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Ensuring representative sample volume predictions in microplastic monitoring

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Abstract

A large body of literature is available quantifying microplastic contamination in freshwater and marine systems across the globe. "Microplastics" do not represent a single analyte. Rather, they are usually operationally defined based on their size, polymer and shape, dependent on the sample collection method and the analytical range of the measurement technique. In the absence of standardised methods, significant variability and uncertainty remains as to how to compare data from different sources, and so consider exposure correctly. To examine this issue, a previously compiled database containing 1603 marine observations and 208 freshwater observations of microplastic concentrations from across the globe between 1971 and 2020 was analysed. Reported concentrations span nine orders of magnitude. Investigating the relationship between sampling methods and reported concentrations, a striking correlation between smaller sample unit volumes and higher microplastic concentrations was observed. Close to half of the studies reviewed scored poorly in quality scoring protocols according to the sample volume taken. It is critical that sufficient particles are measured in a sample to reduce the errors from random chance. Given the inverse relationship with particle size and abundance, the volume required for a representative sample should be calculated case-by-case, based on what size microplastics are under investigation and where they are being measured. We have developed the Representative Sample Volume Predictor (RSVP) tool, which standardises statistical prediction of sufficient sample volumes, to ensure microplastics are detected with a given level of confidence. Reviewing reports in freshwater, we found ~ 12% of observations reported sample volumes which would have a false negative error rate > 5%. Such sample volumes run the risk of wrongly concluding that microplastics are absent in samples and are not sufficient to be quantitative. The RSVP tool also provides a harmonised Poisson point process estimation of confidence intervals to test whether two observations are likely to be significantly different, even in the absence of replication. In this way, we demonstrate application of the tool to evaluate historic data, but also to assist in new study designs to ensure that environmental microplastic exposure data is relevant and reliable. The tool can also be applied to other data for randomly dispersed events in space or time, and so has potential for transdisciplinary use.

Keywords Exposure assessment, Risk assessment, Quality, Environmental sampling, Review, Aquatic environment, Marine, Freshwater, Microplastic concentrations, Plastic pollution

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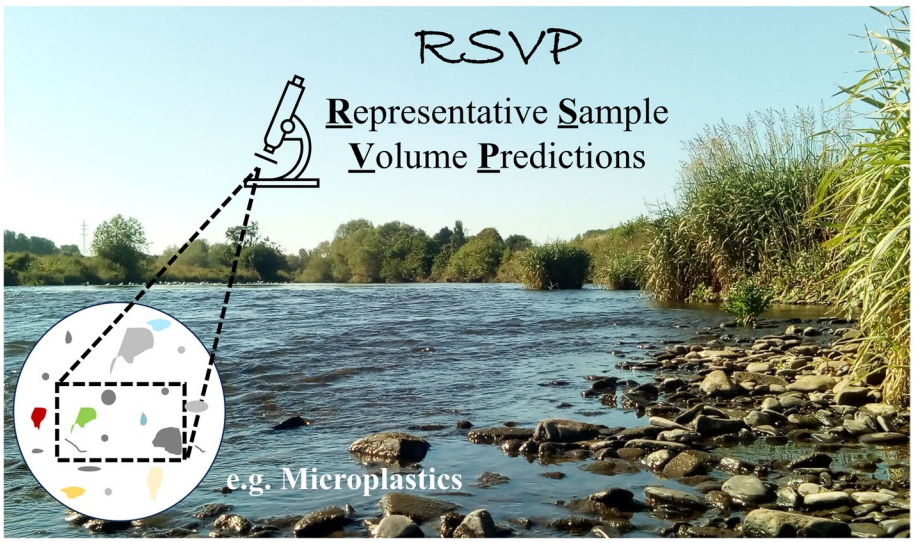
Graphical Abstract



Introduction

The number of studies reporting the global occurrence of microplastic particles (MPs) in marine waters and sediments is reportedly 52 times greater in 2021 than in 2010, with an exponential increase in the publications between 2015–2021 [1]. The increase in the rate of publications is observed to coincide with the listing of microplastics as one of the top 10 global environmental problems by the United Nations Environment Programme (UNEP) in 2014 [2]. Although there is an increasing awareness of the extent of plastic pollution, particularly in the marine environment, there remain continuing concerns regarding the relative quality of the data being generated. There remains an urgent need to develop standardised sampling and analytical methods to assist the evaluation of exposure and risk associated with microplastic exposure to both humans and wildlife [1, 3, 4].

Microplastic concentrations in seawater are observed to range over 8 orders of magnitude, from between 0.006 and 660,000 particles/m^3, with a mean concentration of 20,400 particles/m^3 (standard deviation: 98,300 particles/m^3, [1]). No single analytical technique can quantify microplastics across their diversity of size, shape and polymer types [5, 6], whilst microplastic particle abundance typically increases dramatically in the smaller size ranges as larger items disintegrate into ever smaller fragments [7]. This complicates comparisons of particle number concentrations across studies reporting on different particle size ranges.

Results presented by Tang et al., [1] demonstrate significant spatial heterogeneity in the concentrations of microplastics reported across the world's oceans. A general observation, however, is that regions with higher concentrations are typically associated with semi-enclosed bays located near intensive human activities, which include fishing, shipping, and emissions from wastewater treatment plants. There is a correlation between human activity and relative abundance of microplastics. However, differences with respect to how samples were collected and analysed, as well as inconsistencies in how data are reported (e.g. mass- or particle-based concentrations), make it difficult to compare data collected between different studies [8].

