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Microplastic ingestion in fish larvae in the western English Channel

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Abstract

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

CAPSULE:

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

1. Introduction

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

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

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

Fish stocks have considerable ecological and economic value. Global annual fisheries revenue fluctuates around USD 100 billion supporting about 12% of the world population, and providing 2.9 billion people with 20% of their animal protein (Lam *et al*., 2016). With over 30,000 species of fish worldwide, existing in all of the worlds marine habitats, their abundance and diversity has significant ecological importance for the food chain, nutrient cycling and ecosystem services (Worm *et al*., 2006). Ichthyoplanktonic studies show that unfished taxa account for the majority of fish larvae and contribute significantly to trophic food webs (Baran, 2002). Fish populations are vulnerable to a growing number of anthropogenic pressures, including overfishing, climate change and pollution, resulting in increased mortality and reduced fecundity. Incidence of microplastic consumption by adult fish has been widely reported for pelagic and demersal populations across the globe, including blue whiting (*Micromesistius poutassou*), red gurnard (*Aspitrigla cuculus*), john dory (*Zeus faber*) and dragonet (*Callionymus lyra*) (Lusher *et al*., 2013). However, there is currently no substantial published data regarding microplastic ingestion rates in fish larvae. Fish larvae play a pivotal role in marine food webs (Russell, 1976), and their health, development and survival is fundamental to the long-term sustainability of healthy fish populations. As such, data is urgently required to better assess the risks posed to fish larvae by microplastics *in natura*.

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

2. Methodology

2.1 Field sampling

Field sampling was undertaken on board RV Plymouth Quest in the western English Channel off the coast of Plymouth (UK). Sampling was conducted at stations L4, L5 and E1 (10 km, 19 km and 35 km from shore respectively), which are routinely sampled as part of the Western Channel Observatory (WCO; www.westernchannelobservatory.org.uk). The sampling sites spanned distances of 10-35 km from the city of Plymouth (Figure 1), accounting for habitats with a coastal (L4) and oceanic influence (E1); L5 was added as a reference site because it is a rocky reef known to be a favourable habitat for fish larvae. Eleven samples were collected between 11th April 2016 and 21st June 2016 across the three sites (L4, *n*=5; L5, *n*=3; E1, *n*=3). For each trawl, tow distance and maximal sample depths were recorded using GPS and a Suunto vyper dive computer respectively; maximum depths reached were on average 50 m at L4 and L5, and 65 m at E1. Fish larvae were collected using a 500 µm metal-framed net (1 m^2 square aperture) towed for 20 minutes on an oblique tow. Following the trawl, larvae were passed through a 500 µm sieve and rinsed with filtered (0.22 µm) natural seawater. Subsequently, specimens were transferred into a 1 L Nalgene bottle and preserved in 4% formalin. Microplastics were sampled using a 100 µm WP2 net (47 cm diameter aperture), suspended below the net used for sampling the fish larvae. This concurrent sampling allowed for direct comparison of microplastics ingested by the fish larvae with ‘prey-sized’ microplastics in the surrounding water. Following sampling, the WP2 net was rinsed with filtered seawater and the sample poured through a 100 µm mesh; samples were immediately sealed and subsequently stored in a foil envelope in a -80°C freezer prior to analysis. Control measures included collection of procedural blanks using filtered sea water, and sampling of boat paint for Fourier Transform Infrared Spectroscopy (FT-IR) analysis to ensure false positives were avoided in the plastics count.

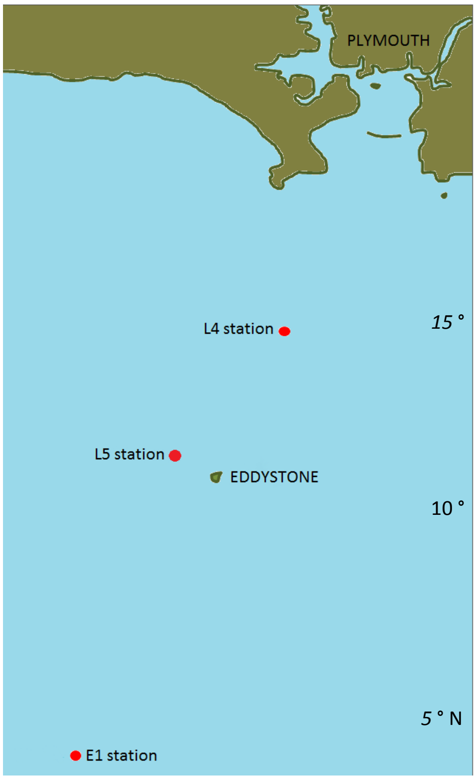

Figure 1. Sampling sites located in the western English Channel. E1: 35 km offshore Plymouth; L5: 19 km offshore; L4: 10 km offshore.

