Microplastics in mussels sampled from coastal waters and supermarkets in the United Kingdom

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Abstract

Global contamination of the marine environment by plastic has led to the discovery of microplastics in a range of marine species, including those for human consumption. In this study, the presence of microplastics and other anthropogenic debris in seawater and mussels (*Mytilus edulis*) from coastal waters of the U.K., as well as supermarket sources, was investigated. These were detected in all samples from all sites with spatial differences observed. Seawater samples taken from 6 locations (in triplicates) displayed 3.5 ± 2.0 debris items/L on average (range: 1.5-6.7 items/L). In wild mussels sampled from 8 locations around the U.K. coastal environment, the number of total debris items varied from 0.7 to 2.9 items/g of tissue and from 1.1 to 6.4 items/individual. For the supermarket bought mussels, the abundance of microplastics was significantly higher in pre-cooked mussels (1.4 items/g) compared with mussels supplied live (0.9 items/g). Micro-FT-IR spectroscopy was conducted on 136 randomly selected samples, with 94 items characterized. The spectra found that 50% of these debris items characterized were microplastic, with an additional 37% made up of rayon and cotton fibers. The microplastic levels detected in the supermarket bought mussels present a route for human exposure and suggests that their quantification be included as food safety management measures as well as for environmental monitoring health measures.

Capsule: Microplastics in seawater, coastal mussels and supermarket mussels

Keywords: Mytilus; microplastics; shellfish; human consumption

Declarations of interests: none.

Highlights

- Coastal mussels sampled from around the United Kingdom all contain microplastics
- Supermarket bought mussels for human consumption also all contain microplastics
- 43% /57% of debris items from coastal/supermarket mussels were microplastics
- Predicted ingestion of 70 microplastic items in 100g processed mussels by consumers

1. Introduction

The global presence of microplastics (defined as particles <5mm in diameter) in the marine environment is well documented. They are found throughout the world's oceans from beaches and coastlines, to subtropical oceanic gyres, polar ice caps and the deep ocean (for review: Wright et al., 2013; Law and Tompson, 2014; Cole et al., 2014), with the U.K. coastal and estuarine waters being no exception (Gallagher et al., 2016; Thompson et al., 2004). Because of their ubiquitous presence and morphological features, microplastics are likely to threaten the life and development of biota via direct and indirect pathways, including ingestion (Desforges et al., 2015), adherence (Kolandhasamy et al, 2018), and trophic transfer (Farrell and Nelson, 2013).

The primary environmental risk associated with microplastics is their availability (Wright et al., 2013; Desforges et al., 2015). Multiple marine species, including their different life stages, have now been reported to ingest plastics from the environment (Thompson et al., 2004; Boerger et al., 2010; Murray and Cowie, 2011; Foekema et al., 2013; Lusher et al., 2013; Devriese et al., 2015; Steer et al., 2017). This includes species of fish and shellfish associated with seafood for human consumption, which presents an exposure route for humans with health implications that are not yet fully understood (Rochman et al., 2015; Van Cauwenberghe and Janssen, 2014).

Mussels have been widely used in biomonitoring of marine environments, including the U.S. Mussel Watch, Assessment and Control of Pollution in the Mediterranean region (MEDPOL), and the North East Atlantic Oslo and Paris Commission (OSPAR) monitoring programmes. Their utility is due to several advantages such as broad geographical distribution, easy accessibility and high tolerance to a considerable range of salinity (O'Connor, 1998). As a representative benthic filter feeder, the blue mussel, *Mytilus edulis,* has been identified as a species susceptible to microplastic uptake (Browne et al., 2008; van Moos et al., 2012; Mathalon and Hill, 2014; Santana et al., 2016; Li et al.,

2016; Catarino et al., 2018). They can filter large volumes of water, with ventilation rates of up to 300 mL·min−1 at 100% O_2 saturation and 15°C, increasing their susceptibility to water-borne substances (Widdows, 1973). Mussels have also been used to study the fate and toxic effects of microplastics in laboratory experimental exposures (Browne et al., 2008; von Moos et al., 2012; Farrell and Nelson, 2013; Avio et al., 2015; Paul-Pont et al., 2016; Silva et al., 2016). Consequently, microplastic contamination in mussels has been proposed as a marine health status parameter (De Witte et al., 2014), and added to the European database on environmental contaminants of emerging concern in seafood (Vandermeersch et al., 2015a). Mussels are thus both vulnerable to microplastic pollution, and are also a vector for transfer of microplastics into the human food chain.

Building on our previous work investigating microplastic abundance and distribution in mussels along the Chinese coastal region and from supermarket sources (Li et al., 2015; Li et al., 2016), we have conducted a parallel survey on microplastics and other anthropogenic debris in mussels from U.K. coastal waters as well as from several supermarkets. This aimed to determine the spatial distribution of microplastics and other anthropogenic debris in the U.K.'s coastal mussel communities, to examine its relationship with concentrations in surrounding seawater, and to compare the tissue burdens with supermarket bought mussels, thus providing both an insight into both wildlife and human exposure via ingestion.

2. Materials and Methods

2.1. Sample collection

M. edulis (n=162 individuals) were collected from 8 sites along the coastal waters of the U.K. from November 2016 to February 2017 (Fig. 1; Table S1). The mussels (n=12-30) from each sampling site

were pooled into six replicates of ~5 g of soft tissue each (n=8 sampling sites with six 5 g replicates)(as in Li et al., 2015; 2016). Surface seawater was collected from the same sampling sites with the exceptions of Edinburgh and Cardiff (n=6 sampling sites with three 5 L replicates samples taken, Fig. 1; Table S1). In addition, farmed, live and processed mussels were purchased at U.K. supermarkets from March to May 2017 (Table S1). In detail, mussels were purchased from 8 different supermarket locations, representing 8 different brands. Some supermarkets sold the mussels live in net bags and others sold the mussels chilled or further processed (cooked) in plastic containers. From each supermarket, either 2 bags of live mussels or 2 containers of chilled/processed mussels were purchased. The mussels from the two bags/containers were then mixed and sub-divided to make a total of 6 replicates for each of the 8 supermarkets/brands. The mussels were transferred to the laboratory and stored at -20 °C until further analysis.

2.2. Hydrogen peroxide treatment of soft tissue and seawater

The extraction methods and analysis of debris from mussels were based on Li et al., (2016). The mussels were rinsed with filtered tap water, and the shell length/weight of each recorded. The soft tissues of 1-5 individual mussels (5 g by weight) were placed in a 1 L conical flask and regarded as a replicate. Six replicates were used for each site. Next, 200 mL of 30% H_2O_2 was added to each conical flask, the bottles were covered (with foil), and placed in an oscillation incubator at 65 °C at 80 rpm for 24 h and then at room temperature for 24 to 48 h depending on the digestion status of the soft tissue. The digestions were terminated once they appeared clear and no obvious particles were visible.

The seawater samples were filtered with a 5 µm pore size, 47 mm diameter cellulose membrane filter (EMD Millipore, Fisher Scientific, U.K.). The substances collected on the filters were washed into glass bottles using 30% hydrogen peroxide to digest any organic matter.

All liquids (tap water, saline solution and hydrogen peroxide) were filtered with a 1 µm filter

paper prior to use to reduce contamination of the samples by airborne microplastic. All of the apparatus used were rinsed three times with filtered tap water. A blank extraction (n=6 replicates) without tissue (or seawater) was performed simultaneously to identify and characterize any procedural contamination.

2.3. Floatation and filtration of microplastics with saline (NaCl) solution

A concentrated saline solution (1.2 g/ml, NaCl) was used to density separate the microplastics and other anthropogenic debris from dissolved liquid of the soft tissue via floatation (Li et al., 2016). Approximately 800 mL of filtered NaCl solution was added to each bottle. The liquid was mixed and left to sediment overnight. The overlying water was gently removed and then filtered with a 5 μm pore size, 47 mm diameter cellulose nitrate membrane filter (EMD Millipore) using a vacuum system. Next, the filter was placed into clean petri dishes with a cover until further analysis.