While there are currently several activities aimed at the development of harmonizing sample collection, analysis and reporting [8–15], there is also a need to strengthen overall confidence and reliability of the data that are reported in the scientific literature [16–18]. Koelmans et al., [16], developed and applied a suite of basic quality assurance and quality control criteria that could be used to provide a transparent critical evaluation of the reliability of microplastic concentrations reported in surface and drinking waters. Others have also proposed similar guidance for measuring microplastics in soils [19], sediments [20] air [21] and biota [22]. In addition to ensuring that monitoring data are reliable, there is also a need to ensure that the data generated are relevant and fit-for-purpose.

When considering the relevance of data, it is first necessary to review the original aim associated with the study. Quantitative exposure assessment allows hotspots to be identified, critical environmental processes to be understood and key sources of microplastics into the environment identified. However, the types of study which are relevant to underpin these different purposes are not one and the same. Identifying which data are relevant for a specific purpose represents an important step when compiling available information [11]. A simple example would be data reporting only particle numbers should not be used to inform a mass balance calculation if the necessary information on particle sizes, and thus mass is absent. Similarly, data that report microplastics > 300 µm are unlikely to give a complete picture of exposure, meaning the risks from smaller particles cannot be assessed without extrapolation (e.g. [23]).

When considering the representativeness of data, the volume of sample represented in an observation is critical. Sufficient volume must be taken to capture enough microplastic particles to be representative of the wider environment sampled. In a patchy, heterogenous world, many assumptions are made when extrapolating from a few millilitres in the laboratory to thousands of meters cubed of water say, in the coastal zones around a country. Whilst such considerations are beginning to be acknowledged in various efforts to standardise methods for microplastic quantification, these necessarily typically focus on sufficient sample volumes for a given sampling method or analytical technique (e.g. including but not limited to [24–28]). A more generic standardised approach to predicting representative sample volumes would allow for harmonisation across the diverse range of methods in use.

We developed the Representative Sample Volume Predictor (RSVP) tool, to assist in standardising sample volume predictions to allow microplastics to be detected with a known level of confidence. The method was developed from a review of historical data in marine and freshwaters (Sect. "Why are environmental observations so variable?"). The methodology behind the RSVP tool (Sect. "RSVP – a tool for representative sample volume predictions") and demonstration of its application in the design of new sampling strategies is presented (Sect. "How can a standardised approach to predicting representative sample unit volumes help resolve outstanding issues with data variability?"). In addition, application of the tool is demonstrated, in this example, to review relevant historic data based on achieving predicted minimum sample volumes to detect microplastics at a given level of confidence (Sect. "Using the RSVP tool to screen for relevant data").

Methods

RSVP – a tool for representative sample volume predictions

The distribution of microplastics within a mixed body of water can be estimated based on an assumption of a random distribution pattern driven by processes such as turbulent dispersion where particles are independent of each other. This random distribution can be estimated following a Poisson point process as demonstrated in [29].

This is not unique to microplastics. These principles apply widely to predicting the number of any discreet objects or "events" that act independently in a fixed period of time or space, such as shooting stars in the night sky, the daily telephone calls received on a hotline, or particular flowers in a quadrat. The chance of counting a specific number in all of these scenarios can be modelled statistically using the Poisson distribution, a discrete probability distribution, that expresses the probability of:

- a given number of discrete events, in this case capturing a given number of microplastic particles;
- occurring in a fixed interval, here a fixed volume of water;
- if these events occur at a known or expected rate, in this case, equivalent to an expected concentration of microplastic particles in the sampled environment.

Adopting the Poisson distribution, the probability (P) that exactly k number of microplastic particles are captured in a given volume is calculated following Eq. 1:

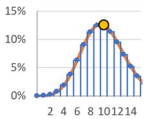
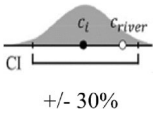
$$P(k) = \frac{\lambda^k e^{-\lambda}}{k!} \tag{1}$$

where $P(k)$ is the probability that k particles will be captured, $k!$ is the factorial of k, e is Euler's number and λ, the rate parameter is the expected number of particles in a given volume i.e. the concentration multiplied by the volume sampled.

In our case, it is not the probability of capturing k particles that is the focus of the RSVP tool, but the prediction of how much volume must be taken to capture at least k particles with a given probability. To perform this assessment, it is convenient to use the related continuous Gamma distribution function. If the shape parameter of the Gamma distribution is set to $\alpha = k + 1$ and rate parameter to $\beta = 1$ the Gamma distribution forms a continuous extension of the Poisson distribution [29].

To make an analogy for the relationship between the Poisson and Gamma distribution, we can think about randomly distributed events in time. The probability of seeing k shooting stars during a night follows a Poisson distribution (where k is an integer ≥ 0), whilst the time

Table 1 Examples of target number of particles required to evaluate data for specific purposes

	Purpose	Target number of particles	Reference
	Monitoring **presence/absence** at a given level of confidence	1	e.g. Figure 4
	To **calculate the sampling error** using the Poisson point process	10	[29]
	To achieve a predicted **95% confidence interval to be within ± 30%** of the total concentration estimates	50	[29]
	To **allow for one additional property** such as polymer identity to be evaluated with an **error of 10%**	96	[30]
	To **allow for one additional property** such as polymer identity to be evaluated with an **error of 5% or less**	384	[30]
	To **simultaneously estimate polymer, colour, size, and morphology** distributions with an error of **5% or less**	620	[30]

between these independent events (the minutes between seeing one shooting star and the next) can be described by a Gamma distribution function (a continuous distribution). To return to measuring microplastics in the environment, the Poisson Distribution describes the probability of finding k microplastics in a given volume, whilst using the Inverse Gamma Cumulative Distribution Function it is possible to calculate how much volume you need to take to capture at least k particles at a given probability.

