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# 1 Microplastic ingestion in fish larvae in the western English Channel

2  
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4  
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## 11 12 **Abstract**

13 Microplastics have been documented in marine environments worldwide, where they pose a  
14 potential risk to biota. Environmental interactions between microplastics and lower trophic  
15 organisms are poorly understood. Coastal shelf seas are rich in productivity but also experience high  
16 levels of microplastic pollution. In these habitats, fish have an important ecological and economic  
17 role. In their early life stages, planktonic fish larvae are vulnerable to pollution, environmental stress  
18 and predation. Here we assess the occurrence of microplastic ingestion in wild fish larvae. Fish larvae  
19 and water samples were taken across three sites (10, 19 and 35 km from shore) in the western  
20 English Channel from April to June 2016. We identified 2.9% of fish larvae ( $n=347$ ) had ingested  
21 microplastics, of which 66% were blue fibres; ingested microfibers closely resembled those identified  
22 within water samples. With distance from the coast, larval fish density increased significantly  
23 ( $P<0.05$ ), while waterborne microplastic concentrations ( $P<0.01$ ) and incidence of ingestion  
24 decreased. This study provides baseline ecological data illustrating the correlation between  
25 waterborne microplastics and the incidence of ingestion in fish larvae.

## 26 27 28 29 **CAPSULE:**

30 We identified 2.9% of fish larvae ( $n=347$ ) had ingested microplastics (predominantly fibres) in the  
31 western English Channel. Ingested microfibers closely resembled those identified in water samples.

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## 35 **1. Introduction**

36 Microplastic (microscopic plastic, 0.1  $\mu\text{m}$ –5 mm) debris has emerged as a persistent environmental  
37 pollutant, recognised within the scientific and political community as a ubiquitous contaminant of  
38 global concern (Thompson *et al.*, 2004). The increasing abundance and widespread distribution of  
39 microplastics has led to concerns over the risks posed to the health of organisms and ecosystem  
40 processes (Clark *et al.*, 2016). Since the emergence of mass-produced plastics in the 1930s (BPF,  
41 2017), production has increased annually, currently reaching in excess of 322 million tonnes per year  
42 globally (PlasticsEurope, 2016). Its durability, low cost and widespread application has made plastic a  
43 popular manufacturing material worldwide (Cole *et al.*, 2011). These same characteristics make it  
44 difficult to dispose of, and once in the environment could be considered a persistent and potentially  
45 hazardous pollutant (Rochman *et al.*, 2013a). Marine plastic debris stems from poor waste  
46 management and accidental losses from fishing, industry, shipping and tourism among other sources  
47 (Jambeck *et al.*, 2015). Microplastic pollution originates from the photooxidative degradation and  
48 subsequent fragmentation of this larger debris (Jambeck *et al.*, 2015), termed secondary  
49 microplastics, and the release of plastics manufactured to be of a microscopic size, such as exfoliates  
50 in cosmetics (Napper *et al.*, 2015), termed primary microplastics. Microplastics in marine waters  
51 were first documented over forty years ago in the North Atlantic subtropical gyre (Carpenter, E. J., *et*  
52 *al.*, 1972). Microplastics have since been found in a diverse range of marine ecosystems, including  
53 deep ocean sediments (Van Cauwenberghe *et al.*, 2013) and Arctic waters (Lusher *et al.*, 2015).  
54 Recent estimates suggest over 5.25 trillion items of floating plastic litter are polluting the world's  
55 oceans, of which the vast majority are microscopic in size (Eriksen *et al.*, 2014).

56

57 Microplastic pollution poses a threat to marine biota through ingestion or entanglement (Wright *et*  
58 *al.*, 2013b). Continuous fragmentation and degradation of microplastics in the marine environment  
59 produces a wide range of particle sizes (Enders *et al.*, 2015), which can be ingested by an equally  
60 wide range of marine organisms, including the Humbolt squid (Braid *et al.*, 2012), blue mussel and  
61 Pacific oyster (Van Cauwenberghe and Janssen, 2014), gooseneck barnacle (Goldstein and Goodwin,  
62 2013), Norway lobster (Murray and Cowie, 2011), brown shrimp (Devriese *et al.*, 2015), zooplankton  
63 (Desforges *et al.*, 2015), harbour seal (Rebolledo *et al.*, 2013) and green turtle (Tourinho *et al.*,  
64 2010). The overlap between microplastics and marine biota is predicted to be most pronounced in  
65 shelf sea regions (Clark *et al.*, 2016), owing to high levels of biological productivity and high  
66 microplastic concentrations stemming from the proximity to terrestrial sources of pollution (e.g.  
67 rivers, estuaries, sewage outfalls) (Browne *et al.*, 2011, Desforges *et al.*, 2014).

68

69 Zooplankton encompass a diverse group of planktonic animals, including the larval stages of  
70 vertebrates and invertebrates. Marine zooplankton predominantly inhabit surface waters when  
71 feeding, where microplastics are found in high abundance (Cozar *et al.*, 2014), increasing the  
72 opportunity for them to ingest microplastics. Under laboratory conditions, zooplankton (e.g.  
73 copepods, urchin larvae, bivalve larvae, decapod larvae) have been observed to readily consume  
74 microplastics (Cole *et al.*, 2013, Cole and Galloway, 2015, Cole *et al.*, 2015, Nobre *et al.*, 2015, Setala  
75 *et al.*, 2014, Lee *et al.*, 2013, Kaposi *et al.*, 2014). Toxicity testing has highlighted the adverse physical  
76 (Wright *et al.*, 2013a) and toxicological effects that microplastic exposure can have on marine biota  
77 (Ogonowski *et al.*, 2016, Peda *et al.*, 2016, Watts *et al.*, 2016, Cole *et al.*, 2015). Experiments using  
78 marine worms and zooplankton have demonstrated that microplastic ingestion can result in reduced  
79 feeding, increased mortality, decreased growth rates, decreased hatching success and reduced  
80 fecundity (Wright *et al.*, 2013a, Cole *et al.*, 2015). Marine zooplankton are a vital source of food for  
81 secondary consumers (e.g. fish, cetaceans), and, as such, may represent a route via which  
82 microplastics enter the food web, posing a risk to secondary producers, apex predators and  
83 potentially human health (Clark *et al.*, 2016). Field observations detailing incidence of microplastic  
84 ingestion by organisms typically relate to larger organisms (e.g. squid, mussels, oysters, adult fish),  
85 owing to the constraints associated with collecting and processing samples (Lusher *et al.*, 2017).  
86 Research by Desforages *et al.* (2014) on zooplankton communities in the North East Pacific has shown  
87 microplastic ingestion ratios of 1 in 17 copepods (*Neocalanus cristatus*), and 1 in 34 euphausiids  
88 (*Euphausia pacifica*), of which 50-68% were fibres. Microplastics have been further identified in  
89 zooplankton communities sampled from the South China Sea, with 70% of identified plastics being  
90 fibrous (Sun *et al.*, 2016). Otherwise, very little is known about ingestion rates of microplastics in  
91 wild zooplankton and the type, source and distribution of plastic being ingested.

