiero et al. [56], such site-dependent distribution likely reflects the combination of the significant inputs from rivers and coastal-based anthropogenic activities (aquaculture farms, tourisms, etc.).

In addition, Gomiero et al. [56] states that size class distribution revealed a marked prevalence of smaller particles (4–100 µm). Conversely, the two size classes mainly found in the area of the Adriatic Sea investigated by us were >500 µm, followed by particles with a size range of 100–500 µm. Smaller size microplastic particles may have been underestimated, as reported other studies [14,42] where recovery rates decrease with decreasing particle size.

5. Conclusions

The findings of this study provide more information on microplastics in shellfish bivalves in the Adriatic Sea, supporting their instrumental role use as an environmental bioindicators for microplastic pollution.

In addition, our outcomes support the possibility of identifying the river discharges as one of the potential transport factors of plastic debris from the mainland to the sea, as for example, the Po river in the northern Adriatic Sea.

Indeed, higher concentrations of potential microplastic items observed in this study were found in the first mussel sampling conducted in December 2019 (1.11 ± 0.92 microplastic items per gram wet weight of mussels′ tissue), after a greater flow of the river Po due to rainfall events recorded in the days immediately before the sampling. It is likely that these events increased the transport of various kinds of debris towards the sea.

In addition, during this period, the industrial and agricultural activities in the analyzed area were carried out at maximum capacity, unlike the second sampling period, in which production was reduced due to the COVID-19 emergency.

In the days before the second sampling, precipitation was practically absent and the Po river flow was lower than in the month of December; therefore, the lower concentration of microplastics observed (0.17 ± 0.21 microplastic items per gram wet weight of mussels′ tissue) might be partly due to more stable weather conditions than those observed in December. Moreover, the second sampling, carried out in May 2020, coincides with the reopening of anthropogenic activities following the three month lockdown in Italy enacted to contain the COVID-19 virus. Such data can lead us to presume that at this particular period, the contribution of plastic pollutants from the Po river to the Adriatic Sea was reduced.

As stated by several authors [44,66,71], the characteristics (i.e., shape, size class and color) of microplastics in bivalves sufficiently reflect the contamination characteristics of both land- and sea-based activities. Moreover, it is commonly found that the contamination of microplastics in bivalves is high in urbanized and industrialized regions with high population densities, where the impact of anthropogenic activities on the environment is considerable [66].

Lastly, bivalves are important as a pathway for transporting anthropogenic pollutants to higher level organisms in food chains [37], and indeed are an important food source for humans.

It is important, therefore, to continue to use bivalves as sentinel species for monitoring microplastic pollution in the marine environment and maximize efforts in order to both improve information concerning the status of microplastic contamination at the local, national, regional, and global scales and to better understand their potential transfer to the food web that currently raises human health concerns.

The data from these assessments will assist in the development of local and global policies, as well as management strategies to reduce and prevent microplastic pollution.

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