Food Research International 153 (2022) 110973

Contents lists available at ScienceDirect

Food Research International

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

Microplastics in seafood: Relative input of *Mytilus galloprovincialis* and table salt in mussel dishes

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ARTICLE INFO

Keywords:
Bay of Biscay
Environmental health
Microplastics
Mytilus galloprovincialis
Risk for consumers

ABSTRACT

Due to current marine pollution, microplastics ingestion through seafood is an increasing risk for consumers. In this study, microplastics from mussels (*Mytilus galloprovincialis*) and table salt employed in popular dishes in Bay of Biscay (Spain) were quantified and analysed by Fourier-Transformed Infrared spectroscopy. Microplastics varied in mussels (mean 0.55–3.20 items/g) depending on the environmental pollution of the collection point (seawater, 0.002–0.015 items/mL; sand, 0.06–0.38 items/g). Microplastics content in table salt (0.1–0.38 items/gr) was much lower than in mussels. Chemical substances found from microplastics in mussels and salt are catalogued as hazardous for human health. Significant correlation between microplastics in sand and mussels was found, suggesting that consumers' risk of microplastics ingestion depends on the harvesting area. Routine microplastics analysis in mussels and disclosure of microplastics content on seafood labels are recommended for conscious, informed consumption.

1. Introduction

Microplastics cause a broad range of ecological problems and currently represent one of the most challenging environmental problems in recent decades (Law & Thompson, 2014). Due to their ubiquity in all aquatic environments and small size (e.g., Hamid, Bhatti, Anuar, Mohan & Periathamby, 2018; Bergmann et al., 2019), microplastics are highly bioavailable to marine animals, from plankton (Botterell et al., 2019) to top predators (Nelms, Galloway, Godley, Jarvis & Lindeque, 2018). Once ingested, microplastics affect marine species through damages to the immune system, neurotoxicity, genotoxicity, reproductive problems, and physical effects, among others (De Sá, Oliveira, Ribeiro, Rocha & Futter, 2018). Microplastics can also act as vectors of other contaminants, and release chemical compounds used in their manufacture, aggravating such problems (Caruso, 2019; Chen et al., 2019). Seafood consumers may be at risk of microplastics ingestion trough the consumption of marine products (e.g., Danopoulos, Jenner, Twiddy & Rotchell, 2020) and sea additives commonly employed to prepare them such as table salt (Lee, Song, Kim & Kim, 2021). Cox et al. (2019) estimated an average consumption of microplastics by the American population of 39,000–52,000 particles per year via diet only, not considering airborne contamination. Although the effects of microplastic ingestion on human health are as yet unknown (Wright and Kelly, 2017; Prata, da Costa, Lopes, Duarte & Rocha-Santos, 2020), a prolonged exposure to microplastics through inhalation has been shown to cause respiratory damages, such as asthma or lung cancer (Vethaak & Legler, 2021). For these reasons, species highly consumed by humans are of special interest, amongst them bivalves, crustaceans and fish are considered to be potential sources of microplastics (Danopoulos et al., 2020).

Bivalves have been the most studied commercial seafood species. As filter-feeding, its microplastics ingestion is well known (e.g., Li et al., 2016; Digka, Tsangaris, Torre, Anastasopoulou & Zeri, 2018; Li et al., 2019). Particularly, mussels of the genus *Mytilus* like the blue mussel (*M. edulis*) have been proposed for biomonitoring microplastics pollution in the ocean, since the microplastics they accumulate reflect those found in sedimentary environmental samples (Kazour & Amara, 2020). Therefore, the concentration of microplastic in these mussels vary depending on the region studied: the amount of microplastics ranged between 0.7 and 2.9 items per gram of tissue (items/g) in the UK (Li, Green, Reynolds, Shi & Rotchell, 2018), while the average found in the Belgian coast was 0.2 ± 0.3 items/g (Van Cauwenberghe, Claessens, Vandegehuchte & Janssen, 2015). In China, these values vary from 0.6 to 4.9 items/g alongside coastal waters, exhibiting higher concentrations in areas with anthropogenic activity (Li et al., 2016). In *M. galloprovincialis*, microplastic content is also variable between and

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https://doi.org/10.1016/j.foodres.2022.110973
Received 30 September 2021; Received in revised form 31 January 2022; Accepted 2 February 2022

within regions, being 3.7 ± 1.1 items/g in the Mediterranean Delta del Ebro or 2.55 ± 2.80 in the Cantabrian coast – south Bay of Biscay (Vandermeersch et al., 2015; Reguera, Viñas & Gago, 2019). The scale at which spatial differences in microplastic pollution occur along the coast is important for the consumer when mussels are harvested from the wild; moreover, even those that are farmed are grown directly in coastal waters. It seems that microplastics may vary at a relatively short scale in *Mytilus*. In southwest England, Scott et al. (2019) found between 1.43 ± 0.30 and 7.64 ± 1.61 items per individual in a few hundred kilometres alongside the coast, but the differences were apparently smaller in northern Ionian Sea where they ranged 2.46–5.26 items/g (Digka et al., 2018).

However, there are some limitations in microplastics studies that make it convenient to be cautious about generalizations regarding their variation among populations and microplastics risks. The abundance of microplastics in bivalves is difficult to compare between studies (Zhang, Man, Mo, Man & Wong, 2020), because microplastics concentration not only depends on the environment but changes with mollusc age, being the retention time higher in smaller mussels (Van Cauwenberghe & Janssen, 2014; Fernández & Albentosa, 2019). A general drawback is a lack of standardisation of methods for microplastic extraction in biota and environmental components, and of the units used for quantification (Besley, Vijver, Behrens & Bosker, 2017). For these reasons, studies based on standard methodologies, with homogeneous units across samples, and performed at different spatial scales are necessary to understand potential risks for consumers of marine mussels associated to microplastics.

