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Dangerous microplastics in topshells and anemones along the north coast of Spain

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Abstract

While levels of microplastics and other pollutants keep increasing in all coastal habitats, seafood is being eaten all over the world. In this research, three edible species were sampled from six points along the central north coast of Spain: *Actinia equina* anemones and *Phorcus lineatus* and *Steromphala umbilicaris* topshells (N = 100). Putative microplastics (N = 2157) were identified, counted, and many analyzed through FT-IR spectroscopy. Herbivorous topshells contained significantly more microplastics than carnivorous anemones. The most common particles were fibers, with transparent, blue and black as most prominent colours. Plastics included PE, polyester, PET, PP, nylon, PS, PVB and acrylic fibers. The sampled items contained several harmful compounds, including PTTC of which even one particle could be fatal if inhaled. This highlights the urgent need for studies regarding the safety of seafood.

Key words: Anemones; Topshells; Microplastic content; Harmful chemicals; FT-IR spectroscopy.

1. Introduction

Microplastics can be defined as “any synthetic solid particle or polymeric matrix, with regular or irregular shape and with size ranging from 1 µm to 5mm, of either primary or secondary manufacturing origin, which are insoluble in water” (Frias & Nash, 2019). Microplastics are extremely endurable, which enables them to persist in the environment for a very long time (Hammer et al., 2012). Consequently, they become increasingly prevalent in the environment and ecosystems, where they accumulate and pose a threat to all living creatures. The ultimate sink of microplastics in the planet is the ocean, where they finally end after making their way through rivers and air (Hale et al., 2020). As a consequence, microplastics have been reported in a wide range of marine organisms, including invertebrates and many species consumed as seafood (de Sá et al., 2018; Van Cauwenberghe & Janssen, 2014; Vandermeersch et al., 2015; Wright et al., 2013).

Not all the marine species are equally sensitive to microplastics pollution, nor take microplastics from the environment at the same rate. This means that different species react in a different way to microplastics pollution. Relationships between amount of microplastics and feeding strategy have been proven. In their review, Wright et al. (2013) described a high level of microplastics intake in detritivores and sediment-feeding organisms, like holothurians; as well as in filter-feeding animals like mussels and in some active planktivores such as ciliates.

Setälä et al. (2016) exposed Balthic invertebrates to different microbeads concentrations and discovered that filter-feeding bivalves ingested significantly higher amounts of beads than other groups (predators, deposit-feeders). Regarding the trophic level, results are still insufficient but would point at a relatively lower content of microplastics at higher levels of the food web (predators and top predators). While species ingest microplastics contained in their preys (Hammer et al., 2016), decrease of microplastic content going up the food web is expected according to the theory of trophic dilution (Alava, 2020). The reason is that, while primary producers (autotrophs, like algae and phytoplankton) cannot do anything to remove microplastics from their tissues, most microplastic items are expulsed in the case of consumers –herbivores, predators, top predators- and not retained in the tissues (Provencher et al., 2019). Naji et al. (2018) did not find any significant increase of microplastics in mollusks with size, supporting the idea of no bioaccumulation of these pollutants. However, this is still insufficiently studied. Nelms et al. (2018) and Zhang et al. (2019), for example, provide evidence that microplastics can be transferred across the food web and be accumulated in top-predators. Therefore, different outcomes are possible.

Humans as seafood consumers are exposed to microplastics ingestion through diet (Smith et al., 2018). Possible health effects of microplastics for humans may arise either from the physical characteristics of microplastics and from the pollutants that attach to their surface (Campanale et al., 2020). Some types of plastic material are more toxic to humans than others, and their effects also depend on how the microplastics enter the body (Ma et al., 2020), and what happens inside of it. Polystyrene for example, is known to cause pulmonary diseases when inhaled (Dong et al., 2020), but seems to cause little harm when brought into contact in vitro with different types of human cells and tissues (Hwang et al., 2020). The greatest danger lies in additives that are supplemented to fibers to make the polymers stronger, heat resistant and/or more flexible (Hammer et al., 2012). Some flame retardants, for example, can disrupt endocrine systems of humans (Meerts et al., 2000), yet overall, release of these molecules in organisms remained very low (Chen et al., 2019). In addition, microplastics could break down to nanoplastics in the acid environment of the stomach, like Dawson et al. (2018) show in their study about krill, which leads to another range of problems and uncertainties. Nonetheless, despite the fact that studies about plastics in seafood are of great value for consumers (Rainieri & Barranco, 2019), thorough studies about health effects of nano- and microplastics are still rare.

Here we focused on invertebrates exploited as seafood. Marine invertebrates are important in the human diet since the Paleolithic. García-Escárzaga and Gutiérrez-Zugasti (2021) emphasized the importance of topshells as staple food that guaranteed survival of human populations in adverse periods, like glaciations. In the Mesolithic these gastropods were also important as part of the human diet, adding variation and nutrition to terrestrial food resources (Álvarez-Fernández, 2015). Indeed they are still eaten now in many countries, despite toxins reported in edible gastropods and some food poisonings (Biessy et al., 2019; Cabral-Oliveira et al., 2015). Many topshells of the Trochidae family that are widely exploited in Europe are herbivores that graze on algae (Templado et al., 2012 in Sousa et al., 2018). They can move slowly in the intertidal zone to look for food and protection. Gutow et al. (2019) demonstrated experimentally that *Littorina* ingests microplastics when foraging on contaminated algae, and Doyle et al. (2019) confirmed uptake of microplastics by wild *Littorina* located in Galway bay. Jones et al. (2020) showed microplastics inside *Gibbula cineraria* grazing on *Zostera marina*. Further, the freshwater gastropod *Lymnaea stagnalis* was confirmed to ingest microplastic spheres when fed with *Lactuca sativa* contaminated with a mixture of ISO medium, plastic spheres (Weber et al., 2021). The physical effects of microplastics on gastropods include clogging of the gastrointestinal tract, and wounding due to sharp fragments (Wright et al., 2013). *Crepidula onyx* showed lower growth rates and sooner establishment when being exposed to high concentrations of microplastics, the effect being maintained after removal of the microplastics (Lo & Chan, 2018). Behavior can be affected as well. Seuront (2018) suggested that the predator flee reaction of *Littorina littorea* is being altered by leaching of contaminants from microplastics. Species that rely on this kind of reaction might suffer tremendously from microplastic contamination, posing a danger to the rest of the food web as well. Nevertheless, not all studies proved a great effect of microplastics on Gastropoda. Doyle et al. (2020), for example, did not find a correlation between microplastics in a concentration as the one currently present in the environment and the reaction of *Littorina littorea* on predator indicatives.

