Science of the Total Environment 915 (2024) 170170

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Microplastics in commercial marine fish species in the UK – A case study in the River Thames and the River Stour (East Anglia) estuaries

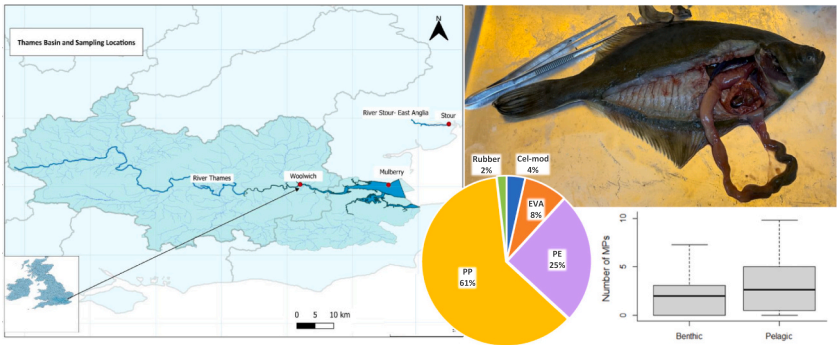
Alice A. Horton [a,*,1], K.D. Isuri Weerasinghe [a,b,c,1], Daniel J. Mayor [a,d], Richard Lampitt [a]

[a] National Oceanography Centre, European Way, Southampton SO14 3ZH, UK
[b] University of Southampton, University Road, Southampton SO17 1BJ, UK
[c] University of Galway, University Road, Galway H91 TK33, Ireland
[d] Biosciences, Geoffrey Pope Building, University of Exeter, Stocker Road, Exeter EX4 4QD, UK

HIGHLIGHTS

- 41.5 % of three commercial fish species contained at least one microplastic particle.
- Mean values were 1.98 MPs/European flounder, 2.46 MPs/whiting and 1.47 MPs/herring.
- River, habitat and species did not contribute to differences in microplastic.
- Fish size significantly influenced MP concentration (bigger fish = more MPs).
- Polymers found correspond with highest commercial production (mainly PP and PE).

GRAPHICAL ABSTRACT



ARTICLE INFO

Editor: Yolanda Picó

Keywords:
Rivers
Ingestion
Organisms
Seafood
Exposure
Contamination

ABSTRACT

The aim of this study was to assess the abundance of microplastics in the gastro-intestinal tracts of three commercially important fish species in the UK, to determine whether catch location, feeding habits and fish size influence the amount of microplastics within fish. Fish were collected from two rivers in the UK: the River Thames and the River Stour (East Anglia). Fish were collected from two sites in the River Thames and one site in the River Stour. Species selected were European flounder (*Platichthys flesus*), whiting (*Merlangius merlangus*), and Atlantic herring (*Clupea harengus*), and were chosen to represent benthic and pelagic feeding habits. Across all locations, 41.5 % of fish had ingested at least one microplastic particle (37.5 % of European flounder, 52.2 % of whiting, and 28.6 % of Atlantic herring). The average number by species was 1.98 (±3.50) microplastics/fish in European flounder, 2.46 (±3.10) microplastics/fish in whiting and 1.47 (±3.17) microplastics/fish in herring. There were no significant differences in the number or mass of microplastics in fish based on river, site, species or habitat. However, the number and mass of microplastics within benthic fish (European flounder) in the River Stour were significantly higher than in benthic fish from the River Thames. By number of microplastics, larger and heavier fish were more highly contaminated. This study enhances our understanding of microplastics in commercially important fish but highlights that fish contamination is not easily predicted by feeding habits or

* Corresponding author.
E-mail address: alihort@noc.ac.uk (A.A. Horton).
[1] These authors contributed equally to this manuscript.

https://doi.org/10.1016/j.scitotenv.2024.170170
Received 27 September 2023; Received in revised form 12 January 2024; Accepted 12 January 2024
Available online 15 January 2024

catch location alone. Exposure and uptake is likely to vary with changing environmental conditions. Fish size tends to be a good predictor of contamination, with larger fish generally containing more microplastics. This is the first study to directly compare concentrations of microplastics in fish from different UK rivers and the first evidence of microplastics in the River Stour.

1. Introduction

Land-based sources are understood to release four times more plastic into the ocean than marine-based sources (Li et al., 2016; Sherrington, 2016). Rivers play a critical role in carrying land-derived plastic waste toward the ocean, with an estimated 2.8–18.6 % of coastal plastic emissions into the ocean occurring via river transport (Lebreton et al., 2017). While river-dominated coasts comprise 0.87 % of global coastlines, they receive a disproportionate 52 % of plastic pollution delivered by rivers (Harris et al., 2021). The amount and timing of waste the rivers carry can depend on population density, stage of urbanisation, industrialisation within catchment areas, rainfall rates, and artificial barriers (Eriksen et al., 2013; Lechner et al., 2014; Mani et al., 2016; Yonkos et al., 2014).

Despite the majority of microplastic studies investigating concentrations within the water surface or water column, it is known that a large proportion of microplastics within aquatic systems will settle to the sediment and become sequestered, with higher concentrations regularly reported in sediments (Yan et al., 2021; Zhang et al., 2017). These microplastics will become more bioavailable to sediment-dwelling and benthic biota, including benthic fish which feed on benthic invertebrates, detritus and vegetation (Borges-Ramírez et al., 2020). Fish can act as important bioindicators of pollution due to their widespread exposure and long lifetimes, but differing sensitivities to pollutants (Naigaga et al., 2011; Okwuosa et al., 2019). Ongoing research efforts demonstrate the ecotoxicological impacts of microplastic contamination on fish, from individual to population-level effects (Bessa et al., 2018; Castro-Castellon et al., 2021). However, the environmental and physiological factors influencing ingestion and accumulation of microplastics are not yet well-understood, and some key estuarine food fish have yet to be studied. Given the importance of fish as a key source of protein for humans, it is strongly recommended to properly evaluate the hazards of emerging pollutants on fish species and aquatic food safety (Wang et al., 2020).

Several research studies have been conducted in recent years with microplastics seen as an emerging concern in the River Thames, where fish species play a significant role. During maximal ebb tides, it has been estimated that 94,000 microplastics s^{-1} flow through the River Thames (UK) at Greenwich, based on a microplastic concentration of 14.2 plastics m^{-3} (Rowley et al., 2020). However, more recent studies on the River Thames have found water concentrations three to four orders of magnitude higher, with one study finding an average of 12,270 microplastics m^{-3} between 2019 and 2021 (Devereux et al., 2023) and (in a separate study) 508,000 microplastics m^{-3} in 2020 (Devereux et al., 2022). The latter contamination was linked specifically to New Year's celebrations, especially a firework display, on the River Thames in Central London. This microplastic concentration range is greater than previously reported in other freshwater settings worldwide (Rowley et al., 2020).

Both freshwater and estuarine/marine species of fish within the River Thames have been shown to be contaminated with microplastics (Horton et al., 2018; McGoran et al., 2017; McGoran et al., 2018). The first evidence of microplastic ingestion by fish in the River Thames was identified in European flounder (*Platichthys flesus*, benthic feeders) and European smelt (*Osmerus eperlanus*, pelagic predators) (McGoran et al., 2017). Plastic fibres were found in the guts of up to 75 % of the European flounder examined, compared to only 20 % of smelt. This distinction was suggested to be due to their different (pelagic vs benthic) feeding habits.

The European flounder (*Platichthys flesus*) (Linnaeus, 1758) is a demersal (benthic) species commonly seen in the upper estuary of the River Thames and is frequently found near contaminant inputs in metropolitan systems (Beaumont and Mann, 1984; McGoran et al., 2017; Williams, 2015). European flounder ingests sediment while feeding in the benthos (McGoran et al., 2017). As a result, flounder will be directly exposed to contaminants deposited in sediments and can be considered an important indicator of the quality of the Thames Estuary. The Atlantic herring (*Clupea harengus*) is one of the world's most commercially valuable marine fisheries targets, with great ecological significance since it connects several trophic levels in the marine food web (Carlson et al., 2021; Mollmann et al., 2004). Multiple abiotic and biotic characteristics and human-induced stressors such as pollution and eutrophication can influence their reproductive success (Moll et al., 2019). However, their interaction with microplastics in UK rivers and estuaries has yet to be investigated. Whiting (*Merlangius merlangus*) is a popular European commercial fish breed in inshore marine environments, sea lochs, and estuaries (Gordon, 1977; Henderson, 2019).

