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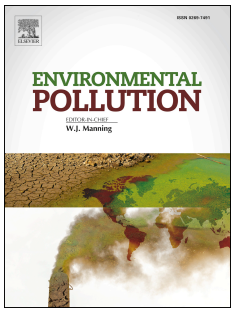

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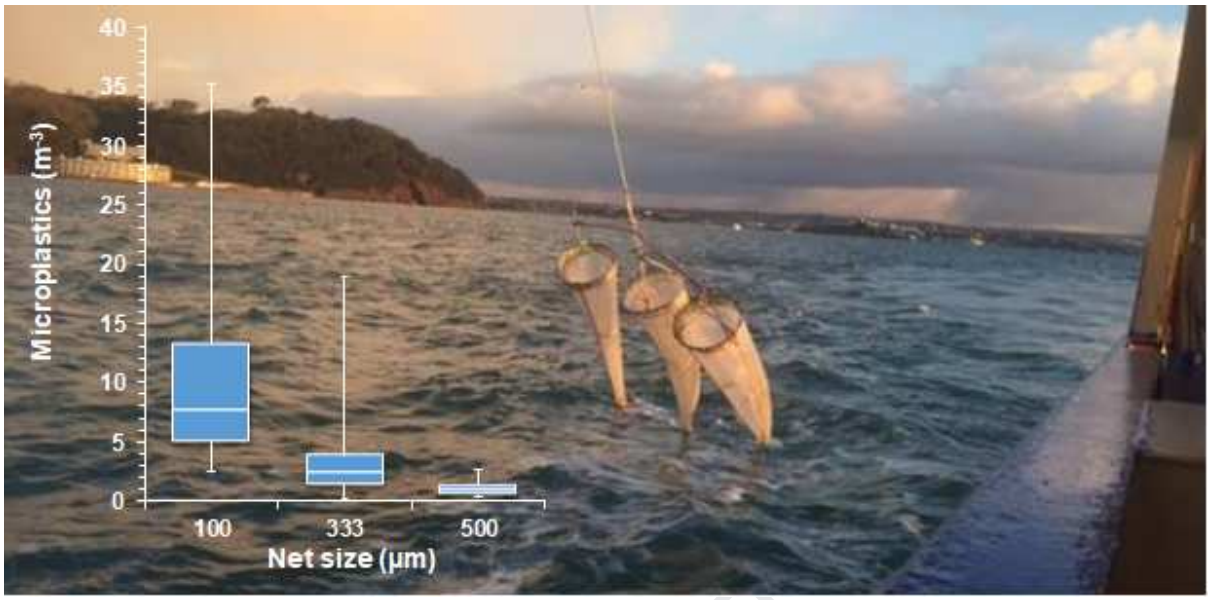
Graphical abstract



Are we underestimating microplastic abundance in the marine environment? A comparison of microplastic capture with nets of different mesh-size.

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Abstract

Microplastic debris is ubiquitous and yet sampling, classifying and enumerating this prolific pollutant in marine waters has proven challenging. Typically, waterborne microplastic sampling is undertaken using nets with a 333 µm mesh, which cannot account for smaller debris. In this study, we provide an estimate of the extent to which microplastic concentrations are underestimated with traditional sampling. Our efforts focus on coastal waters, where microplastics are predicted to have the greatest influence on marine life, on both sides of the North Atlantic Ocean. Microplastic debris was collected via surface trawls using 100, 333 and 500 µm nets. Our findings show that sampling using nets with a 100 µm mesh resulted in the collection of 2.5-fold and 10-fold greater microplastic concentrations compared with using 333 and 500 µm meshes respectively ($P<0.01$). Based on the relationship between microplastic concentrations identified and extrapolation of our data using a power law, we estimate that microplastic concentrations could exceed 3700 microplastics m^{-3} if a net with a 1 µm mesh size is used. We further identified that use of finer nets resulted in the collection of significantly thinner and shorter microplastic fibres ($P<0.05$). These results elucidate that estimates of marine microplastic concentrations could currently be underestimated.

Capsule

US and UK datasets reveal that sampling with a 100 µm net results in the capture of 10-fold greater microplastic concentrations compared with using a 500 µm net

Keywords

Plastic, microplastics, pollution, ocean, net, sampling

1. Introduction

Microplastics are a prolific, persistent and pernicious contaminant, posing an environmental and economic risk to marine ecosystems across the globe (Rochman et al., 2016). Microplastics,

encompassing synthetic plastic particulates, fibres and films, here defined as 1-5000 µm in diameter, have been widely identified in marine ecosystems, including estuaries, coastal biomes, the open ocean and polar waters (Lusher, 2015). Microplastics are either directly manufactured (e.g. cosmetic exfoliates, air blasting media), or derive from the fragmentation of larger plastics over time (Cole et al., 2011). By design, plastics are resistant to degradation and as such are expected to persist in the natural environment for hundreds, if not thousands of years (Andrady, 2015). Owing to their small size, microplastics are bioavailable to a range of organisms across trophic levels, including zooplankton (Steer et al., 2017), bivalves and fish destined for human consumption (Rochman et al., 2015), and marine megafauna (Duncan et al., 2019; Nelms et al., 2019). Exposure studies have highlighted the negative impacts microplastic ingestion can have on marine organisms, including copepods, shellfish, benthic invertebrates and fish, with effects comprising reduced feeding, fecundity, growth and survival, premature moulting, altered behaviour and shifts in ecological functionality (Besseling et al., 2013; Cole et al., 2019; Cole et al., 2015; Cole et al., 2016; Sussarellu et al., 2016; Wegner et al., 2012; Wright et al., 2013). However, it is currently unclear whether such adverse health effects are likely to occur in the natural environment due to the mismatch between the size, type and concentration of microplastics that are traditionally sampled during environmental monitoring studies and those used in exposure studies (Burns and Boxall, 2018). At present, the concentration of bioavailable microplastics in the natural environment, a similar size to natural prey and a similar size to those used in effect studies, is relatively unknown (de Sá et al., 2018).

To comprehensively assess the risks that microplastic debris poses to marine ecosystems requires robust estimates of the size, prevalence and distribution of microplastic within the global ocean. However, accurately quantifying and characterising microplastic debris within environmental samples, and subsequently modelling this data, has proven hugely challenging. Microplastics research is still in its infancy, and over the past decade there has been a multitude of methodological approaches applied when sampling, extracting and identifying microplastic debris, with samples

taken from different ecological compartments (i.e. sediments, water column, biota) each providing their own unique challenges (Lusher et al., 2016; Stock et al., 2019). Thus far, field sampling has predominantly focussed on the subtropical gyres of the northern hemisphere, with data gaps for large swathes of the open ocean, the southern hemisphere, equatorial regions and coastal waters (Clark et al., 2016). One of the most widely applied methods for collecting microplastics at the sea surface has been to conduct trawls using 330-335 µm nets, hereafter referred to as 333 µm, which have traditionally been used for sampling zooplankton (Hidalgo-Ruz et al., 2012; Lusher et al., 2016). Such environmental data has been used to derive initial estimates of oceanic microplastic budgets: for example, van Sebille et al. (2015) estimates that the accumulated number of microplastic particles in 2014, ranged from 15-51 trillion particles, weighing between 93,000 and 236,000 metric tons, with >90% of observations collected using a Manta or Neuston net with 333 µm mesh. A recent review highlighted that over 80% of field studies only sample microplastics >300 µm, and as such microplastics smaller than this size, including 95% of cosmetic microbeads, synthetic microfibres and secondary microplastics with diameters <300 µm, will be absent from datasets (Conkle et al., 2018). As such, we hypothesise current estimates of microplastic pollution at the sea surface are likely to be underestimated.