Another invertebrate seafood resource in expansion today is anemones. *Actinia equina* (Linnaeus, 1758) is an edible anemone typically consumed as a delicacy in the Mediterranean, and also employed to produce food supplements for its anti-inflammatory effects, that compensate some cytotoxicity on the gastrodermis (Lanza et al., 2020; Silva et al., 2017). Anemones ingest microplastics in various ways. *Bunodactis reynaudi* is capable of ingesting big chunks of plastic in one piece (Weideman et al., 2020). Other anemones acquire microplastics indirectly through preys like the brown shrimp *Crangon crangon*, an anemone prey that uptakes microplastic (Devriese et al., 2015). Moreover, some anemones only ingest microplastics in the presence of prey. Romanó de Orte et al. (2019) found that the sea anemone *Aiptasia pallida* directly ingests microplastics when chemical cues of brine shrimp are present in the water, but not without signals of prey presence. In addition, anemones preserve some water during low tide to avoid dehydration, that might also contain microplastics (Morais et al., 2020), thus some items besides those provided by their preys are expected in them. Research about the toxic effects of microplastics in anemones was mainly performed on species that establish a symbiotic relationship with algae (Okubo et al., 2018; Romanó de Orte et al., 2019). In those species, a major danger of microplastic ingestion is the loss of the symbiotic relationship, which leads to bleaching. The anemone *Exaiptasia pallida* exhibits morphological changes when ingesting microplastics (Diana et al., 2020), especially in the crown area that is smaller for anemones that were fed microplastics, while weight reduction occurred for anemones fed with a specific type of plastic (Diana et al., 2020).

This study focuses on the anemone *Actinia equina* (Linnaeus, 1758) and Trochidae topshells (*Steromphala umbilicaris*, Linnaeus, 1758, and *Phorcus lineatus*, da Costa, 1778) exploited as seafood in the north of Spain (south Bay of Biscay). The main objective was to determine the quantity and types of microplastics in these species that are currently understudied, in order to assess the risk of microplastics ingestion through their consumption. From the theory of trophic dilution of microplastics along the food web, we expected a greater accumulation of microplastics in herbivores (topshells) than in carnivores (anemones) in the same location. A location effect (difference between beaches along the coast) was suspected because there are significant differences among beaches regarding microplastic pollution in the region (Masiá et al., 2021; Mendoza et al., 2020). Types of microplastics and adhered compounds were expected to be similar as previous studies (Klasios et al., 2021; Mendoza et al., 2020; Wu et al., 2020), with anthropogenically altered cellulose as the most common material and presence of PP, PET and PE (de Sá et al., 2018; Fang et al., 2021; Naji et al., 2018; Wu et al., 2020).

2. Material and methods

2.1. Ethics statement

This study obtained the permit for sampling from the Principality of Asturias, General Directorate of Marine Fisheries, according to the Spanish Law 15/2002 of 27 of December. Sampling procedures and treatment of animals followed current Spanish legislation about ethics in research with animals. This study aligns with the European Code of Conduct for Research Integrity (All European Academies, Berlin 2017; https://ec.europa.eu/info/funding-tenders/opportunities/docs/2021-2027/horizon/guidance/european-code-of-conduct-for-research-integrity_horizon_en.pdf).

2.2. Sampling area and species sampled

Sampling was conducted on six different beaches along 200 km of the North coast of Spain (central Bay of Biscay) in January and February 2021 (Figure 1). Three of the locations (Gijón, Rodiles and Vega) are situated on the east side of Cape Peñas, where the rocks are calcareous and the water relatively warm; the other three (Otur, Aguilar and Xagó) are situated on the west side of Cape Peñas, where the rocks are siliceous and the water relatively colder (Garcia-Soto et al., 2002).

Figure 1. Sample locations for this study along the northern coast of Spain. Modified from Google maps ©, https://www.google.com/maps/@43.4108907,-5.8113207,10z, accessed 30 June 2021.

The species considered have different feeding strategies. *Actinia equina*, the beadlet anemone, is principally carnivore/detritivore. It mainly consumes insects, crustaceans, mollusks, and organic detritus while sticking to the same spot (Chintiroglou & Koukouras, 1992). The topshells *Phorcus lineatus* (formerly *Osilinus lineatus*) and *Steromphala umbilicaris*, on the other hand, are snails from the family Trochidae. They have the same ecological niche, exhibit the same feeding behavior and are found together in the upper and mid tidal level (e.g. Crothers, 2001).

Catch statistics and the price of these animals in the regional market since 2004 can be found in https://tematico.asturias.es/dgpesca/din/estalonj.php (in Spanish, accessed June 2021) Contrary to the topshells, that have been harvested since the Paleolithic and have always been consumed by humans, we see a recent increase of interest for anemones. The price of anemones has tripled since 2012 according to regional statistics (Gobierno del Principado de Asturias, n.d.), which highlights their importance as an emerging product in the seafood industry today.

