



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

Jiana Li^{a, b}, Christopher Green^c, Alan Reynolds^d, Huahong Shi^a, Jeanette M. Rotchell^{b, *}

^a State Key Laboratory of Estuarine and Coastal Research, East China Normal University, Shanghai 200062, China

^b School of Environmental Sciences, University of Hull, Cottingham Road, Hull, HU6 7RX, United Kingdom

^c Institute of Environment, Health and Societies, Brunel University London, Kingston Lane, Uxbridge, UB8 3PH, United Kingdom

^d Experimental Techniques Centre, Brunel University London, Kingston Lane, Uxbridge, UB8 3PH, 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.

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1. Introduction

The global presence of microplastics (defined as particles <5 mm 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 Thompson, 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*,

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* Corresponding author.

E-mail address: j.rotchell@hull.ac.uk (J.M. Rotchell).

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% O₂ 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% H₂O₂ 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 24–48 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 copper SEM grid. Spectra were acquired with a minimum of 50 scans at a resolution of 4 cm⁻¹ and matched using a series of polymer library databases (PolyATR, AR Polymer Introductory, NDFIBS, RP, CRIME, FIBRES 3, POLY1, POLYADD1 from Perkin Elmer), a hit index of at least 70% match was considered acceptable. Ninety-four samples met this threshold. While working at the limit of the micro-FT-IR's capability, the smallest fibers analysed were 10 µm across. To collect an effective spectra in these cases, the aperture of the IR detector was set to 10 × 50 µm to collect spectra along the length of the fiber. The number of microplastics in individuals were estimated assuming a uniform distribution.

2.5. Statistical analyses

Statistical analyses (ANOVA and linear regression) were performed using SPSS. Any differences of the abundance of total microplastics, and total microfibrils alone, in seawater and mussel tissue samples was determined using One-Way ANOVA with a Dunnett Test. A linear regression analysis was used to determine the relationship between seawater and tissue levels of

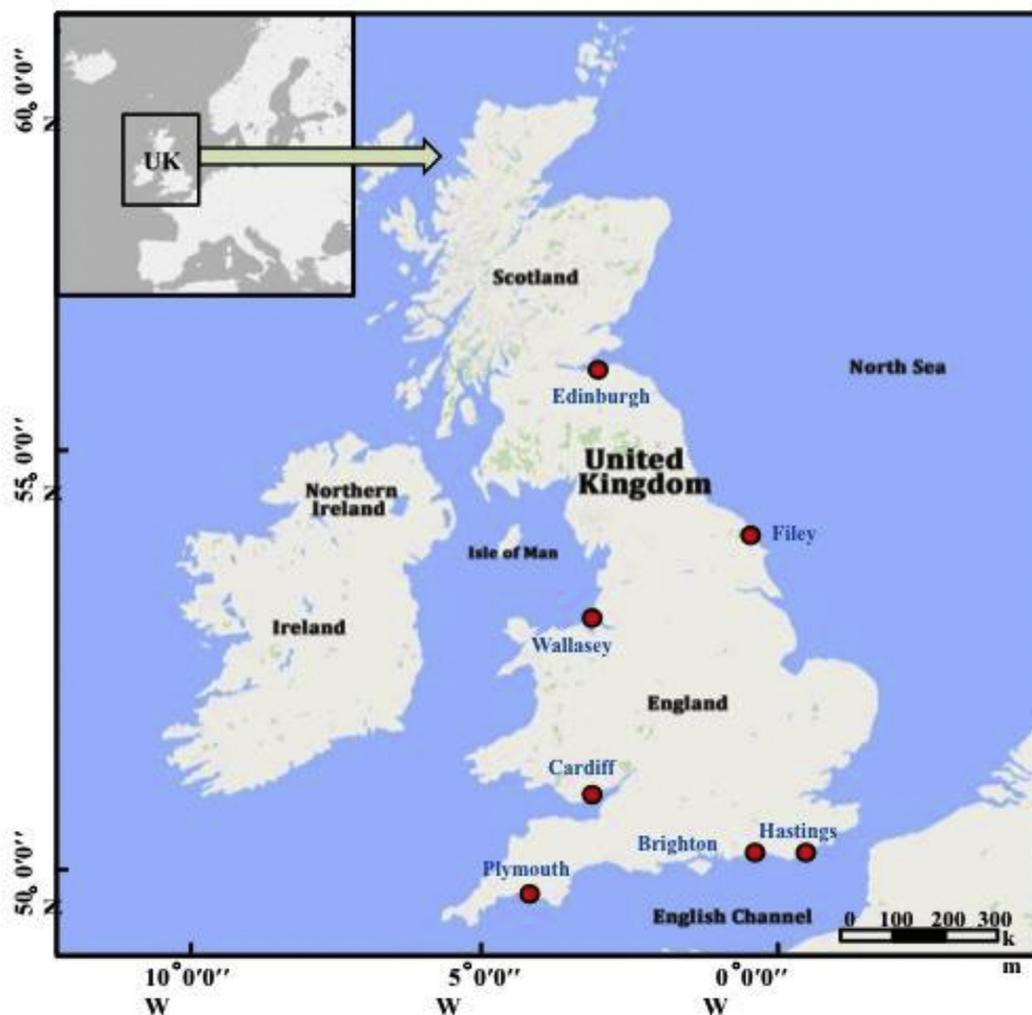


Fig. 1. Sampling sites of mussels along the U.K. coastal waters.

microplastics. Statistical significance was accepted at $* = p < 0.05$, $** = p < 0.01$, $*** = p < 0.001$.

