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What you net depends on if you grab: A meta-analysis of sampling method's impact on measured aquatic microplastic concentration

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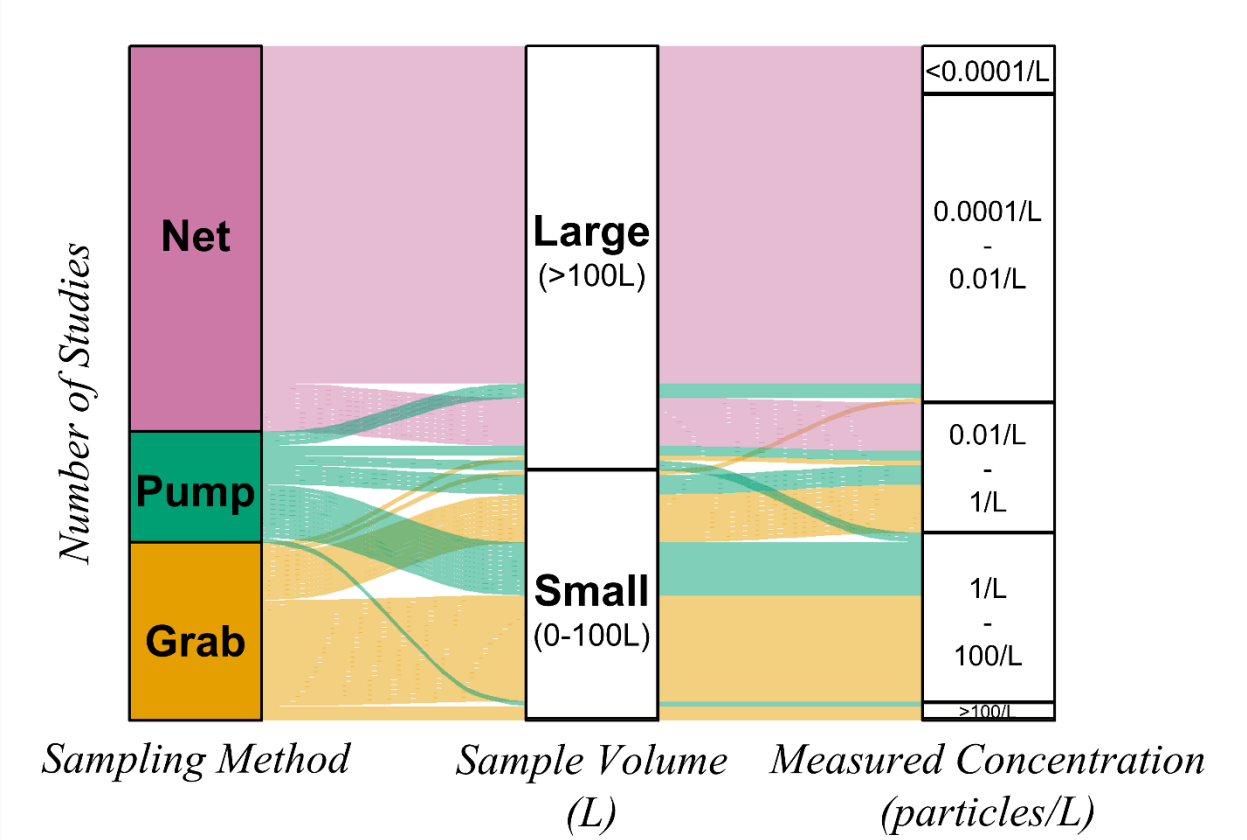

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Abstract

Microplastic pollution is measured with a variety of sampling methods. Field experiments indicate that commonly used sampling methods, including net, pump and grab samples, do not always result in equivalent measured concentration. We investigate the comparability of these methods through a meta-analysis of 121 surface water microplastic studies. We find systematic relationships between measured concentration and sampled volume, method of collection, mesh size used for filtration, and waterbody sampled. Most significantly, a strong log-linear relationship exists between sample volume and measured concentration, with small-volume grab samples measuring up to 10^4 particles/L higher concentrations than larger volume net samples, even when sampled concurrently. Potential biasing factors explored included filtration size ($\pm 10^2$ particles/L), net volume overestimation ($\pm 10^1$ particles/L), fiber loss through net mesh (unknown magnitude), intersample variability ($\pm 10^1$ particles/L), and contamination, the potential factor with an effect large enough ($\pm 10^3$ particles/L) to explain the observed differences. Based on these results, we caution against comparing concentrations across multiple studies or combining multiple study results to identify regional patterns. Additionally, we emphasize the importance of contamination reduction and quantification strategies, namely that blank samples from all stages of field sampling be collected and reported as a matter of course for all studies.

Synopsis

This work uncovers a literature-wide bias in microplastic concentrations, related to sampling method, with steps to remedy the comparability error.

1. Introduction

Microplastics, plastic particles less than 5mm in size, have been detected in water worldwide including systems as pristine as those in the Pyrenees[1], as remote as the deep ocean[2], and seemingly everywhere in between[3]. These particles are either manufactured at sizes less than 5 mm or are the result of breakdown from UV exposure and physical abrasion of larger plastics. Microplastics are of concern because of their observed and hypothesized effects on aquatic organisms[4–6]. In particular, the concern comes from microplastics' propensity to introduce chemical additives into and transport adsorbed contaminants within aquatic environments and organisms[7,8].

The extent of microplastic pollution remains a fundamental question for the field. To answer this, study results from spatial surveys are commonly aggregated to create regional and global pictures of hotspots and average concentrations[3,9–11]. Unfortunately, studies follow a variety of evolving methodologies, and the comparability of results from studies that rely on differing methodologies is generally unknown. Before regulations can be based on an aggregation of regional results, it is imperative to understand how methodological choices affect microplastic measurements.

In this study, we focus on how three different, but commonly used, field sampling methods affect microplastic quantification: nets, bottles, and pumps. These methods largely mimic those used for neustonic plankton sampling, due in part to microplastic contamination being first reported by plankton researchers[12,13].

Net sampling deploys nets for a constant distance (if the net is moving) or time (if water is flowing). Sample volume, typically ~10,000L, varies based the area of submerged net mouth and the stream velocity or length-of-tow (in non-flowing waters). To avoid clogging the net with

organic material during sampling, a relatively large mesh size is used, often ~0.333 mm[14]. Samples are collected at the base of the net, in a removable "cod end", made of the same material as the net, typically nylon. Because of their large size, cleaning nets between samples can be difficult. Currently, they are still the most common sampling equipment used in oceanic settings, as well as in lakes and large streams[15].

