



Microplastics levels in cultured or harvested mollusks non-depurated and commercially depurated at different times

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ABSTRACT

Microplastics (MPs) are emerging pollutants found worldwide, not only in environmental matrices but also in the food web. The present study aimed to establish better removal rates of MPs in cultivated or harvested edible bivalves currently on the market. Samples of three species (mussels, oysters and wedge clams) were collected from a producer at three different depuration times. The most abundant (>90 %) detected morphology corresponded to fibers. Standard depuration rates were 50 %, 26 % and 26 % reduction of MPs in mussels, oysters and wedge clams, respectively. In turn, extending the depuration treatment did not significantly improve the depuration rate. The total ingestion of MPs through the consumption of these species was estimated for the adult population in a range between 2508 and 4692 items, depending on the depuration stage. This means a yearly mean accumulated consumption of 4.5, 2.4, and 2.7 m of fibers for non-depurated, standard and extended depurated mollusks, respectively.

1. Introduction

In recent years, the widespread presence of microplastics (MPs) in the marine environment has raised considerable alarm due to its environmental and human health implications (Tang et al. 2024; Zhou et al. 2024; Auguet et al. 2022). MPs are defined as synthetic or semisynthetic polymeric particles <5 mm in diameter and not soluble in water (UNEP 2015). They are globally released into the marine environment from various sources, such as the fragmentation of larger plastic items, microbeads in personal care products, and synthetic fibers from textiles, among others (Arif et al. 2024). Discharges from wastewater treatment plants are reported to be one of the most important sources of MPs, especially fibers, into the marine environment (Dronjak et al. 2023). Once introduced, these MPs are easily transported, persist for a long time and can be accumulated in marine organisms (Sfriso et al. 2024; Kibria 2023).

The accumulation of MPs in mollusks has attracted particular attention among the scientific community and several regulatory organizations (EFSA, 2016). Mollusks' filter-feeding behavior makes them

vulnerable to the ingestion of environmental pollutants including MPs (Zhang et al. 2020; Expósito et al. 2022). First, the physical presence of MPs can obstruct the digestive tract of bivalves; resulting in reduced feeding efficiency, decrease nutrient uptake and, ultimately, decreased growth and reproductive success of these organisms (Zhang et al. 2020). Secondly, MPs may contain additives, adsorb and/or concentrate toxic pollutants from seawater, which can be transferred to higher trophic levels through consumption of contaminated mollusks (O'Donovan et al. 2018, 2020). Mollusks are consumed whole, so consumers ingest not only MPs but also additives and other pollutants accumulated. The ingestion of MPs through the consumption of mollusks poses several challenges. In that way, MPs are suggested to promote oxidative stress, DNA damages, metabolic disorders, immune response as well as neurotoxicity, reprotoxicity and embryotoxicity (Li et al. 2023). Thus, MPs contamination in mollusks not only threatens marine biodiversity, but also it raises concerns about food safety and human health issues (Unuofin and Igwaran 2023; De-la-Torre 2020).

Minimizing MP levels in mollusks requires a multifaceted approach that addresses both the MPs sources and their pathways into marine

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environments. Strategies to mitigate MPs pollution include regulatory measures to ban or restrict the use of MPs in consumer products, promote the development and adoption of eco-friendly alternatives, including in the textile industry, and implement improved practices of waste management to reduce plastic leakage in the marine environment (Calero et al. 2021; Munhoz et al. 2023). Additionally, targeted efforts to clean up existing MP hotspots and restore degraded habitats can help to reduce the environmental burdens of MPs (Xu et al. 2022).

In order to eliminate microorganisms, marine toxins and sand, the mollusks undergo depuration by submerging them in controlled, disinfected, and filtered seawater for a certain period of time (EU 2019/627; EC 853/2004; ACSA, 2022). Although this process is not designed to specifically eliminate MPs, it may reduce the MPs load of these organisms. At this moment, improving the mollusks MPs depuration through increasing depuration time is the main short-term solution to this food safety problem, if the above-mentioned regulation requirements are fulfilled. In this context, the present study aimed to establish the MPs levels in cultivated (mussels and oysters) or harvested (wedge clam) mollusks in one of the production areas in the NW Mediterranean Sea. In addition, this work investigated the removal efficiency of the current commercial (standard) depuration process, and how the increased depuration time affects this MPs removal rate. To the best of our knowledge, the present study is the first to explore the depuration rate of some of the most consumed mollusks (mussels, oysters and wedge clams) in a real and commercial depuration facility and assess the influence of modifying the depuration time.

2. Material and methods

2.1. Samples and sampling description

MPs were analyzed in three different edible commercial species of bivalves: mussels (*Mytilus galloprovincialis*), oysters (*Crassostrea gigas*) and wedge clams (*Donax trunculus*). These species were cultivated (mussels and oysters) or harvested (wedge clams) in Ebro Delta, Catalonia (NE Spain) and characteristics of the bivalve samples are given in Table 1. Each sample of the three analyzed species was directly taken directly from one producer, from the same racks, zone, or batch, at three different depuration times (non-depurated, standard depuration (24 h for mussels, 2 h for wedge clams and 72 h for oysters) and extended depuration (48 h for mussels, 5 h for wedge clams and 96 h for oysters). The depuration process was performed in the same way by the mollusk producers that distribute them to markets and restaurants, in accordance to the current legislation (EC 853/2004). In producer facilities, marine bivalves were submerged in sand-filtered, UV-disinfected seawater in small scale tanks at controlled salinity (30–36 ‰), pH (7.0–8.4), oxygenation (>5 mg O₂/L), temperature (14–18 °C), and water circulation (>20 L/min). Triplicate samples were analyzed for each organism (mussel, oyster and wedge clam) and depuration times, so a total amount of 27 samples were characterized. For a better

