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Grab *vs.* neuston tow net: a microplastic sampling performance comparison and possible advances in the field†

Abigail P. W. Barrows,*[abc] Courtney A. Neumann,[ab] Michelle L. Berger[a] and Susan D. Shaw[ad]

With the rapid evolution of microplastic research over several decades, there is an urgent need to compare methodologies for quantifying microplastic in aquatic environments. The most common method for sea surface sampling is a neuston net tow. This method captures microplastic from large water volumes, and although is widely employed, it is specifically designed for studying plankton ecology. Its effectiveness for microplastic research is limited by the net's mesh size as well as the likelihood of contamination. In our study, we compared a 1 L surface grab sampling method to a 335 μm neuston net tow. Grab sampling collected over three orders of magnitude more microplastic per volume of water as well as a smaller size range and greater proportion of non-fibrous plastic than sampling with a neuston net. Consequently, solely relying on neuston net samples appears to result in an underestimation of the extent of microplastic pollution. For studies aiming to capture and sort larger microplastics without a microscope, the neuston tow method is preferred, since it samples a greater volume of water, increasing the potential of capturing microplastic pieces. Grab sampling can capture plastic at the micro- and nano-scale and in environments where neuston nets are impractical, but the small volume of water sampled may result in high variability among samples. The comparison of these techniques comes at a critical time when sampling methods need standardization for the accurate measurement of the distribution and composition of microplastic in aquatic environments worldwide.

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Introduction

Plastic pollution is a threat to marine ecosystems. Debris is widely documented in diverse locations including coastlines, surface water, the sea floor, and the poles.[1–7] For the past 50 years, global plastic production has been increasing, with cumulative production estimated at around 5 billion tons.[8] Annual production of 311 million tons is expected to increase to yield a cumulative production of 33 billion tons by 2050.[9,10] An unfortunate result of this mass production is that an estimated 4.8 to 12.7 million metric tons of plastic waste currently enter the oceans each year from land based sources, and if anticipated trends persist, this number will continue to grow.[11,12]

While the amount of plastic entering the marine environment continues to increase, the average size of individual plastic particles appears to be decreasing.[1] Microplastic is most commonly defined as pieces of plastic less than 5 mm in size,[13,14] but there is no widely recognized lower size limit in distribution and abundance studies. In an attempt to further define the size range of microplastic and reduce reporting inconsistencies, previous studies differentiate microplastic into large (1–5 mm), small (<1 mm)[13,15–18] and nano (<100 nm)[19,20] size classes.

Microplastic particles have been documented on the surface waters of every major ocean.[21] The majority of plastic litter originates from the coastal zone[12] and is transported throughout the marine environment *via* wind, currents[22,23] and animal ingestion.[24] Over time, plastics fragment into microplastics *via* chemical, mechanical, and biological processes.[25]

Microplastic research has quickly evolved over several decades, with many studies sampling microplastic in surface waters globally.[22,26,27] To meaningfully synthesize these data or make comparisons, it is necessary to understand the differences in methods for both the sampling and sorting of microplastic.[14,16] The current lack of standardization has hindered effective data sharing, and undermined cohesive understanding of microplastic abundance and potential impacts.[28–30] For example, different techniques result in different reporting units, targeted size ranges of particles, and quality control.[14,29,31,32]

[a]*Marine & Environmental Research Institute, Blue Hill, Maine, USA. E-mail: Abby@adventureandscience.org*

[b]*Adventure Scientists, Bozeman, Montana, USA*

[c]*College of the Atlantic, Bar Harbor, Maine, USA*

[d]*Department of Environmental Health Sciences, School of Public Health, State University of New York at Albany, Albany, New York, USA*