3. Results

3.1. Spatial distribution of microplastics

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

Significantly higher numbers of debris items were detected in seawater samples from all sampling sites ($p < 0.001$ for Filey, Hastings B, Wallasey and Plymouth, $p < 0.05$ for Hastings A), with the exception of Brighton compared with the procedural blank. Filey, Hastings B, Cardiff and Wallasey sampling locations, had significantly more debris items when using Brighton as a reference site (Fig. 2). In mussels, the number of debris items in samples collected from all sampling locations were significantly higher than

the procedural blank samples: Plymouth and Brighton were significant to $p < 0.01$, all other sampling locations to $p < 0.001$. Using Plymouth as a reference site, Brighton mussel tissue samples were not significantly different, while mussels from all the remaining locations were significantly higher (Fig. 3). For the supermarket bought mussels, a similar, widespread level of debris items was detected in all six replicates, with each supermarket source containing at least one debris item and all significantly higher levels than the procedural blank ($p < 0.001$) (Fig. 3). Using sample SM3 as a reference sample, sources SM5 and SM7 contained significantly more debris items compared with the other supermarket sources (Figs. 3., 4C.). The mussels SM5 represent precooked samples from South America, and SM7 were samples that had, according to their packaging, been frozen, then bought chilled and were from the NE Atlantic (Table S1).

3.2. Abundance of microplastics in mussel tissues

In mussels sampled from the coastal locations, the presence of debris items ranged between 0.7 and 2.9 items/g tissue (wet weight) and between 1.1 and 6.4 items/individual (Fig. 3). Seawater samples displayed an average debris abundance of 3.5 ± 2.0 items/L (range: 1.5–6.7 items/L). Linear regression analysis found no

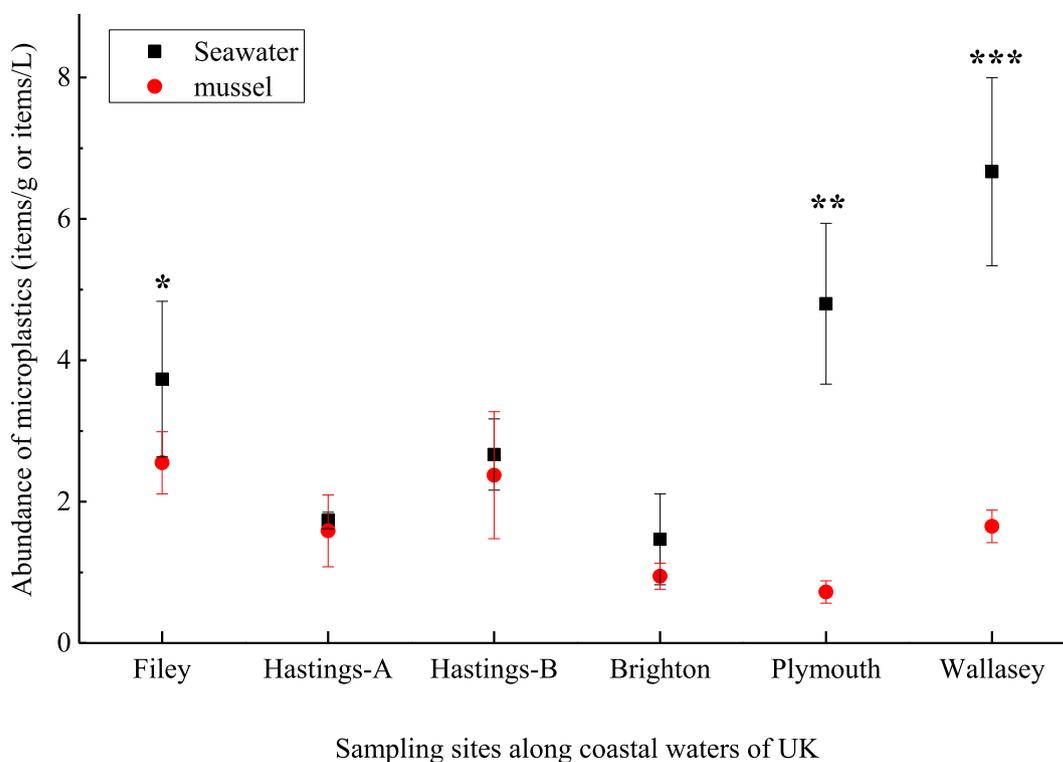


Fig. 2. The relative abundances of debris items contaminants in seawater and mussel tissue samples ($n=6$). For seawater samples: all samples were significantly different ($p<0.001$) from the procedural blank samples with the exceptions of Brighton (no significant difference) and Hastings A ($p<0.05$).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

relationship between the number of debris items in seawater and mussel tissues ($r^2=0.000$). Debris abundance also varied significantly ($p<0.001$ using one-way ANOVA) according to whether the source of the mussels was directly from the coastal environment or from the supermarket (Fig. 4). More debris items per gram of flesh were detected in wild mussels from coastal sites, compared with farmed mussels from supermarkets, yet the farmed mussels were larger in size leading to significantly more items per individual ($p<0.001$)(SM1-4, Table S1) (Fig. 4A and B). Focusing on the supermarket bought mussels; live mussels contained 0.9 items/g on average, compared with an average of 1.4 items/g in processed mussels. The debris abundance was thus significantly higher in pre-cooked processed mussels (samples SM5-SM8) compared to live supermarket bought mussels (SM1-SM4) by weight ($p<0.001$) (Fig. 4C).