2.3. Sampling

On each beach, 8 to 10 samples of each animal group (anemones and Trochidae topshells) were taken randomly from rock surfaces in the intertidal zone. Thus, there are 8 to 10 individuals with the same feeding strategy analyzed from each beach. Upon arrival in the lab, samples were immediately transferred to the freezer (-18°C) for storage until further processing. Topshells of the two target species were taken from each beach. Having the same ecological niche and being harvested and commercialized together under the generic local name of "*bigaros*" in this region, they were treated indistinctly as "topshells" for data analysis.

2.4. Microplastics quantification

Tissue digestion for microplastic extraction was carried out according to a protocol adapted from Li et al. (2015). Samples were taken from the freezer more than 2 hours before for a gentle defrost. Topshells and anemones were then weighed and put in clean glass jars covered with aluminum foil. The shells of the Trochidae were removed before weighing. Thereafter, 30% filtered H_2O_2 was added to each of the animals according to their weight. 20 mL of filtered peroxide was used per gram wet weight of tissue. Subsequently, the samples were put in an oven on 60°C for 3 to 4 days to improve tissue digestion.

After digestion, samples were diluted with filtered distilled water until 1 L. We did this to ease and accelerate the filtering process. Thereafter, the samples were filtered through a 0.45 µm pore size filter of hydrophilic polyether sulfone (Supor membrane disc filters, PALL corporation) with a vacuum pump. The filters provide a white background for counting the microplastics, that are not able to pass the filter. For every sample, 2-5 filters were used based on the flow rate. All filters were stored separately in petri dishes with a cover in a dry, dark, safe box.

After at least three days, when the filters were dry, microplastics on the filters were counted using a Leica ZOOM 2000 binocular on 40X magnification. Microplastics were recognized according to the criteria of Löder and Gerdts (2015).

2.5. FT-IR spectroscopy

Approximately 5% of the counted putative microplastics (145 items), representative of all the morphological types found (shapes and colours), were sent to the 'Servicio Interdepartamental de Investigación de la Universidad Autónoma de Madrid' for Fourier transform infrared spectroscopy or FT-IR spectroscopy, as this method is proven to be very effective for particles bigger than 50µm (Käppler et al., 2016).

2.6. Contamination control

All the used distilled water and hydrogen peroxide were filtered through a 0.2 µm pore filter of hydrophilic polyether sulfone (Supor membrane disc filters, PALL corporation) with a vacuum pump before usage. In addition, benches and all the used material were carefully washed before handling the samples. First, warm tap water was used to wash the materials and benches three times. Then everything was rinsed again three times with filtered distilled water. Glass and metal materials were employed whenever possible. In addition, jars, measuring cylinders, vacuum pumps and other materials were covered with aluminum foil to the greatest extent possible in order to avoid airborne contamination.

Six blanks of 40 mL of filtered H_2O_2 were prepared and handled exactly like the samples to measure contamination in the lab. After the process, the blanks contained 5 to 9 fibers with an average of 7 fibers per blank. The great majority were transparent fibers and just very few coloured. The particles found in the blanks were analyzed by FT-IR spectroscopy.

2.7. Statistical analysis

Datasets were prepared in spreadsheets and analyses was carried out in R. Extreme outliers were identified using an adaptation of the Hampel filter, whereby the lower boundary of the interval is equal to the median minus 9 MAD's (median absolute deviation) and the upper plus 9 MAD's. The Hampel filter was applied for each group separately and extreme outliers were removed from final data analyses. Correlation coefficients were calculated per group to see if the amount of microplastics in an organism increased with their weight.

The quantitative variable employed was microplastics/gram, calculated dividing the raw counts by the wet weight in grams. Means and variances were compared and the variance inflation factor (VIF) was calculated to check whether assumptions for parametric tests were met. Hence, a PERMANOVA was performed to see if the amount of microplastics per gram of tissue could be explained by one of the variables: type of organism (anemones versus topshells) and/or location. Nonmetric multidimensional scaling (NMDS) was performed to visualize the composition of fibers with different colours and fragments, coloured fibers with a total count of <10 were grouped together as 'other fibers'. An ANOSIM test was performed to verify whether the composition of microplastics significantly differed between species and/or location. Fisher's exact test was used to compare the proportion of dangerous and non-dangerous compounds between anemones and topshells, and the distribution of different materials in anemones and topshells.

3. Results

3.1. Microplastics content in the organisms sampled

Raw individual contents of MP are shown in Supplementary table 1. One extreme outlier, out of the median ± 9 MAD's interval, was found and removed from the dataset.

Putative microplastic particles were found in all samples without exception, ranging from 1 to 71 per individual (Supplementary Table 1). In total 2157 particles were counted in the 100 individuals analyzed. The minimum (0.56) count per gram of tissue was found in a topshell on the beach in Vega and the maximum (148.28) in a topshell on the beach in Otur. The group with the highest standard error of the means is Trochidae in Otur (Figure 2).

Correlations between microplastics and individual size (wet weight) within beach and species did not support general bioaccumulation of microplastics in individuals, because the correlation coefficients were positive in some beaches and negative in others. Coefficients for topshells were negative (most not significant), while there was no consistency for anemones (Supplementary Table 2). This result supports Naji et al. (2018) regarding lack of consistent association between wet weight and microplastics counts.

The data did not meet the assumptions for parametric tests as there were still outliers (Figure 2) influencing the distribution. No homogeneity of means and variances was observed and calculated VIF values resulted in 8, 265.51, and 819.36 respectively for the variables organism (anemones versus topshells), location, and the interaction effect. As two of the three

VIF values exceeded 10, our variables show multicollinearity and thus the assumption that variables cannot show collinearity was not met.

