



Quantification is more than counting: Actions required to accurately quantify and report isolated marine microplastics

Molly L. Rivers^{a,*}, Claire Gwinnett^b, Lucy C. Woodall^{a,c}

^a Nekton Foundation, Begbroke Science Park, Begbroke Hill, Woodstock Road, Begbroke, Oxfordshire OX5 1PF, United Kingdom

^b School of Law, Policing and Forensics, Science Centre, Staffordshire University, Leek Road, Stoke on Trent ST4 2DF, United Kingdom

^c Department of Zoology, University of Oxford, Zoology Research and Administration Building, 11a Mansfield Road, Oxford OX1 3SZ, United Kingdom



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ABSTRACT

Research on marine microplastics continues to increase in popularity, with a large number of studies being published every year. However, with this plethora of research comes the need for a standardised approach to quantification and analysis procedures in order to produce comparative assessments. Using data collected from neuston nets in 2016, parameters for quantifying microplastics were compared. Surface area was the most accurate parameter to describe plastic size and should be used to describe plastic quantity (per km² or m³), alongside abundance. Of the two most commonly used methods for calculating plastic concentration (flowmeter and ship's log), ship's log provided consistently smaller abundances, with the exception of one sample, calling for a standardisation in the techniques and measurements used to quantify floating microplastics.

1. Introduction

The number of studies on marine microplastics has rapidly increased in recent years, with microplastic fragments (diameter < 5 mm; Duis and Coors, 2016; Eriksen et al., 2014) being discovered in every marine and coastal environment (Bergmann et al., 2015; Woodall et al., 2014). Within this vast array of studies, the commonest form of sampling is by surface water nets, such as neuston nets and manta trawls (Löder and Gerdtz, 2015; Ryan et al., 2009). Across the many studies using surface-water nets to quantify microplastics, there is great variation in the methods of extraction (Miller et al., 2017), parameters used for quantification and statistics reported (Hidalgo-Ruz et al., 2012). To complicate matters, on many occasions important details of the methodologies used are not reported (e.g. measurement techniques, quantification methodology, replication, etc. [Filella, 2015]). This lack of standardisation in survey design and reporting renders cross-study comparisons of findings impossible (Prata et al., 2018). Therefore it is not surprising to find large discrepancies in global marine plastic abundance estimates, and that this has been suggested as a reason for the difference between the amount of plastic deposited and quantified in marine environments (Cózar et al., 2014).

Few papers describe methods of measuring plastic size, often stating only that size was 'measured', or provide information regarding the presence and distribution of replication, a notable few studies do state their methods in detail however (Mauro et al., 2017; Pedrotti et al.,

2016; Cózar et al., 2014). The most common parameter used, among the studies that state a size parameter, is a measure of 'longest length' (Barrows et al., 2017; Isobe et al., 2015). Yet, of the studies that measured the 'longest length' of fragments, not one defined this parameter. Thus differences in definition are likely, making it impossible to know if size data using the 'longest length' can be reliably used in comparative assessments.

Quantification, in regards to marine microplastics, has traditionally referred to the number of plastic fragments per area or volume of sea water. However, fragmentation, the process of plastic items breaking up into smaller 'fragments', is thought to be the largest producer of microplastics in the marine environment (Andrady, 2011). The heterogeneous nature of these fragments means that two samples with the same abundance are likely to contain different amounts of plastic in terms of size and weight (Qiu et al., 2016). Therefore, count data is inadequate at fully describing quantity and the addition of size measures could be more effective. Yet, when size is measured these data are usually only used to understand the size classes of microplastics found in the marine environment (Filella, 2015). Although this is useful in determining the environmental impact of marine microplastics (Wright et al., 2013), it does not add to our understanding of microplastic abundance. Additionally, the variety of size ranges used across studies makes comparing these data challenging (Cole et al., 2011).

To compile data across studies, consistent parameters need to be reported in a standardised way, however no widely followed

* Corresponding author.

E-mail address: molly@nektonmission.org (M.L. Rivers).