The present study focusses primarily on the River Thames, the second-longest river in the UK at 215 miles (346 km), located in the south of England. The Thames catchment basin is approximately 13,000 km^2, much of which is influenced by the capital city of London and adjoining urban conurbations (McGoran et al., 2017). As a result, the region is defined by human-modified landscapes, resulting in an aquatic ecosystem impacted by widespread urbanisation and intense agricultural operations, receiving wastewater effluent from >13 million people (Horton et al., 2017). The River Stour (East Anglia), also in the east of England and discharging to the North Sea, was selected as a smaller and less-populated comparator (approximate length 47 miles (76 km)), with a catchment area of approx. 1085 km^2 and flowing through no major cities. No microplastics data have yet been published for this river.

The aim of this study was to investigate the abundance of microplastics in the gastrointestinal tract of commercially important food-fish species, including both benthic (European flounder) and pelagic (whiting and Atlantic Herring) habitats, in estuarine locations along two major rivers in the United Kingdom, the River Thames and the River Stour (East Anglia). Specific hypotheses were that the abundance and mass of microplastics in analysed fish would be a) higher in benthic fish than pelagic fish due to greater exposure from sediment b) higher in larger fish, due to higher ingestion of material in general and c) different between the rivers and sites due to different microplastic inputs (e.g. based on different surrounding land use and hydrology) which will likely affect fish exposure.

2. Materials and methods

2.1. Fish collection

Fish were collected by the UK Environment Agency in line with regular fish surveys, between 6th and 8th November 2021. Fish were collected using otter trawl nets. Fish were euthanised and set on ice immediately after capture on site and transported to the laboratory on the day of capture, where they were frozen prior to further analysis. Fish were sampled from two sites on the River Thames (Woolwich and Mulberry) and one site on the River Stour (Fig. 1). Throughout the trawls, the water depths were as follows: Woolwich 11.6–17.1 m, Mulberry 15–15.1 m and Stour 9.1–12.9 m. In some instances, multiple trawls were required to obtain sufficient fish. The tidal state varied throughout the sampling period, and river flow was around average when considering flows throughout Oct-Nov 2021 (average flow during

this time period was 45.7 m^3 s^{-1}, for the sampled dates the flow ranged from 35.2 to 46.9 m^3 s^{-1}). This is also in line with average flows during this time across the decade (average 50.6 m^3 s^{-1} across Oct-Nov 2011–2021), although below average compared to the same period in 2020 (107.3 m^3 s^{-1}) (NRFA, 2022). Rainfall was well below average in November 2021 (but above average in October 2021) (Environment Agency, 2021). The numbers and species of fish collected at each site were based on availability and are shown in Table 1.

2.2. Fish dissection

Frozen fish were placed in a pre-cleaned metal tray (aluminium dissecting pan without wax (Fisher Scientific)) and allowed to thaw for 2 to 3 h inside a laminar flow hood (Bassaire P5VF, Southampton, UK) prior to dissection. Basic measurements, including fish wet weight, were obtained using an electric balance, and the total length was measured using a metal ruler for each specimen. Each specimen was briefly rinsed with Milli-Q water to remove any adhering particles before starting the dissection. The fish dissection was carried out in the aluminium dissecting pan inside the laminar flow hood, using metal scissors, scalpel and forceps. The gastrointestinal tract (GIT, including stomach and intestine) was removed and placed in a pre-weighed glass beaker, covered with aluminium foil, and the weight of the GIT was recorded. The GIT samples were then held in the freezer in the beakers at −20 °C until further processing.

2.3. Digestions and flotation

Fish GIT sample digestion was carried out using pre-filtered 10 % KOH to achieve efficiency in removing biogenic material. GIT samples were taken out of the freezer, and 50 mL of 10 % KOH was added to each sample inside a fume hood (Felcon, UK). The samples were covered using aluminium foil before being placed inside the shaking incubator (SHEL LAB) for 48 h at 40 °C at 100 rpm (Karami et al., 2017). After digestion, the samples were filtered onto 10 μm stainless steel filter discs and then rinsed into clean beakers with MilliQ water.

A canola oil separation process was carried out using a method developed by Crichton et al. (2017). To each sample with Milli-Q, 10 mL of canola oil was added using a glass pipette (Fisher Scientific). The sample was mixed vigorously for 20 s using a stainless-steel spoon until it resembled an emulsion. The sample was left overnight (for 24 h) to settle. After that, the overflow method was carried out using another cleaned 100 mL beaker using Milli-Q water. Afterwards, the combined oil layers containing microplastics were vacuum filtered onto the same 10 μm stainless steel filter. This overflow and filtration process was repeated twice to ensure maximum particle recovery.

A second digestion phase with 30 % H_2O_2 was used to remove any remaining biogenic material in the sample (Avio et al., 2015). For each filtered sample, 20 mL of 30 % H_2O_2 was added, the beakers were covered with Al foil, and they were placed inside the shaking incubator at 40 °C at 100 rpm for 48 h. After the completion of digestion, the samples were filtered onto the same 10 μm stainless steel filter paper using vacuum filtration.

Undiluted Decon90 was used to remove residual oil that might interfere with micro-Fourier Transform Infrared Spectroscopy (μFTIR) analysis (Crichton et al., 2017; Radford et al., 2021). To each filter, 30 mL of undiluted Decon90 was added and left for 24 h. Then the samples were filtered onto a clean 10 μm stainless steel filter and rinsed into a 20 mL glass vial using 50 % ethanol for storage until analysis. For μFTIR analysis, the sample was filtered onto a 3 μm mesh, 25 mm diameter silver filter (Sterlitech, USA), using a silicon washer to restrict the size of the filtered area to 10 mm diameter.