representativeness of the results the highest number possible of individuals per organism was analyzed. For mussels and wedge clams, each sample consisted of approximately 50 g, the maximum amount of organic matter that digestion process is able to eliminate (Expósito et al., 2022). Each mussel sample consisted of 20 individuals (approximately 50 g of soft tissue) or 100 wedge clams (approximately 50 g of soft tissue). However, due to their heavier individual weight (20.5 g/individual) approximately 144 g of soft tissue, 7 oysters, were analyzed. Oysters have a higher water content than mussels or wedge clams and could be also digested with the same procedure used for the others bivalves (Expósito et al., 2022). The difference in the number of organisms that constitute each sample was determined by the individual fresh weight (2.7, 20.5 and 0.5 g for mussels, oysters and wedge clams, respectively).

After depuration, bivalve samples were wrapped in aluminum foil and moved to the laboratory in cooled boxes. Once in the laboratory, samples were rinsed with ultrapure water (distilled water filtered through a 0.45 µm sterilized porous nitrocellulose membrane), external byssus threads and shells were removed, and finally the flesh wrapped in aluminum foil and stored at –20 °C until analysis. Sampling materials were metal or glass and were cleaned away from the sample with distilled water, ultrapure water and/or ethanol to avoid exogenous contamination.

2.2. Microplastics isolation and determination

Analytical determination and characterization of MPs were previously described by Expósito et al. (2022) (Fig. A1 Supplementary materials). Briefly, the isolation stage is fundamental to separate MPs from other flesh components, thus facilitating the identification of their polymeric composition. Firstly, a digestion was conducted to eliminate organic material. This involved a combination of alkaline hydrolysis with sodium hydroxide (KOH, 2 M) for 2 days at 40 °C and surfactants (SDS 10 %) for 2 days at 40 °C with gentle and intermittent agitation. Secondly, a sequential enzymatic hydrolysis with proteases, lipases and cellulases was carried out (adapted from Löder et al. 2017), followed by oxidation with hydrogen peroxide (H₂O₂, 33–35 %) at 40 °C and gentle and intermittent agitation. Finally, enzymatic hydrolysis with chitinase was applied at 40 °C with gentle and intermittent agitation. Once all organic matter was digested, inorganic material (mainly sand) and MPs were separated by density using a saturated solution of ZnCl₂ (1.8 g/mL). More information can be found as Supplementary Material.

Once isolated, MPs were described and identified using both a stereoscopic camera (LEICA® MZ10 coupled to FLEXACAM® C1, 8×-80× magnification, maximum resolution 1.33 µm) and an optical microscope (Olympus® CX41). MPs were classified based on morphology as fibers, films (particles with a two-dimensional shape) and fragments (particles with a three-dimensional shape). Particles of MPs were measured by its longest axis, the largest and medium particles were placed on a clean PTFE filter, and the smallest particles (<0.5 mm) were placed in a 1 cm² square on a calcium fluoride (CaF₂) slide. Photos of the CaF₂ slide and

Table 1
Characteristics of the bivalve samples collected for analysis.

Species	Depuration time (h)	Replicates analyzed	Organisms /sample	Weight (g)* /sample	Weight (g) /individual	Total organisms analyzed
Mussel (<i>Mytilus galloprovincialis</i>)	0 h	3	20	51.4	2.6	60
	24 h	3	20	53.9	2.8	60
	48 h	3	20	52.7	2.6	60
Oyster (<i>Crassostrea gigas</i>)	0 h	3	7	126.2	18.0	21
	48 h	3	7	148.5	21.2	21
	72 h	3	7	156.6	22.4	21
Wedge clam (<i>Donax trunculus</i>)	0 h	3	100	48.1	0.48	300
	2 h	3	100	53.9	0.54	300
	5 h	3	100	49.0	0.49	300
TOTAL		27				1143

Weight per sample and weight per individual of the same specie did not present significant ($p < 0.05$) differences between depuration time.

* Average weight of 3 replicates.

filter were taken, while particle lengths were measured using ImageJ software (size measure calibrate per magnification with a micrometric slide, 1 mm, 1div = 0.01 mm).

Polymer composition analysis was performed on all photographed and measured particles. On one hand, particles larger than 0.5 mm were analyzed using infrared spectroscopy techniques on a Perkin Elmer Frontier instrument with Spectrum software and an Attenuated Total Reflectance (ATR) accessory. The instrument is equipped with a DTGS detector, a Glowbar source and a CsI beam splitter. The analyzed spectral range was between 4000 and 230 cm^{-1} with 4 cm^{-1} accumulations and 4 cm^{-1} spectral resolution. Backgrounding was performed every 6 samples prior to analysis. On the other hand, identification of particles smaller than 0.5 mm on a CaF_2 slide was performed using a Thermo Nicolet® iN10 MX μ -FTIR microscope with Omnic Picta® software. The instrument is equipped with an MCT array imaging detector in transmission mode. The linear array detector measures two lines of 8 pixels (16 pixels at a time). Each pixel has an aperture of $25 \times 25 \mu\text{m}$. IR spectra were recorded with 4 accumulations and 4 cm^{-1} spectral resolution. The spectral range was measured from 4000 to 715 cm^{-1} .

Unknown spectra were compared with OMIC® software libraries database. It includes HR Nicolet® Sampler Library, Hummel Polymer Sample Library, Polymer Laminate Films, Wizard® Library, and an own library with >80 IR spectra (with environmental weathered particles and commercial plastics). Only spectra with greater than or equal to 75 % similarity to reference spectra were accepted. The rejected items were classified as the temporary unidentified category. The unidentified spectra were also identified comparing with BIO-RAD® IR from University of Barcelona (Barcelona, Spain) spectral databases. The spectra with similarity to reference spectra greater than or equal to 75 % were accepted.

