

Article

Microplastics Risk into a Three-Link Food Chain Inside European Hake

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Abstract: Microplastics (MPs) are increasing in the marine environment as well as inside marine organisms, having an important effect on biological diversity. The trophic transfer of MPs was demonstrated under laboratory conditions, but this study is based on the analysis of preys found in stomach contents. MPs from *Merluccius merluccius* individuals caught in the Cantabrian Sea and preys inside their guts (blue whiting, and northern krill inside blue whiting) were analyzed. MPs with different chemical composition occurred inside every hake and their preys, with different damages, from aquatic life hazards with long lasting effects, to allergic skin reactions and respiratory irritation, not only for aquatic species and fishing resources, but also for humans through hake consumption. The similarity of MPs profiles from gills and seawater samples would support seawater as the main source of gill microplastics. The MPs profile of hake GIT was similar to that of hake preys inside. Despite the small sample size, the presence of MPs in all the tissues analyzed of hakes and their preys, together with the evidence of hazard compositions of some of them, highlights the need for policies and actions to reduce plastic and microplastic production and consumption.

Keywords: *Merluccius merluccius*; *Micromesistius poutassou*; *Meganctiphanes norvegica*; microplastics; trophic transfer; chemical hazard



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1. Introduction

Due to its high production and consumption rates and its long decomposition time, plastic pollution has come to represent an added problem to an already stressed environment [1–3]. Plastic production is increasing at a rate of over 8% a year with about 8 million tonnes of plastic debris reaching the sea each year [4]. Such plastic debris interacts with hundreds of marine species, putting their lives at risk [2,3]. Physical agents and UV action trigger the fragmentation of plastic fragments into microplastics (MPs) (<5 mm) [5], increasing the availability of smaller particles to a wider range of organisms, including the lowest levels in marine food webs [2]. MPs have been found in most marine environments where they cause harm to marine organisms in different ways [6], an important one being the effect on biological diversity [7,8]. Due to this ubiquity, the understanding of their presence and relationship with biological communities at different trophic levels is of the utmost importance, introducing the novel concept of “microplastic community”. These “communities” show significant differences between different environments and biological communities, which will be very important for microplastic risk assessment [9]. On the other hand, current studies have highlighted that the presence of MPs increase the abundance of some taxa but decrease the abundance of some other taxa, with the subsequent alteration in community composition [10]. These alterations are highly conserved across

taxonomic ranks. However, the alpha diversity of microbiota is often reduced or increased, depending on the microplastics dose and environmental conditions, suggesting potential threats to biodiversity [10].

If we focus our concern at the individual level, there is abundant evidence of MPs in fish stomach and gut [11,12]. Once MPs are ingested, they can move to other tissues outside the gastrointestinal tract (GIT) through mechanisms, such as intracellular or paracellular endocytosis [13], or the translocation of small-sized particles [14] and adherence [15]. Several studies have demonstrated the presence of MPs in liver and gills [13,14,16,17], whose essential physiological functions can be affected by the presence of these particles [17]. These MPs can also alter the microbial community composition, with subsequent trade-offs among taxa and the altered microbial community in the animal gut [10]. Beyond tissue transfer within individuals, some studies have demonstrated the trophic transfer of MPs in organisms of low trophic levels and under laboratory conditions [18,19]. The extent to which this occurs in the wild and affects top predators is still poorly understood [20,21], since the proportion of individuals that contains MPs greatly varies among studies, adding uncertainty to this issue [22]. Although the number of MPs found in organisms is determined from the different feeding strategies of marine species [23], some studies suggest that trophic transfer is a major route of MPs ingestion by fish and poses threats to organisms at higher trophic levels, with biomagnification across the trophic chain varying among geographical scales and species-specific trophic chains [20,23].

MPs pose risks to marine ecosystems and biodiversity due to their chemical composition. Petrochemicals and their by-products cause a variety of health issues including cancer for living creatures. Research has shown that many chemical additives tested for toxicity are known endocrine disruptors, producing adverse developmental, reproductive (e.g., lower fertility), neurological, and immune effects (e.g., increased risk of cancer) [24,25]. Besides, MPs can adsorb and concentrate contaminants dissolved in seawater, passing them through the food chain [26–28]. MP can release toxic chemicals, such as styrene, metals (e.g., mercury), phthalates, bisphenol A (BPA), polychlorinated biphenyls (PCB), and polycyclic aromatic hydrocarbons (PAHs), that cause neurotoxicity, oxidative damage, and energy-related changes [29]. All those individual damages from MPs might have consequences at the population level, triggering disruptions of the ecosystem dynamics that will affect natural environments as well as our economy and society [30].

In this proof of concept, we have assessed the risks of the ingestion of MPs of different chemical composition considering the MPs in a small sample of hakes *Merluccius merluccius* (Linnaeus, 1758) and their preys in the wild. In other studies, MPs are assessed separately in different organisms of the trophic web [20,21,31], but this study is based on the analysis of preys found in stomach contents. Hakes were caught from the Cantabrian Sea offshore Gijon (Spain), a highly industrialized area with historical events of critical pollution in its surroundings [32]. Fish liver and gills have already been used for aquatic pollution assessment for accumulating environmental pollutants [33]. So, based on this and on the few studies that have assessed the presence of MPs in the gills, liver, and digestive tract at the same time [29,34], we expected particles in gills to have a composition of MPs similar to those present in water since they are directly exposed to water pollution, while particles into the gastrointestinal tract could come from preys.

Therefore, the specific objectives of this proof of concept will be:

1. Analyse the risk of ingestion of MPs with different chemical composition in the *M. merluccius* food chain.
2. Genetic identification of hake preys.
3. Compare the MPs composition present in the different hake organs, preys, and surrounding water.

2. Materials and Methods

2.1. Water and Hake Samples Collection

Three adult hakes (*Merluccius merluccius*) (H1, H2, and H3) collected from the coast of Gijón (Cantabrian Sea in south Bay of Biscay, Asturias, Spain) in late October 2020 (Figure 1) were kindly provided by fishermen and handed to the researchers by Mario Pidal (Nueva Rula de Avilés S. A., Asturias). They had been obtained from a commercial fishing catch. They were identified morphologically. Further genetic analysis was not necessary because *Merluccius merluccius* is the only hake species present in the Cantabrian Sea, and thus misidentification with other hake is not possible. The region is characterized by a temperate Atlantic climate with a dominant eastward current parallel to the coast, the North Atlantic current [35], and the Iberian Poleward current during the winter [36]. The region contains a commercial international port, the Port of Gijón. Tourism during the summer has been increasing in recent decades [37].

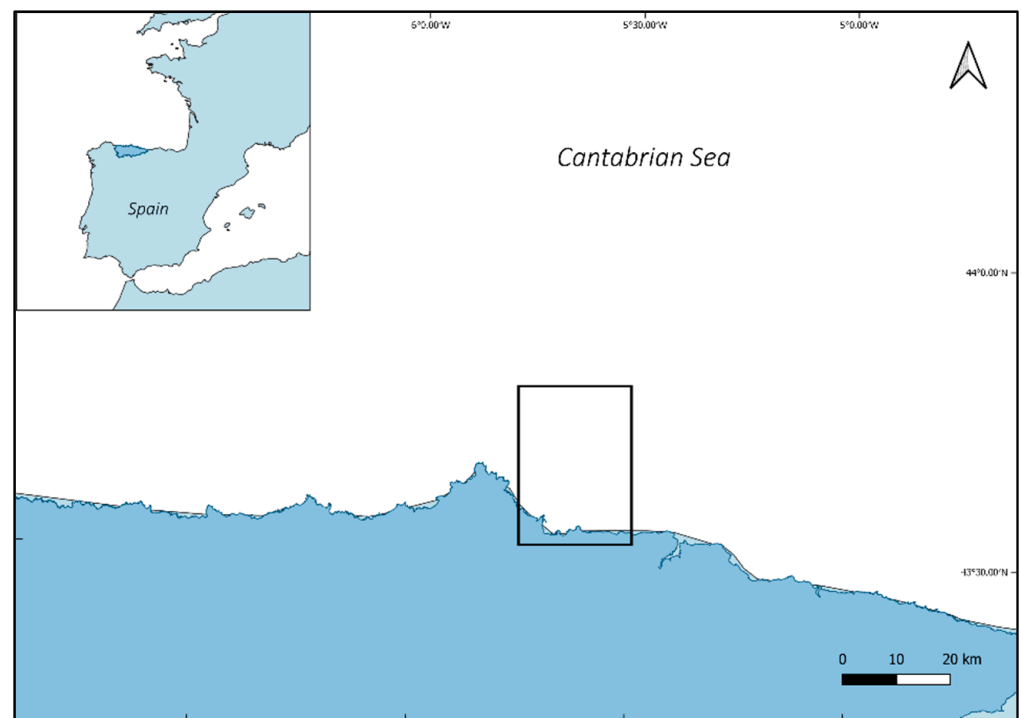


Figure 1. Fishing area where the analysed hakes were caught, and water samples taken (Geographical coordinates 43°35′00.0″ N 5°30′00.0″ W and nearby).