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approaches currently exist. Previous syntheses have reviewed microplastic extraction, isolation and identification techniques used in sampling and processing (Mai et al., 2018; Miller et al., 2017; Underwood et al., 2017). Therefore, this study aims to determine the most appropriate means of quantifying and reporting microplastics. Here we define 'quantification' as the methodologies used to express the quantity of microplastics (i.e. number, weight, size per km^2/m^3 of sea water) after isolation. We compare parameters currently used to quantify sea surface plastic fragments, analyse them using the two commonest methods for calculating particle concentration (flowmeter and ship's log) and address the inconsistencies seen in the reporting of these methodologies and resulting data. The present work highlights areas in which quantification methodologies need standardising and areas of reporting which need elaboration, with the aim to provide assistance in planning sampling and reporting procedures for pelagic plastic research so to allow for the wide scale comparison of future datasets.

2. Materials and methods

Samples were collected by a neuston net (mesh size: 300 μm ; mouth area: 0.5 m^2 [length 1 m, height 0.5 m]) deployed from the *CCGS HUDSON* between 31st July and 11th August 2016 (each deployment was 20 min long with ship speed of 2 knots). Six locations were sampled (2–5 replicates per location [replicate information presented in supplementary material]) between Plantagenet Bank (32°02'43.2"N, 65°06'52.9"W), South of Bermuda, and the Gully submarine canyon west of Nova Scotia (43°49'53.8"N, 58°55'59.9"W) (see Fig. 1).

Whole samples were rinsed into a clean collection bucket using clean water. Using contamination minimisation procedures (Woodall et al., 2015) microplastics visible to the naked eye were handpicked from zooplankton samples on-board ship, placed into sterile petri-

dishes which were sealed immediately and stored together in sealed cardboard boxes until analysis. Once ashore samples were further scanned for microplastics using a stereo-microscope (Motic SMZ 171). Plastic fragments from each sample were then enumerated and bulk weighed using an analytical microbalance (Fisherbrand PS-60, readability = 0.1 mg). Each fragment was photographed, through a stereo-microscope using a TrueChrome Metrics camera (using $\times 0.75$ magnification). Using ImageJ, microplastic photos (taken in RGB colour) were measured, recording the longest length (measured as the longest straight or curved edge of a fragment that is uninterrupted by indents or protrusions) and surface area (see supplementary material). In order to confirm all debris abstracted was plastic, thirteen fragments were selected as a subset with each debris morphotype present being represented by 2–3 fragments (where possible). These were all identified as being plastic and the polymer type for each ascertained where possible by using Attenuated Total Reflectance (ATR) Fourier-Transform Infrared Spectroscopy (FTIR) and in one case, due to the small size and presence of biofouling, using a Mazurek Microtec Polarizing Light Microscope. Polymer type is not a focus of this study, other studies look at this in detail (e.g. Elert et al., 2017; Löder and Gerdt, 2015), however a detailed description of these methodologies and the polymer types identified are included as supplementary material.

Plastic abundance, total surface area (SA), total of the longest length measurements (LL) and total weight were calculated per km^2 using two different methodologies, both commonly used in surface water microplastic studies. Both methodologies calculate the areal concentration by dividing the quantification value by the area sampled. Sampling area was calculated in two different ways, by: (i) using the ship's log to determine vessel speed and deployment duration and multiplying these values by the width of the net (1 m), henceforth referred to as Method A, and (ii) multiplying the total count, produced by an attached