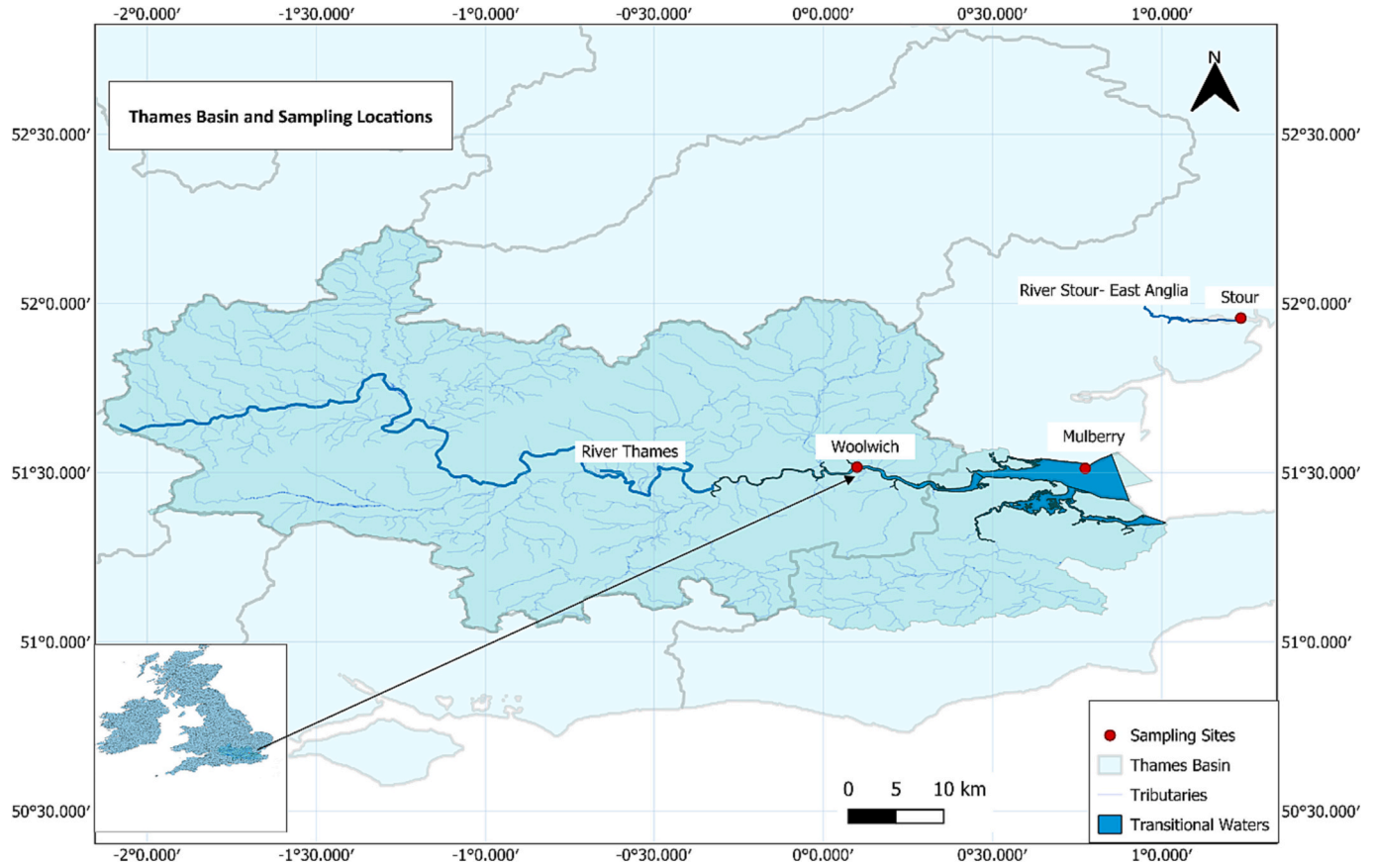


Fig. 1. A map of the UK highlighting the River Thames and the River Stour-East Anglia (Stour), with sampling sites highlighted as red points. The River Thames was sampled at the Woolwich and Mulberry sites.

Table 1
A summary of the fish analysed by site, their characteristics, total and average number and mass of microplastics discovered during GIT analysis.

River	Sampling site	Species	Habitat	Number of individuals	Mean body weight (g) (±SD)	Mean total length (cm) (±SD)	Total number of MPs (LOD corrected)	Average number of MPs/fish (±SD)	Average number of MPs/body weight (g)	Total MP mass (μg) (LOD corrected)	Average MP mass/fish (μg) (±SD)	Average MP mass/fish wet weight (μg/g)
River Stour (East Anglia)	Stour	Flounder	Benthic	5	234.8 (±64.63)	32.3 (±6.65)	23.18	4.64 (±4.99)	0.10	3922.52	784.50 (±1057.27)	3.34
		Whiting	Pelagic	5	149.52 (±45.32)	26.2 (±2.59)	6.71	1.34 (±2.36)	0.04	4.58	0.92 (±2.05)	0.01
River Thames	Woolwich	Flounder	Benthic	6	161.35 (±69.81)	24.83 (±3.78)	0.00	0.00 –	0.00	0.61[a]	0.10 (±0.25)	0.00
		Whiting	Pelagic	10	46.56 (±22.74)	17.52 (±3.63)	31.78	3.18 (±3.89)	0.68	977.63	97.76 (±213.15)	2.10
		Herring	Pelagic	10	14.41 (±20.62)	9.45 (±0.86)	4.45	0.45 (±1.41)	0.31	3.54	0.35 (±0.53)	0.02
	Mulberry	Flounder	Benthic	5	96.58 (±34.40)	20.1 (±1.75)	8.48	1.70 (±2.48)	0.09	2.07	0.41 (±0.43)	0.00
		Whiting	Pelagic	8	119.55 (±26.95)	23.88 (±1.92)	18.04	2.25 (±2.43)	0.15	412.07	51.51 (±145.67)	0.43
		Herring	Pelagic	4	116.38 (±28.58)	23.88 (±1.65)	16.18	4.05 (±5.02)	0.14	905.69	226.42 (±450.31)	1.95
Total		Flounder	Benthic	16	164.06 (±79.01)	25.69 (±6.52)	31.66	1.98 (±3.50)	0.19	3925.21	245.33 (±662.60)	3.35
		Whiting	Pelagic	23	93.44 (±52.70)	21 (±4.69)	56.52	2.46 (±3.10)	0.88	1394.27	85.18 (±222.90)	2.54
		Herring	Pelagic	14	43.54 (±52.61)	13.5 (±6.85)	33.33	1.47 (±3.17)	4.49	909.23	64.95 (±240.89)	1.97

[a] As number and mass of microplastics are corrected for LOD separately, it is the case here that mass is above the LOD, although number is below the LOD.

Where the whole sample could not be transferred onto the silver filter without overloading the filter, a subsample was deposited, and the exact proportion of the sample deposited was calculated by weight.

2.4. Contamination control

Strict contamination control measures were taken throughout the dissection, sample processing, and analysis. Dissection was carried out within a laminar flow hood (Bassaire P5VF, Southampton, UK). Subsequent handling of all the samples, filters, and sampling equipment in the laboratory was carried out within an ISO-5 clean laboratory, either within a laminar flow cabinet (Class II micro-flow biological safety cabinet), which filters air through a 99.999 % high-efficiency particulate air (HEPA) filter (MDH Contamination Control, Hitchings Clinical Services, UK) or a fume hood (Felcon, UK) when digestions were being undertaken. All the equipment was thoroughly washed three times with Milli-Q water before use. PPE cotton or non-shedding Tyvek lab coats and nitrile gloves (Fisher Scientific) were worn all the time to cover clothing when handling samples and equipment.

To avoid contamination from the reagents themselves, all reagents were filtered through a 1.2 μm glass-microfiber filter (4.7 cm diameter, Whatman GF-C) and stored in glass bottles (Fisherbrand) with PTFE lids (ThermoFisher Scientific). All the glassware was washed with Milli-Q water and soaked in a 3 % Decon90 bath overnight before use. Stainless steel filter discs were pre-cleaned with Milli-Q water and placed inside glass petri dishes, which were then covered with aluminium foil and placed inside the muffle furnace at 500 °C for 24 h to remove any particles remaining on the filter. Forceps were cleaned using 70 % ethanol before each filter was handled. All the sampling and processing equipment made of metal or glass were used where possible.

A series of procedural blanks ($n = 11$) without gastrointestinal tract (GIT) samples were performed simultaneously to GIT processing and analysis, to assess airborne plastic contamination and other possible contamination from equipment and reagents. As fish were processed in batches, the processing of the blanks was distributed across these batches (at least one blank sample per batch).

2.5. Micro-FTIR analysis

All microplastics were identified and quantified using a PerkinElmer Spotlight 400 μFTIR Imaging system coupled to a Frontier™ Spectrometer using PerkinElmer SpectrumIMAGE software (PerkinElmer, Llantrisant, UK). Mapping was carried out at a resolution of 8 cm^{-1}, 25 μm pixel size, 2 scans per pixel and an interferometer speed of 2.2 cm/s. The pixel size of 25 μm was selected to give a reasonable compromise between processing time, resulting file size, and resolution. This means that 25 μm was the minimum particle size that could be quantified. Spectra were collected in the range of 4000–750 cm^{-1} wave number in reflectance mode. The infrared mapping area was selected to be 11 mm × 11 mm to ensure the whole 10 × 10 mm area was mapped with some overlap. Atmospheric correction was performed on the resulting .fsm file.

