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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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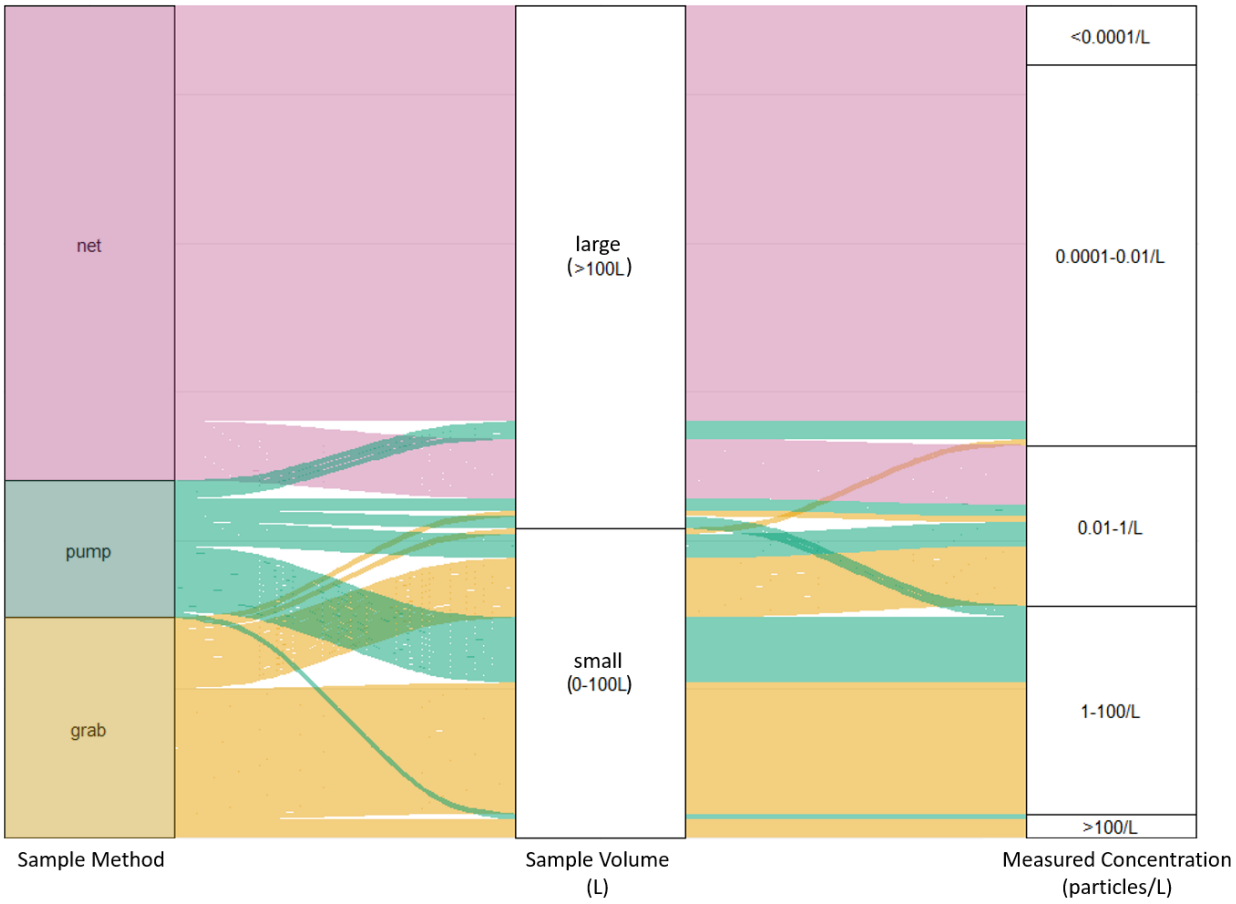
Keywords

Plastic, pollution, surface water, net, grab, pump, contamination, mesh size

Graphical abstract:



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 over one hundred surface water microplastic studies. We find systematic relationships between measured concentration and sampled volume, method of collection, mesh size used for filtration, and water body 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 biases explored included filtration size ($\pm 10^2$ particles/L), net volume overestimation ($\pm 10^1$ particles/L), fiber loss through net mesh (unknown magnitude), and intersample variability ($\pm 10^1$ particles/L). Contamination is the one potential bias with an effect large enough ($\pm 10^3$ particles/L) to explain the observed differences. Based on these results, we caution the practice of comparing concentrations across multiple studies or combining multiple study results to identify regional patterns. Additionally, we reiterate previous recommendations emphasizing the importance of contamination reduction strategies, namely that blank samples be collected, tested, and reported as a matter of course for such 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." 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. 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 very small sieves to capture smaller particles than typical net samples.

Several previous studies have reported dramatically different microplastic concentrations from samples collected using differing methods[16–21], as well as preliminary evidence to suggest systematic trends[22]. 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 body of literature for policymakers and researchers to rely on.

2. Methods

2.1 Literature review:

We performed a literature search of surface water microplastic studies published prior to October 2020. We used Google Scholar searches of the words: “microplastic” + “surface water”, along with (individually) “net”, “pump”, “bulk”, “discrete” and “grab”. Studies were included if they sampled within the top 1m of a waterbody 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 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 overall literature review. One of these datasets was collected specifically for this study (Section 2.2).

