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Article

Quantitative Analysis of Selected Plastics in High-Commercial-Value Australian Seafood by Pyrolysis Gas Chromatography Mass Spectrometry

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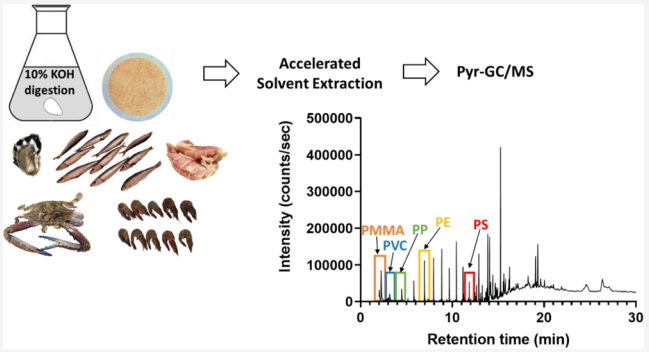
ABSTRACT: Microplastic contamination of the marine environment is widespread, but the extent to which the marine food web is contaminated is not yet known. The aims of this study were to go beyond visual identification techniques and develop and apply a simple seafood sample cleanup, extraction, and quantitative analysis method using pyrolysis gas chromatography mass spectrometry to improve the detection of plastic contamination. This method allows the identification and quantification of polystyrene, polyethylene, polyvinyl chloride, polypropylene, and poly(methyl methacrylate) in the edible portion of five different seafood organisms: oysters, prawns, squid, crabs, and sardines. Polyvinyl chloride was detected in all samples and polyethylene at the highest total concentration of between 0.04 and 2.4 mg g^{-1} of tissue. Sardines contained the highest total plastic mass concentration (0.3 mg g^{-1} tissue) and squid the lowest (0.04 mg g^{-1} tissue). Our findings show that the total concentration of plastics is highly variable among species and that microplastic concentration differs between organisms of the same species. The sources of microplastic exposure, such as packaging and handling with consequent transference and adherence to the tissues, are discussed. This method is a major development in the standardization of plastic quantification techniques used in seafood.



1. INTRODUCTION

The consumption of food and drinks[1] and inhalation[2] are the primary known routes of human exposure to micro- and nanosized plastics in the environment. The ocean is the largest known sink for microplastics[3] with their presence acknowledged as a potential threat to marine organisms.[4] Given the vulnerability of marine organisms to microplastic exposure, the consumption of seafood, in the form of both fish and shellfish, is often considered a leading pathway for microplastic ingestion.[5,6] Several adverse effects have been observed in marine species following ingestion of microplastics, such as physical damage, oxidative stress and damage, effects on feeding and reproduction, or even mortality.[7] Although the risks associated with human plastic ingestion are not yet well understood, an improvement in the currently available analytical methods and reporting of results will help greatly with assessing any potential risk.[8]

Seafood represents approximately 17% of the total animal protein consumed globally.[9] In Australia, the intake of seafood is increasing with prawns, oysters, and crabs being some of the most consumed seafood,[10] while worldwide, sardines and squids are among the most commonly eaten species. Estimates suggest that in Europe, seafood consumers ingest an average of 11,000 microplastic particles per year in countries where a large amount of shellfish is eaten.[11] Recently, it has been further suggested that an average person could be ingesting approximately 5 g of plastic every week based on the consumption of common food and beverages, with shellfish contributing to 0.5 g of the total weekly intake.[12]

Commercially important fish and shellfish, both wild and farmed, contain microplastic particles.[11,13−16] A major concern is seafood where the whole organism is eaten (e.g., bivalves[17]) as any remaining content in the gastrointestinal (GI) tract will also be ingested.[18] Bivalves are the most studied organisms with respect to microplastic concentrations.[6,11,19] Few studies

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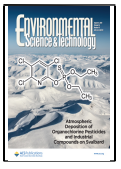

have reported the presence of microplastics in prawns[14] or crabs.[20] Interestingly, there is generally little available information on seafood species other than bivalves or the digestive tissue of fish,[17,21,22] failing to account for the edible portion, as the muscle.

Several challenges arise with respect to the analysis of microplastics in biota. Organisms are composed of a complex mixture that consists, among other things, of organic and inorganic matter, cells, and water.[23] Polymers present in the sample usually suffer from degradation in the natural environment or when ingested by organisms making identification challenging.[24] There is also at present a knowledge and technology gap with respect to the detection of smaller sized microplastics (<150 μm).[16]

The most common approach for microplastic detection in seafood is a primary visual identification of apparent plastic particles. As there can be some difficulty in distinguishing microplastics from other materials,[25] this process is usually followed by a secondary confirmation using spectroscopic analysis.[26] Fourier transform infrared (FT-IR) spectroscopy is the most commonly used technique for the confirmation of the polymer type.[27] Nonetheless, FT-IR spectroscopy has a low precise size resolution because it does not detect particles smaller than 10 μm in size, and its spectral quality can be influenced by external factors, such as the presence of organic matter and/or water[28] making it difficult to match with library spectra.