92

93 Fish stocks have considerable ecological and economic value. Global annual fisheries revenue  
94 fluctuates around USD 100 billion supporting about 12% of the world population, and providing 2.9  
95 billion people with 20% of their animal protein (Lam *et al.*, 2016). With over 30,000 species of fish  
96 worldwide, existing in all of the worlds marine habitats, their abundance and diversity has significant  
97 ecological importance for the food chain, nutrient cycling and ecosystem services (Worm *et al.*,  
98 2006). Ichthyoplanktonic studies show that unfished taxa account for the majority of fish larvae and  
99 contribute significantly to trophic food webs (Baran, 2002). Fish populations are vulnerable to a  
100 growing number of anthropogenic pressures, including overfishing, climate change and pollution,  
101 resulting in increased mortality and reduced fecundity. Incidence of microplastic consumption by  
102 adult fish has been widely reported for pelagic and demersal populations across the globe, including

103 blue whiting (*Micromesistius poutassou*), red gurnard (*Aspitrigla cuculus*), john dory (*Zeus faber*) and  
104 dragonet (*Callionymus lyra*) (Lusher *et al.*, 2013). However, there is currently no substantial  
105 published data regarding microplastic ingestion rates in fish larvae. Fish larvae play a pivotal role in  
106 marine food webs (Russell, 1976), and their health, development and survival is fundamental to the  
107 long-term sustainability of healthy fish populations. As such, data is urgently required to better  
108 assess the risks posed to fish larvae by microplastics *in natura*.

109

110 In this study we investigate the incidence of microplastic ingestion by fish larvae in the productive  
111 shelf-sea waters of the western English Channel, off the coast of Plymouth (UK). We look to test the  
112 hypotheses that: (1) microplastic concentrations increase with proximity to the coast; (2) fish larvae  
113 consume microplastic debris in their natural environment; and, (3) incidence of microplastic  
114 consumption is regulated by the abundance of larvae and the abundance of microplastics. Fish  
115 larvae and microplastics were collected via oblique tows, across three sites with varying distance  
116 from shore; microplastics were isolated using dissection and enzymatic digestion of samples.

117

## 118 **2. Methodology**

119

### 120 **2.1 Field sampling**

121 Field sampling was undertaken on board RV Plymouth Quest in the western English Channel off the  
122 coast of Plymouth (UK). Sampling was conducted at stations L4, L5 and E1 (10 km, 19 km and 35 km  
123 from shore respectively), which are routinely sampled as part of the Western Channel Observatory  
124 (WCO; [www.westernchannelobservatory.org.uk](http://www.westernchannelobservatory.org.uk)). The sampling sites spanned distances of 10-35 km  
125 from the city of Plymouth (Figure 1), accounting for habitats with a coastal (L4) and oceanic  
126 influence (E1); L5 was added as a reference site because it is a rocky reef known to be a favourable  
127 habitat for fish larvae. Eleven samples were collected between 11<sup>th</sup> April 2016 and 21<sup>st</sup> June 2016  
128 across the three sites (L4,  $n=5$ ; L5,  $n=3$ ; E1,  $n=3$ ). For each trawl, tow distance and maximal sample  
129 depths were recorded using GPS and a Suunto vyper dive computer respectively; maximum depths  
130 reached were on average 50 m at L4 and L5, and 65 m at E1. Fish larvae were collected using a 500  
131  $\mu\text{m}$  metal-framed net (1  $\text{m}^2$  square aperture) towed for 20 minutes on an oblique tow. Following the  
132 trawl, larvae were passed through a 500  $\mu\text{m}$  sieve and rinsed with filtered (0.22  $\mu\text{m}$ ) natural  
133 seawater. Subsequently, specimens were transferred into a 1 L Nalgene bottle and preserved in 4%  
134 formalin. Microplastics were sampled using a 100  $\mu\text{m}$  WP2 net (47 cm diameter aperture),  
135 suspended below the net used for sampling the fish larvae. This concurrent sampling allowed for  
136 direct comparison of microplastics ingested by the fish larvae with 'prey-sized' microplastics in the

137 surrounding water. Following sampling, the WP2 net was rinsed with filtered seawater and the  
138 sample poured through a 100 µm mesh; samples were immediately sealed and subsequently stored  
139 in a foil envelope in a -80°C freezer prior to analysis. Control measures included collection of  
140 procedural blanks using filtered sea water, and sampling of boat paint for Fourier Transform Infrared  
141 Spectroscopy (FT-IR) analysis to ensure false positives were avoided in the plastics count.  
142



143  
144 **Figure 1.** Sampling sites located in the western English Channel. E1: 35 km offshore Plymouth; L5: 19 km offshore; L4: 10  
145 km offshore.

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## 149 **2.2 Fish larvae**

150 Fish larvae were isolated by screening the formalin preserved net samples through a 2000 µm sieve.  
151 Specimens were rinsed thoroughly, and the 2000 µm sieve placed in a tray of water to float the  
152 sample inside the sieve. Fish larvae >10 mm were handpicked and placed into a covered beaker  
153 containing ultrapure water. The total number of fish larvae per sample was recorded, and fish larvae  
154 density (individuals m<sup>-3</sup>) calculated using the net dimensions, tow length and depth, and a net  
155 efficiency of 85% (Southward, A. J., 1970). All fish larvae larger than 9 mm were identified to species  
156 level.  
157

158 **2.3 Microplastic ingestion in marine fish larvae**

159 Fish larvae were assessed under a dissection microscope (Wild Heerbruug Switerland M5-49361; 6x-  
160 50x magnification) with gooseneck lighting (Schott KL1500 LCD). Individual fish larvae were placed in  
161 a Petri dish (50 mm) on a polycarbonate filter paper (Whatman cyclopore, 47 mm, 10  $\mu\text{m}$ ) and  
162 identified to species level (Russell, 1976, Munk and Nielsen, 2005). Larval length was recorded,  
163 however, accurate aging was not possible owing to variability in growth rates (Russell, 1976). Prior to  
164 dissection, larvae were checked for microplastics adhered to external surfaces. The jaw, oesophagus,  
165 stomach and intestines were removed using fine tweezers and needle. The digestive tract was  
166 inspected for microplastic particles in accordance with the Norén (2007) protocol: (1) no cellular or  
167 organic structures are visible; (2) if the particle is a fibre, it should be equally thick, not taper  
168 towards the ends and have a three-dimensional bending; (3) homogeneously coloured/clear  
169 particles. If a suspect particle was found, the particle, guts and fish were photographed and the  
170 particle sized (Olympus SZX16 Stereo Microscope with Canon DS126271 camera). A diamond  
171 compression cell (Specac DC2; 2 mm diameter) was used to prepare suspect microplastics prior to  
172 FT-IR analysis; FT-IR was conducted using a Bruker Vertex 70 micro FT-IR coupled with a Bruker  
173 Hyperion 1000 microscope. Spectra were assessed using Bruker Opus 7.5 software.

