Marine Pollution Bulletin 181 (2022) 113843

Contents lists available at ScienceDirect

Marine Pollution Bulletin

journal homepage: www.elsevier.com/locate/marpolbul

Levels of microplastics and their characteristics in molluscs from North-West Mediterranean Sea: Human intake

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ARTICLE INFO

Keywords:
Plastic pollution
Mussels
Seafood
Human exposure
Catalonia (Spain)

ABSTRACT

Microplastics (MPs) are accessible for organisms with active filter feeding strategies, as are many marine molluscs, which live attached or semi-buried in sediments. In the present study, MPs (from 0.02 to 5 mm) concentration, morphology, and composition were determined in consumed mollusc species of the Catalan coast (NW Mediterranean Sea). Microplastic concentrations, morphologic characteristics and composition were studied according to species, catchment zones and depuration condition. Finally, human intake of MPs through molluscs' consumption was determined. >2300 individuals were analysed, being 1460 MPs extracted and their size, and polymeric composition registered. Big oysters and mussels showed the highest MPs concentration by individual, with levels of 22.8 ± 14.4 and 18.6 ± 23.0 MPs/individual, respectively. Mean annual MPs (≥20 μm) consumption for adult population was estimated in 8103 MPs/year, with a 95th percentile of 19,418 MPs/year. It suggests that the consumption of molluscs is an important route of MPs exposure for the Catalan population.

1. Introduction

Microplastics (MPs) are ubiquitous in all environmental marine compartments (Farady, 2019; Expósito et al., 2021). Due to their small size, they are accessible for organisms with active filter feeding strategies as many marine molluscs, which live attached or semi-buried in sediments (Carbery et al., 2018; Zhao et al., 2018; Xiong et al., 2019). After active water filtration, plastic, as well as natural particles (inorganic and organic), are trapped in the mollusc's gill filaments according to their pore size. When an amount of food load is reached, a pre-ingestion selection is made, with labial palps selecting or rejecting part of total filtered particles. Digestion occurs in digestive glandule or in intestine for smallest or largest particles, respectively (Ward et al., 2019a). Intake of different MPs shapes (fragments, beads, fibres, films) by molluscs have been demonstrated (Wegner et al., 2012; Li et al., 2016a,b). In turn, those contaminants present in water are absorbed in the MPs, which are then transferred to aquatic organisms (Li et al., 2019; Kolandhasamy et al., 2018). On the other hand, according to EFSA (2016), the processing and packaging of seafood can be another source of contamination for MPs.

Bivalves are responsible for a large part of human exposure to MPs because they filter a large volume of seawater, being able to accumulate MPs from the environment (Cho et al., 2019). In relation to human exposure through consumption of aquatic species, it will depend on the part of which is consumed. It has been observed that most MPs are retained in the digestive tract and the gills (Carbery et al., 2018).

Microplastic <150 μm in diameter have the potential to translocate into human tissues, trigger a localized immune response (EFSA, 2016; Wright and Kelly, 2017; Prata et al., 2020). Animal studies and "in vitro" assays in human cells lines have shown possible adverse effects from exposure to nano and microplastics (oxidative stress and structural damages, reproductive endocrine disruption system in fishes, as well as toxicity in gastrointestinal, liver and neuronal system) (Wang et al., 2019a,b; Chang et al., 2020; Prüst et al., 2020). Gene expression alteration and genotoxicity, as well as changes in gut microbiota -with seafood being a major medium of exposure- have been also observed (van Raamsdonk et al., 2020; Wang et al., 2020). Although the knowledge regarding toxicity of MPs in humans is still limited, it has been

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https://doi.org/10.1016/j.marpolbul.2022.113843
Received 5 May 2022; Received in revised form 7 June 2022; Accepted 9 June 2022
Available online 18 June 2022

established that the toxicity is primarily influenced by exposure concentration, particle components, adsorbed contaminants, organs involved, and individual susceptibility (Rahman et al., 2021). For this reason, to date neither safety threshold, nor tolerable daily intake, have been established and the risk to humans from dietary exposure must be still assessed.

On the other hand, in recent years studies on MPs in a wide range of marine molluscs, including commercial and wild bivalves, have been conducted (Li et al., 2015; Vandermeersch et al., 2015; Abidli et al., 2019; Ding et al., 2020; Ding et al., 2021; Rochman et al., 2015; Van Cauwenberghe and Janssen, 2014; Reguera et al., 2019; Ward et al., 2019b). However, only few surveys were representative of wide groups of autochthonous molluscs.

Few studies have evaluated the Estimated Daily Intake (EDI) by autochthonous bivalves and own national surveys about molluscs population consumption. Cho et al. (2019), estimated the annual dietary intake of MPs (MPs/year/person) by Korean population via shellfish consumption based on a national's examination surveys about ingestion rates and microplastics analysis of the top four autochthonous commercial bivalve species. Cox et al. (2019), calculated EDI (MPs/day/person) for seafood (fishes and shellfish) combined with others food items including inhalation, focusing on the American caloric intake in relation to their recommended daily intake and the average of number of microplastic particles in food obtained from others fourteen studies.

Danopoulos et al. (2020), estimated EDI as MPs/person/year combining global consumption estimates (FAO, 2020) with the data from outcomes of the statistical summary of MPs in the global seafood samples and conclude that seafood is a major verified vector for human exposure to MPs. Ferrante et al. (2022), considered EDI (MPs/day/kg body weight) for adults and children of autochthonous mussel *Mytilus galloprovincialis* and fishes separately for microplastics less to 3 um from south coast of Mediterranean Sea based on global consumption estimates.

There are also few studies regarding MPs levels in molluscs from the Mediterranean Sea, and especially from the NW Mediterranean coastal area. Catalonia (NE Spain) is an important producer and consumer of molluscs. For that reason, the current investigation was focused on MPs concentrations estimations of commercially relevant molluscs from the Catalan coast (NW Mediterranean Sea). The present study is a first step for estimating the MPs intakes through seafood consumption in Spain. Our study was aimed at: i) to develop an to optimize a methodology for MPs extraction; ii) to determine the concentration, morphology, and composition of MPs, in various highly consumed molluscs' species; iii) to compare MPs concentration and characteristics between species, catchment zones and depuration condition, and finally iv) to establish MPs intakes through molluscs consumption by the Catalan population.

2. Materials and methods

Since the existing protocols for the determination of MPs in molluscs were not adequate, we developed a new protocol, which was adapted to the requirements of the current study. The present protocol was adapted from those reported by various authors (Li et al., 2015, 2018; Masura et al., 2015; Catarino et al., 2018; Courtene-Jones et al., 2017; Karami et al., 2017; Cho et al., 2019; Fang et al., 2019; Jahan et al., 2019; Xu et al., 2020; Wu et al., 2020). The optimized methodology comprises higher sample weight and individuals' number, whole organism treatment, and the use of enzymes for the digestion of specific substrates.

2.1. Sampling

Six molluscs' species were selected among those most widely and frequently consumed by the Catalan population. Wedge clams, *Donax trunculus* (WC), marine snail, *Bolinus brandaris* (S), razor clams *Ensis siliqua* (RC), fine clams *Tapes decussatus* (FC), big oyster *Crassostrea gigas* (BO) and mussels *Mytilus galloprovincialis* (M) were included in this survey. Molluscs' species were sampled in October–November 2017 from biggest anglers' cooperatives along the Catalan coast (377 km). The Catalan areas selected for the study were the following: Barcelona (Central), Girona (North) and Tarragona (South). Different sea zones of catchment of North West Mediterranean (FAO 37-1) (GENCAT, 2021) and depuration grade in molluscs' samples were considered (Table 1; Fig. 1), (see Supplementary materials for coordinates, environmental MPs concentration and anthropogenic activities associated in each subzones Table A2).

2.2. Samples processing

Between 2 and 4 kg of each sample type of molluscs were purchased and acquired by donation fisherman cooperatives. The samples were wrapped in aluminium foils and kept refrigerated during the transport to the laboratory. Subsequently, samples were rinsed with ultrapure water (distillate water filtrate to 0.45 μm through filters), being external byssus threads removed. For representability, >50 individuals were analysed for each sample of mussels, razor clams, fine clams, wedge clams, and snail according to depuration conditions and catchment zones (Dehaut et al., 2016). In turn, up to 28 individuals were analysed for oysters. A number of soft tissues sub-samples (50–70 g) were prepared for analysis, being these sub-samples lyophilized (Telstar Cryodos-50/80) during a period of 48 h in a clean and fibre free beaker. All subsamples were kept at −20 °C until analysis.

2.3. Microplastics extractions

A general protocol for MPs ≥ 20 μm extraction in 50–70 g subsamples of soft tissues was partially -or completely- applied, depending on molluscs sample characteristics. The removal of organic and inorganic matter from the sample was carried out by three phases and an additional density separation step (Fig. A1, Supplementary materials). The first phase required 4 days for complete procedure, while the second one 8 days, and the third one 4 days, with a total of 16 days. The density separation step took one additional day.