The present study aims to estimate the potential ingestion of microplastic derived from consumption of *M. galloprovincialis* in the southern Bay of Biscay. This species was chosen because it is highly consumed worldwide and has a great economic value, but its production is declining in Europe due to adverse environmental changes, amongst them increased plastic and microplastic pollution (Avdelas et al., 2021). Differences in sand microplastic content have been previously found at a short scale in southwest Bay of Biscay beaches (Masiá, Ardura, Gaitán, et al., 2021), thus this region was chosen to illustrate the different risks depending on the harvesting or aquaculture location. To achieve the aims of the current study, the concentration of microplastics in *M. galloprovincialis* from different locations was estimated, the chemical composition of these microplastics were determined by spectroscopy, and their health risks analysed using the European Chemical Agency as information source. The risk of microplastic ingestion by consumers was estimated considering individuals of harvestable size, average serving size, and table salt which is employed in mussels cooking recipes. Our expectations were that microplastic concentration in mussels was correlated with that of the environment, and the risk would be higher when consuming mussels from more polluted beaches, and salt could add a non-negligible risk to the dish since some studies report high microplastic content in some table salt brands (e.g., Yang et al., 2015; Lee et al., 2021).

2. Materials and methods

2.1. Ethics statement

The project was approved by the competent research ethics committee of the Government of Asturias Principality, General Directorate of Maritime Fisheries, project code IDI-2018-00201.

2.2. Sampling region, sampling sites and samples analyzed

The Spanish region of Asturias (southwest Bay of Biscay), where the study was carried out, has artisanal fisheries important for employment in the coast and with cultural and traditional values, which make them one of the most valuables activities in the region (García de la Fuente et al., 2013; García de la Fuente et al., 2020). Fish and seafood are highly consumed (García de la Fuente et al., 2013). The most abundant native mussel species is *Mytilus galloprovincialis*, a member of the *M. edulis* complex.

Ten different brands of commercial salt were purchased from local supermarkets. From the information on the labels, two were of continental origin, seven were marine salt and in one product the origin was not disclosed.

Mussels (*Mytilus galloprovincialis*), water and sand were sampled from seven different beaches distributed irregularly over 130 km along the coast of Asturias. From west to east they were: Arnao, Peñarronda, Otur, Zeluan, Xago, El Puntal and Rodiles (Fig. 1). They are exposed to different anthropogenic stressors and exhibit different levels of microplastic pollution in sand (Masiá, Ardura, Gaitán, et al., 2021). In the beaches considered, where commercial harvesting is not practiced, mussels are taken for personal consumption. The minimum size at catch legally established for mussels ("*mejillón*" in Spanish) is 50 mm in Asturias (see the legal minimum catch sizes of molluscs at the webpage of the General Fisheries Directorate https://tematico.asturias.es/dgpesca/din/tallas.php?tipo=moluscos).

A total of 86 mussels (9–20 per beach) were sampled at random from the intertidal level on March 2019. Mussels of similar size were targeted, thus the differences in the number of samples per beach were due to the availability of the species and to differences in size between sampling sites. Samples were directly taken to the laboratory in coolers, and once in the laboratory, mussels were unfrozen for immediate examination. Five litres of water were taken from the surface, in each beach, close to the spots where mussels were taken from. From each beach, 20 sand samples were taken from five random quadrants (four samples per quadrant) along a 100 m transect parallel to the tidal line, as described in Masiá et al. (2019).

2.3. Microplastics extraction and analysis

For microplastic quantification from commercial salt, 125 g of salt were diluted in 200 mL of distilled water previously filtered, then stirred until dissolved, and then filtered with a vacuum pump, using the same filtration system as for mussels.

Mussels were measured then the whole body was removed from the shells, weighted, and placed in a glass recipient. To digest the tissues, a protocol by Li et al. (2015), slightly modified, was followed. In brief, 200 mL of H_2O_2 (LABKEM- Labbox, Barcelona, Spain) per 10 g of tissue were added, and glasses were placed in the oven at 65 °C for 24 h, followed by 24 h at room temperature. Then, 800 mL of prefiltered distilled water were added to the samples to facilitate the filtration and filtered through 0.45 μm pore size polyethersulphone membranes of 47 mm diameter (Supor® PES Membrane filters, Pall Corporation, Port Washington NY, USA), using a vacuum pump. Four to five filters were used per litre to avoid clogging.

Water samples were directly carried to the laboratory and filtered using the same membranes described above. Quantification of microplastics from sand followed a protocol adapted from Besley et al. (2017), thoroughly described in Masiá et al. (2019). Briefly, sand samples were allowed to dry in the laboratory. Microplastics were separated from the sand by density using an oversaturated solution of NaCl (LABKEM-Labbox, Barcelona, Spain), stirred, then the supernatant was filtered.

To count microplastics filters were placed in petri dishes and observed under the stereomicroscope (40x magnification). Microplastics were counted and classified by shape (fragment or fibre) and colour. A subsample of items from all analysed matrices was randomly selected for chemical identification that was performed by Fourier-Transform Infrared Spectroscopy (FT-IR) with micro-ATR (Attenuated Total Reflection). This analysis was conducted at the Interdepartmental Research Service, Universidad Autónoma de Madrid, Spain. The following settings were applied: spectral range of 4000–550 cm^{-1}, resolution 16 cm^{-1}, opening 50x50 microns. Comparison with reference samples was made using PerkinElmer Spectrum version 10 and the best

