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Microplastic retention in European flat oyster *Ostrea edulis* cultured in two Mediterranean basins

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Oysters, as filter feeders, intercept and retain microplastics (MPs), making them bioindicators of environmental contamination and a potential risk to human health. MP accumulation was assessed in two life stages of cultured *Ostrea edulis* from the Adriatic and Tyrrhenian Sea in the Mediterranean basin. The productive areas share similar environmental conditions highly differing in industrialization level. The results show that human activities directly influence the quantity and quality of MPs found. Oysters from the Tyrrhenian basin showed higher contamination and higher occurrence of spheres, reflecting the inland industrialization level. The MP size class between 50 and 150 μm was the most abundant in both basins, overlapping the diet plankton size and suggesting that species trophic ecology is a key driver in MP interception. This is the first MP-exposure assessment on *O. edulis* in the Mediterranean basin, laying the groundwork for future studies on risk assessment within the "One Health" approach.

Marine filter feeder organisms are particularly exposed to microplastic (MP) ingestion and accumulation[1] due to their trophic ecology. Filter-feeder bivalves filtrate large water volumes to retain nutrients (debris, phyto-, zoo- and mero-plankton), and can therefore ingest a high amount of MPs, favoring their transfer along the food web up to humans[1]. MPs are considered ubiquitous pollutants, found in the soil, air, fresh and sea waters[2]. Global seas and oceans are the main final reservoirs for MPs, and alarming concentrations are reported from the surface to the sea bottoms, the main sink storages[3,4]. MPs have been found at all levels of the marine food web, from tiny plankton to the largest mammals. Filter feeders can easily intercept and retain MPs in marine environments and, for this reason, some oyster species such as *Crassostrea gigas* (Thunberg, 1793) and *Saccostrea glomerata* (Gould, 1850) have been proposed as MP bioindicators[4], as they can reflect site-specific MP contamination. Indeed, the local MP dynamics[5] can be significantly influenced by local MP sources, including inland inputs (e.g., fluvial transport and discharge) as well as marine activities (such as aquaculture farms). The concentration reported in terms of MPs/g in bivalves such as oysters can vary from 0 to more than 20, resulting in an average concentration of 0.06–5.8 items/wet weight (g)[1]. Despite being the second main group of bivalves consumed, from wild and aquaculture farms, oysters are still poorly investigated in fieldwork[6]. Oysters are generally farmed for human consumption, often eaten raw and considered a luxury food with high value organoleptic properties[7]. The production of flat oysters steadily fluctuated at high levels until the mass mortalities caused by *Bonamia ostrea* in the past[8]. In the last decades, aquaculture production has been revived and showed a significant increase in Europe[9] with the major aquaculture producers being France, Spain, Ireland, Croatia, and the United Kingdom[10]. Their production is considered one of the most sustainable aquaculture systems[11] as it does not require external food supply and it provides various ecosystem services such as: carbon sequestration, eutrophication and nutrient control, water quality improvement and biodiversity enhancement by providing substrate for other marine species[10]. The European flat oyster (*O. edulis)* is a sessile bivalve with a great filtering capability, up to 25 L/h[12]. This species is native to the Eastern Atlantic and Mediterranean coasts, living in intertidal areas up to depths of over 30 m. Oysters feed on zoo-, phyto-, and meroplankton through a filtration system based on ciliated gills and labial palps located in the mantle cavity[10]. The coordinated movement of the cilia on the gills creates a water current that enters through the inhaling siphon. Food particles are trapped in mucus threads and moved by the cilia to the labial palps and then to the esophagus. The palps sort the particles by size and density. Unselected particles are expelled as 'pseudofeces,' while selected particles enter in the digestive system and then are excreted as feces[10]. Despite being a keystone species, *O. edulis* is sensitive to several environmental stressors, including organic pollutants, heavy metals[13–15] and emerging contaminants like MPs. Ex situ studies demonstrated that *O. edulis* is vulnerable to experimental MP exposure,

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which can induce feeding and breeding dysfunctions[16–18]. Nowadays, MPs are included in Descriptor 10 of the Marine Strategy Framework Directive (MSFD; 2008/56/EC) for assessing the presence/amount of microlitter in marine environment and its biota. Based on the Shellfish Water Directive (2006/113/EC) and the European Regulation 854/2004 for the safe cultivation of shellfish for human consumption, farming areas are regulated based on microbiological monitoring but without considering local MPs contamination. Hence, bivalves can be directly consumed or may require purification depending on their microbiological contamination but MPs may also represent a potential health risk for human consumption[19]. Depuration and relaying are species-specific processes, and their durations are settled on some pathogens or contaminants to be removed, not considering MP contamination. While 48 h of depuration are considered sufficient for fecal coliform removal[20], laboratory tests showed that 96 h were needed to achieve over 90% depuration of MPs in contaminated *O. edulis* organisms[17]. Considering the potential accumulation of MPs due to the limited self-purification and excretion capacity of oysters[21], more data is needed to establish a solid basis for understanding how oyster plays a key role in conveying MPs to humans. In this scenario, as a necessary first step toward future risk assessment strategies, the aim of this study was to identify MP uptake patterns in relation to the feeding ecology of *O. edulis* reared under different anthropogenic impacts. The evaluation and comparison of the MP retention level were therefore performed in Subadults and Adults European flat oysters, sampled from two Mediterranean productive areas: one located in the Central Adriatic Sea and the other one located in the Central Tyrrhenian Sea.

Results

No MPs were found in the procedural blank filters. A total of 201 MPs and 387 MPs were respectively found in the oysters from the Adriatic and Tyrrhenian productive areas, considering both Subadult and Adult specimens.

MP concentrations in cultured oysters

MPs were found to be more abundant and concentrated in oyster samples from the production area located in the Tyrrhenian Sea, both in terms of items per gram and items per individual. The MP concentration in Tyrrhenian oysters turned out to be significantly higher and almost double with respect to Adriatic oysters, both in terms of items per gram and items per individual ($U_1 = 32, p < 0.05; U_2 = 9, p < 0.0005$). The mean value reached in Tyrrhenian oysters was 1.55 ± 0.81 items/g with respect to the mean value of Adriatic oysters of 0.84 ± 0.56 items/g (Fig. 1a). At the same time, MP concentration expressed as items/individual resulted to be 5.88 ± 1.68 items/individual in Tyrrhenian oysters and 2.60 ± 1.32 items/individual in the Adriatic ones (Fig. 1b). Specifically, comparing MP concentrations among oysters' life stages, Tyrrhenian Subadults showed the highest MP concentrations among groups in terms of items/g, with a mean of 1.98 ± 0.89 (Fig. 1c). The mean concentration (1.13 ± 0.39 items/g) in Tyrrhenian Adults was statistically higher than the Adriatic one (KW test, $H = 5.77$, $p < 0.05$) (Fig. 1c). In terms of items/individual, both Subadults and Adults from the Tyrrhenian site showed significantly greater concentration with respect to the Adriatic ones (KW test, $H = 14.09$, $p < 0.005$) (Fig. 1d). No significant differences emerged between oyster life stages within the same basin.