Contrastingly, bottles are used to collect grab, or "bulk", samples. These samples collect much smaller volumes than a net sample, often 1-10L, but have the benefit of being able to collect even the smallest particles. Small particles are most relevant to ecotoxicity questions, adding special value to methods that allow such particles to be retained[16]. Compared to nets, bottles are a less expensive, more intuitive, and faster method for sample collection, transport and storage. These factors mean they are a frequent choice for citizen science projects, an important approach to research that allows for a greater quantity of data to be collected while also providing opportunities for science education and community dialogue.

For this analysis, we also include studies that use an emerging third option, pumps. These allow for much larger volumes of water than grab samples but can be fitted with or convey water through sieves, which allow them to capture smaller particles than typical net samples. The sieves, tubing, and other pump components may themselves be made of plastic materials and require a source of energy to power them in the field, making them a more challenging choice for some sampling locations.

Several previous studies have reported dramatically different microplastic concentrations from samples collected using differing methods[17–22], as well as preliminary evidence to suggest systematic trends[16,23]. Here we take a wide and thorough look across the literature of surface water studies, including those that pair methods and others that do not, to see how method choice

affects measured microplastic concentration. We then use the relationships uncovered to itemize and quantify potential sources of systematic bias in sampling method.

The objective of this analysis is not to identify the best performing sampling method. Each method is currently in use due to their own context-specific advantages. Our hope, instead, is to shed light on the misalignment of the resulting concentration measurements and help move the microplastics field one step closer to harmonizing methods and creating a comparable, reliable body of literature for policymakers and researchers alike.

2. Methods

We rely on a multi-facetted approach to investigate potential biasing factors of concentration measurement differences. These include (1) a systematic literature review of surface water microplastic samples, (2) a closer look at samples collected in pairs of differing methods, (3) controlled field studies and related works that isolate for particular potential biasing factors, (4) statistical analysis and back-of-the-envelope calculations to identify reasonable bounds on the magnitude or contribution of potential biasing factors.

2.1 Literature review:

We performed a literature search of surface water microplastic studies published prior to October 2020. The review was conducted in September and October 2020. Studies were retrieved from Google Scholar searches of the words: “microplastic” + “surface water”, along with (individually) “net”, “pump”, “bulk”, “discrete” and “grab”. Of the returned results, we included all studies that both sampled within the top 1m of a waterbody (deeper samples were excluded) and reported volume sampled or a means of, at least roughly, calculating volume sampled (e.g. net dimensions and tow distance or speed and time). This strategy of post-hoc

volume calculation accounted for about 1/3 of the included studies. For studies that sampled multiple waterbodies or used multiple methods, results were included for each unique combination of method and waterbody-type. For example, if multiple rivers in a region were sampled with the same method, their results were averaged, while the results of pumping and net methods on a single river were considered separate entries.

Additionally, we identified 15 datasets that measured microplastic concentrations using paired samples of two or more methods ("paired-method") at a single sampling time and location. All but three of these studies, which were omitted due to insufficient data or incompatible sampling depth, were also included in the analysis of literature-wide trends. One of these datasets was collected specifically for this study (Section 2.2).

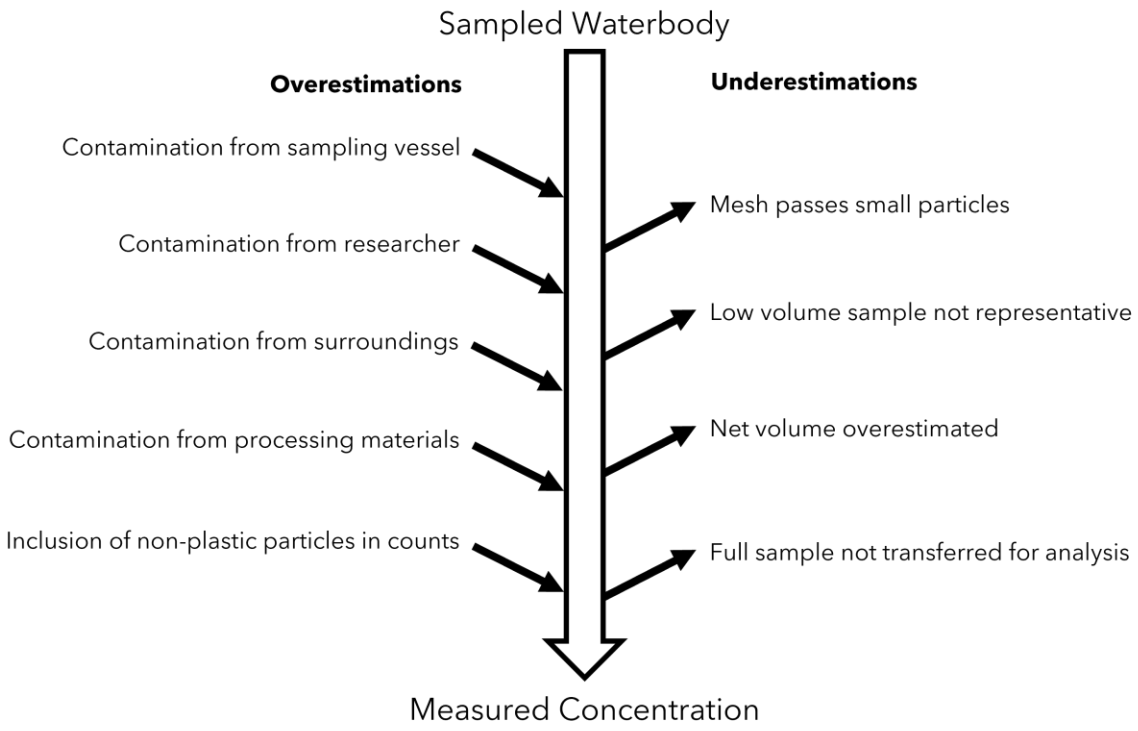

Figure 1. A conceptual diagram of the pathways that may increase (left) or decrease (right) measured concentration, from the sampling of a waterbody to transferring and processing a sample to the quantification of particles in the sample.

