Towards a greater understanding of the presence, fate and ecological effects of microplastics in the freshwater environment

Horton, A.A.

CHAPTER 4

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The influence of exposure and physiology on microplastic ingestion by the freshwater fish *Rutilus rutilus* (roach) in the River Thames, UK

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Abstract

Microplastics are widespread throughout aquatic environments. However, there is currently insufficient understanding of the factors influencing ingestion of microplastics by organisms, especially higher predators such as fish. In this study we link ingestion of microplastics by the roach *Rutilus rutilus,* within the non-tidal part of the River Thames, to exposure and physiological factors. Microplastics were found within the gut contents of roach from six out of seven sampling sites. Of sampled fish, 33% contained at least one microplastic particle. The majority of particles were fibres (75%), with fragments and films also seen (22.7% and 2.3% respectively). Polymers identified were polyethylene, polypropylene and polyester, in addition to a synthetic dye. The maximum number of ingested microplastic particles for individual fish was strongly correlated to exposure (based on distance from the source of the river). Additionally, at a given exposure, the size of fish correlated with the actual quantity of microplastics in the gut. Larger (mainly female) fish were more likely to ingest the maximum possible number of particles than smaller (mainly male) fish. This study is the first to show microplastic ingestion within freshwater fish in the UK and provides valuable new evidence of the factors influencing ingestion that can be used to inform future studies on exposure and hazard of microplastics to fish.

1. Introduction

Microplastics (plastic particles <5 mm) are an emerging environmental contaminant of growing concern due to their abundance and persistence throughout the environment. Microplastics can enter rivers via runoff and drainage systems, effluent input and breakdown of in situ litter. Once in the aquatic environment, it is highly likely that these will be encountered and ingested by pelagic or benthic organisms. In the case of higher trophic organisms such as fish, ingestion may be direct (from the water column or sediment) or indirect (ingestion of organisms that have previously ingested microplastics) (Campbell et al., 2017; Desforges et al., 2015; Setälä et al., 2014). There is a growing body of evidence for microplastic ingestion by freshwater fish (Biginagwa et al., 2016; Peters and Bratton, 2016; Sanchez et al., 2014; Silva-Cavalcanti et al., 2017) with studies finding up to 100% contamination within sampled fish in some areas (Pazos et al., 2017). However, based on a lack of evidence, we are currently unable to determine the extent to which freshwater fish are ingesting microplastics, the complex variety of factors that may influence ingestion and any implications this may have for ecosystems.

Rivers are highly dynamic environments and along its course, a river will be subject to an accumulation of land-derived inputs, for example road runoff, agricultural runoff, wastewater inputs and litter, all of which can contribute to the burden of microplastics within the watercourse (Horton et al., 2017a; Lechner et al., 2014; Morritt et al., 2014; Nizzetto et al., 2016a). The majority of microplastic particles entering the freshwater environment are likely to be derived from the breakdown of larger items, for example single-use packaging items, tyre and road paint particles, or fibres from synthetic fabrics (Boucher and Friot, 2017; Browne et al., 2011; Horton et al., 2017a). It is assumed that a proportion of microplastics (although not all) entering a river will be buoyant and easily transported downstream. Since the sources of (micro)plastic particles are anthropogenic, a site downstream of populated or industrial areas is likely to contain more microplastics than sites that have been subject to little anthropogenic input (Dris et al., 2015b; Horton et al., 2017a; McCormick et al., 2014). As such, sites further from the river source would be expected to be subject to a greater variety of inputs (Mani et al., 2015).

Assuming there is exposure, physiological traits of fish, such as size, may determine whether an individual will ingest microplastics, and the number of particles the fish may ingest. For example, larger roach will consume more in general due to increased energy demands (Hölker

and Breckling, 2001), which increases their potential for ingestion of microplastic particles. Therefore, susceptibility to ingestion and volume of uptake, given exposure, will be determined by physiological characteristics. Combined, these two factors (exposure and likelihood of ingestion) are expected to determine the number of particles that an individual fish can ingest. Microplastics present within the guts of fish may be considered a representation of microplastic pollution within the river, as a proportion of microplastics within the environment are likely to be contained within biota (van Sebille et al., 2015). The higher the number of microplastics an individual ingests, the more likely the particles are to have an adverse health effect, such as reduced capacity for food ingestion and reduced scope for growth (Murray and Cowie, 2011; Watts et al., 2015). Indeed, dose-dependent effects are commonly seen with the most significant effects on organisms following ingestion at the highest exposure concentrations of microplastics (Au et al., 2015; Besseling et al., 2014; Ziajahromi et al., 2017). However, there is a recognised discrepancy between the concentrations within the environment and those used within laboratory exposures, therefore more data is needed from field studies on actual ingestion to inform future laboratory tests (Lenz et al., 2016).

In this study we investigated microplastic ingestion by roach *Rutilus rutilus* (Linnaeus 1758) in the River Thames; the second longest river in the UK. Studies have shown the Thames to be contaminated with both microplastic (Horton et al., 2017a) and macroplastic litter (Morritt et al., 2014), in addition to evidence of microplastic ingestion by marine fish living within the tidal Thames estuary (McGoran et al., 2016). However, no studies to date have yet investigated microplastic ingestion by freshwater fish within the non-tidal Thames. Roach are an indicator species (Havelková et al., 2008; Hellawell, 1972) and abundant throughout the UK in rivers, lakes and ponds. They are omnivorous, eating a wide variety of food from a range of sources including plant matter, benthic invertebrates and zooplankton (Elliott et al., 2015; Wintle, 2011). They are an important component of the aquatic food chain, supporting a number of predatory fish such as pike, and mammals including otters (Bean and Winfield, 1995; Hansson et al., 1998; Webb, 1975).

The aim of this study was to investigate whether wild-caught roach ingest microplastics within the non-tidal part of the River Thames, and how this relates to the location of the sampling site (which may influence exposure to microplastics) and physiological traits of the fish (determining likelihood and volume of ingestion). We hypothesised that exposure of fish to plastic particles will be determined by the distance from the source of the river. Further, we

hypothesised that the number of microplastic particles in the fish will reflect their feeding habits based on energy requirements and will therefore be influenced by size and gender.