We identified a variety of potential factors influencing the concentration trends observed through literature review and solicitation of hypotheses from field experts (Figure 1). We rely on multiple linear regression and backward selection to determine which of the following factors

were significant in predicting measured concentration: sampled volume, sampling method, filtration size, sampled waterbody (freshwater vs. marine), whether visual particle identification was confirmed with a more advanced technique, and whether chemical extraction processes were used. Regression assumptions were checked, and correlation between variables was considered while interpreting results. To determine whether the percentage of fibers differed between paired samples of differing methods, a paired t-test was used. For all statistical tests, we used a p-value upper-bound of 0.05 to determine statistical significance.

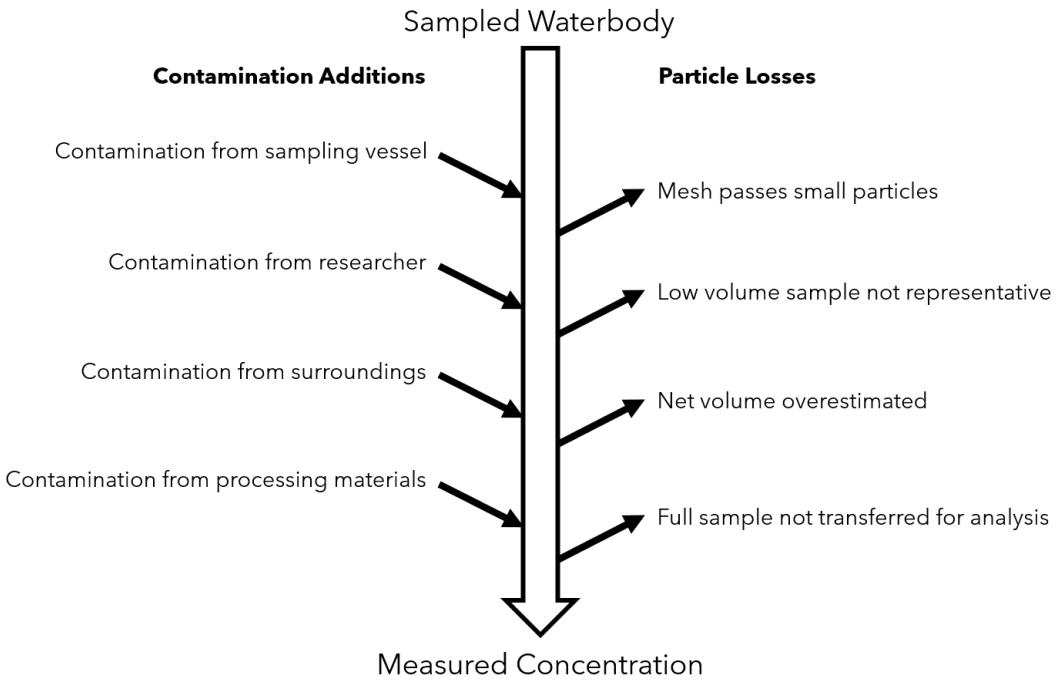


Figure 1. A conceptual diagram of the pathways that may increase (left) or decrease (right) measured concentration, from the sampling of a waterbody to transferring and processing a sample to the quantification of particles in said sample.

To explore the potential effect of an additive factor like contamination on measured concentration, we use Equation 1, to find a rough estimate of the number of contaminating particles, or other additive factor, needed to equate two 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 contamination, *V* is the volume of the sample, and subscripts denote each sample of a pair.

Equation 1 relies on the assumption that there is a level of contamination affecting all processed samples to a similar extent and that there is a true concentration that any paired samples should report. This equation includes two major simplifications: one, that intersample variability is zero (we know side-by-side samples to vary up to $9x^{23}$) and, two, 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.

2.2 Field samples:

To include in the paired 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 grab-net paired 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. Further laboratory processing details, particle identification, Raman confirmation, and contamination reduction are included in the supplementary information.

In addition to typical air and procedural blanks, we also 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.

3. Results and Discussion

A total of 118 studies were included in this literature review. 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[11,16,17,21,22,24–53], 80 using a net method[8,10,13,16,20,21,23,25–28,33,34,42,48,54–115], and 23 of a pump method[20,21,45,60,67,89,101,116–128] 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).