2.2 Field samples:

To include in the paired-method sample analysis with the forementioned published datasets (n=14), we also collected paired grab and net samples in 4 streams (watershed areas: 35km, 73km, 101km, 320km) in Tompkins County, New York. These samples were filtered through the equal size meshes to fill a gap in the literature of paired grab and net samples with equivalent lower-size bounds.

We collected these samples across multiple flow conditions, sampling each river 1-3 times. A grab sample (mean volume: 1.8L) and a neuston net (10min deployment, 1m wide x

0.5m tall x 3m long, 0.335mm mesh; Sea-Gear, Melbourne, FL) were used sequentially to collect microplastics at the surface in the region of highest flow in each river. In the lab, grab samples were poured through a 0.335mm mesh to match the lower size constraint of the net samples. Field data for these samples, as well as further laboratory processing details, particle identification, Raman confirmation, and contamination reduction are included in the supplementary information.

Alongside field samples, deionized water was run through each laboratory processing step, including filtration, drying, digestion, separation, and counting, as procedural blanks to measure potential contamination from laboratory materials. Air blanks were also collected by exposing filter paper to laboratory air for 24 hours. Additionally, we collected a set of "maximum reasonable procedural blanks". These blanks were collected by passing deionized water through single-rinsed mesh, sieves, and beakers. They were designed as "worst-case" blank samples and were intended to quantify an upper-bound on "reasonable" potential contamination levels to compare against concentration discrepancies across sampling method. We collected these blanks after the completion of all laboratory work and after the lab space and equipment had been used extensively for laboratory courses and demonstrations. Results of blanks are included in Table S1. Average air and procedural blank values have been subtracted from reported concentrations.

2.3 Statistical Analysis

We identified *a priori* a variety of potential factors influencing the concentration trends observed through literature review and solicitation of hypotheses from field experts (Figure 1). We use multiple linear regression as a tool to organize these hypotheses and identify which of

these reasonable factors may be more relevant in explaining concentration differences than others. The regression included the following 6 factors: 1) sampled volume; 2) sampling method; 3) filtration or mesh size; 4) sampled waterbody (freshwater vs. marine); 5) whether visual particle counts were confirmed with a more advanced technique to confirm polymer content; and 6) whether measured contamination was subtracted. To avoid problems of collinearity among these predictor variables, the degree of correlation between variables was checked visually and by examining the magnitude of their pairwise correlation. The multiple linear regression used to ascertain the relationship between the response and predictor variables was run in R version 4.0.3[24], with statistical assumptions of normality and homogeneity of variance checked graphically. To determine whether the percentage of fibers differed between paired-method samples, a Wilcoxon signed-rank test was used. For all statistical tests, we used a p-value upper-bound of 0.05 to determine statistical significance.

As we explore potential factors influencing concentration differences, we use a simple equation (Equation 1) to determine a rough magnitude estimate for an additive factor, such as contamination, that may be affecting measured concentration. Equation 1 provides a rough estimate of the number of contaminating particles, or other additive factor, needed to equate two paired-method sample concentrations:

$$\frac{n_1 - k}{V_1} = \frac{n_2 - k}{V_2} \qquad \text{Equation 1}$$

where n is the number of particles counted in the sample, k is the number of introduced particles due to an additive factor (such as contamination), V is the volume of the sample, and subscripts denote each sample of a pair.

Equation 1 assumes that contamination affects samples collected and processed together in a similar way. It also assumes that there is a true environmental concentration that would be reported equivalently by any paired-method samples. This equation includes two major simplifications: 1) that intersample variability is zero (we know side-by-side samples to vary up to 9x[25]); and 2) that the number of introduced particles of contamination will be equal across all samples (more precisely, *k*'s would be sampled from a given distribution). The equation therefore represents the case where an additive effect, like contamination, is the sole factor affecting concentration differences between measurements and volume the sole factor influencing sampling intensity.

3. Results and Discussion

A total of 118 studies were included in the analysis of literature-wide trends. Due to studies that include results from the use of more than one sampling method or sample more than one type of waterbody, 140 unique entries were included (Figure 2). This total includes 37 instances of a grab method[11,16–18,22,26–55], 80 using a net method[8,10,13,17,21,22,25,27–30,35,36,44,50,56–117], and 23 of a pump method[21,22,47,62,69,91,103,118–130] to collect their samples. Of the unique entries, 44% were freshwater (including 39 riverine and 22 limnic systems) and 56% were marine (including 12 estuarine and 65 oceanic systems).

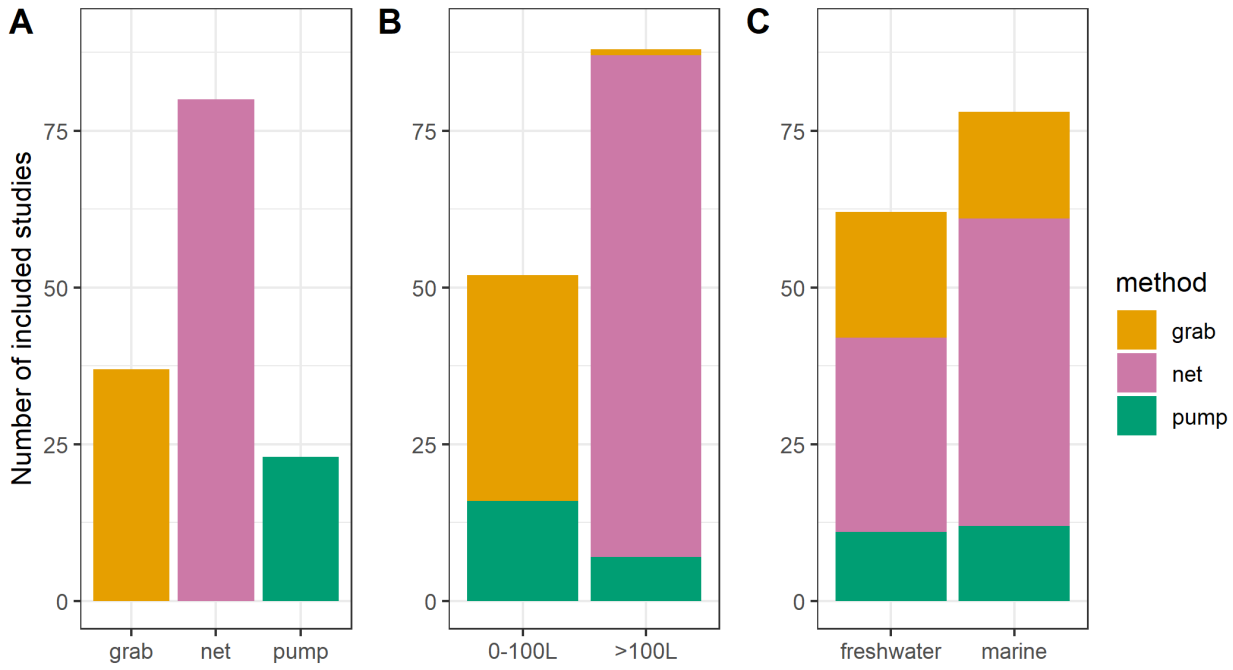

Figure 2. Summary of the unique entries included in this literature review, including sampling method used (A), binned sample volume (B), and sampled waterbody type (C).