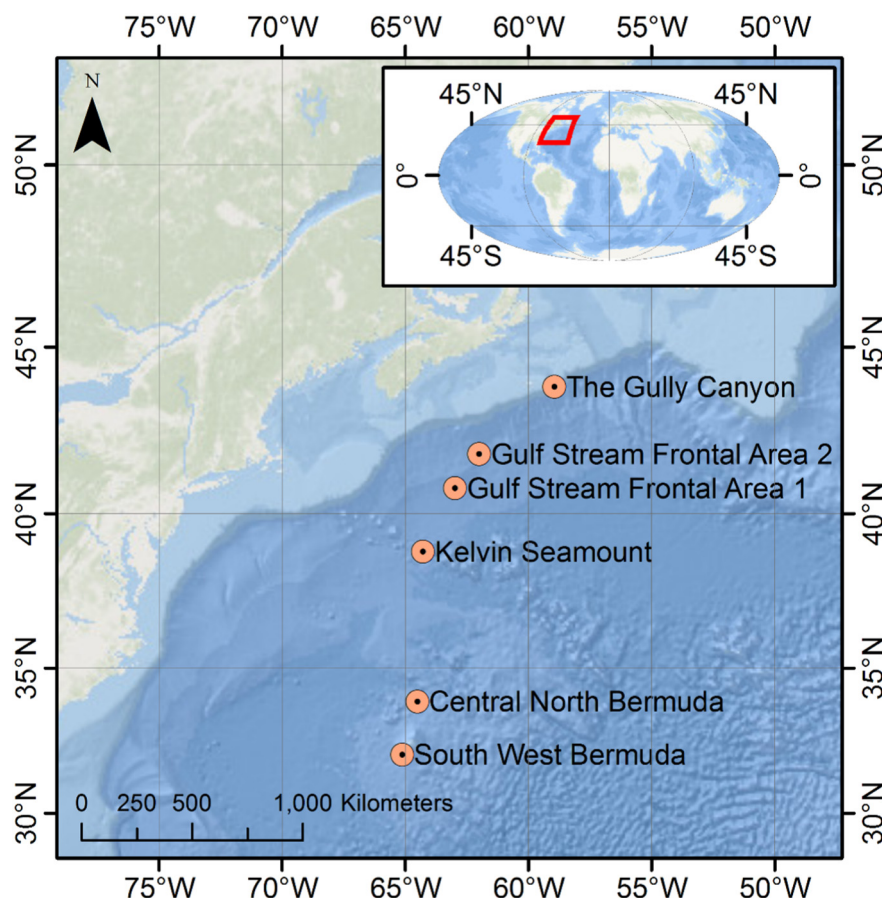


Fig. 1. Map showing the location of survey sites along a transect from Bermuda in the South and Nova Scotia in the North.

flowmeter, by the Impeller Constant (0.245) and by the width of the net, henceforth referred to as Method B. For both methods the area sampled was divided by one million to convert to km^2 . Method B was also used to calculate microplastic concentration per m^3 by multiplying the total count, produced by an attached flowmeter, by the Impeller Constant and the area of the net opening (0.5 m^2) to calculate the sampling volume.

In IBM SPSS v25 (IBM Corp., Armonk, NY), linear regression models (Pearson's correlations) were used to compare all quantification types, and outliers were identified using 25% and 75% Tukey's Hinges percentiles and 1.5 step (extreme outliers were identified as 2×1.5 step). Paired sample *t*-tests were used to compare areal concentration calculation methodologies (flowmeter and ship's log) and to compare SA with abundance and LL. Additionally, the surface area of every plastic fragment, across samples, was used to determine the overall mean surface area of individual microplastics ($= 8.818 \text{ mm}^2$) and was used to calculate the expected SA of each sample. A paired-sample *t*-test was used to test between the expected and actual SA values.

3. Results

Estimated plastic abundance at each study site is presented in Fig. 2. Plastic abundance was consistently lower using Method A compared to Method B (Fig. 2), except in the case of one replicate from the Gully where Method A produced an abundance value ~ 1000 fragments per km^2 greater than Method B. Overall however, paired *t*-tests did not identify a significant difference ($t = 1.333$, $p = 0.199$), which is most likely due to the high variability in plastic abundance across samples. For example, two replicates at Kelvin Seamount had a difference of $\sim 280,000$ fragments km^{-2} when using method B (difference reduced to $\sim 85,000$ fragments when using Method A). Nevertheless, for some deployments there were stark differences between the two methods (e.g. at Kelvin Seamount, method A resulted in a value $\sim 70\%$ smaller than that of method B, see Fig. 2). There were significant correlations between calculated fragment variables (Pearson's correlation): abundance vs SA, $r = 0.894$, $p < 0.001$; abundance vs total weight, $r = 0.987$, $p < 0.001$; abundance vs LL, $r = 0.998$, $p < 0.001$; SA vs LL, $r = 0.897$, $p < 0.001$; SA vs total weight, $r = 0.918$, $p < 0.001$. However, SA and LL show two and three outliers, respectively, all of which are samples with the greatest total surface areas and three have