The first phase (**KS**) is a combination of alkaline hydrolysis (KOH 2 M) (**K**) and surfactants (SDS 10 % (**S**) at 40 °C with agitation. The second phase (**E + H**), consisted in enzymatic hydrolysis with protease, lipases, and celluloses (**E**), followed by an oxidation with hydrogen peroxide 33–35 % (**H**), at 40 °C and gently agitation. The third step (**F + Ch**) involved wet peroxide oxidation (Fenton processes, oxidation in presence of a Fe(II) catalyst to digest labile organic matter) (**F**), and enzymatic hydrolysis with Chitinase (**Ch**) at 40 °C and agitation. Finally, in the case of presence of inorganic matter, density separation (**DS**) with $ZnCl_2$ solution (1.8 g/mL) was carried out (for chemical volume and filtration steps details see Supplementary materials Figs. A1 and A2).

Each phase should be applied based on the amount of organic matter removed in the previous phase through decision steps. Once the organic matter removal has been achieved (based on interference grade for easy particles detection and identification), the process stops. The sample is then ready for density separation steps, or for identification, thereby it is not necessary to complete all phases. The application of alkaline, enzymatic hydrolysis and Fenton processes in phases and sequential steps offer an effective MPs extraction and their different morphologies at less time with less airborne fibres contamination, avoiding significant damages on surface of particles, and reducing costs for chemical reagents. The percentage of removal of organic matter obtained with the application of the three phases is up to 98 %.

The chemical reagents and filters were the following: absolute ethanol (Scharlau, >99,9 %, Barcelona-Spain), potassium hydroxide (Sigma-Aldrich, 99,9 %, Madrid-Spain), hydrogen peroxide stabilized (PamReac AppliChem ITW Reagents 33 % w/v Barcelona-Spain), hydrogen peroxide aqueous solution stabilized (Thermo-Scientific Acros 33–35 % w/v, Madrid-Spain), zinc chloride (Acros Organics, >98,0 %, Madrid-Spain), zinc chloride anhydrous (Sigma-Aldrich, ≥98

Table 1
Molluscs samples description.

Code	Name	Specie	Depuration	Catchment zone	Habitat	Weight (g_{ww}/individual)	N° samples (Total individuals)
WCnd(S)	Wedge clams	*Donax trunculus*	No	South	Suspension/filter feeder	0.263	2 (383)
WCd(S)			48 h	South	Infaunal	0.263	2 (387)
WC(N)			NR	North		0.306	2 (373)
WC(C)			NR	Central		0.219	2 (489)
S(S)	Snails	*Bolinus brandaris*	NR	south	Carnivorous, depredator epifaunal	3.53	4 (68)
S(C)			NR	Central		5.42	5 (55)
RCnd(S)	Razor clams	*Ensis siliqua*	No	South	Suspension/filter feeder Infaunal	5.11	6 (59)
FCd(S)	Fine clams	*Tapes decussatus*	<24 h	South	Suspension/filter feeder Infaunal	2.09	3 (74)
BOnd(S)	Big oyster	*Crassostrea gigas*	No	South	Suspension/filter feeder	12.3	4 (19)
BOd(S)			>72 h	South	Epifaunal	9.78	5 (28)
Md(S)	Mussel	*Mytilus galloprovincialis*	48 h	South	Suspension/filter feeder	2.37	4 (88)
Mnd(S)			No	South	Epifaunal	3.37	4 (62)
Mnd(N)			NO	North		2.90	11 (223)

NR: not reported.

Fig. 1. Sampling areas and molluscs sampled in the Catalan coast (Spain). WC: wedge clams, BO: big oyster, S: snail, RC: razor clams, M: mussels, FC: fine clams.

%, Madrid-Spain), SDS (PamReac AppliChem ITW Reagents, 99 %, Barcelona-Spain), iron (II) sulphate heptahydrate (Sigma-Aldrich, >99,0 %, Madrid-Spain), nitric acid (Scharlau, 2 N, Barcelona-Spain), sodium acetate anhydrous (Scharlau, 99 %, Barcelona-Spain), acetic acid (Scharlau, 96 %, Barcelona-Spain), Tris-HCl (Scharlau, 99 %, Barcelona-Spain), PTFE 5, 10 μm pore size filters (Sartorius, Madrid-Spain). The enzymes used were Chitinase 1000 U/mL, Celullase TXL >1000 U/mL, Lipase FE-01 > 18,000 U/mL, Protease A-01 > 1000 U/mL provide by ASA Spezialenzyme GmbH (Wolfenbüttel-Germany).

2.4. Microplastics quantification and identification

All particles suspected of being MPs were totally quantified on PTFE filters of each subsample using both, a stereoscopic (LEICA MZ10 coupled to FLEXACAM C1) camera, and an optical microscope (Olympus CX41). Microplastics were classified according to the morphology as

fibres (including filaments and fishing line), films (particles with a two-dimensional shape), fragments (particles with a three-dimensional shape), and pellets (solid spheres; solids foam spheres). Some particles suspected of being MPs on the PTFE filter were photographed and marked under a stereomicroscope (LEICA MZ10) equipped with a FLEXACAM C1 digital camera.

Microplastics particles were measured by its longest axis and categorized into nine size categories (0.02–0.05 mm; 0.05–0.125 mm; 0.125–0.25 mm; 0.25–0.5 mm; 0.5–1 mm; 1–2 mm; 2–3 mm; 3–4 mm; 4–5 mm). For MPs measurement, the PTFE filters (47 mm) were divided into ten columns (3.5 mm wide). For each column, all particles of each morphology type, colour, thickness, and size were picked varying the magnification from 8 to 80 X. The biggest and medium particles were placed on a clean PTFE filter, and the smaller ones (<0.5 mm) into a square of 1 cm^2 area on calcium fluoride (CaF_2) slide. For smaller MPs and abundant particles ≤50 μm, the original filters were divided in four quadrants. In each section, four fields from 40 to 80× magnification were randomly chosen, being particles of each morphology and sizes measured. The CaF_2 slide and filter were photographed and the particles length was measured with ImageJ software (Schneider et al., 2012).

Polymeric composition analysis was carried out for all photographed and measured particles. Fragments and films between 0.5 and 5 mm for each subsample were grouped and placed on clean PTFE filters. Fibres, fragments and films from 0.02 to 0.5 mm were arranged on calcium fluoride (CaF_2) slides. Particles size larger than 0.5 mm were analysed by infrared spectroscopy technologies with a Perkin Elmer Frontier instrument with a Spectrum Software and an Attenuated Total Reflectance (ATR) accessory. The instrument has a DTGS detector, Glowbar source and CsI beam splitter. The spectral range analysed was 4000 to 230 cm^{-1} with 4 cm^{-1} accumulations and 4 cm^{-1} of spectral resolution. The background was done before analysis every 6 samples. By contrast, the identification particles smaller than 0.5 mm on a CaF_2 slide was carried out with a μ-FTIR microscope Thermo Nicolet iN10 MX, with Omnic Picta Software. The instrument has a MCT array-imaging detector in transmission mode. Linear array detector measures two lines of 8 pixels (16 pixels at a time). Each pixel has an aperture of 25 × 25 microns. IR spectra were recorded with 4 accumulations and 4 cm^{-1} of spectral resolution (see Supplementary material, Fig. A3). The spectral range was measured from 4000 to 715 cm^{-1}.

For the identification of each polymer, the spectra obtained with the ATR-FTIR and μFTIR techniques were performed, being the unknown spectra 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 (see Supplementary materials). Only match spectra with major -or equal- than 75 % of similarity with reference spectra were accepted. The rejected items were counted 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 matches of similarity major or equal than 75 % were accepted.

For white and transparent films and fragments >0.5 mm, PTFE filter, were examined with spectroscope-microscope Raman Renishaw 20×, 50 X objective magnification microscope-, 785 nm edge, laser state 1 %, 10 %, 50 % intensity, bands from 25 cm-1, 1200 l/mm. The spectra polymer obtained with Raman spectroscopic technique was performed with WIRE 5.3 Software, being the spectra corrected for baseline displacement. The Raman spectra polymer was identified comparing the spectra with WIRE 5.3 polymer libraries database. Only match spectra with major -or equal than 75 % of similarity with the reference spectra were accepted. The spectra with similarities below 75 % were compared with a library of own elaboration taking into account the main plastics products on the market. Only the spectra whose peaks were 60–70 % or more coincident with the peaks of the reference spectra were identified and designated according coincident reference polymer name. The rejected items were also analysed according to their characteristics absorption band. A total of 1460 particles were measured and analysed by spectroscopic methods (see Supplementary materials for particle number analysed by molluscs group, Table A3).

2.5. Quality control and recovery rates

To prevent contamination, distilled water (MilliQ® water), and all solutions prepared, were filtered before the use through sterilized nitrocellulose membrane filters GF/F 0.45 μm (Whatman 47 mm diameter, Maidstone, United Kingdom), and stored in glass bottles. All glassware, sieves and fine tools as scalpels, and stainless steel tweezers, were washed with ultrapure water (distilled water filtrate through 0.45 μm sterilized membrane filters) and alcohol 70 % after washing. All materials were wrapped in aluminium foil and stored in a clean fume hood. All laboratory countertops were cleaned with alcohol 96 % and fibre-free napkins. Clean cotton laboratory coats and latex gloves were used during all processes. All glass material were immediately covered to reduce MPs airborne contamination in extraction and lyophilisation procedures. Samples processing and digestion procedures were conducted in clean fume hood and laminar flow cabinet. Blanks were used in every step of samples processing and digestion. Five and 10 cleaned Petri dish were placed during the samples processing and during MPs visual sorting, respectively, in order to calculate the deposition of airborne fibres. Eleven procedural blank, with the same reagents volume that the samples, were run with every set of samples in digestion processes fulfilling the methodology, step by step and by phases. Finally, three blanks in lyophilisation processes were applied; one for every set of sub-samples. The results were corrected by fibres and particles contaminations. After processing and preparations of sub-samples, lyophilisation procedures and visual sorting of MPs analysis, airborne fibres and particles contamination, were not detected in any blank. However, after the digestion process fibres were found in 91 % of the controls. The fibres in controls assigned to every set were classified by colour, being subtracted from the total quantity found in the respective subsamples. The numbers of fibres in controls ranged from 3 to 17 and the colours were the following: blue, yellow, red, black, grey, green, and transparent.