In this study, we determine the relationship between net mesh size and the abundance and character of captured microplastic, providing an estimate of the extent to which microplastic concentrations may be underestimated using 333 µm nets. Our sampling efforts focus on biologically productive coastal waters on both sides of the North Atlantic (i.e. Gulf of Maine and western English Channel), close to land-based and maritime sources of pollution, where microplastics are predicted to have the greatest influence on marine life (Clark et al., 2016). Microplastic debris was collected via sub-surface trawls using 100, 333 and 500 µm nets to compare microplastic concentrations sampled with nets of differing mesh sizes. The study aims to provide a greater resolution in the determination

of global microplastic budgets, allowing for the risk of microplastic debris to marine ecosystems to be more clearly defined.

2. Materials and Methods

2.1. Environmental sampling

Field sampling was conducted on both sides of the North Atlantic Ocean, focusing on coastal waters of the Gulf of Maine (USA) and the western English Channel (UK). In all cases, sub-surface sampling focused upon the comparison of microplastic concentrations collected by nets towed in parallel. For our US sampling, the use of a sailing vessel limited us to using a maximum of two nets at a time, comprising either two 333 µm nets or a 100 and 500 µm net. For our UK sampling, the use of the RV Quest (Maritime and Coastguard Agency Category 2 workboat) allowed 100, 333 and 500 µm nets to be towed in parallel.

2.1.1. Gulf of Maine (USA)

Fieldwork was conducted throughout July 2013 in the Gulf of Maine (USA), with sampling targeted at sites of upwelling and riverine output around Hurricane Island, Boothbay Harbor, Portland, Kittery, Star Island and Boston (Figure 1; Table S1). Sampling was conducted on-board the RV *American Promise*, with nets deployed from the spinnaker pole to capture sub-surface debris outside of the vessel's wake; nets were maintained half in and half out of the water. Each trawl (250 m transects; 0.7-2.8 knots) used two nets towed in parallel, comprising either: two 333 µm Neuston nets (0.5 m^2 aperture; rectangular, 1 m x 0.5 m); or 100 µm and 500 µm plankton nets (0.2 m^2 aperture; circular, 0.5 m ø). The nets and cod-ends were thoroughly rinsed down, and samples transferred onto clean nylon mesh of corresponding size. Any large pieces of flotsam (e.g. wood, macroalgae) were rinsed with freshwater to remove adhered microplastics, and then removed from the sample. Meshes were rinsed with freshwater and then folded and secured to retain samples and minimise contamination.

Adapting the protocols of Moore *et al.* (Moore et al., 2002), samples were desiccated at 60°C overnight in a food-dehydrator, and stored in sample bags in a desiccating chamber prior to analysis.

2.1.2. English Channel (UK)

Fieldwork was conducted in the western English Channel off the coast of Plymouth (UK) between July and September 2015 (Figure 1; Table S2). Sub-surface sampling was conducted on board the RV *Plymouth Quest* using three Neuston nets (100, 333 and 500 µm; 0.2 m^2 aperture; circular, 0.5 m ø) rigged in parallel and trawled off the beam of the boat (500 m trawl; 0.5—1.5 knots) to avoid down-welling of the debris in the vessel's wake; nets were maintained half in and half out of the water. Each net and cod end were rinsed into a clean bucket with surface seawater collected using the boat's intake system. Any large pieces of flotsam (e.g. wood, macroalgae, feathers) were rinsed with filtered seawater (0.2 µm) to remove adhered microplastics, and then removed from the sample. The bucket contents were poured through a nylon mesh matching the mesh size of the net and rinsed with filtered seawater (0.2 µm). Meshes were folded and secured and then temporarily wrapped in aluminium foil during transit to avoid contamination. Samples were stored at -80 °C and subsequently freeze-dried prior to analysis.

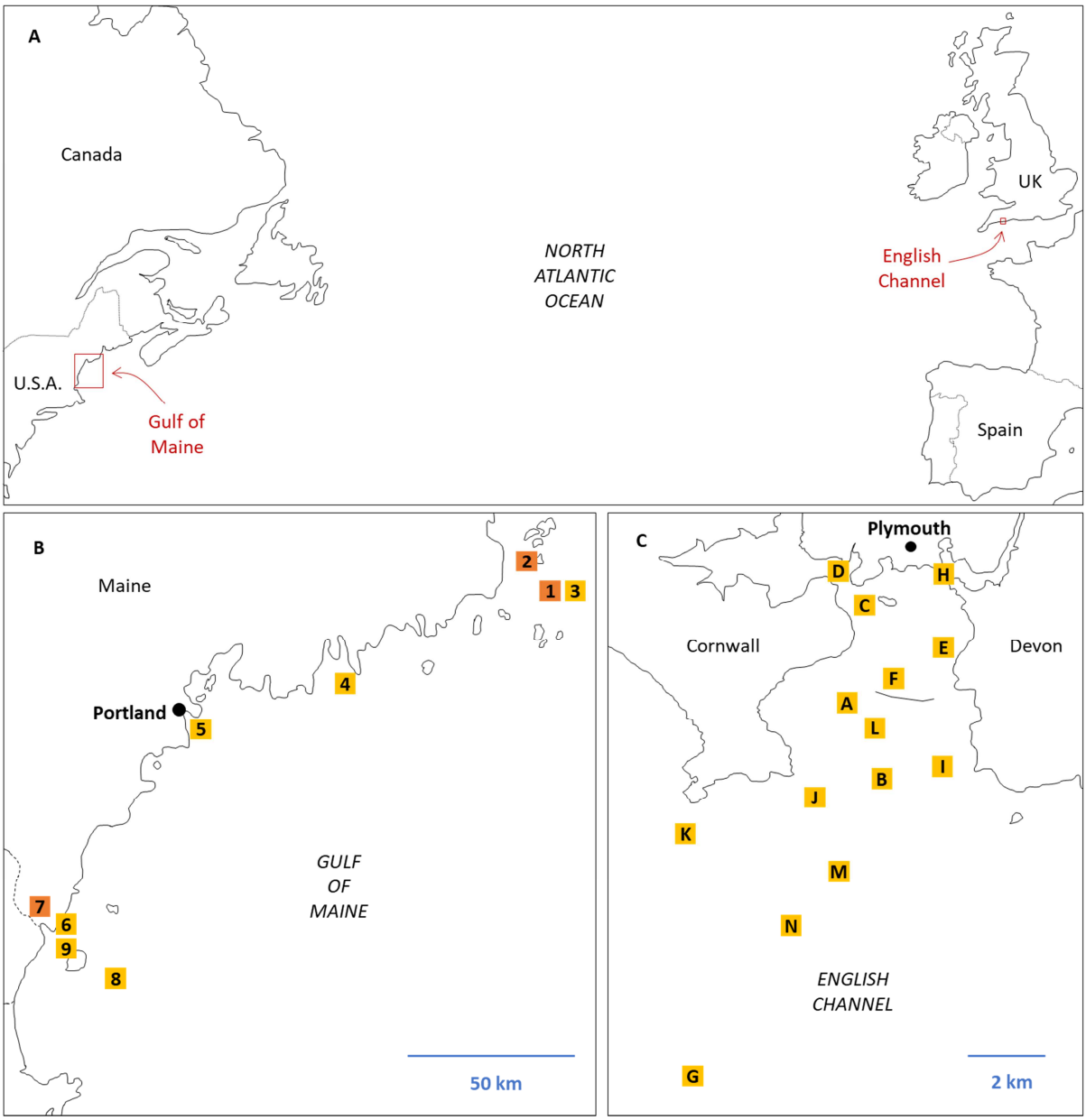

Figure 1. Charts showing locations of sampling sites. (A) North Atlantic Ocean, noting locations of the Gulf of Maine and English Channel. (B) North-eastern US seaboard, relative to Portland (ME), with 50 km scale; yellow boxes denote sites where samples were taken using 100/500 µm nets and 333/333 µm nets, and orange boxes denote where samples were taken using 100/500 µm nets only. (C) Plymouth Sound and western English Channel, with 2 km scale; yellow boxes denote sites sampled with 100/333/500 µm nets.

