Short communications

Presence of microplastic in the digestive tracts of European flounder, *Platichthys flesus*, and European smelt, *Osmerus eperlanus*, from the River Thames

A.R. McGoran [a,*], P.F. Clark [b], D. Morritt [a]

[a] *School of Biological Sciences, Royal Holloway University of London, Egham, Surrey TW20 0EX, UK*
[b] *Department of Life Sciences, The Natural History Museum, Cromwell Road, London SW7 5BD, UK*

* Corresponding author. School of Biological Sciences, Royal Holloway University of London, Egham, Surrey TW20 0EX, UK
E-mail address: alexandra.mcgoran.2012@live.rhul.ac.uk (A.R. McGoran)

Capsule. This study is the first to report of microplastic ingestion by estuarine organisms in the River Thames.

A B S T R A C T

Like many urban catchments, the River Thames in London is contaminated with plastics. This pollutant is recorded on the river banks, in the benthic environment and in the water column. The present study was conducted to assess the extent of microplastic ingestion in two River Thames fish species, the European flounder (*Platichthys flesus*) and European smelt (*Osmerus eperlanus*). Samples were collected from two sites in Kent, England; Erith and Isle of Grain/Sheppey, near Sheerness, with the latter being more estuarine. The results revealed that up to 75% of sampled European flounder had plastic fibres in the gut compared with only 20% of smelt. This difference may be related to their diverse feeding behaviours: European flounder are benthic feeders whilst European smelt are pelagic predators. The fibres were predominantly red or black polyamides and other fibres included acrylic, nylon, polyethylene and polyethylene terephthalate and there was no difference in occurrence between the sites sampled.

Keywords:

Platichthys flesus
Osmerus eperlanus
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Microplastics
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1. Introduction

In the 21st century most consumer products contain and/or are packaged in plastics and the production of plastic has increased by over two orders of magnitude, since the 1950s, to 280 million tonnes in 2011 (Moore, 2008; Wright et al., 2013b). Jambeck et al. (2015) estimated that 2.5 billion tonnes of marine waste was produced in 2010 of which 99.5 million tonnes was plastic with up to ca. 4200kg of plastic waste per day entering the ocean through water ways. Furthermore, these authors predicted that the input of plastics worldwide would increase tenfold by 2025.

Additionally, plastics become fragmented through exposure to UV radiation, oxidation, hydrolysis and contact with the seabed, becoming progressively smaller (Moore, 2008) until eventually being classified as microplastics (<5mm) (Lusher et al., 2013; Wright et al., 2013b; Castañeda et al., 2014). Microplastics comprise two types: those designed primarily to be small in size; and those of secondary origin, degraded from larger sources. Both are widely bioavailable (Wright et al., 2013b; Castañeda et al., 2014). Smaller plastic fragments are more available to lower trophic organisms and can also be eaten by high trophic level organisms as a result of normal feeding rather than selectively. Density and colour can also play a role in the bioavailability of microplastics (Wright et al., 2013b).

Plastic marine debris has potential impacts for both marine wildlife and humans. Moore (2008) reported that the presence of plastics in the ocean has resulted in at least 8 possible negative implications, including entanglement, ingestion and pollutant storage. Humans can be directly impacted by plastic pollution by washed up litter on beaches raising health and safety concerns as well as reducing aesthetics as well as indirectly through impacts to the ecosystem. Moore (2008) also reported that 267 marine species have encountered plastic pollution. More recently, a review by Gall and Thompson (2015) found that, across 340 separate publications, a total of 693 species (invertebrates and vertebrates) have encountered marine debris: 77% of these studies recorded the presence of plastic debris and 92% of the individual organisms encountering debris encountered plastic pieces. There is, however, variability in the reports of plastic abundance in the gut of fish species with Di Beneditto and Awabdi (2014) finding that 1% of *Trichiurus lepturus* (Linnaeus, 1758) ingested marine debris, most of which was plastic, Boerger et al. (2010) reporting 35% in the North Pacific Gyre and Lusher et al., 2013 quoted 37%. Sigler (2014) concluded that this disparity was due to variability in location, fish species and pollution levels. Foekema et al. (2013) found 3% of fish in the North Sea ingested plastic. Whilst in the Mediterranean Sea, 13%, 32% and 13% of swordfish, bluefin tuna and albacore, respectively, had ingested plastic fragments. On average 18% of these predatory fish had ingested 29 plastic pieces (Romeo et al., 2015). These authors proposed that ingestion was more probable when feeding on schools of small prey fish species because, with many prey items to focus on, predators are less selective towards unwanted fragments. It is also possible that plastic could be ingested through prey items and has passed up trophic levels.