To ensure methodology effectiveness in the MPs extraction from the samples, recovery rates were calculated in parallel with samples analysis. Samples of three molluscs were randomly chosen and spiked with coloured virgin plastic spherical particles of polyethylene (PE) spanning from 53 to 500 μm diameter with density 1.02–1.06 g/cm^3 (purchased from Cospheric Inc., California, USA). Subsamples around 50–55 g of soft tissues of molluscs were dissected for spiking. The tissues were carefully cut and inoculated for different parts with coloured PE mix spheres ranging from 53 to 500 μm. The recovery rates were 60 % for 53–63 μm, 60 % for 125–150 μm, 74 % for 250–300 μm, and 99 % for 425–500 μm. Neither damages by agitation (mechanic forces), nor chemical attacks, were found in spheres (Fig. A4, Supplementary materials).

2.6. Dietary exposure

The dietary intake of MPs from mussels, oysters and clams by the Catalan population was estimated using a deterministic method by applying the following equation:

$$DI_t = \sum C_f \times X_{f,t}$$

where DI_t is the dietary intake of MPs of population group t (MPs/day), C_f is the concentration of the MPs in each samples group *f* (mussels, oyster or clams) (MPs/$g_{w.w}$); and $X_{t,f}$ is the mean consumption of samples group *f* (mussels, oyster or clams) by population group t (g/day). Three different population groups were considered: adults, elderly, and pregnant women. The mean consumption was obtained from the EFSA consumption data, taking account only effective consumers surveyed (EFSA, 2021). The mean consumption of each population group was

normalized by dividing the dietary intake by the mean body weight of each population group, which was established in 77 kg for adults, 70.5 kg for elderly and 65 kg for pregnant women (Gonzalez et al., 2019).

2.7. Data analysis

The MPs concentration in molluscs samples was presented as MPs particles per individual (MPs/individual), per gram of wet weight fresh tissue (MPs/$g_{w.w}$), and per gram of dry weight tissue (MPs/$g_{d.w}$). Data were analysed using the SPSS 24.0. Normality tests were carried out on samples grouped by species with Shapiro-Wilk normality test ($n < 50$). Non-parametric Kruskal–Wallis test followed by multiple comparisons was carried out to asses MPs concentrations and morphology differences. A non-parametric Mann-Whitney *U* test was also conducted in order to determine the difference in MPs concentrations and morphology between pairs of molluscs' species. The levels of statistical significance were established at $p < 0.05$.

Regarding the size of the MPs Kolmogorov-Smirnov Test was carried out for samples grouped by species to check normality data distribution (for samples $n > 50$; and significance levels $p > 0.05$). Results indicate that in general MPs' sizes follow a normal distribution with p between 0.109 and 0.200. Levene and ANOVA tests with Tukey post hoc ($p < 0.05$) for independent samples were applied to assess MPs sizes differences between molluscs' species. Hypotheses were contrasted with a significance level of 0.05.

3. Results and discussion

3.1. Samples processing and analysis

Despite the effort of the scientific community for developing methodologies for MPs extraction and analysis from seafood, to date there is not a standardized protocol. The methodologies developed in the current study have advantages such as gentle enzymatic and chemical reagents (for MPs integrity protection). A large number of samples (2310 individual organisms) were analysed and a single protocol was applied to all of them.

The recovery percentages were probably influenced by diameter of 20 μm pore size sieve of 20 cm, as well as the morphology and type of MPs patterns (rounded, smooth surface and virgin). The MPs spiked inside the fresh tissues were probably not wrapped and attached by the fresh tissue like the environmental MPs. Until now, there are not defined standardized methods and materials to determine the recovery percentages for MPs extraction. A change in sieve size to smaller diameters would lead to a higher recovery of MPs, especially the smallest ones.

3.2. Abundance of microplastics in molluscs

Microplastics were detected in all samples of analysed molluscs. Mean MPs levels ranged from 0.32 ± 0.27 to 32.0 ± 13.38 MPs/individual and from 0.54 ± 0.43 to 8.17 ± 10.0 MPs/$g_{w.w.}$ (Table 2). Big oysters, razor clams, and mussels showed the highest concentrations of MPs by individuals with wide range of concentrations from 2.65 to 99.9 MPs/individual. Specifically, in non-depurated big oysters (BOnd(S)), razor clams RCnd (S)), and mussels from north (Mnd(N)), MPs levels ranged from 12.5 to 42.5 MPs/individual, from 2.65 to 37.1 MPs/individual, and from 4.27 to 99.9 MPs/individual, respectively. The lowest MPs concentrations were found in wedge clams from north (WC(N)), ranging from 0.12 to 0.51 MPs/individual. Based on wet weight, the ranges were again wider in non-depurated mussels from north (Mnd (N)), with values ranging between 0.68 and 34.4 MPs/$g_{w.w.}$, followed by mussels depurated from south (Md(S)), ranging from 0.68 to 9.65 MPs/$g_{w.w.}$, and fine clams depurated (FCd(S)), ranging from 1.66 to 10.5 MPs/$g_{w.w.}$. The lowest MPs concentration (wet weight) was found in marine snails from south (S(S)), with levels between 0.19 and 1.06 MPs/$g_{w.w.}$ (Table 2).

The average MPs concentrations by wet weight, by dry weight, and by individual, according to the groups of molluscs are depicted in Fig. 2. Big oysters and mussels showed the highest concentrations: 22.8 ± 14.4 and 18.6 ± 23.0 MPs/individual, respectively. Mussels and fine clams showed the highest level by wet weight with values of 6.47 ± 7.95 and 4.97 ± 4.78 MPs/$g_{w.w.}$, respectively. The lowest concentration by individual was found in wedge clams (0.49 ± 0.23 MPs/individual), and the lowest concentration by wet weight was observed in snails (0.94 ± 0.62 MPs/$g_{w.w.}$). Regarding MPs concentrations by dry weight, values ranged from 3.11 ± 3.11 to 32.7 ± 27.8 MPs/$g_{d.w.}$, for snails and fine clams, respectively.

Fig. 2 shows large significant differences between species when MPs concentrations are expressed either by wet weight, or by individuals. Wedge clams presented significant lower levels ($p < 0.05$) (0.12 MPs/individual) than the remaining species studied. In addition, snails registered significant ($p < 0.05$) lower MPs levels per individual (4.6 MP/individual) than mussels and big oysters (18.6 and 22.8 MPs/individual, respectively). Regarding levels by wet weight, mussels showed significant ($p < 0.05$) higher levels than wedge clams, snails, and big oysters, while fine clams and wedge clams presented significant ($p < 0.05$) higher levels than snails.

3.3. Microplastics size

The sizes of 1460 MPs found in the mollusc samples were analysed. In general, terms, a half of the total particles measured was found in the sizes between 0.02 and 0.5 mm, while 74 % of them were <1 mm

Table 2
Microplastic levels (by wet weight and by individual) in molluscs samples.

Code*	n samples (total individuals analysed)	Wet weight (MPs/$g_{w.w.}$)				Individual (MPs/individual)			
		Mean	SD	Minimum	Maximum	Mean	SD	Minimum	Maximum
WCnd(S)	2 (383)	1.99	0.82	1.41	2.57	0.52	0.22	0.37	0.68
WCd(S)	2 (387)	2.56	1.18	1.72	3.40	0.67	0.31	0.45	0.89
WC(N)	2 (373)	1.04	0.89	0.40	1.67	0.32	0.27	0.12	0.51
WC(C)	2 (489)	2.09	0.51	1.74	2.45	0.46	0.11	0.38	0.54
S(S)	4 (68)	0.54	0.43	0.19	1.06	1.92	1.50	0.67	3.74
S(C)	5 (55)	1.26	0.58	0.59	2.16	6.80	3.17	3.20	11.7
RCnd(S)	6 (59)	2.45	2.59	0.52	7.26	12.5	13.2	2.65	37.1
FCd(S)	3 (74)	4.97	4.78	1.66	10.5	10.4	9.99	3.47	21.9
BOnd(S)	4 (19)	2.60	1.09	1.01	3.45	32.1	13.4	12.5	42.5
BOd(S)	5 (28)	1.58	1.16	0.15	2.85	15.5	11.3	1.49	27.9
Md(S)	4 (88)	4.78	3.82	0.68	9.65	11.3	9.05	1.60	22.9
Mnd(S)	4 (62)	3.50	2.17	1.60	6.62	11.8	7.33	5.41	22.3
Mnd(N)	11 (223)	8.17	10.0	1.47	34.4	23.7	29.0	4.27	99.9

SD: standard deviation. *Code: spice in capital letter: WC: wedge clams, S: snail, RC: razor clams, FC: fine clams, M: mussels, and BO: big oyster. nd: not depurated, d: depurated; between brackets Catchment areas: (S): South, (N): north and (C): central.