Fishermen also took 20 samples of 5 L of water (100 L in total) from the area where the hakes were caught (Figure 1). Water was taken using sterile bottles that were immediately closed, labeled, and stored cold until arrival at the port, where Mario Pidal handed them to the researchers. Water was vacuum filtered with a pump through a 0.45- μ m pore size (Supor[®] 450 Membrane Disc Filters), using the precautions described below for contamination control.

2.2. Contamination Control Measures

In order to avoid any possible sample contamination with airborne synthetic fibres, every step of the process was performed as quickly as possible, and materials were covered when not in use. Samples were manipulated inside a laminar flow cabinet using a cotton lab coat and gloves during the entire process. Plastic materials were avoided during lab manipulation. Dissection was performed with metallic scissors, a scalpel, and forceps. Materials were rinsed at least two times with distilled water. All the products employed in the digestion and treatment of samples and lab material (H₂O₂, distilled water) were filtered with a 0.2- μ m filter (Supor[®] 200 Membrane Disc Filters) to prevent any contamination from

these sources. Three blanks of 100 mL with no tissue were prepared simultaneously to the digestion of tissue samples with the same products, following the same protocol, in the same conditions and during the same time, to detect potential procedural contamination.

2.3. Fish Processing for MP Quantification

The three hakes were covered with foil, preserved in an icebox while transported to the Department of Functional Biology at University of Oviedo, and kept at $-20\text{ }^{\circ}\text{C}$ in the freezer until analysis. Once fish were defrosted and prior to digestion, the total length (cm) and body weight (g) of each specimen were recorded. The gastrointestinal tract (GIT), liver and gills were removed, weighed, and stored in a refrigerator until further MP extraction. In the case of gills, the branchial arch was separated from filaments and discarded. Individuals H1 and H3 had preys in their stomachs that were not yet digested. H1 had a fish (Prey 1.1), and H3 had a fish (Prey 3.1) that had euphausiids in its GIT (Prey 3.1.1). Thus, the latter were second-link hake's prey.

For genetic identification, a small portion of tissue from the dorsal muscle of preys was removed, preserved in ethanol, and stored at $4\text{ }^{\circ}\text{C}$. The remainder was used for MPs analysis.

Glass jars previously cleaned with filtered distilled water were used to digest the tissues. Besides, organs were rinsed too with filtered distilled water before the digestion. Many authors have used H_2O_2 as an effective tool to digest biogenic material [17,22,38]. Thus, we employed the same digestion procedure. Between 200 and 600 mL of 30% hydrogen peroxide (H_2O_2) was added to each jar, depending on the weight of each tissue sample or prey (Supplementary Table S1), to digest the organic matter. Jars were covered with foil and placed in the oven at $60\text{ }^{\circ}\text{C}$ for 48–72 h (depending upon the digestion level) to obtain dissolved solution. The heaviest samples of entire fish with almost intact skeleton and organs needed extra time (up to 7 days of digestion) to allow them to dissolve, always covered and inside the closed cabinet. Afterwards, the solutions were vacuum filtered with a pump through a $0.45\text{-}\mu\text{m}$ pore size (Supor[®] 450 Membrane Disc Filters). The density separation of MPs was not necessary thanks to the complete digestion of the organic matter, favouring the filtering of the entire solution.

2.4. Characterization and Identification of Microplastics

After the filtration process, filters were stored in petri dishes previously cleaned with filtered distilled water and observed through. A LEICA ZOOM 2000 Stereomicroscope at $40\times$ magnification [39] was used to quantify all potential plastic particles caught in the filters. Visual identification of $<5\text{ mm}$ long [40] plastic particles was carried according to the physical characteristics of the particles following Hidalgo Ruz et al. [41], classifying them as fibres (elongated) or fragments (small angular pieces) [31]. Besides, particles were categorized by colour (black, blue, red, and transparent/white). In order to confirm their chemical composition, 6.6% of black, 6.9% of blue, 17.7% of red, 30.4% of transparent/white fibres, and 31.6% of fragments obtained from animal tissue, corresponding to the 12% of the total of the MPs items found, were randomly selected and identified with a micro-Fourier-transformed infrared spectroscopy ($\mu\text{-FT-IR}$). These items represented the most common morphotypes of visually identified particles from all filters (Supplementary Table S2). In addition, the most striking and unique pieces were also selected. All the MP items found from water samples were chemically analysed by $\mu\text{-FT-IR}$. Clean spectra were obtained with no evidence of the mixture of compounds (Supplementary Figure S1).

The potential health danger caused by the materials found in MPs analysis was identified according to the European Chemical Agency (ECHA) with a presence/absence analysis (1/0 respectively). This information is publicly available at the ECHA website (<https://echa.europa.eu/es/>, accessed on 15 December 2021). Pictograms of different chemical hazards associated with the chemical composition of MPs were taken from ECHA in order to present the risk assessment per species with a graphical perspective (Figure 2).

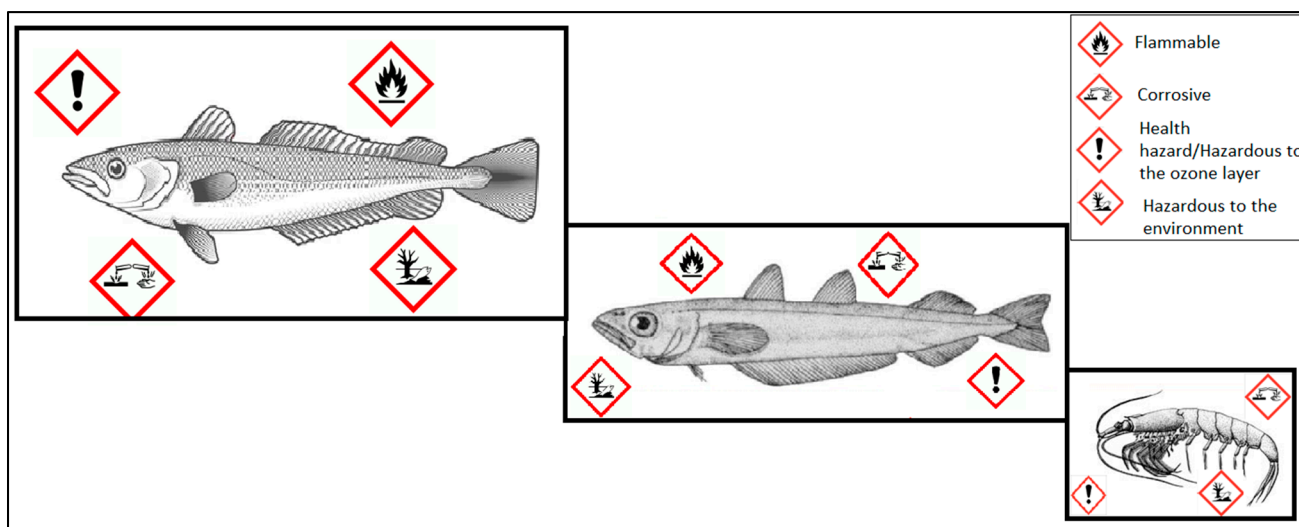


Figure 2. Risk assessment based on the chemicals found in hake, whiting and shrimp (from left to right) in this study, illustrated from hazard pictograms taken from ECHA.

2.5. Genetic Identification of Preys

Preys were first identified *de visu*, then confirmed using DNA because, as seen in Figure 3, they were relatively altered due to the digestion. Due to the different degree of tissue degradation of the preys, different DNA extraction methodologies had to be applied to each of them. A tissue sample of Prey H1.1 was digested with proteinase K, followed by DNA extraction with a filter-based system (DNeasy Tissue Kit, Qiagen Inc., Düsseldorf, Germany) following the manufacturer's instructions. On the other hand, DNA of Prey H3.1 was extracted adapting the protocol based on Chelex[®] resin (Bio-Rad Laboratories, Hercules, CA, USA) developed by Estoup et al. [42]. In both preys, a fragment of the cytochrome oxidase subunit 1 (COI) was amplified by polymerase chain reaction (PCR), employing the universal COI primers for fish published by Ward et al. [43]: COI-Fish-F (5'-TTC TCA ACT AAC CAY AAA GAY ATY GG-3') and COI-Fish-R (5'-TAG ACT TCT GGG TGG CCR AAR AAY CA-3'). PCR conditions were the following: an initial denaturing step of 5 min at 95 °C, followed by 35 cycles of denaturing at 95 °C for 30 s, annealing of 57 °C for 30 s and extension at 72 °C for 30 s, and a final extension at 72 °C for 10 min. All the PCR assays were run in a Thermal Cycler (Applied Biosystems, Waltham, MA, USA, model 2700).