Pyrolysis−gas chromatography mass spectrometry (Py-GC/MS) is a promising technique for analyzing microplastics that are too small to be analyzed by other techniques and particularly where they exist in complex environmental samples.[29,30] Initially, Py-GC/MS was used to detect and identify plastics in environmental samples by first isolating individual particles.[31,32] As such this procedure poses some disadvantages, such as the limited sample mass that must be used, the difficulty in manually transferring the hand-picked particle into a pyrolysis cup, the extensive analysis time, and its destructive nature.[27] This method has been successfully applied to complex environmental samples that have previously endured an extensive cleanup procedure to reduce organic matter and concentrate the plastic material.[33−35] Some studies have reported the identification of different plastic types in environmental samples by selecting specific decomposition products of polymers, their respective indicator ions, and retention times.[33,34,36] Determination of the amount of plastic was only recently accomplished by Okoffo et al.[37] with the identification and quantification of selected plastics in biosolids, after a simple and efficient sample treatment.

Estimates of the concentration level of microplastics in fish and shellfish are constantly reported, but there is no uniformity in reporting these results, which are usually expressed as particles per individual (e.g., Rochman et al.[19] and Li et al.[38]) or particles per gram (e.g., Renzi et al.[39]). There is clearly a need to develop methods to identify and quantify the most abundant types of plastics found in the marine environment, especially in seafood. To overcome extensive sample preparation processes, we investigate the use of an alkaline digestion procedure followed by an accelerated solvent extraction (ASE) method using a solvent at high temperature and pressure to dissolve and extract plastics from seafood. This is an adaptation of the method originally developed by Fuller and Gautam[40] and further optimized by Okoffo et al.[37] Here, we introduce a simple and efficient sample preparation procedure followed by Py-GC/MS analysis to analyze high-commercial-value seafood species. We aim to: (i) validate a microplastic extraction method when applied to seafood, (ii) optimize identification and quantification of microplastics by Py-GC/MS through a mass-related quantification approach, and (iii) for the first time provide an assessment of the plastic concentration a consumer might be exposed to by consuming seafood.

2. MATERIALS AND METHODS

2.1. Chemicals and Reference Materials. A total of six different plastics were selected based on the most common petroleum-based plastics reported to occur in the marine environment:[3,41] polystyrene (PS), poly(methyl methacrylate) (PMMA), polyvinylchloride (PVC), polyethylene (PE), polyethylene terephthalate (PET), and polypropylene (PP). These plastic standards were used to assess the efficiency of the solvent extraction method and develop a bespoke Py-GC/MS library to identify and quantify plastics in seafood. Detailed information on the reference materials and chemicals used for solvent extraction is presented in Table S1 (Supporting Information).

2.2. Sample Collection and Processing. Five different species of raw seafood were purchased from a local fish market: farmed oysters (*Crassostrea gigas; n* = 10), farmed tiger prawns (*Penaeus esculentus; n* = 10), wild blue crabs (*Portunus armatus; n* = 5), wild squid (*Nototodarus gouldi; n* = 10), and wild sardines (*Sardinops neopilchardus; n* = 10). Seafood species were handled exactly the same way as for consumers; thus, no measures were put in place to avoid external contamination by packaging (i.e., handling by workers or transport of seafood in plastic bags).

Each specimen was weighed and washed before dissection to remove any traces of plastics. Only the edible part of each animal was dissected for analysis. Oysters were carefully shucked and kept whole (8−17 g of wet weight per sample). External features of prawns were removed, and the flesh (muscle) was divided into four subsamples. Prawns were not deveined, and one segment of each prawn (the portion of the muscle closest to the head) was used for analysis (3−6 g of wet weight per sample). Crabs were divided into half (two sub samples), and their GI contents and flesh of the legs and claws were placed directly in an Erlenmeyer flask (45−85 g of wet weight per sample). For the squid, the internal shell and head were removed, and the remaining flesh (mantle and fins) was divided into four or five subsamples, depending on the size (4−7 g of wet weight per sample). Sardines were washed, gutted, filleted with the skin on, and divided into four subsamples (muscle). One segment of each sardine (the portion of muscle closest to the head) was used for analysis (4−9 g of wet weight per sample). All of the subsamples (*n* = 10) were prepared in a fume hood to avoid external contamination and rinsed with Milli-Q water prior to digestion to avoid any microplastic adherence to the tissues,[42] wrapped in aluminum foil, and frozen at −20 °C until further analysis.

