

# A comparison of sampling methods for seawater microplastics and a first report of the microplastic litter in coastal waters of Ascension and **Falkland Islands**

Green, D. S., Kregting, L., Boots, B., Blockley, D. J., Brickle, P., da Costa, M., & Crowley, Q. (2018). A comparison of sampling methods for seawater microplastics and a first report of the microplastic litter in coastal waters of Ascension and Falkland Islands. Marine Pollution Bulletin, 137, 695-701. https://doi.org/10.1016/j.marpolbul.2018.11.004

#### Published in:

Marine Pollution Bulletin

# **Document Version:**

Peer reviewed version

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1	A comparison of sampling methods for seawater microplastics and a first report of the					
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4	Dannielle S. Green <sup>1*</sup> , Louise Kregting <sup>2</sup> , Bas Boots <sup>1</sup> , David J. Blockley <sup>3</sup> , Paul Brickle <sup>3,4</sup> ,					
5	Marushka da Costa <sup>5</sup> , Quentin Crowley <sup>5</sup>					
6						
7	<sup>1</sup> Department of Biology, Anglia Ruskin University, Cambridge Campus, East Road,					
8	Cambridge, Cambridgeshire, CB1 1PT, United Kingdom					
9	<sup>2</sup> School of Natural and Built Environment, Queen's University Marine Laboratory, 12-13 The					
10	Strand, Portaferry, BT22 1PF, Northern Ireland, UK					
11	<sup>3</sup> South Atlantic Environmental Research Institute, Stanley Cottage, Ross Road, Stanley,					
12	Falkland Islands, FIQQ 1ZZ					
13	<sup>4</sup> School of Biological Sciences (Zoology), University of Aberdeen, Tillydrone Avenue,					
14	Aberdeen, Scotland, AB24 2TZ, UK					
15	<sup>5</sup> Trinity College Dublin					
16						
17	* Corresponding author at:					
18	*Department of Biology, Anglia Ruskin University, Science Centre, Cambridge,					
19	Cambridgeshire, CB1 1PT, United Kingdom					
20	Email: danniellesgreen@gmail.com					
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# 26 Abstract

To date there is no gold standard for sampling microplastics. Zooplankton sampling methods, 27 such as plankton and Neuston nets, are commonly used to estimate the concentrations of 28 29 microplastics in seawater, but their ability to detect microplastics is limited by their mesh size. We compared different net-based sampling methods with different mesh sizes including bongo 30 nets (>500µm), manta nets (>300µm) and plankton nets (>200µm and >400µm) to 1 litre bottle 31 32 grabbed, filtered (0.45µm) samples. Concentrations of microplastics estimated using net-based methods were ~3 orders of magnitude less than those estimated by 1 litre grab samples. Some 33 34 parts of the world with low human populations, such as Ascension Island and the Falkland Islands, lack baseline data on microplastics. Using the bottle grab sampling method we found 35 that microplastic litter was present at these remote locations and was comparable to levels of 36 37 contamination in more populated coastal regions, such as the United Kingdom.



## 40 Introduction

Microplastics (plastic particles < 5 mm in size) have become the most ubiquitous type of anthropogenic litter contaminating marine habitats worldwide, and due to the increasing production and mismanagement of single-use plastic items and the fragmentation of macroplastic litter, their prevalence is increasing (Jambeck et al. 2015). They can be 'primary', directly produced as micro-sized beads or fragments for use as exfoliants in a range of e.g. personal care products or they can be 'secondary', resulting from the fragmentation of larger plastic items e.g. bags, fishing gear and persist as fragments, films or fibres.

The majority of methods used for quantifying microplastics in marine environmental samples 48 use zooplankton sampling methods with an average mesh size of ~330 µm (Barrows et al. 49 2017). Although these net-based methods have allowed highlighting hotspots of accumulation, 50 the lower limits (based on their aperture) of nets are possibly leading to an underestimation in 51 global concentrations of microplastics. Responding to this concern, Barrows et al. (2017) 52 compared (1 L) grab samples with 335 µm Neuston net tows and found that the grab samples 53 collected over three orders of magnitude more microplastic particles per volume of seawater. 54 This indicates that zooplankton sampling methods do indeed underestimate the environmental 55 concentrations of relatively smaller microplastic particles (< 335 µm) and that further 56 comparison of other commonly employed methods of sampling is required. 57