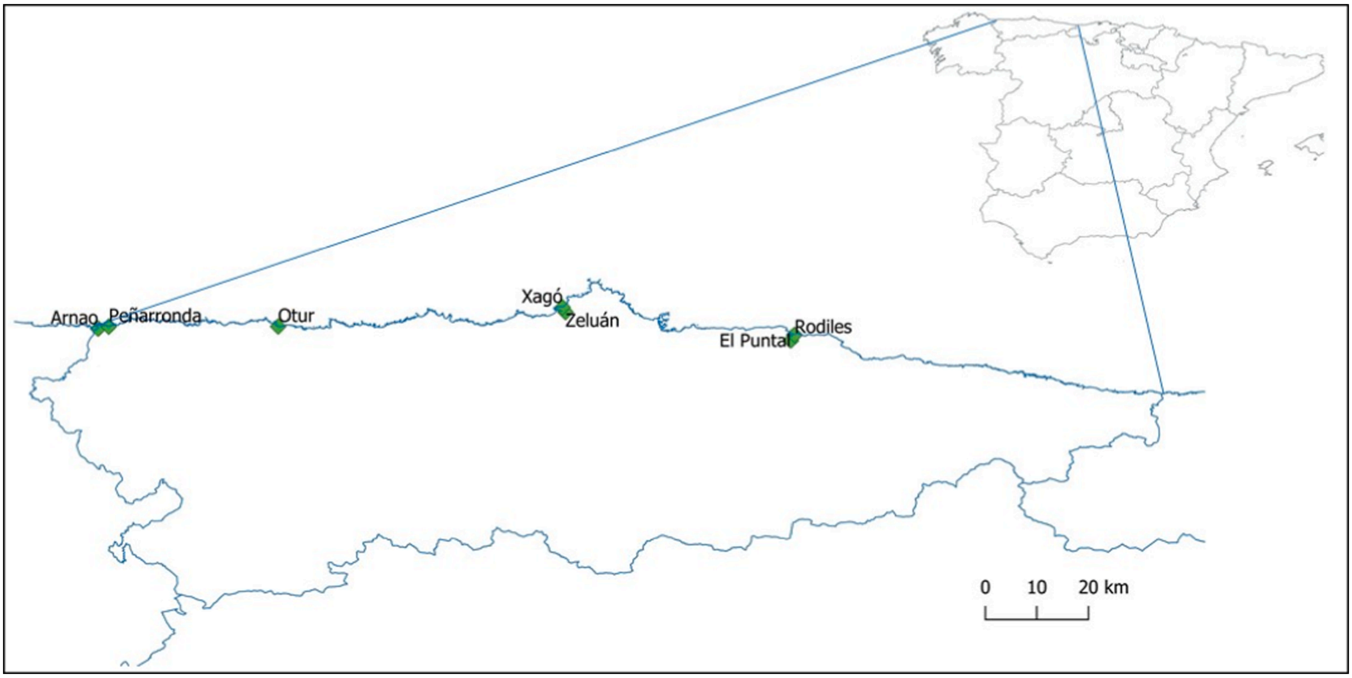

Fig. 1. Map of the considered area (Asturias coast) showing the beaches sampled for mussels *Mytilus galloprovincialis*. Mussels, sand, and water from these beaches were analyzed for microplastics. From west to east: Arnao, Penarronda, Otur, Zeluan, Xago, El Puntal and Rodiles.

hit was retained.

The procedures described above were done under controlled conditions to prevent contamination of the samples. All the material and surfaces were previously washed and rinsed, and samples and equipment kept under a closed laminar flow cabinet to prevent airborne contamination. Samples were manipulated in a closed laminar flow cabinet as well. A procedural blank, consisting of the entire protocol without the sample, was performed for each set of microplastics extraction (water, sand, mussels and commercial table salt) to check for possible contamination. Petri dishes where the filters were placed were closed all the time, being opened only when subsamples were taken for identification by FT-IR.

2.4. *Estimates of microplastic ingestion and health risks*

In Spain, as in many other countries, mussels are commonly cooked using different recipes with salt. For estimations of microplastic ingestion risk (in number of microplastics), servings of 20 mussels of catchable size (>50 mm) were taken as average number of mussels an adult eats in a regular meal. The number of microplastics per serving was calculated multiplying by 20 (serving size) the mean number of microplastics per individual found in each location analysed In addition, 10 g of salt was estimated for cooking. In this case the risk was calculated multiplying the number of microplastics per gram by 10, for each brands of table salt analysed.

Potential health risks due to harmful chemicals of microplastics found in mussels and table salt were checked in the European Chemical Agency (ECHA; www.echa.europa.eu/home, accessed on December 2021). Those health threats are based on harmonized classification and labelling (CLH) aligned with the Regulation (European Commission EC) No 1907/2006 of the European Parliament and of the Council of 18 December 2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH), available at https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:02006R1907-20210101. ECHA has substance information cards that display the substance identity in the EC list and its CLH. Substances are classed by hazard using the following categories: None (no hazard confirmed to date); Warning, or less severe hazard categories; Danger, or severe hazard categories. The types of hazard (like acute toxicity, carcinogenic, environmental damage, etc.) are summarized for each substance. In the present study, health and environmental hazards of mussels and table salt were identified for the substances detected with FT-IR in each matrix. The parent substance may be employed in case of unlisted polymers since it may be produced from degradation.

2.5. *Statistical analysis.*

The variables employed in this study were items/g tissue or items/individual in mussels. Items/L in water, and items/Kg frequently employed for sand, were transformed to items/g to have comparable units in all the matrices. Transformation of items/L to items/g was done considering mean seawater density of 1020 Kg/m^3. Dataset normality was checked with Shapiro-Wilk tests. Pearson's correlation tests were used to determine the association between microplastics in mussels and in environmental matrices. Differences between samples (e.g., between beaches, or between matrices i.e. water, sand, mussels, table salt) for chemical profiles or microplastics type composition were tested for significance using Chi Square tests and Monte Carlo permutations ($n = 9999$). Differences between samples for mean microplastic density –in items/g- were tested using analysis of variance ANOVA (several samples, e.g., mussels, sand and water matrices) or *t*-test (pairs of samples, e.g., table salt of continental versus marine origin). Standard significance threshold of $p < 0.05$ was considered. Data analysis was performed using PAST software version 4.08 (Hammer, Harper & Ryan, 2001).

3. Results

3.1. *Microplastics content in mussels and its spatial variation*

The 86 mussels collected in this study had an average size of 3.08 cm (standard deviation SD = 1.01), with the following proportion of individuals of a harvestable size (>50 mm): 23.5% in Zeluán, 20% in Otur, and 28.5% in El Puntal; none in the other beaches. Microplastics were found in every mussel analysed (mean = 2.65, SD = 1.46 items/individual), ranging from 1.2 items/individual (SD 0.21) in Penarronda, to

5.45 items/individual (SD = 0.68) in Zeluán (Table 1). Regarding microplastic concentrations, the mean of all the mussel samples was 1.62 items/g (SD = 1.0): from 0.6 items/g (SD = 0.09) in Otur to 3.2 items/g (SD = 0.43) in Rodiles (Table 1). From all the microplastic items found in mussels, 87.74% were fibres, 6.37% were fragments of plastic and 5.88% were pellets.

