Ingestion of plastic by fish

McGoran, Alexandra R.; Cowie, Phillip R.; Clark, Paul F.; McEvoy, James P.; Morritt, David

Published in:
Marine Pollution Bulletin

DOI:
10.1016/j.marpolbul.2018.09.054

Published: 07/12/2018

Document Version
Peer reviewed version

Research Paper

Ingestion of plastic by fish: a comparison of Thames Estuary and Firth of Clyde populations

Alexandra R. McGoran [a, c, *], Phillip R. Cowie [b], Paul F. Clark [c], James P. McEvoy [a], David Morritt [a]

[a] *School of Biological Sciences, Royal Holloway University of London, Egham, Surrey TW20 0EX, UK*

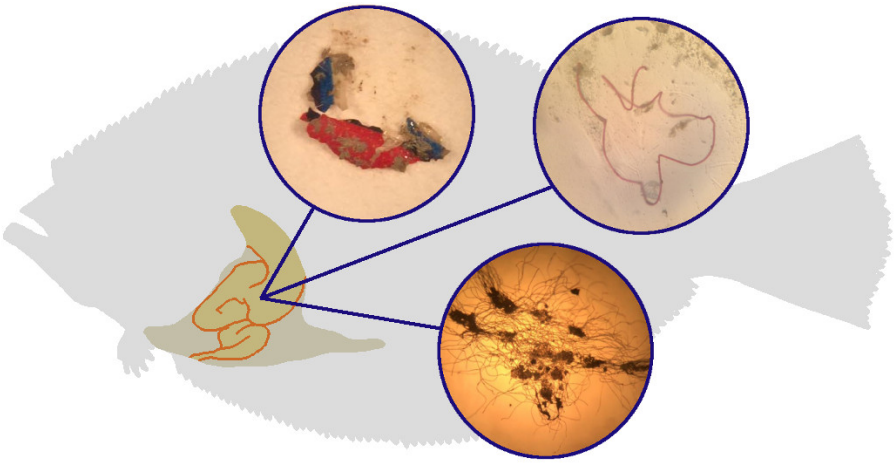
[b] *School of Health and Life Sciences, University of the West of Scotland, Paisley, PA1 2BE, UK*

[c] *Department of Life Sciences, The Natural History Museum, Cromwell Road, London SW7 5BD, UK*

* Corresponding author.

E-mail address: alexandra.mcgoran.2012@live.rhul.ac.uk (A.R. McGoran)

A B S T R A C T



This study compared plastic ingestion between pelagic and benthic fish populations from two UK watersheds: the Thames Estuary and the Firth of Clyde. The alimentary canals of 876 individuals were examined. Of twenty-one estuarine species investigated, fourteen ingested plastics, including predator (fish) and prey (shrimp) species. Overall, 32% of organisms ingested plastic, mostly fibres (88% of total plastics). More flatfish (38%) ingested plastics than other benthic species (17%). In the Thames, more plastic was ingested by pelagic species (average number of plastic pieces ingested: 3.2) and flatfish (average number of plastic pieces ingested: 2.9) than by shrimp (average number of plastic pieces ingested: 1). More fish from the Clyde ingested plastic than similar Thames species (39% compared to 28% respectively); however, the average amount of plastic ingested did not differ between the sites.

ARTICLE INFO

Keywords:

Microplastics; Microfibres; Thames Estuary; Firth of Clyde Fish populations; FTIR Spectroscopy

Funding: This work was partly supported by The University of London Sheina Marshall Memorial Fund.

1. Introduction

Plastic has been mass-produced since the 1940s and is now a huge source of marine pollution world-wide (Galgani et al., 2000; Moore, 2008; Barnes et al., 2009; Browne et al., 2011; Corcoran, 2015; Jambeck et al., 2015). In 2016, 335 million tonnes of plastic were produced globally and production increases yearly (PlasticsEurope, 2018), as does the amount entering the marine environment (Jambeck et al., 2015). In 2010 alone an estimated 12.7 million tons of plastic entered the ocean (Jambeck et al., 2015). Plastic debris has been reported to be ingested by ca. 220 species (Lusher et al., 2017), including marine and freshwater fish (Lusher et al., 2013; Phillips and Bonner, 2015), crustaceans (Murray and Cowie, 2011; Devriese et al., 2015), molluscs (Van Cauwenberghe and Janssen, 2014; Van Cauwenberghe et al., 2015), seabirds (Avery-Gomm et al., 2013) and mammals (Lusher et al., 2015).

It is estimated that, in the marine environment, plastics take hundreds to thousands of years to degrade (Barnes at al., 2009), with Corcoran et al. (2015) reporting the presence of microplastics in lake sediment that had been accumulating for 38 years. Despite this, tide action, photodegradation, biodegradation, thermo-oxidative degradation and hydrolysis can breakdown plastics in the marine environment into ever decreasing smaller fragments (Andrady, 2011). Pieces of plastic less than 5mm in size are referred to as microplastics (Wright et al., 2013) and these have now become an accumulative problem.

Estuaries are hotspots for microplastic accumulation (Browne et al., 2010; Wright et al., 2013). Galgani et al. (2000) noted that litter, largely plastic, on the seafloor around Europe was most concentrated near estuarine inputs. It is also the case in freshwater catchments that plastic concentrates around water inputs (Corcoran, 2015). Rivers and estuaries receive plastics from terrestrial sources and can transport these to marine systems (Cole et al., 2011; Lechner et al., 2014; Jambeck et al., 2015). For example, it is estimated that over 4 tonnes of plastic

58 flows into the sea each day from the River Danube (Lechner et al., 2014). Despite this, research
59 has focussed on marine species (Boerger et al., 2010; Foekema et al., 2013; Lusher et al., 2013).
60 There are only a limited number of studies conducted in estuaries (McGoran et al., 2017;
61 Murphy et al., 2017; Bessa et al., 2018).

62 There are 155 British estuaries, including 35 coastal-plain estuaries (e.g. Thames
63 Estuary; Tinsley, 1998) and 6 fjords (e.g. Firth of Clyde; Jardine, 1986). Reports of plastic
64 pollution in some of these estuaries are escalating. Gallagher et al. (2016) recovered plastics
65 from estuaries in the Solent estuarine complex, Morritt et al. (2014) recorded 8,490 pieces of
66 litter, mainly plastic, during a three-month fyke net fishing programme in the Thames Estuary,
67 and 65% of debris on the shoreline of the Tamar Estuary was found to be in the form of
68 microplastics (Browne et al., 2010).

69 The Thames Estuary and the Firth of Clyde are comparable with respect to potential
70 plastic pollution: both are in close proximity to several microplastic sources, including major
71 cities and shipping traffic. The 16,000 km^2 catchment of the River Thames includes 15 million
72 residents (Environment Agency, 2016) whilst the River Clyde has a catchment of over 3,000
73 km^2 which encompasses 1.7 million people (SEPA, 2015).

74 The Clyde and Thames are ecologically diverse and are important habitats and nurseries
75 for marine fish. The Thames Estuary supports over 950 species, including 112 fish species, and
76 has been recognised as a key habitat for commercial flatfish (Thomas, 1998). The European
77 flounder (*Platichthys flesus*) spends most of its lifecycle in the estuary, and juveniles are able
78 to penetrate the entire tidal reach of the river. Consequently, flounder is a key species to
79 measure the health of the Thames Estuary (Thomas, 1998). Recently McGoran et al. (2017)
80 collected European flounder from two sites in the Thames Estuary to measure the extent of
81 microplastic ingested. The results revealed that up to 75% of sampled *P. flesus* had plastic

fibres in the gut. Scotland's coastline supports ca. 8,000 species (WWF & Scottish Wildlife Trust Joint Marine Programme, 2004), including 59 demersal fish species (The Scottish Government, 2012). The Firth of Clyde is a fjordic system with deep valleys and steep sills (Edwards et al., 1986; Jardine, 1986) and a weak tidal current (less than 0.5ms^{-1}; Wilding et al., 2005; The Scottish Government, 2012) which may aid the accumulation of plastics in the sediment which could be available to these demersal species (Haig, 1986). Prior work in the Firth of Clyde revealed that 83% of *Nephrops norvegicus* had ingested plastic (Murray and Cowie, 2011) whilst less than 30% of fish had consumed plastic (Murphy et al., 2017). At present, Murphy et al. (2017) and McGoran et al. (2017) are the only studies to report plastic ingestion by fish in these two estuaries.

The present study extends a preliminary study in the Thames Estuary by McGoran et al. (2017). The aims were to compare (1) samples collected from Thames Estuary and Firth of Clyde fish populations (2) the samples collected in the Thames Estuary to the previous study by McGoran et al. (2017), in which it was found that 20–75% of fish examined had ingested plastic (3) feeding groups and assess if feeding mode affects plastic ingestion in fish and (4) a common prey species (brown shrimp; *Crangon crangon*) with predator fish species. The data collected in this study were also used to determine whether there were any relationships between gender and plastic ingestion.

2. Materials and methods

2.1 Sampling

Using beam trawls (mesh size: 80 mm), fyke, trammel and shrimp nets eight teleost fish species, two cartilaginous fish species, and one shrimp species were caught in the Thames Estuary. Three sampling sites, downstream of London were used: Thamesmead (51°30.637′N 000°06.591′E), Erith (ca. 51°28.005′N 000°12.122′E) and Isle of Sheppey (51°29.048′N

000°41.800′E; Fig. 1). Sampling was conducted on 17, 18 and 23 November 2015 at Erith, Thamesmead and Isle of Sheppey, respectively. *Crangon* were not caught from the Isle of Sheppey. Fish were identified, dissected, the gut contents searched and analysed, and blank controls (see section 2.3) collected at Royal Holloway, University of London (RHUL) following the method of McGoran et al. (2017) based on that of Lusher at al. (2013). The resulting data sets are thus directly comparable with these studies.

Fifteen teleost and one cartilaginous fish species were caught in beam trawls (mesh size: 50 mm) in the Firth of Clyde (55°46.240′N 4°52.936′W; Fig. 1) on 3 November 2015 and 18 May 2016. Fish were identified, dissected, the gut contents searched, and blanks collected, as described above, at Field Studies Council Millport, Isle of Cumbrae and analysed at RHUL. Appendix A details the sampling sites and equipment used at both sites.