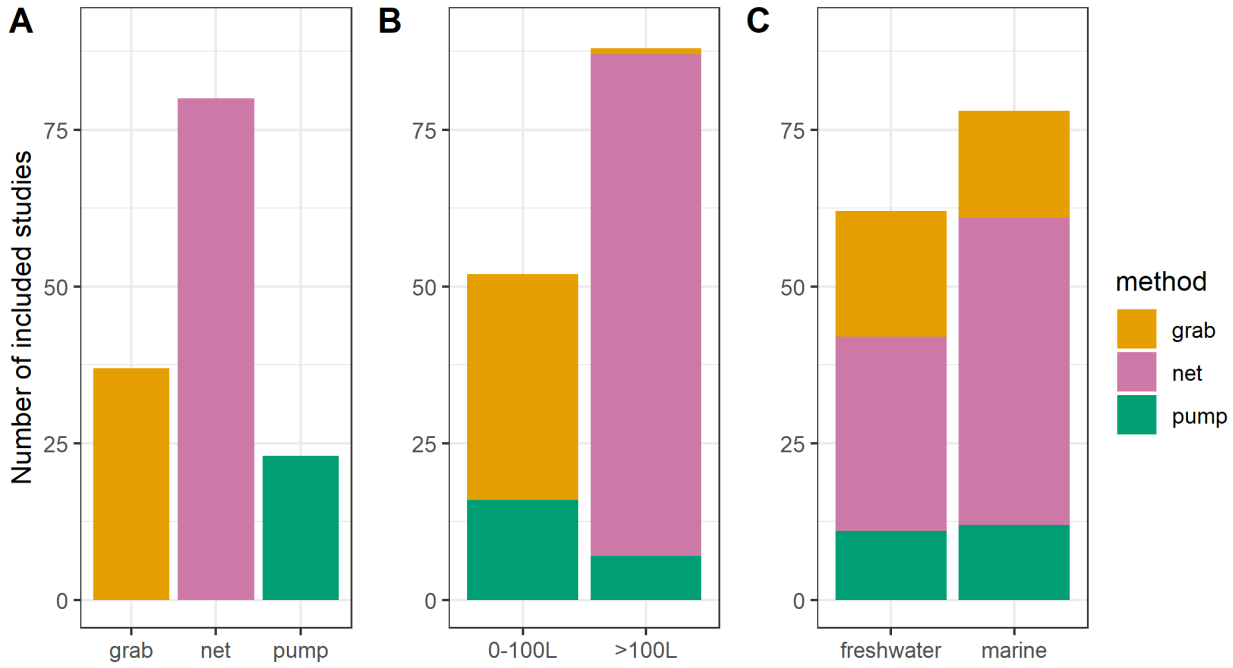


Figure 2. Summary of the unique entries included in this literature review, including sampling method used (A), binned sample volume (B), and sampled waterbody type (C).

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.

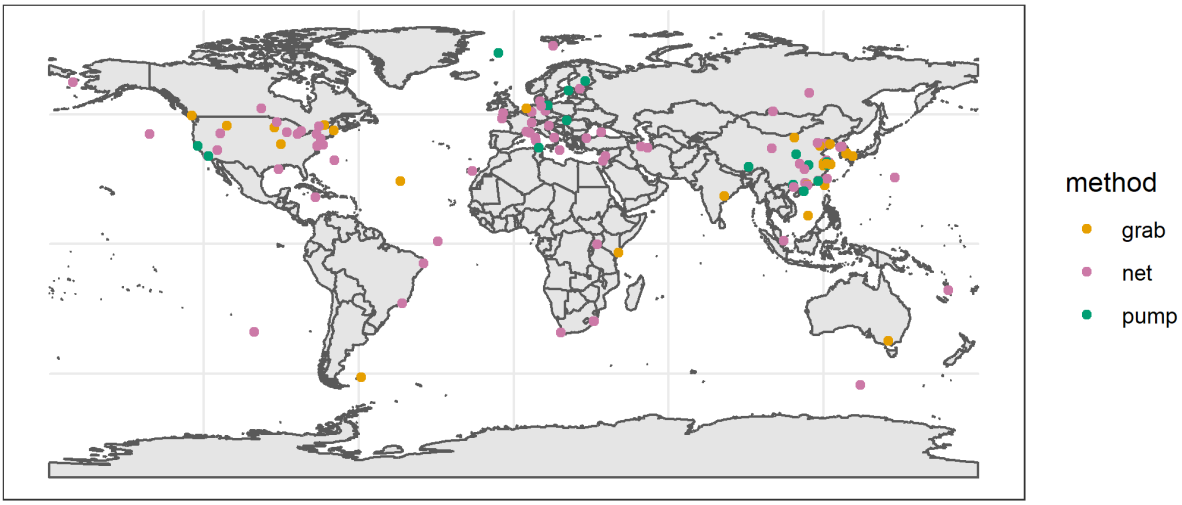


Figure 3. Global distribution of samples included in this analysis.

Across the literature analyzed, volume sampled correlated strongly with measured concentration (Figure 4). Grab samples, relatively low volume, systematically resulted in significantly higher microplastic concentrations than net samples, which sample large volumes of water. Pumped samples, which consist of a wide range of intermediate sample volumes, represented concentrations that overlapped with and fell between grab and net sample concentrations.