These studies span the globe (Figure 3). They also include samples from the 1970's, 2000's and 2010's, with publication dates ranging from 1971-2020 (Figure S1). The studies also rely on a variety of laboratory techniques. Some use wet peroxide oxidation and density separations to first isolate particles, while others simply examine all contents of a sample. Fourier transform-infrared (FTIR), Raman, Nile Red staining and simple visual inspection were all represented.

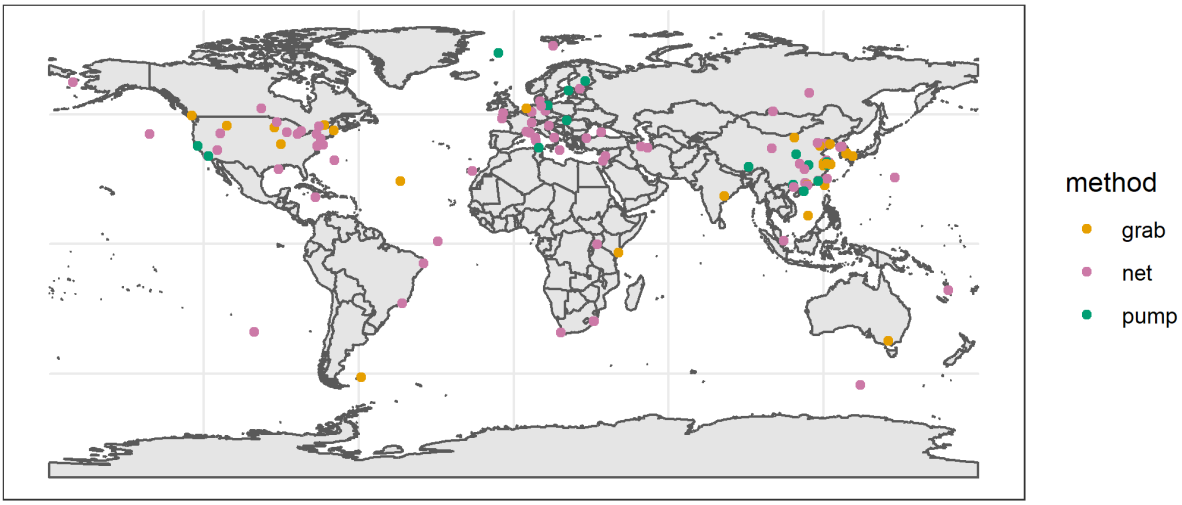

Figure 3. Global[131] distribution of samples included in this analysis.

To help narrow exploration into the main factors that may cause the systematic concentration differences, we use a multiple linear regression run on the overall dataset (Table 1). Across the literature analyzed, volume sampled, mesh size, and waterbody sampled were significant predictors of measured concentration.

Table 1. Summary of coefficients for the multiple linear regression[a] fit to the literature-wide data to predict $\log_{10}$ of measured concentration.

Parameter	Estimate	Standard Error	t value	p-value
Intercept	1.04	0.28	3.75	$3 \cdot 10^{-4}$
Log_{10}(Volume)	-0.50	0.10	-5.20	$9 \cdot 10^{-7}$
Method = Net	-1.10	0.44	-2.52	0.01
Method = Pump	-0.10	0.27	0.40	0.69
Mesh size	-1.51	0.67	-2.24	0.03
Waterbody = Marine	-0.35	0.17	-2.04	0.04
Confirmed polymer	0.20	0.21	0.99	0.33
Subtracted blanks	0.05	0.17	0.28	0.78

[a]Adjusted R squared value for this regression model is 0.77, with an F-statistic of 69 on 7 & 132 degrees of freedom and a p-value of $2 \cdot 10^{-16}$

Volume sampled was the most significant predictor (Figure 4). Grab samples (10^0-10^2 L) systematically resulted in higher microplastic concentrations than net samples (10^2-10^7 L). Pumped samples (10^1-10^4 L) represented concentrations (10^{-4}-10^2 L^{-1}) that overlapped with and fell between grab (10^{-3}-10^3 L^{-1}) and net (10^{-6}-10^{-1} L^{-1}) sample concentrations.