All spectra were analysed using siMPle software v1.1.β developed by Aalborg University, Denmark and the Alfred Wegner Institute, Germany, using the associated database (Liu et al., 2019; Primpke et al., 2019). Number of particles are reported, and the X and Y dimension recorded. Mass was also calculated by the siMPle software, assuming a third (Z) dimension as 0.67 times the minor dimension, and assuming the particle is an ellipsoid. For each particle, these dimensions and the polymer density were used to calculate the particle volume, and thus the mass based on the density of the specific polymer identified.

For reporting, the mass and number of the microplastics were calculated after LOD (limit of detection) blank correction as per Horton et al. (2021), to account for blank contamination. In brief, this consisted of carrying out a blank subtraction based on average number of particles in the blanks by polymer type, with the data only reported if the

resulting number was >3.3 × SD of the blank (otherwise data were reported as 0). Polymers detected in the blanks were polypropylene (PP; average 0.55 ± 0.66 particles per sample), polyamide (PA; 0.45 ± 1.16), polyvinyl chloride (PVC; 0.36 ± 0.48), polyethylene (PE; 0.18 ± 0.47) and ethylene-vinyl-acetate (EVA; 0.09 ± 0.29). If a polymer type was not detected in the blanks it was reported as detected in the sample.

2.6. Statistical analysis

The Shapiro-Wilk normality test was performed to test the normality of count data and mass data. Due to non-normal distribution across all data, non-parametric analyses were used for all tests.

To test the correlation between microplastic mass or microplastic number compared to fish length and wet weight, Kendall's Rank correlation coefficient was used. This approach is non-parametric and robust to outliers. Kendall's Rank correlation coefficient was also carried out to test the correlation between fish length and wet weight.

To test the difference in microplastic mass or number in relation to river, site, habitat and species, Kruskal-Wallis tests were performed. To further explore the data, Kruskal Wallis tests were also carried out on subset data to determine whether there were differences in microplastic ingestion between rivers excluding the influence of habitat (benthic and pelagic fish considered separately), or whether there were differences in microplastic ingestion based only on habitat (each site considered separately).

All statistical analyses were carried out using R (RStudio - version: 4.0.2) statistical software.

3. Results

3.1. Microplastic number

In total, 53 fish were analysed, belonging to three species: European flounder (*Platichthys flesus*), whiting (*Merlangius merlangus*), and Atlantic herring (*Clupea harengus*), representing benthic (European flounder) and pelagic (whiting and Atlantic herring) habitats in both the River Stour and the River Thames. In total, following LOD correction, 121.5 microplastics were detected during gut content analysis (Table 1). Across all sites, 41.5 % of fish had ingested at least one microplastic particle (37.5 % of European flounder, 52.2 % of whiting, and 28.6 % of

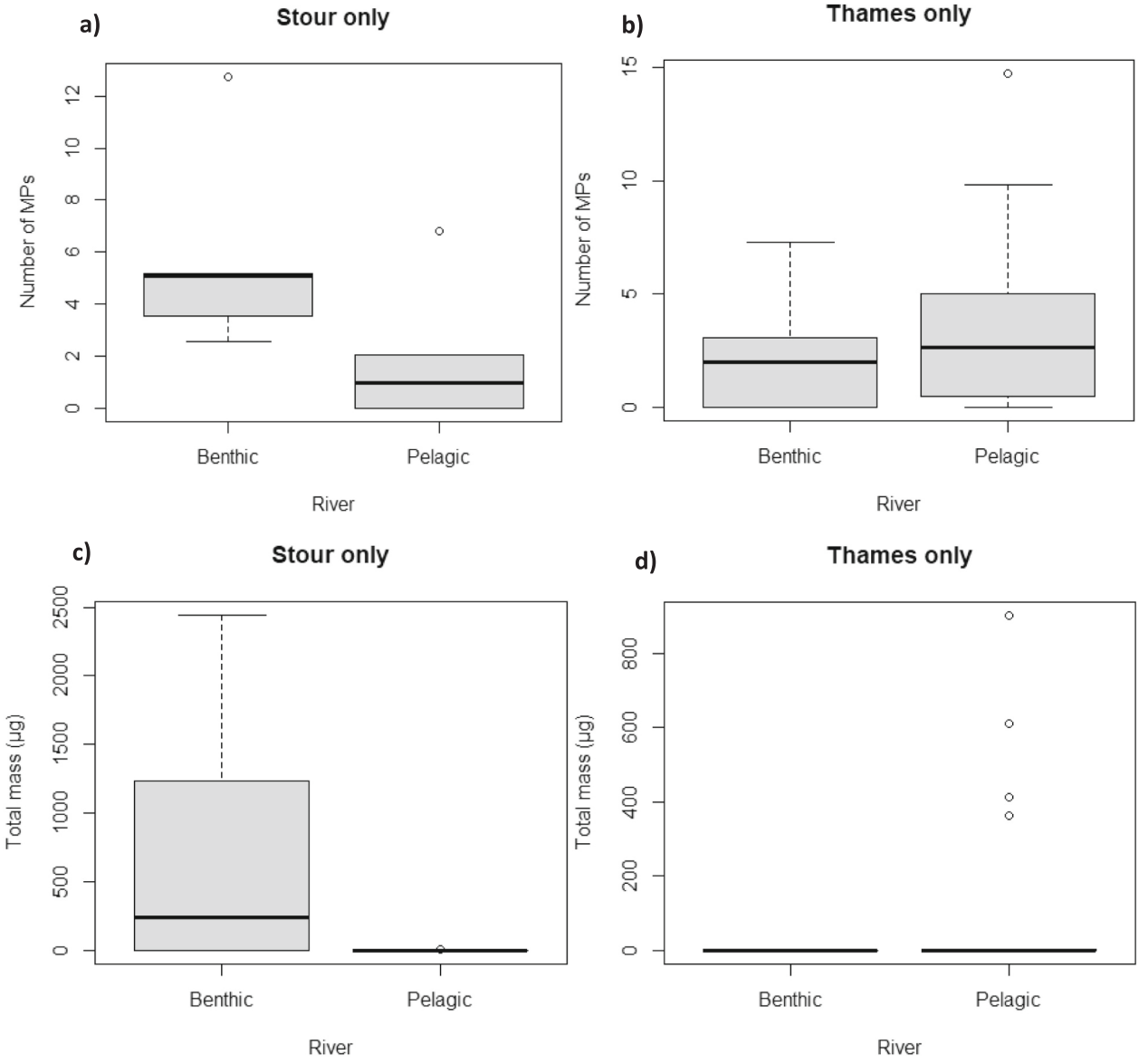


Fig. 2. Boxplots showing the difference in the number (a and b) and mass (c and d) of microplastics (MPs) in fish GIT from the different habitat types when data were subset by river.

Atlantic herring).

Considering the number of microplastics, there were no significant differences in contamination level based on river, site, species or habitat (all Kruskal Wallis, $p > 0.05$). This lack of significance based on species and habitat remained if data were subset into river (Fig. 2a and b) and site. However, the number of microplastics within the GIT of benthic fish from the Stour were significantly higher than those in benthic fish from the Thames, with an average of 4.64 (±4.99 SD) microplastics/fish, compared to an average of 0.77 (±1.8) microplastics/fish across both Thames sites (Kruskal Wallis, $p < 0.05$, Fig. 3a). Pelagic fish showed no significant difference in ingestion between the two rivers (Kruskal Wallis, $p > 0.05$, Fig. 3b).

The number of microplastics ingested were positively correlated to both fish length and fish weight separately (larger and heavier fish were more heavily contaminated, Kendall's Rank correlation, $p < 0.05$), and fish length and weight were correlated with each other (larger fish are heavier, Kendall's Rank correlation, $p < 0.05$).

3.2. Microplastic mass

In total, 6228.72 μg of microplastics were consumed by fish in both rivers. When considering species separately, an average of 245.33 (±662.6) μg of microplastics were ingested per individual European flounder, 85.18 (±222.9) μg per whiting and 64.95 (±240.89) μg per herring (Table 1).