2. Materials and methods

2.1. Sampling sites and fish collection

Rutilus rutilus (roach) were collected from the River Thames between July and October 2013 (following the spawning season) by Environment Agency staff in connection with regular fish population surveys, using electrofishing techniques. Fish were collected from seven sites along the main body of the River Thames, spanning a distance of 203 km, between 36 km and 239 km from the source of the river (Table 1 and Fig. 1). In this study, the source of the river relates to the source of the longest tributary (River Churn). Sampling was conducted between locks, except at the two sites furthest upstream, Cricklade and Castle Eaton, where no locks were present.

A minimum of six roach, which had a minimum fork length (size from the tip of the nose to the middle of the caudal fin rays) of 100 mm each, were collected per site. Caught fish were sacrificed with an overdose of an anaesthetic (0.4 ml/L 2-phenoxyethanol) and their weights and fork lengths recorded. They were then frozen on site by placing them in a liquid nitrogen-cooled container and stored at -80°C until further processing. In order to process the fish, individuals were allowed to warm up to a semi-frozen state and dissected, during which the entire digestive tracts were removed and the gender of the individuals was recorded. Digestive tracts were placed in 15ml centrifuge tubes and stored at -80°C until analysis.

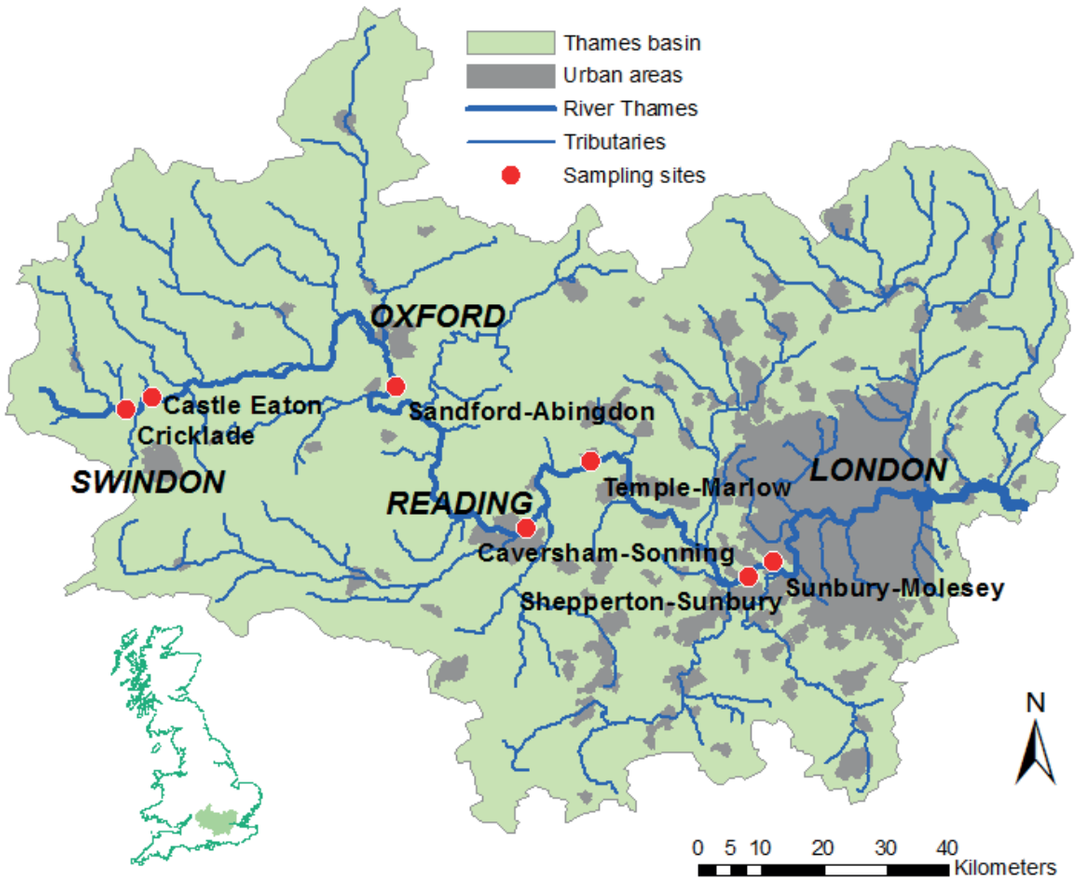

Fig. 1. Map showing locations of sampling sites on the River Thames. Sampling was undertaken in the stretch between locks (detailed by the site name) and therefore markers are placed approximately between the two locks, except for Cricklade and Castle Eaton where there are no locks and the markers denote the exact sampling location. See table 1 and table S2 for further details on sampling sites. The main urban centres are also marked.

2.2. Gut dissection and microplastic extraction

Fish tissues were removed from the freezer one fish at a time, and dissected as the tissue thawed. The entire digestive tract of fish (buccal cavity to anus) was cut open and all contents scraped out with a stainless steel spatula (hereafter referred to as 'gut content' for simplicity). Contents were spread on a Whatman GF/C glass microfibre filter paper (47 mm diameter, 1.2 µm mesh, GE Healthcare Life Sciences, UK) and immediately analysed. To eliminate possible contamination, all filters and tools were examined for particles before gut content analysis. Due to the small amount of gut content in each fish, it was possible to manually and thoroughly sort

through the content and therefore it was not necessary to digest the organic matter. Gut contents were searched under a binocular microscope (Wild Heerbrugg, Switzerland, with Photonic PL2000 cold light source) using a 6x magnification for a maximum of 15 minutes (this time frame based on the amount of time required to thoroughly search the largest volume of gut content), using a stainless steel spatula and forceps to move contents around as necessary. Forceps were used to remove microplastic particles to a clean filter paper. Gut contents were only exposed to the air during this 15 minute period. Following removal of contents, the inside of the gut itself was also examined to check that no particles had been missed. All particles were visibly incorporated into gut content when they were removed and were therefore believed not to be derived from airborne contamination. Between fish, all dissection tools were rinsed thoroughly with deionised water, wiped with ethanol and a lint-free tissue (Kimwipes, Kimtech Science, USA) and observed under the microscope before use to eliminate the possibility of cross-contamination.