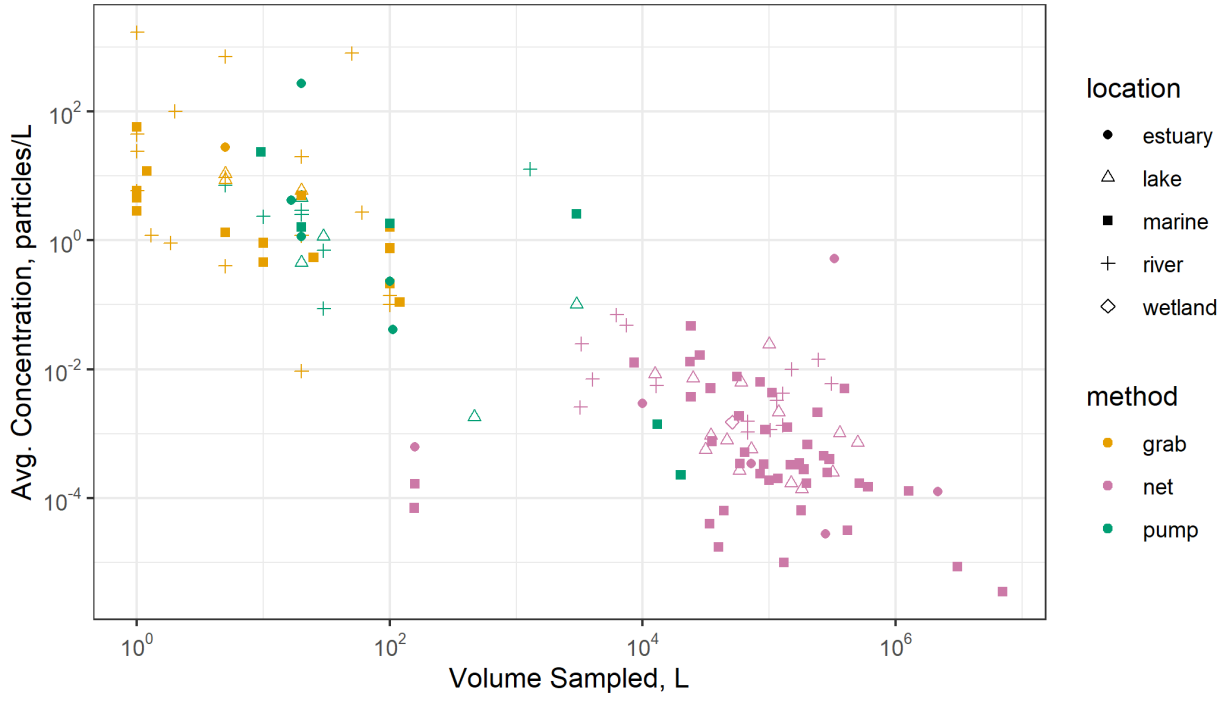

Figure 4. Average volume sampled in studies relying on differing methods (color) and in differing waterbodies (shape) and the average concentration measured in each of those studies.

Method and mesh size, though correlated with sample volume, were found to include enough independent information to also be significant factors in predicting concentration. Correlation between mesh size and volume, for example, as measured with Kendall's Rank Correlation, yields tau = 0.5. Enough variability exists in the relationship between volume and mesh size (Figure S3) that these factors can be examined independently. Pump and grab method

estimates were not statistically significant from one another but were both different from net method estimates after accounting for all other factors (Table 1). For all methods, volume appears to be the driving predictor of measured concentration, more so than the method itself (Figure 4).

The regression also indicated that sampled waterbody type was a significant predictor, with marine samples tending to measure lower concentrations. While the included net samples do tend to be of marine environments (61%) and grab samples of freshwater environments (54%), a further analysis of paired-method samples (Figure 5) highlights that even in the same sampling environment, with the same anthropogenic pressures, the relationship between method and concentration remains. The model fit suggested that visual identification and the use of blanks, as implemented using current, highly variable methods, were not among the strongest predictors of measured concentration.

Among the subset of paired-method studies (n = 15), which sampled at the same time and location with differing methods (Figure 5), the same concentration trend is apparent: low volume samples tend to measure orders of magnitude higher concentrations than high volume samples. A few sample pairs (28 out of 310 paired-method samples) show the opposite trend, specifically when smaller volume sample concentrations are zero, but we believe this to be a demonstration of one of the shortcomings of small sample sizes: that they may miss particles altogether and falsely report zero concentration due to undersampling the system. Koelmans et al. take note of this shortcoming in their review and recommend a minimum sample volume in surface waters of 500L[132]. Replicates of low volume samples can also help mitigate this issue.

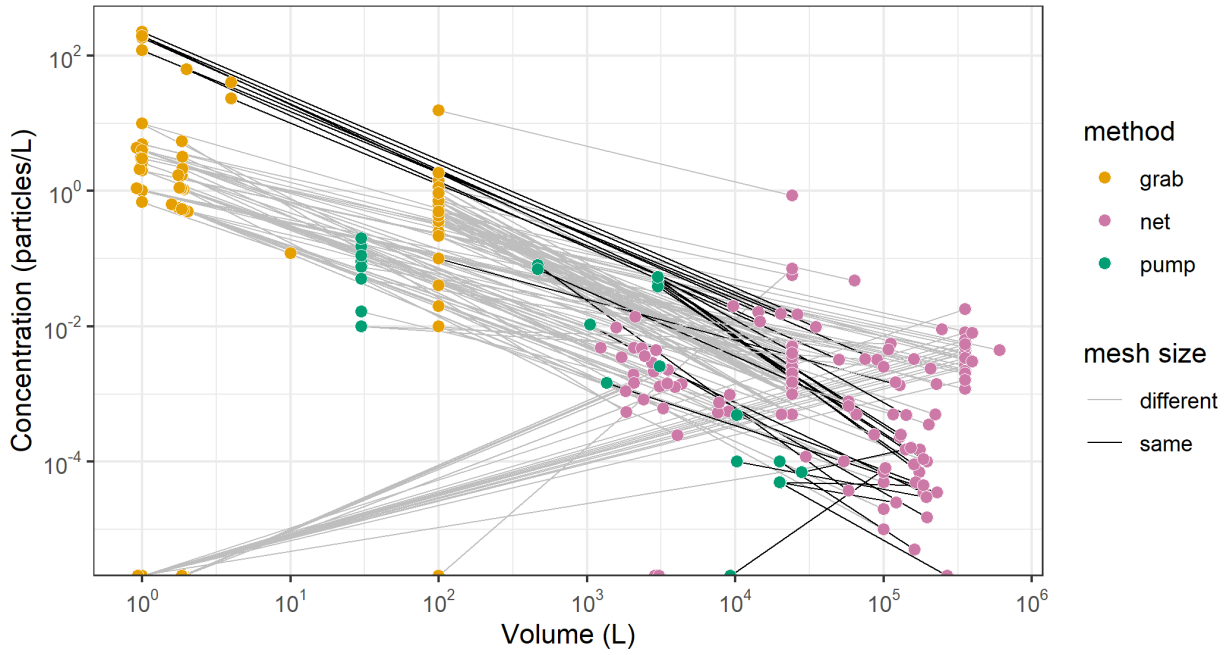

Figure 5. Paired-method samples from 15 datasets, including field data collected for this study. Lines connect sample pairs collected at the same time and location. Black lines indicate pairs were filtered through the same mesh size, while gray lines indicate pairs used two different mesh sizes. Zero concentration is adjusted to 10^{-6} particles/L to account for log-scale limitations and is plotted along the x-axis.