the largest plastic abundance (Fig. 3; see supplementary material). Additionally, all outliers were identified as extreme outliers. Paired sample *t*-tests also found no significant differences between SA and abundance ($t = 1.845$, $p = 0.082$) and SA and LL ($t = 1.813$, $p = 0.086$). Finally, the actual and expected SA were not significantly different ($t = 0.131$, $p = 0.897$), but in one case the actual SA was almost thirteen times the expected SA.

4. Discussion

4.1. Quantification parameters

All parameters (abundance, weight, LL and SA) used to report microplastics in this study, correlated with each other. However, the outliers seen when plotting abundance and LL with SA (Fig. 3) imply that there is a chance of underestimating the extent of plastic contamination when abundance or longest length measurements are used in isolation. This is particularly prevalent for samples containing large amounts of plastic, with three of the four outliers in our data set containing the highest plastic abundance values. In addition, the sample containing the highest plastic abundance value (Fig. 3) had an expected SA almost thirteen times smaller than the actual SA, suggesting an increasing unpredictability with increasing abundance values. Paired *t*-tests compared SA with abundance and LL, finding no significant differences, however they did indicate a need to calculate surface area values as a parameter to describe plastic fragment quantity because abundance values do not describe this variation in fragment shape and size. Taken together, the results indicate that it is important to carefully consider which parameter to use when quantifying microplastic pollution in marine environments and how this might bias the values reported.

Surface area is suggested as a key measurement for quantification, alongside count data, for the following reasons: (i) microplastics are heterogeneous in nature (Andrady, 2011), and abundance values cannot determine between fragments of varying size, weight and shape. Our results showed microplastic abundance and SA in 4/15 samples containing microplastics, did not correlate (Fig. 3; see supplementary material). Therefore, enumeration in isolation cannot fully describe the extent of microplastics in the marine environment, and an additional measure of size (e.g. surface area or length) should also be used. (ii)