Particles were removed as per Horton et al. (2017a) and were required to meet all of the following selection criteria, originally set out by Nor and Obbard (2014): 1) no visible cellular or organic structures, 2) unsegmented, 3) fibres of homogenous width (not tapered) and at least two of the additional criteria: 1) unnaturally coloured or with a brightly coloured coating (e.g. bright orange, blue etc.), 2) appear to be of homogenous texture/material, 3) abnormal (un-natural) shape e.g. perfectly spherical, 4) fibre that remained unbroken if tugged with tweezers, 4) reflective/glassy, 5) flexible without being brittle.

2.3. Polymer identification

Particles removed were quantified and half of the total number of particles (22/44) were analysed by Raman spectroscopy (HR800UV, Jobin Yvon Horiba, France, with integrated Olympus BX41 microscope) using Horiba LabSpec 6 software to give a qualitative representation of chemical composition of the microplastic particles as per Horton et al. (2017a). It was not possible to analyse all particles as some were lost following quantification due to their small size. Acquired spectra were compared to matched reference spectra using BioRad KnowItAll® Informatics System - Raman ID Expert (2015) software and the most appropriate match was selected based on matching peak wavenumber positions and a minimum 80% correlation between unknown and matched spectra (Horton et al., 2017a).

2.4. Data analysis

In this study, we first analysed the maximum likely ingestion for individual fish as a function of distance from the source of the river, as a measure of exposure. Subsequently, we analysed how physiological characteristics influence the actual ingestion compared to the maximum likely ingestion at the location. By dividing the analysis into these steps, we believe to stay close to the true mechanisms of microplastic ingestion and obtain a good understanding of the ingestion by individual fish. Determining an average ingestion at each site would not have provided these insights and would have given a population estimate only.

Firstly to test our hypothesis that the maximum likely ingestion of microplastics was related to the distance downstream from the river source, a quantile regression on the 95% quantile was carried out based on all the raw ingestion data for each fish compared to distance downstream (using the upstream point of the 0-7 km sampling stretch). A quantile regression draws a linear function of an independent variable (here, distance downstream from the river source) such that a given proportion of the observations (in this case, ingestion by individual fish) are below the line. In this instance the upper 95% (τ) was chosen as representing the maximum likely ingestion (Cade et al., 1999). For robustness, the quantile regression was resampled by bootstrapping (999 iterations), a recognised method for testing hypotheses regarding quantile regression models. The significance of the regression coefficients of the quantile regression indicate the significance of the relationship between the fitted line (maximum likely ingestion) and distance from the source. Bootstrapping makes no assumptions and so is particularly suitable when sample sizes are small and/or data are not normally distributed (Fox, 2015).

Second, we tested the hypothesis that the deviation in the actual uptake by an individual from the maximum likely uptake (at a given distance from the river source, based on the 95% quantile regression) is based on physiological traits. This gives a measure of whether fish with certain physiological characteristics are more or less likely to achieve the maximum ingestion at a given exposure. The physiological traits measured were fork length and gender (Fig. 2.). A two-way ANOVA was used to identify whether fork length, gender or their interaction were significantly influencing the deviation in uptake.

Given that sewage is often identified as a significant contributor of microplastics to the freshwater environment, we also carried out ANOVAs to determine whether maximum ingestion (based on resampled data) or average ingestion (based on raw data) were influenced by modelled sewage input. Statistical tests were all carried out using R statistical software.

3. Results and discussion

3.1 Microplastic ingestion

A total of 64 fish, 30 females and 32 males (the genders of two individuals were not identified), were caught at seven sites. The minimum number of fish was six (Sunbury-Molesey) and the maximum was 13 (Temple-Marlow) (Table 1). Caught fish measured between 100 mm and 184 mm and therefore likely represented both adults and juveniles (Table S1). From all sampled fish (64), 32.8% of roach (21) ingested a total of 44 microplastic particles giving a mean ingestion value of 0.69 particles ± 1.25 (SD) per fish (Table 1). Microplastics were observed in the guts of fish from six out of seven sites, whereas at one site (Sandford-Abingdon, 106 km from the source of the river) none of the sampled fish contained plastics.

The majority of particles were fibres (75%), followed by fragments (22.7%) and pellets (2.3%) (Fig. S1 shows a representation of types of particles found). Although particles were not individually measured, all were less than 5 mm and as such considered microplastics. A lower size limit was not set or measured, however all particles observed were of a size that could be removed by hand using forceps. There was limited ability to analyse these particles using Raman spectroscopy. Fifteen out of the 22 analysed particles were unidentifiable due to fluorescence or insufficient spectrum intensity, which are common problems when analysing environmental polymers using Raman spectroscopy (Horton et al., 2017a; Löder and Gerdts, 2015). Of the remaining seven particles, all were of anthropogenic origin and included polyethylene, polypropylene and polyester and a synthetic dye, neolan green (Fig. S2 and table S2). This data can therefore only be considered qualitative, showing the presence of commonly-used polymers. Although it cannot be completely ruled out that some of the unidentified particles may have been organic, or non-polymeric anthropogenic materials, those identified in the study met the criteria from previously successful criteria for microplastic identification (Horton et al., 2017a).

The results presented here complement the results of a recent study by McGoran et al. (2017) who found microplastics within the guts of two different species of marine fish within the estuarine River Thames, also consisting predominantly of fibres. Based on high microplastic inputs to rivers (Horton et al., 2017a; Lechner and Ramler, 2015; Murphy et al., 2016), it is therefore likely that ingestion by freshwater fish is occurring worldwide, especially those in close proximity to, or downstream of, urbanised areas (Dris et al., 2015b; Peters and Bratton, 2016; Sanchez et al., 2014; Silva-Cavalcanti et al., 2017).

Table 1. Site characteristics, sampling undertaken at each site and the numbers of microplastics found. ^Where fish were taken from a stretch between two locks, this distance relates to the upstream end of the stretch. *as calculated using the Low Flows 2000 (LF2000) WQX (Water Quality eXtension) model (Williams et al., 2009). Raw data for each site and individual fish are available in tables S1 and S2.