2.4. Observation and validation of microplastics and other anthropogenic debris

The filters were observed under an Olympus SZX10 Research High-Class Stereo microscope (Olympus Corporation, Japan), and photographed with an Olympus UC30 digital camera. A visual assessment was conducted to identify microplastics and other anthropogenic debris according to the physical characteristics of the particles based on Free et al. (2014). 138 common particles were selected from across samples from seawater and mussels, and their identity confirmed by Fourier-transform infrared microspectroscopy (micro-FT-IR) with a UKAS accredited PerkinElmer Spectrum Spotlight equipped with a mercury−cadmium-telluride focal plane array (FPA) detector (consisting of 16 gold-wired infrared detector elements) cooled with liquid nitrogen (Tagg et al., 2015). Analysis was conducted in transmittance mode with microparticles transferred from filters, using either tweezers or a needle, to be mounted on a potassium bromide disk, and held in place with a 3 mm

151 copper SEM grid. Spectra were acquired with a minimum of 50 scans at a resolution of 4cm^{-1} and
152 matched using a series of polymer library databases (PolyATR, AR Polymer Introductory, NDFIBS,
153 RP, CRIME, FIBRES 3, POLY1, POLYADD1 from Perkin Elmer), a hit index of at least 70% match
154 was considered acceptable. Ninety-four samples met this threshold. While working at the limit of the
155 micro-FT-IR's capability, the smallest fibers analysed were 10 μm across. To collect an effective
156 spectra in these cases, the aperture of the IR detector was set to 10x50 μm to collect spectra along the
157 length of the fiber. The number of microplastics in individuals were estimated assuming a uniform
158 distribution.
159

160 *2.5. Statistical analyses*

161 Statistical analyses (ANOVA and linear regression) were performed using SPSS. Any
162 differences of the abundance of total microplastics, and total microfibers alone, in seawater and
163 mussel tissue samples was determined using One-Way ANOVA with a Dunnett Test. A linear
164 regression analysis was used to determine the relationship between seawater and tissue levels of
165 microplastics. Statistical significance was accepted at *=p <0.05, **=p<0.01, ***=p<0.001.
166

167 ## 3. Results

168 *3.1 Spatial distribution of microplastics*

169 Debris items were detected in all replicate seawater samples from all six locations (Fig. 2), and all
170 replicate mussel tissue samples collected from all coastal sites and supermarkets investigated around
171 the U.K. (Fig. 3). For tissue samples, procedural contamination from airborne fibers was low, with
172 an average of 0.67 ± 0.75 items/filter detected in the procedural blank samples compared with 8.63
173 ± 4.35 items/filter for coastal mussel tissues and 5.70 ± 2.27 items/filter for supermarket bought
174 samples.

175 Significantly higher numbers of debris items were detected in seawater samples from all
176 sampling sites (p=<0.001 for Filey, Hastings B, Wallasey and Plymouth, p=<0.05 for Hastings A),
177 with the exception of Brighton compared with the procedural blank. Filey, Hastings B, Cardiff and
178 Wallasey sampling locations, had significantly more debris items when using Brighton as a reference
179 site (Fig. 2). In mussels, the number of debris items in samples collected from all sampling locations
180 were significantly higher than the procedural blank samples: Plymouth and Brighton were significant
181 to p=<0.01, all other sampling locations to p=<0.001. Using Plymouth as a reference site, Brighton
182 mussel tissue samples were not significantly different, while mussels from all the remaining locations
183 were significantly higher (Fig. 3). For the supermarket bought mussels, a similar, widespread level
184 of debris items was detected in all six replicates, with each supermarket source containing at least
185 one debris item and all significantly higher levels than the procedural blank (p=<0.001) (Fig. 3).
186 Using sample SM3 as a reference sample, sources SM5 and SM7 contained significantly more debris
187 items compared with the other supermarket sources (Fig. 3., Fig. 4C.). The mussels SM5 represent
188 precooked samples from South America, and SM7 were samples that had, according to their
189 packaging, been frozen, then bought chilled and were from the NE Atlantic (Table S1).

190

191 *3.2 Abundance of microplastics in mussel tissues*

192 In mussels sampled from the coastal locations, the presence of debris items ranged between 0.7-
193 2.9 items/g tissue (wet weight) and between 1.1 to 6.4 items/individual (Fig. 3). Seawater samples
194 displayed an average debris abundance of 3.5 ± 2.0 items/L (range: 1.5-6.7 items/L). Linear
195 regression analysis found no relationship between the number of debris items in seawater and mussel
196 tissues (r^2=0.000). Debris abundance also varied significantly (p=<0.001 using one-way ANOVA)
197 according to whether the source of the mussels was directly from the coastal environment or from the
198 supermarket (Fig. 4). More debris items per gram of flesh were detected in wild mussels from coastal
199 sites, compared with farmed mussels from supermarkets, yet the farmed mussels were larger in size

200 leading to significantly more items per individual (p=<0.001)(SM1-4, Table S1) (Fig. 4A, 4B).
201 Focusing on the supermarket bought mussels; live mussels contained 0.9 items/g on average,
202 compared with an average of 1.4 items/g in processed mussels. The debris abundance was thus
203 significantly higher in pre-cooked processed mussels (samples SM5-SM8) compared to live
204 supermarket bought mussels (SM1-SM4) by weight (p <0.001) (Fig. 4C).

205

206 *3.2 Morphology of microplastics in seawater and mussels*

207 Multiple types of debris (based on Free et al., 2014), including fibers, fragments, spheres, flakes,
208 were detected in the seawater and mussel tissues (Fig. 5B and 5D). Fibers were the predominant type
209 of microplastic identified in both seawater (Fig. 5B) and mussels (Fig. 5D) ranging from ~50-90%,
210 followed by fragments ranging from ~5-40%. The size of the debris items varied from 8 μm to 4.7
211 mm, with the smallest size range of 5 μm to 250 μm representing the most particles, followed by the
212 next size range up of 500 μm (Fig. 5A and 5C). Mussel tissues (Fig. 5C) contained relatively more
213 of the smaller sized debris items compared with the seawater samples (Fig. 5A).

214

215 *3.3 Composition of microplastics in mussels*

216 Out of 1048 debris items isolated on filters, a total of 138 debris items (consisting mostly of fibers
217 and a small number of fragments to reflect the overall pattern of debris items) were randomly selected
218 from across all the filters and analysed. From these, 94 particles, ranging in size from 73 μm to 4.7
219 mm, were identified using micro-FT-IR with a spectrum match of over 70% (Table S2), which
220 accounts for ~9% of the total debris items isolated. A half of these particles (50%) were confirmed to
221 be microplastics and included polyester, polypropylene and polyethylene, (Table S2, Fig. 6, Fig. S1).
222 Polyester was the dominant polymer type in both seawater and field mussels, while polypropylene
223 was the most prevalent type in farmed mussels (Fig. 6, Table S2). An additional 37% of debris items

were made up of rayon and cotton fibers as well as a natural/synthetic blend of cotton and olefin and were considered to have an anthropogenic origin, whilst only ~10% were confirmed to be naturally occurring cellulose.

4. Discussion

This study provides a report of microplastics and other anthropogenic debris in mussels from the coastal waters of the U.K. and sold in U.K. supermarkets. This adds to the increasing evidence that effectively ubiquitous contamination of the global marine environment by microplastics and other anthropogenic debris is entering the food chain and affecting commercially important species for seafood consumption. Our results show, in brief, that there is significant and widespread contamination by microplastics and other anthropogenic debris items (relative to the procedural control blank) in coastal seawater samples, coastal mussel tissues and tissues derived from supermarket bought mussels in the U.K. We also observed significant spatial differences in the extent of debris items for both seawater and mussels from coastal locations (Fig. 3). Furthermore, the presence of debris items differed significantly between coastal mussel tissues and farmed mussel tissues sourced from supermarkets (Fig. 4A), whereby shop bought farmed mussels contained less debris items. However, supermarket mussel tissues displayed significantly higher numbers of debris items where samples had been supplied previously processed, either by freezing, chilling or pre-cooking (Fig. 4C). Each of these main findings will be discussed in turn.