3.3. Morphology of microplastics in seawater and mussels

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

3.4. Composition of microplastics in mussels

Out of 1048 debris items isolated on filters, a total of 138 debris

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

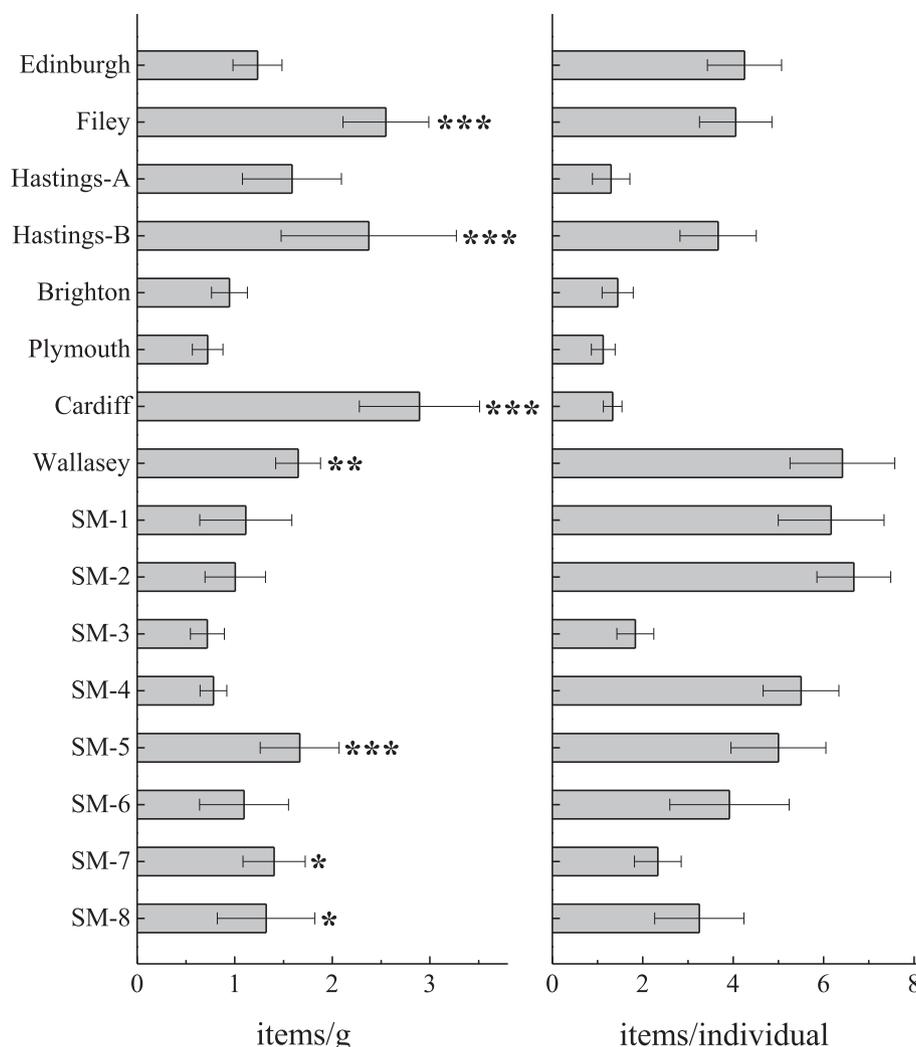


Fig. 3. Abundance of debris items in mussels ($n = 6$). All mussels (coastal and supermarket, SM) contained significantly higher numbers of debris items ($p < 0.001$, with the exceptions of Plymouth, Brighton, Hastings A and Edinburgh (showing no significant difference) compared to the procedural blank. 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.

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 (De Witte 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 Harbor, 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.