Site	Distance from source of river (km)^	Average percentage sewage within the watercourse*	Number of fish	Fork length range (mm)	Gender ratio (M:F)	Number of fish containing microplastics	Percentage of fish containing microplastics	Maximum number of ingested microplastic particles by any individual
Cricklade	36	13.3%	8	147-184	2:6	5	62.5	2
Castle Eaton	43	22.4%	11	106-181	1:10	1	9.1	1
Sandford-Abingdon	106-113	12.9%	7	144-164	4:2 (NA = 1)	0	0	0
Caversham-Sonning	162-166	12.8%	9	123-178	8:1	5	55. 6	3
Temple-Marlow	187-190	14.9%	13	100-153	9:4	3	23.1	3
Shepperton-Sunbury	234-239	15.9%	10	105-161	4:5 (NA = 1)	4	40	3
Sunbury-Molesey	239-243	16.2%	6	122-150	4:2	3	50	6

3.2. Microplastics in fish in relation to environmental factors

Analysis of the quantile regression (the fitted line for maximum ingestion) showed a significant relationship: the maximum ingestion of microplastics by individual roach increased with increasing distance from the source of the River Thames (p <0.005 significance of quantile regression, based on bootstrapped coefficients, Fig. 2). This likely reflects the fact that the number of inputs of microplastics to the river are increasing with distance from the river source, due to increasing urbanisation as the Thames flows towards London. However, given that the abundance of microplastics in surface waters of the River Thames has not yet been determined, it is not possible to directly relate the results of plastic ingestion here to the riverine concentrations of these plastics. A trend of increasing microplastic concentration with increasing distance from the source of the river has previously been observed in the River Danube (Lechner et al., 2014) and the river Rhine (Mani et al., 2015). When looking simply at the size of fish in relation to distance from the source, the size of fish did not significantly change with distance downriver (p=0.85, t test). This implies therefore that the difference in ingestion with distance was independent of any size-related differences. The 'maximum likely ingestion' approach allows for comparison of individual fish and therefore better insights into the factors that may influence ingestion.

The finding that the majority of plastic particles in this study were fibres, in addition to the identification of polyester (derived from synthetic textiles), suggests sewage to be a significant contributor to this contamination. Although sewage inputs can give an indication of population pressures, with greater concentrations of microplastics often found within the environment downstream of effluent outfalls (Estahbanati and Fahrenfeld, 2016; McCormick et al., 2014), these values cannot be used to infer the extent of microplastic pollution as they do not necessarily correlate with environmental concentrations due to inputs from other sources (Boucher and Friot, 2017; Horton et al., 2017a). Indeed this study found no relationship between sewage inputs and microplastic ingestion by fish (P > 0.05, ANOVAs for average and maximum ingestion). However, the range of sewage inputs between these sites is not large (average sewage content of the river flow between 12.8-22.4% depending on the sampling site), therefore if analysing sites with a greater range sewage inputs, this result may be different.

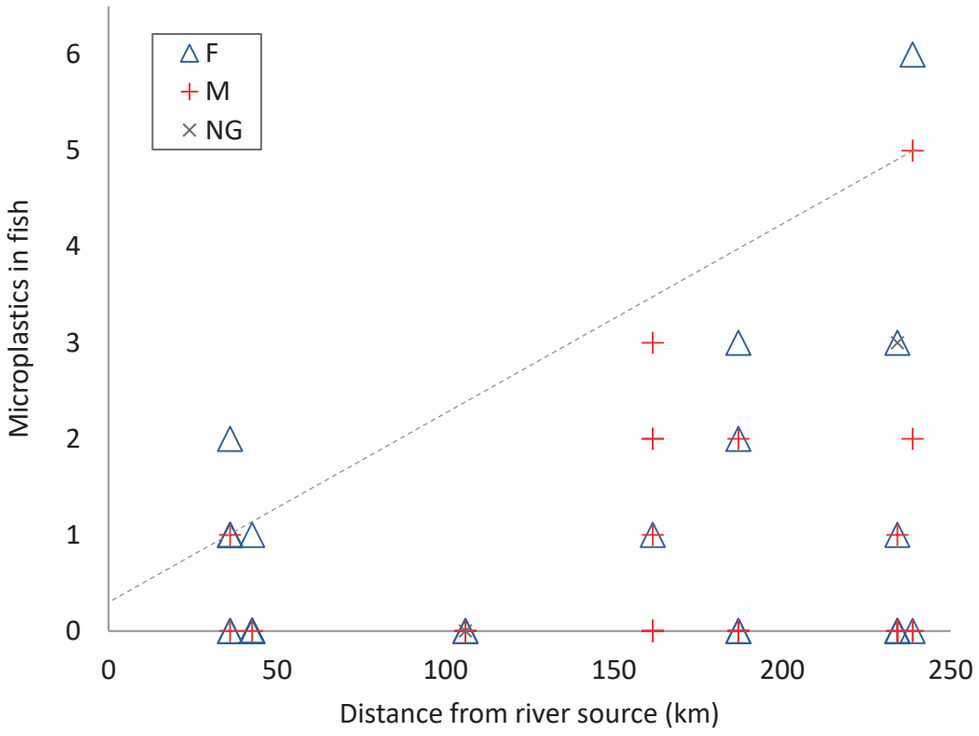

Fig. 2. Number of ingested microplastics in relation to distance from the source of the river. Each data point represents an individual fish, F = female, M = male and NG = no gender (gender not recorded). Some data points overlap therefore there are fewer visible points than fish. The predicted maximum number of microplastics that could be ingested by individual fish at a given distance downstream of the source is shown by the fitted line, which is based on 95% quantile regression. This line therefore represents maximum likely microplastic ingestion based on 95% of fish.

3.3. Microplastics in fish in relation to life history

Although exposure (based on distance from the source of the river) is an important factor determining whether, and to what extent, fish will have the potential to come into contact with and ingest microplastics, ingestion cannot be fully explained by location alone. This is evident in the variability between individuals at each site and the fact that at Sandford-Abingdon (106-113 km from the source of the river) no fish contained microplastics. At a given exposure, physiological characteristics will also influence the likelihood of roach ingesting microplastics, and the number they may consume.