2.3. Quality assurance

To avoid external contamination of the sample, laminar flow hoods were used throughout the process and every few samples ($n = 3$ or 4), a blank sample was analyzed (Expósito et al., 2022). A total of 8 procedural blanks and between 14 and 20 fibers were analyzed; in controls, between 0 and 5 fragments and between 0 and 2 films were detected. From each batch, MPs detected in blanks were subtracted from the total count of MPs found in the analyzed samples taking into account morphology and color.

To ensure the effectiveness of the methodology in extracting MPs from samples, recovery rates were calculated in parallel with sample analysis (Expósito et al., 2022). Approximately 50 g subsamples of mollusk soft tissue were dissected for application. The tissues were carefully cut and inoculated for the different parts with colored PE mixture spheres ranging from 53 to 500 μm . Recovery rates were 60 % for 53–63 μm , 60 % for 125–150 μm , 74 % for 250–300 μm , and 99 % for 425–500 μm . No damage due to agitation (mechanical forces), or chemical attacks on the spheres, was found indicating that sample treatment did not change the MPs present in the samples.

All materials used during the process were glass or metal, and all reagents (described in Supplementary Materials) were filtered (through 5 μm PTFE filters) (Expósito et al., 2022). All glassware, sieves and fine tools such as scalpels and stainless-steel tweezers were washed with ultrapure water and 70 % alcohol after washing. In addition, during the process, the samples and glassware were covered protecting them from the deposition of airborne MPs with aluminum or glass covers (Expósito et al., 2022).

Furthermore, due to potential biases that can occur in visual analyzes during MP identification, the samples were counted and checked by two different analysts. A blind test was performed, as the analysts did not know the type of bivalve or depuration time and samples were coded in a non-correlative way.

2.4. Data analysis

Data analysis was performed using IBM® SPSS software v.29. Firstly, Levene's test was applied to establish the homogeneity of variances (homoscedasticity). Subsequently, either ANOVA or the Kruskal-Wallis test was applied to calculate significant differences, for groups that present homogeneous variances or not, respectively. Differences were considered statistically significant for probabilities <0.05 ($p < 0.05$).

3. Results and discussion

Fig. 1 summarizes MPs numbers and their morphology by organism and weight in bivalves analyzed at three depuration times (Table A1 and A2 Supplementary Materials). In general, depuration time reduces the MPs content in all three organisms, at least from non-depurated (0 h) to standard depuration. This reduction was statistically significant for mussels individually (from 8.76 to 4.38 MPs/individual; $p = 0.003$) and by wet weight (from 2.87 to 1.34 MPs/g; $p = 0.003$), and for oysters by wet weight (from 0.62 to 0.39 MPs/g; $p = 0.023$). By contrast, no significant differences were registered between the standard and extended depuration times regarding levels considered individually ($p = 0.746$) or by wet weight ($p = 0.246$). Similarly, no significant differences in the reduction of MPs levels in wedge clams were noted regarding depuration time based on individual ($p = 0.578$) or on fresh weight ($p = 0.427$). Depuration reduction rates were established at 50 %, 26 % and 26 % in mussels, oysters and wedge clams, respectively, in the standard depuration treatment and referenced by individual. However, extending the depuration period does not significantly (p -value from 0.180 to 0.988) improve the depuration rate. The overall depuration rates from non-depurated to extended depuration were set at 46 %, 43 % and 32 %, for mussels, oysters and wedge clams, respectively.

Comparing the total levels of MPs, mostly fibers, among non-depurated mollusks analyzed showed that wedge clams (1.49 MPs/individual) presented significantly lower levels than mussels (8.76 MPs/individual; $p = 0.001$) and oysters (11.1 MPs/individual; $p < 0.001$). However, taking into account total MPs levels by weight, oysters (0.62 MPs/g) showed significant ($p = 0.003$) lower levels than wedge clams (3.09 MPs/g) and mussels (2.87 MPs/g). Similar significant differences ($p < 0.05$) between organisms were detected in standard depurated organism MPs levels, reported by individual and in extended depuration MPs levels, reported by weight (Table A2 Supplementary Materials). In contrast, regarding extended depuration MPs levels by individual and standard depuration MPs levels by weight reported, only between mussels and wedge clams ($p = 0.016$) and between oysters and wedge clams ($p = 0.006$), respectively, presented significant MPs levels differences between organisms (Table A2 Supplementary Materials).

As previously reported (Expósito et al., 2022), fibers were the most abundant MP morphology, with a contribution of 94, 98 and 99 % of the total MP content in non-depurated mussels, oysters and wedge clams, respectively. Similar fiber contributions to the total MPs content were observed through depuration times; with 94 %, 95 % and 96 % of fiber contribution in mussels, oysters and wedge clams, respectively, for standard depuration; and with 92 %, 96 % and 99 % of fiber contribution in mussels, oysters and wedge clams, respectively, for extended depuration. Similar results were obtained in other studies showing that fibers are the main MPs found in these bivalves (Zhao et al. 2024; Can Tunçelli and Erkan, 2024; Abelouah et al. 2023). In contrast, in other studies conducted in South Korea, Vietnam and Italy respectively (Jeong et al. 2023; Do et al., 2022; Quaglia et al. 2023), fibers were not the main morphology of MPs found in bivalves. However, it should be highlighted that our MPs composition profile is the same as that found in surface waters and sediments along the Catalonia (Spain) coast, close to where the bivalves of the present study were collected (Expósito et al. 2021).