The assumptions of the Gamma (and Poisson) Distribution are that the events are independent (in this case events referring to microplastic particles captured), and that the mean and variance remain constant. The former is likely to apply unless the particles aggregate, but the latter is violated at low numbers because negative counts are not possible, and zeros become more frequent. Thus, these calculations are only recommended for target particle numbers above around 10 particles [29]. In the RSVP tool, an Inverse Gamma Cumulative Distribution Function is implemented to calculate the expected value λ needed to catch at least k particles with a given level of confidence 1-α, i.e. with alpha at 0.05, this would provide 95% confidence that we capture at least k particles.

The output to the user is the volume (v=λ/c) required to capture the target number of microplastics k at the given level of confidence α, assuming the numerical microplastic concentration at the sampling location is c.

The target number of microplastic particles (k) depends on the purpose of the assessment and should be decided *a priori* by the user. For example, if you wish to determine presence or absence of microplastics in a location with 99% confidence, the user would set k to 1, and α as 0.001. The target number of microplastics (k) to be measured for different purposes have been proposed elsewhere e.g. k required to quantify total microplastics [29] or multiple characteristics of microplastics in a sample (e.g. [30], see Sect. "Using the RSVP tool to screen for relevant data", Table 1).

The expected number of particles in a given volume of water λ should be estimated by identifying the most relevant existing data to inform on expected concentrations at the sampling location. Details of the selection criteria used and justification of the relevance of the data should always be clearly reported. Some key criteria to identify relevant data to inform λ are to select data that:

- represents a similar test system to that under investigation (e.g. similar sized river or catchment)
- represents/ integrates similar environmental fate processes
- collected samples using a similar methodology
- processed samples using a similar methodology

- analysed samples using the same analytical technique and so represents the same "analytical window" i.e. region of the microplastic size continuum, polymer types etc.
- scores highly following quality criteria (e.g. for water samples, [16])
- using the arithmetic mean of suitable data is likely to overestimate the concentration in a given sample because one or more very high values can influence the mean unduly. Ideally one would choose a typical value from a large distribution. In most cases there is not enough data to do this, so either erring on the side of caution or choosing a value less influenced by outliers such as the median or the geometric mean is recommended.

We acknowledge that in the absence of data representing microplastic particles in the same size range it is challenging to predict the expected number of microplastic particles in a given volume of water. This is why the first recommendation is to use data from analogous analytical methods to inform λ. Mathematical re-scaling methods have been prosed (e.g. [31]) and have been applied to estimate microplastic concentrations within default size ranges e.g. 1 – 5000 μm, from measurements taken within a restricted size range, using power law distributions (e.g. [20, 32]). The applicability domain of these predictions and the calculated exponents appear dependent on the environmental matrix and as such, particle size/frequency relationships will always be most accurate if calibrated for a specific compartment and set of conditions [33].

The level of confidence α is set by the user, but some common values are automatically provided in the tool. In practical terms the equation above allows the user to ask:

> *How much sample must I collect to capture a given target number of microplastic particles at a given level of confidence?*

In addition, [29] converting the Poisson and related Gamma distribution from "what is the probability to catch exactly *k* particles in a volume *v*, when the concentration and thus the expected value λ was known (calculated by multiplying the concentration with the sampled volume)?" to "what is the probability that λ has a certain value, given that it is known that exactly *k* particles were captured?". For example, if 10 particles were captured in one litre of water, one might assume that the concentration in the sampled water body was 10 particles/L. If the real concentration was actually 8.1 particles/L, there is still a fairly high chance of catching exactly 10 particles (~10.2%) and a higher chance of capturing at least 10 particles (~29.6%). However, the probability of catching exactly 10 particles would be very low if the real concentration was either a lot higher or a lot lower than 8.1 particles/L. For example, the probability of capturing exactly 10 particles in a sample if the true concentration was 3 particles/L is only 0.08%. As can be seen, capturing exactly 10 particles is possible at a whole range of actual environmental concentrations, but gets less likely the further away from 10 the expected value λ is. Thus, for any possible environmental concentration it can be calculated how likely it would be to capture exactly k (in this example exactly 10) particles. From this, a confidence interval of expected values λ around the captured number k can be calculated, provided you know the volume of sample represented in the analysis. To illustrate, if a sample of 5L was analysed and 45 particles captured (i.e. k = 45), we can calculate the 95% confidence interval in which the true river concentration λ is stochastically contained as being between 6.57 and 12.04 particles/L. For more detail of the principles and equations used to calculate these confidence intervals, please refer to [29]. An explanation of this example, along with illustrative figures are provided in Supplementary Material 3, Chapter 1, and in Tab 3 of the excel file, Supplementary Material 1: the RSVP Tool "*3. Illustration*". These calculations are implemented in the RSVP tool to estimate the confidence intervals around single observations without replication and provide an indication of whether these were likely to differ significantly at given levels of confidence. This part of the tool may be used to screen for significance of differences between observations in the absence of replication, a useful tool when range testing a sampling design, or interpreting existing data.

