



Consistent microplastic ingestion by deep-sea invertebrates over the last four decades (1976–2015), a study from the North East Atlantic[☆]

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ABSTRACT

Although evidence suggests the ubiquity of microplastics in the marine environment, our knowledge of its occurrence within remote habitats, such as the deep sea, is scarce. Furthermore, long term investigations of microplastic abundances are even more limited. Here we present a long-term study of the ingestion of microplastics by two deep-sea benthic invertebrates (*Ophiomusium lymani* and *Hymenaster pellucidus*) sampled over four decades. Specimens were collected between the years 1976–2015 from a repeat monitoring site >2000 m deep in the Rockall Trough, North East Atlantic. Microplastics were identified at a relatively consistent level throughout and therefore may have been present at this locality prior to 1976. Considering the mass production of plastics began in the 1940s - 50s our data suggest the relatively rapid occurrence of microplastics within the deep sea. Of the individuals examined (n = 153), 45% had ingested microplastics, of which fibres were most prevalent (95%). A total of eight different polymer types were isolated; polyamide and polyester were found in the highest concentrations and in the majority of years, while low-density polystyrene was only identified in 2015. This study provides an assessment of the historic occurrence of microplastics on the deep seafloor and presents a detailed quantification and characterisation of microplastics ingested by benthic species. Furthermore these data advance our knowledge on the long-term fate of microplastic in marine systems.

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1. Introduction

Since the 1940s - 50s there has been a rapid and world-wide increase in plastic production. The latest data suggest that around 60% of all plastics ever produced are accumulating in landfills or in the environment (Geyer et al., 2017). By their very nature, synthetic plastics are extremely durable (Shah et al., 2008); but under the influence of environmental exposure, including mechanical forces and/or photochemical processes, plastics fragment into microplastics and nanoplastics (Gewert et al., 2015; ter Halle et al., 2016). Several trillion tonnes of plastics are estimated to be afloat in the world's oceans, with microplastics constituting around 92% of this (Eriksen et al., 2014; van Sebille et al., 2015). Plastic is thus a problem of growing environmental concern and has recently been

proposed as a planetary boundary threat. This is defined as having planetary-scale occurrence which is not readily reversible and may cause currently unrecognised disruptive effects to vital Earth system processes (Galloway et al., 2017; Jahnke et al., 2017; Rockström et al., 2009).

With the continued and increasing input of plastic to the oceans (Geyer et al., 2017) it might be expected that surface concentrations would also increase over time, however data reveal inconsistencies. The North Pacific Subtropical Gyre has been documented to be accumulating plastics (Goldstein et al., 2012; Lebreton et al., 2018) while no such trends have been observed in other surface waters (Beer et al., 2017; Law et al., 2014, 2010; ter Halle et al., 2016). Global ocean budgeting identifies large discrepancies between expected and observed surface quantities (Cozar et al., 2014; Eriksen et al., 2014). A number of hypotheses may explain these findings. Firstly, the majority of studies employ surface-towed nets, typically with a mesh size of ~300 μm (Barrows et al., 2017; Hidalgo-ruz et al., 2012). Due to the continual fragmentation of plastics in the

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environment it is possible that a large proportion of small microplastics are not being detected, or have now fragmented to nano-scale which may be missed by these types of sampling. Secondly, wind-driven mixing of the surface layers may vertically redistribute microplastics, resulting in potentially large amounts going unmeasured (Kukulka et al., 2012). Lastly it is thought that the majority of plastic litter does not remain in surface water but sinks until ultimately residing on the seafloor.

Recently Koelmans et al. (2017), undertook a whole ocean mass balance study; their simulations showed that 99.8% of the plastics which had entered the oceans since 1950 had settled below the ocean surface layer and around 8.5 million tonnes of plastics settle annually. The sinking of plastic particles is related to their size, density, physical/chemical properties (Kowalski et al., 2016) and biological interactions. Biofilm formation occurs rapidly in the environment (Lobelle and Cunliffe, 2011; Rummel et al., 2017), altering particle density and hydrophobicity and in turn increasing the rate of sinking. Microplastics may form aggregates with phytoplankton (Long et al., 2015) or marine snow (Porter et al., 2018; Zhao et al., 2016), flocculate and adhere to transparent exopolymers (Engel, 2004; Passow et al., 2001; Summers et al., 2018) or be incorporated into faecal pellets (Cole et al., 2016; Katija et al., 2017). In most cases these processes act to reduce the buoyancy of microplastics and thus increase the sinking rate, redistributing microplastics from surface waters to the ocean floor.

Data quantifying microplastic contamination in the deep sea are sparse; yet those which have, report microplastics in water (Courtene-Jones et al., 2017a; Kanhai et al., 2018), benthic invertebrates (Courtene-Jones et al., 2017a; Taylor et al., 2016) and sediments (Fischer et al., 2015; Van Cauwenberghe et al., 2013; Woodall et al., 2014). Indeed some of the highest concentrations of microplastics reported to date are from deep sediments (42–6595 microplastics kg^{-1}) (Bergmann et al., 2017) supporting the hypothesis that the seafloor may accumulate microplastics. There are some long-term studies which monitor large marine litter on the seafloor (Bergmann and Klages, 2012; Chiba et al., 2018; Galgani et al., 2013, 2010; Maes et al., 2018; Schlining et al., 2013), however we are not aware of any such studies for microplastics. These enquiries are needed to elucidate fundamental questions regarding the behaviour and fate of microplastics within marine systems.