Regarding Prey H3.1.1, DNA was extracted following the above mentioned Chelex[®] resin protocol. In this case, a 158 base pairs fragment of the mitochondrial gene coding for the subunit 1 of NADH dehydrogenase was amplified, employing specific Euphausiacea primers and PCR conditions developed by Zane et al. [44].

PCR products were visualized using 2% agarose gel stained with 10 mg mL⁻¹ Symple-Safe[™] (2.5 µL, EURx, Gdansk, Poland). Amplicons were sent for sequencing at MacroGen Spain, Inc. (Madrid, Spain) using a Sanger sequencing method procedure. Sequences were then edited and trimmed with BioEdit Sequence Alignment Editor software [45]. Finally, the outcome sequences were identified in the GenBank database using the BLAST algorithm (GenBank. Available online: <http://www.ncbi.nlm.nih.gov/genbank/>, accessed on 10 May 2021) and best match criterion.

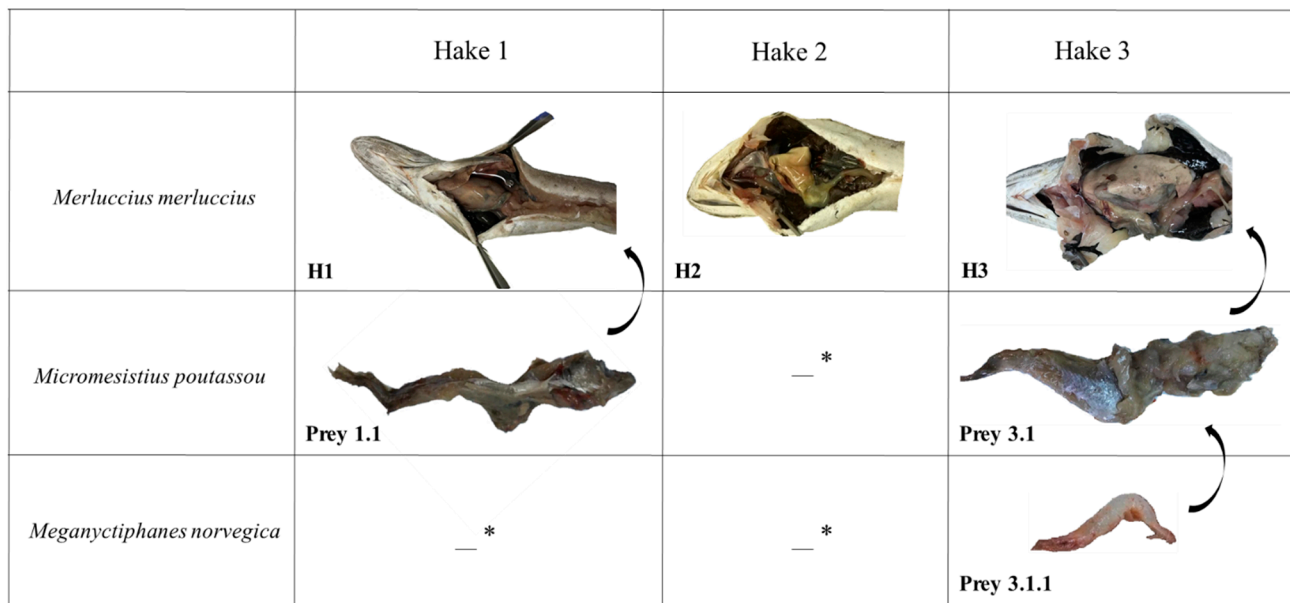


Figure 3. The three individuals of hake (*Merluccius merluccius*) analysed (H1, H2 and H3) and the preys found in the stomachs of H1 (Prey H1.1 *Micromesistius poutassou*) and H3 (Prey H3.1 *M. poutassou*, and Prey H3.1.1 *Meganyctiphanes norvegica* found inside the stomach of Prey H3.1). * No prey was found inside Prey 1.1 and Hake 2.

3. Results

3.1. Microplastic Pollution in Water, Hake Organs and Preys

Table 1 summarises MPs concentration (density) in water, hakes, and the preys inside them. The length and weight of hakes are also given.

Table 1. Length and weight of hakes. Prey species in corresponding to each predator (*M. norvegica* was found inside *M. poutassou*). MPs concentration in MPs/g for hakes, preys and MPs/L for surrounding water from the same fishing area.

		Hake 1	Hake 2	Hake 3
Length (cm)		36	35	39
Weight (g)		378.3	264.25	472.5
MPs density (MPs/g)	GIT	5.19	5.77	0.73
	Liver	0.77	6.4	1.38
	Gills	4.79	8.49	2.29
Prey	<i>Micromesistius poutassou</i>	No prey	<i>Micromesistius poutassou</i> / <i>Meganyctiphanes norvegica</i>	
MPs density of prey (MPs/g)		4.25	-	0.76/1.93
MPs density in surrounding water (MPs/L)			0.0002	

Water samples contained a total of 17 MPs in 100 L corresponding to approximately 0.0002 items/L. MPs occurred in all the hakes and hake's organs analysed (Table 1, Supplementary Table S2). The abundance of MPs in GIT varied from eight to 26 items/individual (i.e., 0.73 to 5.77 items/g), in the liver from seven to 23 (i.e., 0.77 to 6.4 items/g), and in

gills from nine to 15 (i.e., 2.29 to 8.49 items/g) (Table 1). MPs concentrations observed were higher in H2 organs than in the other samples, suggesting differences among individuals.

Blanks contained a few putative MP items (mean 7.6 ± 5.7), with a concentration of about 0.08 MPs/g, which is one order of magnitude lower than the lowest concentration of the animal samples analyzed. This indicates that the results for hake organs and preys were not biased by contamination during the digestion process.

The magnification of MPs in higher trophic levels was not suggested in our results. The total MPs concentrations calculated for H1 (4.37 MPs/g) and H3 (1.26 MPs/g) were similar to those found for their prey (whiting H1.1 and H3.1 being 4.25 and 0.76 MPs/g respectively), while the MPs concentration in the shrimp H3.1.1 (1.93 MPs/g) was higher than that found in its predator H3.1.

Among the MPs recovered in hakes' tissues and prey, fibres were the most abundant (92%), with the other 8% corresponding to fragments (Supplementary Table S2). Black (41.7%) and blue/greenish (40%) were frequent colours in fibres, followed by white/transparent (10.5%) and red (7.8%). In the water samples analysed, fibres were also more abundant than fragments, with blue/greenish and black fibres being the most abundant item types. Blanks exhibited a completely different pattern, with white/transparent fibres being the majority (57%), followed by blue (17%), black (17%), and green (8.7%). From this result and the low densities reported above, a relevant effect of contamination in our results during sample processing can be reasonably discarded.

The profile of MPs was not significantly different between gills ($\chi^2 = 8.09$, 8 d.f., $p = 0.42$), livers ($\chi^2 = 7.76$, 8 d.f., $p = 0.45$), or GIT samples ($\chi^2 = 5.25$, 8 d.f., $p = 0.63$) (Supplementary Table S2). Therefore, the results of the three samples were combined in a single profile by organ. Similarly, since the MPs profile of the two direct hake preys (whiting) were not significantly different to each other ($\chi^2 = 3.83$, 4 d.f., $p = 0.43$), they were also combined. The few MPs found in shrimp (only four) were two black fragments, one red fibre and one blue fibre. Black fibres were the most abundant MP type in hake GIT and liver, while blue/greenish fibres were dominant in water and hake gill samples (Figure 4). Hake GIT were the samples with the lowest proportion of MP fragments. Despite low sample sizes, contingency chi-square comparing the six types of samples was significant ($\chi^2 = 44.27$, 25 d.f., $p = 0.01$) and had a moderate effect size (Cramer's $V = 0.18$). Post-hoc contingency tests revealed significant differences between water and GIT ($\chi^2 = 11.38$, 5 d.f., $p = 0.04$, large effect size $V = 0.41$), but not between water and gills ($\chi^2 = 3.09$, 5 d.f., $p = 0.69$, moderate effect size $V = 0.2$) nor water and liver ($\chi^2 = 6.17$, 5 d.f., $p = 0.29$, large $V = 0.29$).

