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**Microplastic pollution identified in deep-sea water and
ingested by benthic invertebrates in the Rockall Trough,
North Atlantic Ocean.**

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ABSTRACT

Microplastics are widespread in the natural environment and present numerous ecological threats. While the ultimate fate of marine microplastics are not well known, it is hypothesized that the deep sea is the final sink for this anthropogenic contaminant. This study provides a quantification and characterisation of microplastic pollution ingested by benthic macroinvertebrates with different feeding modes (*Ophiomusium lymani*, *Hymenaster pellucidus* and *Colus jeffreysianus*) and in adjacent deep water > 2200 m, in the Rockall Trough, Northeast Atlantic Ocean. Despite the remote location, microplastic fibres were identified in deep-sea water at a concentration of 70.8 particles m⁻³, comparable to that in surface waters. Of the invertebrates examined (n = 66), 48 % ingested microplastics with quantities enumerated comparable to coastal species. The number of ingested microplastics differed significantly between species and generalized linear modelling identified that the number of microplastics ingested for a given tissue mass was related to species and not organism feeding mode or the length or overall weight of the individual. Deep-sea microplastics were visually highly degraded with surface areas more than double that of pristine particles. The identification of synthetic polymers with densities greater and less than seawater along with comparable quantities to the upper ocean indicates processes of vertical re-distribution. This study presents the first snapshot of deep ocean microplastics and the quantification of microplastic pollution in the Rockall Trough. Additional sampling throughout the deep-sea is required to assess levels of microplastic pollution, vertical transportation and sequestration, which have the potential to impact the largest global ecosystem.

Capsule

Microplastics were identified in deep-sea benthic invertebrates and adjacent water > 2200 m deep in the Rockall Trough with quantities comparable to surface concentrations.

INTRODUCTION

Plastic debris is a pervasive anthropogenic contaminant found extensively in the aquatic environment worldwide (Cozar et al., 2014; Hammer et al., 2012). As a major source of marine pollution, plastic debris meets ocean health index criteria and has been recognized as a global threat, joining other marine stressors such as climate change, ocean acidification, overfishing and habitat destruction (Amaral-Zettler et al., 2015; Halpern et al., 2012). The majority of plastic items manufactured have single-use application (Thompson et al., 2009) and between 4.8×10^9 to 12.7×10^9 kg of plastic is estimated to have entered the ocean in 2010 alone (Jambeck et al., 2015); by contrast an estimated 2.7×10^8 kg is afloat in surface waters (Eriksen et al., 2014). The progressive fragmentation of plastic objects into ever smaller and more numerous pieces should lead to the gradual increase of microplastics quantities (Andrady, 2011; Cozar et al., 2014; ter Halle et al., 2016), however global budgeting identifies major discrepancies between the abundance of plastics in surface waters, especially when considering microplastic particles (Cozar et al., 2014; Eriksen et al., 2014).

Microplastics, defined here as particles $1 \mu\text{m}$ - 5 mm in diameter (Arthur et al., 2009) are of particular environmental concern as they are a similar size to prey items and sediment grains and are therefore bioavailable to a wide diversity of organisms. Ingestion is reported in numerous species with documented impacts ranging from lethal to sub-lethal (Browne et al., 2008; Cole et al., 2015; Murray and Cowie, 2011; Welden and Cowie, 2016; Wright et al., 2013a), and trophic transfer of microplastics has been observed (Farrell and Nelson, 2013; Setälä et al., 2014).

64 Additionally, small particles have been shown to translocate within the bodies of crabs and mussels
65 (Browne et al., 2008; Farrell and Nelson, 2013), consequently microplastics potentially have a
66 greater toxological effect than larger plastic items. The high surface area to volume ratio means
67 small particles have a greater area over which to absorb environmental contaminants; these may
68 accumulate in the plastic, however the effect of plastic co-contaminants on biota is not yet clear
69 (Koelmans, 2015).

70 The long-term fate and ‘lifecycle’ of microplastics in the marine environment is poorly
71 understood. Distribution is influenced by abiotic (ocean currents, physical shearing, fragmentation
72 and natural sinking (GESAMP, 2015)) and biotic factors (such as fouling (Fazey and Ryan, 2016),
73 consumption and incorporation in faecal material (Cole et al., 2016) and settling detritus (Long et
74 al., 2015)). These provide vertical transport pathways for microplastics from the sea surface to the
75 benthos, thus it is hypothesized that microplastics are sequestered in the deep sea. There is a severe
76 paucity of knowledge regarding microplastic pollution in the deep sea; however within the last few
77 years microplastics have been documented in deep-sea sediments in regions of the Mediterranean
78 Sea and the Atlantic, Pacific and Indian Oceans (Fischer et al., 2015; Van Cauwenberghe et al.,
79 2013; Woodall et al., 2014), and more recently isolated from deep-sea benthic invertebrates
80 (Taylor et al., 2016).

81 This study aims to provide a thorough assessment and quantification of microplastic
82 ingestion by deep-sea benthic invertebrates displaying different feeding modes and presents the
83 first quantification of microplastic pollution in deep-sea water. To test the hypothesis that
84 microplastics are present at a deep-sea site in the Rockall Trough, Northeast Atlantic Ocean,
85 benthic fauna and water samples were collected from a depth > 2200 m. Samples were analysed

to i) determine whether microplastics occur in this remote deep-sea location and ii) characterise and quantify the microplastics present.

MATERIALS AND METHODS

Sampling location

The Rockall Trough is situated to the west of Scotland, UK. The monitoring site, 'Gage Station M', is located in the Rockall Trough (57.300°N, -10.383°W) near the foot of Anton Dohrn seamount at a depth of 2200 m (Figure 1). During the 2016 research cruise DY052 aboard *R.R.S. Discovery*, four epibenthic sled tows and one Conductivity, Temperature, Depth (CTD) cast for deep-sea water were undertaken.

Field methods

On-board quality assurance/quality control (QA/QC)

QA/QC procedures were designed and employed at all stages to reduce the potential for sample contamination. Standard non-plastic equipment such as metal and glass were used as much as possible; all equipment was cleaned thoroughly by wiping with 70 % ethanol on non-shedding paper three times prior to use. Ships water supplies were fitted with a mesh cartridge filter to remove contaminants, these were tested for efficiency prior- and post-sampling by running water through an 80 µm filter for two hours and examining these under the microscope. Prior to work commencing and between each sled haul the deck was washed down with the ship's fire hose. The number of people working on samples was kept to a minimum. The same personal protective equipment was worn for the duration of sampling and stored separately. Sample fibres from

clothing, along with any potential contaminants from the research vessel such as ropes, piping, mesh screens etc were taken to be analysed alongside the deep-sea samples.