2.2. Enzymatic digestion

To reveal any microplastics obscured by biotic material within the samples, we employed enzymatic digestion per the protocols of Cole *et al.* (2014). Samples were transferred individually into a pre-cleaned porcelain mortar and the weight of the pestle was used to gently break down large structures. Each sample was weighed, transferred to an acid-washed glass vial, and homogenising solution added at a ratio of 15 mL to 0.2 g dry weight sample. Samples were physically homogenised using a 19G needle and 10 mL syringe then incubated at 50°C in an orbital shaker at 100 rpm for 30 minutes. Proteinase K was added to a concentration of 500 µg mL^{-1}, and samples incubated at 50 °C again at 150 rpm for 2 hours. Digested samples were visually examined, and any still containing large quantities of organic material were incubated for a further two hours. Sodium perchlorate (5 M) was then added and each sample homogenised using a 21G needle before mixing at 150 rpm at room temperature for 20 minutes. Finally, samples were incubated at 65 °C for a further 20 minutes. Digested samples were vacuum filtered through 50 µm nylon mesh filters. Samples containing large volumes of material were sub-divided over multiple meshes. All samples were treated identically, irrespective of net size.

2.3. Characterisation

Per the proposed categorisation framework of Hartmann et al. (2019), we look to characterise microplastics by their chemical composition, size, shape and colour. Mesh filters were systematically analysed under a dissection microscope (Olympus SZX16; x40-100 magnification), using a sterilised needle to tease apart the sample. Suspected microplastics were visually identified by their uniformity, colour and form per the guidance of Norén et al. (Norén, 2007). The shape (fibre, fragment or sphere) and colour of all particles was recorded immediately. Owing to the large number of particles present, for each sample 15 particles were randomly selected for sizing and polymeric analysis. Particles were randomly selected by: (1) dividing the mesh into 9 (3 rows x 3 columns); (2) using a random number generator (Microsoft Excel) to determine which section to first select a microplastic from; (3) 15 particles were picked from this first section; (4) where <15 particles

were available, a binary random number was used to determine which section to next sub-sample from (i.e. go sequentially up or down through the grid). Sizing was conducted using CellSens software and light microscope (Olympus SX16) with two-dimensions recorded. Polymeric analysis was conducted on randomly selected particles using either Attenuated Total Reflectance Fourier Transform Infrared spectroscopy (Bruker Alpha ATR-FTIR) or micro ATR (μATR) in Reflectance mode (Perkin Elmer Spotlight 400 FTIR). Owing to the limitations of the Bruker ATR-FTIR, the particles identified using this instrument (n = 355) required one dimension >100 μm for spectral analysis, the remainder of selected particles analysed (Perkin Elmer, n = 416) required a minimum dimension of 11 μm. Spectra were analysed using OPUS 6.5 software (Bruker) and Spectra software (Perkin Elmer). Spectra showing no defined peaks (i.e.; <60% match) were dismissed, otherwise particles were classified as either 'natural' (e.g. chitin, cellulose), or 'microplastic', with further sub-division by polymer: acrylic, polyamide, polyester, polyethylene, polypropylene, polyvinylchloride, biopolymer (e.g. rayon), elastomer (e.g. neoprene, rubber), or other (i.e. copolymers, polystyrene).

2.4. Quality control

Prior to fieldwork and analysis, all participants were instructed on minimising sample contamination via atmospheric deposition, clothing and equipment. During sample collection, nets were trawled to the side of the research vessel to avoid any paint or material from the boat contaminating the sample. Samples were handled by personnel wearing cotton clothing and latex gloves, and procedural blanks using filtered seawater were conducted at each sampling station on each cruise to account for contamination. Samples were enclosed in meshes and stored in sealable containers prior to analysis. To minimise contamination in the laboratory, all analyses were conducted by trained researchers. Further, samples were covered wherever feasible, glassware was used in place of plastic where possible, and all reusable equipment was cleaned thoroughly with ethanol and rinsed twice with Milli-Q water (0.2 μm filtered) prior to use. Sample processing was conducted in positive-pressure (i.e. laminar flow) hoods to prevent airborne contamination. Procedural blanks (*n*=14 for

UK samples; *n*=6 for US samples), containing no sample, but otherwise treated as per the given methodology, were used to quantify contamination of samples during processing.

2.5. Microplastic concentrations

The waterborne concentration of microplastics (microplastics m^{-3}) from each net at each site was calculated using our data adjusted for volume sampled, contamination and mis-identification. The mean number of particles identified in the procedural blanks was subtracted from the total number of particles picked out from each sample; this data was then adjusted to account for the proportion of particles confirmed as plastic following FT-IR. The approximate volume of water sampled (m^3) was calculated by multiplying 50% of the net aperture (m^2), noting nets were half submerged, by length of tow measured as distance (m) over the ground (therefore taking boat speed and tidal stream into consideration), assuming a 95% sampling efficiency (Skjoldal et al., 2013).

2.6 Statistical analyses

Statistical analyses were conducted using R statistical software v3.5.1 (R Core Team, 2019). Normality of data was tested using the Shapiro-Wilk test, and non-parametric data log transformed where applicable. Comparisons between datasets were assessed using a student's t test or ANOVA with post-hoc Tukey test, or a Kruskal-Wallis test for non-parametric data. Significant difference is attributed where $P<0.05$. A power law regression analysis was conducted using pooled mean microplastic concentrations across all UK sites for each net size.

3. Results

3.1. Environmental data

3.1.1. Gulf of Maine (USA)

In total 2,755 particles were isolated from the 100, 333 and 500 µm net samples taken from 9 sites along the coast of the Gulf of Maine. The samples predominantly consisted of fibres (84%), with a smaller quantity of fragments identified (16%); only 12 beads were observed (Figure 2A). Fibres ranged from 5-282 µm in diameter and from 164 µm to >13 mm in length; the diameter of beads and fragments ranged from 57-3585 µm. The majority of fibres were black (62%), blue (15%), red (13%) or transparent (10%; Figure 2B); fragments were predominantly blue (32%) or white/grey (24%), with an otherwise even distribution of colour (Figure 2C). An ATR-FTIR analysis of a randomised sub-sample (*n*=254, excluding particles providing a poor spectral signature) revealed that 85% of the isolated particles were 'microplastic', per the classification criteria set out by Hartmann et al. (2019) (Figure 3A). Almost a third of the plastics identified were biopolymers (30%), of which the majority were Rayon, with co-polymers (21%), polyethylene (13%) and polyester (13%) also well represented in the samples (Figure 3B).

3.1.2. English Channel (UK)

In total 22,666 particles were isolated from the 100, 333 and 500 µm net samples taken from 14 sites in the western English Channel and Plymouth Sound. Across all samples, fibres (77%) were the most common, with smaller quantities of fragments (19%) and beads (4%) identified (Figure 2A). Fibres ranged from 5-350 µm in diameter and from 55 µm to >8 cm in length; the feret diameter of beads and fragments ranged from 15-12,500 µm. Fibres were predominantly black (37%) or blue (32%), with substantial numbers of transparent (15%) and red (10%) filaments (Figure 2B); the vast majority of fragments were blue (73%; Figure 2C). Of the randomised sub-sample of isolated particles (*n*=517, excluding particles providing a poor spectral signature), 94% were microplastic (Figure 3A). The majority of these microplastics were made up of polyester (22%), biopolymers (22%), polypropylene (18%) and acrylic (14%). Also present in substantial quantities was polyethylene (9%) and polyamide (8%), with PVC (2%), elastomers (1%) and others (5%) making up the total (Figure 3B).

259

260 *3.1.3. Procedural blanks*

261 Owing to the strict protocols in place, contamination of procedural blanks was relatively low. For the
262 procedural blanks conducted alongside our Gulf of Maine analysis, we identified a mean of 1.5
263 particles per sample (89% fibres, 11% fragments). For procedural blanks conducted in parallel with
264 the English Channel sampling and analysis, we identified a mean of 9.4 particles per sampling station
265 (75% fibres, 25% fragments).