2.3. Sample Digestion. For digestion, 10 replicates of each seafood species (not mechanically homogenized) were individually placed in an Erlenmeyer flask, and 10 mL per gram of tissue of 10% KOH aqueous solution prefiltered with a glass fiber filter (Whatman GF/D 2.7 μm) was added. The flasks were sealed with aluminum foil and placed in a shaker incubator (Thermoline Scientific Orbital Shaker Incubator) for 24 h at 60 °C with continuous agitation (135 rpm). For every

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batch of five samples digested, an Erlenmeyer flask with KOH solution and no sample was used as a procedural blank. Once the digestion was completed (all the contents were digested and the solution was clear), the samples were removed from the incubator and filtered under vacuum onto a glass microfiber filter (Whatman GF/D 2.7 μm). In this case, the selected pore size of the filters was shown to be the best compromise between a fast filtration without clogging the filter and the minimum pore size possible. Filters were stored in Petri dishes (P5481 Sigma-Aldrich) and covered in aluminum foil to avoid contamination, until analysis.

2.4. Extraction of Plastic Standards Using an ASE Method. The instrumental conditions for ASE of plastic standards were adapted and validated with the study by Okoffo et al.[37] Samples were extracted with dichloromethane (DCM) at 180 °C and 1500 psi. Detailed information on the extraction conditions is presented in the Supporting Information (Table S2).

2.5. Validation of Extraction Efficiency of Plastics in Seafood. The following procedure was implemented to establish the efficiency of the extraction procedure for plastics from digested seafood samples filtered onto a glass fiber filter.

Oysters (n = 6) were individually digested and filtered using the method described in Section 2.3. After digestion, samples were removed from the incubator, cooled, and pooled into a glass-measuring cylinder. The final volume was recorded and equally divided onto glass fiber filters: six oyster filters with no added plastic and 6 × 6 spiked oyster filters that would be later spiked with six different plastics (PS, PE, PET, PMMA, PP, and PVC) (see Figure S1 of the Supporting Information for a schematized explanation). The weight of each filter was recorded and then spiked with 10 to 30 mg of the selected plastic standards. Each filter was then folded and extracted by ASE as outlined above. For each plastic type, a nonspiked filter was run as a blank. After extraction, extracts were reduced to dryness with a stream of nitrogen at 40 °C, and the mass was recorded with an analytical balance. Because the oysters might contain residual plastics and currently there are no plastic-free seafood reference materials, polymer recoveries were estimated by subtracting the amount of residue obtained in the unspiked samples from the spiked samples.

2.6. Py-GC/MS Analysis. The method parameters for analysis were adapted from the study by Okoffo et al.[37] Briefly, samples were pyrolyzed in single-shot mode at 650 °C for 0.2 min (12 s). Pyrolysis products were injected with a split ratio of 5:1, and the pyrolyzer interface temperature was set at 320 °C. Additional details on the single-shot Py-GC/MS conditions are presented in Table S3 of the Supporting Information.

2.7. Plastic Identification and Quantification. Calibration standards (PS, PE, PET, PMMA, PP, and PVC) were analyzed with each batch, and their resultant pyrograms were added to the in-house library and compared with the available literature.[33−35,43]

The detection and quantification of PE are dependent on indicator compounds that can also come from other sources, for example, naturally occurring long alkyl chains that typically produce n-alkanes and n-alkenes upon pyrolysis.[33,44] To overcome this apparent lack of specificity in our approach, a number of steps were performed. (1) Alkaline digestion (with 10% KOH) of the samples prior to ASE and Py-GC/MS analysis. Alkaline digestion is known to remove most phenolic compounds (including tannins and lignins)[45,46] and alkyl chains[47] from the samples reducing the risk of interference. (2) Strict quantification and validation criteria for the presence of PE in seafood samples included: (i) the presence of a characteristic homologous series of PE triplets in the pyrogram (alkadiene, n-alkene, and n-alkane), (ii) a range of homologous series of more than five triplets within C7−C41 of the PE standard, and (iii) the standard deviation of the areas of the individual C10 triplet within two times the standard deviation of the PE standard ($n \geq 5$). (3) The use of a multivariate calibration curve for quantification of PE in different matrices. The intensities of eight peaks in the extracted ion chromatograms of masses 81 Da, 83 Da, and 85 Da of a five-level calibration curve data set were used for the multivariate calibration curve. To identify the most relevant peaks for the quantification of PE in our samples, we performed a principal component analysis (PCA) with two components. The PCA enabled us to reduce the dimensionality of our data set from 24 independent variables (i.e., three m/z values with eight peaks in each) to three representative peaks: 1-decene, 1-dodecene, and 1-tetradecane. The signal intensities of these three peaks in the 83 Da XIC were used in the final multivariate calibration curve. Using this approach, we were able to accurately quantify PE in the complex matrix of the analyzed samples while minimizing background effects.[48,49] For specific details on the multivariate calibration curve and PCA, see the Supporting Information.