4.1 Morphological types of microplastics and other anthropogenic debris observed

Of the debris items detected in seawater and mussel tissue samples, fibers were the most predominant type observed, consistent with other U.K. (Lusher et al., 2014; Cole et al., 2014; Devriese et al., 2015; Steer et al., 2017; McGoran et al., 2017; Murphy et al., 2017; Karlsson et al., 2017), European (DeWitte et al., 2014), and international studies (Rochman et al., 2015; Davidson and Dudas, 2016; Li et al., 2016). Material analysis through micro–FT-IR determined that only 50%

of debris items were microplastics with an additional 36% made up of other anthropogenic fibers, such as rayon and cotton which also have their origin in textiles. Once again this is consistent with other international studies, with microplastics only making up 52% of the debris items recovered from estuarine sediment, macroinvertebrates and seabird faeces in Southern Europe and West Africa (Lourenço et al., 2017) and 53% of debris ingested by three fish species in Sydney Harbour, Australia (Halstead et al., 2018). Other fibers, such as rayon (a semi-synthetic, cellulose based material) have also been detected in marine environments globally. Indeed, in a study of microplastics in coastal waters near Plymouth, U.K., 55% of the analysed particles were found to be rayon or a rayon-plastic polymer mix (Steer et al., 2017). Rayon, along with polyester and nylon, was also commonly found in Northeast Atlantic Ocean seawater surveys (Lusher et al., 2014) and as the most common fiber (53%) detected in True Beaked whales (*Mesoplodon mirus*) stranded on the Irish Coast (Lusher et al., 2015).

Several fibers found in farmed mussels, included acrylic and polyethylene, perhaps from textiles or rope sources used in aquaculture, and this again is consistent with another study conducted in animals from the U.K. Northeast Atlantic (Murphy et al., 2017). The main microplastic contaminant identified in the supermarket bought mussels was polypropylene. Polypropylene has also been highlighted in water samples from the Solent Estuary, U.K. (Gallagher et al., 2016) and recently as the main microplastic identified in canned fish (Karami et al., 2018). Polyethylene has also been previously associated with processing of fish (*Mugil cephalus)* (Avio et al., 2015), and has been detected in seawater and supermarket mussels in this study (Table S2) and others (Gallagher et al., 2016).

4.2 Microplastics and other anthropogenic debris in seawater

Our results show that there is widespread contamination by microplastics and other anthropogenic debris in coastal seawater samples compared with control blank samples (Fig. 2). We also observed significant spatial differences in the extent of debris contamination for seawater locations when using

276 the least impacted location (Brighton) as a reference site (Fig. 2). The microplastic and anthropogenic
277 debris abundances observed in this study are similar with respect to seawater samples reported in the
278 wider literature as follows. The seawater values ranged from 1.5-6.7 items/L which are high compared
279 with 0.4 ± 0.3 particles/L, yet low compared to 27 particles/L reported in two North Sea studies (van
280 Cauwenberghe et al., 2015; Karlsson et al., 2017) perhaps reflecting differing sampling methods or
281 genuine spatial differences.

282 With respect to the relationship between the seawater and tissue sample debris levels, no
283 correlation was found in this study (Fig. 2). Previous work by Browne et al. (2008) suggests rapid
284 translocation of smaller compared to larger polystyrene particles in mussels. The apparent ability of
285 mussels to retain smaller sizes of microplastics is also supported by our finding that mussels contained
286 more (44% - 83%) of the smaller sizes of microplastics (less than 250 μm) compared to seawater with
287 only 30% to 40% (Fig. 5).

288

289 *4.3 Microplastic and other anthropogenic debris in coastal mussel tissues*

290 These results indicate that there is also significant contamination by microplastic and anthropogenic
291 debris in coastal mussel tissues compared with the procedural control (Fig. 3.). We also observed
292 significant spatial differences in the extent of microplastic contamination in mussels from coastal
293 locations using the least impacted location (Plymouth) as a reference site (Fig. 3). With regards to the
294 sampling locations used in this study: Plymouth, Brighton, as well as Hastings A and B are all located
295 in the English Channel, which is considered contaminated with a variety of anthropogenic sources
296 (for review: Tappin and Millward, 2015). The Cardiff sampling site is located within the Severn
297 Estuary, which also has a long legacy of contamination sources, mainly of industrial sources in the
298 past, but also large population sewage effluent discharges (Langston et al., 2010). The Mersey and
299 Forth Estuaries also represent historically contaminated environments but reviews or datasets for
300 metals, hydrocarbons, PCBs and radioactive chemicals for these exist to a lesser extent in the
301 literature (CEFAS Report, 2005). Filey is located on the Holderness coast, in the North Sea region,

adjacent to large coastal fisheries that have collectively been investigated for persistent organic pollutant contamination (FERA Report, 2015).

The microplastic abundances observed in this study are similar with respect to tissue samples reported in the wider literature as follows. Previous U.K. studies have reported an average of 3.0 ± 0.9 microplastics g^{-1} wet weight in Scottish coastal mussels (Catarino et al., 2018) and 0.68 ± 0.55 microplastics g^{-1} wet weight in brown shrimp (*Crangon crangon*) in the southern North Sea/English Channel (Devriese et al., 2015), which represent a similar range (of 0.7-2.9 items/g tissue) to the values reported herein. In this study, microplastic and other anthropogenic debris items were identified in every tissue pool examined (Fig. 3) in line with a report for flounder (*Platichthys flesus*), a bottom feeder flatfish sampled in the Thames Estuary, where 75% contained microplastics (McGoran et al., 2017). In contrast, Steer et al (2017) report that only 2.9% of fish larvae studied in the English Channel had ingested microplastic. Others report significantly lower levels of microplastic contamination in North Sea fish, amounting to only 2 particles in 400 individuals analysed in one study, and 1.2-5.4% abundance range of several species analysed in a second study. The authors attribute low abundances to strict quality assurance criteria in reducing background contamination (Foekema et al., 2013; Hermsen et al., 2017). However, in another study, conducted further offshore, microplastic contamination was reported in 47.7% of fish (n=128, 3 species) sampled from the North East Atlantic around the Scottish coastline (Murphy et al., 2017).

In comparison with other European coastal sampling sites the average abundance of microplastics reported herein (0.7-2.9 items/g tissue wet weight) exceed those reported for coastal mussels (0.2 ± 0.3 g^{-1} wet weight) (Van Cauwenberghe et al., 2015), groyne picked mussels (0.26 fibers/g) and quayside mussels (0.51 fibers/g)(De Witte et al., 2014), as well as for commercial bivalves (0.36 ± 0.07 microplastics/g wet weight) farmed in the North Sea (Van Cauwenberghe & Jannsen, 2014). However, Leslie et al (2017) report significantly higher microplastic contamination in Dutch mussels relative to these U.K. values with 19 microplastics/g dry weight. It is important to highlight that these varying microplastic abundances could be due to differing extraction,

quantification and quality control methods employed, whereby sampling regime (Lusher et al., 2017), the type of tissue digestion (Vandermeersch et al., 2015b; Lusher et al., 2017), or the extent of background contamination (especially airborne) must be considered (Foekema et al., 2013; Dris et al., 2016; Wesche et al., 2017). In this study, a mean of 0.67 ± 0.75 items/filter in the procedural blanks was recorded, which compares favorably with other studies (Wesch et al., 2017; Leslie et al., 2017).

4.4 Implications of microplastic contamination on mussel health

Given the microplastic abundances reported for the seawater and tissues levels herein and their being broad consistency with levels reported globally, it is pertinent to discuss the implications in terms of the mussel health. Previous studies have investigated microplastic uptake in mussels (Browne et al., 2008; Thompson et al., 2004; Van Moos et al., 2012; van Cauwenberghe et al., 2015; Setala et al., 2016) and resulting biological effects, which range from immune impairment (Avio et al., 2015; Van Moos et al., 2012), and various physiological, sub-cellular impacts, including reproductive impairment (Sussarellu et al., 2016) through to reduced growth and trophic transfer (Farrell and Nelson, 2013) in related bivalve or crustacean species. For instance, clams (*Scrobicularia plana*) fed polystyrene beads (1mg/L) for 14 days (plus a 7 day depuration period) showed significantly modified antioxidant capacity, DNA damage, neurotoxicity and oxidative damage (Ribeiro et al., 2017). There is therefore increasing evidence that microplastics are taken up by bivalves (to a greater extent than other species, Setala et al., 2016), and that long-term exposure has detrimental impacts to their health.