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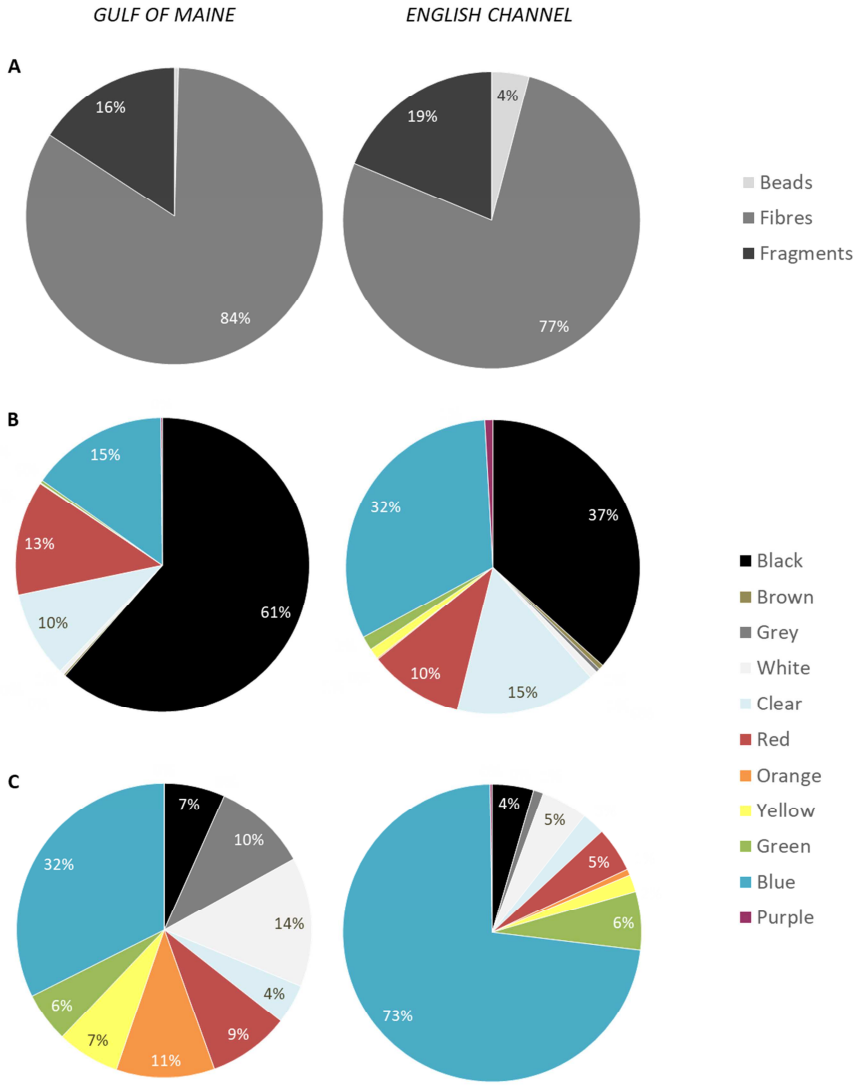

267

268 **Figure 2.** Composition of particles identified in Gulf of Maine (left column; *n*=2,755) and English
269 Channel (right column; *n*=22,666) samples. (A) Breakdown of particles by shape, i.e. fibres,
270 fragments or beads. (B) Colour breakdown of fibres. (C) Colour breakdown of fragments.
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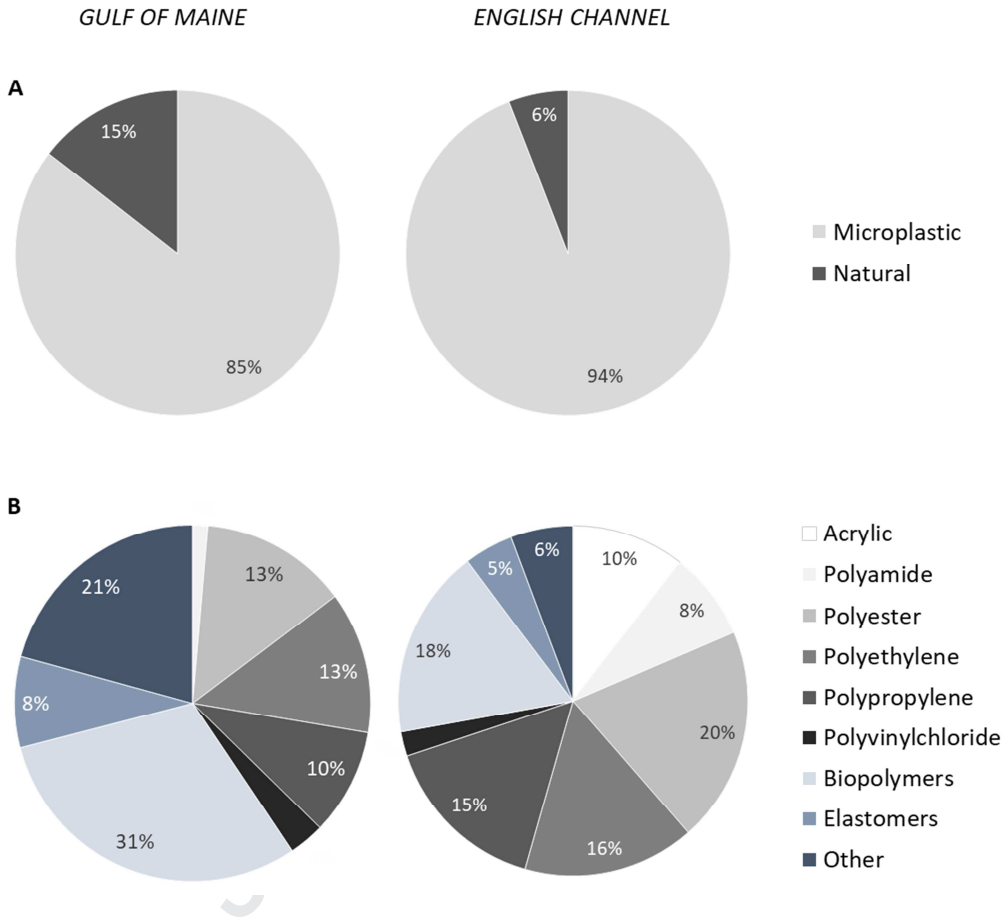

272
273

274 **Figure 3.** Composition of particles picked-out in Gulf of Maine (left column; *n*=254) and English
275 Channel (right column; *n*=517) samples. (A) Composition of material, i.e. naturally occurring or
276 plastic. (B) Breakdown of plastics by polymer type, including biopolymers and elastomers.
277