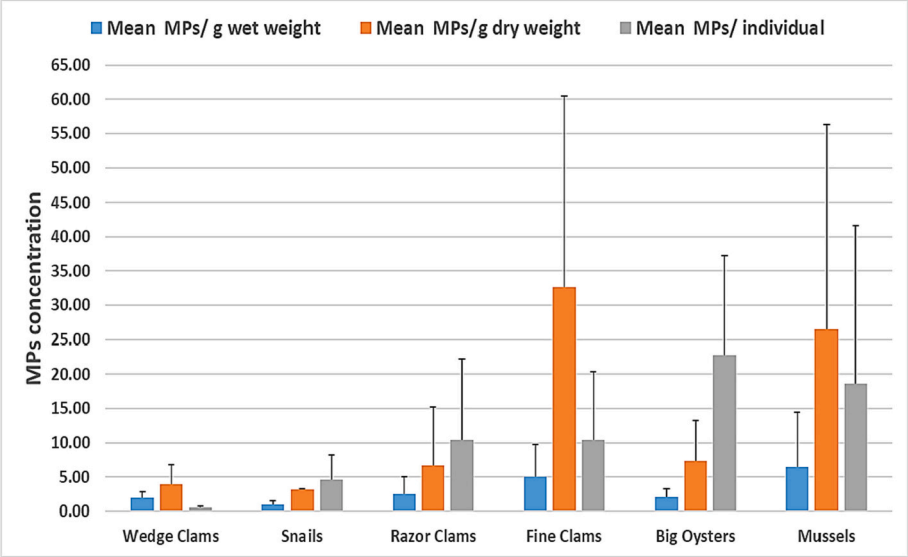

Fig. 2. MPs concentration by molluscs groups. Bars indicate standard deviations.

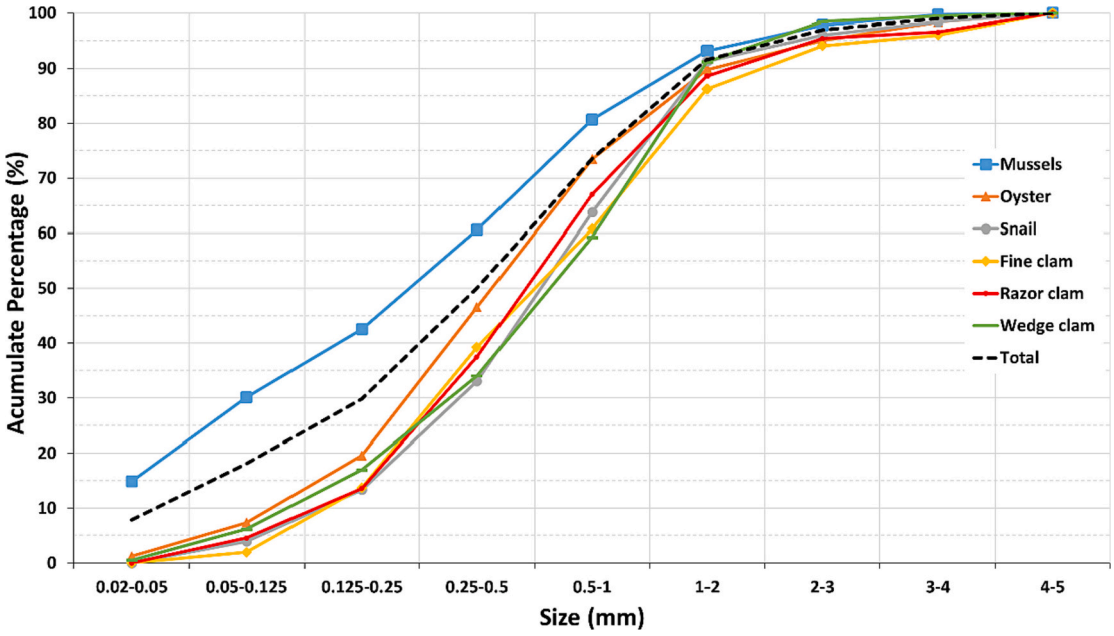

Fig. 3. Microplastics accumulated size distribution globally and according to molluscs' species.

(Fig. 3). The sizes of particles found in the present study were higher than those found by Cho et al. (2019) and Li et al. (2015, 2016a,b), in bivalves from Korean and China markets and their coastal areas. Cho et al. (2019) reported particles sizes smaller than 300 μm, accounting the 65 % of total of MPs. Similarly, Li et al. (2015, 2016a,b), found that MPs sizes below 250 μm constituted up to 84 % of the total MPs in commercial bivalves, and 79 % in coastal mussels.

With respect to species, similar profiles were detected for clams (wedge, fine and razor) and snails, with differences in the distribution of accumulated MP of <10 %. Oysters showed a higher concentration for sizes between 0.02 and 0.125 mm (although non-significant: $p > 0.05$). Only mussels showed a significant ($p < 0.001$) higher concentration in fractions from 0.02 to 1 mm. This difference was due to the fact that samples collected in the north of Catalonia presented a high number of MPs, between 0.02 and 0.125 mm, which counted around 50 % of MPs. Excluding those samples, the mussel size profile was similar to that of the oyster.

3.4. Microplastics morphology

MPs morphology in each sample of molluscs collected from Catalan coast is depicted in Fig. 4. Fibres, fragments and films were detected in molluscs' samples with overall mean proportions of 74 %, 13 % and 13 %, respectively. For all samples, the most predominant type of MPs were fibres, with percentages ranging between 50 and 92 %, excepting the non-depurated Northern Mussels (Mnd(N)), in which the fragments represented 64 % of the total MPs, followed by fibres, 34 %. These samples (Mnd(N)) presented a higher content of small MPs (Section 3.3) in the form of fragments. Interestingly, two PE pellets were found in two subsamples of non-depurated Northern mussel sample. This meant 0.04 % of the total MPs of that sample.

Comparing molluscs samples, differences according to species,

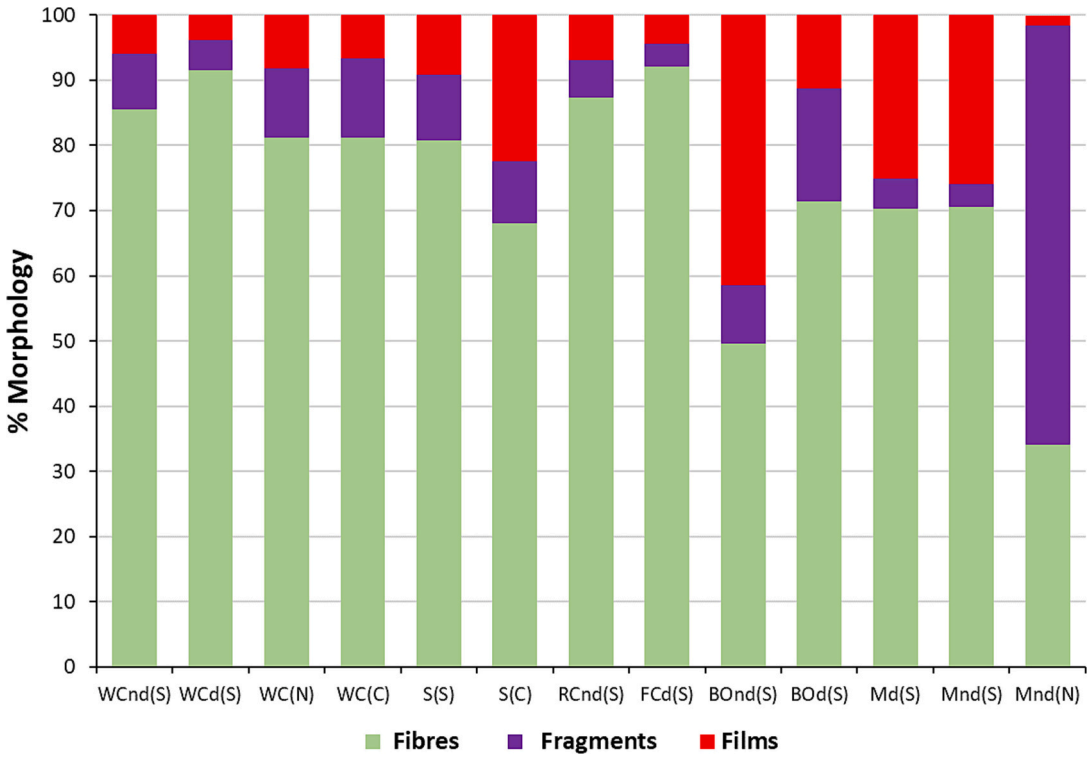

Fig. 4. Morphologic types of microplastics in molluscs' samples.

catchment zone and depuration condition were observed. According to species, wedge clams showed significant ($p < 0.05$) differences with snails regarding fibres and fragments concentration (g wet weight) and also in the percentages of fibres and film. Wedge clams showed also significant ($p < 0.05$) differences with fine clams in relation to film concentration (g wet weight) and fragment percentage. Similarly, snails showed also significant differences with fine clams. Mussels showed significant ($p < 0.05$) differences with snails, razor clams, and oysters with respect to fibres, fragments concentration, and films percentages. Razor clams, fine clams and oysters did not show significant ($p > 0.05$) differences between them, regarding the MPs morphology.