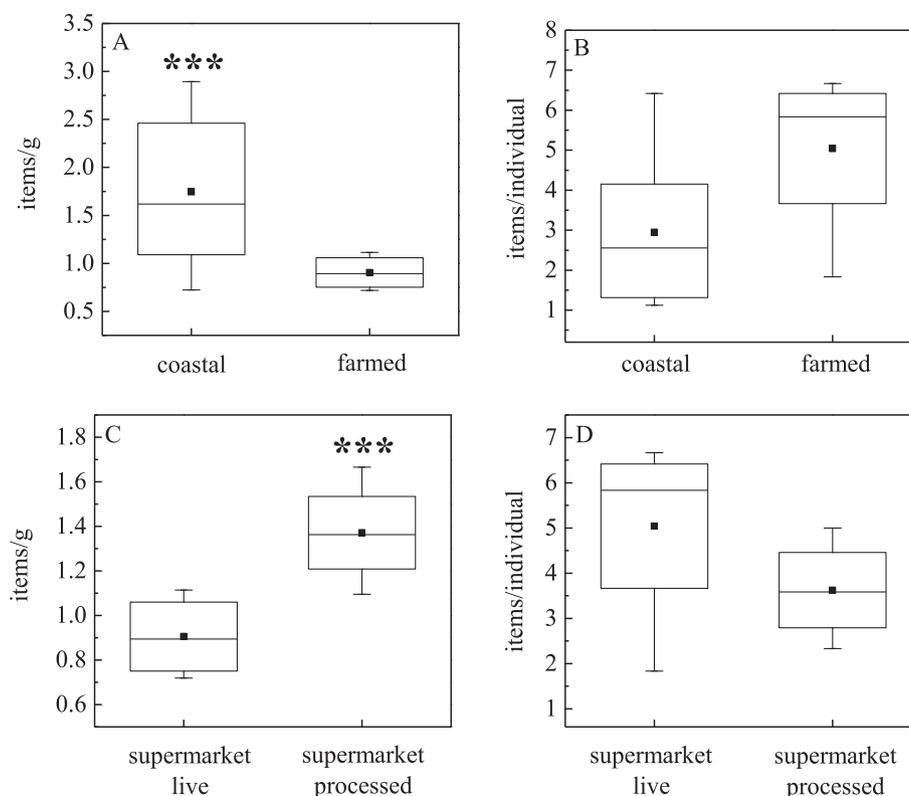


Fig. 4. Relative abundances of debris items in coastal mussels ($n=8$ sites) compared with supermarket sourced farmed mussels ($n=4$), and supermarket live mussels ($n=4$) compared with supermarket processed mussels ($n=4$). *** $p < 0.001$.

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

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

4.3. Microplastic and other anthropogenic debris in coastal mussel tissues

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

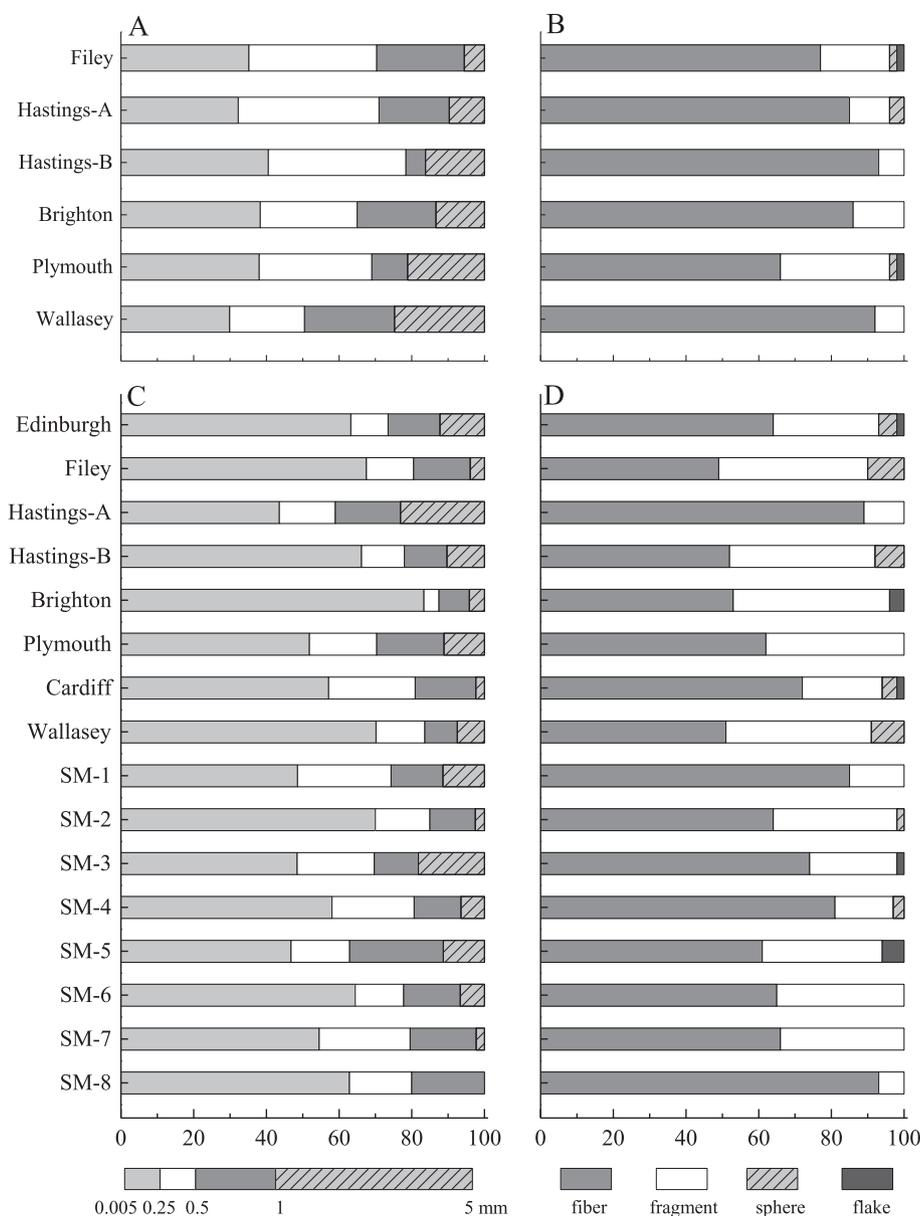


Fig. 5. The sizes and shapes of debris items in seawater (A, B) and mussels (C, D).

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 ($0.2 \pm 0.3 \text{ g}^{-1}$ 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 & Janssen, 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 (Wesche et al., 2017; Leslie et al., 2017).