174

175 **2.4 Waterborne microplastics**

176 Waterborne microplastic samples were removed from storage, and then freeze dried for 72 hours  
177 (Scanvac CoolSafe freeze drier). Desiccated samples were put through an enzymatic digestion  
178 protocol adapted from Cole *et al.* (2014); here, we used the enzymes Proteinase K and cellulase to  
179 remove biotic material, whilst retaining anthropogenic and inorganic material for inspection and  
180 characterisation. In brief: the total weight of each sample was recorded, and if the sample weighed  
181 more than 0.5 g, then a 0.5 g subsample was taken. Each sample was placed in 30 mL of  
182 homogenising solution, physically homogenised and incubated at 50° C for 30 minutes. Next, 1 mL of  
183 20 mg mL<sup>-1</sup> Proteinase K was added and incubated at 50° C overnight. Cellulase was introduced to  
184 the protocol in order to further breakdown any remaining phytoplankton and organic material; 1 mL  
185 of 40 mg mL<sup>-1</sup> cellulase was added and the maintained at 4° C overnight to optimise enzymatic  
186 degradation. Finally, 8.5 mL of 5 M sodium perchlorate was added, the sample physically  
187 homogenised and placed in a water bath at 60° C for 30 minutes. Digested samples were then  
188 vacuum filtered (Whatman cyclopore, 47 mm, 10  $\mu\text{m}$ ) and rinsed thoroughly with ultrapure water.  
189 Filters were analysed on an Olympus SZX16 Stereo Microscope (110 x magnification) and  
190 microplastics identified per the Norén (2007) protocol (see previous section). Suspect microplastics  
191 were quantified and characterised (shape and colour) and a randomly selected subsample of fibres



192 and particles were retained for sizing ( $n=696$ ) and FT-IR analysis ( $n=90$ ), carried-out as described  
193 above. Waterborne microplastic concentrations (microplastics  $m^{-3}$ ) were calculated using the net  
194 dimensions, tow length and depth, and a WP2 net efficiency of 95% (UNESCO, 1968).

195

### 196 **2.5 Incidence of ingestion and encounter rate**

197 Individual fish dissections allowed for 'incidence of ingestion' (number of fish that ingested  
198 microplastic / total number fish dissected) to be calculated. For comparability with other field  
199 studies, where analysis of smaller zooplankton necessitated bulk digestions ([Sun et al., 2016](#),  
200 [Desforages et al., 2015](#)), 'encounter rate' (total number of microplastic particles ingested / number  
201 fish dissected) was also calculated.

202

### 203 **2.6 Contamination controls**

204 Great care was taken during this study to minimise microplastic contamination, with controls set in  
205 place for every stage of the field and laboratory work. Cotton clothing was worn wherever possible  
206 and a white cotton lab coat was worn during laboratory work. The work station was cleaned before  
207 use and lids were placed over samples wherever possible. All Petri dishes and Eppendorfs were  
208 sealed for storage between sessions. Dissection instruments were soaked in ethanol between  
209 samples to avoid cross contamination. Two procedural blanks, using filtered sea water, were  
210 collected on board the RV Plymouth Quest, and subsequently run through the entire laboratory  
211 procedure. During the fish dissections and microscopy, Petri dishes containing dampened  
212 polycarbonate filters (Whatman cyclopore, 47 mm, 10  $\mu m$ ; pre-screened under microscope for  
213 manufacturing debris) were setup to account for airborne contamination ([Lusher et al., 2017](#)); any  
214 suspect microplastics presented on the filter was recorded and accounted for in the data. Finally, the  
215 FT-IR results were used to adjust the plastic count according to the percent success in identification  
216 of plastics versus organic material.

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226 **3. Results**

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228 **3.1. Fish larvae**

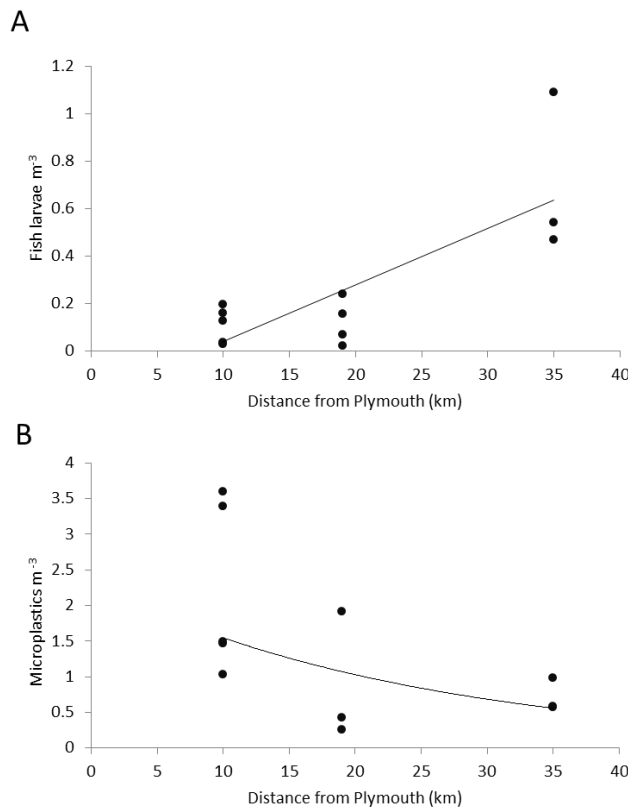
229 Fish larvae concentrations (individuals  $m^{-3}$ ) significantly increased with distance from coast (ANOVA,  
 230  $P<0.05$ ; Figure 2A), with population densities ranging 0.10 fish larvae  $m^{-3}$  at L4, 10 km from  
 231 Plymouth, to 0.70 fish larvae  $m^{-3}$  at E1, 35 km offshore from Plymouth (Table 1).

232

233 **Table 1.** Mean fish larvae data across sites in the western English Channel.