MP shape composition

To characterize the interception and retainment of MPs by the oysters, each item found was classified into shape categories. The shape composition analysis was not only aimed at understanding if there was a selection of MPs in terms of shapes but also to feature how the different anthropic activities along the two basins cause the release of primary and secondary MPs. Primary MPs, typically spherical or pellet-shaped, are intentionally produced in small sizes for use in cosmetics, detergents, coatings, and finishes, while secondary MPs (which may appear as fragments, fibers, films, or foam) are generated from the fragmentation of larger plastic objects through mechanical, biological, and chemical degradation processes[22–24]. In this study, the most found shapes were fragments, fibers, and spheres. Since film and foam were found with a very low frequency (<1 and 3% respectively), they were included within the fragments count, as secondary MPs, because they also derive from the degradation and fragmentation of macroscopic plastic objects. The one-way PERMANOVA test with 9999 permutations revealed significant differences (p-value < 0.05) between shape composition in Adriatic and Tyrrhenian oyster (Fig. 2). Fiber was the most abundant shape found in Adriatic samples (49.25%), while in Tyrrhenian oysters, fragments emerged as the most frequent (44.44%). It is important to notice that the two shape compositions are statistically different because of the fiber and sphere percentages (KW test, $p < 0.05$), the former more frequent in the Adriatic, the latter in the Tyrrhenian Sea.

MP size class composition

The found MPs ranged from 6.3 up to 4487 μm with a median value of 139.21 μm. MPs below 500 μm represented approximately 80% of the total, and MPs with the smallest size range (<100 μm) were the most abundant, representing 29.35% and 43.15% for Adriatic and Tyrrhenian basins, respectively. The size class distribution followed an exponential reduction as the size class increased for both sites (Fig. 3a, $R2 = 0.81$ in Adriatic and $R2 = 0.73$ in Tyrrhenian). The size class composition emerged to be statistically different between the two basins (Fig. 3a), probably due to the greater concentration of the fraction smaller than 100 μm in the Tyrrhenian samples (One-way PERMANOVA, $p < 0.005$). Examining the most abundant fraction (less than 100 up to 500 μm), a more detailed classification was carried out with the aim of relating the retention of certain MP size classes to natural oyster feed uptake. Based on previous findings[25,26], MPs were categorized into four functional groups: 0–50 μm, 50–150 μm, 150–300 μm and 300–500 μm. In both basins, the detailed MP size class distribution showed an asymmetric bell curve, with the size class ranging from 50 to 150 μm being significantly the most abundant (KW test, $H = 31.61$, $p < 0.0001$), representing the 31.34% and 35.41% respectively for Adriatic and Tyrrhenian oysters (Fig. 3b). Regarding the matching between size classes and shapes, spheres were more abundant in the smallest fraction, fragments in the 50–150 μm size class while fibers were predominant in size classes greater than 300 μm. The fraction between 150–300 μm was composed of equal parts fibers and fragments (Fig. 3b).

Discussion

Based on current knowledge, this is the first work reporting MP retention in the flat oyster in the Mediterranean basin. In the present study, specimens of *O. edulis* cultured in two productive areas with different surrounding conditions were compared. The hypothesis was that these varying conditions could influence the quantity and type of MPs released into the environment and bioavailable for oysters' interception.

In this context, the primary focus of this study was to examine the correlation between trophic ecology of oysters and MP shape and size retention, key characteristics that the Nile Red technique effectively enabled to assess and replicate over time[27].

Detecting MPs in complex biological samples, such as oysters, presents several challenges due to interference from natural organic materials and the need for efficient screening methods capable of identifying large quantities of particles. The application of Nile Red staining method, although does not directly identify polymer types, offered an efficient and cost-effective[28] screening technique for detecting high particle counts on a large scale[27,29]. The limitations of this method, including the risk of over- or under-estimating MPs due to co-staining with natural organic materials and interference from the color of MPs[30,31], were carefully addressed in this study through the use of optimized extraction and staining protocols[32,33]. The applied identification protocol[32] was optimized to maximize the fluorescent response and reduce the overestimation of biogenic material by analyzing stained particles under three different fluorescent filters (UV, blue, and green). In this study, the consistent application of the same protocol across all samples from both study areas ensured standardized analysis and minimized bias in the comparison between basins.

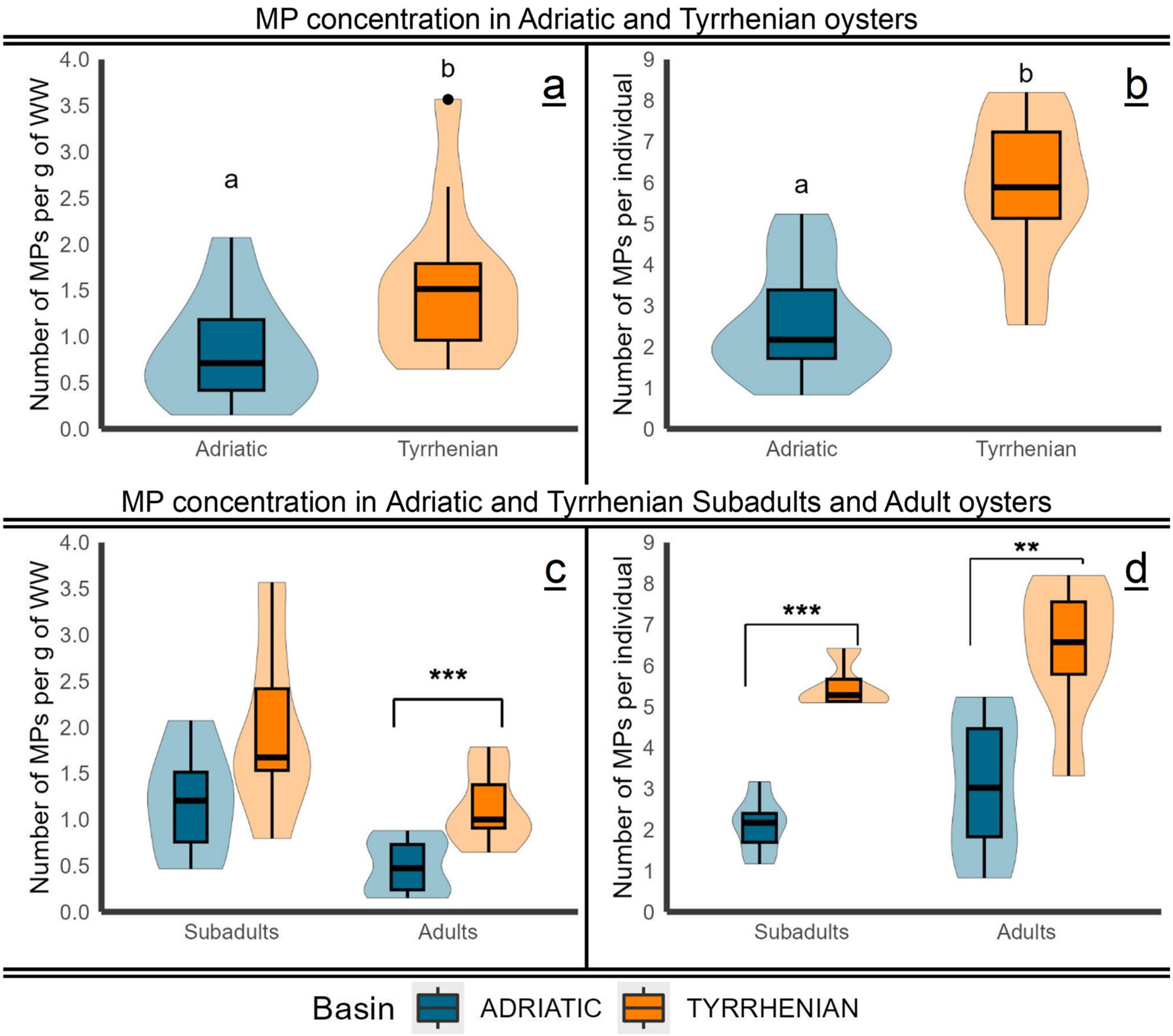
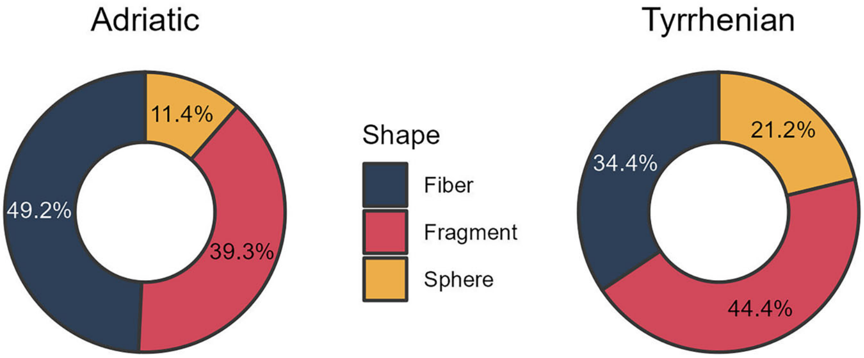