Based on the catchment zones, snails and mussels showed significant ($p < 0.05$) differences in films, fragment, and fibres concentrations, as well as their percentages. Snails from central S(C) and south S(S) zones had average films concentrations of 0.28 ± 0.15 and 0.05 ± 0.03 MPs/$g_{w.w.}$, respectively. In contrast, the percentage of fibres was lower in the central (65 ± 13 %) than in the south (79 ± 5 %) zone. Regarding mussels, fragments were significantly ($p < 0.01$) higher in the north (5.64 ± 8.98 MPs/$g_{w.w.}$) than in the south (0.17 ± 0.08 MPs/$g_{w.w}$). For wedge clams, no significant ($p > 0.05$) differences in morphology proportion between organism from north, south and central catchment zones were observed.

Regarding depuration conditions, wedge clams did not present significant ($p > 0.05$) differences in MPs morphology. However, oysters showed significant ($p < 0.05$) higher levels of films in non-depurated (1.01 ± 0.77 MPs/$g_{w.w.}$) than in depurated (0.18 ± 0.37 MPs/$g_{w.w.}$). That means a significant ($p < 0.05$) reduction from 42 % to 11 % of the total MPs. By contrast, mussels from south (S) showed similar concentrations of films, fragments, and fibres between depurated, Md (S), and non-depurated Mnd (S).

The predominance of MPs in the form of fibres in molluscs is related to their presence in environment fibres. It tends to predominate in seawater and marine sediments in many regions of the world, including the Mediterranean Sea (de Haan et al., 2019; Expósito et al., 2021; Filgueiras et al., 2019; Lots et al., 2017; Reineccius et al., 2020; Sanchez-Vidal et al., 2018; Suaria et al., 2020). In addition, these results are in agreement with those of Ward et al. (2019a), who found long microfibres in mussels and oysters, although mussels ingest fibres regardless of their length.

3.5. Microplastic composition

A total of 1460 particles were analysed for polymeric composition by spectral techniques. Twenty-one different polymers types were identified in the samples of molluscs. The most prevalent polymer was polyethylene (PE), with a contribution of 54 %, followed by polyester (PES), and synthetic cellulose (rayon or viscose), with percentages of 17 % and 12 %, respectively. Other detected polymers were polyvinylidene fluoride (PVDF) (4 %); polypropylene (PP) (3 %); polyamide (PA) (2.4 %); polyacrylonitrile (PAN) (2.2 %); polycarbonate (PC) (0.5 %); polyurethane (PU) (0.3 %) and polystyrene (PS) (0.3 %). Other polymers were detected at lower rates (≤0.2 %): expanded polystyrene (EPS), ethylene vinyl alcohol/ethylene vinyl acetate (EVOH/EVA), polyethylene terephthalate (PET), poly(ethylene-*co*-vinyl acetate) (PEVA), poly(vinyl alcohol) (PVA), polymethyl methacrylate (PMMA), polystyrene acrylate ester, PE-vinylchloride, polyacrylate, polyvinyl chloride (PVC) and PP-PE diene. Around 3 % of man-made polymers, could not be properly identified, being classified as "synthetic polymers".

Only five polymers (polyethylene, polyester, synthetic cellulose, polyamide polypropylene, polyvinylidene fluoride) were detected in all species at different percentages, contributing in >85 % of the total MPs (Fig. 5). The polymer PVDF was frequently detected in oysters of the Catalonia coast, from 17 to 26 %, lower values than the percentages reported by Ding et al. (2020) in oysters from China, where PVDF was frequently detected, accounting for 68 %. The polymer PVDF was also found in razor clams, snails, and mussels from the Catalonia coast, but at low proportions (between 1.5 and 6 %).

Differences in polymer abundance observed between the species of molluscs could be due to the culture methods and the habitat/feeding characteristics of each species. PES fibres were found at high proportions in clams. PES presents higher density (1.26–1.34 g/cm^3) than seawater (1.02 g/cm^3) and could to form hetero-aggregates with organic and

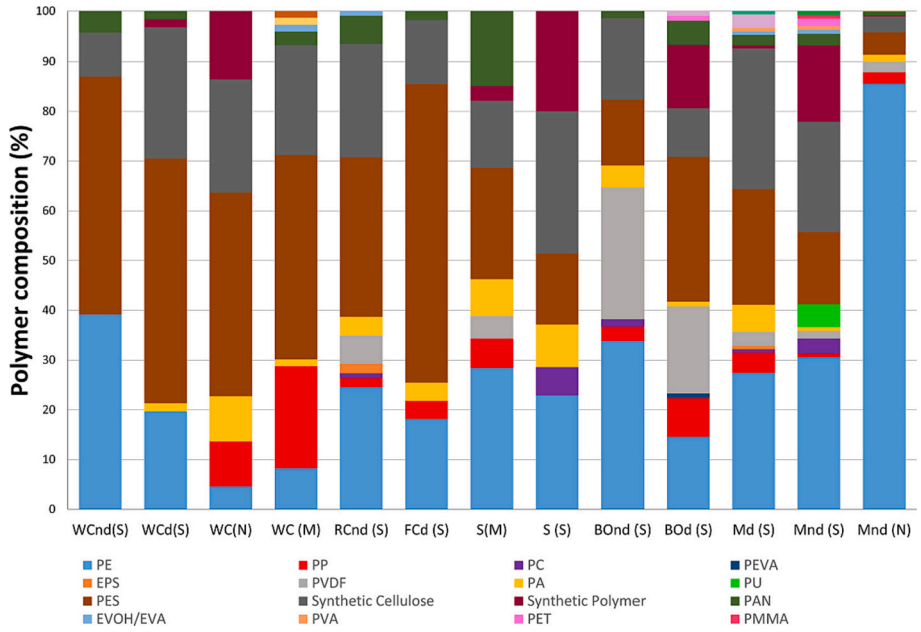

Fig. 5. Microplastics composition in molluscs.

inorganic particles (Wheeler and Lower, 2021), being heavier and deposited on sediment and available to organisms which inhabits in marine bottoms such clams. In mussels cultured in the upper layer of the water column, the polymer PE was found at high quantity due to its low-density (0.92–0.94 g/cm^3) and small particles sizes accessible to mussels' organism. Similarly, Cho et al. (2019), found PES relatively abundant in species grown in the deeper layer of the water column, such as scallops and intertidal sediment as Manila clam. High proportions of PE were observed in all samples of molluscs. It might be due to its massive production and consumption (PlasticsEurope, 2013, 2017, 2019, 2021), as well as its high buoyancy and transportability at seawater based on its low specific density (Cincinelli et al., 2017). The polymer composition predominant of fibres from molluscs' samples was polyester, followed by synthetic cellulose -which is expected to originate from textiles- reflecting the global production of these artificial fibres (PlasticsEurope, 2019).

With respect to the catchment area, a greater diversity of polymers was observed in the southern and central areas (17 polymers) in relation to the north (8 polymers). PE was the predominant polymer found in organisms from northern zone, with 84 % of the total MPs. In contrast, in the central and south areas, MPs composition profile was very similar, with PE, PES, PP, and synthetic cellulose, PVDF (polyvinylidene fluoride), PAN and PA counting 90 % -or even more- of the total MPs.

In the current survey, synthetic cellulose was considered as MP. According to Remy et al. (2015) and Halstead et al. (2018), although these polymers can be rapidly degraded, their intakes can cause adverse biological effects. Yadav and Hakkarainen (2021) reported that the degree of acetylation in cellulose acetate polymer reduces its degradation potential. In spite of this, the methodology followed in the current study, in which the removal of organic matter was carried out through combined alkaline hydrolysis, enzymatic and Fenton processes, the non-degradation of these fibres could be attributable to the large amounts of non-biodegradable compounds that they contain.

3.6. Comparison of microplastic concentration, morphology, and sizes between molluscs' samples

It is probably that MPs filter efficiency depends on bivalve size, mechanism to particles selection, and environment conditions. The molluscs evaluated in the present survey had different feeding habits and habitats (i.e., sediment or water column) (Table 1). Bivalves (clams, oysters and molluscs) are filter feeder of large seawater volumes and ingest the suspended MPs from water column (mussels and oysters), or interface water-sediment (wedge, razor and fine clams) (Li et al., 2015).

Wedge clams, inhabits on sandy beaches, where it forms extensive dense beds living buried in soft bottoms (FAO, 2022). This species showed the lowest MPs concentration by individual, which is due to its low ability to ingest large MPs. According to Ding et al. (2020), features of MPs ingested by shellfish were related to the biometric parameters of shellfish. Fine (*Tapes decussatus*) and razor clam (*Ensis siliqua*) also belong to clams group, which lives buried in soft sand or mud bottoms, where they digging into different depths (infaunal) (FAO, 2022). These species did not show differences in the concentration of MPs nor with the oyster (*Crassostrea gigas*), which is not buried (epifaunal) and stay attached in strong substrate by cementing one of the valves (FAO, 2022). In the current investigation, oysters were cultivated, combining at the first on-off bottom culture, and after suspended culture and harvesting (trays culture), considering it as water-dwelling in adult phase in the Ebro Delta. By localisation habitat, the MPs found in clams seem to come from to bottom resuspension. Ward et al. (2019a) reported abundance of MPs (especially fibres) in clams, being more similar to sediment than to water, a fact that has been also reported by other authors (Su et al., 2018; Ding et al., 2020). It seems that fine clams have lower excretion rates, which makes difficult to excrete microfibres and microfragments (Keisling et al., 2020).