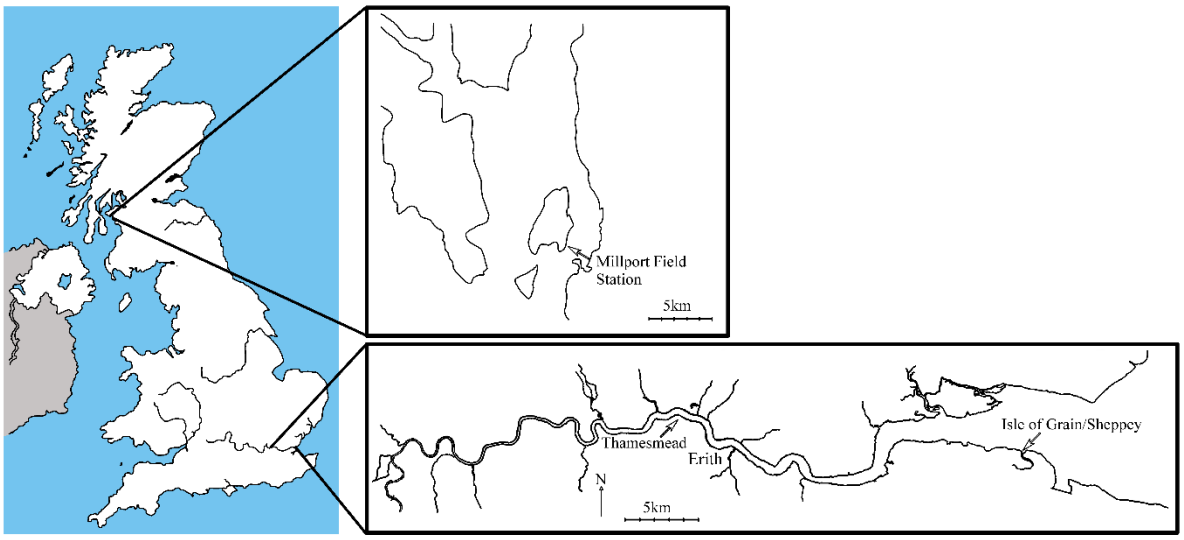


Fig. 1. A map of the UK, highlighting the Firth of Clyde (top) and Thames Estuary (bottom) sampling sites. Sampling in the Thames Estuary was conducted at Thamesmead, Erith and Isle of Sheppey.

121 In total, 876 individuals were examined and 21 species identified (Table B.1; Appendix
122 B). Fish were divided into three functional feeding groups for analysis: flatfish, other benthic
123 fish (excluding flatfish) and pelagic fish. Shrimp were included as a fourth group.

124 *2.2 Quantifying plastic ingestion*

125 Samples were transported to the laboratory, stored in a freezer and identified with
126 reference to Wheeler (1978). Prior to dissection, fish were measured (standard length and
127 height), weighed (using a Sartorius 1413 MP8-1 balance accurate to one decimal place or Tesco
128 Go Cook scales accurate to the nearest gram) and any signs of ill-health (i.e. ulcers; Wright et
129 al., 2013) noted. *Crangon* were also measured (length, tip of rostrum to end of telson, and depth
130 of the carapace) and weighed (using Sartorius 1413 MP8-1 balance). No digestion protocols
131 were implemented to reduce the processing time, with some digestions requiring days or weeks
132 (Foekema et al., 2013; Karami et al., 2017; Kühn et al., 2017; Lusher et al., 2017), and to
133 prevent the degradation of polymers that can be caused by many digestive agents (Lusher et
134 al., 2017). The digestive tract from all species was removed and inspected under a dissection
135 microscope using mounted pins. For shrimp, only the foregut was examined for microplastics.
136 The search time was not standardised for this study because of the variability of the size and
137 volume of the digestive tracts from different fish. Searching was conducted in 1 cm sections of
138 the gut thereby reducing its exposure to potential sources of airborne contamination. Any fibres
139 considered to have originated from airborne sources were removed and not included in the
140 examination. Additional controls against contamination are described in section 2.3. Plastic
141 items were removed from specimens and stored on filter paper in a Petri dish sealed with
142 Parafilm. Over 3,000 particles were recovered from the gut contents of fish and *Crangon*.

143 Gut plastic was initially described by colour and shape. Pale colours, which were
144 difficult to distinguish from one another and fibres without evident pigmentation were grouped

together as “clear fibres”. Several of the potential plastics recovered did not fit into a defined colour category. Plastics with more than one colour were grouped as multi-coloured. Shape was determined as a film, synthetic fibre, sphere or an irregularly shaped fragment.

2.3 Controls against contamination

A clean, white laboratory coat and non-sterile, single-use gloves were worn during dissection procedures and during Fourier Transform Infrared Spectroscopy analyses (see section 2.4). Samples were covered as much as possible to reduce exposure to airborne contamination. Equipment and laboratory space were cleaned with 70% ethanol and white lab roll prior to dissection and searching, as well as between specimens. In addition, empty Petri dishes were placed in each laboratory to monitor environmental contamination. Three replicates were taken, each lasting 30 minutes. Plastics recovered in the Petri dishes were analysed using the methods described for plastics recovered from samples. After FTIR, the limit of detection for each shape and colour plastic was calculated (see below). Plastics were removed from analysis if they did not exceed the limit of detection (LOD). Where the volume of plastic matching the description of a contaminant plastic exceeded that of the LOD, the count was reduced to compensate for contamination (i.e. if the LOD for black fibres was one and a fish ingested three black fibres, only two were reported).

$$LOD = A + SD$$

LOD = Limit of detection, A = Average number of plastics of a particular shape and colour (i.e. clear fibres, black films), SD = Standard deviation.

2.4 FTIR spectroscopy

FTIR spectroscopy is well documented for microplastic analysis (Lusher et al., 2017). Gallagher et al. (2016), however, reported that such analysis is difficult due to the lack of

precise published instructions. As such, detailed methods of FTIR have been included in this paper.

As well as the plastics recovered from the samples, FTIR was conducted on samples of known materials including polyester. As these samples were a known material, it was possible to compare the spectra to the software library (Appendix C, Table C.1) outputs and ensure that identification using these libraries was accurate. Analysis of plastic pieces was undertaken using a Thermo Scientific Nicolet iS5 FTIR spectrometer, with a diamond attenuated total reflection (ATR) cell and a flat-headed pressure clamp.

All pieces were individually analysed and visible organic matter was removed with a mounted pin before FTIR analysis. A background spectrum was made before analysis and updated hourly. For each individual plastic, 16 scans were collected using Thermo Scientific OMNIC 8.3.103 software, the average result was used to generate an absorption spectrum between 500–4000 cm^{-1}. This spectrum was compared to 13 standard software libraries (Appendix C, Table C.1). Identification was informed by Williams and Fleming (1995). Some samples did not precisely match any library spectra and were classified as "spurious results".

Knotted, woven and networks of fibres were all analysed as a whole rather than individually, with the aim of reducing the loss of fibres before analysis. Fibres from tangled knots were rinsed with distilled water, separated and counted after FTIR.

2.4.1 FTIR data processing

Thermo Scientific OMNIC Specta software was used to remove atmospheric CO_2 absorbance peaks, apply ATR correction, and adjust baselines for 400 spectra. Processed spectra were compared to one software library, the Hummel Polymer and Additives Library. The 400 processed spectra were identified either as the single best match from the spectral library, or from a multiple component match with two spectra.

192 Composite matches were found for 37% of corrected spectra, 63% of which produced
193 matches with organic matter and a synthetic compound. Figure D.1b (Appendix D) illustrates
194 the output of a multiple component search. The broad peak in the OH region demonstrates that
195 a large quantity of carbohydrate and protein, probably organic matter from the alimentary
196 canal, was present in the sample; other peaks in the spectrum matched polypropylene.

197 *2.4.2 ATR correction*

198 ATR correction used the following specifications: an angle of incidence of 42°, 1
199 reflection and refractive index of 1.55 (Thermo Fisher Scientific, 2015). The refractive index
200 chosen was an average of refractive indices of three common polymers (nylon 6, polyester and
201 polypropylene) provided by Greaves and Saville (1995), which ranged between 1.496 and
202 1.706. ATR correction using a refractive index between 1.50 and 1.60 showed minimal
203 variation in the output spectra. ATR correction increased the match of a known polyester
204 sample by 20% compared to atmospheric and baseline correction alone.

205 Figure D.1a (Appendix D) shows the processed FTIR spectrum obtained from a clear
206 fibre. Peaks in the fingerprint region closely match polypropylene. The sample spectrum and
207 library spectra did not match perfectly as the sample had been degraded in the environment and
208 / or in the fish gut. ATR correction did not increase the average percentage match of the sample
209 with library spectra (41 ±14.6% before and 37 ±20.2% after) but did increase the maximum
210 recorded match from 87% to 97%. ATR correction, on average, increased the accuracy of a
211 match by 5.3 percentage points, and increased the apparent percentage of synthetic spectra in
212 the sample by 21 percentage points to 58% of the sample. Since a higher percentage match was
213 obtained from corrected spectra, these were used for data collection.

214 *2.5 Statistical Analysis*

215 Statistical analysis was conducted using R version 3.4.2 with R Studio version 1.1.383.
216 Generalised linear models (GLMs) were developed to understand the variables that influenced
217 the number of organisms to ingest plastic and the number of plastic pieces by individuals. The
218 season of sampling, length of organism, gender, feeding group and sample site were
219 investigated. GLMs were compared with AIC and BIC scores so that only reduced models with
220 the main effects were used for analysis. Non-significant variables were removed until eight
221 GLMs were generated (Table 1). Length was skewed, with a higher number of smaller
222 specimens sampled than larger ones. To account for this, length was transformed in the models.
223 To analyse the number of organisms to ingest plastic, binomial models were used. To compare
224 the amount of plastic ingested by individuals, specimens that ingested no plastic were removed
225 from analysis. Shrimp were excluded from comparisons between the Thames and the Clyde as
226 they were only sampled from the Thames. Seasonality could only be considered when
227 analysing fish from the Clyde as Thames samples were all collected in winter. When comparing
228 gender, only the most common flatfish species at each site were analysed. When categorical
229 variables were significant, the results were interpreted using Tukey pairwise comparisons.
230 Similarly, when interaction terms were significant, the results were interpreted by model
231 reductions and ANOVA comparisons.

