Microplastic Analysis in Seawater - Minimum Requirements for Comparative Data Generation

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Scientific, regulatory and societal interests in environmental pollution by microplastics has led to the requirement for quality assured and harmonized approaches to assessing samples for microplastics. Many methods for seawater sampling have emerged with varying degrees of comparability. For example, one of the most widely applied field methods - surface net sampling - is limited in comparative data generation for particles < 300 µm. Other developments using different sampling pumps and automated approaches require method validation and harmonization. Furthermore, there are several different analytical approaches with varying detection limits, costs and technical readiness levels for implementation. This lack of inter-comparison complicates a global understanding of microplastics levels in the marine environment. Encouragingly, methods are continuously being improved to further automatize sampling, sample pretreatment and final analysis with a far greater attention to validation. The minimum requirements for comparative data generation in seawater must include careful consideration of sampling parameters, analytical processes and data treatment all conducted with a high level of QA/QC.

Introduction

Microplastics, or at least particles now considered as microplastics (< 5 mm, GESAMP 2019), were first identified in surface seawater samples dating back to the 1960's during plankton surveys.[1] Investigations which followed generally used similar techniques with nets to sweep surface waters and quantify particles visually[2] and later using a suit of analytical techniques to confirm the presence of plastic polymers, including infrared and Raman spectroscopy and thermal desorption or degradation coupled to mass spectrometry.[3] Surface net sampling has proven valuable in the establishment of long-term data sets.[4-5] Unfortunately, this method is hampered in adverse weather conditions and other limitations related to the determination of accurate sample volumes, lower size limit of particles (mostly > 300 µm) size detection and procedural contamination which can compromise the results. Researchers began to look at alternative methods of analysis, such as the use of seawater intakes on research vessels, submersible pumps and use advanced sensor systems (e.g. FerryBox) to collect large volume samples of which several have shown promise for their use.[6-9]

Academic, non-profit, government and non-governmental organizations have all become engaged in microplastic research: through the development of methods as well as the inclusion of microplastic sampling into ongoing monitoring programs and strategic projects. As the knowledge surrounding microplastic assessment has increased, researchers have turned their attention to defining methods which allow the collection of robust data with quality an essential consideration for project design.[10-12] There is a strong focus towards the production of quality controlled and quality assured (QA/QC) data, with limited sample manipulation and a general need for automated methods of detection.[13, 14] Currently no sensors or on-line measuring technologies exist due to the large sample volumes needed, pre-concentration of the samples and often large amounts of biological interferences. Therefore, data generation relies on sound sampling methodologies and analytical processing in the laboratory. Limitations to comparative data generation cover themes such as the inclusion and exclusion of certain sizes, sample contamination, inconsistent units of reporting, lack of validation in processing methods, accuracy or representativeness of samples and validation of observed/visual results using

analytical techniques.[15] In the following document, the approaches to seawater microplastic assessment are presented with a view towards methods harmonization and minimum requirements for comparative data generation.

Field sampling for microplastics in seawater

Collection of representative samples is of upmost importance. Much literature has assessed the use of different sampling approaches to seawater (Table 1), and the current limiting factor is the collection of large enough volumes to generate a representative sample. Further, when sampling in areas of high biological activity, the samples may be compromised by large amounts of organic matter clogging the collection devices. This is especially true when using small mesh sizes.[12, 16]

Net sampling

This approach is by far the most commonly applied technique for sampling surface water (manta or neuston nets), subsurface waters and the water column (neuston or bongo nets). Nets are towed in surface waters or the water column for a set duration, rinsed on deck and any anthropogenic particles are categorized by morphology (size, shape, color) and sometimes weighed[4, 5]. Many studies using nets sampling focus on the visible identification of the larger fraction of particle 1-5 mm, often these methods do not use spectroscopic confirmation and plastics are only identified with the naked eye. Although net sampling methods enable sampling of large volumes, the disadvantages are discriminating particles smaller than the nominal mesh size, sampling water volumes passing through the net can only be estimated, nets often bounce on the water surface in adverse weather conditions and it can be very difficult to prevent contamination from working on deck of vessels. Further, when clogging occurs the sample may not be representative. Therefore, it is important that samplers can as accurately as possible, estimate the volume of water which has passed through the net, with a flow meter, or through the calculation of distance travelled.[12]