Table 2 summarizes the levels and characteristics (size, morphology and composition) of MPs in mussels, oysters and wedge clams from various studies conducted in different countries between 2022 and

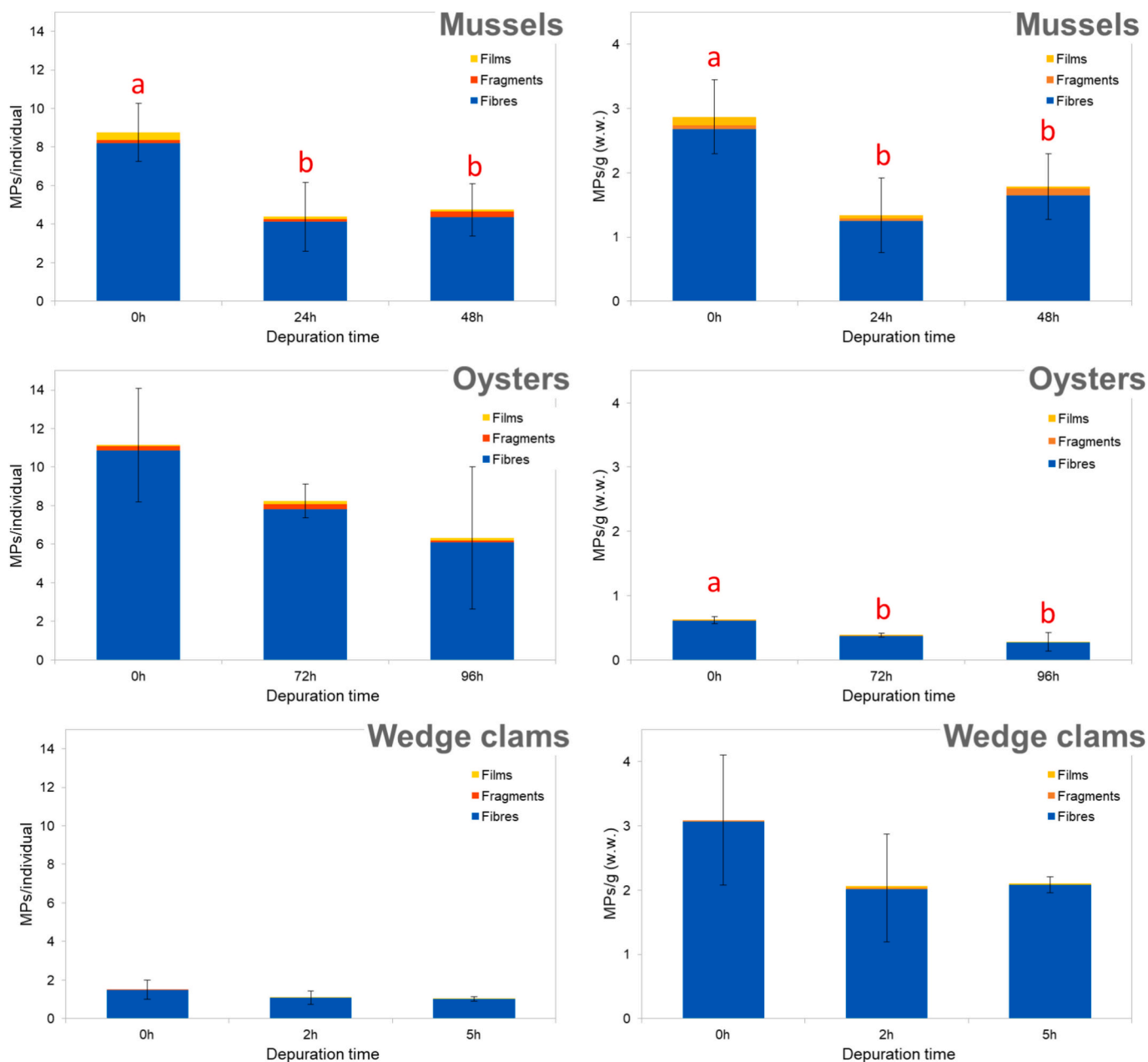


Fig. 1. Mean number (\pm Standard Deviation) of microplastics (MPs) by individual and by wet weight (w.w.) in mussels, oysters and wedge clams as a function of depuration time and their morphology (films, fragments, and fibres). Depuration time that do not share a common letter are significantly ($p < 0.05$) different.

2024. None of these studies reported on the depuration status of the different species, except for the study conducted on the Mediterranean coast of Spain (Expósito et al. 2022).

It should be noted the comparison of MPs levels among different studies is rather complicated because of the lack of standard methods; therefore, differences in analyzed parameters, such as MPs size or materials, may lead to a potential analytical bias. However, the MP levels in the mussels of the present study were higher than those observed in other recent investigations (Table 2), except for those published by our research group (Expósito et al. 2022), with levels around 11 MPs/individual, regardless of their depuration status. Contrastingly, similar results were obtained for oysters when comparing the current results with other studies, excepting our previous study (Expósito et al. 2022). Very limited data on wedge clams are currently available in the scientific literature.