4.5 Food supply contamination by microplastics and other anthropogenic debris

The presence of microplastics and other debris differed significantly between coastal mussel tissues and farmed mussel tissues sourced from supermarkets (Fig. 4A), whereby shop bought farmed mussels contained less debris. However, supermarket mussel tissues displayed significantly higher

numbers of debris items where samples had been supplied previously processed, either by freezing, chilling or pre-cooking (Fig. 4C). Many studies have previously reported a difference in microplastics abundance between wild and farmed/commercially-sourced mussels. In this study, there was significantly more microplastic (1.6 items/g, 3.0 items/individual) in wild mussels from coastal sites, compared with (larger sized) farmed mussels from supermarkets (1.1 items/g, 4.7 items/individual) (SM1-4, Table S1) (Fig. 4A, 4B). This abundance pattern is very similar to the findings of others whereby 2.7 fibers/g in wild mussels were reported compared with ~1.6 fibers/g on average for farmed mussels from Halifax Harbor, Nova Scotia, and Chinese coastal regions respectively (Mathalon and Hill 2014; Li et al., 2016). It is possible that depuration at the end of farming and the point of sale at a supermarket could account for these apparently lower values of debris per gram of flesh. In contrast, work by Li et al (2015) detected higher levels of microplastic contamination in Chinese commercially bought bivalves which ranged from 2.1-10.5 items/g. Higher microplastic levels were also reported for farmed clams (*Venerupis philippinarum*) relative to wild clams (ranging from 0.07-5.47 microplastics/g but with no significant difference in the mean values) in British Columbia, Canada (Davidson and Dudas, 2016).

An interesting further significant difference was observed in the supermarket-sourced mussels depending on whether they were alive or pre-processed at point of purchase (Fig. 4C and 4D). The types of pre-processing of the mussels bought at the supermarkets in this study involved either being pre-frozen and chilled, or cooked-frozen-chilled (SM5-SM8)(Table S1). Processed mussels contained significantly more debris items compared to the live mussels from farmed sources (Fig. 4C, 4D), which has also been observed in other processed foodstuffs such as canned fish containing polypropylene (Karami et al., 2018). It has been suggested that, for fish, the food manufacturing processing methods may cause the translocation of microplastics from the gut area to the edible meat tissues (Avio et al., 2015), suggesting that microplastics may be introduced via de-shelling and insufficient cleaning processes rather than entirely uptake from the environment.

The presence of microplastics in wild mussels and those sold in all supermarkets sampled in this

study indicates that microplastics consumption by seafood eaters in the U.K. is likely to be common and widespread. This is not only an issue for U.K. consumers given the global spread of microplastics in the marine environment, highlighted by the discovery of microplastics in mussels from South America sold in U.K. supermarkets. Similar studies have detected microplastics in bivalve species in supermarkets in France and Belgium (DeWitte et al., 2014; Van Cauwenberghe and Janssen, 2014) and fish markets in China and the United States (Li et al., 2015, Rochman et al., 2015). Annual dietary exposure for the average European shellfish consumer has been estimated to amount to 11,000 microplastics per year, based on the number of microplastics recovered from mussels from French supermarkets (Van Cauwenberghe and Janssen, 2014). In this study of U.K. supermarkets, consumers purchasing live mussels would be expected to ingest around ~100 debris particles, based on an adult consumption of a 100 g mussel portion. This is higher for frozen, chilled or processed mussels at ~140 particles per 100 g portion. If accounting for a 50% representation for actual microplastics found in this study, this results in ~70 microplastic particles per 100 g portion of processed mussels. A recent EFSA statement on the subject states that only microplastics smaller than 150 μm may translocate across the human gut epithelium (EFSA CONTAM Panel, 2016), which equates to an estimated ~40-60% of particles recovered from supermarket brought mussels (Fig. 5), and the absorption of these penetrating organs may be limited to ≤ *0.3%* (EFSA CONTAM Panel, 2016).

4.6 Wider implications concerning human health and public perception of seafood contamination from microplastics

The human health consequences of consumption of microplastics in seafood are unknown and not possible to risk assess in the absence of sufficient exposure and toxicological data (EFSA CONTAM Panel, 2016). The potential impacts have been subject to a number of reviews and broadly include particle toxicity, chemical and microbial hazards (GESAMP 2015, EFSA CONTAM Panel, 2016; Galloway, 2015; Rochman 2015; Vethaak and Leslie, 2016; Kirstein et al., 2016). In finding microplastics in mussel seafood, it is worth considering the public perception of risk from

406 microplastics, especially since their impacts are receiving increasing attention in the media. Public
407 awareness of the problem, revulsion and perception of risk, whether it exists in reality or not, can
408 influence consumption behavior as was demonstrated in the case of genetically modified foods
409 (Gaskell et al., 2004). If the presence of microplastics in seafood is off-putting to consumers, it has
410 been postulated that this could reduce the value of seafood products (GESAMP, 2016). Whilst some
411 studies have demonstrated that depuration of microplastics can occur, perhaps offering a way to
412 "clean out" the animals prior to sale, this will also add additional costs to fisheries or retailers
413 (GESAMP, 2015). Nonetheless, seafood is only one route of human exposure by ingestion since
414 microplastics have been identified in other food sources (EFSA CONTAM Panel, 2016) and in
415 drinking water (Schymanski et al., 2017), whilst airborne microplastics can be inhaled (Wright and
416 Kelly, 2017). Furthermore, a recent study provides evidence that such low levels of microplastics in
417 mussels, which are ingested by humans, are minimal compared to exposure via household fibers that
418 may fallout from the surrounding air while consuming a meal (Catarino et al., 2018).
419
420 *Conclusion*
421 It is becoming increasingly evident that global contamination of the marine environment by plastic
422 litter is impacting wildlife and its entry into the food chain is providing a pathway for the waste that
423 we dispose of to be returned to us through our diet. The U.K. is clearly no exception to this paradigm.
424 This study provides further evidence of this route of exposure and continued research will hopefully
425 drive effective human risk assessment. Currently, whilst there is regulation of some chemical
426 contaminants in food, the same cannot be said for microplastics. In the long term, however, global
427 regulatory solutions to this problem are needed.
428
429

Figure and Table Legends

Figure 1. Sampling sites of mussels along the U.K. coastal waters.

Figure 2. The relative abundances of debris items contaminants in seawater and mussel tissue samples (n=6). For seawater samples: all samples were significantly different (p=<0.001) from the procedural blank samples with the exceptions of Brighton (no significant difference) and Hastings A (p=<0.05). Using the lowest seawater levels detected (at Brighton) as reference samples: the following significance values for seawater samples highlighted are: * p=<0.05, ** p=<0.01, *** p=<0.001.

Figure 3. Abundance of debris items in mussels (n=6). All mussels (coastal and supermarket, SM) contained significantly higher numbers of debris items (p=<0.001, with the exceptions of Plymouth, Brighton, Hastings A and Edinburgh (showing no significant difference) compared to the procedural blank. Using Plymouth tissues as reference samples: the following significance values for seawater samples highlighted are: * p=<0.05, ** p=<0.01, *** p=<0.001. Mussels from SM1- SM4 were bought as live mussels in net bags. SM6-SM8 were mussels that were sold dead: either frozen or chilled. SM5 were mussels that had been cooked and then frozen or chilled prior to sale. Using SM3 mussels as a reference sample, SM5, SM7-8 are highlighted as containing significantly high numbers of debris items.