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The most common method used for sampling the sea surface is a neuston net tow.[22,33–36] Originally designed for plankton collection, neuston tows sample a large volume of surface water. Neuston nets collect plastic pieces as they sieve through the water and can be coupled with plankton monitoring.[37] The widespread use of surface nets allows for easy comparison between data sets, but the units of measurement for calculating microplastic concentration vary from surface area (m^2 and km^2) to volume of water sampled (m^3). Moreover, the lower size range of plastic captured by this technique is fixed by the mesh size of the net (most commonly 333 μm or 335 μm). Use of a smaller mesh size significantly increases the amount of plastic collected;[38,39] therefore, many studies are likely underestimating plastic concentrations in aquatic environments. In addition, a common form of microplastic is the microfiber, defined in this study as a threadlike piece of plastic with a length between 100 μm and 5 mm and a width approximately 1.5 orders of magnitude shorter. Microfibers are debatably the dominant type of microplastic in aquatic environments[39,40] and are highly susceptible to passing through a net due to their small diameter.

Sample contamination is a constant concern in microplastic research.[14,41] Significant concerns for neuston tow sampling include air exposure time, microplastic in rinse water, and microplastic contamination from the net. Many of the contamination issues may not apply to neuston tow studies focusing on naked-eye identification of larger pieces of microplastic. Microplastic studies are increasingly reporting steps taken to address or record quality control measures in the laboratory[3,26,42,43] but are still infrequent in the field.

Seeking to advance this field of research by testing new alternative methods, we compare sampling with a neuston net to an alternative sampling method. The grab method entails filling a 1 L non-plastic container with surface water *in situ* and then filtering the sample in a laboratory. Less water is analyzed using this grab method, but it allows for filtering at the micron scale and can be extended to a greater diversity of sampling locations, including wastewater outfall sites, intertidal zones, and very shallow aquatic habitats. Additionally, the field portion of the grab methodology can be easily integrated into long-term or citizen science monitoring initiatives due to its simplicity and low-cost equipment demands.

The aim of our study is to compare these two sampling methods and make recommendations to improve future quantification of microplastics in aquatic environments worldwide.

Materials and methods

Sample collection

We collected paired neuston tow and grab samples over three days in October, 2014 in surface waters (top 45 cm) off the Maine coast. Sea conditions ranged between 0 and 2 on the Beaufort scale. We recorded rainfall in the previous 48 hours and ambient wind speeds at least two times during each tow.

Neuston tow

The neuston net had a rectangular opening 45 cm high × 95 cm wide, a 4 meter-long net, and a 31 cm high × 9 cm diameter, 335 μm mesh cod end. We collected two 0.5 nautical mile neuston tow samples on each sampling date. The net was towed along the surface on the downwind side of the vessel, outside of the ship's wake. The net sampled the sea surface microlayer (SML, the top 1 mm) to a depth of 45 cm. Tow contents were washed from the outside of the net with a seawater hose into glass sample jars with aluminum foil-lined lids. Jars were rinsed with tap water in the lab three times before sampling, immediately capped, and rinsed three times *in situ* with seawater at the time of sampling. Jars were transported to the laboratory for processing. For this study, the number of particles per volume of water sampled by each method was compared. For neuston tows, we estimated the volume of water sampled by multiplying the tow length by the area of the submerged opening. For comparison with other neuston tow studies, we calculated particle abundance per square kilometer for the neuston tow samples by multiplying the tow length by the trawl width.[33,38]

Grab sampling

In conjunction with each neuston tow, one liter of surface water was collected at 0, 0.25, and 0.5 nautical miles making for a total of three grab samples per tow and six per sampling date. Samples were collected in glass jars with foil-lined lids. We rinsed the jars three times with tap water in the lab pre-sampling, immediately applied the caps, and then rinsed them three times *in situ* with seawater at the time of sampling. Samples were taken immediately after the seawater rinse and capped underwater to reduce air exposure time. Minimizing air exposure time reduces potential airborne contamination of the sample. Samples were taken on the downwind side of the boat in the top 45 cm of the water. Grab sampling collected water from the SML down to 45 cm. Jars were transported to the laboratory for processing.