For these reasons, non-parametric tests were performed. The PERMANOVA resulted in a significant effect of organism (anemones versus topshells) (p= 0.001; F= 13.88; df=1). The location (p=0.65; F=0.74 ; df=5) and interaction (p=0.17; F=1.46 ; df=5) effects were both non-significant. Therefore, topshells contained significantly more putative microplastics per gram tissue than anemones in this study (Figure 3, Table 2), while significant differences among beaches could not be demonstrated in these samples for this variable using this approach.

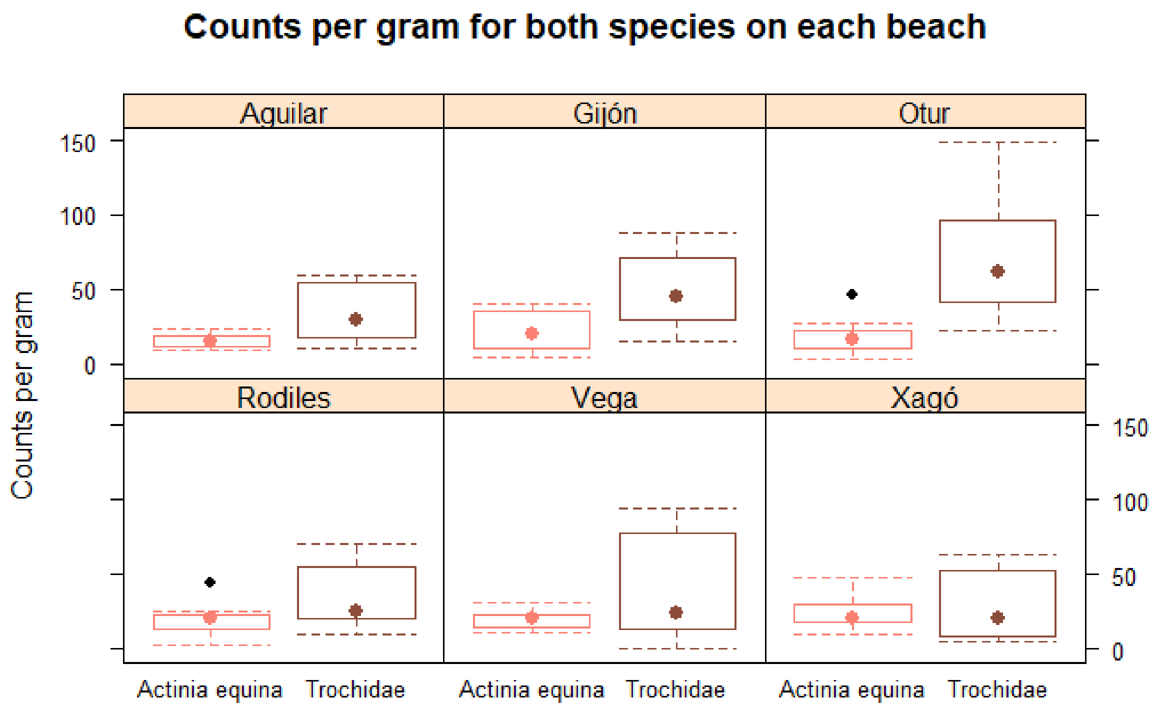


Figure 2. Counts of putative microplastics per gram wet weight for Actinia equina and the topshells Phorcus lineatus and Steromphala umbilicaris (Trochidae), grouped per location. Outliers are marked as black diamonds.

Mean content by type of MP and population are presented in Supplementary table 3. Overall, fibers made up 88% of all particles. The most prominent fiber colours were transparent, black and blue (Figure 3), but fibers that were green purple, red, yellow and other colours were also found, suggesting multiple sources. The remaining 12% of particles contained flakes, small plastic fragments, aggregates of smaller fragments, and a spherical pellet, that were grouped together as “fragments” in Supplementary table 3.

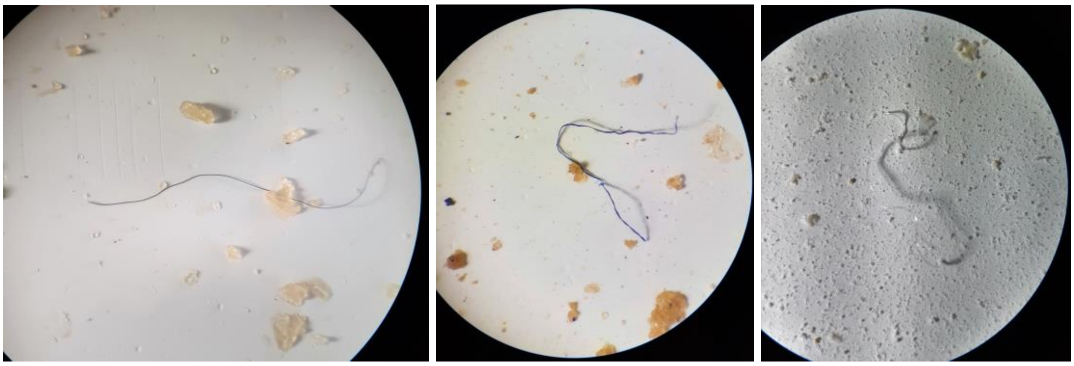
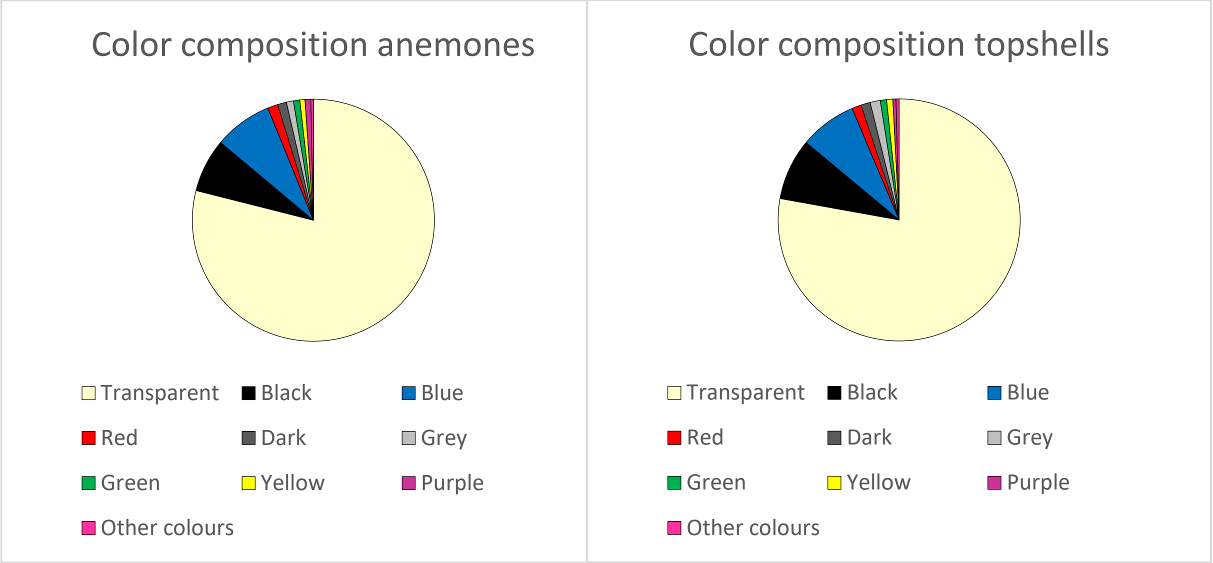