Figure 4. Relative abundances of debris items in coastal mussels (n=8 sites) compared with supermarket sourced farmed mussels (n=4), and supermarket live mussels (n=4) compared with supermarket processed mussels (n=4). *** p =< 0.001.

Figure 5. The sizes and shapes of debris items in seawater (A, B) and mussels (C, D).

Figure 6. Light microscope images, IR spectra, and match statistics (in brackets) of the most frequently observed microparticles: (**A**) polypropylene, (**B**) polyester, (**C**) polyethylene, (**D**) rayon, (**E**) cotton, (**F**) cellulose, (**G**) acrylic mix, (**H**) acrylic, (**I**) nitrile rubber, (**J**) cotton/olefin, (**K**)

453 polypropylene/polyethylene copolymer.

Supplemental Figure and Table Legends

Table S1. The characteristics of sampling sites and the size of mussels. [a]W, wild mussels; F, supermarket bought farmed mussels; [b]SM, supermarket bought mussels; [c]supplied pre-shelled, frozen and kept chilled; [d]supplied pre-cooked, frozen and chilled.

Table S2. Types of debris items identified with micro-FT-IR for the particles randomly selected from seawater, wild mussels and supermarket bought mussels.

461 Figure 1. Sampling sites of mussels along the U.K. coastal waters.

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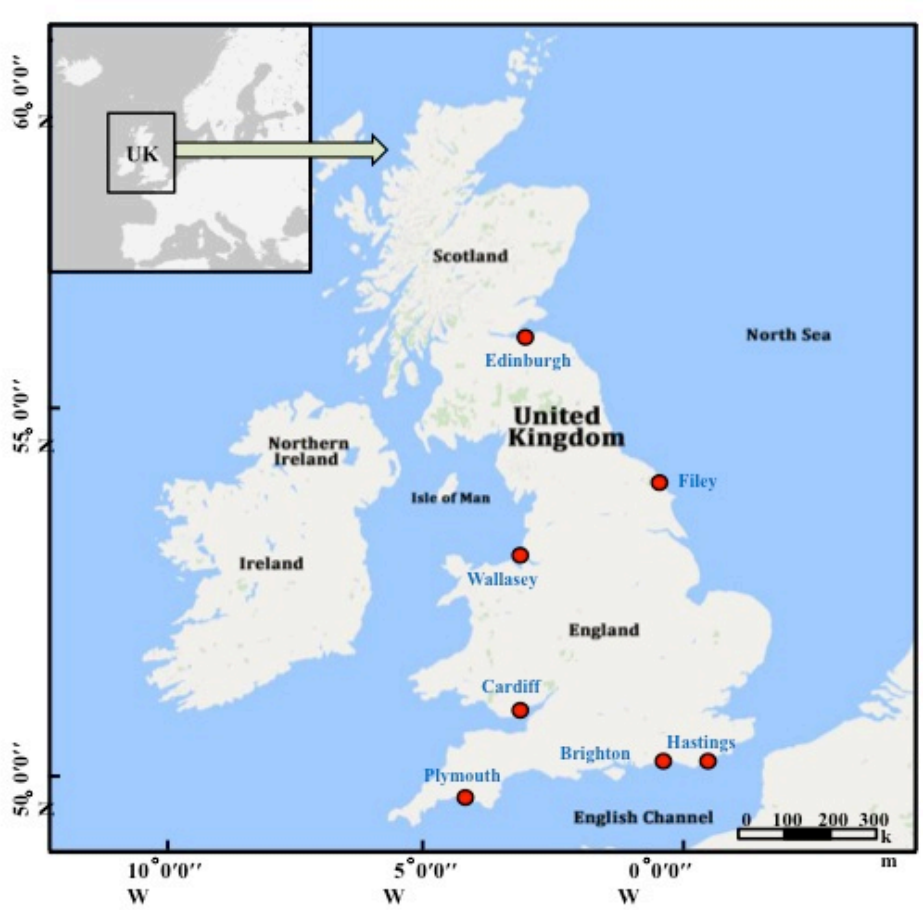

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Figure 2. The relative abundances of debris items contaminants in seawater and mussel tissue samples (n=6). For seawater samples: all samples were significantly different (p=<0.001) from the procedural blank samples with the exceptions of Brighton (no significant difference) and Hastings A (p=<0.05). Using the lowest seawater levels detected (at Brighton) as reference samples: the following significance values for seawater samples highlighted are: * p=<0.05, ** p=<0.01, *** p=<0.001.

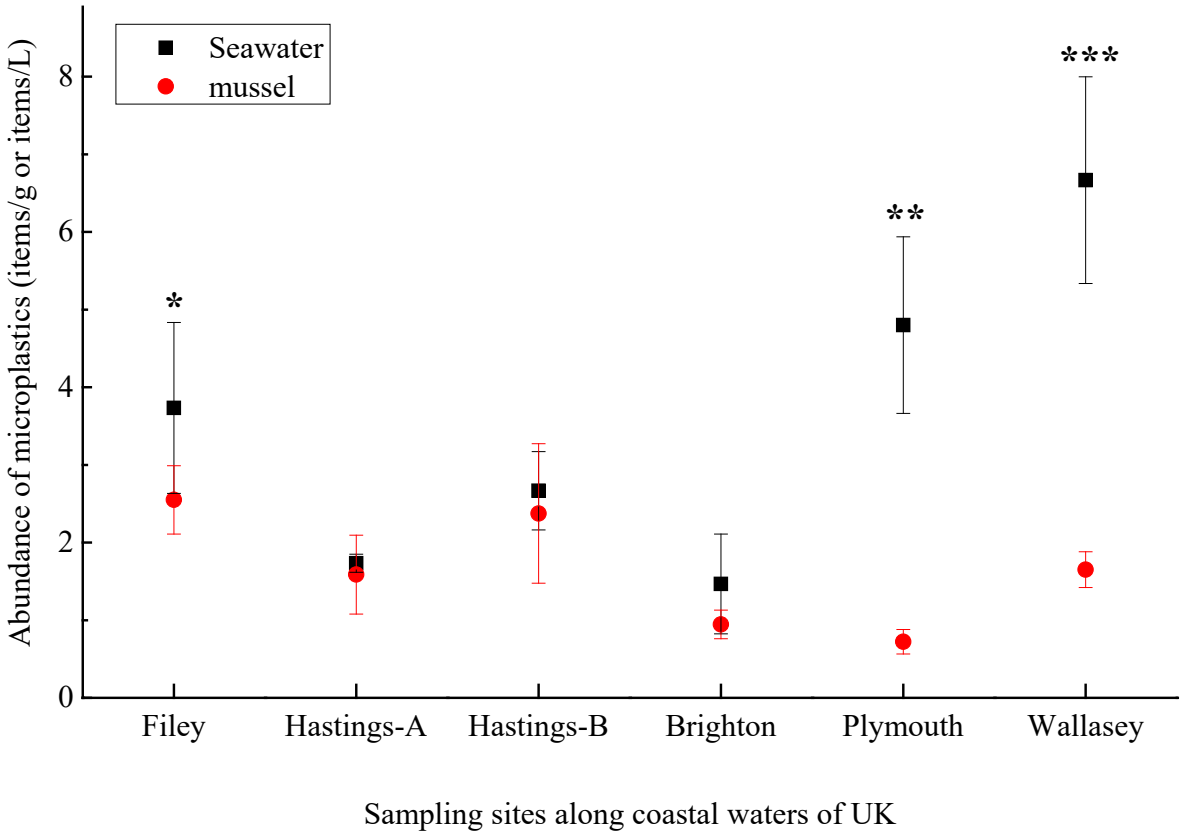


Figure 3. Abundance of debris items in mussels (n=6). All mussels (coastal and supermarket, SM) contained significantly higher numbers of debris items (p=<0.001, with the exceptions of Plymouth, Brighton, Hastings A and Edinburgh (showing no significant difference) compared to the procedural blank. Using Plymouth tissues as 'reference' samples for comparison purposes: the following significance values for seawater samples highlighted are: * p=<0.05, ** p=<0.01, *** p=<0.001. Mussels from SM1- SM4 were bought as live mussels in net bags. SM6-SM8 were frozen/chilled, and SM5 were cooked/frozen/chilled mussels. Using SM3 mussels as a reference sample, SM5, SM7-8 are highlighted as containing significantly high numbers of debris items.