The snails showed lower MPs concentration (wet weight). Gastropod species such as *Bolinus brandaris* are demersal organism inhabitants sandy-muddy (detrital) and soft bottoms with carnivorous predators' habits. MPs can be indirectly ingested from prey already containing MPs particles (Abidli et al., 2019). These species showed significant lower concentration (wet weight) compared with clams. It suggests that the presence of MPs in the area of the selected prey was low, or their MPs excretion capacity was high.

Mussels are called “true surface dwellers” and the organisms here analysed came from suspended culture attached to the substratum, by threads or “byssus” (Piarulli et al., 2020; FAO, 2022). Mussels showed higher MPs concentration (wet weight) compared to other species as wedge clams, snails and oysters (razor clam showed only differences in fragments concentrations). It indicates that the habitats or location in water column-sediment environment, and the shell size, have an

influence on the accumulation of MPs in the organisms. In this same line, Ding et al. (2020) found that MPs intake of water-dwelling shellfish was significantly different from that of sediment-dwelling shellfish. Mussels trap a higher number of MPs due to their size, high pumping and filtration rates (9.3 ± 1.7 L/day) (Catarino et al., 2018; Rist et al., 2019). According to Ward et al. (2019a), the particles trapped in the gill filaments are translocated after a selection process to the digestive glandule and intestine. Mussels are exposed to the sea-surface microlayer, where small particles and most of the high buoyancy particles -including MPs- are periodically resuspended from sediments and sand by currents and waves to water column. Intake of different plastic particle sizes (30 nm to several millimetres) and shapes (fragments, beads, fibres and films) have been observed in mussels (Wegner et al., 2012; Li J et al., 2016 cited by Rist et al., 2019). In turn, Kolandhasamy et al. (2018) showed the adherence and ingestion as the mainly process of MPs accumulation in mussels. MPs uptake is observed in multiple organs of mussels, including foot and mantle, being the intestine, the organ containing the highest level of MPs. Another organ containing MPs is the byssus, but merged instead of adhered (Li et al., 2019). According to bivalves feeding habits, gills may also be another organ that may contain MPs. In the case of the oysters, in this study is a water dwelling as mussels, and the differences regarding MPs concentration were probably due to ingestion-egestion-rejection differences between species and MPs pollution of cultivation zone.

Regional differences were observed between snails (from the central and south zones) and mussels (from the north and southern zones) regarding MPs morphology and size, which was probably due to the different MP pollution in these zones of the Catalan coast.

MPs burden from the northern zone of the Catalonia coast is influenced by a marine current that transports MPs from the northern Mediterranean Sea (France). WWTP effluents, as well as surface runoff of urban soils and discharges of mismanaged plastic, already weathered from industrialized and densely populated coastal areas (Constant et al., 2019; de Haan et al., 2019) -such as Barcelona and Badalona- are responsible for these high levels of pollution (Andrady, 2011; Jambeck et al., 2015; Cózar et al., 2015). The central area of the Catalonia coast receives influence of MPs, and mismanaged plastics discharged by the Rivers Llobregat and Besòs (both basins receptors of WWTP effluents from urban and industrialized zones, agriculture run off) into the Mediterranean coast, and distributed by principal current and local hydrodynamics to shoreline (Schirinzi et al., 2020; Dalmau-Soler et al., 2021). Finally, the southern zone is highly influenced by the Ebro River and the Ebro Delta River activities. The Ebro River discharges affect surrounding environments as estuarine sediments, sandy beaches (northern edge) and seawater surface, which is evidenced by MPs higher concentration in three environment matrices, being the fibres the predominant morphology with presence of secondary MPs (fragments and films). Furthermore, agriculture activities in the Ebro Delta (intensive rice production) could promote the release of MPs accumulated in the soil (mainly fibres) matrix enriched with biosolids to the aquatic environment (Simon-Sánchez et al., 2019; Sun et al., 2019). Fishing and aquaculture activities in the Ebro Delta could be also other potential sources of MPs. Both activities use materials made of fibres and polymers synthetic (nylon and polyethylene) as ropes, nets, floats and mesh (Andrady, 2011; Covernton et al., 2019).

MPs sizes in mussels were significantly smaller than those in clams, oysters and snails. Ding et al. (2020) also found that sizes of MPs in the sediment-dwelling shellfish were significantly larger than those in the water-dwelling shellfish species. In turn, Qu et al. (2018) reported a positive quantitative correlation between abundance of plastic particles in two species of mussels and its surrounding waters, with more incidence of smaller MPs intake. The size of particles partially determines preferential rejection or ingestion. Thus, particles up to 200–300 μm are efficiently captured and ingested, and small particles are only efficiently captured if they are incorporated into aggregates, or are highly agglomerated (Ward et al., 2019a). There is a maximum limit of particle size that can be ingested, which is probably in the range of 600–900 μm. However, the particle selection is never 100 % efficient, and therefore, fibres can be ingested by various bivalve species (Ward et al., 2019a). In some cases, bivalves simply find and engulf the particulate matter. In fact, as other invertebrates, the size of the oral opening in bivalves is not fixed and can stretch. For example, it has been reported that oysters ingested larvae of nine different invertebrate species, measuring between 100 and >500 μm in length, at efficiencies of about 80 % (Ward et al., 2019a).

In this study, between 60 and 85 % of the MPs in bivalves had sizes between 20 μm and 1 mm. This means that most MPs were probably ingested. Microplastics from 2 to 5 mm accounted only an 8 %,thus, its presence in bivalves could be due to oral stretching and not rejection for a special condition of organism, which led to engulf plastic particles (e.g., starving), as well as an inefficient particle selection combined with adherence in others body parts. Specifically for mussels, mussels not depurated from the North zone showed up to 85 % of MPs with a size below 1 mm, while mussels from the South (depurated and not depurated) showed up to 80 % of MPs for the same range size (<1 mm) stating that MPs could be mostly ingested.

The depuration processes effects on MPs concentration were not observed in wedge clams from southern, mussels from southern and northern, and oysters from southern. Significant differences in MPs morphology were only observed in oysters, and MPs morphology and sizes in mussels. Furthermore, differences in specific polymers were observed between non-depurated and depurated organisms, although the number of different polymers was similar. These results are attributable to individual MPs ingestion-egestion dynamics and differences in MPs pollution along the Catalan coast. The depuration time was perhaps not long enough for the efficient removal of MPs from the organisms. Depuration times were 48 h for wedge clams and mussels and > 72 h for oysters. Nevertheless, van Cauwenberghe and Janssen (2014) found significant differences in depurated and non-depurated oysters and mussels with a time of 72 h. According to Ward et al. (2019a), after 168 h (7 days) of depuration, only residual amounts of plastic particles were found in bivalve tissues. Birnstiel et al. (2019) reported that a depuration time of 93 h was quite effective in removing blue fibres in wild and farmer mussels. However, mussels still presented high abundance of MPs even after depuration, suggesting that depuration process was not sufficiently efficient for completing MPs removals. The depuration processes in bivalves could be influenced by the digestion processes of plastic particles in the organisms. Zhao et al. (2018), observed that the residence time of MPs in clams, mussels, and oysters in the hind gut is of the order of hours, while the residence time in the digestive diverticula is of the order of days. Furthermore, it is probably that bivalves' contamination by MPs occurs after depuration during packaging and processing of live depurated organisms (EFSA, 2016).

3.7. Comparison of microplastic concentration in molluscs with others similar studies

Microplastic levels comparison among different studies it is not an easy task due to lack of a standardized analysis, the MPs size considered in each study, as well as the identification techniques used (Ding et al., 2020). Sampling area and cultivation techniques can further influence the MPs concentration, morphology and composition. Notwithstanding, the concentrations reported by different studies are an indicative of the MP contamination levels in different regions and type of molluscs (wild, commercial, or farmed).

Table 3 summarizes the MPs levels found in the present study, which were compared with those recently reported in the scientific literature. The highest MPs values by individuals were observed in wild mussels (*Mytilus edulis*) from Nova Scotia (Halifax-Harbor), Canada, with levels ranging between 34 and 178 MPs/individuals (Mathalon and Hill, 2014). However, the levels of MPs by individuals of molluscs purchased from markets of Catalonia coast tend to be higher than molluscs from

Table 3
Summary of global investigation on microplastics in shellfish samples.