2.2 Fish larvae

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

2.3 Microplastic ingestion in marine fish larvae

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

2.4 Waterborne microplastics

Waterborne microplastic samples were removed from storage, and then freeze dried for 72 hours (Scanvac CoolSafe freeze drier). Desiccated samples were put through an enzymatic digestion protocol adapted from Cole *et al*. (2014); here, we used the enzymes Proteinase K and cellulase to remove biotic material, whilst retaining anthropogenic and inorganic material for inspection and characterisation. In brief: the total weight of each sample was recorded, and if the sample weighed more than 0.5 g, then a 0.5 g subsample was taken. Each sample was placed in 30 mL of homogenising solution, physically homogenised and incubated at 50° C for 30 minutes. Next, 1 mL of 20 mg mL^{-1} Proteinase K was added and incubated at 50° C overnight. Cellulase was introduced to the protocol in order to further breakdown any remaining phytoplankton and organic material; 1 mL of 40 mg mL^{-1} cellulase was added and the maintained at 4° C overnight to optimise enzymatic degradation. Finally, 8.5 mL of 5 M sodium perchlorate was added, the sample physically homogenised and placed in a water bath at 60° C for 30 minutes. Digested samples were then vacuum filtered (Whatman cyclopore, 47 mm, 10 µm) and rinsed thoroughly with ultrapure water. Filters were analysed on an Olympus SZX16 Stereo Microscope (110 x magnification) and microplastics identified per the Norén (2007) protocol (see previous section). Suspect microplastics were quantified and characterised (shape and colour) and a randomly selected subsample of fibres and particles were retained for sizing (*n*=696) and FT-IR analysis (*n*=90), carried-out as described above. Waterborne microplastic concentrations (microplastics m^{-3}) were calculated using the net dimensions, tow length and depth, and a WP2 net efficiency of 95% (UNESCO, 1968).

2.5 Incidence of ingestion and encounter rate

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

2.6 Contamination controls

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

3. Results

3.1. Fish larvae

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

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 (*n*)	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

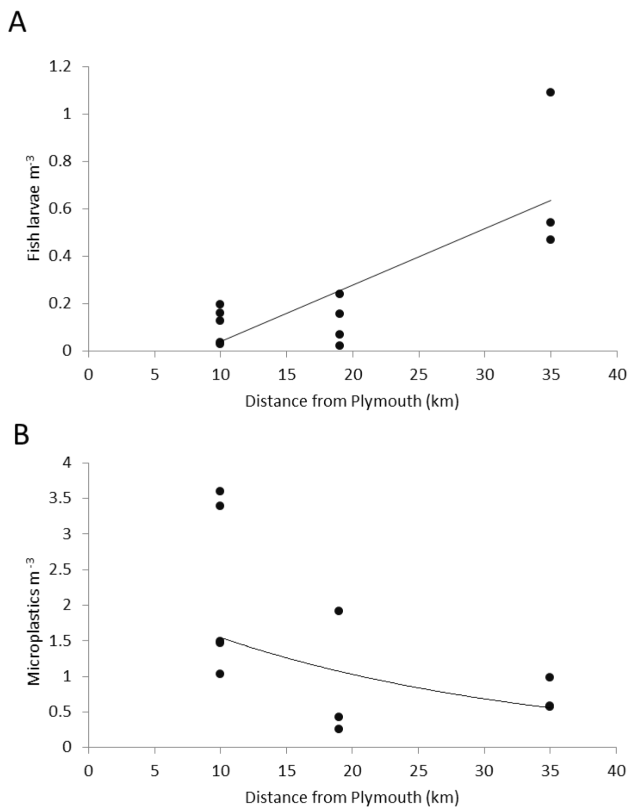

Figure 2. Relationships between distance from Plymouth (km) and: (A) Fish larvae density (individuals m^{-3}), linear regression (black line), R^2=0.63, P<0.05, *n*=12; (B) Waterborne microplastics concentrations (microplastics m^{-3}), exponential regression (black line), R^2=0.84, *P*<0.01, *n* = 11.