Site	L4	L5	E1
Distance from Plymouth (km)	10	19	35
Number of fish larvae sampled ( <i>n</i> )	135	75	137
Fish larvae concentration (mean individuals $m^{-3}$ )	0.10	0.12	0.70
Incidence of ingestion (no. fish that ingested microplastic / no. fish dissected)	3.7 %	5.3 %	0.7 %
Encounter rate (no. microplastic particles ingested / no. fish dissected)	5.2 %	5.3 %	0.7 %
Waterborne microplastic concentration (mean number $m^{-3}$ )	2.43	0.96	0.79
Ratio fish larvae : microplastic ( $m^{-3}$ )	1:27	1:9	1:1

234



235

236 **Figure 2.** Relationships between distance from Plymouth (km) and: (A) Fish larvae density (individuals  $m^{-3}$ ), linear  
 237 regression (black line),  $R^2=0.63$ ,  $P<0.05$ ,  $n=12$ ; (B) Waterborne microplastics concentrations (microplastics  $m^{-3}$ ), exponential  
 238 regression (black line),  $R^2=0.84$ ,  $P<0.01$ ,  $n = 11$ .

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**3.2 Microplastic ingestion in marine fish larvae**

A total of 347 fish larvae across 23 species were examined for microplastic ingestion, with 10 larvae (2.9%) confirmed to contain microplastic particles in their digestive tract. Ingestion was observed in five species (Table 2A): whiting (*Merlangius merlangus*; n=5; Figure 3a), thickback sole (*Microchirus variegatus*; n=2; Fig 3b), poor cod (*Trisopterus minutus*; n=1), common dragonet (*Callionymus lyra*; n=1; Figure 3c), and European eel (*Anguilla anguilla*; n=1). Encounter rates generally reflected the species composition of the net catches (Table 2B) with the exception of thickback sole and the European eel elver. At Station L4 thick back sole made up just over 2% of the species composition of fish larvae over 9 mm in length and yet showed the highest encounter rate, however, this trend was not repeated at Stations L5 or E1. Fish larvae containing ingested microplastics averaged 10±2.38 mm in length (excluding the 1240 mm European eel larvae), indicating they were likely to be no more than two months old (Russell, 1976). The microplastics ingested by fish larvae consisted of blue or red fibres (83%) and blue fragments (17%); fragments ranged from 50-100 µm in size, with fibres ranging from 100-1100 µm in length. FT-IR analysis confirmed that ingested particles consisted of either nylon, a polyester-polyamide composite or synthetic bioplastic (Rayon). Two fish larvae contained two particles, whilst eight larvae contained just one each.

**Table 2. (A)** Fish larvae (n=10) containing microplastic debris, detailing numbers, type, colour, polymer and size of ingested microplastic, **(B)** species composition of all fish caught over 9 mm in size (excluding sprat) and encounter rate.

Species	Site	Characterisation	Polymer	Size (µm)
Common dragonet	L4	2 blue fibres	Nylon	220, 230
European eel	L5	1 blue fragment	Polyamide-polypropylene	100 x 50
Poor cod	L4	1 blue fragment	Unknown*	50 x 50
Thickback sole	L4	1 red fibre	Rayon	270
	L4	2 blue fibres	Unknown*	250, 250
Whiting	L4	1 blue fibre	Rayon	300
	L5	1 blue fibre	Rayon	310
	L5	1 blue fibre	Rayon	450
	L5	1 red fibre	Rayon	1100
	E1	1 blue fibre	Rayon (elastic )	100

\*Owing to difficulties in transferring the plastic to the microscope slide for analysis, plastics were unable to be verified using FT-IR

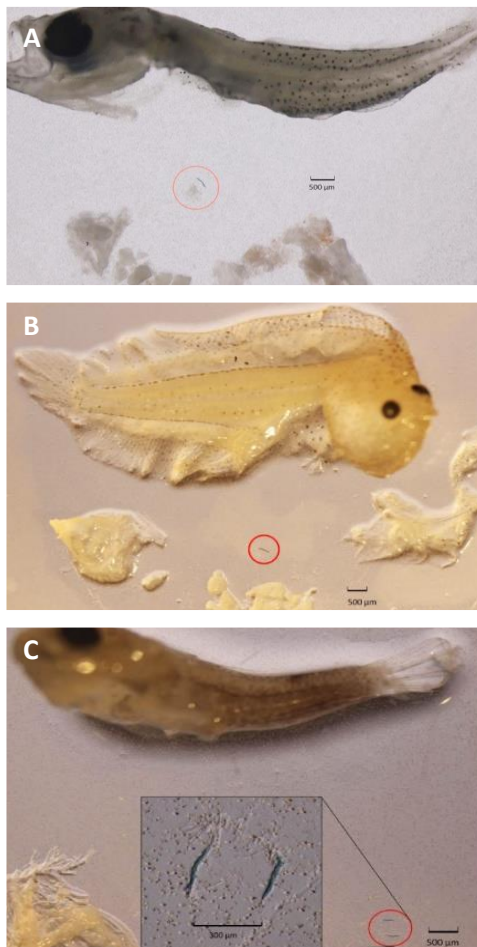
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**B**

Species	L4 (n=197)		L5 (n=67)		E1 (n=198)	
	% composition	encounter rate %	% composition	encounter rate %	% composition	encounter rate %
Whiting	31	0.5	50.3	4.5	42.6	0.51
Poor Cod	17.3	0.5	4.3	0	8	0
Thickback Sole	2.1	1.5	2	0	5	0
Common Drag	13.9	1	22.6	0	21.8	0
European eel	0	0	1.5	1.5	0	0
Other	35.7	0	19.3	0	22.6	0