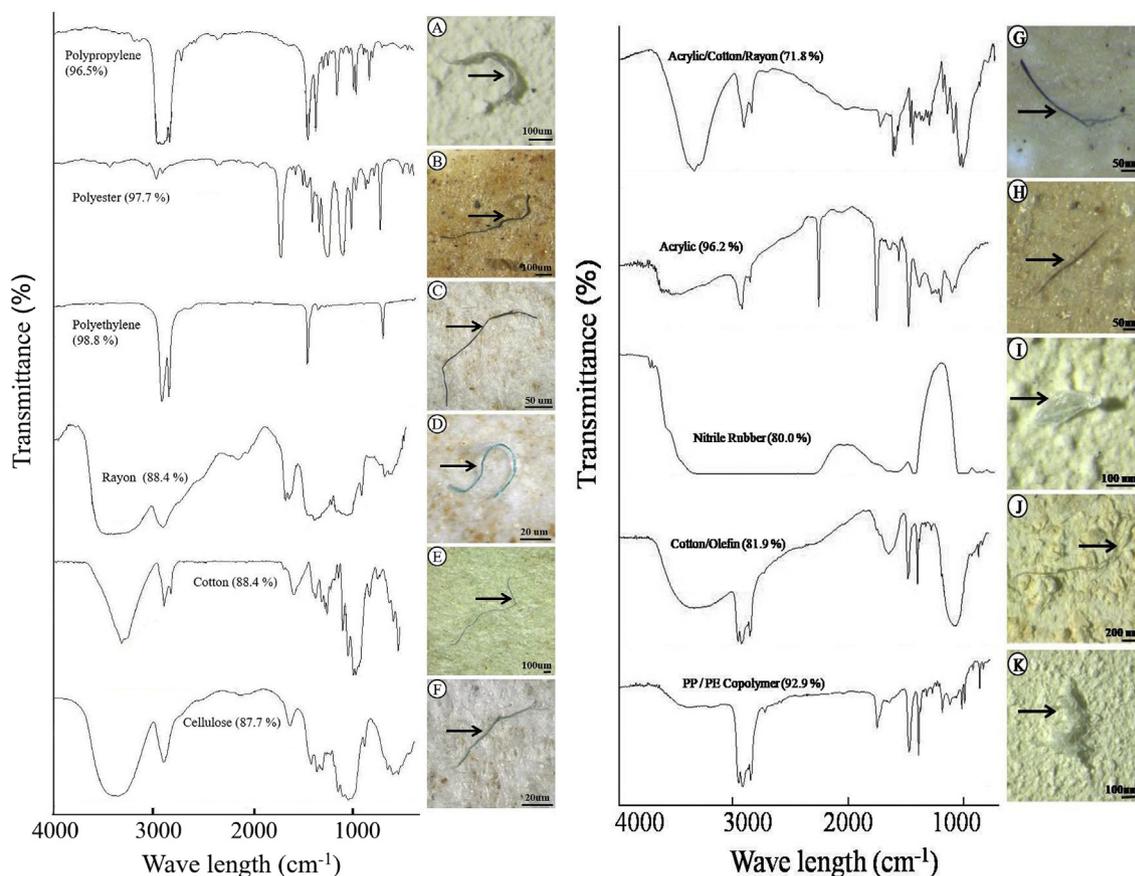


Fig. 6. Light microscope images, IR spectra, and match statistics (in brackets) of the most frequently observed microparticles: (A) polypropylene; (B) polyester; (C) polyethylene; (D) rayon; (E) cotton; (F) cellulose; (G) acrylic mix; (H) acrylic; (I) nitrile rubber; (J) cotton/olefin; (K) PP/PE copolymer.

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 (1 mg/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 and B). 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 to 10.5 items/g. Higher microplastic levels were also reported for farmed clams (*Venerupis philippinarum*) relative to wild clams (ranging from 0.07 to 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 D). 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 and D), 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 (De Witte 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 $\leq 0.3\%$ (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; Veethak and Leslie, 2016; Kirstein et al., 2016). In finding microplastics in mussel seafood, it is worth considering the public perception of risk from microplastics, especially since their impacts are receiving increasing attention in the media. Public awareness of the problem, revulsion and perception of risk, whether it exists in reality or not, can influence consumption behavior as was demonstrated in the case of genetically modified foods (Gaskell, 2004). If the presence of microplastics in seafood is off-putting to consumers, it has been postulated that this could reduce the value of seafood products (GESAMP, 2016). Whilst some studies have demonstrated that depuration of microplastics can occur, perhaps offering a way to “clean out” the animals prior to sale, this will also add additional costs to fisheries or retailers (GESAMP, 2015). Nonetheless, seafood is only one route of human exposure by ingestion since microplastics have been identified in other food sources (EFSA CONTAM Panel, 2016) and in drinking water (Schymanski et al., 2017), whilst airborne microplastics can be inhaled (Wright and Kelly, 2017).

Furthermore, a recent study provides evidence that such low levels of microplastics in mussels, which are ingested by humans, are minimal compared to exposure via household fibers that may fallout from the surrounding air while consuming a meal (Catarino et al., 2018).

5. Conclusion

It is becoming increasingly evident that global contamination of the marine environment by plastic litter is impacting wildlife and its entry into the food chain is providing a pathway for the waste that we dispose of to be returned to us through our diet. The U.K. is clearly no exception to this paradigm. This study provides further evidence of this route of exposure and continued research will hopefully drive effective human risk assessment. Currently, whilst there is regulation of some chemical contaminants in food, the same cannot be said for microplastics. In the long term, however, global regulatory solutions to this problem are needed.

Declarations of interests

None.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at <https://doi.org/10.1016/j.envpol.2018.05.038>.