For environmental samples, microplastic concentration in water varied from 2.3 items/L (SD 3.37) in Otur, to 14.66 items/L (SD 8.93) in El Puntal (mean 7.61, SD = 3.86 items/L) (Table 1). From them, 98.12% were fibres, 1.25% plastic fragments and 0.63% were pellets. For sand samples, values ranged from 382 (SD 65.88) items/Kg in Rodiles, to 58 (SD 8.33) items/Kg in Otur (mean of 263.57, SD = 112.11 items/Kg) (Table 1). Of all the microplastics found in sand samples 98.9% were fibres, 1.19% plastic fragments, and 0.32% pellets.

Blanks (two per matrix –mussel tissue, water, sand) provided a few fibres, with a mean concentration of 0.667 (SD = 0.327) items per sample. Thus, contamination due to external factors could be considered very small or negligible. The numbers of microplastics found for each category (fibres, fragments, pellets) and colour are in Supplementary table 1.

In every sampling site, the concentration of microplastics was greater for mussels than for sand (items/g); and in sand it was greater than in water (items/g); see Table 1. The difference between an ecosystem matrix and the next was around one order of magnitude (means of 1.62 items/g in mussels, 0.26 items/g in sand, 0.076 items/g in water). Shapiro-Wilk test showed a normal distribution of samples for total microplastics concentration; thus, ANOVA was used in comparisons among matrices and Pearson coefficient in correlation tests. The difference between matrices was highly significant (ANOVA with $F_{2,18} = 15.05$, $p = 0.0001$; Table 2).

The spatial variation in the concentration of microplastics in mussels occurred at a very short scale. For example, mussels inhabiting the neighboring beaches Arnao and Penarronda – located a few kilometers apart- exhibited 0.2 and 2.4 microplastics/g respectively, and Rodiles and El Puntal, less than two kilometers distant, had 1.36 and 3.2 respectively (Table 1). This short-scale variation was parallel in mussels and sand. Microplastics concentration in mussels was strongly correlated with the concentration in sand (r = 0.95; d.f. = 5, $p = 0.001$), but no correlation was found between any pair of variables involving water (water-mussels: r = 0.28, d.f. = 5, $p = 0.54$; water–sand: r = 0.29; d.f. = 5, $p = 0.53$).

The items found in water, mussels and sand exhibited similar but not identical shape and colour patterns (Fig. 2). As explained above, for every matrix and sampling site, the vast majority of items were fibres (95.18% of all samples), followed by plastic fragments (4.2%) and pellets (2.2%). The most abundant type were blue fibres (34.74% of all samples), followed by white (34.86%) and black (19.36%) fibres. Pellets were found only in Zeluán's mussels and water, and in Xagó and Penarronda sand. However, the proportion of types of items differed significantly between matrices and sampling sites (global Chi Square = 710.8, 120 d.f., $p \ll 0.001$; Monte Carlo permutations with $p \ll 0.0001$).

Table 1
Summary of the concentration of microplastics found in the three matrices analyzed (water, sand, and mussels), per beach. Results are presented as mean (standard deviation) of microplastic items per gram. N = number of individuals analyzed for microplastics. Sand results are also found in Masiá, Ardura, Gaitán et al., (2021).

	Water	Sand	Mussels	Mussels N
Arnao	0.008 (0.002)	0.237 (0.039)	0.55 (0.094)	12
Penarronda	0.007 (0.001)	0.353 (0.055)	2.40 (0.42)	20
Otur	0.002 (0.003)	0.058 (0.008)	0.57 (0.09)	10
Zeluán	0.006 (0.0008)	0.27 (0.044)	1.164 (0.146)	9
Xagó	0.006 (0.001)	0.344 (0.05)	2.20 (0.367)	10
El Puntal	0.015 (0.009)	0.201 (0.034)	1.36 (0.177)	9
Rodiles	0.010 (0.002)	0.382 (0.066)	3.20 (0.431)	16

Table 2
Analysis of variance comparing microplastics concentration among ecosystem matrices (mussels, sand, and water) in the seven beaches considered.

Source of variation	Sum of squares	d.f.	Mean square	F	*p* (same)
Between matrices:	10.396	2	5.198	15.05	0.00014
Within matrices:	6.218	18	0.345		
Total:	16.614	20			

Although water samples were not significantly different among beaches (Chi Square = 51.59 for 36 d.f. with $p = 0.045$, Monte Carlo permutations with $p = 0.052 > 0.05$ NS), the spatial difference (among beaches) was highly significant for both sand (Chi Square = 177, d.f. 36 with $p \ll 0.001$, same significance for Monte Carlo permutations) and mussels (Chi Square 100.9, 36 d.f., $p \ll 0.001$ also for Monte Carlo). Clear differences between the microplastics profiles of mussels in pairs of neighbouring beaches can be observed (Fig. 2), emphasizing the variation between samples at a short spatial scale. For example, Penaronda and Arnao differed significantly in their microplastics profiles (Chi Square 13.9, 4 d.f., $p = 0.008$).

Sand and mussels items profiles did not differ significantly to each other in Penarronda (Monte Carlo $p = 0.378$ NS), Xagó (Monte Carlo $p = 0.164$ NS) and Otur (Monte Carlo $p = 0.476$); all Chi Squares not significant (data not shown). However, they were significantly different in the other four beaches (data not shown), thus drawing general conclusions about of microplastics profiles in mussels from sand is not possible with these data.

3.2. Microplastic content in salt

In table salt samples (Table 3) microplastics ranged from 0.1 (SD 0.02) items/g to 0.38 (SD 0.05) items/g (total mean 0.24, SD 0.09 items/g). The main type of item found was fibres (94.4%), and a few plastic fragments (1.6%).

Considering only the brands displaying geographic information on the labels (Table 3), salts of continental origin exhibited significantly higher concentration of microplastics than those of marine origin (means of 0.34 with SD = 0.017 and 0.168 with SD = 0.09, respectively; t = 2.46, $p = 0.04$).

3.3. Consumption risk estimates

For a serving of 20 mussels (around 80 g of fresh mussel tissue for small mussels like those sampled in the present study), consumers would ingest between 24 microplastics per serving if harvested from Penarronda to 109 (108.9) if harvested from Zeluan, in average (Table 4). Using 10 g of table salt for cooking the mussels, consumers could ingest between 1 and 4 (3.8) microplastics per serving depending on the brand (Table 4), in the least favourable case of all the items in the salt adhering to mussels' bodies while cooking. This would make up to 113 microplastics per dish serving eating mussels from Zeluan. From these estimates, the risk of microplastics ingestion posed by mussel consumption in this case would be around 27-fold higher than the risk derived from the addition of salt to the dish.