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.

A

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*

B

	L4 (n=197)		L5 (n=67)		E1 (n=198)	
Species	**% 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

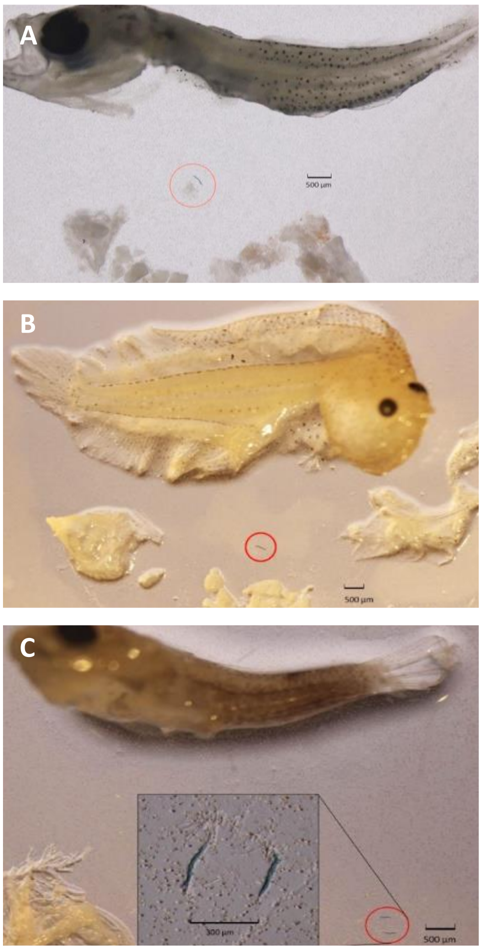

Figure 3. Photographs of dissected fish larvae that had ingested microplastics (circled), viewed under an Olympus SZX16 Stereo Microscope. (A) Whiting (12 mm in length) with 310 µm rayon fibre; (B) Thickback sole (10.5 mm in length) with 270 µm rayon fibre; (C) Common dragonet (9 mm in length) with 2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

3.3. Waterborne microplastics in the water column

We observed a trend of decreasing microplastic concentrations with distance from shore (exponential regression, *P*<0.01; Figure 2B). Microplastic concentrations were highly variable, ranging 0.26–3.79 m^{-3} across sites, with an average microplastic concentration across all three study sites of 1.39 particles m^{-3}. The microplastic debris predominantly consisted of fibres (77%) and fragments (23%), with no significant difference in shape between sites (ANOVA, P=0.485); Figure 4; Figure 5A). Out of a total 2772 microplastic particles observed, only one bead was identified. Across all three sites, approximately 50% of the microplastics were blue (Figure 5B), with black (21.5%), clear (10%) and red (9.5%) plastics also well represented. Of the microplastics analysed: 63% were mixtures of plastic compounds (co-polymers) and 36% were single polymers. The majority (55%) of analysed particles were either rayon or a rayon mix (primarily rayon with polyurethane); polyethylene, nylon and acrylic were also commonly identified, both as singular or co-polymers. We further identified a significant, exponential relationship between microplastic size and relative abundance (exponential regression, R^2=0.84, P<0.05; Figure 5C), with a trend of increasing numbers of particles with decreasing size. For size fractions between 100-500 µm a relationship was less evident (Figure 5D). No significant difference in microplastic size was identified between any of the three sample sites (ANOSIM, P=0.24).