Deep-sea benthic sampling

Two Woods Hole Oceanographic Institution-pattern epibenthic sleds, rigged with main and extension nets of mesh size 0.5 mm were used to obtain samples following historical methods. The sleds were deployed individually down to the seafloor with the doors open and trawled along the seabed for ~60 minutes before the sled doors closed by a pre-set timer mechanism and the net hauled slowly to the surface. Once on-board the net was opened and material was emptied into lidded plastic buckets, before being washed over stacked sieves of mesh sizes 4 mm, 0.5 mm and 0.42 mm. Macrofauna retained on the 4 mm sieve were individually wrapped in aluminium foil, placed in lidded buckets separated by taxonomic groups and frozen at -20°C to be utilized in this study.

Deep-sea water sampling

Two-hundred and forty litres of water were collected using a Sea-Bird 24-way CTD system with stainless steel frame. All 24 niskin bottles were fired 7 m from the seabed at a depth of 2227 m. On deck prior to sampling, the spigot of each niskin bottle was cleaned by rinsing it thoroughly with deionised water and all water filters and hosing were examined carefully to ensure they were free from contaminants. Niskin bottles were systematically sampled by running the entire volume of water through an 80 µm mesh filter until water flow completely ceased. All sampling was carried out by one individual who remained downwind of the filter throughout. Upon completion,

filters were placed in a clean petri dish, sealed with tape and labelled for analysis once back in the laboratory.

Laboratory methods

Laboratory QA/QC

Samples were prepared and analysed in a separate small laboratory only used by the scientist carrying out the analysis. Air vents were sealed and the door remained closed for the duration of the experiment to reduce air-borne contamination sources. The work benches were cleaned with 70 % ethanol on non-shredding paper and allowed to dry fully; this was repeated three times prior to commencing work. Standard non-plastic equipment i.e. glass and metal, were used wherever possible and consumables were used directly from sterile packaging. All apparatus was washed with deionised water prior to use and equipment was inspected under a dissecting microscope. The samples were kept covered to minimize exposure risk. Natural fibre clothes were worn under a clean 100 % cotton laboratory coat, these clothes were stored in the laboratory to avoid contact with external synthetic fibres.

Background laboratory contamination was assessed in two ways based on (Courtene-Jones et al., 2017). Dampened filter paper (30 mm diameter, Whatman No. 1) was placed into a clean petri dish and left exposed for the duration of the experiment to monitor air-borne fibres, these were then sealed and labelled for further analysis. Tape lift screening (TLS) was employed to test for surface microplastics; after the benches had been cleaned, a 5 cm² piece of adhesive tape was cut and placed on the bench surface in three random locations before being placed on an acetate sheet and examined under a microscope, this process was carried out before and after laboratory procedures. Samples of putative contaminants, such as the sterile packaging, adhesive tape and

acetate sheet used for TLS, natural fibre clothing and filter paper used were taken to be analysed alongside the deep sea samples.

Inspection of deep-sea water filters

The 80 µm mesh filters were transferred to individual lidded glass petri dishes. The gauze were systematically and thoroughly examined under a dissecting microscope (Wild M5); any potential microplastics were removed using forceps and transferred to a small petri dish containing a 30 mm diameter of filter paper (Whatman No. 1). The samples remained covered when not in use to reduce airborne contamination.

Enzymatic digestion of deep-sea macroinvertebrates

Fauna > 4 mm were identified to species level in covered glass petri dishes; individuals of *Ophiomusium lymani* (n = 40), *Hymenaster pellucidus* (n = 19) and *Colus jeffreysianus* (n = 7) were used for microplastics analysis (Figure SI 1).

Specimens were removed from the freezer and allowed to defrost while wrapped in aluminium foil for 45 minutes. The length of the central disc (*H. pellucidus* and *O. lymani*), or the shell (*C. jeffreysianus*) were measured with metal dial calipers and the mass of the entire specimen was recorded (Sartorius electronic balance) to the nearest 0.0001 g. Specimens were rinsed thoroughly in a flow of deionised water prior to dissection. Dissections varied slightly between species; for *O. lymani* the central disc was opened in a clean glass petri dish and all tissue was removed from the exoskeleton. For *H. pellucidus* the central disc was opened along with each of the five arms and the tissue was dissected from the body cavity. The shell of *C. jeffreysianus* was crushed by applying pressure and the complete tissue mass was removed. For all species the soft

tissue was weighed using a Sartorius electronic balance and placed in a glass beaker containing 20 ml of 0.3125 % concentration trypsin solution, prepared using Gibco™ trypsin diluted with deionised water (Courtene-Jones et al., 2017). Beakers were covered with glass covers and placed on heated magnetic stirrers set to stir at 250 rpm at 38-42°C and left to digest for 25 minutes.

The resulting mixture was poured through 52 µm mesh gauze before being transferred to a covered glass petri dish. The gauze was thoroughly examined under a Wild M5 dissecting microscope and any potential microplastics were transferred to a small petri dish containing 30 mm diameter filter paper (Whatman No. 1), samples remained covered when not in use to reduce risk of aerial contamination. After all potential microplastics had been transferred to the petri dish it was sealed and labelled for further analysis.

Microplastic identification

The length of each microplastic particle was measured using the ocular scale of a Wild M5 dissecting microscope. Potential microplastics obtained from the water sample and extracted from fauna, along with putative contaminants from the ship and laboratory QA/QC procedures were identified using a Perkin-Elmer Spectrum 100 Fourier Transformation Infrared spectroscope coupled with a universal Attenuated Total Reflectance accessory (ATR-FTIR) equipped with a diamond detector. Each spectra produced was the result from a series of four high resolution scans in the wavelength range 600 - 4000 cm⁻¹ with a spectral resolution of 4 cm⁻¹. Spectra were visualised in OMNIC 9.2.98 (Thermo Fisher Scientific Inc.) with use of the inbuilt libraries to aid identification. The reference library spectra represent clean samples not typically found in the environment. Additional references were generated from plastics from non-typical sources such as beach debris, consumer products and textiles samples to provide more environmentally relevant

samples. As well as using these libraries (in-built and user generated), the characteristic functional group signals were examined visually to confirm the identity of the materials being assessed.