232 **3. Results**

233 *3.1 Contamination*

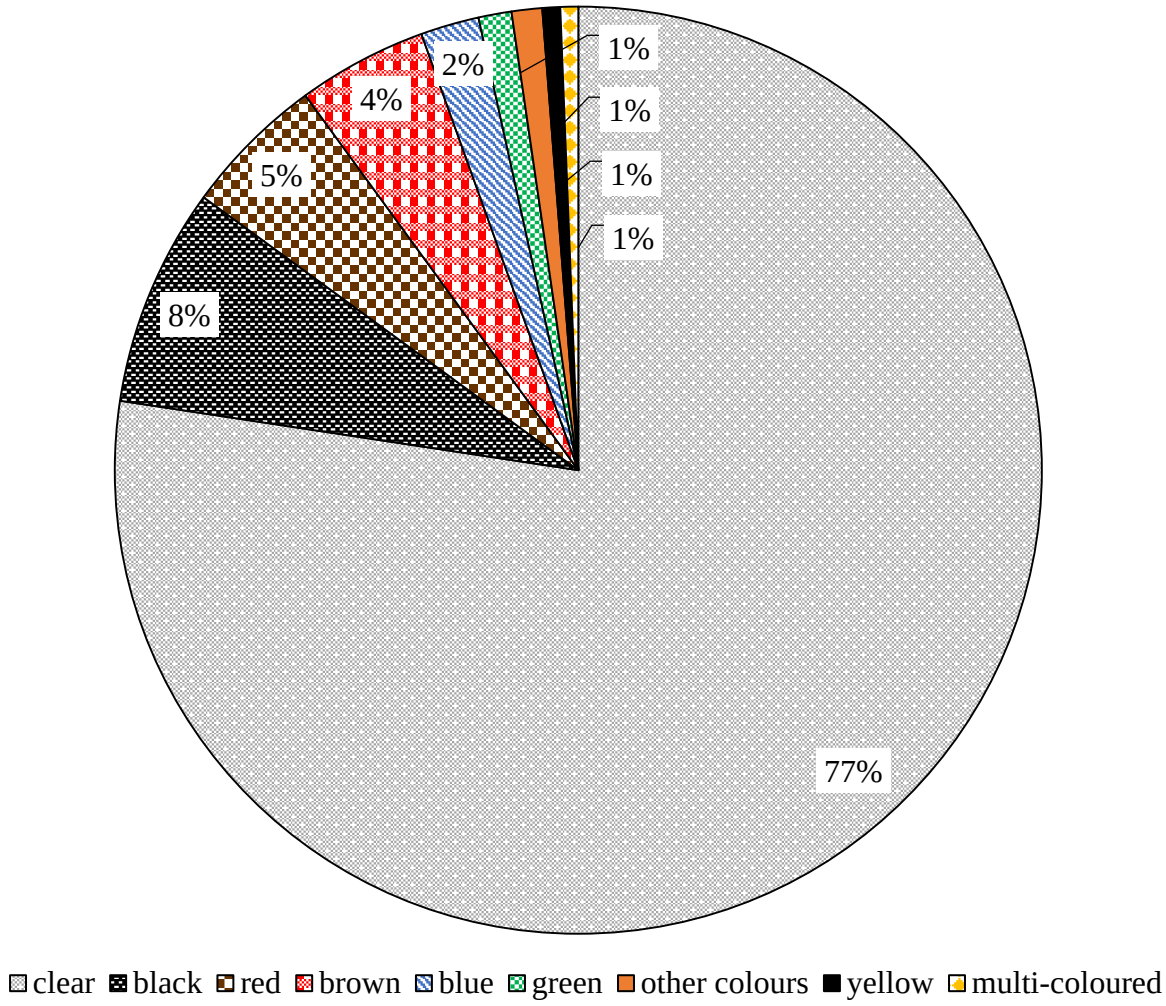
234 Airborne contamination was reported in both laboratories (see Table 2). Fibres were
235 identified as cotton and polyester; films were also identified as polyester. All clear, red, black
236 or blue fibres and black films at or below the LOD were removed from analysis.

237 *3.2 Plastic abundance*

Prior to FTIR, 3,427 potential plastic pieces (Thames Estuary: 850; Firth of Clyde: 2,577) were collected. Fibres were the most abundant plastic, occurring as single filaments and tangled knots. Spheres, films (including sheets of woven fibres), fragments and joined networks of filaments were also recovered.

Fibres lost or destroyed prior to FTIR could not be analysed. FTIR was conducted on the remaining 2,649 particles. Of this subset, 1,285 (48.5%) were confirmed to be synthetic by FTIR analysis when compared to library spectra of known polymers. This volume decreased to 1,128 pieces of plastic when contamination was considered. Among these samples, 26 different polymers and polymer mixes were identified (Appendix E). The most common polymers were polyester (polymers grouped together; 33%), nylon (polyamide 6 + polyamide 6.6; 20%) and polypropylene (15%).

A variety of coloured plastics were collected, 12 in total (Fig. 2). Tangled knots that contained more than one colour fibre were treated as multi-coloured. The most commonly recorded colours were clear (77% of plastics), black (8%), brown (5%) and red (4%).



252

253 **Fig. 2.** The colours of plastics recorded from both estuaries and all samples. Other colours

254 included grey, white, purple, orange and pink.

Tangled fibres were recorded in 31 specimens (7: Thames Estuary; 24: Firth of Clyde) with only two fish, both from the Firth of Clyde, containing more than one knot. Tangled knots ranged from 2–51 fibres per knot. Tangled knots contained 1–5 colours, but most comprised only one, commonly clear fibres.

3.3 Plastic prevalence in fish and shrimp

After FTIR analysis, the number of fish species (both benthic and pelagic) that had ingested plastics was confirmed as 13 (Thames Estuary: 8; Firth of Clyde: 6). Plastic was also ingested by brown shrimp (*Crangon crangon*). Overall, 32% of estuarine organisms (36% of fish and 6% of *Crangon*) had ingested plastic, a total of 278 individuals.

Table 3 shows the proportion of individuals to ingest plastic and the average consumption of plastic in each feeding group. In the Thames Estuary, 33% of flatfish, 19% of other benthic fish, 14% of pelagic fish and 6% of *Crangon* ingested plastic. An average of 2.93, 1.50 and 3.20 plastic pieces were ingested by Thames flatfish, other benthic fish and pelagic fish, respectively. The most common polymer recovered was nylon, which made up 33% of recovered plastics. In the Firth of Clyde, 39% of flatfish, 14% of other benthic fish and 60% of pelagic fish ingested plastic. On average Clyde flatfish, other benthic fish and pelagic fish ingested 3.92, 2.00 and 5.83 plastic pieces respectively. In Clyde fish, polyester was the most common polymer (37% of plastic pieces).

At Thamesmead, Erith and Isle of Sheppey 18%, 34%, and 32% of fish ingested plastic, respectively. An average of 2.2, 3.6 and 1.6 particles were ingested per fish at each site respectively. Comparatively few *Crangon* ingested plastic: 7% and 5% of *Crangon* from Erith and Thamesmead, respectively. The most common polymer recovered from *Crangon* was nylon (43% of plastic pieces). An average of 0.07 and 0.05 pieces of plastic were recorded in the stomach of Erith and Thamesmead *Crangon*, respectively.

279 *3.4 Statistical Analysis*

280 Generalised Linear Model (GLM) 1 and 5 (Table 1) included fish from both the
281 Thames and the Firth of Clyde. The models revealed that significantly more fish from the Firth
282 of Clyde ingested plastic than from the Thames ($p < 0.001$, 39% of fish from the Clyde ingested
283 plastic compared to 28% of fish from the Thames; Fig. 3). The average number of plastic pieces
284 ingested by fish, however, did not differ between the two sites. Additionally, significantly more
285 flatfish ingested plastic than other benthic fish ($p < 0.05$, 38% of flatfish ingested plastic
286 compared to 17% of other benthic fish; Fig. 4). Also, other benthic fish ingested significantly
287 less plastic than both pelagic fish and flatfish ($p < 0.001$, on average flatfish ingested 3.8 pieces
288 of plastic, pelagic fish ingested 4.6 pieces and other benthic fish ingested 1.7 pieces; Fig. 4).
289 Analysis of the amount of plastic ingested by individual fish demonstrated that length only
290 influenced the number of plastic pieces ingested and not the number of fish which consumed
291 plastic. Larger fish ingest significantly more pieces of plastic ($p < 0.001$, slope: 1.1384,
292 intercept: -3.9373).

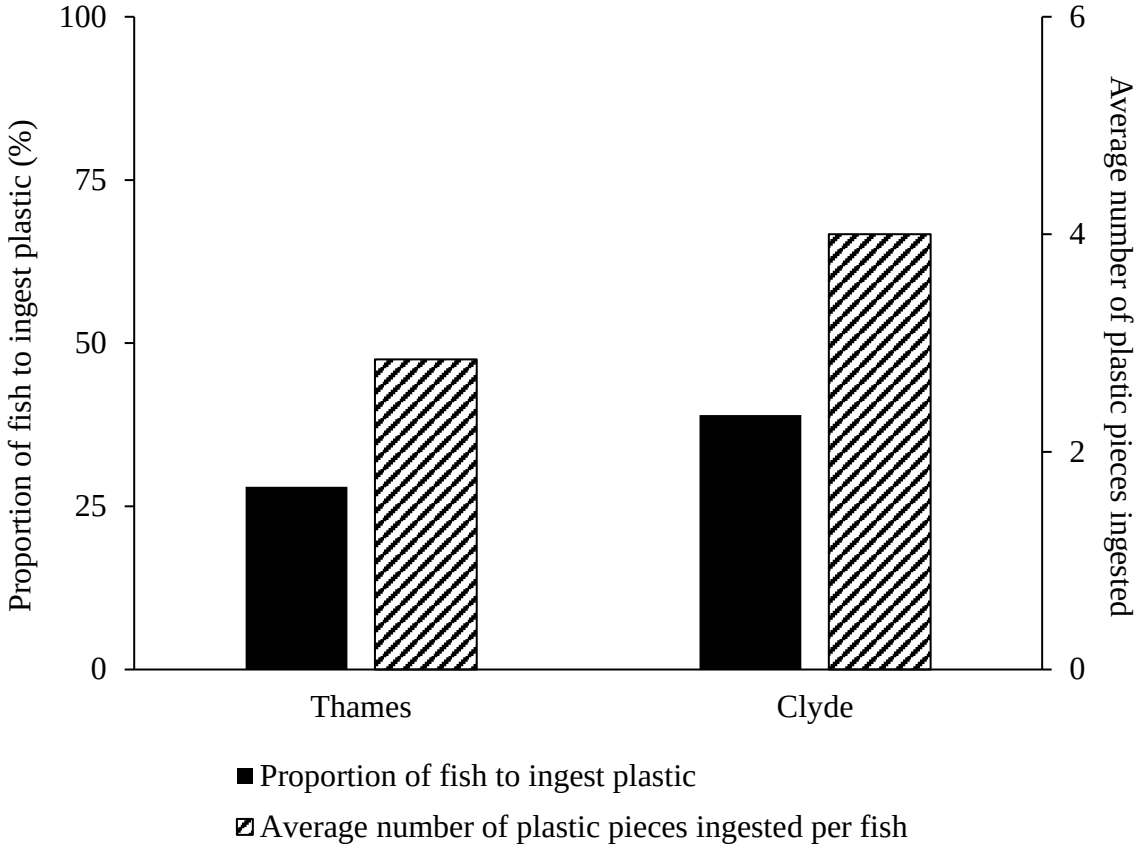


Fig. 3. A greater proportion of fish from the Clyde ingested plastic when compared to fish from the Thames. The average number of plastic pieces ingested per fish did not differ between the sites.