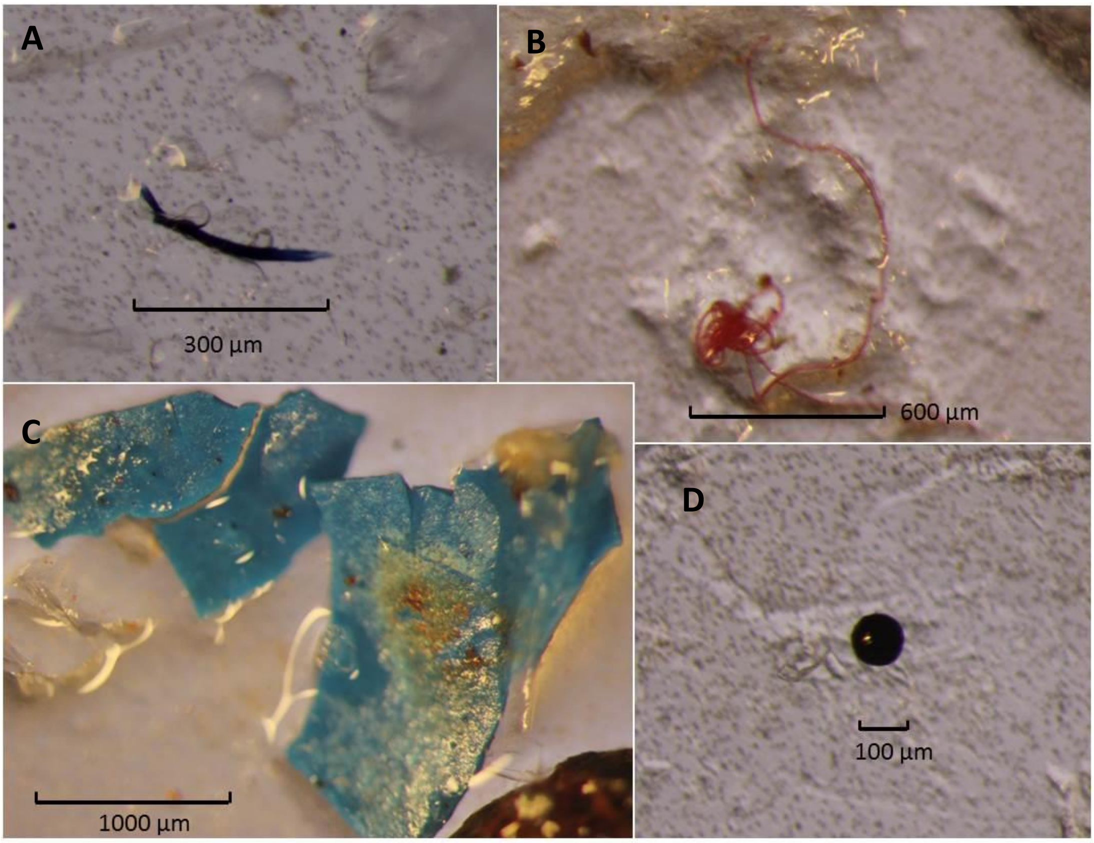

Figure 4. Selection of microplastics from water samples. (A) Blue fibre, 310 µm, rayon; (B) Red fibre, knotted (2000 µm length), polyester; (C) Blue fragments, 1100–1400 µm diameter, acrylic/polyethylene/nylon copolymer; (D) Black bead, 100 µm diameter. Image Credit: M Steer