Scanning Electron Microscope imaging

A sub-sample of the microplastic fibres extracted from deep-sea water (polyester n = 6) and invertebrates (polyester and acrylic n = 8), along with pristine acrylic and polyester fibres obtained from known textile samples (n = 2) were sputter coated with gold-palladium and imaged using a JOEL JSM-6390 Scanning Electron Microscope (SEM) with a 20kV electron accelerating velocity. A series of SEM images, ensuring an overlap of ~80 % between each, were taken of each fibre.

Three-dimensional fibre reconstruction and surface area quantification

Three-dimensional reconstructions of the fibre sub-samples imaged with the SEM were rendered using Agisoft Photoscan Professional V1.2.6 photogrammetry software (Agisoft LLC). The software produces high-resolution three-dimensional surface models, from which surface area quantification of complex objects can be achieved as described in Burns et al., (2015) (Summarised in supplementary information). Models were calibrated against objects of known length and by point-to-point measurements, the resolution of the models were 0.01 μm .

As fibres visually appeared twisted and flattened, estimates of baseline surface area were calculated for each of the fibres by multiplying length by width, thus assuming particles were analogous to smooth rectangles. These calculations provide an estimation of surface area for each specific sized particle and surface areas achieved with photogrammetric methods are reported as a ratio relative to the baseline.

222

223 **Statistical analysis**

224 Data was tested for normality using the Shapiro-Wilk normality test and for homogeneity of
225 variance with the Fligner-Killeen test and was found not to meet the criteria for parametric
226 statistics. To assess microplastic abundance, analysis was performed both using the raw
227 microplastic abundance data and after standardising microplastic quantities by the wet weight (w.
228 w.) tissue mass of an individual. Kruskal-Wallis tests were performed on each of these raw and
229 standardised datasets to investigate differences between species, with subsequent posthoc analysis
230 with a Dunn's test. Microplastic surface area data was not normally distributed, therefore a
231 Wilcoxon rank sum test was used to compare baseline to measured surface areas for the deep sea
232 (fauna and water) and pristine fibres.

233 Generalized linear modeling (GLM) was conducted to relate the response variable (the
234 number of ingested microplastics) to the five factors (organism mass, length, tissue mass, feeding
235 mode and species). Log transformations of organism mass, tissue mass and length were undertaken
236 and the Poisson distribution was used since the response variable was count data. Prior to running
237 the model, collinearity was checked using the Pearson correlation coefficient (indicated by values
238 > 0.6 (Zuur et al., 2010) and the variance inflation factor (VIF; by sequentially removing the
239 variable with the highest value, until all remaining VIFs were below the suggested value of 2 (Zuur
240 et al., 2010)). Those variables found to be collinear (length and weight) were not included in the
241 model, consequently the variables species, feeding mode and tissue weight were retained and
242 considered in relation to the response variable (the number of microplastics). Models with and
243 without interaction effects between all variables (species, feeding mode, tissue weight) were

considered and optimisation was achieved by sequentially removing the least significant variable or interaction term (determined by the highest p-value). Model overdispersion was tested using the dispersion test in the *AER* package and by calculating the residual deviance of the model divided by the degrees of freedom. All statistical analysis was performed in RStudio V 0.99.892 (R Core Team, 2016) with use of the *PMCMR* (Pohlert, 2014), *dunn.test* (Dinno, 2017) *VIF* (Lin, 2015) and *AER* (Kleiber and Zeileis, 2017) libraries.

RESULTS

QA/QC

No microplastics were identified on the filters fitted to the ships water supply. When analysed with ATR-FTIR spectroscopy none of the potential contaminants sampled from the ship (ropes, filters, clothing) or laboratory (sterile consumable packaging, clothing) had spectra which matched that of material found in deep water or invertebrates samples. Laboratory controls yielded similar results; of the 5 fibres found on the atmospheric controls all were identified as cellulose. The number of fibres on TLS varied from a mean of 6.56 ± 2.60 particles prior to laboratory work commencing, to 10.22 ± 4.18 particles after all laboratory work was undertaken. All fibres were blue, red or white and identified as cellulose/cotton with a distinctive ribbon like morphology when examined under the microscope (Figure SI 2).

Identification of microplastics in deep-sea water

ATR-FTIR analysis was performed on 78 potential microplastics obtained from 240 l of deep-sea water; 17 of which were positively identified as synthetic, 28 as cellulose and 33 yielded unclear spectra. This equates to an abundance of 0.0708 synthetic fibres per litre ($70.8 \text{ particles m}^{-3}$) of deep-sea water. All microplastics were monofilament fibres of the colours blue ($n = 13$), red ($n = 2$) and transparent ($n = 2$). Five polymers were identified (Figure 2) with polyester comprising the majority of those identified. Sizes of microfibrils ranged widely from a minimum of 0.4 mm recorded for Polyethylene Terephthalate (PET) to a maximum of 8.3 mm for an acrylic fibre.

Identification of microplastics in deep-sea invertebrates

A total of 359 potential microplastics were extracted from three benthic macroinvertebrate species ($n = 66$ individuals), of which 45 were identified as synthetic from their specific transmission spectra, 165 were identified as cellulose and the remaining 149 did not produce usable spectral data. A total of nine polymers were identified, of which acrylic was most abundant (Figure 2). The majority of synthetic material were monofilament fibres ($n = 39$, 87 %) and the remaining items were fragments ($n = 6$, 13 %). Items were predominantly blue and red in colour ($n = 9$, each accounting for 42 % of the total), however black, green, orange, transparent and multi-coloured items were also identified. Mean particle length ranged from a maximum of 6.25 mm recorded for a polyacrylonitrile fibre to a minimum of 0.023 mm for an acrylic fragment, both ingested by *O. lymani* individuals. Overall mean particle length was 1.191 ± 0.0756 mm across all species.

 Ingested microplastic quantities varied between individuals and species; considering those individuals from which microplastics were extracted, *O. lymani* ingested the greatest number of polymer types and *H. pellucidus* contained the greatest overall abundance with a mean of 1.582 ± 0.448 SE microplastics g^{-1} w.w. tissue (Table 1). There were significant differences between the

number of microplastics ingested between species ($H = 9.7988$, $df = 2$, $p = 0.007$) explained by a highly significant difference between *O. lymani* and *H. pellucidus* (Dunn's test $p = 0.002$) and between *H. pellucidus* and *C. jeffreysianus* (Dunn's test $p = 0.009$) The standardized number of microplastics per gram of tissue also differed significantly between species ($H = 7.0629$, $df = 2$, $p = 0.0293$), again explained by differences between *O. lymani* and *H. pellucidus* (Dunn's test $p = 0.010$) and between *H. pellucidus* and *C. jeffreysianus* (Dunn's test $p = 0.016$) (Figure 3).