The Scottish Association for Marine Science (SAMS) has conducted deep-sea research at a permanent location 2200 m deep in the Rockall Trough since the mid-1970s. Operations ceased during the period 1995–2013 due to a lack of funding; however this collection, spanning four decades represents one of the longest time-series of deep ocean specimens. Organisms were originally collected largely for the analysis of community structure and were preserved intact, giving a unique opportunity to study temporal trends in ingested microplastics. The present study assesses microplastic ingestion over four decades by two species of benthic echinoderms; *Ophiomusium lymani* and *Hymenaster pellucidus*, to examine long-term trends of microplastic abundance and polymer types. *O. lymani* is a benthic deposit feeder and facultative predator (Iken et al., 2001; Pearson and Gage, 1984) while *H. pellucidus* predares on invertebrates and planktonic fallout; both species are mobile and feed at the surface of the sediment. Additionally, both species have previously been reported to ingest microplastics (Courtene-Jones et al., 2017a) making them suitable for inclusion in this study.

The aims of this research are (i) to determine whether the first occurrence of microplastic pollution can be detected in the Rockall Trough, and (ii) to examine long-term temporal trends in overall microplastic abundance and constituent polymer types. Utilising this unique archival collection we aim to provide insights regarding the long-term fate of marine microplastics and their historical occurrence on the deep seafloor.

2. Method

2.1. Sample locations and species

'Gage Station M' is situated at a depth of 2200 m in the Rockall Trough (57.300°N, 10.383°W), North East Atlantic Ocean (Fig. 1). Deep-sea sampling began at this location in 1976 and has been conducted on an annual basis since, except during an 18 year period between the years 1995–2013 where no deep-sea operations were undertaken. The methods to collect samples have remained constant during four decades of operation. The primary historical sampling method entails use of a Woods Hole Oceanographic Institution-pattern epibenthic sled, rigged with polyamide (nylon) nets of mesh size 0.5 mm to sample both the epifauna and infauna. Early sampling (1970s) was also conducted with an Agassiz trawl, which is similar to the epibenthic sled (and also utilise a polyamide net), however it is more suitable for the collection of benthic macro/megafauna (Jamieson et al., 2013). Historic samples were all processed in the same way, involving the contents of the nets being washed through stacked stainless steel sieves (mesh sizes 0.42 mm, 0.5 mm and 1 mm) to separate the invertebrates into different size classes. Each size fraction was transferred to separate lidded buckets and were fixed using 4% buffered formaldehyde before being stored in 70% ethanol. Upon return to the laboratory faunal assemblages were identified to species levels and stored thereafter in 70% ethanol (Gage et al., 1980) in sealed containers.

Macro-benthic species assemblages in this region are dominated by echinoderms and in particular *Ophiomusium lymani* and *Hymenaster pellucidus* are found in relatively high abundance (Gage, 1986). Both species have previously been identified to ingest microplastics (Courtene-Jones et al., 2017a) thus for these reasons *O. lymani* ($n=90$) and *H. pellucidus* ($n=63$) were selected to investigate microplastic loads (SI Fig. 1). Specimens from the years 1976, 1980, 1985, 1990, 1995, 2013 and 2015 where possible were analysed, with sample sizes varying slightly between years based on the number of individuals available (details summarised in SI Table 1).

2.2. Quality assurance/quality control

Well documented laboratory quality assurance/quality control (QA/QC) procedures were followed as detailed in Courtene-Jones et al. (2017a) and summarised in the supplementary information. All scientific equipment (scalpels, forceps, glass beakers, filter manifold etc.) were covered/wrapped in aluminium foil and examined under the microscope prior to use. All samples remained covered when not in use to reduce risk of contamination from aerial sources.

At the time of field sampling the same rigorous contamination measures implemented today were not enforced. The same equipment (metal epibenthic sled/Agassiz trawl with a polyamide net (white in colour) see SI Fig. 2) has been used throughout the four decades of deep-sea operations. The net was sampled and analysed spectroscopically as well as examined closely under the microscope. While we cannot completely rule out historic contamination at the time of collection, thorough washing of each specimen with a flow of deionised water followed by close visual examination prior to dissection was undertaken to remove any external microplastics which may confound results. As only microplastics internalised within the organisms' soft tissues were assessed the likelihood of contamination arising from sampling are reduced.