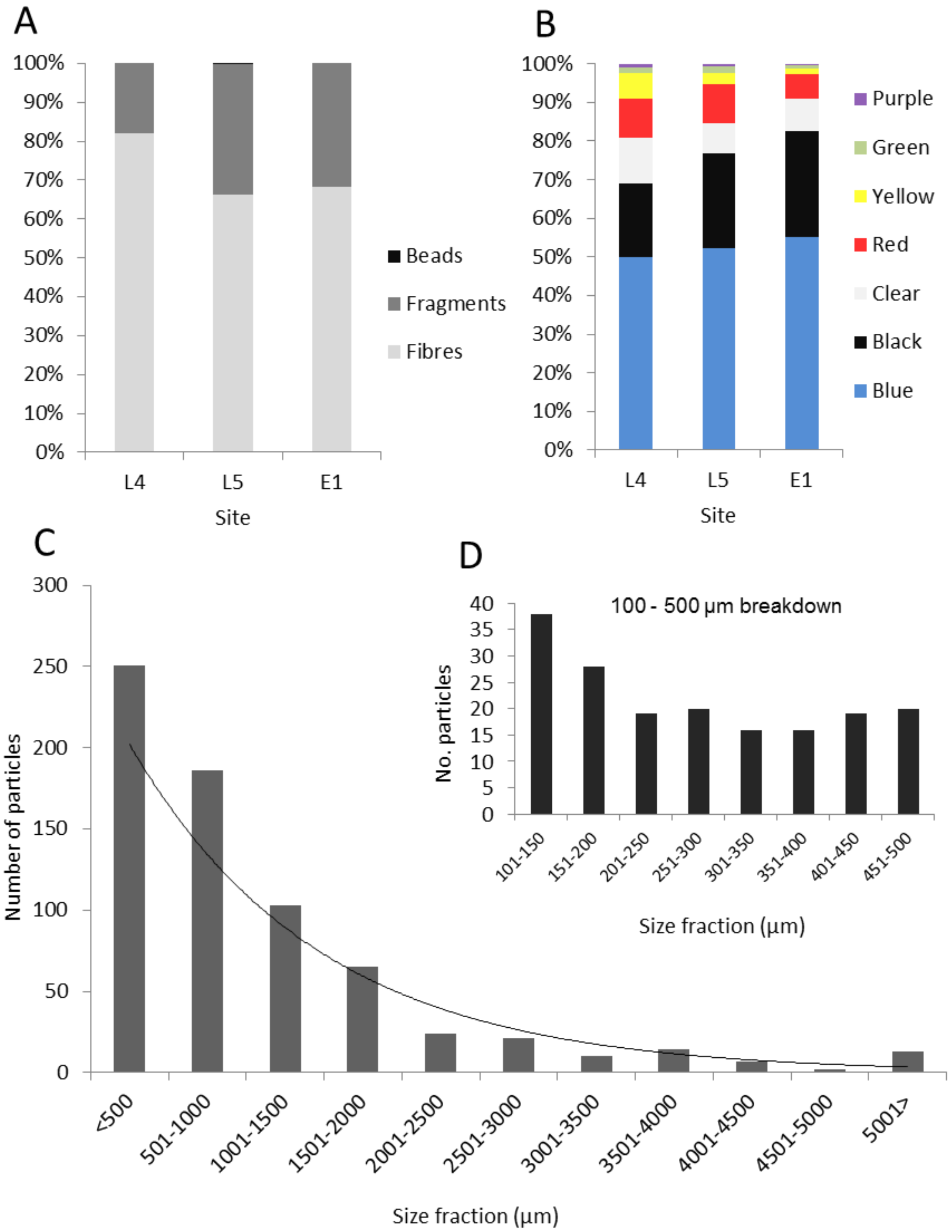

Figure 5. Waterborne microplastic debris sampled from the western English Channel. (A) Proportion (%) of fibres, fragments and beads in water samples per site; (B) Proportional (%) colour composition of microplastic assemblage by site; (C) Frequency distribution of size classes (µm) of microplastics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

3.4 Incidence of ingestion and encounter rate

No significant difference in 'incidence of ingestion' (Table 1; ANOVA, *n*=13, *P*=0.24) or 'encounter rate' (Table 1; ANOVA, n=13, *P*=0.42) was observed between sites. The highest microplastic encounter rate (number microplastic particles ingested / number fish dissected) was at site L5 (5.28%), closely followed by L4 (5.15%) with E1 noticeably lower at 0.72% (Table 2; Figure 6). There was significant variance in fish larvae concentrations between sites (Figure 6; ANOVA, P<0.05), with E1 showing significantly higher fish larval numbers than at L4 and L5. No significant difference in microplastic concentrations (Figure 6; ANOVA, P=0.11) was observed between sites, although a trend of decreasing concentrations with distance from the coast was noted.