The final GLM included species and the log of tissue mass as an offset of the response variable, the number of microplastics ingested. No interaction terms were included in the model as these had negligible effects on the results. The model, with a Poisson distribution was found to be slightly overdispersed, thus a quasipoisson distribution was applied to the final model to account for the overdispersion. The GLM results identified that the number of microplastics ingested was related to species and not to the other factors (weight, length or feeding mode). The GLM indicated a significant negative relationship ($p = 0.0376$) between ingested microplastics offset by tissue mass and *C. jeffreysianus*, indeed this species had a factor of 1.94 less microplastics than other species, however it must be noted that only two individuals ingested microplastics. A positive relationship was found between *O. lymani* and the number of microplastics ingested and the model predicted a factor of 1 times more than in *C. jeffreysianus*, however this result was not significant ($p = 0.2949$). The number of microplastics ingested by *H. pellucidus* was greater, by a factor of 1.67, for a given tissue weight, this positive relationship was significant at the 0.1 level ($p = 0.0845$).

Visualisation of microplastics and quantification of surface area

Scanning Electron Microscope (SEM) imaging revealed microplastics extracted from deep-sea invertebrates and water to be degraded, with much cracking, pitting, fraying and flaking apparent on the microplastic surface, producing a highly rugose exterior. By comparison, pristine fibres appeared to have a relatively smooth, uniform surface structure (Figure 4 and SI 3). These discrepancies were corroborated by the quantification of fibre surface area. The mean ratio of measured surface area relative to the baseline for pristine fibres was 1.792 ± 0.415 SE. Surface area ratios for fibres extracted from deep-sea samples were more than double that of pristine microplastics; 4.157 ± 0.921 SE and 4.331 ± 1.247 SE for fibres extracted from invertebrates and deep-sea water respectively, this was significantly different from baseline values ($V(15) = 12$, $p = 0.0021$). Baseline surface area values were calculated for a rectangular object as fibres appeared elongated and flattened. Acknowledgment is made that baseline values are only estimates, and fibres are assumed to be analogous to rectangles, however cross-checking these results by computing the ratio of surface area derived from a rectangular object to that of half a cylinder results in $\pi / 2$ which is consistent with the values obtained for the pristine fibres. Therefore, no difference was found if baseline values were calculated for a rectangle or half cylinder.

DISCUSSION

The presence of microplastics in deep-sea water and the benthic invertebrate community is clearly demonstrated here, providing further evidence for the widespread distribution of anthropogenic microplastics in the marine environment. Microplastics are heterogeneously distributed in surface waters with concentrations ranging between $0.02 - > 100$ particles m^{-3} in the Northeast Atlantic Ocean (reviewed in Lusher, 2015). The present study provides the first quantification of microplastic pollution in deep ocean water and found the concentration to be on the same order as

in surface waters (70.8 particles m⁻³). While it is possible that microplastics may have been re-suspended from the sediment during sampling, no sediment grains were found on the mesh used to filter the seawater. The CTD was suspended 7 m from the seafloor limiting any potential seabed disturbance and sampling of re-suspended microplastics, giving confidence that the microplastics originated from and are contained within deep water. It is duly noted that this data is based on a single sampling point and thus provides only an initial snapshot of microplastic content in deep water. Many additional bottom water samples are required to more adequately assess the abundance of microplastics present in the deep ocean and provide estimates of deep ocean concentrations; however, this work still represents the first attempt to quantify microplastics in this realm.

Microplastics were identified in all three deep-sea benthic macroinvertebrate species from the phylum Echinodermata and Mollusca examined in this study, with an incidence of ingestion (number of individuals with microplastics / total number of individuals sampled) of 48 % across all species; this, while lower than some coastal invertebrates (Devriese et al., 2015; Welden and Cowie, 2016) is still within the range of incidence values documented for a number of inshore species (Desforges et al., 2015; Foekema et al., 2013; Lusher et al., 2013). Taylor et al., (2016) reported the presence of microplastics in species of deep-sea Echinodermata, Arthropoda and Cnidaria from the Atlantic and Indian Oceans, however singularly sampled species precluded the quantification of ingested microplastics. In the present study, proteolytic enzymes were used to digest soft tissue and extract internalised microplastics without detrimentally impacting the polymers present (Courtene-Jones et al., 2017), allowing for a thorough investigation of ingested microplastics. The quantities enumerated from deep-sea fauna are on the same order as those reported in wild coastal species from a range of taxa (Foekema et al., 2013; Van Cauwenberghe et

al., 2015; Van Cauwenberghe and Janssen, 2014). It is important to note that while visual sorting found potential microplastics in all individuals except one *H. pellucidus* specimen, only a small percentage of particles analysed (12 % for fauna and 22 % for the water sample) were positively identified as synthetic polymers by ATR-FTIR spectrometry. Microplastic quantities presented here are therefore likely to be under-reported, due to the small size of particles and the challenges associated with current analytical methods (Löder and Gerdt, 2015). Technological developments will allow for increased accuracy when investigating micro- and nano-sized plastics ingested by wild fauna.

Microplastic ingestion is demonstrated to vary interspecifically, with significant differences in microplastic abundance between *H. pellucidus* and *O. lymani* and between *H. pellucidus* and *C. jeffreysianus*. The surface deposit feeder and facultative predator *O. lymani* (Iken et al., 2001; Pearson and Gage, 1984) was identified to contain the greatest number of polymer types, however the predatory sea star *H. pellucidus* (Wagstaff et al., 2014) contained the highest median number of microplastics. Indeed, statistical modelling found *H. pellucidus* contained 1.67 times more microplastics per given tissue mass, while a factor of 1.94 less microplastics were internalised by *C. jeffreysianus* than other species. Feeding mode has previously been shown to influence microplastic ingestion in coastal species (Mizraji et al., 2017; Setälä et al., 2015); however in this study the GLM only identified a relationship between species and ingested microplastic quantities and not with feeding mode. It is not possible to speculate why these species specific differences in microplastic levels occur; it is possible however that the small dataset may have precluded any further relationships from being identified, or there may be some other, as yet unidentified, factors involved in microplastic ingestion and retention in an individual's body.