Bulk water samples

There are many approaches to collecting bulk water samples. A volume-reduced water sample consists of pumping water (manually or using a motor) through a filter and out through a flow meter. These samples can be collected from a variety of sampling platforms: large or small vessels, from static platforms and the shoreline. These approaches are generally used when targeting microplastics in the smaller size ranges < 300 µm. QA/QC procedures are fundamental from sample collection into processing, including field and laboratory procedural blanks, which are easier to achieve using bulk water samples. In most cases, samples may be volume reduced in the field where necessary, but the analysis of samples is conducted under controlled laboratory conditions. Researchers began taking bulk water samples using seawater intakes on research vessels in many of the world's oceans[7-9] and have developed to filtering apparatus being incorporated into other seawater monitoring set up, such as the FerryBox system which are deployed on vessels of opportunity.[6] The standard FerryBox system collects continuous data on temperature, salinity, florescence, turbidity, as well as nutrient analysis, continuous plankton recording. The incorporation of microplastics into these systems will allow comparative data generation which can be accurately coupled to the environmental parameters at the time of sampling. Another example of using vessels of opportunity was the inclusion of filtration apparatus on sailing vessels participating in the Volvo Ocean Race, 2017. Samples (n = 68) were collected on board Team AkzoNobel. The analysis was performed in the laboratory used a combination of Raman spectroscopy to identify the particles, and a camera for microplastics particle size.[17] Smaller bulk water samples include the collection of seawater in CTD rosettes.[9]

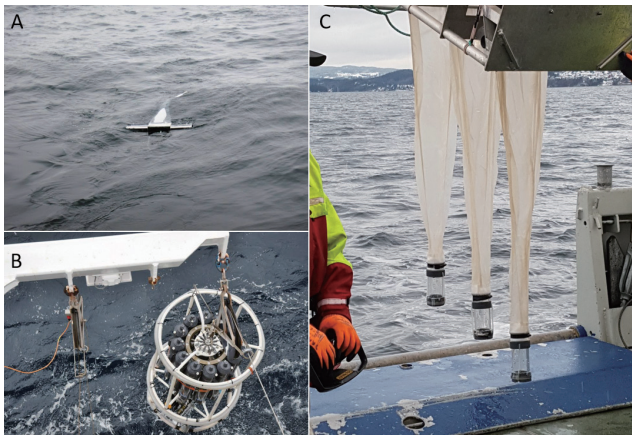

Figure 1 Sampling devices used for microplastic sampling in sea water, A: Manta net; B: CTD rosette bottles, C: multinet

Challenges of continuous measurement

Automated methods, such as continuous measurements, are advantageous as they can collected data without intervention. The representativeness of samples is often complicated by the distance travelled on large vessels or the volume sampled restricted by small water intakes (~in the range of a few cm). As often large volumes of water have to be sampled (> 1000 L), infrequent changing of filters could result in relatively large areas sampled (> 100 km). This is both an advantage, large trajectories can be sampled, but also a disadvantage if a small-scale special resolution is needed. Another challenge includes the presence

of organic material which can cause clogging and further compromise collected samples.[12] Coupled with the need for changing filters, it is paramount that QA/QC is strict during sampling. Field blanks can be carried out to monitor the levels of airborne particles in samples, as well as the risk of contamination from the sampler or the equipment. Without these controls it is impossible for researchers to discern the true levels of microplastics in seawater as levels in the marine environment often are low. In summary, the steps made towards automated/semi-automated sample collection are promising, but far from having a readiness for worldwide implementation.

Table 1 Advantages and limitations of sampling approaches to seawater

	Advantages	Limitations
Surface water nets	Long term data sets Visual sorting possible with fraction >1 mm Sample large area	Surface samples only Weather dependent Estimated flow/volume Lower size limit often misses smaller particles (e.g. 300 µm)
Pumping systems - seawater intake e.g. FerryBox	Accurate flow/volume Large volume sampled	Subsurface only
Pumping systems - *In situ pumps*	Can be deployed at variable depths in the water column Accurate flow/volume Large volume sampled	Weather dependent
Bulk water samplers - CTD rosettes	Can be deployed at variable depths in the water column Can collect replicates	Low water volume (~20 litres)

Laboratory processing of seawater samples

Once samples have been collected from seawater, the analytical steps taken are critical to producing robust and comparative data. As with sample collection, a high level of QA/QC is recommended for studies reliant on laboratory and microscopy analysis. This allows the researchers to check the validity of any processing steps introduced before assessment and continue to monitor sources of procedural contamination. Depending on the interfering material collected on the sampling filters and the processing steps needed before the analysis are not discussed herein, the reader is referred to recent reviews on the matter.[18]