Plastic pollution has been reported in the UK, with plastics being recovered from the Solent estuarine complex, Hampshire (Gallagher et al., 2016) and the shoreline of the Tamar Estuary, Plymouth (Browne et al., 2010). In the Clyde Sea, Scotland 83% of the decapod crustacean *Nephrops norvegicus* (Linnaeus, 1758) had ingested plastic, mainly tangled nylon fibres (Murray and Cowie, 2011). Also in UK waters, Lusher et al. (2013) discovered that plastics, mostly rayon, were ingested by fish; 37% of fish in the English Channel had ingested plastic fragments.

The River Thames, in the south of England, has a catchment area of 13,000km^2 that includes 13 million residents. The river flows through 16 towns and cities including the capital, London (British Geological Survey; Dunk and Arikans; Thames River Trust). The present study looks at two sites in the River Thames to compare samples from upstream in the estuary (Erith) with samples downstream near the sea (Isle of Grain/Sheppey). At upper estuarine sites such as Erith (with a river width of 700–785m), plastic pollution might be more concentrated and organisms are consequently exposed to a greater concentration of plastic than larger water bodies, for instance Isle of Sheppey (9.8km width) or the English Channel (195km width) (Map Magic, 2015). The strong currents at Erith could also result in higher fragmentation of plastics and thus a higher concentration of microplastics.

Although Morritt et al. (2014) quoted that 239 tons of rubbish were removed from the River Thames in 2012 by the Port of London Authority and provided evidence for large amounts of plastics moving sub-surface in the river, little is known about the effects this pollutant has on the local biota. Thus the current study was conducted to assess the extent of ingestion in two River Thames fish species namely European smelt, *Osmerus eperlanus* (Linnaeus, 1758), and European flounder, *Platichthys flesus* (Linnaeus, 1758). These species differ markedly in body form and feeding habit. The European smelt is a pelagic, estuarine fish and primarily feed on crustaceans and fish. This is a relatively rare species in the UK and it is on the decline nationally. The population in the River Thames is of national importance and numbers have increased since the River has become cleaner (Greater London Authority, 2007; Maitland, 2012). The European flounder is a demersal species, present in the upper estuary, feeding on small fish, molluscs, worms and crustaceans (Seafish, 2014). European flounder are highly abundant in the River Thames and, in such urban systems, are often found near inputs of pollutants (Jarrah, 1992).

The research presented here aims to test three hypotheses: firstly, fish in narrower, more riverine parts of the river, such as Erith, will encounter more plastics than further out into the estuary, at Isle of Grain/Sheppey; secondly, pelagic and demersal fish will encounter plastics at different rates or of different types; and thirdly, seasonality may impact plastic ingestion due to differences in plastic inputs or animal behaviour. Seasonality may also affect the prey items consumed by fish and, as such, dietary analysis was conducted. This could reveal links between specific prey items and plastic ingestion. Equally other factors may affect the ingestion of plastics, and some of these are considered in this study. The colour of potential plastics consumed by fish relates to the likelihood of that fibre being synthetic and is thus useful when quantifying ingestion. It was assumed that the narrower reaches of the river would act to concentrate the microplastics in the river and increase the encounter rate between fish and microplastics. It is also believed that plastics may sink and accumulate in the sediment due to factors such as biofilms (Barnes et al., 2009) or that turbulence and water flow keep particles suspended in the water column, resulting in differences in ingestion between pelagic and benthic fish (Browne et al., 2010). The present study also makes a direct comparison with Lusher et al. (2013), another recent UK based study of both pelagic and demersal fish.