Fig. 1 | Microplastic concentration in cultured oysters from the Adriatic and Tyrrhenian Seas. Box and whisker violin plots comparing MP concentrations in the Adriatic and Tyrrhenian productive areas: (**a**) items/gram; (**b**) items/individual, and across oyster life stages: (**c**) items/gram; (**d**) items/individual. The colors are blue for Adriatic oysters and orange for Tyrrhenian oysters. The box plots represent the 1st and 3rd quartiles separated by the median, with outliers plotted as individual black dots. The violin plots show the distribution and density of the data, with the width of each "violin" indicating the data density at different values. In graphs (**a**) and (**b**), letters indicate significance levels from pairwise comparisons ($p < 0.05$). In graphs (**c**) and (**d**), asterisks denote statistical significance, with ** indicating $p < 0.01$ and *** indicating $p < 0.005$.

Fig. 2 | Microplastic Shape Composition. Relative abundance of MP composition by shape in oysters from the Adriatic and Tyrrhenian productive areas. The shapes are color-coded as follows: dark blue for fibers, coral red for fragments, and golden for spheres.



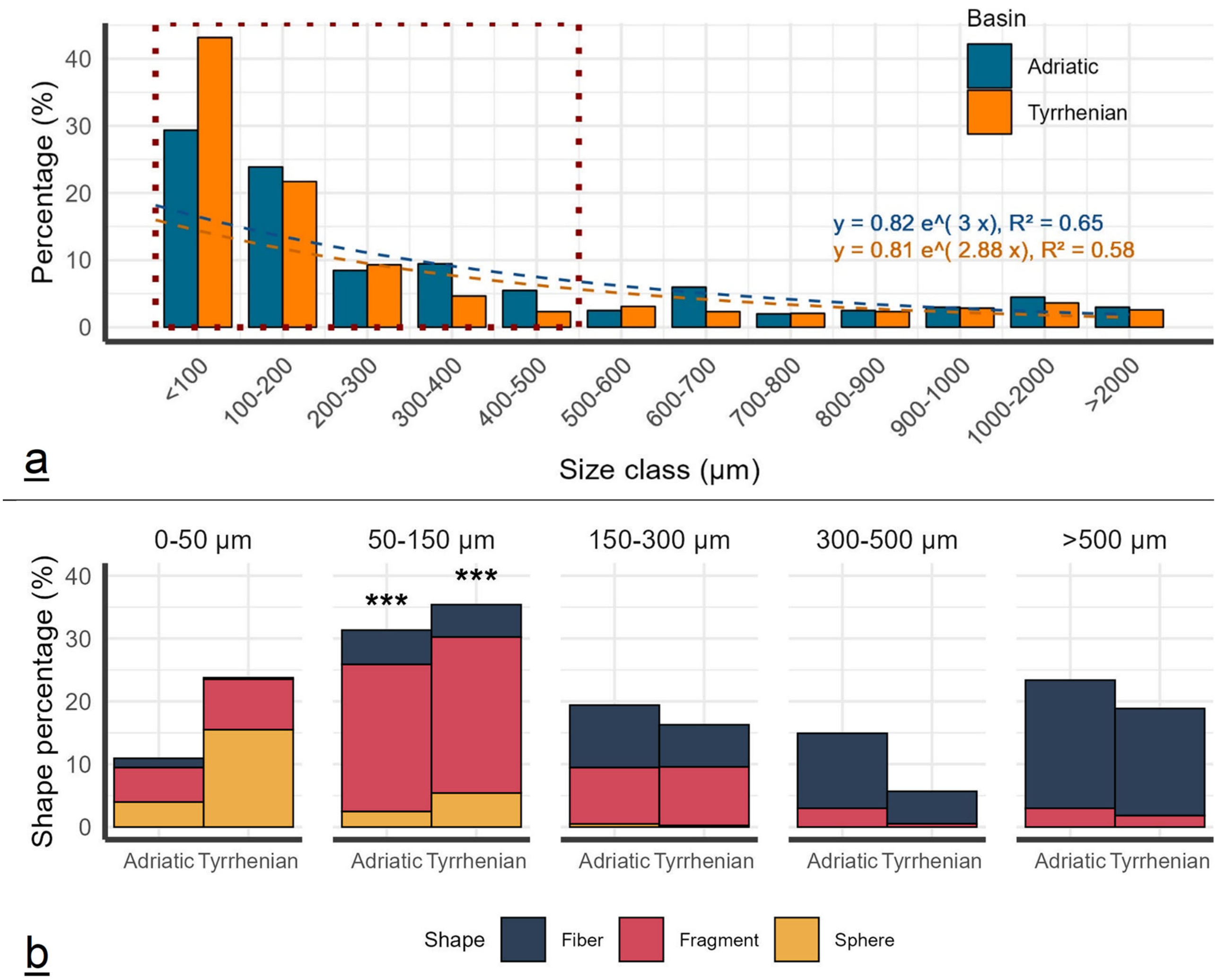


Fig. 3 | Microplastic Size Class Composition. a Relative abundance of MP size distribution in oysters from the Adriatic and Tyrrhenian Seas. Histogram colors are blue for Adriatic oysters and orange for Tyrrhenian oysters. **b** Relative abundance of MP shapes in different functional size classes from the Adriatic and Tyrrhenian basins. Asterisks (***) above the 50–150 μm size class indicate significant differences ($p < 0.0001$). The shapes are color-coded as follows: dark blue for fibers, coral red for fragments, and golden for spheres.