When considering simply presence or absence of microplastics within the gut, there was no significant difference between males and females ($p > 0.05$, Wilcox test). However, the

deviation in actual uptake from the predicted maximum exposure was significantly dependent on gender ($p < 0.05$, ANOVA; Fig. 2). On average, male fish had three particles fewer than the maximum whereas female fish had 1.8 fewer particles on average. Female ingestion was therefore higher (based on less deviation from the maximum). The main effect of fork length was significant ($p < 0.05$, ANOVA): as fork length increases, deviation decreases, therefore larger fish are more likely to attain the maximum ingestion (fig. 3). Although females in this study were significantly bigger than males, with an average size of 148 mm (± 23.3 mm, SD) compared to a male average size of 136 mm (± 19.5 mm, SD) ($p < 0.05$, t-test), gender and fork length effects were not related ($p > 0.05$, interaction effect of the two-way ANOVA) indicating that both gender and fork length influenced ingestion independently.

The increase in ingestion of microplastics with increased fish size correlates with an increased volume of food required to meet the higher energy demands of larger fish (Hölker and Breckling, 2001) leading to a greater chance of direct or indirect ingestion of microplastics. This also suggests that smaller fish are far less likely to reach the maximum ingestion than larger fish at the same exposure. Other studies relating fish size to microplastic ingestion show varying results (Foekema et al., 2013; Peters and Bratton, 2016). This implies that life stage may also influence particle ingestion due to feeding habits.

It is not fully understood why gender would influence microplastic ingestion; this difference could not be explained simply by the larger female size. It could be that gender-specific differences due to the previous spawning event led to greater energy requirements by females (Foltz and Norden, 1977; Lambert and Dutil, 2000) and therefore a greater volume of food consumed (and thus incidental microplastic ingestion). Studies have shown that even water quality can lead to gender-specific differences in fish feeding (Horppila et al., 2011). This is a more complex matter than can be addressed within this study, so this should be another subject for future investigation.

In the current study, in addition to filamentous algae and plant matter, shells were also observed in the guts of some roach indicating the ingestion of molluscs such as bivalves and gastropods. Given the potential for filter-feeding molluscs to ingest microplastic fibres (Farrell and Nelson, 2013; Van Cauwenberghe and Janssen, 2014), there is the possibility that observed particles were ingested by means of food-chain transfer rather than direct ingestion. A recent study on a range of freshwater fish species found that gut microplastic burden varied significantly between species depending on feeding habits and trophic transfer, with apex predators containing the

highest numbers of microplastics, presumably due to ingestion via trophic transfer (Campbell et al., 2017). The presence of filamentous algae indicates that fibrous material will be ingested and that plastic fibres may therefore be unintentionally ingested along with visually similar filamentous algae.

As far as we are aware, this study is the first to relate ingestion of microplastics in a freshwater fish species to gender, fish size and distance from the source of the river. These results suggest that physiological characteristics may be equally as important as environmental characteristics for influencing ingestion of microplastics by fish. Ingestion (likelihood and volume) is therefore a result of a complex combination of factors.

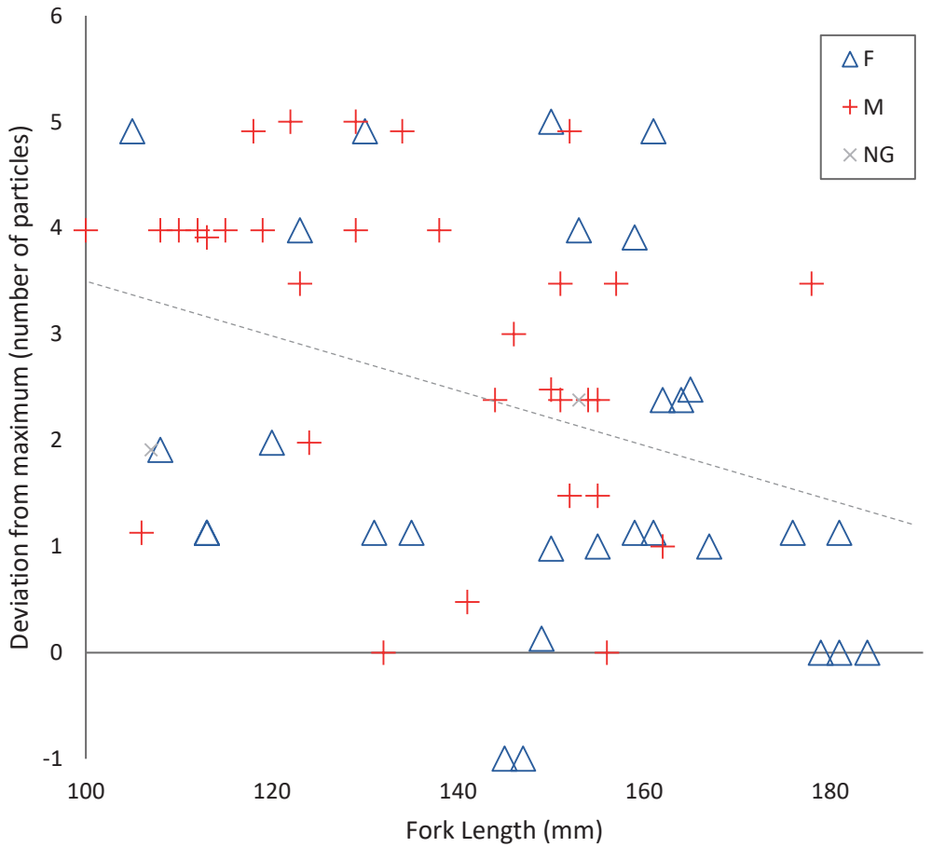

Fig. 3. Deviation from the predicted maximum ingestion (based on 95% quantile regression) compared to fish fork length. Each data point represents an individual fish, F = female, M = male and NG = no gender (gender not recorded). The fitted line was derived from the intercept and slope calculated by ANOVA.

3.4. Implications of microplastic ingestion

Recent studies highlight the potential for damaging effects of microplastics on fish health and fitness. These include changes to immunity (Greven et al., 2016), metabolism (Mattsson et al., 2014), neurotransmission (Oliveira et al., 2013), endocrine function and reproduction (Rochman et al., 2014), and behaviour (Espinosa et al., 2016; Mattsson et al., 2014). Lu et al. (2016) found particles less than 5 μm led to oxidative stress and inflammation within the liver. If plastic particles become nano-sized, they have the potential to cross the blood-brain barrier leading to brain damage and changes in behaviour (Mattsson et al., 2017). Individually or combined, these effects could have severe consequences on fish populations long-term, with significant implications for ecosystems.