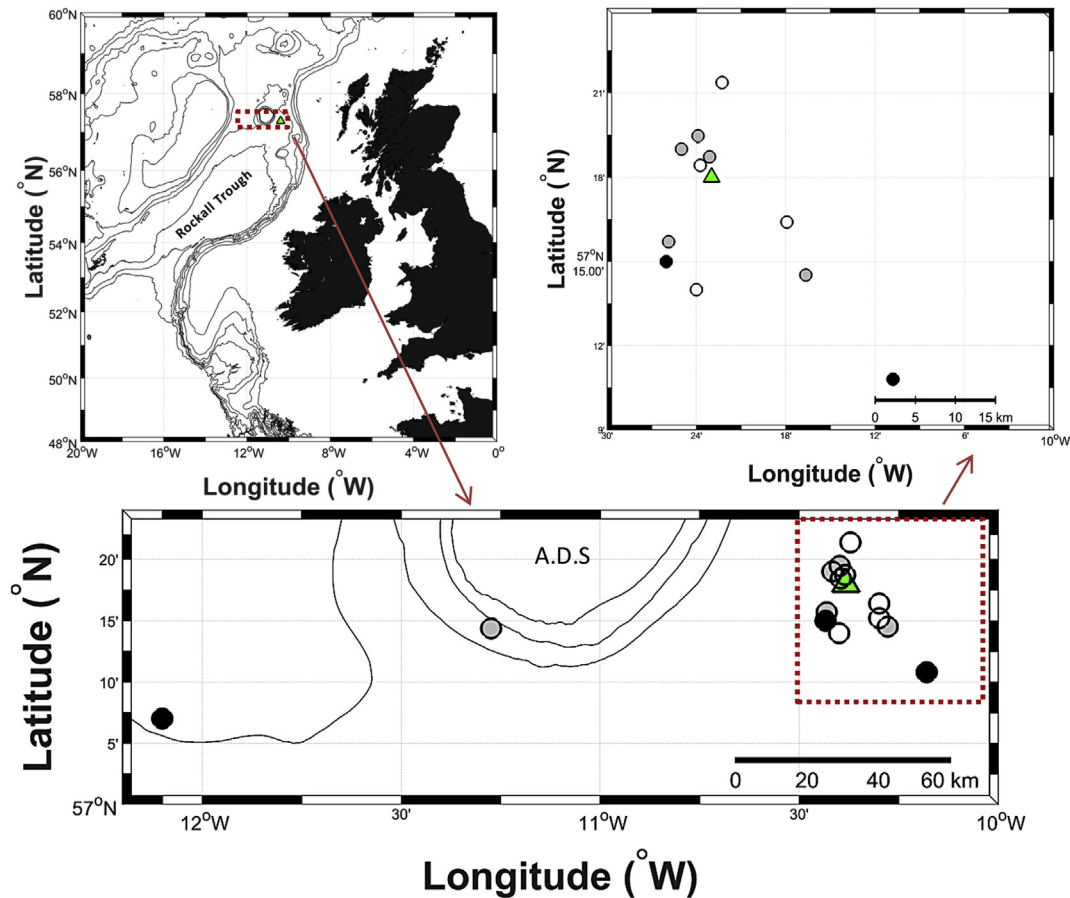


Fig. 1. Map showing the sampling locations around the long-term monitoring site ‘Gage Station M’ (green triangle) to the east of the Anton Dohrn seamount (A.D.S.) in the Rockall Trough; West of the United Kingdom. The area shown in the hatched box is depicted in the subsequent panel, and shows the sampling locations of *O. lymani* (black points), *H. pellucidus* (white points) and both species (grey points). Bathymetry is contoured at 500 m intervals from depths of 500 m–3500 m (MATLAB R2015b using GEBCO_2014 bathymetry data). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

2.3. Enzymatic digestion of invertebrates

Specimens from each sampling year were removed from ethanol and the width of the central disc was measured using metal dial calipers. Individuals were washed thoroughly with a flow of deionised water and placed into clean lidded glass petri dishes. The exoskeleton was carefully examined under a dissecting microscope to ensure there were no microplastics present, and any found were removed with forceps. Next, the central disc was dissected and all soft tissue was removed from the exoskeleton; in the case of *H. pellucidus*, soft tissue extending into each of the five arms (part of the digestive and reproductive systems) was also removed. The soft tissue was then weighed using a Sartorius analytic electronic balance (4 d.p.) before being placed into separate 100 ml glass beakers which were covered with aluminium foil. Enzymatic digestions with the proteolytic digestive enzyme trypsin were carried out following the protocol outlined by Courtene-Jones et al. (2017b).

The 52 μm mesh gauze, through which the solution was filtered, was systematically and thoroughly examined under a dissecting microscope three times and any potential microplastics were removed and placed into a small petri dish containing a 30 mm diameter filter paper (Whatman Grade 1). Once all potential microplastics had been transferred, the 30 mm petri dish was sealed and labelled for later spectroscopic analysis.

2.4. Microplastics identification

A Perkin-Elmer One Fourier Transformation Infrared (FTIR) microscope in transmission mode was used to identify potential microplastic particles. Samples were placed onto gold coated glass slides and infrared radiation in the wavelengths 600 - 4000 cm^{-1} was used. Each spectra produced was the average from 16 scans, a variable aperture size was used and the spectral resolution was 4 cm^{-1} . Background scans were taken between each sample and were automatically subtracted from the sample's spectrum. Spectra were visualised in OMNIC 9 (Thermo Fisher Scientific Inc.) with use of the inbuilt Hummel polymer library to aid identification. Additional user generated libraries from the collaborative University of the West of Scotland and Scottish Association for Marine Science ‘UWS/SAMS’ library and the Alfred Wegener Institute ‘AWI’ (Pimpke et al., 2018) were utilised to enable the comparison with a wider variety of polymers as well as those having undergone environmental weathering. As well as using these three libraries (Hummel, ‘UWS/SAMS’ and ‘AWI’), the characteristic functional group signals from each spectra acquired were examined manually to confirm the identity of the materials being assessed.

2.5. Data analyses

The percentage incidence of ingestion was calculated as the

number of individuals that ingested one or more microplastic particle/total number of dissected individuals $\times 100$. This was calculated for each species and when species were grouped for each sampling year. To take into account differences in specimen weights, microplastic ingestion was standardised per gram of wet weight (w.w.) tissue; these data were tested for normal variance structure using a Shapiro and Levene's test. Data were subsequently \log_{10} transformed to meet the criteria of normal distribution and homoscedasticity.