As a result, MPs were found in all samples from both productive areas, with all specimens having at least 1 MP each. It means an occurrence of 100%, slightly higher than the oyster literature average, estimated around 94%[4], ranging from a minimum of 63% in Pacific oyster (*Crassostrea gigas)* from the Salish Sea (United States) and a maximum of 100% in Natal rock oyster (*Saccostrea cucullata)* from the Gujarat coast (India)[21,34]. It is important to highlight that, given the relatively unexplored nature of this field, it is not currently possible to make thorough comparisons within the same species. Moreover, it is necessary to take into account the species-specific characteristics and the different environmental conditions in which specimens grew up. Both wild and cultured oysters are subject to MP contamination, and their concentration is expected to depend on external surrounding inputs[6,35]. In this study, oysters from the Tyrrhenian basin showed significantly higher concentrations (1.55 ± 0.81 items/g; 5.88 ± 1.68 items/individual) with respect to the mean value of Adriatic oysters (0.84 ± 0.56 items/g; 2.60 ± 1.32 items/individual). The MP concentration in the Tyrrhenian Sea is double that of the Adriatic Sea. Marine MP dispersion can act synergistically with local anthropization in increasing the bioavailability of MPs for oysters[21,27,36–39]. These dynamics are further intensified by river discharges, which play a key role in increasing or mitigating the bioavailability of MPs. Riverine transport is directly influenced by the land use practices in the catchment basin[40], the level of urbanization and industrialization, and the types of human activities occurring. Depending on these factors, rivers can concentrate MPs, leading to the transport of significant amounts. Similarly, the higher MP concentrations in Tyrrhenian oysters, compared to those in the Adriatic, can be attributed to the higher urbanization and industrialization occurring along the Garigliano River catchment, which flows into the Gulf of Gaeta (Fig. 4b). In contrast, the Neretva River catchment is characterized by lower urban density and more agricultural land use (Fig. 4c), which results in a less concentrated transport of MPs.

Compared to the global average[4], oysters from the Tyrrhenian Sea have slightly higher MP concentrations, while those from the Adriatic Sea are moderately lower (1.03 ± 0.33 items/g). The Mediterranean Sea is considered one of the high-risk areas for the accumulation of MPs. This is due to its semi-closed nature of the basin, limited water exchange with the Atlantic, and the high population density in the region[41,42]. Comparing the results from this study with those found in other Mediterranean basins, it emerged that European oysters from both areas have a much lower average than other oyster species sampled in Greece[35] and in Spain[43] (Table 1). For both basins, the MP concentration in terms of items per gram emerged to be greater in Subadults rather than the Adult oysters. This result is coherent with the hypothesis that small oysters may accumulate more MPs probably due to higher metabolism and filtration rate. This inverse correlation

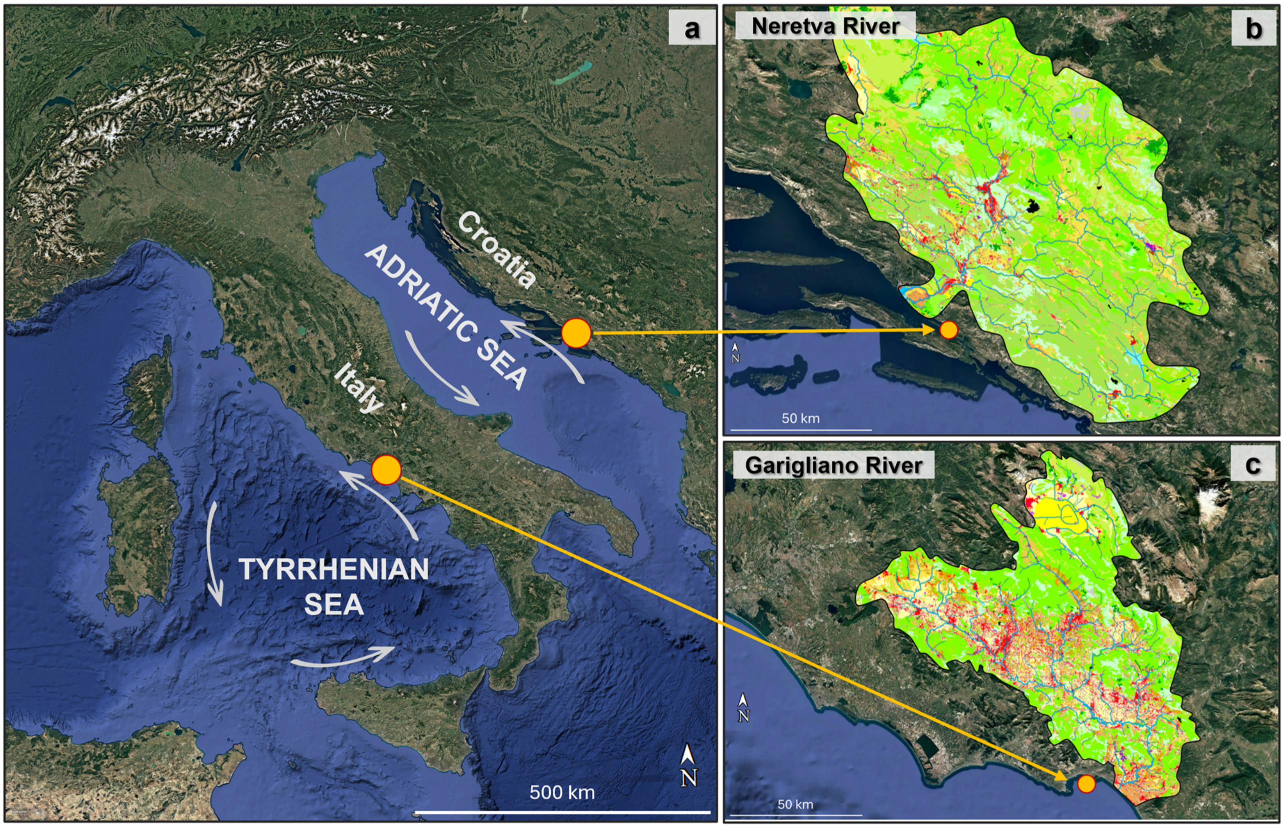


Fig. 4 | Study Areas and Riverine Land Use. **a** Localization of the study areas in the Tyrrhenian and Adriatic Seas with orange circles indicating their respective productive areas; **b** Neretva River catchment basin with land use classification; **c** Garigliano River catchment basin with land use classification. Shades: green for rural areas, yellow for agricultural areas, and red for urban and industrial areas. Images in (**b**) and (**c**) modified from the CORINE Land Cover database (Copernicus Land Monitoring Service, © European Union, 2018).

between MP concentration and shell length/soft tissue weights was previously observed also in other oysters' species, such us Vietnamese *Crassostrea gigas*, Indian and Chinese *Saccostrea cucullata* and Chinese *Crassostrea spp*. (Table 1).