Location	Species	Morphology	Size (μm)	MPs/g wet weight	MPs/individual	Predominant composition	Reference
Commercial molluscs from Catalonia coast (Spain)	*Mytilus galloprovincialis, Tapes decussatus, Crassostrea gigas, Bolinus brandaris, Ensis siliqua, Donax trunculus*	Fib, Frg, Fil	20–5000	Range: 0.19–34.43 (Mean range 0.54–8.17)	Range:0.12–99.85 (Mean range 0.32–32.1)	PE (4–85 %), PES (4–60 %), synthetic cellulose (3–29 %), PVDF (0–27 %), PP (0–20 %), PAN (0–14 %), PA (0–9 %), PC, PU, PS (0–6 %)	This study
Commercial shellfish from Qingdao and Xiamen (China)	*Mytilus galloprovincialis, Perna viridis, Ruditapes philippinarum, Crassostrea gigas, Sinonovacula constricta, Scapharca subcrenata, Meretrix lusoria, and Busycon canaliculatum*	Fib, Frg, Fil	10–5000	0.8–4.4 (digestive system)	1.2–6.0 (in digestive system)	Rayon (42–44 %), Chlorinated Polyethylene (12–14 %), PET (5–16 %) PVC (7–10 %), PVDF (24 %)	Ding et al., 2020
Fishery market of Shanghai (China)	*Scapharca subcrenata; Tegillarca granosa; Mytilus galloprovincialis; Patinopecten yessoensis; Alectryonella plicatula; Sinonovacula constricta; Ruditapes philippinarum; Meretrix lusoria; Cyclina sinensis* (Bivalves)	Fib, Frg, Pell	5–5000	2.1–10.5	4.3–57.2	–	Li et al., 2015
Commercial bivalves from Qingdao (China)	*Chlamys farreri, Mytilus galloprovincialis*	Fib, Frg	25–5000	2.0–12.8 (digestive system)	1.9–19.4 (digestive system)	–	Ding et al., 2018
Commercial molluscs from Seoul, Gwangju, and Busan (South Korea)	*Mytilus edulis; Patinopecten yessoensis; Tapes philippinarum; Crassostrea gigas* (Bivalves)	Fib, Frg, Fil	43–4720	0.15 ± 0.20	0.97 ± 0.74	Frg: PE (24 %), PP (23 %), PS (22 %), PEVA (4 %), PET (2 %), PU (2 %), acrylic (2 %) Fib: PES (82 %), PP (6 %), acrylic (6 %), nylon (3 %).	Cho et al., 2019
Wild molluscs of Persian Gulf (Iran)	*Amiantis umbonella; Amiantis purpuratus; Pinctada radiata; Cerithidea cingulata; Thais mutabilis*	Fib, Fil, Frg, Pell	10–5000	0.2–21.0 (mean)	3.7–17.7 (mean)	–	Naji et al., 2018
Supermarket from Brittany, France Farm in Germany from the North Sea	*Crassostrea gigas* *Mytilus edulis* (depurated and not depurated)	Frg	>25	0.47 ± 0.16 (*C. gigas* nd) 0.35 ± 0.05 (*C. gigas* d 72 h) 0.36 ± 0.07 (*M. edulis* nd) 0.24 ± 0.07 (*M. edulis* d 72 h)	NA	–	Van Cauwenberghe and Janssen, 2014
France, Belgium, and Netherland coast	*Mytilus edulis*	Frg	20–90	0.2 ± 0.3	NA	–	Van Cauwenberghe et al., 2015
Italian natural stocks and mariculture plants	*Mytilus galloprovincialis*	Fib	750–6000	4.4–11.4 (mean)	3.6–12.4 (mean)	–	Renzi et al., 2018
Ionian Sea (Greece)	*Mytilus galloprovincialis*	Frg, Fib	40–737 55–620	Wild: 5.3 ± 0.5 Farmed: 2.5 ± 0.3	Wild: 1.7 ± 0.2 Farmed: 2.0 ± 0.2	PE 75 %, PP 12.5 % and PTFE 12.5 %	Digka et al., 2018
Loire Estuary, Atlantic coast (France)	*Crassostrea gigas*	Frg, Fib	20–1300	0.18 ± 0.16	2.10 ± 1.71	PE 47 %, PP 25 %, ABS 15 %	Phuong et al., 2018
United Kingdom coast	*Mytilus edulis*	Fib, Frg, sphere, Fil	8–4700	0.7–2.9	1.1–6.4	PES 43 %, Rayon 26 %, Cellulose 14 %	Li et al., 2018
Norway coast	*Mytilus* spp.	Fib, Frg, Foa, Pell	70–3870	0.97 ± 2.61 (range: 0–7.9)	1.5 ± 2.3 (range: 0–6.9)	Cellophane 64 %, "parking lot tar" and EVA 19 %, PET 9.9 %, Acrylic 2.9 %, PP 1.2 %, PE 1 %	Bråte et al., 2018
Coastal lagoons on the North Adriatic (Italy)	*Cerastoderma glaucum, Mytilus galloprovincialis, Limecola balthica, Scrobicularia plana*	Fib, Frg, Foa	60–3000	NA	Range:0.01 ± 0.01 to 0.25 ± 0.12	PES 98 %, LDPE 1.5 %, PP 0.25 %, PAN 0.25 %	Piarulli et al., 2020
Commercial molluscs of Bizerte Lagoon (Tunisia)	*Mytilus galloprovinciali, Ruditapes decussatus, Crassostrea gigas, Bolinus brandaris*	Fib, Frg, Fil	50–5000	0.70 ± 0.11 to 1.48 ± 0.02	NA	Fib: PP 100 % Frg: PP 60 %, PE 40 % Fil: PE 50 %, PP 50 %	Abidli et al., 2019
Farmed and wild mussels of Halifax Harbor (Canada)	*Mytilus edulis (farmed and wild)*	Fib	>0.8 μm	–	Wild: 34–126 Farmed: 75–178	–	Mathalon and Hill, 2014

(*continued on next page*)

Table 3 (*continued*)

Location	Species	Morphology	Size (μm)	MPs/g wet weight	MPs/individual	Predominant composition	Reference
Vancouver island coast (Canada)	*Crassostrea gigas* *Mytilus edulis*	Fib, Frg, Pell	<530	*C. gigas* (Wild): 77 ± 126 *C. gigas* (Farmed): 213 ± 154 *M. edulis* (Wild): 138 ± 202 *M. edulis* (Farmed): 259 ± 114	NA	–	Murphy, 2018
Wild mussels of Ria of Vigo and Cantabrian Sea, Atlantic coast (Spain)	*Mytilus* spp	Fib, Frg, Pell	20–5000	Ria of Vigo: 1.59 ± 1.28 Cantabrian Sea: 2.55 ± 2.80	Ria of Vigo 2.19 ± 1.57 Cantabrian Sea 2.81 ± 2.80	–	Reguera et al., 2019
Farmed and wild from Jiaozhou Bay (China)	*Ruditapes philippinarum* *Crassostrea gigas (wild and farm)*	Fib, Frg	500–8201	*C. gigas*: 0.92 ± 0.80 *R. philippinarum* 1.51 ± 1.27 Range: 0.16–12.09	*C. gigas*: 2.34 ± 1.80 *R. philippinarum*: 2.00 ± 1.99 Range: 1–9	Cellophane 48 %, PET 21 %	Zhang et al., 2022
North Sea (Netherlands)	*Mytilus edulis*	Fib, Frg	30–2000	37 MPs/$g_{d.w.}$	NA	–	Karlsson et al., 2017
North Sea (Netherlands)	*Littorina littorea; Mytilus edulis; Crassostrea gigas*	Fib, Pell, Fil	10–5000	20–105 MPs/$g_{d.w.}$	NA	–	Leslie et al., 2017
British Columbia coast (Canada)	*Crassostrea gigas*	Fibre, Frg, Pell	10–5000	0.04 ± 0.06 MPs/$g_{d.w.}$	0.22 ± 0.28	Fib: Polyester, Nylon	Covernton et al., 2019

NA: not assessed. Fib: Fibres, Frg: Fragments, Film: Fil, Pell: pellets or spheres; Foa: Foam; nd: no depurated; d: depurated.

Iran, China, South Korea, Italy, Greece, France, UK, Norway and Canada (British Columbia) (Naji et al., 2018; Ding et al., 2018, 2020; Li et al., 2015; Cho et al., 2019; Piarulli et al., 2020; Digka et al., 2018: Phuong et al., 2018; Li et al., 2018; Bråte et al., 2018; Covernton et al., 2019).

Morphology distribution and sizes range of MPs in molluscs from the Catalonia coast were similar than those found in China, South Korea, Iran, North Sea, Canada, the Atlantic coast of Spain, and Tunisia (Ding et al., 2018, 2020; Cho et al., 2019; Naji et al., 2018; Abidli et al., 2019; Leslie et al., 2017; Covernton et al., 2019; Reguera et al., 2019). Predominant MPs sizes found in the current survey (between 125 μm and 1 mm) were higher than sizes reported in Belgium, French, UK, the Dutch coastline and North Sea (van Cauwenberghe and Janssen, 2014; Digka et al., 2018; Murphy, 2018; Li et al., 2018). However, size range here found was in range with those reported in Atlantic French and Norway coast (Phuong et al., 2018; Bråte et al., 2018).

According to the MPs composition, Catalonia molluscs presented the same polymers found in molluscs from China, South Korea, UK, coastal lagoons of North Adriatic, Tunisia and Canada (Ding et al., 2020; Cho et al., 2019; Abidli et al., 2019; Covernton et al., 2019; Li et al., 2018; Piarulli et al., 2020). Predominant polymers found in the current investigation (PE and PES) were the same than those reported in South Korea, United Kingdom and the Adriatic Sea (Cho et al., 2019; Li et al., 2018; Piarulli et al., 2020). It is important to remark that the EVA polymer was only found here and in the coastline of Norway (Bråte et al., 2018).