There were no significant differences in the ingested mass of microplastics based on river, site, species or habitat (all Kruskal Wallis, $p > 0.05$). However, considering the Stour only there was a significantly greater mass of microplastics within benthic fish compared to pelagic fish (Kruskal Wallis, $p < 0.05$, Fig. 2c). There was also a significantly greater mass of microplastics ingested by benthic fish in the Stour compared to benthic fish in the Thames (Kruskal Wallis, $p < 0.05$, Fig. 3c). Pelagic fish showed no significant difference in ingestion between the two rivers (Kruskal Wallis, $p > 0.05$, Fig. 3d). Unlike particle number, the mass of microplastics in their GITs was not significantly correlated with fish length or weight (Kendall's Rank correlation, $p > 0.05$).

3.3. Polymer type

By number of microplastics, polypropylene (PP) was the dominant polymer across all samples (61 %, found in fish from both rivers and habitats), followed by polyethylene (PE, 25 % found in benthic fish from the Stour and pelagic fish from the Thames (Fig. 4)), then ethylene vinyl acetate (EVA, 8.3 %), artificially modified cellulose (Cel-mod, 3.5 %) and rubber (1.8 %). By mass, the proportional percentage across the polymers is very different, with PE making up 60 % of the microplastic mass, followed by EVA (39 %).

Interestingly, the Thames had the greatest variation in polymers between benthic and pelagic fish, with contamination of benthic fish only by PP, while pelagic fish contained all five polymers. However, in the Stour, benthic fish contained a greater diversity of polymers (five polymers) compared to pelagic fish (two polymers). There were no differences in polymer type based on river or habitat (both Kruskal

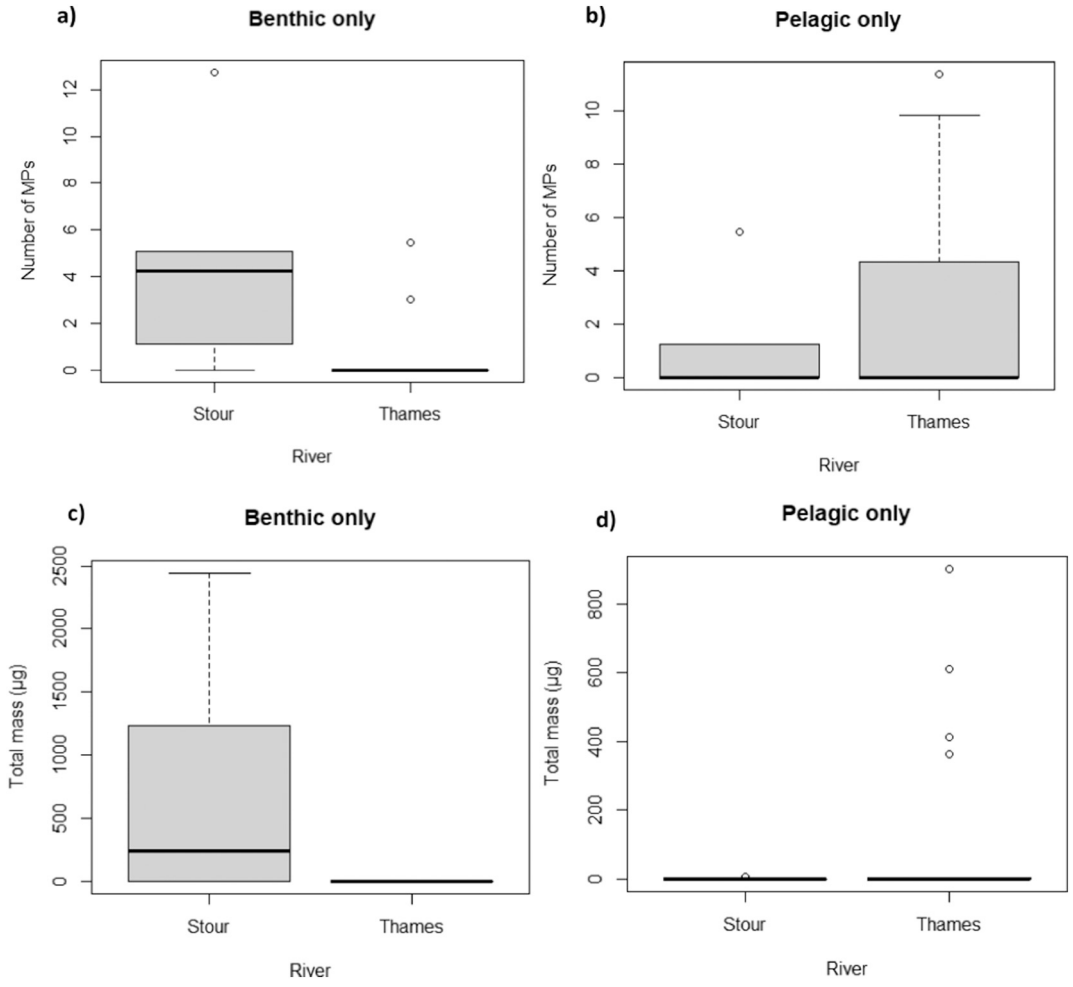


Fig. 3. Boxplots showing the number (a and b) and the mass (c and d) of microplastics (MPs) in fish GIT in the River Thames and River Stour when data were subset into habitat type.

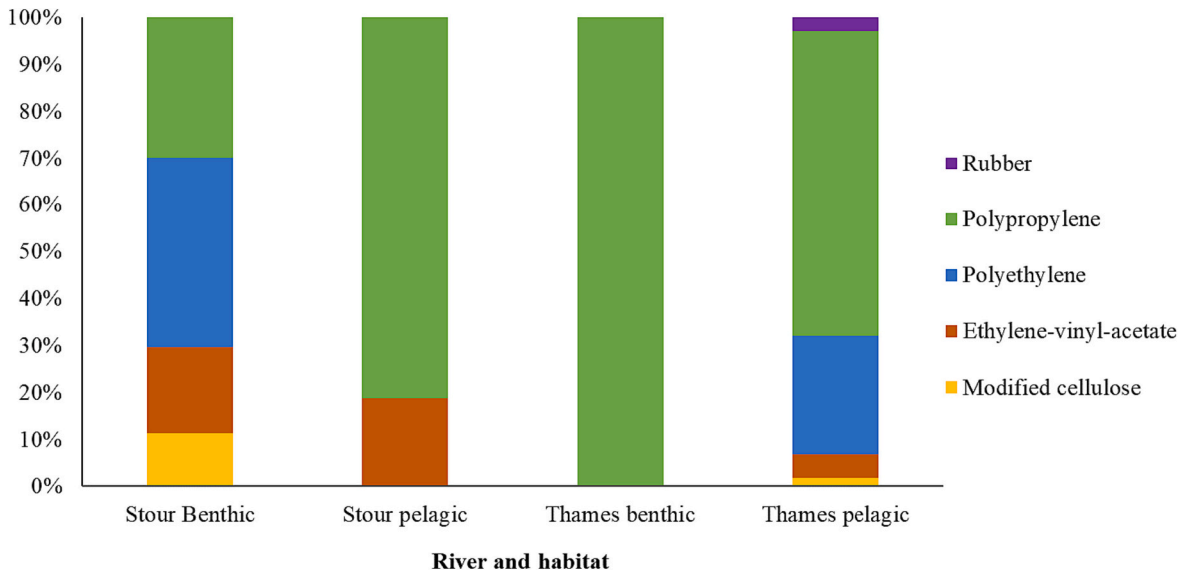


Fig. 4. Proportion of synthetic polymers above LOD across all fish species according to river and habitat.

Wallis, $p > 0.05$, Fig. 4). Although some additional other polymers were detected in the original analysis, these were excluded from the final analysis due to not exceeding the LOD.