To evaluate potential health risks, a subsample of 59 items (7 from water, 10 from sand, 20 from mussels and 22 from table salt), corresponding to 7% of the total of items detected in each matrix, was randomly selected for FT-IR analysis. A few items were identified as cellulose or other natural element (e.g., linseed oil film) and were not considered in the present study. The majority of artificial items found in water (40%) were composed of polyethylene, followed by polyester, methylsulphonyl aniline hydrochloride and rayon in the same proportion (Fig. 3). In sand, items were of rayon (40%), polyvinyl chloride, polyethylene, polyester, and polystyrene (Masiá, Ardura, Gaitán, et al., 2021). In mussels, 45% of the 17 fibres and 3 pellets analysed were of rayon, followed by polyester, polystyrene, polyethylene, and poly

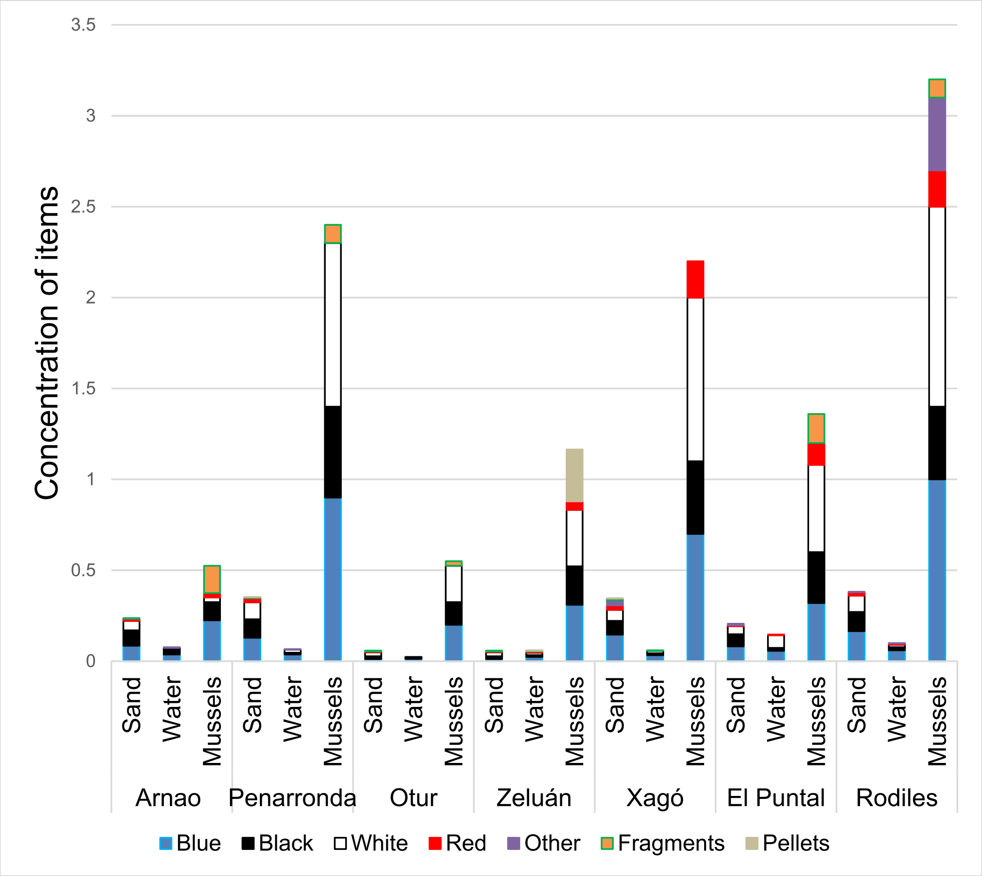

Fig. 2. Proportion of different types of microplastics found in different matrices (sand, water and mussels) analyzed in the seven beaches considered in southwest Bay of Biscay. Microplastic particles are classified by shape (fibers, plastic fragments, pellets), and the fibers by color as blue, black, white, red, and other (colors). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 3
Proportion of different types of microplastics found from ten brands of commercial salt sold in Spain (A-J), and number of microplastics per gram of salt in the 125 g of salt analyzed (item/g). Blue, Black, White, Red and Other refers to fiber colors; fragments are microplastic fragments of any color. Brand names are replaced by capital letters (A-J). Origin: geographic origin as displayed on the product label, as C, continental; M, marine; ND, not disclosed.

Brand	Blue	Black	White	Red	Other	Fragments	Item/g
A-ND	0.25	0.27	0.25	0.21	0.02	0.00	0.38
B-M	0.46	0.15	0.38	0.00	0.00	0.00	0.10
C-M	0.44	0.11	0.30	0.07	0.04	0.04	0.22
D-M	0.50	0.17	0.17	0.10	0.03	0.03	0.24
E-C	0.37	0.20	0.24	0.12	0.02	0.05	0.33
F-M	0.43	0.13	0.35	0.04	0.04	0.00	0.18
G-M	0.40	0.24	0.32	0.00	0.00	0.04	0.2
H-C	0.50	0.20	0.25	0.05	0.00	0.00	0.35
I-M	0.57	0.23	0.14	0.03	0.03	0.00	0.28
J-M	0.68	0.11	0.16	0.05	0.00	0.00	0.15

(diallyl phthalate). The difference in microplastics type between water, sand and mussels was not significant (Chi Square = 12.8 with 12 d.f., $p = 0.38 > 0.05$). Rayon, polyethylene and polyester occurred in water, sand, and mussels, and polystyrene in mussels and sand (Fig. 3).

Commercial table salt (7.2% of the total number of items found in salt samples) contained a few fibres of cellulose and one of cotton that were not considered in this analysis. The most abundant artificial material was rayon (56%), followed by polyester (19%) (Fig. 3). Substances like chloroquine and acetaldehyde found in some items are not properly plastic; they most likely come from degradation of the microplastic items analysed or were deposited on them.

Table 4
Risk estimates of microplastics ingestion from mussels and salt, for each location and brand of table salt analyzed. Calculated as the mean number of microplastics ingested for a serving of 20 mussels and 10 g of salt. Samples with mussels of harvestable size, marked in bold.