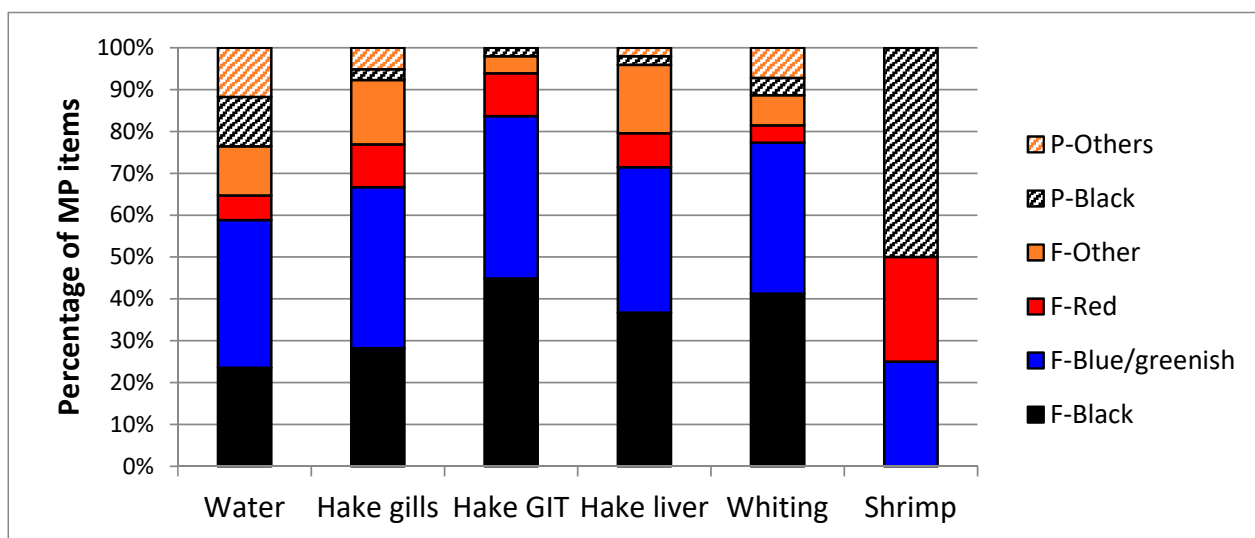


Figure 4. Types of microplastics in water, hake organs and prey. Items are classified as fibres (=F) or fragments (=pieces, P), by colour.

3.2. Microplastic Composition and Risk

μ -FT-IR analysis supported visual identification to confirm plastic materials and discard natural ones. Cellulose (two items) and rayon (six items in total, four of them in water) were removed from further analysis because they are not plastic. Rayon is a synthetic fibre made from natural sources of regenerated cellulose, such as wood and related agricultural products, but not plastic. In total, 16 plastic types were identified, the most abundant being polyester, acrylic, and polyethyleneimine cellulose (transformed cellulose with chelating properties stable in water, widely used from coffee filters to water decontamination in treatment plants [34]). Vinyl, polyvinyl, polyvinyl butyral, polyalkylene oxide, ethylene-vinyl acetate copolymer (EVA), polyethylene terephthalate (PET), polyacrylamide, polyacrylonitrile, polyethylene (PE), polyethylene glycol, styrene-isoprene copolymer, poly(propylene:ethylene), and tetrahydrophthalimide were also identified (Table 2).

Table 2. Polymer types identified by μ -FT-IR from items found in water, hake tissues and hake preys that might encompass risks. Presence in a sample is marked with X. Substances labelled by the European Chemical Agency as dangerous for health, are marked with the letter D. GIT, gastrointestinal tract; Aq Env: specific risk for aquatic environments. Presence in hake tissues: L-liver, G-gills, GIT-gastrointestinal tract.

Chemical Composition	Organ or Environment Damaged					Organism		
	Respiratory	Eye	Skin	GIT	Aq Env	Hake	Whiting	Shrimp
Acrylic	D	D	D	D	D	L	X	
Ethylene-vinyl acetate copolymer	Under evaluation/Pre-registration process					L		
Polyacrylamide				D			X	
Polyacrylonitrile	Under evaluation/Pre-registration process						X	
Polyalkylene oxide		D	D		D	L		
Polyester	D	D	D	D	D	G, GIT	X	
Polyethylene glycol			D					X
Polyethylene terephthalate	Under evaluation/Pre-registration process					L		
Polyethyleneimine		D	D	D	D	G		X
Polyvinyl/Vinyl	Under evaluation/Pre-registration process					L	X	
Tetrahydrophthalimide		D		D				X

ECHA employs hazard pictograms to summarize visually adverse effects of chemical substances, e.g., explosive, flammable, corrosive, serious health hazard (damage to organs, carcinogenic, cause genetic defects etc.), health hazards (respiratory irritation, harmful if swallowed, skin irritation, eye irritation, harm to public health through ozone destruction), acute toxicity, environmental hazards (specifically to aquatic life), and others. While it is true that the risk is also determined by doses of MPs ingested, in the individuals analysed in this study, some of the substances encompassed several of those risks that are summarized in Figure 2, which could be dangerous for the resource and final consumer.

According to the ECHA, seven of the 16 identified chemicals are harmful for health, or are under evaluation, and may cause problems in the individuals carrying them (Table 2). To mention those that can damage aquatic organisms, polyalkylene oxide (found in hake) is harmful to aquatic life with long lasting effects, causing serious eye and skin irritation (Table 2). Polyethyleneimine, found in hake and shrimp (Table 2), is toxic to aquatic life with long lasting effects, is harmful if swallowed, causes serious eye damage, and may cause an allergic skin reaction. Acrylic and polyester were found in hake and whiting (Table 2). Acrylic causes severe skin burns and eye damage, is very toxic to aquatic life, is harmful if swallowed, may cause respiratory irritation, and may cause an allergic skin reaction. Polyester causes severe skin burns and eye damage, is very toxic to aquatic life, is

harmful if swallowed, is harmful in contact with skin, is harmful if inhaled and may cause respiratory irritation.

3.3. Genetic Identification of Preys

The DNA sequences obtained from the preys are available in BE2SHARE, <http://doi.org/10.23728/b2share.3b8180d694fd4ce79a6e33c98241a27a> (accessed on 15 December 2021). Visual and morphological identification were consistent. Direct preys (Prey H1.1 and Prey H3.1) found in Hake 1 and Hake 3 (Figure 1) were identified as blue whiting *Micromesistius poutassou* (Risso, 1827). Several indirect preys of Hake 3 were found in the GIT of Prey H3.1, all small shrimps (Figure 2). They were in different states of digestion, and we took for analysis the least degraded with all tissue's integers. Prey H3.1.1 was identified as Northern krill *Meganyctiphanes norvegica* (Sars, 1856), through DNA identification, belonging to the Euphausiidae family. Analysis in BLAST of the consensus barcode sequence for the three preys revealed a high percentage of nucleotide identity matches (100% for H1.1, 100% for H3.1 and 99.35% for H3.1.1).

4. Discussion

This study, although based on a limited number of samples, demonstrates empirically the simultaneous occurrence of MPs in three steps of the trophic web: a prey inside a predator inside a higher predator, such as an all-inclusive pack-krill inside whiting inside hake. The trophic transfer of MPs has been documented in recent studies in the wild [46] and under laboratory conditions [18,19], but to our knowledge, not as in the present case where the higher predator is contained inside MPs carried by preys as well as preys of preys, in the manner of a Russian doll. Due the importance of the trophic transfer of MPs, studies developed by trophic ecologists have used stable isotope analyses to quantify the trophic niche in freshwater ecosystems [47]. Comparing this methodology with traditional ones, such as the analysis of stomach contents that represent only a snapshot into the diet of organisms, stable isotope analyses provide an integrative quantification, over several weeks to months depending on the tissue analyzed, of the diet of individuals [47–49]. Specifically, stable isotope analyses of carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) provide for the assessment of the origin of the preys and their trophic position in the food chain, respectively, and are commonly used in freshwater ecology, notably to quantify the consequences of global changes [49–51]. On this path, the use of stable isotope analyses, together with the analysis of the simultaneous occurrence of MPs in the trophic web and the species behavior with different factors influencing MPs consumption, e.g., feeding strategy, trophic level transfer, and environmental concentrations, represents a promising approach to understand the microplastic contamination in aquatic food webs.

As expected, water was MP-polluted as in other regions [41]. The water MPs profile was not different to that of gills, and the same happened in the case of the liver, two tissues with recognized pollutants accumulation in fish [29]. A high concentration of MPs in the hake gills is consistent with other studies [26]. This organ is especially vulnerable to environmental pollution because it is in direct and permanent contact with polluted water. Complete gills, without previous rinse, were digested, not differentiating the inside and the outside. However, from the water circulation in the gill system, the most likely origin of the microplastics found in the gills is the seawater the fish breathe. Acquiring microplastics in the gills from the blood during the counter current gas exchange would imply a previous translocation of ingested microplastics to the circulatory system, which seems less probable. The similarity of microplastics from gills and seawater samples would support seawater as the main source of gill microplastics. Zhang et al. [31] have suggested that the number of MPs in gills might be directly related with the pollution characteristics of the contaminated habitats where fish live, leading to a wide variety of results in the literature. On the other hand, the MPs profile of hake GIT was similar to that of hake preys inside, as expected. In other studies, the quantity of particles in GIT is highly variable among individuals and

species, depending on the type and amount of consumed food as well as the digestion and excretion time [12].