Laboratory processing

In the laboratory, neuston tow samples and grab samples were processed similarly. Prior to opening samples, work surfaces were wiped with a brightly colored sponge so that any microplastic fragments from the sponge could be easily identified in case of contamination. Hands, forearms, Petri dishes, and tools were thoroughly rinsed three times under high pressure tap water. Water samples were poured directly (grab samples), or pipetted (neuston tow samples) into a glass filtration apparatus (Buchner funnel and vacuum flask) and vacuum pumped through a 0.45 μm filter (Whatman mixed cellulose nitrate, 47 mm diameter). The only processing variation between the two sampling techniques was a two-step filtration process and pipetting for the neuston tow samples (*versus* direct pouring) due to the dense layer of settled biological material that would be agitated if poured. For neuston tow samples, the original supernatant was pipetted to the filtration apparatus. Then, a 500 mL hyper-saline solution (250 g NaCl per L 0.45 μm

filtered seawater) was added to the remaining solution for flotation of plastic particles[44] and once settled, the resultant supernatant was pipetted into the filtration apparatus. While there was a minimal amount of sample material left in the neuston tow samples that was too dense to filter, the use of the hyper-saline floatation combined with the small pore size of the filters captured more microplastic than traditional neuston tow studies. Most microplastics have a density of <1.2 kg L^{-1} (ranging from 0.8–1.4 kg L^{-1}) and will float in a hyper-saline solution.[14] Also, it is unlikely that there were any plastics denser than seawater (or the hyper-saline solution) captured in the neuston net due to the site location (surface, offshore) and lack of wind on all sampling days. The lowest size limit used for net sampling to our knowledge thus far is 50 μm.[38] Quantifying this difference is important since this is over two orders of magnitude larger than the 0.45 μm lower limit for samples collected with the grab technique. Post filtration, all filters were stored in glass Petri dishes and left covered to dry at room temperature. Filters remained covered during the drying period to prevent airborne contamination from the laboratory.

To assess contamination, we did several types of blanks in the lab. Two blank filters left uncovered for more than 24 hours contained an average of 6.5 microplastics. To determine potential laboratory water contamination, we processed a filtered seawater (through a 0.45 μm filter) and a distilled water blank. One red and one blue plastic fiber were quantified in 1.1 L of filtered sea water and two blue and one clear fiber were quantified in 1 L of distilled water, resulting in a total of 5 microplastics counted in 2.1 L of water. From these assessments, the amount of time filters were exposed to air and the amount of water used to rinse containers and filters, we estimated that the average filter received less than 0.5 pieces of microplastic due to lab contamination.

Filters were examined at 45× magnification under a stereo microscope. Microplastic pieces were identified visually based on strict guidelines: no cellular or organic structures visible and equal thickness throughout.[14] We did not have access to FT-IR or Raman spectrometers to positively confirm a polymer, so additional steps were taken when particles were more difficult to identify. In this case, questionable particles were subject to the hot needle test.[45,46] If still unresolved, the piece was removed and inspected under a compound microscope. If after both tests, composition was still questionable, the piece was discarded and not considered as plastic. Questionable particle inspection occurred for approximately 30% of the pieces and most likely resulted in conservative counts for both methodologies as the majority of pieces less than 100 μm could not be confidently counted as plastic.[7] Microplastic pieces were categorized by color (blue, transparent, other color), shape (fiber, other shape), and size (100 μm to 1.5 mm, 1.6–3.2 mm, 3.3–9.6 mm). We estimate the smallest particles identifiable through 45× magnification were approximately 10 μm wide and 180 μm long based on measurements obtained with ImageJ. The size categories were chosen so that filter grid lines (grid length is 3.2 mm) could be used for size reference, resulting in slightly non-standard size class increments. Plastic length was measured by the longest dimension. A subsample of fibers from both techniques ($N = 39$) was measured using a calibrated micrometer scaled eye piece on a stereo microscope. Filters from each technique were randomly selected ($N = 1$ to 32 filters per sampling effort) and the first fiber encountered was measured.

Statistical analyses

Chi-square tests were used to determine if collected microplastic pieces differed by size category, color, or shape between the grab sampling and neuston tow sampling. Among the subsampled fibers selected for precise measurement, Mann–Whitney *U*-tests were used to test for differences in microfiber lengths and widths between the two sampling techniques.