Ultimately, the RSVP tool provides for several useful functions:

1. How much sample must I collect to capture at least a given target number of microplastic particles at a given level of confidence?
2. In the absence of replication, are two values likely to differ at a given level of confidence?
3. Both functions can be applied either to the total number of microplastics when that is of interest, or to subsets of interest, e.g. by polymer, shape, size colour etc.

A downloadable Excel Worksheet of the tool is provided in the Supplementary Information which guides the user in performing these calculations and interpreting the results.

This does of course only deal with the mathematical implications related to the Poisson process and not with the various other issues of to do with sampling, processing/cleanup, detection/quantification. While the RSVP

tool is designed in the context of predicting representative sample volumes for microplastic exposure assessment, its principles can be applied to any count-based data, provided the assumptions behind the Poisson distribution are met.

A database of marine and freshwater microplastic concentrations

As a proof-of-concept, we have applied the RSVP tool to evaluate data that have reported microplastics in marine and freshwater environments. A previous literature search was leveraged including all dates up to June 2020, using the PubMed search engine, provided by the National Center for Biotechnology Information (Bethesda, United States of America), and the keyword 'microplastic' was used, for which > 2600 publications were obtained. The search ended in 2020 as this pre-existing dataset is being leveraged here to demonstrate the research need and the tool, rather than conclude on the current status of the literature. Adoption and initial definition of the term 'microplastic' in the scientific literature, however, only emerges in 2004 [34], whereby microplastics are defined as synthetic plastic particles < 5mm [35]. Recognizing that synthetic plastic particles < 5mm in size have been reported in various environmental matrices since the 1960's, additional references are included based on a review of relevant citations used in review articles after 2004. As of 30 September, 2020, an Endnote database of 3417 publications had been assembled, including studies related to ecotoxicology and human health effects, monitoring data, environmental fate and distribution, and other microplastic and/or particle-relevant research. This represents the primary source of information used in summarizing monitoring data of microplastics in the aquatic environment.

From the literature review of microplastic research, 168 studies have reported concentrations of microplastic particles in various marine and estuarine systems, with concentrations above limits of detection ranging from around 0.001 to 1.5×10^5 MPs/m^3. Data reported include all major oceans and seas. When considering freshwater systems, 75 studies were identified in the literature review, including lakes, rivers, and ponds. Concentrations of microplastics above limits of detection were reported as ranging from 0.0001 to 2.08×10^6 MPs/m^3.

Statistical analysis of the existing data

Regression analysis was conducted between sample volume and the concentration of MPs, as well as with respect to the minimum reported particle size and concentration. All data were log-transformed for the analysis, with subsequent observations grouped by collection method (i.e., grab, pump and net sampling) and differentiated between marine and freshwater systems. Redundancy Analysis (RDA, "vegan" and "ggfortify" packages) was performed on the full dataset (marine and freshwater), with an emphasis on evaluating whether sample volume or minimum reported particle size represented possible parameters that could be used to explain the variation in concentrations reported. Microplastic concentrations, sample volume and particle size were log-transformed prior to running constrained ordinations.

As an additional level of assessment, we also considered the relationship between the relative quality of studies, based on results obtained from an evaluation using the quality scoring criteria [16] and the concentrations of microplastics reported in both marine and freshwater systems. Full details of the QA/QC criteria are provided in the original publication and are therefore not repeated here. Briefly, however, there are nine separate QA/QC criteria, with each criterion assigned a score of '0', '1', or '2' depending on how a specific criterion is reported in the study. Guidance on assigning a score is provided in [16]. All criteria are assigned equal weight, with a fully reliable study being defined when all nine criteria receive a score of at least '1'. Using the results of the scoring for each of the nine criteria, we performed a Levene's test for homogeneity of variance with a Nemenyi post hoc test. The results of which are then complemented using Principal Components Analysis (PCA, "devtool" and "ggfortify" packages). This considered the relationship between concentration and the quality scoring data, as well as with respect to various sampling parameters (e.g. sample volume and minimum particle size), which are grouped by collection method, water body type and analytical identification method used. All statistics were performed in RStudio, version 4.3.2.

Results and discussion

Why are environmental observations so variable?

Consistent with the recent review by [1], the concentration of microplastics compiled as part of the literature review (with no data treatment or alignment, thus representing different size regions across studies) reveals that reported concentrations across both marine and freshwater environments span ten orders of magnitude, from 0.0001 MP/m^3 to 2,083,500 MP/m^3.

Three major sample collection methods are identified. These include the use of various types of trawling nets, pumped filtration, and grab samples. Nets and pumped filtration introduce lower size limits of particles captured based on the mesh sizes. Grab samples in bottles may allow for the entire size continuum to be captured, but are usually limited in the volume of sample that can be captured to a few litres. Whilst this may be sufficient to be representative of very small and more abundant

particles, it is unlikely to be representative of larger particles that may be the focus of analysis of samples captured with nets or pumped filtration. The relevance and reliability of different sample collection methods is therefore dependent on the purpose of the assessment and in particular the size of particles for quantification.

Most analytical techniques were count based measurements, consisting of vibrational spectroscopy (FTIR, NIR, Raman), and visualisation approaches which do not confirm polymer identity e.g. SEM or stereomicroscopy. These techniques can resolve particle size, and in some cases, shape and polymer identity. Each of these techniques quantifies microplastics within different, but sometimes overlapping regions of the size distribution. The polymers quantified also depends on the analytical technique, the sample preparation, and the libraries of known polymers used in the data analysis. Other analytical instruments, such as gas chromatography—mass spectrometry (GC–MS), do not provide size information, although coupled with a sample fractionation method such as a series of sieves, some size information by fractions can be inferred. Occasionally, the measurement technique was not reported. It is the combination of both the sample collection method and analytical instrument which determines the definition of a microplastic in a given study, and further complicates the challenge of interpreting data generated using different methodologies.