278 *3.2. Net comparisons*
279

280 *3.2.1. Gulf of Maine (USA): 333 µm nets*

281 Average microplastic concentrations (mean ± standard error) collected via two 333 µm nets, towed
282 in parallel at five sites in the Gulf of Maine, were 0.54 ± 0.2 and 0.46 ± 0.3 microplastics m^{-3}, with no
283 statistically significant difference in microplastic concentrations identified (t-test; P=0.406; Figure
284 4A). However, looking at individual site data (Figure 4B), it is evident that there can be clear
285 differences in microplastic concentrations collected using two nets towed in parallel (i.e. Site 5).
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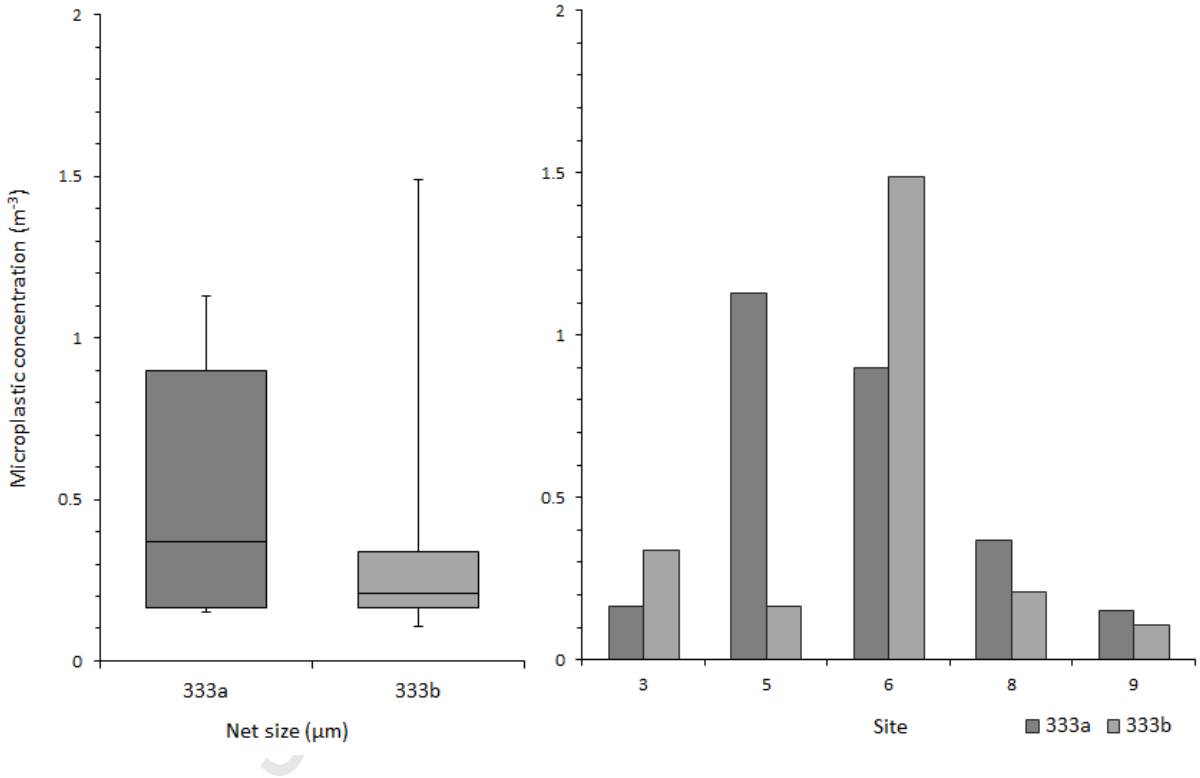


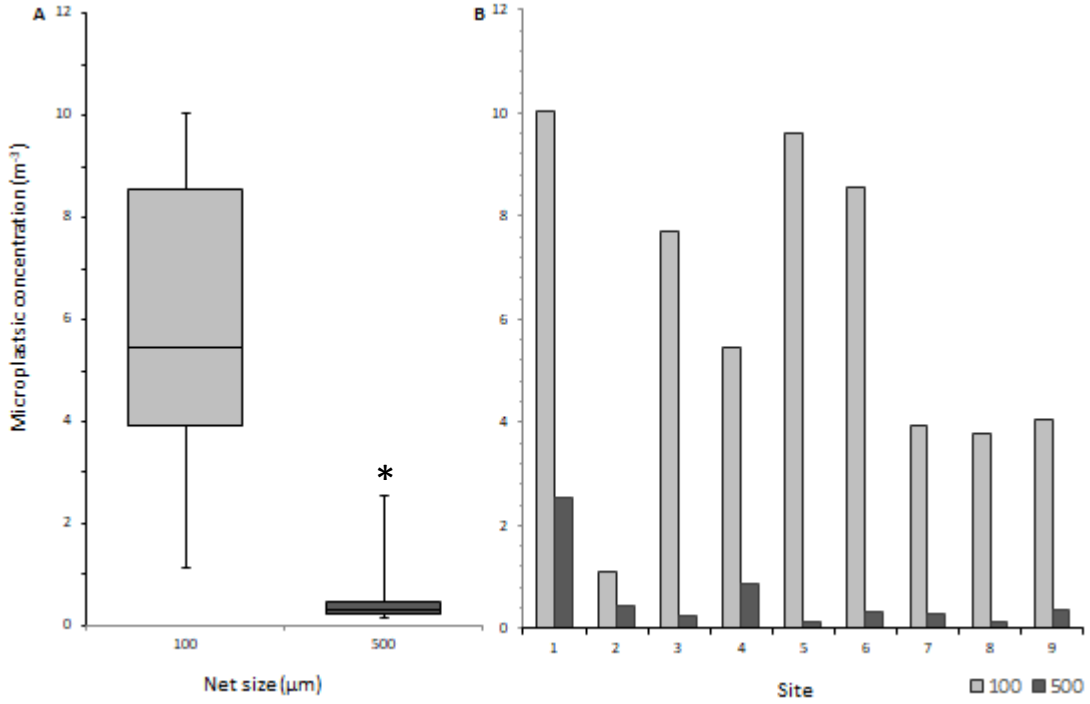
287

288 **Figure 4.** Waterborne concentrations of microplastics (items m^{-3}) in the Gulf of Maine using two 333
289 µm nets towed in parallel. (A) Box and whisker plots showing median concentrations across sites and
290 (B) bar chart displaying concentrations found at each site.
291

292 *3.2.2. Gulf of Maine (USA): 100 and 500 µm nets*

293 Based on parallel tows conducted at nine sites in the Gulf of Maine, we identified average
294 microplastic concentrations of 6.03 ± 1.03 microplastics m^{-3} (100 µm net) and 0.60 ± 0.25
295 microplastics m^{-3} (500 µm net). On average, sampling with a 100 µm net revealed 10-fold higher
296 microplastic concentrations compared with using a 500 µm net (t-test; P<0.001; Figure 5A). Highest

297 microplastic concentrations, as sampled using a 100 µm net, were identified at Site 1 (Outer
298 Penobscot Bay; 10.0 microplastics m^{-3}; Figure 5B).



299

300 **Figure 5.** Waterborne concentrations of microplastics (items m^{-3}) in the Gulf of Maine using 100 µm
301 and 500 µm nets towed in parallel; *denotes significant difference (t-test p = < 0.05). (A) Box and
302 whisker plots showing median concentrations across sites and (B) bar chart displaying
303 concentrations found at each site.

304

305 *3.2.3. English Channel (UK): 100, 333 and 500 µm nets*

306 Sampling efforts across 14 sites in the western English Channel and Plymouth Sound revealed mean
307 microplastic concentrations of 10.03 ± 2.21 microplastics m^{-3} (100 µm net), 4.08 ± 1.32 microplastics
308 m^{-3} (333 µm net) and 1.03 ± 0.16 microplastics m^{-3} (500 µm net). Mesh size was a significant factor in
309 resulting microplastic concentrations (ANOVA, P<0.001; Figure 6A, displaying median and
310 interquartile values), with no significant influence of Site (ANOVA, P=0.79). On average, a 100 µm
311 net revealed 2.5-fold higher microplastic concentrations than using a 333 µm net (ANOVA, P<0.05)
312 and 10-fold greater microplastic concentrations than using a 500 µm net (ANOVA P<0.001); using a

333 µm net resulted in sampling 4-fold greater microplastic concentrations as when a 500 µm net was employed (Tukey Post-hoc; P<0.05). However, at some sites this trend was not apparent, for example: at Site N (Outside Breakwater 4, 7 km offshore; Figure 6B) microplastic concentrations collected using a 333 µm net exceeded those collected via 100 µm net by two-fold; and at Site A (seaward side of Plymouth breakwater) and Site K (Rame Head), use of a 500 µm net revealed marginally greater microplastic concentrations than collected via 333 µm nets. The highest waterborne microplastic concentration, collected using a 100 µm net, was found at Site H (mouth of the River Plym; 35.5 microplastics m^{-3}).