Results

Grab sampling collected over three orders of magnitude more microplastic per volume of water than the neuston tow sampling. Grab sampling collected on average 5.9 ± 4.4 (mean ± SD) microplastics per liter, whereas the neuston tow sampling collected 0.005 ± 0.004 (mean ± SD) microplastics per liter (Table 1). Our neuston tows averaged 213 709 microplastic pieces per km^2 of water or 188 pieces per 0.5 nautical mile tow.

In comparing methodologies, grab sampling collected a higher proportion of small microplastic (100 μm to 1.5 mm), whereas neuston tow sampling collected a higher proportion of large microplastic (3.2–9.6 mm) (Fig. 1a and b, Chi-square test, $p < 0.00001$). Grab sampling collected over three orders of magnitude more microplastic per volume of water than the neuston tow samples in all size categories. The neuston tow method collected three fibrous plastics between 9.6 mm and 16 mm which is larger than the defined size range for microplastics. These were not included in the reported results. Neither method collected plastic larger than 16 mm.

While the majority of plastic pieces collected by both methods were microfibers (91% of 117 pieces and 98% of 1128 pieces in grab samples and neuston tow samples, respectively), grab sampling collected a significantly greater proportion of non-fibrous plastic compared to the neuston tow (Chi-square test, $p < 0.001$).

There was no significant difference between the ratios of colors collected by the two methods (Chi-square test, $p = 0.73$). Both methods found transparent or blue microplastic to comprise ≥90% of microplastic sampled (Fig. 1c and d).

Table 1 Total microplastic pieces per liter (mean ± standard deviation), date and number of samples collected

	Grab sampling		Neuston net sampling	
Date	*N*	Mean ± SD	*N*	Mean ± SD
10/6/14	6	3.4 ± 3.6	2	0.003 ± 0.002
10/13/14	5[a]	10 ± 5.2	2	0.003 ± 0.003
10/28/14	6	4.9 ± 1.1	2	0.008 ± 0.007
Total	**17**	**5.9 ± 4.4**	**6**	**0.005 ± 0.004**

[a] $N = 5$ on 10/13/2014 due to sample loss during laboratory processing.