The MPs concentration and plastics pollution sources in marine environment of molluscs catchment zones worldwide regions are specified in Table A4 (Supplementary materials). The levels of MPs in fresh tissues of molluscs purchased from markets of Catalonia tend to be higher than wild ones from other coastal zones of Spain. Present study shown higher levels than those molluscs from other Mediterranean Sea coasts (Tunisian and Greece), as well as other regions worldwide such as, China, South Korea, United Kingdom, France, Belgium, Netherlands, Norway and North Sea (Reguera et al., 2019; Abidli et al., 2019; Digka et al., 2018; Zhang et al., 2022; Ding et al., 2018, 2020; Li et al., 2015; Cho et al., 2019; Li et al., 2018; Phuong et al., 2018; Van Cauwenberghe and Janssen, 2014; Bråte et al., 2018; Van Cauwenberghe et al., 2015). However, the average levels found in present study were lower than those found in other regions of Italy (natural stock and mariculture from Tyrrhenian Sea, Ligurian Sea, Central Adriatic Sea), Iran and Canada coastline (Vancouver island coast) (Renzi et al., 2018; Naji et al., 2018; Murphy, 2018). Regarding MPs concentration per dry weight ($g_{d.w}$), in general, values found here were lower (from 3.11 ± 0.12 to 32.67 ± 27.78 MPs/g.d.w) than found in North Sea by Karlsson et al. (2017) (37 MPs/g.d.w) and by Leslie et al. (2017) (20–105 MPs/$g_{d.w}$). However, for *C. gigas* the values were higher of 7.30 ± 5.93 MPs/g.d.w compared with obtained by Covernton et al. (2019) on *C. gigas* from Canada (British Columbia).

The variation of MPs concentration and composition in molluscs was related to the difference in regional MPs pollution status, differences in ingestion-egestion-rejection capacity by species, and the influence of differences in analytical methods. For a reliable comparison of results for the monitoring of MPs contamination in edible marine organisms, standardized and optimized methods are clearly necessary.

3.8. Estimated microplastic daily intake through consumption of molluscs

Taking into account the levels of MPs detected in depurated bivalves (mussels, clams and oysters) and the consumption of molluscs by the Spanish population (adults, elderly and pregnant women) from EFSA consumption data (taking account only effective consumers surveyed) (EFSA, 2021), the daily and yearly MPs intake by that population through molluscs' consumption was estimated (Table 4).

The MPs intake through consumption of molluscs was 22.2, 20.4 and 9.67 MPs/day for adult, elderly and pregnant women, respectively. In turn, the adult annual consumption of MPs was set at 8103, with a 95th percentile of 19,418 MPs/year. Mussels were the main contributor to the intake of MPs, with >60 % of the total intake. It is important to highlight that these values are obtained from the population who are certainly effective molluscs' consumers, but they do not represent the total population surveyed; that is to say, consumers and non-consumers.

The current results are 40-fold higher than those found in Korean population through shellfish consumption (212 MPs/person/year) (Cho et al., 2019). Both, consumption rates and MPs present in shellfish, were lower than those of our survey. The intake of MPs by a Chinese population (Zhang et al., 2022) was found to be 1270 MPs/year, which is between 6 and 7 times lower than that of the current study. Danopoulos et al. (2020), estimated the intake of MPs/year combining global consumption estimates by the FAO/UN (Food and Agriculture Organization of the United Nations) with data from outcomes of applying meta-

Table 4
MPs daily and yearly intake (MPs/day, MPs/year, and MPs/kg/day) through consumption of depurated molluscs by Spanish consumers.

	Adults	Elderly	Pregnant women
Mollusc consumption (g/day)[a]			
Clams	1.14 (2.10)	1.20 (2.22)	0.60 (0.60)
Mussels	2.89 (8.95)	3.02 (10.5)	1.40 (4.20)
Oysters	1.69 (NR)	NR	NR
MPs consumption (MPs/day)			
Clams	5.67 (10.4)	5.96 (11.0)	2.98 (2.98)
Mussels	13.8 (42.8)	14.4 (50.2)	6.69 (20.1)
Oysters	2.67 (NR)	NC	NC
Total	22.2 (53.2)	20.4 (61.2)	9.67 (23.1)
MPs consumption (MPs/year)			
Total	8103 (19,418)	7446 (22,338)	3530 (8431)
MPs consumption (MPs/kg/day)			
Total	0.287 (0.690)	0.290 (0.868)	0.148 (0.353)

NR: not reported; NC: Not calculated. Results expressed as mean (95th percentile).
[a] (EFSA, 2021).

analysis of the levels of MPs contamination in seafood (mussels, crustaceans, fish and echinoderms). It was concluded that seafood is a major verified vector for human exposure to MPs. According to the results of Danopoulos et al. (2020) (6306 MPs/person/year), the intake for adults was a little bit smaller than this study mean value. By contrast, in Italy Ferrante et al. (2022) reported an adult MPs yearly intake through mussels' consumption of 5,709,330 MPs/year (15,642 MPs/day). However, in that study MPs sizes analysed ranged between 1.8 and 2.5 μm. In accordance with the results of the present study, Van Cauwenberghe and Janssen (2014) estimated a European annual MPs dietary intake of 11,000 MPs/year with data of MPs concentration found in mussels and oyster from France and Germany. In Northern Tunisia, Abidli et al. (2019) estimated a dietary intake of MPs through the consumption of bivalves and gastropods between 23 and 40 MPs/year for the general population, and between 2556 and 4920 MPs/year from local fishermen.

4. Conclusions

In the present study, a method of analysis of MPs, with different sequential steps, including alkaline hydrolysis, surfactant use, oxidation, enzymatic hydrolysis, density separation and spectroscopic techniques (IR/RAMAN), has been proposed. It has shown to be effective for the extraction, quantification and chemical characterization of MPs in molluscs. All analysed samples of commercial molluscs from the Catalan coast, contained MPs. Intra- and inter-species variability was high due to the different feeding strategies of each species, the location in the sediment/water column zone, the shell size, as well as the specific local MPs pollution. Microplastics size and shape could affect the ingestion or egestion rate of each organism. Wedge clams and snails showed the lowest concentration of MPs: 0.49 ± 0.23 MP/individual and 0.94 ± 0.62 MP/$g_{w.w}$, respectively. On the other hand, big oysters and mussels showed the highest concentration per individual (22.8 ± 14.4 and 18.6 ± 23.0 MPs/individual, respectively), while mussels showed the highest (wet weight) level (6.47 ± 7.95 MPs/$g_{w.w}$). In general, fibres were the predominant morphology. Of the total particles measured (1460), a 74 % showed sizes smaller than 1 mm, whereas 20 % were between 0.02 and 0.150 mm, a critical range for passage through the human intestinal barrier. PES polymer, synthetic cellulose, PVDF and PA, were abundant in depurated organisms, with PE in non-depurated organisms. A similar number of polymers for both conditions were observed. In terms of localization, PE was the predominant polymer in the organisms from the Northern zone, while PES, PP, PAN and PA were mainly found in the organisms from the Central zone. Finally, synthetic cellulose, PVDF and the remaining MPs (PC, PMMA, PVC, PET, PVA, PEVA, EVOH/EVA, PS and EPS) were predominant in the organisms from the Southern coast of Catalonia. The bivalve depuration process did not remove MPs from the organisms. Only changes in their morphology, size and composition were observed. The time of depuration could be a key parameter. Regarding human exposure, the daily intake of MPs through molluscs' consumption was estimated to be 22.2, 20.4 and 9.67 MPs, for adults, elderly and pregnant women, respectively. The mean annual MPs (≥20 μm) consumption for the adult population was estimated in 8103 MPs, with 95th percentile of 19,418 MPs/year. It suggests that consumption of molluscs is as an important route of exposure to MPs for the population of Catalonia. Anyway, the potential human health risks are still unknown. Therefore, further investigations are clearly required.

CRediT authorship contribution statement

Nora Expósito: Methodology, Investigation, Writing – original draft. **Joaquim Rovira:** Writing – review & editing, Conceptualization, Supervision. **Jordi Sierra:** Methodology, Investigation, Visualization, Writing – review & editing. **Gemma Gimenez:** Investigation, Conceptualization, Writing – review & editing. **José L. Domingo:** Writing – review & editing, Funding acquisition. **Marta Schuhmacher:** Writing – review & editing, Funding acquisition.

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.

Data availability

Data will be made available on request.

Acknowledgements

Nora Expósito received funds through AGAUR 2018 FI_B 01029 fellowship cofound by Secretaria d'Universitats i Recerca del Departament d'Empresa i Coneixement de la Generalitat de Catalunya and European Union. Joaquim Rovira received postdoctoral fellowship from "Juan de la Cierva-incorporación" program of the Spanish "Ministerio de Ciencia, Innovación y Universidades" (IJC 2018-035126-I). The authors wish to acknowledge the help received from Núria Ferrer and Pilar Hermo from the Scientific and Technological Centers of the University of Barcelona for the realization of the IR maps. The authors thank also the Catalan Fishermen's Cooperatives for the donation of part of samples of molluscs.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.marpolbul.2022.113843.

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