References

- Avio, C.G., Gorb, S., Milan, M., Benedetti, M., Fattorini, D., d'Errico, G., Paoletto, M., Bargelloni, L., Regoli, F., 2015. Pollutants bioavailability and toxicological risk from microplastics to marine mussels. *Environ. Pollut.* 198, 211–222.
- Boerger, C.M., Lattin, G.L., Moore, S.L., Moore, C.J., 2010. Plastic ingestion by planktivorous fishes in the North Pacific central gyre. *Mar. Pollut. Bull.* 60 (12), 2275–2278.
- Browne, M.A., Dissanayake, A., Galloway, T.S., Lowe, D.M., Thompson, R.C., 2008. Ingested microscopic plastic translocates to the circulatory system of the mussel, *Mytilus edulis* (L.). *Environmental Science & Technology* 42, 5026–5031.
- Catarino, A.I., Macchia, V., Sanderson, W.G., Thompson, R.C., Henry, T.B., 2018. 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.* 237, 675–684.
- CEFAS Report C2436/01, 2005. Contaminant Status in the Irish Sea- Final Report. Ed. W. Reynolds, Crown Copyright, U.K).
- Cole, M., Webb, H., Lindeque, P.K., Fileman, E.S., Halsband, C., Galloway, T.S., 2014. Isolation of microplastics in biota-rich seawater samples and marine organisms. *Sci. Rep.* 4, 4528.
- Davidson, K., Dudas, S.E., 2016. Microplastic ingestion by wild and cultured Manila clams (*Venerupis philippinarum*) from Baynes Sound, British Columbia. *Arch. Environ. Contam. Toxicol.* 71, 147–156.
- Desforges, J.P.W., Galbraith, M., Ross, P.S., 2015. Ingestion of microplastics by zooplankton in the Northeast Pacific Ocean. *Arch. Environ. Contam. Toxicol.* 69, 320–330.
- Devriese, L.I., van der Meulen, M.D., Maes, T., Bekaert, K., Paul-Pont, I., Frère, L., Robbens, J., Vethaak, A.D., 2015. Microplastic contamination in brown shrimp (*Crangon crangon*, Linnaeus 1758) from coastal waters of the southern North Sea and Channel area. *Mar. Pollut. Bull.* 98, 179–187.
- De Witte, B., Devriese, L., Bekaert, K., Hoffman, S., Vandermeersch, G., Cooreman, K., Robbens, J., 2014. Quality assessment of the blue mussel (*Mytilus edulis*):