Interspecific differences between the numbers of ingested microplastics/g tissue and the abundance of polymer types ingested were examined by two sample *t*-test. Polymer diversity was computed based on Shannon-Weiner (*H'*) diversity index (log base transformed data) and species specific and grouped diversity indices were used for Spearman's rank correlation test with year. Differences in the abundance of each polymer type ingested/g tissue between years were compared using the Kruskal-Wallis test (as data failed to meet normal variance structure) and where a significant difference was found a post-hoc Dunn's test was carried out. As the polymer types polyvinylchloride-copolymer and polystyrene were only present during one sampling year these polymers were not included. The polymer composition and abundance between years was also compared by hierarchical cluster analysis based on group average linkage of Bray-Curtis similarity of microplastic/g polymer data. All statistical analysis were performed in R Studio V 0.99.892 (R Core Team, 2016) with the use of the packages *car* (John et al., 2017) *vegan* (Oksanen et al., 2018) and *dunn.test* (Dinno, 2017).

3. Results

3.1. Polymer composition

Eight different polymers were identified (SI Fig. 3); overall, polyamide and polyester were the most abundant (Fig. 2). Three of the polymer types were ingested by both species, namely polyamide, polyester and acrylic. Alkyd and polyethylene were only

isolated from *O. lymani* and PVC-copolymer, polystyrene and polyacrylonitrile were only present in *H. pellucidus*. The mean abundance of microplastics/g of each polymer type did not differ significantly between the two species ($t = -1.096$, $df = 18.72$, $p = 0.287$).

Polymer diversity, measured using the Shannon-Weiner index (Table 1), varied between years and species. When species were grouped, the years 1980 followed by 1985 had the greatest polymer diversity (H' 1.367 and 1.225 respectively) and 1990 had the lowest polymer diversity (H' 0.534). No correlation was found between polymer diversity and year (*H. pellucidus*: $r_s = -0.700$, $p = 0.233$; *O. lymani*: $r_s = 0.321$, $p = 0.498$; species grouped: $r_s = -0.107$, $p = 0.840$). Cluster analysis based on group average linkage of Bray-Curtis dissimilarity further illustrates the lack of trend between polymer type and abundance between years (SI Fig. 4).

3.2. Interspecific and inter-annual microplastic ingestion

Microplastic ingestion varied between species with *O. lymani* having a higher incidence of ingestion than *H. pellucidus* (51% and 22% across all years respectively, Table 1). Examining each year separately revealed the highest ingestion occurred in 1995 for *O. lymani* (80%) and in 1985 for *H. pellucidus* (40%) and when species were grouped (45%).

Microplastics were identified in all years and remained at relatively consistent levels throughout the time series. The abundance of ingested microplastics was between 1.96 ± 0.66 – 4.61 ± 3.62 microplastics/g w.w. (mean \pm SD) when species were grouped; and between 1.96 ± 0.66 – 3.43 ± 1.35 microplastics/g w.w. (mean \pm SD) for *O. lymani* and 0.48 ± 0 – 9.10 ± 4.21 microplastics/g w.w. (mean \pm SD) for *H. pellucidus* (Fig. 3). Total abundance of ingested microplastics did not statistically differ between years for either *H. pellucidus* ($F(1, 14) = 0.195$, $p = 0.666$), *O. lymani* ($F(1, 38) = 0.047$, $p = 0.83$), or when species were grouped ($F(1, 54) = 0.011$, $p = 0.918$). Also, there were no significant differences in the abundance of ingested polymer types/g w.w. tissue between years (Fig. 4).

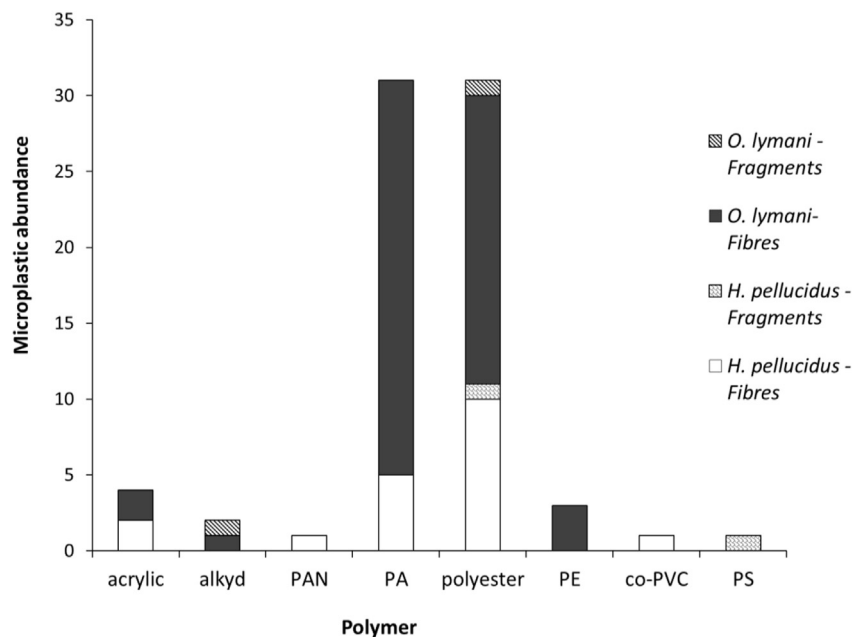


Fig. 2. Total abundance of different polymers and particle types ingested by *O. lymani* and *H. pellucidus* across all years. The quantities of fibres are indicated by the solid filled bars and fragments by the patterned bars.