Figure 3. Black, blue and transparent fiber, under a binocular on 40X magnification.

Pooling together all the beaches, anemones and topshells seemed to display very similar patterns (Figure 4).

Figure 4. Colour composition of microfibers over all samples in anemones (left) and topshells (right).



In the NMDS plot, the topshell samples were nested within the anemones (Figure 5). The ordination had a stress value of 0.15 and thus is being considered a weak fit; therefore, an ANOSIM test was performed to verify the visual prospects. The ANOSIM test showed significant distinction in particle composition between the two groups of species (R statistic= 0.25; p= 0.024), but not by location (R statistic= -0.1333; p= 0.745).

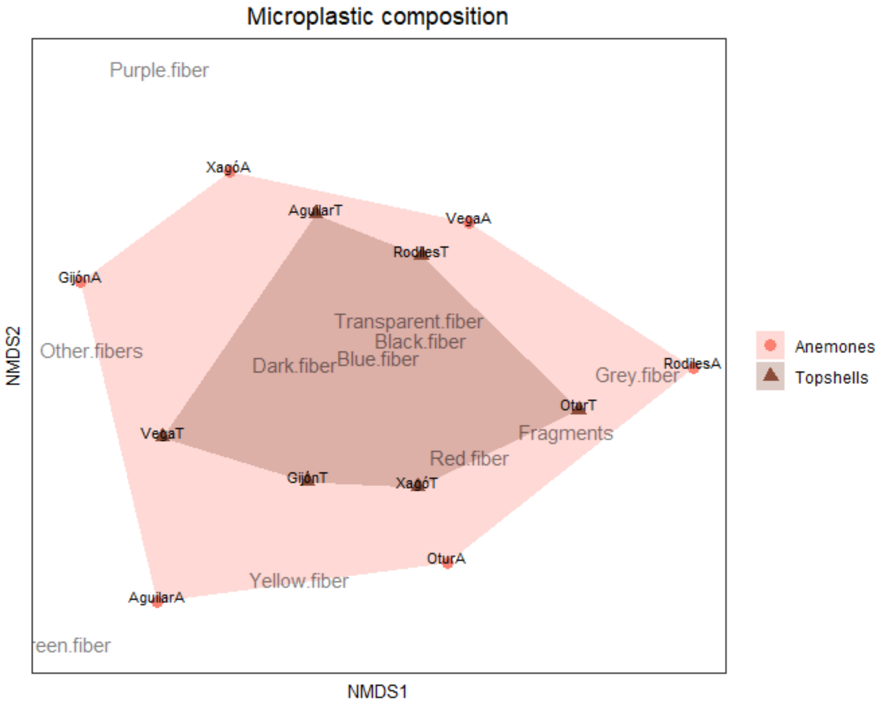


Figure 5. Visualization of microplastic composition by fiber types for the anemones and topshells analysed in this study.

3.2. Chemical composition of particles

The FT-IR spectroscopy on 94 particles from 34 animal samples identified 32% plastic particles, 59% non-plastic artificial particles and 9% natural particles (Table 1). Plastic particles included polyethylene (PE), polyester, nylon, acrylic fibers, polypropylene (PP), polyethylene terephthalate (PET), polystyrene (PS), and polyvinyl butyral (PVB). As many as 84% of the artificial particles that were not plastic, (49% of the total number of particles analyzed) were anthropogenic-transformed cellulose, and 13% (7% of total) were artificial compounds attached to fibers. The category ‘anthropogenic-transformed cellulose’ contained here rayon, carboxy methyl cellulose (CMC), hydroxyethyl cellulose (HEC), and hydroxypropyl cellulose (HPC). Within the category ‘artificial compounds attached to fibers’, the following substances were included: chlorofluorocarbon (CFC), diglycolic acid (DGA), ethylenediamine triacetic acid (EDTA), detergents, glyceraldehyde, and phosphorothioic trichloride (PTTC). Lastly, unknown human made compounds contained two particles, one identified as ‘berries extract film’, and another as ‘passion flowers extract film’. Natural compounds included cellulose, cotton, and chitin.