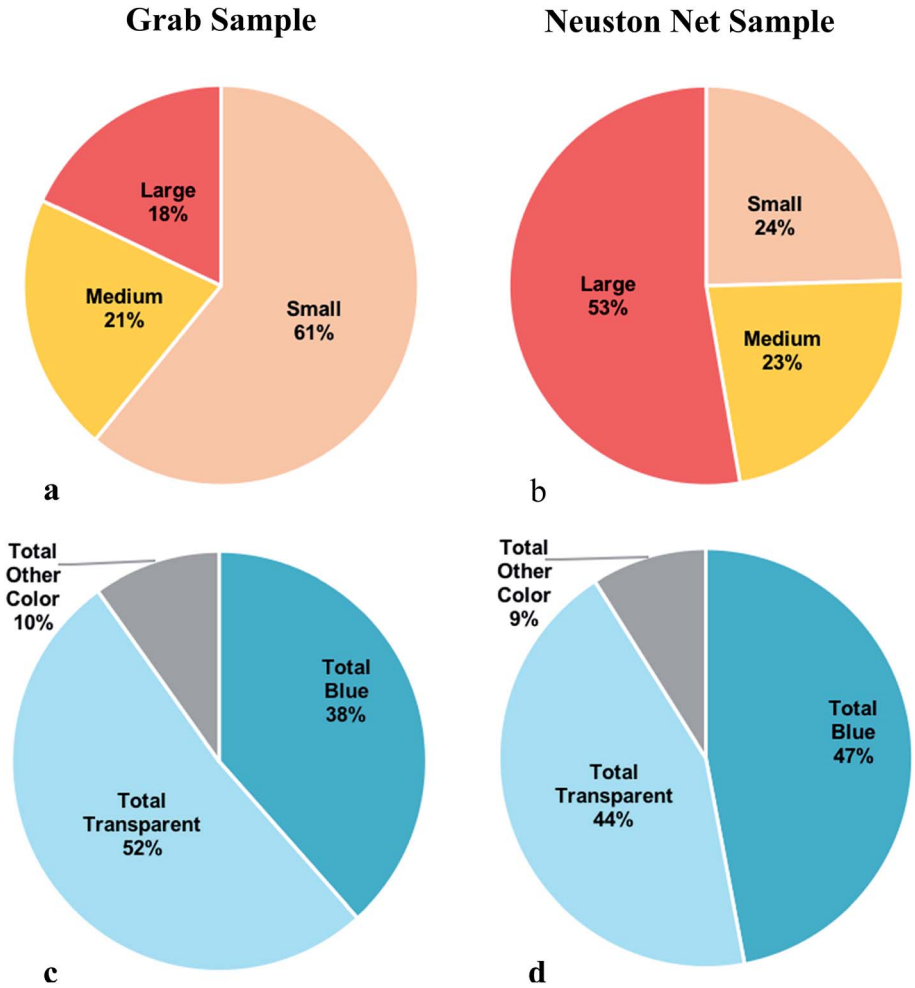

Fig. 1 Microplastic composition proportions by size (a and b) and color (c and d). Microplastic size categories: small (100 μm to 1.5 mm), medium (1.6–3.2 mm), large (3.3–9.6 mm). Total plastic particles: grab sample ($N = 117$), neuston tow sample ($N = 1128$).

A randomly chosen subsample of microfibers from each sample date (total $N = 39$) were measured by length (Fig. 2a) and width (Fig. 2b). A Mann–Whitney U-test showed that microfibers from neuston tow samples were significantly wider (35 ± 23 μm) than fibers from grab samples (21 ± 6 μm, $p = 0.0065$). No significant difference ($p = 0.108$) was found in fiber length between the two sampling techniques (1163 ± 869 μm for tow *versus* 827 ± 594 μm for grab sampling).

There were no significant correlations between average microplastic count and rainfall in previous 48 hours, or wind speed.

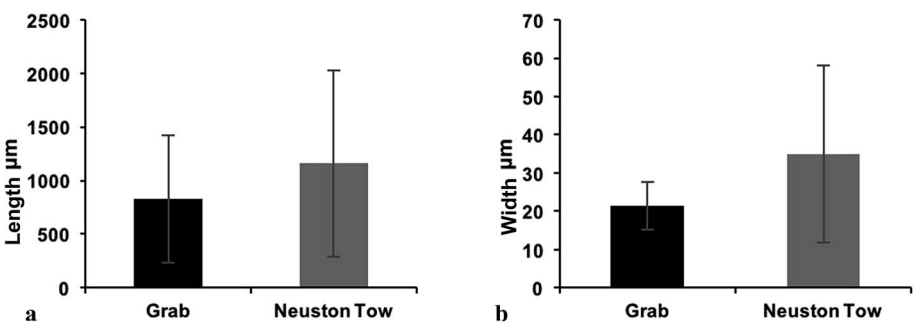

Fig. 2 Microfiber length and width from grab sampling ($N = 18$) and neuston tow sampling ($N = 21$). (a) Mean fiber length of the two sampling techniques showed no significant difference ($p = 0.108$). (b) Mean fiber width showed neuston tow sampling captured significantly wider microfibers ($p = 0.0065$). Error bars show standard deviation.

Discussion

Grab sampling collected over three orders of magnitude more microplastic per volume of water than the neuston tow sampling. Grab sampling also collected more non-fibrous pieces and a higher proportion of plastic in the smallest size class. Solely relying on the neuston tow samples would have resulted in a gross underestimation of the amount of microplastic in Maine coastal waters. The laboratory controls show a low estimate of microplastic contamination, indicating the significant difference between the two technique results is due to the loss of plastic through the neuston net and not due to inflated rates in the grab samples due to contamination. Our results suggest that neuston tow studies and studies extrapolating neuston tow data to worldwide abundances[21,27,47] may similarly be underestimating microplastic concentrations in aquatic environments.