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

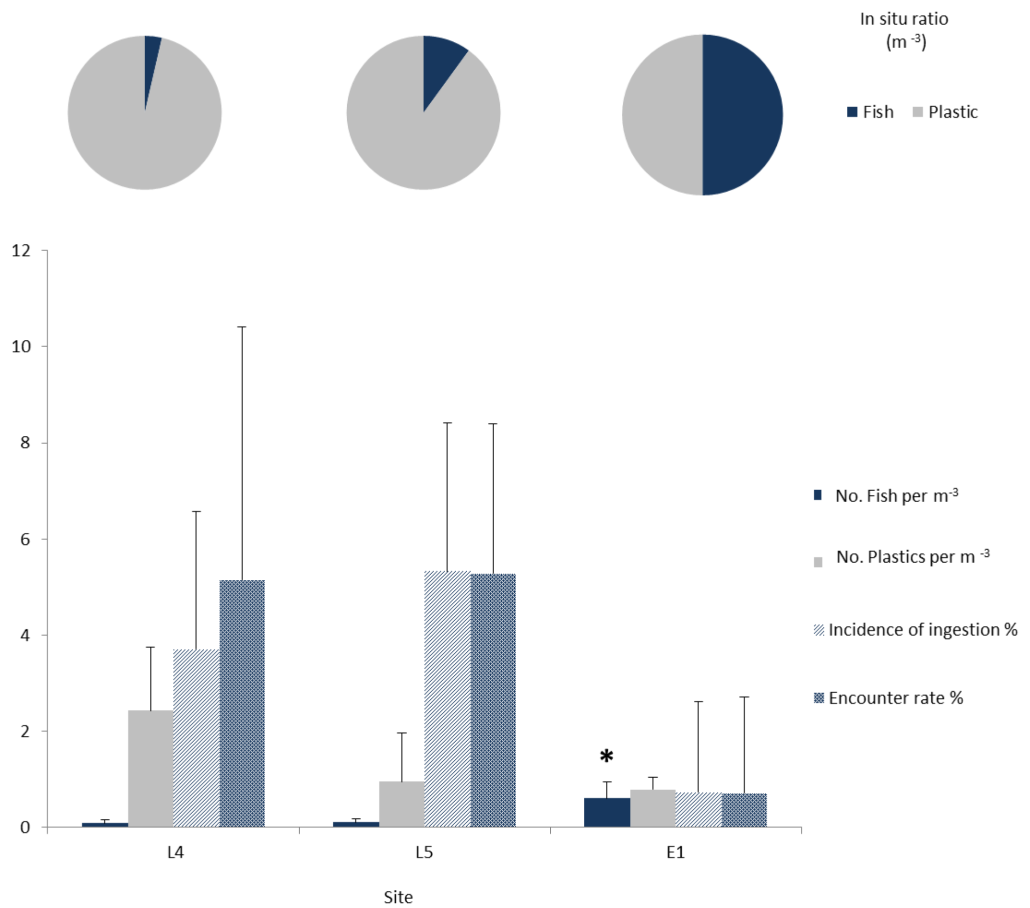

Figure 6. ABOVE: The ratio between concentration of microplastics and fish larvae in the water column at each site is displayed. BELOW: Comparison between plastic concentrations (number m $^{-3}$), fish larvae concentrations (individuals m-3), incidence of ingestion (number of fish with ingested particles/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

4. Discussion

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

4.1. Prevalence of microplastic ingestion

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

Here, we have identified that microplastics are ingested by a number of different species of fish larvae (meroplankton) in their natural environment. Fish larvae spend their entire planktonic stage in the pelagic zone and are unselective feeders. When prey concentration is low they not only pursue all prey sizes encountered but also increase their swimming activity and are much less selective (Munk and Nielson, 2005). By dissecting individual fish larvae, we were able to calculate ‘incidence of ingestion’, which ranged 3.2-5.5% across sites. For comparison with other studies on zooplankton, where bulk digestions have been used to extract microplastics, we also calculated ‘encounter rates’. Our analysis of fish larvae in the western English Channel has demonstrated an encounter rate with microplastics of between 0.7-5.3%. At L4, 3.7% of fish larvae ingested plastic; comparatively, 36.5% of adult fish sampled from L4 (June 2010–July 2011) had ingested microplastics (Lusher *et al*. (2013). Research by Rummel *et al*. (2016) recorded significantly higher ingestion percentages in pelagic fish (10.7%) compared to demersal (3.4%). The post larval stages of fish examined in this study were approximated to be between 5 days and 2 months old, excluding the European eel elver at less than a year old (Russell, 1976); microplastics would therefore have been encountered over a considerably shorter time frame than in adult fish, which may account for the lower proportion of individuals containing plastic observed alongside potential differences in gut retention times. All of the fish species that had ingested microplastics in this study (excluding the European eel larvae) have also been identified to consume microplastics as adults (Lusher *et al*., 2013). Further work is required to gauge how long fish larvae will retain ingested microplastics in order to better predict the likely impact of ingestion of the individual (i.e. are ingested plastics transient or do they have long residence times).