293

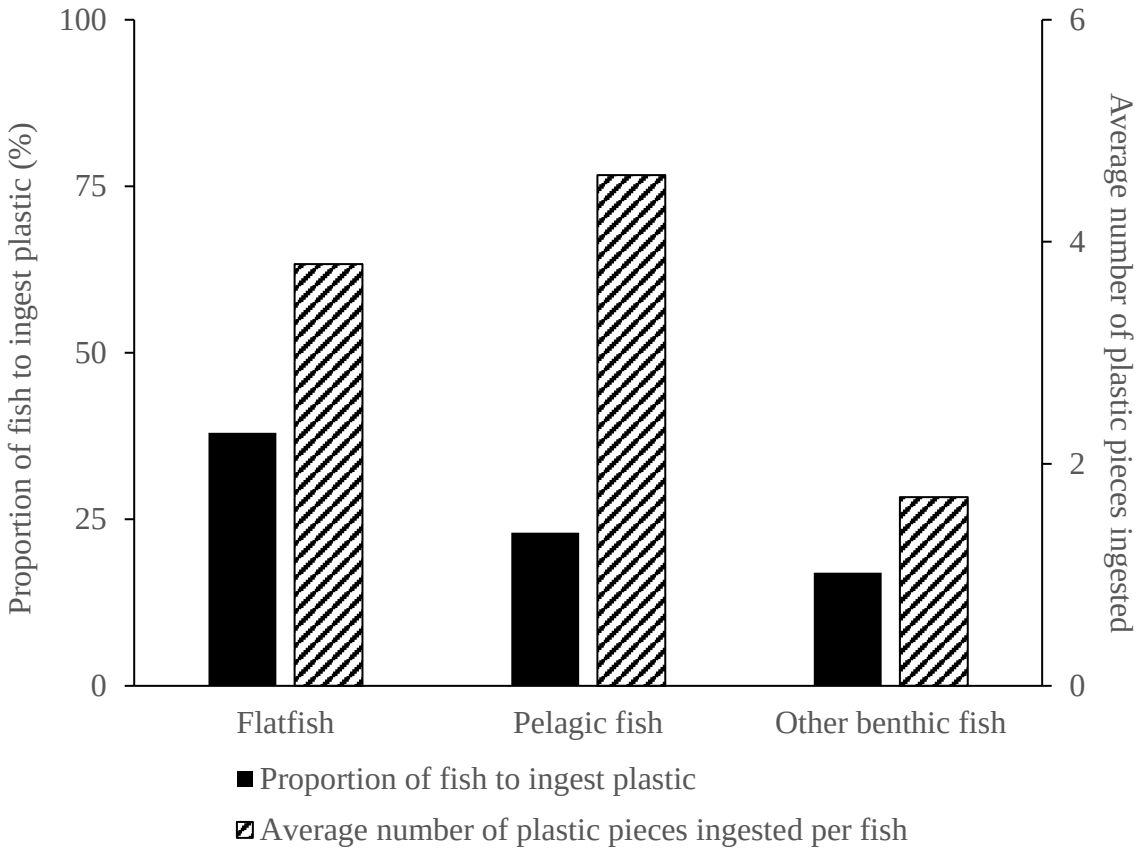


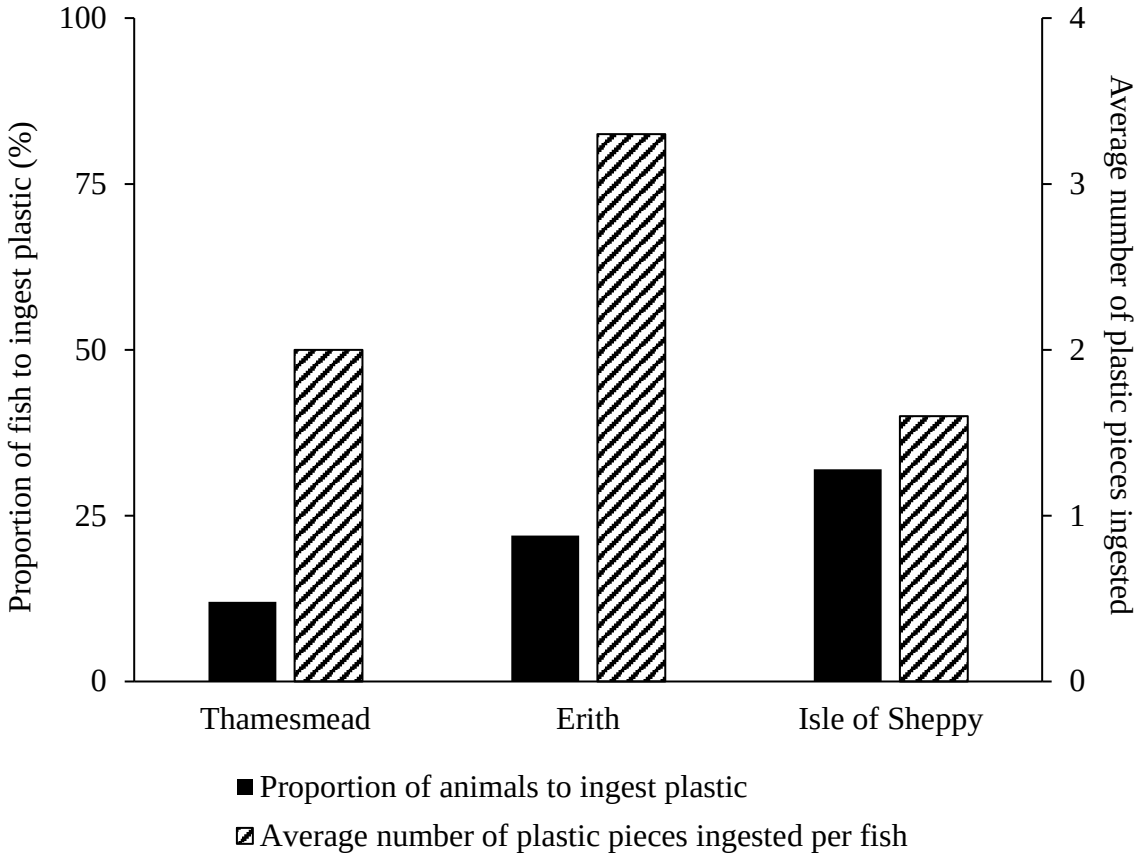
Fig. 4. A greater proportion of flatfish ingested plastic than other benthic fish, but not pelagic fish. Both flatfish and pelagic fish ingested, on average, more pieces of plastic than other benthic fish.

294

295 For model 2 and 6 (Table 1), Thames individuals were analysed separately from Clyde
296 fish. This allowed for the analysis of shrimp (only collected in the Thames) and the sub-
297 sampling sites in the Thames (Thamesmead, Erith and Isle of Sheppey). A greater proportion
298 of animals from Erith ingested plastic compared to Thamesmead ($P<0.05$, Erith: 22%,
299 Thamesmead: 12%; Fig. 5). Animals sampled from Erith also ingested more pieces of plastic
300 on average ($p < 0.05$, the average plastic ingestion per individual from Erith was 3.3 pieces,
301 compared to 2 in Thamesmead and 1.6 in the Isle of Sheppey; Fig. 5). Model 2 revealed that

larger organisms in the Thames tended to ingest plastic ($p < 0.001$, the average length of organisms to ingest plastic was 221.7 mm compared to 131.8 mm for organisms that did not ingest plastic). In the Thames, a greater proportion of flatfish ingested plastic than other benthic fish, the other feeding groups did not significantly differ from each other ($p < 0.05$, 33% of flatfish in the Thames ingested plastic compared to 14% of pelagic fish, 19% of other benthic fish and 6% of shrimp). Pelagic fish and flatfish also ingested more pieces of plastic than shrimp did ($p < 0.05$, average plastic ingestion by flatfish was 2.9 pieces, for pelagic fish was 3.2 and for other benthic fish was 1.5).

A



B

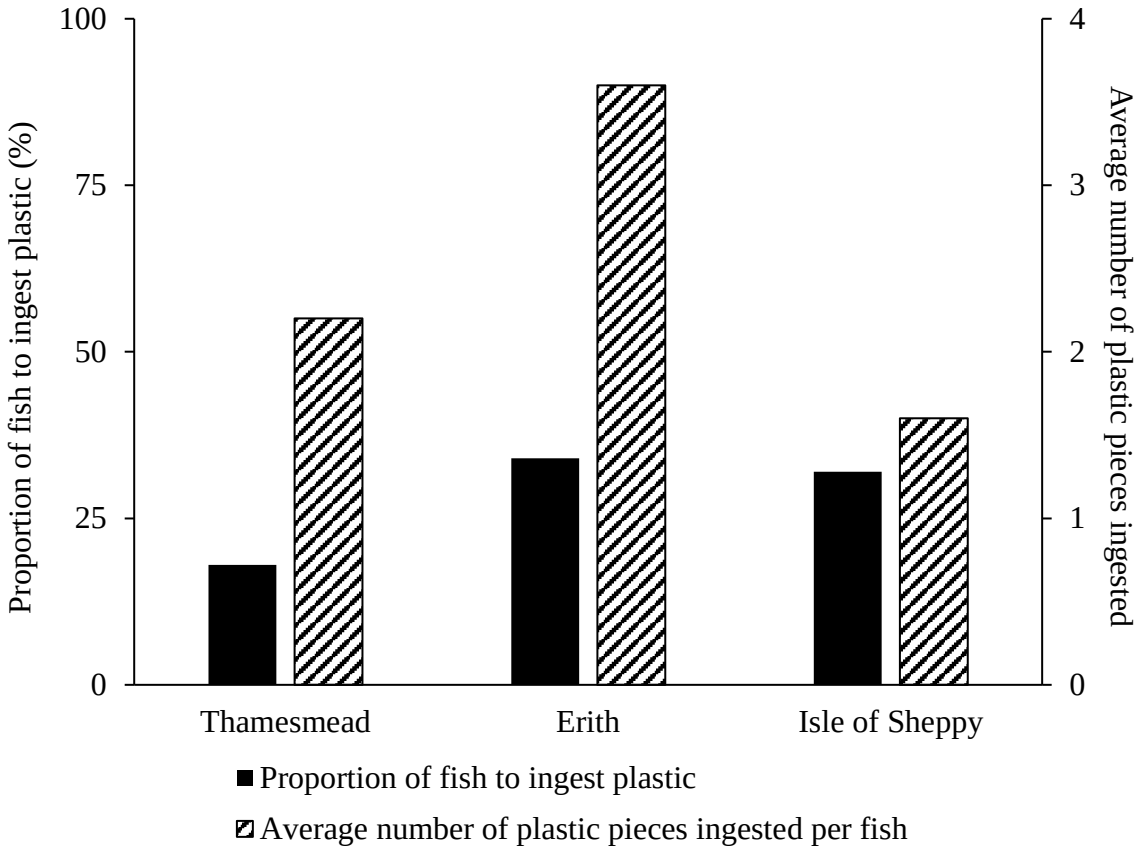


Fig. 5. The amount of A) animals (including both fish and shrimp) and B) fish (only) to ingest plastic and the average amount of plastic ingest by individual was greatest at Erith.

310

311 Models 3, 4, 7 and 8 (Table 1) compared the number of flounder in the Thames and
312 dab in the Clyde found to ingest plastic as well as the amount of plastic ingested by these
313 species. The models highlighted differences in the significance of gender on plastic ingestion.
314 For Thames flounder, gender did not significantly affect the number of fish found to ingest
315 plastic or the number of plastic pieces ingested ($p > 0.05$). However, in the Clyde, 59% of
316 female dab ingested plastic compared to 43% of males ($p < 0.05$; Fig. 6), on average ingesting
317 6 pieces of plastic and 2.9 pieces, respectively ($p < 0.01$; Fig. 6). The season of sampling also
318 had a significant impact on plastic ingestion by Clyde dab. A higher proportion of fish sampled

in the summer ingested plastic ($p < 0.001$, 71% compared to 6% in winter). Additionally, fish from the summer samples ingested an average 4.6 pieces of plastic per individual compared to 1.2 pieces ingested by winter fish ($p < 0.05$).