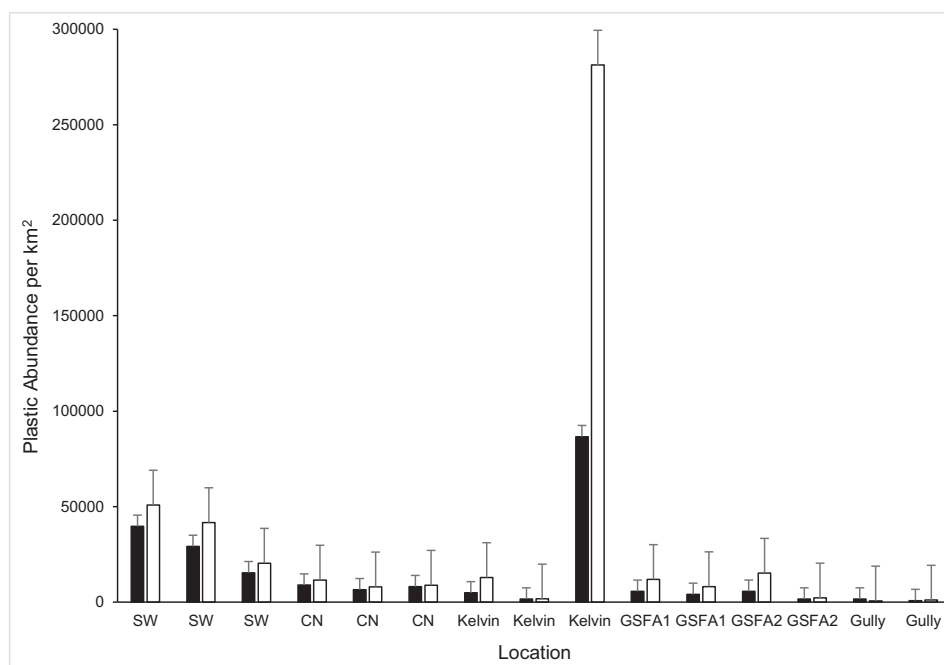


Fig. 2. Plastic abundance (fragments per km^2), across each studied location, calculated using the ship's log (black bars), and flowmeter (white bars), respectively. Error bars denote standard error. Samples with no plastic were excluded, including 3 samples from GSFA2 and 2 samples from Gully. Location abbreviations: SW — Southwest Bermuda, CN — central North Bermuda, Kelvin — Kelvin Seamount, GSFA1 — Gulf Stream frontal area 1, GSFA2 — Gulf Stream frontal area 2 and Gully — Gully canyon.

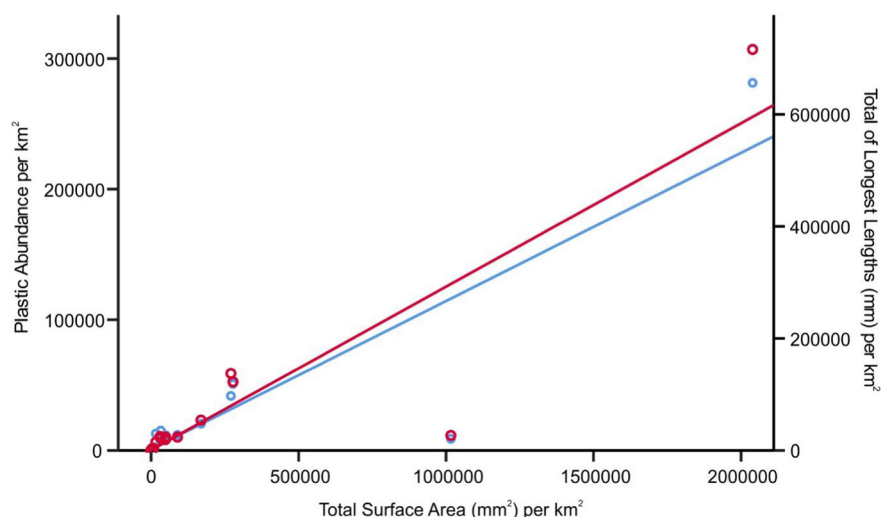


Fig. 3. Scatter plot showing the relationships between abundance and total surface area of plastic debris ($F_{1,18} = 67.624$, $p < 0.001$, $r = 0.894$), and between total surface area and total of the longest lengths ($F_{1,18} = 70.131$, $p < 0.001$, $r = 0.897$), calculated using a flowmeter. Blue data points and trend line represent plastic abundance, red data points and trend line represent total plastic longest lengths. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Total weight showed a very strong correlation with SA, thus, the variations in fragment mass, provides some information regarding the heterogeneity of fragment size that enumeration does not. However, because of the extremely small size and weight of microplastic fragments most samples must be bulk weighed to produce detectable values. This results in values that cluster around zero, increasing the influence of larger fragments and decreasing the chance of trends and differences being detected. (iii) While measuring the longest length of each fragment in this current study, it became apparent that for many irregularly shaped fragments, identifying and measuring length is difficult and highly subjective. This suggests the possible unreliability of this measurement in portraying the true size of plastic fragments. Additionally, LL showed a weaker correlation with SA than with abundance and multiple outliers. (iv) Surface area values can be easily and non-subjectively calculated from images using software such as ImageJ and is less likely to produce clustering, therefore can show trends and outliers more clearly. An alternative method to processing the plastic fragments is to use a digital scanner with software such as ZooScan and Zoolmage, which can be used to count, measure (e.g. ferret diameter and surface area) and photograph microplastics (Pedrotti et al., 2018; 2016; Gilfillan et al., 2009).