	Beach	Item/mussel	SD	Ingestion risk
Mussels	Arnao	1.75	0.29	35
	Peñaronda	1.2	0.21	24
	Otur	**2,2**	**0.38**	**44**
	Zeluan	**5.44**	**0.68**	**108.89**
	Xagó	2,2	0.37	44
	Puntal	**3.78**	**0.49**	**75.56**
	Rodiles	2	0.27	40
Salt	Brand	Item/g		Ingestion risk
	A	0.384		3.84
	B	0.104		1.04
	C	0.216		2.16
	D	0.24		2.4
	E	0.328		3.28
	F	0.184		1.84
	G	0.2		2
	H	0.352		3.52
	I	0.28		2.8
	J	0.152		1.52

Seven of the substances identified from FT-IR in mussels and salt are classified as hazardous by the ECHA (Table 5), with Warning or Danger signals. Mussels contained aniline derivatives, diallyl phthalate, polyester and polystyrene resin. The two first are harmful if swallowed; aniline derivatives and polyester can damage organs (such as the eye)

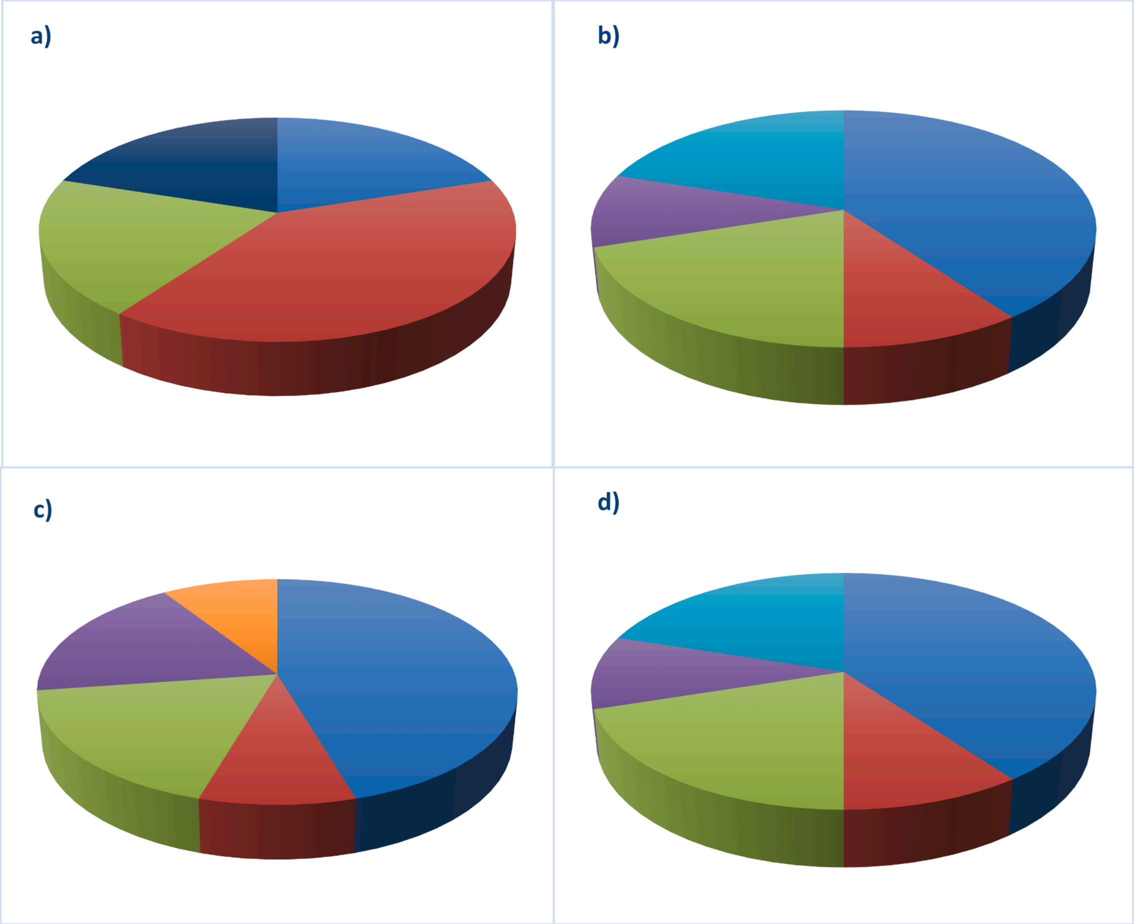

Fig. 3. Chemical fingerprint of the microplastic items examined using FT-IR, from the four matrices considered. Results are presented as the proportion of items of each material found. a) Water (n = 7); b) Sand (n = 10); c) Mussels (n = 20); d) Salt (n = 22).

Table 5
Health hazards of substances identified from FT-IR in the microplastics found from mussels and table salt in this study. Pre-registration means that the substance has not been evaluated by the European Chemical Agency yet. X = confirmed harm; ? = not evaluated.

					Health hazard			Environmental hazard	
	EC list number	Mussels	Table salt	Warning signal	Harmful if swallowed	Damage to organs	Cancer	Acute toxicity	Long lasting effects
Acetaldehyde	686-082-4		X	Danger	X	X	X		
Aniline derivatives	951-019-4	X		Warning	X	X		X	X
Chloroquine	200-191-2		X	Warning	X				
Diallyl phthalate	205-016-3	X		Warning	X			X	X
Polyether polymer	946-213-0		X	Warning	X	X			
Polyester	630-340-0	X	X	Warning		X			
Polyethylene	618-339-3	X		No hazards classified					
Polyethylene terephthalate	607-507-1		X	No hazards classified					
Polystyrene resin	935-499-2	X		Warning					X
Rayon	612-376-9	X	X	Pre-registration	?	?	?	?	?

and cause skin irritation; polystyrene causes long lasting harm to the environment (Table 5). The table salt analysed contained three substances harmful if swallowed (acetaldehyde, chloroquine and polyether), and polyester. Acetaldehyde is tagged with the signal of Danger in the ECHA list because it can damage organs and cause cancer as well (Table 5).