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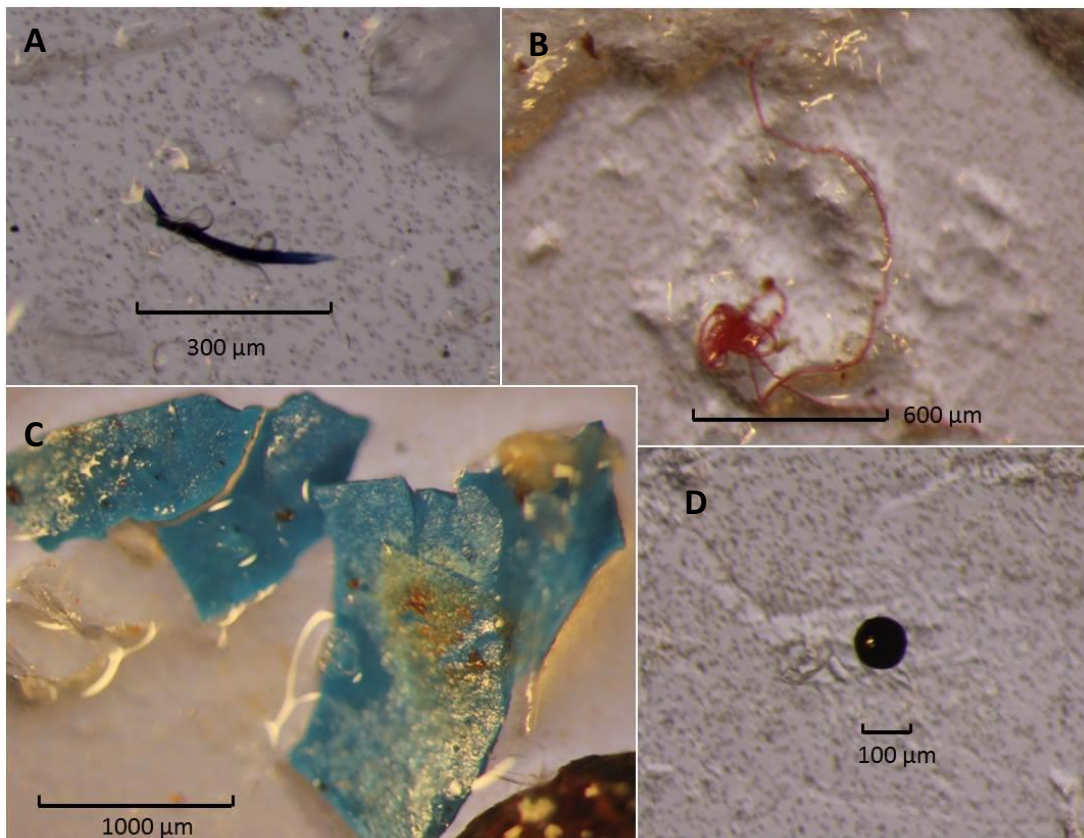
269 **Figure 3.** Photographs of dissected fish larvae that had ingested microplastics (circled), viewed under an Olympus SZX16  
 270 Stereo Microscope. (A) Whiting (12 mm in length) with 310 µm rayon fibre; (B) Thickback sole (10.5 mm in length) with 270  
 271 µm rayon fibre; (C) Common dragonet (9 mm in length) with 2 blue nylon fibres (220 µm and 230 µm). Image credit: M  
 272 Steer.

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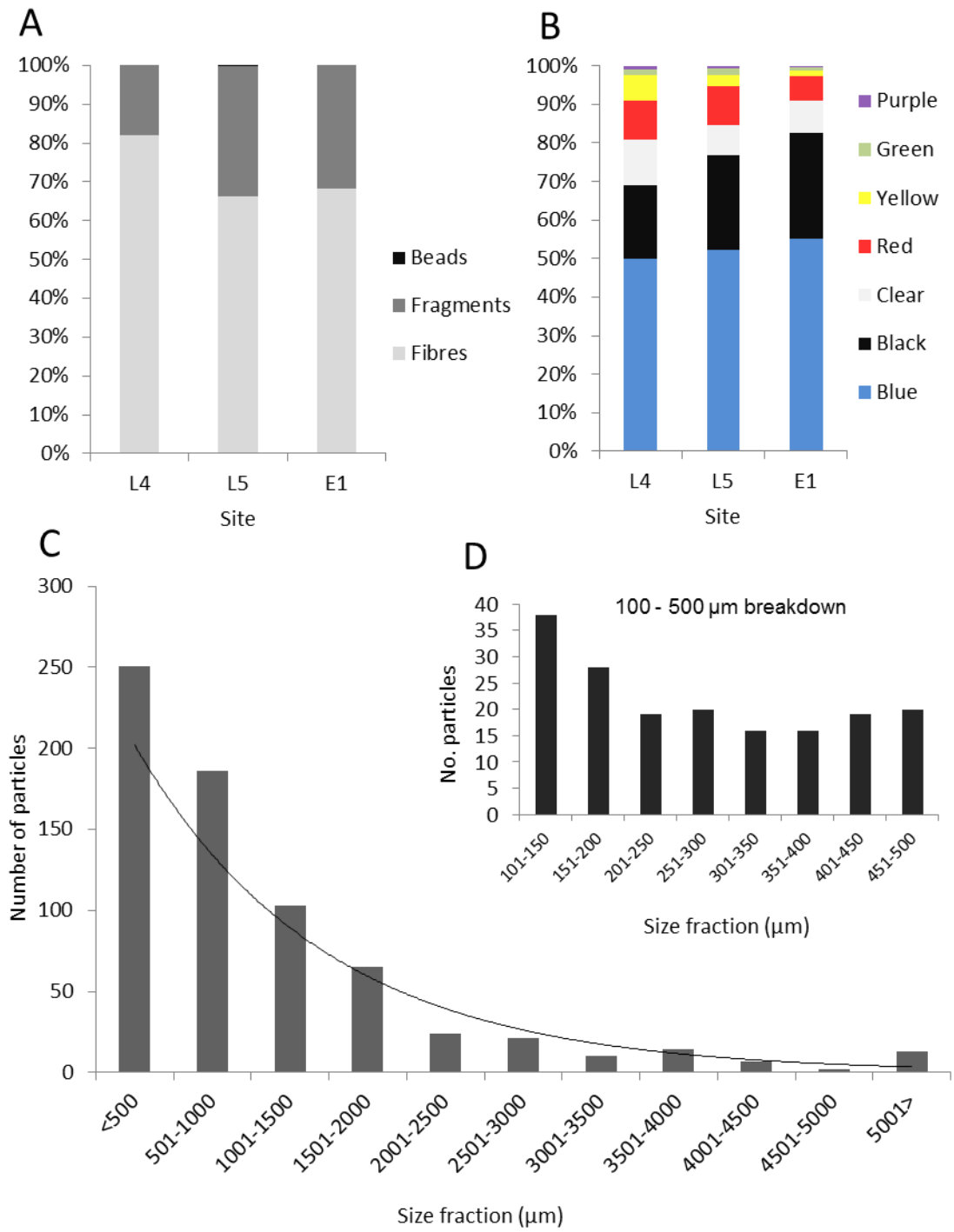
274 **3.3. Waterborne microplastics in the water column**

275 We observed a trend of decreasing microplastic concentrations with distance from shore  
 276 (exponential regression,  $P < 0.01$ ; Figure 2B). Microplastic concentrations were highly variable,  
 277 ranging  $0.26\text{--}3.79\text{ m}^{-3}$  across sites, with an average microplastic concentration across all three study