PAN = polyacrylonitrile, PA = polyamide, PE = polyethylene, PS = polystyrene, co-PVC = polyvinylchloride-copolymer.

Table 1

Sampling information along with the abundance of microplastics per gram of wet weight soft tissue (MP/g w.w.), incidence of microplastic ingestion, polymer diversity (Shannon-Weiner H') and total number of unique polymer types ingested by each species and when species were grouped from the different sampling years. Values presented after \pm symbol represents standard deviation. n/a indicates the absence of individuals within the sampling year.

Year	Species	No. individuals sampled	Mean tissue w.w. (g)	Incidence of ingestion	Mean MP/g w.w. tissue	No. unique polymers	Polymer diversity (H')
2015	<i>O. lymani</i>	20	0.547 \pm 0.195	30%	2.57 \pm 1.53	3	1.053
	<i>H. pellucidus</i>	20	0.773 \pm 0.520	20%	1.27 \pm 0.73	2	0.462
	Species grouped	40	0.660 \pm 0.404	25%	2.11 \pm 1.42	4	1.115
2013	<i>O. lymani</i>	20	0.449 \pm 0.168	25%	2.93 \pm 1.34	2	0.692
	<i>H. pellucidus</i>	20	0.228 \pm 0.173	10%	9.10 \pm 4.21	2	0.597
	Species grouped	40	0.338 \pm 0.202	18%	4.61 \pm 3.62	2	0.660
1995	<i>O. lymani</i>	10	0.585 \pm 0.139	80%	2.27 \pm 0.97	4	1.207
	<i>H. pellucidus</i>	3	1.863 \pm 0.211	33%	0.48 \pm 0	1	0.000
	Species grouped	13	0.880 \pm 0.579	70%	2.11 \pm 1.07	4	1.197
1990	<i>O. lymani</i>	10	0.476 \pm 0.157	30%	2.48 \pm 0.50	2	0.584
	<i>H. pellucidus</i>	n/a	n/a	n/a	n/a	n/a	n/a
	Species grouped	10	0.476 \pm 0.157	30%	2.48 \pm 0.50	2	0.584
1985	<i>O. lymani</i>	10	0.627 \pm 0.130	50%	2.46 \pm 1.23	3	0.670
	<i>H. pellucidus</i>	10	0.838 \pm 0.231	40%	2.05 \pm 1.05	3	1.278
	Species grouped	20	0.732 \pm 0.212	45%	2.55 \pm 1.59	4	1.225
1980	<i>O. lymani</i>	10	0.346 \pm 0.069	50%	3.43 \pm 1.35	3	1.063
	<i>H. pellucidus</i>	10	1.039 \pm 0.455	30%	1.36 \pm 0.27	4	1.371
	Species grouped	20	0.693 \pm 0.476	40%	2.51 \pm 1.46	5	1.367
1976	<i>O. lymani</i>	10	0.484 \pm 0.142	40%	1.96 \pm 0.66	2	0.640
	<i>H. pellucidus</i>	n/a	n/a	n/a	n/a	n/a	n/a
	Species grouped	10	0.484 \pm 0.142	40%	1.96 \pm 0.66	2	0.640

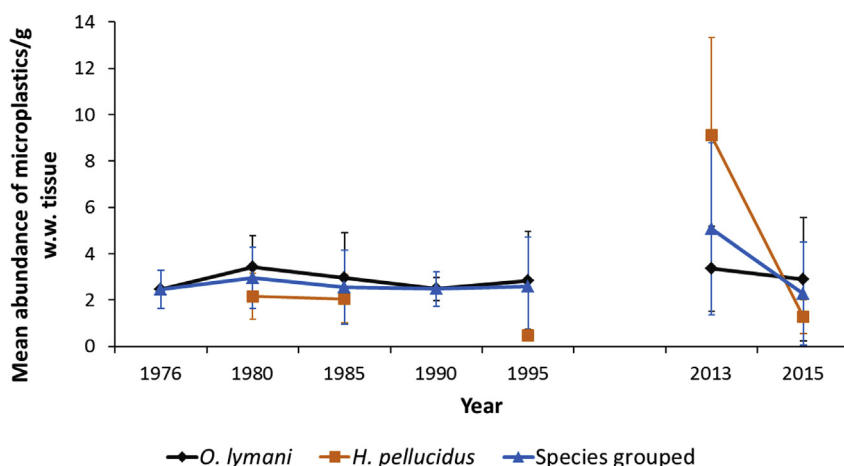


Fig. 3. Mean abundance of microplastics/g of wet weight tissue across the time period for *O. lymani* (black line), *H. pellucidus* (orange line) and when species are grouped (blue line). Error bars show standard deviation. This plot is a summary of values in presented in the 'Mean MP/g w.w. tissue' column of Table 1. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

3.3. Characterisation of microplastics

FTIR was performed on 470 potential microplastic items; of which 74 were identified as synthetic polymers, 294 items were natural (cellulose ($n = 292$)/calcium carbonate ($n = 2$)) and 102 items yielded low quality and unusable spectral data. Thus, 16% of items analysed were confirmed to be microplastics. The majority of plastic items were fibres ($n = 70$, 95%) with only a very low number of fragments identified ($n = 4$, 5%). A variety of colours were detected (SI Fig. 5) and example microplastics are shown in SI Fig. 6. Fragments ranged in diameter from a minimum of 0.08 mm to a maximum of 0.67 mm (mean \pm SD: 0.34 mm \pm 0.26 mm) and fibres ranged in length from 0.21 mm–4.90 mm (mean \pm SD: 1.20 mm \pm 0.98 mm). All microplastics were less than 5 mm and the size distributions varied only marginally between years (SI Fig. 6).