2. Materials and Methods

A total of 66 European flounder and 10 European smelt were collected from two sites in the Thames Estuary: the more riverine Erith, south-east London and the estuarine Isle of Grain/Sheppey, near Sheerness in July and November 2014 (Fig. 1), using fyke nets. Two trawls were collected on an opportunistic basis from Erith (ca. 51.480° N 0.1778° E) to check for any seasonal differences. The first trawl contained 40 European flounder and the second trawl contained 12 European flounder and 10 European smelt. The Isle of Grain/Sheppey trawl (51°28.416' N 000°46.168' E), contained 14 European flounder. All specimens were mature adults and iced immediately after capture. Samples were then collected and transported to the laboratory where they were frozen prior to dissection and were identified based on site (Erith July, riverine site 1; Erith November, riverine site 2; Isle of Sheppey, estuarine site; Erith European smelt) species and order of dissection. Stomach and gut were separated to assess whether plastics were successfully passed through the alimentary canal or whether the pyloric sphincter prevented the movement of ingested plastic. The methodology was based on that of Lusher et al. (2013) and followed this as closely as possible including the measurements taken for each specimen, e.g. length, width, and weight in order to make direct comparisons with their data.

To prevent contamination a clean laboratory coat and non-sterile, single-use gloves were worn. Dissecting instruments were examined under a microscope prior to dissection and the investigation of the digestive tract. The organs were placed in individual sealable bags and stored frozen.

Stomach and gut contents were examined separately. The gut section was cut longitudinally and opened, and examined under a dissecting microscope with a downward projecting light. The digestive tract was opened in small sections to reduce the chance of airborne contamination. Using pins, a thorough search was then undertaken of the opened gut section. Plastics were removed and stored on filter paper in Petri dishes that were sealed with Parafilm after dissection. The food was then carefully transferred and stored in ethanol for dietary analysis. Diet samples were searched under dissection microscope and sorted into separate Petri dishes for each dietary element. During this process, any plastics found were removed and stored separately on filter paper in a labelled Petri dish. Once analysed, samples were placed in a 60°C oven for three days and then weighed. The stored plastics were then grouped based on colour and shape. Shape was divided into three broad categories: fibres (thread-like plastic), sheets (flat plastic films) and fragments (irregular shaped plastics that were not fibres or sheets). Fourier Transform Infrared Spectroscopy (FT-IR) was conducted, using a Thermo Scientific Nicolet iS5 FT-IR spectrometer with a diamond attenuated total reflection cell. Background spectra were generated every hour. Plastics were pooled based on colour and consecutive samples were taken until there was enough material to cover a reasonable area of the reflection cell when compressed by a flat head. Plastics were not separated between the stomach and gut at this point as plastics could clearly pass through the digestive tract. Plastic fragments were too small to allow analysis using the available FT-IR equipment, so were excluded from analysis. Plastic abundance was calculated as percentage occurrence in fish and all statistical analyses were performed using IBM SPSS 21 for Windows.

3. Results and Discussion

3.1 *Plastic ingestion*

July-collected European flounder from Erith (riverine site 1) were found to have ingested plastic fragments and fibres in 90% of fish (n = 40); plastics were present in the gut and stomach. July-collected European flounder from the Isle of Sheppey (estuarine site) had ingested plastic in 71% of fish (n = 14), 85 plastic items were recovered. The November-collected European flounder from Erith (riverine site 2) had ingested 38 fragments across 83% of specimens (n = 12). Of the European smelt sampled from Erith, 20% had ingested plastic (n = 10). Figure 2a shows the distribution of plastic colours and types between fish (Fig. 2b) shows the proportion of particles removed from samples that were fibres and the proportion of fibres that were each colour.