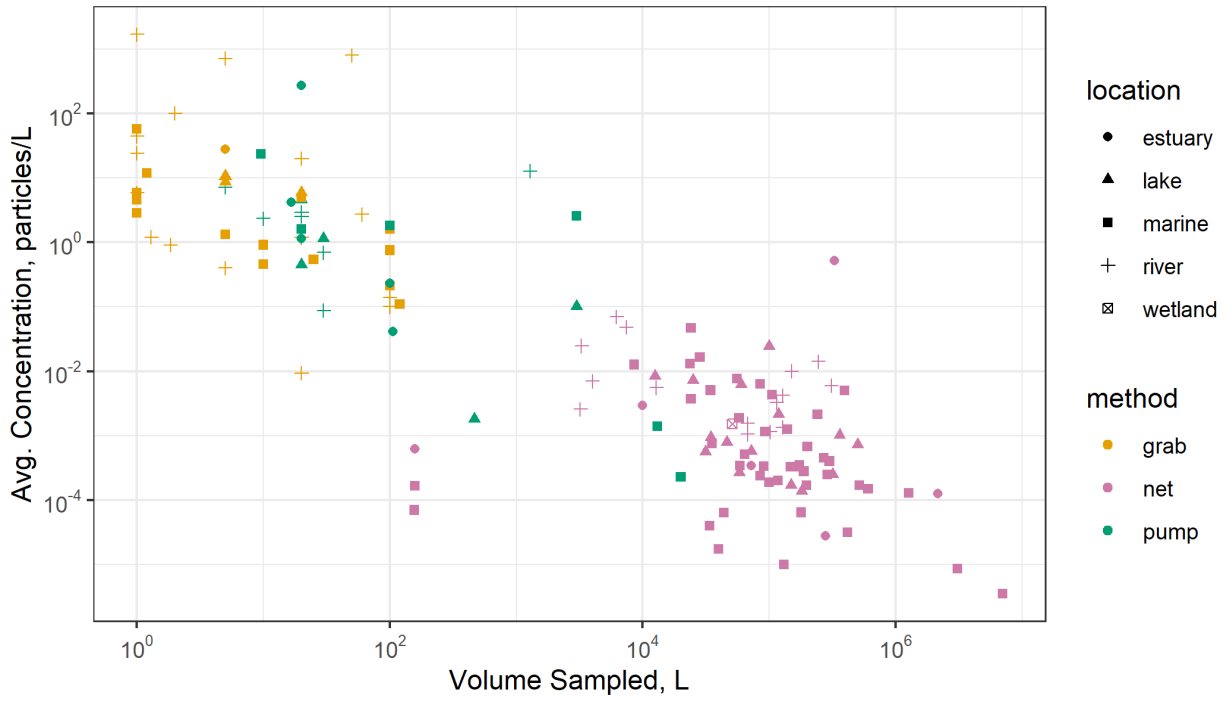


Figure 4. Average volume sampled in studies relying on differing methods (color) and in differing waterbodies (shape) and the average concentration measured in each of those studies.

A multiple linear regression run on the overall dataset indicated volume was the most significant predictor of concentration (Table 1). Method and mesh size are correlated with sample volume, but they were found to include enough independent information to also be significant factors in predicting concentration. Pump and grab sample estimates were not statistically significant from one another but were both different from net sample estimates after accounting for all other factors (Table 1). As is also evidenced in Figure 4, pumped sample concentrations appear to be affected only by their volume and not uniquely by the method itself.

The regression also indicated that sampled waterbody type was a significant predictor, with marine samples tending to measure lower concentrations. The performance of a chemical extraction process was highly colinear with sampled waterbody type, likely due to the fact that

both freshwater sampling and more advanced laboratory methods have come about in more recent years. We have chosen to include waterbody type in the best fit regression (Table 1) because it offers a more defensible relationship to 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	1.26	0.19	6.68	$6 \cdot 10^{-10}$
Log_{10}(Volume)	-0.51	0.10	-5.20	$7 \cdot 10^{-7}$
Method = Net	-1.11	0.44	-2.55	0.012
Method = Pump	-0.10	0.27	0.37	0.71
Smallest mesh size	-1.61	0.66	-2.43	0.017
Waterbody = Marine	-0.38	0.17	-2.26	0.026

[a]Adjusted R squared value for this regression model is 0.78, with an F-statistic of 97.0 on 5 & 134 degrees of freedom and a p-value of $2 \cdot 10^{-16}$.

When looking specifically at the 15 studies that collected pairs of samples using differing methods (Figure 5), the same trend is apparent: grab samples tend to measure orders of magnitude higher concentrations than net samples. A few sample pairs (28 out of 310 paired 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. Koelman et al. take note of this shortcoming in their review and recommend a minimum sample volume in surface waters of 500L[129].

One explanation of the volume-concentration relationship disproven by paired 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 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).