Grab sampling captures small microplastic that neuston tows fail to capture as efficiently because they can pass through the net.[48,49] Similarly, Song *et al.* (2014) found four times greater microplastic abundance in 100 L bulk water (mean 0.21 L^{-1}, $N = 3$) than in 330 μm net tow samples (mean 0.047 L^{-1}, $N = 20$).[38] Studies with plankton-sampling nets have found that the concentrations of microplastic decrease as their size approaches the lower sampling limit.[31] Fibers can become more easily entangled than other plastic shapes. Thus fibers may be selectively collected in neuston tow samples. Alternatively, since the average diameter of the fibers collected in this study are far smaller than the net mesh size, even when bent tenfold, it is likely that many fibers were still not captured. Increasing the number of smaller size categories (for example five categories between 0.45 μm and 1.5 mm) during analysis would allow for a better comparison of sampling effect on microplastic concentration. In our study, the lower size limit of plastic in the neuston tow samples was 335 μm, the mesh size of the net, while in the grab samples, the lower size limit was 100 μm, the smallest size visible under the microscope. It is important to consider that the addition of visual sorting using a microscope can greatly increase the amount of misidentified microplastics.[7] Microplastic down to 0.45 μm could be quantified with this method if higher powered magnification or other analysis was used. This size difference allowed for collection of smaller microplastic using grab sampling, an important step for future studies that aim to characterize plastics using microscopy and verification instrumentation. A study by Song *et al.* (2014) found no significant difference between mean abundances in bulk water and 50 μm hand-net samples,[38] indicating that reducing mesh net size can successfully capture an environmentally representative sample of microplastic abundance. However, it is important to note that reduction of mesh size greatly increases the quantity of biological material captured, making isolation of plastic more difficult. Thus, sampling large volumes of water has many benefits for understanding large-scale microplastic distribution but presents difficulties in processing the sample without using chemical digestion or sieve techniques, which can potentially introduce contamination.

In a field where contamination is an ongoing issue and the presence of microplastic particles in the atmosphere may be significant,[41] sampling techniques need to incorporate contamination controls. A neuston tow is difficult to rinse in a methodologically controlled way prior to each sampling, while the glass (or metal) sample jars used for grab sampling can be heated to 500 °C or are easily rinsed in the laboratory and in the field prior to each sampling. A neuston tow must be hauled onto the boat and rinsed into a storage container for transport; grab samples are capped while still underwater, thus dramatically reducing exposure time to potential air or sampler contamination. For studies solely relying on non-microscope visual sorting of large size class microplastics, airborne contamination may be less of an issue. In this case, neuston net sampling can be a powerful technique for quantification *in situ* and/or without laboratory facilities. In this study, we rinsed the outside of the net with an unfiltered seawater hose, another potential source of contamination, as the hose is made of a plastic blend and the seawater could contain microplastic. The amount of hose contamination may be negligible and easily controlled for with a field blank in future studies. Neuston tow nets are comprised of plastic mesh that may shed and contaminate samples. In contrast, we have been able to minimize contamination in grab samples. They can be taken in glass or stainless steel to minimize possible collection and storage contamination, they can be filtered with a one-step process in the laboratory to reduce contamination from equipment and air exposure, and the only introduced liquid is 0.45 μm filtered water. Lastly, neuston tow samples typically take multiple processing steps. Each step adds potential for more contamination and increased opportunity for loss of plastics; thus, minimizing steps is extremely valuable.

The neuston tow has the benefit of being able to sample very large volumes of water and is easily coupled with other surface studies such as phytoplankton monitoring, but it must be acknowledged that the required mesh size probably lets a large proportion of the smallest microplastics pass through. The neuston tow collects greater amounts of detritus than grab sampling due to the large volume of water sampled, which often results in the need for additional filtering. Multiple studies used a series of sieves to filter samples[15,40,50,51] causing a probable reduction of plastics captured due to loss through the smallest mesh sieve, or plastic sticking to the sieve sides. Additional equipment introduces more opportunities for contamination from the sieves themselves (many studies use plastic sieves), and increased air exposure. Destruction of biological material with hydrogen peroxide or other strong acids or bases[38,43,52,53] has been suggested and used as a technique for eliminating biological material that interferes with efficient filtering. These strong acids have been shown to decrease the size of microplastic and cause bleaching, making visual identification more difficult.[43,52] In neuston tow studies aiming to sort larger microplastics without a microscope, air exposure and the introduction of chemical digestion may be less of an issue due to the size of the plastics quantified.