4. Discussion

When considering all data, neither the river, the site, the habitat or the species influenced the number or mass of microplastics ingested by fish. The number of microplastics was significantly influenced by both fish mass and length, with larger and heavier fish ingesting more microplastics. This is as would be expected and supports our hypothesis, given that larger fish will consume more in general, and thus are more likely to ingest microplastics, either intentionally or accidentally. This is also in line with previous studies in the Thames (Horton et al., 2018) and further afield (Alomar et al., 2017; Munno et al., 2022; Park et al., 2022) showing that larger fish contained higher numbers of microplastics. However, this is not a consistent trend across the literature and often no correlation between fish size and microplastics ingestion is reported (Güven et al., 2017; Parvin et al., 2021).

Despite a difference in number of microplastics in the fish GITs, fish wet weight and length did not significantly influence the mass of microplastics present. The fact that the difference in number was significant, while the difference in mass was not, implies that the more numerous particles in larger fish were likely smaller, based on a comparable mass. A difference in polymer types leading to a different density in particles cannot be an explanation due to the lack of significant difference in the polymers present within benthic and pelagic fish. However, based on the limited fish available in this study, and the risk of overanalysing the data, all species were combined for the analysis of microplastics ingestion vs fish length and mass and thus we did not we did not delve into an analysis of particle sizes by species. The reasons why larger fish would contain microplastics with different characteristics compared to smaller fish are not clear but warrants further investigation. This result shows the importance of reporting and analysing both number and mass of particles, given these contrasting findings depending on the metric reported.

When considering the data across both rivers, no difference was observed in microplastic abundance between benthic and pelagic species. When considering each river separately, the GITs of benthic fish (flounder) in the River Stour contained a significantly higher number and mass of microplastics than pelagic fish. However, it should be noted that benthic fish in the River Stour are also on average the largest and heaviest across all the sites and species (Table 1). Given that larger fish contain more microplastics, this higher number and mass of microplastics therefore cannot solely be attributed to location. This trend was not observed in the River Thames. Interestingly, while benthic fish in the River Stour had the highest contamination level by number and mass of any of the other species and site (average 4.64 MPs/fish, 784.5 μg/fish based on five fish), the same number of flounder in the River Thames Mulberry site contained no microplastics at all. This suggests that there are factors in the River Stour which lead to a higher proportional contamination of sediment or benthos with microplastics, compared to the River Thames. Data on sediment concentrations at these sites were not possible to obtain with this study but would be needed to further explore these site-specific differences in benthic species uptake.

It has previously been identified that the habitat and feeding habits of species can influence their exposure to, and thus ingestion of, microplastics. Benthic species have been suggested to be more exposed and thus more likely to consume microplastics due to the settling behaviours of microplastics, and higher concentrations in sediments (Borges-Ramírez et al., 2020; Parvin et al., 2021; Wang et al., 2019). In another River Thames study, differences observed between European flounder (*Platichthys flesus*), and European smelt (*Osmerus eperlanus*) were assumed to be due to feeding habits (McGoran et al., 2017). Although the result is not clear-cut in this study, further studies correlating the feeding behaviours of fish, with assessing microplastic partition throughout environmental matrices would enable a greater understanding of the factors influencing fish uptake of microplastics.

The lack of difference observed between rivers and sites is likely due to the dynamic nature of these environments, with microplastic abundance depending on multiple independent environmental, hydrographic and seasonal variables. Fish were collected during normal river flow conditions, however it is difficult to determine the influence of these multiple additional factors on microplastic uptake by fish with samples taken only from a single time point. While microplastics can settle and become sequestered in sediments during low flows, during flooding these particles can become resuspended and flushed from the river (Hurley et al., 2018), therefore if taking multiple samples in time it would be useful to consider flow conditions when analysing data. Nonetheless, although site-specific data can help interpretation of results, it is worth noting that all three fish species analysed here are capable of travelling many kilometres, migrating between inland waters and the open ocean (Bekkevold et al., 2005; Gordon, 1977; Morais et al., 2011). They therefore will have been exposed to microplastics from a

range of sources and locations, not just those at the site where they were caught.

Fish are widely acknowledged as important bioindicators of water quality (Okwuosa et al., 2019). Analysing fish instead of environmental samples such as water and sediment will reduce the spatial and temporal variability which would be encountered if conducting one-off measurements of other matrices. Given that microplastics can be retained in in the gut of fish for a number of days (Grigorakis et al., 2017), microplastics detected in these fish at these sites may have been ingested elsewhere and/or in previous days. Considering only environmental data from the specific time point and location at which fish were sampled would therefore not provide sufficient insight into the wider biological and environmental processes affecting microplastic uptake by fish. Previous studies have found that both exposure and fish characteristics significantly influence ingestion (Horton et al., 2018; Koraltan et al., 2022; Park et al., 2022).

The most prominent polymer types manufactured globally are PE (26.9 % combining LD-PE and HD-PE) and PP (19.3 %, data from 2021), which are used as packing materials due to their excellent mechanical properties and low price (Geyer et al., 2017; PlasticsEurope, 2023; Rothman and Ryan, 2023). The prevalence of these synthetic polymers discovered during the analysis of fish here can therefore be attributed to the high demand, production, and subsequent disposal of these polymers. This study's findings correspond with a study carried out in freshwater fish (*Rutilus rutilus*) in the non-tidal part of the River Thames where PE and PP were detected, alongside polyester (Horton et al., 2018). In the Baltic Sea and the North Sea approximately 40 % of particles were PE in benthic and pelagic fish (Rummel et al., 2016). Atlantic specimens, including commercially important marine teleost fish, were mainly composed of PP, PE and polyamide (PA) (Bottari et al., 2019). Further, in Japanese Anchovies (*Engraulis japonicus*) caught from Tokyo Bay, PE and PP were the most prominent synthetic polymer forms in the gastrointestinal tract (Tanaka and Takada, 2016). PVC is not represented as frequently in environmental samples, despite also being one of the most widely produced polymers (12.9 %), possibly because it is not used in the same way for single-use packaging materials (PlasticsEurope, 2023). The absence of PET (polyester) is surprising due its widespread use in drinks bottles and textiles.

Polymer density has been suggested to affect the amount and types of polymers that pelagic and demersal fish consume (Lusher et al., 2013). However virgin polymer density alone is not a sufficient predictor of exposure, as the most common supposedly buoyant lower-density polymers PP and PE often become fouled or aggregate, subsequently sinking (Harris, 2020). This makes them accessible to many benthic organisms (Porter et al., 2019), hence observing polypropylene in benthic fish from both rivers in this study. The lack of difference in polymers contained in benthic and pelagic fish GITs (Fig. 4) indeed suggests that these polymers are distributed throughout the water column regardless of initial density, due to varying levels of fouling and aggregation with organic matter.

The lowest diversity of polymers was found in benthic fish of the Thames (PP only) compared to the highest diversity in benthic fish of the Stour (five polymers). As the benthic fish in both rivers are the same species (European flounder), this suggests the difference in ingestion lies in the exposure to these polymers, and not species-specific selection of particles. In order to make better assessments of differences, it is advised that more data considering additional rivers and species, and a greater number of individuals and timepoints are included in this type of assessment in the future.

In this study we were not specifically looking for evidence of harm posed by the observed microplastics within these fish species, however observations of harm caused by microplastics to fish are widespread (Bhuyan, 2022; Jovanović, 2017). Given the contamination of these commercially-important fish with microplastics in these studied locations, it is possible that negative effects on individuals and populations may occur, based on chronic exposure. To understand implications for fish populations it will be crucial to link environmental microplastic concentrations and subsequent internalisation of microplastics with the likely harm, at both current and predicted future levels of contamination. This is especially important where fish are relied upon as a commercial resource for human consumption or aquaculture. Where these fish are eaten, either by other species or by humans, there is the possibility of trophic transfer. However, health implications of ingested microplastics for humans are, as yet, not well-understood (Blackburn and Green, 2022; Ghosh et al., 2023).