5. Conclusions

Microplastics are being ingested by roach, and it is therefore likely that many other species of freshwater fish in the River Thames will also ingest microplastics. The number of microplastic particles in the guts of individuals is understood to be the result of two processes, exposure (which is likely to increase with distance downstream) and physiological characteristics of the fish. In this study, larger, female fish were more likely to reach a maximum ingestion at a given exposure, believed to be a result of increased energy requirements and thus feeding. This understanding gained from this study will help in interpreting findings from future studies data on the occurrence of microplastics in guts of fish worldwide, as well as identifying which fish are most likely to consume microplastics.

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CHAPTER 4
SUPPLEMENTARY INFORMATION

Table S1. Raw data showing site details and physiological characteristics of fish in relation to number and types of microplastics found within individuals. Where fish were sampled between two locks, the distance from the source of the river is given as the range between these two locations. For analysis and presentation of data, the upstream distance was used. *'NG' refers to 'no gender' *i.e.* the gender of the fish was not recorded.

Location	Distance from source (km)	Fishing date	Fork length (mm)	Gender	Fragments	Fibres	Pellets	Total particles in fish
Cricklade	36	11th Oct 2013	147	F	0	2	0	2
			155	F	0	0	0	0
			156	M	0	1	0	1
			162	M	0	0	0	0
			167	F	0	0	0	0
			179	F	1	0	0	1
			181	F	0	1	0	1
			184	F	0	1	0	1
Castle Eaton	43	11th Oct 2013	106	M	0	0	0	0
			113	F	0	0	0	0
			113	F	0	0	0	0
			113	F	0	0	0	0
			131	F	0	0	0	0
			135	F	0	0	0	0
			149	F	0	1	0	1
			159	F	0	0	0	0
			161	F	0	0	0	0
			176	F	0	0	0	0
			181	F	0	0	0	0
Sandford-Abingdon	106-113	2nd Jul 2013	144	M	0	0	0	0
			151	M	0	0	0	0
			153	NG*	0	0	0	0
			154	M	0	0	0	0
			155	M	0	0	0	0
			162	F	0	0	0	0
			164	F	0	0	0	0
Caversham-Sonning	162-166	11th Jul 2013	123	M	0	0	0	0
			141	M	0	3	0	3
			150	M	0	1	0	1
			151	M	0	0	0	0

Location	Distance from source (km)	Fishing date	Fork length (mm)	Gender	Fragments	Fibres	Pellets	Total particles in fish
			152	M	1	1	0	2
			155	M	0	2	0	2
			157	M	0	0	0	0
			165	F	0	0	1	1
			178	M	0	0	0	0
Temple-Marlow	187-190	2nd Sep 2013	100	M	0	0	0	0
			108	M	0	0	0	0
			110	M	0	0	0	0
			112	M	0	0	0	0
			115	M	0	0	0	0
			119	M	0	0	0	0
			120	F	0	2	0	2
			123	F	0	0	0	0
			124	M	0	2	0	2
			129	M	0	0	0	0
			138	M	0	0	0	0
			150	F	3	0	0	3
			153	F	0	0	0	0
Shepperton-Sunbury	234-239	9th Sep 2013	105	F	0	0	0	0
			107	NG*	1	2	0	3
			108	F	3	0	0	3
			113	M	0	1	0	1
			118	M	0	0	0	0
			130	F	0	0	0	0
			134	M	0	0	0	0
			152	M	0	0	0	0
			159	F	0	1	0	1
			161	F	0	0	0	0
Sunbury-Molesey	239-243	10th Sep 2013	122	M	0	0	0	0
			129	M	0	0	0	0
			132	M	1	4	0	5
			145	F	0	6	0	6
			146	M	0	2	0	2
			150	F	0	0	0	0

Table S2. Locations of relevant locks on the River Thames

Lock name	Latitude (degrees, minutes, seconds)	Longitude (degrees, minutes, seconds)
Cricklade	51° -21' -20.448"	-1° 9' - 9.988"
Castle Eaton	51° -20' -16.953"	-1° 13' -28.063"
Sandford	51° -18' 29.719"	-1° -14' 1.748"
Abingdon	51° -20' 13.856"	-1° -16' - 8.556"
Caversham	51° 28' -21.500"	-0° 2' 9.170"
Sonning	51° 28' 22.516"	-0° 5' - 4.951"
Temple	51° -27' 7.424"	-0° 12' 21.804"
Marlow	51° -26' 2.096"	-0° 14' - 7.670"
Shepperton	51° 23' - 4.762"	-0° -28' 27.451"
Sunbury	51° 24' 18.344"	-0° -24' -21.768"
Molesey	51° 24' 17.322"	-0° -21' 14.498"