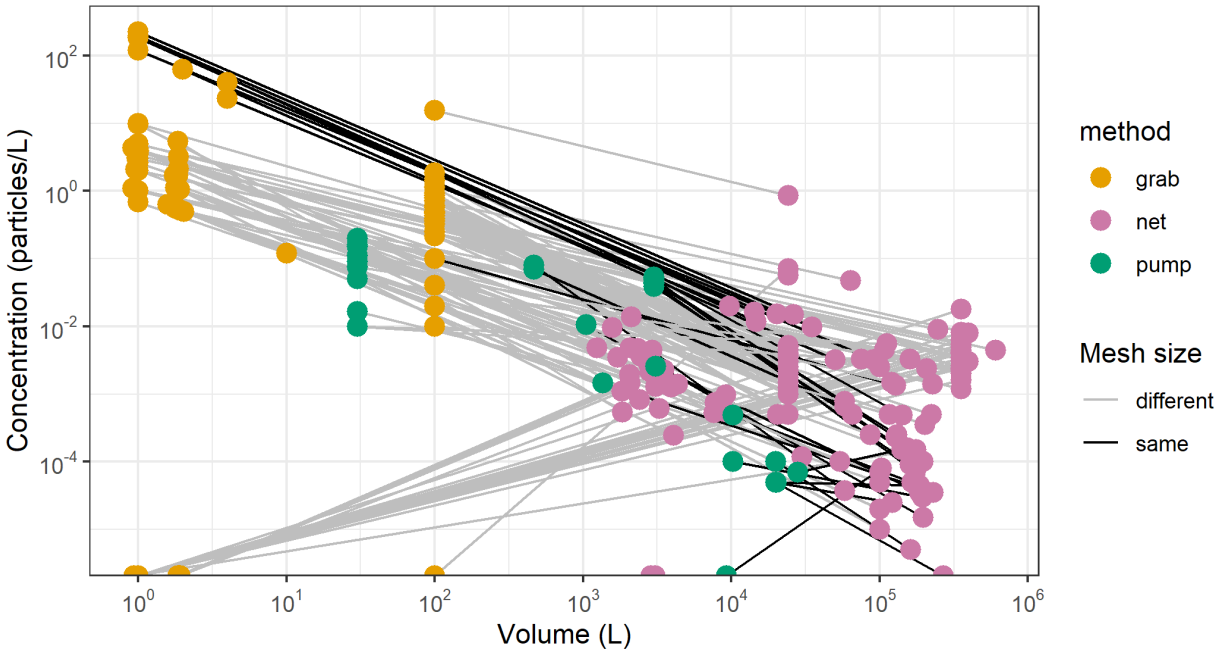


Figure 5. Studies that collected pairs of samples at given sites using differing methods. Lines connect a pair of samples collected at the same time and place. Black lines connect paired samples that are filtered through the same mesh size, while gray lines connect those that used two different mesh sizes. Zero concentration is adjusted to 10^{-6} particles/L to account for log-scale limitations and is plotted along the x-axis.

3.1 Potential contribution of mesh size differences

What this paired sample analysis makes clear is that the systematic, orders of magnitude differences in concentration cannot be explained by mesh size differences alone (Figure 5). To some extent, mesh size does matter: when you allow smaller particles to be in your sample, you will likely collect more particles overall[20,60,130,131]. Smaller volume samples, specifically grab and

some pump samples, allow for a smaller mesh or filtration size to be used. Across a variety of mesh size comparisons, for both pump and net methods, there is evidence that mesh size affects concentration, with, in the case of Lindeque et al. 2020, 100µm mesh measuring 10x higher concentration than 500µm mesh[130]. Mesh size should therefore be noted in methods and considered when comparing between studies. The concentration differences reported by studies pairing these kinds of studies is still dwarfed, however, by the magnitude of concentration differences across sample volume and method.

To better support the fact that volume is a greater determinant of measured concentration than mesh size is, we turn to paired studies. Paired samples of equal filtration size, including our own field samples, still resulted in different concentration (black lines, Figure 5). Net samples measured lower concentrations than those sampled by grab, despite both being filtered through the same size mesh. Across the broader literature review, few mesh sizes are represented by differing methods. For those that do overlap, however, net concentrations are again lower than grab and pump samples (Figure S2).

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.

3.2 Overestimating 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%[120]. 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 S3); 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 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. Fibers are the most dominant type of particle in the included studies, followed by fragments (Figure S4). One unsupported explanation for lower net concentrations is that fibers are lost through the mesh of nets at greater rates than from pump or grab samples due to extended water pressure during net sampling. 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[132]. This study finds no significant evidence of that type of fiber loss: paired studies that included particle type showed statistically similar percentages of fibers between differing method samples (paired t-test, p-value = 0.56). While our literature review found a majority of net studies reporting fragments as the most common particle type, followed closely by fibers (Figure S4), too many compounding factors exist for this evidence to contradict the controlled paired studies that show no such trend. The lack of evidence may be explained by the fact that some fibers small enough to escape through net mesh are actually retained[21], suggesting that the fibers unintentionally lost may be negated by those unintentionally captured, an overall null effect on concentration.

One additional way microplastics could be lost from net samples is by being captured in the net, but not transferred into the sample. 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. There is potential for interaction between plastic net mesh and microplastic particles, so we encourage future studies to examine net mesh before and after sampling to add to this body of knowledge.

3.4 Potential contribution of intersample variability

Given that all waterbodies are heterogeneous to some extent, it is reasonable to assume that no two water samples will hold the exact same contents. For this reason, one suggested explanation for differing concentrations holds that it is actually the replication and not necessarily the methods themselves that create the variance observed in paired grab-net studies. To some extent, this is disproven by the systematic differences observed across unpaired studies of varying methods (Figure 4).

To investigate whether this may, however, play even a minor role in the differences observed in paired-sampling studies, we rely on existing studies which have measured the concentration variance between replicate studies of the same sampling method. Lindeque et al. towed two nets (0.333mm mesh-size manta trawls) in parallel and found no significant difference between the measured concentrations (0.54 and 0.46 microplastics m^{-3})[130]. Schmidt et al. found triplicate net samples taken within 2 hours of each other varied up to 9x[23]. Hung et al. found duplicate net samples had a standard deviation of less than 15%, while duplicate grab samples varied by 2x[21]. 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).

3.5 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.

The majority of studies included in this review (71%) mentioned running blank or control samples to measure contamination. 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[133].

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[21]. 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[134]. 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[135]. 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), 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[75]. 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[29]. 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)[21]. 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 (1982), 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[136]. 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[137]. 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)[138].

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[20,60,101,130,131]	$0\text{-}10^{2}$
Net Volume Overestimation[14,120]	$0\text{-}10^{1}$
Particles that enter net not captured in sample	Insufficient data
Intersample variability[21,23,130]	$0\text{-}10^{1}$
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 samples collected at same time and location from various published studies.
[c] Calculated using Equation 1 on paired 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 the studies that collected paired samples of differing methods (Figure 5), including only values not yet corrected by blank measurements. We find that for the majority of published 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) 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 sample studies: those that 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[21].

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. Focused research on potential sources of error and contamination (Figure 1) are crucial to an eventual ability to compare concentrations across studies and methods. Until then, all reported microplastics concentrations should be accompanied by a limit of quantification, paired with clear and thorough descriptions of the types of blanks used to determine it.