278 sites of  $1.39 \text{ particles m}^{-3}$ . The microplastic debris predominantly consisted of fibres (77%) and  
279 fragments (23%), with no significant difference in shape between sites (ANOVA,  $P=0.485$ ); Figure 4;  
280 Figure 5A). Out of a total 2772 microplastic particles observed, only one bead was identified. Across  
281 all three sites, approximately 50% of the microplastics were blue (Figure 5B), with black (21.5%),  
282 clear (10%) and red (9.5%) plastics also well represented. Of the microplastics analysed: 63% were  
283 mixtures of plastic compounds (co-polymers) and 36% were single polymers. The majority (55%) of  
284 analysed particles were either rayon or a rayon mix (primarily rayon with polyurethane);  
285 polyethylene, nylon and acrylic were also commonly identified, both as singular or co-polymers. We  
286 further identified a significant, exponential relationship between microplastic size and relative  
287 abundance (exponential regression,  $R^2=0.84$ ,  $P<0.05$ ; Figure 5C), with a trend of increasing numbers  
288 of particles with decreasing size. For size fractions between 100-500  $\mu\text{m}$  a relationship was less  
289 evident (Figure 5D). No significant difference in microplastic size was identified between any of the  
290 three sample sites (ANOSIM,  $P=0.24$ ).  
291



292  
293 **Figure 4.** Selection of microplastics from water samples. (A) Blue fibre, 310  $\mu\text{m}$ , rayon; (B) Red fibre, knotted (2000  $\mu\text{m}$   
294 length), polyester; (C) Blue fragments, 1100–1400  $\mu\text{m}$  diameter, acrylic/polyethylene/nylon copolymer; (D) Black bead, 100  
295  $\mu\text{m}$  diameter. Image Credit: M Steer



296

297 **Figure 5.** Waterborne microplastic debris sampled from the western English Channel. (A) Proportion (%) of fibres,  
 298 fragments and beads in water samples per site; (B) Proportional (%) colour composition of microplastic assemblage by site;  
 299 (C) Frequency distribution of size classes (µm) of microplastics sampled ( $n=694$ ) with exponential regression ( $R^2=0.84$ ,  
 300  $P=0.00$ ,  $n=11$ , black dotted line); (D) frequency distribution within the 100-500 µm size range ( $n=251$ ).

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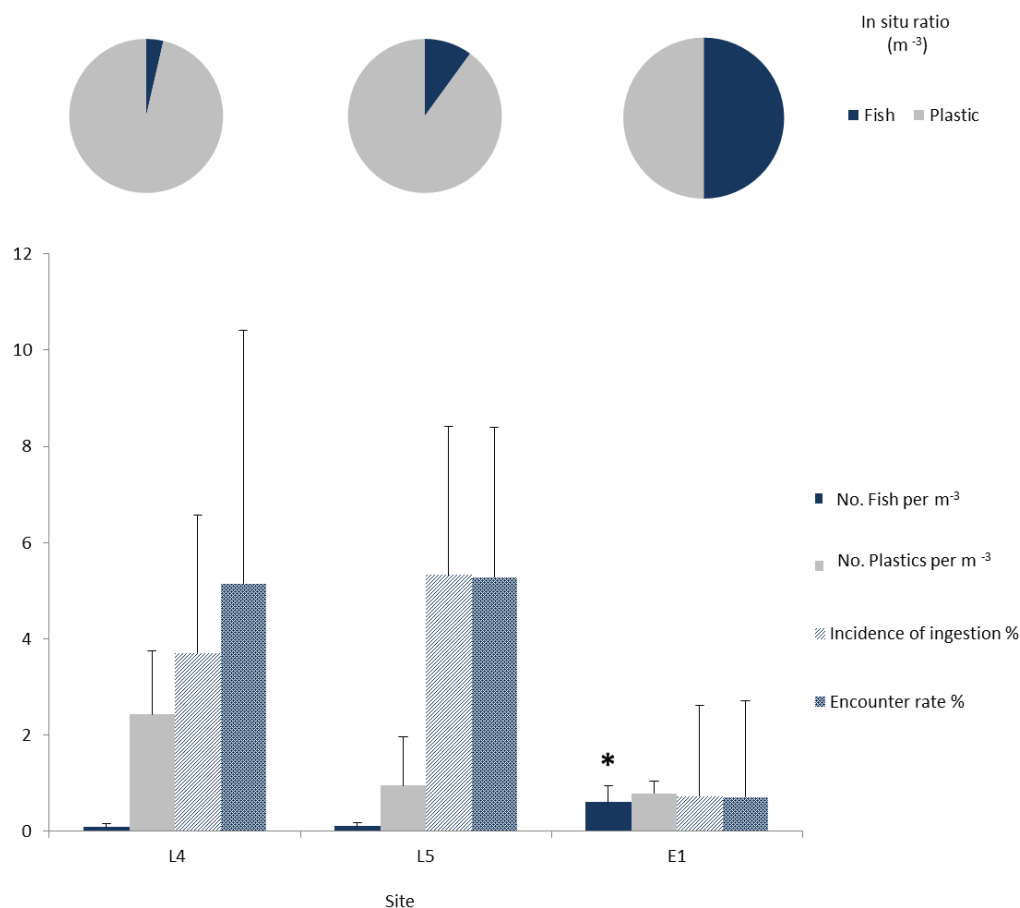
302 **3.4 Incidence of ingestion and encounter rate**

303 No significant difference in ‘incidence of ingestion’ (Table 1; ANOVA,  $n=13$ ,  $P=0.24$ ) or ‘encounter  
 304 rate’ (Table 1; ANOVA,  $n=13$ ,  $P=0.42$ ) was observed between sites. The highest microplastic

305 encounter rate (number microplastic particles ingested / number fish dissected) was at site L5  
 306 (5.28%), closely followed by L4 (5.15%) with E1 noticeably lower at 0.72% (Table 2; Figure 6). There  
 307 was significant variance in fish larvae concentrations between sites (Figure 6; ANOVA,  $P < 0.05$ ), with  
 308 E1 showing significantly higher fish larval numbers than at L4 and L5. No significant difference in  
 309 microplastic concentrations (Figure 6; ANOVA,  $P = 0.11$ ) was observed between sites, although a  
 310 trend of decreasing concentrations with distance from the coast was noted.

311

312 When comparing fish larvae concentrations with waterborne microplastic concentrations, the site  
 313 closest to Plymouth (10 km) had a ratio of 27 microplastic particles per single fish larvae in the  
 314 water. This decreased to a 1:1 ratio at E1, 35 km offshore (Table 2). Although fish larvae  
 315 concentrations were at their lowest at L4 station (closest proximity to Plymouth), microplastics  
 316 concentrations were at their highest, accounting for the maximum value of incidence of ingestion  
 317 recorded (Table 1).



318

319 **Figure 6.** ABOVE: The ratio between concentration of microplastics and fish larvae in the water column at each site is  
 320 displayed. BELOW: Comparison between plastic concentrations (number m<sup>-3</sup>), fish larvae concentrations (individuals m<sup>-3</sup>),  
 321 incidence of ingestion (number of fish with ingested particles/ number of fish dissected) and encounter rate (number  
 322 microplastic particles ingested/ number fish dissected) per site; \* denotes significant difference from other sites.