2, 4-Dimethyl-1-heptene (m/z 126) was selected as an indicator ion for PP and methyl methacrylate (m/z 100) for PMMA. Styrene (m/z 104) is the most common indicator ion from the pyrolysis of PS; however, it is also an indicator ion for other plastics (e.g., PVC); thus, a styrene dimer (m/z 130) was chosen as the PS specific indicator compound. Benzene (m/z 78) was selected as the indicator compound for PVC because of its high peak intensity and sensitivity.

The selected indicator ions for the detection of different plastics and pyrogram information are summarized in Table S4 and Figure S2 of the Supporting Information, respectively.

2.8. Sample Preparation of Selected Seafood for Py-GC/MS Analysis. Seafood samples were individually extracted by ASE using the same method and parameters described in Section 2.5. Samples were run in batches of five filters along with a procedural blank (KOH filter). Because there are no regulatory measures or standard protocols for the use of blank samples on seafood analysis for microplastics, for this study, the KOH filters used as procedural blanks for alkaline digestion were also used as a procedural blank for solvent extraction (10 procedural blanks in total). Whether contamination occurred during digestion or solvent extraction, contamination was possible to assess by analyzing these filters the same way as the seafood samples. At the beginning of each solvent extraction, two empty cells were run, to avoid contamination from previous runs. Immediately after ASE, 80 μL of each sample was directly injected into sample cups (in triplicate) and loaded into a pyrolysis autosampler.

2.9. Method Validation and Performance. Standard solutions for the selected plastics were prepared by extracting plastics by ASE using the extraction method described in Section 2.4.

To check the dissolution and stability of plastics in DCM after ASE, the selected plastics were extracted and left in the collection bottles (n = 3) for a total period of 2.5 h and sampled into pyrolysis cups every 15 min for Py-GC/MS analysis starting at time 0. The peak area on the specific

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indicator ions of each plastic was recorded (see detailed information in Supporting Information Figure S3).

To assess the linearity of the instrument when using different split ratios, the selected plastics (PS, PE, PP, PVC, and PMMA) were run at equal concentrations (0.4 μg mL^{-1}) and at different split ratios (50:1, 20:1, 10:1, and 5:1) (specific details in Supporting Information Figure S4).

For repeatability, polymer concentration variability was tested by analyzing the selected plastics on the same day and on different days (n = 5), by calculating the relative standard deviation (RSD, %) with precision between 3 and 12. To assess variability among runs, a known concentration of PS standard solution (1 mg mL^{-1}) was run with every batch of samples (n = 10).

External calibration curves were obtained by analyzing different concentrations of the standard solutions (0.02−10 μg on column). The limit of detection (LOD) and limit of quantification (LOQ), expressed in μg of plastic per gram of tissue, were determined for each plastic at the lowest available concentration from the calibration curve (concentration = 0.02 μg L^{-1}; n = 7). The standard deviation of the measured response was multiplied by 3.14 (LOD) and 10 (LOQ), respectively. LOD and LOQ values were then divided by the average fresh weight (g) of each tested sample.

Each seafood sample extract was analyzed in triplicate. Little variability was observed among the triplicate analysis (RSD < 20%). The final plastic concentration in each sample was expressed in μg per gram of fresh weight tissue. When a value was below the LOQ, half of the value of the LOD was used.

2.10. Quality Assurance and Quality Control (QA and QC). Hermsen et al.[50] recently stressed the need for stricter QA when analyzing microplastics in biota samples. Special care was taken in this study to minimize contamination by microplastic particles from the laboratory environment during sample preparation and handling. During all steps, clean devices were used to collect and store the samples. All sample containers were rinsed thoroughly three times with Milli-Q water prior to use. Plastic items were avoided to avoid contamination. Laboratory coats made of 100% cotton were worn during all steps of analysis procedures. The work was performed in a fume hood when possible, to minimize contamination by airborne microplastics. Whenever samples were not being processed, they were covered with aluminum foil. Every Whatman GF/D filter used in this study was individually wrapped with aluminum foil and heated at 450 °C for 4 h in a muffle furnace (Thermo Scientific Thermolyne Furnace Benchtop Muffle Type 47900) to remove any traces of organic matter.[51] Before every ASE, cells were washed with acetone and sonicated three times for 30 min followed by a DCM wash to guarantee that there were no traces of plastic. Hydromatrix was prewashed with DCM under the same conditions described above. A procedural blank of prewashed hydromatrix was used in every extraction to guarantee that no contamination was from the ASE itself. All the plastic components from the ASE were analyzed by Py-GC/MS to determine if any of the decomposition products is the same as the target compounds of this study. No plastic contamination was observed because none of the target compounds were detected.