The significant variability in total reported concentrations raises the question of why? This may be a true reflection of the existence of hotspots and cold spots globally of microplastic pollution, or it could be due to differences in study design, quality, and analytical capabilities. Quality criteria have been proposed for monitoring and measuring microplastics in waters [16]. The marine and freshwater databases were quality scored according to this system (Supplementary Material 2).

Reliability scores are weakly correlated with microplastic concentrations

To understand whether data quality and reliability influence the high variability observed in freshwater and marine observations, an assessment of critical criteria relating to sample collection, preparation and analysis are reviewed. These criteria are found to be only weakly correlated with microplastic concentrations (Supplementary Material 3, Chapter 1). Principle component analysis provides insights into which explanatory variables combine to best explain the variance in the data (Supplementary Material 3, Figure SI2). The concentration of microplastics is observed to be negatively correlated with the sample method and volume scores, implying that higher concentrations of microplastics are reported in studies with lower quality scores for these critical aspects of sample collection and sample volume (i.e. smaller volume samples). It is not possible to conclude whether this is due to studies which took smaller sample volumes, targeting smaller and so more abundant microplastics. Interestingly, the results suggest that the strength in relationship between the different quality criteria and the factors is generally quite low, indicating that the various QA/QC criteria only weakly correlate with the reported concentrations of MPs.

Observations are influenced by the volume of sample captured and the size of microplastics analysed

Redundancy analysis of the explanatory variables concluded that the sample volume and minimum particle size explain 73% of the variance in the reported microplastic concentrations across marine and freshwaters (Supplementary Material 3, Figure SI4). To understand whether data can be interpreted across different studies it is therefore important to consider the implications of variable sampling volume and the targeted microplastic size on the reported concentration.

A significant negative correlation between the sample volume and the reported concentration is observed in both marine and freshwaters (Fig. 1, marine: adjusted $R^2 = 0.4$, $p < 0.01$; freshwater: adjusted $R^2 = 0.66$, $p < 0.01$).

On closer inspection, it is notable that this correlation between sample volume and concentration is not apparent for net samples. Samples collected by nets seem to be in quite good agreement, typically reporting < 100 microplastics/m^3, irrespective of the volume captured. Nets typically capture larger particles, usually > 300 μm in size, which relates to the pore size of most nets used for such sampling. The relative consistency in the observations for net data with sample volume (adjusted $R^2 = 0.03$, $p = 0.17$) may be due to these observations generally quantifying similar sized microplastic particles. The restriction of most net measurements to quantification of quite large microplastics > 300 μm suggests they are less susceptible to the correlation between smaller particles and more frequent detections that are observed in the grab and pumped filtration samples.

Indeed, when considering the entire data set, there is a negative correlation between minimum particle size and the reported concentration of microplastics in both marine waters, adjusted $R^2 = 0.37$, $p < 0.01$ and in freshwaters, adjusted $R^2 = 0.31$, $p < 0.01$ (Fig. 2 and Supplementary Material 3, Table SI2). When the same sample collection method was used, and the same minimum particle size analysed, reported concentrations are typically within around 4 orders of magnitude of one another (Fig. 2). It is challenging to conclude whether this reflects true differences between hotspots and more background

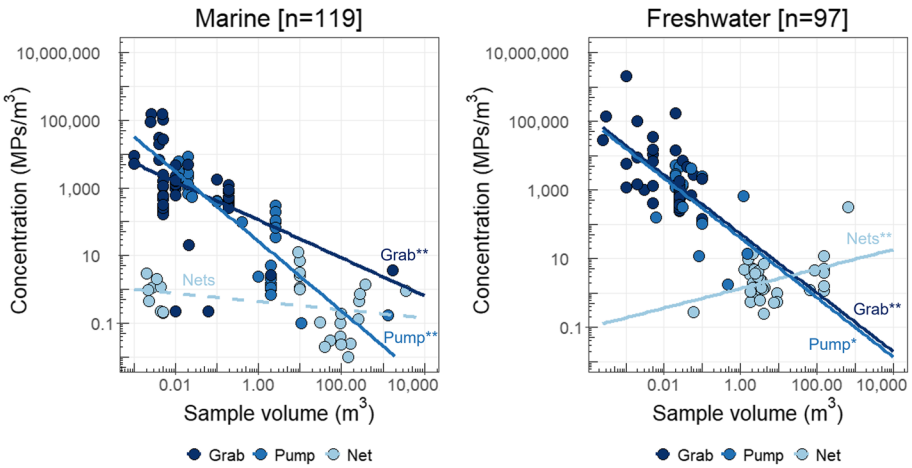


Fig. 1 Regression plots of concentration against the sample volume captured in marine and freshwaters. The collection method is denoted by different coloured points, for grab, net and pumped filtration sampling. Modelled fits for the regressions are presented for each of the three collection methods. Solid lines represent statistical significance (* = $p < 0.05$, ** = $p < 0.01$) whilst dotted lines were not statistically significant

concentration locations, or if this is due to analytical uncertainties. Such assessment is possible between specific studies through detailed evaluation and comparison of study designs. Adhering to reporting criteria such as [16] will assist in making the relevant data and meta data around microplastics monitoring available for such detailed assessments.