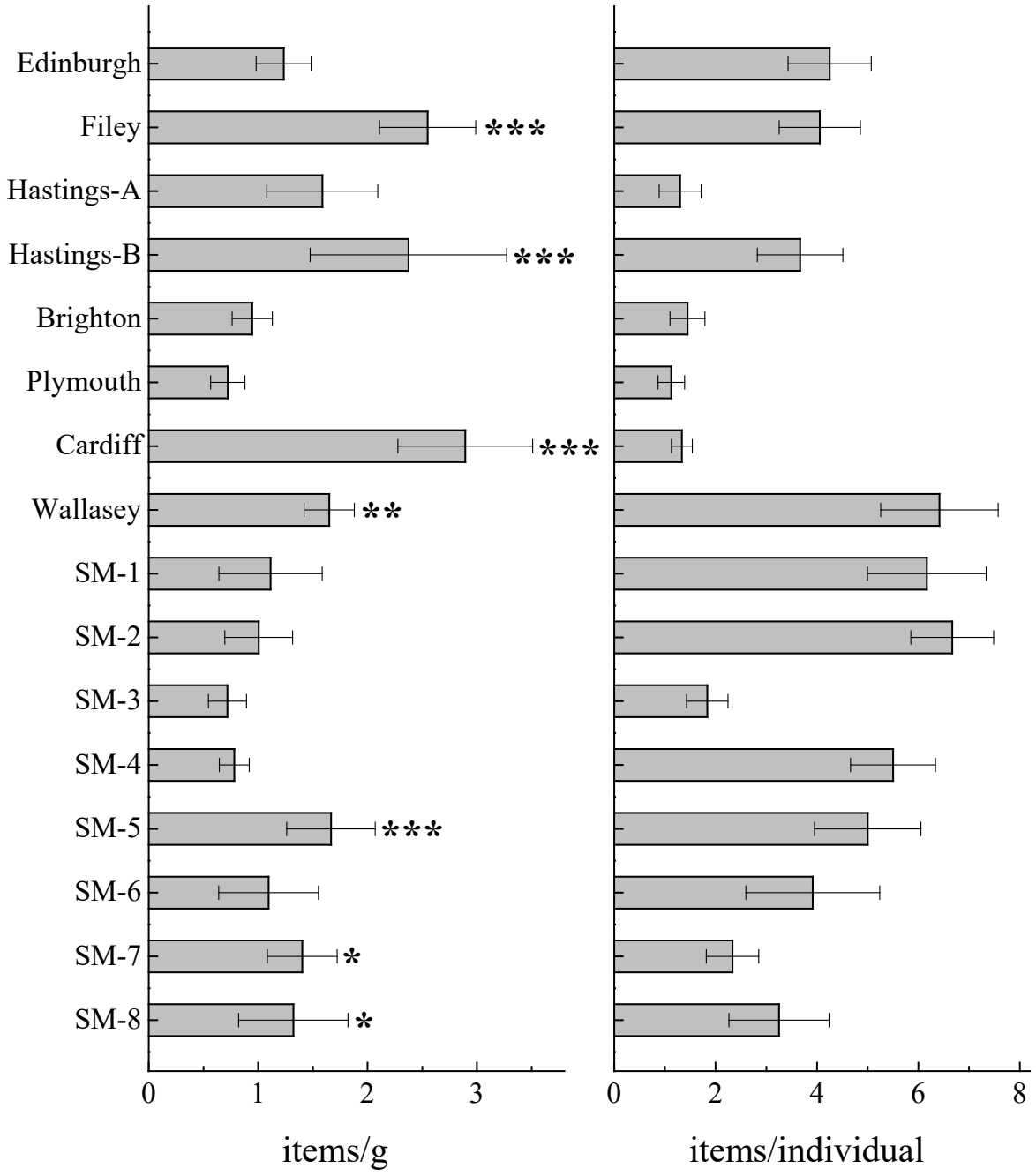
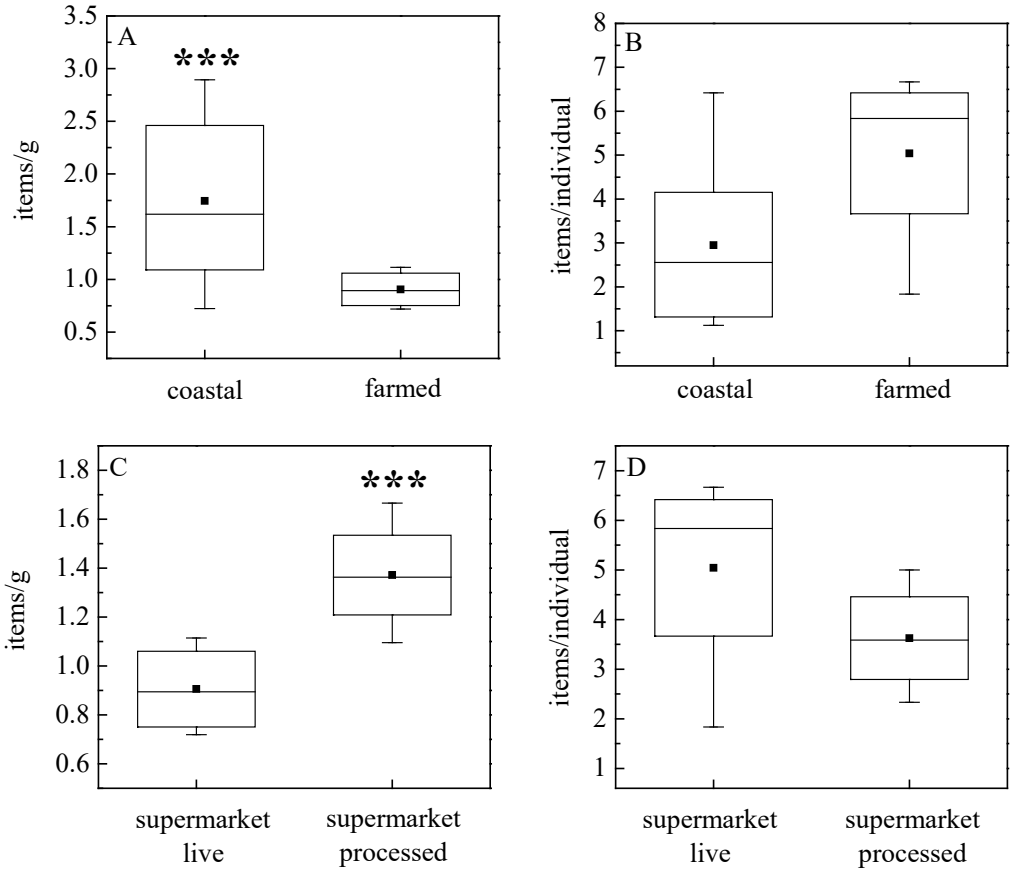


Figure 4. Relative abundances of debris items in coastal mussels (n=8 sites) compared with supermarket sourced farmed mussels (n=4), and supermarket live mussels (n=4) compared with supermarket processed mussels (n=4). *** p =< 0.001.