The abundance of plastics in riverine site 1 and riverine site 2 was compared in order to test for a temporal change. Neither the plastic abundance in riverine site 1 nor riverine site 2 was normally distributed (Shapiro-Wilk: W = 0.631, df = 40, p <0.01 and W = 0.765, df =

12, $p < 0.01$, respectively). There was no significant difference in the quantity of ingested plastic in European flounder between riverine site 1 and riverine site 2 (Mann-Whitney U: U = 228, z = -0.267, $p > 0.05$).

Riverine site 1 and estuarine site were compared to test for a difference between plastics at sites of varying distance from the sea; although plastic abundance in fish from estuarine site were normally distributed (Shapiro-Wilk: W = 0.880, df = 14, $p > 0.05$), riverine site 1 was not, as previously stated. No significant difference was found between the samples (Mann-Whitney U: U = 203.5, z = -1.538, $p > 0.05$).

The occurrence of plastic was compared in European smelt and riverine site 2. A significant difference was recorded between the two species at Erith (Mann-Whitney U: U = 21, z = -2.769, $p < 0.01$) with European flounder having a much higher presence of plastic in the digestive tract.

3.1.1 *Plastic analysis*

With reference to fibres, 73% of European flounder from riverine site 1, 71% of European flounder at estuarine site, 75% of European at riverine site 2 and 20% of European smelt had ingested plastics. An average of 0.43 ± 0.75 SD fibres were consumed per fish from riverine site 1. The averages at the estuarine site, riverine site 2 and in European smelt were as follows: 0.85 ± 1.17 SD, 0.33 ± 0.49 SD and 0.2 ± 0.42 SD. FT-IR identified black fibres as polyamides (Fig. 3) or PET, blue fibres as polyester-nylon mixes; clear fibres as polyester, brown fibres as organic (Fig. 4), and red fibres as acrylic, nylon, polyethylene or polyethylene terephthalate (PET) (Fig. 5).

Over 70% of River Thames European flounder examined during the present study had ingested plastic fibres, which is high compared to previously published estimates of plastic ingestion by fish (Boerger et al., 2010; Foekema et al., 2013; Lusher et al., 2013; Di Beneditto and Awabdi, 2014; Sigler, 2014; Romeo et al., 2015). These microplastics could have been ingested during normal feeding behaviour, with the sediment ingested when feeding on benthic invertebrates. This is as opposed to mistaken identity for prey. In comparison, for example, Lusher et al. (2013) reported that 37% of fish in the English Channel had ingested plastics.

Plastic abundance in riverine site 1 had no significant association to length ($R^2 = 0.012$, F = 0.448, df = 1, 38, $p > 0.05$). Riverine site 2 also showed no association ($R^2 = 0.098$, F = 1.085, df = 1, 10, $p > 0.05$). However, there was a highly significant positive association (r = 0.802) in estuarine site ($R^2 = 0.643$, F = 21.607, df = 1, 12, $p < 0.01$), producing the regression equation: plastic frequency = -13.147 + 0.071(length). There was no association between plastic frequency and size in European smelt ($R^2 = 0.084$, $F = 0.730$, df = 1, 8, $p > 0.05$).

3.1.2 *Pelagic vs benthic feeding*

Wright et al. (2013b) reported that plastic fibres were the most abundant form of plastic in the marine realm, and the current estuarine study supports this conclusion. Fibres of all colours were found in the gut of European flounder, but black fibres were predominant. The potential sources of these fibres are discussed later.