Although there has been extensive monitoring of microplastic contamination of the open oceans (Moore, 2008; Law et al., 2014), microplastics are likely to be more abundant in and around coastal areas (Browne et al. 2010; Wright et al. 2013; Zhao et al. 2014). Microplastics are an issue in coastal habitats as they can be ingested by a wide range of organisms. Effects on the health of individual organisms is well documented from laboratory experiments (for review see: Lusher et al. 2017, Wright et al. 2013). In addition, recent evidence suggests that at high concentrations (~1000 particles L<sup>-1</sup> which is around 2 orders of magnitude greater than 65 currently reported environmental levels), microplastic contamination in coastal water columns 66 may also settle or be deposited onto shallow water benthic habitats and can alter faunal and 67 floral communities and reduce primary productivity (Green 2016, Green et al. 2017). It is, 68 therefore, vital to monitor the levels of contamination in coastal habitats in order to prevent 69 these areas from reaching critical levels for negative impacts to occur (Gago et al. 2016).

Although it seems intuitive that greater levels of contamination will occur in locations close to 70 large coastal populations of humans, such as the Mediterranean (1 to 10 particles  $m^{-2}$  using a 71 200 µm neuston net, Cózar et al. 2015), the East Asian sea (surface waters sampled with a 350 72  $\mu$ m plankton net had an average (±S.D.) of 3.7 ± 10.4 particles m<sup>-3</sup>, Isobe et al. 2015) and the 73 south-eastern coast of Korea (~7 particles  $L^{-1}$  when using a net with 50 µm mesh size, Kang et 74 al., 2015), there is also evidence that relatively remote areas with sparse human populations 75 are also contaminated with microplastic litter, for example, coastal sediments of marine 76 protected areas in the Balearic Islands were more contaminated with microplastics than more 77 urbanised areas (>800 particles kg<sup>-1</sup> dry sediment, Alomar et al., 2016), trapped in Arctic Sea 78 ice (up to 234 particles m<sup>-3</sup> of ice, Obbard et al. 2014) and in surface & subsurface waters of 79 the Arctic Sea (0 to 1.31 particles m<sup>-3</sup> using a 333 µm manta net, Lusher et al. 2015). Plankton 80 81 net trawls from surface waters of the Southern Ocean between Australia and Antarctica also found microplastics of 3.1 x 10<sup>-2</sup> m<sup>-3</sup>100,000 pieces km<sup>-2</sup>, mainly consisting of fibres (Isobe et 82 83 al. 2017). Different sampling methods inevitably lead to a range of different units of concentration being used, which if not able to be converted, can make it difficult to make 84 comparisons. Standardisation of analytical protocols for quantifying microplastics would help 85 solve this issue (Mai et al. 2018). 86

For some parts of the world, however, there is very little or no baseline information on
microplastic concentrations. For example, Ascension Island and the Falkland Islands have no
data on their coastal microplastic litter. Data on the abundance and distribution of stranded

90 (Otley and Ingham, 2003) and floating (Barnes and Milner, 2005) macroplastic debris in these
91 areas suggest that, perhaps due to the fragmentation of these larger items, microplastic litter
92 may also be prevalent and therefore it is important to monitor this.

In order to quantify the level of under-estimation of microplastic concentrations obtained by current common methods of microplastic sampling in seawater, we compared the abundances of microplastics recorded by three common sampling methods (bongo, manta and plankton nets) with those obtained by of 1 L filtered seawater obtained with bottle grabs. Furthermore, we used bottle grab sampling to quantify the abundance of microplastic litter around the coastal surface waters of Ascension Island and the Falkland Islands and compared it to abundances found in more densely populated regions of the world.

100

## 101 **2. Materials and Methods**

# 102 2.1. Prevention and quantification of airborne contamination

103 Inadvertent contamination from the air or from the synthetic clothing of researchers is a common problem thought to lead to an over-estimation of microplastic fibres in environmental 104 samples (Wesch et al. 2017). In order to prevent contamination of samples from their own 105 clothing, researchers wore tightly woven cotton jackets instead of synthetic fleeces whilst 106 107 sampling and white, cotton laboratory coats during sorting in the laboratory. Glass sample 108 bottles (1 L, metal caps) were thoroughly rinsed (three times with tap water followed by three times with ultra-pure water) and checked for contamination by filling with pre-filtered (0.45 109 µm aperture) water and processing this filtered water using the same method as for the 110 111 environmental samples. All equipment used was rinsed with ultra-pure or deionised water 112 before covering with clean tinfoil. All bench tops and microscopes were cleaned prior analysis of the filtered samples. In order to quantify levels of potential contamination with airborne 113 microplastics during filtration, pre-filtered water was passed through a clean GF/C filter paper 114

to check for contamination of the filtering apparatus. Filtered samples were placed immediately into covered Petri dishes while the time exposed to open air was less than 5 seconds. No contamination was found in the filtering apparatus nor in the glass bottles. In addition, to quantify airborne contamination in the laboratory during sample processing, 3 moist filter papers were placed in Petri dishes and exposed to the air within the fume hood and on the laboratory benches during each instance of sample processing.

121

122 2.2. Sampling using common zooplankton methods versus one litre grab samples.

123 In the Summer of 2015 at three different locations; Stanley Harbour in the Falkland Islands (51°41'20.4"S; 57°50'55.3"W), Plymouth Sound in England, UK (50°20'57.3"N; 124 4°08'41.8"W) and Strangford Narrows in Strangford Lough, Northern Ireland, UK 125 126 (54°25'28.4"N; 5°35'49.8"W), one or two commonly used zooplankton net sampling techniques were compared with bulk sampling using one litre bottles. The methods used at 127 each site were selected based on what sampling equipment was available at that location. These 128 three sampling events were treated as separate surveys and, as such, are presented and analysed 129 separately (Table 1). All samples were processed by the same person to reduce analyst bias 130 when comparing sampling methods. 131

132

**Table 1.** Summary of sampling methods compared and the location in which they occurred.

Location	Methods compared
Stanley Harbour	Bongo net vs bottle grab
Plymouth Harbour	Manta net vs bottle grab
Strangford Lough	Plankton nets (one coarse and one fine) versus bottle grab

134

135 2.2.1. East Falklands; bottle versus bongo nets (500µm)

Bongo nets with 500 µm mesh and a diameter of 30 cm were deployed off the back of a vessel

and towed for exactly 5 minutes at 5 knots, maintained at a depth of 1 m in Stanley Harbour.

Stanley Harbour is a large inlet on the east coast of East Falkland Island. Calibrated flow meters 138 in the mouths of the nets allowed the volume of water that passed through to be calculated 139 accurately, resulting in  $\sim 30 \text{ m}^3$  of water sampled each time. On deck, after towing, the contents 140 of the cod end was rinsed out using distilled, filtered water, into 500 ml glass sample jars. 141 During the tow, in between each bongo net sample, seawater samples from the sub-surface 142 (~50 cm) of the water were collected by hand in one litre glass bottles from the back of the 143 144 vessel. These samples were capped whilst still being held under water in order to avoid airborne contamination. In the laboratory, water samples were filtered through 0.45 µm glass fibre filters 145 146 (GF/F) and were visually sorted under a dissecting microscope. Particles that appeared to be plastic, according to criteria suggested by Hidalgo-Ruz et al. (2012), were then recorded and 147 classified as either 'fibres', 'films', 'fragments' or 'beads'. Although visual identification of 148 microplastics is prone to error (either under- or over- estimating the abundance of 149 microplastics; Song et al. 2015), training and experience is likely to lower the error rates of 150 visual identification (Lusher et al. 2017) and in the current study an experienced researcher 151 undertook all visual sorting and a subset of microplastics were confirmed using FT-IR analysis 152 (see section 2.3). Filters were placed in clean, lidded, glass petri dishes and, once dry, were 153 observed under a dissecting microscope (magnification x 40) in a systematic manner using a 154 longitudinal top to bottom traverse method starting from top left hand corner and a 1 cm<sup>2</sup> grid 155 drawn onto the petri dish. A total of six samples were collected for each method (N = 12). 156

157

# 158 2.2.2. Plymouth Sound; bottle versus Manta (300 μm)

A manta net with a rectangular opening 50 cm wide x 15 cm deep lined with a 3 m long 300  $\mu$ m net fitted with a 30 x 10 cm<sup>2</sup> screw-fit collecting bag was used to sample the surface layer (top 15 cm) of the water in Plymouth Sound. Plymouth Sound is a bay on the English Channel at Plymouth in England. The manta was fixed onto a frame and was trawled alongside the 163 vessel for 5 minutes at 5 knots. Material caught in the cod end of the net was rinsed into 500 164 mL glass sample jars which was filtered onto cellulose filter paper (retention of 11  $\mu$ m) and 165 visually sorted under a dissecting microscope in a laminar flow cupboard. This was compared 166 with bottle grab samples collected and processed as detailed previously in section 2.2.1 and 167 were also processed within the laminar flow cupboard. A total of ten samples were collected 168 for each method (N= 20). Appropriate controls were included throughout as described in 169 section 2.1 and no airborne contamination was observed.

170

# 171 2.2.3. Strangford Narrows; bottle versus 200µm and 400µm plankton nets

To compare plankton nets (with a diameter of 50 cm) of two mesh sizes (200 µm or 400 µm) 172 with samples collected in bottles of 1 litre, the survey was conducted in the Strangford Narrows, 173 Strangford Lough, a fast flow channel. Strangford Lough on the Island of Ireland is connected 174 to the Irish Sea located between the two landmasses of the UK and Ireland. Plankton nets were 175 deployed off the side of a moored barge during flood tide at the location for exactly 5 minutes. 176 In order to monitor flow velocity a Nortek Aquadopp 2 MHz (Acoustic Doppler Current 177 Profiler) was mounted alongside the nets at 2 m below the barge to calculate the volume using 178 average velocities at the depth of the nets. After each tow the cod ends were rinsed with 179 distilled, filtered water, into 500 mL glass sample jars and a bottle sample was taken. Samples 180 were processed as described in 2.2.2. A total of seven samples were collected for each method 181 (N=21) at this location. 182

183

184 The volume V (m<sup>3</sup>) of water sampled for each net method (bongo, manta and plankton) was 185 estimated using the net entrance surface area A (m<sup>2</sup>) and the length of the tow:

$$V = A * L$$

188 2.2.4. Quantification of microplastic litter in coastal waters of Ascension Island and the East
189 Falklands

In August 2015, surveys for microplastic litter were done at 6 sites on Ascension Island and at 11 sites on the Falkland Islands (East Falklands only). At each site, 5 seawater samples were taken from the surface (top ~5 cm) of the water in one litre glass bottles, giving a total of 85 samples (30 at Ascension Island and 55 at the Falkland Islands). Glass bottle samples were collected and processed as detailed in 2.2.1.

195

# 196 2.3. Characterisation of polymers from microplastic particles

197 A Perkin Elmer 200i Spotlight Microscope FT-IR spectrometer was used to characterise the 198 polymers of microplastics from a randomly selected subset (10%) of the samples. To maximise 199 the resolution of the readings microplastics were first subjected to 30% (v/v) solution of  $H_2O_2$ 200 overnight to avoid any interference from biological material and were then directly mounted 201 onto the crystal surface of the FTIR.

202

# 203 2.4. Statistical data analysis

204 For statistical analysis, the concentrations of microplastics obtained from each sampling method were converted to number of particles per litre. The data did not conform to parametric 205 206 assumptions of normality and homogeneity of variance, therefore non-parametric tests (Wilcoxon rank sum tests) were used to compare the bottle versus bongo nets and the bottle 207 versus manta net methods. Similarly, Kruskal-Wallis rank sum tests with Wilcoxon tests for 208 pairwise comparisons were used to compare the bottle versus coarse or fine plankton nets and 209 also to compare the concentrations of microplastics found with the bottle method amongst the 210 four locations (Ascension Island, the Falkland Islands, Plymouth Sound and Portaferry). 211

212 Statistical significance were assumed at  $\alpha = 0.05$ . All statistical analyses were done using the 213 R environment (R v3.1.3; R core team 2015).

214

# 215 **3. Results**

# 216 *3.1. Sampling using one litre bottles versus common zooplankton methods*

In each of the three locations, the bottle grab method yielded between 3 and 4 orders of magnitude greater abundances of total microplastic particles  $L^{-1}$  and these differences were statistically significant in all three surveys (Table 2), but varied depending on the type of microplastic.

In the Falkland Islands, the number of microplastic films did not significantly differ between 221 sampling methods (P = 0.774), however the number of microplastic fragments found was 222 223 greater (P = 0.028) when using Bongo nets than when using the bottle grab method. On the contrary, the number of fibres was significantly greater (P = 0.005) in samples collected using 224 the bottle method than by using Bongo nets (Table 2). In Plymouth Sound, there were no 225 microplastic films found using either method and there was no significant difference between 226 the number of microplastic fragments found using the Manta net compared with the bottle 227 method (P = 0.455). On the contrary, the average number of fibres found was significantly 228 greater when using the bottle method compared with the Manta net (P = <0.001). In addition, 229 a total of 17 meso-plastics (> 5 mm) were found using the Manta net, representing an average 230 of 1.09 x 10<sup>-4</sup> (SE = 6.63 x 10<sup>-5</sup>) L<sup>-1</sup>. It is worth noting that no meso-plastics were found in 231 bottle grab samples and analysis was only done to compare the abundance of microplastics (<5 232 mm). Finally, in Strangford Narrows, the average number of microplastic films was 233 234 significantly greater when using a fine plankton net than when using a coarse plankton net or the bottle method (P = 0.027). There were no significant differences in the number of 235 microplastic fragments amongst the methods (P = 0.810), but the number of microplastic fibres 236

found was significantly greater when using the bottle method or the fine plankton net than when using the coarse plankton net (P = 0.002; Table 2).

239 From the three surveys to compare methods, a subset of 11 samples (29 microplastic particles) were identified and confirmed with FTIR spectrometry. From the Falkland Islands, 4 out of a 240 possible 12 replicate samples (6 individual microplastics) were identified, from these; 3 were 241 polyethylene, 1 was monocrystalline cellulose, 1 was regenerated cellulose and 1 was 242 243 undetermined polyamide (nylon). In Plymouth, 3 out of 20 samples (14 microplastics) were identified, from these; 5 were polypropylene, 5 were polyethylene terephthalate and 4 were 244 245 polyethylene. Finally, in Strangford Narrows, 4 out of 21 samples (9 microplastics) were identified, from these; 2 were acrylic, 2 were polypropylene, 2 were polyvinyl chloride, 1 was 246 neoprene, 1 was polyethylene and 1 was polyvinyl acetate. 247

248

# 249 3.3. Microplastic litter in coastal waters of Ascension Island and The Falkland Islands

Microplastic litter was found at every site sampled around the coastal waters of Ascension 250 251 Island (Figure 1) and the East Falklands (Figure 2), and concentrations ranged from 0.4 to 9 particles L<sup>-1</sup>. The majority (94 %) of microplastics collected were fibres, with films accounting 252 for  $\sim 5$  % and fragments representing only <1 % (Table 3). A subset of 11 out of 55 samples 253 (15 microplastics) from the Falklands were further identified using FTIR analysis. Of these, 6 254 were polyethylene, 3 were polyethylene terephthalate and the following six polymers 255 256 constituted 1 microplastic each; monocrystalline cellulose, nylon, polyester, polymethyl methacrylate, polystyrene and regenerated cellulose. 257

The concentrations of microplastics found using the bottle method significantly differed (W = 20.41, d.f. = 3, P < 0.001) amongst the four locations in this study, with the Falkland Islands having greater abundances of microplastics than Portaferry (P < 0.001) or Plymouth (P = 0.0398), but not differing to Ascension Island (P = 0.127). The concentration of microplastics found at Ascension Island also did not significantly differ to those found at Plymouth (P = 0.295) or Portaferry (P = 0.097).

264

# 265 4. Discussion

This set of comparative studies indicates that three common zooplankton sampling methods (manta, bongo and plankton nets), frequently used to sample microplastics, may underestimate the concentrations of microplastic fibres by 3 to 4 orders of magnitude compared to when using the grab method. Other types of microplastic, however, such as fragments and films were underestimated in some cases by the grab method when compared with Bongo nets or a fine (200  $\mu$ m) plankton net.

Estimating and monitoring the concentrations of microplastics is vital for understanding the 272 current and future implications of microplastic litter for marine ecosystems worldwide (as 273 274 recommended by national and international policies, and legislation such as the EU Marine 275 Strategy Framework Directive (2008/56/EC) and the NOAA Marine Debris Programme). The desired method of choice may depend upon the context and aims of the sampling regime, for 276 example, if the aim of the sampling regime is to capture and sort meso- and larger micro-277 plastics in-situ without a microscope, zooplankton tow methods will yield better results because 278 they sample a larger volume of water and therefore increase the potential to capture these 279 280 pieces. Due to the small filter pore size  $(0.45 - 11 \,\mu m)$ , the grab method is more likely to capture smaller pieces of microplastics which zooplankton nets (>200 µm) will miss, however, the 281 small volume of water sampled may omit larger micro- and meso- plastics (> 5 mm). On the 282 other hand, the need to measure flow speeds in order to estimate the volume of water processed 283 and the act of cleaning the net in between each tow is likely to introduce uncertainty into 284 measurements taken using zooplankton methods. As recommended by Barrows et al. (2017) a 285

combination of methods is likely to lead to a greater overall understanding of the concentrations
of larger mesoplastics (using zooplankton nets) and smaller microplastics (using grab samples).

Coastal regions are vitally important economically (providing valuable ecosystem services; 288 Costanza et al. 2014) and ecologically (supporting unique biodiversity; Ray, 1991, UNEP, 289 290 2006) and they provide habitat for over a third of the world's human population, and as such, 291 are under pressure from a myriad of anthropogenic threats (including habitat loss, overfishing, 292 invasive species, climate change, eutrophication and pollution). There is, therefore, a critical need to standardise sampling methods in order to allow environmental managers to accurately 293 track levels of contamination and to prioritise areas most at risk from microplastic pollution. 294 Due to the lack of no specialist equipment required and replicability of the grab method, it is a 295 very promising approach to e.g. facilitate citizen science programmes aimed at monitoring 296 microplastic concentrations at large spatial scales. Indeed, citizen science using the grab 297 298 method has recently been utilised by Barrows et al. (2018) in a global assessment of microplastic litter in seawater samples and it was found that the samples contained an average 299 of  $11.8 \pm 24.0$  particles L<sup>-1</sup> with an average of  $13.4 \pm 0.9$  particles L<sup>-1</sup> for the Atlantic Ocean, 300 similar to the estimate for the coastal waters of the Falkland Islands of 9.8  $\pm$  1.5 particles L<sup>-1</sup> 301 reported in the current study. There is evidence that the grab method is an appropriate way to 302 monitor microplastic contamination that could be paired with existing environmental surveys 303 304 with relatively little effort leading to a standardised monitoring protocol. Based on the current study it is recommend that this method be utilised, perhaps combined with a citizen science 305 approach, thereby raising public awareness of microplastic pollution whilst also improving the 306 reliability of datasets to record patterns of microplastic contamination over space and time. 307

This study found that the coastal waters of two remote islands with very small populations, Ascension Island (no official inhabitants, but a transient population of ~800 people in 2016) and the East Falklands (~3200 people in the 2016 census), are subject to similar (and even

greater) levels of contamination of microplastics as coastlines with a greater human population 311 density such as the United Kingdom (~263,100 people in Plymouth and ~100,000 people in 312 the towns surrounding Strangford Lough, Northern Ireland). This is not entirely surprising 313 given recent discoveries of high levels of microplastic contamination in other remote locations 314 such as Antarctica (Waller et al. 2017) and the Arctic (Lusher et al. 2015; Cózar et al. 2017). 315 Identifying the source of microplastics is currently difficult and speculative, but some of the 316 317 fibres found in this study had the appearance of weathered fragments of ropes or fishing nets (Figure 3). Other researchers have correlatively linked increasing microplastic debris to 318 319 increasing numbers of fishing vessels (in the Arctic (Tekman et al. 2017) or to increasing mariculture activity (in the Xiangshan Bay in China (Chen et al. 2018). Production of fishery 320 and aquaculture has increased approximately eightfold since 1950 with these food products 321 accounting for 17% of animal protein intake by the world's population. The development and 322 success of this industry has been largely due to plastic. Synthetic materials are stronger, more 323 durable and weigh less than natural materials and, as such, are used in almost all elements of 324 the industry including the construction of boats, ropes, fishing gear and seafood packaging 325 (FAO, 2017). Although, at present, there are no current global estimates of the contribution of 326 fisheries and aquaculture to microplastic litter in marine environments, it is a possibility since 327 larger plastic items from fisheries and aquaculture regularly contaminate surface waters (Cózar 328 et al., 2014; Thiel et al., 2003) or the seafloor (Iñiguez et al. 2016) that these could degrade 329 330 into microplastics. In addition to potentially contributing to marine microplastic debris, there is concern for food safety of fisheries and aquaculture products due to contamination with 331 microplastics and their associated toxins (Rochman et al., 2015; Wardrop et al., 2016). The 332 Falkland Islands has a relatively large fishery with a total annual catch (last 5 years) of 270,000 333 tonnes (Falkland Islands Government, 2018) and given that contamination of important 334 fisheries species with microplastics has been found in other parts of the Atlantic Ocean 335

(including *Scomber japonicus* offshore Portugal; Neves et al. 2015, in *Atherinella brasiliensis*offshore Brazil; Alves et al. 2016 and in *Engraulis encrasicolus* in the Mediterranean; Collard
et al., 2017) it is important to know the potential for this to occur by assessing the distribution
and abundance of microplastics in fisheries grounds.

In conclusion, there is a lack of data describing the spatial and temporal variability of the concentrations of microplastics and the impacts that they might have in remote locations such as Ascension Island and the Falklands. Future research should focus on implementing standardised routine monitoring of coastal waters (ideally using a grab bottle method), in order to more fully understand the extent of microplastics contamination.

345

# 346 Acknowledgements

Our appreciation extends to the South Atlantic Environmental Research Institute as well as a Queen's University Belfast fellowship awarded to LK for funding this research and to the Falkland Islands fisheries for allowing us to use their vessel and facilities, to Richard Ticehurst for assisting with fieldwork in Plymouth, Carwyn Frost and Ian Benson for assisting with sample collection from the mooring barge in the Strangford Narrows and to Ilaria Marengo and Megan Tierney for their assistance in the field in the Falklands and to SAERI's IMS-GIS Centre for the maps.

354

# 355 Author contributions

DSG conceived the ideas for the methods comparison. DJB conceived the idea for sampling
Ascension and Falklands. LK, BB, DJB, PB and DSG carried out the work. MC and QC carried
out FTIR analysis. DSG wrote the paper and all authors helped with edits. All authors approve
the final version of the manuscript.

360

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# 513 Figures and Tables





**Figure 1.** Map of Ascension Island showing average concentrations of microplastics (particles

516  $L^{-1}$ ) obtained with 1L bottle grab sampling.



Figure 2. Map of the Falkland Islands showing average concentrations of microplastics
(particles L<sup>-1</sup>) in the East Falklands obtained with 1L bottle grab sampling.



- **Figure 3.** Photographs of microplastic fibres found in Ascension Island (a, b) or the East
- 522 Falklands (c, d).

# 523 Tables

**Table 1.** Median (+ Inter Quartile Range (IQR)) number  $L^{-1}$  of microplastic films, fragments, fibres and total microplastics determined using different sampling methods including bulk one litre samples (Bottle) versus towing bongo nets (Bongo), Manta nets (Manta) or Plankton nets with either a 400µm (Coarse) or a 200µm (Fine) mesh. Results of non-parametric statistical analyses Wilcoxon rank sum test (W) with d.f. = 5 for Falklands and 9 for Plymouth and Kruskal-Wallis rank sum test (K) with d.f. = 2. Significant differences (in bold) are considered when P values <0.05. In the Portaferry data, subscript letters denote significant differences revealed by pairwise Dunn tests. Mean (±S.E.) values are also included

529 to allow for easy comparison with other values reported in the literature.

Location	Method	Films	Fragments	Fibres	Total
Falklands	Bottle	0.00 (0.00 - 0.74)	0.00 (0.00 - 0.00)	9.00 (7.25 - 13.00)	9.00 (8.00 - 13.00)
	Bongo	$0.00 (0.00 - 1.34 \ge 10^{-6})$	9.23 x 10 <sup>-6</sup> (1.42 x 10 <sup>-6</sup> – 2.8 x 10 <sup>-6</sup> )	8.00 x 10 <sup>-5</sup> (2.70 x 10 <sup>-5</sup> – 1.8 x 10 <sup>-4</sup> )	$1.04 \ge 10^{-4} (3.26 \ge 10^{-5} - 2.10 \ge 10^{-4})$
		W=16, P=0.774	W=30, P=0.028	W=78, P=0.005	W=78, P=0.005
Mean (S.E)	Bottle	<i>3.33</i> (±2.11)	0	9.50 (±1.63)	9.83 (±1.47)
	Bongo	$6.22 \ x \ 10^{-7} \ (\pm 3.94 \ x \ 10^{-7})$	$1.47 \ x \ 10^{-5} \ (\pm 6.66 \ x \ 10^{-6})$	$1.02 \ x \ 10^{-4} \ (\pm 3.86 \ x \ 10^{-5})$	$1.19 \ x \ 10^{-4} \ (\pm 4.23 \ x \ 10^{-5})$
Plymouth	Bottle	0	0.00 (0.00 - 0.75)	2.00 (1.00 - 2.00)	2.00 (1.25 - 3.00)
	Manta	0	$0.00\ (0.00 - 0.00)$	$6.43 \ge 10^{-4} (0.00 - 1.20 \ge 10^{-3})$	$6.4 \ge 10^{-4} (0.00 - 1.24 \ge 10^{-3})$
		N/A	W=58, P=0.455	W=100, P<0.001	W=100, P<0.001
Mean (S.E)	Bottle	0	<i>3.00</i> (±1.53)	2.30 (±5.59)	2.60 (±5.42)
	Manta	0	$1.16 \ x \ 10^{-4} \ (\pm 7.76 \ x \ 10^{-5})$	$6.67 \ x \ 10^{-4} \ (\pm 2.09 \ x \ 10^{-4})$	$7.83 \times 10^{-4} (\pm 2.66 \times 10^{-4})$
Portaferry	Bottle	0.00 (0.00 - 0.00)	0.00 (0.00 - 1.00)	0.00 (0.00 - 0.50)	1.00 (0.00 – 1.00)
	Coarse	0.00 (0.00 - 0.00)	$0.00 (0.00 - 2.12 \text{ x } 10^{-4})$	$1.70 \ge 10^{-4} (0.00 - 2.12 \ge 10^{-4})$	2.12 x 10 <sup>-4</sup> (1.56 x 10 <sup>-4</sup> – 3.54 x 10 <sup>-4</sup> )
	Fine	2.12 x 10 <sup>-4</sup> (0.00 - 4.24 x 10 <sup>-4</sup> )	1.70 x 10 <sup>-4</sup> (0.00 – 3.18 x 10 <sup>-4</sup> )	8.49 x 10 <sup>-4</sup> (7.43 x 10 <sup>-4</sup> – 1.13 x 10 <sup>-3</sup> )	<b>1.36 x 10<sup>-3</sup> (1.17 x 10<sup>-3</sup> – 1.60 x 10<sup>-3</sup>)</b>
		K=7.25, P=0.027	K=0.43, P=0.810	K=12.22, P=0.002	K=17.78, P<0.001
Mean (S.E)	Bottle	<sub>a</sub> 0	7.14 ± 4.21	$_{a}1.14~(\pm 0.34)$	$_{a}1.29~(\pm 8.08)$
	Coarse	$_{a}2.02 \ x \ 10^{-5} \ (\pm 2.02 \ x \ 10^{-5})$	$1.52 \ x \ 10^{-4} \ (\pm 8.92 \ x \ 10^{-5})$	$_{b}1.25 \ x \ 10^{-4} \ (\pm 4.60 \ x \ 10^{-5})$	$_{b}2.97 \ x \ 10^{-4} \ (\pm 1.04 \ x \ 10^{-4})$
	Fine	$_{b}2.73 \ x \ 10^{-4} \ (\pm 1.29 \ x \ 10^{-4})$	$1.96 \ x \ 10^{-4} \ (\pm 8.52 \ x \ 10^{-5})$	$_{a}9.38 \ x \ 10^{-4} \ (\pm 1.41 \ x \ 10^{-3})$	$_{c}1.41 \ x \ 10^{-3} \ (\pm 1.58 \ x \ 10^{-4})$

**Table 2.** Average ( $\pm$ S.E) number L<sup>-1</sup> of microplastic films, fragments and fibres determined bulk one litre samples using glass bottles (n = 5).

Location	Site	Films	Fragments	Fibres	Total
Ascension Island	Long beach	-	-	0.4 (± 0.24)	0.4 (± 0.24)
	Pan Am A	$0.8 (\pm 0.58)$	-	7.2 (± 2.75)	8.0 (± 2.51)
	Pan Am B	-	-	3.2 (± 1.32)	3.2 (± 1.32)
	Boatswain Bird Island	$1.0 (\pm 0.55)$	-	6.8 (± 3.46)	7.8 (± 3.89)
	North East Bay	$1.2 (\pm 0.73)$	-	$2.8 (\pm 0.86)$	4.0 (± 1.30)
	English Bay	$0.4 (\pm 0.40)$	-	3.8 (± 1.68)	4.2 (± 2.06)
Falklands Islands	Bleaker Island	-	-	3.6 (± 0.81)	3.6 (± 0.81)
	New Haven	-	-	5.6 (± 2.78)	5.6 (± 2.78)
	Bertha's Beach	-	-	2.8 (± 1.11)	2.8 (± 1.11)
	Fitzroy	-	-	$3.6 (\pm 0.67)$	3.6 (± 0.67)
	Goose Green	-	-	4.6 (± 1.91)	4.6 (± 1.91)
	Elephant Beach	$0.8 (\pm 0.37)$	-	7.2 (± 2.27)	8.0 (± 2.41)
	San Carlos	-	-	$8.8 (\pm 0.80)$	8.8 (± 0.80)
	Teale Inlet	-	-	5.8 (± 1.65)	5.8 (± 1.65)
	Green Patch	-	-	7.8 (± 1.16)	7.8 (± 1.16)
	Stanley Harbour	0.2 (± 0.20)	-	2.8 (± 0.49)	3.2 (± 0.66)
	Cape Dolphin	0.2 (± 0.20)	0.8 (±0.2)	7.4 (± 1.07)	8.4 (± 1.21)
	Port William	$0.3 (\pm 0.21)$	-	9.5 (± 1.63)	9.8 (± 1.47)