All the cups used for Py-GC/MS were new and run on the instrument before adding any sample to avoid any possible contamination. Empty cups were run between each batch of samples to avoid cross contamination.

3. RESULTS AND DISCUSSION

3.1. Recovery of the Selected Plastics for Standards and Spike Samples by ASE. The overall recovery of the selected plastics was between 87 and 98% for standards and between 78 and 100% for spiked seafood samples (Tables S5 and S6 of the Supporting Information), except for PET in spiked samples (mean recovery of 32 ± 11%; RSD (%) = 34). Because of these poor recoveries, PET was not quantified in the seafood samples.

3.2. Method Validation and Performance. No significant difference was found in the peak area of the selected indicator compounds over time (RSD < 16%) providing evidence that the extracted plastics are stable in solution in DCM after ASE (Figure S3 of the Supporting Information).

No significant variability of the peak area of the PS dimer was observed between runs, with % RSD = 17% (Table S7 of the Supporting Information). The calibration range for each plastic was from 0.02 to 10 μg on a column, where $R^2 \geq 0.98$ (Table S8). LOQ values (μg per g of tissue) for each tested species are included in Table S9. The observed procedural blank levels are given in Table S10 of the Supporting Information. It should be noted that the plastic concentration reported for the different seafood species was not corrected for the average blank concentrations.

3.3. Mass Concentration of Plastics in Seafood Samples. A new method for the identification and quantification of five different plastics (PS, PE, PVC, PP, and PMMA) was successfully applied to the edible portions of commercially available seafood that are commonly consumed by humans. Plastics were detected in every species of seafood analyzed (Figure 1). Different mass concentrations were determined between species and individuals of the same species (Figure 1 and Table S11−Supporting Information). Squid contained the lowest concentration of total plastic (Σ

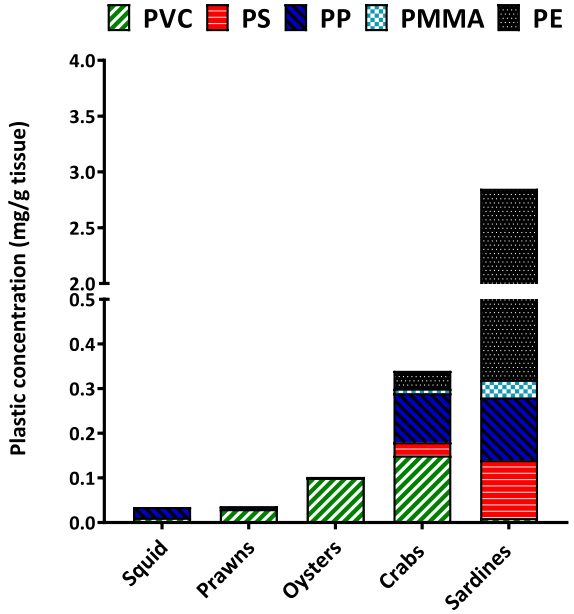

Figure 1. Total plastic concentration (n = 10) (in mg per g of tissue) of the five plastic types (PVC, PS, PP, PMMA, and PE) quantified in five different organisms (squid, prawns, oysters, crabs, and sardines). Polystyrene (PS), polyethylene (PE), polyvinyl chloride (PVC), polypropylene (PP), and poly(methyl methacrylate) (PMMA). When plastic concentration < LOQ, 1/2 LOD value was used.

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0.04 mg g^{-1} of tissue) followed by prawns (Σ 0.07 mg g^{-1}), oysters (Σ 0.1 mg g^{-1}), crabs (Σ 0.3 mg g^{-1}), and sardines (Σ 2.9 mg g^{-1}). PVC was the only plastic detected in all of the species analyzed (0.3−75 μg g^{-1}). PMMA (0.7−27 μg g^{-1}), PS (0.3−100 μg g^{-1}) and PE (43−2.4 × 10^3 μg g^{-1}) were present in two organisms−sardines and crabs. PP was quantified in four of the five seafoods tested: squid, prawns, crabs, and sardines (0.3−60 μg g^{-1}).