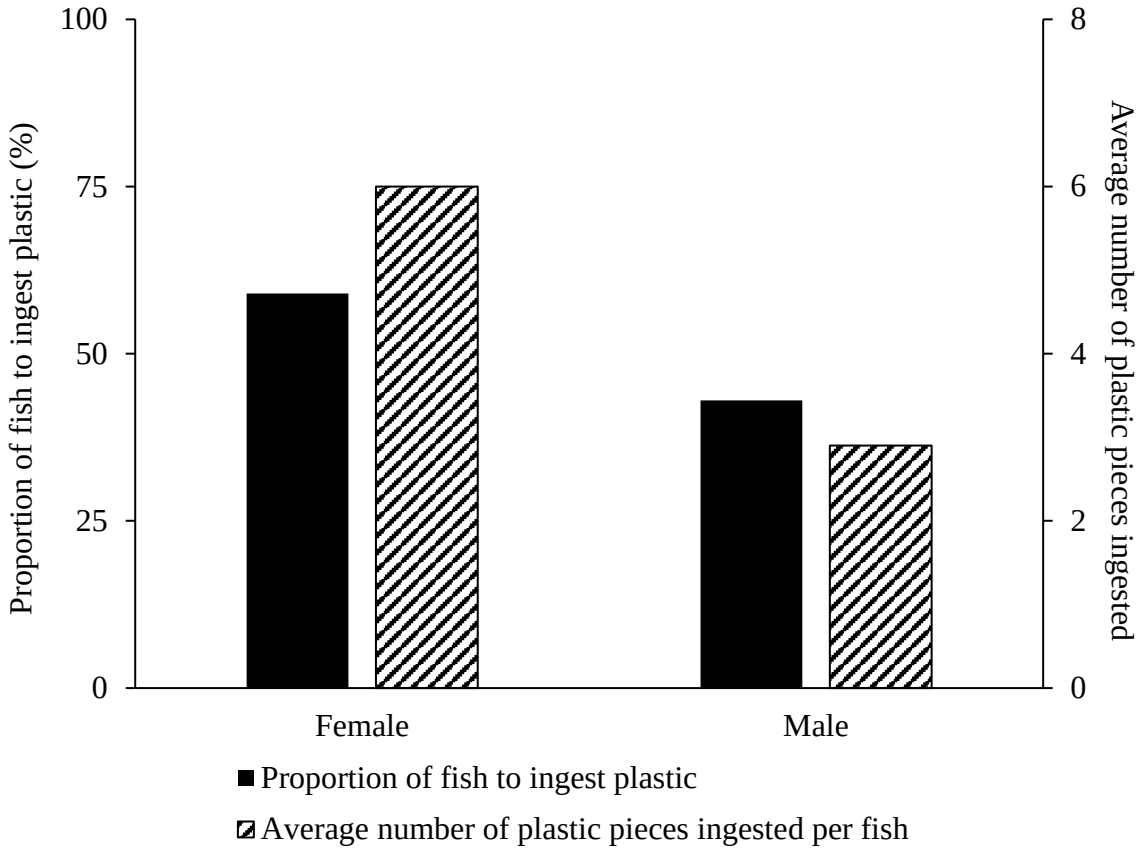


Fig. 6. More female dab, *Limanda limanda* (Linnaeus, 1758), in the Firth of Clyde ingested plastic than males; females also consumed significantly more plastic on average.

4. Discussion

4.1 Polymer diversity

The types of polymers recovered varied between the Firth of Clyde and the Thames Estuary. Nylon was the most abundant polymer recovered from the Thames Estuary whilst polyester was the most abundant polymer in the Firth of Clyde. Both nylon and polyester are used in the textile industry. Nylon is also used in fishing industry and polyester is a major

329 component of wet wipes. Products and by-products of these industries could be responsible for
330 much of the pollution in the Thames Estuary and Firth of Clyde. Indeed, Thames21 (2018)
331 have recovered over 5,450 wet wipes from the foreshore of the Thames. Despite the difference
332 in polymer type between the sites, the colours recovered were the same at both sites. Clear
333 fibres were the most abundant followed by black plastics.

334 *4.2 Limitations of FTIR*

335 Nylon samples produce similar spectra to those of organic polyamides and there is a
336 possibility of misidentification of both nylon and organic samples. If all nylon samples were
337 removed from this study, a minimum of 33% of fish would have ingested plastic – a similar
338 proportion of ingestion was reported in freshwater fish in the Thames (Horton et al., 2018).
339 The statistical analyses used in the present study assume that FTIR is accurate in its
340 identification of this synthetic polymer. Micro-FTIR had it been available in the present study,
341 would have likely increased the proportion of fibres accurately matched with library spectra.

342 *4.3 Plastic ingestion*

343 A lower proportion of fish from both sites ingested plastic than in the preliminary
344 study (McGoran et al., 2017), 36% compared to up to 75%. However, plastic was ingested by
345 fish at all sites and in all feeding groups, including previously poorly studied fish, such as
346 elasmobranchs (Smith, 2018). By including a greater diversity of species and a larger sample
347 size, the present study may better represent the state of plastic pollution in UK estuaries, but it
348 must also be noted that the methodology used in both studies only provides a snapshot of the
349 situation and that plastic ingestion may fluctuate. It is important to be aware that, in both the
350 Thames and the Clyde, sample size for pelagic fish and other benthic fish was relatively low
351 (other benthic: 21 in Thames, 14 in Clyde; pelagic fish: 37 in Thames, 10 in Clyde). This could
352 impact the strength of statistical analysis.

353 Larger individuals ingested more plastic than smaller ones irrespective of site of origin,
354 feeding group or gender. This is to be expected as larger animals will have greater energetic
355 requirements and will require a greater intake of food, increasing their chances of consuming
356 plastic. It may also be possible that the prey items of larger individuals, which differ from that
357 of smaller specimens (Schückel et al., 2012), are visually more similar to the plastics and are,
358 as such, ingested more frequently in larger fish. Schückel et al. (2012) reported that as dab
359 (*Limanda limanda*) and plaice (*Pleuronectes platessa*) grew, their diet focussed on larger prey,
360 such as polychaetes. Prey selection with regards to plastic ingestion was not examined in the
361 present study and is a topic for future analysis.

362 Although only a few species were present in both the Thames and the Clyde, the
363 similarity in their feeding strategies was used to overcome the differences in species
364 assemblages. Overall, more fish from the Firth of Clyde ingested plastic than in the Thames,
365 but the average number of plastic pieces ingested by fish did not differ between sites. Estuaries
366 are complex systems and it is not possible in the present study to determine the factors which
367 influence plastic ingestion. But, the Clyde and Thames represent different types of estuary
368 (fjord and coastal-plain respectively) and geological and hydrodynamic factors are likely
369 having an impact. Fjordic systems are defined by deep valleys in the riverbed. These ridges
370 may capture and accumulate microplastics. The Clyde was dominated by flatfish species,
371 which could be exposed to large quantities of plastic on the benthos. Bottom water in the Firth
372 of Clyde takes ca. 1 month to pass out to sea (Edwards et al., 1986) and plastics may therefore
373 be retained in the sediment for a long time. The slower flowing waters in the Clyde may
374 therefore help to explain why more plastic was consumed by fish in this catchment compared
375 to the Thames Estuary.

376 As estuaries are routes to the sea, it could be argued that microplastics should
377 accumulate downstream in the estuary (Isle of Sheppey). Indeed, Lee et al. (2013) found this

to be the case and Browne et al. (2010) noted that the high flow rate and turbulence in estuaries can keep high-density plastics suspended until they reach the sea. In the Thames, however, both a greater proportion of fish ingested plastic and the average amount of plastic ingested was greater in fish from Erith than in fish from Thamesmead and the Isle of Sheppey. Similarly, Morritt et al. (2014) also found that plastic in the Thames did not move downstream. The tidal nature of the Thames Estuary may lead to long-term upstream retention of microplastics. Additionally, plastic abundance increases with proximity to urban centres (Barnes et al., 2009; Corcoran, 2015). Perhaps a combination of the movement of plastics downstream and the tide pushing plastics upstream has resulted in plastics accumulating downstream of London and Thamesmead at Erith, but upstream of Sheppey. Additionally, a greater number of wastewater treatment plants are present near Erith (Westlake, 2016). Wastewater effluents are well documented as large microplastic inputs to rivers (as discussed in section 4.5). Large waste tips in the area could also be responsible for some of the plastic entering the estuary.

More flatfish ingested plastic than other benthic fish, which could highlight differences in their feeding strategies which makes flatfish more prone to ingesting plastic. It is possible that plastics may accumulate in the sediment; thus, plastic may be more available to bottom-dwelling organisms. Flatfish are closely associated with the sediment and can act as ambush predators. Some flatfish species are known to consume sediment with their prey (Hurst et al., 2007). This could be a route of exposure to microplastics. Flatfish may mistake plastic, especially fibres for prey, such as bivalve siphons and polychaetes. The digestive tracts of other benthic fish were also found to contain less plastic than those of pelagic fish and flatfish, but it must be noted that this is based on a small sample size of only 35 benthic fish. In the Thames, 33% of flatfish ingested plastic whilst only 14% of pelagic fish and 19% of other benthic fish consumed plastic. McGoran et al. (2017) also reported that flatfish in the Thames Estuary ingested more plastic than pelagic fish. In comparison however, some studies have reported no

403 such difference (Lusher et al., 2013). Analysis of sediment samples could provide evidence for
404 the retention of plastics in such deposits and go some way to explaining plastic ingestion in
405 flatfish.

406 The density of plastics, currents, turbulence, inflows, seabed topography and
407 hydrodynamics determine the depth distribution of plastic pieces in the water column (Cole et
408 al., 2011). High-density plastics and plastics coated in biofilms occur lower in the water column
409 and are expected to be prominent in the diet of benthic fish (Barnes et al., 2009; Cole et al.,
410 2011; Corcoran, 2015). In this study, polyvinyl chloride, acrylic and polyester, all of which are
411 high-density plastics, were found exclusively in flatfish. On the other hand low-density
412 microplastics (e.g. polyethylene and polystyrene), along with those plastics re-suspended by
413 turbulence, float near the water surface and are available to smaller organisms such as plankton
414 (Cole et al., 2011) as well as pelagic fish. Polystyrene (and polystyrene mixes) and
415 polyethylene were more abundant in benthic species. It is possible that consumption of
416 plankton by invertebrates or fish results in trophic transfer and bioaccumulation. Thompson et
417 al. (2004) proposed that polymer density does not influence the distribution of plastics,
418 recording various polymers in both the water column and the sediment. Many other factors can
419 influence the distribution of plastics. For example, plastic density can be impacted by biological
420 and environmental factors such that plastics can flocculate, increasing density, and sink (Barnes
421 et al., 2009). Song and Andrady (1991) reported that biofouling aids the sinking of plastics in
422 the marine environment. Tangled knots were only recorded in flatfish, as they likely sank with
423 the combined density of the fibres. Alternatively, they may have formed in the stomach of the
424 fish. In *N. norvegicus*, most ingested plastic was tangled filaments, and Murray and Cowie
425 (2011) suggested these balls originated from the sediment or were ingested through trophic
426 transfer. In addition, knots of fibres were observed in the gastric mill of Chinese mitten crabs,
427 *Eriocheir sinesis*, from the Thames (Emma Powell, pers. comm.) and perhaps formed due to

428 the action of the gastric mill. Native crab species were observed in the diet of flounder and
429 were perhaps a source of knotted fibres.