European smelt from Erith had ingested far fewer plastics than European flounder from the same site (sampled at the same time of year), suggesting that European flounder are exposed to more microplastics. European flounder are benthic feeders, ingesting large quantities of sediment and, this is the mostly likely route of plastic ingestion. Sediment was noted in the alimentary canals of most specimens, but no measurements of the quantity of sediment ingested were recorded in this study. This would account for the large array of coloured fibres in their diet. As a pelagic predator, European smelt, which only ingested black fibres, is a naturally more selective feeder, not feeding on silt. Benthic environments retain microplastics that sink to the ocean floor or river bed, with fragments being caught on or between grains of sediment. Indeed, Katsnelson (2015) reported that microplastics accumulate on the deep sea floor at densities some four times higher than they do at the surface. Plastics could sink due to numerous factors, including plastic density, biofouling, adhesion of minerals to the plastic surface and through incorporation of plastic into faecal pellets (for example, by zooplankton) (Corcoran, 2015). Interestingly the one prey species that occurred in the guts of both fish species in this study was the brown shrimp *C. crangon* and recent work has demonstrated the occurrence of microplastics in the gut of 63% of samples of this species from the Southern North Sea and English Channel (Devriese et al., 2015). Plastics, however, could be ingested through prey items and trophic cascade, bioaccumulating higher in the food chain.

3.1.3 *River vs estuary vs sea*

No difference was found between the River Thames sites, with respects to European flounder, whilst a far greater percentage of fish were recorded to have ingested plastic in the River Thames than the English Channel. Lusher et al. (2013) recorded plastics in 37% (185) of pelagic and demersal fish, whereas this study found that 73% (51) of fish studied in the

River Thames had ingested plastic fibres. Like Lusher et al. (2013), this study found that both fish in the water column and in the benthos consumed plastics but, in contrast to Lusher et al. (2013), this present study found that the benthic species, European flounder, ingested the most plastics. This could be due to the limited number of species sampled and the bias in sample sizes favouring European flounder.

3.2 *Diet*

The amphipod, *Corophium volutator* (Pallas, 1766), and polychaetes were recorded most frequently as prey items in Erith European flounder (Fig. 6a, b) whereas juvenile *Ensis* sp. were recorded most frequently in Isle of Sheppey European flounder (Fig. 6c). Only *Crangon crangon* (Linnaeus, 1758) was present in diets from both sites. The European smelt population mainly consumed fish (Fig. 6d). No relationship was found between plastic abundance and dry mass of gut contents at any site (riverine site 1: $R^2 = 0.024$, F = 0.175, df = 1.7, p >0.05; riverine site: $R^2 = 0.073$, F = 1.733, df = 1.22, p >0.05; estuarine site: $R^2 = 0.208$, F = 1.843, df = 1.7, p >0.05; European smelt: $R^2 = 0.222$, F = 1.423, df = 1.5, p >0.05).

3.3 *Source of plastic*

Browne et al. (2011) also reported large numbers of nylons and polyesters in the marine environment, particularly in the vicinity of highly populated areas. By testing washing machine outputs, it was determined that many microplastic fibres originate from domestic washing. Clothing such as synthetic fleece garments produce 1,900 fibres every wash per garment. It is likely that many of the fibres present in this study also originate from washing machines although not exclusively from this source. The fibres are so small that they are not removed by filters and sewage systems.

3.4 *Impacts*

The large proportion of fish that ingested plastic fibres in this study raises questions about the impacts these could have on the health of the fish. The impacts of ingested plastics on macrofauna is not fully understood (Phillips and Bonner, 2015). Without knowing the residence time of plastics in the gut it is difficult to accurately infer the consequences of their presence. In this study, tangled fibres were found in the gut and it is this ability to clump that is likely to result in false satiation, reduced fitness, stomach abrasions and even death (Wright et al., 2013b). None of the fish sampled from the River Thames were observed to have abrasions or blockages in the digestive tract. Rochman et al. (2013) also found low numbers of fibres in the stomach, suggesting that they do not accumulate, or block the digestive tract.