As one of the most common types of MP debris worldwide and in the marine environment [52], black and blue fibers were the most abundant type in our study, despite not being possible to know their origin, and this is in line with previous studies [20,53]. Wastewaters, textiles, hygiene, and cosmetic products as well as the fishing industry are some of the main sources of MPs pollution [40]. Organisms inhabiting coastal environments are especially susceptible to MP contamination due to the inputs from land [53]. Moreover, large, urbanized areas are responsible for abundant marine litter [54]. The coast in front of the catch area of the hakes analyzed here is a highly industrialized and populated area where high levels of MP pollution, especially fibers, have been reported in water and sediments from previous studies [55]. The European hake, being a demersal species, will likely encounter MPs, since the seabed has been revealed as a major sink for MP debris [56]. Altogether, this could explain the high concentration of MPs found in all tissues and preys analyzed here, being higher than the amount found in fishes from other areas such as Asia [57], with a range of 0–0.56 items/gram in commercial dried fish [58], or 0.0002–3.64 items per gram in fishes from Indian coast [59]. However, biomagnification would not play a main role here. Always considering the issue of limited sample size, our results would not support the magnification [23,60] or dilution [20,61] of MPs along the trophic chain, because prey MPs contents were always within the range of MP contents in the predators. In addition, it is important to highlight that a higher number of steps in the food chain would offer us a broader view, since PM can be transported from lower trophic levels to the hake, through intermediate prey.

Regarding the risk of ingestion that was the main focus of our study, we found a wide variety of plastics in the MPs analyzed, with polyester and acrylic being the most abundant followed by different types of vinyl. As seen in Table 2, several of those compounds are labeled as dangerous for animal health by the ECHA, with important hazards for the individual and the environment (Figure 4). These hazards are consistent with many studies that have demonstrated adverse effects of MPs to animals and humans (e.g., carcinogenic, endocrine disruption, neurotoxic) [24–27,29,62], even at a very high hazard level considering the health hazard ranking published by Lithner et al. [62], such as polyacrylamide in second position with a hazard level of 12,379 or polyvinyl in fifth position with a level of danger of 10,551 (out of a maximum of 13,844 in the first position of the ranking). MPs can cause histological damage in the intestine of adult zebrafish [63], may diminish the chances of capturing prey or escaping the predators [64], and encompass negative effects at the population level [65]. These and other adverse effects of MPs may be expected in hakes given that they already contain a noticeable density of MPs in functionally essential organs, such as the gills and liver, as is consistent with previous studies that reveal the real human health risks posed by MPs in seafood [66], also emphasizing that, although the GIT of fish are usually discarded before consumption, the edible parts of fish could be contaminated by the MPs contained in the GIT during food preparation or through translocation, raising potential health concerns [13]. Authors warn about the potential of MPs to contribute to biodiversity losses and the emergence of human and animal diseases [67], compromising ecological processes as well as food security and health [29]. If MPs really cause disruptions in hake populations, this would affect the local economy and perhaps compromise this type of seafood [61].

The presence of plastic in the human blood is a current sad reality [68]. However, few data are available to assess the ecological risk of marine MPs to human health through seafood consumption [69]. Therefore, there is currently no legal framework regulating admissible levels of MP pollution in seafood [70,71]. Fish that are consumed whole (including the GIT), such as anchovies and sardines, and shrimps included in our case study, pose risk of MPs ingestion to consumers [13,72]. Although hakes are normally eviscerated before consumption, it seems that removing the digestive tract does not eliminate the risk of MPs intake by consumers [34]. Some studies have detected MPs in the muscle of commercially

important fish [14,73] and a crustacean [10]. Once ingested, >90% of MPs were reported to be excreted in feces [74], especially large particles > 150 μm . However, smaller particles may be absorbed systematically. It has been reported that MPs 0.1–10 μm in size can cross the blood–brain barrier and the placenta [75], particles < 150 μm can cross gastrointestinal epithelium, and particles < 2.5 μm can enter the systemic circulation through endocytosis [13]. However, there is still little certainty about the transfer of and risk posed by MPs to humans through diet [70]. Some studies suggest limited health risks of seafood to humans [76], proposing minimal MP uptake through seafood consumption compared to other sources of exposure, such as dust [77], plastic-packaged food [70], or airborne MPs [77]. Nevertheless, the presence of hazard components in widely consumed species, such as the hake, whiting, and shrimps analyzed here, together with their great popularity among consumers (0.94 and 1.51 kg/pers/year for hake and shrimps respectively, ECHA. Available online: <https://ec.europa.eu/>, accessed on 15 December 2021), make necessary more and deeper studies to unravel the real ingestion of MPs and their implications for consumer's health through the seafood.

Finally, there is difficulty in comparing MPs studies without a standardized methodology [66], since it hinders the possibility to equate the results regarding the amount of plastics found. We found MPs in all the hake tissues and preys analyzed. Indeed, MPs have also been found in GITs, livers, and gills of many species in other studies [15–17,30]. Despite the fact they are usually reported in the gastric tract [78] and the alleged translocation in most cases of such large particles is difficult to explain with the current knowledge on the translocation pathways for MPs in fish [79], the translocation of MP particles to other tissues, such as the liver [68,80] and muscle [14,81], is a reality [79]. Therefore, as the gastrointestinal tract is especially important to the entry of MPs to the organism, their translocation and dietary transfer into the trophic chain, exploring the microplastic transfer inside and between organisms into the trophic web, is essential [82], highlighting the need for more in-depth studies.

On the other hand, the literature shows varied results regarding the plastics found in the different organs, sometimes due to technical or methodological issues. Here, we used H_2O_2 for tissue digestion, but other studies use different digestion agents (HNO_3 , KOH , etc.) that allow for a different recovery of MPs and may even dissolve some types [83]. Besides, using different digestion agents will produce changes in the MPs bleaching: while the bleaching is not expected using H_2O_2 , or in a smaller way, as it has been presented in previous studies [84], the bleached of different materials is present in samples exposed to KOH [85]. Another source of variation between studies is the filter pore. Decreasing the filter pore size, the number of MPs recovered per liter increases by several orders of magnitude [86]. In this study, we have used filters with a small pore size (0.45 μm), while other studies [17,20] use filters with a much larger pore size, making comparisons difficult. For instance, Su et al. [17] did not find MPs in fish livers and suggested that this could be due to methodological limitations, (i.e., they used a 20 μm pore size filter, in comparison with the 0.45 μm pore size filters employed in our study), thus demonstrating the urgent needed of a standardized methodology.

5. Conclusions and Recommendations

Our research demonstrates the occurrence of MPs through a three-link food chain in wild hakes. It seems that MPs concentration would not be magnified in higher trophic level organisms. To confirm this, studies with more individuals are recommended.

Despite the small sample size, small water volume, and small MPs chemical composition analyzed, the presence of MPs in all the tissues analyzed of hakes and their preys, together with the evidence of hazard compositions of some of them, highlights the need for policies and actions to reduce plastic and microplastic production and consumption.

Finally, standardized protocols are recommended in order to make comparison among different works concerning MPs quantifications for an accurate risk assessment [60].

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/d14050308/s1>, Figure S1: Clean spectra obtained from some dangerous polymer identified by μ -FT-IR (Table 2), with no evidence of mixture of compounds. In different colours the sample analysed, in black the reference pattern. (A) Acrylic, (B) Polyester and (C) Tetrahydrophthalimide.; Table S1: H₂O₂ volume in mL employed to digest the samples analysed in this study. GIT, gastrointestinal tract. More volume was employed when hard tissue was present; Table S2: Number and type of microplastics found in each sample analysed. GIT, gastrointestinal tract.

Author Contributions: P.C. wrote the original draft and developed the research idea, microplastic collection, and data analysis. E.G.-V. and A.A. (Alba Ardura) developed the research idea and supervision. A.A. (Alba Ardura) developed the DNA analysis. A.A. (Andrés Arias) developed the taxonomical identification. S.A. was in charge of MPs in seawater. P.M. developed the dissection of individuals and lab work. E.G.-V. developed the data analysis and funding acquisition. The manuscript was written through the contributions of all authors. All authors have read and agreed to the published version of the manuscript.