430 Female dab in the Clyde ingested more plastic than the males, but this trend was not
431 seen in Thames European flounder. Horton et al. (2018) also found that female freshwater fish
432 in the UK ingested more plastic than males. It has been suggested that water quality may impact
433 plastic ingestion differently for males and females, and that females may have larger energy
434 requirements than males; leading to increased food consumption and greater exposure to plastic
435 (Horton et al., 2018). Likewise, Vassilopoulou and Haralabous (2008) reported that female
436 flatfish have a lower condition factor than males, especially during the breeding season, due to
437 greater energy demands. Additionally, female dab grow quicker than males (Wheeler, 1969)
438 and may metamorphose earlier, becoming a substrate feeder sooner and having a higher
439 exposure to plastics. Females also take an additional year to become sexually mature (Wheeler,
440 1969), which could enable them to forage more whilst males compete for mates. Schückel et
441 al. (2012) demonstrated that diet changes as fish grow. It is possible that female dab, which
442 grow quicker, may be switching to a prey source that is visually more similar to plastic, such
443 as polychaetes, sooner than males. Additionally, size differences between dab and flounder
444 may facilitate resource partitioning, resulting in differences in plastic ingestion. On average,
445 Thames flounder ingested 3.1 pieces of plastic, whilst dab from the Clyde ingested a mean of
446 4.5 pieces of plastic (Table B.1, Appendix B). Differences in the behaviour of flounder and dab
447 may explain why only dab demonstrated variation in ingestion between genders. Hurst et al.
448 (2007) reported that the foraging behaviour of three co-existing flatfish species, which occupy
449 the same ecological guild, was determined by distribution, foraging times, habitat use and
450 differences in prey. The diets of flounder and dab differ, despite the species sharing some
451 common prey items. Adult flounder have a mostly mollusc-based diet, whereas dab have a
452 wider diet that mostly consists of crustaceans and polycahetes (Wheeler, 1969; Schückel et al.,

453 2012). Each niche could result in different exposures to plastic pollution. Although dab and
454 flounder are present in both the Thames and the Clyde, they are not equally abundant at both
455 sites. This makes comparisons difficult. Additionally, summer samples were collected from the
456 Firth of Clyde but not from the Thames. Seasonality had a significant impact on plastic
457 ingestion in the Clyde, with a much higher proportion of fish in summer ingesting plastic, on
458 average almost 4 times as much in winter. The same might have been true in the Thames.
459 Vassilopoulou (2006) found that the diet of *Lepidorphombus boscii* varied seasonally, with
460 individuals ingesting less material but a greater variety of prey items during winter and spring
461 compared to summer and autumn. This could result in variations in the amount or variety of
462 plastic ingested. Wheeler (1969) noted that flounder is a more active feeder during the warmer
463 months and in mid-winter can almost completely stop feeding. Additionally, both dab and
464 flounder spawn between February and June (Wheeler, 1969). By sampling dab in May, the
465 present study may have highlighted variation in foraging behaviour during the spawning
466 period. Too few trawls were taken, however, to accurately identify temporal differences in
467 plastic ingestion. Gender differences in plastic ingestion could not be fully explained in this
468 paper and are a subject for future analysis.

469 *4.4 Impacts of plastic ingestion*

470 Although plastic ingestion has the potential to cause many ill effects to aquatic organisms,
471 including abrasions, ulcers, false satiation and blockages in the digestive tract (Wright et al.,
472 2013), previous experiments into the effects of microplastics have used unrealistic
473 concentrations. Additionally, it is likely that many microplastics are passed through the
474 alimentary canal of a fish without complication (Jovanović et al., 2018). Large prey items, such
475 as shrimp and bivalves were found to have been ingested by most fish and it is likely that
476 plastics would be egested with waste remains of these prey items. No tangled fibres were large
477 enough to cause blockages or lead to false satiation since prey items bigger than the knots were

478 recorded. Grigorakis et al. (2017) demonstrated that neither microbeads nor microfibres were
479 retained in the gut longer than digesta, concluding that microplastics did not accumulate in the
480 gut over successive meals. Gut morphology is known, however, to impact the retention of
481 plastics (Jabeen et al., 2017). Equally, Jovanović et al. (2018) reported that virgin microplastics
482 did not accumulate in the alimentary canal of adult fish. After 45 days of exposure to
483 microplastics no stress or ill-effects were reported.

484 *4.5 Trophic transfer*

485 In the present study, pelagic fish and flatfish ingested more plastic than shrimp. This
486 could be indicative of bioaccumulation in the food chain. Plastics available to lower trophic
487 level organisms, such as crustaceans and bivalves, could also be accessible to higher trophic
488 level organisms, such as fish, through ingestion. Welden and Cowie (2016) found that *N.*
489 *norvegicus* ingested plastic and reported that an average 74% of their diet consisted of
490 crustaceans and bivalves. Similarly, *C. crangon* feed on a range of organisms including
491 molluscs (Devriese et al., 2015) and it is well documented that molluscs ingest plastics (Van
492 Cauwenberghe and Janssen, 2014; Van Cauwenberghe et al., 2015). Research by Farrell and
493 Nelson (2013) suggests that the plastic load of molluscs could be passed on to *Crangon* via
494 ingestion. Welden and Cowie (2016) reported that smaller langoustine retained more plastic in
495 the foregut, likely due to the morphology of the gastric mill plates. It is possible that the same
496 is true for *Crangon* and that plastics from the shrimp could potentially be transferred to the
497 fish. Brown shrimp make up a large part of the diet of the two most common fish in the present
498 study, dab and flounder (Wheeler, 1969), and were found in the gut of many of the fish in this
499 study (including flounder, pouting, sole, whiting, roker and eel).

500 In this study, 6% of *Crangon* ingested plastics, whilst a much higher proportion of fish
501 ingested plastics (36%). The difference in consumption of plastic by fish and shrimp may be

due to bioaccumulation. In a study by Devriese et al. (2015), 63% of *Crangon* ingested plastics, which could result in a high plastic exposure for fish through their diet. Furthermore, the acid digestion protocol used by Devriese et al. (2015) may have recovered plastics that were left undiscovered in the present study, where only the stomach was searched. In fact, Devriese et al. (2015) reported the multipart intestinal tract of *Crangon* as a key factor in the storage of plastics. Future analysis of *Crangon* should use a digestion protocol or investigate the whole digestive system to ensure that all microplastics are recovered. Moult stage, size and sex also impact plastic retention in crustaceans (Welden and Cowie, 2016). Shellfish, including the white furrow shell, *Abra alba*, and polychaetes were also recorded in the diets of flatfish in the present study and the literature (Wheeler, 1969), whilst cod, dogfish and eels all ingested fish, in some cases whole. Dab have also been recorded as feeding on fish (Wheeler, 1969). While evidence of dogfish (*Scyliorhinus canicula*) ingesting plastic is limited. The present study produces an estimate of ingestion in line with the only other published study to include this species: 14% compared to 15% by Smith (2018). As an opportunistic predator, feeding on crustaceans and fish, as well as many other prey items, dogfish could be exposed to plastic through their prey and through the water column. The present study indicates that fish consume plastic and previous studies have established plastic ingestion in bivalves and polychaetes (Van Cauwenberghe et al., 2015). Trophic transfer has been demonstrated in laboratory studies (Murray and Cowie, 2011; Farrell and Nelson, 2013; Watts et al., 2014), suggesting that these could act as potential sources of plastic for predatory fish species. It should be noted that trophic transfer may be having a minimal effect on plastic retention (Chagnon et al., 2018).

4.5 Sources of plastic

The Clyde and Thames Estuaries are major shipping channels (Port of London Authority, no date). The considerable maritime traffic associated with these two catchments potentially results in significant inputs of plastic litter from shipping (Haig, 1986; Tivy, 1986),

527 which could break down and account for many of the fibres recorded in this present study. In
528 the Adriatic Sea, 53% of plastic originated from fisheries and the aquaculture industry
529 (Strafella et al., 2015). Cole et al. (2011) reported that fishing gear, typically made of nylon,
530 could be found at variable depths in the sea, becoming available to both pelagic and benthic
531 fish. This was supported by the present study, which found that benthic fish, including flatfish,
532 and pelagic fish had ingested nylon. In addition, *Crangon* had also ingested nylon filaments.
533 Plastic fibres have also been linked to sewage works (Dubaish and Liebezeit, 2013; Free et al.,
534 2014), as shown by Browne et al. (2011) who reported that sites which have been used for
535 sewage disposal contained 250% more plastic, mostly fibres, compared to locations which did
536 not have sewage deposits. Many wastewater treatment plants do not have filters small enough
537 to remove microplastics (Mourgkogiannis et al., 2018). Some of these plants release only a
538 small number of particles per litre of effluent (Ziajahromi et al., 2017). However, the large
539 volumes of effluent released result in many thousands of particles entering waterways.
540 Additionally, particles removed from wastewater accumulate in sewage sludge, tens of
541 thousands per kg of dry sludge (Li et al., 2018). This sludge is often applied to agricultural
542 land, providing both a route to terrestrial systems, but also back into aquatic systems through
543 run off. The sewage works in the Thames Estuary are likely responsible for many of the fibres
544 in this study. Browne et al. (2011) found a similar abundance of synthetic fibres in wastewater
545 effluents at sites used for sewage disposal and expected that these originated from washing
546 machine outputs. The researchers also demonstrated that some textiles can shed nearly 2,000
547 fibres per wash (Browne et al., 2011).

548 Woven fibres were identified as polypropylene by FTIR, although some produced
549 spurious results. These likely originate from larger sources (e.g. sanitary products, which are
550 abundant in the Thames; Morritt et al., 2014). Plastic films also originate from larger sources,
551 such as carrier bags.

In conclusion, this study reveals that plastics are ingested by both benthic and pelagic fish populations from two UK estuaries, although to a lesser degree than expected, when compared to McGoran et al. (2017). More fish from the Clyde and more flatfish ingested plastic. Our results highlight the severity of estuarine plastic pollution in the UK and the need for more research into freshwater and estuarine ecosystems.

Acknowledgements

The authors would like to acknowledge the following people for their assistance with sampling for this study: Dave Pearce, Duncan Fraser, Tom Stevenson and Stuart Cape. We would also like to thank Dr James Hourston, Dr Lena Grinsted, Dr Sarah Papworth and Dr Ferran Palero for their contributions to statistical analysis. We would also like to thank an anonymous reviewer for their comments which have helped to greatly improve the quality of the manuscript.

Funding: This work was partly supported by The University of London Sheina Marshall Memorial Fund.

Author Contributions (initials in alphabetical order)

ARM, DM and PRC conceived and designed the study, with ARM and PFC undertaking data collection. ARM and JPM analysed the data. Materials and analysis tools were contributed by JPM and PRC. The paper was written and approved by all authors: ARM, DM, JPM, PFC, PRC.