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 containers, mesh, and spaces that samples will be run through, (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 for concentration comparisons by future studies.

While literature context typically helps inform new concentration measurements, the analysis presented in this paper indicates that sampling method, in particular sampled volume, affects measured concentration to such an extent that these broader comparisons, particularly across differing sampling methodologies, are misleading. This also 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. Until specific experiments can be performed to isolate and remedy the precise cause of the systematic differences in concentration observed in this work, cross-study or multi-method comparisons and compilations should be avoided when possible. Instead, we recommend comparisons be made only between concentrations that use the

same sampling method and have corrected measured concentrations by contamination estimates determined from equivalent blanks. Designing sampling strategies that allow spatial analyses to be performed on relative abundances within a given sampling campaign may help avoid misleading inter-study concentration comparisons, as well.

Data Availability

All data used in this study will be made available prior to publication.

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Supplementary Information

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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Table of Contents

A. Field sample processing methods

After samples were returned to the laboratory, both types of samples were processed in the same way: wet peroxide oxidation, density separation, and visual inspection of all particles under a dissecting microscope[1]. The Marine & Environmental Research Institute's visual "Guide to Microplastic Identification"[2] was used to identify potential plastic items, in addition to a hardness test performed by probing suspected particles with forceps[3]. Particles were then characterized by their particle shape and color. This identification process was performed by an average of 2 researchers, in sequence, per sample. Counts were then averaged.

A subsample of suspected microplastic particles were validated using Raman microscopy (WITec Alpha300R Confocal Raman Microscope) at 20x magnification. A 532nm laser was used at 1-2mW power to produce spectra. The resulting spectra were then compared with spectra in the Bio-Rad KnowItAll Informatics System (2018) spectral database. Sensitivity of this validation was 100% and precision was 88%, indicating that our visual identifications matched spectral identifications well.

To reduce contamination during this process, in the field, samplers remained downstream of the sampling site at all times. All vessels, including field and lab containers and sieving mesh were triple rinsed before coming in contact with the sample. In the lab, researchers wore white cotton lab coats and blue nitrile gloves at all times. Samples were kept covered when not in use.

Three types of blanks were collected to measure laboratory contamination: (1) "Air blanks" consisting of three filter papers left exposed to laboratory air for 24hr, (2) "Procedural blanks" consisting of five deionized water samples run alongside stream samples through each step and container the laboratory process, (3) "Maximum reasonable blanks" were also collected

after the completion of this work and after the lab space and equipment had been used extensively for laboratory courses and demonstrations.

B. Field sample blanks results

Table S1. Results of blank samples collected in the laboratory alongside the processing of field samples that were collected for this study.

	Air Blank	Ordinary lab procedural blank	Worst-case lab procedural blank[a]
Blank 1	4	2	66
Blank 2	7	1	45
Blank 3	5	0	59
Blank 4	NA[a]	0	NA
Blank 5	NA	1	NA

[a]NA: Not applicable. (only 3 blanks were collected for air and for worst-case blanks)

C. Figure S1: Overview of publications included in literature review (by sampling year).

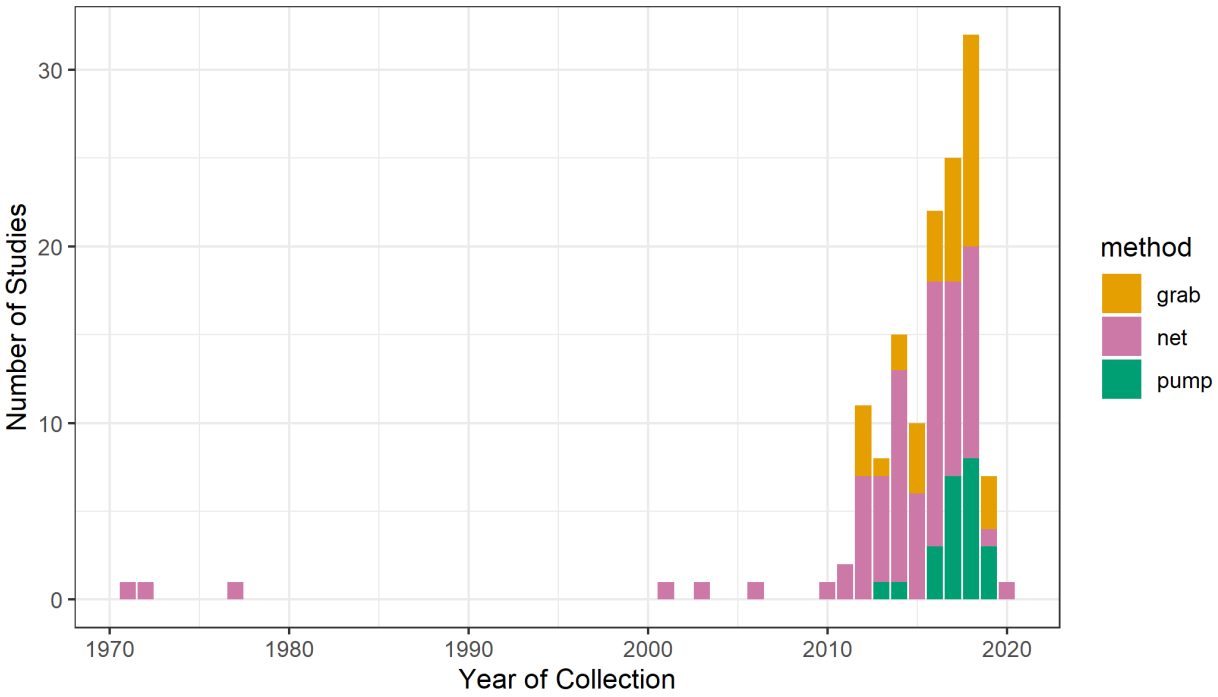

Figure S1. Histogram of the publications included in this literature review. Multi-year studies are included on final year of collection. Studies that did not report sampling dates are included in their year of publication.