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References

1. Derraik, J.G.B. The pollution of the marine environment by plastic debris: A review. *Mar. Pollut. Bull.* **2002**, *44*, 842–852. [[CrossRef](#)]
2. Arthur, C.; Baker, J.E.; Bamford, H.A. *Proceedings of the International Research Workshop on the Occurrence, Effects, and Fate of Microplastic Marine Debris*; National Oceanic and Atmospheric Administration (NOAA): Washington, DC, USA, 2009.
3. Azevedo-Santos, V.M.; Brito, M.F.G.; Manoel, P.S.; Perroca, J.F.; Rodrigues-Filho, J.L.; Paschoal, L.R.P.; Gonçalves, G.R.L.; Wolf, M.R.; Blettler, M.C.M.; Andrade, M.C.; et al. Plastic pollution: A focus on freshwater biodiversity. *Ambio* **2021**, *50*, 1313–1324. [[CrossRef](#)] [[PubMed](#)]
4. Jambeck, J.R.; Geyer, R.; Wilcox, C.; Siegler, T.R.; Perryman, M.; Andrady, A.; Narayan, R.; Law, K.L. Plastic waste inputs from land into the ocean. *Science* **2015**, *347*, 768–771. [[CrossRef](#)] [[PubMed](#)]
5. Geyer, R.; Jambeck, J.R.; Law, K.L. Production, use, and fate of all plastics ever made. *Sci. Adv.* **2017**, *3*, e1700782. [[CrossRef](#)]
6. Gall, S.C.; Thompson, R.C. The impact of debris on marine life. *Mar. Pollut. Bull.* **2015**, *92*, 170–179. [[CrossRef](#)]
7. de Souza Machado, A.A.; Lau, C.W.; Kloas, W.; Bergmann, J.; Bachelier, J.B.; Faltin, E.; Becker, R.; Görlich, A.S.; Rillig, M.C. Microplastics Can Change Soil Properties and Affect Plant Performance. *Environ. Sci. Technol.* **2019**, *53*, 6044–6052. [[CrossRef](#)]
8. Maes, T.; Jel, J.V.D.D.; Vethaak, A.D.; Desender, M.; Bendall, V.A.; Van Velzen, M.; Leslie, H.A. You Are What You Eat, Microplastics in Porbeagle Sharks From the North East Atlantic: Method Development and Analysis in Spiral Valve Content and Tissue. *Front. Mar. Sci.* **2020**, *7*, 273. [[CrossRef](#)]
9. Li, C.; Gan, Y.; Zhang, C.; He, H.; Fang, J.; Wang, L.; Wang, Y.; Liu, J. “Microplastic communities” in different environments: Differences, links, and role of diversity index in source analysis. *Water Res.* **2020**, *188*, 116574. [[CrossRef](#)]
10. Agathokleous, E.; Iavicoli, I.; Barceló, D.; Calabrese, E.J. Ecological risks in a ‘plastic’ world: A threat to biological diversity? *J. Hazard. Mater.* **2021**, *417*, 126035. [[CrossRef](#)]
11. Adeogun, A.O.; Ibor, O.R.; Khan, E.A.; Chukwuka, A.V.; Omogbemi, E.D.; Arukwe, A. Detection and occurrence of microplastics in the stomach of commercial fish species from a municipal water supply lake in southwestern Nigeria. *Environ. Sci. Pollut. Res.* **2020**, *27*, 31035–31045. [[CrossRef](#)]
12. Romeo, T.; Pietro, B.; Pedà, C.; Consoli, P.; Andaloro, F.; Fossi, M.C. First evidence of presence of plastic debris in stomach of large pelagic fish in the Mediterranean Sea. *Mar. Pollut. Bull.* **2015**, *95*, 358–361. [[CrossRef](#)]
13. Collard, F.; Gilbert, B.; Compère, P.; Eppe, G.; Das, K.; Jauniaux, T.; Parmentier, E. Microplastics in livers of European anchovies (*Engraulis encrasicolus*, L.). *Environ. Pollut.* **2017**, *229*, 1000–1005. [[CrossRef](#)]

14. Abbasi, S.; Soltani, N.; Keshavarzi, B.; Moore, F.; Turner, A.; Hassanaghaei, M. Microplastics in different tissues of fish and prawn from the Musa Estuary, Persian Gulf. *Chemosphere* **2018**, *205*, 80–87. [[CrossRef](#)] [[PubMed](#)]
15. Kolandhasamy, P.; Su, L.; Li, J.; Qu, X.; Jabeen, K.; Shi, H. Adherence of microplastics to soft tissue of mussels: A novel way to uptake microplastics beyond ingestion. *Sci. Total Environ.* **2017**, *610–611*, 635–640. [[CrossRef](#)] [[PubMed](#)]
16. Lu, Y.; Zhang, Y.; Deng, Y.; Jiang, W.; Zhao, Y.; Geng, J.; Ding, L.; Ren, H.-Q. Uptake and Accumulation of Polystyrene Microplastics in Zebrafish (*Danio rerio*) and Toxic Effects in Liver. *Environ. Sci. Technol.* **2016**, *50*, 4054–4060. [[CrossRef](#)] [[PubMed](#)]
17. Su, L.; Deng, H.; Li, B.; Chen, Q.; Pettigrove, V.; Wu, C.; Shi, H. The occurrence of microplastic in specific organs in commercially caught fishes from coast and estuary area of east China. *J. Hazard. Mater.* **2019**, *365*, 716–724. [[CrossRef](#)]
18. Farrell, P.; Nelson, K. Trophic level transfer of microplastic: *Mytilus edulis* (L.) to *Carcinus maenas* (L.). *Environ. Pollut.* **2013**, *177*, 1–3. [[CrossRef](#)]
19. Batel, A.; Linti, F.; Scherer, M.; Erdinger, L.; Braunbeck, T. Transfer of benzo [a] pyrene from microplastics to *Artemia nauplii* and further to zebrafish via a trophic food web experiment: CYP1A induction and visual tracking of persistent organic pollutants. *Environ. Toxicol. Chem.* **2016**, *35*, 1656–1666. [[CrossRef](#)] [[PubMed](#)]
20. Nelms, S.E.; Galloway, T.S.; Godley, B.J.; Jarvis, D.S.; Lindeque, P.K. Investigating microplastic trophic transfer in marine top predators. *Environ. Pollut.* **2018**, *238*, 999–1007. [[CrossRef](#)]
21. Chagnon, C.; Thiel, M.; Antunes, J.; Ferreira, J.L.; Sobral, P.; Ory, N.C. Plastic ingestion and trophic transfer between Easter Island flying fish (*Cheilopogon rapanouiensis*) and yellowfin tuna (*Thunnus albacares*) from Rapa Nui (Easter Island). *Environ. Pollut.* **2018**, *243*, 127–133. [[CrossRef](#)]
22. Jabeen, K.; Su, L.; Li, J.; Yang, D.; Tong, C.; Mu, J.; Shi, H. Microplastics and mesoplastics in fish from coastal and fresh waters of China. *Environ. Pollut.* **2016**, *221*, 141–149. [[CrossRef](#)] [[PubMed](#)]
23. Miller, M.E.; Hamann, M.; Kroon, F.J. Bioaccumulation and biomagnification of microplastics in marine organisms: A review and meta-analysis of current data. *PLoS ONE* **2020**, *15*, e0240792. [[CrossRef](#)] [[PubMed](#)]
24. Diamanti-Kandarakis, E.; Bourguignon, J.P.; Giudice, L.C.; Hauser, R.; Prins, G.S.; Soto, A.M.; Zoeller, R.T.; Gore, A.C. Endocrine-disrupting chemicals: An Endocrine Society scientific statement. *Endocr. Rev.* **2009**, *30*, 293–342. [[CrossRef](#)] [[PubMed](#)]
25. Campanale, C.; Massarelli, C.; Savino, I.; Locaputo, V.; Uricchio, V.F. A Detailed Review Study on Potential Effects of Microplastics and Additives of Concern on Human Health. *Int. J. Environ. Res. Public Health* **2020**, *17*, 1212. [[CrossRef](#)] [[PubMed](#)]
26. Joo, S.H.; Liang, Y.; Kim, M.; Byun, J.; Choi, H. Microplastics with adsorbed contaminants: Mechanisms and Treatment. *Environ. Chall.* **2021**, *3*, 100042. [[CrossRef](#)]
27. Amelia, T.S.M.; Khalik, W.M.A.W.M.; Ong, M.C.; Shao, Y.T.; Pan, H.-J.; Bhubalan, K. Marine microplastics as vectors of major ocean pollutants and its hazards to the marine ecosystem and humans. *Prog. Earth Planet. Sci.* **2021**, *8*, 12. [[CrossRef](#)]
28. Bhagat, J.; Nishimura, N.; Shimada, Y. Toxicological interactions of microplastics/nanoplastics and environmental contaminants: Current knowledge and future perspectives. *J. Hazard. Mater.* **2020**, *405*, 123913. [[CrossRef](#)]
29. Barboza, L.G.A.; Vieira, L.R.; Branco, V.; Figueiredo, N.; Carvalho, F.; Carvalho, C.; Guilhermino, L. Microplastics cause neurotoxicity, oxidative damage and energy-related changes and interact with the bioaccumulation of mercury in the European seabass, *Dicentrarchus labrax* (Linnaeus, 1758). *Aquat. Toxicol.* **2018**, *195*, 49–57. [[CrossRef](#)]
30. Galloway, T.S.; Cole, M.; Lewis, C. Interactions of microplastic debris throughout the marine ecosystem. *Nat. Ecol. Evol.* **2017**, *1*, 1–8. [[CrossRef](#)]
31. Zhang, F.; Wang, X.; Xu, J.; Zhu, L.; Peng, G.; Xu, P.; Li, D. Food-web transfer of microplastics between wild caught fish and crustaceans in East China Sea. *Mar. Pollut. Bull.* **2019**, *146*, 173–182. [[CrossRef](#)]
32. Greenpeace, 2018. Destrucción a toda costa 2008. Informe de Greenpeace Sobre la Situación del Litoral Español. Available online: <https://archivo-es.greenpeace.org/espana/Global/espana/report/costas/informe-completo-destruccion.pdf> (accessed on 15 September 2021).
33. Bernet, D.; Schmidt, H.; Meier, W.; Burkhardt-Holm, P.; Wahli, T. Histopathology in fish: Proposal for a protocol to assess aquatic pollution. *J. Fish Dis.* **1999**, *22*, 25–34. [[CrossRef](#)]
34. Karami, A.; Golieskardi, A.; Bin Ho, Y.; Larat, V.; Salamatinia, B. Microplastics in eviscerated flesh and excised organs of dried fish. *Sci. Rep.* **2017**, *7*, 5473. [[CrossRef](#)] [[PubMed](#)]
35. Domínguez-Cuesta, M.J.; Valenzuela, P.; Rodríguez-Rodríguez, R.; Ballesteros, D.; Jimenez-Sanchez, M.; Piñuela, L.; Garcia-Ramos, J.C. Cliff Coast of Asturias. In *The Spanish Coastal Systems*; Morales, J.A., Ed.; Springer: Cham, Switzerland, 2019.
36. De Castro, M.; Gómez-Gesteira, M.; Álvarez, I.; Crespo, A.J.C. Atmospheric modes influence on Iberian Poleward Current variability. *Cont. Shelf Res.* **2011**, *31*, 425–432. [[CrossRef](#)]
37. Mendoza, A.; Osa, J.L.; Basurko, O.C.; Rubio, A.; Santos, M.; Gago, J.; Galgani, F.; Peña-Rodríguez, C. Microplastics in the Bay of Biscay: An overview. *Mar. Pollut. Bull.* **2020**, *153*, 110996. [[CrossRef](#)]
38. Li, J.; Qu, X.; Su, L.; Zhang, W.; Yang, D.; Kolandhasamy, P.; Li, D.; Shi, H. Microplastics in mussels along the coastal waters of China. *Environ. Pollut.* **2016**, *214*, 177–184. [[CrossRef](#)]
39. Masiá, P.; Ardura, A.; Garcia-Vazquez, E. Microplastics in special protected areas for migratory birds in the Bay of Biscay. *Mar. Pollut. Bull.* **2019**, *146*, 993–1001. [[CrossRef](#)]
40. Andrady, A.L. Microplastics in the marine environment. *Mar. Pollut. Bull.* **2011**, *62*, 1596–1605. [[CrossRef](#)]
41. Hidalgo-Ruz, V.; Gutow, L.; Thompson, R.C.; Thiel, M. Microplastics in the Marine Environment: A Review of the Methods Used for Identification and Quantification. *Environ. Sci. Technol.* **2012**, *46*, 3060–3075. [[CrossRef](#)]