Summing the hazards of the products contained in microplastics of this potential dish, mussels would pose nine (five environmental and four of health) and salt seven (Table 5). Not only for the amount of microplastics ingested but also for the number of potential hazards, mussels would be more hazardous than table salt for consumers in this particular case. Besides, the ingestion of microplastics from salt would be less probable because the amount of salt employed in the dish is much smaller, in weight, than that of mussels.

Since the minimum harvestable size for mussels in Asturias is 50 mm, in the present case only those sampled from Otur, Zeluán, and El Puntal could be collected for consumption. Zeluán was the location with the highest number of microplastics per individual, and El Puntal was the second one (Table 4). Frequent consumers of wild mussels of harvestable size from these locations should be aware of the number of microplastics ingested.

4. Discussion

This study presents important novelties in the fields of food safety and environmental health. As expected, the estimated risk of microplastic ingestion from mussel consumption was shown to depend on the specific collection location, differing significantly at a very short scale (Masiá, Ardura, Gaitán, et al., 2021). The risk was also a little bit different depending on the type of salt employed for cooking. Microplastic ingestion estimates from 25 (24 from mussels + 1 from salt) to 113 (109 from mussels and 4 from salt) are high in comparison with the average daily consumption of 142 microplastics by American male adults (Cox et al., 2019). A single standard serving of mussels from this Bay of Biscay region would represent between 18% and 80% of the total number of microplastics acquired normally via diet by male adults in a full day, being up to 96% of the ingestion in female adults (126 in average following Cox et al., 2019) and the 100% in the case of American male children (Cox et al., 2019). Although high, the risk estimated is not exceptional because the concentrations of microplastics in mussels found in this study (between 0.55 and 3.20 items/gram and ranging from 1.2 to 5.45 items/individual) fall within the range published for south Bay of Biscay (between 0 and 8.90 items/g, Reguera et al., 2019) or the French Atlantic coast (between 0 and 8 items/individual, Phuong, Poirier, Pham, Lagarde & Zalouk-Vergnoux, 2018). However, it must be pointed out that unlike in those studies, in the particular zone studied here, all the individuals had at least one microplastic item, emphasizing high ingestion risk.

The contribution of table salt to the risk of microplastics consumption was relatively low in this study (mean of all brands of 0.24 items/g, SD 0.09), in comparison with other studies (e.g., Yang et al., 2015; Lee et al., 2021). According to the results, only two microplastics will be added per serving in average, supporting the idea of Karami et al. (2017) about very small or intake of microplastics throughout sea salt. Lower microplastics content in common sea salt than in brands of continental origin found in this study would concur with other publications, where regular sea salt contains fewer microplastics than some speciality products (e.g., Fischer, Goßmann & Scholz-Böttcher, 2019). However, due to limited number of salts analysed here and the lack of harmonisation in analytical procedures in this field (Lee et al., 2021), this interpretation should be taken with caution.

Regarding specific risks for human health, the continuous exposure to microplastics in humans can cause damages such as granulomatous lesions and cancer in lungs (Wright & Kelly, 2017). Health risks derived from consumption may be serious too. Microplastics can enter the gastrointestinal tract and the circulatory system trough endocytosis, and may interact with different organs and cells, producing inflammation, cytotoxicity, or haemolysis, among other toxicological effects (Campanale, Massarelli, Savino, Locaputo & Uricchio, 2020). Moreover, microplastics ingested through seafood can release harmful chemicals employed in manufacturing or be adsorbed to them (Smith, Love, Rochman & Neff, 2018). In the current study, some of the chemical constituents of the microplastics found in mussels are catalogued as hazardous for humans, like the diallyl phthalate and aniline derivatives found in some items, or polyester, according to the European Chemicals Agency (ECHA). Likewise, microplastics found in table salt in this study contained chemicals that are dangerous for the consumers, as recognized by authoritative agencies. If consumed on a regular basis, with or without mussels, the brands of salt with higher microplastic concentrations like A, F or H in the present study could represent a significant diet-borne risk.

In addition to the risk posed to consumers, microplastics accumulation in mussels is harmful for the mussel itself, and consequently for the fishing resource. Although mussels can easily eliminate big microplastics in faecal pellets (Kinjo, Mizukawa, Takada & Inoue, 2019; Piarulli & Airoldi, 2020), small microplastics can translocate within epithelial cells causing damage to the organism (Von Moos, Burkhardt-Holm & Koehler, 2012; de Sá et al., 2018; Fernández and Albentosa, 2019). Effects of microplastics reported in *M. galloprovincialis* are varied, from alteration of the immune system, upregulation of some genes, DNA strand breaks in haemocytes, DNA degradation in gills and reduction of the nutritional status, and changes in their condition factor among many others (Capolupo, Franzellitti, Valbonesi, Lanzas & Fabbri, 2018; Pittura et al., 2018; Masiá, Ardura, & García-Vázquez, 2021). Although these results are based on experimental studies and use much smaller particles than those found in the present study, sometimes at environmentally unrealistic concentrations (Paul-Pont et al., 2018), high microplastics content in mussels could be considered a risk for the resource (Chen, Lu, Yang & Liao, 2021; Shang et al., 2021). The uptake of microplastic by mussels has also wider ecological implications, as their faeces containing microplastics can sink entering the benthic environment and becoming available for other organisms (Fernández & Albentosa, 2019; Piarulli & Airoldi, 2020).

An interesting result of this study was that, as it happens in sand (Masiá, Ardura, Gaitán, et al., 2021), spatial differences in mussels' microplastic pollution occurred at a very short scale of a few kilometres, at both microplastics concentration and microplastic types. In other words, mussels collected from different locations –even very near- pose different risks of microplastic ingestion to the consumer. The scale at which mussel microplastics pollution differs significantly between locations was smaller in this study than in previous works (e.g., Digka et al., 2018; Scott et al., 2019; Kazour & Amara, 2020), highlighting the importance of local pollution sources in the amount of microplastics in this species.