One explanation of the volume-concentration relationship disproven by paired-method sample results is that researchers may be intentionally choosing to sample larger volumes when they visit areas where lower concentrations are anticipated. What these paired-method samples show instead is that even at the same time and location, higher sample volumes measure lower concentrations, just as in the literature-wide trend (Figure 4).

One important note is that samples containing a large volume of water naturally will contain more particles, thus diminishing the influence of any single particle captured on the overall mean per volume. For small sample volumes like 1L grab samples, concentration values

have a resolution of 1 particle/L. Contrastingly, for larger sample volumes, such as 10,000L net samples, this resolution is much finer; each additional plastic particle would alter the concentration by only 0.0001 particle/L. Larger volumes also have the effect of reducing variability between samples[133].

3.1 Potential contribution of mesh size differences

Mesh size differences alone are insufficient to explain the orders of magnitude differences in concentration alone. Paired-method studies that use the same mesh size still find small volumes to measure lower concentrations than large volumes (black lines, Figure 5). When filtered through the same size mesh, net samples measured an average of 10^4-times lower concentrations than those sampled by a paired grab and 10^3-times lower concentrations than a paired pump.

Net samples tend to use larger filtration sizes than grab and pump samples do. This is largely an intentional design choice to avoid clogging. However, only a few mesh sizes are commonly used for sampling nets, which allows volume, which varies widely, to remain somewhat independent of mesh size within a given sampling method and, therefore, be examined separately (Figure S3). For example, the studies from our broader literature review that sampled using a net with 300-350μm mesh still show a strong volume-concentration relationship (Figure S4). In contrast, when looking only at grab samples with 1L sample volume, there is no evident mesh size – concentration relationship (Figure S5).

To some extent, mesh size certainly does matter: when you allow smaller particles to be in your sample, you will likely collect more particles overall[21,62,134,135]. Smaller volume samples, specifically grab and some pump samples, allow for a smaller mesh or filtration size to be used

without the issue of clogging. In the included studies, grab sample mesh or filtration sizes ranged from 0.4-335 µm, pump samples from 4-300 µm and net samples from 50-947 µm.

Existing field measurements in the literature have quantified the change in measured concentration when volume is controlled for while varying mesh sizes (Table S2). In the case of Lindeque et al., 100µm mesh measured 10x higher concentration than 500µm mesh[134]. Based on the literature, Koelmans et al. develop a concentration conversion factor to account for mesh size differences[136]. At its maximum, to convert from a measured particle size range of 333-5000µm, as is common to net samples, to a broader range of 1-5000µm, the calculated conversion is a factor of 40. Paired-method samples in our analysis, where at a given time and location samples of differing methods are collected, measure concentrations that differ by 2 orders of magnitude or more. Accounting for mesh size is therefore an important, but insufficient step in rectifying measured concentration differences between methods.

3.2 Potential contribution of overestimated net volumes

One possible explanation for net samples measuring lower concentrations is due to how sample volumes are measured. Grab sample and some pumped sample volumes can be precisely measured based on the sampling vessel. In contrast, net samples, and some pump sampling techniques, require calibrated flowmeters for accurate sample volume measurement. Without one, net volumes are prone to overestimation. Overestimated sample volumes result in measured concentrations lower than true system concentrations.

Karlsson et al. found that net sample volumes calculated without a flowmeter incorporate a volume error of at least 1%[122]. They observed that the water level in the net mouth fluctuates during towing, making sampled depth an inconsistent metric. One in three studies included in our

analysis lacked flowmeter results and required us to calculate volume sampled, by relying on average mouth depth and a given boat speed or GPS distance. We found that calculated volumes did have a steeper volume-concentration relationship than studies with volumes given (Figure S6); however, removing studies that required ad-hoc volume calculations did not affect the predictors included in a best-fitting regression model.

Another way that a net volume calculation can be inaccurate is due to fluid dynamic principles, which result in water bypassing the net due to flow resistance (drag) from the mesh itself. A typical strategy for calculating volume is to multiply a tow length by net dimensions (for river samples, tow length is time of deployment multiplied by river velocity). This provides a theoretical volume that ignores drag, assuming no water bypasses the net. The relationship between actual volume sampled and theoretical volume sampled is known as "filtration efficiency". This factor can change dramatically even for the same equipment. It is affected by the speed at which water is being forced through the net, the mesh size and the abundance of biological material in the sampled water body.

At a filtration efficiency of 85%, which is an acceptable value in plankton tows[14], measured concentration would be underestimated from "true" system concentration by 15%. While noteworthy, this percent decrease is dwarfed by the differences observed in the paired-method studies analyzed, where net concentrations were 75-100% lower than grab concentration and 45-100% lower than pump concentrations. This indicates filtration efficiency, while important, cannot solely explain the concentration differences observed.

3.3 Potential for fiber loss between sampling and processing

It is aspirational to assume that all particles that enter through the net are captured and collected in the cod-end. Likely some particles, fibers especially, may be trapped in the mesh itself or pass through the net entirely and return to the surrounding waters. The majority of included studies found fibers to be the most prevalent particle-type captured, followed by fragments. When examined by method, however, fibers' dominance was only true for grab and pump samples; in the majority of net samples, fragments were the most prevalent particle-type (Figure S7). This points to one of two potential hypotheses. The first is that a significant portion of fibers are being lost from the net. Lusher et al. provide evidence for this by putting sieves in series and discovering particles in secondary and tertiary sieves, an indication that some number slip through a primary sieve[137]. Another way fibers may be lost from net samples is if they are captured during sampling, but not transferred to the vessel processed in the lab. Too few studies have looked for and quantified residual microplastic particles in the net mesh for this work to investigate whether lower net concentrations could be caused by this kind of particle loss. We encourage future studies to examine net mesh before and after sampling to add to this body of knowledge. The second hypothesis relies on the observation that contaminating particles are largely fibers[22,77]. If small-volume sample counts are dominated by contamination, perhaps fibers' dominance in grab and pump samples reflects contamination and not environmental conditions. Without knowing the true, relative prevalence of particle-types in the sampled environment and because this meta-analysis looks at studies from across the globe, we cannot confirm either hypothesis with this dataset.