42. Estoup, A.; Largiader, C.R.; Perrot, E.; Chourrout, D. Rapid one-tube DNA extraction for reliable PCR detection of fish polymorphic markers and transgenes. *Mol. Mar. Biol. Biotechnol.* **1996**, *5*, 295–298.
43. Ward, R.D.; Zemlak, T.S.; Innes, B.H.; Last, P.R.; Hebert, P.D. DNA barcoding Australia's fish species. *Philos. Trans. R. Soc. B Biol. Sci.* **2005**, *360*, 1847–1857. [[CrossRef](#)]
44. Zane, L.; Ostellari, L.; Maccatrozzo, L. Genetic differentiation in a pelagic crustacean (*Meganyctiphanes norvegica*: Euphausiacea) from the North East Atlantic and the Mediterranean Sea. *Mar. Biol.* **2000**, *136*, 191–199. [[CrossRef](#)]
45. Hall, T.A. BioEdit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symp.* **1999**, *41*, 95–98.
46. Garcia, F.; De Carvalho, A.R.; Riem-Galliano, L.; Tudesque, L.; Albignac, M.; Ter Halle, A.; Cucherousset, J. Stable Isotope Insights into Microplastic Contamination within Freshwater Food Webs. *Environ. Sci. Technol.* **2021**, *55*, 1024–1035. [[CrossRef](#)]
47. Layman, C.A.; Araujo, M.S.; Boucek, R.; Hammerschlag-Peyer, C.M.; Harrison, E.; Jud, Z.R.; Matich, P.; Rosenblatt, A.; Vaudo, J.J.; Yeager, L.; et al. Applying stable isotopes to examine food-web structure: An overview of analytical tools. *Biol. Rev.* **2011**, *87*, 545–562. [[CrossRef](#)]
48. Fry, B. *Stable Isotope Ecology*; Springer: New York, NY, USA, 2006. [[CrossRef](#)]
49. Jackson, M.C.; Donohue, I.; Jackson, A.L.; Britton, R.; Harper, D.M.; Grey, J. Population-Level Metrics of Trophic Structure Based on Stable Isotopes and Their Application to Invasion Ecology. *PLoS ONE* **2012**, *7*, e31757. [[CrossRef](#)] [[PubMed](#)]
50. Cucherousset, J.; Boulètreau, S.; De Martino, A.; Roussel, J.-M.; Santoul, F. Using stable isotope analyses to determine the ecological effects of non-native fishes. *Fish. Manag. Ecol.* **2011**, *19*, 111–119. [[CrossRef](#)]
51. Gago, J.; Carretero, O.; Filgueiras, A.V.; Viñas, L. Synthetic microfibers in the marine environment: A review on their occurrence in seawater and sediments. *Mar. Pollut. Bull.* **2018**, *127*, 365–376. [[CrossRef](#)]
52. Montoto-Martínez, T.; Hernández-Brito, J.J.; Gelado-Caballero, M.D. Pump-underway ship intake: An unexploited opportunity for Marine Strategy Framework Directive (MSFD) microplastic monitoring needs on coastal and oceanic waters. *PLoS ONE* **2020**, *15*, e0232744. [[CrossRef](#)]
53. Browne, M.A.; Galloway, T.S.; Thompson, R.C. Spatial Patterns of Plastic Debris along Estuarine Shorelines. *Environ. Sci. Technol.* **2010**, *44*, 3404–3409. [[CrossRef](#)]
54. Gago, J.; Henry, M.; Galgani, F. First observation on neustonic plastics in waters off NW Spain (spring 2013 and 2014). *Mar. Environ. Res.* **2015**, *111*, 27–33. [[CrossRef](#)]
55. Masiá, P.; Ardura, A.; Gaitán, M.; Gerber, S.; Rayon-Viña, F.; Garcia-Vazquez, E. Maritime ports and beach management as sources of coastal macro-, meso-, and microplastic pollution. *Environ. Sci. Pollut. Res.* **2021**, *28*, 30722–30731. [[CrossRef](#)] [[PubMed](#)]
56. Woodall, L.C.; Sanchez-Vidal, A.; Canals, M.; Paterson, G.L.J.; Coppock, R.; Sleight, V.; Calafat, A.; Rogers, A.D.; Narayanaswamy, B.E.; Thompson, R.C. The deep sea is a major sink for microplastic debris. *R. Soc. Open Sci.* **2014**, *1*, 140317. [[CrossRef](#)] [[PubMed](#)]
57. Piyawardhana, N.; Weerathunga, V.; Chen, H.-S.; Guo, L.; Huang, P.-J.; Ranatunga, R.; Hung, C.-C. Occurrence of microplastics in commercial marine dried fish in Asian countries. *J. Hazard. Mater.* **2021**, *423*, 127093. [[CrossRef](#)] [[PubMed](#)]
58. Veerasingam, S.; Ranjani, M.; Venkatachalapathy, R.; Bagaev, A.; Mukhanov, V.; Litvinyuk, D.; Verzhvetskaia, L.; Guganathan, L.; Vethamony, P. Microplastics in different environmental compartments in India: Analytical methods, distribution, associated contaminants and research needs. *TrAC Trends Anal. Chem.* **2020**, *133*, 116071. [[CrossRef](#)]
59. Saley, A.; Smart, A.; Bezerra, M.; Burnham, T.; Capece, L.; Lima, L.; Carsh, A.; Williams, S.; Morgan, S. Microplastic accumulation and biomagnification in a coastal marine reserve situated in a sparsely populated area. *Mar. Pollut. Bull.* **2019**, *146*, 54–59. [[CrossRef](#)]
60. Walkinshaw, C.; Lindeque, P.K.; Thompson, R.; Tolhurst, T.; Cole, M. Microplastics and seafood: Lower trophic organisms at highest risk of contamination. *Ecotoxicol. Environ. Saf.* **2019**, *190*, 110066. [[CrossRef](#)]
61. Wang, T.; Hu, M.; Xu, G.; Shi, H.; Leung, J.Y.; Wang, Y. Microplastic accumulation via trophic transfer: Can a predatory crab counter the adverse effects of microplastics by body defence? *Sci. Total Environ.* **2021**, *754*, 142099. [[CrossRef](#)]
62. Lithner, D.; Larsson, Å.; Dave, G. Environmental and health hazard ranking and assessment of plastic polymers based on chemical composition. *Sci. Total Environ.* **2011**, *409*, 3309–3324. [[CrossRef](#)]
63. Lei, L.; Wu, S.; Lu, S.; Liu, M.; Song, Y.; Fu, Z.; Shi, H.; Raley-Susman, K.M.; He, D. Microplastic particles cause intestinal damage and other adverse effects in zebrafish *Danio rerio* and nematode *Caenorhabditis elegans*. *Sci. Total Environ.* **2017**, *619*, 1–8. [[CrossRef](#)]
64. Besseling, E.; Foekema, E.M.; Van Franeker, J.A.; Leopold, M.F.; Kühn, S.; Rebolledo, E.B.; Hebe, E.; Mielke, L.; Ijzer, J.; Kamminga, P.; et al. Microplastic in a macro filter feeder: Humpback whale *Megaptera novaeangliae*. *Mar. Poll. Bull.* **2015**, *95*, 248–252. [[CrossRef](#)]
65. Ferreira, P.; Fonte, E.; Soares, M.E.; Carvalho, F.; Guilhermino, L. Effects of multi-stressors on juveniles of the marine fish *Pomatoschistus microps*: Gold nanoparticles, microplastics and temperature. *Aquat. Toxicol.* **2016**, *170*, 89–103. [[CrossRef](#)] [[PubMed](#)]
66. Fang, C.; Zheng, R.; Chen, H.; Hong, F.; Lin, L.; Lin, H.; Guo, H.; Bailey, C.; Segner, H.; Mu, J.; et al. Comparison of microplastic contamination in fish and bivalves from two major cities in Fujian province, China and the implications for human health. *Aquaculture* **2019**, *512*, 734322. [[CrossRef](#)]
67. Zettler, E.R.; Mincer, T.J.; Amaral-Zettler, L.A. Life in the “plastisphere”: Microbial communities on plastic marine debris. *Environ. Sci. Technol.* **2013**, *47*, 7137–7146. [[CrossRef](#)] [[PubMed](#)]