Furthermore, there is a high chance that plastics absorb and then leach chemical pollutants from the sea to the gut. Persistent organic pollutants in water are often hydrophobic being attracted to plastic fragments and easily absorbed (Wright et al., 2013b). Phillips and Bonner (2015) found that both freshwater and marine fish species ingested plastic. Toxic monomers were present in all plastics that they recovered. When such plastic fragments accumulate in the digestive tract, the absorbed toxins may then leach and could have detrimental effects. The monomers in plastics such as polyester can act as endocrine disruptors and carcinogens and also cause irritation of the respiratory system (Rochman et al., 2013; Wright et al., 2013b; Phillips & Bonner, 2015). Rochman et al. (2013) reported that persistent bioaccumulative and toxic substances absorbed by plastics, which were fed to fish, resulted in liver stress and severe depletion of glycogen in 74% of fish. Wright et al. (2013a) exposed *Arenicola marina* to sediment containing polyvinyl chloride. The exposed organisms had a reduction of up to half their energy reserves, exhibited signs of inflammation and had impaired fitness. The chemicals leached from plastics could potentially bioaccumulate (Rochman et al., 2013; Wright et al, 2013b), although Phillips and Bonner (2015) recommend further research into the topic. Contrary to these findings it has been suggested that the concentration of organic pollutants ingested is too low to have any considerable effect and that there is a cleaning mechanism in place and that longer living organisms, such as fish, may suffer more from the bioaccumulation of chemical pollutants (Koelmans et al., 2014). Further research is needed into the effects of plastic to resolve the discrepancies between studies.

3.5 *Limitations*

This study had a limited sample size and did not collect European flounder and European smelt all year round. Fragments too small for analyses were not considered and thus the study may underestimate the abundance of ingested plastic. Microscopy or differential scanning calorimetry as used by Castañeda et al. (2014), could remove speculation over the identity of these smaller fragments. FT-IR analysis was conducted on grouped samples, perhaps enabling organic fibres to be misidentified as synthetic. In future, fibres should be analysed individually. This study fails, as have previous studies (Lusher et al., 2013; Castañeda et al., 2014), to show the impact of its consumption and ingestion. No estimate of residence time can be made from the collected sample and thus any observed abnormalities may be due to alternative factors.

4. Conclusion

This study reports the first evidence of plastic in the guts of River Thames fish species and further informs the debate on the input of plastics to the marine environment via riverine sources. Further research should explore other trophic levels, trophic transfer, impacted habitats and the potential negative effects on the organisms that ingest microplastics.

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Captions

Fig. 1. The sampling sites used during the present study; E = Erith, S = Isle of Grain/Sheppey.

Fig. 2. (a) Percentage of fish; The first bar (green) represents the percentage of the sampled fish that had one or more plastic fibres in each sample. The subsequent bars (red, black, blue, clear) show the percentage of the sampled digestive tracts at each site which contained the different colour fibres. Some fish ingested several different coloured fibres and thus the accumulation of bars does not equate to 100%. (b) Percentage of fibres; the percentage of fibres that were red, black, blue and clear in each sample.

Fig. 3. Absorbance spectrum from collated sample of black fibres generated through FT-IR. All black samples, bar two, produced identical spectra. The carbonyl-region peaks identify the fibres as a polyamide (secondary amide).

Fig. 4. FT-IR absorbance spectra for brown fibres compared to cellulose, which has a similar structure. Brown fibres are thus disregarded as organic because they are likely to be plant debris.

Fig. 5. FT-IR absorption spectra for a collective of red fibres. (a) has sharp C-H bond peaks that stretch and vibrate resembling polyethylene (polythene), (b) is poly(acrylonitrile), with a clear carbon-nitrogen triple bond just above 2200cm^{-1}, a peak which are present in library spectra, (c) is polyester with its ester linkage expressed through a carbonyl peak above 1700cm^{-1}.

Fig. 6. European flounder: (a) diet from Erith caught in July, (b) Diet from Erith caught in November, (c) diet from Isle of Grain/Sheppey. European smelt (d) diet from Erith.