Analysis of the paired-method data within this study, where differing methods are being sampled from the same environment, finds no significant evidence of fiber loss from net

389 samples: paired studies that included particle-type showed statistically similar percentages of
390 fibers between samples of differing method (Wilcoxon signed-rank test, p-value = 0.37). Perhaps
391 this points to an additional interaction where fibers are not only lost from the net, but also gained
392 in similar quantities when fibers small enough to escape through net mesh are actually retained[22].
393 Controlled field studies will be needed to fully understand and quantify fiber losses and gains
394 through mesh.
395
396 *3.4 Potential contribution of intersample variability*
397 Given that all waterbodies are heterogeneous to some extent, it is reasonable to assume
398 that no two water samples will hold the exact same contents. For this reason, one suggested
399 explanation for differing concentrations holds that it is actually the replication and not
400 necessarily the methods themselves that create the variance observed in paired grab-net studies.
401 To some extent, this is disproven by the systematic differences observed across unpaired studies
402 of varying methods (Figure 4).
403 To investigate whether this may, however, play even a minor role in the differences observed
404 in paired-method studies, we rely on existing studies which have measured the concentration
405 variance between replicate studies of the same sampling method. Lindeque et al. towed two nets
406 (0.333mm mesh-size manta trawls) in parallel and found no significant difference between the
407 measured concentrations (0.54 and 0.46 microplastics m^{-3})[134]. Schmidt et al. found triplicate net
408 samples taken within 2 hours of each other varied up to 9x[25]. Hung et al. found duplicate net
409 samples had a standard deviation of less than 15%, while duplicate grab samples varied by 2x[22].
410 From this evidence, we conclude that heterogeneity plays only a negligible role in the multiple

orders of magnitude concentration differences observed among methods reported in this analysis (e.g., Figure 4).

Another possible influence of heterogeneity is in patchy distribution of particles at the sampling site, where researchers may be selecting for higher concentration areas. In rivers, for example, many researchers choose to sample the thalweg, but in smaller streams, a net may sample additional flow outside of the true thalweg, while a pump or grab sample would be able to sample more narrowly from only that zone. This may result in higher concentrations from more focused sampling methods. Additional investigations into the patchiness the distribution of particles at various sampling locations will help quantify the role of location selection in the volume-concentration relationship.

3.5 Potential contribution of contamination

Contamination as an explanation fits the systematic concentration differences observed due to the relationship between count, volume, and concentration. When a count is inflated in a small volume sample, the concentration is affected much more dramatically than if the count of a larger volume sample were inflated by the same number of particles.

A wide range of approaches were used to measure contamination. Of the datasets included in the literature-wide review, 28% neglected to run or report any blank samples alongside field samples. Less than 5% measured for contamination throughout the sampling processing (including from field instruments, ambient air, and laboratory processing methods and supplies). The most common type of blanks run was “procedural”, or “method”, blanks, where some quantity of filtered water was run through laboratory equipment and processes in parallel with field samples. Beyond the measurement of blanks, strategies for reporting and

accounting for the measured contamination varied widely. Of those that measured blanks, 16% failed to report how many particles were found during the process and only 28% removed contamination, if found, from reported concentrations. This is an improvement from previous reviews, such as Hanvey et al., who found only 7% of the microplastic studies included procedural blanks[138].

Despite the increasing prevalence of measuring contamination in the laboratory processes, not all potential pathways are being quantified. For example, field blanks are still uncommon[22]. As one rare example, Ryan et al. used a neuston net fitted with mesh at its mouth to exclude introduced particles and still captured 28 particles ($0.1/m^3$), assumed to be originating from the plastic net itself[139]. The contribution of sampler's clothing, pump tubing, or plastic lids on grab sample vessels are all still potential sources that require more investigation. Other laboratory-based sources of error are possible and understudied as well. Recent work by Witzig et al. indicates that even plastic gloves used for personal protection during lab work may be contributing to an overestimation of sample polymer content[140].

Inflated counts unrelated to contamination are also a concern. For instance, when visual counts are used to calculate concentrations, counts are often inflated by organic materials mistaken as plastics. 23% of the studies included in our overall literature analysis did not use any advanced microscopy or material identification methods to confirm polymer content of counted particles. As an example of the shortcoming of visual counting technique, Lenz et al. visually identified 1279 items as plastic but found through Raman spectroscopy that only 64% matched known polymer signals[141]. While visual overestimation would influence concentrations in a volume-sensitive way, we do not see systematic differences between studies that confirm particle

material. Additional unexpected pathways of inflated counts, false positives and contamination should be an emphasis of future work.

Contamination in the laboratory is typically minimal, but regularly present. It derives from fibers settling out of laboratory air (in our own 24-hour air blanks, we detected an average of 6 particles, all fibers), contamination of reagents, and particles in or on the variety of equipment and containers that typical multi-step processing requires. Procedural contamination would be consistent across all sample types run in a lab, but the same number of introduced particles would alter the concentration of a small volume sample to a greater extent than of a large volume sample.

In the literature, reported contamination ranges from zero particles in a blank to dozens. The actual number of particles measured in a blank likely depends not only on laboratory protocols, but also on the volume of water processed for a given blank, the duration of processing and the number of vessel transfers performed. It also is highly dependent on exactly what parts of the sampling, processing, and counting the blank undergoes.

Because of these inputs, it is difficult to compare blank values across studies directly. More commonly, they are put in the context of sample counts. For example, while Cable et al. measured an average of 42 particles in three blanks, mean sample counts in their high volume net samples ranged from 8 particles to 17,146 particles[77]. Similarly Scircle et al. detected an average of 35 particles in nine procedural blank samples, compared against particle counts within grab samples that ranged from 0 to 151 particles[31]. Hung et al. chose to omit all pumped samples from their analysis because of how similar blank and measured particle counts were (287 blank particles vs. 192 sample particles)[22]. For context, when we attempted to create and measure a

highest reasonable bound of procedural contamination by avoiding the careful cleaning and protections typical across the literature, we measured as many as 66 particles.