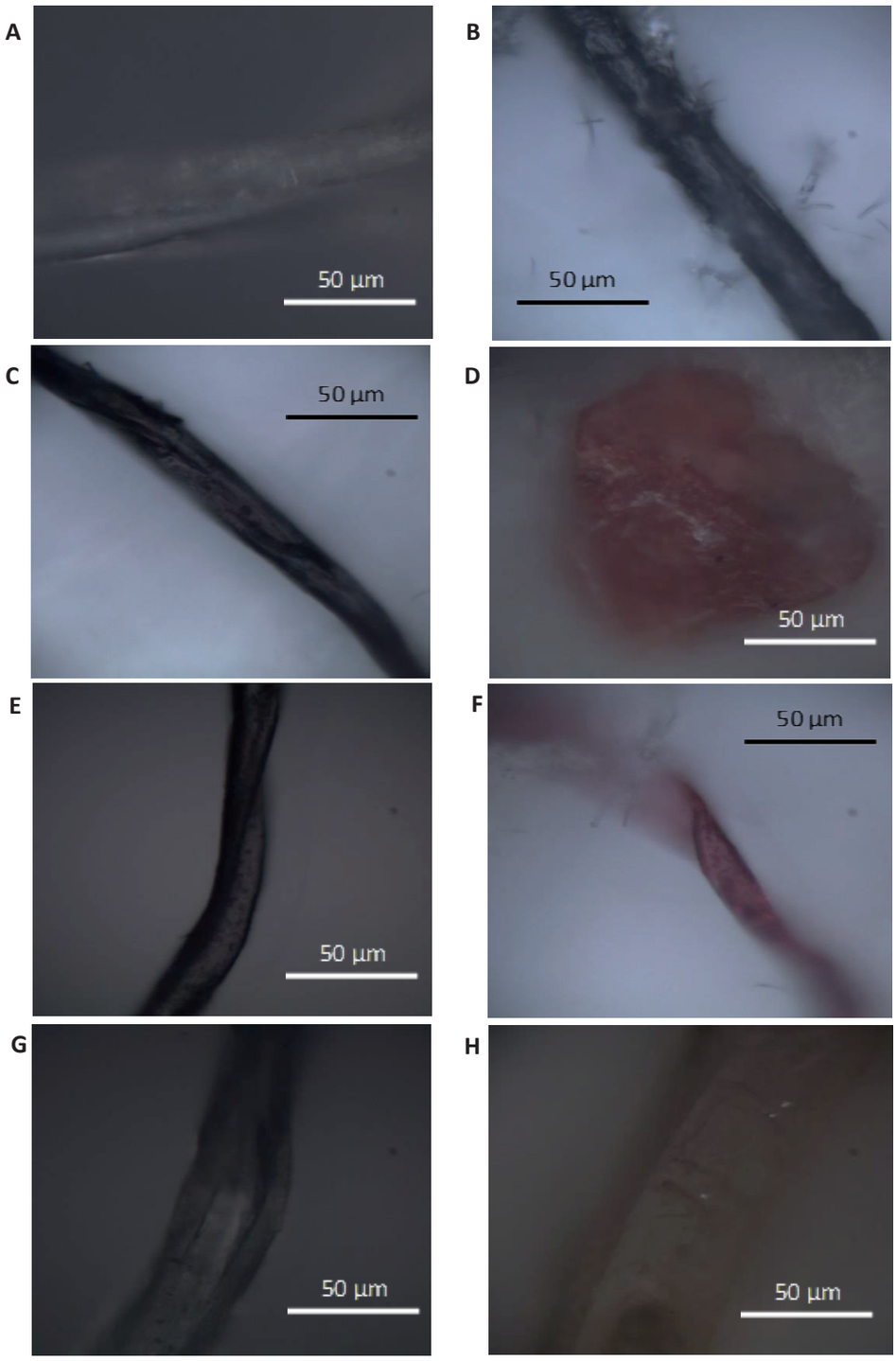
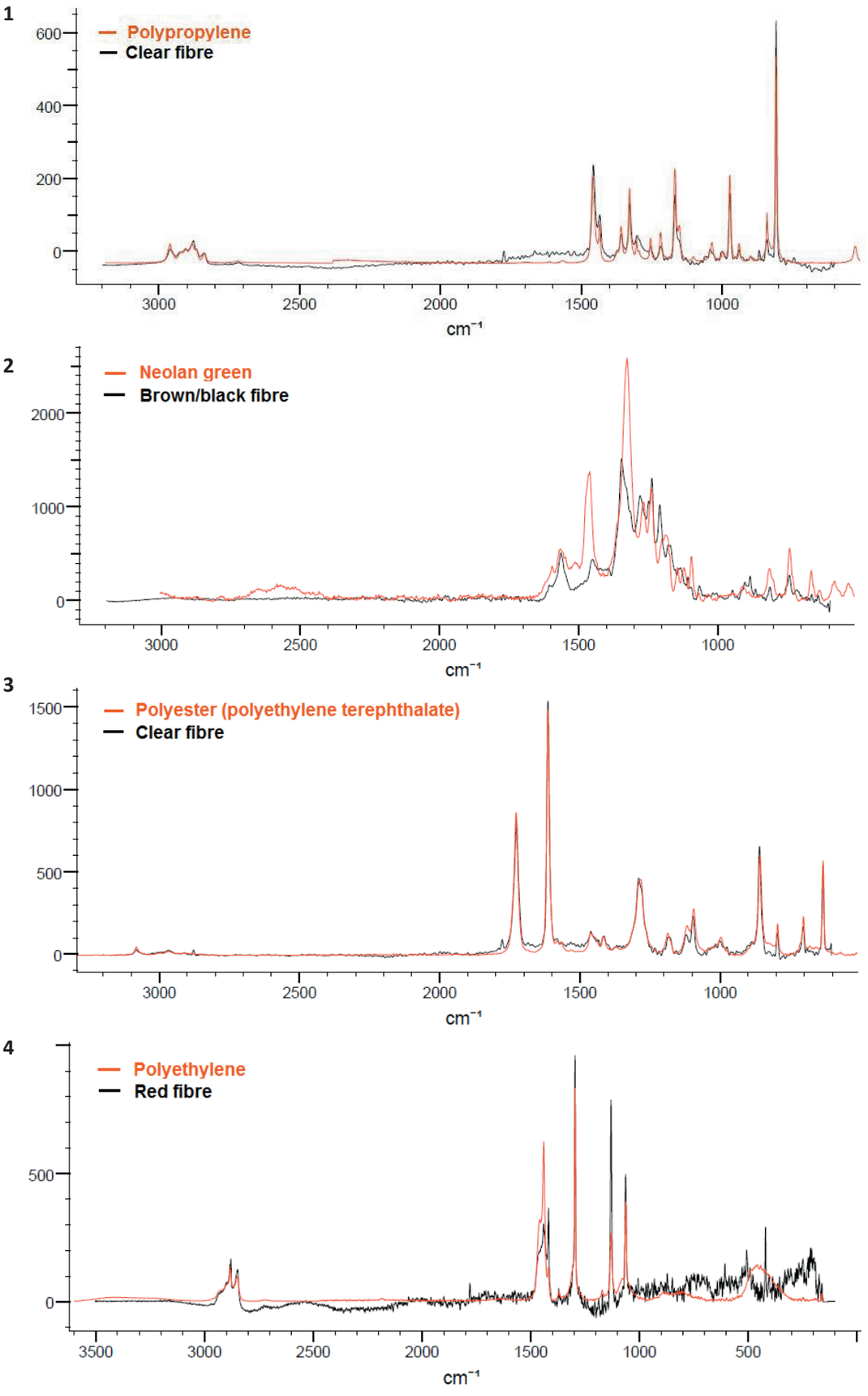


Fig S1. Images of a selection of representative particles at 50x magnification. Images A-C represent particles from fish collected between Caversham-Sonning. Images D-F represent particles from Temple-Marlow. Image G represents a particle from Sunbury-Molesey. Image H represents a particle from Castle Eaton. These images therefore show particles found within fish throughout the length of the non-tidal River Thames. Particles A, C G and H could be accurately identified and correspond to spectra 1, 2, 3 and 5 below (Fig. S2 and table S2).