4.2. Flowmeter vs. ships log

Studies using the ship's log to calculate particle abundance report values per km^2 , while studies using flowmeter readings usually report concentration values per m^3 (Eriksen et al., 2018; Kang et al., 2015). Therefore, data comparisons between studies using different calculation methods are generally impossible. Although not significantly so, our results showed that plastic abundances were consistently lower when using Method A, except in one case (Fig. 2). This is congruent with findings from a previous study (Maes et al., 2017) that found flowmeter readings resulted in shorter distances than those of on-board instruments. The flow meter measures water passing through the net, while the ship's log measures water passing the hull, so the net being deployed on the ships quarter, several meters (horizontally and vertically) from the ships log, means it is likely that water movement measured would be different for each method. Therefore the particularly large difference seen in the sample with the highest microplastic abundance (at Kelvin Seamount, see Fig. 2) is not surprising as this sample contained substantially more microplastics than other samples, causing the discrepancy between the two methods to be more evident. Surface water nets also often partially breach the water surface, preventing accurate calculations of water sampled when using ship's log due to uncertainty of the portion of the net that passed through the water (Welden, 2015).

Maes et al. (2017) also suggested the bow wave effect may cause less water to be filtered through the net than would be calculated using the ship's log, and result in an underestimation of plastic abundance when not using a flowmeter. Although our data do not show a significant difference between the sampling methods, most likely due to the high variability in abundance values and our relatively small sample size, the consistently higher abundances seen when using a flow meter do correspond with the only previous study to investigate this (Maes et al., 2017). Therefore, we propose the use of flowmeters to report plastic abundance both per km^2 and per m^3 .

4.3. Reporting

This study presents the overall mean and range values for all parameters measured in Table 1, allowing for limited text to be allocated to this information (more detailed summary data is provided as supplementary material). Studies with different research aims and hypotheses often report results differently. The particle abundance mean and range are commonly noted, but may be presented per size class, type, geographical location, etc., while other statistics are also used in conjunction or in place of these aforementioned figures, such as: median abundance, total abundance, relative percentages, etc. (Cózar et al., 2014; Lima et al., 2014; Goldstein et al., 2013). The presentation of findings necessarily reflects the aim of each study; however, universal reporting of the overall mean and range plastic abundance, size and mass measurements (if applicable), in addition to other statistics, would allow for better comparative assessments across publications. Alternatively providing the raw data or detailed summary data as supplementary material, such as: Eriksen et al. (2014), Gewert et al. (2017) and Pedrotti et al. (2016), would also allow for such assessments to be made. Some previous studies have provided summary statistics in

Table 1

Plastic fragment characteristics based on total data. For the minimum estimates, we excluded samples with no plastic. All values are expressed per km^2 (and m^3 in brackets), calculated using flowmeter.

	Abundance	Total surface area (mm^2)	Total weight (g)	Total of the longest lengths (mm)
Mean	25,067.727 (0.050)	213,493.501 (0.321)	216.033 (< 0.000)	62,868.319 (0.125)
Minimum	623.521 (0.001)	912.729 (0.002)	0.405 (< 0.000)	804.609 (0.002)
Maximum	281,274.357 (0.563)	2,039,034.046 (4.078)	2641.613 (0.005)	716,150.800 (1.432)

graphs or other schematics (Cózar et al., 2014; Lima et al., 2014), however this does not provide extractable values suitable for data comparisons.

5. Conclusion

This study shows the importance of using multiple parameters to quantify microplastics and details some of the current challenges to large scale microplastics data comparison. The suggestions put forth are intended to provide guidance for future studies to allow for a holistic view of the state of microplastic pollution within aquatic environments. Indeed similar standardised approaches to parameters collected, analysis performed and the statistics reported is required across all studies of anthropogenic impacts on marine environments (Woodall et al., 2018; Turner and Renegar, 2017; Foden et al., 2008). In order for widespread data comparison to be possible, care must be taken to include detailed reporting of methodologies and inclusive statistics, representing the entire data set. Only then will it be possible to obtain a greater understanding of the extent and effects of microplastics on aquatic environments.

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Declarations of interest

None.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.marpolbul.2018.12.024>.

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