Considering the MP concentration per individual, it emerged greater in both Adult groups, suggesting a possible and progressive accumulation effect over time, crucial for assessing the potential risk to the health of organisms and for human consumption. Using both units of measure for MPs concentration can provide a more complete understanding of the contamination and the ecological implications on marine bivalves. Moreover, these units can be used for monitoring, risk assessment and developing effective mitigation strategies. Indeed, the unit item/g can be ideal for assessing the level of contamination among species with variable size and growth rates, while item/individual can be useful for assessing the absolute impact of the total amount accumulated over the time.

Shape of MPs is a key indicator of their sources. The most found shapes were fragments, fibers and in a smaller proportion spheres. These results are coherent with MPs found in marine bivalve globally[4,36,39,44]. Specifically, in Adriatic oysters, fibers represented 49.25%, fragments 39.3%, while spheres 11.44%. In Tyrrhenian ones, fragments were the most abundant (44.44%), followed by fibers (34.37%) and finally spheres (21.19%). In both sites, spheres were found in higher percentages with respect to Pacific Oysters from Canada and similar to those found in Pearl Oyster from Greece (Table 1). The MP shape composition emerged as statistically different between the two Mediterranean basins, particularly due to the higher occurrence of fiber in Adriatic oysters and spheres in Tyrrhenian ones. Aquaculture activities can be a contributary input to these differences. Indeed, MP fibers can be released into the marine environment through the weathering of farming materials such as lanterns, ropes, and other equipment representing an effective source of MPs in each productive area[45]. However, since these structures are present in both sites, this potential source is expected to impact both areas similarly. The differences found are hence more likely attributed to MP transport via riverine discharges, influenced by different surrounding anthropogenic activities[37]. In fact, the Gulf of Gaeta (central Tyrrhenian Sea) is impacted by the Garigliano River, which flows through highly urbanized and industrialized areas. As a result, the higher percentage of spheres found in the Tyrrhenian oysters is likely related to the substantial discharges into the river catchment area from these industrialized areas, which are typically associated with high MP contamination levels, particularly in pellet-shaped and spherical MPs[46]. On the other hand, Mali Ston Bay (central Adriatic Sea) is influenced by the Neretva River, which flows mostly through less urbanized, rural, and agricultural areas. In this context, the greater concentration of fibers in the Adriatic basin is mainly attributed to the different land use practices in the river catchment basin. Fibers generally originate from agricultural run-off and the washing of synthetic textiles, while fragment from photo-bio-physical degradation of macroscopic plastic objects[22,23].

MP size class can represent a driver in understanding the role of species trophic ecology in MP retention. In this study, MPs smaller than 500 µm represented approximately 80% of the total with the fraction less than 100 µm being the most represented for both sites. Upon analyzing the occurrence of MPs across dimensional ranges of 100 µm, a decreasing exponential trend was observed, accordingly to other studies[36,38,47]. Similar results were reported in oysters from China coast[44], where portions less than 100 µm represented around the 38% and size range between 100 and 300 µm (rather than 0–50 µm) being the most frequently detected. Results here showed the following similarly, with the 50–150 µm fraction representing around 33% and the 50–300 µm fraction around 50%. Ingestion is the primary pathway for MP accumulation in bivalves[47], and smaller MPs are generally more easily intercepted by filter feeders due to their feeding mechanism, which retain and select planktonic-sized particles[21,48]. The efficiency and selectivity of the filtration system strongly depends on the species anatomy and physiology. Oysters have species-specific gills and labial palps suitable for up taking some dimensional ranges of particles[38,49]. From intestinal contents emerged that *O. edulis* feeds mainly on phytoplankton and zooplankton with seasonal variations. During winter, different groups of phyto and zooplankton were found in *O. edulis*: diatoms and dinoflagellates for the first and bivalve larvae with copepods nauplii for the second one[25,26]. Based on these findings, the distribution of MPs across different plankton functional size groups, reflecting the available sources for oysters, was investigated. The 0–50 μm size class includes debris and most microphytoplankton, such as diatoms and dinoflagellates. The 50–150 μm size class contains meroplankton and microzooplankton, including bivalve larvae and copepod nauplii. The 150–300 μm size class is primarily composed of copepodites, while the 300–500 μm size class mainly includes adult copepod species. The size class 50–150 μm (second functional group) resulted in being the most abundant, followed by fraction 0–50 μm, according to the findings on oyster winter diet[26]. These results suggest that MP retention in oysters may be strongly influenced by the species trophic ecology. Specifically, the differences in the abundance of MPs found in various size classes of prey items highlight the role of dietary preferences in MP accumulation. Oysters feeding on smaller microphytoplankton and veliger larvae may ingest a higher number of MPs compared to those consuming larger prey, like copepods and larger diatoms. This selective feeding behavior indicates that MP retention is not a passive process but also closely linked to the specific trophic interactions and feeding strategies of the oysters. Further research should explore how seasonal variations in diet and environmental conditions affect MP retention, providing a more comprehensive understanding of the ecological factors driving MP accumulation in oysters. Moreover, by integrating the information on shapes and sizes, it emerged that the smaller sizes are characterized mainly by spheres and fragments, while the larger ones are characterized more by fibers. The filamentary shape of the fibers, generally characterized by thin thicknesses, explains how very large particles can be intercepted and retained despite being outside the feeding dimensional range. This finding suggests that the structural characteristics of MPs, such as shape and thickness, play a significant role in their retention and accumulation in marine organisms. The ability of oysters to trap larger fibers indicates a complex interaction between the physical properties of MPs and the biological mechanisms of filter-feeding. Further research should focus on investigating how different shapes and sizes of MPs affect the feeding efficiency and health of marine organisms, shedding light on the broader ecological implications of MP pollution.

In conclusion, this study provided the first evaluation on MP retention in the European flat oyster (*Ostrea edulis*) from Mediterranean productive areas, influenced by diverse levels of anthropogenic impact. The results confirmed that MP contamination is widespread, with all oysters analyzed containing MPs. The observed MP concentrations indicated a significantly high level of contamination (Table 1), suggesting the need to further investigate the potential MP retention over time to better understand the implications for ecosystem health and human consumption. MP shape composition varied between the two basins, reflecting regional differences in anthropogenic inputs. Higher concentrations of fibers in the Adriatic and spheres in the Tyrrhenian Sea aligned with distinct sources of MP pollution, such as wastewater discharges and industrial activities. The size distribution of MPs in both basins, predominantly in the 50–150 μm range, reflected the flat oysters' effective retention of MPs, being influenced by species trophic ecology. In conclusion, these results highlighted the widespread contamination and subsequent potential for oysters to act as vectors for MP ingestion in humans. From this it emerged an urgent need to fully understand the mechanisms of MP retention in marine bivalves, integrating knowledge on oyster feeding ecology with MP uptake patterns. This preliminary step is crucial not only for ecological and environmental risk assessment but also for evaluating potential risks to human health through seafood consumption, especially in highly contaminated regions like the Mediterranean Sea.