D. Filtration techniques used by included studies

There are three main ways in which the bottom of a sample's size range gets enforced. For net samples, this is always plastic mesh, which makes up the net itself. Pump and grab samples rely mainly on either metal sieves or filter paper. This literature review contained studies relying on each of these methods (9% filter, 25% sieve, 66% plastic mesh) and found no difference between them in terms of concentration measured. Theoretically, however, it is possible that the way particles interact with each of these filtration strategies differs in terms of potential for contamination, propensity for sticking to and within the mesh, and fluid dynamics through differing shaped or spaced orifices.

E. Figure S2: Filtration size used, as it relates to concentration measured

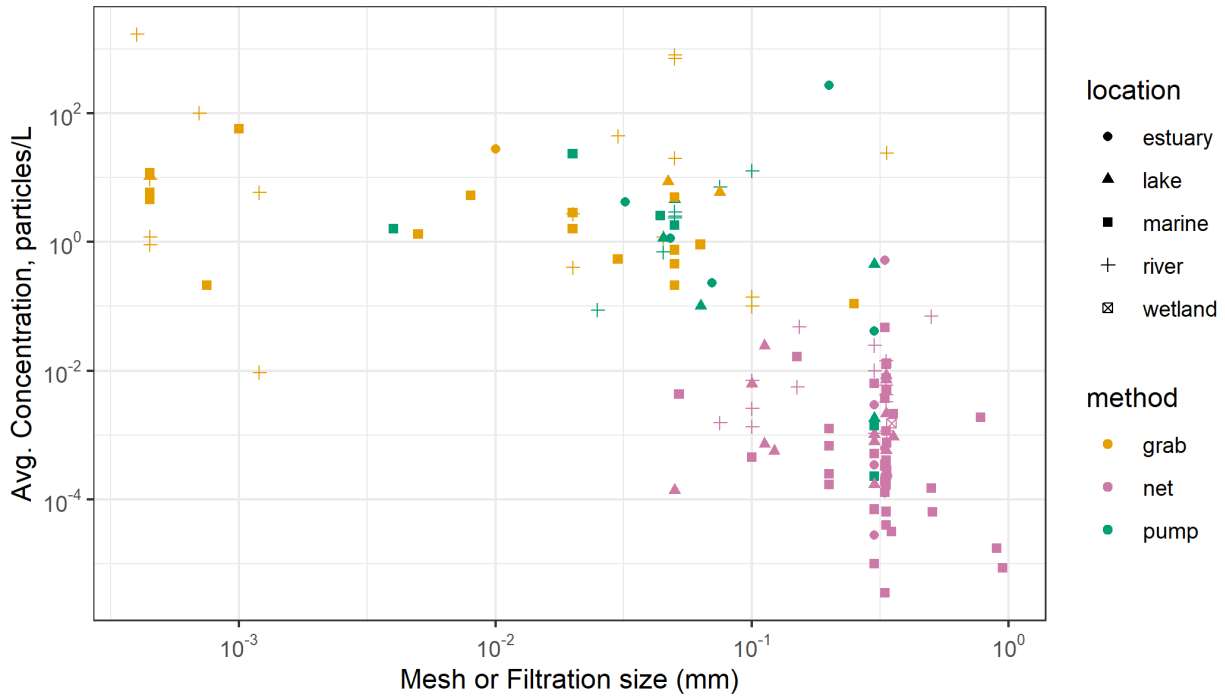

Figure S2. In the few instances where differing methods use the same mesh size, there still appears to be a measurable divide between concentrations measured from pump and grab methods versus those from net samples. Additional, targeted work will need to be done in order to confirm this further, as replications at this point are limited to only a few studies with overlapping mesh sizes.