499 Figure 5. The sizes and shapes of debris items in seawater (A, B) and mussels (C, D).

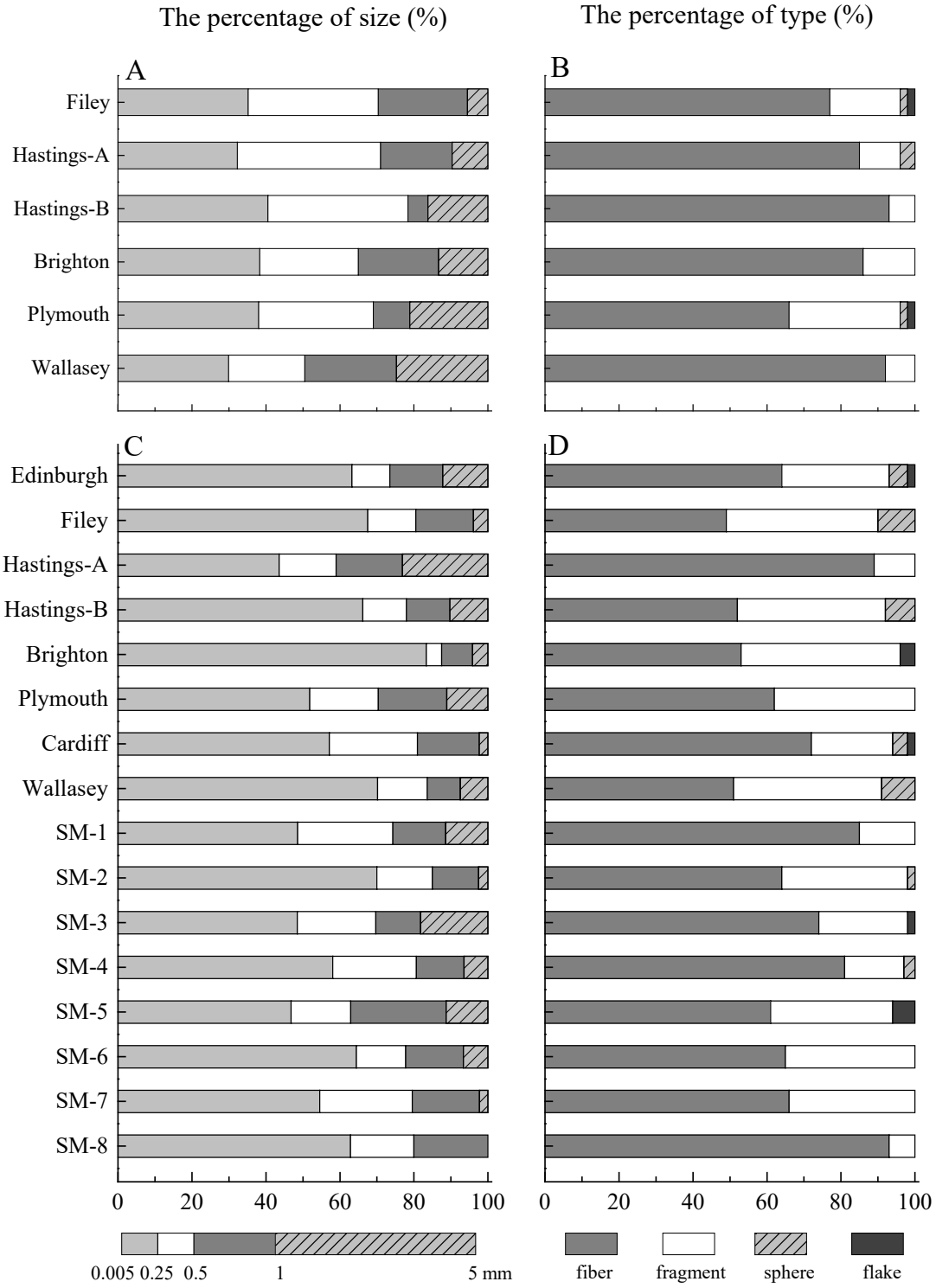


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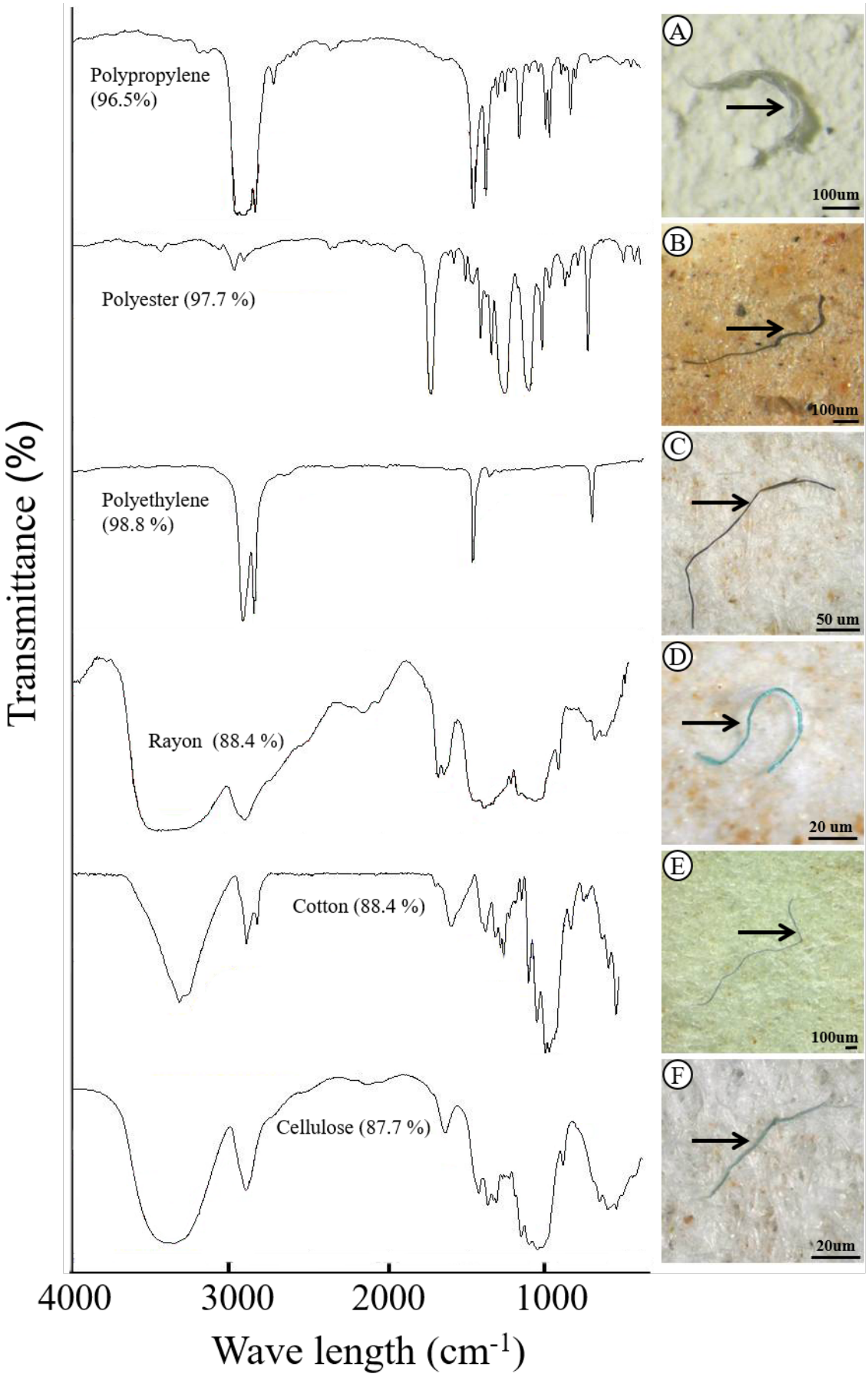
Figure 6. Light microscope images, IR spectra, and match statistics (in brackets) of the most frequently observed microparticles: (**A**) polypropylene; (**B**) polyester, (**C**) polyethylene, (**D**) rayon, (**E**) cotton, (**F**) cellulose, (**G**) acrylic mix, (**H**) acrylic, (**I**) nitrile rubber, (**J**) cotton/olefin, (**K**) PP/PE copolymer.