Methods

Study areas

The oysters examined in this study were cultivated in suspended lanterns at the two aquaculture areas, the Mali Ston Bay in the Central Adriatic Sea and the Gulf of Gaeta in the Central Tyrrhenian Sea (Fig. 4a). *O. edulis* aquaculture in the Adriatic basin is extensive, covering an area of over 1.6 km² and producing a total of 70 tons of oysters/year[57]. Whereas in the Tyrrhenian basin, the bivalve productive area is less extensive. It covers 0.8 km² and produces approximately 50 tons of bivalves annually[58], making it one of the most important productive areas in the Tyrrhenian Sea. Both areas have suitable conditions for the cultivation of bivalves due to their water quality and nutrient availability, which are significantly influenced by the discharge of two rivers located approximately 15–20 km from the aquaculture sites. These rivers, which play a crucial role in supplying freshwater and in distributing nutrients, contribute significantly to the ecosystem balance. In Mali Ston Bay the Neretva River creates a dynamic environment influenced by anticyclonic marine currents[59]. Similarly, the hydrodynamics of the Gulf of Gaeta are determined by the interaction between the flow of the Garigliano River and the gulf cyclonic current entering from the southeast-central Tyrrhenian Sea[60,61]. The river that flows into the Adriatic basin is the Neretva River which originates in the Dinaric mountains and crosses through various rural, agricultural, and urban areas with low industrial presence (Fig. 4b). The Garigliano River originates in the central Apennine mountains and flows into the Tyrrhenian basin traversing rural, agricultural, and urban areas presenting high industrialization within the catchment area (Fig. 4c).

Specimens sampling

Both samplings were conducted in January 2023. For each sampling, 70 specimens were randomly collected from the lanterns (Fig. 5a), washed to remove any residue adhering to the shell, and placed in stainless steel thermally insulated containers with dry ice, hermetically sealed. Samples were transported to the Laboratory and stored at −80 °C until the analysis. All the oysters collected were thawed and divided according to the concave valve length: specimens with valves less than 60 mm were classified as "Subadults", while those larger were classified as "Adults". A total of 140 specimens were selected for the investigation, 35 Subadults and 35 Adults from each area. For each oyster life stage, specimens were grouped in 5 specimen pools. For each specimen, the shell length (Fig. 5b), the total body wet weight (grams of shell and soft tissue), and the wet weight of the soft tissue (g) were recorded (Table 2). The whole soft tissue of each pool was freeze-dried before being analyzed.

Quality assurance and quality control

For preventing external contamination, the standard guidelines[62] were applied. Plastic tools were avoided, preferring steel and glass materials. All materials and surfaces were cleaned with 95% ethanol, and glassware washed with dH_2O and dried in an oven at 60 °C. For avoiding airborne contamination, lab cotton coats were worn, and protocol procedures were performed under a fume hood. All solutions and reagents used were pre-filtered with Schleicher & Schuell 5891 filter papers. Moreover, external valves of specimens were thoroughly washed with dH_2O before processing. To assess the effectiveness of prevention actions, procedural blank samples were performed in parallel with laboratory procedures[63]. In case if MP items were found in the blank samples, their number was subtracted from the sample count to avoid overestimation.

Sample processing

In order to digest the organic matter, a modified protocol among the most used was applied[1]. The modified protocol was previously assessed in a subgroup of 30 mixed oysters (Subadults and Adults). The organic digestion efficiency was calculated[64] and was found to be 96.73 ± 2.3%.

Table 1 | Summary of MP characteristics in oysters across different sampling areas and conditions

Sampling area	Species	Wild/ cultured	Suspended /Bottom	Anthropic impact	Oysters' size	Method	Mesh pore size (mm)	MP Concentration		MP Shape (%)			MP Size class		Ref.
								MPs/ind	MPs/g	Fiber	Secondary MPs	Primary MPs	range	%	
Mali Stone Bay (Adriatic Sea–Mediterranean)	*O. edulis*	Cultured	Suspended	rural, agricultural, and urban, low industrialization	46–73.5 mm	Nile Red	0.45	2.6 ± 1.32	0.84 ± 0.56	49.25	39.3	11.44	50−150 µm	31.34	present study
Gulf of Gaeta (Tyrrhenian Sea–Mediterranean)	*O. edulis*	Cultured	Suspended	rural, agricultural, and urban areas presenting high industrialization	47.8–71.2	Nile Red	1.45	5.88 ± 1.68	1.55 ± 0.81	34.37	44.44	21.19	50−150 µm	35.4	present study
Tampa Bay (Gulf of Mexico)	*C. virginica*	n.r.	Bottom	river discharges, high industrialization, high urbanization, high population density, tourism, fishing	70.4 ± 11.5 mm	Nile Red	5	13.8 ± 17.1	5.2 ± 6.6	5.2	94.8	0	n.r.	n.r.	27
Mississippi Gulf Coast (Gulf of Mexico)	*C. virginica*	n.r.	n.r.	river discharges, industrialization, high urbanization	n.r.	Nile Red and µ-FTIR	30	n.r.	30.7 ± 11.5 to 4.7 ± 0.25	n.r.	n.r.	n.r.	30–90 µm	80	48
Salish Sea (Pacific Ocean)	*C. gigas*	Cultured	Bottom	aquaculture	106.5 ± 13.6 mm	Visual and µ-FTIR-ATR	8	0.22 ± 0.28	0.04 ± 0.06 (dt)	90.5	2.4	7.1	100−499 µm	50	52
Jiaozhou Bay (Yellow Sea)	*C. gigas*	Cultured	n.r.	population density, fishery, tourism	7.71–9.34 cm	Visual and µ-FTIR	0.7	1.2-3.3	0.34–2.95	45	51	4	<500 µm	36	47
Danang Bay (East China Sea)	*C. gigas*	Cultured	Suspended	activity of building and repairing ships, domestic and industrial WWTP input	n.r.	Visual and µ-FTIR	0.7	18.54 ± 10.08	1.88 ± 1.58	73.71	25.84	0.45	<100 µm	77.3	36
Catalonia (Balearic Sea–Mediterranean)	*C. gigas*	Cultured	Both	Ebro river, WWTP input, industrial activities and urban runoff	n.r.	Visual, ATR-FTIR and µ-FTIR	10	22.8 ± 14.4	2.6 ± 1.09	50	50	0	0.02–0.125 mm	n.r.	43
Gujarat (Arabian Sea)	*S. cuccullata*	Wild	Bottom	fishing activities, tourism, industrialization, and urbanization (Rabari	38–100 mm	Visual and ATR-FTIR	20	n.r.	2.72 ± 1.98	84	16	0	1-2 mm	67	21
Todos Santos, Mexico (Pacific Ocean)	*M. gigas*	Cultured	n.r.	population density, port, WWTPs, agricultural runoff, river discharge	>6 cm	µ-FTIR-ATR	n.r.	n.r.	0.07 ± 0.03	92	8	0	<1 mm	65[a]	37
San Quintin, Mexico (Pacific Ocean)	*M. gigas*	Cultured	n.r.	low population density	>6 cm	µ-FTIR-ATR	n.r.	n.r.	0.04 ± 0.02	93	7	0	<1 mm	45[a]	37
Gulf of Sagiada (Ionian Sea–Mediterranean)	*P. imbricata radiata*	Cultured	Suspended	n.r.	commercial	Visual and Raman	0.8	n.r.	3.56 ± 0.35	15.82	80.13	4.05	0.8–1500 µm	n.r.	35
Evoikos Gulf (Aegean Sea–Mediterranean)	*P. imbricata radiata*	Cultured	Suspended	n.r.	commercial	Visual and Raman	0.8	n.r.	2.55 ± 0.83	34.98	50.58	14.44	0.8–1500 µm	n.r.	35
Elounda Bay (Aegean Sea–Mediterranean)	*P. imbricata radiata*	Wild	Bottom	n.r.	commercial	Visual and Raman	0.8	n.r.	3.03 ± 0.54	11.57	86.87	1.56	0.8–1500 µm	n.r.	35