It is important to note that while this study presents novel findings, small sample sizes of benthic invertebrates, particularly for *C. jeffreysianus* and *H. pellucidus*, and deep ocean water prevents robust estimates of microplastic pollution from being made. Numerous logistical and methodological challenges and costs are associated with sampling the deep sea. This study utilised the full number of samples collected during deep-sea operations in the time available during the DY052 research cruise. Concurrent sediment cores were not within the scope of the research cruise and thus prevented the quantification and subsequent comparison of microplastic levels between all three potential deep-sea ‘reservoirs’. Additional sampling at this site and other regions within the Rockall Trough, along with the inclusion of sediment cores would strengthen the dataset and yield cross site and/or temporal replication not available during the DY052 cruise.

Close visual inspection of microplastics extracted from deep-sea samples showed high levels of degradation, including surface cracks, pitting, flaking and fragmentation; producing a mean surface area significantly different to baseline values and in excess of double that of pristine fibres. The duration of microplastics in the environment and the associated degradation has a number of consequences of biological concern. The large surface area to volume ratio, high surface reactivity and small size of particles makes them dynamic in the environment (Mattsson et al., 2015) and can increase toxicity (Jeong et al., 2016). Increased surface area of small degraded particles provides a greater area for the establishment of biofilms which influence sinking velocity (Lobelle and Cunliffe, 2011) and provides increased area for the colonisation of bacteria, including pathogenic species (Kirstein et al., 2016; Zettler et al., 2013). Persistent organic pollutants (POPs) may accumulate in microplastics, and as polymers degrade chemical additives breakdown and leach from the plastic (Engler, 2012), further increasing the toxic effects to organisms.

While factors involved in the horizontal transport of microplastics near the sea surface are relatively well documented (Law et al., 2014), the processes affecting vertical transport of microplastics to the benthos are potentially more complex and not well understood. Sinking velocities are influenced by a number of factors and microplastic behaviour in part is affected by particle size, shape and polymer density (Ballent et al., 2012; Kowalski et al., 2016). The quantity identified in deep-sea water by this study, akin to surface water concentrations indicates processes distributing microplastics throughout the water column. The majority of polymers identified had densities greater than seawater, such as polyester, acrylic and polyamide. Of note, is the presence of positively buoyant polymers, such as polyethylene, which has a specific density of 0.91 - 0.94 g cm⁻³ (Andrady, 2015). In addition to physical properties, microplastic sinking rates are also influenced by interactions with marine organisms, including biofouling (Fazey and Ryan, 2016; Lobelle and Cunliffe, 2011), incorporation into faecal pellets (Cole et al., 2016) and marine aggregates (Long et al., 2015; Ward and Kach, 2009; Wright et al., 2013b; Zhao et al., 2016). These biological processes alter the settling velocity of microplastics by as much as an order of magnitude (Clark et al., 2016; Long et al., 2015). Furthermore species of zooplankton undertake diel vertical migrations (Williamson et al., 2011) which could further redistribute microplastics in the oceans. It cannot be affirmed whether the microplastics isolated from the deep sea in this study arise from the degradation and fragmentation of larger items already located in the deep ocean, or are transported by physical and biological processes through the water column to the seafloor.

Conclusion

This study demonstrates the presence of microplastics in deep-sea benthic fauna and water in the Rockall Trough. Further sampling of water and fauna, along with the addition of sediment cores are necessary to assess ecosystem-wide microplastic pollution in this region and monitor temporal changes. While this study focuses on the Northeast Atlantic Ocean, we hypothesize that microplastics are present throughout the global deep-sea. Further attention and sampling efforts should be directed to the deep oceans globally to establish the prevalence of microplastic pollution in this remote and still largely unstudied ecosystem. The deep sea is vulnerable to a number of anthropogenic pressures (Ahnert and Borowski, 2000; Glover and Smith, 2003; Puig et al., 2012) and now microplastic pollution may be added to these threats, raising concern for ongoing ecosystem functioning. Future steps must work towards understanding the susceptibility and potential impacts of microplastic ingestion by deep-sea species assemblages, and elucidate spatial and temporal vertical transport routes by which microplastics enter and are sequestered in the deep sea.

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Appendix A. Supplementary data

REFERENCES

- Ahnert, A., Borowski, C., 2000. Environmental risk assessment of anthropogenic activity in the deep-sea. *J. Aquat. Ecosyst. Stress Recover.* 7, 299–315. doi:10.1023/A:1009963912171
- Amaral-Zettler, L.A., Zettler, E.R., Slikas, B., Boyd, G.D., Melvin, D.W., Morrall, C.E., Proskurowski, G., Mincer, T.J., 2015. The biogeography of the Plastisphere: Implications for policy. *Front. Ecol. Environ.* 13, 541–546. doi:10.1890/150017
- Andrady, A.L., 2015. Persistence of Plastic Litter in the Oceans, in: Bergmann, M. (Ed.), *Marine Anthropogenic Litter*. pp. 57–72. doi:10.1007/978-3-319-16510-3
- Andrady, A.L., 2011. Microplastics in the marine environment. *Mar. Pollut. Bull.* 62, 1596–1605. doi:10.1016/j.marpolbul.2011.05.030
- Arthur, C., Baker, J., Bamford, H., 2009. Proceedings of the International Research Workshop on the Occurrence, Effects, and Fate of Microplastic Marine Debris. NOAA Tech. Memo. NOS-OR&R-48 530.
- Ballent, a., Purser, A., de Jesus Mendes, P., Pando, S., Thomsen, L., 2012. Physical transport properties of marine microplastic pollution. *Biogeosciences Discuss.* 9, 18755–18798. doi:10.5194/bgd-9-18755-2012

467 Browne, M.A., Dissanayake, A., Galloway, T.S., Lowe, D.M., Thompson, R.C., 2008. Ingested
 468 microscopic plastic translocates to the circulatory system of the mussel, *Mytilus edulis* (L.).
 469 Environ. Sci. Technol. 42, 5026–5031. doi:10.1021/es800249a

470 Burns, J.H.R., Delparte, D., Gates, R.D., Takabayashi, M., 2015. Utilizing underwater three-
 471 dimensional modeling to enhance ecological and biological studies of coral reefs. Int. Arch.
 472 Photogramm. Remote Sens. Spat. Inf. Sci. - ISPRS Arch. 40, 61–66.
 473 doi:10.5194/isprsarchives-XL-5-W5-61-2015

474 Clark, J.R., Cole, M., Lindeque, P.K., Fileman, E., Blackford, J., Lewis, C., Lenton, T.M.,
 475 Galloway, T.S., 2016. Marine microplastic debris: a targeted plan for understanding and
 476 quantifying interactions with marine life. Front. Ecol. Environ. 14, 317–324.
 477 doi:10.1002/fee.1297