References

Andrady, A.L., 2011. Microplastics in the marine environment. Mar. Poll. Bull. 62, 1596–1605. https://doi.org/10.1016/j.marpolbul.2011.05.030.

574 Avery-Gomm, S., Provencher, J.F., Morgan, K.H., Bertram, D.F., 2013. Plastic ingestion in
575 marine-associated bird species from the eastern North Pacific. Mar. Poll. Bull. 72,
576 257–259. https://doi:10.1016/j.marpolbul.2013.04.021.

577 Barnes, D.K.A., Galgani, F., Thompson, R.C., Barlaz, M., 2009. Accumulation and
578 fragmentation of plastic debris in global environments. Phil. Trans. R. Soc. B 364,
579 1985–1998. https://doi:10.1098/rstb.2008.0205.

580 Bessa, F., Barría, P., Neto, J.M., Frias, J.P.G.L., Otero, V., Sobral, P., Marques, J.C., 2018.
581 Occurrence of microplastic in commercial fish from a natural estuarine environment.
582 Mar. Poll. Bull. 128, 575–584. https://doi.org/1016/j.marpolbul.2018.01.044.

583 Boerger, C. M., Lattin, G.L., Moore, S. L. & Moore, C. J., 2010. Plastic ingestion by
584 planktivorous fishes in the North Pacific Central Gyre. Mar. Poll. Bull. 60, 2275–
585 2278. https://doi.org/10.1016/j.marpolbul.2010.08.007.

586 Browne, M.A., Crump, P., Niven, S.J., Teuten, E., Tonkin, A., Galloway, T., Thompson, R.,
587 2011. Accumulation of microplastic on shorelines worldwide: sources and sinks.
588 Envir. Sci. Technol. 45, 9175–9179. https://doi:10.1021/es201811s.

589 Browne, M.A., Galloway, T.S., Thompson, R.C., 2010. Spatial patterns of plastic debris
590 along estuarine shorelines. Environ. Sci. Technol. 44, 3404–3409.
591 https://doi:10.1021/es903784e.

592 Chagnon, C., Thiel, M., Antunes, J., Ferreira, J.L., Sobral, P., Ory, N.C., 2018. Plastic
593 ingestion and trophic transfer between Easter Island flying fish (*Cheilopogoc*
594 *rapanouiensis*) and yellowfin tuna (*Thunnus albacares*) from Rapa Nui (Easter
595 Island). Environ. Pollut. 243, 127 – 133. https://doi.org/10.1016/j.envpol.2018.08.042.

596 Cole, M., Lindeque, P., Halsband, C., Galloway T.S., 2011. Microplastics as contaminants in
597 the marine environment: a review. Mar. Poll. Bull. 62, 2588–2597.
598 https://doi:10.1016/j.marpolbul.2011.09.025.

599 Corcoran, P.L., 2015. Benthic plastic debris in marine and freshwater environments. Environ.
600 Sci. Proc. Imp. 17, 1363–1369. https://doi:10.1039/c5em00188a.

601 Corcoran, P.L., Norris, T., Ceccanese, T., Walzak, M.J., Helm, P.A., Marvin, C.H., 2015.
602 Hidden plastics of Lake Ontario, Canada and their potential preservation in the
603 sediment record. Envir. Poll. 204, 17–25. https://doi:10.1016/j.envpol.2015.04.009.

604 Devriese, L.I., van der Meulen, M.D., Maes, T., Bekaert, K., Paul-Pont, I., Frère, L.,
605 Robbens, J., Vethaak, A.D., 2015. Microplastic contamination in brown shrimp
606 (*Crangon crangon*, Linnaeus 1758) from coastal waters off the Southern North Sea
607 and Channel area. Mar. Poll. Bull. 98, 179–187.
608 https://doi:10.1016/j.marpolbul.2015.06.051.

609 Dubaish, F., Liebezeit, G., 2013. Suspended microplastics and black carbon particles in the
610 Jade system, southern North Sea. Water Air Soil Poll. 224, 1352.
611 https://doi:10.1007/s11270-012-1352-9.

612 Edwards, A., Baxter, M.S., Ellett, D.J., Martin, J.H.A., Meldrum, D.T., Griffiths, C.R., 1986.
613 Clyde Sea Hydrography. Proc. R. Soc. Edinb. B 90, 67–83.

614 Environment Agency, 2016. Water for life and livelihoods. Part 1: Thames river basin district
615 River basin management plan.

616 FAO, 2016. The state of world fisheries and aquaculture 2016. Contributing to food security
617 and nutrition for all. Rome. pp. 1–200.

618 Farrell, P., Nelson, K., 2013. Trophic level transfer of microplastic: *Mytilus edulis* (L.) to
619 *Carcinus maenas* (L.). Envir. Poll. 177, 1–3.
620 https://doi:10.1016/j.envpol.2013.01.046.

621 Foekema, E.M., De Gruijter, C., Mergia, M.T., van Franeker, J.A., Murk, A.J., Koelmans,
622 A.A., 2013. Plastic in North Sea fish. Environ. Sci. Technol. 47, 8818–8824.
623 https://doi.org/10.1021/es400931b.

624 Free, C.M., Jensen, O.P., Mason, S.A., Eriksen, M., Williamson, N.J., Boldgiv, B., 2014.
625 High-levels of microplastic pollution in a large, remote, mountain lake. Mar. Poll.
626 Bull. 85, 156–163. https://doi:10.1016/j.marpolbul.2014.06.001.

627 Galgani, F., Leate, J.P., Moguedet, P., Souplet, A., Verin, Y., Carpentier, A., Goraguer, H.,
628 Latrouite, D., Andral, B., Cadiou, Y., Mahe, J.C., Poulard, J.C., Nerisson, P., 2000.
629 Litter on the sea floor along European coasts. Mar. Poll. Bull. 40, 516–527.
630 https://doi:10.1016/S0025-326X(99)00234-9.

631 Gallagher, A., Rees, A., Rowe, R., Stevens, J., Wright, P., 2016. Microplastics in the Solent
632 estuarine complex, UK: an initial assessment. Mar. Poll. Bull. 102, 243–249.
633 https://doi:10.1016/**j**.marpolbul.2015.04.002.

634 Greaves, P.H., Saville, B.P., 1995. Microscopy of textile fibres. Royal Microscopical Society
635 Microscopy handbooks 32. Bios Scientific Publishers.

636 Grigorakis, S., Mason, S.A., Drouillard, K.G., 2017. Determination of the gut retention of
637 plastic microbeads and microfibers in goldfish (*Carassius auratus*). Chemosphere
638 169, 233–238. https://doi.org/10.1016/j.chemosphere.2016.11.055.

639 Haig, A.J.N., 1986. Use of the Clyde Estuary and Firth for the disposal of effluents. Proc. R.
640 Soc. Edinb. B 90, 393–405. https://doi.org/10.1017/S026972700000511X.

641 Horton, A.A., Jürgens, M.D., Lahive, E., van Bodegom, P.M., Vijver, M.G., 2018. The
642 influence of exposure and physiology on microplastic ingestion by the freshwater fish
643 Rutilus rutilus (roach) in the River Thames, UK. Environ. Pollut. 236, 188–194.
644 https://doi.org/10.1016/j.envpol.2018.01.044.

645 Hurst, T.P., Ryer, C.H., Ramsey, J.M., Haines, S.A., 2007. Divergent foraging strategies of
646 three co-occurring north Pacific flatfish. Mar. Biol. 151, 1087–1098.
647 https://doi.org./10.1007/s00227-006-0541-8.

648 ICES [online], 2015. ICES Special request advice northeast Atlantic and Arctic Ocean.
649 1.6.6.1 OSPAR request on development of a common monitoring protocol for plastic
650 particles in fish stomachs and selected shellfish on the basis of existing fish disease
651 surveys. Available at:
652 www.ices.dk/sites/pub/Publication%20Reports/Advice/2015/Special_Requests/OSPA
653 R_PLAST_advice.pdf [Accessed 4th April 2018].

654 Jabeen, K., Su, L., Li, J., Yang, D., Tong, C., Mu, J., Shi, H., 2017. Microplastics and
655 mesoplastics in fish from coastal and fresh waters of China. Environ. Pollut. 221,
656 141–149. https://doi.org/10.1016/j.envpol.2016.11.055.

657 Jambeck, J.R., Greyer, R., Wilcox, C., Siegler, T.R., Perryman, M., Andrady, A., Narayan,
658 R., Law, K.L., 2015. Plastic waste inputs from land into the ocean. Science 347, 768–
659 771. https://doi:10.1126/science.1260352.

660 Jardine, W.G., 1986. The geological and geomorphological setting of the Estuary and Firth of
661 Clyde. Proc. R. Soc. Edinb. B 90, 25–41.
662 https://doi.org/10.1017/S0269727000004851.

663 Jovanović, B., Gökdag, K., Güven, O., Emre, Y., Whitley, E.M., Kideys, A.E., 2018. Virgin
664 microplastics are not causing imminent harm to fish after dietary exposure. Mar. Poll.
665 Bull. 130, 123–131. https://doi.org/10.1016/j.marpolbul.2018.03.016.
666 Karami, A., Golieskardi, A., Choo, C.K., Romano, N., Ho, Y.B., Salamatinia, B., 2017. A
667 high-performance protocol for extraction of microplastics in fish. Sci. Total Environ.
668 578, 485–495. https://doi.org/j.scitotenv.2016.10.213.
669 Kühn, S., van Werven, B, van Oyen, A., Meijboom, A., Bravo Rebolledo, E.L., van Franeker,
670 J.A., 2017. The use of potassium hydroxide (KOH) solution as a suitable approach to
671 isolate plastics ingested by marine organisms. Mar. Poll. Bull. 115, 86–90.
672 https://doi.org/10.1016/j.marpolbul.2016.11.034.
673 Lechner, A., Keckeis, H., Lumesberger-Loisl, F., Zens, B., Krusch, R., Tritthart, M., Glas,
674 M., Schludermann, E., 2014. The Danube so colourful: a potpourri of plastic litter
675 outnumbers fish larvae in Europe's second largest river. Envir. Poll. 188, 177–181.
676 https://doi:10.1016/j.envpol.2014.02.006.
677 Lee, J., Hong, S., Song, Y.K., Hong, S.H., Jang, Y.C., Jang, M., Heo, N.W., Han, G.M., Lee,
678 M.J., Kang, D., Shim, W.J., 2013. Relationships among the abundances of plastic
679 debris in different size classes on beaches in South Korea. Mar. Poll. Bull. 77, 349–
680 354. https://doi:10.1016/j.marpolbul.2013.08.013.
681 Li, X., Chen, L., Mei, Q., Dong, B., Dai, X., Ding, G., Zeng, E.Y., 2018. Microplastics in
682 sewage sludge from the wastewater treatment plants in China. Water Res. 142, 75–85.
683 https://doi.org/10.1016/j.watres.2018.05.034.
684 Lusher, A.L., Hernandez-Milian, G., O'Brien, J., Berrow, S., O'Connor, I., Officer, R., 2015.
685 Microplastic and macroplastic ingestion by deep diving, oceanic cetacean: the True's

686 beaked whale *Mesoplodon mirus*. Envir. Poll. 199, 185–191.
687 https://doi:10.1016/**j**.envpol.2015.01.023.