Confirming departure expectations, the concentration of microplastics in mussels was significantly correlated with sedimentary environmental microplastics pollution. Masiá, Ardura, Gaitán, et al. (2021) found a gradient of microplastics contamination in the seven beaches here considered consistent with: Rodiles > Penarronda > Xago ≫ Zeluan > Arnao > El Puntal > Otur. The results in mussels almost mirrored that gradient, especially in the most polluted part of the list (Table 1): Rodiles > Penarronda > Xago ≫ El Puntal > Zeluan > Otur > Arnao. However, as in other studies, mussel microplastics content was not correlated with that of water, only with sand microplastics (e.g., Li et al., 2018). Seawater is renewed with each tide, transporting microplastics from the sea to the beach back and forth and microplastics tend to form aggregates with organic matter and sink rapidly from the water column, accumulating in sediments (Davis & Murphy, 2015; Summers, Henry & Gutierrez, 2018). Thus, water samples would be less representative of the general level of pollution in a beach than sand, where particles will be accumulated and stay for a longer time. On the other hand, the majority of microplastics found in mussels were microfibers. Although other studies reported higher contents of fragments than microfibers in mussels (Hermabessiere et al., 2019; Kazour & Amara, 2020), other current studies have reported fibres as the most common shape of particle (Marques et al., 2021); in this particular study, more microfibers could be explained from an overwhelming proportion of microfibers in the coastal environment in this region (Masiá, Ardura & Garcia-Vazquez, 2019, Masiá, Ardura, Gaitán, et al., 2021).

Importantly, the amount of microplastics in mussels (items/g) found in this study, was ten times greater than the amount of microplastics found in sediment in the same area. This supports the idea of these organisms bioaccumulating microplastics from the environment (Karlsson et al., 2017), and therefore, their consumption in highly polluted areas may represent a real risk for human health. On the other hand, selective retention of artificial cellulose-modified fibres (rayon) while less polyvinyl items in mussels, proposed by Scott et al. (2019), could not be confirmed in the present study since significant differences in microplastic materials between mussels and environmental matrices were not found.

About the possible weaknesses of this work, the present study has a limitation due to relatively small sample sizes (number of mussels analyzed) in some beaches. However, since the samples are biologically homogeneous belonging to the same species, trophic level, ecoregion and feeding type, they could be considered a single unit following Hermsen et al. (2018). These authors recommend a minimum of 50 individuals per unit to be analysed in microplastics studies, and in this study the total number of individuals analysed was 86, which, although split in different locations, could be considered sufficient for the small region considered. From the technical point of view another possible weakness is the detection limit; being analysed visually under the stereomicroscope, the smallest particles can escape the present study: fractions of fragments down to 10 μm. Knowing this limitation, all procedures were performed by a single researcher to avoid further bias when extracting particles for chemical identification. Although new analytical methodologies have been developed (Stock, Kochleus, Bänsch-Baltruschat, Brennholt & Reifferscheid, 2019), the methodology chosen in this work has been carried out in order to be able to compare between the different samples collected and previous studies developed, and it is a fast, cheap and valid technique, which is still in use (e.g., Pazos, Spaccesi & Gómez, 2020).

The results of this study suggest some recommendations for consumers. First, the ingestion of microplastics should be limited since, as seen in this study, some of them contain harmful chemicals. Therefore, harvesting seafood in areas with high levels of microplastic pollution should be restricted, as it is already established for other contaminants and toxic compounds (Shuval, 2003; Chigbu, Gordon & Tchounwou, 2005; Evans, Athearn, Chen, Bell & Johnson, 2016). Although the present results about microplastics show different pollution in different beaches, they are inconclusive regarding pollutants like POPs or metals dissolved in the water, that behave very differently of microplastics (Kögel, Refosco & Maage, 2020). Analysis of microplastics at a local scale would be necessary, as microplastic pollution has been shown to be not homogeneously distributed alongside the coast, and even in nearby beaches the content can vary. Consequently, microplastics should be regularly monitored in harvesting areas, as is done with other toxic elements like heavy metals and with faecal contamination, in order to provide safe seafood.

Secondly, as for many other contaminants such as heavy metals or persistent organic pollutants (POPs), safe thresholds for consumption should be investigated (Bezerra, Lacerda & Lai, 2019; Johnson et al., 2013). If a precautionary approach is adopted regarding this emerging pollutant, microplastics analysis of seafood and table salt is recommended. Including microplastics content in seafood labels is suggested to give the consumer the opportunity to make conscious choices. In addition, in order to set a basis to monitor for management, field studies need to move on further from semiquantitative studies to quantitative studies, including recovery and measurement uncertainty analysis.

Overall, although some of the microplastics found can suppose a risk for human health, the real risk is still unknow, and benefits from consuming sea products are still favourable to the consumer (e.g., Thomsen et al., 2021). Seafood has been more studied for microplastics than other types of food; however, it should not be avoided from diet or substituted based only on present results. Further investigations should be done in this and other commercial species in order to determine the general risks for human consumption of seafood in the region.

5. Conclusion

In the present study, microplastics risks associated to mussel consumption were estimated for the first time in south Bay of Biscay. Results indicated that consumption of those microplastic-polluted mussels can put human health at risk from the chemical constituents of microplastic items. Differences in the abundance of microplastics in mussels at a short spatial scale have been seen for the first time alongside Asturias coast. The quantitative analysis of microplastics in *Mytilus galloprovincialis* showed a significant correlation between sediments and mussels, being ten times greater in organisms than in sediments and supporting the value of this species for microplastics biomonitoring. Due to the significant differences between neighbouring sampling sites, to monitor microplastics at a local scale in harvesting areas is recommended, as well as the disclosure of microplastics concentration in seafood labels. Further investigations on safe consumption thresholds for microplastics is also recommended, as well as to broaden studies regarding microplastics in marine commercial species in the studied area and other regions.

CRediT authorship contribution statement

Paula Masiá: Investigation, Methodology, Resources, Validation, Writing - original draft. **Alba Ardura:**Conceptualization, Data curation, Formal analysis, Investigation, Resources, Supervision, Writing - review & editing. **Eva Garcia-Vazquez:** Conceptualization, Data curation, Formal analysis, Investigation, Resources, Supervision, Writing - review & editing, Project administration, Funding acquisition.

Funding Sources

This study has been funded from the Spanish Ministry of Science and Innovation, Grant GLOBALHAKE PID2019-108347RB-I00 and MCIU-19-EIN2019-103189, and from the Government of Asturias Grant IDI-2021-000040.

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.

Acknowledgments

We are grateful to Thomas E. Holloway for proofreading the manuscript.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodres.2022.110973.

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