3.6 Lessons from a related field: plankton population research

Much of the sampling methods used for microplastics were adapted from plankton sampling. There are many parallels in terms of particle shape, size and distribution between the two sample targets. The results of plankton studies that perform similar paired-method comparisons, on plankton concentrations instead of plastic ones, report mixed results. Some, such as Cada and Loar, find no difference between icthyoplankton (4-10mm) densities sampled with net (100,000L) or with pump (16,700L) despite the pumped samples allowing smaller particles[142]. While icthyoplankton differ from microplastics in that they are able to actively avoid net capture, this comparison took place at night when avoidance is minimal. Others, such as Masson et al., report zooplankton (>0.053mm) concentrations being somewhat, though not statistically, higher when sampled with a pump (2-20L) vs. nets (10-220L) of the same mesh size[143]. And still others, such as Appel, found about two orders of magnitude higher concentration for zooplankton (>0.061mm) collected pumps (12L) or grab samples (2L) as opposed to those collected with nets (5,000-11,500L)[144].

We were unable to find any plankton method comparison studies with orders of magnitude concentration differences comparable to those we see in microplastics research (Table 2). This suggests the concentration differences in microplastic research are largely from factors unique to plastics. Contamination is one such explanation that fits. It is, for example, much easier to discern between zooplankton and lake debris than between a sampled plastic particle and a contaminating one. More targeted research is required to know for certain whether the

contributing factor truly is more easily concealed contamination, unique interactions with sampling equipment or another factor not yet identified.

Table 2. (A) A summary of the concentration ranges observed across the synthesized literature in this study, as well as (B) the observed and calculated concentration differences produced by potential biasing factors.

(A)

Method	Measured Concentration (particles/L)
Grab	$9.3 \cdot 10^{-3} – 1.7 \cdot 10^{3}$
Pump	$2.3 \cdot 10^{-4} – 2.7 \cdot 10^{2}$
Net	$3.5 \cdot 10^{-6} – 5.1 \cdot 10^{-1}$

(B)

Potential Biasing Factor	Orders of magnitude explained[a,b]
Mesh/filtration size[21,62,103,134,135]	$0\text{-}10^{2}$
Net Volume Overestimation[14,122]	$0\text{-}10^{1}$
Particles that enter net not captured in sample	Insufficient data
Intersample variability[22,25,134]	$0\text{-}10^{1}$
Contamination[c]	$0\text{-}10^{3}$

[a] Note: Values included for each biasing factor are not necessarily independent. Each assumes the entire observed concentration difference is due to a single factor, when in reality, no study method fully isolates for the tested factor. For example, concentration differences from two side-by-side samples may be driven by the patchiness of the sampled waterbody, but may also be driven by contamination additionally.
[b] Values are the ratio of concentrations from paired-method samples collected at same time and location from various published studies.
[c] Calculated using Equation 1 on paired-method samples included in (A) to find concentration differences that could be accounted for with a reasonable k (i.e. $k <$ sample count).

3.7 Assessment

We use Equation 1 to find the value of k that explains the difference in concentrations for paired-method studies (Figure 5). We start by looking only at reported values not yet corrected by blank measurements. We find that for the majority of published, non-blank-corrected paired-method studies, the introduction of only a few particles can explain the difference between grab and net concentrations (median: 3.4, mean±standard error: 39±1.4) and between pump and net

concentrations (median: 3.9, mean±standard error: 36±1.9). These values for the theoretical number of introduced particles (*k*), even at their highest, are well within the range of values reported in the literature (Section 3.5). The skewed results for *k*, however, reinforces the observation that the number of introduced particles varies substantially among studies.

For a more study-specific test of our contamination-alone assumption of Equation 1 and to assess whether *k* is reasonable within individual studies, we focus on 11 of the paired-method studies that both ran blanks and report the number of particles found in those blanks. For each study, we compare the particle counts measured in blanks run within the given study against the theoretical number of introduced particles (*k*) needed to satisfy Equation 1. For the seven grab-net studies and the two pump-net studies with available blank counts, theoretical contamination differed from actual measured blank counts by less than one particle (an average of 0.57 particles and 0.60 particles, respectively). These preliminary values indicate contamination alone (or in conjunction with another additive affect) can explain nearly all of the observed concentration differences observed between samples of differing methods and volumes. It also suggests, however, that current contamination quantification methods are not universally sufficient for identifying and removing contamination introduced into each sample, given studies like Hung et al., which remove a standard blank count from sample counts and still find incompatible concentrations[22].

A combination of the examined factors, including contamination, could also be at play. Though the values included in Table 2B are not fully independent of each other, in sum and at their extreme, they can cumulatively account for the full concentration discrepancies observed. To determine with certainty the factors at play and identify adequate methodological

interventions to correct for them, these biasing factors must be isolated further through targeted research.

3.8 Recommendations

Differentiating between plastics from environmental samples and from contamination is impossible with current methods, which makes precautions to avoid contamination at all times and measuring blanks throughout processing imperative to reliable results.

Based on limited existing data, we can recommend that blanks be (1) run repeatedly throughout the processing of a pool of related samples, (2) run through all items and spaces in contact with samples, including mesh and steps completed in the field (3) adjusted, when reported, for relevance to sampled volumes, exposure times, and particle counts, and (4) thoroughly described such that a true "methodological peer" can be identified by future studies for concentration comparisons.

Cross-study or multi-method comparisons and compilations should be avoided when possible, until specific experiments can be performed to isolate and remedy the systematic differences in concentration observed. This has broader implications in terms of policy decisions that rely on a compilation of various studies; describing regional trends from a combination of individual studies or creating forecasting models based on disparate studies is a risky endeavor at this time. We also encourage study designs that allow relative abundance comparisons within a sampling campaign, as this analysis strategy can control for biasing factors and avoid misleading inter-study concentration comparisons.

Until standard methods for contamination quantification are developed, we recommend large sampling volumes be used, regardless of method choice, to mitigate the influence particle

count inflation can have on overall sampling volume. We, unfortunately, were unable to detect a volume threshold above which samples were unaffected, and thus, we are unable to recommend a specific volume. Correcting for mesh size[136], collecting repeat samples, selecting sampling sites randomly, and confirming visual counts with advanced techniques are all important steps to accurate microplastic quantification, as well. We remain hopeful that researchers and citizen scientists will continue to be able to use sampling methods that best suit their needs so long as proper corrections, considerations, and contamination quantification protocols are followed.

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Supporting Information

1) Document of details on field methods used and blank sample measurements, as well as additional visualizations of the synthesized data from this review (all included tables and figures are referenced directly in text). 2) Data used for this study in zipped folder, including our own

field sample results and a spreadsheet of all studies and attributes used for this meta-analysis.

This information is available free of charge via the Internet at http://pubs.acs.org.

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