- comparison between commercial and wild types. *Mar. Pollut. Bull.* 85, 146–155.
- Dris, R., Gasperi, J., Saad, M., Mirande, C., Tassin, B., 2016. Synthetic fibers in atmospheric fallout: a source of microplastics in the environment? *Mar. Pollut. Bull.* 140, 290–293.
- EFSA CONTAM Panel (EFSA Panel on Contaminants in the Food Chain), 2016. Statement on the presence of microplastics and nanoplastics in food, with particular focus on seafood. *EFSA Journal* 14 (6), 4501–4531. <https://doi.org/10.2903/j.efsa.2016.4501>.
- Farrell, P., Nelson, K., 2013. Trophic level transfer of microplastic: *Mytilus edulis* (L.) to *Carcinus maenas* (L.). *Environ. Pollut.* 177, 1–3.
- FERA Report, 2015. In: Fernandes, A., Rose, M., Smith, F., Panton, S. (Eds.), Geographical Investigation for Chemical Contaminants in Fish Collected from UK and Proximate Marine Waters: Report to the Food Standards Agency. DEFRA, Crown Copyright, U.K.
- Foekema, E.M., De Groot, C., Mergia, M.T., van Franeker, J.A., Murk, A.J., Koelmans, A.A., 2013. Plastic in North Sea fish. *Environ. Sci. Technol.* 47, 8818–8824.
- Free, C.M., Jensen, O.P., Mason, S.A., Eriksen, M., Williamson, N.J., Boldgiv, B., 2014. High levels of microplastic pollution in a large, remote, mountain lake. *Mar. Pollut. Bull.* 85, 156–163.
- Gallagher, A., Rees, A., Rowe, R., Stevens, J., Wright, P., 2016. Microplastics in the Solent estuarine complex, U.K.: an initial assessment. *Mar. Pollut. Bull.* 102, 243–249.
- Gaskell, G., 2004. Science policy and society: the British debate over GM agriculture. *Curr. Opin. Biotechnol.* 15, 241–245.
- Galloway, T.S., 2015. Micro- and nano-plastics and human health. In: Bergmann, M., Gutow, L., Klages, M. (Eds.), *Marine Anthropogenic Litter*. Springer Open. <https://doi.org/10.1007/978-3-319-16510-3>, 2015.
- GESAMP, 2015. Sources, fate, and effects of microplastics in the marine environment: a global assessment. (Kershaw, P.J., ed.). In: *IMO/FAO/UNESCO-IOC/UNIDO/WMO/IAEA/UN/UNEP/UNDP Joint Group of Experts on Scientific Aspects of Marine Environmental Protection*. Rep. Stud. GESAMP No.90. 96pp.
- GESAMP, 2016. Sources, fate and effects of microplastics in the marine environment: Part 2 of a global assessment. In: Kershaw, P.J., Rochman, C.M. (Eds.), *IMO/FAO/UNESCO-IOC/UNIDO/WMO/IAEA/UN/UNEP/UNDP Joint Group of Experts on the Scientific Aspects of Marine Environmental Protection*, p. 220. Rep. Stud. GESAMP No. 93.
- Halstead, J.E., Smith, J.A., Carter, E.A., Lay, P.A., Johnston, E.L., 2018. Assessment tools for microplastics and natural fibres ingested by fish in an urbanised estuary. *Environ. Pollut.* 234, 552–561.
- Hermesen, E., Pompe, R., Besseling, E., Koelmans, A.A., 2017. Detection of low numbers of microplastics in North Sea fish using strict quality assurance criteria. *Mar. Pollut. Bull.* 122, 253–258.
- Karami, A., Golleskardi, A., Choo, C.K., Larat, V., Karbalaee, S., Salamatinia, B., 2018. Microplastic and mesoplastic contamination in canned sardines. *Sci. Total Environ.* 612, 1380–1386.
- Karlsson, T.M., Vethaak, A.D., Almroth, B.C., Ariese, F., van Velzen, M., Hasselov, M., Leslie, H.A., 2017. Screening for microplastics in sediment, water and marine invertebrates and fish: method development and microplastic accumulation. *Mar. Pollut. Bull.* 122, 403–408.
- Kirstein, I.V., Kirmizi, S., Wichels, A., Garin-Fernandez, A., Erler, R., Loder, M., Gerdts, G., 2016. Dangerous hitchhikers? Evidence for potentially pathogenic *Vibrio* spp. on microplastic particles. *Mar. Environ. Res.* 120, 1–8.
- Kolandhasamy, P., Su, L., Li, J., Qu, X., Jabeen, K., Shi, H., 2018. Adherence of microplastics to soft tissue of mussels: a novel way to uptake microplastics beyond ingestion. *Sci. Total Environ.* 610, 635–640.
- Langston, W.J., Jonas, P.J.C., Millward, G.E., 2010. The severn estuary and bristol channel: a 25 year critical review. *Mar. Pollut. Bull.* 61 (1–3).
- Law, K.L., Thompson, R.C., 2014. Microplastic in the seas. *Science* 345, 144–145.
- Leslie, H.A., Brandsma, S.H., van Velzen, M.J.M., Vethaak, A.D., 2017. Microplastics en route: field measurements in the Dutch river delta and Amsterdam canals, waste water treatment plants, North Sea sediments and biota. *Environ. Int.* 101, 133–142.
- Li, J., Yang, D., Li, D., Jabeen, K., Shi, H., 2015. Microplastics in commercial bivalves from China. *Environ. Pollut.* 207, 190–195.
- Li, J., Qu, X., Su, L., Zhang, W., Yang, D., Kolandhasamy, P., Li, D., Shi, H., 2016. Microplastics in mussels along the coastal waters of China. *Environ. Pollut.* 214, 177–184.
- Lourenço, P.M., Serra-Gonçalves, C., Ferreira, J.L., Catrya, T., Granadeiro, J.P., 2017. Plastic and other microfibers in sediments, macroinvertebrates and shorebirds from three intertidal wetlands of southern Europe and west Africa. *Environ. Pollut.* 231, 123–133.
- Lusher, A.L., McHugh, M., Thompson, R.C., 2013. Occurrence of microplastics in the gastrointestinal tract of pelagic and demersal fish from the English Channel. *Mar. Pollut. Bull.* 67 (1), 94–99.
- Lusher, A.L., Burke, A., O'Connor, I., Officer, R., 2014. Microplastic pollution in the Northeast Atlantic: validated and opportunistic sampling. *Mar. Pollut. Bull.* 88, 325–333.
- Lusher, A.L., Hernandez-Milian, G., O'Brien, J., Berrow, S., O'Connor, I., Officer, R., 2015. Microplastic and macroplastic ingestion by a deep diving, oceanic cetacean: the True's beaked whale *Mesoplodon mirus*. *Environ. Pollut.* 199, 185–191.
- Lusher, A.L., Welden, N.A., Sobral, P., Cole, M., 2017. Sampling, isolating and identifying microplastics ingested by fish and invertebrates. *Analytical Methods* 9, 1346–1360.