Polypropylene
(96.5%)
Polyester (97.7 %)
Polyethylene
(98.8 %)
Rayon (88.4 %)
Cotton (88.4 %)
Cellulose (87.7 %)
Transmittance (%)
4000
3000
2000
1000
Wave length (cm-1)
A
100um
B
100um
C
50 um
D
20 um
E
100um
F
20um

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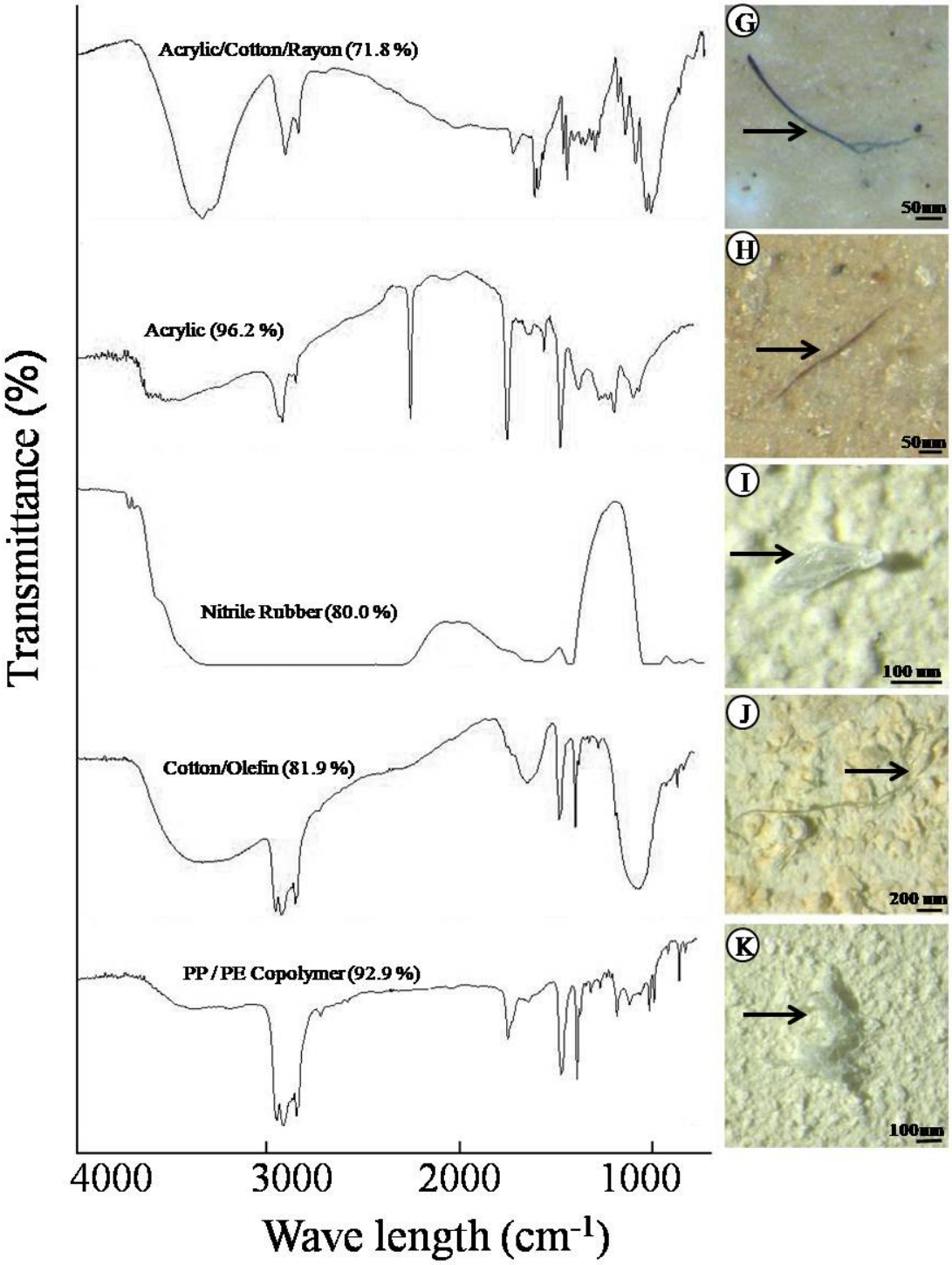
Acrylic/Cotton/Rayon (71.8 %)
Acrylic (96.2 %)
Nitrile Rubber (80.0 %)
Cotton/Olefin (81.9 %)
PP / PE Copolymer (92.9 %)
Transmittance (%)
4000
3000
2000
1000
Wave length (cm-1)
G
50nm
H
50nm
I
100 nm
J
200 nm
K
100nm

509

510

511 Table S1. The characteristics of sampling sites and the size of mussels. [a]WM, wild/coastal mussels;
512 FM, supermarket bought farmed mussels; [b]SM, supermarket bought mussels; [c]supplied pre-shelled,
513 frozen and kept chilled; [d]supplied pre-cooked, frozen and chilled.

Site	Source	Location (coordinates)	No.	Mean Shell Length (cm)	Mean Shell weight (g/individual)	Mean Soft tissue weight (g/individual)
Edinburgh, Forth Estuary	WM[a]	Musselburgh mussel bed (55.949840,-3.055463)	12	4.80±0.31	11.63±1.15	3.43±0.24
Filey, Holderness Coast	WM	rocky outcrop (54.12600, 01.72101)	18	3.35±0.27	7.18±0.93	1.59±0.05
Hastings-A, English Channel	WM	beach groins, less public (50.51422, 00.36156)	30	3.21±0.19	3.69±0.85	0.82±0.05
Hastings-B English Channel	WM	rocky outcrop, more public (50.51061, 00.33849)	18	4.03±0.38	8.00±1.64	1.63±0.34
Brighton, English Channel	WM	beach groins (40.5781, 73.9597)	18	3.64±0.23	7.20±1.46	1.52±0.14
Plymouth, English Channel	WM	Freathy, rocky outcrop (50.345903, -4.254810)	24	3.54±0.42	6.52±1.82	1.57±0.23
Cardiff, Severn Estuary	WM	harbour wall (51.464053, -3.159434)	30	3.25±0.36	1.98±0.65	0.47±0.06
Wallasey, Mersey Estuary	WM	shipping post (53.426521, -3.066215)	12	4.60±0.18	12.89±1.94	3.90±0.57
SM[b]-1	FM	Scotland	6	5.88±0.	13.58±1.84	6.03±1.54
SM-2	FM	Scotland	6	6.4±0.2	15.00±2.69	7.03±1.68
SM-3	FM	Scotland	18	4.86±0.	6.69±1.05	2.58±0.32
SM-4	FM	Scotland	6	6.43±0.	14.65±2.41	7.13±1.11
SM-5[c]	FM	South America	6	pre-		3.04±0.33
SM-6[c]	WM	North Sea	12	pre-		3.79±0.89
SM-7[c]	WM	NE Atlantic	18	pre-shelled		1.67±0.18
SM-8[d]	FM	South America	12	pre-shelled		2.56±0.43

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Table S2. Types of microplastics identified with micro-FT-IR for the particles randomly selected from seawater, wild mussels and supermarket bought mussels. [1]Olefin copolymer of polypropylene/polyethylene.

Sample source	Composition of particles	Number	Percentage (%)
seawater	**particles measured**	**36**	**100**
	plastic particles	**19**	**53**
	anthropogenic-natural	**15**	**42**
	natural/other particles	**2**	**6**
	Polyester	*17*	*47*
	Rayon	*9*	*25*
	Cotton	*6*	*17*
	Polyethylene	*2*	*6*
	Cellulose	*2*	*6*
coastal mussels	**particles measured**	**35**	**100**
	plastic particles	**15**	**43**
	anthropogenic-natural	**14**	**40**
	natural/other particles	**6**	**17**
	Polyester	*15*	*43*
	Rayon	*9*	*26*
	Cotton	*5*	*14*
	Cellulose	*5*	*14*
	Acrylic/cotton/rayon mix	*1*	*3*
supermarket mussels	**particles measured**	**23**	**100**
	plastic particles	**13**	**57**
	anthropogenic-natural	**6**	**26**
	natural/blend/other	**4**	**17**
	Polypropylene	*4*	*17*
	Polyester	*4*	*17*
	Rayon	*4*	*17*
	Acrylic	*3*	*13*
	Cellulose	*2*	*9*
	Cotton	*2*	*9*
	Polyethylene	*1*	*4*
	Propylene glycol ricinoleate	*1*	*4*
	Nitrile rubber	*1*	*4*
	Cotton/olefin[1]	*1*	*4*

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