Several studies have explored the effect of depuration on MPs

concentration in bivalves, confirming that there is a pronounced reduction of MPs after the depuration process. Graham et al. (2019) found that the Pacific oysters (*C. gigas*) had released approximately 85 % of the absorbed polystyrene (PS) microspheres after 72 h of depuration. (Choi et al.2022) observed that 65 % of PS fragments previous ingested were egested after 48 h of depuration period by Pacific oysters (*C. gigas*), however 35 % of PS fragments remain inside their body after 7 days (168 h) of depuration. The results indicate a higher rate of MPs removal, which is consistent with our observations that during the early stages of depuration, removal rates were significantly increased. However, a portion of the MPs found in oysters persists even after prolonged periods. It is important to note that the studies referenced utilized MPs fragments or microspheres rather than fibers, which are the predominant form identified in the current research. For instance, Lins et al. (2024) exposed oysters (*C. gasar*) to polyester microfibers for 24 h and noted nearly complete removal after 48 h of depuration. This outcome

Table 2
Numbers and characteristics of MPs in mussels, wedge clams, and oysters in others studies.

MPs/ organism	MPs/g	% Fibers	Size (mean or range)	Composition	Country	Ref.
<i>Mussel (Mytilus galloprovincialis)</i>						
nd: 11.8 ± 7.33 d: 11.3 ± 9.05	nd: 3.50 ± 2.17 d: 4.78 ± 3.82	50–92	20 µm–1 mm (60–85 %)	PE 54 %, PES 17 % and RY 12 %	Spain	Expósito et al. 2022
2.5 ± 0.3–6.6 ± 0.62	0.6 ± 0.07–1.9 ± 0.18	37.0	5–500 µm (69 %)	PA 33.3 %, NY 8.33 % and PU 8.33 %	Italy	Quaglia et al. 2023
0.542 ± 1.028	0.217 ± 0.441	72	1316 ± 1063 µm	CMFs 35 % > PAN = PP 14 % > PS 10 % > PA = PES 8 % > LDPE = polycloprene 6 %	Portugal	Ferreira et al. 2023
2.52 ± 1.1	NR	63.7	<0.1 mm (5,6 %); 0.1–0.5 mm (26.2 %); 0.5–1.0 mm (18 %) and 1.0–5.0 mm (49.8 %)	PE 29.2 %, PP 21.5 %, PET 21.3 %, PA 13.9 %, PVC 5.9 %	Montenegro	Bošković et al. 2023
NR	Morocco:1.27 ± 0.42 Tunisia 0.90 ± 0.51	75	402–4860 µm 33–3113 µm	Morocco: PET 41 %,PP 32 %,PVC 15 %, PA 11 %, PS 0.5 % and CP 0.5 % Tunisia: PP 40 %, PET 37 %, PE 20 % and PS 3 %	Morocco and Tunisia	Abelouah et al. 2023
1.24 ± 0.56	0.36 ± 0.14	18	41–1691 µm	Acrlate 20 %, PP 18 %,PE 9 %, PET 8 %	South Korea	Jeong et al. 2023
2.28 ± 0.67	1.33 ± 0.86	79.8	0.1–0.5 mm	EPDM = EPR > PS=PMP=PA 6 > PET.	Turkey	Can Tunçelli and Erkan 2024
3.30 ± 1.96	1.09 ± 0.76	98.8	1114.35 ± 951.62 µm	RY 58.3 %, PET 27.3 %, PA 6.1 %, PE 2.5 %, PP 2.2 %, PAM 1.6 %, PMMA 1.2 % and PVC 0.8 %	China	Zhao et al. 2024
8.76; 4.38; 4.75	2.87; 1.34; 1.78	94; 94; 92	Mean: 957; 986; 912 µm	PES; Cel; PA, PE, PU; PP; others	Spain	Present study
<i>Oyster (Crassostrea gigas)</i>						
18.54 ± 10.08 nd: 32.1 ± 13.4 d: 15.5 ± 11.3	1.88 ± 1.58 nd: 2.60 ± 1.09 d: 1.58 ± 1.16	26 50–92	22.4–1318.8 µm 20 µm–1 mm (60–85 %)	NY PE 54 %, PES 17 % and RY 12 %	Vietnam Spain	Do et al. 2022 Expósito et al. 2022
8.2 ± 0.45	1.24 ± 0.26	100	300–1000 µm 54.6 % - 63.0 % 1000–2000 µm 22.3 %–33.3 %	PP 52 %, PE 33.1 %, PS 10.2 %, PES 3.9 % and PVC 0.79 %.	Vietnam	Doan et al. 2023
12 ± 2.65–18.8 ± 2.6	0.41 ± 0.12–0.71 ± 0.16	28.2	5–500 µm 70.74 %	PP 50 %	Italy	Quaglia et al. 2023
6.4 ± 3.0; 1.7 ± 2.1	0.6 ± 0.36; 0.2 ± 0.2	99	27.8–16,523 µm	47 % natural fibers; 20.9 % NY	Ireland	Paul et al. 2023
11.1; 8.24; 6.34	0.62; 0.39; 0.28	98; 94; 96	Mean: 1040; 999; 1375 µm	PES; Cel; PA, PE, PS; PP; others	Spain	Present study
<i>Wedge clam (Donax trunculus)</i>						
nd: 0.52 ± 0.22 d: 0.67 ± 0.31	nd:1.99 ± 0.82 d: 2.56 ± 1.18	50–92	20 µm–1 mm (60–85 %)	PE 54 %, PES 17 % and RY 12 %	Spain	Expósito et al. 2022
0.56 ± 0.1–0.0	NR	100	1–0.1 mm (42.8 %)	PET 60 %; PVC (20 %); NY (20 %)	Italy	Olivieri et al. 2022
NR	0.23 ± 0.17	52.9	0.1–0.5 mm (56 %)	PE (60 %) PET (30 %)	Italy	Malloggi et al. 2024
1.49; 1.11; 1.02	3.09; 2.06; 2.10	99; 97; 99	Mean: 1137; 1003; 1069 µm	PES; Cel; PA, PE, PP; PVC; PMMA: others	Spain	Present study