68. Leslie, H.A.; van Velzen, M.J.M.; Brandsma, S.H.; Vethaak, D.; Garcia-Vallejo, J.J.; Lamoree, M.H. Discovery and quantification of plastic particle pollution in human blood. *Environ. Int.* **2022**, 107199. [[CrossRef](#)]
69. Rist, S.; Almroth, B.C.; Hartmann, N.B.; Karlsson, T.M. A critical perspective on early communications concerning human health aspects of microplastics. *Sci. Total Environ.* **2018**, 626, 720–726. [[CrossRef](#)]
70. EFSA Panel on Contaminants in the Food Chain (CONTAM). Presence of microplastics and nanoplastics in food, with particular focus on seafood. *EFSA J.* **2016**, 14, e04501.
71. Ziccardi, L.M.; Edgington, A.; Hentz, K.; Kulacki, K.J.; Driscoll, S.K. Microplastics as vectors for bioaccumulation of hydrophobic organic chemicals in the marine environment: A state-of-the-science review. *Environ. Toxicol. Chem.* **2016**, 35, 1667–1676. [[CrossRef](#)]
72. Compa, M.; Ventero, A.; Iglesias, M.; Deudero, S. Ingestion of microplastics and natural fibres in *Sardina pilchardus* (Walbaum, 1792) and *Engraulis encrasicolus* (Linnaeus, 1758) along the Spanish Mediterranean coast. *Mar. Pollut. Bull.* **2018**, 128, 89–96. [[CrossRef](#)]
73. Smith, M.; Love, D.C.; Rochman, C.M.; Neff, R.A. Microplastics in Seafood and the Implications for Human Health. *Curr. Environ. Health Rep.* **2018**, 5, 375–386. [[CrossRef](#)]
74. Vethaak, A.D.; Leslie, H.A. Plastic debris is a human health issue. *Environ. Sci. Technol.* **2016**, 50, 6825–6826. [[CrossRef](#)]
75. Catarino, A.I.; Macchia, V.; Sanderson, W.G.; Thompson, R.C.; Henry, T.B. Low levels of microplastics (MP) in wild mussels indicate that MP ingestion by humans is minimal compared to exposure via household fibres fallout during a meal. *Environ. Pollut.* **2018**, 237, 675–684. [[CrossRef](#)] [[PubMed](#)]
76. Prata, J.C. Airborne microplastics: Consequences to human health? *Environ. Pollut.* **2018**, 234, 115–126. [[CrossRef](#)] [[PubMed](#)]
77. Neves, D.; Sobral, P.; Ferreira, J.L.; Pereira, T. Ingestion of microplastics by commercial fish off the Portuguese coast. *Mar. Pollut. Bull.* **2015**, 101, 119–126. [[CrossRef](#)] [[PubMed](#)]
78. De Sales-Ribeiro, C.; Brito-Casillas, Y.; Fernandez, A.; Caballero, M.J. An end to the controversy over the microscopic detection and effects of pristine microplastics in fish organs. *Sci. Rep.* **2020**, 10, 12434. [[CrossRef](#)] [[PubMed](#)]
79. Avio, C.G.; Gorbi, S.; Regoli, F. Experimental development of a new protocol for extraction and characterization of microplastics in fish tissues: First observations in commercial species from Adriatic Sea. *Mar. Environ. Res.* **2015**, 111, 18–26. [[CrossRef](#)] [[PubMed](#)]
80. Jovanović, B.; Gökdağ, K.; Guven, O.; Emre, Y.; Whitley, E.M.; Kideys, A.E. Virgin microplastics are not causing imminent harm to fish after dietary exposure. *Mar. Pollut. Bull.* **2018**, 130, 123–131. [[CrossRef](#)]
81. O'Connor, J.D.; Murphy, S.; Lally, H.; O'Connor, I.; Nash, R.; O'Sullivan, J.; Bruen, M.; Heerey, L.; Koelmans, A.A.; Cullagh, A.; et al. Microplastics in brown trout (*Salmo trutta* Linnaeus, 1758) from an Irish riverine system. *Environ. Pollut.* **2020**, 267, 115572. [[CrossRef](#)]
82. Lusher, A.L.; Welden, N.A.; Sobral, P.; Cole, M. Sampling, isolating and identifying microplastics ingested by fish and invertebrates. In *Analysis of Nanoplastics and Microplastics in Food*; CRC Press: Boca Raton, FL, USA, 2020; pp. 119–148.
83. Welden, N.; Abylkhani, B.; Howarth, L. The effects of trophic transfer and environmental factors on microplastic uptake by plaice, *Pleuronectes platessa*, and spider crab, *Maja squinado*. *Environ. Pollut.* **2018**, 239, 351–358. [[CrossRef](#)]
84. Ehlers, S.; Manz, W.; Koop, J. Microplastics of different characteristics are incorporated into the larval cases of the freshwater caddisfly *Lepidostoma basale*. *Aquat. Biol.* **2019**, 28, 67–77. [[CrossRef](#)]
85. Dawson, A.L.; Motti, C.A.; Kroon, F.J. Solving a Sticky Situation: Microplastic Analysis of Lipid-Rich Tissue. *Front. Environ. Sci.* **2020**, 8, 163. [[CrossRef](#)]
86. Lenz, R.; Enders, K.; Nielsen, T.G. Microplastic exposure studies should be environmentally realistic. *Proc. Natl. Acad. Sci. USA* **2016**, 113, E4121–E4122. [[CrossRef](#)] [[PubMed](#)]