F. Figure S3: Comparison of slopes for net sample volumes given & calculated

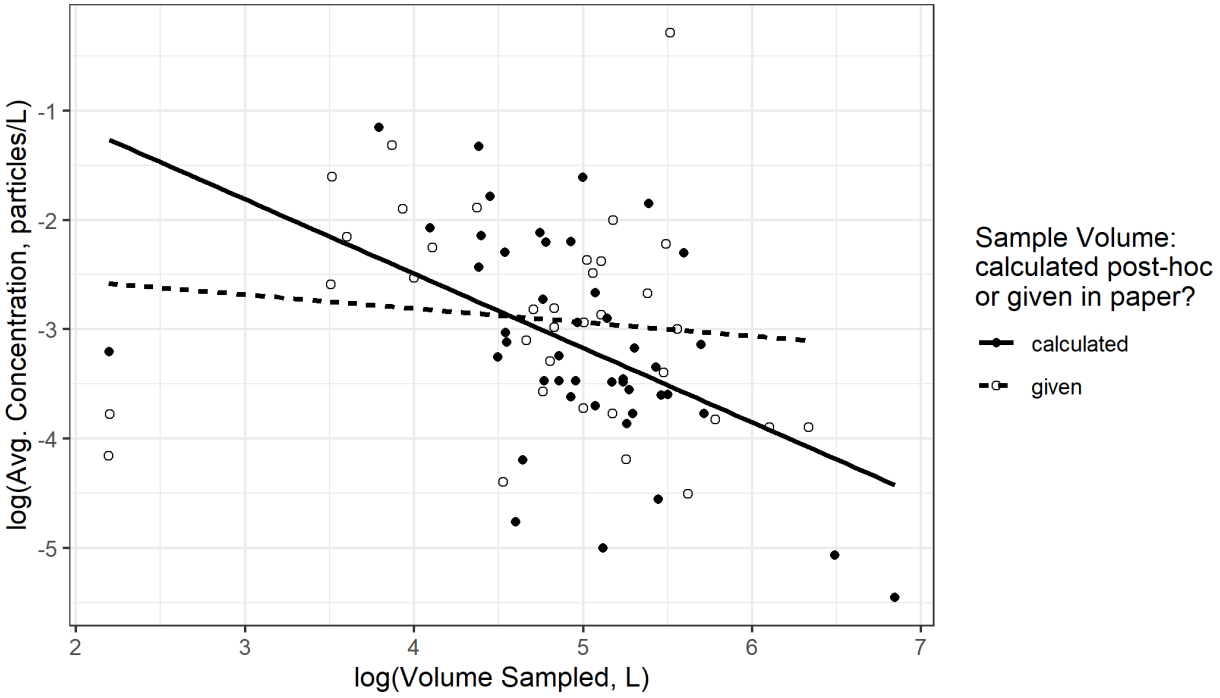

Figure S3. All net samples included in this analysis, filled according to the source we used to determine sample volume. Lines are a simple linear relationship for those studies that reported sample volume (dashed) and those where we calculated sample volume based on an average sample depth and a given boat speed, distance or area.

All studies using grab and pump samples included in this analysis reported sample volume used, so this assessment of our post-hoc volume calculation (Figure S3) focused solely on net studies. Clearly the relationships between volume and concentration differ between the two groups of net samples: samples where volume was calculated post-hoc showed a stronger relationship, driven largely by the outliers. A more shallow slope actually results in net samples being more distinct than grab and pump samples in a piece-wise style, which means that sample method ends up as a bigger influence than sample volume. Statistically, our regression (Table 1)

fit remains unchanged even when removing studies where post-hoc volume calculations were needed, which suggests that in practice, this potential contribution of error does not alter our results or conclusions.

G. Figure S4: The most frequently identified particle type for reviewed literature

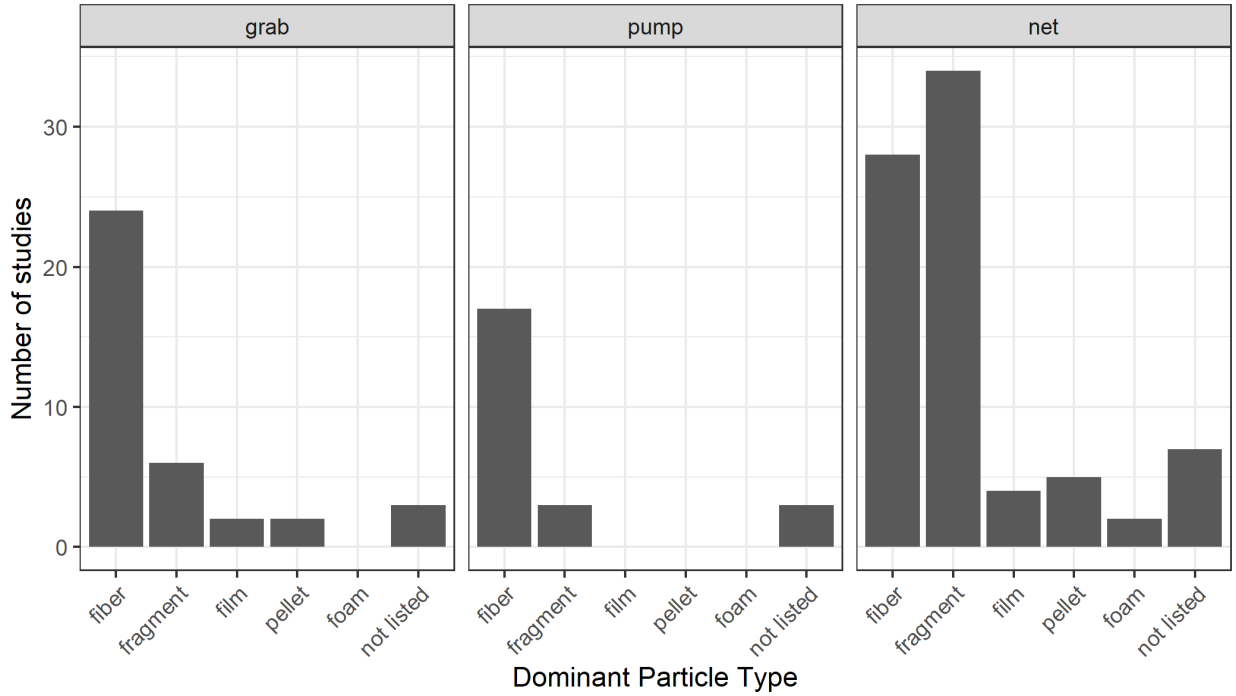

Figure S4. A summation of studies in the analyzed literature the report a given particle type as the most common found in their samples.