3.4. QA/QC

No particles were found on any of the 22 atmospheric controls performed. The quantities of microplastics on Tape Lift Screening ($n = 22$), used to assess background contamination levels, varied; there was 2.25 fibres \pm 1.58 (mean \pm S.D.) after cleaning and prior to commencing laboratory work, and 3.50 fibres \pm 2.27 (mean \pm S.D.) after laboratory work. These fibres were red, blue and white in colour and identified as cellulose/cotton in their composition by FTIR analysis. Additionally none of the ingested fibres were found to match the characteristics (white polyamide) of the Agassiz/epibenthic sled netting.

4. Discussion

A consistent abundance of microplastics were identified

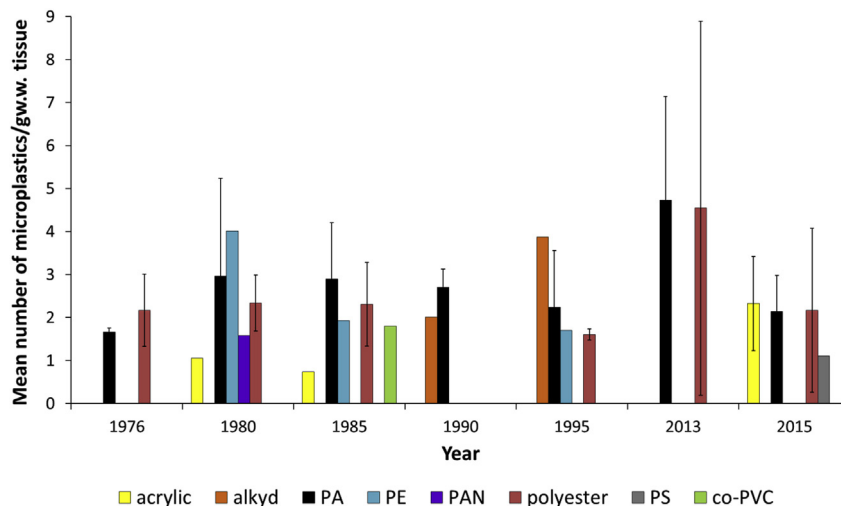


Fig. 4. Composition and mean abundance of each polymer type ingested per gram of wet weight soft tissue across years. Error bars show standard deviation and are absent for those polymers where only a single microplastic was present within the sampling year.

PAN = polyacrylonitrile, PA = polyamide, PE = polyethylene, PS = polystyrene, co-PVC = polyvinylchloride-copolymer.

throughout the entire specimen series spanning a period of four decades. The present dataset represents a quantification of historic microplastic ingestion and develops our knowledge of the occurrence of this pollutant on the deep seafloor. While the number of positively identified microplastics from each sampling year was relatively low, no trends were observed between the overall abundance or the polymer types ingested between years, demonstrating not only the consistent levels of ingested microplastics but that this pollutant likely arrived at the study site prior to 1976.

Global production and use of synthetic plastics have increased exponentially since their mass production in the 1950s (Geyer et al., 2017; PlasticsEurope, 2017), resulting in an increase in the flux of plastic waste entering the environment by as much as 120% between the years 1975–2010 (Jambeck et al., 2015). These decadal trends have been observed in some surface waters (Goldstein et al., 2012; Lebreton et al., 2018; Thompson et al., 2004) and in coastal sediments around Belgium between the years 1993–2008 (Claessens et al., 2011). It is not well established how long it will take for macroplastics to break down into microplastics and subsequently escape surface waters (GESAMP, 2015) but high concentrations of microplastics in marine sediments indicate this as a potential sink for these small particles (Gago et al., 2018; Woodall et al., 2014). Given the uncertainty surrounding microplastic deposition rates, it was initially hypothesised that this study may observe the first occurrence of microplastics on the Rockall Trough seafloor and identify temporal trends in abundance and/or polymer types using benthic invertebrates as a proxy.

It was realised that the occurrence of ‘the plastic age’ (Thompson et al., 2009) within the Rockall Trough pre-dates our earliest sample, dating from 1976; sampling was not undertaken prior to this preventing the analysis of earlier samples. To some degree this is not entirely surprising. Larger plastics have been documented on the seafloor (100–200 m depth) as early as the mid-1970s (Holmström, 1975), and recent experimental and modelling data indicate the relatively rapid transit of microplastics through the water column (Long et al., 2015; Zhang, 2017). Theoretical modelling by Kooi et al. (2017) showed that microplastics with densities greater than seawater (i.e. negatively buoyant), such as polyamide, polyester and PVC start to settle immediately and always sink to the seafloor. For example, a 0.1 mm PVC particle may only take 10 days to sink to a depth of 4000 m (Kooi et al., 2017). By

this rationale most of the plastics entering the oceans since the 1950s may now reside on the seafloor (Koelmans et al., 2017).