RY: Rayon, PET: Polyethylene terephthalate, PA: Polyamide, PE: Polyethylene, PP: Polypropylene, PAM: Polyacrylamide, PMMA: Polymethyl methacrylate, PVC: Polyvinyl chloride, EPDM: Ethylene Propylene Diene Monomer, EPR: Ethylene Propylene Rubber, PS: Polystyrene, PMP: Polymethylpentene, CMFs: cellulose microfibrils, PAN: polyacrylonitrile, PES: polyester, LDPE: low-density polyethylene LDPE, CP: Cellophane, NY: nylon, nd: non-depurated and d: depurated. NR: Not reported.

contrasts with our findings and may be due to the short exposure duration in Lins et al.'s study, as opposed to the lower concentrations and longer exposure times, across organism growth, that oysters undergo in the present investigation. Further research under realistic conditions is necessary to better understand these variables. Similarly, Paul et al. (2023) studied oysters (*Magallana gigas*, formerly *C. gigas*) cultivated in a natural environment and subsequently depurated them in laboratory settings. They reported a predominance of fibers (99 %) and a depuration rate of 77 % after 96 h. These results, despite not being conducted in actual commercial facilities, are more aligned with those observed in the current study. In mussels (*M. galloprovincialis*), Pizzurro

et al. (2024) and (Fernandez and Albentosa 2019) documented reductions in MPs of 80 % and 85 % after 7 and 6 days of depuration, respectively. Additionally, (Heo et al. 2022) reported an elimination rate of 95 % after 2 h and 7 days for MPs with diameters of 10 µm and 90 µm, respectively. These findings emphasize the efficacy of depuration as a method to reduce MP load in bivalves, although the specific conditions that enhance effectiveness and the varying responses related to MP morphology and size require further investigation. Furthermore, it is essential to conduct relevant studies that utilize more realistic mixtures of microplastics and simulate how these MPs have been integrated, particularly focusing on low-level exposures throughout the

development of organisms.

Fig. 2 depicts the polymeric composition of fibers and particles (the sum of fragments and films) present in the three organisms analyzed at different depuration times. Regarding fibers, similar results were found in the three organisms with only three polymers (polyester (PES), synthetic cellulose and polyamide (PA)), counting for >90 % of total MPs detected. These are the main polymers used by the textile industry. Other fiber polymers detected were polypropylene (PP), acrylic and polyethylene (PE). No trend was detected in fiber polymeric composition through the depuration process in any of the organisms studied. Most of these fibers reach the coastal marine environment, where these mollusks are cultivated or harvested, through urban-agricultural run-off and wastewater treatment plants (WWTPs) that cannot completely

remove this MPs type in effluents (Dronjak et al. 2023).

Regarding the MPs particles (fragments plus films), a large variety of polymers, and with more variability among the depuration times or bivalve species, was noted. On one hand, this variety was assumed to be linked with the huge heterogeneity of polymeric materials that can be fragmented and end up into the marine environment. The most frequent polymers found were polyethylene terephthalate (PET), PP, PE, polymethylmethacrylate (PMMA), polytetrafluoroethylene (PTFE), polyisobutylene (PIB), and PS. On the other hand, the variability between depuration times could be due to the small number of particles found compared to MPs fibers. Because of this, the detection of few numbers of particles can change the proportions of polymers, and decrease in the others, in a significant way.