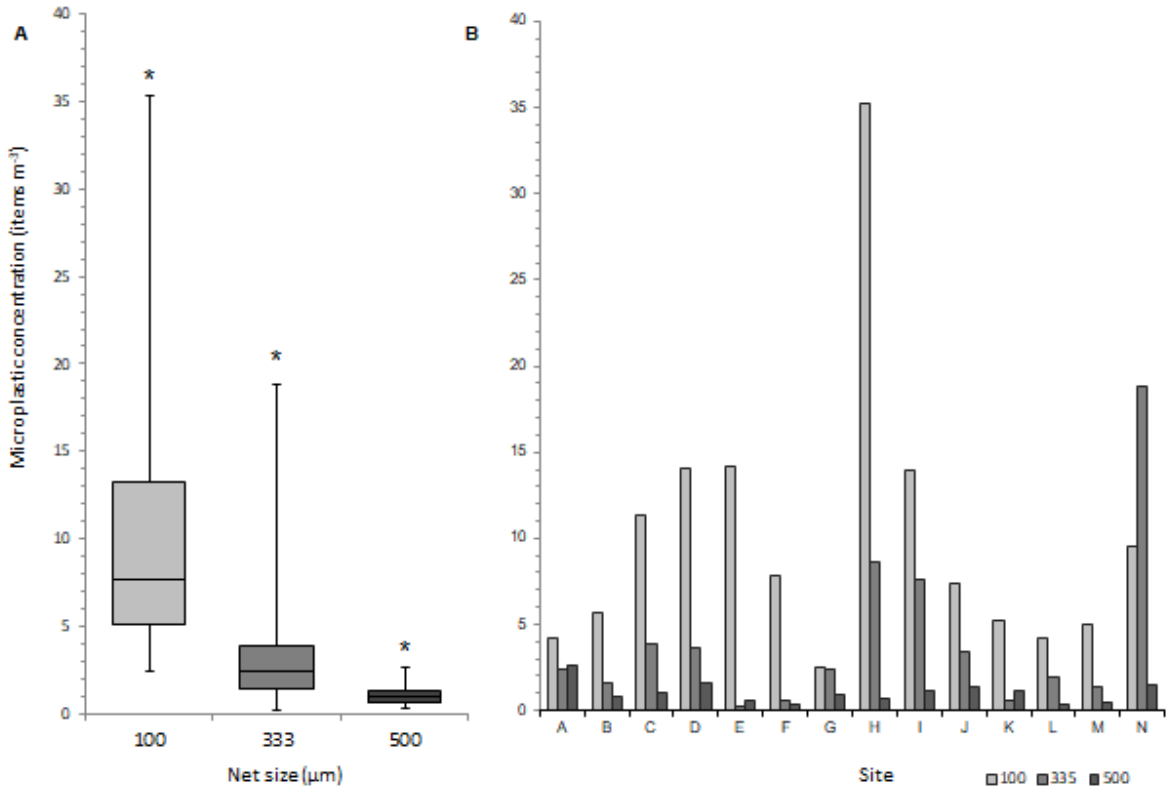


Figure 6. Waterborne concentration of microplastics (items m^{-3}) in the western English Channel, as sampled using 100, 333 or 500 µm nets. (A) Box and whisker plots showing median concentrations across sites; *denotes significant difference (ANOVA, p = < 0.05). (B) Bar chart displaying microplastic concentrations for each net found at each site.

328 Fibres captured with a 100 µm net were significantly shorter than those sampled with a 333 and 500
329 µm net, with a significantly smaller diameter than those sampled with a 500 µm net (Kruskal-Wallis,
330 $P<0.05$; Figure 7). Mean fragment/bead diameter was far greater in the 500 µm net samples (575
331 µm) than the 100 µm (121 µm) or 333 µm (133 µm) net samples, however these differences were
332 not statistically significant (Kruskal-Wallis, 100v500, $P=0.07$; 333v500, $P=0.08$). Fibres were the
333 dominant particle shape characterised across all nets, comprising 75% in the 100 µm net, 81% in 333
334 µm net and 83% in 500 µm net (Figure 7). Beads were only observed in the 100 µm net whilst
335 fragments made up the remaining particle shape across all nets. Blue, black, clear and red were the
336 predominant particle colours across all net sizes, recording similar concentrations in each net size.
337 Extrapolation of mean microplastic concentrations from pooled data across all sites provided
338 estimates of concentrations using different mesh sizes (Figure 8), estimating a mean concentration
339 of 11.4 microplastics m^{-3} when using a 100 µm mesh size, 207.1 microplastics m^{-3} with a 10 µm
340 mesh and increasing to 3700 microplastics m^{-3} if using a 1 µm mesh.
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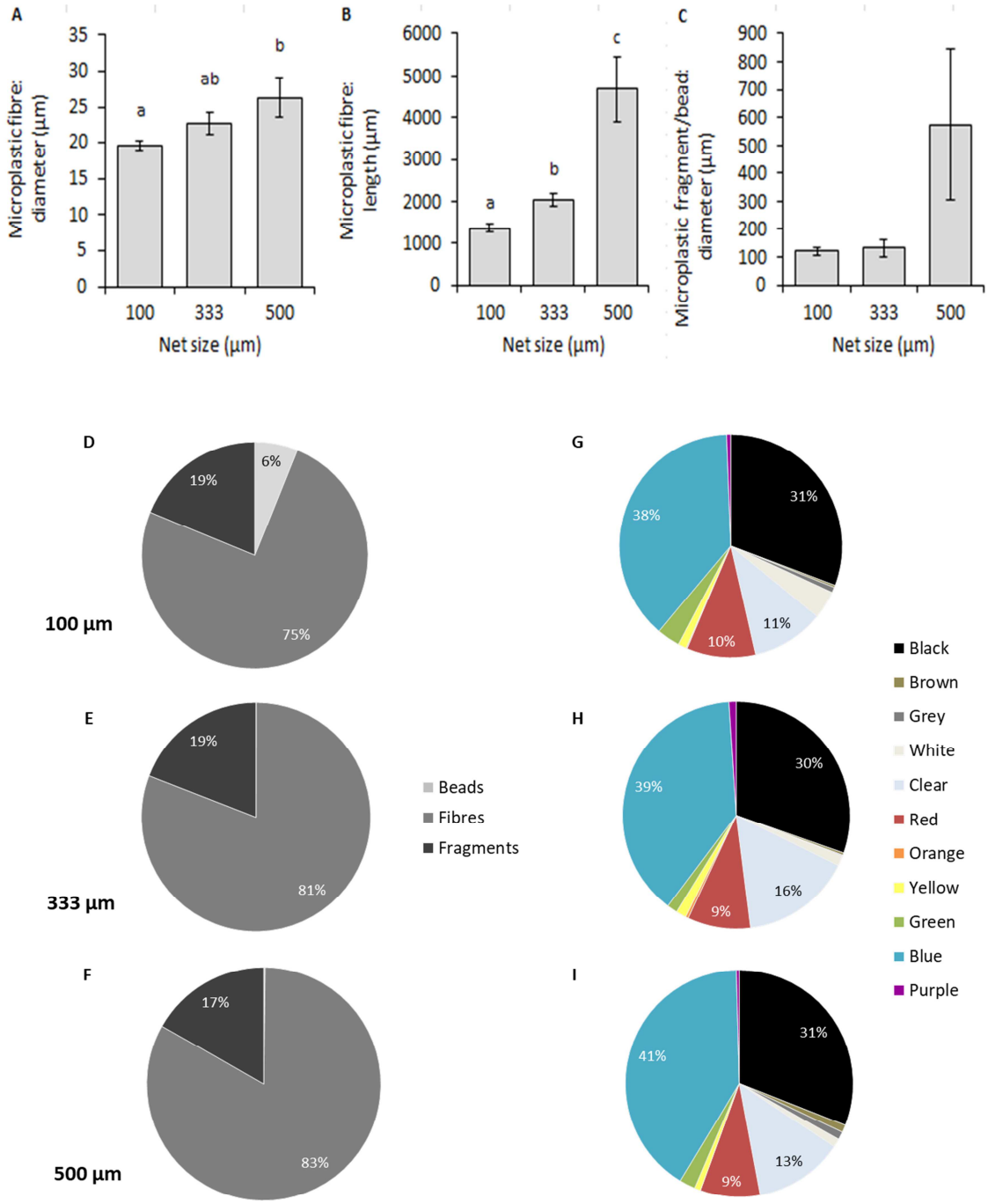

343

344 **Figure 7.** Average size of microplastics identified in UK coastal samples collected using nets with
345 different mesh size. (A) Microplastic fibre diameter; (B) Microplastic fibre length; (C) Fragment/bead
346 diameter. Data presented as mean ± standard error. A Kruskal-Wallis test was applied to compare
347 datasets, with significance attributed where $P < 0.05$. Proportion of UK characterised particles by
348 shape (D,E,F) and colour (G,H,I) for each net size; 100 µm (D,G), 333 µm (E,H), 500 µm (F,I).