1
Polypropylene
Clear fibre
600
400
200
0
3000
2500
2000
1500
1000
cm⁻¹
2
Neolan green
Brown/black fibre
2000
1000
0
3000
2500
2000
1500
1000
cm⁻¹
3
Polyester (polyethylene terephthalate)
Clear fibre
1500
1000
500
0
3000
2500
2000
1500
1000
cm⁻¹
4
Polyethylene
Red fibre
500
0
3500
3000
2500
2000
1500
1000
500
cm⁻¹

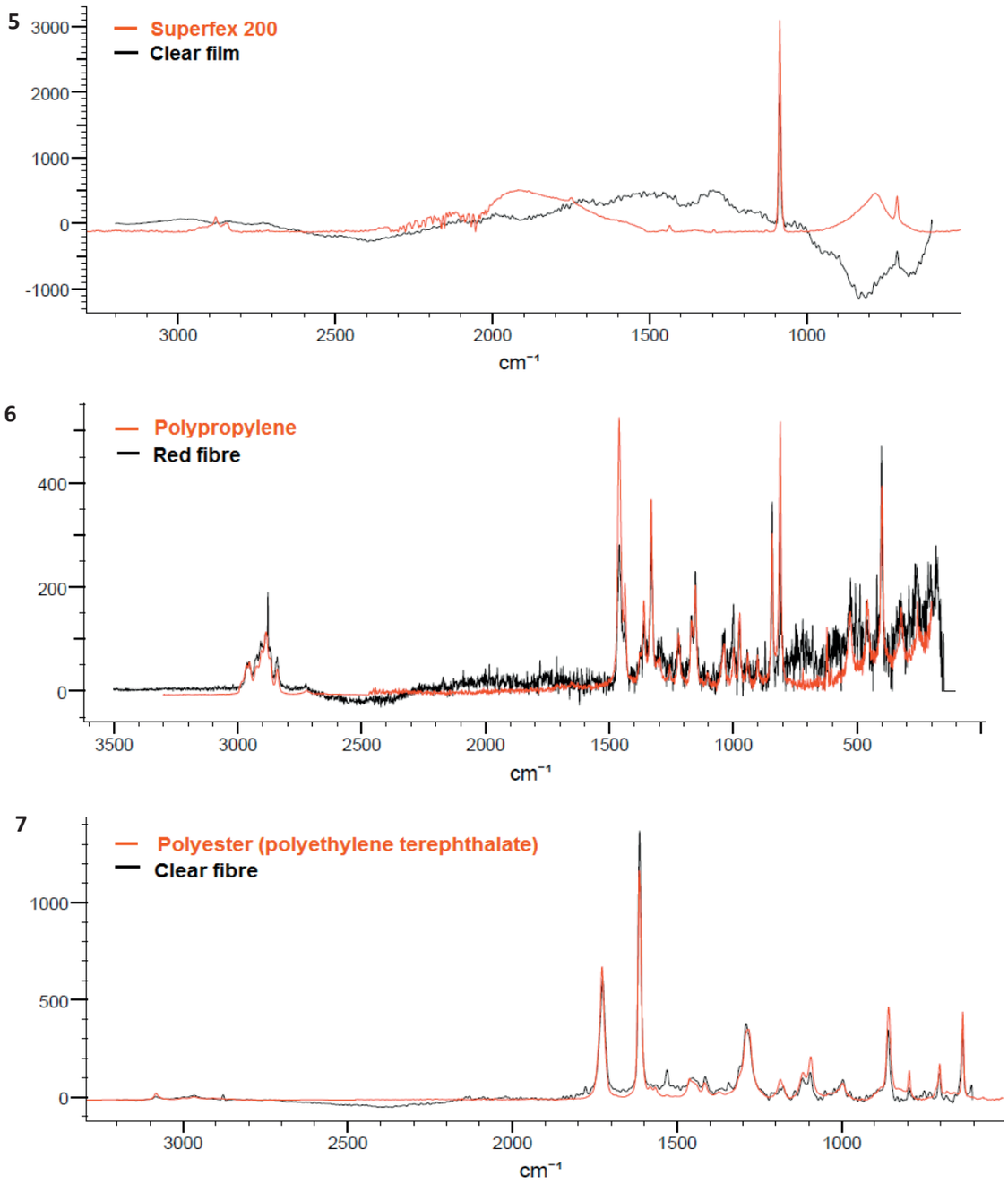


Fig S2. Spectra of identifiable particles and matched compounds using BioRad KnowItAll® Informatics System - Raman ID Expert (2015) software. Further particle information is available in table S3.

Table S3. Information associated with particles identifiable using Raman spectroscopy

Spectrum	Particle type	Particle colour	From (location)	% match	Substance name	Classification
1	Fibre	Clear	Caversham-Sonning	92	Polypropylene	Polymer
2	Fibre	Brown/black	Caversham-Sonning	87	Neolan Green	Dyestuff
3	Fibre	Clear	Sunbury-Molesey	97	Polyester	Polymer
4	Film	Clear	Shepperton-Sunbury	88	Superfex 200	Fluoropolymer
5	Fibre	Red	Shepperton-Sunbury	82	Polyethylene	Polymer
6	Fibre	Red	Castle Eaton	90	Polypropylene	Polymer
7	Fibre	Clear	Cricklade	95	Polyester	Polymer