The majority of the polymers identified in the present study (polyester, polyamide, acrylic, alkyd, PAN and PVC) had densities greater than seawater (Andrady, 2011; Crawford and Quinn, 2017), corroborating the findings of other benthic research (Bergmann et al., 2017; Courtene-Jones et al., 2017a; Woodall et al., 2014) and indicating these polymers likely sink directly and relatively rapidly to the seafloor, where they are bioavailable to the benthic community. Positively buoyant microplastics were also isolated; non-expanded polystyrene (1.04 g/cm^3 (Crawford and Quinn, 2017)), was only present in 2015, and polyethylene which was one of the lowest density polymers identified in this study (density $0.92\text{--}0.97 \text{ g/cm}^3$ (Crawford and Quinn, 2017)) was identified post-1980. This may indicate that low density polymers sink over longer time scales than their high density counterparts. Experimental work showed polystyrene particles decreased their sinking velocity with increasing salinity (Kowalski et al., 2016). In natural marine waters the density of seawater typically increases with increasing depth; in the Rockall Trough seawater potential density (σ_θ) changes from around 1.0272 g/cm^3 in the surface layer to 1.0278 g/cm^3 at 2500 m depth (Holliday et al., 2000). It has been suggested that microplastic particles will remain in suspension when reaching a depth where water and particle density are similar (Wang et al., 2016). However ocean processes are complex and other factors will influence the sinking of microplastics. Synthetic polymers vary in their elasticity (Young’s modulus) from around 0.15 GPa for low density polyethylene to 4.5 GPa for polyester (measured at 20°C and atmospheric pressure) (Ashby and Jones, 2006). The increasing pressure associated with depth will cause microplastics to compress; the depth at which this occurs and the amount of compression will depend on the specific Young modulus. It is likely that certain microplastic polymers will compress to a greater degree than the surrounding seawater (2.21 GPa at atmospheric pressure at 20°C) (Liley et al., 2007). This will result in the microplastics increasing in density relative to the surrounding seawater, and in this way facilitate their sinking.

The occurrence of these low density polymers on the seafloor may also be explained by the hetero-aggregation of small plastic particles with other inorganic material. Coagulation is driven by the size, shape and density, along with the surface character of a

particle, in turn influencing the fate of these aggregates in marine systems (Filella, 2015). In this study, 95% of the microplastics isolated were fibres. Particles such as fibres and films have high surface area to volume ratios and as such have a higher rate of aggregation/biofouling causing them to sink sooner and over relatively shorter time scales than larger plastic fragments (Ryan, 2015). Theory predicts that small sized particles will coagulate quickly (Filella and Buffle, 1993) and even buoyant material may be transported to the deep ocean relatively quickly in this way (Logan and Hunt, 1987), offering a mechanism by which microplastics may be removed from surface waters (Cozar et al., 2014). Furthermore not all sources of plastic may be introduced from land or at the ocean surface. Shipping activity, fishing equipment, offshore industries such as oil and gas and other maritime activities may introduce microplastics directly into the water column (Browne, 2015) or to the seabed and consequently reduce the time taken for microplastics to be sequestered to the seafloor. Human influences, such as deep-water bottom trawling can be traced back to 1960s in International waters to the west of Rockall Plateau (Basson et al., 2001), and by the mid-1980s the Rockall Trough had become the major deep-water bottom trawl fishing area in the northern North East Atlantic Ocean (Gordon, 2003; Heymans et al., 2011). The deep waters of the Rockall Trough are mainly comprised of Labrador seawater and tend to be slow moving (Holliday et al., 2000). Waters deeper than 1200 m are constrained due to the local bathymetry and can only exit via the southern approach of the trough (New and Smythe-Wright, 2001). These factors may promote the accumulation of microplastics within the Rockall Trough, however further work would be needed to corroborate this.

Interestingly no clear trends in overall ingested abundances or polymer abundances were observed across the time series. There was a slight increase in microplastics in 2013, however this was not statistically significant. The gap in sampling between the years 1995–2013 was beyond the control of the present study. To try and overcome this and enable a more in-depth assessment of microplastic load post-sampling break, 20 specimens from each species were analysed from the years 2013 and 2015. While the analysis of a complete time-series would be beneficial in drawing more robust conclusions about microplastic prevalence in the deep sea; the dataset obtained indicates stability in microplastic abundance across sampling years and additional sampling is unlikely to reveal different conclusions. Species distribution in the deep-sea is heterogeneous (Zeppilli et al., 2016) and as such the sample sizes varied between species and years. *O. lymani* dominates macrofauna assemblages in this region (Gage, 1986) enabling ≥ 10 individuals to be analysed for microplastics throughout the time series. *H. pellucidus* was the next most abundant species collected by the sled trawls (*pers obs.*), however specimens were absent during the years 1976 and 1990 preventing their inclusion in the study and only a low number ($n = 3$) were present in 1995. It is possible that the difference in sample numbers may have influenced overall microplastic abundances or polymer diversity, however these years do not show such reductions, giving confidence to the results reported. No other macroinvertebrate species occurred in high enough abundance across the time period to be included within this study. Additional species and sample numbers would, of course, strengthen the dataset presented and facilitate drawing conclusions on the multi-decadal ingestion of microplastics.