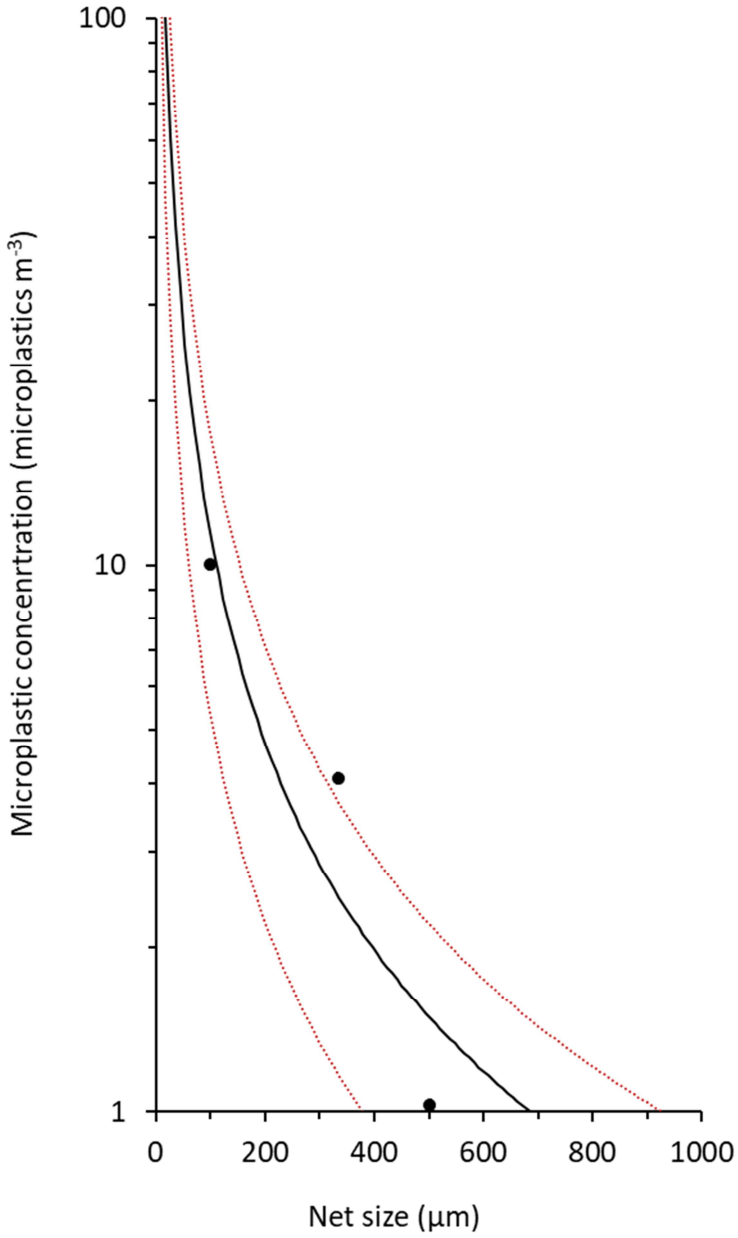

Figure 8. Extrapolation of microplastic concentrations (logarithmic scale) based on our UK coastal samples collected using nets with 100, 333 or 500 µm mesh (black dots), using a power law (black line); 95% confidence intervals shown with dotted red lines.

4. Discussion

Our results demonstrate that sampling with a smaller sized mesh yields a significantly higher concentration of microplastics compared to sampling with larger mesh sizes; a consistent result seen

across a series of biologically productive coastal stations on both sides of the North Atlantic. Both our US and UK datasets reveal that sampling with a 100 µm net results in the capture of 10-fold greater microplastic concentrations compared with using a 500 µm net. Further, our UK sampling regime revealed a 2.5-fold increase in microplastic concentrations sampled with a 100 µm mesh compared to a 333 µm mesh. We believe this to be the first study directly comparing microplastics captured with different size mesh using nets towed concurrently. Our results demonstrate that using a traditional 333 µm mesh can result in the underestimation of waterborne microplastic concentrations owing to smaller microplastics and microfibres being missed. Several other studies have indicated this trend, for example: Enders et al. (2015) identified a greater abundance of smaller microplastic particles sampled in the smaller fraction of a staggered underway intake filtration set-up in the North Atlantic ocean; comparing discrete water samples with towed nets Norén (2007) found concentrations of microplastics up to 1,000 times higher when water column samples were concentrated onto an 80 µm mesh, as opposed to using a 450 µm mesh Neuston net; in the Nakdong River mouth in the Southern Sea of Korea, Kang et al. (2015) identified 0.62-860 microplastics m^{-3} using a 330 µm Manta trawl, and 21-15,560 microplastics m^{-3} using a 50 µm hand net; and Barrows et al. (2017) demonstrated that a surface grab collected over three orders of magnitude more microplastic per volume of water than sampling with a Neuston tow net; and lastly, a study by Covernton et al. (2019) demonstrated microplastic concentrations determined by filtering a 1 L bulk sample through an 8 µm filter was on average approximately 5.8 times greater (per L of water) than a 10 L bucket sample sieved through 63 µm mesh. All the above recent studies concur that microplastic concentration increases significantly with decreasing mesh size. As 80% of microplastic sampling campaigns focus only on the collection of >300 µm plastic debris (Conkle et al., 2018), we conclude that current estimates of marine microplastic pollution is being vastly underestimated.

Global estimates of floating microplastic debris, modelled on data primarily ascertained from 333 µm net samples, is in the order of 5-50 trillion particles (Eriksen et al., 2014; van Sebille et al., 2015).

Based on the relationship between microplastic concentrations identified with 100 and 333 µm nets as detailed in this study, we surmise that for buoyant microplastics >100 µm, the global plastic reservoir is in the order of 12.5—125 trillion particles. We can further extrapolate our data using a power law as prescribed elsewhere (Cózar et al., 2014; Lenz et al., 2016), to estimate how many microplastics might be sampled by nets with even smaller mesh sizes (Figure 8). Based on this extrapolation, in the waters around Plymouth (UK) we estimate the use of a 10 µm mesh net would yield on average approximately 207 microplastics m^{-3}, and by using a 1 µm mesh microplastic concentrations could exceed 3700 microplastics m^{-3}. Appreciably there are wider considerations to any such extrapolation; for example, we know microplastics can be "removed" from surface waters through coastal deposition (Hinata et al., 2017), rapid nano-fragmentation (Andrady, 2015), ingestion by biota (Cole et al., 2013), and repackaging of microplastics in faeces (Cole et al., 2016; Coppock et al., 2019) and marine snow (Porter et al., 2018). However, such a model supports our hypothesis that smaller plastics are underestimated based on traditional sampling. Such a model may also be useful in providing estimates of bioavailable microplastic concentrations for exposure studies (Lenz et al., 2016). A more accurate description of the size and number of microplastics present in the environment, is essential to guide the concentration, shape and size of particles used in exposure experiments in order to identify the mechanisms of interaction between microplastics and organisms, to yield more realistic estimates of sub-lethal effects, and better understand the risk of microplastic pollution to aquatic ecosystems. On average, our results show an increase in microplastic particles sampled with a smaller mesh size, however inconsistencies to this trend are evident at individual sites. This was most notable at site N (UK), where the 333 µm net sample contained twice as many microplastics as the 100 µm net. A small variation in the general trend was also observed at sites A, E, and K (UK), with the 500 µm nets collecting slightly more microplastics than the 333 µm nets, however the differences here are negligible. Potentially, in these highly productive waters, this was a consequence of the 100 µm net becoming clogged with organic material (e.g. localised *Phaeocystis* blooms), thereby decreasing the efficiency of the net and