Our results show that different species accumulate different plastic types at highly variable concentrations. Replicates for oysters, prawns, and squid showed many null values or below the LOQ (Table S11), which reduces the statistical power of the reported results and may give under or overestimates of the real total plastic concentration. Plastic was detected in most individual samples of crabs and sardines, yet differences between results from subsamples representing replicates of the same organism were relatively high (RSD = 58% (crabs) and 257% (sardines)) (Table S11), which highlights the need of a larger sample size to increase the robustness of the results.

3.3.1. Squid (N. gouldi). Each of the analyzed seafood species of this study has different biological, physiological, and anatomic features and lives in different compartments of the marine environment, which influences the uptake and potential accumulation of microplastics. Squid live in the deeper areas of the ocean, in the mesopelagic zone at least 300 m below the surface and feed mainly on small fish, crabs, and shrimp. The squid's mouth has a small beak that breaks the prey into smaller pieces while it is still alive. Food then passes down to the stomach and from there to the liver where it is absorbed for energy and growth. Because all the internal contents of the squid were removed and only the mantle (edible part) was analyzed, it was expected that most of the plastic present in the squid would be in the digestive system and gills, which may explain the low total concentration of plastic found in *N. gouldi* (0.04 mg g^{-1} tissue). In the marine environment, the abundance of microplastics may possibly decrease with the depth, so this may be another reason why squid had the lowest microplastic content of all the tested organisms.[52] This is the first study on the microplastic content in squid, and thus, comparison with other literature studies is not possible. Our preliminary results show that a consumer can potentially be exposed to 0.7 mg of plastic when considering a serving of 100 g (Table 1). Nevertheless, further studies would be recommended to support these results.

Table 1. Potential Plastic Ingestion by Seafood Consumption

seafood	average plastic content (mg g^{-1})	average serving	average serving weight (g)	potential plastic exposure (mg)
squid	0.01	4	100	0.7
oysters	0.01	10	50	0.7
prawns	0.01	5	75	1.1
crabs	0.03	1	100	3.0
sardines	0.3	3	100	30

3.3.2. Tiger Prawns (P. esculentus). Prawns feed on particulate matter and are mostly found in the intertidal zone being in close contact with particulate matter. They have an open circulatory system, which means that the blood flows freely into the body cavities (haemocoels) and makes direct contact with the tissues. For this study, the head of the prawn was not analyzed because it is not usually consumed. As the hepatopancreas and most of the vascular system are located in that region, a large proportion of potential plastic present in there can be lost. Even so, the edible part of a prawn (muscle) still has remnants of the aorta artery and intestine that may contain microplastics. Previous studies reported approximately 0.80 ± 0.12 particles g^{-1} tissue in the muscle of *Alepes djedaba*[53] 2.7 particles/individual in *Penaeeus indicus*[17] and synthetic fibers in *Crangon crangon* (1.23 ± 0.99 microplastics/shrimp).[14] Although not comparable to our results because of different units of quantification, these concentrations are relatively low. In the present study, the total plastic content in the tested prawns was 0.07 mg g^{-1} tissue (also a low concentration compared to the other species). Thus, considering a serving of five king prawns, a consumer could be ingesting approximately 1 mg of plastic per serving (Table 1).

3.3.3. Pacific Oysters (C. gigas). Oysters are deposit feeders, which means that they filter particulate matter (including microplastics) from the water and sediments. The particles are first taken up through the inhalant siphon from the surrounding water and trapped in the gills, which is the first contact organ. In the gills, the particulate matter transfers to the haemolymph that goes to the heart and from there it is distributed to the rest of the body because of oyster's open circulatory system. Bivalves are usually eaten whole without removal of the digestive tract and hypothetically represent a scenario of microplastic exposure. The European Food Safety Authority (EFSA) reported the possible scenario of ingestion of mussels: after the consumption of 225 g (approximately 10 mussels) and using the highest reported microplastic concentration so far, it would give a total exposure to 900 pieces of plastic. Assuming spherical microplastics with a diameter of 25 μm and density of 0.92 g cm^{-3}, the exposure would be a total mass of 7 μg per 10 mussels eaten. On the other hand, the latest report of the World Wide Fund (WWF) for Nature[12] states that an average seafood consumer could be ingesting up to 0.5 g of plastic a week, when considering shellfish intake. In 10 oysters analyzed, we had a total plastic concentration of 0.1 mg g^{-1} tissue. Following the ingestion of 10 oysters, the average mass of plastic intake per serving would be approximately 0.7 mg (depending on the oyster's weight) (Table 1), which is a much higher value than what the EFSA reports and lower than the WWF one. From all the tested seafood organisms in this study, oysters were expected to have the highest plastic content because of their filter feeding nature and habitat. Furthermore, a recent review suggests that lower trophic organisms, such as bivalves, are at higher risk of contamination of microplastics because, besides their filter feeding nature, the available research data suggest that microplastics do not biomagnify in the food web.[22] Oysters have been widely reported for microplastics contamination. Studies have divulged the presence of microplastics in oysters purchased from farms and local markets. Teng et al.[15] found an average of 0.62 microplastics per gram (wet weight) in farmed oysters from 17 different sites in China. Rochman et al.[19] reported 0.6 ± 0.9 microplastics per individual in *C. gigas* purchased from a market in USA, and Van Cauwenberghe and Janssen[11] found 0.47 particles per gram of tissue in oysters acquired from a supermarket in France. A possible explanation for the "low" plastic content found in the present oysters (at least when compared to what was expected being a filter feeder) can be their source. The oysters in this study originated