Table 1 (continued) | Summary of MP characteristics in oysters across different sampling areas and conditions

Sampling area	Species	Wild/ cultured	Suspended /Bottom	Anthropic impact	Oysters' size	Method	Mesh pore size (mm)	MP Concentration		MP Shape (%)			MP Size class		Ref.
								MPs/ind	MPs/g	Fiber	Secondary MPs	Primary MPs	range	%	
Rhodes (Aegean Sea–Mediterranean)	*P. imbricata radiata*	Cultured-block	Suspended	n.r.	commercial	Visual and Raman	0.8	n.r.	2.06 ± 0.51	6.46	92.07	1.47	0.8–1500 µm	n.r.	35
Rhodes (Aegean Sea–Mediterranean)	*P. imbricata radiata*	Cultured - baskets	Suspended	n.r.	commercial	Visual and Raman	0.8	n.r.	1.54 ± 0.63	13.01	80.05	6.94	0.8–1500 µm	n.r.	35
Salish Sea (Pacific Ocean)	*C. gigas*	Wild	Bottom	strait, small creeks, population density	n.r.	RMS, ATR-FTIR, and µ-FTIR	5	0.69-3	0.02–0.3	96	4	0	621.93 µm (mean)	n.r.	34
Aughinish Bay (Celtic Sea)	*O. edulis*	Wild	Bottom	shellfish production area	7.2 ± 0.8 cm	Visual and µ-FTIR	1.2	5.6 ± 4.5	0.4 ± 0.4	99	1	0	1000–5000 µm	46	17
Aughinish Bay (Celtic Sea)	*M. gigas*	Cultured	Suspended	shellfish production area	7.8 ± 0.6 cm	Visual and µ-FTIR	1.2	6.4 ± 3.0	0.6 ± 0.3	99	1	0	1000–5000 µm	37.5	17
North Chinese Coast	*C. gigas, C. angulate, C. hongkongensis, C. sikamea*	Cultured	n.r.	low impact	4.45-11.26 cm	Visual and µ-FTIR	1	1.46-4.12	0.14–0.83	60.67	30.22	9.11	<500 µm	38.57	38
South Chinese Coast	*C. gigas, C. angulate, C. hongkongensis, C. sikamea*	Cultured	n.r.	high impact	4.44-10.02	Visual and µ-FTIR	1	1.05-9.08	0.12–2.35	60.67	30.22	9.11	<500 µm	39.57	38
South China Sea	*S. cucullata*	Wild	Bottom	Pear river Estuary	9.26 ± 1.96 cm	Visual and µ-FTIR-ATR	2.7	n.r.	0.14–7.90	51.6	45.1	3.3	101–500 µm	34.47	44
Great Australian Bight	*C. gigas, S. glomerata*	Cultured	n.r.	n.r.	75.2–94.8 mm	µ-FTIR	38 and 1000	0.83 ± 0.08	0.09 ± 0.01	61.8	37.7	0	38 µm–1 mm	59	4
Pearl River Estuary (South China Sea)	*S. cucullata*	Wild	Bottom	Pear river Estuary, urban, rural, fishery, aquaculture, tourisms	n.r.	Visual and µ-FTIR	20	1.4–7.0	1.5–7.2	69.4	20	10.6	<100 µm	75.6-89.7	50
Brittany (Atlantic Ocean)	*C. gigas*	Cultured	n.r.	n.r.	9.0 ± 0.5 cm	µ-Raman	5	n.r.	0.47 ± 0.16	n.r.	n.r.	n.r.	>25 µm	42	53
Taiwan coast (Pacific Ocean)	*C. angulate and S. spp.*	Wild	Bottom	fishery, aquaculture, tourisms	2.61 ± 0.09 cm to 8.93 ± 0.20 cm	Raman	5	n.r.	9.32 ± 12.84	28.55	68.72	2.73	<100 µm	53	54
Taiwan coast (Pacific Ocean)	*C. angulate and S. spp.*	Cultured	n.r.	fishery, aquaculture, tourisms	2.61 ± 0.09 cm to 8.93 ± 0.20 cm	Raman	6	n.r.	1.93 ± 2.11	28.55	68.72	2.73	<100 µm	53	54
Gulf of Manner (Arabic Sea)	*M. bilineata*	Wild	Bottom	fishery, wastewater	2-16 cm	Visual and FTIR-ATR	0.8	6.9 ± 3.84	0.81 ± 0.45	61	39	0	0.25–0.5 mm	44.6	55
St. Catherines Island (Atlantic Ocean)	*C. virginica*	Wild	Bottom	non-urbanized area, no riverine inputs	>7.5 cm	Visual	35	n.r.	7.61 ± 3.26	49.3	50.2	0.5	n.r.	n.r.	51
Masan Bay (East Sea)	*C. gigas*	Wild	Bottom	urban, petrochemical, heavy metal, electrical, and plastic industries	4–9 cm	µFT-IR	20	0.36 ± 0.72	1.2 ± 2.4[a]	10 ± 14	90 ± 14	0	<300 µm	78	56

Table 1 (continued) | Summary of MP characteristics in oysters across different sampling areas and conditions

Sampling area	Species	Wild/ cultured	Suspended /Bottom	Anthropic impact	Oysters' size	Method	Mesh pore size (mm)	MP Concentration		MP Shape (%)			MP Size class		Ref.
								MPs/ind	MPs/g	Fiber	Secondary MPs	Primary MPs	range	%	
Jinhae Bay (East Sea)	*C. gigas*	Cultured	Suspended	aquaculture	4–9 cm	µFT-IR	20	0.67 ± 1.48	0.9 ± 1.6[a]	9 ± 13	91 ± 13	0	<300 µm	78	56
Geoje Islan (East Sea)	*C. gigas*	Wild	Bottom	rural, no industries nor aquaculture farm	4–9 cm	µFT-IR	20	0.11 ± 0.24	1.2 ± 1.5[a]	4 ± 7	96 ± 7	0	<300 µm	78	56
Bizerte Lagoon (Mediterranean Sea)	*C. gigas*	n.r.	Bottom	fishing, aquaculture, urban, highly urbanized	86.83 ± 1.36 mm	FTIR-ATR	1	n.r.	1.48 ± 0.02	90	10	0	0.1–1 mm	88	69
Yantai (Yellow Sea)	*C. gigas*	Cultured	Bottom	fishing industry	11–13 cm	Visual and µ-FTIR	5	n.r.	4.53–7.12; 24.49–35.6 (dt)	n.r.	n.r.	n.r.	<0.2 mm	n.r.	70
Chesapeake Bay (Atlantic Ocean)	*C. virginica*	n.r.	n.r.	urban and suburban	8.9–9.3 cm	Raman	0.45	n.r.	5.6–7	9–12	80–88	2–6	n.r.	n.r.	71

n.r. not reported.