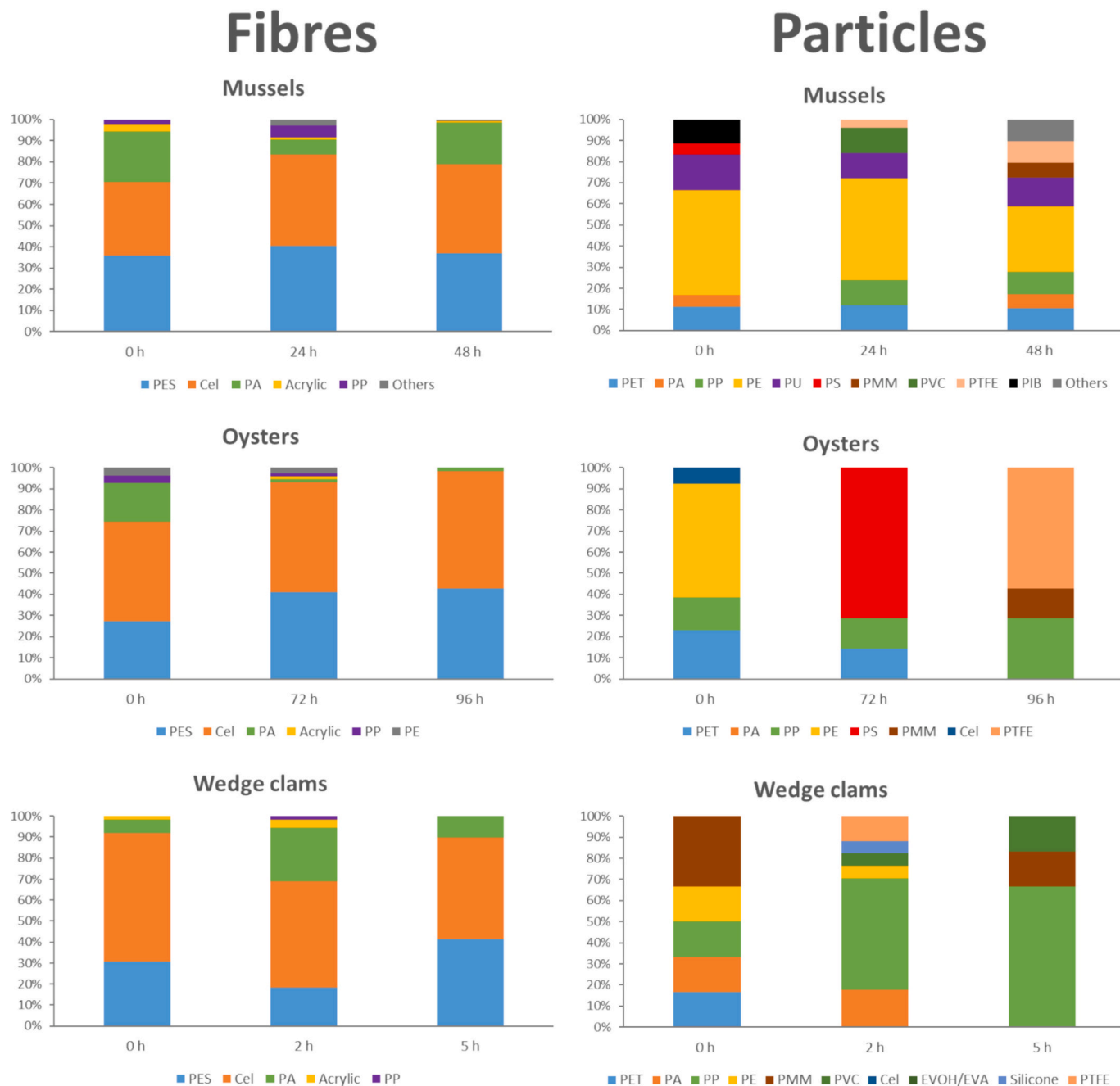


Fig. 2. Polymeric composition of microplastics (MPs) fibers at left side, and particles (fragments plus films), at right side, in mussels, oysters and wedge clams at three depuration treatment times. PES: Polyester; Cel: synthetic cellulose; PA: polyamide; PP: polypropylene; PET: polyethylene terephthalate; PE: polyethylene; PU: polyurethane; PS: polyethylene; PMMA: polymethylmethacrylate; PTFE: polyethylene tetrafluoride; EVOH/EVA: Poly(ethylene vinyl alcohol/acetate); and PIB: polyisobutylene.

The size of MPs (fibers, films and fragments together, but taking into account that fibers mean >90 % of the total MPs) reached a mean value of 950, 1050 and 1100 μm in mussels, wedge clams and oysters, respectively (median of 750, 910 and 890 μm, respectively) (Table 3, Fig. A2 Supplementary Materials). Fibers' diameter mean value was 18.3 ± 6.90 μm with a range from 5.75 to 60.1 μm. No significant differences (p = 0.990) in particle size were detected based on the organism or the duration of depuration. Although no clear trend was identified concerning depuration time overall, oysters exhibited a tendency for larger particle sizes during different depuration statuses, suggesting a propensity to eliminate smaller particles. However, additional research is required to validate this observation. In addition, wedge clams probably due to their habitat, burrowing lifestyle, and size, incorporate MPs with maximum sizes around 1000 μm smaller than those found in oysters and mussels. In contrast, oysters had the largest maximum MPs sizes ranging 4400 to 5400 μm.

Table 4 shows the MPs ingestion by the adult population, taking into account the MPs concentrations in mollusks and the most updated Spanish consumption rates of mussels, oysters and clams. According to (EFSA 2024), Spanish adults have a daily intake of 2.89 g/day (95th percentile of 8.95 g/day) for mussels, 1.14 g/day (95th percentile of 2.10 g/day) for clams, and 1.69 g/day (with no reported 95th percentile) for oysters. In addition, the total length of yearly ingestion was obtained by multiplying MPs yearly ingestion, the ratio of fibers regarding total MPs found and mean length of a fiber obtained in the present study (Table 4). Daily and yearly MPs ingestion were estimated at 12.86 (P95: 33.21) MPs/day and 4692 (P95: 12,121) MPs/year for non-depurated mollusks. These values were approximately halved if the mollusks were depurated as usual (24 h, 72 h and 2 h, for mussels, oysters and wedge clams, respectively). Extended depuration (48 h, 96 h and 5 h, for mussels, oysters and wedge clams, respectively) did not result in a decrease of MPs ingestion, although a slightly increase of MPs in mussels was noted compared to standard depuration. The MPs ingestion through the consumption of mussels means around 56–65 % of the total intake. The intake of MPs through oysters and wedge clams accounted for 6–10 % and 27–34 %, respectively, of the total ingestion. MPs are heterogeneous pollutants in terms of morphology, composition, and size. The amount of ingested MPs can be also expressed as total length of fibers, being a useful term when there is a high proportion of fibers (>90 %). The total length of yearly intake of fibers due to the consumption of mussels, oysters and wedge clams was estimated in 4.54 (P95: 11.44), 2.37 (P95: 5.81) and 2.73 (P95: 6.82) m for non-depurated, standard depurated and extended depurated organisms, respectively, with a mean diameter of 18.3 ± 6.90 μm.

Despite all the efforts of the scientific community, it is not currently

Table 3

Size distribution of microplastics (MPs) in μm in mussel, oyster and wedge clam samples depending on depuration time.

DT	Mean ± SD	Minimum	P5	P25	P50	P75	P95	Maximum
<i>Mussel (Mytilus galloprovincialis)</i>								
0 h	957 ± 784	90	194	410	758	1243	2858	4437
24 h	986 ± 787	122	154	417	812	1304	2460	4208
48 h	912 ± 900	75	139	310	664	1141	3039	4799
<i>Oyster (Crassostrea gigas)</i>								
0 h	1040 ± 878	109	201	514	782	1095	2856	5175 ^a
72 h	999 ± 751	101	228	525	829	1243	2502	4495
96 h	1375 ± 1053	115	322	721	1040	1711	3573	5467 ^a
<i>Wedge clam (Donax trunculus)</i>								
0 h	1137 ± 749	194	251	633	947	1426	2886	3673
2 h	1003 ± 666	69	144	597	857	1296	2297	3227
5 h	1069 ± 708	111	239	592	924	1350	2440	3619

SD, P5, P25, P50, P75 and P95: Standard deviation, 5th, 25th, 50th, 75th and 95th percentiles, respectively.