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from the west coast of Australia, where the microplastic content of surface waters is reported to be lower than in other regions of the world such as Asia or Europe.[54,55] A future comparison of the microplastic content by Py-GC/MS analysis among oysters from different geographical locations may help to further elucidate any such differences.

3.3.4. Blue Crabs (P. armatus). Crabs are omnivores, primarily feeding on algae and other food, including molluscs, worms, other crustaceans, and detritus.[56] Crab's foregut encloses a complex gastric mill that grinds and mixes ingested food. Because the foregut of the crab is also used in many seafood dishes, the abdomen content was analyzed in this study along with the flesh of the legs and claws. The blue crab *P. armatus* had a total plastic content of 0.34 mg g^{-1} tissue. The relatively high polymer content found in this species is not a surprise because several factors make crabs prone to accumulate plastic in their abdomen. According to Watts et al.,[57] PS microspheres are retained in the foregut of *Carcinus maenas* because of adherence to the hair-like setae. *C. maenas* is also able to take up microplastics by direct ingestion or by contaminated prey[58] and from inspiration through the gills.[57] Gut content analysis in specimens of spider crab *Maja. squinado* collected from the Celtic Sea revealed microplastic contamination in 42.5% of 23 individuals, with 1.39 ± 0.79 microplastics per individual.[59] Looking at our results, if we consider a serving of one blue crab per person assuming that both the flesh of the claws/legs and abdomen are eaten, a consumer can be potentially exposed to 3 mg of plastic (Table 1).

3.3.5. Sardines (S. neopilchardus). Sardines mainly feed on zooplankton, and their gill rack enables the filtration of particles from the water.[60] They have a closed circulatory system, which means that the blood is transported from the heart to the organs by a capillary system. Hence, distribution of microplastics to edible tissues (e.g., muscle) is not likely to occur. The total plastic concentration found in sardines was 2.9 mg g^{-1} (Figure 1 and Table S11). In terms of what a consumer can be exposed to, considering a serving of 3 sardines per person, the average human intake would be 30 mg of plastic (Table 1).

Sardines were the most surprising result of this study because it was expected that eviscerated fish would have less microplastic content than crabs or oysters, for example. The high concentration of microplastics in sardines could be explained by the uptake of particles from the water or by the ingestion of contaminated prey such as planktonic organisms.[61] However, only the muscle and the skin of the fish were analyzed, which does not support such a theory. Microplastics are thought to bioaccumulate in the food chain,[62−64] which could be another possible explanation for the high concentration found. However, recent studies suggest the opposite because they state that microplastics do not biomagnify up in the food web.[22]

Most literature highlights that the biggest concern is seafood that we eat whole, such as bivalves[17] and that the presence of micro or nanoplastics in the GI tract of fish does not pose any harm because it is not usually consumed.[18] According to existing information, microplastic concentration in muscle tissue should be lower than what has been reported in the GI tract of bony fish.[65] However, few studies have actually analyzed nondigestive tissues for microplastics. Karami et al.[66] investigated the presence of microplastics in edible dried fish tissues. In two species *Chelon. subviridis* and *Johnius. belangerii,* the results show that the flesh actually had a higher microplastic content than the excised organs. Microplastics were also found in every muscle tissue samples of the fish *Serranus scriba* in all sampling sites in Tunisia (1.78 ± 0.26 items/g of tissue).[67] This evidence suggests that evisceration probably does not eliminate the risk of microplastics' ingestion by humans and that the particles found in the flesh could have been translocated from the GI tract.[66] However, information on accumulation and translocation of microplastics into organs and other tissues is still scarce.[18,68] In order for translocation to occur, it is likely that the particles need to be less than tens of micrometers in diameter, to be able to pass through gills or the GI tract through cell internalization and subsequent translocation.[17] Although in this scenario it does not seem the best plausible explanation for the plastic content in sardines, this hypothesis cannot be discarded.