478 Cole, M., Lindeque, P., Fileman, E., Halsband, C., Galloway, T.S., 2015. The impact of
 479 polystyrene microplastics on feeding, function and fecundity in the marine copepod *Calanus*
 480 *helgolandicus*. Environ. Sci. Technol. 49, 1130–1137. doi:10.1021/es504525u

481 Cole, M., Lindeque, P.K., Fileman, E., Clark, J., Lewis, C., Halsband, C., Galloway, T.S., 2016.
 482 Microplastics alter the properties and sinking rates of zooplankton faecal pellets. Environ.
 483 Sci. Technol. 50, 3239–3246. doi:10.1021/acs.est.5b05905

484 Courtene-Jones, W., Quinn, B., Murphy, F., Gary, S.F., Narayanaswamy, B.E., 2017. Optimisation
 485 of enzymatic digestion and validation of specimen preservation methods for the analysis of
 486 ingested microplastics. Anal. Methods 9, 1437–1445. doi:10.1039/C6AY02343F

487 Cozar, A., Echevarria, F., Gonzalez-Gordillo, J.I., Irigoien, X., Ubeda, B., Hernandez-Leon, S.,
 488 Palma, A.T., Navarro, S., Garcia-de-Lomas, J., Ruiz, A., Fernandez-de-Puelles, M.L., Duarte,
 489 C.M., 2014. Plastic debris in the open ocean. Proc. Natl. Acad. Sci. 111, 10239–10244.
 490 doi:10.1073/pnas.1314705111

491 Desforges, J.W., Galbraith, M., Ross, P.S., 2015. Ingestion of Microplastics by Zooplankton in
 492 the Northeast Pacific Ocean. Arch. Environ. Contam. Toxicol. 3, 320–330.
 493 doi:10.1007/s00244-015-0172-5

494 Devriese, L.I., van der Meulen, M.D., Maes, T., Bekaert, K., Paul-pont, I., Frère, L., Robbens, J.,
 495 Vethaak, A.D., 2015. Microplastic contamination in brown shrimp (*Crangon crangon*,
 496 Linnaeus 1758) from coastal waters of the Southern North Sea and Channel area. *Mar. Pollut.*
 497 *Bull.* 98, 179–187. doi:10.1016/j.marpolbul.2015.06.051

498 Dinno, A., 2017. Dunn’s Test of Multiple Comparisons Using Rank Sums.

499 Engler, R.E., 2012. The complex interaction between marine debris and toxic chemicals in the
 500 ocean. *Environ. Sci. Technol.* 46, 12302–12315. doi:10.1021/es3027105

501 Eriksen, M., Lebreton, L.C.M., Carson, H.S., Thiel, M., Moore, C.J., Borerro, J.C., Galgani, F.,
 502 Ryan, P.G., Reisser, J., 2014. Plastic pollution in the world’s oceans: more than 5 trillion
 503 plastic pieces weighing over 250,000 tons afloat at sea. *PLoS One* 9, e111913.
 504 doi:10.1371/journal.pone.0111913

505 Farrell, P., Nelson, K., 2013. Trophic level transfer of microplastic: *Mytilus edulis* (L.) to *Carcinus*
 506 *maenas* (L.). *Environ. Pollut.* 177, 1–3. doi:10.1016/j.envpol.2013.01.046

507 Fazey, F.M.C., Ryan, P.G., 2016. Biofouling on buoyant marine plastics: An experimental study
 508 into the effect of size on surface longevity. *Environ. Pollut.* 210, 354–360.
 509 doi:10.1016/j.envpol.2016.01.026

510 Fischer, V., Elsner, N.O., Brenke, N., Schwabe, E., Brandt, A., 2015. Plastic pollution of the Kuril–
 511 Kamchatka Trench area (NW pacific). *Deep Sea Res. Part II Top. Stud. Oceanogr.* 111, 399–
 512 405. doi:10.1016/j.dsr2.2014.08.012

513 Foekema, E.M., De Gruijter, C., Mergia, M.T., van Franeker, J.A., Murk, A.J., Koelmans, A. a,
 514 2013. Plastic in north sea fish. *Environ. Sci. Technol.* 47, 8818–24. doi:10.1021/es400931b

515 GESAMP, 2015. Sources, fate and effects of microplastics in the marine environment: A global
 516 assessment. *Reports Stud. GESAMP* 90, 96.

517 Glover, A.G., Smith, C.R., 2003. The deep-sea floor ecosystem: current status and prospects of
 518 anthropogenic change by the year 2025. *Environ. Conserv.* 30, 219–241.
 519 doi:10.1017/S0376892903000225

520 Halpern, B.S., Longo, C., Hardy, D., McLeod, K.L., Samhour, J.F., Katona, S.K., Kleisner, K.,
521 Lester, S.E., O’Leary, J., Ranelletti, M., Rosenberg, A.A., Scarborough, C., Selig, E.R., Best,
522 B.D., Brumbaugh, D.R., Chapin, F.S., Crowder, L.B., Daly, K.L., Doney, S.C., Elfes, C.,
523 Fogarty, M.J., Gaines, S.D., Jacobsen, K.I., Karrer, L.B., Leslie, H.M., Neeley, E., Pauly, D.,
524 Polasky, S., Ris, B., St Martin, K., Stone, G.S., Sumaila, U.R., Zeller, D., 2012. An index to
525 assess the health and benefits of the global ocean. *Nature* 488, 615–620.
526 doi:10.1038/nature11397

527 Hammer, J., Kraak, M.H.S., Parsons, J.R., 2012. Plastics in the Marine Environment: The Dark
528 Side of a Modern Gift, in: *Reviews of Environmental Contamination and Toxicology*. pp. 1–
529 44. doi:10.1007/978-1-4614-3414-6

530 Iken, K., Brey, T., Wand, U., Voigt, J., Junghans, P., 2001. Food web structure of the benthic
531 community at the Porcupine Abyssal Plain (NE Atlantic): A stable isotope analysis. *Prog.*
532 *Oceanogr.* 50, 383–405. doi:10.1016/S0079-6611(01)00062-3

533 Jambeck, J.R., Geyer, R., Wilcox, C., Siegler, T.R., Perryman, M., Andrady, A., Narayan, R., Law,
534 K.L., 2015. Plastic waste inputs from land into the ocean. *Science* (80-.). 347, 768–771.