4.2. Potential health effects

Very little is known regarding the effects of ingesting microplastics on wild fish. There are substantial difficulties in assessing physiological or behavioural responses to ingestion in the wild, largely due to the inability to assess gut retention times or monitor chronic health effects arising from a single stressor. Laboratory studies on fish have illustrated significant physiological (gut blockage, decrease in food intake due to less gut space) and toxicological (inflammatory responses, oxidative stress, hepatic stress, decreased energy availability) damage can result from consumption of plastics (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 example, we note that the concentrations and types of microplastics used in the aforementioned exposure studies are largely unrepresentative of the microplastics identified at our study sites. We advocate that microplastics used in experiments need to reflect what is found in the field more closely as this information becomes available; the use of environmentally aged fibres (i.e. with 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 fate and effects of microplastics in the marine ecosystem. Ecologically relevant data is essential in order to address the impacts of microplastics on animal populations, communities and ecosystems.

Laboratory experiments using juvenile fish or fish larvae are currently limited in scope and number. Owing to the susceptibility of fish larvae to environmental stressors during development, it is imperative that the effects of microplastic exposure on key health parameters (i.e. growth rate, feeding) in juvenile fish is given due attention. de Sa *et al*. (2015) revealed that developmental conditions may influence a fish's ability to distinguish plastic from prey; it would therefore be intriguing to evaluate whether community fitness has a bearing on a fish larvae's ability to select prey over plastic.

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

4.3. Comparison between waterborne and ingested microplastics

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

Constituting over 50% of the microplastics found in our water samples, Rayon is a semi synthetic bioplastic used in clothing, furnishing, female hygiene products and nappies; Cole (2014) also found Rayon in the surface waters at L4 and close to the Plymouth sound (October 2013). Bioplastics (i.e. Rayon) are rarely represented in toxicity testing of microplastics, and should be considered an area requiring further testing. The large number of microscopic synthetic fibres found in the water suggests sewage outlets might be a prominent source of microplastic pollution observed across our sampling sites (Browne *et al*., 2010). Polyester and polyurethane (PU) were also identified in the waterborne samples; both polymers are used in resin systems for boat hulls, PU is found in numerous marine paints and polyester is a popular material for commercial marine rope including fishing nets in conjunction with nylon. There was a notable absence of microbeads in our samples, however this may be an artefact of our sampling protocol: Fendall and Sewell (2009) report that two thirds of cosmetic brands use <100 µm microbeads, therefore in using a 100 µm net we would be unlikely to capture spherical particles below this size threshold. The most abundant size range of waterborne microplastics was the <500 µm category, with abundance decreasing exponentially as particle size increased; a trend also reported in open ocean samples by Cozar *et al*. (2014). These plastics are of a similar size to microzooplankton which form a key component of the diet of fish larvae.

4.4. Relationship between distance and uptake

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

The abundance of fish larvae increased with increasing distance from shore. This study targeted spring spawning boreal species residing throughout the water column and at their most abundant and diverse in May (Russell, 1976). Fish larvae remain planktonic until adolescence when they move to their preferred habitat (e.g. Gadoids to rocky shores, flatfish to the benthos). Until this time they remain planktonic and in deeper water, with only surface dwelling larvae prone to onshore drift by prevailing winds; thus explaining the lower numbers recorded close to shore. Conversely microplastic concentrations decreased with distance from shore and as such, the ratio of fish:plastic decreased and directly correlated with the frequency of microplastic consumption. It is generally hypothesised that biota in coastal regions will experience a greater impact from microplastic ingestion. Furthermore we suggest that spatial and temporal overlap is key to the degree of impact observed at population level. Microplastic concentrations are spatially and temporally variable, influenced by local currents, accumulation spots and climate events among others. If these hotspots overlie spawning grounds for adult fish and areas where planktonic larvae fish are abundant then there will be far greater incidence of ingestion and therefore significantly higher encounter rate observed than during this study. It is the identification of these areas alongside a drive towards to producing ecologically relevant data that should be the focus of future research efforts in order to target prevention, policy and legislation (Rochman, 2016). The emphasis should now be on encouraging the use of preventative measures rather than the need for expensive clean-up operations.

5. Conclusion

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

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