- Mathalon, A., Hill, P., 2014. Microplastic fibers in the intertidal ecosystem surrounding Halifax harbor, Nova Scotia. *Mar. Pollut. Bull.* 81, 69–79.
- McGoran, A.R., Clark, P.F., Morrill, D., 2017. Presence of microplastic in the digestive tracts of the European flounder, *Platichthys flesus*, and the European smelt, *Osmerus eperlanus*, from the River Thames. *Mar. Pollut. Bull.* 220, 744–751.
- Murphy, F., Russell, M., Ewins, C., Quinn, B., 2017. The uptake of macroplastic and microplastic by demersal and pelagic fish in the Northeast Atlantic around Scotland. *Mar. Pollut. Bull.* 122, 353–359.
- Murray, F., Cowie, P.R., 2011. Plastic contamination in the decapod crustacean *Nephrops norvegicus* (Linnaeus, 1758). *Mar. Pollut. Bull.* 62 (6), 1207–1217.
- O'Connor, T.P., 1998. Mussel Watch results from 1986 to 1996. *Mar. Pollut. Bull.* 37 (1–2), 14–19.
- Paul-Pont, I., Lacroix, C., Fernández, C.G., Hégaret, H., Lambert, C., Le Goïc, N., Frère, L., Cassone, A.L., Sussarellu, R., Fabioux, C., Guymarch, J., 2016. Exposure of marine mussels *Mytilus* spp. to polystyrene microplastics: toxicity and influence on fluoranthene bioaccumulation. *Environ. Pollut.* 216, 724–737.
- Ribeiro, F., Garcia, A.R., Pereira, B.P., Fonseca, M., Mestre, N.C., Fonseca, T.G., Ilharco, L.M., Bebianno, M.J., 2017. Microplastic effects in *Scrobicularia plana*. *Mar. Pollut. Bull.* 122, 379–391.
- Rochman, C.M., 2015. Micro- and nano-plastics and human health. In: Bergmann, M., Gutow, L., Klages, M. (Eds.), *Marine Anthropogenic Litter*. Springer Open. <https://doi.org/10.1007/978-3-319-16510-3>, 2015.
- Rochman, C.M., Tahir, A., Williams, S.L., Baxa, D.V., Lam, R., Miller, J.T., The, F.-Y., Werorilangi, S., Teh, S.J., 2015. Anthropogenic debris in seafood: plastic debris and fibers from textiles in fish and bivalves sold for human consumption. *Science Reports* 5, 14340.
- Santana, M.F.M., Ascer, L.G., Custódio, M.R., Moreira, F.T., Turra, A., 2016. Microplastic contamination in natural mussel beds from a Brazilian urbanized coastal region: rapid evaluation through bioassessment. *Mar. Pollut. Bull.* 106, 183–189.
- Schymanski, D., Goldbeck, C., Humpf, H.U., Fürst, P., 2017. Analysis of microplastics in water by micro-Raman spectroscopy: release of plastic particles from different packaging into mineral water. *Water Res.* 129, 154–162.
- Silva, P.P.G.E., Nobre, C.R., Resaffe, P., Pereira, C.D.S., Gusmão, F., 2016. Leachate from microplastics impairs larval development in brown mussels. *Water Res.* 106, 364–370.
- Setälä, O., Norkko, J., Lehtiniemi, M., 2016. Feeding type affects microplastic ingestion in a coastal invertebrate community. *Mar. Pollut. Bull.* 102, 95–101.
- Steer, M., Cole, M., Thompson, R.C., Lindeque, P.K., 2017. Microplastic ingestion in fish larvae in the western English Channel. *Environ. Pollut.* 226, 250–259.
- Sussarellu, R., Suquet, M., Thomas, Y., Lambert, C., Fabioux, C., Pernet, M.E.J., Le Goïc, N., Quillien, V., Mingant, C., Epelboin, Y., Corporeau, C., Guymarch, J., Robbens, J., Paul-Pont, I., Soudant, P., Huvet, A., 2016. Oyster reproduction is affected by exposure to polystyrene microplastics. *Proceedings of the National Academy of Sciences, U.S.A.* 113, 2430–2435.
- Tagg, A.S., Sapp, M., Harrison, J.P., Ojeda, J.J., 2015. Identification and quantification of microplastics in wastewater using focal plane array-based reflectance micro-FT-IR imaging. *Anal. Chem.* 87, 6032–6040.
- Tappin, A.D., Millward, G.E., 2015. The English Channel: contamination status of its transitional and coastal waters. *Mar. Pollut. Bull.* 95, 529–550.
- Thompson, R.C., Olsen, Y., Mitchell, R.P., Davis, A., Rowland, S.J., John, A.W.G., McGonigle, D., Russell, A.E., 2004. Lost at sea: where is all the plastic? *Science* 304, 838–838.
- Van Cauwenberghe, L., Janssen, C.R., 2014. Microplastics in mussels cultured for human consumption. *Environ. Pollut.* 193, 65–70.
- Van Cauwenberghe, L., Claessens, M., Vendegehuchte, M.B., Janssen, C.R., 2015. Microplastics are taken up by mussels (*Mytilus edulis*) and lugworms (*Arenicola marina*), living in natural habitats. *Environ. Pollut.* 199, 10–17.
- Vandermeersch, G., Lourenço, H.M., Alvarez-Muñoz, D., Cunha, S., Diogène, J., Cano-Sancho, G., Sloth, J.J., Kwadijk, C., Barcelo, D., Allegaert, W., Bekaert, K., Fernandes, J.O., Marques, A., Robbens, J., 2015a. Environmental contaminants of emerging concern in seafood – European database on contaminant levels. *Environ. Res.* 143 (B), 29–45.
- Vandermeersch, G., Van Cauwenberghe, L., Janssen, C.R., Marques, A., Granby, K., Fait, G., Kotterman, M.J.J., Diogène, J., Bekaert, K., Robbens, J., Devriese, L., 2015b. A critical review on microplastic quantification in aquatic organisms. *Environ. Res.* 143, 46–55.
- Veethak, A.D., Leslie, H.A., 2016. Plastic debris is a human health issue. *Environ. Sci. Technol.* 50, 6825–6826.
- von Moos, N., Burkhardt-Holm, P., Kohler, A., 2012. Uptake and effects of microplastics on cells and tissue of the blue mussel *Mytilus edulis* L. after an experimental exposure. *Environ. Sci. Technol.* 46, 11327–11335.
- Wesche, C., Elert, A.M., Worner, M., Braun, U., Klein, R., Paulus, M., 2017. Assuring quality in microplastic monitoring: about the value of clean air devices as essentials for verified data. *Sci. Rep.* 7, 5424.
- Widdows, J., 1973. Effect of temperature and food on the heart beat, ventilation rate and oxygen uptake of *Mytilus edulis*. *Mar. Biol.* 20, 269–276.
- Wright, S.L., Thompson, R.C., Galloway, T.S., 2013. The physical impacts of microplastics on marine organisms: a review. *Environ. Pollut.* 178, 483–492.
- Wright, S.L., Kelly, F.J., 2017. Plastic and human health: a micro issue? *Environ. Sci. Technol.* 51, 6634–6647.