[a]extracted value, dt = dry tissue.

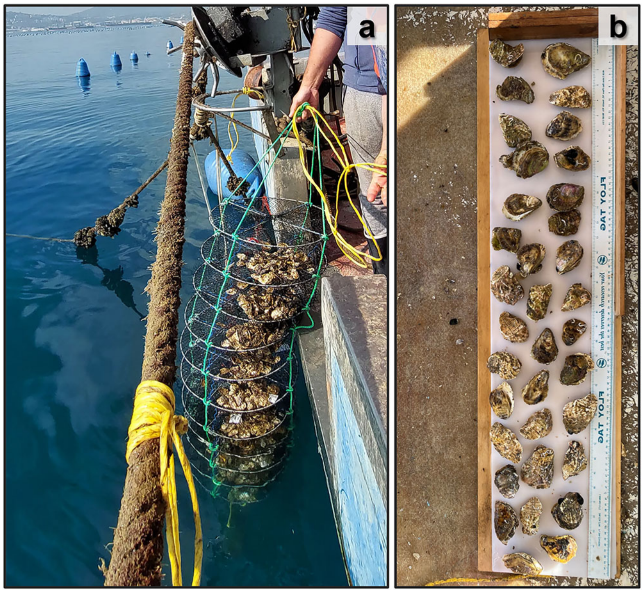


Fig. 5 | Oyster Rearing and Collection. a Oysters aquaculture in rearing lanterns; **b** Oysters collection and biometric acquisition.

The digestion protocol was initiated by placing the soft oyster tissue into a 150 ml glass beaker, to which 10 ml of 10% KOH was added per gram of sample dry weight. The KOH solution was left to react under a fume hood at room temperature (25°C). After 24 h, the KOH solution was diluted up to 2% using distilled water, and the reaction continued for an additional 24 hours. Because of the high inorganic debris amount in the samples, after the organic digestion, the sample underwent density separation phase by adding a zinc chloride ($ZnCl_2$–1.7 kg/L) saturated solution[65]. The $ZnCl_2$ solution was added directly to the glass beaker containing the digested soft tissue until it reached 1 cm from the top edge. The resulting mixture solution was stirred by a sequence of 5 min stirring, and 5 min rest, repeated three times. The solution was left decanting overnight, and when settled, the supernatant was filtered with a Millipore vacuum pump equipped with a Buchner funnel (XX1004724 Millipore; HAWP04700 Millipore 0.45 µm filter). The filters were inserted into petri dishes and placed in an oven at 30 °C and left to dry. To carry out the qualitative and quantitative characterization of the extracted MPs, the Nile Red staining technique was applied (NR, Carlo Erba Reagents Srl). NR is a hydrophobic, meta-chromatic, and photochemically stable dye with a good binding affinity for a wide range of polymers allowing a fast screening of MPs even those smaller than 20 µm[30,66]. Following staining protocol[32], filters to stain were inserted in the vacuum filtration system and a 1 ml of NR (10 µg/mL acetone) was poured and left for 15 min on the sample. Subsequently, the filtration system was activated to remove the excess NR. Finally, 1 ml of acetone was added to clean the surface of the filter from dye residues. This process was repeated twice.

MP identification and characterization

Filters were examined at 112x magnification under the Axiozoom V.16 fluorescent stereomicroscope (Zeiss, Germany) equipped with a 5MP CCD camera, the software ZEN 3.8 DLic and a motorization for automatic fluorescence photo acquisitions. Three different filter sets were used: UV (Filter Set 02, G 365, LP 420 nm), blue (Filter Set 38, EX 470/40 nm, BP 525/50 nm), and green (Filter Set 20, EX 546/12, BP 575–640 nm). Combined use of multiple filter sets allowed the accurate identification of generally less visible MPs[32]. Microscope setting used: intensity 100%, gain: 1.0×; saturation: 1.50; and gamma: 0.60. To identify the items as MPs, these should have high and homogeneous fluorescence intensity and well-defined edges. Moreover, standard identification criteria were applied[67]. Fluorescent MP items were acquired and

Table 2 | Mean weight of soft tissue (wet weight) and mean shell length of Subadults and Adults specimens from the Adriatic and Tyrrhenian productive areas

Adriatic specimens		
	Subadults	**Adults**
Mean weight of soft tissue (g)	1.95 ± 0.41	6.48 ± 1.12
Mean shell length (mm)	46.0 ± 0.88	73.5 ± 0.66
Tyrrhenian specimens		
	Subadults	**Adults**
Mean weight of soft tissue (g)	2.94 ± 0.53	5.78 ± 1.07
Mean shell length (mm)	47.8 ± 0.43	71.2 ± 0.13

analyzed using the software Image J (Fiji, version 1.53t); for each item, the Feret's diameter was recorded. These parameters were used to characterize MPs in terms of shape (spheres, fibers, foam, films, and fragments) and size class compositions.

Statistical analysis

Results regarding MP concentration in oysters are shown as mean ± standard deviation both in terms of item/individual and items/g (grams of wet weight of soft tissue). The MP results obtained were compared first to verify the differences between oysters coming from two productive areas and then, more specifically, between subadult and adult pools of the same area. Prior to conducting the full statistical analysis, data were tested for normality and homogeneity of the variance with the Shapiro-Wilk and Levene's tests. Since data were not normally distributed, the Mann-Whitney U test was utilized to compare MP concentrations between productive areas. Moreover, for investigating differences among the oysters' life stages in the two areas, the Kruskal-Wallis (KS) test was applied, followed by pairwise Dunn's post hoc test for multiple comparisons with the Bonferroni correction. Furthermore, a one-way PERMANOVA with fixed factors and 9999 permutations was conducted to test differences in MP compositions in terms of shape and size classes between the groups. The significance level was set at 0.05, and all tests were performed on PAST software (version 4.13[68]).

Data availability

The data that support the findings of this study are available from the corresponding author, A.R., upon reasonable request.

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Author contributions

G.P. managed and curated the data, performed formal analysis, conducted research, developed the methodology, and wrote the original draft of the paper. A.R. was responsible for the conceptualization, acquisition of funding, project administration, provision of resources, supervision, and review and editing of the manuscript.

Competing interests

The authors declare no competing interests.

Additional information

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