688 Lusher, A.L., McHugh, M., Thompson, R.C., 2013. Occurrence of microplastics in the
689 gastrointestinal tract of pelagic and demersal fish from the English Channel. Mar.
690 Poll. Bull. 67, 94–99. https://doi.org/10.1016/j.marpolbul.2012.11.028.

691 Lusher, A.L., Welden, N.A., Sobral, P., Cole M., 2017. Sampling, isolating and identifying
692 microplastics ingested by fish and invertebrates. Anal. Methods 9, 1346–1360.
693 https://doi:10.1039/C6AY02415G.

694 McGoran, A.R., Clark, P.F., Morritt, D., 2017 Presence of microplastic in the digestive tracts
695 of flounder, *Platichthys flesus*, and smelt, *Osmerus eperlanus*, from the River
696 Thames. Envir. Poll. 220 (Part A), 744–751.
697 https://doi.org/10.1016/j.envpol.2016.09.078.

698 Moore, C.J., 2008. Synthetic polymers in the marine environment: A rapidly increasing, long-
699 term threat. Envir. Res. 108, 131–139. https://doi:10.1016/j.envres.2008.07.025.

700 Morritt, D., Stefanoudis, P.V., Pearce, D., Crimmen, O.A., Clark, P.F., 2014. Plastic in the
701 Thames: a river runs through it. Mar. Poll. Bull. 78, 196–200.
702 https://doi.org/10.1016/j.marpolbul.2013.10.035.

703 Mourgkogiannis, N., kalavrouziolis, I.K., Karapanagioti, H.K., 2018. Questionnaire-based
704 survey to managers of 101 wastewater treatment plants in Greece confirms their
705 potential as plastic marine litter sources. Mar. Poll. Bull. 133, 822–827.
706 https://doi.org/10.1016/j.marpolbul.2018.06.044.

Murphy, F., Russell, M., Ewins C., Quinn, B., 2017. The uptake of macroplastic & microplastic by demersal and pelagic fish in the Northeast Atlantic around Scotland. Mar. Poll. Bull. 122, 353–359. https://doi.org/10.1016/j.marpolbul.2017.06.073.

Murray, F., Cowie, P.R., 2011. Plastic contamination in the decapod crustacean *Nephrops norvegicus* (Linnaeus, 1758). Mar. Poll. Bull. 62, 1207–1217. https://doi:10.1016/j.marpolbul.2011.03.032.

Phillips, M.B., Bonner, T.H., 2015. Occurrence and amount of microplastic ingested by fishes in watershed of the Gulf of Mexico. Mar. Poll. Bull. 100, 264–269. https://doi:10.1016/j.marpolbul.2015.08.041.

PlasticsEurope, 2018. Plastics – the facts 2017 [online]. Available at: http://www.plasticseurope.org/Document/plastics---the-facts-2017.aspx [Accessed 25th January 2018].

Port of London Authority. Part of daily life [online], no date. Available at: https://www.pla.co.uk/Port-Trade/Part-of-daily-life [Accessed 2nd February 2018].

Reisser, J., Shaw, J., Wilcox, C., Hardesty, B.D., Proietti, M., Thums, M., Pattiaratchi, C., 2013. Marine plastic pollution in waters around Australia: characteristics, concentrations, and pathways. PLoS ONE 8, e80466. https://doi:10.1371/journal.pone.0080466.

Schückel, S., Sell, A.F., Kröncke, I., Reiss, H., 2012. Diet overlap among flatfish species in the southern North Sea. J. Fish. Biol. 80, 2571–2594. https://doi.org/10.1111/j.1095-8649.2012.03309.x.

Scottish Environmental Protection Agency (SEPA), 2015. Flood risk management strategy. Clyde and Loch Lomond local district plan [online]. Available at:

http://apps.sepa.org.uk/FRMStrategies/pdf/lpd/LPD_11_Sources.pdf [Accessed 14th April 2016].

Smith, L.E., 2018. Plastic ingestion by *Scyliorhinus canicular* trawl captured in the North Sea. Mar. Poll. Bull. 130, 6–7. https://doi.org/10/1016/j.marpolbul.2018.03.001.

Song, Y.E., Andrady, A.L., 1991. Fouling of floating plastic debris under Biscayne Bay exposure conditions. Mar. Poll. Bull. 22, 608–613. https://doi.org/10.1016/0025-326X(91)90249-R.

Strafella, P., Fabi, G., Spagnolo, A., Grati, F., Polidori, P., Punzo, E., Fortinuoni, T., Marceta, B., Raicevich, S., Cvitkovic, I., Despalatovic, M., Scarcella, G., 2015. Spatial pattern and weight of seabed marine letter in northern and central the Adriatic Sea. Mar. Poll. Bull. 91, 120–127. https://doi.org/10.3354/meps12002.

Thames21, 2018. Record number of wet wipes found on Thames foreshore [online]. Available at: https://www.thames21.org.uk/2018/04/record-number-wet-wipes-found-thames-foreshore/ [Accessed 22nd September 2018].

The Scottish Government, 2012. Clyde ecosystem review [online]. Scottish Marine and Freshwater Science 3. Available at: http://www.gov.scot/Publications/2012/06/7562/0 [Accessed 22nd November 2016].

Thermo Fisher Scientific, 2015. iD5 ATR Accessory for the Nicolet™ iS5 Spectrometer [online]. Available at: https://www.thermofisher.com/order/catalog/product/IQLAADGAAGFAJAMAYY?ICID=search-product [Accessed 27th July 2016].

Thomas, M., 1998. Temporal changes in the movements and abundance of Thames estuary fish populations. In: Attrill, M.J. (Ed.) A rehabilitated estuarine ecosystem: the

753 environment and ecology of the Thames Estuary. London: Kluwer Academic
754 publishers, pp. 115–139.

755 Thompson, R.C., Olsen, Y., Mitchell, R.P., Davis, A., Rowland, S.J., John, A.W.G.,
756 McGonigle, D. Russel, A.E., 2004. Lost at sea: where is all the plastic? Science 304,
757 838. http://doi:10.1126/science.1094559.

758 Tinsley, D., 1998. The Thames estuary: a history of the impact of humans on the environment
759 and a description of the current approach to environmental management. In: Attrill,
760 M.J. (Ed.) A rehabilitated estuarine ecosystem: the environment and ecology of the
761 Thames Estuary. London: Kluwer Academic publishers, pp. 5–26.

762 Tivy, J., 1986. The geography of the Estuary and Firth of Clyde. Proc. R. Soc. Edinb. B 90,
763 7–23.

764 Van Cauwenberghe, L., Claessens, M., Vandegehuchte, M.B., Janssen, C.R., 2015.
765 Microplastics are taken up by mussels (*Mytilus edulis*) and lugworms (*Arenicola*
766 *marina*) living in natural habitats. Envir. Poll. 199, 10–17.
767 http://doi:10.1016/j.envpol.2015.01.008.

768 Van Cauwenberghe, L., Janssen, C.R., 2014. Microplastics in bivalves cultured for human
769 consumption. Envir. Poll. 193, 65–70. https://doi.org/10.1016/j.envpol.2014.06.010.

770 Vassilopoulou, V., 2006. Dietary habits of the deep-sea flatfish *Lepidorphombus boscii* in
771 north-eastern Mediterranean waters. J. Fish Biol. 69, 1202–1220.
772 https://doi.org/10.1111/j.1095-8649.2006.01199.x.

773 Vassilopoulou, V., Haralabous, J., 2008. Effects of sexual maturity and feeding on condition
774 of a deep-sea flatfish, *Lepidorhombus boscii*, in north-eastern Mediterranean waters.
775 J. Nat. Hist. 42, 695-720. https://doi.org/10.1080/00222930701835647.

776 Watts, A.J.R., Lewis, C., Goodhead, R.M., Beckett, S.J., Moger, J., Tyler, C.R., Galloway,
777 T.S., 2014. Uptake and retention of microplastics by the shore crab *Carcinus maenas*.
778 Envir. Sci. Technol. 48, 8823–8830. https://doi.org/10.1021/es501090e.

779 Welden, N.A.C., Cowie, P.R., 2016. Environment and gut morphology influence microplastic
780 retention in langoustine, *Nephrops norvegicus*. Envir. Poll. 214, 856–865.
781 https://doi:10.1016/j.envpol.2016.03.067.

782 Westlake, S.-J., 2016. Thames Water's approach to managing wastewater [online]. Available
783 at: https://www.ceh.ac.uk/sites/default/files/20160217%20Sarah-
784 Jane%20Westlake%20-%20Thames%20Water%20Wastewater%20Management.pdf
785 [Accessed 23rd September 2018].

786 Wheeler, A., 1969. The Fishes of the British Isles and North-west Europe. London:
787 Macmillan.

788 Wheeler, A., 1978. Key to the fishes of Northern Europe: over 350 species described.
789 London: Frederick Warne.

790 Wilding, T.A., Duncan, J., Nickell, L.A., Hughes, D.J., Gontarek, S.J., Black, K.D., Sayer,
791 M.D.J., 2005. Synthesis of information on the benthos of SEA 6 Clyde Sea Area.
792 Report to the Department of Trade and Industry, Scottish Association of Marine
793 Science, Oban, Scotland, PA37 1QA

794 Williams, D.H., Fleming, I., 1995. Spectroscopic methods in organic chemistry, fifth edition.
795 London: McGraw-Hill Book Company

796 Woodall, L.C., Sanchez-Vidal, A., Canals, M., Paterson, G.L.J., Coppock, R., Sleight, V.,
797 Calafat, A., Rogera, A.D., Narayanaswamy, B.E., Thompson, R.C., 2014. The deep

798 sea is a major sink for plastic debris. R. Soc. Open Sci. 1, 140317.
799 https://doi:10.1098/rsos.140317.

800 Woodall, L.C., Robinson, L.F., Rogers, A.D., Narayanaswamy, B.E., Paterson, G.L.J., 2015.
801 Deep-sea litter: a comparison of seamounts, banks and a ridge in the Atlantic and
802 Indian Oceans reveal both environmental and anthropogenic factors impact
803 accumulation and composition. Front. Mar. Sci. 2, 3.
804 https://doi.org/10.3389/fmars.2015.00003

805 Wright, S.L., Thompson, R.C., Galloway, T.S., 2013. The physical impacts of microplastics
806 on marine organisms: a review. Envir. Poll. 178, 483–492.
807 https://doi:10.1016/j.envpol.2013.02.031.

808 WWF & Scottish Wildlife Trust Joint Marine Programme (JMP), 2004. The tangle of the
809 Clyde: why we must reform the management of Scotland's marine environment.

810 Ziajahromi, S., Neale, P.A., Rintoul, L., Leusch, F.D.L., 2017. Wastewater treatment plants
811 as a pathway for microplastics: development of a new approach to sample wastewater-
812 based microplastics. Water Res. 112, 93–99.
813 https://doi.org/10.1016/j.watres.2017.01.042.