Similar to the results presented here, Beer et al. (2017) found no trends in the abundance of microplastics ingested by pelagic fish from the Baltic sea over a 28 year dataset (1987–2015), however the authors did not characterise polymer types preventing comparison with our findings. In fact, the current study appears to be the first long-term (decadal/multi-decadal) investigation to characterise microplastic polymers. A total of eight different polymer

types were identified; polymer diversity (H') remained relatively stable throughout the time-series, with two polymer types identified in 1976, 1990 and 2013, four polymer types in 1995 and 2015; and a maximum of 5 polymer types in 1980 and 1985. Three polymers were unique to just one sampling year; polyacrylonitrile in 1980, PVC-copolymer in 1985 and polystyrene in 2015. Acrylic, alkyd and polyethylene were found sporadically within the time series, and both polyamide and polyester fibres were present in all years sampled, with the exception of polyester in 1990. Polyester fibres are documented to be one of the most prevalent and widespread microplastics in the environment (Browne et al., 2011). Polyester and polyamide dominate fibre production, with polyester use in the textile sector seeing an annual growth of 7% since 1990; this polymer now accounts for nearly half of the global fibre market (Carr, 2017; Chemical Economics Handbook, 2016). An estimated 600 metric tons of polyester, polyamide and acrylic fibres have been discarded and are accumulating in landfill sites or the natural environment (Geyer et al., 2017), in addition to this up to 0.1 mg fibres/g polyester textile can be shed during washing (Hernandez et al., 2017), providing a source of fibre release to waste water treatment plants and to the environment (Murphy et al., 2016).

The two species examined have previously been reported to ingest microplastics (Courtene-Jones et al., 2017a). *O. lymani* is a surface deposit feeder and facultative predator on small crustaceans and polychaetes (Iken et al., 2001; Pearson and Gage, 1984), and *H. pellucidus* predaes on small benthic invertebrates and planktonic fallout (Wagstaff et al., 2014); however despite having different feeding modes and diets these factors have not been linked to the abundance of microplastic they ingest (Courtene-Jones et al., 2017a). Additionally, prior work found no detrimental effects to microplastic polymers when using 4% formaldehyde as a fixative, followed by storage in 70% ethanol (Courtene-Jones et al., 2017b), indicating the suitability of these specimens for this study. The abundances of microplastics ingested across years (1.96 ± 0.66 – 4.61 ± 3.62 microplastics/g (mean \pm SD) depending on sampling year) are within a range comparable to coastal species (Devriese et al., 2015; Foekema et al., 2013; Li et al., 2016; Van Cauwenberghe et al., 2015), providing further evidence for the high levels of microplastics at the seafloor. Sample contamination is a key issue within the field of microplastics research (Wesch et al., 2017); at the time of sampling the same rigorous contamination measures put in place today would not have been used. While we cannot completely rule out historic contamination at the time of collection, thorough washing of each specimen with deionised water followed by visual examination prior to dissection was undertaken, to remove any external microplastics which may confound results. This study only assesses microplastic levels contained within the internal tissues of these fauna where the chances for contamination arising from sample collection are minimal. Strict protocols were followed in the laboratory where samples were dissected and background controls revealed only low numbers of cellulose fibres were detected, giving confidence to the results reported here.

Only 16% of all the putative microplastics analysed were confirmed to be synthetic; thus the data presented may in fact be an under estimation of ingested quantities. The majority of particles (63%) were identified as natural based on their FTIR spectrum despite having a brightly coloured appearance (primarily blue or red). Other studies have found similar results, attributing spectra to the semi-synthetic polymer Rayon, which has an almost identical FTIR spectrum to cellulose (Lusher et al., 2015, 2014; Sadri and Thompson, 2014). To conclude whether the material is natural or Rayon would require further analysis of these particles; thus to prevent confounding our results, all cellulosic fibres (as identified with FTIR) were discounted from this study.

Microplastics have the potential to be retained and accumulate within the bodies of biota (GESAMP, 2015). Watts et al. (2014) demonstrated that following laboratory exposure crabs retained microplastics in their tissues for up to 14 days, while Browne et al. (2008) found microplastics remained in the circulatory system of mussels 48 days after high-concentration exposure. The longest study of its kind evidenced that langoustine retained microplastic fibres following low-concentration chronic exposure, which were then lost during moult, a process occurring every 6–12 months (Welden and Cowie, 2016). With the exception of these studies little work has been undertaken to determine the retention times, clearance rates and associated influencing factors in different organisms. Microplastics clearly have the potential to remain in the bodies of organisms which have been suggested as a short-term sink (Güven et al., 2017) however may not be retained long term due to processes of egestion. Different environmental matrices (sediment/water/fauna) likely store microplastics differently and while sediment and water may continually accumulate microplastics, it is possible that organisms may not accumulate microplastics in the same quantities as in their ambient environment. In fact it has been suggested that there may be a point at which an organism becomes saturated (Ryan et al., 2009) and does not continue to accumulate microplastics internally. This may explain why the abundances of ingested microplastics remains relatively stable in this study, despite the continued inputs of plastics into the marine environment (Geyer et al., 2017; Jambeck et al., 2015); however this is a matter requiring further work.

5. Conclusions

The dataset shows, for the first time, the long-term prevalence of microplastic pollution in the deep sea and documents a relatively stable abundance over the last four decades. Previous studies from marine systems have tended to provide only a snapshot in time and there is a lack of quantitative long-term data on microplastic pollution. This study provides one such assessment and indicates that microplastics may have been present on the seafloor at this locality prior to 1976. Questions remain regarding the rates of vertical transport and the dynamic processes influencing the sinking of microplastics to the benthos and we suggest this as a future priority area. Furthermore, this study shows the merit of accessing archival specimen collections to determine the historical presence of microplastic pollution. We would therefore encourage those with access to such repositories to consider their application within this field to broaden our knowledge of the long-term fate and behaviour of microplastics within marine systems.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.envpol.2018.10.090>.

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