The average amount of fibers in blanks (7.0) was indeed lower than that of any group of samples (see Figure 2). From chemical analysis, some materials like Alkyl-aryl siloxane (AAS) and Styrene/ isoprene copolymer – an alkylated silicone (SIS)- were found only in the blanks (Supplementary table 4). Rayon was the most prominent material in the blanks (55%), pointing at some contamination originating from clothes despite careful treatment of samples, researchers always wearing cotton lab coats. Except for one PS fiber, no harmful materials were found in the blanks, indicating that the dangerous particles found in the samples originated from the animals analyzed.

Category	*Material*	*Anemones (n=56 items)*	*Topshells (n=38 items)*
Plastic	PE	0.268	0.079
	Polyester	0.036	0.026
	Nylon	0.036	0
	PP	0.018	0.026
	PET	0	0.026
	PVB	0	0.026
	PS	0	0.026
	Acrylic fiber	0	0.053
	Rayon	0.393	0.474
Artificial non-plastic	HPC	0.018	0.053
	HEC	0.036	0
	CMC	0.018	0
	DGA	0.018	0
Attached compounds	Glyceraldehyde	0.018	0
	detergent	0.018	0.026
	extract	0.018	0.026
	CFC	0	0.026
	PTTC	0	0.026
	EDTA	0	0.026
Natural materials	Cellulose	0.071	0.026
	Cotton	0.018	0.053
	Chitin	0.018	0

Table 1. Result of the FT-IR spectroscopy, showing the proportion of items of each type of material found in particles extracted from anemones and topshells in this study.

The distribution of the different types of materials in anemones and topshells were apparently different, with more PE items in anemones, more rayon in topshells, and most materials represented only by one item. However, the difference was not statistically significant (Fisher's exact test with p = 0.161). Removing PE (21% of blank items, being 21.4% in the anemones and only 7.9% in the topshells) and rayon particles (55% of blank items, being 39% and 47% in anemones and topshells respectively) to control for possible contamination, the distributions were still not significantly different (p = 0.418 for Fisher's test).

3.3. Harmful compounds

Possible harmfulness of particles was checked on the European Chemical Agency website (https://echa.europa.eu). A total of eight materials found in the particles analyzed are listed as harmful or potentially harmful in the list of compounds analyzed by the agency: polyester, nylon, PET, PS, EDTA, DGA, PTTC and glyceraldehyde (Table 2). These harmful, sometimes toxic compounds represented 10.7% and 13.3% of the total analyzed particles found in anemones and topshells respectively, a difference not statistically significant (p = 1 for Fisher's test). The other compounds have not been found to be harmful by research so far (https://echa.europa.eu, accessed June 2021).

	Irritation	*Toxicity*	*Mutagenicity*	*Environmental hazard*	*Carcinogenesis*

Polyester	V	V		V	
Nylon	O	O	O	O	O
PET				O	
PS	V			O	O
EDTA	V				
DGA	V				
PTTC	V	V			
Glyceraldehyde	O	O		O	O

Table 2. Summary of harmful effects of the materials identified from particles in this study. V: verified effect. O: suspected effect (European Chemical Agency, 2021).

4. Discussion

To our knowledge, this is the first study on the safety of these species as seafood, regarding microplastics and attached compounds in European Atlantic waters. The level of microplastics found, being considerable, falls within the range of results published for other species of the same taxonomic groups (e.g. Diana et al., 2020, for the anemone *Exaiptasia pallida*; Jones et al., 2020, for the topshell *Gibbula cineraria*). Perhaps the most striking result of this study was to confirm that these species also contain plastic particles and compounds attached to fibers that are harmful for humans, aquatic life and the environment. An average of 12.7% of the items analyzed in the two species were found to be harmful. One of the compounds, PTTC, is even fatal when inhaled (ECHA, 2020). These results stress the importance of chemical compounds that attach to the surface of microplastics (Frias et al., 2010; Hammer et al., 2012; Koelmans et al., 2014).

Here it was statistically demonstrated that topshells (herbivores) contained a greater amount of microplastics than anemones (mainly carnivores). Significant difference in microplastics per gram of wet weight was found between the two groups of species. Being sampled from the same sites, this result would support the hypothesis of dilution –opposite to bioaccumulation- of microplastics at higher trophic levels, suggested by Provencher et al. (2019) and supported by Alava (2020) models. Different feeding strategy (anemones catch prey with their tentacles while topshells graze) could also explain this difference, as suggested by Setälä et al. (2016) for invertebrates and Lopes et al. (2020) for fish. However, Xu et al. (2020) and Naji et al. (2018) found no overall significance between species with different feeding strategies. The results of this study cannot distinguish if the feeding strategy (grazing versus active catch), the trophic level (herbivores versus carnivores) or both, are the cause of the difference between anemones and topshells.

On the other hand, Xu et al. (2020) proposed that the impact of the environment is higher than that of feeding strategies. Differences between beaches regarding environmental levels of plastics and microplastics have been reported in the study area, being Xagó the most and Otur the least polluted (Masiá et al., 2021; Mendoza et al., 2020), but those differences were not reflected on anemones and topshells in this study. It is possible that the spatial variations observed in sediments and water in a region moderately microplastics-polluted (Masiá et al., 2019, 2021; Mendoza et al., 2020) are not sufficiently large to differentiate the populations inhabiting therein. Further studies could investigate if and in what conditions these species reflect the environmental level of microplastics, including a higher number of individuals.