Assuming that the concentration of microplastics is expected to follow a power law relationship between decreasing size and increasing concentration (see for instance, [33]), it would be anticipated that a significant negative correlation between these size and concentration would be observed across all sample collection methods and in both environments. When split by sample collection method, results obtained only suggest a significant negative correlation for net samples in marine waters (adjusted $R^2 = 0.26$, $p < 0.01$), and for pump samples in freshwater (adjusted $R^2 = 0.44$, $p < 0.01$). Results for all other combinations of collection method in marine and freshwater are observed to be not significant at $p > 0.05$, with respect to decreasing particle size and concentration (Supplementary Material 3, Table SI2). This result is surprising and speaks once more of the challenges when comparing data from different sources. There are various sources of uncertainty which may contribute to this rather

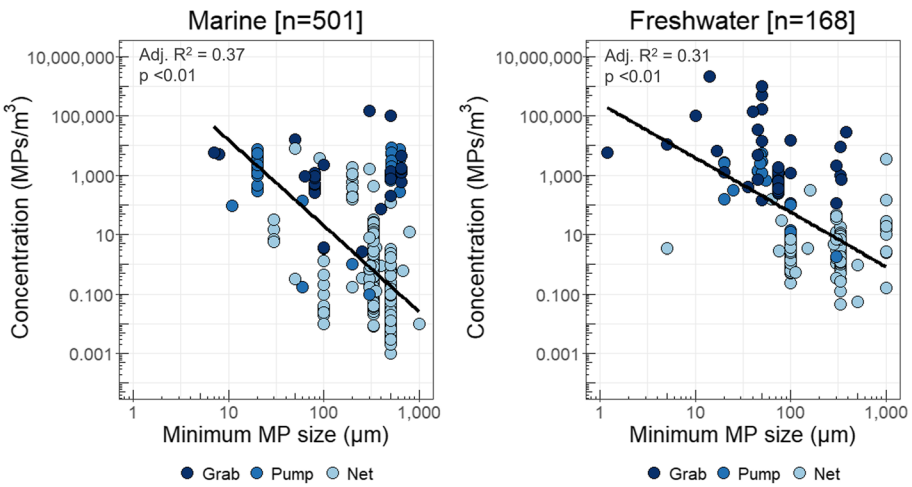


Fig. 2 Regression plots of concentration against the minimum microplastic particle size captured in marine and freshwaters. The collection method is denoted by different coloured points, for grab, net and pumped filtration sampling. The regression lines represent the regression for all datapoints, irrespective of the sampling method

weak correlation between particle size and reported concentration:

- In the assessment it is not possible to interrogate the sensitivity of each analytical technique across the range of sizes measured and reported in each study. No comprehensive assessment of sensitivity of analytical techniques across particle sizes is available and so the impact of analytical sensitivity differences between studies is unknown. Current efforts to generate representative test materials with consistent and known properties (e.g. [36–38]) represent an opportunity for future systematic recovery and sensitivity assessments across analytical techniques through interlaboratory comparisons (e.g. [15, 39–41]).
- In some cases, minimum particle size quantified could be extracted from the data. Where such information was not available, the minimum particle size was assumed equivalent to the physical limits of the sampling method e.g. net mesh size or filter pore sizes are used in the assessment. There may be some uncertainty introduced by this assumption.
- The ability of different analytical techniques to resolve aggregated particles and report on their constituent particle number is unknown.
- Whilst it is evident that degradation and fragmentation processes will lead to ever more abundant smaller particles in the environment, it may be that very small fragments are generated preferentially. For example, if weathering causes particles to shed small fragments from their surfaces, rather than breaking up into a continuous distribution of particle sizes (e.g. [7, 42]). Such fragments may be underrepresented in the data due to analytical techniques being unable to quantify these sizes to date in environmental samples, with most reports concerned with microplastics > 10 µm in size.

How can a standardised approach to predicting representative sample unit volumes help resolve outstanding issues with data variability?

Microplastic particles in the environment are heterogenous, exhibiting a variety of physicochemical properties such as polymer type, size, and shape. Particles may also be distributionally heterogenous, experiencing variations in concentrations across time and space due to environmental fate processes. It is therefore necessary to minimise the influence of these two sources of variability in order to generate a representative understanding of the environmental exposure when designing any sampling strategy (Fig. 3).

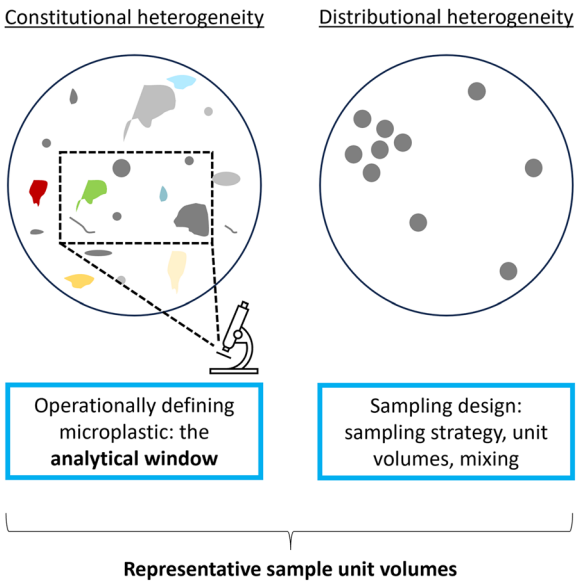


Fig. 3 Predicting representative sample volumes is critical to resolve constitutional and distributional heterogeneity of microplastics

In traditional theory of sampling, "comminution" of the collected particles through cutting, crushing, or grinding is often used to resolve constitutional heterogeneity [43]. However, this solution is not suitable when quantifying microplastic particle number concentrations, as disintegration of the existing microplastic particles into smaller fragments would mean they no longer have integrity to the original sampled environment. An alternative is needed. Increasing the number of particles that can be quantified in a single sampling unit can reduce the impact of this constitutional heterogeneity inherent to environmental microplastic samples.