323

#### 324 **4. Discussion**

325 The results demonstrate 2.9% of fish larvae found at the study sites in the western English Channel  
326 had ingested microplastics. Of the ingested particles, 83% were fibrous and 83% were blue,  
327 mirroring the assemblage of microplastics concurrently sampled from the water column. Fish larvae  
328 abundance increased with distance from shore, while waterborne microplastic concentrations  
329 decreased. At L4 and L5, within the designated 19 km coastal water zone (UN convention), a 5.2%  
330 encounter rate was observed alongside a fish to microplastic ratio per cubic meter of water of 1:27  
331 and 1:9 respectively; at E1, 35 km from the coast, this decreased to 0.72% and a ratio of 1:1.

332

##### 333 **4.1. Prevalence of microplastic ingestion**

334 Exposure studies have revealed zooplankton are capable of ingesting microplastics (Cole *et al.*,  
335 2013), however evidence of microplastic consumption *in natura* is less evident. In Portuguese  
336 coastal waters 61% of zooplankton (n=152, species not determined) had ingested microplastics (Frias  
337 *et al.*, 2014). In the Northeast Pacific, calanoid copepods (*Neocalanus cristatus*) and euphausiids  
338 (*Euphausia pacifica*) exhibited a microplastic encounter rate of 2.6% and 5.8% respectively  
339 (Desforges *et al.*, 2015). Until recently, the uptake of microplastics by meroplankton (planktonic for a  
340 single stage of life cycle) in the field has been severely understudied. Recent research on incidence  
341 of microplastic ingestion across five zooplankton groups, including fish larvae, sampled from the  
342 South China sea revealed an encounter rate of 120% (Sun *et al.*, 2016); however, in that study  
343 sampling was limited to “several larvae”, with no concurrent waterborne microplastic data recorded.

344

345 Here, we have identified that microplastics are ingested by a number of different species of fish  
346 larvae (meroplankton) in their natural environment. Fish larvae spend their entire planktonic stage  
347 in the pelagic zone and are unselective feeders. When prey concentration is low they not only  
348 pursue all prey sizes encountered but also increase their swimming activity and are much less  
349 selective (Munk and Nielson, 2005). By dissecting individual fish larvae, we were able to calculate  
350 ‘incidence of ingestion’, which ranged 3.2-5.5% across sites. For comparison with other studies on  
351 zooplankton, where bulk digestions have been used to extract microplastics, we also calculated  
352 ‘encounter rates’. Our analysis of fish larvae in the western English Channel has demonstrated an  
353 encounter rate with microplastics of between 0.7-5.3%. At L4, 3.7% of fish larvae ingested plastic;  
354 comparatively, 36.5% of adult fish sampled from L4 (June 2010–July 2011) had ingested  
355 microplastics (Lusher *et al.* (2013)). Research by Rummel *et al.* (2016) recorded significantly higher  
356 ingestion percentages in pelagic fish (10.7%) compared to demersal (3.4%). The post larval stages of



357 fish examined in this study were approximated to be between 5 days and 2 months old, excluding  
358 the European eel elver at less than a year old (Russell, 1976); microplastics would therefore have  
359 been encountered over a considerably shorter time frame than in adult fish, which may account for  
360 the lower proportion of individuals containing plastic observed alongside potential differences in gut  
361 retention times. All of the fish species that had ingested microplastics in this study (excluding the  
362 European eel larvae) have also been identified to consume microplastics as adults (Lusher *et al.*,  
363 2013). Further work is required to gauge how long fish larvae will retain ingested microplastics in  
364 order to better predict the likely impact of ingestion of the individual (i.e. are ingested plastics  
365 transient or do they have long residence times).

366

#### 367 **4.2. Potential health effects**

368 Very little is known regarding the effects of ingesting microplastics on wild fish. There are substantial  
369 difficulties in assessing physiological or behavioural responses to ingestion in the wild, largely due to  
370 the inability to assess gut retention times or monitor chronic health effects arising from a single  
371 stressor. Laboratory studies on fish have illustrated significant physiological (gut blockage, decrease  
372 in food intake due to less gut space) and toxicological (inflammatory responses, oxidative stress,  
373 hepatic stress, decreased energy availability) damage can result from consumption of plastics  
374 ([Rochman \*et al.\*, 2013b](#), [Oliveira \*et al.\*, 2013](#), [Mazurais \*et al.\*, 2015](#), [de Sa \*et al.\*, 2015](#), [Karami \*et al.\*, 2016](#)). However, the environmental relevance of such laboratory studies are often limited. For  
375 example, we note that the concentrations and types of microplastics used in the aforementioned  
376 exposure studies are largely unrepresentative of the microplastics identified at our study sites. We  
377 advocate that microplastics used in experiments need to reflect what is found in the field more  
378 closely as this information becomes available; the use of environmentally aged fibres (i.e. with  
379 adsorbed POPs, biofilms and dimethyl sulphide ([Ziccardi \*et al.\*, 2016](#), [Wardrop \*et al.\*, 2016](#), [Jang \*et al.\*, 2016](#), [Lambert \*et al.\*, 2014](#), [Savoca \*et al.\*, 2016](#))) would give a much better understanding of the  
381 fate and effects of microplastics in the marine ecosystem. Ecologically relevant data is essential in  
382 order to address the impacts of microplastics on animal populations, communities and ecosystems.

384

385 Laboratory experiments using juvenile fish or fish larvae are currently limited in scope and number.  
386 Owing to the susceptibility of fish larvae to environmental stressors during development, it is  
387 imperative that the effects of microplastic exposure on key health parameters (i.e. growth rate,  
388 feeding) in juvenile fish is given due attention. [de Sa \*et al.\* \(2015\)](#) revealed that developmental  
389 conditions may influence a fish's ability to distinguish plastic from prey; it would therefore be

390 intriguing to evaluate whether community fitness has a bearing on a fish larvae's ability to select  
391 prey over plastic.

392

393 The encounter rates observed in this study are relatively low when compared to previous studies on  
394 zooplankton, partly due to the fact that as larval concentrations increased, microplastic  
395 concentrations decreased (with distance from shore). We would expect that higher encounter rates  
396 would be observed where high microplastic concentrations overlap with high fish larval  
397 concentrations; in these instances, we might reasonably expect that negative health effects on  
398 individuals could extend to the population as a whole. Fish produce high numbers of eggs in order to  
399 account for the high mortality rates in larvae, therefore the relationship between larval survival and  
400 population dynamics is complex.