A combination of approaches, from visual assessment with the naked eye through to automated spectroscopic methods can be used for the final analysis. A very recent critical assessment of the analytical methods associated to harmonized and coast efficient analysis of microplastics has been published.[3] This review presented the available techniques which includes naked eye detection, optical microscopy, uses of dyes and stains, flow cytometry, Fourier-Transform Infrared (FT-IR) spectroscopy and microscopy, Raman spectroscopy and microscopy and thermal degradation/desorption coupled to gas-chromatography/mass spectrometry. The methods chosen will impede the level of comparison between investigations. Especially when the aim is to quantify the presence of different plastic types. For example, when working with samples contaminating particles from 1-5 mm in size, researchers can use the unaided visual identification with a high level of confidence to identify plastics, but particles < 1 mm require more supported techniques (microscope plus analytical validation) to determine the presence of synthetic polymers as error values can reach up to 70%.[3, 11, 12] Without polymer identification, this may lead to a high level of misidentification, especially when particles size is below 50-100 µm. As such, the use of spectroscopic methods is strongly recommended when working with the identification of microplastics < 1 mm, and fundamental for particles < 100 µm.

FT-IR and Raman spectroscopic methods allow the identification of particle composition by producing a fingerprint spectrum which is unique to different materials. Such that plastic spectra can be differentiated from those produced by natural materials. FT-IR and Raman are both complementary techniques, as molecular vibrations which are inactive with FT-IR, can be active for Raman, and vice versa. Spectroscopic methods can also be coupled to microscope set ups, allowing the application of polymer identification to small particles (µFT-IR ca. 10 µm, µRaman ca. 1 µm).[3] When particles are preselected for FT-IR/Raman using optical light-microscopy by the operator, this can introduce a bias in the analysis, and in some instances transparent or translucent particles as well as very small particles might be overlooked during the preselection process.[3] Hence, a reduced proportion of operator interference is encouraged, and researchers continue to seek advancements in µFT-IR and µRaman approaches. Often,

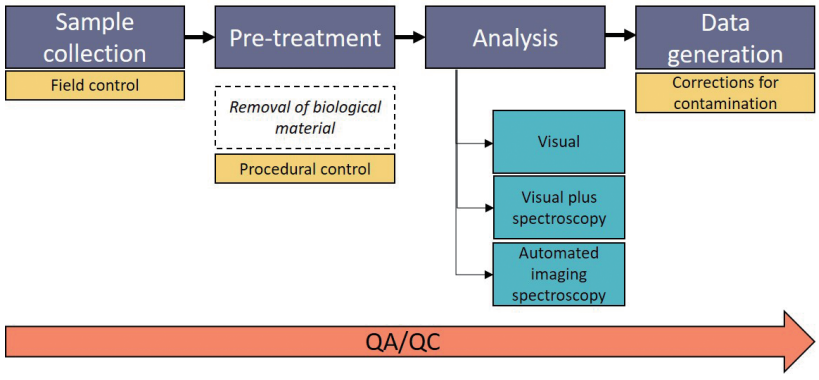

Figure 2 Schematic of steps requires for comparative data generation

the use of spectroscopic methods is costly and time consuming, as such many studies use subsamples of representative particles.

To counteract this, automated spectral methods are being developed to enable high throughput of samples but are currently still limited to low sample volumes. Furthermore, the automatic samples analysis requires significant sample preparation and clean-up to be effective. Nevertheless, μFT-IR has been seen to be a powerful tool and the atomization reduces time and demand of data generation.[3] Providing the researchers are clear in their approach, choice of methods and use clear reporting guidelines, they can generate comparative data.

Conclusion

In the microplastics field of research there are many different sampling approaches and technologies available to investigate seawater microplastics. Developments are hampered by procedural contamination as microplastics and fibres can be introduced by the sampler, the air or sample equipment. This requires a thorough understanding of potential sources of error and effort to minimize intervention with samples in the field. A high level of QA/QC is required from collection through to data generation. This is especially important when studies reliant are reliant on laboratory/microscopy analysis. Method development is continuously ongoing to further automatize sampling, sample pretreatment and final analysis with a far greater attention to validation.

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* This content is based on our investigation at the year of issue unless otherwise stated.

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