3.4. Sources of Plastic Contamination in Seafood. A possible source of plastic contamination in the sardines may be the potential transfer of microplastics from the GI tract to flesh during food processing and handling. As most microplastics are found in the GI tract of fish, it is expected that gutting would decrease microplastics' exposure, unless gutting is carried out incorrectly.[69,70] There are no studies that we are aware of on the fate of microplastics during seafood handling and processing to be able to evaluate this.[69]

Contamination of seafood can also be originating from the surrounding environment where the tested species were caught. Catarino et al.[71] used deployed caged mussels in Edinburgh (UK) as biomonitors for environmental contamination of microplastics and observed an increase in plastic content in mussels following deployment of 2 weeks.

It is possible that contamination could also occur from airborne particles,[72] machinery, equipment, and textiles,[50,69,73] handling,[74] and/or from fish transport. The number of plastic particles found in edible flesh samples from different fish species from the Atlantic ocean showed no significant differences from the number of particles found in the respective procedural blanks.[75] Kolandhasamy et al.[42] found microfibers in the foot and mantle of mussels—organs that do not participate in the digestion process and suggested the adherence of microplastics to tissues as a possible explanation for that result. In the present study, possible particles originated from external contamination might have attached to the tissues.

The elevated concentration of PE in the sardine samples raises some questions. Most of the packaging used for the seafood purchased for this study was made of low-density PE (plastic bags), a potential source of PE. Recent studies have demonstrated that just opening plastic containers or bags can generate between 0.5 and 250 microplastic/cm^2,[74] and food trays contaminate packaged meat with microplastic levels ranging from 4.0 to 18.7 particles per kg.[76] In purchasing seafood wrapped in plastic, as is common in many fish markets, we may have identified a potential source of PE contamination in fish that is not from the natural environment but a function of how we handle fish as consumers. There is a clear need to investigate this potential pathway further. Quantification of microplastics in seafood has been suggested as an essential component of food safety management systems.[69,70] It is recommended that future studies should investigate the packaging and handling in relation to seafood contamination,[77] rather than solely looking for particles in fish stomachs and accumulation in bivalves.

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3.5. Method Limitations and Areas of Improvement.

We acknowledge that this methodology has certain limitations and areas of improvement that need to be considered before it can become a standardized technique for micro- and nanoplastic analysis. We further recognize that this is a destructive method and that it cannot provide information on the color, shape, or size of microplastics. However, it can be a complementary method to spectroscopy techniques. In this case, a preanalysis could be conducted before solvent extraction because the samples are placed on glass fiber filters.

Unfortunately, the fractions of micro- and nanoplastics below the 2.7 μm pore size of the filter have not been quantified. For future work, it would be useful to improve the present method by analyzing smaller microplastic fractions by either using small pore-size filters or whole freeze-dried tissues without a previous digestion procedure. In the second scenario, a double shot Py-GC/MS analysis could be optimized to prevent the extracted organic material from interfering with plastic pyrolyzates.

Concentrating the samples by extracting less volume of solvent (using smaller ASE cells) or pooling samples might be something to consider in future experiments. Single-ion method detection is a strategy that could be utilized to further lower the plastics' LODs. The use of deuterated standard materials for the determination of specific products of polymer degradation is highly recommended, to increase confidence in polymer identification and subsequent quantification. Optimization of the Py-GC/MS method for other plastic types and additives would be beneficial for future studies.

Although contamination was avoided at all times, traces of plastic were detected in some procedural blanks (Table S10). We think that this represents an important feature of this paper not only because we found a way of having procedural blanks for all steps of sample analysis but also because the plastic concentration in blanks was not omitted. We highly encourage other researchers to start reporting contamination in procedural blanks. We understand in some cases the particles are too small to be seen or are missed because of the method used for analysis. Nevertheless, it would help other laboratories work on understanding what is the source of contamination and what QA/QC measurements can be improved for future standardized techniques.

Reporting plastic mass-related concentrations is a step forward toward standardizing methods for microplastic detection and reporting in food as well as in other environmental samples with complex matrices.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.est.0c02337.

Additional information on conditions for ASE, Py-GC/MS analysis, recovery and spike experiments on ASE, dissolution data, calibration curves, and pyrograms of extracted standards and seafood samples (PDF).

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Notes

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