401

#### 402 **4.3. Comparison between waterborne and ingested microplastics**

403 The characteristics of the microplastics ingested by fish larvae were representative of those found in  
404 the water column, with 8 blue fibres out of 12 particles, reflecting the 77% fibre and 50% blue  
405 composition of the microplastics in the water. [Desforges \*et al.\* \(2015\)](#) also found fibrous  
406 microplastics were predominant in euphausiids (68% fibres) and copepods (50% fibres). Black  
407 microplastics accounted for 21.5% of the water samples in this study whilst red just 9.5%, however  
408 red fibres constituted 17% of the ingested particles whereas black wasn't ingested at all. If we are to  
409 successfully advise on policy for microplastic production, use and disposal, it is advisable that future  
410 laboratory experiments also assess the possibility of feeding selectivity taking place on microplastic  
411 colour and shape.

412

413 Constituting over 50% of the microplastics found in our water samples, Rayon is a semi synthetic  
414 bioplastic used in clothing, furnishing, female hygiene products and nappies; Cole (2014) also found  
415 Rayon in the surface waters at L4 and close to the Plymouth sound (October 2013). Bioplastics (i.e.  
416 Rayon) are rarely represented in toxicity testing of microplastics, and should be considered an area  
417 requiring further testing. The large number of microscopic synthetic fibres found in the water  
418 suggests sewage outlets might be a prominent source of microplastic pollution observed across our  
419 sampling sites (Browne *et al.*, 2010). Polyester and polyurethane (PU) were also identified in the  
420 waterborne samples; both polymers are used in resin systems for boat hulls, PU is found in  
421 numerous marine paints and polyester is a popular material for commercial marine rope including  
422 fishing nets in conjunction with nylon. There was a notable absence of microbeads in our samples,  
423 however this may be an artefact of our sampling protocol: Fendall and Sewell (2009) report that two

424 thirds of cosmetic brands use <100 µm microbeads, therefore in using a 100 µm net we would be  
425 unlikely to capture spherical particles below this size threshold. The most abundant size range of  
426 waterborne microplastics was the <500 µm category, with abundance decreasing exponentially as  
427 particle size increased; a trend also reported in open ocean samples by Cozar *et al.* (2014). These  
428 plastics are of a similar size to microzooplankton which form a key component of the diet of fish  
429 larvae.

430

#### 431 **4.4. Relationship between distance and uptake**

432 Shelf-sea ecosystems have been highlighted as regions with high likelihood of microplastic-biotic  
433 interaction. In coastal regions close to urban centres (e.g. Plymouth) microplastic concentrations will  
434 be higher owing to their proximity to a source of input. Likewise, biological productivity is higher in  
435 shelf-seas because of increased nutrient and organic carbon input from land (Clark *et al.*, 2016). Our  
436 data concurs with this hypothesis, showing a microplastic encounter rate of 5.2% in fish larvae  
437 within 19 km of shore, while fish larvae 35 km from shore encountered far less (0.72%). We  
438 observed a decrease in waterborne microplastic concentrations with increasing distance from the  
439 coastline. The high degree of temporal variability in the L4 microplastic assemblage (standard  
440 deviation for L4= 1.26, L5= 0.97, E1= 0.24) could be accounted for by its proximity to Plymouth and  
441 the variations in input which can fluctuate depending on runoff, tidal regime, sewage input, weather  
442 and pollution incidents. Furthermore there is the possibility of seasonal variability in the transport of  
443 microplastics from Plymouth sound out to sea. Only a fraction of the particles released from the  
444 sound are likely to reach L4 – instead they are swept westward close to the coastline (J Clark,  
445 Plymouth Marine Laboratory, personal comms). This could account for the decreased microplastic  
446 concentrations experienced with increased distance from shore; alongside a dilution effect. The  
447 observed homogeneity in colour and shape of particles across all three sites suggests consistent  
448 sources of contamination (i.e. sewage outfall, maritime activity); however this isn't necessarily from  
449 a geographically similar source. E1 has oceanic water influence therefore it is perhaps unlikely that  
450 large numbers of microplastics from a source in Plymouth would reach the site. Similar sources of  
451 microplastic contamination (i.e. sewage, maritime and industrial) exist along the south coast of  
452 England and it is these inputs that are more likely to be the key influence on the assemblage of  
453 plastics outside of the coastal zone.

454

455 The abundance of fish larvae increased with increasing distance from shore. This study targeted  
456 spring spawning boreal species residing throughout the water column and at their most abundant  
457 and diverse in May (Russell, 1976). Fish larvae remain planktonic until adolescence when they move

458 to their preferred habitat (e.g. Gadoids to rocky shores, flatfish to the benthos). Until this time they  
459 remain planktonic and in deeper water, with only surface dwelling larvae prone to onshore drift by  
460 prevailing winds; thus explaining the lower numbers recorded close to shore. Conversely  
461 microplastic concentrations decreased with distance from shore and as such, the ratio of fish:plastic  
462 decreased and directly correlated with the frequency of microplastic consumption. It is generally  
463 hypothesised that biota in coastal regions will experience a greater impact from microplastic  
464 ingestion. Furthermore we suggest that spatial and temporal overlap is key to the degree of impact  
465 observed at population level. Microplastic concentrations are spatially and temporally variable,  
466 influenced by local currents, accumulation spots and climate events among others. If these hotspots  
467 overlie spawning grounds for adult fish and areas where planktonic larvae fish are abundant then  
468 there will be far greater incidence of ingestion and therefore significantly higher encounter rate  
469 observed than during this study. It is the identification of these areas alongside a drive towards to  
470 producing ecologically relevant data that should be the focus of future research efforts in order to  
471 target prevention, policy and legislation ([Rochman, 2016](#)). The emphasis should now be on  
472 encouraging the use of preventative measures rather than the need for expensive clean-up  
473 operations.

474

475

## 476 **5. Conclusion**

477 Although the observed ingestion rate for microplastics in fish larvae was low at 2.9% we must  
478 remember that these meroplankton have in fact only been in the pelagic zone as plankton for a  
479 matter of weeks. Based upon the existing evidence, we suggest that ingestion of microplastics is  
480 likely to be detrimental to these individuals, however it is currently unclear whether the low  
481 incidence of ingestion would be sufficient to contribute to negative impacts at the population level.  
482 There are difficulties in assessing the pattern of ingestion due to the low number of individuals  
483 found to contain microplastic; further investigation is required to determine whether fish larvae  
484 exhibit selective behaviour towards microplastics of differing shape and colour. Concurrent water  
485 sampling allowed an invaluable insight into the microplastic assemblage in the water at the time of  
486 ingestion; this novel data highlights the spatial and temporal overlap of larvae and microplastics.  
487 There can be no doubt that zooplankton, including meroplankton, are ingesting microplastics and  
488 biomicroplastics. This study has shown that higher encounter rates occur where microplastic  
489 concentrations exceed those of fish larvae. We therefore expect incidence of ingestion to be  
490 greatest in productive habitats which experience high concentrations of microplastics.

491

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