DT: Depuration time. Size in μm.

^a Despite not classifiable as MPs (>5 mm), they are included in the present study.

Table 4

Daily and yearly MPs and the length of the fibers ingested for Spanish adult population that consume mussels, oysters and wedge clams, the consumption being established by (EFSA 2024).

	DT	Daily ingestion (MPs/day)		Yearly ingestion (MPs/year)		Fiber length ingested yearly (m)	
		mean	P95	Mean	P95	mean	P95
Mussels	0 h	8.29	25.68	3027	9373	2.71	8.38
	24 h	3.86	11.95	1409	4362	1.30	4.03
	48 h	5.16	15.97	1882	5829	1.58	4.89
Oysters	0 h	1.04	–	380	–	0.39	–
	72 h	0.66	–	241	–	0.23	–
	96 h	0.47	–	171	–	0.23	–
Wedge clams	0 h	3.52	6.49	1285	2367	1.45	2.67
	2 h	2.35	4.33	859	1582	0.84	1.55
	5 h	2.39	4.41	873	1608	0.92	1.70
Total*	ND	12.86	33.21	4692	12,121	4.54	11.44
	StD	6.87	16.94	2508	6184	2.37	5.81
	ExtD	8.02	20.84	2926	7608	2.73	6.82

DT: Depuration time. P95: 95th percentile. ND: Non-depurated; StD: Standard depuration (24 h, 72 h and 2 h, for mussels, oysters and wedge clams, respectively); ExtD: Extended depuration (48 h, 96 h and 5 h, for mussels, oysters and wedge clams, respectively). *Taking into account mussels, oysters and wedge clams altogether.

possible to perform an accurate assessment of the risk to human health from MPs, as these must be considered as a mixture of pollutants (Arredondo-Navarro et al. 2024). Size, morphology, and polymeric composition with different additives contained, linked or not to polymeric matrix and the environmental pollutants that can be adsorbed or attached to them, play a role in MPs toxicity. This complexity makes the risk assessment process particularly difficult and the setting of a safety intake threshold challenging. In addition, it should be highlighted that >90 % of the MPs present in bivalves here analyzed were fibers coming from textiles, including clothes, single use wipes or home apparel. It is well known that these fabrics and fibers may contain harmful substances, including trace elements and a number of organic compounds, such as polychlorinated biphenyls, bisphenol A and analogues, or azo dyes, identified as precursors of carcinogenic aromatic amines (Herrero et al. 2022, 2023a, 2023b; Souza et al. 2023a, 2023b).

Considering that MPs predominant morphology (>90 %) in the analyzed bivalves consists of fibers made of polyester, polyamide, and synthetic cellulose, it is essential to enhance emission control measures, innovate new materials and designs, and focus legislative efforts on the fibers released during textile washing, from fishing ropes and nets, and single-use wipes (Alex et al. 2024; Mghili et al. 2024).

4. Conclusions

MPs levels decreased when bivalves were subjected to standard depuration (24 h, 72 h and 2 h), individually referenced by 50 %, 26 % and 26 % in mussels, oysters and wedge clams, respectively. The MPs reduction was actually statistically significant in standard-depurated mussels and oysters. In addition, the extended depuration (48 h, 96 h and 5 h) of mussels, oysters and wedge clams did not significantly reduce MPs levels. Therefore, it is evident that a prolonged depuration does not significantly reduce MPs levels in the mollusks here analyzed. The predominant (>90 %) MPs found in the mollusks analyzed were polyester, polyamide and synthetic cellulose fibers. The significant reductions were observed exclusively in this morphology, owing to the elevated number of fibers relative to the other morphologies. Considering the consumption of these species, mussels were the main contributor to the total MPs intake by the adult population, with an estimated yearly exposure of 4692 and 2508 MPs for non-depurated or standard depurated organisms, respectively. Translating these values to the length of total fibers consumed annually, the adult population ingests 4.5 m for the non-depurated organism consumption and approximately 2.6 m for the standard and extended depurated mollusks consumption. It is important to emphasize that MPs were not assessed in the depuration facility, where their presence in the water or suspended and later on deposited from the air could potentially be ingested by mollusks. The present study provides relevant data on the contamination levels of MPs in commercially important bivalve species after depuration processes performed by commercial producers and distributors that provide mollusks to the general population.

CRediT authorship contribution statement

Nora Expósito: Writing – review & editing, Methodology, Investigation. **Andrea Barrientos-Riosalido:** Writing – review & editing, Visualization, Investigation. **Saul Santini:** Writing – review & editing, Methodology, Investigation. **Alessandra Cincinelli:** Writing – review & editing, Supervision, Investigation. **Laura Alcalde:** Writing – review & editing, Supervision, Resources, Conceptualization. **Victoria Castell:** Writing – review & editing, Supervision, Resources, Conceptualization. **Martí Nadal:** Writing – review & editing, Supervision, Resources. **Jordi Sierra:** Writing – review & editing, Supervision, Investigation, Conceptualization. **Joaquim Rovira:** Writing – original draft, Supervision, Resources, Project administration, Methodology, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.marpolbul.2025.117568>.

Data availability

Data will be made available on request.

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