5. Conclusion

River, site, habitat and species did not explain differences in microplastic abundance between the three analysed fish species, although there were some intra-site differences. Fish characteristics (weight and length) were shown to influence the number and mass of microplastics ingested. Overall, these results imply that in these locations, the influence of fish size is more significant than external environmental factors or where they forage. The polymers detected are those that would be expected based on their widespread use: predominantly polyethylene and polypropylene, with small proportions of artificially modified cellulose, rubber and ethylene-vinyl-acetate. These data, corresponding with previous studies, suggests that those polymers with the greatest global usage are also those which require the most urgent attention with respect to disposal and waste management.

Disclaimer

The views and opinions expressed here reflect only the authors' views, not necessarily those of the European Commission or the European Research Executive Agency (REA).

CRediT authorship contribution statement

Alice A. Horton: Writing – review & editing, Writing – original draft, Supervision, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization. **K.D. Isuri Weerasinghe:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis. **Daniel J. Mayor:** Writing – review & editing, Visualization, Validation, Supervision, Methodology. **Richard Lampitt:** Writing – review & editing, Supervision, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgements

We are grateful to the Environment Agency, especially Gabrielle Garland and Mike Fraser, for providing the fish and associated site data. We also thank Katsia Pabortsava for laboratory support and training. This work was funded by the European Union's Horizon 2020 research and innovation programme under grant agreement No 101003954 (LABPLAS project). IW was supported by an Erasmus Mundus Scholarship in the Marine Environment and Resources (MER+) 2020-2022 programme.

References

Alomar, C., Sureda, A., Capó, X., Guijarro, B., Tejada, S., Deudero, S., 2017. Microplastic ingestion by Mullus surmuletus Linnaeus, 1758 fish and its potential for causing oxidative stress. Environ. Res. 159, 135–142.

Avio, C.G., Gorbi, S., Regoli, F., 2015. Experimental development of a new protocol for extraction and characterization of microplastics in fish tissues: first observations in commercial species from Adriatic Sea. Mar. Environ. Res. 111, 18–26.

Beaumont, W.R.C., Mann, R.H.K., 1984. The age, growth and diet of a freshwater population of the flounder, *Platichthys flesus* (L.), in southern England. J. Fish Biol. 25 (5), 607–616. https://doi.org/10.1111/j.1095-8649.1984.tb04907.x.

Bekkevold, D., André, C., Dahlgren, T.G., Clausen, L.A.W., Torstensen, E., Mosegaard, H., Carvalho, G.R., Christensen, T.B., Norlinder, E., Ruzzante, D.E., 2005. Environmental correlates of population differentiation in Atlantic Herring. Evolution 59 (12), 2656–2668.

Bessa, F., Barría, P., Neto, J.M., Frias, J.P.G.L., Otero, V., Sobral, P., Marques, J.C., 2018. Occurrence of microplastics in commercial fish from a natural estuarine environment. Mar. Pollut. Bull. 128, 575–584.

Bhuyan, M.S., 2022. Effects of microplastics on fish and in human health. Front. Environ. Sci. 10, 250.

Blackburn, K., Green, D., 2022. The potential effects of microplastics on human health: what is known and what is unknown. Ambio 51 (3), 518–530.

Borges-Ramírez, M.M., Mendoza-Franco, E.F., Escalona-Segura, G., Osten, J.R.-v., 2020. Plastic density as a key factor in the presence of microplastic in the gastrointestinal tract of commercial fishes from Campeche Bay, Mexico. Environ. Pollut. 267, 115659.

Bottari, T., Savoca, S., Mancuso, M., Capillo, G., GiuseppePanarello, G., MartinaBonsignore, M., Crupi, R., Sanfilippo, M., D'Urso, L., Compagnini, G., Neri, F., Romeo, T., Luna, G.M., Spanò, N., Fazio, E., 2019. Plastics occurrence in the gastrointestinal tract of *Zeus faber* and *Lepidopus caudatus* from the Tyrrhenian Sea. Mar. Pollut. Bull. 146, 408–416.

Carlson, A.K., Rubenstein, D.I., Levin, S.A., 2021. Modeling Atlantic herring fisheries as multiscalar human-natural systems. Fish. Res. 236, 105855.

Castro-Castellon, A.T., Horton, A.A., Hughes, J.M.R., Rampley, C., Jeffers, E.S., Bussi, G., Whitehead, P., 2021. Ecotoxicity of microplastics to freshwater biota: considering exposure and hazard across trophic levels. Sci. Total Environ. 816, 151638.

Crichton, E.M., Noël, M., Gies, E.A., Ross, P.S., 2017. A novel, density-independent and FTIR-compatible approach for the rapid extraction of microplastics from aquatic sediments. Anal. Methods 9 (9), 1419–1428.

Devereux, R., Westhead, E.K., Jayaratne, R., Newport, D., 2022. Microplastic abundance in the Thames River during the New Year period. Mar. Pollut. Bull. 177, 113534.

Devereux, R., Ayati, B., Westhead, E.K., Jayaratne, R., Newport, D., 2023. "The great source" microplastic abundance and characteristics along the river Thames. Mar. Pollut. Bull. 191, 114965.

Environment Agency, 2021. Monthly Water Situation Report - November 2021. Kingdom, United.

Eriksen, M., Mason, S., Wilson, S., Box, C., Zellers, A., Edwards, W., Farley, H., Amato, S., 2013. Microplastic pollution in the surface waters of the Laurentian Great Lakes. Mar. Pollut. Bull. 77 (1–2), 177–182. https://doi.org/10.1016/j.marpolbul.2013.10.007.

Geyer, R., Jambeck, J.R., Law, K.L., 2017. Production, use, and fate of all plastics ever made. Sci. Adv. 3 (7), e1700782.

Ghosh, S., Sinha, J.K., Ghosh, S., Vashisth, K., Han, S., Bhaskar, R., 2023. Microplastics as an emerging threat to the global environment and human health. Sustainability 15 (14), 10821.

Gordon, J., 1977. The fish populations in inshore waters of the west coast of Scotland. The food and feeding of the whiting (*Merlangius merlangus* L.). J. Fish Biol. 11 (6), 513–529.

Grigorakis, S., Mason, S.A., Drouillard, K.G., 2017. Determination of the gut retention of plastic microbeads and microfibers in goldfish (Carassius auratus). Chemosphere 169, 233–238.

Güven, O., Gökdağ, K., Jovanović, B., Kıdeyş, A.E., 2017. Microplastic litter composition of the Turkish territorial waters of the Mediterranean Sea, and its occurrence in the gastrointestinal tract of fish. Environ. Pollut. 223, 286–294.

Harris, P.T., 2020. The fate of microplastic in marine sedimentary environments: a review and synthesis. Mar. Pollut. Bull. 158, 111398.

Harris, P.T., Westerveld, L., Nyberg, B., Maes, T., Macmillan-Lawler, M., Appelquist, L.R., 2021. Exposure of coastal environments to river-sourced plastic pollution. Sci. Total Environ. 769, 145222.

Henderson, P.A., 2019. A long-term study of whiting, Merlangius merlangus (L) recruitment and population regulation in the Severn Estuary, UK. J. Sea Res. 155, 101825.

Horton, A.A., Svendsen, C., Williams, R.J., Spurgeon, D.J., Lahive, E., 2017. Large microplastic particles in sediments of tributaries of the River Thames, UK–Abundance, sources and methods for effective quantification. Mar. Pollut. Bull. 114 (1), 218–226.

Horton, A.A., Jürgens, M.D., Lahive, E., van Bodegom, P.M., Vijver, M.G., 2018. The influence of exposure and physiology on microplastic ingestion by the freshwater fish *Rutilus rutilus* (roach) in the River Thames, UK. Environ. Pollut. 236, 188–194.

Horton, A.A., Cross, R.K., Read, D.S., Jürgens, M.D., Ball, H.L., Svendsen, C., Vollertsen, J., Johnson, A.C., 2021. Semi-automated analysis of microplastics in complex wastewater samples. Environ. Pollut. 268, 115841.

Hurley, R., Woodward, J., Rothwell, J.J., 2018. Microplastic contamination of river beds significantly reduced by catchment-wide flooding. Nat. Geosci. 11 (4), 251–257.