There was little variance in sea conditions and wind during our sampling, and in this fledgling field we believe that covariates that are easy to measure and may affect microplastic sampling should be recorded. There were no significant trends between quantity of microplastic pieces collected and field conditions in this study. Strong winds,[54,55] rain,[51,56,57] and sea state[35,58,59] may all affect microplastic distribution in surface layers, thus potentially affecting microplastic collection. Therefore, consistent field conditions, or at minimum, recording conditions for future reference, will validate spatial-temporal comparisons.

Grab sampling of microplastic can be used in a variety of citizen science initiatives that have access to a basic laboratory. This method allows for point- and non-point source pollution sampling: at wastewater treatment plants, factories, storm drains, streams, and rivers. Targeted source sampling and collections from shallow freshwater streams can be efficiently accomplished with grab samples. The main limitation to grab sampling is that it only allows for a small amount of water to be collected and analyzed, and is more subject to distribution anomalies on a local scale. Because of the small volume, the method is vulnerable to variance caused by small-scale physical patterns that create uneven distribution of plastics over the sampling scale. Measurements are unbiased with respect to the mean density of microplastics, but may have high variance among samples, leading to less precision than methods that sample larger volumes. It is recommended that future studies test for sample volume effect, *e.g.* a comparison of microplastic abundance in 0.5, 1.0, 10, 50, 100 L samples, which may help to scale plastic estimates over a larger area. Increasing the volume of water would also allow this method to be effectively coupled with other research, similar to the neuston tow net. As such, studies aimed at documenting pollution distribution on a larger, more general scale should require a site-relevant representative number of grab samples, an increase in the sample volume, or a combination of the two techniques (using a smaller mesh net when possible). Combining the two techniques through collecting grab samples along a net tow transect or towing in deeper water and using grab sampling in adjacent areas where towing is more difficult, may be the most precise way of quantifying microplastic distribution in aquatic environments.

There are several ways that sampling methods can be improved moving into the future. Both sampling methods can be strengthened when field and laboratory conditions allow by using Milli-Q® filtered water for rinsing all equipment that will contact the sample. Lab and field blanks should be taken at set increments to evaluate the extent of contamination. Lastly, techniques like pyrolysis gas chromatography/mass spectrometry, micro-Raman or micro-Fourier-transform infrared spectroscopy are useful for verifying and identifying plastics, especially pieces too small to be quantified visually. In the Lagoon of Venice, Italy, a study found microplastic <1 mm in every sediment sample, the majority between 30 and 500 μm,[60] supporting the observation that small microplastic contamination is widespread. These improvements will not be possible for every study, but wherever feasible, they would improve the accuracy of the results.

Conclusion

This study demonstrates the power and limitations of two sampling techniques in microplastic field research. For studies aiming to capture and sort larger microplastics without a microscope, the neuston tow method is preferred, since it can easily sample a greater volume of water, increasing the potential of capturing larger plastic pieces. However, our results confirm the ability of the grab sampling method to capture a larger density and more diverse sampling of microplastic and to minimize contamination with the correct laboratory and field procedures. Underestimation of microplastic, especially with regard to global projections, is a crucial issue in the field today.[31,61] Grab sampling alone or combined with a neuston tow net can facilitate synthesis and comparison of datasets and is a more accurate, flexible technique for microplastic sampling. Grab sampling can capture plastics at the micro- and nano-scale and in a wide variety of environments that are difficult to sample with tow nets. The application of this technique comes at a critical time when methods need to be more consistent to facilitate accurate measurement of the distribution and composition of microplastic in aquatic environments worldwide.

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