The sample unit volume must be optimised to achieve a sufficient number of particles analysed in the measurement to be representative of the constitutional heterogeneity of the population/environment targeted. Statistical guidance is provided elsewhere on the number of particles needed to be captured to describe the constitutional variability in microplastic samples e.g. [30]. To generate representative data for more than one aspect of microplastic characteristics, for example to measure sufficient particles to describe not only polymer identities but also shapes and colour with a given level of error, increasing numbers of particles must be measured within a sample. It should be noted, that as no single analytical technique can be quantitative of the constitutional diversity of microplastic in its entirety, any such analysis is constrained by the analytical window of the technique and so an operational definition of "microplastics" in each study must be reported.

In the laboratory, the influence of distributional heterogeneity of microplastics can be reduced through

mixing or blending of the sample. However, in the field, the only recourse is through sampling design and increasing the sample volume collected. For systems in which particles are expected to be randomly distributed, for example in a turbulent river sample, this random distribution can be estimated following a Poisson point process. This has been used to estimate sampling errors when counting microplastic particles (e.g. [29]), whilst others have also used bootstrapping methods to estimate sampling error when only characterising a subsample of microplastics in a population (e.g. [44]). In all cases, increasing the number of particles quantified in an analysis reduces the sampling error and thus provides a more robust estimate of the total particle number concentration of microplastics.

From this understanding of both the constitutional and distributional heterogeneity of microplastics in aquatic samples, it may be assumed that the smaller the volume taken, the larger the impact of these heterogeneities on the reported concentration. Thus, higher variability can be expected in data where lower sample unit volumes were collected. Indeed, in our analysis, the absolute variance in data was reduced for data associated with higher quality scores for sample volume size and better documentation of sample preparation methods (Supplementary Material 3, Figure SI3), both of which are key factors to address the constitutional and distributional heterogeneity of microplastics in aquatic dispersions.

Using the RSVP tool to screen for relevant data

As an example of the application of the RSVP tool as a screening tool for historic data, here we predict the minimum volume required to detect microplastic particles with a given confidence of 95%, using the concentration reported in the study as the nominal concentration in the sampled environment (Fig. 4).

Data points in the graph are scaled to cube-root transformed concentrations, as reported concentrations range across many orders of magnitude and could not be visualised without transformation. Of 97 datapoints that reported the sample volume captured for freshwaters, 84 data points captured sufficient volume to report detections (presence/absence) of microplastics with 95% confidence. For 13 datapoints the probability of catching at least 1 particle in the given volume was < 95%. Interestingly, two of these studies would have scored 2 in the quality scoring system for sample volumes. This demonstrates the utility of the tool in providing a retrospective assessment of sample volume sizes on a case-by-case basis, enabling a more robust evaluation of study quality. For those datapoints which did not pass the minimum sample volume requirement, this does not mean that the reported microplastic in those studies are not "real" observations of microplastic presence. Rather, there is a greater than 5% chance of false negatives, i.e. of collecting no microplastic particles at all, even though they are present in the sampled environment. Thus, were the researchers to repeat this sampling many times from an environment with the reported concentration, on more than 5% of the occasions, they would wrongly conclude there were no microplastic particles.

Further considerations are:

- Blank correction and limits of detection: In this calculation the reported data was taken as given. Whether reported data is statistically significantly

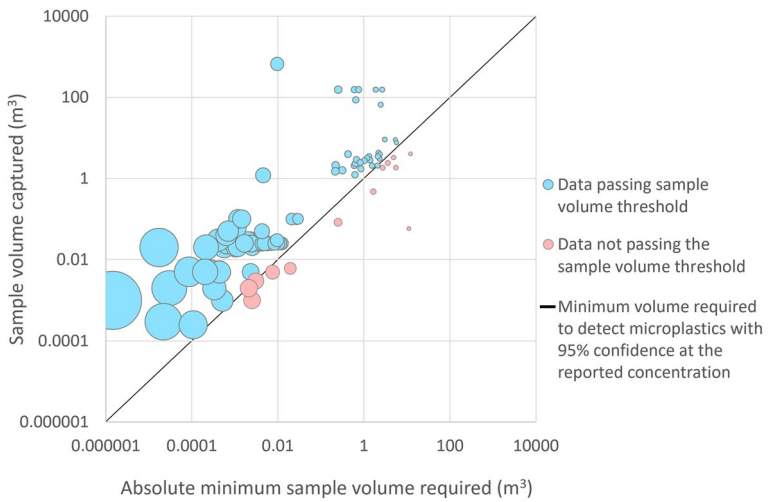


Fig. 4 Captured sample volumes versus predicted minimum sample volume requirements. Blue circles indicate studies which sampled a volume sufficient to detect microplastics with > 95% confidence, whilst red circles indicate data which did not meet this requirement. The size of the circles represents the concentration reported. Note for visualisation purposes, the cube-root of the concentration was used to scale the size of the circles

different from the background is a separate question, commonly addressed by calculating detection limits with regards to the standard deviation of the blanks. In some cases, contamination is not (adequately) accounted for when using procedural blanks. The reported concentration is inclusive of an unaccounted-for background contamination and may not be truly above the limits of detection of the study. Only one of the six studies where datapoints did not pass the minimum required sample volume scored highly for clean air, with all others scoring 0. There were mixed quality scores for negative controls across these studies.