Regarding microplastic types, the global results obtained in this study were similar to other studies about microplastics in marine invertebrates, in which the most prominent particles are usually fibers (de Sá et al., 2018; Fang et al., 2021; Gallagher et al., 2016; Karlsson et al., 2017; Naji et al., 2018; Wu et al., 2020; Xu et al., 2020). Likewise, blue, black and transparent fibers are the commonest in the environment and within organisms (Fang et al., 2021; Gallagher et al., 2016; Karlsson et al., 2017; Zaki et al., 2021). Here a wide variation of particles of less abundant colours was also found, a phenomenon interpreted by Naji et al. (2018) as due to varied sources of microplastics in the studied area. Kühn et al. (2015) addressed the issue of weathering of the fibers in the stomach and intestines that can make the colour of fibers change. Our results with more transparent fibers in anemones could suggest that perhaps colours fade differentially in the digestive tracts of anemones and topshells, but this cannot be confirmed in our study given the relatively high number of transparent fibers in the blanks. Another explanation could be different preferences regarding the colour of microparticles, in case the studied species would actively catch microplastics. Colour selection has been suggested in previous studies on other species (Kühn et al., 2015; Wright et al., 2013), but in most cases it is due to microplastics to be confounded with preys of similar size and colour. It is unlikely the case with the topshells, grazing on algae, or even the anemones that catch actively quite large preys while they do not catch microplastics actively (Romanó de Orte et al., 2019), although preference could not be totally ruled out for the studied organisms.

The most common type of polymers in our samples was rayon, an artificial cellulose fiber, which is in conformity with other studies about microplastics in marine organisms (Klasios et al., 2021; Wu et al., 2020). The types of plastic found were also consistent with the results of chemical composition of microplastics in other studies about marine organisms (de Sá et al., 2018; Fang et al., 2021; Naji et al., 2018; Wu et al., 2020). Xu et al. (2020) found many cellophane particles which were not detected in our samples, nor in the study area in previous studies (Masiá et al., 2019, 2021; Mendoza et al., 2020), thus there is no reason to expect them in the organisms therein.

On the technical side the methodology used is seemingly efficient to quantify microplastics in the studied species. However, suspected contamination with a few fibers in the blanks was a limitation of this study. Authors emphasize the importance of controlling contamination during sampling, transport and laboratory treatment of the samples (e.g., Lusher et al., 2017), and our results indeed confirm that need.

4.1. Conclusions and recommendations

In conclusion, *Actinia equina*, *Steromphala umbilicaris,* and *Phorcus lineatus* in the Bay of Biscay contain particles and fibers with compounds that can be irritant, toxic, mutagenic, carcinogenetic, and environmental hazards Thus these species are not totally safe for human consumption when harvested from places contaminated with microplastics; moreover, these contaminants might pose a threat to the populations of anemones and topshells and the ecosystem as a whole. Higher microplastics content in topshells than in anemones would suggest an effect of the trophic level or feeding behavior in the ingestion of microplastics in these species.

This investigation illustrates research gaps regarding the safety of seafood. Coastal marine organisms like topshells and anemones are highly exposed to microplastics while representing an important resource. Further research could address other regions with different contamination levels to investigate if these species could be bioindicators of microplastic pollution. On the other hand, the real quantity of harmful compounds in microplastics ingested by exploited invertebrates is not known yet, and our results suggest that may be very high. There is a urgency for more research on plastic and microplastic contents in exploited coastal species.

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Supplementary data

Supplementary table 1. Raw data per individual, presenting species, location, wet weight of each individual, putative particle count per individual and concentration of putative particles per individual.

Individual	Species	Location	Wet weight (in gram)	Absolute counts	Counts per gram
1	Actinia equina	Xagó	1.65	44	26.67
2	Actinia equina	Xagó	2.04	36	17.65
3	Actinia equina	Xagó	1.49	65	43.62
4	Actinia equina	Xagó	1.94	58	29.90
5	Actinia equina	Xagó	1.41	67	47.52
6	Actinia equina	Xagó	1.73	31	17.92
7	Actinia equina	Xagó	2.37	38	16.03
8	Actinia equina	Xagó	1.54	32	20.78
9	Actinia equina	Xagó	2.68	26	9.70
10	Actinia equina	Gijón	2.76	13	4.71
11	Actinia equina	Gijón	1.2	7	5.83
12	Actinia equina	Gijón	0.46	18	39.13
13	Actinia equina	Gijón	0.45	15	33.33
14	Trochidae	Aguilar	0.46	25	54.35
15	Trochidae	Aguilar	0.89	9	10.11
16	Trochidae	Aguilar	0.54	32	59.26
17	Trochidae	Aguilar	0.58	21	36.21
18	Trochidae	Aguilar	0.68	37	54.41
19	Trochidae	Aguilar	1.27	29	22.83
20	Actinia equina	Rodiles	1.44	30	20.83
21	Actinia equina	Rodiles	0.89	19	21.35
22	Actinia equina	Rodiles	3.04	36	11.84
23	Actinia equina	Rodiles	0.81	20	24.69
24	Actinia equina	Rodiles	1.61	71	44.10
25	Actinia equina	Aguilar	1	13	13.00
26	Actinia equina	Aguilar	1.12	21	18.75
27	Trochidae	Aguilar	1.1	20	18.18
28	Trochidae	Aguilar	1.17	20	17.09
29	Actinia equina	Gijón	0.87	17	19.54
30	Actinia equina	Gijón	0.73	29	39.73
31	Actinia equina	Gijón	0.72	26	36.11
32	Actinia equina	Gijón	0.81	9	11.11
33	Actinia equina	Gijón	1.8	18	10.00
34	Trochidae	Rodiles	0.82	22	26.83
35	Trochidae	Rodiles	0.69	17	24.64
36	Trochidae	Rodiles	0.61	16	26.23
37	Trochidae	Rodiles	0.38	27	71.05
38	Trochidae	Rodiles	0.31	19	61.29
39	Trochidae	Rodiles	0.7	16	22.86