410 resulting in a decrease of water volume sampled (personal observations). Alternatively, highly
411 localised spatial variation may have resulted in these discrepancies. On average, there was no
412 difference in the concentration of microplastics collected by two 333 µm nets towed in parallel,
413 however there were clear discrepancies between individual samples, highlighting the heterogeneity
414 of microplastic concentrations at such small spatial scales; for example in Outer Portland Bay (Site 5)
415 microplastic concentrations were 0.2 and 1.1 microplastics m^{-3} between nets trawled just metres
416 apart. Reasons for this heterogeneity may include aggregation of microplastics around or within
417 biological material or small scale local eddies and currents. Further, the high-density sampling
418 around Plymouth Sound provides further evidence of the spatial and temporal variability in
419 microplastic concentrations within localised waters, with values of 2.5 – 35.3 microplastics m^{-3}
420 identified within a region of just 50 km^2. This calls into question how frequently in time and space
421 one must sample to gain an accurate picture of localised microplastic concentrations. Sampling
422 practices may also influence the accuracy of collected data; for example, sea state and primary
423 productivity can both influence the position of the net in the water, causing inaccuracies in
424 estimating the volume of water sampled. While not applied here, sea state data can be used to
425 compensate for wind-driven mixing of microplastics (Kooi et al., 2016; Kukulka et al., 2012).
426
427 Considering the geographical distance between our US and UK sampling sites, the number of
428 microplastics sampled on both sides of the north Atlantic with a 100 µm mesh net were remarkably
429 similar, with average concentrations of 6.03 ± 1.03 microplastics m^{-3} in the US and 10.03 ± 2.21
430 microplastics m^{-3} in the UK. All samples were taken from coastal waters, influenced by run-off from
431 land and riverine input (Smyth et al., 2015). The slightly higher concentration of microplastics
432 sampled in the UK is likely due to the sites' proximity to the coast, with the furthest site sampled in
433 the UK being 6.5 km from shore and the furthest site sampled in the US being 24 km from the shore.
434 A previous study in the same UK region showed that the concentration of microplastics decreased
435 with distance from the shore (Steer et al., 2017). Highest microplastic concentrations in our US

436 samples were associated with the outflows of the Penobscot and Piscataqua rivers, and in our UK
437 samples the greatest abundance of microplastics (35.3 particles m^{-3}) was found at the mouth of the
438 River Plym (Site H). Rivers, which receive inputs from agriculture, industry, storm water drains and
439 sewage outflow, are hugely important transport pathways of plastic from land to sea (Lebreton and
440 Andrady, 2019; Lebreton et al., 2017). Sampling at site H occurred after a storm event, and we
441 hypothesise that the high microplastic concentrations observed were associated with high rainfall
442 potentially resulting in the flushing out of roads, drainage systems and agricultural land, and the
443 possible overflow of wastewater treatment works (Horton et al., 2017; Moore et al., 2002).
444
445 In addition to sampling a greater number of microplastics with a smaller size mesh, the fibres that
446 were sampled were also significantly smaller. Sampling with a smaller mesh net therefore not only
447 gives a better indication of the microplastic budget but also gives a better estimation of the
448 abundances of microplastic particles of a size that are bioavailable to small marine organisms such as
449 zooplankton (Botterell et al., 2018). Microplastics can be ingested by a range of marine organisms,
450 including zooplankton (Desforges et al., 2015; Steer et al., 2017; Sun et al., 2017), deep sea
451 invertebrates (Courtene-Jones et al., 2019), bivalves, and fish destined for human consumption
452 (Rochman et al., 2015; Walkinshaw et al., 2020), with the capacity to impact upon the health of the
453 organism and potentially their ecosystem functionality (Galloway et al., 2017; Green, 2016). Using
454 smaller meshed nets will allow researchers to better sample and estimate the abundance and
455 bioavailability of microplastics, in turn allowing more accurate evaluations of the risks microplastics
456 pose to biota, biodiversity, ecosystem function and productivity. The fact that microplastics less than
457 100 µm in size were sampled with a 100 µm mesh net is indicative of some of these plastics
458 becoming trapped in organic material (e.g. exopolymeric agglomerations, phytoplankton; Long et al.,
459 2015; Summers et al., 2018).
460

Fibres were the predominant type of microplastic identified in all our environmental samples (84% USA; 77% UK), being principally black or blue in colour. Microplastic fibres can stem from the breakdown of larger plastic items (e.g. rope) (Welden and Cowie, 2017) or the release of microfibres from synthetic garments during washing cycles (Napper and Thompson, 2016). Abrasion from clothing is also likely to be a significant source of fibre pollution, demonstrated by high quantities observed in atmospheric fallout (Dris et al., 2016) and run off from snow melts (Bergmann et al., 2019). Rayon (biopolymer), polypropylene and polyester are widely used in textiles, providing further evidence that wastewater effluent (containing microfibres from clothes washing (Napper and Thompson, 2016)) and degradation of fishing gear (Welden and Cowie, 2017) are substantial sources of microplastics in coastal waters (Murphy et al., 2016; Napper and Thompson, 2016). The elastomers identified in the UK samples may be associated with vehicle tyre wear (Kole et al., 2017), with inputs stemming from highway drainage (e.g. A38, Tamar bridge). A better understanding of the detailed characteristics of microplastics in the marine environment may help elucidate the origin of these particles, as discussed above, which in turn can help influence societal behaviour and drive future policy intervention.

In recent years there have been calls for harmonisation of microplastic sampling methods (Frias and Nash, 2019; Hartmann et al., 2019; Hidalgo-Ruz et al., 2012), to facilitate comparability between data sets. For example, collection may be via discrete sampling such as using a Niskin bottle (Courtene-Jones., 2017) or via a more continuous sampling method such as a Manta trawl (Sadri et al., 2014) or ships underway system (Lenz et al., 2015), all with differences in error rate and sampling efficiency. Differences in laboratory processing such as methods to digest biotic material, sub-sampling, characterisation and polymeric analysis further serve to make comparisons challenging. Despite these harmonisation calls however, a huge range of different techniques for sampling and quantifying plastics, each championed by different research groups, continue to be used. Furthermore, polymeric analysis of samples would ideally be carried out using automated detection

of particles, such as Focal Plane Array (FPA) or image mapping using FT-IR. Whilst this is the clear way forward in microplastic research, when these methods have been used to date, samples have tended to be very 'clean', and not yet suitable for complex, biologically rich samples such as those obtained in this study.

Conclusion

We have demonstrated that the 333 µm nets commonly used for microplastics sampling underestimate microplastic abundance, particularly for <333 µm microplastics that are within the optimal prey size range of numerous marine organisms. Where possible, sampling should aim to collect the fullest range of microplastics present, with an appreciation that sampling with larger mesh size nets will not give an accurate estimate of abundance or a full account of the microplastics present within the water column. However, we also appreciate that when sampling there needs to be a balance between efficiency, accuracy and detail. We surmise that sampling with smaller sized mesh nets (i.e. 100 µm) gives a better representation of microplastic concentrations in the natural environment and helps to ascertain more reliable estimates of microplastic budgets. In turn this effort allows for better assessment of the current level of risk posed to the marine environment, better guiding monitoring efforts, and providing a clearer benchmark against which to judge the effectiveness of future management scenarios.

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Highlights

- Microplastic concentration using a 100 µm net is 10-fold greater than a 500 µm net.
- UK data revealed 2.5-fold increase in microplastics using 100 compared to 333 net.
- Power law extrapolation of our data enables guidance for exposure experiments.
- Our results suggest underestimation of smaller plastics based on traditional sampling.

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Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: