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

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Abstract

To date there is no gold standard for sampling microplastics. Zooplankton sampling methods, such as plankton and Neuston nets, are commonly used to estimate the concentrations of 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 nets (>500μm), manta nets (>300μm) and plankton nets (>200μm and >400μm) to 1 litre bottle 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 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 that microplastic litter was present at these remote locations and was comparable to levels of contamination in more populated coastal regions, such as the United Kingdom.

Keywords: grab samples, nets, anthropogenic litter, remote, Atlantic Ocean.

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

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^{-1} which is around 2 orders of magnitude greater than currently reported environmental levels), microplastic contamination in coastal water columns may also settle or be deposited onto shallow water benthic habitats and can alter faunal and floral communities and reduce primary productivity (Green 2016, Green et al. 2017). It is, therefore, vital to monitor the levels of contamination in coastal habitats in order to prevent 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 large coastal populations of humans, such as the Mediterranean (1 to 10 particles m^{-2} using a 200 µm neuston net, Cózar et al. 2015), the East Asian sea (surface waters sampled with a 350 µm plankton net had an average (±S.D.) of 3.7 ± 10.4 particles m^{-3}, Isobe et al. 2015) and the south-eastern coast of Korea (~7 particles L^{-1} when using a net with 50 µm mesh size, Kang et al., 2015), there is also evidence that relatively remote areas with sparse human populations are also contaminated with microplastic litter, for example, coastal sediments of marine protected areas in the Balearic Islands were more contaminated with microplastics than more urbanised areas (>800 particles kg^{-1} dry sediment, Alomar et al., 2016), trapped in Arctic Sea ice (up to 234 particles m^{-3} of ice, Obbard et al. 2014) and in surface & subsurface waters of the Arctic Sea (0 to 1.31 particles m^{-3} using a 333 µm manta net, Lusher et al. 2015). Plankton net trawls from surface waters of the Southern Ocean between Australia and Antarctica also found microplastics of 3.1 x 10^{-2} m^{-3}100,000 pieces km^{-2}, mainly consisting of fibres (Isobe et 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 comparisons. Standardisation of analytical protocols for quantifying microplastics would help solve this issue (Mai et al. 2018).

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

(Otley and Ingham, 2003) and floating (Barnes and Milner, 2005) macroplastic debris in these areas suggest that, perhaps due to the fragmentation of these larger items, microplastic litter 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.

2. Materials and Methods

2.1. Prevention and quantification of airborne contamination

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 samples (Wesch et al. 2017). In order to prevent contamination of samples from their own clothing, researchers wore tightly woven cotton jackets instead of synthetic fleeces whilst sampling and white, cotton laboratory coats during sorting in the laboratory. Glass sample 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 µm aperture) water and processing this filtered water using the same method as for the environmental samples. All equipment used was rinsed with ultra-pure or deionised water 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 microplastics during filtration, pre-filtered water was passed through a clean GF/C filter paper 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.

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

In the Summer of 2015 at three different locations; Stanley Harbour in the Falkland Islands (51º41’20.4”S; 57º50’55.3”W), Plymouth Sound in England, UK (50º20’57.3”N; 4º08’41.8”W) and Strangford Narrows in Strangford Lough, Northern Ireland, UK (54º25’28.4”N; 5º35’49.8”W), one or two commonly used zooplankton net sampling techniques were compared with bulk sampling using one litre bottles. The methods used at each site were selected based on what sampling equipment was available at that location. These three sampling events were treated as separate surveys and, as such, are presented and analysed separately (Table 1). All samples were processed by the same person to reduce analyst bias when comparing sampling methods.

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

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 in the mouths of the nets allowed the volume of water that passed through to be calculated accurately, resulting in ~30 m^3 of water sampled each time. On deck, after towing, the contents of the cod end was rinsed out using distilled, filtered water, into 500 ml glass sample jars. During the tow, in between each bongo net sample, seawater samples from the sub-surface (~50 cm) of the water were collected by hand in one litre glass bottles from the back of the 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 (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 classified as either ‘fibres’, ‘films’, ‘fragments’ or ‘beads’. Although visual identification of microplastics is prone to error (either under- or over- estimating the abundance of microplastics; Song et al. 2015), training and experience is likely to lower the error rates of visual identification (Lusher et al. 2017) and in the current study an experienced researcher undertook all visual sorting and a subset of microplastics were confirmed using FT-IR analysis (see section 2.3). Filters were placed in clean, lidded, glass petri dishes and, once dry, were observed under a dissecting microscope (magnification x 40) in a systematic manner using a longitudinal top to bottom traverse method starting from top left hand corner and a 1 cm^2 grid drawn onto the petri dish. A total of six samples were collected for each method (N = 12).

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 µm net fitted with a 30 x 10 cm^2 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 vessel for 5 minutes at 5 knots. Material caught in the cod end of the net was rinsed into 500 mL glass sample jars which was filtered onto cellulose filter paper (retention of 11 μm) and visually sorted under a dissecting microscope in a laminar flow cupboard. This was compared with bottle grab samples collected and processed as detailed previously in section 2.2.1 and were also processed within the laminar flow cupboard. A total of ten samples were collected for each method (N= 20). Appropriate controls were included throughout as described in section 2.1 and no airborne contamination was observed.

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

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

$$V = A * L$$

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

2.3. Characterisation of polymers from microplastic particles

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

2.4. Statistical data analysis

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 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 versus manta net methods. Similarly, Kruskal-Wallis rank sum tests with Wilcoxon tests for pairwise comparisons were used to compare the bottle versus coarse or fine plankton nets and also to compare the concentrations of microplastics found with the bottle method amongst the four locations (Ascension Island, the Falkland Islands, Plymouth Sound and Portaferry).

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

3. Results

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 sampling methods (P = 0.774), however the number of microplastic fragments found was 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 the bottle method than by using Bongo nets (Table 2). In Plymouth Sound, there were no microplastic films found using either method and there was no significant difference between the number of microplastic fragments found using the Manta net compared with the bottle method (P = 0.455). On the contrary, the average number of fibres found was significantly greater when using the bottle method compared with the Manta net (P = <0.001). In addition, a total of 17 meso-plastics (> 5 mm) were found using the Manta net, representing an average of 1.09×10^{-4} (SE = 6.63×10^{-5}) L^{-1}. It is worth noting that no meso-plastics were found in bottle grab samples and analysis was only done to compare the abundance of microplastics (<5 mm). Finally, in Strangford Narrows, the average number of microplastic films was 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 microplastic fragments amongst the methods (P = 0.810), but the number of microplastic fibres 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).

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 possible 12 replicate samples (6 individual microplastics) were identified, from these; 3 were polyethylene, 1 was monocrystalline cellulose, 1 was regenerated cellulose and 1 was 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 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 neoprene, 1 was polyethylene and 1 was polyvinyl acetate.

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 Island (Figure 1) and the East Falklands (Figure 2), and concentrations ranged from 0.4 to 9 particles L^{-1}. The majority (94 %) of microplastics collected were fibres, with films accounting for ~5 % and fragments representing only <1 % (Table 3). A subset of 11 out of 55 samples (15 microplastics) from the Falklands were further identified using FTIR analysis. Of these, 6 were polyethylene, 3 were polyethylene terephthalate and the following six polymers constituted 1 microplastic each; monocrystalline cellulose, nylon, polyester, polymethyl methacrylate, polystyrene and regenerated cellulose.

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 ($P = 0.097$).

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 µm) plankton net.

Estimating and monitoring the concentrations of microplastics is vital for understanding the current and future implications of microplastic litter for marine ecosystems worldwide (as recommended by national and international policies, and legislation such as the EU Marine 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 example, if the aim of the sampling regime is to capture and sort meso- and larger micro-plastics in-situ without a microscope, zooplankton tow methods will yield better results because they sample a larger volume of water and therefore increase the potential to capture these pieces. Due to the small filter pore size (0.45 - 11 µm), the grab method is more likely to capture smaller pieces of microplastics which zooplankton nets (>200 µm) will miss, however, the small volume of water sampled may omit larger micro- and meso- plastics (> 5 mm). On the other hand, the need to measure flow speeds in order to estimate the volume of water processed and the act of cleaning the net in between each tow is likely to introduce uncertainty into measurements taken using zooplankton methods. As recommended by Barrows et al. (2017) a 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; Costanza et al. 2014) and ecologically (supporting unique biodiversity; Ray, 1991, UNEP, 2006) and they provide habitat for over a third of the world's human population, and as such, are under pressure from a myriad of anthropogenic threats (including habitat loss, overfishing, 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 track levels of contamination and to prioritise areas most at risk from microplastic pollution. Due to the lack of no specialist equipment required and replicability of the grab method, it is a very promising approach to e.g. facilitate citizen science programmes aimed at monitoring microplastic concentrations at large spatial scales. Indeed, citizen science using the grab 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 of 11.8 ± 24.0 particles L^{-1} with an average of 13.4 ± 0.9 particles L^{-1} for the Atlantic Ocean, similar to the estimate for the coastal waters of the Falkland Islands of 9.8 ± 1.5 particles L^{-1} reported in the current study. There is evidence that the grab method is an appropriate way to monitor microplastic contamination that could be paired with existing environmental surveys 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 approach, thereby raising public awareness of microplastic pollution whilst also improving the reliability of datasets to record patterns of microplastic contamination over space and time.

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 density such as the United Kingdom (~263,100 people in Plymouth and ~100,000 people in the towns surrounding Strangford Lough, Northern Ireland). This is not entirely surprising given recent discoveries of high levels of microplastic contamination in other remote locations such as Antarctica (Waller et al. 2017) and the Arctic (Lusher et al. 2015; Cózar et al. 2017). Identifying the source of microplastics is currently difficult and speculative, but some of the 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 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 and aquaculture has increased approximately eightfold since 1950 with these food products accounting for 17% of animal protein intake by the world´s population. The development and success of this industry has been largely due to plastic. Synthetic materials are stronger, more durable and weigh less than natural materials and, as such, are used in almost all elements of the industry including the construction of boats, ropes, fishing gear and seafood packaging (FAO, 2017). Although, at present, there are no current global estimates of the contribution of fisheries and aquaculture to microplastic litter in marine environments, it is a possibility since larger plastic items from fisheries and aquaculture regularly contaminate surface waters (Cózar et al., 2014; Thiel et al., 2003) or the seafloor (Iñiguez et al. 2016) that these could degrade 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 microplastics and their associated toxins (Rochman et al., 2015; Wardrop et al., 2016). The Falkland Islands has a relatively large fishery with a total annual catch (last 5 years) of 270,000 tonnes (Falkland Islands Government, 2018) and given that contamination of important fisheries species with microplastics has been found in other parts of the Atlantic Ocean (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.

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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.

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Figures and Tables

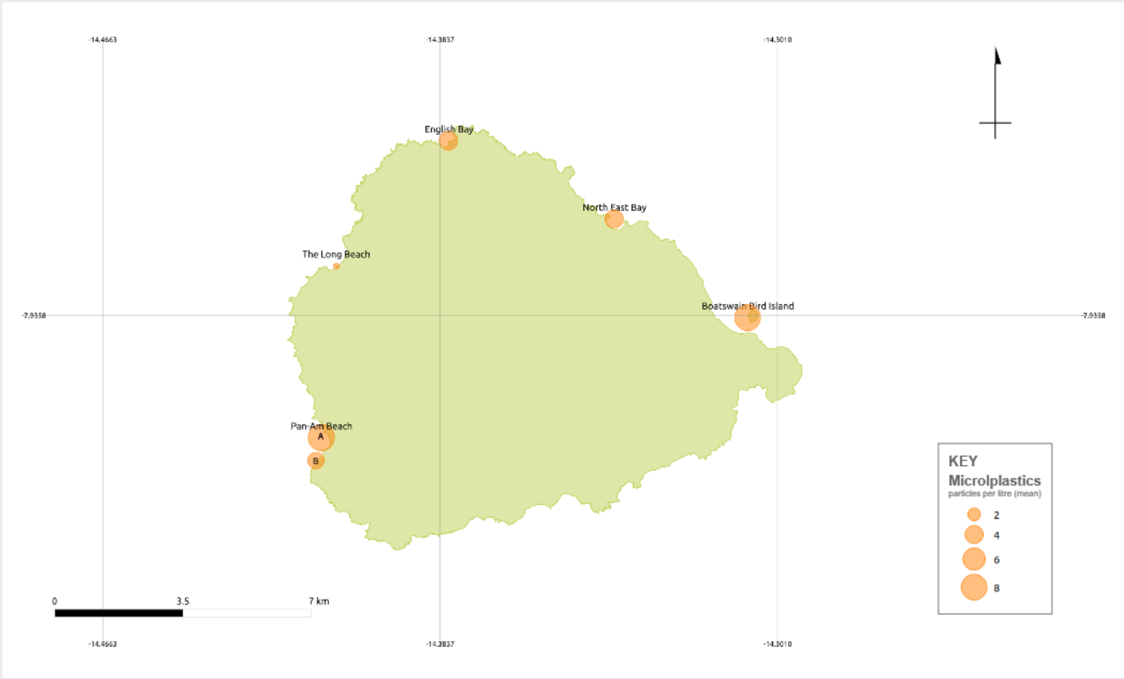

Figure 1. Map of Ascension Island showing average concentrations of microplastics (particles L^{-1}) obtained with 1L bottle grab sampling.

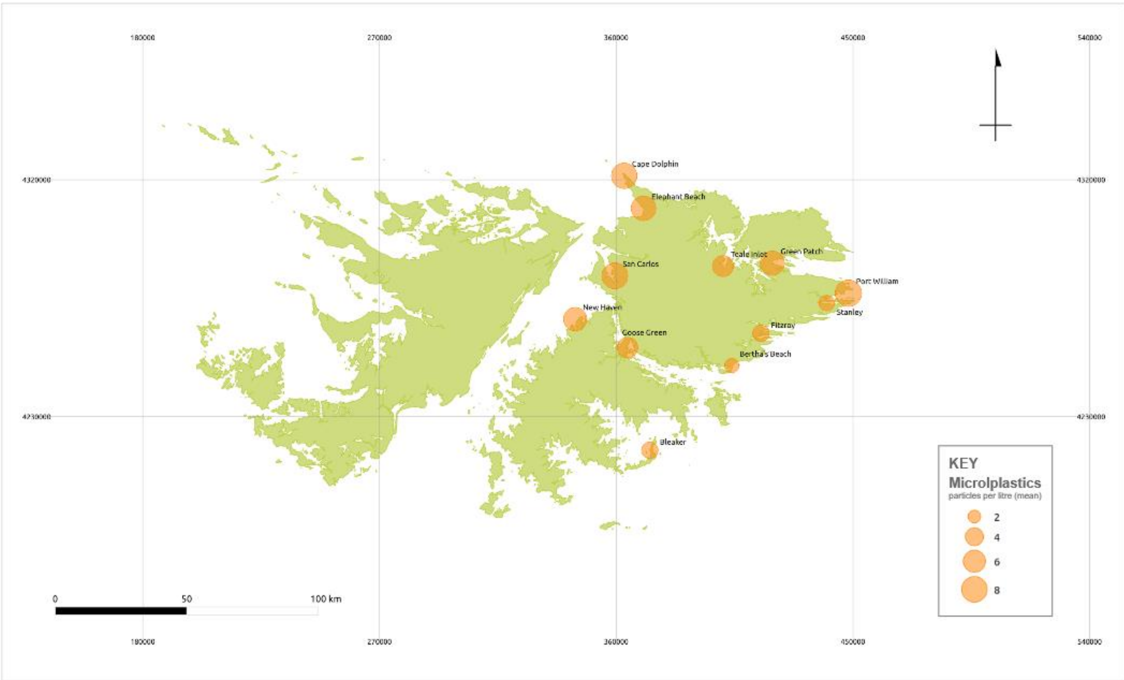

Figure 2. Map of the Falkland Islands showing average concentrations of microplastics (particles L^{-1}) in the East Falklands obtained with 1L bottle grab sampling.

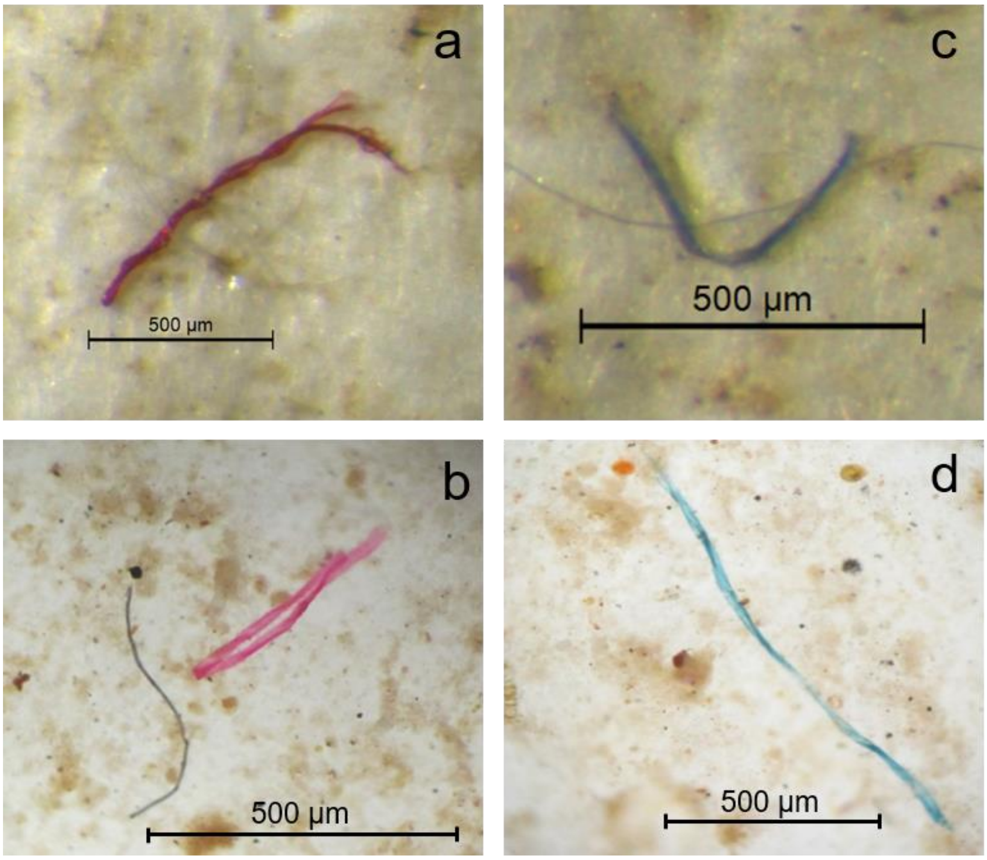

Figure 3. Photographs of microplastic fibres found in Ascension Island (a, b) or the East Falklands (c, d).

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 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 x 10^{-6})	**9.23 x 10^{-6} (1.42 x 10^{-6} – 2.8 x 10^{-6})**	**8.00 x 10^{-5} (2.70 x 10^{-5} – 1.8 x 10^{-4})**	**1.04 x 10^{-4} (3.26 x 10^{-5} – 2.10 x 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*	*3.33 (± 2.11)*	*0*	*9.50 (± 1.63)*	*9.83 (± 1.47)*
	Bongo	*6.22 x 10^{-7} (± 3.94 x 10^{-7})*	*1.47 x 10^{-5} (± 6.66 x 10^{-6})*	*1.02 x 10^{-4} (± 3.86 x 10^{-5})*	*1.19 x 10^{-4} (± 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 x 10^{-4} (0.00 – 1.20 x 10^{-3})**	**6.4 x 10^{-4} (0.00 – 1.24 x 10^{-3})**
		N/A	W=58, P=0.455	W=100, P<0.001	W=100, P<0.001
Mean (S.E)	*Bottle*	*0*	*3.00 (± 1.53)*	*2.30 (± 5.59)*	*2.60 (± 5.42)*
	Manta	*0*	*1.16 x 10^{-4} (± 7.76 x 10^{-5})*	*6.67 x 10^{-4} (± 2.09 x 10^{-4})*	*7.83 x 10^{-4} (± 2.66 x 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 x 10^{-4})	**1.70 x 10^{-4} (0.00 – 2.12 x 10^{-4})**	**2.12 x 10^{-4} (1.56 x 10^{-4} – 3.54 x 10^{-4})**
	Fine	**2.12 x 10^{-4} (0.00 – 4.24 x 10^{-4})**	1.70 x 10^{-4} (0.00 – 3.18 x 10^{-4})	**8.49 x 10^{-4} (7.43 x 10^{-4} – 1.13 x 10^{-3})**	**1.36 x 10^{-3} (1.17 x 10^{-3} – 1.60 x 10^{-3})**
		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*	*$_a$0*	*7.14 ± 4.21*	*$_a$1.14 (± 0.34)*	*$_a$1.29 (± 8.08)*
	Coarse	*$_a$2.02 x 10^{-5} (± 2.02 x 10^{-5})*	*1.52 x 10^{-4} (± 8.92 x 10^{-5})*	*$_b$1.25 x 10^{-4} (± 4.60 x 10^{-5})*	*$_b$2.97 x 10^{-4} (± 1.04 x 10^{-4})*
	Fine	*$_b$2.73 x 10^{-4} (± 1.29 x 10^{-4})*	*1.96 x 10^{-4} (± 8.52 x 10^{-5})*	*$_a$9.38 x 10^{-4} (± 1.41 x 10^{-3})*	*$_c$1.41 x 10^{-3} (± 1.58 x 10^{-4})*

Table 2. Average (±S.E) number L^{-1} 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 (± 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 (± 0.55)	-	6.8 (± 3.46)	7.8 (± 3.89)
	North East Bay	1.2 (± 0.73)	-	2.8 (± 0.86)	4.0 (± 1.30)
	English Bay	0.4 (± 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 (± 0.67)	3.6 (± 0.67)
	Goose Green	-	-	4.6 (± 1.91)	4.6 (± 1.91)
	Elephant Beach	0.8 (± 0.37)	-	7.2 (± 2.27)	8.0 (± 2.41)
	San Carlos	-	-	8.8 (± 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 (± 0.21)	-	9.5 (± 1.63)	9.8 (± 1.47)