40	Trochidae	Rodiles	0.93	9	9.68
41	Trochidae	Rodiles	0.87	18	20.69
42	Trochidae	Rodiles	0.27	15	55.56
43	Trochidae	Rodiles	0.66	13	19.70
44	Trochidae	Otur	0.43	11	25.58
45	Trochidae	Otur	0.52	32	61.54
46	Trochidae	Otur	0.04	19	475.00
47	Trochidae	Otur	0.42	24	57.14
48	Trochidae	Otur	0.29	43	148.28
49	Trochidae	Otur	0.76	17	22.37
50	Trochidae	Otur	0.23	28	121.74
51	Trochidae	Otur	0.4	28	70.00
52	Actinia equina	Rodiles	0.98	14	14.29
53	Trochidae	Vega	0.24	20	83.33
54	Trochidae	Vega	0.26	19	73.08
55	Trochidae	Vega	0.18	17	94.44
56	Trochidae	Vega	0.63	16	25.40
57	Trochidae	Vega	0.77	17	22.08
58	Trochidae	Vega	0.85	14	16.47
59	Trochidae	Vega	0.94	9	9.57
60	Trochidae	Vega	1.8	1	0.56
61	Trochidae	Xagó	0.49	31	63.27
62	Trochidae	Xagó	0.75	18	24.00
63	Trochidae	Xagó	0.55	29	52.73
64	Trochidae	Xagó	0.53	28	52.83
65	Trochidae	Xagó	0.63	7	11.11
66	Trochidae	Xagó	1.04	18	17.31
67	Trochidae	Xagó	1.94	13	6.70
68	Trochidae	Xagó	1.71	8	4.68
69	Actinia equina	Aguilar	1.03	19	18.45
70	Actinia equina	Aguilar	1.64	16	9.76
71	Actinia equina	Aguilar	1.54	21	13.64
72	Actinia equina	Aguilar	1.45	35	24.14
73	Actinia equina	Aguilar	0.75	12	16.00
74	Actinia equina	Aguilar	1.41	15	10.64
75	Actinia equina	Rodiles	1.01	20	19.80
76	Actinia equina	Rodiles	1.76	5	2.84
77	Actinia equina	Otur	1.15	18	15.65
78	Actinia equina	Otur	0.87	13	14.94
79	Actinia equina	Otur	1.33	23	17.29
80	Actinia equina	Otur	0.72	20	27.78
81	Actinia equina	Otur	0.37	17	45.95
82	Actinia equina	Otur	0.89	16	17.98
83	Actinia equina	Otur	1.81	7	3.87
84	Actinia equina	Otur	3.94	20	5.08
85	Trochidae	Gijón	0.33	25	75.76
86	Trochidae	Gijón	0.55	24	43.64
87	Trochidae	Gijón	0.38	18	47.37

88	Trochidae	Gijón	0.44	8	18.18
89	Trochidae	Gijón	0.48	20	41.67
90	Trochidae	Gijón	0.96	15	15.63
91	Trochidae	Gijón	0.17	15	88.24
92	Trochidae	Gijón	0.35	23	65.71
93	Actinia equina	Vega	0.91	18	19.78
94	Actinia equina	Vega	1.42	17	11.97
95	Actinia equina	Vega	0.55	17	30.91
96	Actinia equina	Vega	0.5	11	22.00
97	Actinia equina	Vega	0.87	15	17.24
98	Actinia equina	Vega	1.24	13	10.48
99	Actinia equina	Vega	0.87	21	24.14
100	Actinia equina	Vega	0.5	11	22.00

Supplementary table 2. Correlation coefficients (number of microplastics and wetweight) per group. Significant results marked with an asterisk.

CORRELATION COEFFICIENT	***ANEMONES***	***TOPSHELLS***
OTUR	0.09	-0.49
AGUILAR	0.42	-0.26
XAGÓ	-0.58	-0.63*
GIJÓN	-0.27	-0.14
RODILES	0.31	-0.37
VEGA	0.34	-0.94*

Supplementary table 3. Concentration (per gram wet weight) of coloured fibers and fragments per group.

Organism	**Location**	***Transparent***	***Black***	***Blue***	***Red***	***Dark***	***Grey***	***Green***	***Yellow***	***Purple***	***Other***	***Fragments***
Anemones	**Xagó**	18.16	1.78	2.2	0.12	0.47	0.12	0	0	0.36	0.12	0.24
	Rodiles	14.3	1.39	1.3	0.26	0	0.43	0	0	0	0	0.95
	Gijón	12.55	0.92	1.22	0.2	0	0	0.2	0.1	0.2	0.1	0
	Vega	14.14	1.46	1.46	0.29	0.15	0.15	0	0	0.15	0	0.15
	Otur	8.94	0.9	0.81	0.46	0.18	0.18	0.18	0.18	0	0	0.27
	Aguilar	11.87	0.91	0.8	0.2	0.3	0.1	0.6	0.1	0	0.10	0.3
Topshells	**Xagó**	12.83	2.36	1.7	0.52	0.26	0.52	0.26	0.13	0	0.13	1.18
	Rodiles	21.15	3.04	0.8	0.16	0.32	0.48	0.16	0.16	0.32	0	0.96
	Gijón	27.32	4.1	4.64	0.27	0.82	0.27	0.27	0.82	0	0.27	1.64
	Vega	13.76	1.41	2.47	0.35	0.18	0	0.53	0.18	0.18	0	0.88
	Otur	46.89	2.95	4.59	0.98	0.33	0.66	0	0.33	0	0	3.28
	Aguilar	24.36	1.5	1.2	0.15	0.45	0.3	0	0	0.15	0.15	0.6

Supplementary table 4. Overview of types of material found in the blanks (in %).

Material	**% of items in the blanks**
PEI	21
PS	2
RAYON	55
HEC	2
CMC	2
DETERGENT	2
CELLULOSE	8
AAS	2
NACS	2
PDP	2
SIS	2