535 Jeong, C.-B., Won, E.-J., Kang, H.-M., Lee, M.-C., Hwang, D.-S., Hwang, U.-K., Zhou, B.,
536 Souissi, S., Lee, S.-J., Lee, J.-S., 2016. Microplastic size-dependent toxicity, oxidative stress
537 induction, and p-JNK and p-P38 activation in the monogonont rotifer (*Brachionus koreanus*).
538 *Environ. Sci. Technol.* 50, 8849–8857. doi:10.1021/acs.est.6b01441

539 Kirstein, I. V., Kirmizi, S., Wichels, A., Garin-Fernandez, A., Erler, R., Löder, M., Gerdts, G.,
540 2016. Dangerous hitchhikers? Evidence for potentially pathogenic *Vibrio* spp. on
541 microplastic particles. *Mar. Environ. Res.* 120, 1–8. doi:10.1016/j.marenvres.2016.07.004

542 Kleiber, C., Zeileis, A., 2017. *Applied Econometrics with R*.

543 Koelmans, A.A., 2015. Modeling the role of microplastics in bioaccumulation of organic
544 chemicals to marine aquatic organisms. A critical review, in: Bergmann, M. (Ed.), *Marine*
545 *Anthropogenic Litter*. pp. 309–324. doi:10.1007/978-3-319-16510-3

546 Kosyan, A.R., 2007. Morphological features , ecology, and distribution of poorly studied

547 molluscan genera of the Colinae subfamily (Gastropoda , Buccinidae) from the far eastern
548 seas of Russia. *Oceanology* 47, 571–576. doi:10.1134/S0001437007040108

549 Kowalski, N., Reichardt, A.M., Waniek, J.J., 2016. Sinking rates of microplastics and potential
550 implications of their alteration by physical, biological, and chemical factors. *Mar. Pollut.*
551 *Bull.* 109, 310–319. doi:10.1016/j.marpolbul.2016.05.064

552 Law, K.L., Morét-Ferguson, S.E., Goodwin, D.S., Zettler, E.R., Deforce, E., Kukulka, T.,
553 Proskurowski, G., 2014. Distribution of surface plastic debris in the eastern pacific ocean
554 from an 11-year data set. *Environ. Sci. Technol.* 48, 4732–4738. doi:10.1021/es4053076

555 Lin, D., 2015. VIF Regression: A Fast Regression Algorithm For Large Data.

556 Lobelle, D., Cunliffe, M., 2011. Early microbial biofilm formation on marine plastic debris. *Mar.*
557 *Pollut. Bull.* 62, 197–200. doi:10.1016/j.marpolbul.2010.10.013

558 Löder, M.G.J., Gerdts, G., 2015. Methodology Used for the Detection and Identification of
559 Microplastics—A Critical Appraisal, in: *Marine Anthropogenic Litter*. pp. 201–227.
560 doi:10.1007/978-3-319-16510-3

561 Long, M., Moriceau, B., Gallinari, M., Lambert, C., Huvet, A., Raffray, J., Soudant, P., 2015.
562 Interactions between microplastics and phytoplankton aggregates: Impact on their respective
563 fates. *Mar. Chem.* 175, 39–46. doi:10.1016/j.marchem.2015.04.003

564 Lusher, A., 2015. Microplastics in the marine environment: Distribution, interactions and effects,
565 in: Bergmann, M. (Ed.), *Marine Anthropogenic Litter*. pp. 245–307. doi:10.1007/978-3-319-
566 16510-3

567 Lusher, a. L., McHugh, M., Thompson, R.C., 2013. Occurrence of microplastics in the
568 gastrointestinal tract of pelagic and demersal fish from the English Channel. *Mar. Pollut. Bull.*
569 67, 94–99. doi:10.1016/j.marpolbul.2012.11.028

570 Mattsson, K., Hansson, L.-A., Cedervall, T., 2015. Nano-plastics in the aquatic environment.
571 *Environ. Sci. Process. Impacts* 17, 1712–1721. doi:10.1039/C5EM00227C

572 Mizraji, R., Ahrendt, C., Perez-Venegas, D., Vargas, J., Pulgar, J., Aldana, M., Ojeda, F.P., Duarte,
573 C., Galban-Malagon, C., 2017. Is the feeding type related with the content of microplastics
574 in intertidal fish gut? *Mar. Pollut. Bull.* doi:10.1016/j.orgel.2006.09.004

575 Murray, F., Cowie, P.R., 2011. Plastic contamination in the decapod crustacean *Nephrops*
576 *norvegicus* (Linnaeus, 1758). *Mar. Pollut. Bull.* 62, 1207–17.
577 doi:10.1016/j.marpolbul.2011.03.032

578 Pearson, M., Gage, J.D., 1984. Diets of some deep-sea brittle stars in the Rockall Trough. *Mar.*
579 *Biol.* 82, 247–258.

580 Pohlert, T., 2014. The Pairwise Multiple Comparison of Mean Ranks Package (PMCMR). R
581 package.

582 Puig, P., Canals, M., Company, J.B., Martín, J., Amblas, D., Lastras, G., Palanques, A., Calafat,
583 A.M., 2012. Ploughing the deep sea floor. *Nature* 489, 286–289. doi:10.1038/nature11410

584 R Core Team, 2016. R: A language and environment for statistical computing. R Foundation for
585 Statistical Computing.

586 Setälä, O., Fleming-Lehtinen, V., Lehtiniemi, M., 2014. Ingestion and transfer of microplastics in
587 the planktonic food web. *Environ. Pollut.* 185, 77–83. doi:10.1016/j.envpol.2013.10.013

588 Setälä, O., Norkko, J., Lehtiniemi, M., 2015. Feeding type affects microplastic ingestion in a
589 coastal invertebrate community. *MPB* 102, 95–101. doi:10.1016/j.marpolbul.2015.11.053

590 Taylor, M.L., Gwinnet, C., Robinson, L.F., Woodall, L.C., 2016. Plastic microfibre ingestion by
591 deep-sea organisms. *Sci. Rep.* 6. doi:10.1038/srep33997

592 ter Halle, A., Ladirat, L., Gendre, X., Goudouneche, D., Pusineri, C., Routaboul, C., Tenailleau,
593 C., Duployer, B., Perez, E., Goudounèche, D., Pusineri, C., Routaboul, C., Tenailleau, C.,
594 Duployer, B., Perez, E., 2016. Understanding the Fragmentation Pattern of Marine Plastic
595 Debris. *Environ. Sci. Technol.* 50, 5668–5675. doi:10.1021/acs.est.6b00594