- Confidence level: Here we applied a confidence level of 95% for presence/absence in the sampled volume. A less stringent level of confidence may be acceptable, depending on the purpose of the assessment.
- Complete mixing and independence of events: The study area may not conform to the assumptions of the RSVP tool. The tested system should represent microplastics that are behaving independently of one another and randomly distributed within the environment sampled, for example through turbulent dispersion in a flowing river. Under conditions that violate the assumptions of the Poisson distribution (e.g. independence of events) the RSVP prediction will be invalid.

The desired number of particles to detect is dependent on the purpose of the assessment. There are additional costs with increasing the sample volume, particularly in the clean-up and extraction of microplastics from environmental samples. Therefore, it is not always desirable to capture the maximum possible sample size for a study, rather the representative volume required may be tailored to the purpose of the study. Some general rules of thumb can be found in contemporary studies that are useful as a guide to the number of particles required to be captured and analysed for a given purpose (Table 1).

As can be seen, the desired or target number of particles to obtain in a sample is a critical parameter in the RSVP tool and is dependent on the purpose of the assessment (Table 1). Equally the level of confidence required in the assessment could also foreseeably be dependent on the purpose of the assessment (as has been proposed for health-based thresholds for risk management [45]). For example, for environmental managers, detection or quantification of microplastics at a lower level of confidence might trigger exploratory work, whilst higher levels of confidence might be needed to trigger routine monitoring or for compliance of other more defined activities.

It should be cautioned that the RSVP tool is applicable only when the assumptions underpinning the Poisson distribution are adhered too. In particular, assumptions that particles are randomly distributed and acting independently must be met. These conditions have been demonstrated to be met when randomly sampling rivers under turbulent flow (e.g. [46]). Validation in other scenarios is needed. Justification is required when using the tool in other environments, for example in soils to understand terrestrial risks or air when evaluating human exposure. Providing access to the underlying data such as the volume captured and the total number of particles quantified as standard practice in future studies would allow for such conditions to be tested and would further increase the applicability of the RSVP tool.

Conclusions

A review of historic data reporting on microplastic concentrations in marine and freshwater environments revealed concentrations spanning several orders of magnitude. Less variance is observed in data with larger sample volumes. It is necessary to have a harmonised approach to estimate representative sample volumes in any given environment which can be tailored to the study needs and environment sampled.

To help support future studies and to evaluate historical data we recommend applying the RSVP tool, which allows for a documented approach to predict representative sample volumes when quantifying microplastics in the environment. This can be used both prospectively to aid the design of new studies, or retrospectively, to review the relevance of existing data for a given purpose. The considerations when using the RSVP tool are as follows:

1. Problem formulation – define your problem. What do you want to measure in the environment? Where do you want to measure? What sample collection and analytical techniques do you have at your disposal?
2. Identify relevant data – using the analogy of the analytical window, select the most relevant existing data for your purpose. Consider the relevance of the system that is sampled, the hypothesis that is addressed, the sampling collection method, and the analytical technique used in existing studies. Each of these aspects define the analytical window of a measurement and operationally define "microplastics" in a study. Relevant data must be representative of a similar operational definition of microplastics in the source data as your new study design. Find the closest analogous study to your own design you have described during the problem formulation and assess against quality criteria (e.g. [16, 20, 21]) to best inform study design *a priori*.

3. Evaluate selected data in the RSVP tool – two options present themselves, a prospective evaluation of a new study design or a retrospective evaluation of existing data:

    a. Prospective prediction of representative sample volumes – calculations for representative sample volumes required to capture a given number of particles at a given level of confidence are provided. Guidance on target number of particles for analysis and the level of confidence required are proposed, linking to other relevant contemporary approaches.
    b. Retrospective evaluation of existing data – these same calculations can be used to review and screen existing data. For example, they can identify relevant data which achieved a desired level of confidence or predicted sampling error, based on the volume captured and the total number of particles quantified. Or they can be used to estimate whether two concentrations are likely to differ at a given level of confidence.

It is hoped that these steps will help the community generate more reliable monitoring data on microplastics in the environment.

Supplementary Information

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Supplementary Material 1. The RSVP Tool_v1.0 – Representative Sample Volume Predictor.

Supplementary Material 2. Database quality scores.

Supplementary Material 3. Supporting Information - Ensuring Representative Sample Volume Predictions in microplastic monitoring.

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Authors' contributions
R.K.C.: Writing – Original draft, Methodology, Formal analysis, Conceptualization, Writing – review & editing. S.L.R.: Writing – review & editing, Visualization, Formal analysis. M.D.J.: Writing – review & editing, Methodology, Visualization, Formal analysis. A.C.J.: Writing – review & editing. C.W.D.: Writing – review & editing, Conceptualization. T.G.: Writing – review & editing, Conceptualization, Methodology, Formal analysis.

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Data availability
Data is provided within the manuscript or supplementary information files.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Consent for publication is hereby given by the authors.

Competing interests
Richard Cross, Sarah Roberts, Andrew Johnson and Monika Jürgens declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. Craig Davis is employed by ExxonMobil Biomedical Sciences, Inc. Todd Gouin is a member of the Editorial Board of Microplastics and Nanoplastics, but was not involved in the journal's review of, or decisions related to, this manuscript.

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