Jovanović, B., 2017. Ingestion of microplastics by fish and its potential consequences from a physical perspective. Integr. Environ. Assess. Manag. 13 (3), 510–515.

Karami, A., Golieskardi, A., Choo, C.K., Romano, N., Ho, Y.B., Salamatinia, B., 2017. A high-performance protocol for extraction of microplastics in fish. Sci. Total Environ. 578, 485–494.

Koraltan, İ., Mavruk, S., Güven, O., 2022. Effect of biological and environmental factors on microplastic ingestion of commercial fish species. Chemosphere 303, 135101.

Lebreton, L.C., Van Der Zwet, J., Damsteeg, J.W., Slat, B., Andrady, A., Reisser, J., 2017. River plastic emissions to the world's oceans. Nat. Commun. 8 (1), 15611.

Lechner, A., Keckeis, H., Lumesberger-Loisl, F., Zens, B., Krusch, R., Tritthart, M., Glas, M., Schludermann, E., 2014. The Danube so colourful: A potpourri of plastic litter outnumbers fish larvae in Europe's second largest river. Environ. Pollut. 188, 177–181. https://doi.org/10.1016/j.envpol.2014.02.006.

Li, W.C., Tse, H.F., Fok, L., 2016. Plastic waste in the marine environment: a review of sources, occurrence and effects. Sci. Total Environ. 566-567, 333–349.

Liu, F., Olesen, K.B., Borregaard, A.R., Vollertsen, J., 2019. Microplastics in urban and highway stormwater retention ponds. Sci. Total Environ. 671, 992–1000. https://doi.org/10.1016/j.scitotenv.2019.03.416.

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 (1–2), 94–99. https://doi.org/10.1016/j.marpolbul.2012.11.028.

Mani, T., Hauk, A., Walter, U., Burkhardt-Holm, P., 2016. Microplastics profile along the Rhine River. Sci. Rep. 5 (1), 17988 https://doi.org/10.1038/srep17988.

McGoran, A., Clark, P., Morritt, D., 2017. Presence of microplastic in the digestive tracts of European flounder, *Platichthys flesus*, and European smelt, *Osmerus eperlanus*, from the River Thames. Environ. Pollut. 220, 744–751.

McGoran, A.R., Cowie, P.R., Clark, P.F., McEvoy, J.P., Morritt, D., 2018. Ingestion of plastic by fish: a comparison of Thames Estuary and Firth of Clyde populations. Mar. Pollut. Bull. 137, 12–23.

Moll, D., Kotterba, P., Jochum, K.P., von Nordheim, L., Polte, P., 2019. Elemental inventory in fish otoliths reflects natal origin of Atlantic herring (*Clupea harengus*) from Baltic Sea juvenile areas. Front. Mar. Sci. 6, 191.

Mollmann, C., Kornilovs, G., Fetter, M., Koster, F.W., 2004. Feeding ecology of central Baltic Sea herring and sprat. J. Fish Biol. 65 (6), 1563–1581. https://doi.org/10.1111/j.0022-1112.2004.00566.x.

Morais, P., Dias, E., Babaluk, J., Antunes, C., 2011. The migration patterns of the European flounder *Platichthys flesus* (Linnaeus, 1758) (Pleuronectidae, Pisces) at the southern limit of its distribution range: ecological implications and fishery management. J. Sea Res. 65 (2), 235–246.

Munno, K., Helm, P.A., Rochman, C., George, T., Jackson, D.A., 2022. Microplastic contamination in Great Lakes fish. Conserv. Biol. 36 (1), e13794.

Naigaga, I., Kaiser, H., Muller, W.J., Ojok, L., Mbabazi, D., Magezi, G., Muhumuza, E., 2011. Fish as bioindicators in aquatic environmental pollution assessment: a case study in Lake Victoria wetlands, Uganda. Physics and Chemistry of the Earth, Parts A/B/C 36 (14), 918–928.

NRFA (2022) National River Flow Archive (NRFA), 39001 - Thames at Kingston. Hydrology, U.C.f.E.a. (ed).

Okwuosa, O.B., Eyo, J.E., Omovwohwovie, E.E., 2019. Role of fish as bioindicators: a review. Iconic Res. Eng. J 2, 1456–8880.

Park, T.-J., Kim, M.-K., Lee, S.-H., Lee, Y.-S., Kim, M.-J., Song, H.-Y., Park, J.-H., Zoh, K.-D., 2022. Occurrence and characteristics of microplastics in fish of the Han River, South Korea: factors affecting microplastic abundance in fish. Environ. Res. 206, 112647.

Parvin, F., Jannat, S., Tareq, S.M., 2021. Abundance, characteristics and variation of microplastics in different freshwater fish species from Bangladesh. Sci. Total Environ. 784.

PlasticsEurope, 2023. Plastics - The Facts 2022.

Porter, A., Smith, K.E., Lewis, C., 2019. The sea urchin *Paracentrotus lividus* as a bioeroder of plastic. Sci. Total Environ. 693, 133621.

Primpke, S., Dias, P.A., Gerdts, G., 2019. Automated identification and quantification of microfibres and microplastics. Anal. Methods 11 (16), 2138–2147. https://doi.org/10.1039/C9AY00126C.

Radford, F., Zapata-Restrepo, L.M., Horton, A.A., Hudson, M.D., Shaw, P.J., Williams, I.D., 2021. Developing a systematic method for extraction of microplastics in soils. Anal. Methods. 13 (14), 1695–1705.

Rothman, R., Ryan, A.J., 2023. Plastic Pollution in the Global Ocean, pp. 21–46.

Rowley, K.H., Cucknell, A.-C., Smith, B.D., Clark, P.F., Morritt, D., 2020. London's river of plastic: high levels of microplastics in the Thames water column. Sci. Total Environ. 740, 140018 https://doi.org/10.1016/j.scitotenv.2020.140018.

Rummel, C.D., Löder, M.G.J., Fricke, N.F., Lang, T., Griebeler, E.-M., Janke, M., Gerdts, G., 2016. Plastic ingestion by pelagic and demersal fish from the North Sea and Baltic Sea. Mar. Pollut. Bull. 102 (1), 134–141. https://doi.org/10.1016/j.marpolbul.2015.11.043.

Sherrington, C., 2016. Plastics in the Marine Environment. Eunomia.

Tanaka, K., Takada, H., 2016. Microplastic fragments and microbeads in digestive tracts of planktivorous fish from urban coastal waters. Sci. Rep. 6 (1), 34351.

Wang, J., Wang, M., Ru, S., Liu, X., 2019. High levels of microplastic pollution in the sediments and benthic organisms of the South Yellow Sea, China. Sci. Total Environ. 651, 1661–1669.

Wang, W., Ge, J., Yu, X., 2020. Bioavailability and toxicity of microplastics to fish species: A review. Ecotoxicol. Environ. Saf. 189, 109913 https://doi.org/10.1016/j.ecoenv.2019.109913.

Williams, P., 2015. The Sea Fish Industry Authority Annual Report and Accounts 2014/2015. HM Government.

Yan, M., Wang, L., Dai, Y., Sun, H., Liu, C., 2021. Behavior of microplastics in inland waters: aggregation, settlement, and transport. Bull. Environ. Contam. Toxicol. 107 (4), 700–709.

Yonkos, L.T., Friedel, E.A., Perez-Reyes, A.C., Ghosal, S., Arthur, C.D., 2014. Microplastics in Four Estuarine Rivers in the Chesapeake Bay, U.S.A. Environ. Sci. Technol. 48 (24), 14195–14202. https://doi.org/10.1021/es5036317.

Zhang, K., Xiong, X., Hu, H., Wu, C., Bi, Y., Wu, Y., Zhou, B., Lam, P.K.S., Liu, J., 2017. Occurrence and characteristics of microplastic pollution in Xiangxi Bay of Three Gorges Reservoir, China. Environ. Sci. Technol. 51 (7), 3794–3801.