596 Thompson, R.C., Swan, S.H., Moore, C.J., vom Saal, F.S., 2009. Our plastic age. *Philos. Trans.*

597 R. Soc. Lond. B. Biol. Sci. 364, 1973–1976. doi:10.1098/rstb.2009.0054

598 Van Cauwenberghe, L., Claessens, M., Vandegehuchte, M.B., Janssen, C.R., 2015. Microplastics
599 are taken up by mussels (*Mytilus edulis*) and lugworms (*Arenicola marine*) living in natural
600 habitats. *Environ. Pollut.* 199, 10–17. doi:10.1016/j.envpol.2015.01.008

601 Van Cauwenberghe, L., Janssen, C.R., 2014. Microplastics in bivalves cultured for human
602 consumption. *Environ. Pollut.* 193, 65–70. doi:10.1016/j.envpol.2014.06.010

603 Van Cauwenberghe, L., Vanreusel, A., Mees, J., Janssen, C.R., 2013. Microplastic pollution in
604 deep-sea sediments. *Environ. Pollut.* 182, 495–499. doi:10.1016/j.envpol.2013.08.013

605 Wagstaff, M.C., Howell, K.L., Bett, B.J., Billett, D.S.M., Brault, S., Stuart, C.T., Rex, M.A., 2014.
606 β -diversity of deep-sea holothurians and asteroids along a bathymetric gradient (NE Atlantic).
607 *Mar. Ecol. Prog. Ser.* 508, 177–185. doi:10.3354/meps10877

608 Ward, J.E., Kach, D.J., 2009. Marine aggregates facilitate ingestion of nanoparticles by
609 suspension-feeding bivalves. *Mar. Environ. Res.* 68, 137–142.
610 doi:10.1016/j.marenvres.2009.05.002

611 Welden, N.A.C., Cowie, P.R., 2016. Environment and gut morphology influence microplastic
612 retention in langoustine, *Nephrops norvegicus*. *Environ. Pollut.* 214, 859–865.
613 doi:10.1016/j.envpol.2016.03.067

614 Williamson, C.E., Fischer, J.M., Bollens, S.M., Overholt, E.P., Breckenridge, J.K., 2011. Towards
615 a more comprehensive theory of zooplankton diel vertical migration: Integrating ultraviolet
616 radiation and water transparency into the biotic paradigm. *Limnol. Oceanogr.* 56, 1603–1623.
617 doi:10.4319/lo.2011.56.5.1603

618 Woodall, L.C., Sanchez-Vidal, A., Canals, M., Paterson, G.L.J., Coppock, R., Sleight, V., Calafat,
619 A., Rogers, a. D., Narayanaswamy, B.E., Thompson, R.C., 2014. The deep sea is a major
620 sink for microplastic debris. *R. Soc. Open Sci.* 1, 140317.

621 Wright, S.L., Rowe, D., Thompson, R.C., Galloway, T.S., 2013a. Microplastic ingestion decreases
622 energy reserves in marine worms. *Curr. Biol.* 23, R1031–R1033.

doi:10.1016/j.cub.2013.10.068

- Wright, S.L., Thompson, R.C., Galloway, T.S., 2013b. The physical impacts of microplastics on marine organisms: A review. *Environ. Pollut.* 178, 483–492. doi:10.1016/j.envpol.2013.02.031
- Zettler, E.R., Mincer, T.J., Amaral-Zettler, L.A., 2013. Life in the “Plastisphere”: microbial communities on plastic marine debris. *Environ. Sci. Technol.* 47, 7137–7146.
- Zhao, S., Danley, M., Ward, J.E., Li, D., Mincer, T.J., 2016. An approach for extraction, characterization and quantitation of microplastic in natural marine snow using Raman microscopy. *Anal. Methods* 1359–1366. doi:10.1039/C5AY03217B
- Zuur, A.F., Ieno, E.N., Elphick, C.S., 2010. A protocol for data exploration to avoid common statistical problems. *Methods Ecol. Evol.* 1, 3–14. doi:10.1111/j.2041-210X.2009.00001.x

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Figure 1. Map showing the deep-sea sampling locations to the west of the United Kingdom (U.K) and Northern Ireland (N.I). The CTD deep water sampling location (blue circle) and four epibenthic sled trawls (red tracks) are shown around the regular monitoring site ‘Gage Station M’ (green triangle) to the east of Anton Dohrn (A.D.) Seamount in the Rockall Trough. Area within the dashed line box is shown in more detail in the adjacent panel. Bathymetry is contoured at 500 m intervals from depths of 500 m to 3500 m (MATLAB R2015b using GEBCO_2014 bathymetry data).

Figure 2. Example microplastic fibres found in (a & b) deep sea water and (c & d) extracted from benthic invertebrates, along with the proportion, as a percentage, of polymer (e) fibres identified in deep-sea water (n = 17); (f) fibres (n = 39) and (g) fragments (n = 6) extracted from deep-sea benthic macroinvertebrates. Differences in relative abundance and polymer diversity are observed between water and invertebrates; polyester is the dominant polymer in deep-sea water, while acrylic accounts for the majority of ingested microplastics by benthic fauna.

Figure 3. Number of microplastic particles standardized per gram of w.w. tissue ingested by each of the three invertebrate species. Thick black line indicates median value, boxes depict the first and third quartiles and the whiskers show the interquartile range. Outliers are shown by the open points and letters denotes significant differences between species groups.

695 **Figure 4.** SEM images of pristine fibres and those isolated from deep-sea water and benthic
 696 macroinvertebrates. Fibres from the deep sea show visible surface cracking, pitting, flaking and
 697 fragmentation when compared to pristine fibres which are smooth and uniform in appearance.

Table 1. Number of individuals (ind.) sampled and with microplastics internalised, weights and feeding mode for each invertebrate species, along with the mean number of microplastics extracted g⁻¹ of wet weight (w. w.) tissue and the total number of polymers ingested.

Species	No. of ind sampled / No. of ind. with microplastics	w.w. tissue mass range (g)	Specimen mass range (g)	Feeding mode	Mean microplastics g ⁻¹ w.w. tissue	No. polymers ingested
<i>Ophiomusium lymani</i>	40 / 16	0.532 – 2.503	4.296 - 7.050	Deposit feeder/ facultative predator (Iken et al., 2001)	1.153 ± 0.278 SE	9
<i>Hymenaster pellucidus</i>	19 / 14	0.267 - 3.441	0.691 – 12.533	Predator: benthic invertebrates and planktonic fallout (Wagstaff et al., 2014)	1.582 ± 0.448 SE	6
<i>Colus jeffreysianus</i>	7 / 2	1.385 – 3.076	3.129 – 6.980	Predator: burrowing amphipods and bivalves (Kosyan, 2007)	0.678 ± 0.044 SE	2